

Theoretical and Neutron Scattering Approaches for Understanding the Behavior of Single-Chain Nanoparticles

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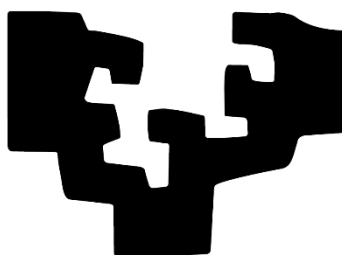
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ABSTRACT

The ability to design and manipulate matter at the nanometric scale has opened unique opportunities for the development of advanced functional materials. Within this context, single-chain technology has emerged as a powerful approach to construct discrete soft nano-objects by working at the level of individual polymer chains. Among the different approaches accessible through this strategy, single-chain nanoparticles (SCNPs) have attracted particular attention: unimolecular nanostructures formed through the intra-molecular folding or collapse of individual synthetic polymer chains, typically displaying dimensions below 20 nm, with tunable size, morphology and internal structure. Depending on the precursor architecture and folding conditions, SCNPs can adopt a variety of conformations — from sparse or globular morphologies to more complex architectures such as tadpole-shaped or Janus nanoparticles — each with distinct structural and functional properties. From a theoretical perspective, the Elastic SCNP Model (ESM) provides a quantitative framework to describe SCNP size and conformation as a function of chain length and intra-molecular cross-linking degree.

Among the available synthesis strategies, the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC), one of the most outstanding click chemistry reactions, has proven particularly powerful for SCNP formation, enabling precise intra-molecular cross-linking under mild and controlled conditions. Photoactivated folding via ultraviolet (UV) irradiation constitutes an attractive alternative, allowing bond formation to be triggered on demand without the need for metal catalysts. Both approaches are relevant to this work, either as direct synthetic strategies or as reference systems for comparison. The nature and density of the resulting intra-molecular bonds play a decisive role in determining not only SCNP size and morphology, but also their structural and dynamical behavior in the bulk state.

Beyond isolated nanoparticles, incorporating SCNPs into polymeric matrices gives rise to all-polymer nanocomposites— composite materials in which both the matrix and the dispersed phase are polymeric —, which provide an ideal platform to investigate how chain topology influences polymer properties at the molecular level, while keeping the chemistry essentially unchanged.

In this framework, this thesis aims to provide a comprehensive understanding of the structural and dynamical behavior of SCNP-based systems, approached from both a theoretical and an experimental perspective.

The manuscript begins with a general introduction to polymers and the key physical concepts underlying these systems, progressively deepening into the fields of nanoscience, single-chain technology, and more specifically, SCNPs. Their synthesis methods are presented, along with the different morphologies that SCNPs can adopt by tuning the synthesis parameters of both the precursor and the nanoparticles themselves, as well as their dependence on environmental conditions. These morphological differences serve as a starting point for the theoretical study of SCNPs, approached through models such as the ESM. From an experimental perspective, all-polymer nanocomposites are also introduced as systems where the incorporation of SCNPs proves particularly interesting for investigating their properties.

Chapter 2 presents a theoretical study of three representative scenarios for SCNPs within the ESM framework: surface-grafted brushes, tadpole-like conformations, and severe crowding

conditions. For SCNP brushes, scaling laws are derived for key structural properties, revealing that brush thickness can be tuned through the intrachain cross-linking degree, opening possibilities for drug delivery applications. Simple structure-size relationships are also established for tadpole-shaped SCNPs, showing good agreement with experimental data, while a predictive method for SCNP size under crowding is proposed, with implications for the study of intrinsically disordered proteins.

Before presenting the experimental results, Chapter 3 provides an overview of the characterization techniques employed throughout this work. These include chemical characterization by Size Exclusion Chromatography (SEC), Nuclear Magnetic Resonance (NMR), Elemental Analysis (EA) and Fourier Transform Infrared Spectroscopy (FTIR), thermal analysis by Differential Scanning Calorimetry (DSC), and mechanical characterization by rheology. Scattering techniques are also covered, ranging from WAXS to neutron scattering, with special emphasis placed on the latter, including an introduction to their fundamental concepts, given their unique ability to probe structure and dynamics at the microscopic scale. Several instruments at the Institut Laue-Langevin (ILL) are also described, covering a wide range of time and length scales, making them particularly valuable for the investigation of SCNPs and all-polymer nanocomposites.

Chapter 4 details the synthesis of the precursor polymers P(THF-ran-ECH) (hPrec) and its deuterated counterpart P(TDF-ran-ECD) (dPrec), as well as the preparation of the corresponding SCNPs and nanocomposite samples. Molecular weights of 22 kDa and 36.5 kDa are obtained for hPrec and dPrec respectively, both narrowly distributed, with an ECH/ECD content of approximately 27%, and are subsequently functionalized with azide groups for the formation of SCNPs via click chemistry. Through DSC, rheology, and WAXS, the main effects of intra-molecular crosslinking are identified: a broadening and upward shift of the glass transition, a slower structural relaxation, and no significant changes in the short-range structural organization. Neutron scattering techniques provide deeper microscopic insight into these effects. Neutron Spin Echo (NSE) and Quasi Elastic Neutron Scattering (QENS) measurements reveal that intra-molecular crosslinking leaves local dynamics largely unaffected while inducing pronounced slowing down and enhanced heterogeneity at intermediate length and time scales, with effects more pronounced in click-synthesized SCNPs compared with UV-SCNPs from earlier studies. In all-polymer nanocomposites, isotopic labeling enables selective probing of each component, revealing that SCNPs and matrix chains develop contrasting dynamical behaviors that compensate at the macroscopic level.

Finally, Chapter 5 summarizes the main conclusions of this thesis, which combines two complementary but independent approaches: a theoretical study of SCNP structural properties under different conditions and an experimental investigation of the structural and dynamical consequences of intramolecular cross-linking in polymer melts and all-polymer nanocomposites.

RESUMEN

La capacidad de diseñar y manipular la materia a escala nanométrica ha abierto oportunidades únicas para el desarrollo de materiales funcionales avanzados. En este contexto, la tecnología de cadena única ha surgido como una poderosa estrategia para construir nano-objetos blandos discretos trabajando a nivel de cadenas poliméricas individuales. Entre los diferentes enfoques accesibles mediante esta estrategia, las nanopartículas de cadena única (SCNPs, del inglés Single-Chain Nanoparticles) han atraído especial atención: nanoestructuras unimoleculares formadas mediante el plegamiento o colapso intramolecular de cadenas poliméricas sintéticas individuales, con dimensiones típicamente inferiores a 20 nm y tamaño, morfología y estructura interna ajustables. Dependiendo de la arquitectura del precursor y de las condiciones de plegamiento, las SCNPs pueden adoptar diversas conformaciones — desde morfologías dispersas o globulares hasta arquitecturas más complejas como nanopartículas en forma de “renacuajo” o de tipo Janus — cada una con propiedades estructurales y funcionales distintas. Desde una perspectiva teórica, el Modelo Elástico de SCNPs (ESM, del inglés Elastic SCNP Model) proporciona un marco cuantitativo para describir el tamaño y la conformación de las SCNPs en función de la longitud de cadena y el grado de entrecruzamiento intramolecular.

Entre las estrategias de síntesis disponibles, la cicloadición azida-alquino catalizada por cobre(I) (CuAAC, del inglés Copper(I)-catalyzed Azide-Alkyne Cycloaddition), una de las reacciones de química *click* más destacadas, ha demostrado ser especialmente poderosa para la formación de SCNPs, permitiendo un entrecruzamiento intramolecular preciso en condiciones suaves y controladas. El plegamiento fotoactivado mediante irradiación ultravioleta (UV) constituye una alternativa atractiva, permitiendo desencadenar la formación de enlaces bajo demanda sin necesidad de catalizadores metálicos. Ambas estrategias son relevantes en este trabajo, ya sea como rutas de síntesis directas o como sistemas de referencia para comparación. La naturaleza y densidad de los enlaces intramoleculares resultantes desempeñan un papel decisivo en la determinación no solo del tamaño y la morfología de las SCNPs, sino también de su comportamiento estructural y dinámico en estado *bulk*.

Más allá de las nanopartículas aisladas, la incorporación de SCNPs en matrices poliméricas da lugar a nanocompuestos “todo-poliméricos” — materiales compuestos en los que tanto la matriz como la fase dispersa son poliméricas —, que constituyen una plataforma ideal para investigar cómo la topología de cadena influye en las propiedades del polímero a nivel molecular, manteniendo esencialmente la misma química.

En este marco, el objetivo de esta tesis es proporcionar una comprensión profunda del comportamiento estructural y dinámico de sistemas basados en SCNPs, abordado desde una perspectiva tanto teórica como experimental.

El manuscrito comienza con una introducción general a los polímeros y a los conceptos físicos fundamentales que subyacen a estos sistemas, profundizando progresivamente en los campos de la nanociencia, la tecnología de cadena única y, más concretamente, las SCNPs. Se presentan sus métodos de síntesis, junto con las diferentes morfologías que las SCNPs pueden adoptar ajustando los parámetros de síntesis tanto del precursor como de las propias nanopartículas, así como su dependencia de las condiciones ambientales. Estas diferencias morfológicas sirven como punto de partida para el estudio teórico de las SCNPs, abordado mediante modelos como el ESM. Desde una perspectiva experimental, se introducen también los nanocompuestos “todo-

poliméricos” como sistemas en los que la incorporación de SCNPs resulta especialmente interesante para investigar sus propiedades.

El Capítulo 2 presenta un estudio teórico de tres escenarios representativos para las SCNPs en el marco del ESM: cepillos injertados en superficies, conformaciones en forma de “renacuajo” y condiciones de confinamiento severo. Para los cepillos de SCNPs, se derivan leyes de escala para propiedades estructurales clave, revelando que el espesor del cepillo puede ajustarse mediante el grado de entrecruzamiento intracadena, abriendo posibilidades para aplicaciones de liberación de fármacos. Se establecen también relaciones estructura-tamaño para SCNPs en forma de “renacuajo”, mostrando un buen acuerdo con datos experimentales, mientras que se propone un método predictivo para el tamaño de SCNPs en condiciones de confinamiento, con implicaciones para el estudio de proteínas intrínsecamente desordenadas.

Antes de presentar los resultados experimentales, el Capítulo 3 ofrece una visión general de las técnicas de caracterización empleadas a lo largo de este trabajo. Estas incluyen caracterización química mediante Cromatografía de Exclusión por Tamaño (SEC), Resonancia Magnética Nuclear (RMN), Análisis Elemental (EA) y Espectroscopía Infrarroja por Transformada de Fourier (FTIR), análisis térmico mediante Calorimetría Diferencial de Barrido (DSC) y caracterización mecánica mediante reología. También se cubren las técnicas de dispersión, desde WAXS hasta la dispersión de neutrones, con especial énfasis en esta última, incluyendo una introducción a sus conceptos fundamentales, dada su capacidad única para estudiar la estructura y la dinámica a escala microscópica. Se describen asimismo varios instrumentos del Institut Laue-Langevin (ILL), que cubren un amplio rango de escalas temporales y espaciales, lo que los hace especialmente valiosos para la investigación de SCNPs y nanocomposites “todo-poliméricos”.

El Capítulo 4 detalla la síntesis de los polímeros precursores P(THF-ran-ECH) (hPrec) y su homólogo deuterado P(TDF-ran-ECD) (dPrec), así como la preparación de las correspondientes SCNPs y muestras de nanocomposites. Se obtienen pesos moleculares de 22 kDa y 36,5 kDa para hPrec y dPrec respectivamente, ambos con distribución estrecha y un contenido de ECH/ECD de aproximadamente el 27%, que son posteriormente funcionalizados con grupos azida para la formación de SCNPs mediante química *click*. A través de DSC, reología y WAXS, se identifican los principales efectos del entrecruzamiento intramolecular: un ensanchamiento y desplazamiento hacia valores más altos de la transición vítrea, una relajación estructural más lenta y ningún cambio significativo en la organización estructural a corto alcance. Las técnicas de dispersión de neutrones proporcionan una visión microscópica más profunda de estos efectos. Las medidas de eco de spin de neutrones (NSE) y dispersión cuasielástica (QENS) revelan que el entrecruzamiento intramolecular apenas afecta a la dinámica local, mientras que induce una pronunciada ralentización y una mayor heterogeneidad a escalas intermedias de longitud y tiempo, con efectos más pronunciados en las SCNPs sintetizadas por química *click* en comparación con las UV-SCNPs de estudios previos. En los nanocomposites “todo-poliméricos”, el marcaje isotópico permite estudiar selectivamente la dinámica de cada componente, revelando que las SCNPs y las cadenas de la matriz desarrollan comportamientos dinámicos contrastantes que se compensan a nivel macroscópico.

Finalmente, el Capítulo 5 resume las principales conclusiones de esta tesis, que combina dos enfoques complementarios pero independientes: un estudio teórico de las propiedades estructurales de las SCNPs en diferentes condiciones y una investigación experimental de las consecuencias estructurales y dinámicas del entrecruzamiento intramolecular en fundidos poliméricos y nanocomposites “todo-poliméricos”.

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CHAPTER 1

Introduction

1.1. Polymers: Definition and General Aspects

Polymers are macromolecular structures formed by the covalent bonding of repeating molecular units known as monomers.^[1] The term *polymer* includes not only synthetic materials but also natural macromolecules such as proteins or nucleic acids.

When classifying different types of polymers, several parameters or characteristics must be considered. On one hand, depending on the nature of the monomers present in the chain, homopolymers contain only one type of monomer, whereas heteropolymers may contain two (copolymer) or more different types of monomers. Heteropolymers can in turn be classified according to the arrangement of monomers along the chain, as shown in Fig. 1.1.

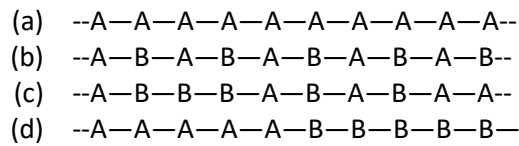


Figure 1.1. Polymeric structures, where A and B are different monomers. (a) homopolymer, (b) alternating copolymer, (c) random copolymer, and (d) block copolymer.

A second classification of polymers is based on their structure, distinguishing mainly between linear and branched polymers. The former are the simplest, since they consist of a sequence of monomers arranged one after another, possessing only one initial and one terminal end. In contrast, branched polymers contain side chains attached to the main chain, resulting in a wide variety of possible architectures.

Polymers can exhibit either amorphous or semi-crystalline morphologies. In amorphous polymers, the molecular chains are randomly oriented and entangled, whereas in semi-crystalline polymers, the chains pack into ordered regions known as crystallites, which are embedded within an amorphous matrix. A polymer cannot be 100% crystalline.

Unlike small molecules or proteins, the molecular weight of synthetic polymers does not have a single value but instead follows a distribution that depends on the synthesis process.^[1,2] To characterize or parametrize this distribution, different types of average molecular weights can be defined.

The number-average molecular weight, M_n , is the arithmetic mean of the mass

$$M_n = \frac{\sum_i N_i M_i}{\sum_i N_i} \quad (1.1)$$

where M_i is the molecular mass of chain i and N_i is the number of chains with molecular weight M_i .

The weight-average molecular weight, M_w , represents the weight-averaged mass of the different chains:

$$M_w = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i} \quad (1.2)$$

The ratio between M_w and M_n is known as the dispersity index ($\mathcal{D}$). High $\mathcal{D}$ values correspond to a broad size distribution, and viceversa.

1.1.1. Polymer Physics: Size and Scaling Laws

Polymer physics provides a conceptual framework to describe how the characteristic size of a polymer chain emerges from simple scaling arguments, largely independent of its microscopic composition.

One of the simplest idealizations of a flexible polymer chain consists of representing it as a random walk on a lattice where the polymer is described as a succession of N steps of fixed length α , starting from one end of the chain and reaching an arbitrary endpoint.^[3] At each step, the walk may proceed toward any of the nearest-neighbor sites with equal statistical weight.

More generally, when focusing on large-scale conformational properties, polymers are conveniently described within a coarse-grained framework. In this approach, a polymer chain is modeled as a sequence of N monomers, represented as beads located at positions $\vec{r}_i$ whose conformation is fully determined by the set of bond vectors connecting successive beads. The overall size of the chain can then be characterized by the end-to-end vector:

$$\vec{R}_e = \vec{r}_N - \vec{r}_1 \quad (1.3)$$

Its modulus, $|\vec{R}_e|$ provides a direct measure of chain extension. In practice, this magnitude is averaged over the ensemble of accessible chain conformations, yielding the average or mean quantity R_e .

In practice, both the squared end-to-end distance and the squared radius of gyration are averaged over the ensemble of accessible chain conformations, yielding the mean squared quantities $\bar{R}_e^2$ and $\bar{R}_g^2$.

Soft matter systems such as polymer solutions are structurally and dynamically complex, and their theoretical description is often highly elaborate and not always quantitative. Nevertheless, valuable physical insight can be obtained by analyzing how macroscopic observables depend on a small number of relevant variables, while neglecting numerical prefactors. This viewpoint leads to scaling laws, which capture the functional dependance between physical quantities and play a central role in polymer physics.

In the ideal case of a fully flexible polymer, where bond vectors are uncorrelated and can orient freely in space, the chain behaves as a three-dimensional Gaussian random walk.^[3] Within this model, the characteristic average size R of the chains scales with the number of monomers N according to

$$R \sim N^\nu \quad (1.4)$$

with the Flory exponent $\nu = 1/2$. Here, R refers to e.g. the above introduced end-to-end distance R_e or to other characteristic sizes as the average radius of gyration the average hydrodynamic radius (see definitions later). This behavior is representative of polymers under θ -solvent conditions, where attractive and repulsive interactions effectively cancel, and for a polymer in melt state.

Real polymer chains, however, cannot self-intersect due to excluded-volume interactions between monomers. In good solvents, these repulsive interactions lead to chain swelling and invalidate the Gaussian description. A more realistic representation is provided by the self-avoiding walk model, for which the scaling exponent increases to $\nu = 3/5$. This result can be rationalized within Flory theory, which balances the entropic elasticity of the chain against

excluded-volume interactions and yields an approximate but remarkably accurate prediction for the scaling behavior of polymer size in three dimensions.

While the average end-to-end distance offers a useful measure of chain dimensions, polymer sizes are more commonly characterized experimentally using quantities such as the radius of gyration (R_g) and the hydrodynamic radius (R_H).

The radius of gyration $R_g = \sqrt{\langle R_g^2 \rangle}$ is defined in terms of the sum of the squared distances between the individual beads and the center of mass of the chain R_{cm} :

$$R_g^2 = \frac{\sum_{i=1}^N m_i (\vec{r}_i - \vec{R}_{cm})^2}{\sum_{i=1}^N m_i} \quad (1.5)$$

where m_i is the mass of the bead and R_{cm} is given by:

$$\vec{R}_{cm} = \frac{\sum_{i=1}^N m_i \vec{r}_i}{\sum_{i=1}^N m_i} \quad (1.6)$$

If the mass of a monomer m_i is assumed to be constant (polymers composed of identical monomers), Eq. (1.5) can be simplified to:

$$R_g^2 = \frac{1}{N} \sum_{i=1}^N (\vec{r}_i - \vec{R}_{cm})^2 \quad (1.7)$$

As for the end-to-end distance, the average radius of gyration in a real system is obtained by averaging over all the different chains.

The dimension of a polymer chain in solution can be also characterized by the hydrodynamic radius R_H , which is defined as the radius of a hypothetical sphere that diffuses through a solvent at the same rate as the polymer. Accordingly, a polymer with hydrodynamic radius R_H exhibits the same center-of-mass diffusion coefficient as a sphere of radius R_H . Once the diffusion coefficient D_{CM} is measured, the hydrodynamic radius can be obtained from the Stokes–Einstein relation

$$R_H = \frac{k_B T}{6\pi\eta_s D_{CM}} \quad (1.8)$$

where η_s is the viscosity of the solvent, k_B the Boltzmann constant and T is the temperature.

The scaling concepts and characteristic length scales introduced in this section will be taken as a starting point for the theoretical description of the SCNPs investigated in this thesis. In the following section, these ideas are extended by introducing an elastic model that exploits the balance between chain elasticity and effective interactions to describe SCNPs conformations and size-related observables.

1.1.2. Polymer Dynamics

Because of their macromolecular character, the structural and dynamic processes of polymers strongly depend on the length and time scales under observation (Fig. 1.2).^[4] At lengths comparable to the R_g , the chain character of the macromolecule prevails over the role of pendant side groups or the specific features of the polymer backbone. The characteristic

dynamical processes present at this level (chain diffusion, Rouse dynamics and reptation) depend on the chain length and can be very slow.

At inter-molecular length scales ($\approx 10 \text{ \AA}$), the main dynamical process is the so-called α relaxation, which characteristic time increases dramatically as the temperature approaches the glass transition temperature T_g . At small (molecular) length scales ($\approx 1 \text{ \AA}$), the motions are usually localized and activated. Among these dynamical processes, one can find vibrations, such as the Boson peak,^[5] and secondary relaxations like the β -relaxation.

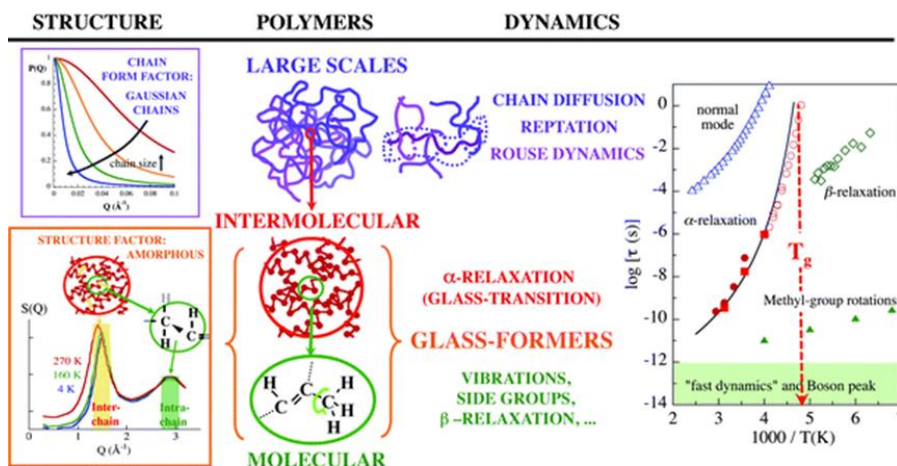


Figure 1.2. Relevant polymer features as a function of the observation length scale. On the right, the relaxation map of polyisoprene illustrates the temperature dependence of the relaxation times associated with the main dynamical processes. Q is the scattering vector, a magnitude inversely proportional to the length scale explored. The normal mode is characteristic of chain dynamics (end-to-end vector fluctuations) and depends on the molecular weight. Reproduced from Ref^[4].

The Glass Transition

Due to their long, entangled molecular chains and often irregular microstructures, many polymers are unable to crystallize (even partially) and therefore exhibit predominantly amorphous behavior. One of the most important phenomena associated with the amorphous state is the glass transition, which is not exclusive to polymers but is common to all glass-forming systems, including low-molecular-weight substances.

Upon cooling from the melt, polymer viscosity increases as molecular mobility decreases. If crystallization is avoided below the melting temperature, the material enters a supercooled liquid state. Further cooling leads to a temperature range in which molecular motions become so slow that polymer chains can no longer rearrange on the experimental time scale, resulting in a kinetically frozen, glassy state. The temperature associated with this transition is referred to as the glass transition temperature, T_g .

Unlike first-order phase transitions such as melting, the glass transition occurs over a temperature range and is strongly dependent on the cooling rate. Slower cooling allows greater molecular relaxation, leading to a lower apparent T_g , whereas faster cooling results in a higher T_g . This behavior reflects the kinetic and non-equilibrium nature of the glass transition, which depends on the relationship between molecular relaxation times and experimental time scales. Consequently, the properties of the glassy state depend on the thermal history of the material.

The glass transition can be observed not only in fully amorphous polymers but also in the amorphous regions of semi-crystalline polymers.

From a mechanical perspective, the glass transition strongly influences the viscoelastic behavior of polymers and defines several temperature-dependent regimes.

- Below T_g ($T < T_g$), in the glassy region, polymers are typically stiff and brittle, with molecular motions limited to localized vibrations and short-range rotations, resulting in a high and nearly constant elastic modulus.
- Near T_g ($T \approx T_g$), within the glass transition region, segmental motions become active, leading to a dramatic drop in modulus over a relatively narrow temperature range and producing a tough, leather-like behavior.
- Above T_g ($T > T_g$), polymers enter the rubbery regime, characterized by significant elastic deformation due to enhanced chain mobility. The extent of this region depends on molecular architecture, such as molecular weight, degree of crystallinity, and crosslink density. At sufficiently high temperatures, chain entanglements relax, and the material exhibits rubbery flow and eventually viscous liquid flow.

Relaxation Processes

The glass transition is directly related to the so-called structural or α -relaxation, a fundamental dynamical process common to all glass-forming materials, including polymers. This relaxation governs the transition from the rubbery or viscous state to the glassy state and reflects the dramatic slowing down of molecular mobility as the temperature approaches the glass transition temperature, T_g . In addition to the α -relaxation, polymers also exhibit secondary relaxation processes, commonly referred to as β -, γ -, and higher-order relaxations, which are typically observed below T_g and involve more localized molecular motions.

The Segmental α -Relaxation

The segmental α -relaxation is strongly associated with the glass transition^[6] and arises from cooperative motions of polymer chain segments. As the temperature is reduced toward T_g , these segmental motions slow down drastically, leading to a rapid increase of the characteristic relaxation time and eventually to a kinetically frozen glassy state. The α -relaxation and the glass transition are affected in a similar way by molecular parameters such as molecular weight, crosslink density, and chain architecture. The α -process is generally attributed to conformational rearrangements of the polymer backbone involving long-range cooperative motions.

The relaxation function corresponding to the α -relaxation cannot be described by a single exponential decay. Instead, it is commonly modeled using a stretched exponential function, known as the Kohlrausch–Williams–Watts (KWW) function:

$$\phi(t) \propto \exp \left[- \left(\frac{t}{\tau_w} \right)^\beta \right] \quad (1.9)$$

where β is the stretching exponent and τ_w the characteristic time.

The temperature dependence of the α -relaxation time τ (which may correspond to the previously introduced τ_w or, e.g., to the average relaxation time) is non-Arrhenius and is typically described by the Vogel–Fulcher–Tammann (VFT) equation:^[7-9]

$$\tau(T) = \tau_0 \exp \left[\frac{DT_0}{T - T_0} \right] \quad (1.10)$$

where τ_0 is the relaxation time in the high-temperature limit, D (temperature independent) is the fragility parameter and T_0 is the Vogel temperature, corresponding to the temperature at which the relaxation time diverges. The fragility parameter provides a quantitative measure of how rapidly the dynamics of a material slow down as it approaches T_g . According to Angell's classification, materials exhibiting strong deviations from Arrhenius behavior are termed fragile,^[10] whereas those following an Arrhenius-like temperature dependence are classified as "strong".^[11] Most glass-forming polymers generally show a fragile behavior.

Secondary Relaxation Processes

In addition to the primary α -relaxation, polymers exhibit secondary relaxation processes that are active mainly in the glassy state. These relaxations, commonly labeled as β - and γ -relaxations, are associated with localized molecular motions such as rotations of side groups, motions of chain ends, or conformational changes involving small portions of the polymer backbone. Below T_0 , where long-range cooperative motions of the main chain are frozen, these localized motions can still occur independently.

Secondary relaxations generally take place on shorter time scales and exhibit much weaker intensity (e.g. dielectric strength) compared to the α -relaxation. Their temperature dependence in the glassy state is typically well described by an Arrhenius law:

$$\tau(T) = \tau_0 \exp \left[\frac{E_a}{k_B T} \right] \quad (1.11)$$

where τ_0 is the pre-exponential factor, E_a is the activation energy of the process, and k_B is Boltzmann's constant. Depending on the chemical structure of the polymer, these processes may involve varying degrees of cooperativity, but they remain fundamentally local in nature.

1.2. Nanoscience

Nanoscience is the study of materials, structures and phenomena that occur at the nanometric scale, that is, at dimensions typically ranging from 1 to 100 nm. At this length scale, materials often exhibit physical, chemical and biological properties that differ fundamentally from those observed in their bulk counterparts. These differences arise from size confinement effects, quantum phenomena, and the increased importance of surface-related interactions due to the high surface-to-volume ratio characteristic of nanoscale systems.^[11]

Although nanoscience emerged as a distinct field only in the second half of the 20th century, its conceptual foundations can be traced back to Richard Feynman's seminal 1959 lecture, in which he envisioned the possibility of manipulating matter at the level of individual atoms and molecules. Since then, advances in fabrication, characterization and analytical techniques have enabled the controlled study and design of nanoscale systems, leading to the rapid expansion of nanoscience and nanotechnology during the last decades.^[12]

Nanoscience is inherently interdisciplinary, spanning chemistry, physics, biology, materials science and engineering. In chemistry, it is closely linked to colloids, micelles, polymer chains,

supramolecular assemblies and phase-separated nanodomains in block copolymers. From this perspective, chemistry may be regarded as the “ultimate nanotechnology”, as chemical synthesis allows the construction of new forms of matter by assembling atoms and molecular building blocks with subnanometer precision^[13]. In physics and electrical engineering, nanoscience is frequently associated with quantum confinement effects and the behavior of electrons and photons in nanoscale structures, which underpin the operation of nanodevices and nanoelectronic components. Meanwhile, in biology and biochemistry, many fundamental building blocks of life—including DNA, proteins, viruses and subcellular organelles—naturally fall within the nanoscale regime, making nanoscience highly relevant for understanding and controlling biological processes.^[14]

A defining feature of nanoscale materials is the dramatic increase in the fraction of atoms located at or near the surface as the material size decreases. For bulk materials larger than approximately one micrometer, surface contributions are negligible compared to the bulk volume. In contrast, at the nanoscale, surface effects dominate and can give rise to unexpected mechanical, optical, magnetic or catalytic properties. These size-dependent characteristics are responsible for many of the unique functionalities that make nanomaterials attractive for technological applications.^[15]

Thanks to these features, nanoscience has become a rapidly growing research area worldwide, driving breakthroughs in fields such as nanoelectronics, nanomedicine, catalysis and advanced materials. This sustained growth is reflected in the increasing number of scientific publications and the significant investment in nanoscience-related research and development over the past decades.^[16]

1.3. Single-Chain Technology

Single-chain technology has become a distinct area within nanoscience, focusing on the construction of individual soft nano-objects by working at the level of a single polymer chain. This approach enables the generation of ultra-small unimolecular nanostructures, typically with sizes of a few to several tens of nanometers, placing single-chain technology at the interface of polymer chemistry,^[17-19] materials science,^[20] physics^[21] and biology.^[22]

Contrary to the traditional view of polymers as collective assemblies, advances in macromolecular synthesis have demonstrated that a single polymer chain can be engineered to behave as a discrete nano-object with well-defined structure and properties. Inspired by natural macromolecules, single-chain technology aims to transform synthetic polymers into compact, functional nanostructures through controlled intra-molecular folding or collapse. This is most commonly accomplished under highly dilute conditions, which favor intra-molecular over inter-molecular interactions, through the use of covalent cross-linking,^[23-25] supramolecular interactions,^[26, 27] or dynamic covalent-bonding strategies.^[28]

Using this methodology, a wide variety of soft nano-objects have been obtained via chain compaction, highlighting the versatility of single-chain technology. Representative examples include dynamic (stimuli-responsive),^[29-32] letter-shaped,^[33,34] and compositionally unsymmetrical single rings,^[35,36] complex multi-ring systems formed from connected or pre-formed rings,^[37-41] as well as single-chain nanoparticles with sparse or globular conformations.^{[42-}

^{47]} In addition, more elaborate architectures such as single-chain dumbbells,^[48] tadpoles^[49-51] and hairpins^[52,53] have also been reported. A schematic overview of these structures is shown in Fig. 1.3.

Among these nano-objects, single-chain nanoparticles (SCNPs) constitute one of the most extensively investigated systems and represent the central focus of this work.

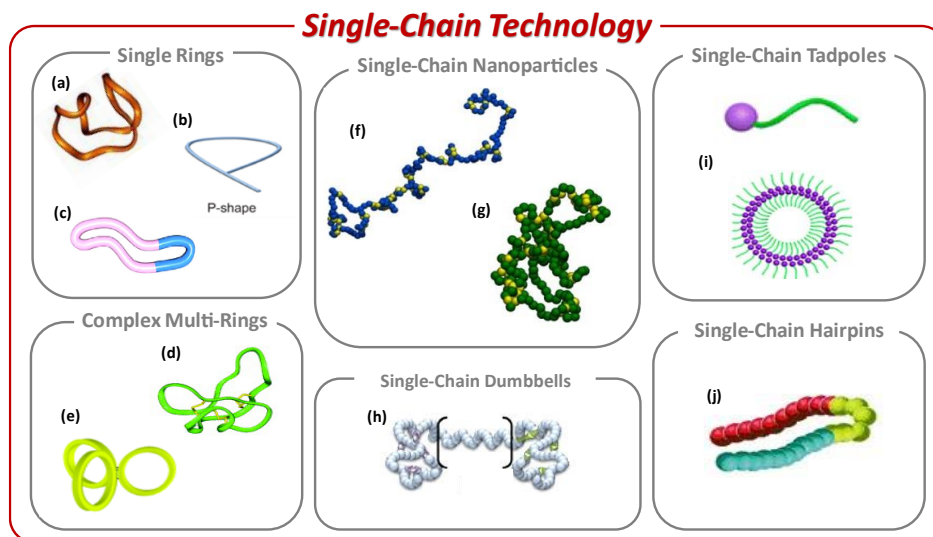


Figure 1.3. Schematic representation of different soft nano-objects obtained by single-chain technology via polymer chain compaction, including single rings: (a) dynamic (stimuli-responsive), (b) letter-shaped and (c) compositionally unsymmetrical; complex multi-rings based on (d) connected rings and (e) from pre-formed rings; single-chain nanoparticles (f) sparse and (g) globular; (h) single-chain dumbbells, (i) tadpoles (self-assembled in micelles) and (j) hairpins.

1.3.1. Single-Chain Nanoparticles (SCNPs)

Single-chain nanoparticles (SCNPs) are ultra-small soft nano-objects formed through the intra-molecular folding or collapse of individual synthetic polymer chains. As unimolecular entities, SCNPs typically display dimensions below 20 nm and combine high surface-to-volume ratios with excellent stability in solution. These features make them particularly attractive for applications requiring well-defined size, structural integrity and homogeneous dispersibility.

The intra-molecular cross-linking of linear polymer chains was first reported by Kuhn and Balmer in 1962,^[54] and subsequently revisited both theoretically and experimentally during the 1980s^[55-57] and 1990s.^[58,59] The modern concept of SCNPs, understood as discrete unimolecular nanoparticles obtained via controlled intrachain folding, was formally introduced at the beginning of the 21st century.^[60] Since then, SCNPs have emerged as an active and rapidly growing research topic at the interface of polymer science, nanotechnology and biology.

The folding of a single synthetic polymer chain into a compact nanoparticle is often compared to the folding of proteins into their native, functional conformations. While current SCNPs still lack the sequence precision, structural uniformity and folding accuracy characteristic of natural biomacromolecules, this bioinspired analogy has provided a strong conceptual framework for the field. As a result, considerable effort has been devoted to the development of increasingly sophisticated synthetic strategies aimed at controlling chain architecture, intra-molecular interactions and final nanoparticle morphology.

One of the most distinctive features of SCNPs is the presence of internal compartmentalization and confined domains, which resemble enzymatic pockets in natural biomacromolecules. These confined environments can strongly influence accessibility, local concentration and reactivity, making SCNPs attractive platforms for bioinspired applications such as catalysis, molecular recognition and sensing. Consequently, SCNPs represent one of the most promising outcomes of single-chain technology and constitute a versatile platform for both fundamental studies and applied research.

The final size, morphology and internal structure of SCNPs are determined by several key parameters, including the molar mass of the polymeric precursor, the degree and distribution of functional groups along the chain, the nature of the interactions driving the folding or collapse process, and the solvent quality during nanoparticle formation, whether good or selective. Each of these aspects is addressed through the successive steps of i) precursor synthesis, ii) functionalization and iii) intrachain folding/collapse which will be discussed in detail in the following sections.

i) Precursor synthesis

As SCNPs are unimolecular nanostructures derived from individual polymer chains, the characteristics of the precursor polymer critically determine the properties of the final nanoparticles. In particular, precise control over molar mass, dispersity, composition and architecture is essential to obtain reproducible and well-defined SCNPs. For this reason, controlled or “living” polymerization techniques are typically employed for precursor synthesis.

Among the polymerization methods most commonly used to prepare SCNPs are reversible addition–fragmentation chain transfer (RAFT) polymerization,^[24,25,28,45,50,51] nitroxide-mediated polymerization (NMP),^[48,61,62] atom transfer radical polymerization (ATRP)^[30,39,46] and ring-opening polymerization (ROP).^[52,60,63-67] These techniques allow the synthesis of polymers with predictable molecular weights, narrow molar mass distributions and well-controlled incorporation of functional monomers.

Ring-opening polymerization (ROP) is particularly attractive for the preparation of SCNPs precursors, especially when polyethers, polyesters or related backbones are desired.^[2] ROP involves the polymerization of cyclic monomers through ring opening, driven by a combination of thermodynamic and kinetic factors. Depending on the nature of the monomer and the propagating species, ROP can proceed through cationic, anionic, radical or zwitterionic mechanisms. In particular, zwitterionic ring-opening polymerization (ZROP) catalyzed by strong Lewis acids has been shown to afford well-defined polyether structures under mild conditions, offering additional opportunities for precursor design.

In this work, special attention is devoted to ZROP. In particular, the Lewis acid–catalyzed ZROP of cyclic ethers using tris(pentafluorophenyl)borane, $B(C_6F_5)_3$, has been extensively investigated and shown to be a powerful strategy for the synthesis of well-defined polyether backbones.^[63,65-67]

In the $B(C_6F_5)_3$ -catalyzed ZROP mechanism, polymerization proceeds via the formation of a zwitterionic active species, generated by the interaction between the catalyst and the monomer. Chain propagation occurs through successive ring-opening of cyclic monomers, while termination and side reactions are minimized under appropriate reaction conditions (Fig. 1.4). This mechanism enables good control over molar mass and polymer architecture without the

need for metal-based catalysts, which is advantageous for subsequent functionalization and folding steps.

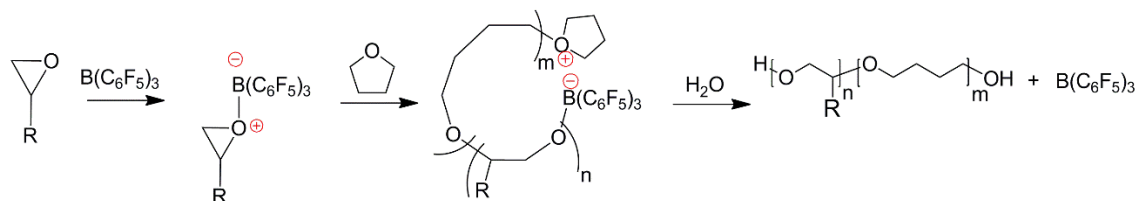


Figure 1.4. Simplified mechanism of $B(C_6F_5)_3$ -catalyzed ZROP of a generic glycidic monomer with THF as described in Ref.^[63].

The use of ZROP provides access to polymer precursors with tailored backbone structures and functional group compatibility, making it particularly well suited for single-chain technology. The polyethers obtained through this approach exhibit the flexibility and solubility required for efficient intrachain folding, as well as chemical handles that can be further exploited during precursor functionalization and SCNP formation.

ii) Precursor functionalization

In many cases, polymers obtained directly from controlled polymerization techniques do not contain functional groups suitable for intra-molecular cross-linking. As a result, an intermediate functionalization step is often required to introduce reactive moieties along the polymer chain. This process, commonly referred to as post-polymerization modification, provides access to a wide range of functional precursor architectures without the need for complex monomer synthesis.

A variety of efficient and selective chemical reactions have been employed to functionalize SCNP precursors. These include nucleophilic substitution reactions such as e.g. azidation,^[23, 24] alkylation^[26] or esterification,^[50] among others. The choice of functionalization strategy depends on factors such as reaction efficiency, compatibility with the polymer backbone and suitability for subsequent folding chemistry.

Alternatively, precursor functionalization can be integrated directly into the polymerization step by copolymerizing inert monomers with a controlled fraction of functional monomers. This approach allows reactive groups to be randomly distributed along the polymer chain, eliminating the need for post-polymerization modification and simplifying the synthetic procedure.

In all cases, the degree and spatial distribution of functional groups along the precursor chain play a decisive role in determining the folding efficiency, compaction degree and final morphology of the SCNPs.

iii) Intrachain folding and collapse

The intrachain folding or collapse of the precursor polymer constitutes the defining step in the synthesis of SCNPs, as it directly determines their size, internal structure and properties. To favor intra-molecular reactions over undesirable inter-molecular cross-linking or aggregation, folding reactions are typically carried out under highly dilute conditions. The choice of solvent is also critical, as solvent quality influences the initial conformation of the polymer chain prior to folding.

Folding strategies can be categorized according to both the connectivity and the nature of the interactions involved, as depicted in Fig. 1.5. Based on connectivity, three main approaches are commonly employed: intrachain homocoupling, intrachain heterocoupling and external cross-linker-induced collapse. In intrachain homocoupling, identical functional groups located along the same polymer chain react pairwise to form cross-links. In intrachain heterocoupling, complementary functional groups react selectively with each other. In cross-linker-induced collapse, an external multifunctional molecule promotes folding by bridging different sites along the chain.

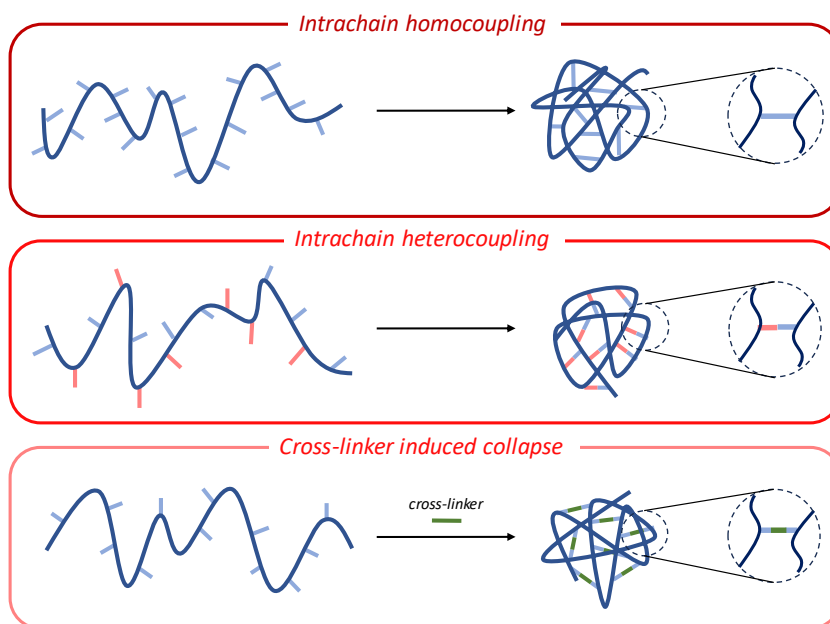


Figure 1.5. Schematic illustration of intrachain homocoupling, intrachain heterocoupling, and external cross-linker-induced collapse strategies for single-chain nanoparticle (SCNP) formation.

Folding mechanisms can be classified according to the type of interaction driving compaction. Covalent folding strategies lead to permanently cross-linked SCNPs with high thermal and chemical stability.^[23-26,51,60,62,63] Non-covalent or supramolecular folding relies on weaker interactions such as hydrogen bonding, metal–ligand coordination or π – π interactions, allowing for dynamic and reversible SCNP structures.^[45,46,52,53,61] Dynamic covalent bonds combine features of both approaches, enabling reversible folding that can respond to external stimuli such as pH, temperature or redox conditions.^[28,30]

The extent of folding and degree of chain compaction depend on multiple parameters, including precursor molar mass, functional group density, solvent quality and the nature of the cross-linking chemistry. In general, SCNPs formed through covalent bonds exhibit higher degrees of compaction than those assembled via non-covalent interactions from identical precursors. These differences translate into distinct conformations, often described as sparse or globular morphologies, which strongly affect the behavior of SCNPs in solution and their performance in applications such as catalysis or sensing.

Within the broad range of covalent folding strategies, click chemistry has emerged as a particularly powerful and reliable approach for the synthesis of single-chain nanoparticles.^[24-26,30-33,36,38-40,62,63] Owing to its high efficiency, selectivity, and excellent compatibility with polymeric systems, click chemistry enables efficient intrachain cross-linking under the dilute

conditions required for SCNP formation. Among the different click reactions, the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) stands out as one of the most widely employed methodologies.^[31-33,36,38,40]

In addition to purely chemical activation, photoactivated folding strategies triggered by UV light constitute an attractive alternative route toward SCNP formation.^[68] The use of light as an external stimulus allows folding to be initiated on demand and, in certain cases, reversibly controlled by adjusting the irradiation conditions. Both CuAAC-based and photoactivated folding approaches are therefore discussed in detail below, as they play a central role in this work, either as direct synthetic strategies or as reference systems for comparative analysis.

Click chemistry

The term click chemistry was introduced by Sharpless and co-workers in 2001 to describe a set of highly efficient, selective and reliable reactions designed to rapidly assemble complex structures from simple building blocks under mild conditions.^[69] Independently, similar concepts were developed by Meldal, emphasizing carbon–heteroatom bond formation through robust and high-yielding reactions.^[70]

A chemical transformation is generally classified as a click reaction when it combines operational simplicity with high selectivity and quantitative yields, shows broad functional-group tolerance, proceeds under mild conditions, and allows straightforward product isolation without extensive purification. When polymeric systems are involved, additional requirements such as fast reaction kinetics, equimolarity and scalable purification also become crucial. These features make click reactions particularly well suited for intra-molecular coupling processes, where efficient and controlled bond formation is essential to avoid inter-molecular aggregation.

Click reactions can be broadly grouped into four main categories: cycloadditions, nucleophilic substitutions, non-aldol carbonyl reactions and additions to carbon–carbon multiple bonds. Among these, cycloaddition reactions (and especially the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC)) have played a dominant role in polymer chemistry and, more specifically, in the synthesis of SCNPs.

Click chemistry contributes to SCNP formation through the different folding pathways discussed previously, including intrachain homo- and hetero-coupling as well as cross-linker-induced collapse. Its high efficiency and orthogonality enable precise control over the location and density of cross-links, which are critical parameters governing the final size, morphology and internal structure of the resulting nanoparticles. For these reasons, CuAAC has become one of the most widely used reactions for SCNP synthesis and is the focus of the following section.

Copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC)

The CuAAC reaction represents the archetypal click transformation, involving the 1,3-dipolar cycloaddition between azides and alkynes to form 1,2,3-triazoles. While the thermal azide–alkyne cycloaddition originally reported by Huisgen requires harsh conditions and yields mixtures of regioisomers, the introduction of copper(I) catalysis dramatically improved both reaction rate and regioselectivity, affording exclusively the 1,4-disubstituted triazole under mild conditions.

This reaction perfectly fulfills the principles of click chemistry: it is highly selective, tolerant to a wide range of functional groups, insensitive to water and oxygen, and proceeds in high yields under relatively simple experimental conditions. These attributes make it particularly attractive

for intra-molecular crosslinking reactions in dilute solution, where efficient folding must compete against undesired inter-molecular processes.

Its application to the formation of single-chain nanoparticles (SCNPs) was first reported in 2008 by Ruiz de Luzuriaga and co-workers,^[71] who demonstrated efficient intrachain heterocoupling between azide- and alkyne-functionalized polymer precursors at room temperature. Subsequent studies extended this strategy to multifunctional styrene-based^[72] and temperature-responsive polymer systems,^[73] enabling the preparation of SCNPs with tunable degrees of crosslinking and stimuli-responsive behavior.

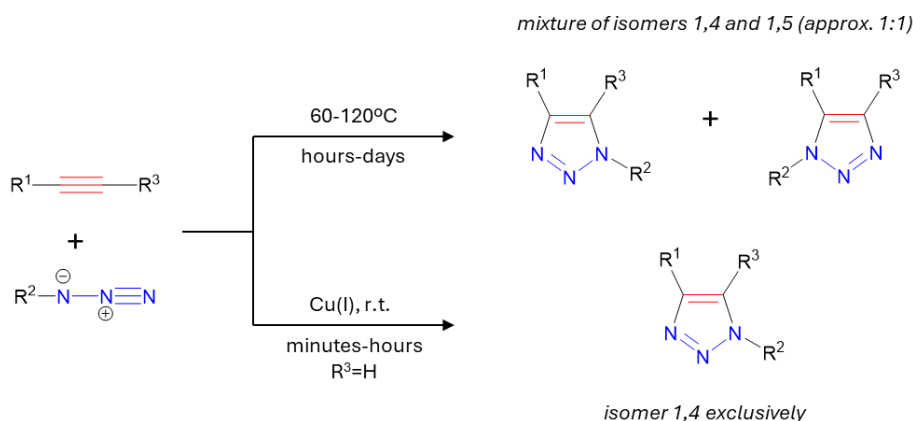


Figure 1.6. Scheme of 1,3-dipolar cycloaddition between azides and alkynes under thermal and copper(I)-catalyzed conditions.

