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Hydrophobic Actuation of a DNA Origami Bilayer Structure**

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Abstract: *Amphiphilic compounds have a strong tendency to form aggregates in aqueous solutions. It is shown that such aggregation can be utilized to fold cholesterol-modified, single-layered DNA origami structures into sandwich-like bilayer structures, which hide the cholesterol modifications in their interior. The DNA bilayer structures unfold after addition of the surfactant Tween 80, and also in the presence of lipid bilayer membranes, with opening kinetics well described by stretched exponentials. It is also demonstrated that by combination with an appropriate lock and key mechanism, hydrophobic actuation of DNA sandwiches can be made conditional on the presence of an additional molecular input such as a specific DNA sequence.*

The ability to induce large conformational changes within supramolecular assemblies in response to specific environmental conditions is highly desirable for a wide variety of applications in nanotechnology and biomedicine. For instance, controlled delivery systems that release compounds in response to an external trigger have been under intense investigation in pharmaceutical chemistry and nanomedicine over the past years. Intelligent release systems are seen as a promising area of application for DNA-based nanostructures, and recently a variety of switchable, container-like structures have been described.[1] We here introduce a novel hydrophobic switching mechanism for such DNA nanocontainers that utilizes the varying strength of the hydrophobic interactions of cholesterol moieties in different environments. Hydrophobic modifications of DNA molecules have been developed for a variety of different applications. Lipid–DNA conjugates have been used to facilitate cellular uptake,[2] to induce vesicle fusion,[3] and to build vesicle-based nanocontainers[4] and immobilize them on surfaces.[5] Recently, several groups attached DNA-based nanostructures to lipid bilayer membranes[6] and even created artificial DNA-based membrane channels.[7] Another motivation comes from structural DNA nanotechnology, where hydrophobic effects can expand the repertoire of interactions available for the construction of DNA-based nanostructures by self-assembly.[8] Structural switching of DNA nanostructures has been achieved by various means.[9] Next to the commonly used DNA strand displacement approach,[10] changes in pH,[11] photochromic *cis–trans* isomerization,[12] and aptamer-based allosteric mechanisms[11f,13] have been most often applied for the realization of DNA-based molecular switches.

Here, we utilize the strong aggregation of DNA–cholesterol conjugates in aqueous solution to introduce a "hydrophobic switching" mechanism for DNA-based nanostructures. Amphiphiles such as DNA–lipid conjugates and DNA block-copolymers with hydrophobic blocks have a strong tendency to associate into higher order structures such as micelles and bilayer membranes,[14] which poses a problem for some applications, while it may be desired in others. The hydrophobic switching concept is demonstrated here using twist-corrected single-layered DNA origami sheets[15] consisting of 24 parallel DNA double helices (dimensions 65 nm × 90 nm), which were modified with up to 35 cholesterol moieties (Figure 1 a). Locally, such a high concentration of cholesterol units exceeds the critical micelle concentration (CMC) of cholesterol-bearing amphiphiles by several orders of magnitude. The CMC of cholesterol alone is roughly 30 nM.[16] Attached to the origami sheet, the cholesterol units are confined to a cylinder with radius $r = 32.5$ nm and length

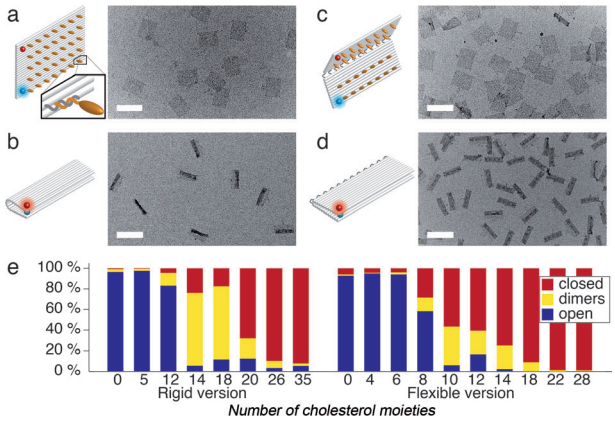

Figure 1. a) Schematic depiction and positive-stain TEM images of bare DNA origami sheets comprising 24 parallel double helices (nominal dimensions 65 nm × 90 nm). Also indicated are the positions of a donor (blue) and acceptor (red) dye used for FRET studies. Attached cholesterol-modified oligonucleotides are shown in orange. b) Folded origami structures, held together by hydrophobic interactions between 35 cholesterol moieties placed on one side, resulting in a 20 nm × 90 nm bilayer structure. c,d) Alternative origami bilayer structure with a flexible hinge along the long symmetry axis of the DNA sheet in its open (c) and closed conformation (d). e) Statistical analysis of TEM images of DNA origami sheets with different numbers of cholesterol modifications for both flexible and rigid design. Scale bars: 100 nm.

