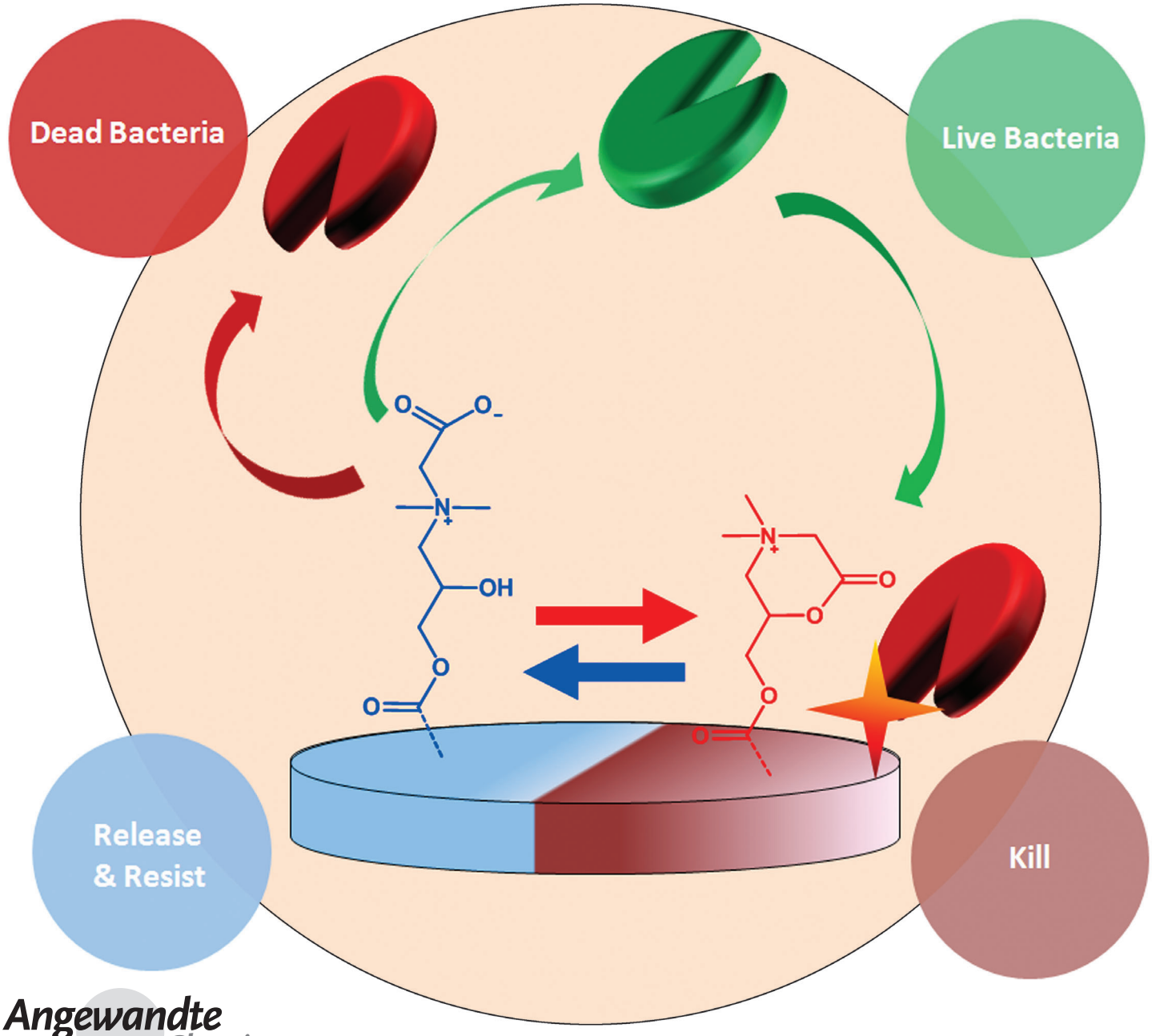
Smart Materials

DOI: 10.1002/anie.201106466

Reversibly Switching the Function of a Surface between Attacking and Defending against Bacteria**

