

Nano-Donuts from pH-Dependent Block Restructuring in Amphiphilic ABA Triblock Copolymer Vesicles at the Air-Water Interface

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1. Experimental

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1. Experimental

1.1 Materials.

Poly(2-vinylpyridine)-*block*-poly(d-styrene)-*block*-poly(2-vinylpyridine) (P2VP-*b*-dPS-*b*-P2VP) triblock copolymer used for this work was synthesized via anionic polymerization¹ and its characteristics are summarized in Table S1. Freshly distilled toluene was used as a spreading solvent for the LB experiments, and Milli-Q water (>18 M Ω -cm at 25°C) was used as subphase. Hydrogen chloride (HCl) and sodium hydroxide (NaOH) of ACS reagent grade were used for pH adjustment in water subphase.

Table S1. P2VP-*b*-dPS-*b*-P2VP triblock copolymers used in this experiment.

Polymer	Total M_n (g/mol)($\times 10^3$)	PDI (M_w/M_n)	N_{dPS}	N_{P2VP}	Composition (dPS:P2VP)
P2VP- <i>b</i> -dPS- <i>b</i> - P2VP	TB125K	1.4	558	595	1:1

1.2 Langmuir-Blodgett (LB) monolayer

A KSV-2000 system (KSV Instruments, Finland) was used to investigate surface properties and stability of the polymer. Without any spreading solution, several suction operations were done to clean the pure water surface for impurity removal during every compression process, where two teflon-coated mobile barriers move inward to maximum position. Instead of chloroform which is well known as a spreading solvent, toluene (1mg/ml; a good solvent for the PS blocks) was used to dissolve the triblock copolymer. The spreading solution was filtered using PTFE filters (pore size: 0.22 μ m) prior to usage. A solution of the polymer, 50 μ l (1.0 mg/mL), was carefully dropped with 5-7 μ l each droplet in several spots on the water surface to make it well-dispersed on the LB trough. The solvent was then allowed to evaporate for about 15 min prior to compression. For π -A isotherms the barrier speed was set at a speed of 5 mm/min in all experiments. The ionization constant for pyridine in solution (pKa=5.2) was assumed to be identical with that on the water surface.² Pyridine residues can be expected to be completely ionized (positively charged) at pH 2.67, which is enough to submerge the outer P2VP blocks into water subphase. On the other hand, all the pyridine units on the P2VP chain are water-insoluble and surface-active at the interface being in their neutral forms at high pH (12.9).

After investigation of the langmuir monolayers, LB deposition conditions were optimized to make films for AFM imaging analysis. For the LB deposited films, clean SiO₂ substrates were prepared using the following cleaning procedure: The substrates were sonicated in acetone (15 min), milli-Q water (15 min), and isopropanol (10 min), dried under a stream of N₂ and then immersed into piranha solution, (70/30 by volume H₂SO₄/H₂O₂) (CAUTION: strong oxidizing solution) for 50 min. They were then washed with H₂O, sonicated in isopropanol and milli-Q water, respectively, and dried in oven at 120 °C. In order to deposit LB films on the clean hydrophilic surfaces (SiO₂ substrate), two different surface pressures (2 and 8 mN/m) were applied and the triblock copolymer LB monolayer was transferred onto the substrates by pulling it out of the water subphase (the LB lift-up method). Lift-up speed was kept at a speed of 5 mm/min in all experiments.

1.3 Atomic Force Microscopy

Atomic Force Microscopy (Agilent Technologies, Agilent 5500 AFM/SPM System) was used to investigate surface morphologies and surface analysis. The AFM measurements were carried out with a piezoscanner capable of scanning an area of 9 \times 9 μ m² at room temperature. Commercially available tapping mode tips (Nano ProbeTM, SPM Tips) were used on cantilevers with a resonance frequency in the range of 290-410 kHz. All images (AFM topography, tapping mode) were flattened, filtered, and analyzed by using SPIP software (Scanning Probe Image Processor, Imagemet.com). All data in the dimensions of surface aggregates were collected and averaged by at least 20 measurements from the line profile of AFM images.

