Self-assembled biomimetic nanoreactors I: Polymeric template

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ABSTRACT: The variety of nanoarchitectures made feasible by the self-assembly of alternating copolymers opens new avenues for biomimicry. Indeed, self-assembled structures allow the development of nanoreactors which combine the efficiency of high surface area metal active centers to the effect of confinement due to the very small cavities generated by the self-assembly process. A novel self-assembly of high molecular weight alternating copolymers is characterized in the present study. The self-assembly is shown to organize into nanosheets, providing a 2 nm hydrophobic cavity with a 1D confinement.

1. Introduction

Some of the most exciting innovations of recent decades relate to molecular self-assembly and supramolecular catalysis¹⁻³. Biomimicry is an attractive approach to dynamic nanomaterials given the ubiquity of stable, self-assembling nanoreactors in nature, yet the design and control of such materials for industrial use remains a formidable challenge³⁻⁶. Broadly speaking, mimicry is attempted either by replicating natural structures⁷ or by reproducing the physical and chemical properties of the system by other means⁸. Given the sensitivity of bioreactors to harsh environmental conditions, the latter approach may permit the synthesis of robust analogues for application outside of biological pH, temperature, or ionic concentrations⁹.

Enzymes generate extremely high reaction rates partly by the concerted action of two complementary effects: catalysis and confinement. Biological catalysis consists of the forced geometric arrangement of substrates into relative position that minimizes activation energy¹⁰. This is accomplished by the placement of complementary amino acids to bind intermediate reaction structures, the 'lock and key' of enzymatic catalysis, and, in cases of redox reactions, coordination with a metal centre¹⁰. In aqueous media, the confinement effect refers to the autonomous increase in effective molarity and compression of non-polar molecules within a nanoscale hydrophobic domain⁸. High substrate concentration at the reactive site increases the kinetic rate of reaction by increasing local electron density but also affects reaction thermodynamics by reducing the entropic cost of synthetic reaction.

To develop a system capable of mimicking the confinement and catalysis features of biological nanoreactors, a template must be selected as an analogue to their macromolecular structure. Characterization of a suitable candidate would include evaluation of its pH, ionic, and temperature stability, its ability to host active metal centers, its internal reaction dynamics, and the dimensions and hydrophobicity of its confinement spaces. The material used for the present development of a biomimetic system is poly(styrene-*alt*-maleic acid) (SMA). SMA is an alternating, amphiphilic copolymer whose chains self-assemble into a pH sensitive cylindrical micelle nanostructure in aqueous solution at neutral pH. Dynamic light scattering (DLS) in aqueous solution revealed that the hydrodynamic radius of the SMA association presented a maximum at pH 6.5; the size of the association is reduced as pH rises or falls¹¹. This pH corresponds to the monodeprotonated form of the diacid which theoretical models showed to produce a linear chain conformation at pH 6.5 that does not appear at pH 3 or 12. Individual chains then associate by π - π interaction to form dynamic supramolecular nanotubes with 14 Å interior and 21 Å exterior radii¹². The nanotubes were also found to be insensitive to temperature variation between 15° and 75°C and to ionic strength at polymer to salt concentrations below 1/80¹³.

The hydrophobic interior of the SMA nanotubes was found to be capable of exploiting the confinement effect to promote otherwise unfavourable reactions. Indeed, pyrrole in 1% w/w SMA solution was observed to preferentially concentrate within the SMA nanostructure and spontaneously polymerize. Extensive investigation determined that the confined dimensions reduced pyrrole dynamics such that the reaction thermodynamics permitted thermal initiation¹⁴.

The importance of the polymer molecular weight on the nanoarchitecture is known to allow tuning of the thickness and stability of self-assembled block copolymers¹⁵. The importance of this factor on the self-assembly of the alternating copolymer, SMA, has however only been studied over a very narrow molecular weight range (1,600 - 40,000) and no change in nanostructure was observed¹¹. Here, we present the in situ characterization using small angle neutron scattering and transmission electron microscopy of a nov-

el nanostructure obtained by self-assembly at pH 7 of the pH sensitive alternating copolymer with a high molecular weight of 350,000. Molecular modeling was also performed to provide precise information on the atomic level polymeric structure. The nanoarchitecture provides a hydrophobic cavity optimal for confinement effect as observed in biological systems and is a very good candidate template for the synthesis of biomimetic nanoreactors.