*Zhiqiang Cao, Luo Mi, Jose Mendiola, Jean-Rene Ella-Menye, Lei Zhang, Hong Xue, and Shaoyi Jiang**



There are two major approaches to prevent the microbial colonization and biofilm formation on a surface. The active approach is to "attack", by killing bacteria with a wide range of antimicrobial materials or drugs including but not limited to cationic polymers, antimicrobial peptides, antibiotics, silver ions, nitrous oxide.[1–4] However, poor biocompatibility or drug resistance is of great concern. The passive approach is to "defend", by resisting bacteria by using nonfouling coatings, such as poly(ethylene glycol) (PEG), zwitterionic, and their derivative coatings.[3–9] However, once bacteria are attached on a surface, there is no mechanism to kill them. Ideally, a surface is able to perform the interswitchable functions each at a time, such as to kill attached bacteria, release killed microbes, and either return to the initial killing state or maintain the final nonfouling state. We reported a "kill-and-release" strategy based on a cationic ester.[8] The cationic ester surface was able to kill attached bacteria and release dead microbes upon the hydrolysis of ester groups, resulting in the nonfouling zwitterionic surface.[8] Unfortunately, this is a one-time action and the surface cannot be regenerated back to its original form, because the hydrolysis of ester groups is not reversible. It is highly desirable but challenging to develop a single material that switches reversibly and easily between attacking (e.g., cationic) and defending (e.g., zwitterionic) forms.

Herein we overcome these challenges with a smart polymer capable of switching repeatedly between its two equilibrium states, a cationic *N*,*N*-dimethyl-2-morpholinone (CB-Ring) and a zwitterionic carboxy betaine (CB-OH, Figure 1) to achieve attacking and defending functions in a controlled manner. Herein, we show that a CB-Ring surface kills over 99.9% of *Escherichia coli* K12 (*E. coli* K12) attached on it under dry conditions. In neutral or basic aqueous environments, CB-Ring is hydrolyzed to CB-OH immediately. The CB-OH surface releases dead bacteria and at the same time resists bacteria adhesion in the aqueous media. CB-OH can finally be converted back to CB-Ring under acidic conditions, thereby regenerating the bacteria-killing function. As compared with the "one-time-switch" surface mentioned above, switching of this smart material is fully reversible and triggered under practical conditions; the bacteria-killing function can be regenerated with weak acid (e.g. acetic acid), and in physiological environment the function immediately shifts to releasing and resisting bacteria. We have reported the synthesis of CB-OH and studied its equilibrium with CB-Ring driven at acidic and basic conditions.[10] Herein we propose and demonstrate the use of this smart material in killing, releasing, and resisting bacteria in a reversible manner.

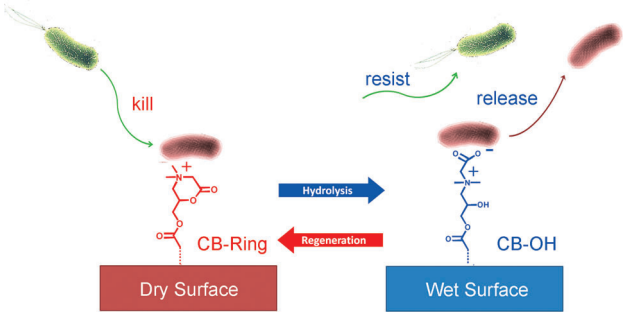


Figure 1. A smart polymer coating repeatedly switches between the attacking function (CB-Ring, to kill bacteria under dry conditions) and defending function (CB-OH, to release and resist bacteria under wet conditions). CB-Ring can be hydrolyzed to CB-OH in neutral or basic aqueous solutions and can be regenerated by dipping CB-OH in acidic media.