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$l = 90$ nm, or a volume of $V \approx 0.3$ aL, which for 35 moieties results in a concentration of about 200 μM. Hydrophobic interactions between the cholesterol units were found to be strong enough to induce bending of the origami sheet into structures with reduced dimensions of roughly 20 nm × 90 nm, as shown in the TEM micrograph in Figure 1b. This reduced size is consistent with the folding of the origami sheets along their long axis, that is, parallel to the axis of the constituent DNA helices. Importantly, we found that in the presence of a surfactant such as polyoxyethylene(20)sorbitan monooleate (Tween 80) DNA bilayer formation did not occur, as this apparently weakened the cholesterol-induced intra-origami interactions and replaced them with more favorable ones. TEM micrographs indicated that the surfactant did not denature or otherwise distort the DNA origami sheets.

In order to rationally direct the bilayer-folding process, we also generated hinged structures by introducing two additional thymidine spacers as flexible elements into the crossover connections between the two central helices. For the hinged origami sheets, the central row of cholesterol moieties had to be removed, resulting in a maximum number of 28 cholesterol units per sheet (Figure 1c,d). In the closed state (in the absence of surfactant), the flexible structures appeared slightly larger (ca. 26 nm × 90 nm) than the folded rigid structures. This supports the hypothesis that the flexible structures are folded along the central hinge, whereas the rigid structures are bent.

We statistically quantified the extent of bending or folding as a function of the number of cholesterol modifications and the flexibility of the sheets based on TEM images of a large number of DNA structures (Figure 1e). Instead of the more commonly used negative staining procedure, we applied positive staining for this analysis, which enabled identification and classification of individual single-layer structures. We observed almost no folded structures for small numbers of cholesterol moieties and an increased fraction of closed structures for larger numbers. With equal numbers of modifications, the hinged origami sheets were observed more often in the closed state than the rigid versions. Furthermore, rigid origami sheets tended to dimerize more often for intermediate numbers of cholesterol moieties. This finding is corroborated by the lower electrophoretic mobility observed for rigid origami sheets with an intermediate number of cholesterol units, potentially caused by origami oligomerization (Figure S6). Notably, imaging of cholesterol-modified origami sheets using atomic force microscopy on mica substrates resulted in a different apparent folding efficiency (Figure S7). We generally observed less closed structures than in TEM studies, and also a dependence on salt concentration, which indicates that electrostatic interactions between mica and the origami structures overwhelm the hydrophobic interactions within the bilayers.

In order to study surfactant-induced opening of folded origami bilayer structures unaltered by interactions with a charged surface, we performed fluorescence resonance energy transfer (FRET) experiments in solution. As indicated in Figure 1, to this end the structures were labeled with the fluorophores ATTO 532 and ATTO 647-N, which form a FRET pair with a Förster radius of about 6 nm. Formation

