Liposome Functionalization

International Edition: DOI: 10.1002/anie.201511785
German Edition: DOI: 10.1002/ange.201511785

Anisotropic Self-Assembly of Citrate-Coated Gold Nanoparticles on Fluidic Liposomes

Kouta Sugikawa, Tatsuya Kadota, Kazuma Yasuhara, and Atsushi Ikeda**

Abstract: *The behavior of self-assembly processes of nanoscale particles on plasma membranes can reveal mechanisms of important biofunctions and/or intractable diseases. Self-assembly of citrate-coated gold nanoparticles (cAuNPs) on liposomes was investigated. The adsorbed cAuNPs were initially fixed on the liposome surfaces and did not self-assemble below the phospholipid phase transition temperature (T_m). In contrast, anisotropic cAuNP self-assembly was observed upon heating of the composite above the T_m, where the phospholipids became fluid. The number of self-assembled NPs is conveniently controlled by the initial mixing ratio of cAuNPs and liposomes. Gold nanoparticle protecting agents strongly affected the self-assembly process on the fluidic membrane.*

The interaction of inorganic nanoparticles (NPs) with phospholipid membranes is a central aspect of targeted drug-delivery, nanotoxicity studies, and functional hybrid materials that combine liposome softness with the hard colloidal properties of NPs. Thus, there have been many studies on detailed interactions of phospholipid membranes and NPs.[1–9] However, except for a few theoretical studies, self-assembly of NPs on lipid membranes has not been adequately investigated.

Assembly of biomacromolecules (such as proteins, DNA, and viruses) on lipid membranes has been extensively studied.[10] Lipid membranes are generally very flexible, and under thermal perturbation they undergo deformations that may induce interactions with membrane-bound particles. Cacciuto and Saric have revealed that, for a wide range of bending rigidities, NPs on fluid membranes theoretically self-assemble in a linear fashion when the binding energy between NPs is high enough.[11] Furthermore, Wang et al. have shown experimentally that charged NPs can self-assemble into anisotropic structures when the sum of attractive van der Waals and dipolar interaction forces overcome repulsion between NPs.[12] However, in most experimental studies stable anionic NPs have been used, which exhibit mutual strong electrostatic repulsion on liposome surfaces. The electrostatic repulsion of NPs actually inhibited adhesion between liposomes and enhanced their stabilization.[2,8,13] Thus, it was assumed that the negligible binding energy between NPs cannot be used for self-assembly on fluid lipid membranes.

Herein, we describe use of citrate-coated AuNPs (cAuNPs) to facilitate anisotropic self-assembly enabled by fluidization of lipid membranes. Liposomes were decorated with cAuNPs by simply mixing both components, as previously reported for anionic NP-liposome systems.[1,2,8] Anisotropic self-assembly was induced by heating of the lipid membrane up to the fluid-phase transition temperature (T_m). The self-assembly process was extensively characterized with UV/Vis absorption spectra and cryogenic transmission electron microscopy (cryo-TEM). Furthermore, the relationship between fluidization of the lipid membrane and NP self-assembly was investigated with liposomes possessing a range of T_m values. Finally, we used AuNPs coated with α-lipoic acid to examine how protecting agents affect AuNP self-assembly.

The cAuNP-liposome composites were prepared as follows. Liposomes composed of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC, $T_m = 41\,°C$; Figure 1a) were prepared by extrusion (see Experimental Section for details).[14]

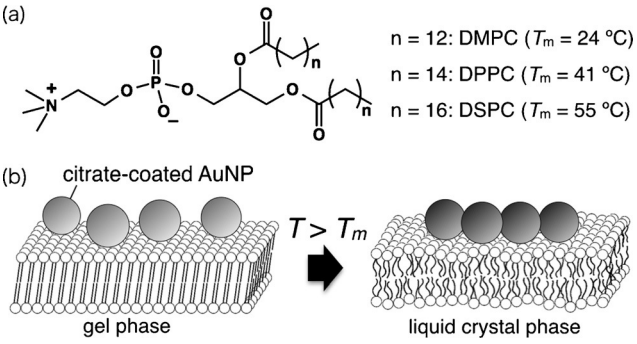

Figure 1. a) Phospholipid structures and their respective phase transition temperatures (T_m). b) Anisotropic self-assembly of cAuNPs on liposomes induced by the gel-liquid phase transition of the lipid membrane.