UV-Photoactivation

Photochemical reactions offer a powerful and versatile tool for the controlled formation of SCNPs, as light in the UV–visible range can promote molecules to excited electronic states and trigger chemical transformations under very mild conditions. In contrast to thermally activated processes, photoinduced reactions often proceed without the need for high temperatures or aggressive reagents, enabling precise temporal and spatial control over bond formation. These features have made photochemistry increasingly attractive in polymer science, particularly for the design of functional nanostructures.

In the context of single-chain technology, photoactivation has emerged as an alternative strategy to induce the intra-molecular folding or collapse of individual polymer chains under dilute conditions.^[68] Conceptually, photoactivated SCNP formation closely mirrors chemically driven approaches, as the folding process can be achieved through intrachain homocoupling, intrachain heterocoupling, or collapse induced by external cross-linkers. The key difference lies in the nature of the stimulus used to trigger bond formation: instead of chemical reagents or catalysts, light acts as the external trigger, allowing folding to be initiated on demand.

Several photochemical mechanisms have been successfully employed for SCNP synthesis. Intrachain homocoupling strategies include (i) photodimerization,^[50,74-77] (ii) photocyclization,^[77,78] (iii) photoisomerization^[77,79,80] and the (iv) photogeneration of highly reactive species, such as nitrenes or carbenes,^[81,82] which can rapidly induce chain compaction. In parallel, photoinduced heterocoupling reactions such as (i) photoactivated Diels–Alder (DA) reactions,^[83-85] (ii) nitrile imine-mediated tetrazole–ene cycloadditions (NITEC)^[86-88] and (iii) nitrile imine–carboxylic acid ligations (NICAL),^[89] enable efficient and selective intra-molecular

cross-linking. In addition, photodeprotection strategies can trigger self-assembly or folding by unveiling reactive or interactive sites along the polymer backbone.^[90-92] Beyond intrachain photoinduced coupling strategies, SCNPs can also be formed via photogenerated collapse mediated by external cross-linkers.^[93,94]

Among these approaches, UV-induced nitrene formation from azide-functional polymers has been explored for SCNP preparation, leading to rapid and efficient intrachain cross-linking. Previous studies have demonstrated that such photoactivated methods provide access to robust SCNPs with sizes comparable to those obtained through purely chemical folding routes, while offering additional advantages in terms of reaction control and functional tunability (e.g., fluorescence or photoresponsiveness).^[81,82,94]

In this work, photoactivated SCNPs through nitrene formation are briefly presented because they provide a relevant benchmark for comparison with the chemically folded SCNPs synthesized via click chemistry. This comparison allows the influence of the folding mechanism (photoinduced versus chemically induced) on SCNP size, structure and properties to be critically assessed.

1.3.2. Morphology and Architectures

Single-chain nanoparticles can adopt different morphologies depending on the polymer precursor, synthesis method, and environmental conditions. Their final architecture can be finely tuned by controlling parameters such as polymer composition, solvent, and temperature. In general, two main types of precursor copolymers lead to distinct SCNP morphologies: (i) block copolymers (chemically distinct monomers arranged in blocks) and (ii) random copolymers (different monomers randomly distributed along the chain) (Fig. 1.7).^[95]

Block copolymers can collapse into Janus SCNPs, characterized by two chemically distinct regions resulting from the independent collapse of each block. These anisotropic nanoparticles often exhibit self-assembly behavior, giving rise to higher-order structures such as micelles, lamellae, or vesicles. Depending on the collapse efficiency, twin Janus or tadpole Janus SCNPs can be formed.^[96,97]

In contrast, random copolymers collapse into either sparse or globular SCNPs and generally do not display self-assembly behavior. Sparse SCNPs are typically obtained in good solvents, where short-range intrachain cross-linking dominates, leading to non-compact, heterogeneous structures with extended chain segments.^[21,98,99] Notably, their morphology closely resembles that of intrinsically disordered proteins (IDPs), which also show broad distributions in size and topology (Fig. 1.7).^[100]

Globular SCNPs are often obtained from more complex folding or collapse synthesis routes that promote the formation of a global core-shell morphology, closely resembling that of native globular proteins such as enzymes. One effective strategy to promote compact globular conformations relies on enhancing long-range intra-molecular loop formation through the use of bifunctional precursors and relatively long cross-linkers, which favor increased chain compaction.^[93] The solvent also plays an important role in the collapse process and the resulting SCNP morphology.^[101,102] Alternative strategies to achieve nearly globular SCNPs rely on the self-assembly of amphiphilic random copolymers.^[103-106]

Overall, these results demonstrate that single-chain technology enables precise control over SCNP morphology, expanding the range of protein-inspired applications.

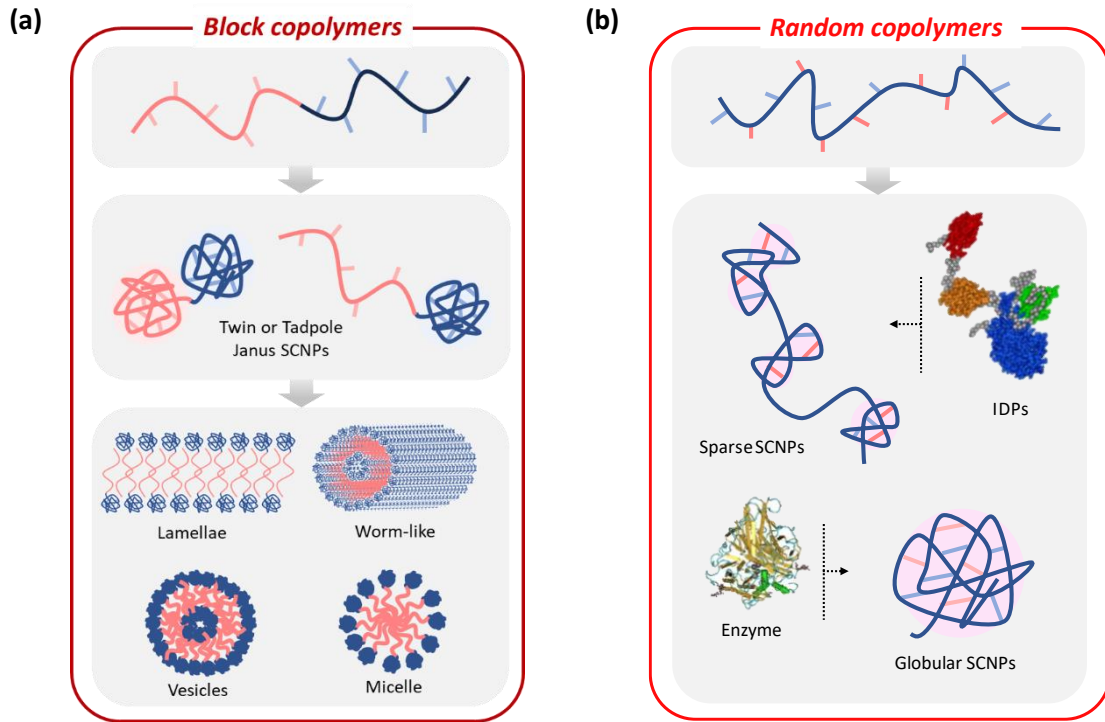


Figure 1.7. Scheme of the different morphologies of SCNPs from (a) block copolymer precursor and from (b) random copolymer precursor. Self-assembled structures of Twin or Tadpole SCNPs are shown, together with the morphological similarities between sparse SCNPs and intrinsically disordered proteins (IDPs), and globular SCNPs and enzymes.

1.3.3. Elastic SCNP Model (ESM)

From a theoretical perspective, the elastic SCNPs model (ESM), introduced in recent years, establishes a quantitative relationship between the size of SCNPs, the number of chain segments, and the fraction of reactive groups in the precursor polymer through a parameter associated with the elasticity of the unimolecular network.^[107]

In this simple model, individual SCNPs are formed through intrachain cross-linking of a fully flexible linear polymer precursor consisting of N monomers. The precursor consists of two randomly distributed types of monomers: reactive monomers (A) and unreactive monomers (B), with a fraction x representing the proportion of A monomers. It is assumed that the precursor, of size R_0 , follows a scaling law such as:^[3]

$$R_0 \approx aN^{\nu_0} \quad (1.12)$$

where a is proportional to the monomer size and ν_0 is the corresponding scaling exponent. For the precursor in good solvent conditions, $\nu_0 = \nu_F = 3/5 = 0.6$ (0.588 according to renormalization group theory).^[3]

During SCNP formation, assuming very high dilution, the A monomers on each chain dimerize to create "A-A" intra-molecular bonds. The resulting SCNP, with a size R , is regarded as an elastic unimolecular network made up of elastic strands connected by cross-links.

In this simplified model, the Flory-type free energy (F) of elastic SCNPs consists of two main components: (i) the elastic free energy (F_{el}) resulting from the elastic strands connected by cross-links, and (ii) the excluded volume contribution (F_{ex}) arising from the balance between monomer-monomer and monomer-solvent interactions, such as

$$F = F_{el} + F_{ex} \quad (1.13)$$

The SCNP free energy can be expressed as:

$$\frac{F(R)}{k_B T} \approx \frac{F_{el}(R)}{k_B T} + \frac{F_{ex}(R)}{k_B T} \quad (1.14)$$

where k_B is the Boltzmann constant and T the absolute temperature. The elastic free energy is given by

$$\frac{F_{el}(R)}{k_B T} \approx \frac{R^2}{R_0^2} + KR^2 \quad (1.15)$$

where $R_0 \approx aN^{1/2}$, as given in Eq. (1.15), a is related to the monomeric segment length, K is an elasticity parameter proportional to x such as $K = Ax$, and A is the corresponding proportionality constant. In the limit $x \rightarrow 0$ (i.e., $K \rightarrow 0$) Eq. (1.13) reduces to the well-known Flory expression for a flexible linear polymer chain. On the other hand, for elastic SCNPs it can be seen that $K \gg R_0^{-2}$, so that Eq. (1.15) becomes

$$\frac{F_{el}(R)}{k_B T} \approx KR^2 = AxR^2 \quad (1.16)$$

When this model was compared to experimental data of real SCNPs in Ref.^[107], the assumption $K \gg R_0^{-2}$ proved to be a very good approximation. For instance, in the case of polystyrene (PS) SCNPs the value of A was estimated to be 47.6 nm^{-2} , meaning the condition $K = Ax > R_0^{-2}$ was satisfied even at relatively low values of N and very low values of x (e.g., for $N=500$ and x as low as 0.025).^[107] Therefore, the former condition holds in most of experimental SCNPs (typically $0.1 < x < 0.4$).

On the other hand, the contribution to $F(R)$ from excluded volume interactions changes depending on the nature of the solvent:

i) Good solvent regime

For the case of very good solvent conditions (i.e., athermal solvent), the excluded volume contribution to the free energy is given by Ref.^[3].

$$\frac{F_{ex}(R)}{k_B T} \approx \frac{N^2 a^3}{R^3} \quad (1.17)$$

so the free energy of an elastic SCNP (Eq. (1.14)) becomes

$$\frac{F(R)}{k_B T} \approx KR^2 + \frac{N^2 a^3}{R^3} \quad (1.18)$$

and the equilibrium configuration of the elastic SCNP in good solvent conditions, as obtained by minimizing Eq. (1.18) with respect to R , is given by

$$R \approx a^{3/5} K^{-1/5} N^{2/5} \quad (1.19)$$

Equation (1.16) has been used to fit reliable experimental size data of polystyrene (PS) SCNPs providing an average deviation between calculated and experimental data of only 4.2%.^[108]

ii) *Theta-solvent regime*

For the case of θ -solvent conditions, F_{ex} is given by Ref.^[3]

$$\frac{F_{ex}(R)}{k_B T} \approx \frac{N^3 a^6}{R^6} \quad (1.20)$$

so the free energy of elastic SCNPs (Eq. (1.14)) becomes

$$\frac{F(R)}{k_B T} \approx K R^2 + \frac{N^3 a^6}{R^6} \quad (1.21)$$

and the equilibrium configuration of the elastic SCNP in θ -solvent conditions, as obtained by minimizing Eq. (1.21) with respect to R , is given by

$$R^\theta \approx a^{3/4} K^{-1/8} N^{3/8} \quad (1.22)$$

1.4. Polymer Nanocomposites

Polymer nanocomposites are multicomponent materials in which a polymer matrix incorporates dispersed entities with at least one characteristic dimension in the nanometer range. The introduction of nanoscopic components leads to a marked increase of the interfacial area and to characteristic length scales that are comparable to those governing polymer conformations and dynamics. As a consequence, polymer nanocomposites often exhibit properties that differ significantly from those of the neat polymer, even at relatively low filler concentrations. These differences arise not only from changes in static structure, but also from modifications of the molecular dynamics across a wide range of time and length scales.^[109-111]

In recent years, all-polymer nanocomposites have attracted particular attention as model systems to investigate fundamental aspects of polymer physics.^[112-118] In these materials, both the matrix and the dispersed phase are polymeric, which largely suppresses strong chemical incompatibilities and extreme stiffness contrasts typical of inorganic fillers. This allows one to focus on the role of macromolecular architecture, topology, and confinement effects on polymer dynamics. Among the different classes of polymeric nanofillers, single-chain nanoparticles (SCNPs) constitute a unique case.^[114-118] When SCNPs are embedded in a matrix of their linear precursors, the resulting all-polymer nanocomposites provide an ideal platform to disentangle the mutual influence of matrix and nanocomponent dynamics while keeping chemistry essentially unchanged.

Previous studies have demonstrated that blending SCNPs with linear polymer matrices can lead to a phenomenology that differs markedly from that observed in conventional polymer blends. For instance, SCNP-based nanocomposites such as PS-SCNPs blended with PVME^[119-121] or PMMA-SCNPs blended with PEO^[114-116] have shown pronounced changes in calorimetric, dielectric, structural, and dynamic properties compared to analogous systems containing only linear chains. In several of these systems, confinement effects and modifications of segmental and chain dynamics have been reported, often in the presence of a strong dynamic asymmetry between the components arising from differences in their glass transition temperatures.

Interestingly, even in all-polymer nanocomposites where such dynamic asymmetry is not expected (such as blends of linear PS chains with PS-based SCNPs) significant changes in macroscopic properties have been observed, including reductions in viscosity and glass transition temperature relative to the linear polymer melt.^[122,123]

These findings indicate that the presence of SCNPs alone, through their distinct topology and internal structure, can influence the glass transition behavior of the material. Since the glass transition is intimately related to the segmental or α -relaxation process, a detailed understanding of the underlying atomic-scale motions is essential to rationalize the observed behavior.

While the mutual influence of the components on segmental dynamics has been extensively studied in blends of linear polymers, much less is known when one of the components consists of SCNPs. From an experimental perspective, addressing this issue is particularly challenging because it requires component-selective access to molecular motions. In this regard, quasielastic neutron scattering (QENS) combined with selective isotopic deuteration provides a unique and powerful approach, as it enables the direct investigation of the segmental dynamics of each component within the same nanocomposite at the molecular level.^[114-116]

1.5. Aim and Outline of this Thesis

The aim of this thesis is to gain a comprehensive understanding of the structural and dynamical behavior of systems containing or based on single-chain nanoparticles (SCNPs) through theoretical modeling and experimental investigation.

On the theoretical side, this work applies the Elastic SCN Model (ESM) to describe the structure of SCNPs under different conditions that strongly influence their behavior, such as surface grafting, anisotropic architectures (tadpoles), and environments of strong crowding.

On the experimental side, well-defined SCN systems synthesized through different intra-molecular cross-linking strategies (click chemistry vs. azide photodecomposition) are investigated using a comprehensive multi-technique approach, with particular emphasis on neutron scattering methods. The experimental part focuses on how the nature and density of intra-molecular cross-links influence structural relaxation, segmental dynamics, mechanical response, and glass transition behavior in bulk SCN systems and in more complex polymer-based materials, including all-polymer nanocomposites.

Ultimately, this work aims to provide a coherent multiscale description of SCNPs and to contribute to a deeper understanding of the relationship between intra-molecular architecture and macroscopic material properties.

This thesis is organized into five chapters. The present introductory chapter outlines the main concepts required for the understanding of the work, with particular emphasis on SCNPs, which constitute the core subject of this study.

Chapter 2 presents the theoretical framework and discusses the structural properties of SCNPs under different representative conditions within the ESM approach.

Chapter 3 describes the experimental techniques employed throughout the thesis, with emphasis on neutron scattering methods.

Chapter 4 reports the main experimental results of this work and their discussion.

Finally, Chapter 5 summarizes the main conclusions of the thesis and outlines the key findings and perspectives derived from this work.

1.6. References

- [1] P. J. Flory, *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, NY, **1953**.
- [2] G. Odian, *Principles of Polymerization*, 4th ed., Wiley-Interscience, New York, **2004**.
- [3] M. Rubinstein and R. H. Colby, *Polymer Physics*; Oxford University Press, Oxford, U.K., **2003**.
- [4] A. Arbe, F. Alvarez and J. Colmenero, *Soft Matter*, **2012**, *8*, 8257.
- [5] A. R. Denton, *Phys. Rev. E*, **2003**, *67*, 011804.
- [6] A. Alegría, E. Guerrica-Echevarría, L. Goitiandia, I. Tellería and J. Colmenero, *Macromolecules*, **1995**, *28*, 1516.
- [7] A. Narros, A. Arbe and F. Alvarez, *J. Chem. Phys.*, **2008**, *128*, 224905.
- [8] F. Alvarez, A. Alegría and J. Colmenero, *Phys. Rev. B*, **1993**, *47*, 125.
- [9] S. Havriliak and S. Negami, *J. Polym. Sci., Part C: Polym. Symp.*, **1966**, *14*, 99.
- [10] C. A. Angell, *Proc. Natl. Acad. Sci. U.S.A.*, **1995**, *92*, 6675.
- [11] M. D. Ediger, C. A. Angell and S. R. Nagel, *J. Phys. Chem.*, **1996**, *100*, 13200.
- [12] R. P. Feynman, *Eng. Sci.*, **1960**, *23*, 22.
- [13] G. M. Whitesides, *Angew. Chem. Int. Ed.*, **2003**, *42*, 2221.
- [14] C. M. Niemeyer and C. A. Mirkin, *Nanobiotechnology: Concepts, Applications and Perspectives*, Wiley-VCH, Weinheim, Germany, **2004**.
- [15] A. P. Alivisatos, *Science*, **1996**, *271*, 933.
- [16] M. C. Roco, C. A. Mirkin and M. C. Hersam, *J. Nanopart. Res.*, **2011**, *13*, 897.
- [17] D. Arena, C. Nguyen, L. M. A. Ali, M. Gary-Bobo and J. A. Pomposo, *Adv. Healthcare Mater.*, **2024**, *13*, 2401683.
- [18] M. A. M. Alqarni, C. Waldron, G. Yilmaz and C. R. Becer, *Macromol. Rapid Commun.*, **2021**, *42*, 2100035.
- [19] O. Altintas and C. Barner-Kowollik, *Macromol. Rapid Commun.*, **2016**, *37*, 29.
- [20] J. A. Pomposo, A. J. Moreno, A. Arbe and J. Colmenero, *Prog. Polym. Sci.*, **2022**, *133*, 101593.
- [21] A. J. Moreno, F. Lo Verso, A. Sanchez-Sanchez, A. Arbe, J. Colmenero and J. A. Pomposo, *Macromolecules*, **2013**, *46*, 9748.
- [22] A. P. P. Kröger and J. M. J. Paulusse, *J. Control. Release*, **2018**, *286*, 326.
- [23] M. González-Burgos, A. Alegría, A. Arbe, J. Colmenero and J. A. Pomposo, *Polym. Chem.*, **2016**, *7*, 6570.
- [24] A. Blázquez-Martín, S. Bonardd, E. Verde-Sesto, A. Arbe and J. A. Pomposo, *ACS Polymers Au*, **2024**, *4*, 140.
- [25] A. P. P. Kröger, J.-W. D. Paats, R. J. E. A. Boonen, N. M. Hamelmann and J. M. J. Paulusse, *Polym. Chem.*, **2020**, *11*, 6056.

- [26] Y. Shao, Y.-L. Wang, Z. Tang, Z. Wen, C. Chang, C. Wang, D. Sun, Y. Ye, D. Qin, Y. Ke, F. Liu and Z. Yang, *Angew. Chem. Int. Ed.*, **2022**, *61*, e202205183.
- [27] E. Guazzelli, E. Masotti, M. Calosi, M. Kriechbaum, F. Chlig, G. Galli and E. Martinelli, *Polymer*, **2021**, *231*, 124107.
- [28] A. Sanchez-Sanchez, D. A. Fulton and J. A. Pomposo, *Chem. Commun.*, **2014**, *50*, 1871.
- [29] M. K. Miller, F. A. Chapa-Villarreal, H. F. Oldenkamp, M. G. Elder, A. K. Venkataraman and N. A. Peppas, *J. Control. Release*, **2023**, *361*, 246.
- [30] C. Cheng, D. Lee, Z. Liao and J. Huang, *Polym. Chem.*, **2016**, *7*, 6164.
- [31] J. Willenbacher, B. V. Schmidt, D. Schulze-Suenninghausen, O. Altintas, B. Luy, G. Delaittre and C. Barner-Kowollik, *Chem. Commun.*, **2014**, *50*, 7056.
- [32] D. Danilov, C. Barner-Kowollik and W. Wenzel, *Chem. Commun.*, **2015**, *51*, 6002.
- [33] M. Zamfir, P. Theato and J.-F. Lutz, *Polym. Chem.*, **2012**, *3*, 1796.
- [34] B. V. Schmidt, N. Fechner, J. Falkenhagen and J.-F. Lutz, *Nat. Chem.*, **2011**, *3*, 234.
- [35] J. E. Poelma, K. Ono, D. Miyajima, T. Aida, K. Satoh and C. J. Hawker, *ACS Nano*, **2012**, *6*, 10845.
- [36] B. Zhang, H. Zhang, Y. Li, J. N. Hoskins and S. M. Grayson, *ACS Macro Lett.*, **2013**, *2*, 845.
- [37] K. Hagita and T. Murashima, *Polymer*, **2021**, *223*, 123705.
- [38] F. Amir, M. D. Hossain, Z. Jia and M. J. Monteiro, *Polym. Chem.*, **2016**, *7*, 6598.
- [39] O. Altintas, E. Lejeune, P. Gerstel and C. Barner-Kowollik, *Polym. Chem.*, **2012**, *3*, 640.
- [40] Z. Jia, D. E. Lonsdale, J. Kulis and M. J. Monteiro, *ACS Macro Lett.*, **2012**, *1*, 780.
- [41] B. J. Ree, Y. Satoh, T. Isono and T. Satoh, *Macromolecules*, **2021**, *54*, 4120.
- [42] C. K. Lyon, A. Prasher, A. M. Hanlon, B. T. Tuten, C. A. Tooley, P. G. Frank and E. B. Berda, *Polym. Chem.*, **2015**, *6*, 181.
- [43] M. Huo, N. Wang, T. Fang, M. Sun, Y. Wei and J. Yuan, *Polymer*, **2015**, *66*, A11.
- [44] M. Artar, E. Huerta, E. W. Meijer and A. R. A. Palmans, in *Sequence-Controlled Polymers: Synthesis, Self-Assembly, and Properties*, J.-F. Lutz, T. Y. Meyer, M. Ouchi and M. Sawamoto (Eds.), ACS Symp. Ser., **2014**, *1170*, 313.
- [45] S. Basasoro, M. Gonzalez-Burgos, A. J. Moreno, F. Lo Verso, A. Arbe, J. Colmenero and J. A. Pomposo, *Macromol. Rapid Commun.*, **2016**, *37*, 1060.
- [46] E. Guazzelli, E. Masotti, M. Kriechbaum, F. Uhlig, G. Galli and E. Martinelli, *Macromol. Chem. Phys.*, **2023**, *224*, 2200360.
- [47] S. Liao, L. Wei, A. E. Bouchez and F. Stellacci, *Chem*, **2023**, *9*, 1562.
- [48] R. K. Roy and J.-F. Lutz, *J. Am. Chem. Soc.*, **2014**, *136*, 12888.
- [49] J. Cui, W. Zhang, Y. Han, Y. Wang and W. Jiang, *Langmuir*, **2023**, *39*, 13335.
- [50] S. Thanneeru, W. Li and J. He, *Langmuir*, **2019**, *35*, 2619.

- [51] Y. Vo, R. Raveendran, C. Cao, L. Tian, R. Y. Lai and M. H. Stenzel, *J. Mater. Chem. B*, **2024**, *12*, 12627.
- [52] J. Romulus and M. Weck, *Macromol. Rapid Commun.*, **2013**, *34*, 1518.
- [53] J. Lu, N. ten Brummelhuis and M. Weck, *Chem. Commun.*, **2014**, *50*, 6225.
- [54] W. Kuhn and G. Balmer, *J. Polym. Sci.*, **1962**, *57*, 311.
- [55] J. E. Martin and B. E. Eichinger, *Macromolecules*, **1983**, *16*, 1345.
- [56] J. E. Martin and B. E. Eichinger, *Macromolecules*, **1983**, *16*, 1350.
- [57] C. A. Finch, in *Chemistry and Technology of Water-Soluble Polymers*, C. A. Finch (Ed.), Springer, Boston, MA, **1983**, 81.
- [58] W. Funke, O. Okay and B. Joos-Müller, in *Microencapsulation, Microgels, Iniferters*, Springer, Berlin, Heidelberg, **1999**, 139.
- [59] M. Liu, R. Cheng and C. Wu, *Eur. Polym. J.*, **1999**, *35*, 1907.
- [60] D. Mecerreyes, V. Lee, C. J. Hawker, J. L. Hedrick, A. Wursch, W. Volksen and R. D. Miller, *Adv. Mater.*, **2001**, *13*, 204.
- [61] J.-F. Lutz, J.-M. Lehn, E. W. Meijer and K. Matyjaszewski, *Nat. Rev. Mater.*, **2016**, *1*, 16024.
- [62] S. C. Solleder, R. V. Schneider, K. S. Wetzel, A. C. Boukis and M. A. R. Meier, *Macromol. Rapid Commun.*, **2017**, *38*, 1600711.
- [63] N. G. Engeli, A. Anastasaki, G. Nurumbetov, N. P. Truong, V. Nikolaou, A. Shegiwal, M. R. Whittaker, T. P. Davis and D. M. Haddleton, *Nat. Chem.*, **2017**, *9*, 171.
- [64] F. S. Bates and G. H. Fredrickson, *Phys. Today*, **1999**, *52*, 32.
- [65] G. H. Fredrickson and F. S. Bates, *Annu. Rev. Mater. Sci.*, **1996**, *26*, 501.
- [66] L. Leibler, *Macromolecules*, **1980**, *13*, 1602.
- [67] M. W. Matsen and F. S. Bates, *Macromolecules*, **1996**, *29*, 1091.
- [68] I. W. Hamley, *The Physics of Block Copolymers*, Oxford University Press, Oxford, U.K., **1998**.
- [69] F. S. Bates, M. A. Hillmyer, T. P. Lodge, C. M. Bates, K. T. Delaney and G. H. Fredrickson, *Science*, **2012**, *336*, 434.
- [70] Y. Mai and A. Eisenberg, *Chem. Soc. Rev.*, **2012**, *41*, 5969.
- [71] A. R. de Luzuriaga, N. Ormategui, H. J. Grande, I. Odriozola, J. A. Pomposo and I. Loinaz, *Macromol. Rapid Commun.*, **2008**, *29*, 1156.
- [72] L. Oria, R. Aguado, J. A. Pomposo and J. Colmenero, *Adv. Mater.*, **2010**, *22*, 3038.
- [73] N. Ormategui, I. Garcia, D. Padro, G. Cabanero, H. J. Grande and I. Loinaz, *Soft Matter*, **2012**, *8*, 734.
- [74] H. Frisch, J. P. Menzel, F. R. Bloesser, D. E. Marschner, K. Mundsinger and C. Barner-Kowollik, *J. Am. Chem. Soc.*, **2018**, *140*, 9551.
- [75] I. Dashan, D. K. Balta, B. A. Temel and G. Temel, *Eur. Polym. J.*, **2019**, *113*, 183.

- [76] J. T. Offenloch, E. Blasco, S. Bastian, C. Barner-Kowollik and H. Mutlu, *Polym. Chem.*, **2019**, *10*, 4513.
- [77] T. Ma, B. Mu and W. Tian, *Angew. Chem. Int. Ed.*, **2025**, *137*, e202500354.
- [78] B. Zhu, G. Qian, Y. Xiao, S. Deng, M. Wang and A. Hu, *J. Polym. Sci., Part A: Polym. Chem.*, **2011**, *49*, 5330.
- [79] L. Greb, H. Mutlu, C. Barner-Kowollik and J.-M. Lehn, *J. Am. Chem. Soc.*, **2016**, *138*, 1142.
- [80] A. E. Izuagbe, V. X. Truong, B. T. Tuten, P. W. Roesky and C. Barner-Kowollik, *Macromolecules*, **2022**, *55*, 9242.
- [81] G. Li, F. Tao, L. Wang, Y. Li and R. Bai, *Polymer*, **2014**, *55*, 3696.
- [82] J. Rubio-Cervilla, P. Malo de Molina, B. Robles-Hernández, A. Arbe, A. J. Moreno, A. Alegría, J. Colmenero and J. A. Pomposo, *Macromol. Rapid Commun.*, **2019**, *40*, 1900046.
- [83] O. Altintas, J. Willenbacher, K. N. R. Wuest, K. K. Oehlenschlaeger, P. Krolla-Sidenstein, H. Gliemann and C. Barner-Kowollik, *Macromolecules*, **2013**, *46*, 8092.
- [84] E. Blasco, B. Yameen, A. S. Quick, P. Krolla-Sidenstein, A. Welle, M. Wegener and C. Barner-Kowollik, *Macromolecules*, **2015**, *48*, 8718.
- [85] M. Spicuzza, S. P. Gaikwad, S. Huss, A. A. Lee, C. V. Craescu, A. Griggs and E. Elacqua, *Polym. Chem.*, **2024**, *15*, 1833.
- [86] J. Willenbacher, K. N. R. Wuest, J. O. Mueller, M. Kaupp, H.-A. Wagenknecht and C. Barner-Kowollik, *ACS Macro Lett.*, **2014**, *3*, 574.
- [87] C. M. Geiselhart, J. T. Offenloch, H. Mutlu and C. Barner-Kowollik, *ACS Macro Lett.*, **2016**, *5*, 1146.
- [88] K. N. R. Wuest, H. Lu, D. S. Thomas, A. S. Goldmann, M. H. Stenzel and C. Barner-Kowollik, *ACS Macro Lett.*, **2017**, *6*, 1168.
- [89] C. Heiler, J. T. Offenloch, E. Blasco and C. Barner-Kowollik, *ACS Macro Lett.*, **2017**, *6*, 56.
- [90] E. J. Foster, E. B. Berda and E. W. Meijer, *J. Am. Chem. Soc.*, **2009**, *131*, 6964.
- [91] T. Mes, R. van der Weegen, A. R. A. Palmans and E. W. Meijer, *Angew. Chem. Int. Ed.*, **2011**, *50*, 5085.
- [92] G. M. Ter Huurne, A. R. A. Palmans and E. W. Meijer, *CCS Chem.*, **2019**, *1*, 64.
- [93] I. Perez-Baena, I. Asenjo-Sanz, A. Arbe, A. J. Moreno, F. Lo Verso, J. Colmenero and J. A. Pomposo, *Macromolecules*, **2014**, *47*, 8270.
- [94] J. De-La-Cuesta and J. A. Pomposo, *ACS Omega*, **2018**, *3*, 15193.
- [95] A. Nitti, R. Carfora, G. Assanelli, M. Notari and D. Pasini, *ACS Appl. Nano Mater.*, **2022**, *5*, 13985.
- [96] E. Harth, B. Van Horn, V. Y. Lee, D. S. Germack, C. P. Gonzales, R. D. Miller and C. J. Hawker, *J. Am. Chem. Soc.*, **2002**, *124*, 8653.
- [97] X. Ji, Y. Zhang and H. Zhao, *Chem. Eur. J.*, **2018**, *24*, 3005.

- [98] J. A. Pomposo, I. Perez-Baena, F. Lo Verso, A. J. Moreno, A. Arbe and J. Colmenero, *ACS Macro Lett.*, **2014**, *3*, 767.
- [99] A. Sanchez-Sanchez, S. Akbari, A. Etxeberria, A. Arbe, U. Gasser, A. J. Moreno, J. Colmenero and J. A. Pomposo, *ACS Macro Lett.*, **2013**, *2*, 491.
- [100] A. M. Stadler, L. Stingaciu, A. Radulescu, O. Holderer, M. Monkenbusch, R. Biehl and D. J. Richter, *J. Am. Chem. Soc.*, **2014**, *136*, 6987.
- [101] F. Lo Verso, J. A. Pomposo, J. Colmenero and A. J. Moreno, *Soft Matter*, **2015**, *11*, 1369.
- [102] C. H. Liu, L. D. Dugas, J. I. Bowman, T. Chidanguro, R. F. Storey and Y. C. Simon, *Polym. Chem.*, **2020**, *11*, 292.
- [103] J. B. Beck, K. L. Killops, T. Kang, K. Sivanandan, A. Bayles, M. E. Mackay, K. L. Wooley and C. J. Hawker, *Macromolecules*, **2009**, *42*, 5629.
- [104] T. Akagi, P. Piyapakorn and M. Akashi, *Langmuir*, **2012**, *28*, 5249.
- [105] M. Chen, C. J. Riddles and M. R. Van De Mark, *Langmuir*, **2013**, *29*, 14034.
- [106] T. Terashima, T. Sugita, K. Fukae and M. Sawamoto, *Macromolecules*, **2014**, *47*, 589.
- [107] J. De-La-Cuesta, E. González, A. J. Moreno, A. Arbe, J. Colmenero and J. A. Pomposo, *Macromolecules*, **2017**, *50*, 6323.
- [108] S. Mavila, O. Eivgi, I. Berkovich and N. G. Lemcoff, *Chem. Rev.*, **2016**, *116*, 878.
- [109] A. C. Balazs, T. Emrick and T. P. Russell, *Science*, **2006**, *314*, 1107.
- [110] S. K. Kumar, B. C. Benicewicz, R. A. Vaia and K. I. Winey, *Macromolecules*, **2017**, *50*, 714.
- [111] S. C. Tjong, *Mater. Sci. Eng., R*, **2006**, *53*, 73.
- [112] A. Moyassari, M. Unge, M. S. Hedenqvist, U. W. Gedde and F. Nilsson, *J. Chem. Phys.*, **2017**, *146*, 204901.
- [113] E. J. Bailey and K. I. Winey, *Prog. Polym. Sci.*, **2020**, *105*, 101242.
- [114] D. Bhowmik, J. A. Pomposo, F. Juranyi, V. García-Sakai, M. Zamponi, Y. Su, A. Arbe and J. Colmenero, *Macromolecules*, **2014**, *47*, 304.
- [115] D. Bhowmik, J. A. Pomposo, F. Juranyi, V. García-Sakai, M. Zamponi, A. Arbe and J. Colmenero, *Macromolecules*, **2014**, *47*, 3005.
- [116] A. Arbe, J. A. Pomposo, I. Asenjo-Sanz, D. Bhowmik, O. Ivanova, J. Kohlbrecher and J. Colmenero, *Macromolecules*, **2016**, *49*, 2354.
- [117] P. Zhao, Z. Yan, Y. Liang and R. Zhang, *Macromolecules*, **2025**, *58*, 3478.
- [118] J. Qu, T. Yue, H. Zhao, Y. Chen, L. Zhang and J. Liu, *Macromolecules*, **2025**, *58*, 5395.
- [119] J. A. Pomposo, A. R. de Luzuriaga, I. García, A. Etxeberria and J. Colmenero, *Macromol. Rapid Commun.*, **2011**, *32*, 573.
- [120] B. Robles-Hernández, X. Monnier, J. A. Pomposo, M. Gonzalez-Burgos, D. Cangialosi and A. Alegría, *Macromolecules*, **2019**, *52*, 6868.

- [121] B. Robles-Hernández, M. González-Burgos, J. A. Pomposo, J. Colmenero and A. Alegría, *Polymers*, **2019**, *11*, 533.
- [122] M. E. Mackay, T. T. Dao, A. Tuteja, D. L. Ho, B. Van Horn, H.-C. Kim and C. J. Hawker, *Nat. Mater.*, **2003**, *2*, 762.
- [123] A. Tuteja, M. E. Mackay, C. J. Hawker and B. Van Horn, *Macromolecules*, **2005**, *38*, 8000.

CHAPTER 2

Theoretical Method Development for Single-Chain Nanoparticle Structure-Size Analysis

2.1. Introduction

In the last decades, single-chain technology has emerged as a fascinating research topic, enabling the creation of various functional soft nano-objects through chain compaction. In particular, single-chain nanoparticles (SCNPs), formed via intrachain cross-linking of a linear polymer precursor, have garnered significant attention.^[1-5]

In a SCNP, the own folding process induces the formation of locally compact domains and, at the end, it gives a notable degree of global chain compaction as sketched in Fig. 2.1.^[6] Remarkably, the local domains of SCNPs can be used to immobilize e.g., catalysts, sensing probes or drug molecules paving the way to a variety of potential applications.^[5] In this sense, the folding of a synthetic polymer chain to a SCNP resembles the folding of certain biomacromolecules to their native, functional state. Nevertheless, SCNPs produced from precursors in good solvent conditions often show a conformation far from the globular one displayed e.g., by compact 3D-structured proteins. In fact, the morphology of these SCNPs closely resembles that typically exhibited by intrinsically disordered proteins (IDPs)^[6] and some works explore this relationship between the phase behavior and size of IDPs in more detail.^[1,2]

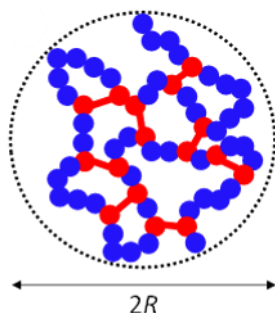


Figure 2.1. Schematic illustration of an elastic single-chain polymer nanoparticle (SCNP), depicted as a network of elastic strands (blue) connected by cross-links (red), showing an isolated SCNP at high dilution with a radius R .^[7,8]

To understand the structure of SCNPs in different scenarios,^[3] a combination of theory, simulations and experimental techniques has been very successful. From the theoretical side, the Elastic SCNPs Model (ESM), presented in Chapter 1,^[7,8] provides a connection between the SCNP size, number of segments and fraction of reactive groups in the precursor polymer in terms of a parameter related to the elasticity of the unimolecular network. When combined with scaling concepts, the ESM allows one to estimate the size of locally compact domains in SCNPs,^[4] and the translocation of SCNPs through nanopores and nanoslits,^[8] for instance.

Throughout this chapter, and applying the premises proposed by the ESM, the structural properties of SCNPs will be described under different conditions that significantly influence their behavior and applications. Specifically, we will focus on three key scenarios:

1. Grafted onto a surface, forming polymer brushes (Fig. 2.2).

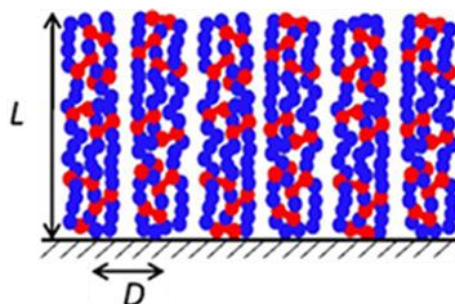


Figure 2.2. Brush composed of densely grafted elastic SCNPs on a flat surface (brush thickness: L ; distance between grafting points: D). The red color represents the reactive “A” groups.

2. Adopting a tadpole-like conformation (Fig. 2.3).

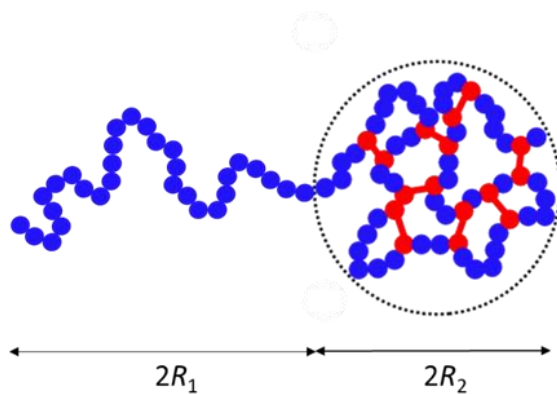


Figure 2.3. Tadpole-shaped single-chain nanoparticle (TSCNP) formed via intrachain cross-linking from a diblock copolymer containing reactive groups (red color).

3. Under conditions of severe crowding (Fig. 2.4).

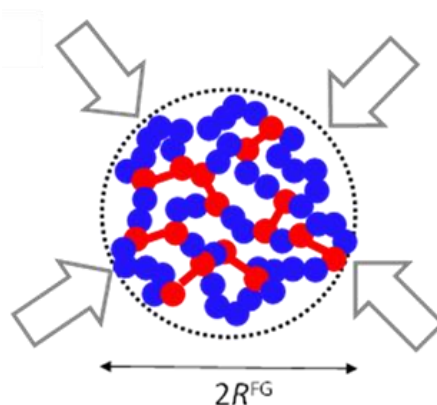


Figure 2.4. Size reduction experienced by a SCNP on passing from high diluted conditions in the presence of solvent molecules to a highly crowded environment. The red color represents the reactive “A” groups.

2.2. Structural Properties of Brushes of Elastic SCNPs on Flat Surfaces

2.2.1. Introduction

The study of polymer brushes formed by a sufficiently dense assembly of polymer chains tethered by one end to a surface extends into many fields, including chemistry, physics, and bionanotechnology.^[9-11] Polymer brushes allow the tuning of many surface properties such as biocompatibility, wettability, friction and, for metal surfaces, even corrosion resistance.^[12,13] Hence, surfaces decorated with brushes of linear polymers have attracted significant interest for a variety of practical applications,^[14-18] including colloid stabilization, development of protein-resistant layers, new adhesives, etc.

Linear polymer brushes on solid substrate surfaces have been the subject of many different studies in the last decades, covering from experiment to theory and simulation, so their structural properties are nowadays well comprehended. The interest to reach a similar level of understanding for brushes of polymers with increased level of topological complexity^[19-31] (i.e., star-like polymers, dendrons, comb-like polymers, cycled macromolecules) is growing in recent years. This interest is motivated to some extent by the complex topology found in many extracellular brush-like layers that govern cell adhesive and interactive properties (e.g., polysaccharides) so unraveling the effect of topology from the effect of specific interactions is of paramount importance.

Different theoretical models, from mean-field and scaling methods to analytical and numerical self-consistent field (SCF) theories, have been developed to understand how the macromolecular architecture mediates the brush thickness, limiting grafting density, polymer density profile, and distribution of free ends in these complex brushes. Interestingly, the scaling approach initially proposed by Alexander^[32] and de Gennes^[33] for brushes of linear chains has been found useful to understand the structural properties (thickness, maximum grafting density) of brushes formed by polymers with more complex architectures than the linear one (e.g., randomly branched polymers). More refined SCF theories^[34,35] have been formulated to ascertain the vertical monomer density profile and distribution of free ends in brushes of polymers with certain affordable topologies. When computationally affordable, the accuracy of the different models has been tested by molecular dynamics (MD) simulations.^[23, 36, 37]

Very recently, surfaces displaying molecular recognition motifs have been decorated with brushes of responsive SCNPs and transformed into a cross-linked polymeric monolayer,^[38] thus paving the way to controlled nanofilm formation around bioactive surfaces (e.g., virus capsids).

This section aims to explore the structural properties of brushes formed by topologically complex SCNPs on flat surfaces, and to compare them with those exhibited by brushes of other macromolecular architectures, such as branched polymers, symmetric cyclic macromolecules, and linear chains. The equilibrium structure of a planar brush of SCNPs grafted onto a solid, inert surface was analyzed up to the limiting maximum grafting density using a model of elastic SCNPs.^[7,8] Scaling-law expressions for the brush thickness, local domain size and free energy per chain were derived for brushes of elastic SCNPs on flat surfaces in contact with (i) a good solvent, (ii) a θ -solvent, and (iii) in the melt state. In addition, the unfolding of SCNP brushes induced by the progressive random breaking of intrachain cross-links was investigated for all the above mentioned regimes.

In the Results and Discussion part, a summary of all the derived scaling law expressions will be presented. Subsequently, a comparison of SCNPs with other macromolecular architectures will be conducted for the three cases previously discussed: i) good solvent, ii) θ -solvent, and iii) melt regime. Additionally, a derivation of the limiting scaling laws at maximum grafting density will be presented, as well as an analysis of the progressive unfolding via random breaking of the intrachain cross-links of SCNP brushes. Furthermore, a comparison of model predictions with experimental results will also be included.

2.2.2. Method Development

This study focuses on the most interesting case of brushes composed of densely grafted SCNPs on a flat surface. As shown in Fig. 2.2, the elastic nanoparticles are arranged on a flat surface in a brush-like manner, where the parameters L and D represent the length (or brush thickness) and the average distance between two grafting sites, respectively. Depending on the solvent conditions of this system, three scenarios can be considered: i) good solvent regime (athermal solvent), ii) θ -solvent regime, and iii) melt regime.

These scenarios will be examined in more detail, including the determination of expressions for brush thickness (L), local domain size (ξ), grafting density (σ), and free energy per chain, if possible, as well as other magnitudes associated with each case.

i) Good solvent regime

A) Brush Thickness

In contact with a good solvent, each SCNP in the brush is stretched with a length $L > R$, where R represents the size (radius) of the SCNP. Since for densely grafted SCNPs the volume available per SCNP is $\approx D^2 L$, the free energy expression, (Eq. (1.14), from Chapter 1) becomes

$$\frac{F(L)}{k_B T} \approx KL^2 + \frac{N^2 a^3}{D^2 L} \quad (2.1)$$

where K represents an elasticity parameter, N is the number of monomers in the chain (degree of polymerization), and a is the monomeric segment length. Eq. (2.1) can be rewritten in terms of the grafting density $\sigma = a^2/D^2$, such as

$$\frac{F(L)}{k_B T} \approx KL^2 + \frac{N^2 a \sigma}{L} \quad (2.2)$$

After minimization, we obtain an expression that relates the brush thickness L with a , K , N and σ , such as

$$L \approx a^{1/3} K^{-1/3} N^{2/3} \sigma^{1/3} \quad (2.3)$$

B) Local Domain Size

Each SCNP in the brush can be viewed as a string of blobs, where each blob has a local domain size ξ and consists of g segments. The volume fraction ϕ is then expressed as

$$\phi \approx \frac{Na\sigma}{L} \approx \frac{ga^3}{\xi^3} \quad (2.4)$$

Inside each blob (good solvent conditions, no confinement inside the blob) we can assume:^[40]

$$\xi \approx a^{3/5} K^{-1/5} g^{2/5} \quad (2.5)$$

Hence, by combining Eqs. (2.3)–(2.5) we obtain an expression for ξ as a function of a , K , N and σ , such as

$$\xi \approx a^{5/3} K^{1/3} N^{-2/3} \sigma^{-4/3} \quad (2.6a)$$

Alternatively, ξ can be expressed as a function of a , σ and L :

$$\xi \approx a^2 (\sigma L)^{-1} \quad (2.6b)$$

C) Grafting Density Limits in the Brush Regime.