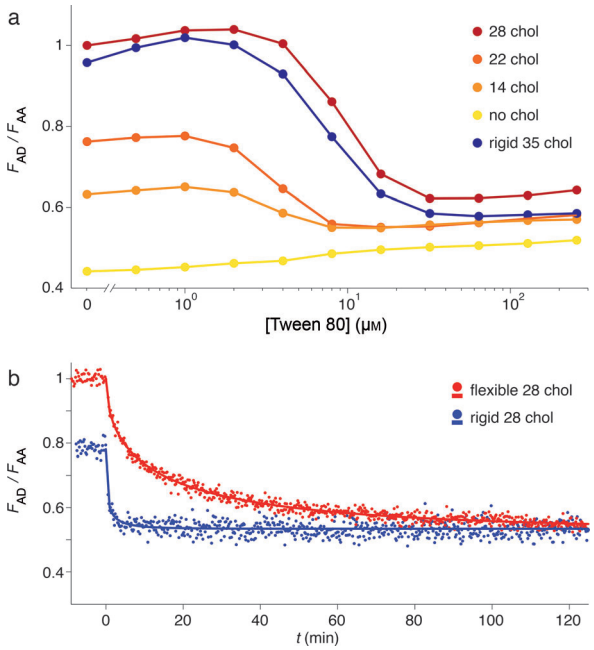

Figure 2. a) Opening of folded origami sheets with increasing concentration of surfactant Tween 80, measured by FRET (quantified by FRET-mediated acceptor emission F_{AD} normalized by the directly excited acceptor fluorescence F_{AA}). b) Kinetics of opening upon addition of 6 μM Tween at $t = 0$ for folded structures with and without a flexible hinge for the same number of cholesterol moieties. The opening process is described well by stretched exponential kinetics as indicated by the continuous lines (fit parameters: flexible: $\beta = 0.52$, $k = 0.069$ min; rigid: $\beta = 0.44$, $k = 1.91$ min).

of DNA bilayer sheets results in a high FRET efficiency, while opening the structures leads to a reduction of FRET. As shown in Figure 2a, the FRET signal sharply decreases above critical concentrations of Tween 80 in the micromolar range. The unfolding transition appeared steeper and occurred at slightly higher concentrations for structures with larger numbers of cholesterol modifications. In order to compare FRET signals from the various structures, we normalized the acceptor fluorescence excited by FRET (F_{AD}) with the acceptor fluorescence obtained from direct excitation (F_{AA}). Normalized FRET values of bilayer sheets were found to be higher for larger numbers of cholesterol modifications, indicating tighter closing of the structures. We also compared the opening kinetics of flexible and rigid bilayer sheets with the same number of cholesterol moieties upon addition of a critical amount of Tween 80. As shown in Figure 2b, the initial FRET value of a flexible structure was higher, while its opening kinetics was considerably slower. This indicates that the rigid structure was closed less tightly initially, and instantaneously sprung open when surfactant was added.

The opening process does not obey first-order kinetics, but is very well described by a stretched exponential $\exp[-(kt)^{\beta}]$ (Figure 2b). This indicates the presence of disorder in the system, perhaps caused by microscopic variations of cholesterol interactions within the origami sheets. In fact, non-exponential relaxation kinetics observed in proteins has been previously associated with the existence of large numbers of conformational substates of similar

energy.[17] Notably, the strength of hydrophobic closing and the kinetics of opening depended strongly not only on the number of cholesterol modifications, but also on their exact chemical nature and mode of attachment to the origami structures (Figures S1–S5).

We surmised that opening of the DNA bilayer structures might also be promoted by favorable hydrophobic interactions between the cholesterol units and the lipid membranes. Indeed, mixing of closed structures with small unilamellar vesicles (SUVs) made from palmitoyl-oleoyl-phosphatidylcholine (POPC) resulted in a strong decrease in the FRET signal over time (Figure 3a). Signals recorded for DNA origami sheets without cholesterol were not influenced by the SUVs, ruling out the direct interaction of the fluorophores with the lipid membranes. As the opening of the DNA bilayers might also be caused by electrostatic interactions between DNA and PC lipids,[18] we performed experiments under various buffer conditions. For low concentrations of monovalent ions, experiments with unmodified DNA origami sheets on supported lipid bilayers and giant unilamellar vesicles (GUVs) indicate unspecific binding in the presence of divalent ions. At elevated salt concentrations, however, origami structures are released from the membrane unless they are attached through cholesterol anchors. We therefore also performed FRET unfolding experiments in the absence of Mg^{2+}, but at high ionic strength ([NaCl] = 1 M) at which the origami structures were still stable (Figures S8 and S10). Again we observed a strong reduction in FRET efficiency in the presence of SUVs, strongly supporting a scenario in which the origami sheets indeed unfold and bind to the lipid bilayer membranes through the cholesterol functions. Using confocal laser microscopy, the binding of origami sheets to GUVs (Figure 3b,c, Figure S9) can be directly visualized.

