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Self-assembly of block copolymers via micellar intermediate states into vesicles on time scales from milliseconds to days

Carolin Fürst [a], Peng Zhang [b], Stephan V. Roth [b], Markus Drechsler [a], Stephan Förster [a, *]

[a] Physical Chemistry I, University of Bayreuth, Universitätsstr.30, 95447, Bayreuth, Germany
[b] HASYLAB/DESY, Notkesstr. 85, 22607, Hamburg, Germany

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ABSTRACT

Block copolymer micelles and vesicles are mostly prepared by the solvent mixing method, where the block copolymer is first dissolved in a common solvent for both blocks, which is then mixed with a selective solvent, mostly water, to induce self-assembly into the desired structure. Using a combination of microfluidic flow-focusing and capillary interdiffusion experiments combined with *in-situ* small-angle X-ray scattering (SAXS) and cryo-transmission electron microscopy (cryo-TEM) we investigated the structural evolution during solvent mixing from single block copolymers into spherical and cylindrical micellar intermediate structures into vesicles. We find that micelle formation is very fast and diffusion-limited, occurring on time scales of a few milliseconds. The development of an ordered lyotropic micellar phase is completed within 1 s. The structural transformation into cylindrical micelles occurs over several hours, which subsequently evolve into vesicles over time scales of days. Whereas the first two steps are transport-limited, the two latter processes involve large activation energies related to micellar fusion against the sterically stabilizing micellar coronas, which corresponds to much longer time scales of self-assembly.

1. Introduction

Amphiphilic block copolymers in solution can self-assemble into a variety of micellar and vesicular structures, depending on their relative block lengths and block chemistries [1]. This allows one to tune self-assembly to prepare well-defined structures with good control of size and topology for a variety of different applications in materials [2] and life science [3].

The methods that are used to prepare block copolymer assemblies often differ and have to be adapted to the specific block copolymer/solvent system. In the simplest case the block copolymers can be directly dissolved in the solvent to form the desired structure. However, in most cases this method fails, because the block copolymer forms undesired partially solvated, ill-defined structures or does not dissolve at all. This problem is avoided by dissolving the block copolymer in a good solvent for both polymer blocks. This solution is subsequently mixed with or dialyzed into a selective solvent, mostly water, which induces self-assembly to obtain the targeted structure. The obtained structure may indeed be the expected equilibrium structure, but could also represent a morphology which was trapped during the preparation process, and represents a metastable non-equilibrium state.

It is thus important to monitor the actual structural evolution during solvent mixing. Here it becomes essential to consider the time scales of mixing and self-assembly. The mixing time depends on the mixing procedure. Generally, mixing of two liquids firstly involves convection, e.g. by stirring, to intersperse and break down the fluid volumes of each liquid to micrometer dimensions, on which length scale interdiffusion becomes effective to eventually homogenize the solution on a molecular scale. The time scales of convection and interdiffusion must then be compared and adjusted to the time scales of self-assembly.

The mixing process can be simplified and made more efficient if liquid streams of micrometer dimensions are mixed. Then convection is not needed, and interdiffusion is the only relevant mixing process. With the advent of microfluidic technology, micrometer scale mixing is possible with a choice of many different channel designs [4]. In microchannels flow is characterized by low Reynolds numbers and is laminar such that very well defined mixing

* Corresponding author.
E-mail addresses: carolin.fuerst@uni-bayreuth.de (C. Fürst), peng.zhang@desy.de (P. Zhang), stephan.roth@desy.de (S.V. Roth), markus.drechsler@uni-bayreuth.de (M. Drechsler), Stephan.foerster@uni-bayreuth.de (S. Förster).

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conditions can be realized. A simple and versatile continuous mixing design is based on flow-focusing [5], where a central stream (e.g. the block copolymer solution) is focused with two side streams (e.g. the selective solvent) to micrometer width in a downstream mixing channel. The time a solvent molecule needs to diffuse across the width w of the focused central stream is the mixing time, which e.g. for a focused stream width of $w = 10$ μm is ca. 50 ms, becoming much smaller for smaller stream widths.

With respect to block copolymer solution mixing, flow-focusing establishes a well-defined stationary concentration gradient across the two solvents streams. Within this concentration gradient the block copolymer self-assembles. Each position x downstream the microfluidic mixing channel is then related to a certain polymer/solvent composition and a certain time t after first contact of the two liquids. Thus the time-evolution of the self-assembly process is mapped onto different positions along the mixing channel. The structural evolution at each position can then be monitored by scattering, spectroscopy or microscopy techniques. The achievable time resolution depends only on the spatial resolution Δx of the technique and the flow velocity v as $\Delta t = \Delta x/v$. With typical flow velocities of the order of $v = 10$ mm/s and spot sizes of $\Delta x = 10$ μm this then results in a time resolution of ~1 ms. The maximum detectable time t_{max} is given by the residence time of the fluids in the mixing channel as $t_{max}=L/v$, such that for typical lengths of $L \sim 5$ cm maximum time scales of $t_{max} \sim 5$ seconds can be assessed.