The maximum (or limiting) grafting density, σ_{max} , is obtained from the condition of the highest possible packing of the SCNPs in the brush, $\phi \approx \frac{Na\sigma_{max}}{L} = 1$, and from Eq. (2.3) it is given by

$$\sigma_{max} \approx a^{-1} (KN)^{-1/2} \quad (2.7)$$

The lower limit of grafting density in the brush regime can be determined from the boundary between the mushroom regime ($D > 2R$) and the brush regime ($D < 2R$), which is given by the condition: $D = 2R$. Consequently, in the brush regime we have

$$\sigma \geq \frac{a^{4/5} K^{2/5}}{4N^{4/5}} = \sigma_{min} \quad (2.8)$$

D) Free Energy per Chain

The change in free energy upon confinement of a SCNP in a planar brush in contact with a good solvent (F_B^c) can be estimated as^[8]

$$\frac{F_B^c}{k_B T} \approx \frac{D^2 L}{\xi^3} = \frac{a^2 L}{a \xi^3} \quad (2.9)$$

so from Eqs. (2.3) and (2.6) we obtain

$$\frac{F_B^c}{k_B T} \approx a^{-8/3} K^{-4/3} N^{8/3} \sigma^{10/3} \quad (2.10a)$$

$$\frac{F_B^c}{k_B T} \approx \left(\frac{L}{a}\right)^4 \sigma^2 \quad (2.10b)$$

E) Mapping to Self-Consistent Field (SCF) Analytical Results - Correlation with Analytical Results from Self-Consistent Field (SCF) Theory

According to SCF analytical results, a topological coefficient k (a *universal* parameter to characterize polymer topology) determines the brush thickness L and polymer density distribution $\Psi(z)$, where z denotes the distance from the surface, for a variety of brushes of different topology under good solvent conditions,^[21] according to:

$$\frac{L}{a} = \left(\frac{2N\sigma}{k^2} \right)^{1/3} \quad (2.11)$$

$$\Psi(z) = \frac{3}{4} \left(\frac{k}{a} \right)^2 (L^2 - z^2) \quad (2.12)$$

For instance, for a symmetric cycled macromolecule with q linkers ($q \gg 1$) and fixed total number of monomers N , the SCF topological coefficient is given by $k_{cyclic} = q^{1/2}/N$, whereas for a linear chain: $k_{linear} = \pi/(2N)$.^[21] By performing a mapping of Eq. (2.3) to Eq. (2.11) we can obtain an *apparent* SCF topological coefficient k for SCNPs, such as

$$k_{SCNPs} = a \left(\frac{2K}{N} \right)^{1/2} \quad (2.13)$$

By combining Eq. (2.12) and Eq. (2.13), an expression for the SCF density profile is obtained:

$$\Psi(z) = \frac{3}{2} \left(\frac{K}{N} \right) (L^2 - z^2) \quad (2.14)$$

F) *Unfolding via Sequential Breaking of Intrachain Cross-Links*

The case of the unfolding of SCNP brushes in a good solvent into linear chains via the progressive random breaking of intrachain "A-A" cross-links (see Fig. 2.2) was then considered. For this case, by retaining the complete expression for the elastic free energy (Eq. (1.15) from Chapter 1) and taking into account that R_0 represents the size of the SCNPs when unfolded, the modified expression is obtained by changing $K \rightarrow K + R_0^{-2}$ in Eq. (2.3)

$$L \approx a^{1/3} ((a^2 N)^{-1} + Ax)^{-1/3} N^{2/3} \sigma^{1/3} \quad (2.15)$$

Eq. (2.15) can be used to predict the evolution of L upon decreasing the value of the intrachain cross-linking degree x (i.e., to estimate the unfolding pathway).

ii) *Theta-solvent regime*

A) *Brush Thickness*

The focus is now shifted to the case of a brush composed of grafted elastic SCNPs on a flat surface in contact with a θ -solvent. Following an analysis similar to that followed in the case of good solvent conditions (now replacing $N^2 a^3 D^{-2} L^{-1}$ by $N^3 a^6 D^{-4} L^{-2}$ in Eq. (2.1)) it is found that the brush thickness -denoted as L^θ - is given by

$$L^\theta \approx a^{1/2} K^{-1/4} N^{3/4} \sigma^{1/2} \quad (2.16)$$

Consequently, the ratio of the brush thickness in a θ -solvent (Eq. (2.16)) to the brush thickness in a good solvent (Eq. (2.3)) is given by

$$\frac{L^\theta}{L} \approx (a\sigma)^{1/6} (KN)^{1/12} \quad (2.17)$$

B) *Local Domain Size*

For a brush composed of grafted elastic SCNPs on a flat surface in contact with a θ -solvent, it can be shown that ξ^θ is given by

$$\xi^\theta \approx a^{3/2} K^{1/4} N^{-3/4} \sigma^{-3/2} \quad (2.18)$$

The former expression has been obtained by combining Eqs. (2.6b) and (2.16) and the scaling of Eq. (1.22) (Chapter 1) for the blob size in θ -conditions. Then, the ratio of the local domain size in a θ -solvent (Eq. (2.18)) to that in a good solvent (Eq. (2.6a)) is

$$\frac{\xi^\theta}{\xi} = (a\sigma)^{-1/6} (KN)^{-1/12} \quad (2.19)$$

C) *Grafting Density Limits in the Brush Regime*

It can be easily shown (by using $\phi \approx \frac{Na\sigma_{max}}{L} = 1$ and Eq. (2.16)) that σ_{max}^θ is given by the same expression derived for a brush composed of grafted elastic SCNPs on a flat surface in contact with a good solvent (Eq. (2.7)), so $\sigma_{max}^\theta/\sigma_{max} \equiv 1$. Concerning the lower limit of grafting density, by using $D = 2R^\theta$ and Eq. (1.22) (Chapter 1), the following condition is obtained

$$\sigma^\theta \geq \frac{a^{1/2} K^{1/4}}{4N^{3/4}} = \sigma_{min}^\theta \quad (2.20)$$

D) *Free Energy per Chain*

The change in free energy upon confinement of a SCNP in a planar brush in contact with a θ -solvent ($F_B^{c,\theta}$) is given by

$$\frac{F_B^{c,\theta}}{k_B T} \approx a^{-2} K^{-1} N^3 \sigma^4 \quad (2.21)$$

The former expression has been obtained by combining the condition $\frac{F_B^{c,\theta}}{k_B T} \approx D^2 L \xi^{-3}$, with Eqs. (2.16) and (2.18).

Consequently, the ratio of $F_B^{c,\theta}$ (Eq. (2.21)) to F_B^c (Eq. (2.10a)) is

$$\frac{F_B^{c,\theta}}{F_B^c} \approx \left(\frac{KN}{a^2} \right)^{1/3} \sigma^{2/3} \quad (2.22)$$

E) *Unfolding via Sequential Breaking of Intrachain Cross-Links*

For the case of unfolding of brushes of SCNPs in a θ -solvent to linear chains via progressive random breaking of the intrachain “A-A” cross-links we obtain the following expression (by setting $K \rightarrow K + R_0^{-2}$ in Eq. (2.16))

$$L^\theta \approx a^{1/2}((a^2N)^{-1} + Ax)^{-1/4}N^{3/4}\sigma^{1/2} \quad (2.23)$$

iii) *Melt regime*

A) *Brush Thickness*

The analysis is extended to a brush composed of a melt of densely grafted elastic SCNPs on a flat surface. Following a procedure similar to that introduced by Milner,^[41] the free energy of the system can be written as

$$\frac{F(L)}{k_B T} \approx KL^2 + \frac{\gamma}{\sigma} \quad (2.24)$$

where the second term is an interfacial tension contribution. From the incompressibility condition ($D^2L = Na^3$), we obtain $L \approx aN\sigma$, so Eq. (2.24) becomes

$$\frac{F(L)}{k_B T} \approx KL^2 + \frac{aN\gamma}{L} \quad (2.25)$$

After minimization, we obtain an expression of the brush thickness in the melt regime, L^m , as a function of a , K , N and γ such as

$$L^m \approx \left(\frac{aN\gamma}{K} \right)^{1/3} \quad (2.26)$$

B) *Grafting Density*

From $\phi \approx \frac{Na\sigma}{L^m} = 1$ and Eq. (2.26), we obtain

$$\sigma \approx (aN)^{-2/3} \left(\frac{\gamma}{K} \right)^{1/3} \quad (2.27)$$

C) *Unfolding via Sequential Breaking of Intrachain Cross-Links*

For the case of unfolding of brushes of SCNPs in the melt to linear chains *via* progressive random breaking of intrachain cross-links we obtain (by replacing $K \rightarrow K + R_0^{-2}$ in Eq. (2.26))

$$L^m \approx \left(\frac{aN\gamma}{(a^2N)^{-1} + Ax} \right)^{1/3} \quad (2.28)$$

2.2.3. Results and Discussion

Table 2.1 provides a summary of the scaling laws obtained in this work for brushes of densely grafted elastic SCNPs on flat surfaces in contact i) with a good solvent, ii) with a θ -solvent, and iii) in the melt. The scaling expressions reported in Table 2.1 are valid only in the brush regime, i.e., in the grafting density range $\sigma_{min} \leq \sigma \leq \sigma_{max}$.

Brushes of elastic single-chain nanoparticles versus brushes of other macromolecular architectures

To establish a comparison of elastic SCNPs with other architectures, the Alexander-de Gennes (AdG) model has been used. The scaling results obtained within the framework of the AdG model for the most relevant cases of brushes of randomly branched polymers (BPs)^[31] and brushes of linear polymers (LPs)^[32,33] are summarized in Tables 2.2 and 2.3.

Table 2.1. Summary of scaling law expressions for brushes of densely grafted elastic single-chain nanoparticles on flat surfaces^a.

Magnitude	Brush of SCNPs in a good solvent	Brush of SCNPs in a θ -solvent	Brush of SCNPs in the melt
Brush thickness	$L \approx a^{1/3} K^{-1/3} N^{2/3} \sigma^{1/3}$	$L^\theta \approx a^{1/2} K^{-1/4} N^{3/4} \sigma^{1/2}$	$L^m \approx K^{-1/3} (aN\gamma)^{1/3}$
Local domain size	$\xi \approx a^{5/3} K^{1/3} N^{-2/3} \sigma^{-4/3}$	$\xi^\theta \approx a^{3/2} K^{1/4} N^{-3/4} \sigma^{-3/2}$	-
Free energy per chain	$\frac{F_B^c}{k_B T} \approx a^{-8/3} K^{-4/3} N^{8/3} \sigma^{10/3}$	$\frac{F_B^{c,\theta}}{k_B T} \approx a^{-2} K^{-1} N^3 \sigma^4$	-
Grafting density range	$\left(\frac{a^{4/5} K^{2/5}}{4N^{4/5}} \right) \leq \sigma \leq a^{-1} (KN)^{-1/2}$	$\left(\frac{a^{1/2} K^{1/4}}{4N^{3/4}} \right) \leq \sigma \leq a^{-1} (KN)^{-1/2}$	$\sigma \approx (aN)^{-2/3} \left(\frac{\gamma}{K} \right)^{1/3}$

^a K = SCNP elasticity parameter.

a = Monomer size.

N = SCNP total number of monomers.

σ = SCNP grafting density.

γ = SCNP brush interfacial tension.

Table 2.2. Scaling law expressions for brushes of densely grafted branched polymers (BPs) on flat surfaces^a.

Magnitude	Brush of BPs in a good solvent	Brush of BPs in a θ -solvent	Brush of BPs in the melt
Brush thickness	$L \approx ab^{1/6}N^{5/6}\sigma^{1/3}$	$L \approx ab^{1/8}N^{7/8}\sigma^{1/2}$	$L^m \approx ab^{1/6}N^{1/2}\gamma^{1/3}$
Local domain size	$\xi \approx ab^{-1/30}N^{-1/6}\sigma^{-2/3}$	$\xi \approx ab^{-1/40}N^{-7/40}\sigma^{-7/10}$	-
Free energy per chain	$\frac{F_B^c}{k_B T} \approx b^{4/15}(N\sigma)^{4/3}$	$\frac{F_B^c}{k_B T} \approx b^{1/5}N^{7/5}\sigma^{8/5}$	-
Grafting density range	$\left(\frac{1}{4b^{1/5}N}\right) \leq \sigma \leq \left(\frac{b}{N}\right)^{1/4}$	$\left(\frac{1}{4b^{1/8}N^{7/8}}\right) \leq \sigma \leq \left(\frac{b}{N}\right)^{1/4}$	$\sigma \approx b^{1/6}N^{-1/2}\gamma^{1/3}$

^a a = Monomer size.

b = Average number of monomers between consecutive branching points.

N = Total number of monomers.

σ = Grafting density.

γ = Brush interfacial tension.

Table 2.3. Scaling law expressions for brushes of densely grafted linear polymers (LPs) on flat surfaces^a.

Magnitude	Brush of LPs in a good solvent	Brush of LPs in a θ -solvent	Brush of LPs in the melt
Brush thickness	$L \approx aN\sigma^{1/3}$	$L^\theta \approx aN\sigma^{1/2}$	$L^m \approx aN^{2/3}\gamma^{1/3}$
Local domain size	$\xi \approx a\sigma^{-1/2}$	$\xi^\theta \approx a\sigma^{-1/2}$	-
Free energy per chain	$\frac{F_B^c}{k_B T} \approx N\sigma^{5/6}$	$\frac{F_B^c}{k_B T} \approx N\sigma$	-
Grafting density range	$\left(\frac{1}{4N^{6/5}}\right) \leq \sigma \leq 1$	$\left(\frac{1}{4N}\right) \leq \sigma \leq 1$	$\sigma \approx \left(\frac{\gamma}{N}\right)^{1/3}$

^a a = Monomer size.

N = Total number of monomers.

σ = Grafting density.

γ = Brush interfacial tension.

i) Good Solvent Regime

Figure 2.5 illustrates the significant effect of the specific macromolecular topology on the dependence of L (normalized by $\sigma^{1/3}$) on N for elastic SCNPs with different values of the elasticity parameter K , randomly branched polymers (BPs) (Table 2.2) and linear polymers (LPs) (Table 2.3) in good solvent conditions.

Remarkably, the scaling exponent of L on N is $2/3$ (≈ 0.67) for SCNPs, $5/6$ (≈ 0.83) for BPs (see Table 2.2) and 1 for LPs (Table 2.3). As illustrated in Fig. 2.5, for a given value of N , the elasticity parameter K of the SCNPs, which is related to the intrachain cross-linking degree x and the elasticity constant A ($K \approx Ax$), is an additional parameter for tuning the brush thickness when compared to traditional brushes of linear polymers, as canonical systems. As an example, a small cross-linking degree (e.g., $x = 0.02$ from $K = 1$ by assuming^[7] $A = 47.6 \text{ nm}^{-2}$) is enough to notably decrease the brush thickness in the case of SCNPs when compared to LPs or BPs.

Table 2.4 provides values of the topological ratio $\eta \equiv k/k_{linear} = 2kN/\pi$ (for $N = 100$, $a = 1 \text{ nm}$),^[21] symmetric cycled macromolecules with different number of linkers ($q = 4$ and $q = 6$; $k_{cyclic} = q^{1/2}/N$)^[21] and brushes of SCNPs with different elastic characteristics ($K = 1, 5, 15$ and 35 ; $k_{SCNPs} = \left(\frac{2K}{N}\right)^{1/2}$, see Eq. (2.13)). Obviously, the linear chain is taken as the reference case, for which $\eta \equiv 1$. According to Eq. (2.11), upon increasing the value of η a decrease in brush thickness is expected. For tree-like polymers and cycled chains the interpenetration length of opposing brushes (and hence the friction between sliding brushes) is predicted to decrease upon an increase in the topological ratio η .^[21] Therefore, a similar effect on tribological properties is expected for brushes of SCNPs according to the η data displayed in Table 2.4.

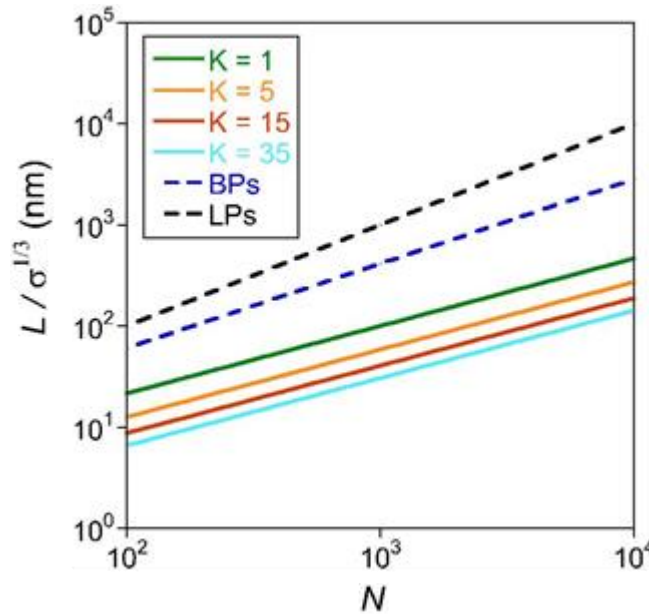


Figure 2.5. Illustration of the effect of macromolecular topology on brush thickness in a good solvent normalized by the grafting density as a function of the total number of monomers N as predicted from: $L/\sigma^{1/3} \approx a^{1/3}K^{-1/3}N^{2/3}$ (Eq. (2.3)) for elastic SCNPs with different values of the elasticity parameter K ; randomly branched polymers (BPs), as calculated from (see Table 2.2): $L/\sigma^{1/3} \approx ab^{1/6}N^{5/6}$ with $b = 5$ and linear polymers (LPs), as estimated from (Table 2.3): $L/\sigma^{1/3} \approx aN$. For the sake of clarity, $a=1 \text{ nm}$ has been selected in all cases.

ii) Theta-Solvent Regime

The brush thickness in a θ -solvent L^θ (normalized by $\sigma^{1/2}$) as a function of N for elastic SCNPs, randomly BPs and LPs is shown in Fig. 2.6. Overall, the same trends as observed for the case of good solvent conditions are found in the θ -solvent regime, with only minor changes in the scaling exponents for SCNPs and BPs. Hence, the scaling exponent of L^θ on N is $3/4$ ($= 0.75$) for SCNPs, $7/8$ (≈ 0.88) for BPs (Table 2.2) and 1 for LPs (Table 2.3).

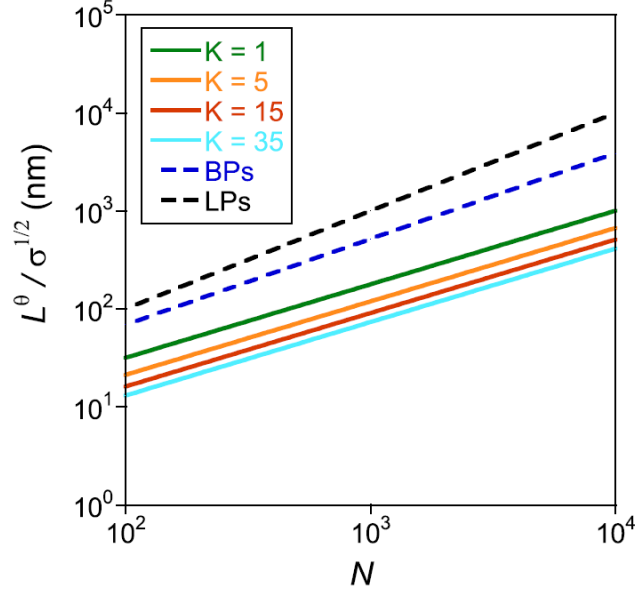


Figure 2.6. Illustration of the effect of macromolecular topology on brush thickness in a θ -solvent normalized by the grafting density as a function of the total number of monomers N as predicted from: $L^\theta / \sigma^{1/2} \approx a^{1/2} K^{-1/4} N^{3/4}$ (Eq. (2.16)) for elastic SCNPs with different values of the elasticity parameter K ; randomly BPs, as calculated from (Table 2.2): $L^\theta / \sigma^{1/2} \approx ab^{1/8} N^{7/8}$ with $b = 5$; and LPs, as estimated from (Table 2.3): $L^\theta / \sigma^{1/2} \approx aN$. For the sake of clarity, $a = 1$ nm has been selected in all cases.

iii) Melt Regime

Figure 2.7 illustrates the dependence of brush thickness in the melt L^m (normalized by $\gamma^{1/3}$) on N for elastic SCNPs, randomly BPs and LPs. In the melt regime, the scaling exponent of brush thickness on N is $1/3$ (≈ 0.33) for SCNPs, $1/2$ ($= 0.5$) for BPs (Table 2.2) and $2/3$ (≈ 0.67) for LPs (Table 2.3).

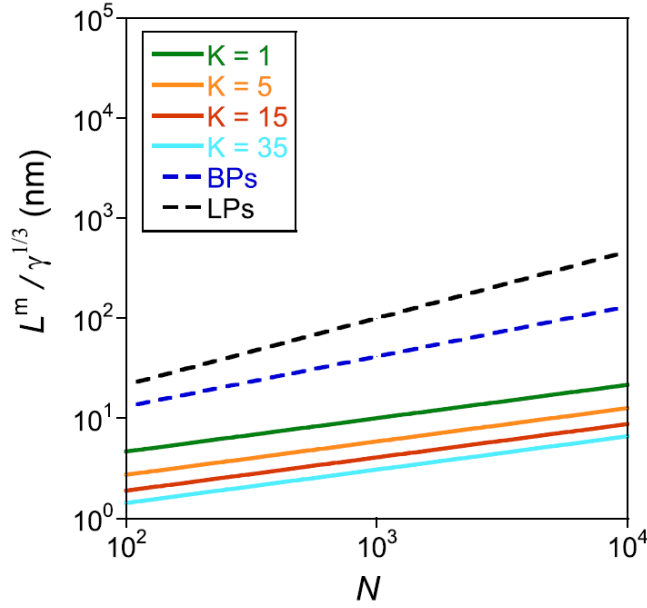


Figure 2.7. Dependence of brush thickness in the melt normalized by the interfacial tension on the total number of monomers N as predicted from: $L^m/\gamma^{1/3} \approx (aN/K)^{1/3}$ (Eq. (2.26)) for elastic SCNPs with different values of K ; randomly branched polymers, as calculated from (Table 2.2): $L^m/\gamma^{1/3} \approx ab^{1/6}N^{1/2}$ with $b = 5$; and linear polymers, as estimated from (Table 2.3): $L^m/\gamma^{1/3} \approx aN^{2/3}$. For the sake of clarity, $a = 1$ nm has been selected in all cases.

Table 2.4. Comparison of topological ratio (η) values for brushes symmetric cycled macromolecules^[21] and single-chain nanoparticles with different representative values of q and K parameters.

Macromolecular Architecture	Topological ratio ^a , $\eta \equiv 2kN/\pi$
Symmetric cycled chains, $q = 4$	1.3
Symmetric cycled chains, $q = 6$	1.6
Single-chain nanoparticles, $K = 1 \text{ nm}^{-2}$	9.0
Single-chain nanoparticles, $K = 5 \text{ nm}^{-2}$	20.1
Single-chain nanoparticles, $K = 15 \text{ nm}^{-2}$	34.9
Single-chain nanoparticles, $K = 35 \text{ nm}^{-2}$	53.3

^a Calculated for $N = 100$ and $a = 1$ nm in all cases (see text).

Limiting scaling laws at maximum grafting density for brushes of elastic single-chain nanoparticles in solution

It is worth mentioning that the brush thickness, local domain (blob) size and free energy per chain at maximum grafting density, σ_{max} , are given by the following expressions ($a = 1$ nm), which are valid both for good solvent and θ -solvent conditions:

$$L_{\sigma_{max}} \approx \left(\frac{N}{K}\right)^{1/2} \quad (2.29)$$

$$\xi_{\sigma_{max}} \approx K \quad (2.30)$$

$$\left(\frac{F_B^c}{k_B T}\right)_{\sigma_{max}} \approx K^{-3} N \quad (2.31)$$

Accordingly, the thickness and free energy per chain of brushes of elastic SCNPs at maximum grafting density ($L_{\sigma_{max}}$ and $\left(\frac{F_B^c}{k_B T}\right)_{\sigma_{max}}$, respectively) depend on the total number of monomers N and the elasticity parameter K of the SCNP, whereas the blob size $\xi_{\sigma_{max}}$ scales only with K (i.e., it is harder to deform a tightly cross-linked SCNP than a slightly collapsed one) but (unlike for randomly BPs, see Table 2.2) it does not depend on N .

Figure 2.8 illustrates the dependence of $L_{\sigma_{max}}$ on N for selected values of K according to Eq. (2.29). Taking into account that a representative value of A for polystyrene (PS) SCNPs^[7] is 47.6 nm², the values of $K = 1, 5, 15$ and 35 displayed in Fig. 2.8 should correspond to fractional cross-linking degrees of $\chi = 0.02, 0.11, 0.32$ and 0.74, respectively. Hence, as illustrated in Fig. 2.8, at a constant value of N , $L_{\sigma_{max}}$ can be adjusted by appropriate selection of SCNP cross-linking degree χ and, therefore, elasticity parameter K .

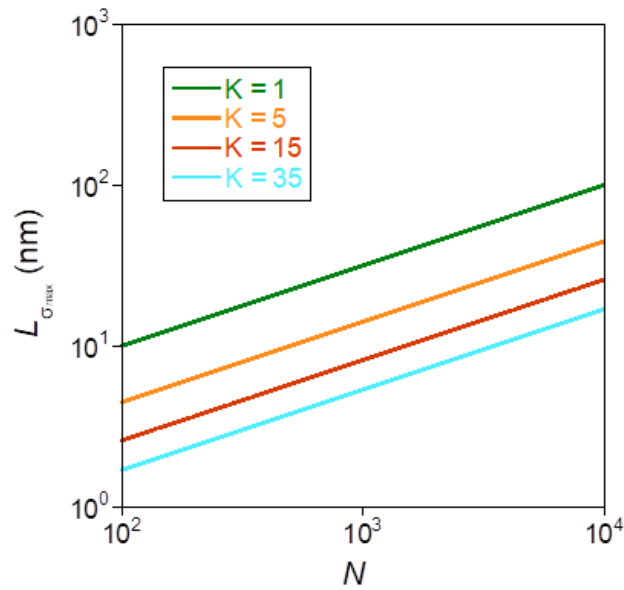


Figure 2.8. Illustration of the dependence of brush thickness at maximum grafting density σ_{max} , $L_{\sigma_{max}}$, on the total number of monomers N and elasticity parameter K for brushes of elastic SCNPs (see text, and Eq. (2.29)). For the sake of clarity, $a = 1$ nm has been selected in all cases.

Unfolding of brushes of elastic single-chain nanoparticles on flat surfaces

In the following, the expected behavior upon progressive unfolding via random breaking of the intrachain cross-links of brushes of elastic SCNPs on flat surfaces is compared for systems in contact i) with a good solvent, ii) with a θ -solvent, and iii) in the melt, previously presented above. From a practical point of view, this could be the case of SCNPs containing e.g., intrachain enamine covalent bonds, which can be broken at low pH values.^[42] This study is of great interest for the potential design of brushes of SCNPs with drug delivery characteristics based on stimulus-mediated rupture of intrachain cross-links.

Panels (a), (b) and (c) of Fig. 2.9 show the predicted unfolding pathway for brushes of elastic SCNPs of $N = 1000$ and $A = 47.6$ nm² in good solvent, θ -solvent and in the melt, respectively, upon decreasing the cross-linking degree χ , according to Eqs. (2.15), (2.23) and (2.28), respectively. In all the three cases, a large increase in brush thickness is observed upon progressive random breaking of intrachain cross-links (normalized -see figure caption-, the

thickness of a brush of linear chains of identical N , given by the respective expressions in the Table 2.3).

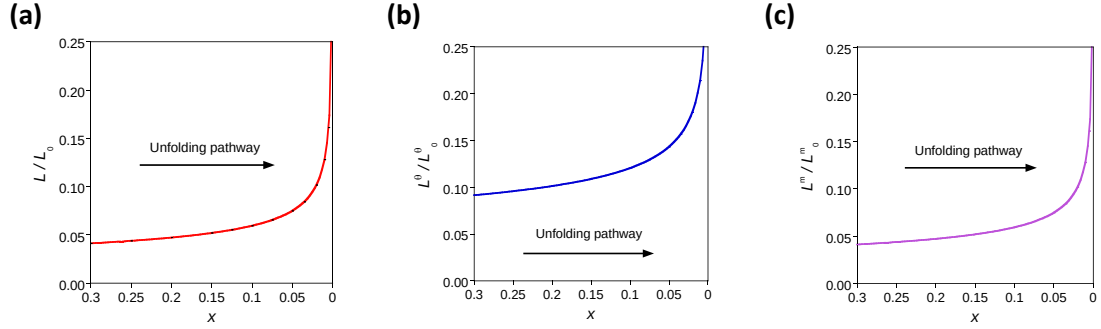


Figure 2.9. Unfolding pathway of brushes of SCNPs ($N = 1000$, $A = 47.6 \text{ nm}^{-2}$, $a = 1 \text{ nm}$) in good solvent (a), θ -solvent (b), and melt (c) regimes via progressive random breaking of intrachain cross-links as calculated from Eqs. (2.15), (2.23) and (2.28). L_0 , L_0^θ and L_0^m are the thicknesses of brushes of linear chains of identical N in good solvent (a), θ -solvent (b), and melt (c) regimes, respectively (Table 2.3).

Comparison of model predictions with experimental results

Concerning experimental data, a study by Fulton and co-workers^[38] reporting responsive SCNP brushes that can be transformed into a cross-linked polymer nanolayer was found. A rough estimation of the expected brush thickness for this system can be obtained based on Eqs. (2.3) and (2.7). By using the experimental data reported by Fulton *et al.*, the size of the linear precursor polymer in good solvent ($N = 88$, $x = 0.17$) is estimated to be around 2.7 nm and, hence, $a \approx 0.18 \text{ nm}$.^[43] Assuming $K \approx 1$ as a reasonable value for Fulton's SCNPs, $\sigma_{max} \approx 0.59$ is obtained from Eq. (2.7) and $L \approx 9.4 \text{ nm}$ from Eq. (2.3), which is in good agreement with $L_{exp} \approx 10 \text{ nm}$.^[38] Instead, a brush of the linear precursor polymer is expected to show a thickness of $L \approx 0.18 \times 88 = 15.8 \text{ nm}$.

2.3. Structure-Size Relationships for Tadpole-Shaped SCNPs (With Long and Short Flexible Tails Unveiled)

2.3.1. Introduction

Hybrid nano-objects with complex topology such as polymer-tethered nanoparticles have attracted significant interest for applications in a variety of fields, including colloidal and material science, as well as nanotechnology. The surfactant behavior of amphiphilic polymer-tethered nanoparticles with hydrophilic and hydrophobic components is well-known, endowing these nano-objects with unique interfacial properties.^[44-46] In fact, understanding the solution properties and self-assembly behavior of polymer-tethered nanoparticles is of great scientific and industrial interest. For nanotechnology applications, polymer-tethered nanoparticles can be considered as elemental building blocks to construct advanced self-assembled structures.

Beyond research in polymer-tethered inorganic nanoparticles,^[47-49] in recent years, the construction of polymer-tethered organic nanoparticles has become a new way to obtain totally soft building blocks for different applications. Among them, the most versatile ones are tadpole-shaped single-chain nanoparticles (TSCNPs) comprising a flexible polymer chain tethered to an intra-molecularly folded/collapsed single-chain polymer nanoparticle.^[3] TSCNPs can be considered as an output of so-called single chain technology.^[50] TSCNPs are often synthesized (see Fig. 2.10(a)) from a diblock copolymer precursor containing an inert block and a reactive block^[51] in a good solvent for both blocks at high dilution (i.e., below the chain overlap concentration, c^*).^[50] A variety of intrachain cross-linking chemistries have been used to

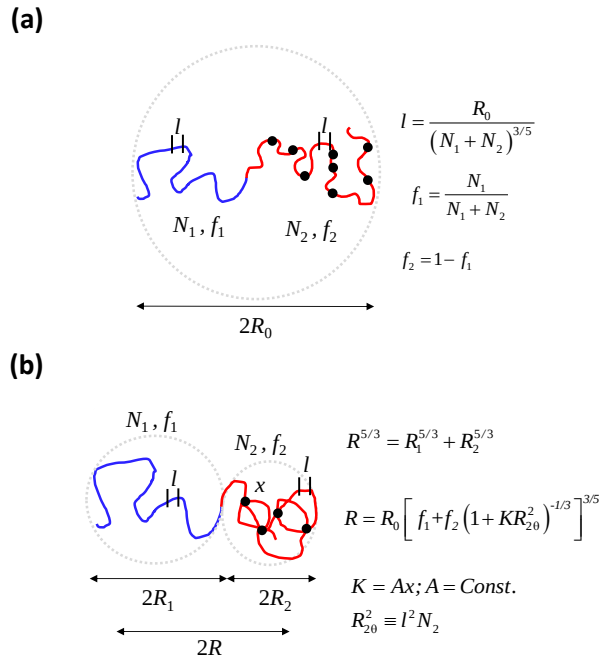


Figure 2.10. (a) Schematic illustration of a diblock copolymer comprising an inert block (denoted as **1**) with N_1 monomers, and a reactive block with xN_2 cross-linkable monomers as well as $(1-x)N_2$ non-reactive monomers. The average monomer length is given by $l = R_0/(N_1 + N_2)^{3/5}$ where R_0 is the size of the diblock copolymer precursor at high dilution in a good solvent for both blocks (i.e., in a non-selective solvent). The number fraction of monomers in blocks **1** and **2** are: $f_1 = N_1/(N_1 + N_2)$ and $f_2 = 1 - f_1$, respectively. (b) Cartoon picture of a tadpole-shaped single-chain nanoparticle (TSCNP) with an intrachain cross-linking degree x prepared via intrachain cross-linking from the diblock copolymer described in (a) showing the size of the inert copolymer block **1** (R_1) and that of the intrachain cross-linked copolymer block **2** (R_2). TSCNP size, as derived from $R^{5/3} = (R_1)^{5/3} + (R_2)^{5/3}$, depends in a non-trivial manner on N_1 , N_2 , x , l and an A constant, A (see text).

promote the folding/collapse of the reactive block in the TSCNP precursor.^[52-63] Herein, we report on two useful limiting expressions derived for TSCNPs with either long or short flexible tails, allowing predicting their size before synthesis in a good solvent, as a function of simple structural parameters of the initially selected block copolymer. Powerful predictive tools are hence disclosed for rational design of TSCNPs as useful, totally soft building blocks for nanotechnology.

2.3.2. Method Development

The main initial assumption in this work is that upon intra-molecular cross-linking of a diblock copolymer (see Fig. 2.10(b)) in a good solvent for both blocks (i.e., in a non-selective solvent) at high dilution, the size of the resulting TSCNP (R) can be related to the size of the inert copolymer block (R_1) and that of the folded/collapsed block (R_2) by means of the following expression:

$$R^{5/3} = R_1^{5/3} + R_2^{5/3} \quad (2.32)$$

The rationale for this expression is that it provides with the expected values when $R_1 \rightarrow 0$ (single-chain nanoparticle with N_2 monomers), $R_2 \rightarrow 0$ (linear chain with N_1 monomers) and $x \rightarrow 0$ (linear block copolymer with $N_1 + N_2$ monomers), where x is the TSCNP intrachain cross-linking degree. Structural parameters of the precursor are the number of monomers in the inert copolymer block denoted as **1** (N_1), the number of reactive monomers in block **2** (xN_2), the number of inert monomers in block **2** ($[1 - x]N_2$) and the average monomer length, l , as given by^[40] $l = R_0/(N_1 + N_2)^{3/5}$, where R_0 is the size of the TSCNP precursor in good solvent for both blocks at high dilution. The number fraction of monomers in blocks **1** and **2** are: $f_1 = N_1/(N_1 + N_2)$ and $f_2 = 1 - f_1$, respectively.

On one hand, the size of the flexible TSCNP tail in good solvent, R_1 , can be estimated – to a first approximation – as that of a self-avoiding polymer chain, according to:^[40]

$$R_1 = lN_1^{3/5} \quad (2.33)$$

On the other hand, as discussed previously in Section 2.2 of this chapter, a reasonable estimate of the size of the TSCNP folded/collapsed block, R_2 , is provided by the ESM in good solvent at high dilution, such as:^[7-8]

$$R_2 = l^{3/5} K^{-1/5} N_2^{2/5} \quad (2.34)$$

where $K = Ax$, and A is an elasticity constant. As an example, for poly(styrene) single-chain nanoparticles Eq. (2.34) provides an average deviation between calculated and experimental size data lower than 5%.^[7] By combining Eqs. (2.32)–(2.34), the global size of the TSCNP, R , is given by:

$$R = R_0 \left[f_1 + f_2 (1 + KR_{2\theta}^2)^{-1/3} \right]^{3/5} \quad (2.35)$$

where $R_{2\theta}^2 \equiv l^2 N_2$. Based on previous works about elastic single-chain nanoparticles,^[7,8] we expect $KR_{2\theta}^2 \gg 1$.

It is worth mentioning that when $f_2 \rightarrow 0$ Eq. (2.35) reduces to the following, extremely simple limiting expression:

$$R = R_0 f_1^{3/5} \quad [\text{TSNPs, long tails}] \quad (2.36)$$

Note that an identical result is obtained when $K^{1/3} R_{2\theta}^{2/3} \gg f_2$ even if $f_2 > 0$. Eq. (2.36) is expected to be valid for TSNPs with relatively long flexible tails (i.e., $f_1 \geq f_2$).

Considering now the opposite case of TSNPs with very short flexible tails (i.e., $f_1 < f_2$), and assuming that $K R_{2\theta}^2 = A x R_{2\theta}^2 \gg 1$ from Eq. (2.35), the following expression is obtained:

$$R = R_0 \left[f_1 + f_2 (K R_{2\theta}^2)^{-1/3} \right]^{3/5} \quad (2.37)$$

And if TSNPs with short tails are considered equivalent to SCNPs (i.e., by assuming $f_1 = 0$ and $f_2 = 1$ in Eq. (2.37)), then, to a first approximation, this equation can be further simplified to the following limiting expression:

$$R = R_0 (5 x l^2 N_2)^{-1/5} \quad [\text{TSNPs, short tails}] \quad (2.38)$$

2.3.3. Results and Discussion

i) Long flexible tails

A compilation of the experimental size data, $R(\text{exp})$, available in the literature for TSNPs with $f_1 \geq f_2$ is summarized in Table 2.5.

Figure 2.11 illustrates a comparison of data calculated according to Eq. (2.36) and experimental results displayed in Table 2.5. An average deviation of about 10% between the reported and calculated data is found, probably arising from the assumptions made to derive Eq. (2.36), as well as from the own uncertainties associated to size data derived from size exclusion chromatography and light scattering measurements, which for some TSNP systems are as high as 7% (see Table 2.5, line 1). Interestingly, the good overall agreement observed validates Eq. (2.36) as a useful predictive expression to obtain *a priori* estimates of the size in good solvent at high dilution of TSNPs with relatively long flexible tails (i.e., $f_1 \geq f_2$). Due to the different TSNPs included in Table 2.5, the validity of Eq. (2.36) to predict TSNP size (even for $f_1 \simeq f_2$) could be attributed – to some extent – to the validity of the condition $K^{1/3} R_{2\theta}^{2/3} = (A x)^{1/3} R_{2\theta}^{2/3} \gg f_2$ for these systems. It is worth mentioning that Eq. (2.36) resembles the expression obtained in ref. 64 through a Flory-like argument for the case of SCNPs with reversible intrachain cross-links: $R = R_0 (1 - x)^{3/5}$. In both cases, the level of chain compaction predicted seems to be determined by the ratio of the “inert” monomers to the total monomers involved. In fact, for SCNPs with reversible intrachain cross-links having xN reactive monomers and $(1 - x)N$ inert monomers, the number fraction of inert monomers is simply given by: $f_1 = (1 - x)N / [(1 - x)N + xN] = (1 - x)$ so, similar to the case of TSNPs with long tails, we can also write $R = R_0 f_1^{3/5}$ for the case of reversible SCNPs. Behind the validity of Eq. (2.36) for TSNPs with long tails is the fact that, in this case, the global size is mainly determined by the dominant contribution arising from the tail (R_1) although normalized – via the factor f_1 – by the presence of the folded/collapsed copolymer block. It would be interesting to determine experimentally for TSNPs with long tails (e.g., by means of small-angle X-ray/neutron scattering

(SAXS/SANS) experiments) the scaling exponent of TSCNP size in good solvent with the total number of monomers, since according to Eq. (2.36) it is expected to remain that of the block copolymer precursor, that is $3/5 = 0.6$ (note, however, that best estimate of Flory exponent is^[40] 0.588 instead of 0.6).

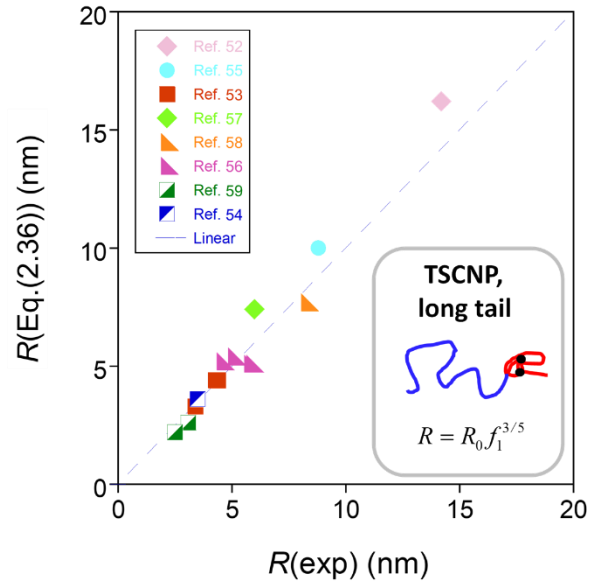


Figure 2.11. Comparison of experimental size data, $R(\text{exp})$, from the literature^[52-59] for TSCNPs with long flexible tails ($f_1 \geq f_2$) with predicted size data obtained from the limiting expression derived in this work, $R = R_0 f_1^{3/5}$ (Eq. (2.36)).

Table 2.5. Compilation of available experimental size data (R) for TSCNPs with relatively long flexible tails (i.e., $f_1 \geq f_2$) taken from the literature.^[52-59]

Block Copolymer Precursors							TSCNPs				
#	Inert Block ^a	N_1^b	N_2^c	f_1^d	$R_0^{SEC}(\text{nm})^e$	$R_0^{LS}(\text{nm})^f$	Intrachain Reaction ^g	$R^{SEC}(\text{nm})^e$	$R^{LS}(\text{nm})^h$	$R^{CAL}(\text{nm})^i$	Ref.
1	PS	902	281	0.76	-	19.1 ^f	Cinnamoyl dimerization	-	14.2	16.2	52
2	PS	297	74	0.80	5.0	-	Alkylamino quaternization	4.4	-	4.4	53
3		297	74					4.3	-	4.4	
4		151	15	0.91	3.4	-		3.4	-	3.2	
5	PS	169	30	0.85	4.0	-	Alkylamino quaternization	-	3.5	3.6	54
6	PS/PEO	2165	475	0.82	-	11.3	Pyridine quaternization	-	8.8	10.0	55
7	PEO	455	445	0.51	8.1	-	BCB dimerization	5.1	-	5.4	56
8			342	0.57	7.2	-		5.9	-	5.1	
9			338	0.57		-		5.8	-	5.1	
10			334	0.58		-		4.6	-	5.1	
11	PMMA	2250	286	0.88	-	8.0	Radical coupling	-	6.0	7.4	57
12	PtBA	510	420	0.88	11.1	-	Cinnamoyl dimerization	8.3	-	7.7	58
13	PNAM	100	100	0.55	-	3.9	Boronate ester formation	-	3.1	2.6	59
14		100	100	0.50	-	3.3		-	2.5	2.2	

^a PS = Poly(styrene); PEO = Poly(ethylene oxide); PMMA = Poly(methyl methacrylate); PtBA = Poly(*tert*-butyl acrylate); PNAM = poly(4-acryloylmorpholine). ^b N_1 is the number of monomers in the inert block.

^c N_2 is the number of monomers in the reactive block.

^d f_1 is the number fraction of monomers in the inert block.

^e For the block copolymer precursors: $R^{SEC}(\text{nm}) = 1.44 \times 10^{-2} M_w^{0.561}$ (see ref 64); for the TSCNPs: $R_H^{SEC}(\text{nm}) = 1.44 \times 10^{-2} (M_w^{app})^{0.561}$ where M_w is the weight average molecular weight of the precursor and M_w^{app} is the apparent weight average molecular weight of the TSCNPs, as determined by size exclusion chromatography (SEC).

^f Data from dynamic light scattering (DLS) measurements (unless otherwise stated).

^g BCD = Benzocyclobutene.

^h Data from static light scattering (SLS) measurements.

ⁱ $R^{CAL} = R_0^i c^{3/5}$ (i = SEC or LS) (Eq. 2.36 in main text).

ii) Short flexible tails

Figure 2.12 illustrates a comparison of the experimental size data reported in the literature for TSCNPs with $f_1 < f_2$ (Table 2.6) and predicted size data according to Eq. (2.37) with a constant value of the elasticity constant $A = 5 \text{ nm}^2$ in all cases. Even by assuming that A takes the same value for TSCNPs of very different intrachain cross-linking chemistries, the average deviation between reported and calculated data in Fig. 2.12(a) is only around 11%. Based on the value of $A = 5 \text{ nm}^2$, it can be shown that the condition $AxR_{2\theta}^2 \gg 1$, as well as the condition $(Ax)^{1/3}R_{2\theta}^{2/3} \gg f_2$ for TSCNPs with long tails, are met for most of the TSCNP systems compiled here. Additionally, we have included in Fig. 2.12(a) predictions from Eq. (2.36) corresponding to TSCNPs with long flexible tails to illustrate that this limiting expression does not provide, as expected, a good agreement for the case of TSCNPs with short tails. Conversely, it can be shown that treating the TSCNPs with short tails as equivalent to SCNPs (i.e., by assuming $f_1 = 0$ and $f_2 = 1$ in Eq. (2.37)) provides with a similar agreement between theoretical and experimental data, with an average deviation between calculated and experimental data of 11.8% Fig. 2.12(b)).

Hence, on the contrary to the case of TSCNPs with long flexible tails, now the global TSCNP size (R) is mainly determined by the dominant contribution arising from the folded/collapsed block (R_2). Taking into account that x , l and N_2 can be easily determined for new TSCNP systems, Eq. (2.38) can be used in a predictive fashion based on the near constant value of $A = 5 \text{ nm}^2$ as suggested by the results shown in Fig. 2.12 for a variety of TSCNPs.

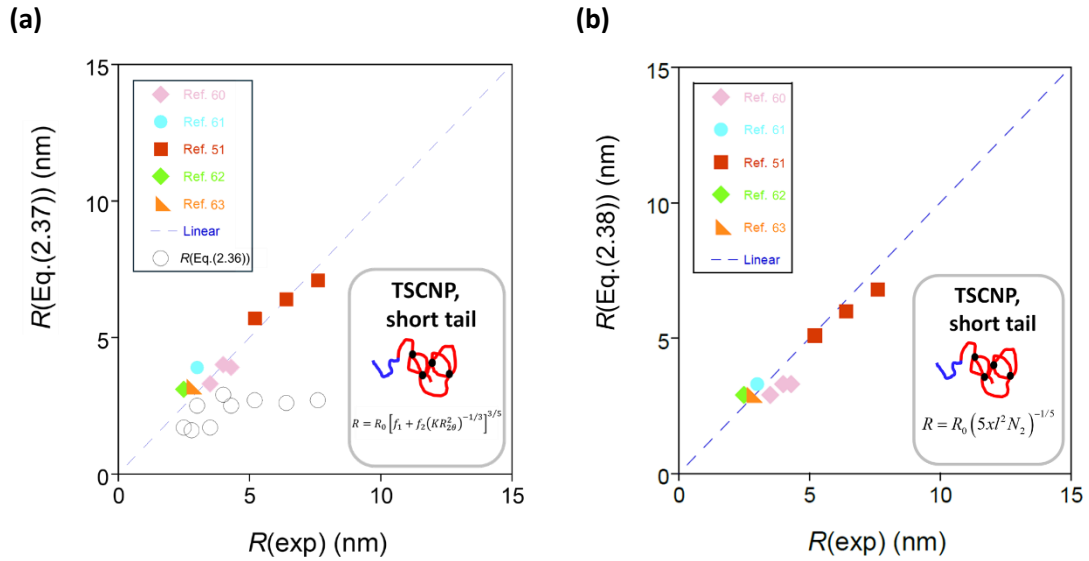


Figure 2.12. Comparison of experimental size data, $R(\text{exp})$, from the literature^[51,60–63] for TSCNPs with (a) short flexible tails ($f_1 < f_2$) with predicted size data (solid symbols) obtained from Eq. (2.37) with $A = 5 \text{ nm}^2$ and (b) short flexible tails ($f_1 < f_2$) with predicted size data (solid symbols) obtained from Eq. (2.38). Open black circles correspond to predicted size data according to Eq. (2.36) (TSCNPs with long flexible tails) which, as expected, does not provide a good agreement for the present case of TSCNPs with short tails.

Table 2.6. Compilation of the size data from the literature for TSCNPs with short tails ($f_1 < f_2$).^[51,60-63]

Block Copolymer Precursors								TSCNPs					
#	Inert Block ^a	N_1^b	N_2^c	f_1^d	$R_0^{SEC}(\text{nm})^e$	$R_0^{LS}(\text{nm})^f$	$l(\text{nm})^g$	Intrachain Reaction ^h	χ^i	$R^{SEC}(\text{nm})^e$	$R^{LS}(\text{nm})^f$	$R^{CAL}(\text{nm})^j$	Ref.
1	PDEA	78	112	0.41	-	5	0.23	Disulfide	0.29	-	4.0	4.0	60
2		52	113	0.32	-	5		exchange	0.28	-	4.3	3.9	
3		28	109	0.20	-	4.5			0.29	-	3.5	3.3	
4	PDMAEMA	245	524	0.32	-	5.0	0.09	Glaser coupling	0.36	-	3.0	3.9	61
5	PEO	114	726	0.14	8.8	-	0.14	BCB	0.05	7.6	-	7.1	51
6			756	0.13	8.9	-		dimerization	0.10	6.4	-	6.4	
7			686	0.14	8.6	-			0.20	5.2	-	5.7	
8	PEO	73	184	0.28	3.6	-	0.14	Diels-Alder reaction	0.17	2.5	-	3.1	62
9	PDMA	35	145	0.19	-	4.2	0.19	Cinnamoyl dimerization	0.24	-	2.8	3.2	63

^a PDA = Poly(2-(diethylamino)ethyl methacrylate); PDMAEMA = Poly(2-(dimethylamino)ethyl methacrylate); PEO = Poly(ethylene oxide); PDMA = poly(*N,N'*-dimethylacrylamide).

^b N_1 is the number of monomers in the inert block.

^c N_2 is the number of monomers in the reactive block.

^d f_1 is the number fraction of monomers in the inert block.

^e For the block copolymer precursors: $R^{SEC}(\text{nm}) = 1.44 \times 10^{-2} M_w^{0.561}$ (see ref. 64); for the TSCNPs: $R^{SEC}(\text{nm}) = 1.44 \times 10^{-2} (M_w^{app})^{0.561}$ where M_w is the weight average molecular weight of the precursor and M_w^{app} is the apparent weight average molecular weight of the TSCNPs, both determined by size exclusion chromatography (SEC).

^f Data from dynamic light scattering (DLS) measurements.

^g l is the average monomer length, given by $l \simeq R_0 / (N_1 + N_2)^{3/5}$, where R_0 is the size of the TSCNP precursor in good solvent for both blocks at high dilution.

^h BCB = Benzocyclobutene.

ⁱ χ is the TSCNP intrachain cross-linking degree.