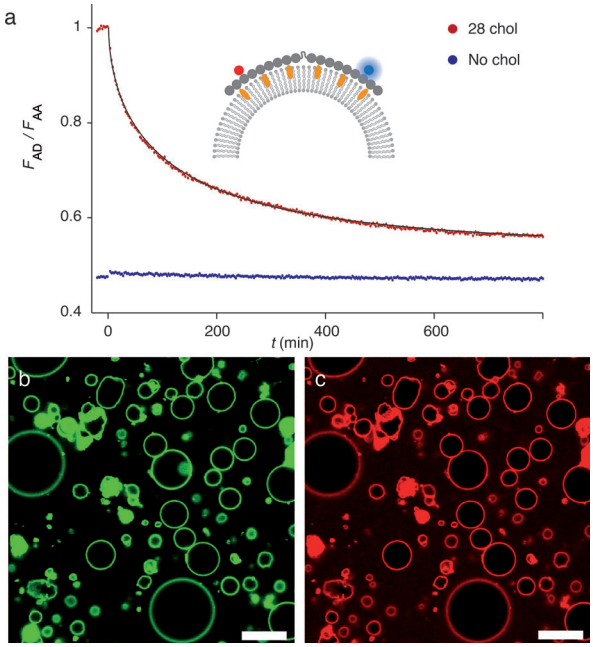

Figure 3. a) Opening kinetics of the structures after mixing with SUVs. The process is very well described by a stretched exponential ($\beta = 0.40$, $k = 0.025$ min). For comparison, structures without cholesterol moieties showed no change in their FRET signal after mixing with SUVs. b) Confocal laser microscopy image of labeled GUVs, imaged by excitation of the lipid label BODIPY FL. c) Confocal image of the same sample obtained by excitation of ATTO647N-labeled, cholesterol-modified DNA origami sheets. Scale bars: 20 μm.

As is the case for other switching mechanisms based solely on changes in buffer conditions (pH variations, addition/removal of metal ions, etc.),[9] hydrophobic switching has the disadvantage of being relatively unspecific. The presence of surfactants or lipids may influence many other processes in a complex environment, and the opening of the DNA bilayer structures may also be triggered erroneously by the "wrong" external stimulus. In order to add specificity to the opening process, we rendered the hydrophobic switching process conditional on an additional molecular "input". Using a simple lock and key strategy as previously utilized, for example, for a DNA origami box,[1d] we fixed the flexible DNA origami sheets with additional DNA duplex "clamps" as shown in Figure 4e. The clamp duplexes were extended with single-stranded toehold sections to which DNA "signal strands" could attach and disrupt the duplexes by toehold-mediated strand displacement. As demonstrated in Figure 4, the "gated" DNA bilayer sheets opened only in the presence of both DNA signals and surfactant molecules.

In conclusion, we have shown that hydrophobic interactions can be utilized to establish a novel switching mechanism for DNA-based nanostructures. The intramolecular aggregation of cholesterol modifications is strong enough to bend and fold DNA origami structures. In the presence of surfactants or

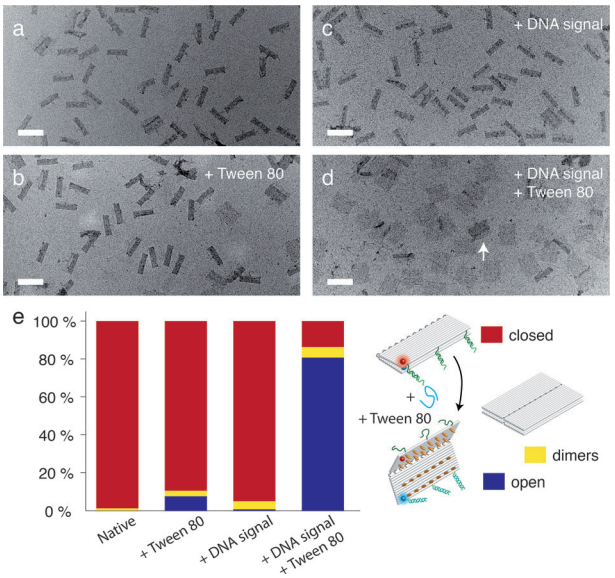

Figure 4. Conditional opening of gated origami bilayer sheets. a) TEM image of a flexible DNA sheet in the closed conformation, additionally locked as indicated in (e). b) TEM image obtained after addition of Tween. c) TEM image obtained after addition of opening signal strands. d) Only upon addition of both Tween 80 and DNA signal are the DNA bilayer sheets opened. e) Statistical analysis of opening obtained from TEM images. The illustration to the right shows the opening in the presence of Tween and signaling DNA. Dimer structures can occur as well, as marked in (d). Scale bars: 100 nm.