2. Experimental

Poly(styrene-*alt*-maleic acid) sodium salt solution, 13% wt in H₂O, 350 kDa average molecular weight (SMA, Sigma-Aldrich) was freeze dried and rehydrated according to each sample specification. Small angle neutron scattering (SANS) experiments were performed on the NG3 30 m Small-Angle Neutron Scattering instrument at the NIST Center for Neutron Research, Gaithersburg, MD. The incident wavelength (λ) used during the experiments was 6 Å. The SANS experiments were carried out at a sample-to-detector distance of 1.00, 6.00, and 13.00 m, corresponding to a scattering vector range of $0.003 \leq q \leq 0.40 \text{ Å}^{-1}$ [$q = 4\pi \sin\theta / \lambda$]. The samples were loaded in quartz cells with a sample aperture of $10 \times 10 \text{ mm}$. To maximize the contrast between the polymer and the solvent, the polymer used was hydrogenated and prepared in deuterated water solution. The spectra obtained were corrected for electronic background and empty cell. The data were reduced at the NIST Center for Neutron Research using Igor Macros package. Analysis of the reduced data was performed using SasView 3.0.0 (<http://www.sasview.org>). The selected model is detailed in the results and discussion section. The morphology of the polymeric template was studied by Transmission Electron Microscopy (TEM) Experiments on a high resolution FEI HR-TEM/STEM Titan 80-300LB microscope operating at 300 kV. Solution samples were stained on carbon-coated copper grids and allowed to air dry. Molecular modeling was completed using Gaussian 09 software¹⁶.

3. Results and Discussion

A combination of molecular simulation and experimental characterization is critical to bridge the length scale needed for a complete structural characterization of nanostructures. The approach detailed in this paper allowed the characterization of polymeric templates to understand and improve the kinetics of reactions occurring within the confined environment of the nanoreactors.

The self-assembly of polymers can be characterized by different experimental methods ranging from dy-

namic light scattering for average large scale information on the hydrodynamic radius of the association, to very precise imaging methods such as transmission electron microscopy or atomic force microscopy usually performed with the polymer interacting with a surface and often in a dry state¹⁷⁻¹⁸. Small angle neutron scattering (SANS) provides a unique approach for the investigation of the association of polymers in solution because this method allows for characterization in solution¹³. The pH sensitivity and structure of the self-assembled structure of SMA with a low molecular weight were previously characterized by SANS¹¹. A concentration dependent characteristic scattering peak was observed in the SANS spectra between 0.05 Å^{-1} and 0.18 Å^{-1} due to the nanotube structure of the self-assembled polymer in its low molecular weight range.

The high molecular weight polymeric samples used in this study were also analyzed by SANS. Information concerning the structures present in a SANS spectra is interpreted as the intensity of q , which relates to the length d of structures in solution as $q \sim 2\pi/d$. Further analysis is possible through the application of model form factors, $P(q)$, whose shape parameters are optimized by least-squares fit to the intensity curve. Indeed, the differential scattering cross section per unit volume can be given by the equation:

$$\frac{d\Sigma(q)}{d\Omega} = n_p P(q)$$

where n_p is the number density of particles and $P(q)$ is the particle form factor.

The SANS spectrum obtained for the high molecular weight polymer at pH7 did not produce the characteristic peak for hollow cylinders that had been seen in low molecular weight samples. Several form factors including those for solid and hollow cylinders and rectangles were tested for fit against the spectra obtained for the high molecular weight polymer at pH7; the model that best fit the low intensity spectra with physically reasonable parameters is a stack of crystalline bi-layer lamella described by:

$$P(q) = 2\pi(\Delta\rho)^2\Gamma_m \frac{P_{bil}(q)}{q^2} Z_n(q)$$

where the form factor of the bi-layer with thickness t is approximated as:

$$P_{bil}(q) = \left(\frac{\sin\left(\frac{qt}{2}\right)}{\frac{qt}{2}} \right)^2$$

and $Z_n(q)$ accounts for the interference of n layers.

The fit to the experiment data is shown in Figure 1 and the parameters obtained from curve fitting are presented in Table 1.

Figure 1. Crystalline lamella model (line) fitted to SANS data of 1% SMA, pH 7.

Low signal intensity is expected for very thin lamellar systems and impacts quality of fit, but as shown in Figure 1 a bi-layer laminar model adequately fits the 1% SMA experimental data. The model was optimized with a chain spacing of 7.0 Å, thickness of 12.3 Å and one bi-layer. This would provide a hydrophobic cavity inside the hydrophobic layer of about 2nm. This model was also employed in subsequent SANS experiments of SMA-metal systems whose higher intensity signal further justified the choice of a bi-layer form factor¹⁹.

Table 1. SANS model fitting parameters.

Parameter		SMA pH 7
Lamina	SLD_{solid}	1.87e-06
	SLD_{solv}	6.38e-06
	Spacing s (Å)	6.99
	Thickness t (Å)	12.34
	Layers n (N)	1.00
Quality of fit (χ^2/n)		37.071

The structure of the self-assembled nanoarchitecture was further investigated by HR-TEM. Images of organic polymers by HR-TEM are not normally visible due to burn off by the intense electron beam. In order to stabilize the structure for characterization by HR-TEM the template was stabilized by platinum nanoclusters that were formed without a reducing agent within the hydrophobic interior in accordance with the method described by Groves¹⁷. Fortunately, by using this method the polymer nanostructures were very stable and observed on images of samples regardless of pH. Figure 2 clearly shows the SMA nanostructure in a sample prepared at pH 7; the parallel lines are individual SMA polymer chains organ-

ized into a sheet by the π - π interactions observed at regular intervals between them. The spacing of the chains is 6.9, s.d.=0.8 Å, the distance between sets of π - π interactions 3.3, s.d.=0.8 Å, and their progression angle relative to the polymer backbone is $70 \pm 4^\circ$.