The average diameter of extruded material was 105 nm, as determined by TEM (Supporting Information, Figure S1a). cAuNPs were synthesized according to a modified method reported by Frens,[15] and were approximately 14.1 nm in size, as determined by TEM (Supporting Information, Figure S1b). To induce cAuNP adsorption on liposomes, cAuNPs and DPPC liposomes were mixed at specific ratios ([AuNP]/[liposome] = 0–8.0) at 25 °C in water. After incubation for several hours the red solution formed sediment, which

[*] Dr. K. Sugikawa, T. Kadota, Prof. A. Ikeda
Graduate School of Engineering, Hiroshima University
Higashi-Hiroshima 739-8527 (Japan)
E-mail: aikeda@hiroshima-u.ac.jp
Dr. K. Yasuhara
Graduate School of Materials Science, Nara Institute of Science and Technology
Nara 630-0192 (Japan)

Supporting information for this article can be found under http://dx.doi.org/10.1002/anie.201511785.

could be reversibly dispersed by gentle mixing (for [AuNP]/[liposome] = 4.0 see the Supporting Information, Figure S2a). These observations indicate formation of cAuNP-DPPC composites. Structural information was obtained with cryo-TEM imaging, revealing DPPC liposomes decorated with various amounts of cAuNPs (Figure 2a,b; Supporting Information, Figure S3). The number of cAuNPs adsorbed per liposome quantitatively agreed with the [AuNP]/[liposome] ratio calculated from the sample composition (Figure 2c). Therefore, in this concentration range, essentially all cAuNPs added to the solution were adsorbed on a lipid membrane.[2] Notably, the average diameter of liposomes decorated with cAuNP remained at 105 nm, even after 1 week of incubation at room-temperature.

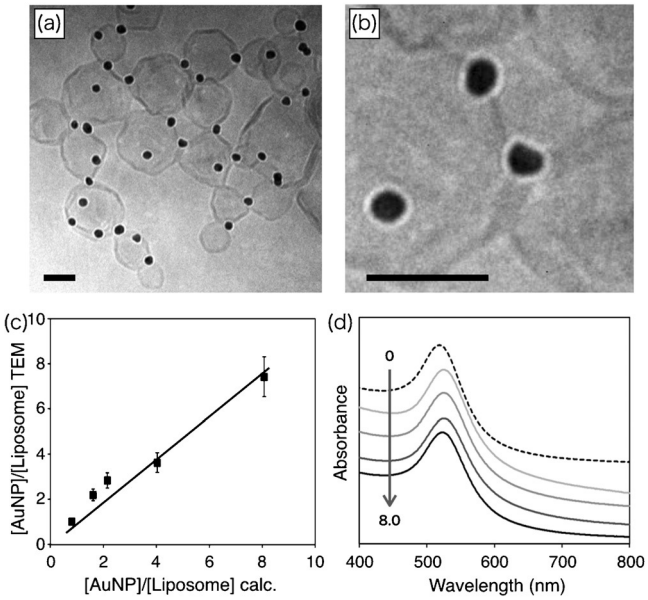

Figure 2. Cryo-TEM images of a) cAuNP-DPPC composites prepared at a [AuNP]/[liposome] ratio of 4.0 at 25 °C (pH 6.5). b) Magnified Cryo-TEM image; 50 nm scale bar. c) Average number of adsorbed cAuNPs per liposome as a function of [AuNP]/[liposome] ratio. Error bars show standard deviations. d) UV/Vis spectra of cAuNP-DPPC composite solutions at 25 °C prepared with [AuNP]/[liposome] ratios of 0.8, 1.6, 2.2, 4.0, and 8.0, at pH values of 6.0, 6.5, 6.6, 6.5, and 6.5, respectively.