lipid bilayer membranes, this aggregation is resolved, resulting in the opening of the structures. Hydrophobic actuation can be combined with other switching mechanisms. This is of interest if the opening and attachment of a DNA nanostructure, for example a nanocontainer, to a lipid bilayer membrane is desired exclusively in the presence of a specific environmental signal. More generally, hydrophobic interactions expand the repertoire of switching mechanisms for reconfigurable nucleic acid based nanostructures and may make it possible to access structural features that are usually found in proteins. Notably, the surfactant-induced unfolding of DNA bilayer structures is analogous to the chemical denaturation of proteins which expose their hydrophobic residues in the presence of denaturants.

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[1] a) R. P. Goodman, M. Heilemann, S. Doose, C. M. Erben, A. N. Kapanidis, A. J. Turberfield, *Nat. Nanotechnol.* **2008**, *3*, 93 – 96; b) A. S. Walsh, H. Yin, C. M. Erben, M. J. A. Wood, A. J. Turberfield, *ACS Nano* **2011**, *5*, 5427 – 5432; c) F. A. Aldaye, H. F. Sleiman, *J. Am. Chem. Soc.* **2007**, *129*, 13376 – 13377; d) E. S. Andersen, M. Dong, M. M. Nielsen, K. Jahn, R. Subramani, W. Mamdouh, M. M. Golas, B. Sander, H. Stark, C. L. P. Oliveira, J. S. Pedersen, V. Birkedal, F. Besenbacher, K. V. Gothelf, J. Kjems, *Nature* **2009**, *459*, 73 – 76; e) S. M. Douglas, I. Bachelet, G. M. Church, *Science* **2012**, *335*, 831 – 834; f) A. Banerjee, D. Bhatia, A. Saminathan, S. Chakraborty, S. Kar, Y. Krishnan, *Angew. Chem.* **2013**, *125*, 6992 – 6995; *Angew. Chem. Int. Ed.* **2013**, *52*, 6854 – 6857.

[2] a) A. Krieg, J. Tonkinson, S. Matson, Q. Zhao, M. Saxon, L.-M. Zhang, U. Bhanja, L. Yakubov, C. Stein, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 1048 – 1052; b) J. Soutschek, A. Akinc, B. Bramlage, K. Charisse, R. Constien, M. Donoghue, S. Elbashir, A. Geick, P. Hadwiger, J. Harborth, *Nature* **2004**, *432*, 173 – 178.

[3] Y.-H. M. Chan, B. van Lengerich, S. G. Boxer, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 979 – 984.

[4] M. Loew, J. Kang, L. Dähne, R. Hendus-Altenburger, O. Kaczmarek, J. Liebscher, D. Huster, K. Ludwig, C. Böttcher, A. Herrmann, A. Arbuzova, *Small* **2009**, *5*, 320 – 323.

[5] S. M. Christensen, D. Stamou, *Soft Matter* **2007**, *3*, 828 – 836.

[6] a) K. Börjesson, E. Lundberg, J. Woller, B. Nordén, B. Albinsson, *Angew. Chem.* **2011**, *123*, 8462 – 8465; *Angew. Chem. Int. Ed.* **2011**, *50*, 8312 – 8315; b) A. Czogalla, E. P. Petrov, D. J. Kauert, V. Uzunova, Y. Zhang, R. Seidel, P. Schwille, *Faraday Discuss.* **2013**, *161*, 31.

[7] a) M. Langecker, V. Arnaut, T. G. Martin, J. List, S. Renner, M. Mayer, H. Dietz, F. C. Simmel, *Science* **2012**, *338*, 932 – 936; b) J. R. Burns, E. Stulz, S. Howorka, *Nano Lett.* **2013**, *13*, 2351 – 2356; c) J. R. Burns, K. Göpfrich, J. W. Wood, V. V. Thacker, E. Stulz, U. F. Keyser, S. Howorka, *Angew. Chem.* **2013**, *125*, 12291 – 12294; *Angew. Chem. Int. Ed.* **2013**, *52*, 12069 – 12072.