^j Calculated from Eq. (2.37) in the main text: $R^{CAL} = R_0^i [f_1 + f_2 (KR_{2\theta}^2)^{-1/3}]^{3/5}$ (i = SEC or LS) where $f_2 = 1 - f_1$, $K = Ax \simeq 5x$ and $R_{2\theta}^2 \equiv l^2 N_2$

As a final validation test of the predictive capabilities of the two limiting expressions disclosed in this work for TSCNPs with long (Eq. (2.36)) and short (Eq. (2.38)) tails, we have selected a system in which TSCNPs are formed through hydrolysis and polycondensation of silane moieties in the folding/collapse copolymer block leading to the formation of hybrid TSCNPs with silica-like heads and flexible polymer tethers. These TSCNPs were prepared from block copolymers of poly(ethylene oxide)- block-[poly(methyl methacrylate)-co-poly(3-(trimethoxysilyl)propyl methacrylate)].^[65] As an example, for a TSCNP with $f_1 = 0.57$ and $R_0 = 3.6$ nm we expect from Eq. (2.36) a size of $R(\text{Eq. (2.36)}) \simeq 2.6$ nm, whereas the experimental size according to data from ref. 65 is $R(\text{exp}) = 3.1$ nm (Table 2.7 and Fig. 2.13).

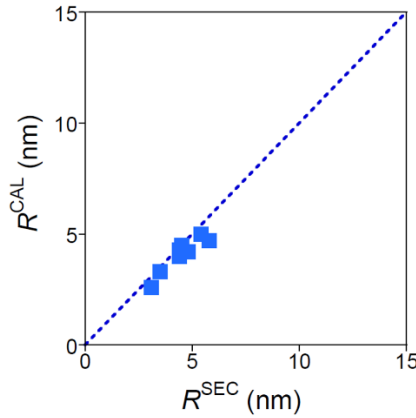


Figure 2.13. Comparison of validation test results taken from the literature (ref. 65) and Tables 2.7 and 2.8 from the text.

On the other hand, for a TSCNP with $x = 0.1$, $l = 0.14$ nm, $N_2 = 470$ and $R_0 = 6.8$ nm a size of R (Eq. (2.38)) $\simeq 5.0$ nm was estimated from Eq. (2.38), whereas $R(\text{exp})$ is 5.4 nm, in good agreement with the prediction (Table 2.8 and Fig. 2.13). The transferability of the constant $A = 5 \text{ nm}^{-2}$ to TSCNPs with silica-like heads suggests that the specific intrachain cross-linking chemistry utilised during TSCNP synthesis does not affect significantly the global TSCNP size. As a result, independent of the specific intrachain cross-linking chemistry, Eq. (2.38) becomes a useful predictive expression to obtain a priori estimates of the size of TSCNPs with relatively short flexible tails (i.e., $f_1 < f_2$) in good solvent at high dilution Table 2.8 and Fig. 2.13).

Table 2.7. Validation test of the predictive capabilities of Eq. (2.36) (TSCNPs with long tails, $f_1 \geq f_2$) for hybrid TSCNPs with silica-like heads and long PEO tethers.^[65]

#	Block Copolymer Precursor				TSCNPs		
	Inert Block ^a	N_1^b	N_2^c	f_1^d	$R_0^{SEC} \text{ (nm)}^e$	Intrachain Reaction	$R^{SEC} \text{ (nm)}^e$ $R^{CAL} \text{ (nm)}^f$ $\epsilon \text{ (%) }^g$
1	PEO	114	87	0.57	3.6	Hydrolysis / Polycondensation	3.1 2.6 16.1

^a PEO = Poly(ethylene oxide).

^b N_1 is the number of monomers in the inert block.

^c N_2 is the number of monomers in the reactive block.

^d f_1 is the number fraction of monomers in the inert block.

^e For the block copolymer precursors: $R^{SEC} \text{ (nm)} = 1.44 \times 10^{-2} M_w^{0.561}$ (see Ref.^[65]); for the TSCNPs: $R^{SEC} \text{ (nm)} = 1.44 \times 10^{-2} (M_w^{app})^{0.561}$ where M_w is the weight average molecular weight of the precursor and M_w^{app} is the apparent weight average molecular weight of the TSCNPs, as determined by size exclusion chromatography (SEC).

^f $R^{CAL} = R_0^{SEC} f_1^{3/5}$ (Eq. (3.36) in main text).

^g $\epsilon \text{ (%) } = (|R^{CAL} - R^{SEC}| / R^{SEC}) \times 100$

Table 2.8. Validation test of the predictive capabilities of Eq. (2.38) (TSCNPs with short tails, $f_1 < f_2$) for hybrid TSCNPs with silica-like heads and short PEO tethers.^[65]

Block Copolymer Precursor							TSCNPs				
#	Inert Block ^a	N_1^b	N_2^c	f_1^d	$R_0^{SEC}(\text{nm})^e$	$l(\text{nm})^f$	Intrachain reaction	χ^g	$R^{SEC}(\text{nm})^e$	$R^{CAL}(\text{nm})^h$	$\varepsilon(\%)^i$
1	PEO	114	470	0.20	6.8	0.14	Hydrolysis / Polycondensation	0.1	5.4	5.0	7.4
2		388	388	0.23	6.5			0.17	4.5	4.5	0.0
3		382	382	0.23	6.7			0.24	4.5	4.3	4.4
4		365	365	0.24	6.8			0.31	4.8	4.2	12.5
5		151	151	0.43	4.4			0.30	3.5	3.3	5.7
6		298	298	0.28	6.1			0.29	4.4	4.0	9.1
7		401	401	0.22	7.0			0.28	4.4	4.3	2.3
8		505	505	0.18	7.8			0.25	5.8	4.7	19.0

^a PEO = Poly(ethylene oxide).

^b N_1 is the number of monomers in the inert block.

^c N_2 is the number of monomers in the reactive block.

^d f_1 is the number fraction of monomers in the inert block.

^e For the block copolymer precursors: $R^{SEC}(\text{nm}) = 1.44 \times 10^{-2} M_w^{0.561}$ (see Ref.^[64]), for the TSCNPs: $R^{SEC}(\text{nm}) = 1.44 \times 10^{-2} (M_w^{app})^{0.561}$ where M_w is the weight average molecular weight of the precursor and M_w^{app} is the apparent weight average molecular weight of the TSCNPs, as determined by size exclusion chromatography (SEC).

^f l is the average monomer length.

^g χ is the TSCNP intrachain cross-linking degree.

^h $R^{CAL} \approx R_0^{SEC} (5\chi l^2 N_2)^{-1/5}$ (Eq. (3.38)).

ⁱ $\varepsilon(\%) = (|R^{CAL} - R^{SEC}|/R^{SEC}) \times 100$.

2.4. Size Determination Approach of SCNPs Under Severe Crowding Conditions

2.4.1. Introduction

The effect of macromolecular crowding on polymer conformations is modulated by chain topology.^[66] In good solvent at high dilution a linear macromolecule composed of N segments adopts a self-avoiding conformation^[40] with chain size $R \sim N^{3/5}$. However, under crowding conditions in a highly concentrated solution and in the melt the macromolecule adopts an ideal, random-walk configuration^[40] such that: $R \sim N^{1/2}$. On the contrary, in a melt of unknotted and unconcatenated ring polymers the strong effect of topology induces significantly more compaction to give fractal, crumpled globule conformations^[67] such that: $R \sim N^{1/3}$ at sufficiently large N . The crumpled globule is characterized by a fractal space-filling-like structure, with a wide range of scales which are self-similar and almost unknotted.^[66]

It is worthy of mention that the collapse process of a linear chain into a self-similar, fractal globule on changing from good to bad solvent conditions was first treated by de Gennes,^[68] and later by Grosberg, Nechaev, and Shakhnovich.^[69] Interestingly, the fractal, crumple globule behavior of ring polymers under crowding was applied as a tentative model to explain the packing of chromatin fibers into segregated “territories” in the cell nucleus.^[70] Moreover, the effect of macromolecular crowding on polymer conformations is of significant interest in the case of other complex topologies (e.g., dendrimers^[71]) for both academic and practical applications (i.e., dendrimers as processing aids in industrial extrusion processes).

Regarding SCNPs, as mentioned in Chapter 1, their synthesis from precursors under good-solvent conditions often results in conformations far from the globular ones typically exhibited by e.g., compact 3D-structured proteins. Accordingly, the morphology of SCNPs is closer to that commonly observed in intrinsically disordered proteins (IDPs).^[6]

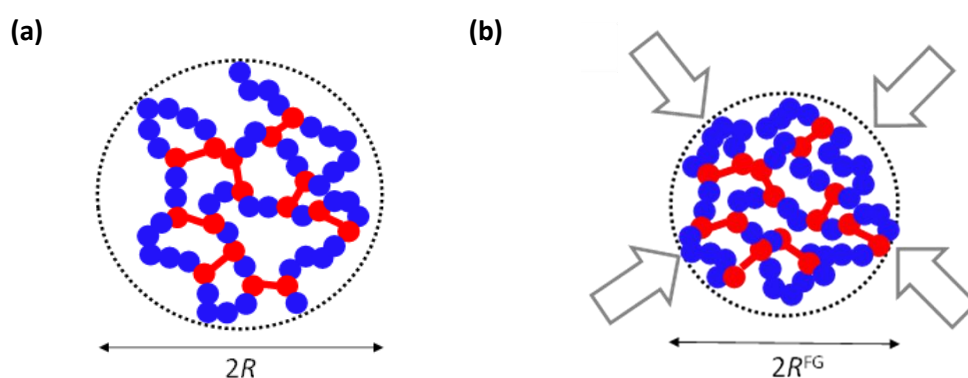


Figure 2.14. (a) An isolated single-chain nanoparticle (SCNP) of size R can be considered as an ultra-fine nanogel composed of a single cross-linked polymer chain (cross-links denoted by red color). (b) Schematic illustration of the size reduction experienced by a SCNP on passing from high diluted conditions in the presence of solvent molecules (a) to a highly crowded environment such as at bulk density in the presence of other SCNPs (b).

The ESM model is a very useful tool for establishing a connection between the SCNP size, the number of segments, and the fraction of reactive groups in the precursor polymer through a parameter related to the elasticity of the unimolecular network. Conversely, the ESM is not able

to reproduce the astonishing conformational behavior of SCNPs under severe crowding conditions (e.g., highly concentrated solutions, all-polymer nanocomposites) and, specifically, the significant size reduction observed in highly crowded solutions of covalent-bonded SCNPs and all-polymer nanocomposites containing SCNPs (see Fig. 2.14(b)).^[72] The full agreement between molecular dynamics (MD) simulations ($R \sim N^{0.37}$) and small-angle neutron scattering (SANS) experiments ($R \sim N^{0.37}$) suggests that SCNPs adopt a “crumpled globule” conformation in highly crowded environments.^[73] Since some of the potential applications of SCNPs rely on the actual size of these soft nano-objects in bulk conditions, a predictive tool providing the size of SCNPs under crowding at bulk density would be very valuable to establish reliable structure–properties relationships.^[3] Moreover, as SCNPs are simple models of IDPs the knowledge generated would be of potential interest to construct models of unstructured polypeptides under crowding.

A method is proposed to estimate the size of SCNPs under strong crowding conditions by combining coarse-grained MD simulation results with simple scaling concepts.^[74]

2.4.2. Method development

In the case of computer simulations, size dimensions are scaled to the bead size that is taken as 1 nm. In the case of experimental systems, the radius of gyration, R , and the domain size, d , are always given in nm.

Coarse-grained MD simulations of SCNPs in terms of the bead spring model provided accurate data of the size reduction experienced by a solution of SCNPs upon increasing concentration up to the bulk concentration.^[73] The simulations, performed by Moreno and coworkers, were carried out at constant temperature via a Langevin thermostat. Chemical details were not accounted for, although the simulations were able to appropriately capture the effect of chain connectivity and excluded volume. Consequently, the results obtained came from the topology of the SCNPs (see Fig. 2.14) and not from their chemical composition or changes in temperature. Due to severe computational costs, only SCNPs with $N = 200$ beads were simulated with very good accuracy from very diluted conditions up to bulk concentration (i.e., $\phi^{bulk} \equiv 1$).

From simple scaling arguments^[40,74] we can write:

$$\frac{R^{FG}}{R_0} = \left(\frac{\phi^{bulk}}{\phi^*} \right)^\alpha = \left(\frac{R_0^3}{b^3 N} \right)^\alpha \quad (2.39)$$

where R^{FG} is the size of the SCNP under highly crowding conditions (i.e., $\phi^{bulk} \equiv 1$), R_0 is the size of the SCNP at high dilution, ϕ^* is the overlap volume fraction^[40] given by

$$\phi^* = b^3 N / R_0^3 \quad (2.40)$$

with b being the effective segment length and α the corresponding positive scaling exponent. ϕ^* is called the overlap volume fraction because it is the concentration at which SCNPs start to “touch” one to another. Taking into account the well-known scaling law^[73] $R_0 = bN^\nu$, we arrive at:

$$\frac{R^{FG}}{R_0} = N^{(3\nu-1)\alpha} \equiv N^\beta \quad (2.41)$$

Where $\beta \equiv (3\nu - 1)\alpha$. According to Eq. (2.41), in the case of a real SCNP under severe crowding conditions one expects:

$$R^{FG} = R_0 N_{eff}^\beta \quad (\text{SCNPs under crowding}) \quad (2.42)$$

where R_0 is the SCNP size in good solvent at high dilution and N_{eff} is the effective number of monomers in the chain (see below). A reliable value of β can be obtained from the extensive MD simulations results of Ref.^[73]. From $R^{FG}/R_0 = 0.698$ and $N = 200$ we obtain $\beta = -0.068$ and $\alpha = \beta/(3\nu - 1) = -0.19$ based on $\nu = 0.45$.^[73] As illustrated in Fig. 2.15, the value of $\alpha \approx -0.2$ provides a good fit of the MD simulation data according to:

$$R(\phi) = R_0 (\phi \leq \phi^*) \quad (2.43a)$$

$$R(\phi) = R_0 \left(\frac{\phi}{\phi^*} \right)^\alpha (\phi \geq \phi^*) \quad (2.43b)$$

where ϕ denotes the volume fraction of SCNPs in the solution. In this sense, the maximum concentration corresponds to $\phi = \phi^{bulk} = 1$ (see Fig. 2.15) and, hence, to the limiting value: $(\phi^{bulk}/\phi^*) = R_0^3/b^3N$

Concerning N_{eff} for real SCNPs, we adopt a mapping of R_0 (actual size of the real SCNP at high dilution) to the SCNP size from the simulations^[73] such as:

$$N_{eff} = 200 \left(\frac{R_0}{7.3} \right)^{1/0.45} \quad (2.44)$$

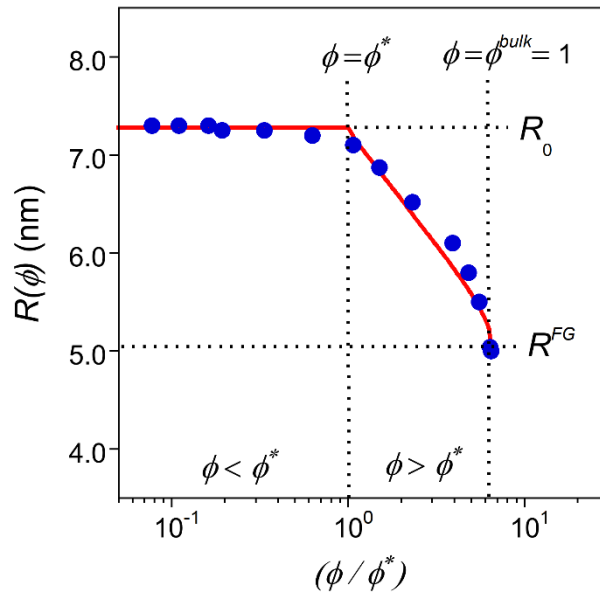


Figure 2.15. Comparison of MD simulations results^[73] (blue solid circles) to predictions from Eq. (2.43) (red traces) for SCNPs in solution as a function (ϕ/ϕ^*) where ϕ is the volume fraction of SCNPs in the system and ϕ^* is the overlap volume fraction (Eq. (2.40)). ϕ^* is the overlap concentration. The maximum concentration corresponds to $\phi = \phi^{bulk} = 1$; i.e., $(\phi^{bulk}/\phi^*) = R_0^3/b^3N$.

Hence, based on available R_0 data, N_{eff} can be obtained from Eq. (2.44), and we can estimate R^{FG} by means of Eq. (2.42) with $\beta = -0.068$ to be compared with the available R^{FG} data of different SCNPs of varying size under severe crowding conditions.

2.4.3. Results and discussion

Predictions for several systems based on SCNPs will be compared to experimental data, and the predictions will be extended to melts of ring polymers and nanostructured Janus-shaped SCNPs.

Crumpled globule single-chain nanoparticles

Predictions from Eq. (2.42) were first compared with literature data for crumpled globule SCNPs (see Table 2.12). Data available result from complementary large-scale MD simulations at both coarse-grained and fully atomistic level as well as experimental data of near-athermal all-polymer nanocomposites containing SCNPs.

Table 2.12. Comparison of the size of SCNPs under severe crowding conditions, as estimated from Eq. (2.42), to available experimental data from the literature.

System ^a	R_0 (lit.)	N_{eff} (Eq. (2.44))	R^{FG} (Eq. (2.42))	R^{FG} (lit.)
PS-SCNPs/PS ^b	2.5 nm	19	2.07 nm	2.1 nm
PS-SCNPs/PS ^c	4.8 nm	79	3.57 nm	2.7 nm
PMMA-SCNPs/PEO ^d	6.8 nm	171	4.80 nm	5.8 nm
PMMA-SCNPs/PMMA-SCNPs ^e	4.5 nm	68	3.38 nm	3.3 nm
	10.1 nm	412	6.71 nm	8.5 nm

^a PS = poly(styrene); PMMA = poly(methyl methacrylate); PEO = poly(ethylene oxide). ^b Ref.^[75]. ^c Ref.^[76]. ^d Ref.^[73]. ^e Ref.^[77].

Crumpled globule conformations of SCNPs were found by Jia *et al.*^[75] in all-polymer nanocomposites composed of poly(styrene) (PS) chains and PS-SCNPs from large-scale MD simulations at both coarse-grained and full atomistic level. Without crowding effects, PS-SCNPs showed $R_0 = 2.5$ nm whereas in the all-polymer nanocomposites the simulations revealed $R^{FG} = 2.1$ nm. From Eq. (2.42) and $N_{eff} = 19$ (Eq. (2.44)) we estimate a value of $R^{FG} = 2.07$ nm (Table 2.12) which is in very good agreement with the reported value of R^{FG} .

PS-SCNPs in blends with deuterated PS chains were investigated by Mackay *et al.*^[76] via small angle neutron scattering (SANS) experiments. PS-SCNPs of $R_0 = 4.8$ nm were found to exhibit a size reduction to $R^{FG} = 2.7$ nm when blended with linear deuterated PS of 63 kDa in molar mass at 50 wt%. From Eq. (2.42) and $N_{eff} = 79$ (Eq. (2.44)) we obtain $R^{FG} = 3.6$ nm (Table 2.12) which is slightly larger than the reported value of R^{FG} . It is worth noting that that value was obtained by assuming that the SCNPs were hard spheres, a rather crude approximation.^[76]

Additionally, as reported by Moreno and coworkers,^[73] poly(methyl methacrylate) (PMMA)-SCNPs synthesized from a precursor of 92 kDa in molar mass displayed $R_0 = 6.8$ nm in good solvent at high dilution, whereas in blends with linear deuterated poly(ethylene oxide) (PEO) of 96 kDa they showed $R^{FG} = 5.8$ nm, as determined from SANS experiments. From Eq. (2.42) and $N_{eff} = 171$ (Eq. (2.44)) we obtain $R^{FG} = 4.8$ nm (Table 2.12) which is in good agreement with the experimental R^{FG} value.

In a more recent study by Ruiz-Bardillo *et al.*,^[77] the impact of bond reversibility on the macromolecular conformation of PMMA-based SCNPs under self-crowding conditions was investigated by means of small-angle neutron scattering. Two molecular weights were explored, low (57 kg/mol) and high (237 kg/mol), and since irreversible cross-links favor the adoption of a crumpled globule conformation under bulk conditions, Michael addition SCNPs are considered for comparison with Eq. (2.42). For low molecular weight precursors, the SCNPs yielded a dilute-state size of $R_0 = 4.5$ nm. Upon determination of the effective number of segments ($N_{eff} = 68$)

through Eq. (2.44) and subsequently Eq. (2.42), a value of $R^{FG} = 3.38$ nm was obtained, in good agreement with the experimental determination under crowding conditions ($R^{FG} = 3.3$ nm). Regarding the high molecular weight system, larger dilute-size was obtained ($R_0 = 10.1$ nm), as expected for a larger precursor chain. After determining $N_{eff} = 412$ by applying Eq. (2.44), the predicted value from Eq. (2.42) ($R^{FG} = 6.71$ nm) deviates more notably from the experimental data ($R^{FG} = 8.5$ nm), although it still exhibits reasonable agreement considering the experimental uncertainties and possible errors (see Table 2.12).

Figure 2.16 (blue symbols) provides a comparison of the size of SCNPs under crowding as calculated from Eq. (2.42) to reported literature values. Further experimental and simulations data will be very useful to establish the general validity of Eq. (2.42) at larger values of N and for SCNPs of different chemical composition.

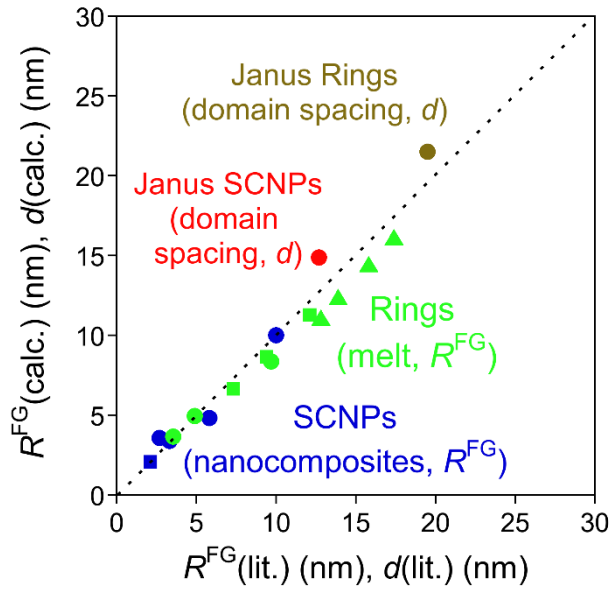


Figure 2.16. Size (R^{FG}) of SCNPs under severe crowding conditions as calculated from Eq. (2.42) (blue symbols), ring polymers in the melt as estimated from Eqs. (2.45) and (2.46) (green symbols), and domain spacing (d) of nanostructured Janus-shaped SCNPs and Janus ring polymers as derived from Eq. (2.47) (red and brown symbols) in comparison to literature values.^[67,74–80]

Crumpled globule ring polymers

Next, we explore the possibility of extending the predictive capability of Eq. (2.42) to provide useful values of the crumpled globule size of unknotted and unconcatenated ring polymers in the melt. These were revealed by the extensive coarse-grained MD simulations of Halverson *et al.*^[67] for large rings with $N \gg N_e$, where N_e is the entanglement length for linear polymers in a melt. By combining Eq. (2.41) and (2.44), we arrive at the following simple expression:

$$R^{FG} = 0.673N^{0.382} \quad (\text{rings in bulk as SCNPs, } N \gg N_e) \quad (2.45)$$

where the prefactor in Eq. (2.45) arises from the effective monomer length.

A comparison of predictions from Eq. (2.45) for ring polymers with $N = 400, 800$ and 1600 to values of R^{FG} obtained through MD simulations is given in Fig. 2.16. Equation (2.45) provides values of R^{FG} (6.64, 8.65 and 11.27 nm) that are systematically lower (see Fig. 2.16, green solid

squares) than the R^{FG} data from MD simulations (7.3, 9.4 and 12.1 nm).^[67] A similar conclusion is reached when predictions from Eq. (2.45) are compared to Monte Carlo simulations reported by Suzuki *et al.*^[78] for ring polymers with $N = 1536, 2048, 3072$ and 4096 (see Fig. 2.16, green solid triangles). The small deviations observed in Fig. 2.16 can be actually attributed to the different topologies of SCNPs and ring polymers. Obviously, the topological difference is more notorious upon increasing the ring polymer size. For the case of real ring polymers in the melt, it is convenient to express Eq. (2.45) in terms of N/N_e such as:

$$R^{FG} = R_e \left(\frac{N}{N_e} \right)^{0.382} \quad (\text{rings in bulk as SCNPs, } N \gg N_e) \quad (2.46)$$

where $R_e = \lambda(N_e/12)^{1/2}$ and λ is the monomer length.^[79] Eq. (2.46) is strictly valid for $N \gg N_e$.

Considering experimental results, for ring PS in bulk with $N/N_e = 20$ ($N_e = 173$, $\lambda = 0.7$ nm)^[81] we estimate $R^{FG} = 8.35$ nm from Eq. (2.46) which compares favourably to $R^{FG} = 9.7 \pm 0.6$ nm obtained by SANS measurements of highly-pure ring PS samples by Iwamoto *et al.*^[81]

For ring PEO in bulk with $N/N_e = 44$ and 20 ($N_e = 45$, $\lambda = 0.6$ nm)^[79] we obtain $R^{FG} \approx 4.93$ and 3.65 nm from Eq. (2.46) in good agreement with the SANS values of highly-pure ring PEO samples reported by Kruteva *et al.* ($R^{FG} = 4.90 \pm 0.14$ nm and 3.54 ± 0.07 nm).^[79]

Crumpled globule Janus-shaped single-chain nanoparticles

Janus-shaped SCNPs have been proposed by Watanabe *et al.*^[80] as valuable nanomaterials for potential applications in ultrahigh resolution block copolymer lithography. Functionalized block copolymers composed of mutually immiscible PS and poly(glycolic acid) (PGA) blocks (PS-b-PGA) were subjected to one-shot intra-block cross-linking by these authors giving to compartmentalized Janus-shaped SCNPs with excellent self-assembling behavior as determined by small angle X-ray scattering (SAXS) measurements. Interestingly, the microphase-separated structure of Janus-shaped SCNPs in the bulk state was found to be up to 47% smaller (domain spacing $d = 12.7$ nm) than that of the precursor block copolymer ($d_0 = 24.0$ nm).^[80] We hypothesize here that the astonishing conformational behavior of SCNPs under severe crowding conditions could contribute, to some extent, to this behavior. Based on the size of the Janus-shaped SCNPs at high dilution determined by Watanabe *et al.*^[80] ($R_0 = 2.2$ nm) we estimate $R^{FG} = 1.83$ nm from Eq. (2.42) as the size of these Janus-shaped SCNPs in the bulk state (a reduction of 27% when compared to the estimated size of the precursor block copolymer in the bulk state).

An estimation of the expected d/d_0 ratio from the ratio of the apparent molar mass (M_0^*) of the precursor block copolymer to the apparent molar mass (M^*) of the Janus-shaped SCNPs can be obtained according to:

$$\frac{d}{d_0} = \left(\frac{M^*}{M_0^*} \right)^{\frac{1}{2}} \quad (\text{Janus-shaped SCNPs}) \quad (2.47)$$

Equation (2.47) is obtained from the well-known dependence of the domain spacing (d) on molecular weight (M) such as: $d \sim M^{2/3}$.^[82] From the reported data by Watanabe *et al.*^[80] – as determined by size exclusion chromatography – ($M_0^* = 23.8$ kDa and $M^* = 11.7$ kDa) we obtain $d/d_0 \approx 0.62$ from Eq. (2.47) and hence $d = 14.9$ nm, which is a value close to the experimental one (see Fig. 2.16). In addition, the predicted domain spacing of nanostructured Janus-shaped ring polymers (cyclic PS-block-PEO copolymers)^[83] is included in Fig. 2.16 for comparison. The

estimated value from Eq. (2.47), $d = 21.5$ nm, based on the values: $M_0^* = 18.35$ kDa, $M^* = 13.90$ kDa and $d_0 = 25.9$ nm reported by Poelma *et al.*,^[83] is also close to the SAXS experimental result, $d = 19.5$ nm.

2.5. Conclusions

Based on the findings presented in this chapter, the following key conclusions can be drawn:

Inspired by recent experiments involving brushes of responsive SCNPs, a model of brushes formed by elastic SCNPs of total number of monomers N and elasticity parameter K ($K \approx Ax$, where x is the intrachain cross-linking degree) has been developed. Given the topological complexity of the system, this represents a first approximation attempt to analyze the equilibrium structure of a planar brush of flexible SCNPs. Based on this model, we have derived valuable scaling law expressions for key properties such as brush thickness, local domain size and free energy per chain for brushes of elastic SCNPs on flat surfaces in the presence of either a good solvent or a θ -solvent, as well as in the melt state, as a function of N and $K(x)$. Furthermore, we have established the grafting density range ($\sigma_{min} \leq \sigma \leq \sigma_{max}$) corresponding to the brush regime (Table 2.1).

When compared to brushes of linear chains or other complex topologies (e.g., branched polymers) at constant N , the thickness of brushes formed by SCNPs is expected to be significantly smaller (Figs. 2.5–2.7). A key advantage of SCNPs lies in their ability to control brush thickness through the elasticity parameter K , which is proportional to x , the intrachain cross-linking degree. According to this model, increasing the value of $K(x)$ leads to a reduction in brush thickness. A mapping to SCF analytical results has provided a relationship between the SCF topological coefficient k and the elasticity parameter K . Due to the large values of topological ratio $\eta \equiv k_{SCNPs}/k_{linear}$ estimated for brushes of SCNPs (Table 2.4), a reduction of the interpenetration length of opposing brushes and, hence, of the friction between sliding brushes composed of SCNPs is expected when compared to that for linear polymer brushes.

The progressive unfolding of brushes of elastic SCNPs on flat surfaces has been explored, providing a plausible scenario for the evolution of brush thickness as intrachain cross-links are randomly broken in a i) good solvent, ii) θ -solvent, and iii) melt conditions (Fig. 2.9). It is worth mentioning that these findings may be useful to assist the potential design of brushes of SCNPs with drug delivery characteristics based on stimulus-mediated rupture of intrachain cross-links.

Regarding tadpole-shaped single-chain nanoparticles TSCNPs, valuable structure-size relationships are disclosed for systems with long ($f_1 \geq f_2$) and short ($f_1 < f_2$) flexible tails, respectively. For TSCNPs with long tails in good solvent conditions at high dilution, the simple expression $R = R_0 f_1^{3/5}$ reproduces (within $\approx 10\%$ error) the size values reported in the literature.^[52-59] For TSCNPs with short tails, the limiting expression $R = R_0 (5x l^2 N_2)^{-1/5}$ is able to predict the size values of TSCNPs prepared utilising a variety of intrachain cross-linking techniques^[60-63] with an average deviation between experimental and calculated data $< 12\%$. To conclude, this work opens an avenue to the a priori, rational design of new TSCNP systems as useful, totally soft building blocks for nanotechnology and other potential applications.

Lastly, we propose a method to predict the size of SCNPs under severe crowding conditions, R^{FG} . A simple expression (Eq. (2.42)) relates R^{FG} to the size of a SCNPs in good solvent at high dilution, R_0 , and two parameters which are estimated in a self-consistent way from available

MD simulations data: a general scaling exponent ($\beta = -0.068$) and the effective number of monomers in the chain, N_{eff} , – connected with R_0 via Eq. (2.44). This method also serves as a preliminary approach for estimating the size of large ring polymers in the melt ($N \gg N_e$) as well as the size of Janus-shaped SCNPs in the bulk state. This work lays the groundwork for establishing structure-property relationships for SCNPs under crowding, with potential relevance for e.g., intrinsically disordered proteins (IDPs).

2.6. References

- [1] X. Zeng, A. S. Holehouse, A. Chilkoti, T. Mittag and R. V. Pappu, *Biophys. J.*, **2020**, *119*, 402.
- [2] H.-Y. Chou and A. Aksimentiev, *J. Phys. Chem. Lett.*, **2020**, *11*, 4923.
- [3] J. A. Pomposo (Ed.), *Single-Chain Polymer Nanoparticles: Synthesis, Characterization, Simulations and Applications*, Wiley-VCH, Weinheim, **2017**.
- [4] J. A. Pomposo, A. J. Moreno, A. Arbe and J. Colmenero, *ACS Omega*, **2018**, *3*, 8648.
- [5] E. Verde-Sesto, A. Arbe, A. J. Moreno, D. Cangialosi, A. Alegría, J. Colmenero and J. A. Pomposo, *Mater. Horiz.*, **2020**, *7*, 2292.
- [6] J. A. Pomposo, I. Perez-Baena, F. Lo Verso, A. J. Moreno, A. Arbe and J. Colmenero, *ACS Macro Lett.*, **2014**, *3*, 767.
- [7] J. De-La-Cuesta, E. Gonzalez, A. J. Moreno, A. Arbe, J. Colmenero and J. A. Pomposo, *Macromolecules*, **2017**, *50*, 6323.
- [8] J. A. Pomposo, J. Rubio-Cervilla, E. Gonzalez, A. J. Moreno, A. Arbe and J. Colmenero, *Polymer*, **2018**, *148*, 61.
- [9] C. Feng and X. Y. Huang, *Acc. Chem. Res.*, **2018**, *51*, 2314.
- [10] W. L. Chen, R. Cordero, H. Tran and C. K. Ober, *Macromolecules*, **2017**, *50*, 4089.
- [11] Q. Yu, L. K. Ista, R. P. Gu, S. Zauscher and G. P. Lopez, *Nanoscale*, **2016**, *8*, 680.
- [12] J. O. Zoppe, N. C. Ataman, P. Mocny, J. Wang, J. Moraes and H. A. Klok, *Chem. Rev.*, **2017**, *117*, 1105.
- [13] T. Sato, G. J. Dunderdale, C. Urata and A. Hozumi, *Macromolecules*, **2018**, *51*, 10065.
- [14] B. Jaquet, D. Wei, B. Reck, F. Reinhold, X. Y. Zhang, H. Wu and M. Morbidelli, *Colloid Polym. Sci.*, **2013**, *291*, 1659.
- [15] M. Krishnamoorthy, S. Hakobyan, M. Ramstedt and J. E. Gautrot, *Chem. Rev.*, **2014**, *114*, 10976.
- [16] H. Jiang and F. J. Xu, *Chem. Soc. Rev.*, **2013**, *42*, 3394.
- [17] P. Costa, J. E. Gautrot and J. T. Connelly, *Acta Biomater.*, **2014**, *10*, 2415.
- [18] J. J. Keating, J. Imbrogno and G. Belfort, *ACS Appl. Mater. Interfaces*, **2016**, *8*, 28383.
- [19] I. V. Mikhailov, F. A. M. Leermakers, O. V. Borisov, E. B. Zhulina, A. Darinskii and T. M. Birshtein, *Macromolecules*, **2018**, *51*, 3315.
- [20] H. Merlitz, C. W. Li, C. X. Wu and J.-U. Sommer, *Polymer*, **2016**, *103*, 57.
- [21] E. B. Zhulina, F. A. M. Leermakers and O. V. Borisov, *Macromolecules*, **2016**, *49*, 8758.
- [22] E. B. Zhulina, F. A. M. Leermakers and O. V. Borisov, *Langmuir*, **2015**, *31*, 6514.
- [23] W. Cui, C. F. Su, H. Merlitz, C. X. Wu and J.-U. Sommer, *Macromolecules*, **2014**, *47*, 3645.
- [24] O. V. Borisov, A. A. Polotsky, O. V. Rud, E. B. Zhulina, F. A. M. Leermakers and T. M. Birshtein, *Soft Matter*, **2014**, *10*, 2093.

- [25] A. A. Polotsky, F. A. M. Leermakers, E. B. Zhulina and T. M. Birshstein, *Macromolecules*, **2012**, *45*, 7260.
- [26] W. M. de Vos, F. A. M. Leermakers, S. Lindhoud and S. W. Prescott, *Macromolecules*, **2011**, *44*, 2334.
- [27] A. A. Polotsky, T. Gillich, O. V. Borisov, F. A. M. Leermakers, M. Textor and T. M. Birshstein, *Macromolecules*, **2010**, *43*, 9555.
- [28] M. Kröger, O. Peleg and A. Halperin, *Macromolecules*, **2010**, *43*, 6213.
- [29] Y. F. Guo, J. D. van Beek, B. Z. Zhang, M. Colussi, P. Walde, A. Zhang, M. Kröger, A. Halperin and A. D. Schluter, *J. Am. Chem. Soc.*, **2009**, *131*, 11841.
- [30] S. V. Panyukov, S. S. Sheiko and M. Rubinstein, *Phys. Rev. Lett.*, **2009**, *102*, 148301.
- [31] E. B. Zhulina and T. A. Vilgis, *Macromolecules*, **1995**, *28*, 1008.
- [32] S. Alexander, *J. Phys. (Paris)*, **1977**, *38*, 983.
- [33] P. G. de Gennes, *Macromolecules*, **1980**, *13*, 1069.
- [34] G. T. Pickett, *Macromolecules*, **2001**, *34*, 8784.
- [35] G. J. Fleer, M. A. Cohen Stuart, J. M. H. M. Scheutjens, T. Cosgrove and B. Vincent, *Polymers at Interfaces*, Chapman & Hall, London, **1993**.
- [36] K. Binder and A. J. Milchev, *J. Polym. Sci., Part B: Polym. Phys.*, **2012**, *50*, 1515.
- [37] L. N. Gergidis, A. Kalogirou and C. Vlahos, *Langmuir*, **2012**, *28*, 17176.
- [38] C. S. Mahon, C. J. McGurk, S. M. D. Watson, M. A. Fascione, C. Sakonsinsiri, W. B. Turnbull and D. A. Fulton, *Angew. Chem., Int. Ed.*, **2017**, *56*, 12913.
- [39] O. Altintas and C. Barner-Kowollik, *Macromol. Rapid Commun.*, **2016**, *37*, 29.
- [40] M. Rubinstein and R. H. Colby, *Polymer Physics*, Oxford University Press, New York, **2003**.
- [41] S. T. Milner, *J. Polym. Sci., Part B: Polym. Phys.*, **1994**, *32*, 2743.
- [42] A. Sanchez-Sanchez, D. A. Fulton and J. A. Pomposo, *Chem. Commun.*, **2014**, *50*, 1871.
- [43] According to data reported by Fulton et al. [38], the average molecular weight of the SCNP precursor is $M \simeq 10.9$ kDa ($N = 88$, $x = 0.17$). From the well-known expression for polystyrene SEC standards $R = 1.44 \times 10^{-2} M^{0.56}$ we estimate $R \simeq 2.6$ nm and, consequently, $a \simeq 0.18$ nm from $a = R/N^{(3/5)}$. [40]
- [44] R. Deng, F. Liang, J. Zhu and Z. Yang, *Mater. Chem. Front.*, **2017**, *1*, 431.
- [45] X. Zhang, W. Lin, L. Wen, N. Yao, S. Nie and L. Zhang, *Phys. Chem. Chem. Phys.*, **2016**, *18*, 26519.
- [46] Y. Zhang and H. Zhao, *Langmuir*, **2016**, *32*, 3567.
- [47] Z. Nie, D. Fava, E. Kumacheva, S. Zou, G. C. Walker and M. Rubinstein, *Nat. Mater.*, **2007**, *6*, 609.
- [48] A. Shavel and L. M. Liz-Marzán, *Phys. Chem. Chem. Phys.*, **2009**, *11*, 3762.

- [49] J. He, Y. Liu, T. Babu, Z. Wei and Z. Nie, *J. Am. Chem. Soc.*, **2012**, *134*, 11342.
- [50] M. Gonzalez-Burgos, A. Latorre-Sanchez and J. A. Pomposo, *Chem. Soc. Rev.*, **2015**, *44*, 6122.
- [51] E. Harth, B. Van Horn, V. Y. Lee, D. S. Germack, C. P. Gonzales, R. D. Miller and C. J. Hawker, *J. Am. Chem. Soc.*, **2002**, *124*, 8653.
- [52] J. Tao and G. Liu, *Macromolecules*, **1997**, *30*, 2408.
- [53] J. Wen, L. Yuan, Y. Yang, L. Liu and H. Zhao, *ACS Macro Lett.*, **2013**, *2*, 100.
- [54] J. Wen, J. Zhang, Y. Zhang, Y. Yang and H. Zhao, *Polym. Chem.*, **2014**, *5*, 4032.
- [55] L. Cheng, G. Hou, J. Miao, D. Chen, M. Jiang and L. Zhu, *Macromolecules*, **2008**, *41*, 8159.
- [56] Y. Kim, J. Pyun, J. M. J. Fréchet, C. J. Hawker and C. W. Frank, *Langmuir*, **2005**, *21*, 10444.
- [57] F. Xu, Z. Fang, D. Yang, Y. Gao, H. Li and D. Chen, *ACS Appl. Mater. Interfaces*, **2014**, *6*, 6717.
- [58] G. Njilang, G. Liu and S. A. Curda, *Macromolecules*, **2008**, *41*, 5697.
- [59] J. Zhang, J. Tanaka, P. Gurnani, P. Wilson, M. Hartlieb and S. Perrier, *Polym. Chem.*, **2017**, *8*, 4079.
- [60] C. Song, T. Lin, Q. Zhang, S. Thayumanavan and L. Ren, *J. Controlled Release*, **2019**, *293*, 1.
- [61] L. Jiang, H. Li and D. Chen, *Chin. J. Chem. Phys.*, **2017**, *30*, 211.
- [62] O. Altintas, J. Willenbacher, K. N. R. Wuest, K. K. Oehlenschlaeger, P. Krolla-Sidenstein, H. Gliemann and C. Barner-Kowollik, *Macromolecules*, **2013**, *46*, 8092.
- [63] S. Thanneeru, W. Li and J. He, *Langmuir*, **2019**, *35*, 2619.
- [64] J. A. Pomposo, J. Rubio-Cervilla, A. J. Moreno, F. Lo Verso, P. Bacova, A. Arbe and J. Colmenero, *Macromolecules*, **2017**, *50*, 1732.
- [65] W. Li, C.-H. Kuo, I. Kanyo, S. Thanneeru and J. He, *Macromolecules*, **2014**, *47*, 5932.
- [66] S. M. Bhattacharjee, M. Mj and A. Bandyopadhyay (Eds.), *Topology and Condensed Matter Physics*, Springer Nature, Singapore, **2017**.
- [67] J. D. Halverson, W. B. Lee, G. S. Grest, A. Y. Grosberg and K. Kremer, *J. Chem. Phys.*, **2011**, *134*, 204904.
- [68] P.-G. de Gennes, *J. Phys. Lett.*, **1985**, *46*, L639.
- [69] A. Y. Grosberg, S. K. Nechaev and E. I. Shakhnovich, *J. Phys.*, **1988**, *49*, 2095.
- [70] A. Grosberg, Y. Rabin, S. Havlin and A. Neer, *Europhys. Lett.*, **1993**, *23*, 373.
- [71] M. Wengenmayr, R. Dockhorn and J.-U. Sommer, *Macromolecules*, **2019**, *52*, 2616.
- [72] M. González-Burgos, A. Arbe, A. J. Moreno, J. A. Pomposo, A. Radulescu and J. Colmenero, *Macromolecules*, **2018**, *51*, 1573.
- [73] A. J. Moreno, F. Lo Verso, A. Arbe, J. A. Pomposo and J. Colmenero, *J. Phys. Chem. Lett.*, **2016**, *7*, 838.

- [74] P.-G. de Gennes, *Scaling Concepts in Polymer Physics*, Cornell University Press, London, **1979**.
- [75] X.-M. Jia, R. Shi, G.-S. Jiao, T. Chen, H.-J. Qian and Z.-Y. Lu, *Macromol. Chem. Phys.*, **2017**, *218*, 1700029.
- [76] M. E. Mackay, T. T. Dao, A. Tuteja, D. L. Ho, B. Van Horn, H.-C. Kim and C. J. Hawker, *Nat. Mater.*, **2003**, *2*, 762.
- [77] A. Ruiz-Bardillo, I. Asenjo-Sanz, E. Verde-Sesto, L. Porcar, J. Kohlbrecher, J. A. Pomposo, A. J. Moreno, A. Arbe and J. Colmenero, *ACS Macro Lett.*, **2025**, *14*, 1389.
- [78] J. Suzuki, A. Takano and Y. Matsushita, *J. Chem. Phys.*, **2009**, *131*, 144902.
- [79] M. Kruteva, J. Allgaier, M. Monkenbusch, L. Porcar and D. Richter, *ACS Macro Lett.*, **2020**, *9*, 507.
- [80] K. Watanabe, N. Kaizawa, B. J. Ree, T. Yamamoto, K. Tajima, T. Isono and T. Satoh, *Angew. Chem., Int. Ed.*, **2021**, *60*, 18122.
- [81] T. Iwamoto, Y. Doi, K. Kinoshita, Y. Ohta, A. Takano, Y. Takahashi, M. Nagao and Y. Matsushita, *Macromolecules*, **2018**, *51*, 1539.
- [82] G. R. Strobl, *The Physics of Polymers*, Springer, Berlin, **1997**.
- [83] J. E. Poelma, K. Ono, D. Miyajima, T. Aida, K. Satoh and C. J. Hawker, *ACS Nano*, **2012**, *6*, 10845.

CHAPTER 3

Experimental Techniques

3.1. Introduction

This chapter presents a comprehensive overview of the characterization techniques employed throughout this work. These methods have been essential in analyzing the properties of the precursors and SCNPs, and have significantly contributed to understanding their structure, composition, and behavior under various conditions.

A broad set of complementary techniques has been employed to provide detailed and multidimensional insights into the materials investigated. These include chromatographic, spectroscopic, thermal, mechanical, and scattering methods. Each technique is briefly described, outlining its fundamental principles, the specific parameters it measures, and the type of instrumentation used.

Special emphasis is placed on neutron scattering techniques, due to their unique ability to probe structural and dynamical properties at the microscopic scale. These techniques have proven particularly valuable in elucidating aspects that are difficult to access through conventional characterization methods, making them a central component of this study.

3.2. Chromatography: Size Exclusion Chromatography (SEC)

Size exclusion chromatography (SEC) is a powerful technique used to characterize polymers based on the separation of molecules by their size, which means by their hydrodynamic radius (RH) or volume (VH).^[1,2] In a SEC process, a mobile phase (solvent), known as liquid carrier, is continuously flowing through the system at a constant flow rate. The sample is injected into this flow and carried through a column packed with a stationary phase composed of porous beads. As the sample travels through the column, molecules are separated based on their VH: larger molecules, unable to enter the pores, elute first, while smaller molecules penetrate the porous network and experience longer retention times.^[3] When the sample elutes from the column, it passes through a detector/series of detectors and the output is often analyzed by a SEC software package on the computer. This size-based separation, depicted in the chromatogram generated, allows for the determination of molecular weight distribution and dispersity, making SEC a valuable tool for polymer characterization.

Regarding SCNP synthesis, the collapse of the polymer precursor and the resulting reduction in VH can be effectively monitored and quantified using SEC.^[4,5] Additionally, shape-related information is crucial for understanding the complex mechanisms of nanoparticle formation and assessing the influence of factors such as polymer composition and solvent effects.

In this work, SEC measurements were performed at 303 K on an Agilent 1200 system equipped with PLgel 5 μ m Guard and PLgel 5 μ m MIXED-C columns from Agilent, and triple detection from Wyatt Technology Corporation: a differential refractive index (RI) detector (Optilab Rex), a multi-angle laser light scattering (MALLS) detector (MiniDawn Treos), and a viscosimetric (VIS) detector (ViscoStar-II) (Fig. 3.1). Data analysis was performed with ASTRA Software (version 6.1) also from Wyatt Technology Corporation. THF was used as eluent at a flow rate of 1 mL/min. A value of $dn/dc = 0.062$ was used for precursors and SCNPs samples, prepared at 4 mg/mL in THF.

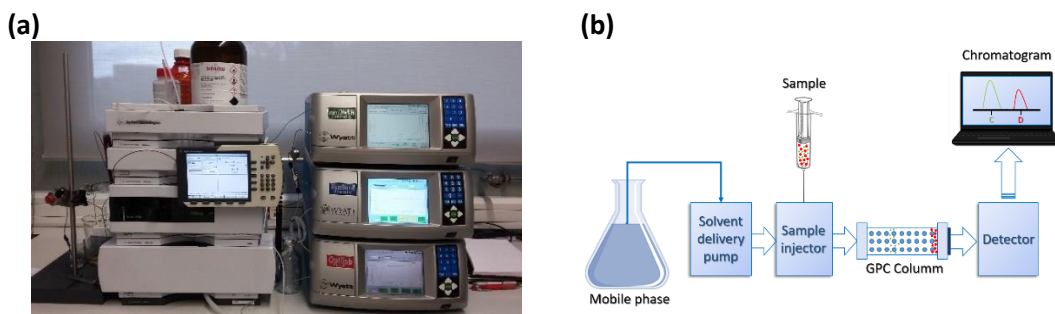


Figure 3.1. Picture of the SEC instrument employed in this work (a) and a schematic representation of the SEC process (b).

3.3. Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear Magnetic Resonance (NMR) is a spectroscopic non destructive technique employed for the elucidation of chemical structures and the investigation of molecular dynamics.

The NMR method is based on the interaction of atomic nuclei with an external magnetic field and a radiofrequency source.^[6] Certain nuclei, such as ^1H and ^{13}C , possess an intrinsic spin and, in the absence of a magnetic field, their spin orientations have the same energy. However, when exposed to a strong magnetic field, these nuclei experience energy level splitting, creating a difference in energy between possible spin orientations.