Considering the rigidity of the DPPC bilayer[16] and the cAuNP diameter (larger than the 5 nm thick lipid bilayer), it is reasonable to assume that cAuNP will not fuse with the lipid bilayer but will remain adsorbed on the surface.[7,9,11] This deduction was confirmed by cryo-TEM observations and differential scanning calorimetry (DSC) measurements. Previously reported, fusion of the lipid bilayer with cAuNP[5] was not observed in high-magnification cryo-TEM images (Figure 2b). Furthermore, no change in the DSC thermograms of DPPC liposomes was observed in the presence of cAuNPs (Supporting Information, Figure S4), also indicating that AuNPs did not fuse with the liposomes.[5,6] Adsorption of anionic NPs onto zwitterionic liposomes is most likely a result of electrostatic attraction with the positively charged N^+ in the P^-–N^+ zwitterionic moiety located perpendicular with respect to the membrane surface.[2,4,6,13,17] To test this notion, we attempted to adsorb cAuNP onto anionic DPPC liposomes containing 5 mol% anionic 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) sodium salt (DPPG). Over 86% of cAuNPs did not adsorb onto the anionic liposomes, as confirmed by cryo-TEM imaging (Supporting Information, Figure S5). This observation suggests that cAuNP electrostatically adsorbs onto the zwitterionic liposome.

The cryo-TEM images in Figure 2a,b and Figure S3 (Supporting Information) revealed isolated cAuNPs that did not self-assemble on the liposome surface. This deduction is strongly supported by UV/Vis spectra of the cAuNP-DPPC solution, where a 520 nm peak attributed to an AuNP plasmon resonance is red-shifted by 10 nm (Figure 2d), indicating a change in the surrounding environment of the cAuNPs.[18] Further red-shifting was not observed when the [AuNP]/[liposome] ratio was increased to 8.0 (Figure 2d). Furthermore, in the absence of liposomes, cAuNPs aggregated immediately upon addition of NaCl because of the salting-out effect (Supporting Information, Figure S6a).[19] By contrast, in the presence of liposomes no change was observed in the UV/Vis spectra of cAuNP solutions (Supporting Information, Figure S6b) after addition of NaCl. These results indicate that cAuNPs adsorbed on the lipid membranes were fixed, preventing further self-assembly below the T_m.

The effect of cAuNPs on liposome stability was also investigated. When cAuNPs were added to liposome dispersions, red precipitates were observed in a few hours (Supporting Information, Figure S7), suggesting that adsorption of cAuNPs onto the bilayer surface promotes their destabilization. Furthermore, the destabilization effect of cAuNPs increased with larger concentrations of cAuNPs. This result is in good agreement with cryo-TEM images that reveal cAuNPs bridging liposomes. In contrast, when anionic AuNPs coated with α-lipoic acid by Au–S bonds (αAuNPs) were added to a liposome dispersion,[20] destabilization of liposomes was observed for low concentrations of αAuNPs, while stabilization was observed at high concentrations (Supporting Information, Figure S7). This characteristic has also been observed for anionic NP-liposome systems, where electrostatic repulsion caused by adsorbed anionic NPs leads to vesicle stabilization.[2] These results indicate that cAuNPs adsorbed on lipid bilayer surfaces do not create enough electrostatic repulsion to inhibit liposome aggregation. They are also consistent with recent experimental and theoretical results that indicate that steric repulsion of the citrate layer, rather than electrostatic repulsion, plays a critical role in the stability of AuNPs.[21,22]

When the cAuNP-DPPC solution was heated above the T_m (41.5 °C), the solution gained a purple hue (Supporting Information, Figure S2b). The intensity of the 520 nm plasmon band also decreased and a new band appeared at 615 nm in the UV/Vis spectra (Figure 3a). This change is attributed to electric dipole–dipole interactions and coupling between plasmons of neighboring gold NPs in assemblies.[23] Furthermore, the appearance of two separate peaks indicates formation of an anisotropic AuNP assembly.[12,24,25] However,