The bacteria-attacking function of CB-Ring was hypothesized based on its structural characteristics, the cationic, quaternary amine group and its similarity to 2-morpholinone. It is well-known that quaternary amine compounds are able to efficiently kill a variety of microorganisms both on surfaces and in bulk solutions.[8,11–14] Moreover, 2-morpholinone derivatives (with unsubstituted nitrogen atoms) have shown pronounced activity against bacterial and fungal species.[15] The preparation of the CB-Ring film followed the method we previously established. CB-OH monomer (Figure 2a) was first synthesized and its thin polymer film (film thickness (26.6 ± 0.6) nm) was developed through surface-initiated atom-transfer radical polymerization from thiol initiators on gold substrates.[10] Since CB-OH has a half-life of 14 min in trifluoroacetic acid (TFA) before being fully converted to CB-Ring,[10] we dipped CB-OH surface in TFA for 20 min to generate a sufficient amount of CB-Ring on the surface and subsequently washed the surface repeatedly with acetonitrile.

The bactericidal activity of the CB-Ring surface was tested on *E. coli* K12 by using an established assay.[8,16] A typical quaternary amine surface known for its bactericidal ability (C8N,[8] film thickness (26.6 ± 0.5) nm) and the zwitterionic CB-OH surface were used as the positive and negative controls, respectively (Figure 2a). It was observed that cationic CB-Ring generated from TFA killed more than 99.9% of the bacteria sprayed on its surface in one hour under dry conditions, similar to the cationic C8N surface (Figure 2b). CB-Ring generated from acetic acid (HAc) had equally good bactericidal activity and will be further explained below. As expected, CB-OH, being zwitterionic, had no bactericidal activity at its dry state (100% live bacteria in Figure 2b), showing roughly the same amount of live bacteria as a bare gold surface.

It should be pointed out that CB-Ring can be regenerated from two methods: with TFA, an established method,[10] or with HAc. Herein we show the use of HAc to convert CB-OH to CB-Ring surface and examine the bactericidal activity of the resulting CB-Ring surface. As a weak acid HAc does not erode away the gold layer on our substrates and can be used

[*] Z. Cao, L. Mi, J. Mendiola, J.-R. Ella-Menye, L. Zhang, H. Xue, Prof. S. Jiang
Department of Chemical Engineering
University of Washington
Seattle, WA 98195 (USA)
E-mail: sjiang@u.washington.edu
Homepage: http://depts.washington.edu/jgroup/Index.htm

[**] This work has been supported by the Defense Threat Reduction Agency (HDTRA1-10-1-0074) and the Office of Naval Research (N000140910137).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201106466.