The polymer shown in Figure 2 contains embedded platinum nanoparticles, appearing as out-of-focus dark patches. The beam was held in place for several minutes before the polymer disintegrated to reveal the platinum nanoclusters within. Figure 3 provides an example of SMA organization at pH 5 shown at a larger scale. The polymer nanostructure remains plainly visible, demonstrating the pH stability of the SMA-Pt system.

Figure 2. Close up of TEM image, SMA-Pt system at pH 7 showing SMA supramolecular association into a bi-layer nanosheet. Regularly spaced points between chains indicate π - π interactions.

Figure 3. TEM image of SMA-Pt system at pH 5 showing Pt nanocluster confinement to SMA sheets and their top-level organization. Scale bar is 10 nm.

The thermodynamic principles relating SMA self-assembly to solution pH was successfully predicted by molecular simulation in the authors' previous work¹². Specifically, the hydrolysis of one acid group at pH 7 provides the opportunity for very strong intramolecular hydrogen bonding to give a rigid, linear conformation to the polymer. This linearity favours the self-association of the chains by removing the otherwise high entropic cost that prevents association. Individual chains had been fully optimized using Density Functional Theory (DFT) with the combination of functional/basis set B3LYP/6-31G. A conformational search as described in the literature was performed to ensure the optimization of the ground state on the polymer chain and to predict its thermodynamic behaviour¹². It was shown for low molecular weight polymers that the association of eight polymer chains forms a nanotube with an internal diameter of ~3nm effective dimension for confinement enhanced reactions inside the hydrophobic cavity.

Using the same linear structure proven to be the most stable for individual polymer chains at pH7, the self-assembly of the SMA chains was modelled as a supramolecular sheet. As shown in Figure 4, the association among optimized chains occurs with interaction between neighbouring styrene rings.

The chirality of the monoprotonated SMA polymer, determined by the direction of styrene groups' progressive rotation along the chain, has been noted previously¹³. In the present predicted conformation, not only must the chains run antiparallel to form a single sheet, but the chains composing paired sheets must also be antichiral to one another demanding a very high level of organization. The π - π interactions between the styrene groups of two polymer chains are observed every 11.7 Å. Staggering two SMA sheets will place the separation between the π - π interactions visible from the top-down view at 5.4Å intervals (HR-TEM=6.3Å). SMA chains of the top and bottom sheets coincide and are spaced 8.5Å apart (HR-TEM=6.9Å). Due to styrene rotation the line of bonds are not perpendicular to the chains but progresses at approximately 69° to the polymer backbone (HR-TEM=76°). All of the above measurements are as shown in Figure 4a and closely correspond to those observed in Figure 2. Figure 4b reveals the primarily hydrophobic interior and hydrophilic exterior of the polymeric bi-layer.

Hence, this careful characterization of the association of SMA chains in aqueous environment shows that the individual chain conformation remains the

same as that observed in previous studies of SMA self-assembly but the supramolecular structure has changed from nanotube to nanosheet. The difference lies in the molar mass, with low molecular weight polymers favouring tubular nanostructures and high molecular weight polymers favouring nanosheet conformation.

Figure 4. Bi-layer model of SMA. a) The top-down view, chains are aligned by π - π bonding between styrene groups. b) the side view, hydrophilic groups (oxygen shown in red) are concentrated on the exterior surface, hydrophobic styrene groups to the interior.

4. Conclusion

The theoretical and experimental results provide evidence of a novel poly(styrene-alt-maleic acid) nanoarchitecture that self-assembles by π - π interactions into highly organized lamina containing confined cavities. This result introduces tunability of the SMA superstructure for fine control over the dimensionality of its confined domains. The laminar structure is an ideal template for the synthesis of unsupported nano-reactors, as illustrated in Figure 5, due to the expected presence of both hydrophobic and hydrophilic confinement spaces located within and between the bi-layers, respectively. This nanostructure opens new opportunities for the development of nanoreactors with different active centres, as well as possibilities for controlled reactions within hydrophilic and hydrophobic cavities of a single nanoreactor in an aqueous

environment.

Figure 5: Schematic representation of the unsupported polymeric nanoreactor in aqueous environment. The tailored nanoreactors can contain hydrophilic (blue, upper) or hydrophobic (green, lower) active centers or a combination of hydrophilic and hydrophobic active centers. The size of the active center cavity is approximately 2nm.

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