Flow-focusing mixing experiments combined with synchrotron SAXS have first been developed to study fast protein folding with down to microsecond resolution [6,7]. It has only recently been applied to study the structural evolution of other molecular structures. We demonstrated that this method can be used successfully to investigate the kinetics of amphiphile self-assembly, where we studied the formation of micellar lyotropic phases using polyimide based flow focusing devices [8]. Polyimide is highly transparent to X-rays, but allows one to realize only simple channel designs like two-dimensional (2D) flow-focusing where the central channel is focused with two side streams [11]. To avoid wall-contact of the mixing streams, which for 2D-focusing occurs on the bottom and ceiling of the mixing channel, and to avoid pre-mixing in the channel junction, three-dimensional (3D) focusing devices employing a buffer layer have been realized [9,10]. For the present study we developed a new three-dimensional hybrid focusing device. It 3D-focuses and locates the polymer and the water stream adjacent to each other, separated by a buffer stream. The streams are additionally separated from the channel walls by sheath streams and focused into an X-ray transparent thin glass capillary as the mixing channel, where high-quality SAXS-data can be recorded.

Using classical 2D-polyimide flow-focusing, the newly developed 3D-hybrid device and a capillary interdiffusion experiment, we investigated the self-assembly pathway of an amphiphilic block copolymer during solvent mixing on time scales from milliseconds to days. We show that the block copolymer, which forms vesicles in water as its equilibrium structure, upon solvent mixing into water structurally evolves via spherical and cylindrical micellar transient structures into, eventually, vesicles.

2. Materials and methods

2.1. Microfluidic chip preparation

For the *in-situ* mixing SAXS experiments we used two different microfluidic devices, a polyimide chip with a simple 2D channel design which has been used previously [8], and a newly developed SIFEL-capillary hybrid chip with a 3D-focusing/buffer layout.

2.1.1. Polyimide device

The polyimide device is made of three different types of commercial available polyimide films. The channels are cut into the films with laser cutting. The explicit preparation of these microfluidic devices is described elsewhere [8]. These microfluidic chips have a low background scattering in SAXS experiments and are resistant to most organic solvents. Fig. 1 shows the simple polyimide chip design with three inlets and one outlet and a perpendicular channel cross. The dimension of the channels is 110 μm in width, 115 μm in height and 31 mm length from the channel cross to the channel outlet.

2.1.2. SIFEL-device

SIFEL is a solvent-resistant perfuoropolymer material (Shin-Etsu SIFEL2610), which consists of a perfluoropolyether backbone with reactive silicone end-groups which can be cured under similar conditions as PDMS (Sylgard) [12–14]. For this chip both chip sides are structured and are bonded together to achieve a 3D-focusing design. The design is schematically shown in Fig. 1b, where grey channels belong to the bottom chip side and the black channels are molded into the upper chip side.

The device has nine inlets and one outlet. Seven inlets are used as buffers (B, dioxane), to separate the reactants from the channel walls and to modify the starting point of the reaction with a buffer stream in the middle between the polymer solution (PS) and water stream (H_2O). The outlet is connected to a glass capillary where the reaction can be followed via SAXS. Fig. 2 shows the different streams in the channel flow, where fluorescein and rhodamine B are used to show the two still separated focused streams in the glass capillary to prove the 3D-focusing/buffer stream design.

2.1.3. Sample preparation

For the SAXS measurements a poly(isoprene-*b*-ethylene glycol) block copolymer was used with a molecular weight of $M_w = 7411$ g/mol and block lengths of 70 and 60 monomer units respectively. The polydispersity of PI_{70}-PEG_{60} is $M_w/M_n = 1.08$. The polymer was synthesized via sequential living anionic polymerization in THF. First the polyisoprene block was synthesized. Therefore, the polymerization of the purified isoprene was initiated with *s*-BuLi. The reaction was terminated with dried ethylene oxide (EO). The PI-OH homopolymer was then activated with diphenylmethyl potassium (DPMK), and then ethylene oxide was condensed into the PI/THF solution. After polymerization the living polymer anions were reacted with acetic acid, and the polymer subsequently precipitated in cold acetone and dried to constant weight. The block copolymer molecular weight (derived from a drawn PI-precursor) and polydispersity were determined by SEC (THF, PI-calibration) and ^{1}H NMR. The microstructure of the PI-block is 11% trans-1,4-addition, 61% 3,4-addition and 28% 1,2-addition. For the experiments, the block copolymer was dissolved in 1,4-dioxane.

2.1.4. Small-angle X-ray scattering

All experiments were performed at the PETRA III MiNaXs Beamline P03 at DESY/HASYLAB or at the in-house rotating anode setup, GANESHA (SAXSLAB). For the measurements in the polyimide device at PETRA III, the beam size was 31 μm in *x*-direction and 22 μm in *y*-direction and had a wavelength of $\lambda = 0.1088$ nm. For the SAXS measurements with the SIFEL-glass-capillary microfluidic chip at PETRA III the wavelength was $\lambda = 0.957$ nm and the beam size was 25 μm in *x*-direction and 15 μm in *y*-direction. For detection a Pilatus 1 M or 300 K (DECTRIS) was used with a pixel size of 172 μm × 172 μm. The sample was placed at a distance of 3.53m-4m to the detector between which an evacuated tube was positioned. The microfluidic chip or the glass capillary was positioned with precision motors in x-, y-, z-direction.