Reference

1. J. Alonzo, Z. Huang, M. Liu, J. W. Mays, R. G. Toomey, M. D. Dadmun and S. M. Kilbey, *Macromolecules*, 2006, **39**(24), 8434.
2. B. H. Chung, M. H. Choi, M. H. Ree, J. C. Jung, W. C. Zin and T. H. Chang, *Macromolecules*, 2006, **39**, 684.

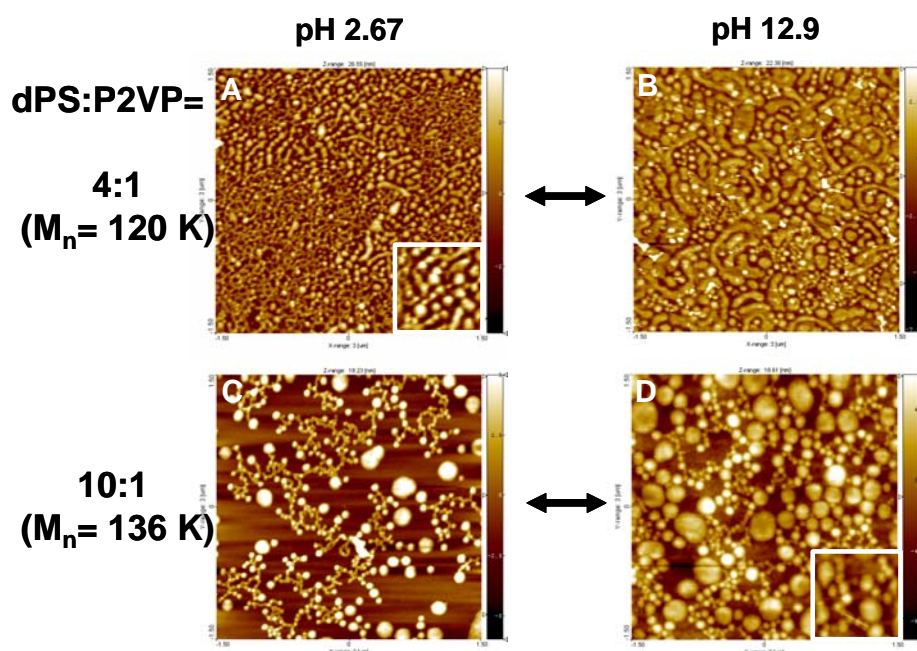


Fig. S1 AFM topographic images of the LB films ($M_n = 120K$, **A, B** and $M_n = 136K$, **C, D**) transferred at 2 mN/m with 50 μ l amounts of spread solution. (**A, C** at pH 2.67, and **B, D** at pH 12.9).

For TB120K (dPS:P2VP=4:1, 80% dPS block content) the LB film transferred at pH 2.67 formed the wormlike interconnected micelles (Figure S1-A) and partially network structure of aggregates were also observed. However, at high pH, the LB film formed as rod-shaped aggregates (4.22 ± 0.54 nm in average height) and partially circular micelles over the entire area (Figure S1-B). Although we show the specific area on the LB film via AFM images, where two aggregates are coexistent, the surface morphology at a mostly wide area of other AFM scanings was observed to be mostly long rod-shaped aggregates (spaghetti shape). At low pH (2.67), upon increasing the PS block content to 94% (TB136K), the LB film represents distinctly extraordinary surface morphological behaviors (necklace-shaped micelles)(Figure S1-C). However, Figure S1-D shows the various sizes of planar aggregates (grains) and necklace-shaped aggregates at pH 12.9. Typically the high content (>94%) of PS blocks contributes to the formation of this shape at high pH, regardless of a type of block copolymer (di/triblock copolymer). Therefore, from these phenomena it is found that both ionization degree of P2VP outer blocks and composition ratio (in other words, the relative length of outer P2VP) might play important roles forming particular surface morphology.