When the nuclei absorb electromagnetic radiation at a specific resonance frequency, they transition from a lower-energy spin state to a higher-energy one. As they return to their original state, they emit energy as a signal that, once detected and analyzed, generates an NMR spectrum. This spectrum reveals essential details about the structure and surroundings of the nuclei within a molecule.

In this thesis ^1H NMR spectroscopy was used to characterize the structure and composition of the samples, as well as to confirm the random nature of the copolymerization. Experiments were performed at r.t. and under inert atmosphere of argon for the kinetic polymerization experiment, on a Bruker spectrometer operating at 500 MHz, using CDCl_3 as the solvent (Fig. 3.2).

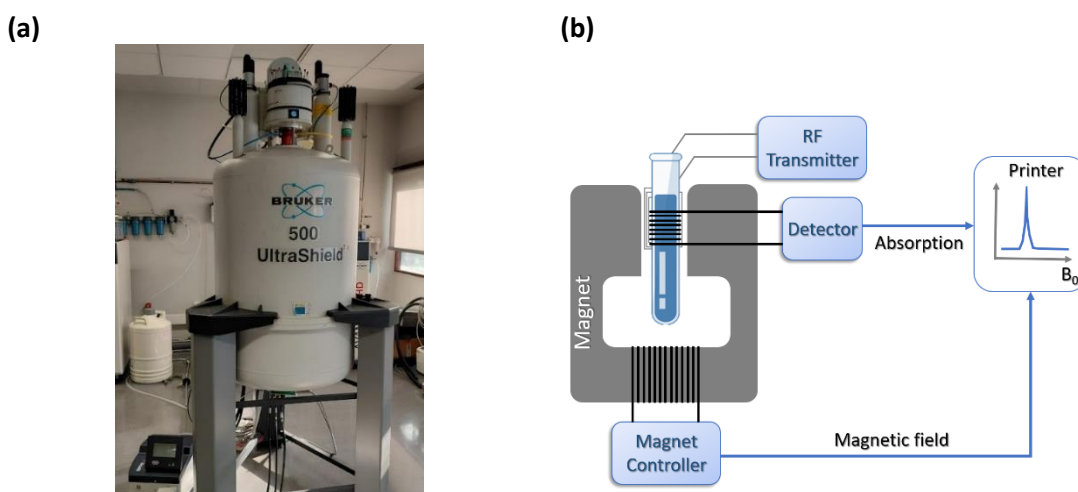


Figure 3.2. Picture of the NMR instrument employed in this work (a) and schematic representation of its functional components (b)

3.4. Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectroscopy studies the interaction between matter and infrared radiation, which corresponds to the region of the electromagnetic spectrum with wavelengths ranging from 0.7 to 1000 μm .^[7] The infrared spectrum originates from the absorption of photons with energy corresponding to the mentioned region, causing a transition between vibrational levels in a molecule within the electronic state in which that species resides. Thus, the frequency of the vibrations can be associated with a particular bond type.^[8] The term Fourier transform infrared spectroscopy originates from the fact that a mathematical process (Fourier transform) is required to convert the raw data into the actual spectrum.

The most common and straightforward method for measuring a sample's spectrum is transmission, applicable to gaseous, liquid, or solid samples. However, when transmission experiments are not feasible, alternative techniques are used, one of which is Attenuated Total Reflectance (ATR). In this method, a special accessory made of an infrared-transparent material is used. The infrared light is directed onto one end of a trapezoidal-shaped crystal at a specific angle, causing it to bounce internally through the crystal until it exits at the other end. Although the light bounces within the crystal, some of it penetrates the surface of the material. If there is a sample on the surface, it will absorb part of the radiation, causing a reduction in the internal reflection. How deeply the radiation penetrates depends on factors like the frequency of the infrared light, the refractive index of the sample and the crystal, as well as the angle of incidence.

In order to accurately characterize the samples, particularly to determine the presence or absence of azide groups, ATR-FTIR spectra were recorded at room temperature using a JASCO 3600 FTIR spectrometer (Fig. 3.3). The samples were analyzed without requiring complex sample preparation.

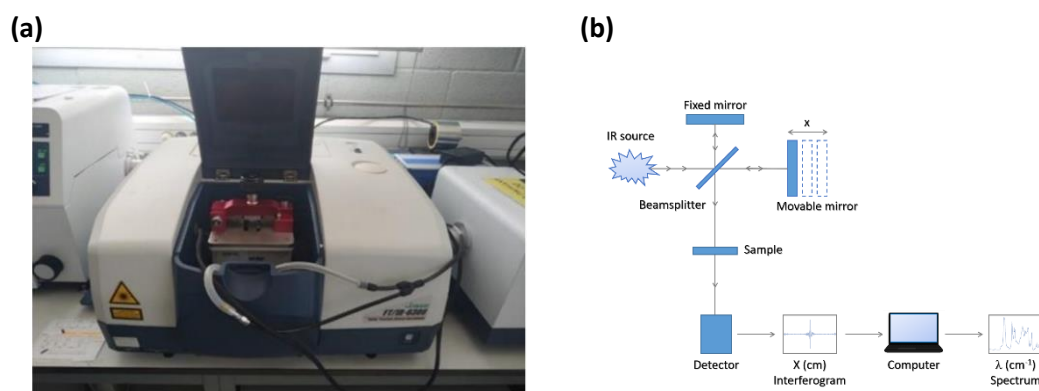


Figure 3.3. Picture of the FTIR equipment used in this work (a) and a scheme of the equipment's set up (b).

3.5. Elemental Analysis (EA)

Elemental analysis is a process that allows for the precise determination of carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) (CHNS) amounts in virtually all existing substances. The most widely used method in organic chemistry and polymer science is microcombustion analysis, which consists of several steps: degasification, high-temperature sample combustion under a pure oxygen flow, rapid separation of the resulting gaseous species, and detection.^[7]

Elemental quantification of the samples was performed using an EA3000 elemental analyzer from EuroVector, provided by the General Research Services at the University of the Basque Country (UPV/EHU) (Fig. 3.4). The prepared calibration and analysis samples were placed in the auto-sampler, from where they were periodically introduced into a vertical quartz reactor heated to 980 °C under a constant helium flow. A few seconds before sample introduction, the helium stream was enriched with high-purity oxygen. The combustion gas mixture was then directed through a reaction zone to ensure complete quantitative oxidation, followed by a reduction step in a copper zone, where nitrogen oxides were converted to nitrogen. The resulting gases (N_2 , CO_2 , and H_2O) were separated in a chromatographic column and detected by the thermal conductivity detector (TCD). The signals, proportional to the amount of eluted gases,^[9] were analyzed using Callidus software.

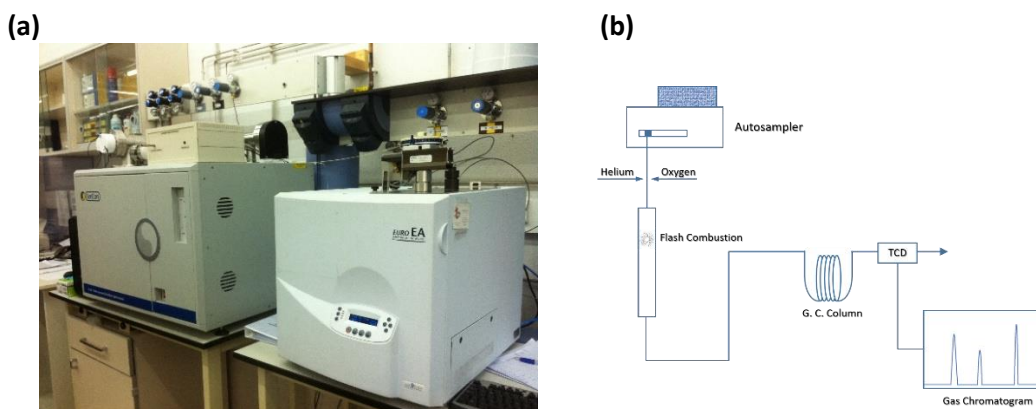


Figure 3.4. Picture of the Elemental Analysis instrument employed in this work (a) and a scheme of the equipment's operating principle (b).

3.6. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) is a thermal characterization technique that measures the differences in the amount of heat absorbed or released between a substance and a reference as a function of temperature when both are subjected to heating, typically at a constant rate.^[10] The fundamental principle behind this technique is that during physical transformations, such as phase transitions, the sample absorbs (endothermic process) or releases (exothermic process) heat to maintain the same temperature as a stable reference material. Beyond thermodynamic phase transitions, DSC can also detect other physical changes, such as glass transitions, making it an essential tool for the physical characterization of polymers in general^[11] and SCNPs.^[12]

The calorimetric glass transition temperature (T_g) of the samples was measured using a differential scanning calorimeter (DSC) TA instrument Q2000 under ultrapure nitrogen flow (Fig. 3.5). Approximately 5 mg of each sample was used for the measurements. For the experiments, first, non-isothermal scans were performed, where the samples were heated up until 350 K. The samples were then kept at this temperature for 3 minutes in order to erase any previous thermal history, and finally, a cooling scan at 20 K/min was recorded to 170 K, followed by a subsequent heating scan also at 20 K/min from this temperature to 350 K. The T_g values were extracted from the onset value of the jump in heat capacity.

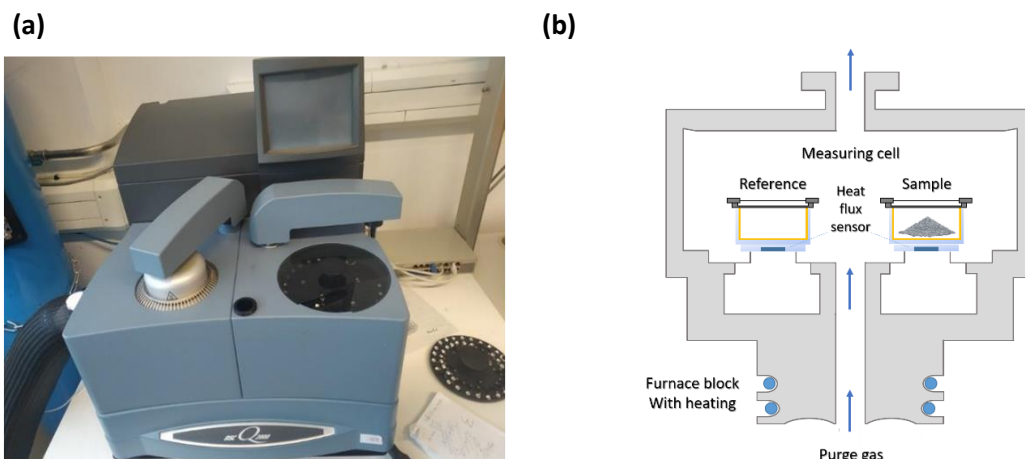


Figure 3.5. Image of the DSC instrument used in this work (a) and schematic representation of its operating principle (b).

3.7. Rheology

Rheology, as a mechanical analysis technique, investigates the deformation and flow behavior of materials under applied forces, offering fundamental insights into their mechanical response. Deformation refers to the change in shape or dimensions of a material when subjected to an external force, whereas flow describes the continuous movement of a fluid or soft material from one point to another. The resistance to flow, known as viscosity, determines how easily a material moves—higher viscosity means lower flow. All materials exist on a spectrum between viscous liquids (including Newtonian fluids) and elastic solids, but it is particularly interesting to study materials whose properties are between purely elastic and Newtonian fluids, as polymers.

Most polymers exhibit viscoelastic behavior when subjected to deformation.^[13] This means they display characteristics of both viscous and elastic materials, leading to strain dependency over time. In polymers, the energy associated with plastic deformation is related to the dynamic shear loss modulus (G''), while elastic deformation is linked to the dynamic shear storage modulus (G') (see Fig. 3.6).

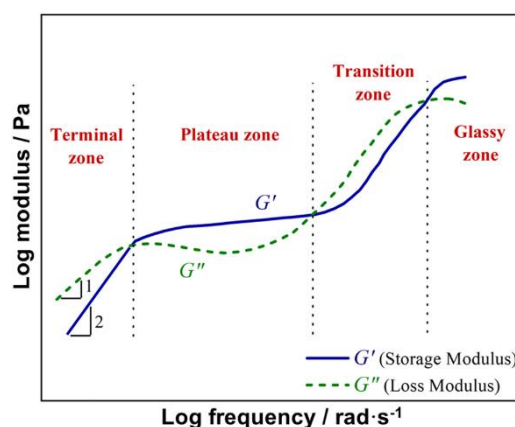


Figure 3.6. Dynamic mechanical spectrum of a polymer showing the storage modulus (G') and loss modulus (G'') as functions of frequency. The plot illustrates the viscoelastic behavior of polymers, where G' represents the elastic (energy-storing) response and G'' the viscous (energy-dissipating) response.

Dynamic mechanical spectroscopy, which involves applying a sinusoidal stress and measuring the resulting strain, enables the determination of these moduli. G' and G'' are fundamental to key polymer properties, such as the glass transition temperature and entanglement molecular weight. The latter can be estimated from the modulus in the plateau region, where G' is proportional to the density of physical and chemical crosslinks per unit volume.

Rheological experiments in the linear regime in the frequency range of ≈ 0.03 –16 Hz were performed by using an ARES torsional rheometer (TA Instruments) with a parallel plate geometry (8 mm diameter) and a gap distance of ≈ 0.4 –0.5 mm (Fig. 3.7). To ensure a linear regime during the experiments, a strain amplitude of 2% was used. All the experiments were carried out under a N_2 atmosphere, and the temperature was set by an LN2 controller. The temperature range measured was $213 \leq T \leq 313$ K for all the samples studied.

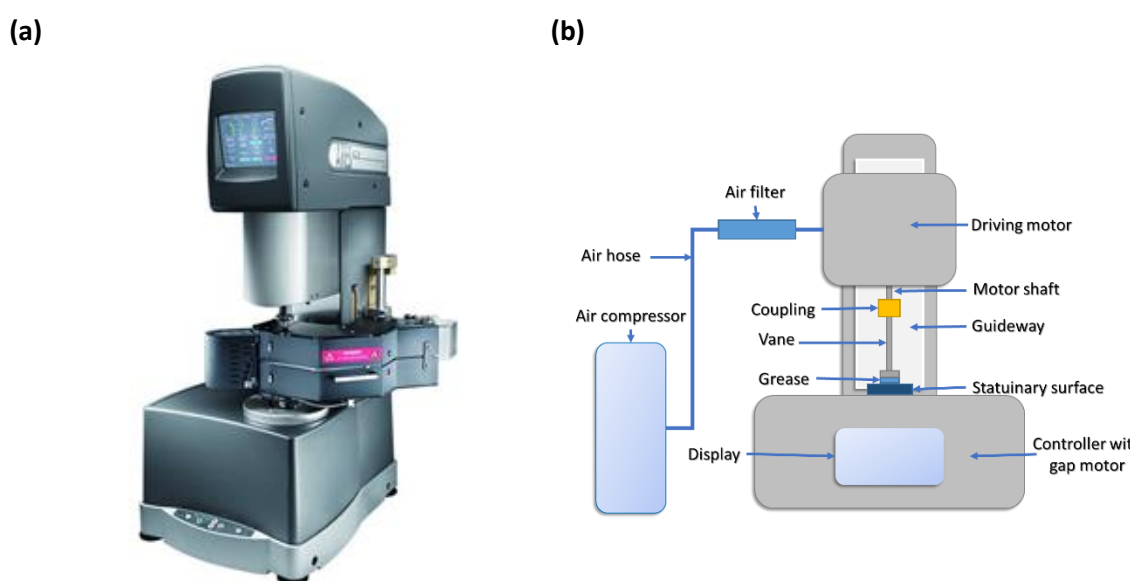


Figure 3.7. Picture of the ARES torsional rheometer used in this work (a) and schematic representation of the instrument (b).

3.8. Neutron Scattering (NS)

Scattering techniques are particularly effective for making significant progress in understanding the structural and dynamic properties of polymers and SCNPs. These experiments offer spatial resolution by examining the wavevector dependence of the measured quantities. By using various probes (mainly photons and neutrons) and different experimental setups, both static and dynamic properties can be studied at the molecular level. Particularly, neutron scattering (NS) is highly valuable for exploring complex soft materials, such as those containing SCNPs. Among its many advantages, including high penetration depth, two are especially critical: the ability to simultaneously access relevant length and time scales, and the flexibility to tune scattering contrast as needed.^[14]

3.8.1 Basic Properties of Neutrons

The neutron is a subatomic particle, a nucleon, consisting of three valence quarks, which are held together by gluons which gives it specific physical properties (Fig. 3.8):

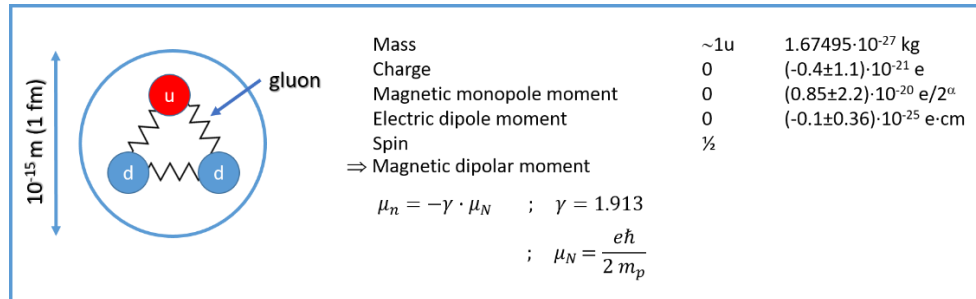


Figure 3.8. Schematics of the neutron being composed of three quarks and gluons and the main quantities characterizing the neutron as a particle.

These specific properties make neutrons highly valuable for condensed matter investigations:

- **Instability outside the nucleus:** Due to the weak interaction, the neutron is not stable outside the nucleus. A free neutron undergoes β -decay with an average lifetime of about 15 minutes, providing ample time for scattering experiments (Eq. (3.1)).



- **Mass and energy transfer:** Unlike the massless photon, the neutron has a mass of approximately $1.675 \times 10^{-27} \text{ kg}$ (about one atomic mass unit). This finite mass is comparable to the mass of a nucleus, allowing significant energy transfer during scattering processes.
- **Charge neutrality and deep penetration:** Neutrons are uncharged particles, which means they do not interact strongly with matter through Coulomb forces. As a result, they have large penetration depths, making them ideal for studying bulk properties of materials and for investigating materials under extreme conditions, such as very low or high temperatures, high pressures, and strong magnetic or electric fields.
- **Nondestructive nature:** Due to their low energy, typically in the meV range, neutrons are nondestructive, enabling samples to be irradiated over long periods (days) and reused after the experiment.
- **Magnetic properties:** The neutron has a nuclear spin of $1/2$, leading to a magnetic dipole moment of $\mu_n = \gamma \mu_N$, where $\gamma = 1.91$ and $\mu_n = 5.05 \cdot 10^{-27} \text{ J/T}$. This magnetic moment allows neutrons to interact with the magnetic fields of unpaired electrons in a sample, resulting in magnetic scattering. As such, neutron scattering can be used to study magnetic structures and excitations.
- **Isotopic sensitivity:** Unlike X-rays or electrons, which interact primarily with the electronic cloud, neutron scattering arises from interactions with atomic nuclei, resulting in high sensitivity to light elements such as hydrogen, a key constituent of most polymers. In addition, neutron scattering can distinguish between neighboring elements in the periodic table, such as magnesium, iron, and chromium, for which X-ray contrast is often limited due

to their similar electron densities. This nuclear contrast enables isotopic substitution strategies, most notably hydrogen–deuterium (H-D) exchange, which can be exploited to selectively highlight specific polymer chains, segments, or functional groups. Through contrast variation, both structural organization and dynamics can be investigated within complex multicomponent soft-matter systems, in close analogy to staining techniques used in light microscopy. In this work, these advantages were exploited to study polymer systems.^[14]

As highlighted above, neutrons provide valuable insight into both the static and dynamic properties of atomic arrangements, which are crucial for understanding the physical properties of materials. The range of accessible scattering wavevectors enables analysis of structural properties and molecular motions on length scales ranging from below an Angstrom to dozens of nanometers. NS probes motions across a wide range of timescales: from momentum distributions on the femtosecond (fs) timescale (eV) to slow dynamics such as the relaxation of polymer chains in the melt at around 100 ns (neV).

Applications of neutron scattering spectroscopy in soft materials can be divided into inelastic scattering (energy transfer peaks at finite energies) to study vibrational modes, complementing techniques like Raman or infrared spectroscopy, and quasi-elastic scattering (small energy transfers centered around zero energy) to study rotational, diffusional, and other relaxation processes. These neutron experiments complement techniques such as dielectric relaxation spectroscopy and NMR. In particular, for soft matter studies, the large contrast achieved by substituting hydrogen with deuterium (without changing the material's intrinsic properties) allows selective study of specific molecular units or components in multicomponent systems.

3.8.2. Fundamental Concepts of Neutron Scattering

In Figure 3.9, a schematic of a generic scattering spectroscopy experiment is shown. A neutron beam, characterized by an initial energy E and an initial wavevector $\vec{k}$, strikes the sample. A detector, covering a solid angle Ω ,^[15-18] records the neutrons scattered by the sample, which arrive with a final energy E' and wavevector $\vec{k}'$, different from the initial ones.

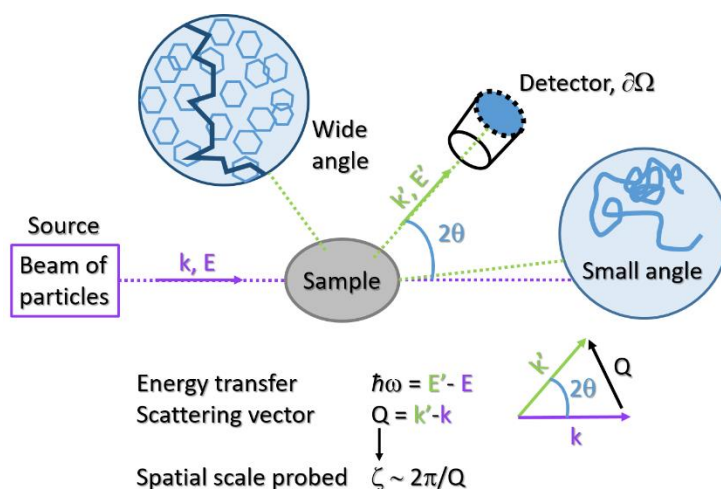


Figure 3.9. Schematic representation of neutron scattering experiment.

The difference between the incident and the final wavevectors is called scattering vector $\vec{Q}$:

$$\vec{Q} = \vec{k}' - \vec{k} \quad (3.2)$$

The modulus of $\vec{Q}$, Q , is determined (under elastic, or close to elastic conditions) as:

$$Q = 4\pi \lambda^{-1} \sin\theta \quad (3.3)$$

where 2θ is the scattered angle and λ is the incident wavelength of the neutron. Thus, the explored Q -value can be varied by adjusting either the scattering angle or the wavelength of the incident neutrons. The difference between the scattered energy E' and the incident E represents the energy transferred from the sample to the neutron:

$$\hbar\omega = E' - E = \frac{\hbar^2}{2m} (k'^2 - k^2) \quad (3.4)$$

There are three different types of scattering events, as shown in Fig. 3.10:

- **Elastic**: when no energy is transferred to the sample ($E' = E$).
- **Inelastic**: when a neutron loses or gains energy and it is scattered with a final energy and wavelength different from the initial ones ($E' \neq E$).
- **Quasi-elastic (QENS)**: this is a type of inelastic scattering that involves very small energy transfers. It occurs when a neutron interacts elastically with a moving particle, causing the neutron's energy to experience a Doppler shift due to the particle's velocity. This effect is observed as a broadening of the elastic peak in the scattering spectrum.

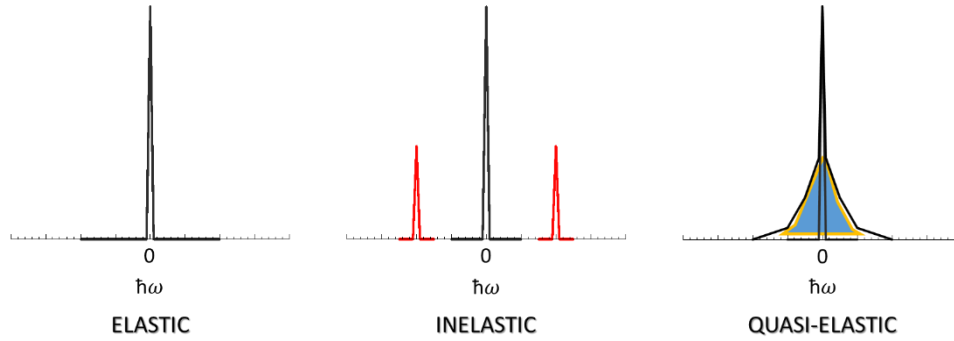


Figure 3.10. Schematic representation of different types of scattering events. The broadening of the elastic peak, compared to a delta function, is due to the finite resolution of the instrument.

In QENS experiments, the double differential scattering cross section $\partial^2\sigma/\partial\Omega\partial\hbar\omega$ is the measured quantity. This is defined as the number of neutrons scattered into a solid angle comprised between Ω and $\Omega + d\Omega$, which have undergone a change in energy between $\hbar\omega$ and $\hbar\omega + d\hbar\omega$, relative to the total number of incident neutrons.

The interpretation of $\partial^2\sigma/\partial\Omega\partial\hbar\omega$ is straightforward in terms of the *correlation functions* defined in the van Hove (1954) formalism:^[16]

$$\frac{\partial^2\sigma}{\partial\Omega\partial\hbar\omega} \propto \sum_{\alpha,\beta} \bar{b}_\alpha \bar{b}_\beta S_{coh}^{\alpha\beta}(\vec{Q}, \omega) + \sum_{\alpha} \overline{\Delta b_\alpha^2} S_{inc}^{\alpha}(\vec{Q}, \omega) \quad (3.5)$$

The indexes α and β represent all the possible kinds of isotopes present in the sample (α, β : H, D, C, O, etc.). Equation (3.5) involves the so-called *scattering length* b , that is the parameter which describes the interaction between the neutron and the scattering nucleus. It is a property specific to each nucleus and depends on both the considered isotope and the relative orientation of the neutron-nuclear spin pairs. Therefore, isotopes of the same atomic species are characterized by different values of the scattering length. In $\partial^2\sigma/\partial\Omega\partial\hbar\omega$, a coherent (coh) and an incoherent (inc) contribution can be identified, that are weighted by the scattering lengths and their deviations from their mean value. The incoherent contribution arises from the random distribution of the deviations of the scattering lengths from their mean value $\overline{\Delta b_\alpha^2} = \overline{b_\alpha^2} - \bar{b}_\alpha^2$. The incoherent and coherent scattering cross section are defined as follows:

$$\sigma_{inc} = 4\pi\overline{\Delta b_\alpha^2}; \sigma_{coh} = 4\pi\bar{b}^2 \quad (3.6)$$

The features ($\vec{Q}$ and ω -dependencies) of both contributions to Eq. 3.5 are determined by the corresponding scattering functions [$S_{coh}^{\alpha\beta}(\vec{Q}, \omega)$ involving nuclei of kinds α and β , and $S_{inc}^\alpha(\vec{Q}, \omega)$ involving nuclei of kind α]. These are related, via Fourier transformation, with the intermediate scattering functions [$S_{coh}^{\alpha\beta}(\vec{Q}, t)$ and $S_{inc}^\alpha(\vec{Q}, t)$] and the van Hove correlation functions [$G^{\alpha\beta}(\vec{r}, t)$ and its self-part $G_{self}^\alpha(\vec{r}, t)$]:

$$S_{coh(inc)}^{\alpha\beta(\alpha)}(\vec{Q}, t) = \hbar \int S_{coh(inc)}^{\alpha\beta(\alpha)}(\vec{Q}, \omega) e^{i\omega t} d\omega \quad (3.7)$$

$$G_{self}^{\alpha\beta(\alpha)}(\vec{r}, t) = \frac{1}{(2\pi)^3} \int S_{coh(inc)}^{\alpha\beta(\alpha)}(\vec{Q}, t) e^{-i\vec{Q}\vec{r}} d\vec{Q} \quad (3.8)$$

In the classical limit, $G^{\alpha\beta}(\vec{r}, t)$ can be written as:

$$G^{\alpha\beta}(\vec{r}, t) = \left\langle \frac{1}{N} \sum_{i\alpha, j\beta}^{N_\alpha, N_\beta} \delta\{\vec{r} - [\vec{r}_{i\alpha}(t) - \vec{r}_{j\beta}(0)]\} \right\rangle \quad (3.9)$$

Here $\vec{r}_{i\alpha}(t)$ [$\vec{r}_{j\beta}(0)$] is the position of the vector i^{th} atom of kind α [j^{th} atom of kind β] at time t [time = 0] and the sum runs over all the different atoms of kinds α and β [$N_\alpha(N_\beta)$: total number of atoms of kind $\alpha(\beta)$; $N = \sum_\alpha N_\alpha$]. Thus, $G^{\alpha\beta}(\vec{r}, t)d\vec{r}$ is the probability that, given a particle of kind β at the origin at time $t = 0$, any particle of kind α is in the volume $d\vec{r}$ at position $\vec{r}$ at time t . It can easily be seen that in the static case $G^{\alpha\beta}(\vec{r}, t = 0) = \delta_{\alpha\beta}(\vec{r}) + g^{\alpha\beta}(\vec{r})$, where $g^{\alpha\beta}(\vec{r})$ is the static pair distribution function. It is important to note that in diffraction experiments, the detection of scattered particles does not depend on their energy. This is equivalent to perform an integral over the ω -variable, i. e., it corresponds to the static $t = 0$ limit. Thus, the accessed magnitude in a diffraction experiment is the sum of the partial structure factors weighed by the corresponding scattering lengths products. On the other hand, the self-part of the van Hove correlation function $G_{self}^\alpha(\vec{r}, t)$ is obtained by restricting the correlations considered in Eq. 3.9 to those relating the positions of a single particle of kind α at different times:

$$G_{self}^{\alpha}(\vec{r}, t) = \langle \frac{1}{N} \sum_{i\alpha}^{N_{\alpha}} \delta\{\vec{r} - [\vec{r}_{i\alpha}(t) - \vec{r}_{i\alpha}(0)]\} \rangle \quad (3.10)$$

$G_{self}^{\alpha}(\vec{r}, t)$ is the Fourier transform of $S_{inc}^{\alpha}(\vec{Q}, t)$ in space: incoherent scattering relates to single-particle motions, and this tells us what a single atom does. Equation 3.5 can thus finally be written as:

$$\begin{aligned} \frac{\partial^2 \sigma}{\partial \Omega \partial \hbar \omega} \propto & \sum_{\alpha, \beta} \bar{b}_{\alpha} \bar{b}_{\beta} \int e^{i\omega t} \langle \sum_{i\alpha, j\beta} e^{-i\vec{Q}[\vec{r}_{i\alpha}(t) - \vec{r}_{j\beta}(0)]} \rangle dt + \\ & + \sum_{\alpha} \overline{\Delta b_{\alpha}^2} \int e^{i\omega t} \langle \sum_{i\alpha} e^{-i\vec{Q}[\vec{r}_{i\alpha}(t) - \vec{r}_{i\alpha}(0)]} \rangle dt \end{aligned} \quad (3.11)$$

The main Eq. 3.5 shows that the weights of the coherent and incoherent contributions to the scattered intensity are determined by the scattering lengths of the isotopes involved. The most important example, due for its implications in the soft matter field, is found for hydrogen and deuterium (Table 3.1).

Table 3.1: Values of the average neutron scattering lengths $\bar{b}_{\alpha}$, their squares $\bar{b}_{\alpha}^2$ and their deviations $\overline{\Delta b_{\alpha}^2}$ for different isotopes α .

Isotope α	$\bar{b}_{\alpha}/fm$	$\bar{b}_{\alpha}^2/fm^2$	$\overline{\Delta b_{\alpha}^2}/fm^2$
1H	-3.7406	13.992	638.78
2H (D)	6.6710	44.502	16.322
^{12}C	6.6511	44.237	0
^{16}O	5.8030	33.675	0

The data presented in Table 3.1 reveal that:

- Due to the large value of $\overline{\Delta b_H^2}$, in hydrogen-containing systems the signal is dominated by the incoherent scattering from hydrogens, revealing their self-motions.
- By substituting H with D, the incoherent contribution is drastically reduced resulting in differently weighed coherent contributions.
- The intensity scattered by fully deuterated samples is mainly coherent and, since $\bar{b}_D \approx \bar{b}_C$, all pair correlations are almost equally weighted.
- Hydrogen and deuterium differ significantly in their average scattering length values ($\bar{b}_D = 6.67 fm$ vs $\bar{b}_H = -3.74 fm$). This large contrast offers a powerful tool for probing soft materials via NS: deuterium labeling. By replacing hydrogen with deuterium, a pronounced difference in scattering contrast is achieved between molecules composed of protonated and deuterated components. This enables deuterium labeling techniques to 'stain' specific molecular regions, making them distinguishable not only in dilute solutions but also in complex, crowded systems—such as concentrated polymer solutions or even the condensed phase.

3.8.3. Sources

Neutron scattering experiments are realized in large facilities including a neutron source (either a nuclear reactor—providing a continuous flux— or a spallation—pulsed— source) and dedicated instrumentation.^[14]

- Neutrons produced by nuclear fission: To produce neutrons through nuclear fission, a slow neutron is captured by a fissile heavy isotope, such as Uranium-235. The fission of the uranium isotope results in the release of 2-3 high-energy neutrons (1.29 MeV), along with lighter elements and the emission of energy. These neutrons can then go on to induce further fission reactions, sustaining a chain reaction. To make these neutrons useful for experiments, they are slowed down by a cooling medium (moderator) to lower their energy, making them more suitable for scattering experiments or other scientific purposes.
- Neutrons produced by spallation: As an alternative to nuclear fission reactors, neutrons can be produced through spallation reactions. In this process, high energy protons ($E \approx 1\text{GeV}$) are accelerated and directed onto a target made of a neutron rich material. Due to their large energy, the protons have an extremely short de Broglie wavelength $\lambda = \sqrt{\frac{h^2}{2mE}}$, causing them to interact with the single nucleus as a whole. The ejected nucleon may either exit the nucleus, initiating an inter-nuclear cascade or it may be scattered by other nucleons, leading to an intra-nuclear cascade. As a result of the initial spallation stage, the nucleus becomes highly excited. In the second stage, this energy is released through the evaporation of various particles, including neutrons.

3.8.4. Instrumentation

In this thesis, several neutron scattering instruments have been used to obtain different types of information from the sample, which are essential for a comprehensive analysis.

- Diffraction with polarization analysis
- Small Angle Neutron Scattering (SANS)
- Neutron Spin Echo (NSE)
- Backscattering (BS) Spectrometer
- Time of Flight (ToF) Spectrometer

Diffraction with polarization analysis

In this thesis, neutron diffraction with polarization analysis was employed to separate coherent and incoherent differential cross sections.^[19, 20]

This technique is commonly used in the study of magnetic systems, as diffuse scattering provides detailed insights into magnetic configurational disorder and its interaction with local atomic defects. However, magnetic disorder scattering tends to be weak and is often accompanied by nuclear disorder scattering and spin-incoherent scattering. To distinguish between these contributions, polarized neutron techniques are required.

The Diffuse Scattering Spectrometer D7, employed in this work and located at the Institut Laue-Langevin (ILL) in Grenoble (France),^[21] is a cold neutron multidetector diffractometer equipped with full XYZ polarization analysis and the option of time-of-flight energy analysis. This setup, shown in Fig. 3.11, is ideal for investigating both nuclear and magnetic short-range order as well as magnetic defect structures.

There are two modes of operation of D7, diffraction and spectroscopy:

- **Diffraction mode:** D7 is most commonly used as a polarized neutron diffractometer, with data typically analyzed under the static approximation.^[22] Depending on the experimental requirements, the neutron polarization at the sample position can be manipulated in the following manners:
 - **Uniaxial Polarization Analysis:** The neutron polarization is fixed perpendicular to the scattering plane, and both spin-flip and non-spin-flip scattering are measured. This mode is primarily used to separate coherent from spin-incoherent scattering in non-magnetic samples. Coherent scattering does not flip the neutron spin, whereas spin-incoherent scattering causes spin flips in 2/3 of the neutrons, enabling clear separation of the two components.
 - **XYZ Polarization Analysis:** In this method, the polarization direction is alternated among three orthogonal axes, and both spin-flip and non-spin-flip scattering events are measured for each orientation. This approach enables the separation of magnetic, incoherent, and coherent nuclear scattering components. It is particularly effective for investigating disordered, antiferromagnetic, or paramagnetic materials.^[22-24]
- **Spectroscopy mode:** D7 can function as a time-of-flight spectrometer thanks to its integrated Fermi chopper, which provides permanent polarization analysis. However, the chopper significantly reduces the neutron flux—by a factor of approximately 150—resulting in much longer acquisition times for spectroscopic measurements.

The experiments were carried out with uniaxial polarization analysis and the incident neutron wavelength was set to $\lambda = 4.89 \text{ \AA}$ to cover a Q -range from 0.15 to $2.5 \text{ \AA}^{-1}$. A Vanadium sheet was used to calibrate the detector efficiency and for the background scattering, a combination of an empty cell and a Cadmium sheet was used. In addition, a quartz plate for the polarization correction was used. The samples (blends) were studied at 300 K and flat aluminum cells were used as sample holders.

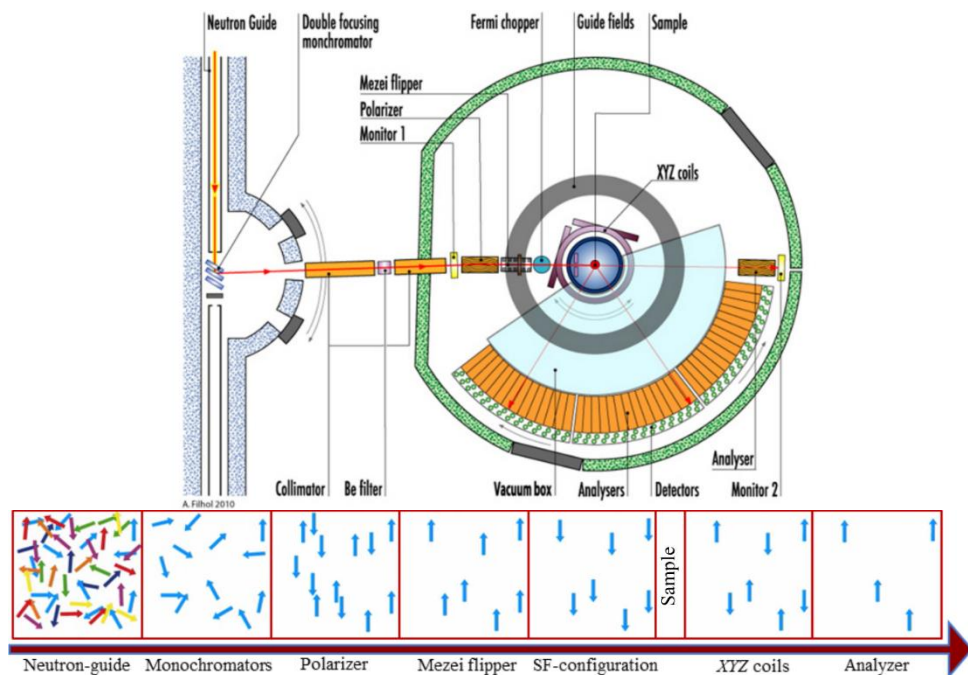


Figure 3.11. Schematic showing of the diffuse scattering spectrometer D7 at the ILL and neutron spin behaviour in spin-flip (SF) configuration.

Small Angle Neutron Scattering (SANS)

Small-Angle Scattering (SAS), including Small-Angle Neutron Scattering (SANS), investigates structures in samples ranging from approximately 0.5 to a few hundred nanometers. This technique can be applied to both isotropic samples, such as blends and liquids, and anisotropic samples, like quasi-crystals. SANS is particularly useful for studying self-assembled polymeric and biological systems, multi-scale systems with large particle size distributions, solutions of (nano-)particles, soft matter systems, protein solutions, and material science applications.^[25-28] The scattered intensity of neutrons is measured at small angles. In addition to structural analysis, SANS can also be used to investigate the spin state and magnetic structure of the sample, enabling further insight into the sample's magnetic properties, such as clusters in alloys, polymers, or biological macromolecules.^[29-31]

A schematic version of the D22 SANS instrument at the Institute Laue Langevin (ILL) in Grenoble,^[32] employed in this thesis, is shown in Fig. 3.12. The SANS experiment follows the four basic steps common to all scattering experiments: monochromatization, collimation, scattering, and detection. The detector's position can typically be adjusted, allowing for the selection of different sample-detector distances, which in turn alters the Q -range investigated for a given incident wavelength.

Experiments on the deuterated samples were performed using $\lambda = 6 \text{ \AA}$. The scattered intensity was recorded with sample-detector distances of 17.6, 5.6, and 2 m to cover a Q -range from 0.003 to $0.5 \text{ \AA}^{-1}$. Four different temperatures were explored: 280, 300, 320, and 360 K.

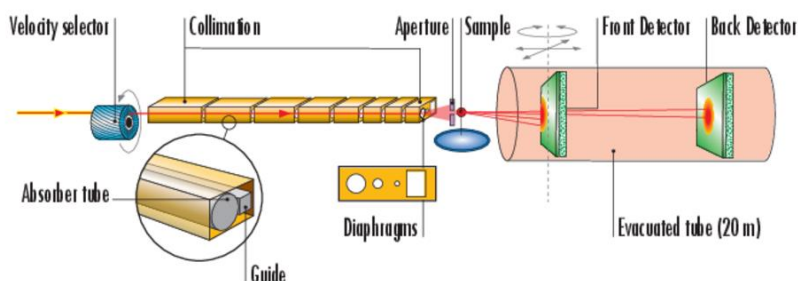


Figure 3.12. Schematic of small angle scattering experiment of D22 instrument.

Neutron Spin Echo (NSE)

Neutron Spin Echo (NSE) is a neutron spectroscopy technique that is sensitive to the QENS signal. The principle behind NSE involves encoding the energy transfer in the scattering process into the spin rotation of each neutron individually. [33]

By applying precession magnetic fields before and after the scattering event, the neutron polarization depends only on the difference in velocity of each neutron, regardless of its initial speed. This method stands out because it provides information directly in the time domain, allowing experimental results to be corrected for resolution effects through simple division. The measured time is proportional to $J \propto \lambda^3$, where J represents the field integral. For a specific wavelength, the Fourier time range is limited to short times (typically around 2 ps) due to spin depolarization from the loss of the guide field, at long times by the maximum field integral that can be applied. This technique also strongly reduces incoherent scattering. In fact, the quantity measured in an NSE experiment is a normalized function:^[33]

$$\tilde{S}_{NSE}(Q, t) = \frac{I_{coh}\tilde{S}_{coh}(Q, t) - \frac{1}{3}I_{inc}\tilde{S}_{inc}(Q, t)}{I_{coh} - \frac{1}{3}I_{inc}} \quad (4.12)$$

Where $\tilde{S}_{coh}(Q, t)$ and $\tilde{S}_{inc}(Q, t)$ are the normalized intermediate pair and self-correlation functions (normalized to their value of $t=0$). I_{coh} and I_{inc} denote the total ('static') coherent and incoherent intensities. The suppression of the incoherent signal can be advantageous for many soft matter questions, where the dynamic structure factor or the single chain dynamic structure factor are on the focus. Details of the experimental setup employed in such instruments can be found in Refs.^[33,34].

The coherent intermediate scattering function or dynamic structure factor of deuterated SCNPs and their precursors in bulk were studied with the NSE spectrometer IN11C also at the ILL.^[32] A schematic picture of the instrument is shown in Fig. 3.13. The multidetector at IN11c covers an angular range of 30° in the horizontal plane. It was placed at 20°, 50°, and 80° scattering angle for its central detector. With an incident wavelength of 5.5 Å, a Q -range of $0.15 \leq Q \leq 1.64 \text{ Å}^{-1}$ and a time range from 5 ps to 0.7 ns were explored. The same temperatures as in SANS were investigated (280, 300, 320 and 360 K).

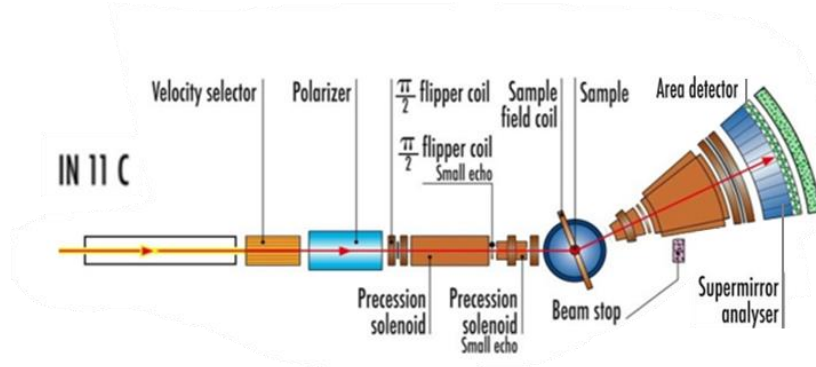


Figure 3.13. Schematic representation of the NSE setup used in the IN11C configuration at the ILL.

Backscattering Spectrometer (BS)

A backscattering spectrometer, specifically the IN16B instrument at the ILL,^[21] was also used (Fig. 3.14). It is a cold neutron spectrometer, that operates as a “variable incident energy, fixed final energy spectrometer,” enabling the precise investigation of material dynamics.

A pulsed beam of neutrons, with an energy range determined by a Doppler machine, is directed at the sample. Once the neutrons interact with the sample, they are scattered in all directions. However, only those neutrons with a specific final energy, set by the backscattering reflection at the analyzer crystals, are reflected back towards the detectors, where they are counted.

IN16B can work in two configurations: In the “low background” setup (LB), the experiment is designed to reduce outside interference, that corresponds to the IN16B in the guide-side position. On the other hand, in the “high flux configuration” (HF) at the guide-end position, the instrument uses a velocity selector to filter the incoming neutron wavelengths, replacing the graphite deflector used in the first setup.

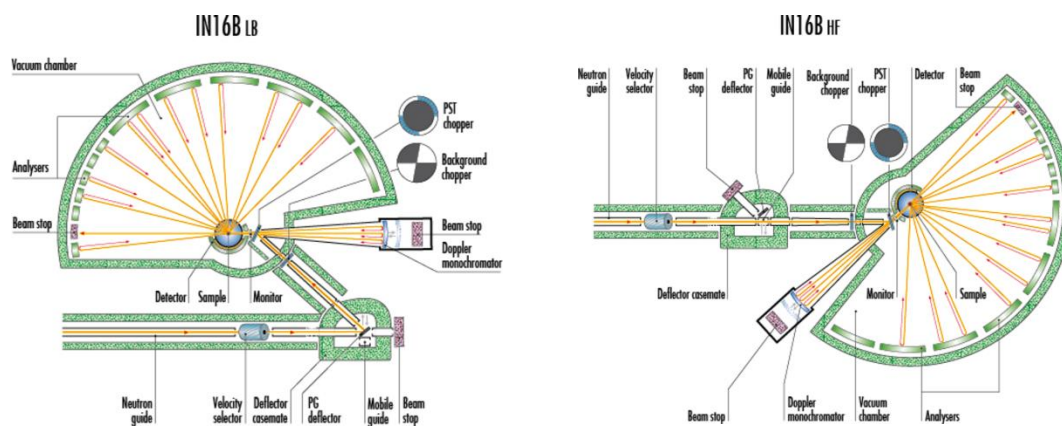


Figure 3.14. Schematic representation of the IN16B instrument at the ILL, in the “low background” (LB) and “high flux” (HF) configuration.

Time of Flight (ToF) Spectrometer

Time-of-flight (ToF) spectrometers measure the number of neutrons arriving at each detector as a function of the time of flight. A continuous neutron flux from the neutron guide is turned into a pulsed monochromatic beam by a set of choppers. These pulsed neutrons are scattered by the sample, which can be in the form of a liquid, powder, or single crystal, and subsequently detected by a large array of detectors covering a wide detection area. By analyzing the time required for the neutrons to reach each detector, a three-dimensional spectrum is obtained, which includes detector position (x , y) and time (t). From this dataset, the double differential scattering cross section $\partial^2\sigma/\partial\Omega\partial\hbar\omega$ (the relevant observable in QENS experiments) can be obtained, as discussed in Section 3.8.2.

The experiments were conducted at the IN5 instrument at the ILL,^[21] the schematic of which is shown in the Fig. 3.15.

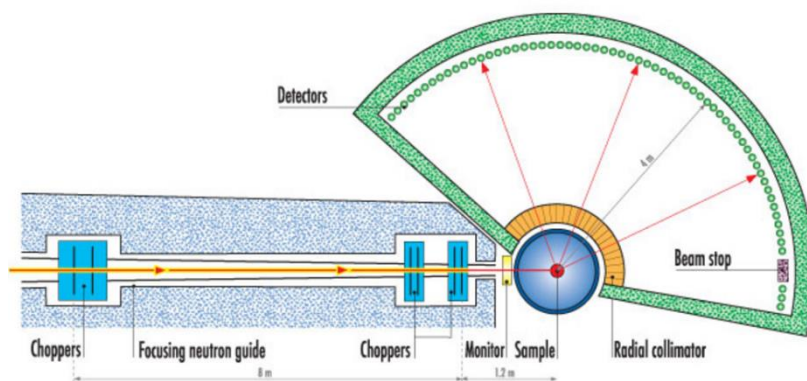


Figure 3.15. Schematic of the IN5 spectrometer at the Institut Laue-Langevin (ILL).