[8] T. G. W. Edwardson, K. M. M. Carneiro, C. K. Mclaughlin, C. J. Serpell, H. F. Sleiman, *Nat. Chem.* **2013**, *5*, 868 – 875.

[9] Y. Krishnan, F. C. Simmel, *Angew. Chem.* **2011**, *123*, 3180 – 3215; *Angew. Chem. Int. Ed.* **2011**, *50*, 3124 – 3156.

[10] a) B. Yurke, A. Mills, *Genetic Programming Evolvable Machines* **2003**, *4*, 111 – 122; b) D. Y. Zhang, G. Seelig, *Nat. Chem.* **2011**, *3*, 103 – 113.

[11] a) D. Liu, S. Balasubramanian, *Angew. Chem.* **2003**, *115*, 5912 – 5914; *Angew. Chem. Int. Ed.* **2003**, *42*, 5734 – 5736; b) W. Wang, Y. Yang, E. Cheng, M. Zhao, H. Meng, D. Liu, D. Zhou, *Chem. Commun.* **2009**, 824 – 826; c) S. Modi, M. G. Swetha, D. Goswami, G. D. Gupta, S. Mayor, Y. Krishnan, *Nat. Nanotechnol.* **2009**, *4*, 325 – 330.

[12] a) X. Liang, H. Nishioka, N. Takenaka, H. Asanuma, *ChemBioChem* **2008**, *9*, 702 – 705; b) F. Tanaka, T. Mochizuki, X. Liang, H. Asanuma, S. Tanaka, K. Suzuki, S.-i. Kitamura, A. Nishikawa, K. Ui-Tei, M. Hagiya, *Nano Lett.* **2010**, *10*, 3560 – 3565; c) Y. Ogura, T. Nishimura, J. Tanida, *Appl. Phys. Express* **2009**, *2*, 025004; d) F. Lohmann, D. Ackermann, M. Famulok, *J. Am. Chem. Soc.* **2012**, *134*, 11884 – 11887; e) J. Cheng, S. Sreelatha, R. Hou, A. Efremov, R. Liu, J. R. C. van der Maarel, Z. Wang, *Phys. Rev. Lett.* **2012**, *109*, 238104; f) Y. Yang, M. Endo, K. Hidaka, H. Sugiyama, *J. Am. Chem. Soc.* **2012**, *134*, 20645 – 20653; g) M. Endo, Y. Yang, Y. Suzuki, K. Hidaka, H. Sugiyama, *Angew. Chem.* **2012**, *124*, 10670 – 10674; *Angew. Chem. Int. Ed.* **2012**, *51*, 10518 – 10522.

[13] a) S. Jhaveri, M. Rajendran, A. D. Ellington, *Nat. Biotechnol.* **2000**, *18*, 1293 – 1297; b) R. Nutiu, Y. F. Li, *J. Am. Chem. Soc.* **2003**, *125*, 4771 – 4778; c) B. Shlyahovsky, L. Di, Y. Weizmann, R. Nowarski, M. Kotler, I. Willner, *J. Am. Chem. Soc.* **2007**, *129*, 3814; d) J. Elbaz, M. Moshe, I. Willner, *Angew. Chem.* **2009**, *121*, 3892 – 3895; *Angew. Chem. Int. Ed.* **2009**, *48*, 3834 – 3837.

[14] J. N. Israelachvili, *Intermolecular and surface forces*, Academic Press, Burlington, **2011**.

[15] P. W. K. Rothemund, *Nature* **2006**, *440*, 297 – 302.

[16] M. E. Haberland, J. A. Reynolds, *Proc. Natl. Acad. Sci. USA* **1973**, *70*, 2313 – 2316.

[17] I. Iben, D. Braunstein, W. Doster, H. Frauenfelder, M. Hong, J. Johnson, S. Luck, P. Ormos, A. Schulte, P. Steinbach, *Phys. Rev. Lett.* **1989**, *62*, 1916 – 1919.

[18] S. Gromelski, G. Brezesinski, *Langmuir* **2006**, *22*, 6293 – 6301.