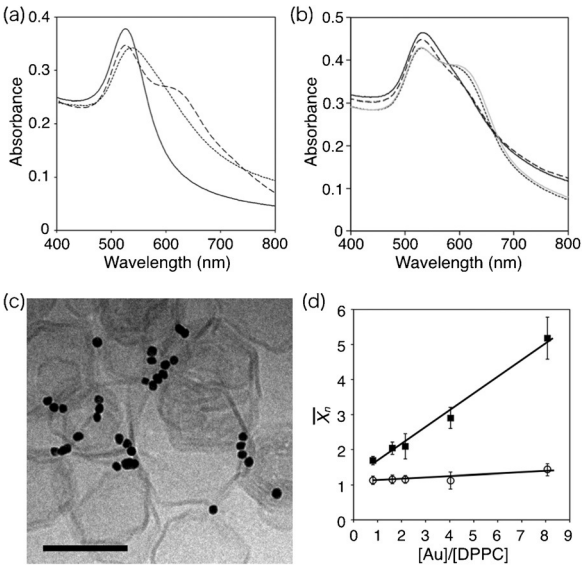

Figure 3. a) UV/Vis absorption spectra of a cAuNP-DPPC solution ([Au]/[DPPC] = 4.0) at 25 °C ((——), pH = 6.5) and at 50 °C in the absence ((-----), pH = 6.2) and presence ((•••••), pH = 6.2) of NaCl (50 mM). b) Spectra of the same solution at 50 °C at pH values of 5.0 (——), 6.1 (-----), 9.5 (•••••), and 10.3 (——). c) Cryo-TEM image of cAuNP-DPPC composites prepared at a [Au]/[DPPC] ratio of 4.0 at 50 °C ([NaCl] = 0 mM, pH = 9.2); 100 nm scale bar. d) Average number of NPs in each aggregated structure (n) as a function of the [AuNP]/[liposome] ratio at 25 °C (○) and 50 °C (■). The pH values of the cAuNP-DPPC composite solutions with [AuNP]/[liposome] ratios of 0.8, 1.6, 2.2, 4.0, and 8.0 were 6.0, 6.5, 6.6, 6.5, and 6.5, respectively. Error bars show standard deviations.

upon heating above the T_m of the lipid membrane; 3) The number of NPs in the self-assemblies can be controlled by the initial concentration of cAuNPs, because all NPs were stably adsorbed on the liposomes.

To further understand the effect of lipid membrane fluidization on cAuNP self-assembly, variation in the 595 nm absorbance of cAuNP-DPPC composites was recorded as a function of temperature (Figure 4; Supporting Information, Figure S11 b). Particles were well-dispersed

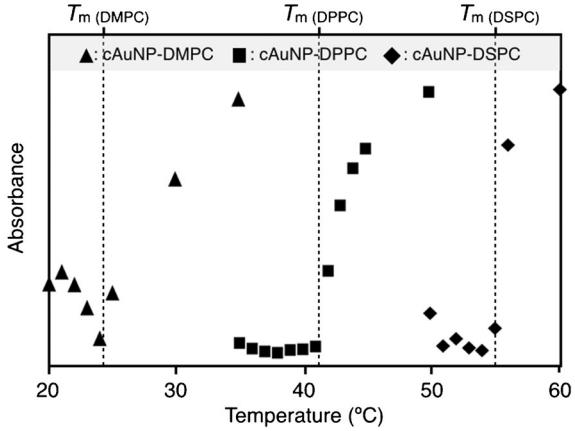

Figure 4. Absorbance of cAuNP-DMPC, -DPPC, and -DSPC composite solutions ([Au]/[phospholipid] = 0.28) at 600 nm, 595 nm, and 587 nm, respectively, as a function of temperature. Dashed vertical lines indicate the transition temperatures of DMPC, DPPC, and DSPC liposomes. The pH values of cAuNP-DMPC, -DPPC, and -DSPC composite solutions were 6.2, 6.2, and 6.7, respectively.

the two peaks gradually coalesce into one broadened peak when NaCl is added to the solution (Figure 3 a). The broad, intense, red-shifted peak indicates an isotropic assembly.[25,26] Finally, the absorbance at 615 nm increased with pH (Figure 3 b), implying decreased repulsion between NPs under basic conditions.[12] All of these results clearly indicate a correlation between the growth of the anisotropic structure and the surface state of cAuNPs.