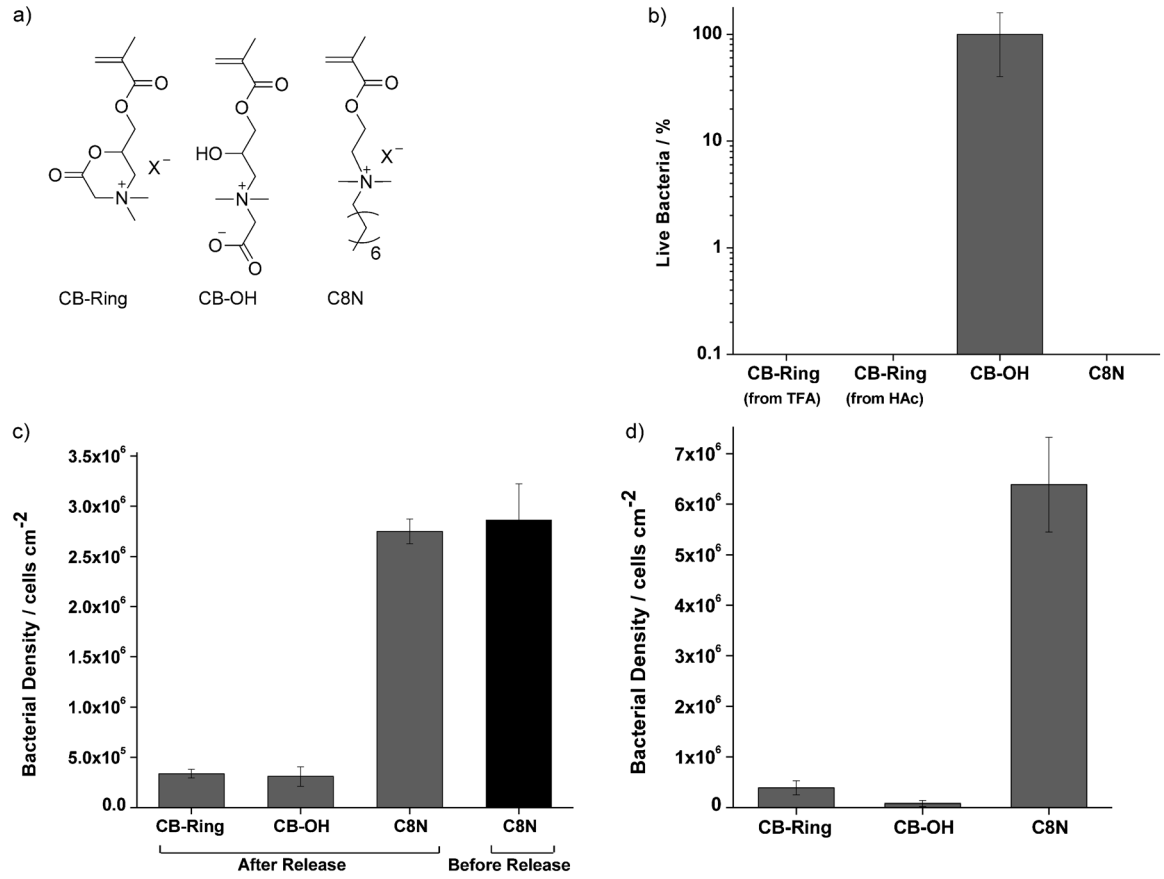


Figure 2. a) Chemical structures of cationic CB-Ring, zwitterionic CB-OH, and cationic C8N monomers. X^-: counterion. b) Bactericidal activity of the surfaces against *E. coli* K12 under dry condition. The y axis represents the percentage of live *E. coli* K12 colonies grown relative to the number of colonies grown on CB-OH control ($n=3$). Note that zwitterionic CB-OH cannot kill the bacteria. In the other three cases more than 99.9% of the bacteria were killed. c) Bacterial cell density of *E. coli* K12 on the surfaces before and after the releasing procedure (gently shaking in PBS for one hour; $n=3$). Initially (before release), bacteria were equally sprayed on CB-Ring, CB-OH, and C8N surfaces and dried for 15 min. Note that only the bacterial coverage on C8N was recorded*. After the releasing procedure, the remaining bacterial coverage was recorded. *The bacterial coverage for CB-Ring and CB-OH surfaces was the same as C8N but could not be directly measured; staining required wet conditions that released bacteria on CB-Ring and CB-OH surfaces. d) Bacterial cell density of *E. coli* K12 attached on the surfaces after incubating with the bacteria under wet conditions (10^8 cells mL^{-1} for 0.5 h; $n=3$). The labels CB-Ring, CB-OH, and C8N represent the types of the surfaces before the incubation with bacteria; note that CB-Ring was converted to CB-OH during the incubation.

to repeatedly treat the surface without destabilizing the polymer coating. The potential conversion from CB-OH to CB-Ring was examined by dissolving CB-OH monomer into [D]HAc at a concentration of 0.2 M and the characteristic peaks in the ^{1}H NMR spectrum of CB-OH and CB-Ring (Figure S1 in the Supporting Information) were compared. It was found that treatment with [D]HAc for 20 h led to 50% conversion of CB-OH to CB-Ring. After 40 h the equilibrated product was precipitated in ethyl ether, dried in vacuum, and placed into pH 7.3 buffer made by titrating a solution of Na_2CO_3 (200 mM) in D_2O with DCl. ^{1}H NMR spectroscopy indicated a fast hydrolysis of CB-Ring back to CB-OH with nearly full conversion in less than 10 min (Figure S2 in the Supporting Information). A mechanism for the equilibrium between CB-Ring and CB-OH was proposed (Figure S3 in the Supporting Information). The conversion from CB-OH to CB-Ring is catalyzed by the protonation of the carboxylate group leading to an intramolecular Fischer esterification involving the neighboring hydroxy group. A strong acid is the ideal catalyst for the reaction as is the case for TFA, which gave almost complete conversion to CB-ring in one hour,[10] whereas HAc, a weak acid, yielded 50% conversion after 20 h. We re-examined the bactericidal activity of CB-Ring surface generated by HAc treatment for 20 h. Even the surface that was not fully converted to CB-Ring was as efficient as the previous TFA-generated CB-Ring surface, by killing more than 99.9% *E. coli* K12 in the dry condition (Figure 2b). Furthermore, we tested the polymer-film stability by switching the surface between CB-OH and CB-Ring repeatedly, through various cycles of treatment with HAc for 20 h and with phosphate buffered saline (PBS) for two hours (CB-OH→CB-Ring and CB-Ring→CB-OH, respectively). No obvious film deterioration was found as indicated by a constant film thickness of CB-OH over seven repeated cycles (Figure S4 in the Supporting Information). For these reasons, we chose HAc as an alternative to TFA in repeatedly manipulating this "smart material".