One common issue in inelastic neutron scattering is that processes are too slow to be observed. Usually, time-of-flight (ToF) spectrometers are limited to an energy resolution of about 10 μeV , which means they can only observe processes up to around 200 ps. This is often not enough for systems with slow, large-scale motions, like polymers, biological materials, or glass-forming substances. For these cases, instruments with much higher energy resolution are needed, such as backscattering (BS) or neutron spin echo (NSE) spectrometers.

The combination of the last two instruments mentioned (IN16B and IN5 spectrometers) gives us the opportunity to access a wide QENS dynamic range (approximately 2 ps < t < 3 ns). With them,

the Q-range covered was from 0.19 to 1.90 Å⁻¹ and the temperatures investigated were 285, 320 and 360 K. We applied this approach to the protonated samples, in order to follow the H-dynamics, and to the labeled blends, where we aimed to selectively address the H-self dynamics of the protonated component. The sample thicknesses used in the experiments were calculated in order to reach around 90% of transmission, and flat aluminum cells were used as sample holders. The incident wavelength λ was 6.271 Å for IN16B spectrometer, while $\lambda = 5$ Å was used for IN5 instrument. The determination of the resolution function $R(Q, \omega)$ was performed by measurements at 2 K. In order to analyze the quasielastic spectra, the data were Fourier-transformed to the time domain and then a deconvolution was done from the instrumental resolution to obtain the intermediate scattering function in the time domain $S(Q, t)$. Due to limited beam time, a low-temperature measurement could not be performed in all samples investigated. Spectra obtained on a Vanadium sample were used to perform the deconvolution in the case of IN16B results. Mismatches when putting them together with the time-of-flight data were corrected applying scaling factors to the backscattering data.

3.9. Wide Angle X-ray Scattering (WAXS)

Among scattering techniques, WAXS (Wide Angle X-ray Scattering) is a powerful and broadly used technique for probing the atomic and molecular structure of materials by means of X-rays, primarily focused on high-angle scattering, typically in the range of $2\theta > 10^\circ$. In this range, diffraction from crystallites or ordered domains occurs. The interaction between X-rays and matter is complex; when an X-ray beam passes through a material, part of it is scattered, another portion is transformed into other types of energy, and the remaining is absorbed by the material. If the scattered radiation has the same wavelength as the incident X-rays, it is called elastic radiation. Coherent elastic scattering is also known as Bragg scattering (diffraction). If the wavelength of the scattered radiation is slightly higher than that of the incident radiation, it is referred to as inelastic radiation, or Compton scattering (incoherent).

In WAXS, X-rays are directed onto a sample, and the scattered X-rays are detected and analyzed to obtain a diffraction pattern. The position and intensity of the diffraction peaks, which arise from Bragg scattering, provide detailed information on the spacing between planes of atoms or molecules (d-spacing) and the symmetry of the crystal lattice or molecular arrangement. The Bragg law forms the basis of X-ray diffraction (see Fig. 3.16) and enables the measurement of interplanar distances by analyzing the diffraction angles, provided the wavelength of the X-rays is known.^[7]

This technique is particularly valuable for analyzing crystalline or partially crystalline materials, including polymers.^[35] It provides insights into the size, shape, and distribution of crystalline domains, as well as structural features such as short-range order in amorphous regions, allows following phase transitions, and is sensitive to defects. WAXS complements SAXS (Small-Angle X-ray Scattering), which focuses on the structural properties at the nanoscale.

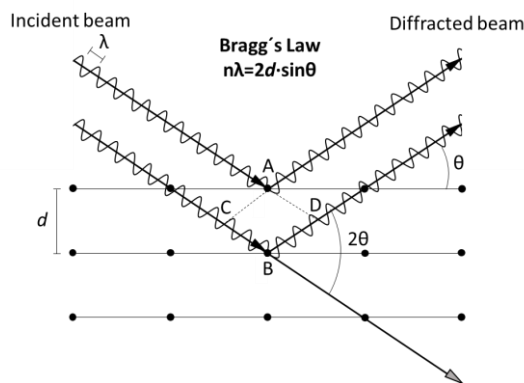


Figure 3.16. X-ray diffraction in successive crystal planes representing Bragg's Law. d is the distance between the planes and θ is the angle between the incident ray and the scattering planes.

The short-range order of the systems was studied by WAXS on a 8 Advance diffractometer (Bruker) working in parallel beam geometry with Cu K_{α} transition photons of wavelength $\lambda = 1.54 \text{ \AA}$, as shown in Fig. 3.17. The experiments were performed at r.t. in reflection mode (θ – 2θ configuration), varying the scattering angle 2θ from 10° to 30° with steps of 0.05° . The scattered intensities are shown as a function of momentum transfer Q , $Q = 4\pi\lambda^{-1} \sin \theta$.

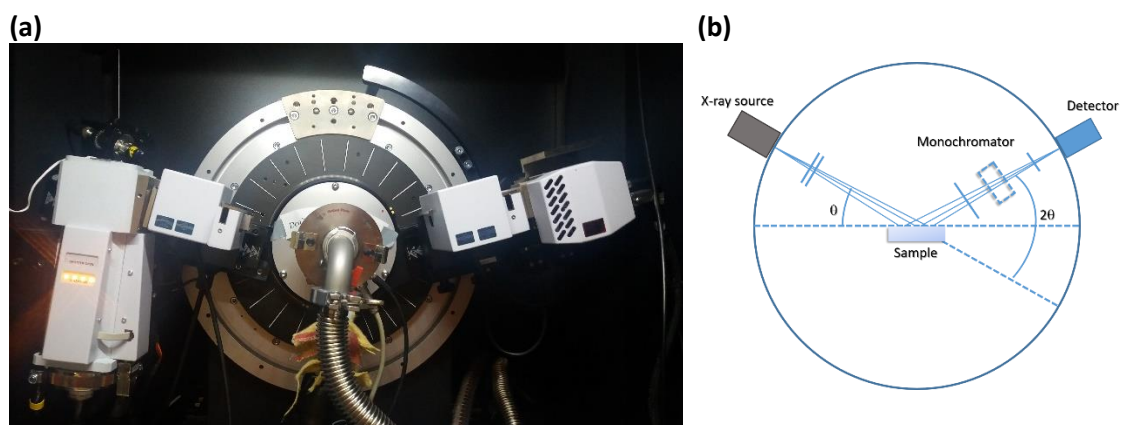


Figure 3.17. Picture of the WAXS instrument employed in this work (a) and schematic representation of its operating principle (b).

3.10. Conclusions

Throughout this chapter, the experimental techniques employed for the characterization of the samples presented in this thesis have been described. The methods comprise chemical, thermal, mechanical, and structural analyses, each providing complementary insights into the properties of the studied polymeric materials.

Chemical characterization was carried out using SEC, NMR spectroscopy, FTIR spectroscopy, and EA. These techniques enabled the determination of molecular weight distributions, verification of chemical composition and SCNPs formation, and confirmation of the successful incorporation of functional units within the copolymer structures, among others.

Thermal and mechanical characterization techniques, DSC and rheology, respectively, were used to evaluate thermal transitions, eventual crystallinity, and viscoelastic behavior, thus

establishing correlations between the molecular structure and macroscopic mechanical properties. Structural analysis at different length scales was performed using Wide Angle X-ray Scattering (WAXS).

Additionally, the structure and dynamics were studied by scattering techniques at microscopic level. For this, WAXS and several NS instruments located at the Institut Laue-Langevin (ILL) were employed. Neutrons are particularly valuable for the investigation of polymeric materials due to their unique interaction with matter and their ability to probe both structure and dynamics over a wide range of time and length scales.

The Diffuse Scattering Spectrometer was used for structural analysis, allowing the separation of coherent and incoherent contributions. This allows establishing the nature of the scattered signal in the particular case of the nanocomposites. The SANS instrument provided structural information at the nanoscale. The structural investigation was completed with WAXS information. Moving to the dynamics, the NSE technique enabled the determination of the intermediate scattering function. QENS experiments were performed using both the ToF and BS instruments, which together covered different time and energy resolution ranges to probe molecular dynamics in the studied systems.

Together, these complementary techniques form a comprehensive experimental framework that enables the correlation of chemical structure, morphology, and dynamic behavior in the studied polymeric systems.

3.11. References

- [1] H. G. Barth, B. E. Boyes and C. Jackson, *Anal. Chem.*, **1998**, *70*, 251.
- [2] S. Mori and H. G. Barth, *Size Exclusion Chromatography*, Springer, Berlin, **1999**.
- [3] B. Trathnigg, *Encycl. Anal. Chem.*, Wiley, Chichester, **2000**.
- [4] M. Gonzalez-Burgos, A. Latorre-Sanchez and J. A. Pomposo, *Chem. Soc. Rev.*, **2015**, *44*, 6122.
- [5] J. A. Pomposo (Ed.), *Single-Chain Polymer Nanoparticles*, Wiley-VCH, Weinheim, **2017**.
- [6] H. Günther, *NMR Spectroscopy*, Wiley, Chichester, **2013**.
- [7] M. Faraldos and C. Goberna (Eds.), *Técnicas de Análisis y Caracterización de Materiales*, CSIC, Madrid, **2003**.
- [8] P. R. Griffiths and J. A. de Haseth, *Fourier Transform Infrared Spectrometry*, Wiley, Hoboken, **2007**.
- [9] F. Pregl, *Quantitative Micro-Analysis of Organic Substances*, Nobel Lectures, World Scientific, Singapore, **1923**.
- [10] G. Höhne, W. F. Hemminger and H. J. Flammersheim, *Differential Scanning Calorimetry*, Springer, Berlin, **2013**.
- [11] A. A. Bershtein and V. M. Egorov, *Differential Scanning Calorimetry of Polymers*, Prentice Hall, London, **1994**.
- [12] A. Arbe, J. Rubio-Cervilla, A. Alegría, A. J. Moreno, J. A. Pomposo, B. Robles-Hernández, P. Malo de Molina, P. Fouquet, F. Juranyi and J. Colmenero, *Macromolecules*, **2019**, *52*, 6935.
- [13] J. D. Ferry, *Viscoelastic Properties of Polymers*, Wiley, New York, **1980**.
- [14] T. Brückel, S. Förster, M. Kruteva, M. Zobel and R. Zorn (Eds.), *Neutron Scattering*, Springer, Berlin, **2015**.
- [15] T. Springer, *Springer Tracts Mod. Phys.*, **1972**, *64*, 1.
- [16] S. W. Lovesey, *Theory of Neutron Scattering from Condensed Matter*, Clarendon Press, Oxford, **1984**.
- [17] M. Bée, *Quasielastic Neutron Scattering*, Adam Hilger, Bristol, **1988**.
- [18] G. L. Squires, *Introduction to the Theory of Thermal Neutron Scattering*, Dover, New York, **1996**.
- [19] D. Bhowmik, J. A. Pomposo, F. Juranyi, V. García-Sakai, M. Zamponi, Y. Su, A. Arbe and J. Colmenero, *Macromolecules*, **2014**, *47*, 304.
- [20] O. Schärpf, *Physica B*, **1992**, *182*, 376.
- [21] A. Arbe, M. Appel, J. Colmenero, B. Frick, J. Maiz, L. Mangin-Thro, A. Mendia, J. Ollivier, J. A. Pomposo and N. Shafqat, *ILL Data*, **2019**, DOI: 10.5291/ILL-DATA.9-11-1901.
- [22] M. Braden, *Acta Crystallogr., Sect. A*, **2012**, *68*, 665.
- [23] O. Schärpf and H. Capellmann, *Phys. Status Solidi A*, **1993**, *135*, 359.

- [24] J. R. Stewart, P. P. Deen, K. H. Andersen, H. Schober, J. F. Barthélémy, J. M. Hillier, A. P. Murani, T. Hayes and B. Lindenau, *J. Appl. Crystallogr.*, **2009**, 42, 69.
- [25] I. Grillo, *IEEE Int. Conf. Syst. Man Cybern.*, **2008**.
- [26] O. D. Velev, E. W. Kaler and A. M. Lenhoff, *ACS Omega*, **2022**, 7, 42163.
- [27] P. Malo de Molina, D. Kafouris, C. S. Patrickios, L. Noirez and M. Gradzielski, *Macromolecules*, **2023**, 56, 8323.
- [28] C. R. López-Barrón, in *Polymer Reaction Engineering of Dispersed Systems*, W. Pauer (Ed.), *Advances in Polymer Science*, Springer Nature (Springer Cham), **2018**, 281, 123.
- [29] S. Bhattarai, H. Adawi, J.-G. Lussier, A. Gebretsadik, M. Dzero, K. L. Krycka and A. Schroeder, *Phys. Rev. B*, **2023**, 107, 054409.
- [30] S. D. Oberdick, J. A. Borchers and K. L. Krycka, *Appl. Phys. Lett.*, **2022**, 120, 052401.
- [31] S. Krueger, *Physica B*, **1998**, 241–243, 1131.
- [32] A. Arbe, J. Colmenero, P. Fouquet, I. Hoffmann, J. Maiz, A. Martel, A. Mendia, J. A. Pomposo, L. Porcar and N. Shafqat, *ILL Data*, **2020**, DOI: 10.5291/ILL-DATA.6-04-277.
- [33] F. Mezei, *Lect. Notes Phys.*, **1980**, 128.
- [34] J. S. Higgins and H. C. Benoit, *Polymers and Neutron Scattering*, Oxford University Press, Oxford, **1997**.
- [35] M. Gomez-Menendez, I. Asenjo-Sanz, E. Verde-Sesto, A. Iturrospe, J. A. Pomposo, A. Arbe and J. Maiz, *Polymer*, **2025**, 335, 128759.

CHAPTER 4

Microscopic and Macroscopic Properties of Materials Based on Single-Chain Nanoparticles
Synthesized by Click Chemistry

4.1. Introduction

Soft matter is characterized by complex structures and dynamics. In particular, polymer melts are intricate systems where the relevant dynamic processes depend on the length scale of observation.^[1,2] Understanding both macroscopic and microscopic polymer dynamics is essential for the design, development, and fabrication of new bulk materials. Generally, physical properties, such as mechanical, thermal, and transport characteristics, are strongly influenced by the various dynamical processes occurring within polymers.^[3]

In this context, single-chain nanoparticles (SCNPs) introduce an additional layer of complexity, as they are formed through the intra-molecular collapse or folding of individual polymer chains, known as precursors. A broad range of synthetic strategies have been developed to promote this intra-chain cross-linking, including covalent cross-linking reactions, dynamic covalent chemistry, and non-covalent interactions, with the former being among the most commonly used approaches.^[4-6] Typically, the formation of SCNPs involves three main steps: (i) synthesis of a precursor polymer, (ii) functionalization of the precursor, and (iii) folding/collapse of the functionalized precursor via intra-chain interactions.^[7]

The properties of SCNPs are largely governed by both the nature and the density of intra-molecular cross-links. Topological and architectural variations can significantly impact molecular behavior. As a result, a wide range of chemical and physical properties (such as density,^[8] hydrodynamic size,^[8-12] viscosity,^[13-17] glass transition temperature,^[18] mechanical strength,^[19] and dynamic behavior^[20]) are affected by the chain topology and architecture of SCNPs.^[21,22]

In this regard, scattering techniques constitute powerful tools to unravel the structural and dynamic properties of soft matter systems, including SCNPs.^[23] The momentum and energy transfer dependence of the measured magnitudes can provide spatial and time resolution, on scales that can be tuned by choosing the proper probe and/or experimental conditions. In particular, neutron scattering (NS) techniques provide two additional advantages for soft matter investigations: i) the possibility to match –simultaneously– the right length and time scales, and ii) the possibility to label selectively the components of a complex material via deuterium labeling.^[24-30]

In a previous work by Jon Rubio and collaborators,^[31,32] the structure and dynamics of SCNPs based on poly(tetrahydrofuran) (PTHF), synthesized through in azide photodecomposition and exposed to UV irradiation (hereafter referred to as “UV-SCNPs”), were thoroughly investigated. Using a combination of NS, dielectric spectroscopy, and rheological measurements, the impact of intra-molecular cross-links on bulk polymer properties was explored. While inter-molecular characteristics, as probed by diffraction and neutron spin echo (NSE) at the position of the first structure factor peak, remained largely unchanged, significant alterations were observed in the rheological response. Moreover, a new relaxation mechanism, associated with internal domains generated by intra-molecular bonding, was identified. This mechanism—detected via dielectric spectroscopy and NSE at intermediate length scales—adds to the complexity of the dynamic behavior in these systems.

In this thesis, the synthesis of poly(tetrahydrofuran-*ran*-epichlorohydrin) (P(THF-*ran*-ECH)) polymers and SCNPs via click chemistry is presented, along with their sample preparation protocol. The SCNPs obtained here by click synthesis will be denoted simply as “SCNPs” (labeled as hSCNPs and dSCNPs for protonated and deuterated samples, respectively) in contraposition to the “UV-SCNPs” previously introduced UV-systems explored in Ref.^[31]. The chapter then focuses on the following main objectives:

1. To present a comprehensive multi-technique experimental investigation that combines chemical, structural, and dynamical characterization methods. The study employs chromatographic, spectroscopic, mechanical, and calorimetric analyses, together with an extensive suite of NS techniques, including quasi-elastic neutron scattering (QENS).
2. To investigate how the nature of intra-molecular cross-links influences the structural relaxation, segmental dynamics, and mechanical response of bulk SCNP systems. This includes a comparison between SCNPs synthesized via click chemistry and those obtained through azide photodecomposition (UV-SCNPs).
3. To selectively characterize the microscopic component dynamics in complex all-polymer nanocomposites by investigating the glass transition and the associated segmental dynamics in a system composed of SCNPs embedded in a linear-chain precursor matrix, through the combined use of calorimetry and QENS techniques.

The experimental techniques employed to achieve these goals are described in detail in the Chapter 3 of the thesis.

4.2. Synthesis and Chemical Characterization of Poly(Tetrahydrofuran-*ran*-Epichlorohydrin) Samples

4.2.1. Materials

The monomers employed, tetrahydrofuran (THF, ≥99.9% (GC), Scharlab) and (±)-epichlorohydrin (ECH, ≥99% (GC), Sigma-Aldrich), were dried using calcium hydride (CaH₂, 95%, Aldrich) as drying agent, degassed by three freeze-pump-thaw cycles, and distilled under reduced pressure at 323 K. For fully deuterated samples, tetrahydrofuran-d₈ (THF-d₈, TDF, 99.5 atom% D, Eurisotop) and (±)-epichlorohydrin-d₅ (ECH-d₅, ECD, ≥98 atom% D, Aldrich) were purified by the same degassing method.

The initiator tris(pentafluorophenyl)borane (B(C₆F₅)₃, 95%, Sigma-Aldrich) was purified by sublimation under reduced pressure at 333 K using a cold-finger condenser. Dichloromethane (CH₂Cl₂, anhydrous, Sigma-Aldrich) was degassed by argon bubbling for 30 minutes prior to use. (+)-Sodium L-ascorbate (BioXtra, ≥99% (NT), Sigma-Aldrich), N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA, 99%, Sigma-Aldrich), propargyl ether (98%, Sigma-Aldrich), N,N-dimethylformamide (DMF, anhydrous, ≥99.8%, Scharlab), sodium azide (NaN₃, ≥99.5%, Sigma-Aldrich), and methanol (MeOH, ≥99.9%, Scharlab) were used as received. Copper(I) bromide (CuBr, 98%, extra pure, Acros Organics) was purified following the method of Keller and Wycoff^[33] by washing five times with 20 mL of glacial acetic acid (extrapure, Fisher Chemical), followed by three washes with 30 mL of absolute ethanol (≥99.8%, Aldrich) and six washes with 15 mL of anhydrous diethyl ether (≥99.0%, anhydrous, Sigma-Aldrich). The resulting solid was dried at 80 °C under vacuum for 2 hours and stored under nitrogen atmosphere.

For characterization purposes, deuterated chloroform (CDCl₃, 99.96 atom% D, containing 0.03% (v/v) tetramethylsilane, TMS, Aldrich) or fully deuterated chloroform (CDCl₃, 100% D, Eurisotop) was used, with 1,2,4,5-tetrabromobenzene (TBB, 97%, Aldrich) as internal standard when required. Tetrahydrofuran for chromatography (THF, HPLC grade, Scharlab) was used as received.

4.2.2. Precursors

Polymer synthesis of both protonated (P(THF-*ran*-ECH)) and fully deuterated P(TDF-*ran*-ECD) polymer precursors followed the same experimental procedure described in previous studies.^[31,32]

The synthesis reactions were carried out in bulk conditions under an argon atmosphere. Borane catalyst (B(C₆F₅)₃) and monomers TDH/ECD or THF/ECH were mixed in a 100 mL Schlenk flask and stirred at room temperature for 72 and 48 hours respectively. The resulting crude products were precipitated in cold MeOH and dried at 333 K under reduced pressure in a vacuum oven for 24 h, getting sticky transparent copolymers. Table 4.1 summarizes the experimental conditions and characteristics of the synthesized polymers.

Table 4.1. Summary of synthesis conditions of P(TDF-*ran*-ECD) and P(THF-*ran*-ECH) copolymers

	B(C ₆ F ₅) ₃		TDF or THF		TCD or ECH		Reaction time (h)	Yield (%)
	mg	mmol	mL	mmol	mL	mmol		
P(TDF- <i>ran</i> -ECD)	93	0.18	23.2	287.0	5.6	71.7	72	68
P(THF- <i>ran</i> -ECH)	200	0.4	25	308.0	6.3	81.0	48	71

In order to obtain the final polymer precursors, the azidation of the previously described copolymers was performed. This chemical modification enables the subsequent formation of single-chain nanoparticles via click chemistry. The resulting precursors are referred to as **dPrec** and **hPrec**, depending on whether they are deuterated or non-deuterated, respectively.

The azidation process for both copolymers was carried out by dissolving 4.0 g of polymer in 160 mL of anhydrous DMF in a 500 mL round-bottom flask. Sodium azide (NaN_3 , 1.7 g) was then added, and the resulting suspension was stirred at 333 K for 24 h. The crude product was filtered to remove NaN_3 excess and concentrated under reduced pressure to approximately 15 mL. The crude product was subsequently precipitated into 400 mL of a cold 1:4 (v/v) $\text{H}_2\text{O}/\text{MeOH}$ mixture and dried under vacuum at 323 K for 24 h to yield the azide-functionalized polymer precursors. The isolated yields were 75% for dPrec and 87% for hPrec.

For a clearer overview of the process, Fig. 4.1 illustrates the schematic representation of the chemical synthesis.

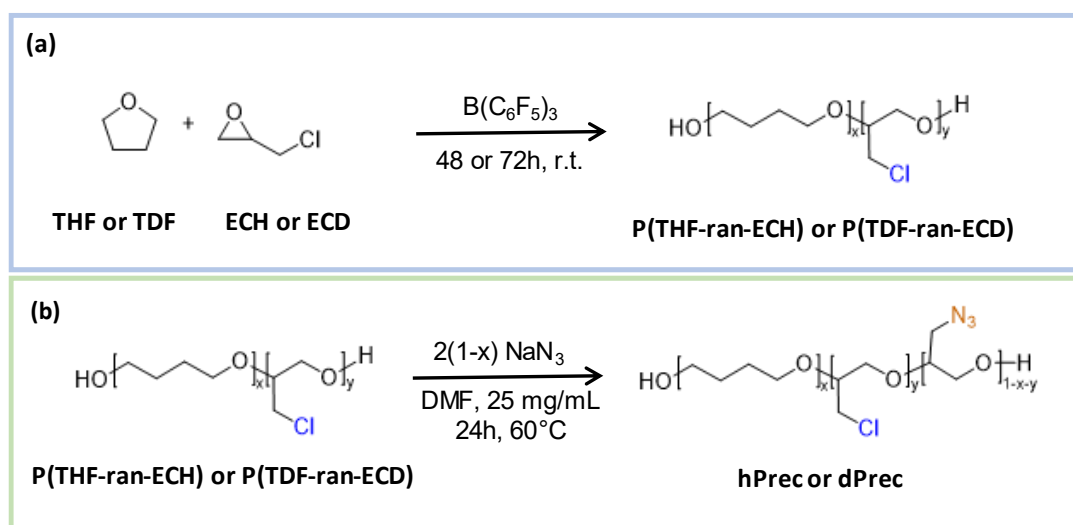


Figure 4.1. Synthesis process of hPrec or dPrec: (a) copolymerization of $\text{P}(\text{THF-ran-ECH})$ or $\text{P}(\text{TDF-ran-ECD})$ and (b) subsequent azidation. Deuterium atoms are omitted for the sake of clarity.

4.2.3. Nanoparticles

The synthesis of single-chain nanoparticles (SCNPs) was carried out via copper (I)-catalyzed azide alkyne cycloaddition (CuAAC) click reaction. A schematic view of the process is shown in Fig. 4.2.

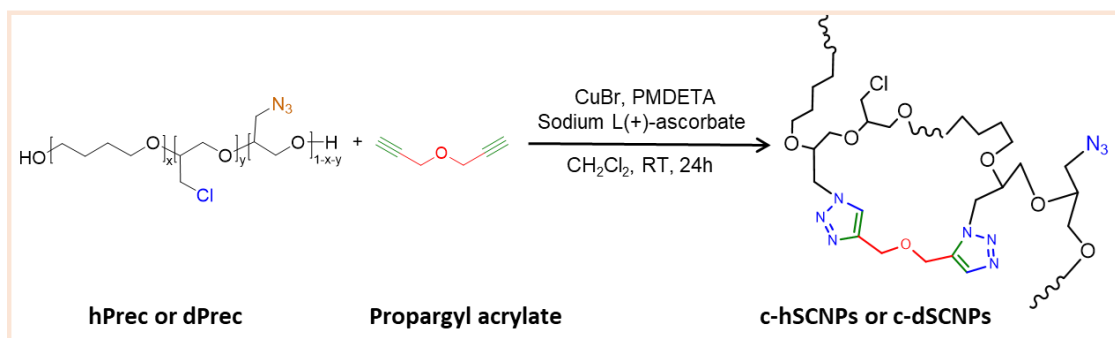


Figure 4.2. Schematic representation of the synthesis of SCNPs via click chemistry reaction using propargyl ether as homobifunctional cross-linker. Deuterium atoms are omitted for the sake of clarity.

For this, CuBr (0.86 g, 6 mmol) and (+)-sodium L-ascorbate (1.190 g, 6 mmol) were added into a round-bottom flask of 250 mL and flushed with argon (Ar) for 15 min. Then, 130 mL of dried and degassed CH₂Cl₂ was added under Ar into the flask following by the addition of PMDETA (1.26 mL, 6 mmol). Finally, a solution of 150 mg of either dPrec or hPrec in 30 mL of dried and degassed CH₂Cl₂ was added into the mixture to get a solution of 1 mg/mL. A solution of propargyl ether (7 μ L, 0.072 mmol) in 7 mL of dried and degassed CH₂Cl₂ was injected to the mixture with a syringe pump at 7 mL/h. After the complete addition of the cross-linker solution, the reaction was stirred at r.t. for 24 h. The copper catalyst was removed by extraction with a saturated solution of NH₄Cl (4 $\times$ 100 mL). The organic phase was dried with anhydrous MgSO₄, filtered and evaporated at reduced pressure to obtain **dSCNPs** and **hSCNPs** (derived from dPrec and hPrec, respectively) as greenish viscous liquids or around 80% yield each. The process described above is illustrated in Fig. 4.3.

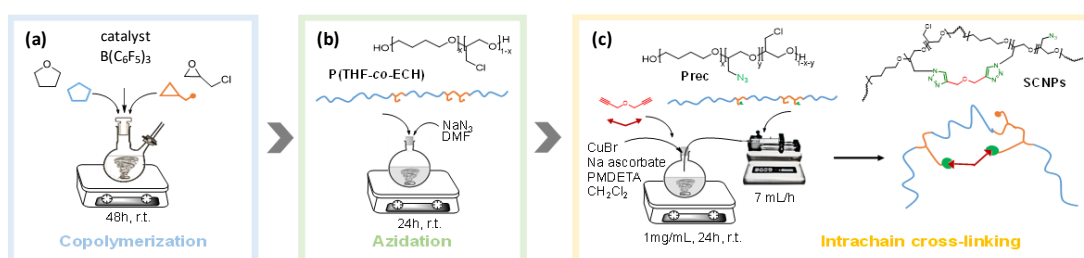


Figure 4.3. Schematic illustration of the synthetic approach followed for the fabrication of the materials presented in this work, involving three key stages: copolymerization (a), azidation (b), and the final intrachain cross-linking and formation of the SCNPs (c).

4.2.4. Sample Preparation

For NS experiments, different types of samples were prepared depending on the experiment conducted.

First, either a precursor or a SCNP solution was stirred in 2 mL of CH₂Cl₂ until completely dissolved. The solution was then drop-casted onto aluminum flat holders used for NS experiments. Afterward, the solvent was slowly evaporated under a fume hood, and the samples were thoroughly dried under vacuum in an oven at 343 K for 24 h.

In the case of nanocomposite samples, mixtures with always a concentration of 75 wt% precursors and 25 wt% SCNPs were investigated. To obtain different contrast for the NS experiments, two different compositions, containing either deuterated or protonated precursor and the inversely labeled SCNPs were prepared. The blends were obtained by mixing the appropriate precursor with the SCNP sample giving rise to the systems referred to as **75hPrec** for the 75hPrec/25dSCNPs mixture and **25hSCNP** for the 75dPrec/25hSCNPs mixture, where h represents protonated samples and d symbolizes deuterated ones. In this case, the precursor and the SCNPs mixtures were stirred until the total dissolution in CH₂Cl₂ during 24 h at room temperature. Then, each mixture was drop-casted onto the aluminum flat holders used for NS experiments. Subsequently, the solvent was slowly evaporated in a fume hood and finally the samples were well-dried in an oven at 343 K for 24 h under vacuum conditions. The appearance of the samples was as that of a viscous fluid. Figure 4.4 presents a schematic representation of the process.

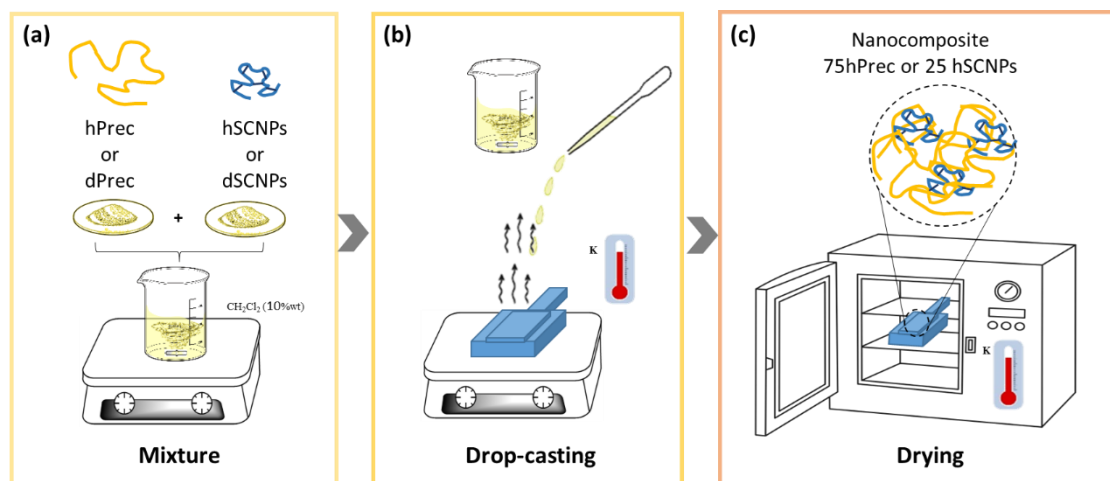


Figure 4.4. Schematic representation of the sample preparation procedure for the nanocomposites: (a) preparation of a 10 wt% CH₂Cl₂ solution containing the appropriate amounts of hPrec or dPrec and dSCNPs or hSCNPs; (b) drop-casting of the resulting solution onto aluminum flat holders followed by slow solvent evaporation; and (c) final drying of the samples under vacuum at 343 K (see text for details).

4.2.5. Chemical Characterization

The synthesized polymers were first characterized by Size Exclusion Chromatography (SEC) and Nuclear magnetic Resonance (NMR) spectroscopy. SEC provided the molecular weight and dispersity ($\bar{D}$) of the copolymers. The deuterated copolymer, P(TDF-*ran*-ECD), exhibited a weight-average molecular weight (M_w) of 36.5 kDa with a dispersity of 1.20, while its protonated counterpart, P(THF-*ran*-ECH), showed a M_w of 22 kDa and a dispersity of 1.24. These results confirm the successful synthesis of well-defined copolymers with relatively narrow molecular weight distributions.

To complement these findings, NMR spectroscopy was performed to determine the copolymer composition. The ¹H NMR spectra revealed that the content of ECH or ECD in the respective copolymers was approximately 27 mol%, confirming the incorporation of both monomers into the polymer backbone. Furthermore, kinetic studies using ¹H NMR were carried out to investigate the copolymerization behavior of the monomer pairs. As shown in Fig. 4.5, the spectra indicated that THF and ECH began polymerizing simultaneously and followed similar reaction kinetics throughout the process. However, THF exhibited a slightly higher conversion rate compared to ECH by the end of the polymerization. This difference suggests that THF was incorporated at a marginally faster rate. Nonetheless, the close similarity in their conversion profiles and incorporation rates supports the conclusion that the copolymerization proceeds with a largely random monomer sequence distribution.

After the azidation process, elemental analysis was performed to determine the nitrogen content in the copolymers, dPrec (deuterated) and hPrec (protonated), confirming the success of the process. The degree of azidation was found to be 21% in dPrec and 21% in hPrec, ensuring the correct substitution of some -Cl group with -N₃, which is essential for the formation of SCNPs via click chemistry. Table 4.2 provides a summary of the reported results.

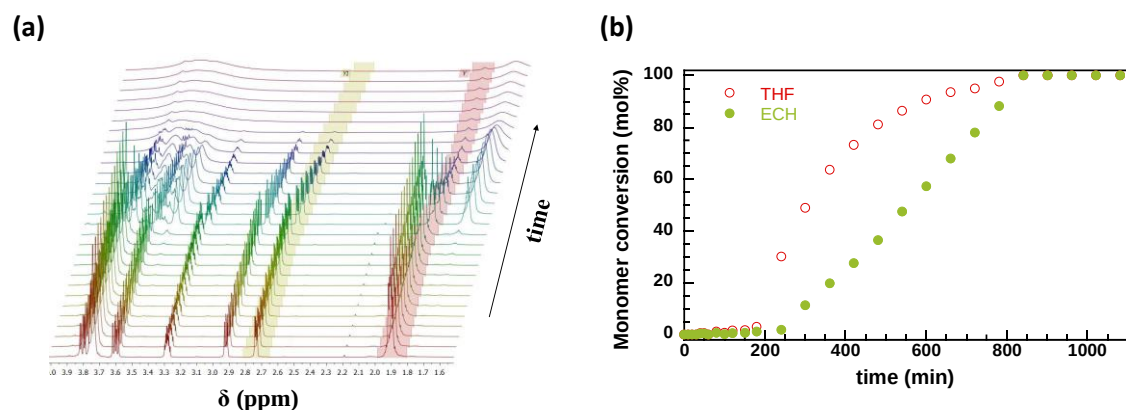


Figure 4.5. (a) Real-time ^1H NMR spectra and (b) monomer conversion as a function of time obtained during bulk copolymerization of THF and ECH with a feed composition of 50/50 mol%.

Size exclusion chromatography experiments also confirm that the SCNPs are formed. As shown in Fig. 4.6, the SCNPs obtained have longer retention time at the SEC peak maximum compared to their precursor. This therefore indicates a reduction in the radius of gyration while maintaining a constant molar mass distribution. This behavior is consistent with the intra-molecular collapse expected during the formation of SCNPs, where the overall chain length remains unchanged but the conformation becomes more compact.

Table 4.2. Characterization after the azidation process in hPrec and dPrec copolymers.

	Starting polymer	M_w (kDa) ^a	$\bar{D}$ ^a	ECD/ECH (mol%) ^b	Azidation degree (%) ^c
dPrec	P(TDF- <i>ran</i> -ECD)	36.5	1.20	27	21
hPrec	P(THF- <i>ran</i> -ECH)	22	1.24	27	21

^a Determined by GPC/SEC ^b Determined by ^1H NMR spectroscopy ^c Calculated by elemental analysis (E.A); E.A. calculated: C(%) cal. 58.94, H(%) cal. 9.61, N(%) cal. 3.36 and E.A. found: C(%) exp. 58.30, H(%) exp. 9.21, N(%) exp. 3.62.

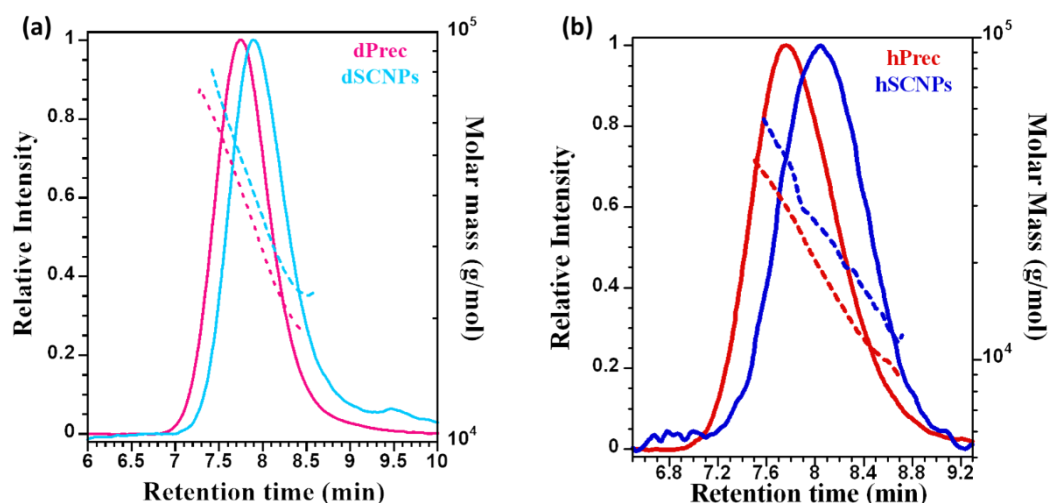


Figure 4.6. SEC chromatograms of precursors and SCNPs showing the shift of the SEC retention peak (solid lines) and molar mass of precursors upon chain compaction via click chemistry to produce SCNPs (dashed lines) for (a) dPrec and dSCNPs and (b) hPrec and hSCNPs.

Complementarily, the Fourier Transformed Infrared (FTIR) spectra (Fig. 4.7) show that the protonated copolymer P(THF-*ran*-ECH) do not exhibit the characteristic azide band around 2100 cm^{-1} . However, this signal is present in their azide-functionalized derivative, hPrec. Upon

comparison with their corresponding hSCNPs, the azide signal disappears once again, indicating that this functional group is responsible for the formation of SCNPs via click chemistry. In the case of the deuterated samples, however, the characterization by this technique is more complex, as the spectral region where the azide group typically absorbs overlaps with deuterium-related bands.^[34] As a result, confirming the presence or consumption of the azide group through FTIR is not feasible in these samples. Nevertheless, as has already been shown and will be further demonstrated, multiple complementary characterization techniques have been employed to provide a broader perspective and to ensure and monitor the chemical process throughout each step.

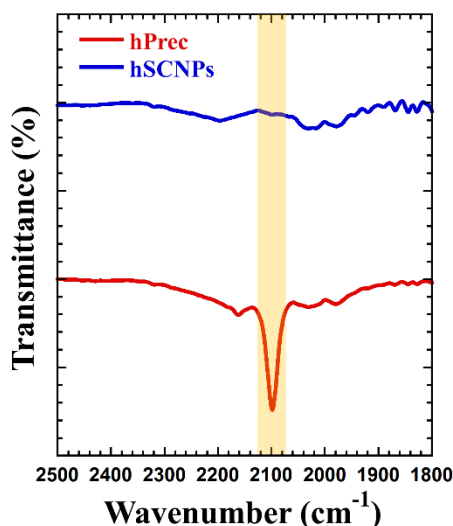


Figure 4.7. FTIR spectra for protonated samples P(THF-*ran*-ECH) and its derivatives hPrec and hSCNPs. The yellow-marked area highlights the region of interest for the study.

4.3. Effect of intra-molecular cross-linking. Homopolymer Characterization.

This section presents the experimental characterization of deuterated and protonated homopolymer precursors and their corresponding SCNPs, with the aim of assessing the impact of cross-linking chemistry on the resulting nanoparticle properties. To this end, calorimetric, mechanical, and scattering measurements are reported. The scattering study encompasses WAXS as well as NS experiments.

4.3.1. Calorimetric Characterization

Differential Scanning Calorimetry (DSC) characterization reveals the glass transition temperatures (T_g) of the precursors and the SCNPs, and the nanocomposite mixtures under study (Fig. 4.8). In the pure melts, intra-molecular cross-links produced by the click chemistry employed here induce a clear broadening of the glass transition, together with a shift of the average T_g toward higher values. This macroscopic effect is evident for both isotopically labeled systems: while the dPrec and hPrec exhibit T_g values of 202 K, these increase to 207 K and 210 K for dSCNPs and hSCNPs, respectively. This increase in T_g suggests, for a given temperature, slower motions at the segmental level in the SCNPs. The broadening implies a wider distribution of characteristic relaxation times, which is also reflected at the microscopic level as it will be shown below: local atomic dynamics exhibit clear signatures of these constraints, as directly observed by quasielastic neutron scattering (QENS) at temperatures well above the respective T_g s of the neat samples. Subsequently, in Section 4.4.1, these results will be compared with those obtained from nanocomposites prepared using pure melts.

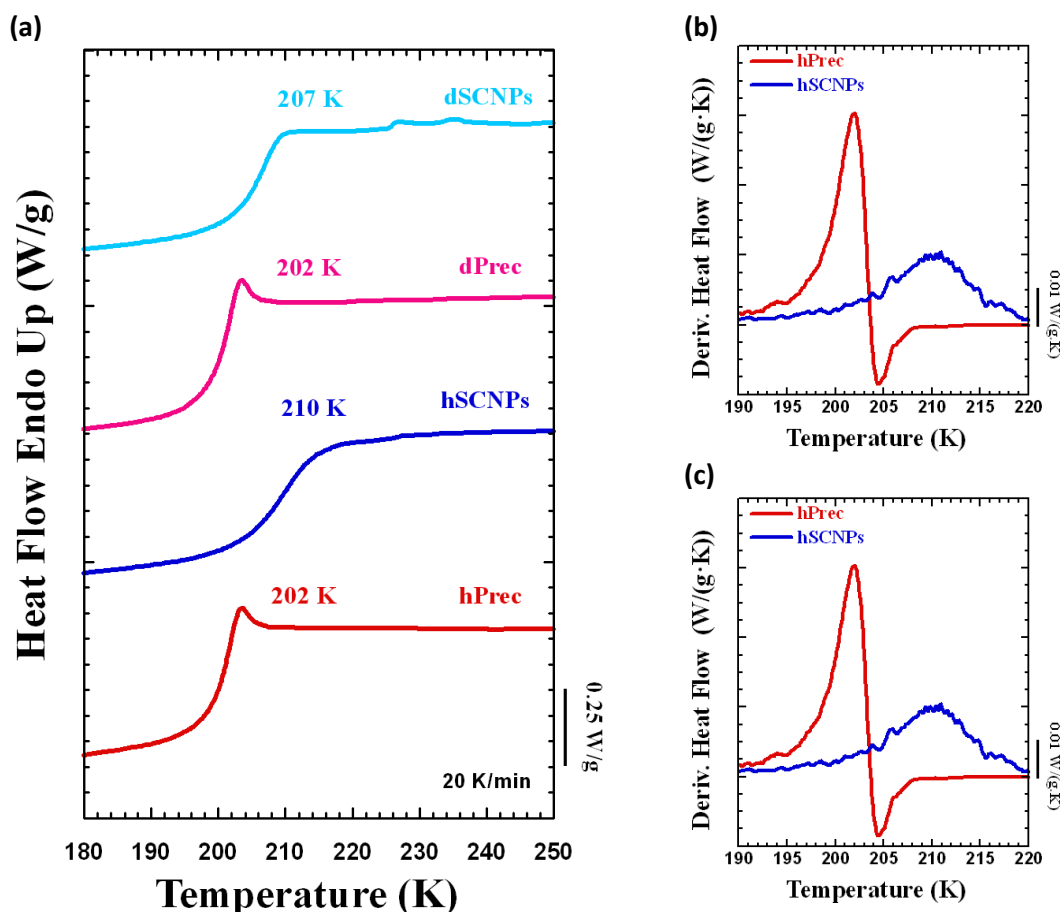


Figure 4.8. (a) DSC heating scans at 20 K/min after the previous cooling process of the reported protonated and deuterated precursors and click SCNP. Temperature evolution of the derivative of the heat capacity of the (b) protonated and (c) deuterated samples.

4.3.2. Mechanical Characterization

The mechanical response of the dSCNPs and hSCNPs, in comparison to their linear precursors, was studied through rheological experiments. The principal effect of changing the temperature was found to be a rescaling of time: temperature variations shift the viscoelastic functions along the modulus and time (or frequency) axes without altering their shapes. This behavior, known as time-temperature superposition (TTS)^[35] allowed the construction of master curves for the real and imaginary parts of the shear modulus of the samples, as shown in Fig. 4.9. These curves were generated by applying the TTS principle to isothermal measurements of $G'(\omega)$ and $G''(\omega)$ with a reference temperature of $T_{\text{ref}} = 293$ K.

The dPrec sample exhibits the characteristic behavior of long linear chains, which is commonly observed in polymer melts with linear and other molecular topologies.^[36,37] At intermediate frequencies, the elastic modulus $G'(\omega)$ is above $G''(\omega)$, reflecting a solid-like behavior. The curves cross at two points: (i) at high frequencies (about 10^7 rad/s), where the onset of entanglement effects on the mechanical response is reflected, and (ii) at low frequencies (3×10^2 rad/s) where chains disentangle. For the dSCNPs sample, the plateau in the shear modulus smears out and no disentanglement point can be identified in the experimental window accessed. This indicates that, at the temperatures and frequencies probed, the system doesn't flow, reminding the behavior of a gel. It is also noted that, in the case of the dSCNPs the obtained absolute values

for the moduli are rather low. This effect could be due to uncertainties in the geometry of the sample.

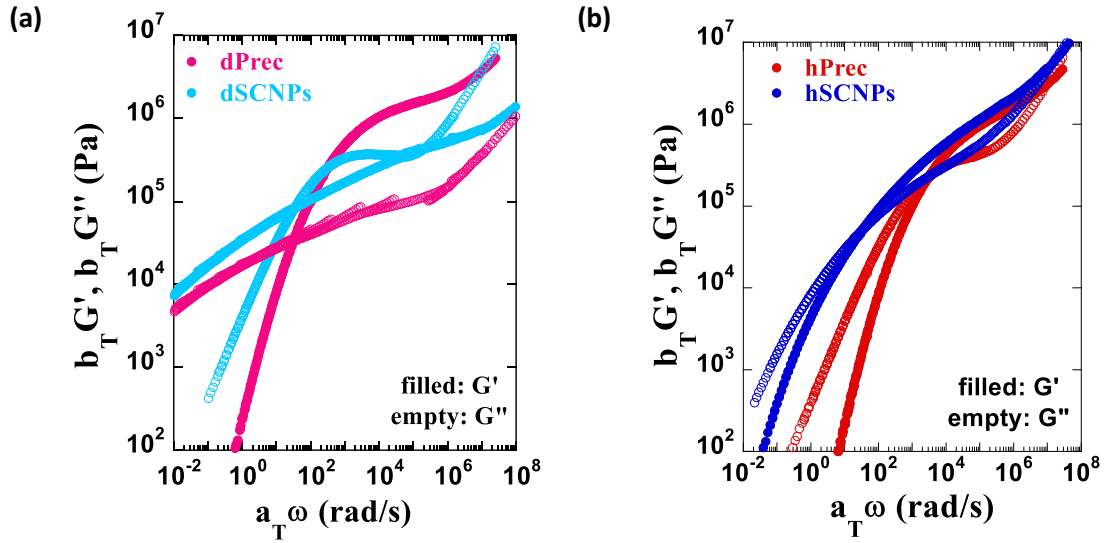


Figure 4.9. Rheological master curves (reference temperature: 293 K) for (a) dPrec and dSCNPs and (b) hPrec and hSCNPs.

The mechanical response of hPrec and its corresponding hSCNPs is shown in Fig. 4.9(b), resulting in smooth curves, which is also consistent with the behavior of linear polymer melts. The curves in hPrec exhibit two distinct crossover points: (i) at high frequencies (about 10^7 rad/s), where the onset of entanglement effects is reflected, and (ii) at low frequencies (3×10^3 rad/s), where disentanglements of chains are detected. For monodisperse polymer melts below the low frequency crossing point, the moduli are expected to scale as $G'(\omega) \sim \omega^2$ and $G''(\omega) \sim \omega$. Here, where the samples presented have dispersity values higher than 1 ($\mathcal{D} = 1.24$), the crossing point is shifted towards higher frequencies. In the case of the hSCNPs sample, the high-frequency crossover occurs at a similar value (around 10^7 rad/s), but the low-frequency crossover is shifted to 3×10^1 rad/s, and the transition to the terminal regime happens at significantly lower frequencies. This shift indicates a slower and broader structural relaxation of the hSCNPs compared to the precursor.

The observed rheological behavior of the hSCNPs, characterized by a broader and slower relaxation compared to the linear precursor, highlights the impact of the internal cross-links on the macroscopic dynamics of the material. This trend, however, contrasts with the behavior reported for UV-SCNP samples^[31, 32], where the apparent disappearance of entanglements was observed and the disentanglement frequency was shifted to higher values relative to the precursor melt. A plausible explanation for this observation would be the presence of residual cross-linkers or other impurities.