As seen in the cryo-TEM images, NPs clearly self-assemble into linear structures in the middle and at the edges of liposomes in the absence of NaCl (Figure 3 c; Supporting Information, Figure S9). In contrast, assemblies formed in the presence of NaCl were isotropic close-packed two-dimensional (2D) or three-dimensional (3D) structures (Supporting Information, Figure S8), in good agreement with UV/Vis spectra (Figure 3 a).

Quantitative analysis of the anisotropic self-assemblies revealed that the number of NPs adsorbed per liposome remained constant, even after heating (Supporting Information, Figure S10). Furthermore, the average number of cAuNPs in each assembly ($\bar{X}_n$) stayed in the range 1.0–1.2 at 25 °C, despite the fact that the number of adsorbed cAuNPs increased linearly with the [AuNP]/[liposome] ratio at 50 °C (Figure 3 d). These results indicate three important facts: 1) NPs adsorbed on a lipid bilayer never leave the liposome surface during self-assembly; 2) NPs adsorbed on lipid membranes self-assemble anisotropically (not randomly) below 41 °C, suggesting that NP assembly does not occur below the T_m. Absorbance increased rapidly above 42 °C. The critical temperature for cAuNP assembly could be controlled by changing the lipids in the membrane (Figure 4; Supporting Information, Figure S11). When DMPC (1,2-diimyristoyl-*sn*-glycero-3-phosphocholine) or DSPC (1,2-distearoyl-*sn*-glycero-3-phosphocholine) liposomes (Figure 1 a) were used, the critical temperature for AuNP assembly was identical to the respective 24 °C and 55 °C T_m values. When lipids with a T_m much lower than room-temperature were used, such as egg phosphatidylcholine and DLPC (1,2-dilauroyl-*sn*-glycero-3-phosphocholine), NP anisotropic self-assembly occurred soon after mixing cAuNPs and liposomes in water at 25 °C (Supporting Information, Figure S12). Hence, fluidization of the lipid membrane is an essential requirement for anisotropic cAuNP self-assembly.[11]

When mixing is performed above the lipid phase transition temperature, prolonged contact between particles and fluid membranes might lead to internalization (endocytosis) instead of adsorption. However, internalization occurs on a much longer time scale than the short contact periods described here, and can therefore be ignored.[1,9,27]

Finally, we investigated the effect of protecting agents upon self-assembly of AuNPs on liposomes. αAuNPs were mixed with DPPC liposomes to obtain αAuNP-DPPC composites. Cryo-TEM images revealed αAuNPs adsorbed on liposomes, similar to the examples of cAuNP adsorption

described above, and that of other reported anionic NPs (Supporting Information, Figure S13a). However, after heating the solution above the T_m, self-assembly of αAuNPs on the liposome surface was not observed (Supporting Information, Figure S13b). This was confirmed with UV/Vis spectra (Supporting Information, Figure S14). These results indicate that protecting agents can strongly affect the linear self-assembly of AuNPs (Figure 1b).

Self-assembly of charged NPs into one-dimensional (1D) structures can be understood in terms of repulsive (V_{rep}), attractive van der Waals (V_{vdW}), and attractive dipolar interaction potentials (V_{dip}).[12,28] Using Derjaguin–Landau–Verwey–Overbeek (DLVO) theory, Zhang et al. revealed that a decrease in V_{rep} and an increase of attractive V_{dip} are essential for the anisotropic self-assembly of NPs. The value of V_{dip} for citrate-coated AuNPs is usually negligible because citrates form stable and uniform structures on AuNP surfaces in water. Therefore, weakening V_{rep} between cAuNPs by adding salts usually causes random aggregation, eventually forming precipitates with irregular shapes. However, once the uniform structure of citrate is deformed by adsorption of molecules or exchange of protecting ligands, the dipole moment can be greatly enhanced.[12,26,29] Enhancement of V_{dip} often induces anisotropic assembly of NPs, especially when V_{rep} decreases. Shumaker-Parry and Park recently reported that steric repulsion of the citrate layer on AuNP (rather than electrostatic repulsion) is critical for cAuNPs stability,[21] which explains the immediate aggregation and precipitation of cAuNPs at high pH. In corroboration, our experimental findings demonstrate increased anisotropic self-assembly of AuNPs at higher pH (Figure 3b). Zhang et al. also reported that NPs start to attach to the side of NP chains to generate 2D or 3D structures when V_{rep} between NPs and the sides of NP chains becomes weaker than V_{vdW}.[12] This assessment is consistent with our experimental results, where addition of NaCl induces random isotropic assembly of cAuNPs (Figure 3a; Supporting Information, Figure S8).