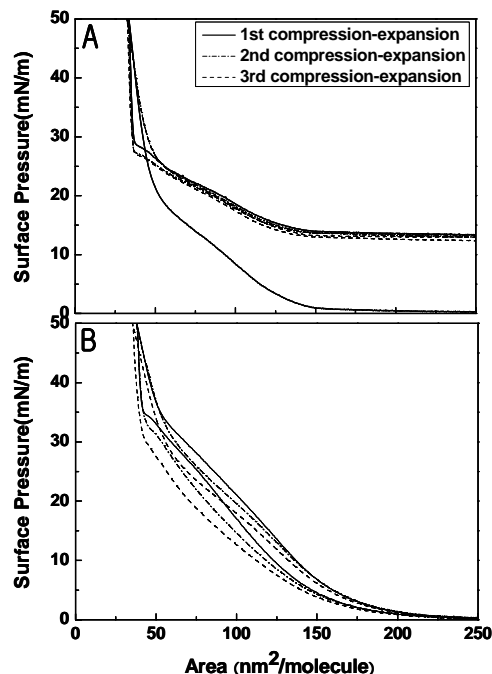


Fig. S2 The compression and expansion isotherms of P2VP-*b*-dPS-*b*-P2VP triblock copolymers ($M_n = 125K$, 1mg/ml) at **A**, pH 2.2 and **B**, pH 12.9. (compression and expansion speed: 5 mm/min, 295 K).

Hysteresis Measurements: Compression-expansion studies were performed to gain insight into the stability of the donut-shaped aggregates. After reaching a high surface pressure ($\pi=50$ mN/m) with compression, the monolayer was decompressed back to the maximum area position. The surface pressure for the film at pH 2.2 (when the barriers move back to the maximum area position after first compression) retained a value of 13.5mN/m and not to zero. This irreversible process represents the fact that the monolayer undergoes rearrangement phenomenon in which spherical particles are formed with the ionized P2VP primarily in the water subphase. On second and third expansion, the surface pressure returns to the identical value, possessed at maximum area position after the first decompression at 13.5 mN/m. This indicated that the rearrangement produced a more stable film in which a surface energy balance is achieved between aggregation of the dPS core and ionized P2VP electrostatic interaction. On the contrary, at pH 12.9 the monolayer shows some hysteresis with compression-expansion cycles. The plot shows the same initial surface pressure (0mN/m) at the maximum position despite several compression/expansion cycles. This indicates that the monolayer is very stable to begin with and that even with compression to a high surface pressure, the surface interaction is a reversible process. Since the P2VP blocks are surface-active at high pH and by AFM was confirmed to form donut-shaped aggregates, the stability is essentially obtained even at the beginning of compression (1st cycle).