4.3.3. Short-Range Order Characterization by WAXS

Wide Angle X-ray Scattering (WAXS) experiments were performed on both dPrec and hPrec as well as their corresponding SCNPs to obtain information on the short-range order of the samples. The results, presented in Figs. 4.10(a) and (b), show that all four samples exhibit a dominant peak in the Q -range between 1.0 and $2.0 \text{ \AA}^{-1}$, centered around $1.4 \text{ \AA}^{-1}$. This peak is attributed to inter-main chain correlations, corresponding to an average distance of $d_{\text{chain}} = 2\pi/Q_{\text{max}} \approx 4.4 \text{ \AA}$.^[38] Notably, no significant differences are observed between the deuterated and

protonated samples, nor is the peak position affected by the introduction of internal cross-links during the SCNP formation via the click chemistry process. These findings suggest that the short-range order remains essentially unchanged across all samples.

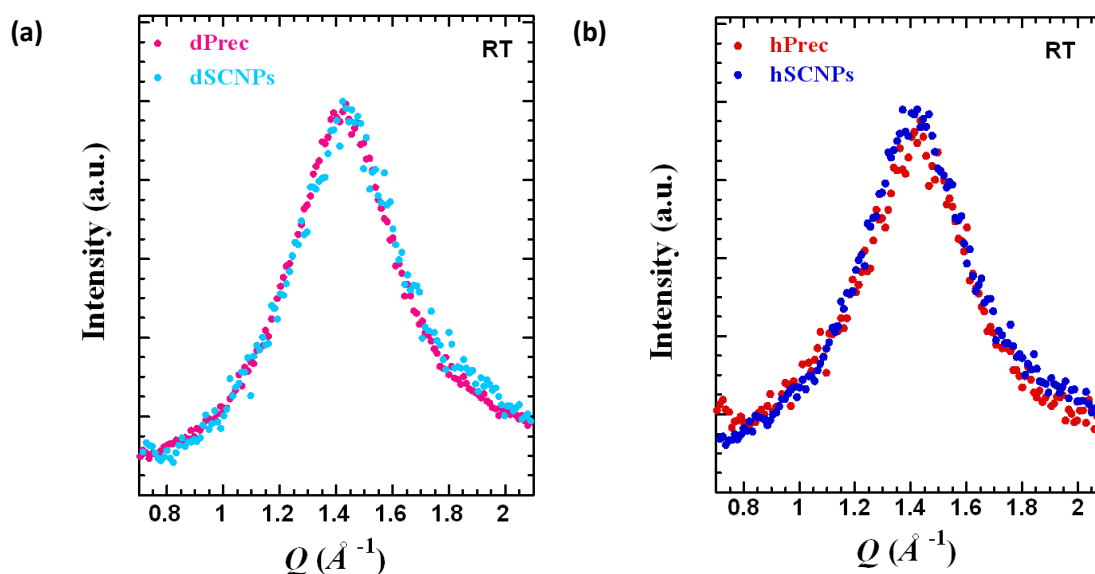


Figure 4.10. WAXS diffraction patterns determined at room temperature for (a) dPrec and dSCNPs and (b) hPrec and hSCNPs. The intensities have been scaled to match at their respective maxima.

After the characterization of the samples in terms of their chemical composition, thermal transitions, mechanical properties, and short-range order structural features, NS techniques were employed to gain deeper insight into their molecular structure and dynamics. In the following, the corresponding results including quasielastic neutron scattering (QENS) data are presented, organized separately for protonated and fully deuterated systems. First, the collective features addressed by coherent scattering on the deuterated samples are considered.

4.3.4. Collective Features: Neutron Scattering on Deuterated Homopolymer Samples

While X-rays are sensitive to the electronic probability density, neutron diffraction on fully deuterated samples addresses the total structure factor $S(Q)$, where all nuclei pair correlations are equally weighted. IN11c in diffraction mode delivers this function (Fig. 4.11). The peak position here is also unaffected by the presence of internal cross-links. The Q -range covered by this instrument extends toward lower Q -values than the WAXS measurements accessing longer spacial scales. In the low- Q range explored, the coherent scattering of the dSCNPs exhibits a marked increase with decreasing Q -value that is not present in the precursor melt. The region of Q -values where this effect is noticed is already located in the regime where we explore the so-called intermediate length scales (ILS)—the range of length scales larger than the inter-molecular distances but not yet in the hydrodynamic regime.

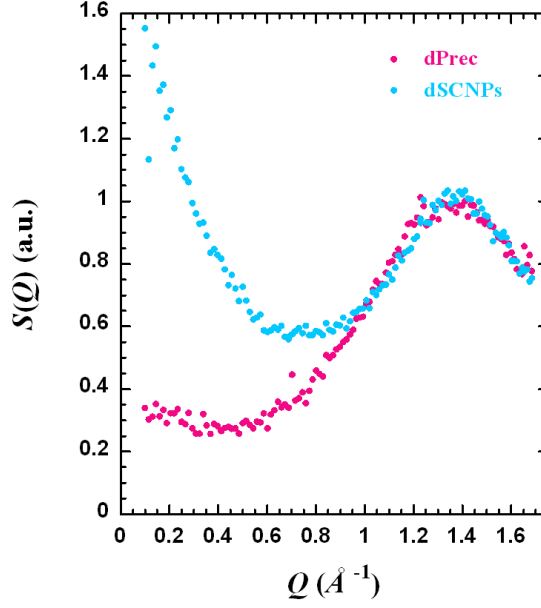


Figure 4.11. Static structure factor obtained by IN11c on dPrec and dSCNPs at 320 K. Data was scaled to the peak maximum.

To gain further insight into the low- Q features observed in the diffraction data, complementary measurements were carried out using small-angle neutron scattering (SANS). Figure 4.12 displays the differential cross sections for both dPrec and dSCNPs. To describe this region of $S(Q)$ in the precursor, a constant function has been considered, as expected for a homogeneous system at large length scales with respect to the inter-molecular distances. This describes well the behavior down to $\approx 0.08 \text{ \AA}^{-1}$, value below which a strong increase of the intensity is observed. Such a low- Q contribution can be accounted for by a law $\propto Q^{-x}$, with $x = 3.5$. This additional scattering is usually found in fully deuterated polymer melts and could be attributed to the presence of microbubbles. The combination of these two contributions well describes the precursor results. As observed by IN11c, in the Q -region around $0.1 \text{ \AA}^{-1}$ the dSCNPs show an excess of the scattered intensity compared to the precursor sample. This difference can be accounted for by an Ornstein–Zernike (OZ) expression:

$$I_{OZ}(Q) = \frac{I_{OZ}(0)}{1 + (\xi Q)^2} \quad (4.1)$$

where $I_{OZ}(0)$ is the amplitude or $Q \rightarrow 0$ value of this function and ξ is the correlation length. To give account for the intensity scattered by the dSCNPs in the whole range accessed by SANS, this contribution was added to the power law and constant background contributions as considered for the dPrec sample. As can be seen in Fig. 4.12, the resulting total function (solid line) describes well the experimental results. There, the three separate contributions are also displayed. The value of x in the power law found was the same as for dPrec, $x = 3.5$. Regarding the OZ contribution, a value of 0.7 cm^{-1} was obtained for the amplitude, and $\xi \approx 15 \text{ \AA}$ for the characteristic length. The SANS results were insensitive to temperature, in the investigated range.

To discuss the origin of these effects on the different properties, it is very helpful to consider in a comparative way some previous works on bulk SCNPs obtained via intra-molecular azide photodecomposition process (UV-SCNPs) from similar precursors^[31,32]. In those studies, an insensitivity of the inter-molecular distances and an increase in scattered intensity at ILS were also observed. This excess of scattering, also described using an OZ function, yielded an

amplitude of 0.5 cm^{-1} and a correlation length of 9 Å for this contribution. Thus, the excess of density fluctuations induced by the cross-links is more pronounced – according to the larger amplitude – and the associated characteristic length is larger when employing the click chemistry method. Such an excess reveals structural heterogeneities in the melt. The origin of these heterogeneities can be attributed to the internal compartmentation of the SCNPs into 'domains'^[42]. According to molecular dynamics simulations^[39], the presence of chain portions forming loop-like structures within the chains ('domains') is expected, with broadly distributed sizes. The results presented here would suggest the presence of larger domains on average in the case of the click synthesis route, involving the generation of relatively large oxybis(methylene)-bistriazole cross-linking moieties. It is noted that the size of these moieties is already about 1 nm ^[40], compared to the atomic bond size (on the order of $1\text{--}2 \text{ Å}$) of the links in the UV-SCNPs.

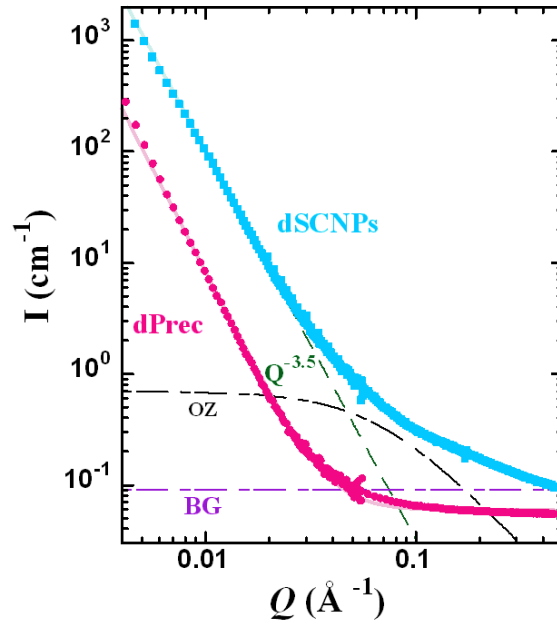


Figure 4.12. Differential cross section of dPrec (pink circles) and dSCNPs (bright blue squares). The light pink solid line is a description of the data for dPrec at intermediate length scales, consisting of a $\propto Q^{-x}$ law with $x = 3.5$ and a constant background. The pale blue solid line describes the dSCNPs data, including an Ornstein-Zernike contribution (black dashed-dotted line), a $\propto Q^{-x}$ law with $x = 3.5$ (green dashed line) and a constant background (purple dashed-dotted line).

Neutron Spin Echo (NSE) experiments on the deuterated samples reveal collective dynamics. As indicated by the previous structural results (WAXS and the polarization data from IN11c), the structure factor presents an amorphous halo centered at $\approx 1.4 \text{ Å}^{-1}$. At this value (Q_{max}) NSE directly monitors the temporal evolution of inter-chain correlations. Figures 4.13(a) and (b) show the NSE results on the normalized dynamic structure factor $S(Q,t)/S(Q,0)$ at a Q -value close to Q_{max} and the different temperatures investigated on dPrec and dSCNPs, respectively. At first sight, we can observe a clear slowing down of the dynamic structure factor when the sample contains internal cross-links in the whole T-range studied. The curves can be well described by Kohlrausch–Williams–Watts (KWW) functions

$$\frac{S(Q,t)}{S(Q,0)} \propto \exp \left[- \left(\frac{t}{\tau_w} \right)^\beta \right] \quad (4.2)$$

where β is the stretching exponent and τ_w the characteristic time. The fits of Eq. (4.2) to the experimental data show a variation of β with temperature. In the temperature range investigated ($285 < T < 360$ K), it increases from 0.47 to 0.6 for dPrec and from 0.39 to 0.69 for dSCNPs. In Fig. 4.13(c) the temperature dependence of the collective characteristic time at this Q -value—the usually called structural relaxation time—is plotted for both samples. As expected from the simple inspection of the NSE curves, the structural times are longer for the dSCNPs than for their precursor counterparts for all T -values studied, in accordance with the higher value of the glass-transition temperature T_g observed by DSC. The precursor times can be well described by the Vogel-Fulcher-Tamman (VFT) function

$$\tau(T) = \tau_{\infty} \exp \left[\frac{B}{T - T_0} \right] \quad (4.3)$$

with the values of the Vogel temperature T_0 and the energetic term B as determined by dielectric spectroscopy for the α -relaxation described in a previous work^[31], $T_0 = 161.4$ K and $B = 1125$ K. The prefactor used is $\tau_{\infty} = 9 \times 10^{-5}$ ns. The dSCNPs results can be reasonably described by keeping the same values of τ_{∞} and B , and assuming a shift in the Vogel temperature. This is found to be of 17 K, i.e., larger than the shift observed for the glass-transition temperature.

Although the short-range order (average distances between adjacent chains) is not significantly disturbed by the cross-links, the structural relaxation is notably slowed down, especially at lower temperatures. This is in agreement with the increase in the Vogel temperature, suggesting that while cross-links minimally affect short-range order, they profoundly influence the collective dynamics. As it has been shown before, these effects are also reflected in the rheological behavior of the sample, where the presence of cross-links leads to changes in the material's response.

The difference in the dynamical behavior is even more evident when the comparison of the NSE results is performed at Q values below Q_{max} , in the intermediate length scales (ILS) regime. This is illustrated in Fig. 4.13(d) for a Q -value of $0.27 \text{ \AA}^{-1}$. While for the linear chains the decay of the density correlation function is faster than at Q_{max} , for the dSCNPs the dynamic structure factor displays a much more pronounced stretching and decays at much longer times (more than one decade) than the inter-molecular correlations. The blue circles in Figs. 4.14(a)–(c) show that this effect is found over a large Q -range below the structure factor peak. In particular, collective motions appear to be frozen in the accessed dynamic window for $Q=0.15 \text{ \AA}^{-1}$. Thus, the presence of cross-links strongly hampers the relaxation of collective motions of the chains in this regime.

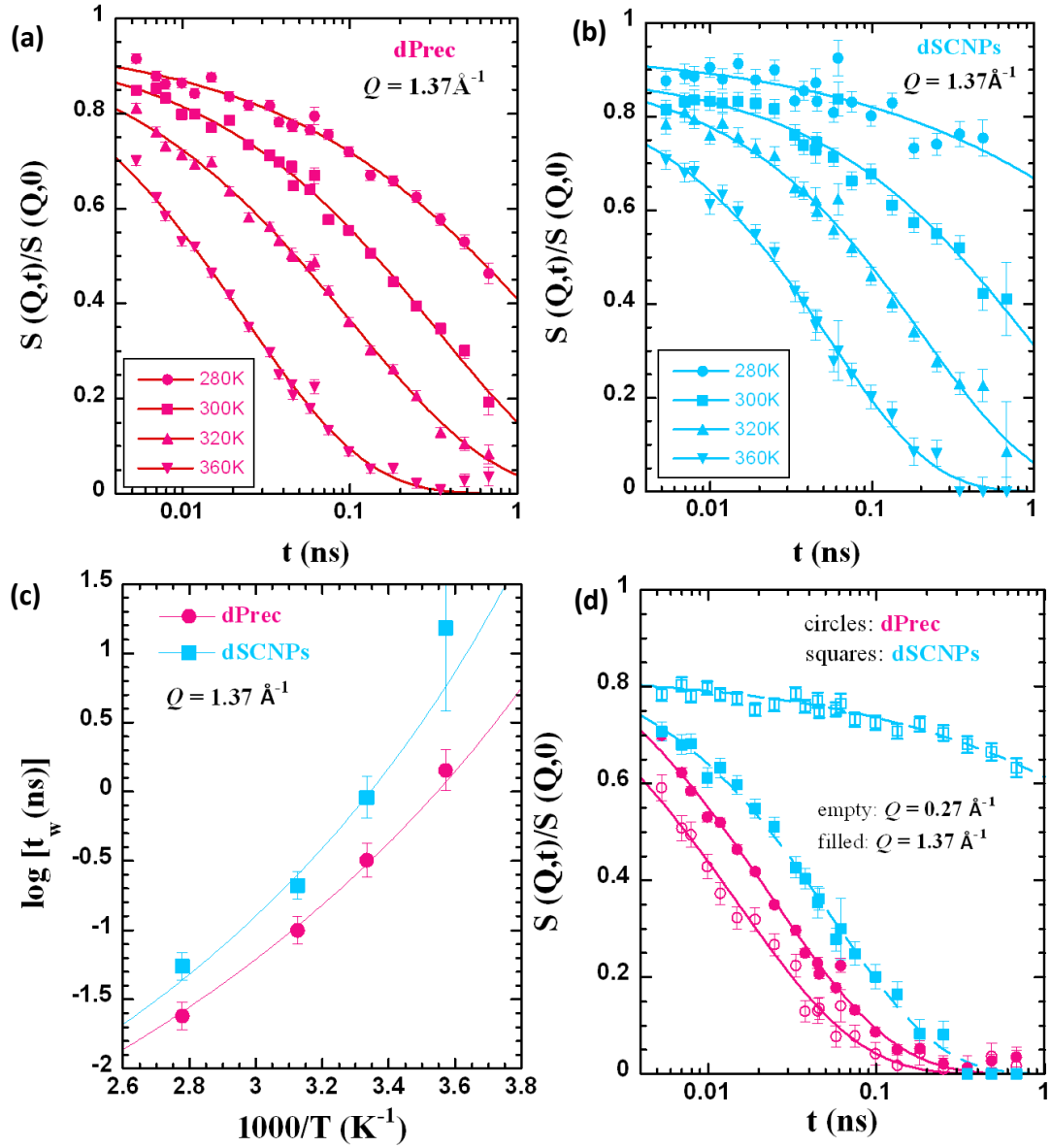


Figure 4.13. Normalized dynamic structure factor at $Q \approx Q_{max}$ of (a) dPrec and (b) dSCNPs samples at different temperatures, (c) temperature dependence of the collective characteristic times from NSE at $Q = 1.37 \text{ \AA}^{-1}$ and (d) a comparative between dPrec and dSCNPs samples at $Q \approx Q_{max}$ and a representative Q value in the ILS region at 360 K. Lines are fits of stretched exponentials for the dynamic structure factor and of the Vogel-Fulcher equation for the characteristic times.

To further illustrate these effects, Fig. 4.14 also compares the behavior of the dSCNPs with that of UV-SCNPs. In contrast to the click samples, the UV-SCNPs exhibit the same dynamical features as their linear precursor counterparts, and no shift in the T_g -value is observed. This finding suggests that the structural dynamics of UV-SCNPs are faster than those of the present dSCNPs. In the comparison shown in Fig. 4.14, corresponding to the highest temperature investigated, the filled symbols represent the Q -value close to Q_{max} . The data from both systems nearly coincide when a relative shift factor of 1.6 is applied to the time scales, corresponding to a structural time ratio of $\tau_{click} = 1.6 \tau_{UV}$, at 360 K. This indicates that the nature of the cross-linker significantly affects the dynamics at the inter-molecular level. Specifically, the presence of a relatively large oxybis(methylene)-bistriazole cross-linking moieties significantly slows down the decay of the inter-molecular correlations compared to the case when covalent bonds directly link chain monomers (see Fig. 4.2). This difference becomes more pronounced with decreasing

temperature, consistent with the thermal behavior observed for the structural relaxation time (Fig. 4.13(c)).

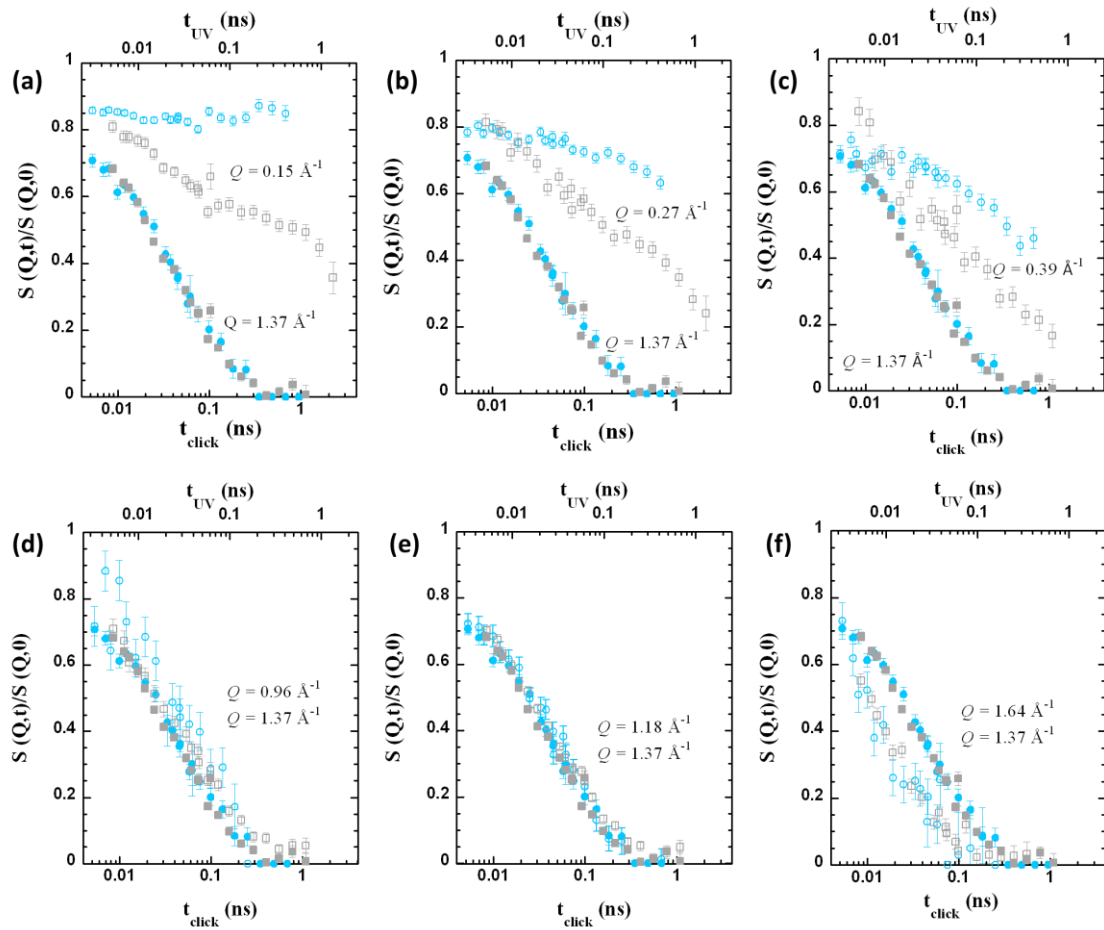


Figure 4.14. Normalized dynamic structure factor at $Q \approx Q_{max}$ (filled symbols) and a different Q values (empty symbols), (a) $Q = 0.15 \text{ \AA}^{-1}$, (b) $Q = 0.27 \text{ \AA}^{-1}$, (c) $Q = 0.39 \text{ \AA}^{-1}$, (d) $Q = 0.96 \text{ \AA}^{-1}$, (e) $Q = 1.18 \text{ \AA}^{-1}$, and (f) $Q = 1.64 \text{ \AA}^{-1}$ at 360 K for SCNPs samples synthesized via click chemistry (dSCNPs, pale blue circles) and via intra-molecular azide photodecomposition (UV-SCNPs, grey squares). They are plotted vs the corresponding Fourier time scale (lower x-axis for the dSCNPs, upper x-axis for the UV-SCNPs).

Regarding the impact of cross-links at other length scales, the different panels in Fig. 4.14 show the NSE results for dSCNPs and UV-SCNPs samples at various Q -values, always compared with the data of $Q = 1.37 \text{ \AA}^{-1}$ as reference, at 360 K. The above commented shift in the time applied in the figures allows appreciating how different is for the two samples the relative behavior at a given Q -value with respect to the structural relaxation at the first peak. It can be seen that, around the maximum of the peak (explored Q -values above approx. $1 \text{ \AA}^{-1}$) the behavior of the dynamic structure factor is identical, within the experimental uncertainties, for both bulk systems (see Figs. 4.14(d)-(f)). If we move toward lower Q -values, however, the behavior starts to differ. Entering the ILS region, as shown in Figs. 4.14(a)-(c) for $Q = 0.15, 0.27$, and $0.39 \text{ \AA}^{-1}$, respectively, the dSCNPs present a clear slowing down of the dynamic structure factor, compared with the UV-SCNPs. It is noted that the latter also exhibit a clear effect of internal cross-links in this regime, consisting of an enhanced stretching and a slowing down with respect to the linear precursor chains. However, in this case, the impact is less pronounced than for the dSCNPs bulk. For both melts, these effects could be attributed to the emergence of new constraints related to the internal domain topology. This internal structure would lead certain correlations to persist over longer times. Close to cross-links, the conformation of the chain and

the packing would be different from the average in the melt. These properties would also change from one domain to another. Segments chemically connected by the cross-links would feel an extra friction to relax. This would provoke the retardation of the decay of the density fluctuations at the corresponding length scales (as deduced from the SANS results, in the nanometric range). As it was proposed for the UV-SCNPs, the complete decay of the dynamic structure factor would only be reached through additional mechanisms for relaxation not present in the linear precursor chains. Due to the topological complexity induced by the cross-linking synthesis, a hierarchy of relaxation mechanisms would be in fact expected to occur in order to reach the full relaxation of the dSCNPs in the melt. It has been reported from dielectric spectroscopy studies on the UV-SCNPs bulk that an additional dynamic contribution to dipolar relaxation emerges, located between the α -process and the Rouse regime, and associated with the relaxation of internal domains within the SCNPs^[31]. These effects appear to be even more pronounced in the dSCNPs, likely due to the larger size of the cross-links involved in this system, which could account for this observation.

As mentioned above, the absence of flow in the dynamic window explored by the rheological experiments suggests a kind of gel-like behavior for the deuterated dSCNPs. This behavior is a priori not expected in the cases where there is exclusively intra-chain bonding as it has been found, for instance, in ring polymer melts that have a smaller disentanglement time and lower viscosity than their linear chain counterparts.^[41] Similarly, the work on UV-SCNP melts showed a much lower relaxation time compared to its linear precursor,^[31] suggesting in fact a suppression of entanglements with respect to the linear case. A gel-like behavior as that here observed for the dSCNPs would be expected when inter-chain bonding occurs, i.e., in a 3D network.

However, SEC results clearly show that the polymer molecular weight does not increase significantly upon cross-linking reaction (Fig. 4.7(a)) confirming that the formed bonds are exclusively intra-molecular after synthesis. On the other hand, in principle, the type of internal cross-linking is not expected to affect the long-time rheological behavior. Therefore, the anomalous response may instead originate from residual copper ions remaining from the catalytic reaction. These ions, together with the possible presence of a remaining fraction of pending (partially unreacted) cross-linkers, could give rise to the formation of some inter-molecular crosslinks among different SCNPs when they are condensed in a bulk system. Even if the inter-molecular bridges are extremely scarce, they can lead to a huge impact in the mechanical properties of the melt.

These additional constraints to flow would also explain the slowing down of the dynamic structure factor of the present dSCNPs with respect to the UV-SCNPs at ILS above discussed. It was also noticed that the dSCNPs system could not be dissolved and diluted after the experiments, to perform a posteriori SEC analysis. This observation further confirms the formation of a gel-like structure after the condensation of these nanoparticles in a bulk material with reminiscences of copper ions.^[42,43] Furthermore, the overlap of the $G''(\omega)$ in the master curve is clearly worse than in the precursor melt which may be attributed to a complex underlying superposition of relaxation processes with different activation energies. This interpretation is consistent with the scenario proposed here to explain the particularly pronounced slowing down of the dynamic structure factor in the dSCNP melt, especially at ILS. In contrast, the protonated hSCNPs do not exhibit such flow suppression in rheology; however, the dominance of incoherent scattering in the protonated system prevents a direct microscopic comparison between both isotopic systems of the collective dynamics by NSE.

4.3.5. Self H-motions: Neutron Scattering on Protonated Homopolymer Samples

Following the results obtained for the deuterated samples, the protonated counterparts are now considered. In this case, QENS is particularly valuable because it provides microscopic insight into the individual hydrogen dynamics in the high-frequency range, while also offering spatial resolution. Further details on the QENS measurement and the role of isotope-dependent scattering lengths can be found in Chapter 3.

In protonated samples, like those investigated in this work, the overwhelming contribution to the measured scattered intensity corresponds to incoherent scattering from the hydrogens, revealing correlations between the position of a given proton at different times, averaged over all the hydrogens in the sample. Figure 4.15 shows some representative examples of the incoherent dynamic structure factor measured in the frequency domain, normalized to its value at $\hbar\omega = 0$ to enable a direct comparison between datasets. The selected Q -value is $1.18 \text{ \AA}^{-1}$, and results at the three investigated temperatures are shown. The upper panels (Figs. 4.15(a)–(c)) correspond to measurements performed on the time-of-flight spectrometer, which probes shorter timescales than the backscattering spectrometer, which results are shown in Figs. 4.15(d)–(f).

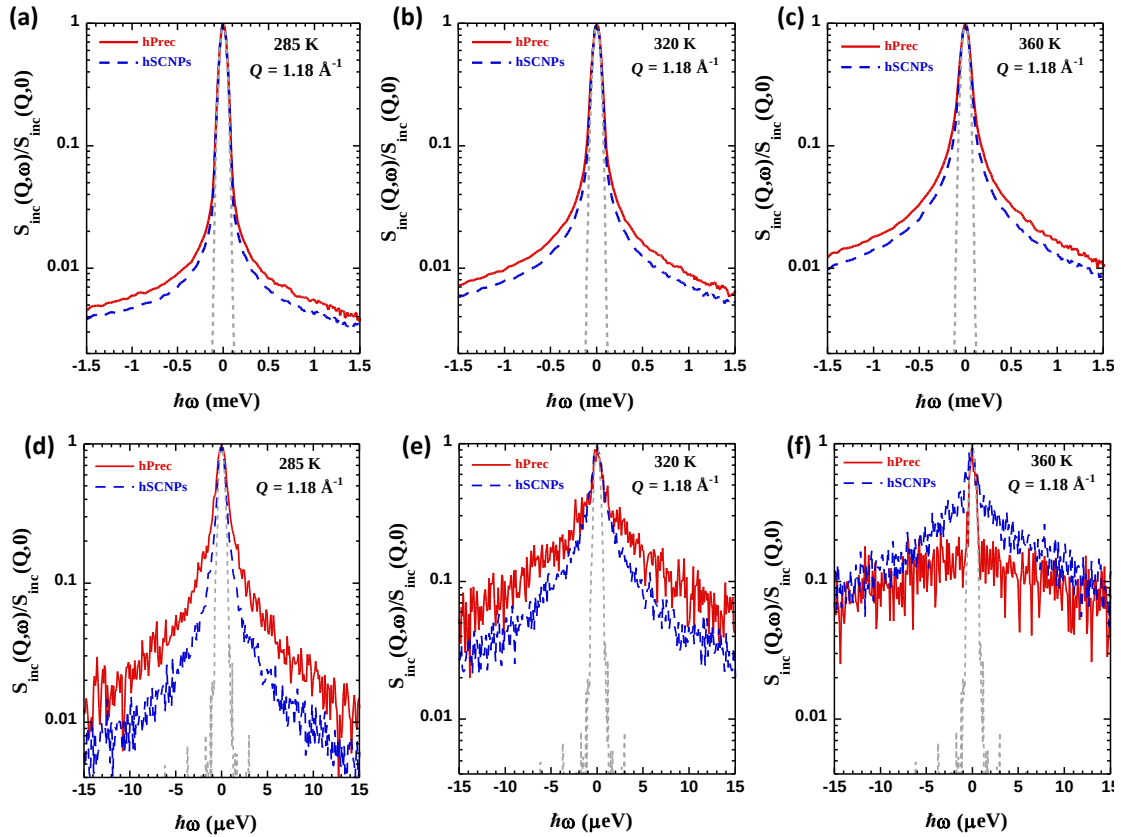


Figure 4.15. Normalized IN5 (a)–(c) and IN16B (d)–(f) spectra obtained at $Q = 1.18 \text{ \AA}^{-1}$ and the different temperatures indicated for hPrec (solid line) and hSCNPs (dashed line). The dotted line shows the instrumental resolution function.

A clear broadening with respect to the resolution function can be observed for all samples and temperatures measured. The width of a quasielastic spectrum is related with the inverse of the characteristic time of motion probed by the instrument. From this direct comparison, already a difference is observed between the hPrec and hSCNP samples. At the highest temperature

investigated, the spectra appear extremely broad within the IN16B window (Fig. 4.15(f)). It has to be noted that and the comparison based on the normalization of the data in the elastic peak region is highly sensitive to background subtraction. Taking this into account, it can be seen that, in general, the spectra corresponding to the hSCNPs sample are narrower for all temperatures, suggesting a slower dynamics in both dynamic windows explored when compared to its linear precursor sample.

The spectra measured in the frequency domain are affected by instrumental resolution through convolution. Therefore, the analysis of the quasielastic spectra was done by Fourier transforming the data to the time domain and then deconvoluting from the instrumental resolution to obtain the intermediate incoherent scattering function in the time domain $S_{inc}(Q,t)$. Some representative examples are shown in Fig. 4.15. Figure 4.15(a) corresponds to the analysis done at a Q -value of $1.18 \text{ \AA}^{-1}$ and three different T -values, and Fig. 4.15(b) considers a fixed T -value of 320 K (the intermediate T investigated) and four representative Q -values explored.

Following the previous analysis of the deuterated samples based on the normalized intermediate scattering function $S(Q,t)/S(Q,0)$ (Eq. 4.2), a similar description was applied here. The QENS results can be well described above ≈ 2 ps by means of Kohlrausch-Williams-Watts (KWW) or stretched exponential functions

$$S_{inc}(Q,t) = A(Q) \left\{ \exp \left[- \left(\frac{t}{\tau_w^s} \right)^\beta \right] + BG \right\} \quad (4.4)$$

with a stretching exponent β close to 0.5. The prefactor $A(Q)$ accounts for the decay of the correlation function at shorter times due to vibrational and other fast contributions. A small elastic contribution (BG) was also included in the fits, which had a value of ≈ 0.02 , and < 0.05 in all cases. This component is able to account for an inaccurate background subtraction in the experiment. As observed in Fig. 4.16, the description works well for both series of samples in the covered Q -range. The compilation of the results obtained for the prefactor $A(Q)$, the β parameter, and the average characteristic times are shown in Figs. 4.17(a)-(c), respectively. In the case of a KWW function, as expressed in Eq. (4.4), the average characteristic time is given by $\langle \tau \rangle = \tau_w^s \Gamma(1/\beta)/\beta$.

The prefactor values could be well described, as seen in Fig. 4.17(a), by a Debye–Waller-Factor like expression

$$A(Q) \approx \exp[-\langle u^2 \rangle Q^2/3] \quad (4.5)$$

The values obtained for the mean squared radius of the “thermal cloud” explored in the fast regime $\langle u^2 \rangle$ can be found in Table 4.3. They increase with temperature and are slightly larger for the hydrogens in the hPrec sample. The same occurs with the values of the β -parameter averaged over the Q -values explored, indicated by the horizontal lines in Fig. 4.17(b) and listed in Table 4.3.

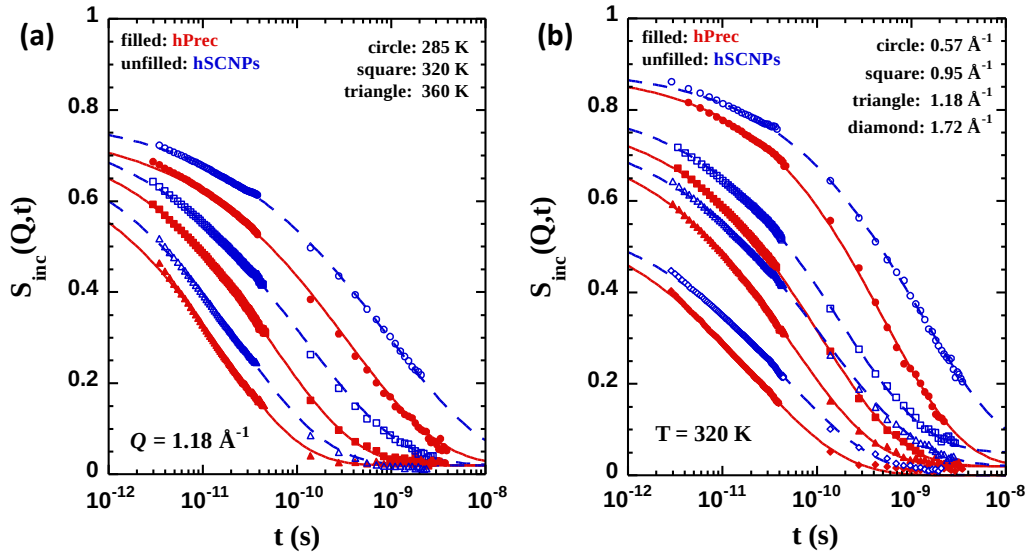


Figure 4.16. Fourier transformed and deconvoluted QENS spectra obtained from IN5 (sets of data for times shorter than 10^{-10} s) and IN16B (sets of data for times longer than 10^{-10} s) on the hPrec (red) and hSCNP (blue) samples at (a) three different temperatures investigated: 285 K (circle), 320 K (square), and 360 K (triangle) and a Q -value of $1.18 \text{ \AA}^{-1}$; and (b) for $T = 320 \text{ K}$ at four different Q -values: $0.57 \text{ \AA}^{-1}$ (circle), $0.95 \text{ \AA}^{-1}$ (square), $1.18 \text{ \AA}^{-1}$ (triangle), and $1.72 \text{ \AA}^{-1}$ (diamond). Solid lines are KWW fits with $\beta \approx 0.5$ (exact values shown in Fig. 4.17(b) to the results above 2 ps).

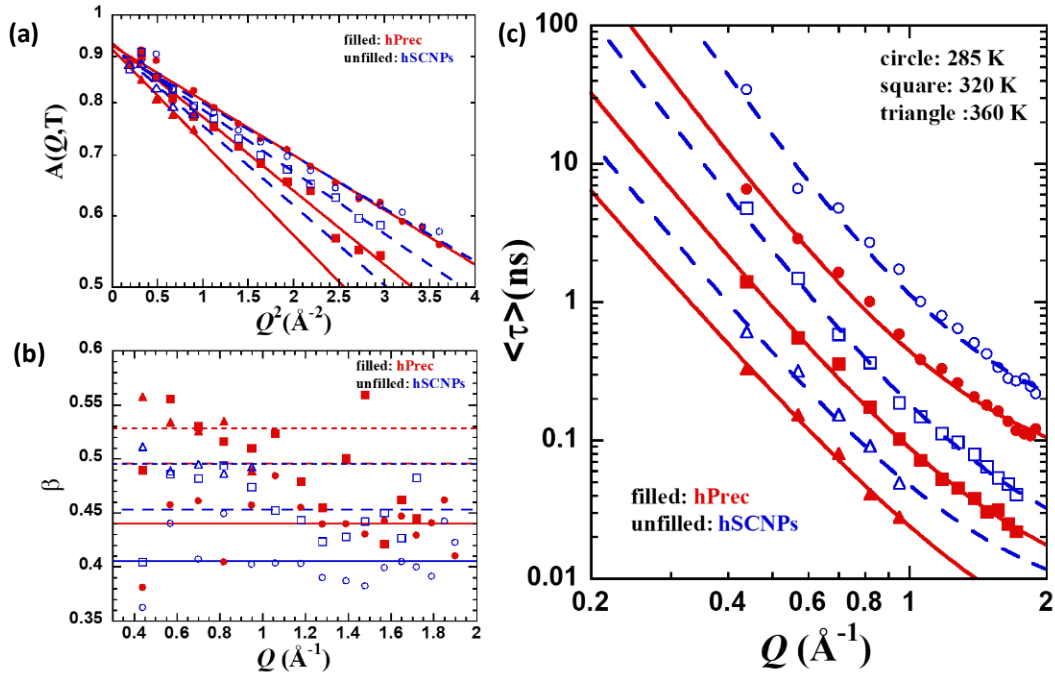


Figure 4.17. Scattering vector dependence of the parameters obtained from KWW fits (Eq. (4.4)) to the hPrec (filled red symbols) and hSCNPs (empty blue symbols) QENS data at 285 K (circles), 320 K (squares), and 360 K (triangles). (a) Prefactors A on a log scale versus Q^2 . Lines are fits of a Debye–Waller-Factor like expression (solid: hPrec; dashed: hSCNPs) (b) stretching parameter. Horizontal lines show the average value for each temperature (solid: 285 K; dashed: 320 K; dotted: 360 K) and each sample in the corresponding color, and (c) average characteristic time $\langle \tau \rangle = \tau_w^S \Gamma(1/\beta) / \beta$ for H-self motions obtained for hPrec (filled symbols) and hSCNPs (empty symbols) at 285 K (circles), 320 K (squares), and 360 K (diamonds), imposing a fixed average β -value indicated by the horizontal lines in (b) (see also Table 4.3). Lines are fits of Eq. (4.6) to the hPrec (solid lines) and hSCNPs (dashed lines).

As was observed before in glass-forming systems,^[32,44-46] in the low Q -range explored, the characteristic time for H-self motions was found to follow a power law $\langle\tau\rangle \propto Q^{-2/\beta}$ as dictated by the Gaussian prediction. At high Q s, deviations from this Gaussian behavior manifest. In jump diffusion models, finite jump lengths tend to cause a bending of the dispersion for the diffusive relaxation times away from the Q^{-2} law, which is valid for simple diffusion at low Q . Based on this explanation, the generalization by the anomalous jump diffusion (AJD) model to the case of subdiffusive behavior predicts the characteristic time for the self-correlation function as

$$\tau_s(Q) = \tau_{s,0} \left[1 + \frac{1}{Q^2 l_0^2} \right]^{\frac{1}{\beta}} \quad (4.6)$$

where l_0 is the preferred jump length and $\tau_{s,0}$ is the time between jumps.^[46] This model was applied to the results on the two systems investigated, using the average values of the stretching parameter listed in Table 4.3 for each temperature. The resulting values of the model parameters are also summarized in Table 4.3.

Table 4.3. Values of the parameters involved in the KWW description and AJD fitting of the hPrec and hSCNPs results.

Sample	T (K)	$\langle u^2 \rangle$ ($\text{\AA}^2$)	β	$\tau_{s,0}$ (ps)	l_0 ($\text{\AA}$)
hPrec	285	0.44±0.02	0.44±0.03	37±8	0.68±0.06
	320	0.55±0.02	0.50±0.04	5.8±0.7	0.57±0.04
	360	0.73±0.02	0.53±0.04	3.1±0.3	0.69±0.02
hSCNPs	285	0.41±0.02	0.40±0.04	87±12	0.73±0.03
	320	0.51±0.02	0.45±0.03	11.6±1.2	0.63±0.02
	360	0.73±0.02	0.49±0.03	3.6±0.4	0.64±0.03

Figure 4.18 explores the temperature dependence of the characteristic times observed by QENS. As a representative value, $Q = 0.95 \text{ \AA}^{-1}$ was selected. These data are compared with the rheological shift factors obtained on both protonated samples. The latter have been multiplied by suitable factors τ_R to coincide with the QENS results in the overlapping temperature range. For the hPrec sample, the combined sets of data have then been fitted to a VFT function with the same B and T_0 parameters as obtained from the dielectric relaxation data reported elsewhere.^[31] The prefactor value obtained now is $\log[\tau_\infty(s)] = -13.06 \pm 0.03$. Fixing this prefactor value for the hSCNPs, the fit of a VFT function yields $B = 1152 \pm 46 \text{ K}$, $T_0 = 178 \pm 3 \text{ K}$. Thus, the T_0 values deduced for both samples differ by 20 K. The temperature dependence displayed by the values of $\tau_{s,0}$ from the AJD model is very similar to that shown by the characteristic times at $Q = 0.95 \text{ \AA}^{-1}$, as can be deduced from the good description of the data provided by the corresponding VFT laws properly shifted, as shown in the Fig. 4.18. They have been determined by keeping the same B and T_0 values as used for $Q = 0.95 \text{ \AA}^{-1}$ and fitting the prefactors, yielding $\log[\tau_\infty(s)] = -14.29 \pm 0.05$ and -14.38 ± 0.13 for hPrec and hSCNPs respectively.

All these results demonstrate that QENS allows extending the investigation of the segmental dynamics accessible by “conventional” techniques (as broadband dielectric spectroscopy) to significantly higher temperatures/shorter times. Combining these different kinds of techniques provides access to a very broad dynamic range. When combined with rheological and dielectric data, a consistent description of the temperature dependence of the structural relaxation is obtained, showing that it follows the same behavior as the H-self dynamics.

A closer inspection of the model parameters provides additional microscopic insight into the role of the internal cross-links. The stretching exponent β is slightly smaller for the hSCNPs than

for the precursor, revealing a broader distribution of relaxation times. This enhanced stretching reflects the emergence of dynamical heterogeneities induced by the cross-linked architecture, consistent with a spatially non-uniform mobility at the segmental scale.

Regarding the jump diffusion analysis, the jump length l_0 is in general slightly larger for the hSCNPs. This trend points to stronger deviations from Gaussian behavior in the cross-linked system, suggesting that relaxation involves somewhat larger effective displacements, possibly associated with the need to overcome local topological constraints imposed by the intra-molecular bonds. In contrast, $\langle u^2 \rangle$ is not appreciably modified by the presence of cross-links. This indicates that the short-time localized motions remain essentially unaffected, and that the impact of cross-linking predominantly manifests at longer times.

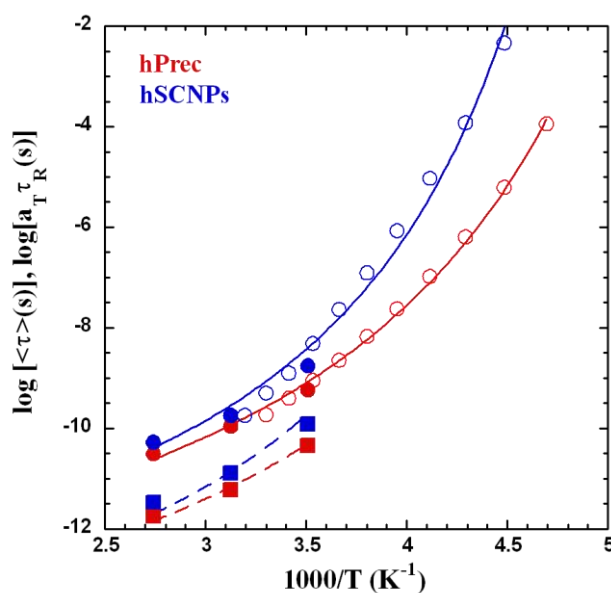


Figure 4.18. Temperature dependence of the average characteristic times obtained by QENS for a representative Q -value of $0.95 \text{ \AA}^{-1}$ (filled circles) and resulting values of $\tau_{s,0}$ (filled squares). The former have been combined with the corresponding rheological results (empty circles). Lines are fits of VFT functions (see text).

4.4. Dynamics in the All-Polymer Nanocomposites

4.4.1. Calorimetric Characterization

This section presents the DSC results of the two nanocomposite systems prepared as described in Section 4.2.4: 75hPrec and 25hSCNP. In Fig. 4.19(a), the DSC thermograms of the nanocomposites are compared with those of the pure melts already presented in Fig. 4.8.

Independently of the isotopic label, for the the composition of the nanocomposite investigated (75 wt% precursor and 25 wt% SCNPs), the T_g value is equal to the initial precursor value (202 K). The similarity applies not only to the average value of the glass transition, but also to its width. This can be better appreciated in Figs. 4.19(b) and (c), where the derivative of the specific heat is compared for the mixture and its pure components. Figure 4.19(b) shows these functions for the 75hPrec sample and Fig. 4.19(c) for the inverse labeling (25hSCNPs).

The results on the pure materials (Section 4.3.1., Fig. 4.8) indicate that internal cross-links induce a weak dynamic asymmetry —the difference in glass transition temperatures of the homopolymers— between the pure components of the system. In mixtures where the components present a strong dynamic asymmetry (such as PMMA and PEO) two distinct glass transitions associated with each component are typically detected by DSC.^[47] This behavior has been reported both for blends of linear polymers and for all-polymer nanocomposites composed of linear PEO chains as matrix and PMMA-SCNPs as fillers,^[48] including systems of comparable composition (75% linear matrix / 25% SCNPs). In contrast, no such double transition is observed in the present nanocomposites. The glass transition temperature of the mixture essentially coincides with that of the precursor, which is the majority component. Both the average T_g and the breadth of the transition are nearly indistinguishable from those of the pure precursor melt. A closer inspection of the derivative of the heat capacity (Figs. 4.19(b) and 4.19(c)) reveals only a very small shift in the position of the maximum and a slightly smoother overall shape in the mixtures. Consequently, any clear influence of the SCNPs on the matrix glass transition or evidence of an independent vitrification process for the SCNPs cannot be resolved from the present DSC data.