On the basis of the reported theory and our experimental results, we propose the following mechanism for anisotropic self-assembly of cAuNPs on lipid membranes (Figure 5). cAuNPs are stable in water because of steric repulsion between citrate molecules (Figure 5a).[21] When NPs interact with the lipid membrane the citrate layer deforms and the dipole moment is greatly enhanced. As a result, attractive forces determined by the sum of V_{vdW} and V_{dip}, overcome V_{rep}, because the latter is weakened on the lipid membrane. A weak V_{rep} is confirmed by improved stability of cAuNPs, and leads to coupling of neighboring NPs. The anisotropic character of the dipolar interaction translates to anisotropic coupling of AuNPs as well. Below the T_m, cAuNPs are disposed toward self-assembly but are prevented from doing so because of the rigid gel phase of the lipid membrane (Figure 5b). This is confirmed by UV/Vis spectra in Figure 2d and Figure S4 (Supporting Information). Once the membrane is warmed above the T_m, AuNP mobilities are increased and they are able to self-assemble into anisotropic structures (Figure 5c,d).

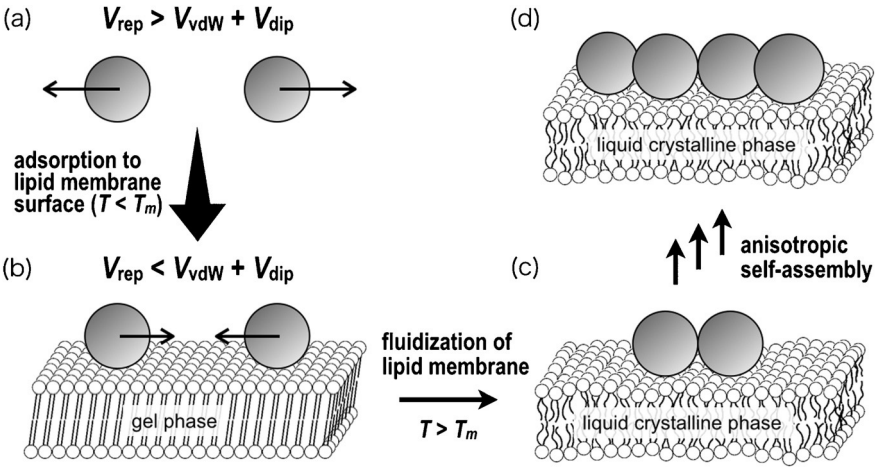

Figure 5. Adsorption and aggregation of cAuNPs on a zwitterionic lipid membrane surface.

In conclusion, we have decorated liposomes with cAuNPs by adsorption in water. The number of NPs adsorbed on the liposomes was controlled by simply altering the NP/liposome mixing ratio. When the lipid membrane was heated above the T_m, the adsorbed AuNPs self-assembled into anisotropic structures. Self-assembly was sensitive to fluidization of the lipid membrane, and the T_m was easily adjusted by changing the lipid components. We also investigated the effect of protecting agents on the self-assembly of AuNPs and revealed that cAuNP destabilization on the liposome was key to linear self-assembly. In summary, these results probe the fundamental interactions between NPs and liposomes, and reveal that liposomes are flexible scaffolds for controlled 1D self-assembly of NPs. Overall, these processes should be applicable to other self-assemblies of nanoscale adsorbents on liposomes.