For the bacteria-defending function, we examined the ability of this "smart material" to release the previously attached/dried (killed) bacteria in wet conditions and to further resist bacteria attachment. To test the bacteria-releasing capability, surfaces previously sprayed with *E. coli* K12 and dried for 15 min, were gently shaken in PBS for one hour. Bacteria were stained with FM® 1-43 and their densities on the surface before and after the shaking (releasing) were monitored under a fluorescence microscope (quantitative data in Figure 2c; representative images in Figure S5 in the Supporting Information). It was found that CB-Ring surface was able to release 90% of the previously attached/dried bacteria, similar to CB-OH. It was expected that upon the contact with PBS, cationic CB-Ring was quickly converted to CB-OH within minutes (Figure S2 in the Supporting Information). Previously was shown that CB-OH surface was ultra-low-fouling with respect to resisting fibrinogen and undiluted human plasma, with less than 5 ng cm^{-2} adsorbed proteins.[10] As a reference, a monolayer of protein results in a surface coverage of 100–500 ng cm^{-2}.[4] The nonfouling nature of CB-OH accounts for the efficient release of the attached/dried bacteria. In contrast, the permanent cationic C8N exhibited no observable bacteria release ($p > 0.6$, one-way analysis of variance (ANOVA), Figure 2c).

To test the bacteria resistance in wet conditions, surfaces were incubated with *E. coli* K12 solution (10^8 cells mL^{-1} in PBS) for 0.5 h and shaken in fresh PBS for one hour to remove unbound bacteria. Bacteria that remained on the surfaces were stained with FM 1-43 for visualization. It was found that the smart surface in its initial state at both CB-Ring and CB-OH effectively resisted the bacteria adhesion, whereas C8N had large amounts of bacteria adhesion owing to its cationic and hydrophobic nature (quantitative data in Figure 2d; representative images in Figure S6 in the Supporting Information). An established ultra-low-fouling zwitterionic surface made from polycarboxy betaine (PCBMA)[8,9] was tested as a negative control (film thickness (32.2 ± 0.3) nm), showing an extremely low bacterial coverage of $(6.4 \pm 3.2) \times 10^4$ cells cm^{-2}. The density of bacteria on the CB-OH surface was $(7.8 \pm 5.8) \times 10^4$ cells cm^{-2}, which was as low as on the PCBMA control ($p > 0.6$, one-way ANOVA). As a reference, a single observable bacterial cell under the field of the microscope in our setup represented a bacterial density of 3.2×10^4 cells cm^{-2}.

Finally, we examined the bacteria-attacking ability of CB-Ring after one cycle of switch. The CB-Ring surface was sprayed with *E. coli* K12, dried for one hour, and shaken in PBS for another hour to release the killed bacteria. The resulting CB-OH surface was treated with HAc for 20 h to regenerate the CB-Ring surface. The resulting CB-Ring surface was tested for bactericidal activity and was found to be as good as it was in the previous cycle (i.e., it killed more than 99.9% of *E. coli* K12).