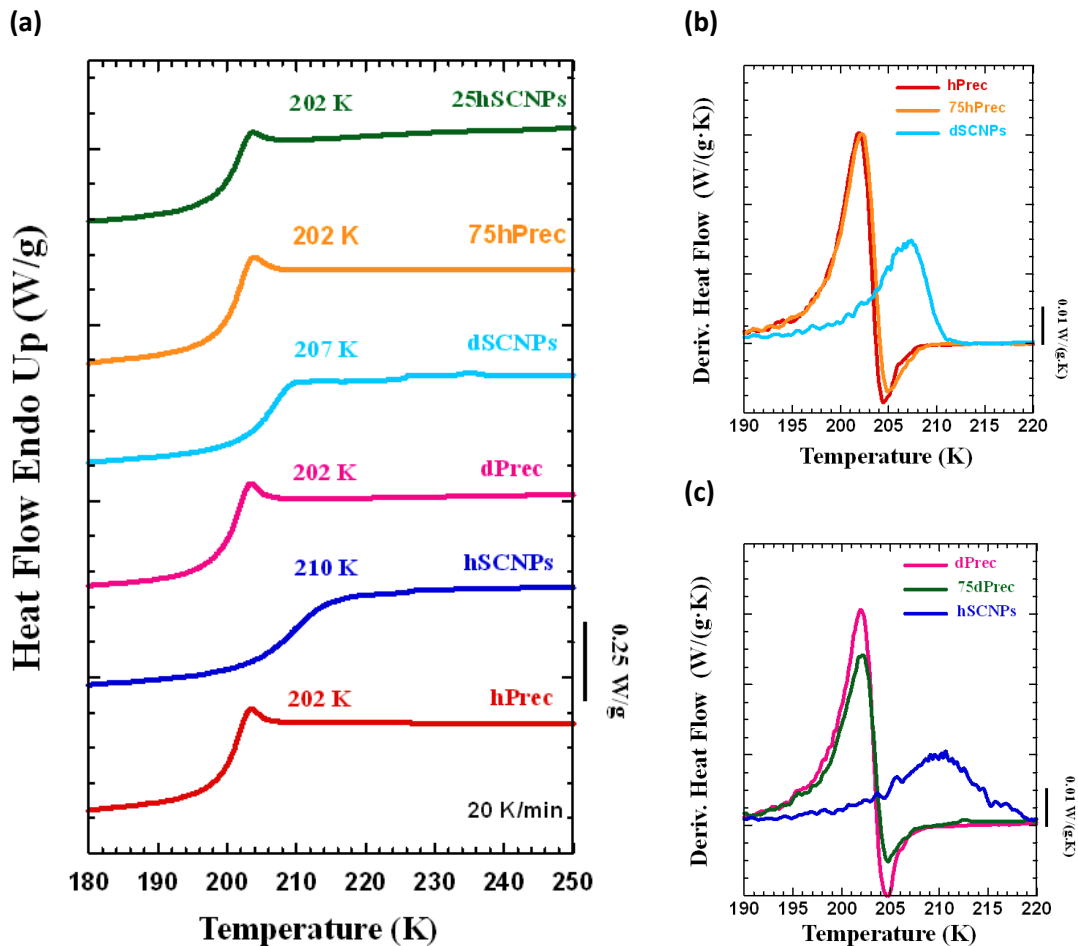


Figure 4.19. (a) DSC heating scans at 20 K/min after the previous cooling process of protonated and deuterated precursors and click SCNPs, and the nanocomposites investigated. Temperature evolution of the derivative of the heat capacity of the (b) 75hPrec sample and its pure components and (c) 75dPrec sample and its pure components.

It should be noted that the macroscopic dynamic asymmetry in these samples is only about 5–8 K, i.e., much smaller than in previously studied PMMA-SCNPs/PEO nanocomposites, where the difference reached approximately 50 K.^[49] The effects induced by such a modest asymmetry are therefore expected to be too subtle to be detected by conventional DSC measurements.

4.4.2. Isolating Component Dynamics: Neutron Scattering on Labelled Nanocomposites

The purpose of this contrast variation strategy is to selectively probe the motion of a specific component by means of isotopic labeling. Specifically, one component is deuterated to largely suppress its incoherent scattering, thereby enabling the dynamics of the hydrogenated component to be followed. Coherent scattering contributions remain, which importance can be evaluated thanks to polarization analysis.

The information provided by the diffraction experiments with polarization analysis is first considered, as it allows for the separation of coherent and incoherent contributions to the total intensity. Figure 4.20 illustrates the relative contribution of incoherent scattering to the total differential cross section for the two samples investigated. The results show that the incoherent contribution is dominant in all the Q -range investigated. The sample with dPrec shows a higher amount of coherent scattering but always below 50%. The modulation of the curves shown in Fig. 4.20 is due to this coherent scattering. Since the incoherent differential cross section is Q -independent, the results above $Q \approx 0.5 \text{ \AA}^{-1}$ reflect the inverse of the partial structure factor (coherent scattering). This shows a main peak in the Q -range between 1 and $2 \text{ \AA}^{-1}$ centered around $1.4 \text{ \AA}^{-1}$ for both samples. This peak is well-known from previously reported X-ray experiments (Fig. 4.10) and it is associated with the inter-main chain correlations (see Sections 4.7.1 and 4.7.2). The short-range order, thus, seems to be not modified by the composition. The decrease of the relative incoherent contribution at low Q -values ($Q < 0.5 \text{ \AA}^{-1}$) is due to the coherent small angle scattering arising mainly from the concentration fluctuations in the mixture. It is noted that this low- Q coherent contribution is generally present in mixtures of isotopically labelled samples. From the D7 results it can be concluded that in the Q -range explored with the current QENS experiments, the sensitivity is primarily to the incoherent scattering of the protons in the hydrogenated component of the mixture. However, it has to be kept in mid that the results below approximately $0.5 \text{ \AA}^{-1}$ and in the range $1.2 \leq Q \leq 1.7 \text{ \AA}^{-1}$ might be significantly affected by coherent contributions.

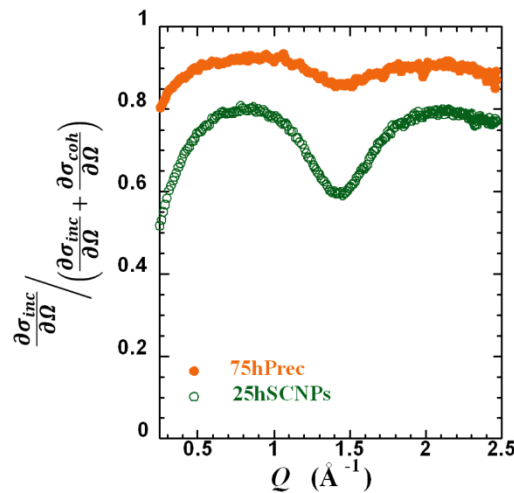


Figure 4.20. Ratio between incoherent and total differential cross sections obtained by D7 at 300 K on samples with protonated and deuterated nanocomposites.

Moving further to QENS experiments, and taking into account the diffraction results that show the dominance of incoherent contributions, it can be assumed that the signal is predominantly due to the incoherent scattering function $S_{\text{inc}}(Q, \omega)$ of the hydrogens in the labelled (protonated) component. As mentioned above, this function reveals correlations between the positions of individual protons at different times within one of the nanocomposite components. Figure 4.21 presents representative QENS spectra. To compare directly data obtained from different samples the curves are normalized to their value at $\hbar\omega = 0$. Results at $T = 320$ K have been chosen for four representative Q -values in the range where the coherent contribution is minimal. At these Q -values the incoherent signal amounts to 70-80% of the total intensity in the 25hSCNPs sample and to about 90% in the 75hPrec sample, as can be seen in Fig. 4.20.

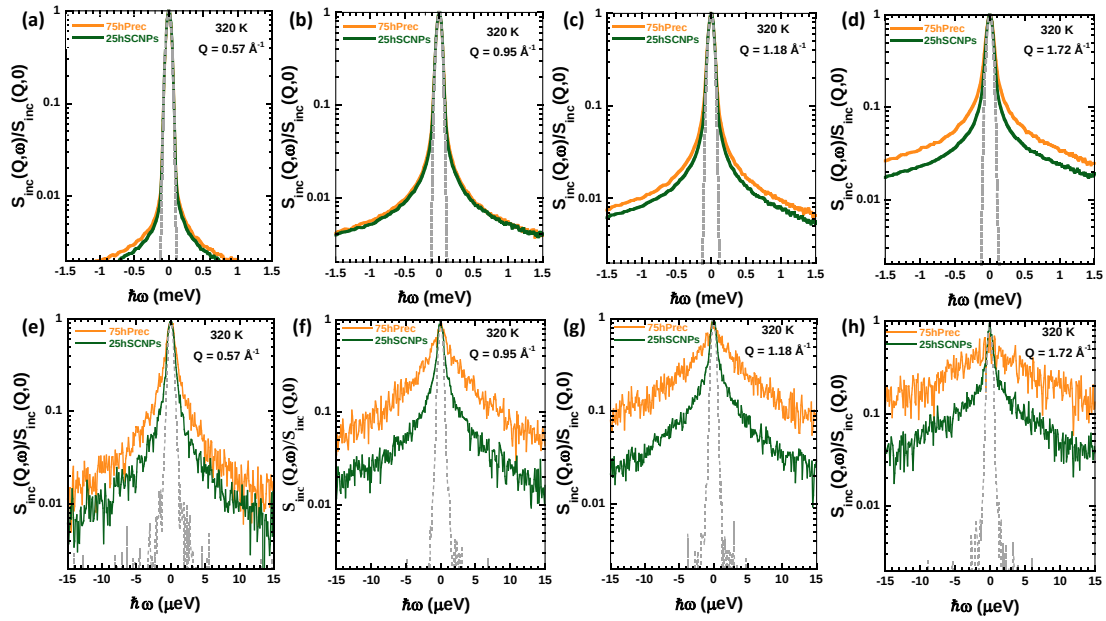


Figure 4.21. Normalized IN5 (a)-(d) and IN16B (e)-(h) spectra obtained at 320 K and the different Q -values indicated for different nanocomposites 75hPrec (orange line) and 25hSCNPs (green line). The dotted line shows the instrumental resolution function. Upper panels show the IN5 results, whereas lower panels display the IN16B data.

As previously mentioned, the width of the quasielastic spectrum is related to the inverse of the characteristic time of motion probed by the instrument. At all the temperatures investigated (285, 320 and 360 K) and, in the Q -range explored ($0.57 < Q < 1.72 \text{ \AA}^{-1}$), a quasielastic broadening is observed. For a given temperature, as can be observed in Fig. 4.21 for the case of 320 K, this broadening becomes more pronounced with increasing Q -value, which suggests diffusive-like behavior of the protons also within the nanocomposite. Moreover, a clear difference can be observed between both samples, 75hPrec and 25hSCNPs. In general, the spectra corresponding to the 25hSCNPs sample are narrower for all Q -values, suggesting a slower dynamics of the SCNPs with respect to the linear precursor component in both dynamic windows studied.

Following the procedure described earlier for the protonated samples, the intermediate incoherent scattering function in the time domain $S_{\text{inc}}(Q, t)$ was obtained thanks to the Fourier transformation of the data and subsequent deconvolution from the instrumental resolution. In Fig. 4.22 the results of this procedure are shown for spectra corresponding to the same representative Q -values as chosen for Fig. 4.21. From Fig. 4.22(a) to (c), the curves correspond

to the 75hPrec sample at fixed T -values of: 285 K (Fig. 4.22(a)), 320 K (Fig. 4.22(b)) and 360 K (Fig. 4.22(c)). From Fig. 4.22(d) to (f) the results are from the 25hSCNPs sample at the same fixed T -values of: 285 K (Fig. 4.22(d)), 320 K (Fig. 4.22(e)) and 360 K (Fig. 4.22(f)). The results clearly show that the characteristic time for hydrogen motions (which, as a first approximation, can be defined as the time where $S_{inc}(Q,t)$ decays to $1/e$) becomes shorter with increasing T for a given Q -value, and with increasing Q -value at a given temperature, as already inferred from the frequency-domain analysis. Also, it can be appreciated that the curves exhibit a more stretched functional form than a single exponential function. On the other hand, the intermediate incoherent scattering functions obtained for the two nanocomposite samples are compared in Fig. 4.23(a) with both pure precursor and hSCNPs samples under the same conditions (a Q -value of $0.95 \text{ \AA}^{-1}$ and a fixed T -value of 320 K). As commented in the previous section an also patent in this figure, the dynamics of the hSCNPs in the pure melts appear slower than those of the precursor; in the mixture, this difference not only remains evident but also seems to be amplified at long times.

The analysis of the intermediate incoherent scattering function $S_{inc}(Q,t)$ of the components based on the same approach outlined earlier, using KWW or stretched exponential functions (Eq. 4.4) to describe deviations from exponential behavior. This includes considering the prefactor $A(Q)$, the stretching exponent β , and the characteristic times. Again, a small elastic contribution (BG) was also allowed in the fits. This component may account for an inaccurate subtraction of the background in the experiment -as it was assumed for the homopolymers-, though, in this case, it could also result from the presence of some immobile protons (within the experimental window) in the case of pronounced heterogeneous behavior. Figure 4.22 and Figure 4.23(a) show that this function works well for both nanocomposites as well as for the neat protonated polymers in the studied Q -range. The values found for the BG were again ≈ 0.02 , and < 0.05 in all cases. Thus, if a fraction of protons remains extremely slow in the nanocomposites, it is very small. The characteristic times are displayed in Fig. 4.22(b) and 4.23. Table 4.4 compiles the values obtained for the mean square radius of the thermal cloud and the stretching parameter β .

The AJD model previously applied for pure components was used to describe the results of the two nanocomposites investigated (see descriptions in Figs. 4.23(b) and Fig. 4.24). In order to avoid the influence of coherent scattering, in the fit of the results on the nanocomposites, data corresponding to the region $1.2 \leq Q \leq 1.65 \text{ \AA}^{-1}$ was not considered, where this contribution is not negligible (see Fig. 4.20); as can be appreciated in Figs. 4.23(b) and 4.24, the times in this Q -regime show a modulation that prevents for an accurate determination of the values of the fit parameters. With this restriction, the model describes well the results on the nanocomposites (see Figs. 4.23(b) and 4.24). The values obtained for the parameters involved in the model (I_0 and $\tau_{s,0}$) are listed in Table 4.4. It is noted that for 360 K and high Q the decay of the intermediate scattering function of the 75hPrec takes place with very short characteristic times, hardly distinguishable from the first fast decay below 2 ps; results above $1.2 \text{ \AA}^{-1}$ were therefore not considered, and consequently the AJD model could not be applied under these conditions.

Table 4.4. Values of the parameters involved in the KWW description and the AJD fitting of the nanocomposite mixture components' results.

Sample	T (K)	$\langle u^2 \rangle$ ($\text{\AA}^2$)	β	$\tau_{s,0}$ (ps)	l_0 ($\text{\AA}$)
75hPrec/25dSCNPs	285	0.36 ± 0.04	0.44 ± 0.04	15 ± 4	0.63 ± 0.05
	320	0.54 ± 0.04	0.50 ± 0.05	4.8 ± 0.4	0.64 ± 0.02
	360	0.57 ± 0.04	0.53 ± 0.03	--	--
75dPrec/25hSCNPs	285	0.30 ± 0.04	0.38 ± 0.04	51 ± 9	0.76 ± 0.04
	320	0.37 ± 0.04	0.40 ± 0.06	8.9 ± 0.6	0.76 ± 0.02
	360	0.59 ± 0.04	0.44 ± 0.02	3.5 ± 1.0	0.73 ± 0.06

Examination of the data presented in Table 4.4 in comparison with those of Table 4.3 (reference homopolymers) reveals that the presence of SCNPs influences the dynamics of the precursor chains mainly through a decrease of the residence time $\tau_{s,0}$. In contrast, both the stretching parameter β and the jump length l_0 remain essentially unchanged, within the uncertainties, compared to the corresponding bulk values reported in Table 4.3. Interestingly, this effect is somewhat counterintuitive, as the incorporation of slower entities leads to an apparent acceleration of the local segmental motions in the linear chains. This behavior can be rationalized in terms of interfacial effects, where the soft and deformable nature of the SCNPs may induce enhanced mobility in their surroundings, leading to a local dynamical acceleration of the precursor chains at the interface. Additionally, a possible interpenetration of precursor chains into the SCNP structure may further contribute to the observed behavior.

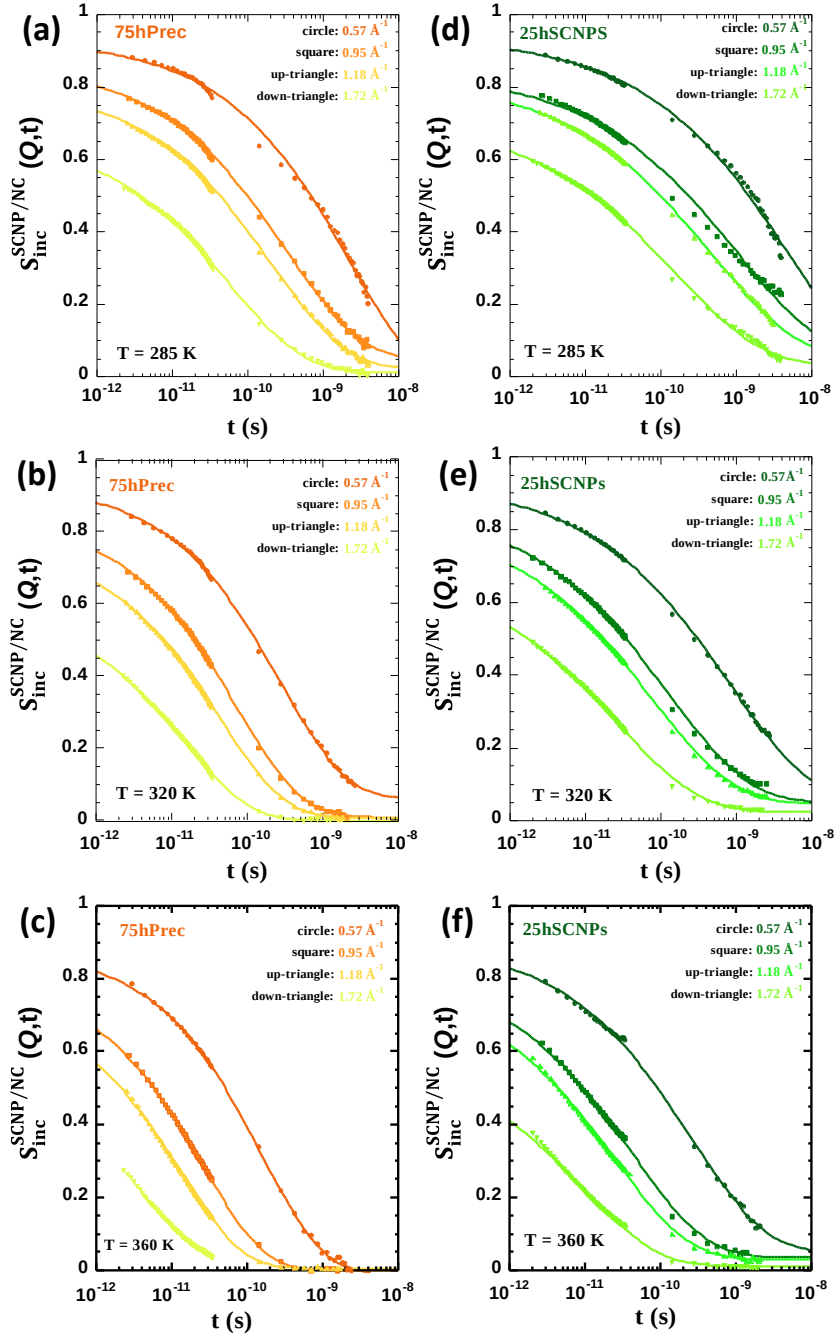


Figure 4.22. Fourier transformed and deconvoluted QENS spectra obtained from IN5 (sets of data for times shorter than 10^{-10} s) and IN16B (sets of data for times longer than 10^{-10} s) on the 75hPrec (a)-(c) and 25hSCNPs (d)-(f) samples at the three different temperatures investigated: 285 K (a,d), 320 K (b,e), and 360 K (c,f) and at four different Q -values: $0.57 \text{ \AA}^{-1}$ (circle), $0.95 \text{ \AA}^{-1}$ (square), $1.18 \text{ \AA}^{-1}$ (up-pointing triangle), and $1.72 \text{ \AA}^{-1}$ (down-pointing triangle). Solid lines are KWW fits with the β -values shown in Table 4.4 to the results above 2 ps.

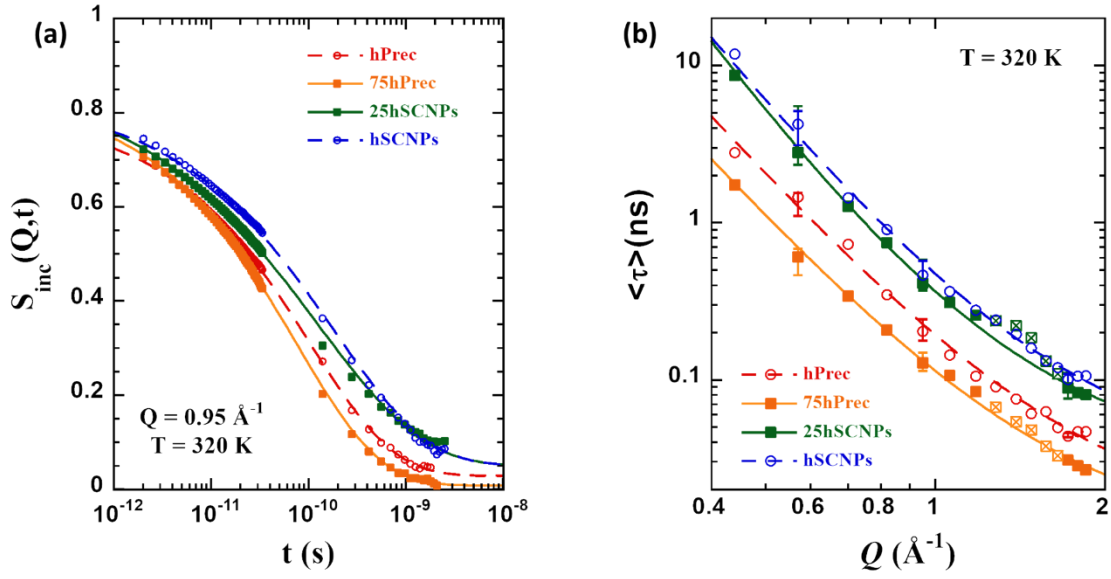


Figure 4.23. (a) Fourier transformed and deconvoluted QENS spectra of precursor (hPrec) and hSCNPs (empty circles) and nanocomposites (full squares) at 320 K and $Q = 0.95 \text{ \AA}^{-1}$. Dashed (hPrec and hSCNPs) and solid (nanocomposites) lines are KWW fits with the β -values shown in Table 4.4 to the results above 2 ps. (b) Scattering vector dependence of the average characteristic time $\langle \tau \rangle = \tau_s \Gamma(1/\beta)/\beta$ for H-self motions obtained for precursor and hSCNPs (empty circles) and nanocomposites (squares) at 320 K. Lines are fits of Eq. (4.8). Results of nanocomposites in the range $1.2 \leq Q \leq 1.65 \text{ \AA}^{-1}$ (represented by crossed squares) have been ignored for the fit of the AJD model. Estimated error bars are included for the nanocomposite results at $Q = 0.57, 0.95$ and $1.72 \text{ \AA}^{-1}$.

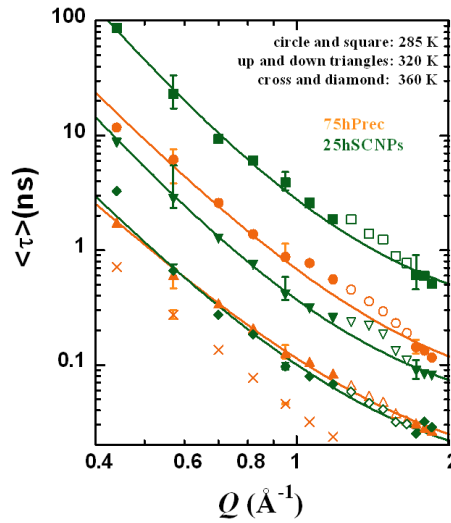


Figure 4.24. Scattering vector dependence of the average characteristic time $\langle \tau \rangle = \tau_s \Gamma(1/\beta)/\beta$ for H-self motions obtained for 75hPrec (orange symbols) and 25hSCNPs (green symbols) at 285 K (circle and square), 320 K (up-triangle and down-triangle), and 360 K (cross and diamond) (fits shown in Fig. 4.21). Lines are fits of Eq. (4.4) to the 75hPrec (orange lines) and 25hSCNPs (green lines), using the β -values listed in Table 4.4 for each case. Results of nanocomposites in the range $1.2 \leq Q \leq 1.65 \text{ \AA}^{-1}$ (represented by empty symbols) have been ignored for the fit of the AJD model. Error bars have been included at $Q = 0.57, 0.95$ and $1.72 \text{ \AA}^{-1}$.

At this stage, the results can be considered as a whole, connecting microscopic and macroscopic perspectives. The latter, provided by DSC results, reflect the averaged behavior of the sample but do not resolve the contributions of individual components within the NC. In contrast,

isotopic labeling enables separate characterization of the intermediate scattering functions of each component $S_{\text{inc}}^{\text{Prec/NC}}(Q,t)$ and $S_{\text{inc}}^{\text{SCNP/NC}}(Q,t)$ via QENS. These functions can be combined to reconstruct the total intermediate scattering function for all hydrogens, $S_{\text{inc}}^{\text{NC}}(Q,t)$, representing the behavior that would be observed in a hypothetical fully protonated sample:

$$S_{\text{inc}}^{\text{NC}}(Q,t) = 0.75 S_{\text{inc}}^{\text{Prec/NC}}(Q,t) + 0.25 S_{\text{inc}}^{\text{SCNP/NC}}(Q,t) \quad (4.8)$$

This function gives the averaged response of the system. Eq. (4.8) was calculated using the KWW fitting functions describing the experimental results on $S_{\text{inc}}^{\text{Prec/NC}}(Q,t)$ and $S_{\text{inc}}^{\text{SCNP/NC}}(Q,t)$ (lines in Fig. 4.22). The results of this calculation are shown in Fig. 4.25 as lines. For comparison, the intermediate scattering function of the pure precursor chains in the melt under the same conditions is also plotted. As can be seen in this figure, the calculated function for the whole nanocomposite perfectly matches the results observed in the linear precursor melt, in full agreement with the observations above reported from DSC measurements. These findings demonstrate that the QENS results on the microscopic dynamics well above the glass transition temperature are perfectly compatible with the calorimetric observation that the properties of the mixture appear to mimic completely the pure matrix behavior.

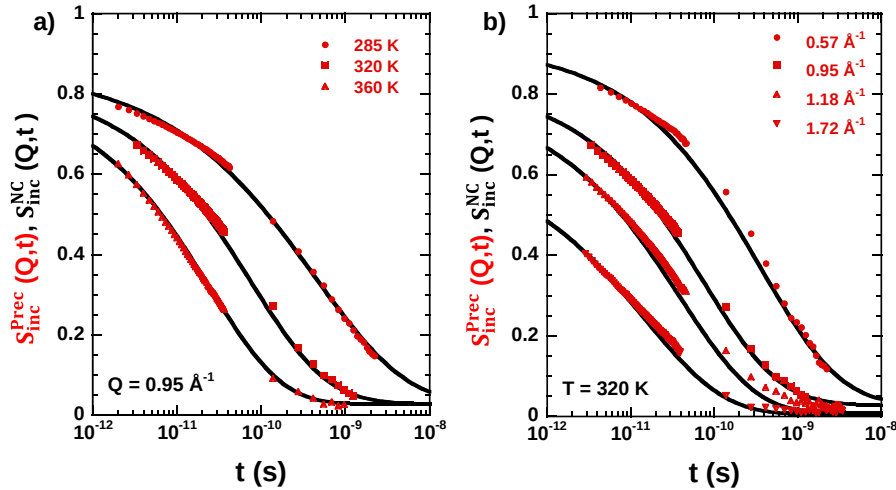


Figure 4.25. Intermediate scattering function calculated for all the hydrogens in the nanocomposite (Eq. (4.8)) (lines) starting from the KWW functions describing the component behavior (solid lines in Fig. 4.22) compared with the experimentally determined intermediate scattering function of the precursor melt (symbols). Panel (a) shows the results for $Q = 0.95 \text{ \AA}^{-1}$ at the different temperatures explored and panel (b) at 320 K for the different Q -values investigated.

In the seminal work of Mackay et al.,^[50] both a reduction in T_g and in viscosity were reported for PS-based nanocomposites. In the mixtures studied in that work, the two neat components exhibited the same T_g , unlike in the present case. Given that segmental mobility of the precursor is accelerated by the presence of SCNPs, a decrease in the nanocomposite T_g would have been expected if the T_g of the SCNPs remained the same as for the linear precursor chains. Therefore, the present results are consistent with the trends reported in Ref. 51^[51] regarding glass transition effects. The interfacial acceleration of matrix segmental relaxation, observed here through component-selective QENS measurements and predicted by MD simulations^[51,52], likely underlies the T_g reduction reported in Ref. 50. On the other hand, the present experiments do not address viscosity effects; however, some MD simulations suggest an unexpected amplification of viscosity reduction with increasing polymer molecular weight in the matrix,^[53] a subject that requires further investigation.

4.5. Conclusions

With a combined methodology that includes thermal analysis, rheological measurements, X-Ray diffraction and NS, the structural and dynamical consequences of introducing intra-molecular cross-links in polymer melts have been thoroughly characterized. For this purpose, the selected polymer precursors have been P(TDF-*ran*-ECD) (deuterated) and P(THF-*ran*-ECH) (protonated) which were first functionalized with azide groups, giving rise to dPrec and hPrec respectively. A synthesis route based on click chemistry was then employed to generate intra-molecular connections between functionalized monomers. This procedure leads to the formation of single-chain nanoparticles through the incorporation of relatively bulky oxybis(methylene)-bistriazole moieties acting as cross-linking agents, whereby dPrec yields to dSCNPs and hPrec to hSCNPs. The approach allows for a detailed comparison between the structural and dynamical behavior of the SCNPs and that of their linear precursor chains, providing direct insight into the effects of chain topology modification in the melt state. Comparison with analogous systems prepared via intra-molecular azide photodecomposition enables the distinction between generic effects arising from intra-molecular bonding and those specifically related to the chemical nature and size of the cross-linking moieties.

The results reveal that intra-molecular crosslinks have little influence on the average inter-molecular distances or on the local and fast dynamics of the chains, as indicated by WAXS and QENS measurements. Nonetheless, they exert a pronounced impact at intermediate length and time scales, where structural heterogeneities of nanometric size and complex relaxation patterns emerge. These features indicate that intra-molecular crosslinking enhances dynamic heterogeneity and restricts large-scale conformational rearrangements. Consequently, the glass-transition temperature of the SCNPs shifts toward higher values compared to their linear precursors. The extent of these effects depends on the character of the cross-linking chemistry: bulky linkers introduced through click chemistry cause a more significant slowdown than direct covalent bonds formed by UV irradiation, where cross-linking moieties are absent.

In some cases, traces of copper ions remaining from the catalytic process can promote additional inter-molecular reactions, generating a small fraction of permanent bridges between chains. Even in small quantities, such inter-molecular bonds dramatically alter the rheological behavior, imparting gel-like features and further suppressing the decay of density fluctuations at intermediate length scales. These effects are opposite to the entanglement suppression observed in UV-synthesized SCNPs, where crosslinking remains strictly intra-molecular.

The combination of QENS and isotopic labeling has proven especially powerful in disentangling the microscopic dynamics of the different components in complex systems, such as all-polymer nanocomposites consisting of SCNPs dispersed in their linear precursor matrix. In such nanocomposites, dynamic asymmetry and heterogeneity develop with increasing timescale: while the SCNPs display slower and more heterogeneous motions associated with the relaxation of internal loops, the surrounding linear chains exhibit accelerated segmental dynamics, particularly near the SCNP–matrix interface. These compensating effects result in an averaged macroscopic behavior similar to that of the pure precursor melt, as supported by DSC data. Molecular dynamics simulations^[52] of analogous systems corroborate this scenario, attributing the accelerated motions of the matrix chains to the thermal fluctuations of the soft SCNP surface.

Overall, the findings demonstrate that intra-molecular crosslinking profoundly modifies polymer dynamics at intermediate and large length scales, giving rise to structural and dynamic

heterogeneities without altering significantly the local organization of the melt. The ability to modulate these effects through the type and density of the internal crosslinks provides a versatile tool for tailoring the viscoelastic and relaxation properties of polymeric materials. Such control is particularly relevant for the design of advanced systems, including all-polymer nanocomposites, adaptive networks, and functional materials for catalysis or sensing applications.

4.6. References

- [1] M. Rubinstein and R. H. Colby, *Polymer Physics*, Oxford University Press, New York, **2003**.
- [2] D. Richter, M. Monkenbusch, A. Arbe and J. Colmenero, *Neutron Spin Echo in Polymer Systems*, Springer, Berlin, **2005**.
- [3] E. J. Bailey and K. I. Winey, *Prog. Polym. Sci.*, **2020**, *105*, 101242.
- [4] C. K. Lyon, A. Prasher, A. M. Hanlon, B. T. Tuten, C. A. Tooley, P. G. Frank and E. B. Berda, *Polym. Chem.*, **2015**, *6*, 181.
- [5] S. Mavila, O. Eivgi, I. Berkovich and N. G. Lemcoff, *Chem. Rev.*, **2016**, *116*, 878.
- [6] A. M. Hanlon, C. K. Lyon and E. B. Berda, *Macromolecules*, **2016**, *49*, 2.
- [7] E. Verde-Sesto, A. Arbe, A. J. Moreno, D. Cangialosi, A. Alegría, J. Colmenero and J. A. Pomposo, *Mater. Horiz.*, **2020**, *7*, 2292.
- [8] P. A. Klonos, N. Patelis, E. Glynos, G. Sakellariou and A. Kyritsis, *Macromolecules*, **2019**, *52*, 9334.
- [9] A. Arbe, J. A. Pomposo, A. J. Moreno, F. Lo Verso, M. González-Burgos, I. Asenjo-Sanz, A. Iturrospe, A. Radulescu, O. Ivanova and J. Colmenero, *Polymer*, **2016**, *105*, 532.
- [10] J. A. Pomposo, J. Rubio-Cervilla, A. J. Moreno, F. Lo Verso, P. Bačová, A. Arbe and J. Colmenero, *Macromolecules*, **2017**, *50*, 1732.
- [11] M. González-Burgos, I. Asenjo-Sanz, J. A. Pomposo, A. Radulescu, O. Ivanova, S. Pasini, A. Arbe and J. Colmenero, *Macromolecules*, **2020**, *53*, 8068.
- [12] J. A. Pomposo, A. J. Moreno, A. Arbe and J. Colmenero, *ACS Omega*, **2018**, *3*, 8648.
- [13] I. Pérez-Baena, A. J. Moreno, J. Colmenero and J. A. Pomposo, *Soft Matter*, **2014**, *10*, 9454.
- [14] F. Khabaz and R. Khare, *J. Chem. Phys.*, **2014**, *141*, 214904.
- [15] R. De and B. Das, *J. Mol. Struct.*, **2020**, 1199, 126992.
- [16] R. De and B. Das, *Polym. Int.*, **2014**, *63*, 1959.
- [17] X. Zhu, Y. Zhou and D. Yan, *J. Polym. Sci. Polym. Phys.*, **2011**, *49*, 1277.
- [18] M. K. Singh, M. Hu, Y. Cang, H.-P. Hsu, H. Therien-Aubin, K. Koynov, G. Fytas, K. Landfester and K. Kremer, *Macromolecules*, **2020**, *53*, 7312.
- [19] P. Bačová, F. Lo Verso, A. Arbe, J. Colmenero, J. A. Pomposo and A. J. Moreno, *Macromolecules*, **2017**, *50*, 1719.
- [20] A. Arbe, J. A. Pomposo, I. Asenjo-Sanz, D. Bhowmik, O. Ivanova, J. Kohlbrecher and J. Colmenero, *Macromolecules*, **2016**, *49*, 2354.
- [21] A. M. Hanlon, I. Martin, E. R. Bright, J. Chouinard, K. J. Rodriguez, G. E. Patenotte and E. B. Berda, *Polym. Chem.*, **2017**, *8*, 5120.
- [22] C. Heiler, S. Bastian, P. Lederhose, J. P. Blinco, E. Blasco and C. Barner-Kowollik, *Chem. Commun.*, **2018**, *54*, 3476.

- [23] P. Lindner and T. Zemb, *Neutrons, X-rays and Light: Scattering Methods Applied to Soft Condensed Matter*, North-Holland, Amsterdam, **2002**.
- [24] T. Springer, *Quasielastic Neutron Scattering for the Investigation of Diffusive Motions in Solids and Liquids*, Springer, Berlin, **1972**.
- [25] S. W. Lovesey, *Theory of Neutron Scattering from Condensed Matter*, Clarendon Press, Oxford, **1984**.
- [26] M. Bée, *Quasielastic Neutron Scattering*, Adam Hilger, Bristol, **1988**.
- [27] G. L. Squires, *Introduction to the Theory of Thermal Neutron Scattering*, Dover Publications, New York, **1996**.
- [28] J. S. Higgins and H. C. Benoit, *Polymers and Neutron Scattering*, Oxford University Press, Oxford, **1997**.
- [29] R. J. Roe, *Methods of X-Ray and Neutron Scattering in Polymer Science*, Oxford University Press, Oxford, **2000**.
- [30] B. Gabrys, *Applications of Neutron Scattering to Soft Condensed Matter*, Gordon and Breach, Amsterdam, **2000**.
- [31] A. Arbe, J. Rubio-Cervilla, A. Alegría, A. J. Moreno, J. A. Pomposo, B. Robles-Hernández, P. Malo de Molina, P. Fouquet, F. Juranyi and J. Colmenero, *Macromolecules*, **2019**, 52, 6935.
- [32] A. Arbe, J. Rubio, P. M. de Molina, J. Maiz, J. A. Pomposo, P. Fouquet, S. Prevost, F. Juranyi, M. Khaneft and J. Colmenero, *J. Appl. Phys.*, **2020**, 127, 044305.
- [33] R. N. Keller, H. D. Wyckoff and L. E. Marchi, *Copper(I) Chloride. In Inorganic Syntheses*, Wiley-Blackwell, Hoboken, NJ, USA, **1946**, 1.
- [34] J. Ma, I. M. Pazos, W. Zhang, R. M. Culik and F. Gai, *Annu. Rev. Phys. Chem.*, **2015**, 66, 357.
- [35] J. D. Ferry, *Viscoelastic Properties of Polymers*, John Wiley & Sons, New York, NY, USA, **1980**.
- [36] M. Abdel-Goad, W. Pyckhout-Hintzen, S. Kahle, J. Allgaier, D. Richter and L. J. Fetters, *Macromolecules*, **2004**, 37, 8135.
- [37] Y. Doi, K. Matsubara, Y. Ohta, T. Nakano, D. Kawaguchi, Y. Takahashi, A. Takano and Y. Matsushita, *Macromolecules*, **2015**, 48, 3140.
- [38] B. Frick, D. Richter and C. Ritter, *Europhys. Lett.*, **1989**, 9, 557.
- [39] A. J. Moreno, F. Lo Verso, A. Arbe, J. A. Pomposo and J. Colmenero, *J. Phys. Chem. Lett.*, **2016**, 7, 838.
- [40] *ChemDraw Suite* version 23.1.2, Revvity Signals Software, Inc., Waltham, MA, USA, **2025**.
- [41] R. Pasquino, T. C. Vasilakopoulos, Y. C. Jeong, H. Lee, S. Rogers, G. Sakellariou, J. Allgaier, A. Takano, A. R. Brás, T. Chang, S. Gooßen, W. Pyckhout-Hintzen, A. Wischnewski, N. Hadjichristidis, D. Richter, M. Rubinstein and D. Vlassopoulos, *ACS Macro Lett.*, **2013**, 2, 874.

- [42] J. Rubio-Cervilla, E. González and J. A. Pomposo, *Applications of Single-Chain Polymer Nanoparticles*, in *Single-Chain Polymer Nanoparticles: Synthesis, Characterization, Simulations, and Applications*, J. A. Pomposo (Ed.), Wiley-VCH, Weinheim, Germany, **2017**.
- [43] S. Thanneeru, S. S. Duay, L. Jin, Y. Fu, A. M. Angeles-Boza and J. He, *ACS Macro Lett.*, **2017**, 6, 652.
- [44] J. Colmenero, A. Alegria, A. Arbe and B. Frick, *Phys. Rev. Lett.*, **1992**, 69, 478.
- [45] A. Arbe, J. Colmenero, F. Alvarez, M. Monkenbusch, D. Richter, B. Farago and B. Frick, *Phys. Rev. Lett.*, **2002**, 89, 245701.
- [46] A. Arbe, J. Colmenero, F. Alvarez, M. Monkenbusch, D. Richter, B. Farago and B. Frick, *Phys. Rev. E*, **2003**, 67, 051802.
- [47] A. Arbe, F. Alvarez and J. Colmenero, *Soft Matter*, **2012**, 8, 8257.
- [48] T. P. Lodge, E. R. Wood and J. C. Haley, *J. Polym. Sci. Part B: Polym. Phys.*, **2006**, 44, 756.
- [49] D. Bhowmik, J. A. Pomposo, F. Juranyi, V. García-Sakai, M. Zamponi, Y. Su, A. Arbe and J. Colmenero, *Macromolecules*, **2014**, 47, 304.
- [50] M. Mackay, T. Dao, A. Tuteja, D. Ho, B. Van Horn, H.-C. Kim and C. J. Hawker, *Nat. Mater.*, **2003**, 2, 762.
- [51] X.-M. Jia, R. Shi, G.-S. Jiao, T. Chen, H.-J. Qian and Z.-Y. Lu, *Macromol. Chem. Phys.*, **2017**, 218, 1700029.
- [52] T. Chen, H.-J. Qian, Y.-L. Zhu and Z.-Y. Lu, *Macromolecules*, **2015**, 48, 2751.
- [53] R. Zorn, M. Mayorova, D. Richter, A. Schönhals, L. Hartmann, F. Kremer and B. Frick, *AIP Conference Proceedings*, **2008**, 982, 79.

CHAPTER 5

Conclusions

5.1. General conclusions

This thesis has explored the structural and dynamical behavior of systems containing or based on single-chain nanoparticles (SCNPs) through two complementary approaches: theoretical modeling and experimental characterization. Although the theoretical and experimental studies consider different systems and conditions, both focus on understanding how intramolecular cross-linking and SCNP topology influence the structure and dynamics of polymer-based materials across different length and time scales.

The main conclusions of this work can be summarized as follows:

- A scaling model describing brushes formed by elastic SCNPs grafted onto flat surfaces has been developed. Analytical expressions for brush thickness, local domain size, and free energy per chain have been derived as a function of the number of monomers and the elasticity parameter related to the intrachain cross-linking degree. The model predicts that SCNP brushes are significantly thinner than brushes formed by linear chains or other complex topologies with the same molecular weight, and that their thickness can be tuned through the elasticity parameter. The large predicted topological ratios with respect to linear polymer brushes suggest reduced interpenetration between opposing brushes and potentially lower friction in sliding systems composed of SCNP brushes. In addition, the progressive unfolding of SCNP brushes upon the rupture of intrachain cross-links has been explored under different solvent conditions, providing a conceptual framework for stimulus-responsive SCNP-based brush systems.
- Structure–size relationships have been established for tadpole-shaped SCNPs with flexible tails. Simple analytical expressions describing systems with long and short tails reproduce experimental data reported in the literature with good accuracy, demonstrating the capability of the model to capture the essential structural features of these anisotropic architectures. These results provide a simple way to estimate the overall size of tadpole-like SCNPs from their structural parameters and offer useful guidelines for the rational design of such systems.
- A simple method has been proposed to estimate the size of SCNPs under strong crowding conditions. The resulting expression relates the size of a SCNP in crowded environments to its size in good solvent at high dilution and parameters obtained from molecular dynamics simulations. This approach provides a practical framework to predict SCNP dimensions in dense environments and may also be extended to other topologically constrained macromolecular systems, such as ring polymers, complex SCNP architectures, or highly flexible macromolecules in crowded media, including IDPs.
- On the experimental side, two precursor copolymers were synthesized: protonated P(THF-*ran*-ECH) (hPrec) and deuterated P(TDF-*ran*-ECD) (dPrec). They were functionalized, and used to prepare hSCNPs and dSCNPs, respectively, via click chemistry (specifically via the CuAAC reaction). Both the precursor polymers and the resulting SCNPs were comprehensively characterized using complementary techniques. Chemical characterization by SEC, NMR spectroscopy, FTIR spectroscopy, and elemental analysis confirmed the molecular weight distribution, chemical composition, successful incorporation of functional groups, and the formation of SCNPs. In addition, a combined experimental approach including thermal analysis, rheology, X-ray diffraction, and neutron scattering techniques enabled a detailed investigation of the structural and dynamical consequences of intramolecular cross-links in polymer melts.

These results were analyzed in comparison with previously reported SCNP systems synthesized without bulky cross-linkers (UV-SCNPs), providing insight into the behavior of SCNP-based systems across a wide range of length and time scales.

- Internal cross-links do not significantly modify local structure or fast dynamics in polymer melts. This is evidenced by the preservation of average intermolecular distances observed by WAXS and by the amplitude of atomic displacements at sub-nanosecond timescales detected by QENS. In contrast, pronounced effects appear in the segmental dynamics associated with the α -relaxation, reflected in slower characteristic times and broader dynamic responses within the time window accessed by QENS. These features indicate the development of additional dynamic heterogeneities at intermediate length scales, consistent with SANS observations. Consequently, SCNPs exhibit higher T_g s than their precursors, as confirmed by DSC and rheological measurements.

- The nature of the internal bonds plays a key role in structural relaxation. Bulky cross-linking units introduced via click chemistry are associated with a pronounced slowdown of the segmental dynamics related to the α -relaxation, whereas direct covalent bonds formed by UV-induced cross-linking produce little effect. Furthermore, trace amounts of residual copper from the catalytic reaction can promote the formation of inter-molecular bridges, which drastically alter the rheological response and lead to gel-like behavior. These additional bonds may also hinder the decay of density fluctuations at intermediate scales, as detected by NSE, while SANS measurements reveal larger correlation lengths and enhanced scattering contributions, suggesting structural heterogeneities associated with cross-link chemistry and possible inter-molecular bonding.

- All-polymer nanocomposites were prepared by mixing linear precursors and SCNPs to achieve a 75/25 wt% composition. This resulted in two systems: 75hPrec (consisting of 75hPrec/25dSCNPs) and 25hSCNP (consisting of 75dPrec/25hSCNPs). QENS combined with such isotopic labeling proved to be a powerful approach for disentangling component-specific dynamics in complex polymeric systems. Using this methodology, SCNPs were found to exhibit slower and more heterogeneous motions, while the surrounding linear matrix chains showed accelerated segmental dynamics, particularly near the SCNP–matrix interface. These compensating effects produce macroscopic behavior similar to the pure precursor melt, consistent with DSC observations. Overall, the results demonstrate that intra-molecular cross-linking profoundly modifies polymer dynamics at intermediate and long scales without affecting local organization, and that tuning the type and density of cross-links offers a versatile tool to control viscoelastic and relaxation properties in advanced polymeric materials.

5.2. Publications

The work presented in this thesis has been published in the following articles:

- Probing the influence of composition and cross-linking degree on single-chain nanoparticles from poly (tetrahydrofuran-ran-epichlorohydrin) copolymers: Insights from neutron scattering, calorimetry, and dielectric spectroscopy.
E. Verde-Sesto, I. Asenjo-Sanz, F. Juranyi, J. A. Pomposo, J. Maiz. *Journal of Colloid and Interface Science*, 2025, 679, 785-797.
- Segmental dynamics of poly (tetrahydrofuran) cross-linked by click-chemistry: Single-chain nanoparticles vs cross-linked networks.
S. Sharifi, I. Asenjo-Sanz, E. Verde-Sesto, J. Maiz, J. A. Pomposo, A. Alegría. *Polymer*, 2023, 287, 126422.
- Disentangling component dynamics in an all-polymer nanocomposite based on single-chain nanoparticles by quasielastic neutron scattering.
J. Maiz, E. Verde-Sesto, I. Asenjo-Sanz, L. Mangin-Thro, B. Frick, J. A. Pomposo, A. Arbe, J. Colmenero. *Macromolecules*, 2022, 55 (6), 2320-2332.
- Impact of composition on the crystal texture and on the dynamics of P (THF-co-ECH) copolymers.
J. Maiz, E. Verde-Sesto, I. Asenjo-Sanz, F. Juranyi, J. A. Pomposo, A. Arbe, J. Colmenero. *EPJ Web of Conferences*, 2022, 272, 01005.
- A method to estimate the size of single-chain nanoparticles under severe crowding conditions.
I. Asenjo-Sanz, E. Verde-Sesto, J.A. Pomposo. *RSC Advances*, 2022, 12 (3), 1571-1575.
- Dynamic Processes and Mechanisms Involved in Relaxations of Single-Chain Nano-Particle Melts.
J. Maiz, E. Verde-Sesto, I. Asenjo-Sanz, P. Malo de Molina, B. Frick, J.A. Pomposo, A. Arbe, J. Colmenero. *Polymers*, 2021, MDPI 13, 2316.
- Collective Motions and Mechanical Response of a Bulk of Single-Chain Nano-Particles Synthesized by Click-Chemistry.
J. Maiz, E. Verde-Sesto, I. Asenjo Sanz, P. Fouquet, L. Porcar, J. A. Pomposo, P. Malo de Molina, A. Arbe, J. Colmenero. *Polymers*, 2021, MDPI 13, 50-64.
- Valuable Structure-Size Relationships for Tadpole-Shaped Single-Chain Nanoparticles with Long and Short Flexible Tails Unveiled.
I. Asenjo-Sanz, E. Verde-Sesto, J. A. Pomposo. *Physical Chemistry Chemical Physics*, 2019, 21, 10884-10887.
- Brushes of Elastic Single-Chain Nanoparticles on Flat Surfaces.
I. Asenjo-Sanz, A.J. Moreno, A. Arbe, J. Colmenero, J.A. Pomposo. *Polymer*, 2019, 169, 207-214.

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