Acknowledgements

This work was supported by the Electric Technology Research Foundation of Chugoku and the Grant-in-Aid for Scientific Research (B) (Grant no. 25288037). The authors would like to express their deepest gratitude to S. Fujita, Graduate School of Materials Science, Nara Institute of Science and Technology for providing technical assistance with the cryo-TEM imaging.

Keywords: gold nanoparticles · lipid bilayer · liposomes · phase transition temperature · self-assembly

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 4059–4063
Angew. Chem. **2016**, *128*, 4127–4131

[1] R. Michel, E. Kesselman, T. Plostica, D. Danino, M. Gradzielski, *Angew. Chem. Int. Ed.* **2014**, *53*, 12441 – 12445; *Angew. Chem.* **2014**, *126*, 12649 – 12653.
[2] R. Michel, T. Plostica, L. Abezgauz, D. Danino, M. Gradzielski, *Soft Matter* **2013**, *9*, 4167 – 4177.
[3] a) J. W. Liu, E. Luijten, *Phys. Rev. Lett.* **2004**, *93*, 247802; b) D. Pornpattananangkul, S. Olson, S. Aryal, M. Sartor, C.-M. Huang, K. Vecchio, L. Zhang, *ACS Nano* **2010**, *4*, 1935 – 1942; c) M. Schulz, A. Olubummo, W. H. Binder, *Soft Matter* **2012**, *8*, 4849 – 4864; d) C. Kojima, Y. Hirano, E. Yuba, A. Harada, K. Kono, *Colloids Surf. B* **2008**, *66*, 246 – 252.
[4] a) T. Cha, A. Guo, X. Y. Zhu, *Biophys. J.* **2006**, *90*, 1270 – 1274; b) I. Hoffmann, R. Michel, M. Sharp, O. Holderer, M. S. Appavou, F. Polzer, B. Farago, M. Gradzielski, *Nanoscale* **2014**, *6*, 6945 – 6952.
[5] S. Savarala, S. Ahmed, M. A. Ilies, S. L. Wunder, *Langmuir* **2010**, *26*, 12081 – 12088.
[6] S. Savarala, S. Ahmed, M. A. Ilies, S. L. Wunder, *ACS Nano* **2011**, *5*, 2619 – 2628.
[7] a) T. Hamada, M. Morita, M. Miyakawa, R. Sugimoto, A. Hatanaka, M. C. Vestergaard, M. Takagi, *J. Am. Chem. Soc.* **2012**, *134*, 13990 – 13996; b) J. Agudo-Canalejo, R. Lipowsky, *ACS Nano* **2015**, *9*, 3704 – 3720.
[8] S. Thamphiwatana, V. Fu, J. Y. Zhu, D. N. Lu, W. W. Gao, L. F. Zhang, *Langmuir* **2013**, *29*, 12228 – 12233.
[9] A. H. Bahrami, M. Raatz, J. Agudo-Canalejo, R. Michel, E. M. Curtis, C. K. Hall, M. Gradzielski, R. Lipowsky, T. R. Weikl, *Adv. Colloid Interface Sci.* **2014**, *208*, 214 – 224.
[10] a) H. Aranda-Espinoza, A. Berman, N. Dan, P. Pincus, S. Safran, *Biophys. J.* **1996**, *71*, 648 – 656; b) M. Goulian, R. Bruinsma, P. Pincus, *Europhys. Lett.* **1993**, *22*, 145 – 150.
[11] A. Saric, A. Cacciuto, *Phys. Rev. Lett.* **2012**, *108*, 118101.
[12] H. Zhang, D. Wang, *Angew. Chem. Int. Ed.* **2008**, *47*, 3984 – 3987; *Angew. Chem.* **2008**, *120*, 4048 – 4051.
[13] Y. Yu, S. M. Anthony, L. Zhang, S. C. Bae, S. Granick, *J. Phys. Chem. C* **2007**, *111*, 8233 – 8236.