Many smart polymers have been developed to modulate various properties (e.g., permeability and wettability as well as adhesive, mechanical, and optical properties, etc.).[17] But none of these established materials was able to switch between bacteria-attacking and -defending functions repeatedly. It is also worth noting that the smart material studied herein can be readily implemented on different types of surfaces (e.g., metal, glass, polymer, etc.). An adhesive anchoring moiety will be chosen depending on the nature of these substrates, and the polymer coating can be obtained by either "graft-from" or "graft-to" methods.[6]

Received: September 13, 2011
Revised: October 10, 2011
Published online: December 30, 2011

Keywords: antimicrobial activity · biological activity · surface chemistry · switchable materials · materials science

[1] L. Zhang, D. Pornpattananangkul, C. M. J. Hu, C. M. Huang, *Curr. Med. Chem.* **2010**, *17*, 585–594.
[2] M. Ramstedt, B. Ekstrand-Hammarstrom, A. V. Shchukarev, A. Bucht, L. Osterlund, M. Welch, W. T. S. Huck, *Biomaterials* **2009**, *30*, 1524–1531.
[3] X. Khoo, M. W. Grinstaff, *MRS Bull.* **2011**, *36*, 357–366.
[4] B. D. Ratner, A. S. Hoffman, F. J. Schoen, J. E. Lemons, *Biomaterials Science an Introduction to Materials in Medicine*, 2nd ed., Elsevier, Amsterdam, **2004**.
[5] E. Ostuni, R. G. Chapman, M. N. Liang, G. Meluleni, G. Pier, D. E. Ingber, G. M. Whitesides, *Langmuir* **2001**, *17*, 6336–6343.
[6] S. Jiang, Z. Q. Cao, *Adv. Mater.* **2010**, *22*, 920–932.
[7] G. Cheng, Z. Zhang, S. F. Chen, J. D. Bryers, S. Y. Jiang, *Biomaterials* **2007**, *28*, 4192–4199.
[8] G. Cheng, H. Xue, Z. Zhang, S. Chen, S. Jiang, *Angew. Chem.* **2008**, *120*, 8963–8966; *Angew. Chem. Int. Ed.* **2008**, *47*, 8831–8834.
[9] G. Cheng, G. Z. Li, H. Xue, S. F. Chen, J. D. Bryers, S. Y. Jiang, *Biomaterials* **2009**, *30*, 5234–5240.
[10] Z. Q. Cao, N. Brault, H. Xue, A. Keefe, S. Y. Jiang, *Angew. Chem.* **2011**, *123*, 6226–6228; *Angew. Chem. Int. Ed.* **2011**, *50*, 6102–6104.
[11] E. J. Kramer, S. Krishnan, R. J. Ward, A. Hexemer, K. E. Sohn, K. L. Lee, E. R. Angert, D. A. Fischer, C. K. Ober, *Langmuir* **2006**, *22*, 11255–11266.
[12] T. Abel, J. I. Cohen, R. Engel, M. Filshtinskaya, A. Melkonian, K. Melkonian, *Carbohydr. Res.* **2002**, *337*, 2495–2499.
[13] S. B. Lee, R. R. Koepsel, S. W. Morley, K. Matyjaszewski, Y. J. Sun, A. J. Russell, *Biomacromolecules* **2004**, *5*, 877–882.
[14] A. M. Klibanov, *J. Mater. Chem.* **2007**, *17*, 2479–2482.
[15] R. Agarwal, M. H. Ansari, M. W. Y. Khan, M. Ahmad, K. D. Sharma, *J. Am. Oil Chem. Soc.* **1989**, *66*, 825–827.
[16] J. C. Tiller, C. J. Liao, K. Lewis, A. M. Klibanov, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 5981–5985.
[17] M. A. C. Stuart, W. T. S. Huck, J. Genzer, M. Muller, C. Ober, M. Stamm, G. B. Sukhorukov, I. Szleifer, V. V. Tsukruk, M. Urban, F. Winnik, S. Zauscher, I. Luzinov, S. Minko, *Nat. Mater.* **2010**, *9*, 101–113.