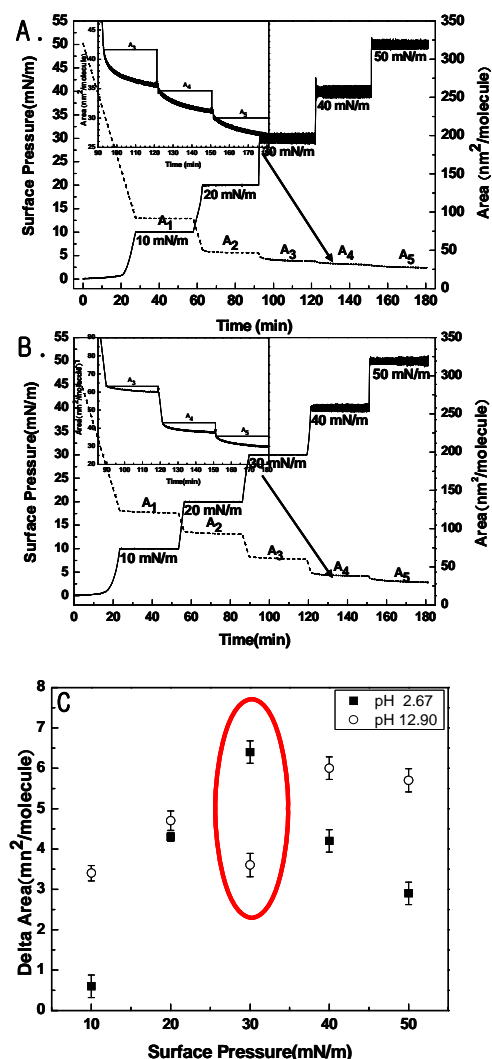


Fig. S3 The kinetic study of the P2VP-*b*-dPS-*b*-P2VP monolayer ($M_n = 125K$) at (a) pH 2.67, (b) pH 12.9 at 295 K and (c) area change per molecule at various pressures (10, 20, 30, 40, and 50 mN/m) at two different pHs.

Isobaric creep measurements and Kinetic studies of the LB monolayers: The LB films were compressed to the specified surface pressures (10, 20, 30, 40, and 50 mN/m) and was held for 30 min while monitoring the change in mean molecular area. These experiments were performed at low (2.67) and high (12.9) pH to examine the major influence of the hydrophilic P2VP units on the stability of the surface films. When compressed up to 30 mN/m, the observable barrier shift (6.5 nm² per molecule) is a maximum at the low pH film, compared to the film at higher pH. This indicates that much more P2VP outer blocks are lost into the water subphase and rearranged with highly packed spherical particle formation (as seen by AFM) at this surface pressure. However, despite the same surface pressure, the mean molecular area at pH 12.9 represents almost a minimum in occupied area per molecule (3.6 nm² per molecule). Thus this confirms the relatively higher stability for this Langmuir monolayer. In this case, the surface-active P2VP outer blocks provide greater stability under the given surface pressure in a nano-donut shaped particle compared to the spherical particles.

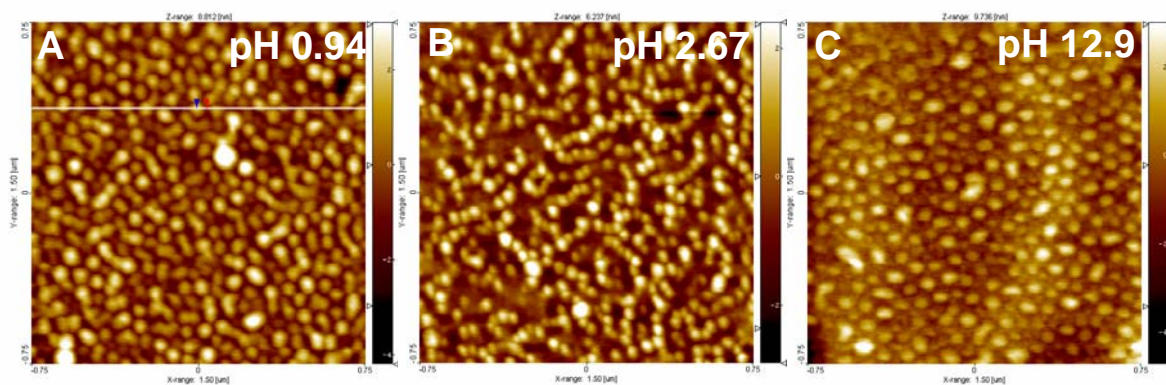


Fig. S4 AFM topographic images of the LB films transferred at 8 mN/m under **A.** pH 0.94, **B.** pH 2.67, and **C.** pH 12.9.

The LB samples were obtained as transferred at 8 mN/m under different pH conditions (0.94, 2.67, and 12.9). At pH 2.67 a morphological difference shows that the aggregates consist of pearl necklace-shaped interconnected micelles. Such intermicellar distance is dramatically reduced along with necklace-shaped micellar conformation since the area occupied by surface active P2VP corona at the air-water interface decreases due to their complete submersion into water at low pH, i.e. increase in solubility on hydrophilic blocks. As the pH decreases down to 0.94, the necklaced surface micelles disappeared and the surface density of such micelles significantly increases, compared to the other pHs. At a high pH, the donuts seem to coalesce to form a uniform field of corona surrounded by a shell.