[14] a) Y. Doi, A. Ikeda, M. Akiyama, M. Nagano, T. Shigematsu, T. Ogawa, T. Takeya, T. Nagasaki, *Chem. Eur. J.* **2008**, *14*, 8892 – 8897; b) A. Ikeda, T. Hida, T. Nakano, S. Hino, K. Nobusawa, M. Akiyama, K. Sugikawa, *Chem. Lett.* **2014**, *43*, 1551 – 1553.
[15] a) K. C. Grabar, R. G. Freeman, M. B. Hommer, M. J. Natan, *Anal. Chem.* **1995**, *67*, 735 – 743; b) G. Frens, *Nat. Phys. Sci.* **1973**, *241*, 20 – 22.
[16] a) N. Delorme, A. Fery, *Phys. Rev. E* **2006**, *74*, 030901; b) C. H. Lee, W. C. Lin, J. P. Wang, *Phys. Rev. E* **2001**, *64*, 020901.
[17] B. Seantier, B. Kasemo, *Langmuir* **2009**, *25*, 5767 – 5772.
[18] a) M. R. Dewi, G. Laufersky, T. Nann, *RSC Adv.* **2014**, *4*, 34217 – 34220; b) K. Sugikawa, Y. Furukawa, K. Sada, *Chem. Mater.* **2011**, *23*, 3132 – 3134; c) C. Yu, L. Varghese, J. Irudayaraj, *Langmuir* **2007**, *23*, 9114 – 9119.
[19] M. Y. Lin, H. M. Lindsay, D. A. Weitz, R. C. Ball, R. Klein, P. Meakin, *Nature* **1989**, *339*, 360 – 362.
[20] S. Y. Lin, Y. T. Tsai, C. C. Chen, C. M. Lin, C. H. Chen, *J. Phys. Chem. B* **2004**, *108*, 2134 – 2139.
[21] J.-W. Park, J. S. Shumaker-Parry, *J. Am. Chem. Soc.* **2014**, *136*, 1907 – 1921.
[22] a) S. Biggs, P. Mulvaney, C. F. Zukoski, F. Grieser, *J. Am. Chem. Soc.* **1994**, *116*, 9150 – 9157; b) P. Wagener, A. Schwenke, S. Barcikowski, *Langmuir* **2012**, *28*, 6132 – 6140.
[23] a) Y. Takeuchi, T. Ida, K. Kimura, *Surf. Rev. Lett.* **1996**, *3*, 1205 – 1208; b) U. Kreibig, L. Genzel, *Surf. Sci.* **1985**, *156*, 678 – 700; c) K. Liu, A. Lukach, K. Sugikawa, S. Chung, J. Vickery, H. Therien-Aubin, B. Yang, M. Rubinstein, E. Kumacheva, *Angew. Chem. Int. Ed.* **2014**, *53*, 2648 – 2653; *Angew. Chem.* **2014**, *126*, 2686 – 2691.
[24] a) E. Dujardin, L. B. Hsin, C. R. C. Wang, S. Mann, *Chem. Commun.* **2001**, 1264 – 1265; b) Z. Y. Zhong, S. Patskovskyy, P. Bouvrette, J. H. T. Luong, A. Gedanken, *J. Phys. Chem. B* **2004**, *108*, 4046 – 4052.
[25] L. Jiang, J. Guan, L. Zhao, J. Li, W. Yang, *Colloids Surf. A* **2009**, *346*, 216 – 220.
[26] S. Lin, M. Li, E. Dujardin, C. Girard, S. Mann, *Adv. Mater.* **2005**, *17*, 2553 – 2559.
[27] A. H. Bahrami, R. Lipowsky, T. R. Weikl, *Phys. Rev. Lett.* **2012**, *109*, 188102.
[28] C. Xi, P. F. Marina, H. Xia, D. Wang, *Soft Matter* **2015**, *11*, 4562 – 4571.
[29] P. Crespo, R. Litran, T. C. Rojas, M. Multigner, J. M. de la Fuente, J. C. Sanchez-Lopez, M. A. Garcia, A. Hernando, S. Penades, A. Fernandez, *Phys. Rev. Lett.* **2004**, *93*, 087204.

Received: December 20, 2015
Revised: January 26, 2016
Published online: February 23, 2016

