Smart Materials

DOI: 10.1002/ange.201100004

Manipulating Sticky and Non-Sticky Properties in a Single Material**

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Non-sticky and sticky properties are two highly desired characteristics of materials. The former property refers to the ability to efficiently resist non-specific adsorption of biomolecules and microorganisms, while the latter enables the covalent attachment of biomolecular recognition elements. The presence of both properties permits significant advancements in numerous applications, such as biosensing, drug delivery, and tissue engineering.[1–4] However, conventional wisdom prevents these two distinct properties from coexisting within a single material. For example, to achieve sticky properties, non-sticky materials must be either reacted to introduce functionalizable groups (for example carboxylate moieties) or reacted with coupling agents (for example carbodiimides) so that they can be further conjugated with biomolecular recognition elements. Such chemistry has been applied to many materials, such as dextran,[5] polyethylene glycol (PEG),[6–8] and zwitterionic polymers.[4,9,10] To the best of our knowledge, a single material containing a controllable sticky and non-sticky dual functionality has not been reported.

Herein we present an answer to this challenge. The design was inspired by a type of molecule with an acid/base-driven equilibrium between two chemical states: a lactone ring structure and an acidic open-ring structure, such as illustrated by the drug camptothecin.[11–13] Combining this idea with the established non-fouling properties of zwitterionic materials,[4] we present a new monomer that can switch reversibly between an open carboxylate form (CB-OH) and a six-membered lactone ring (CB-Ring; Scheme 1, dashed box). We hypothesize that this new material can alternate between these two equilibrium states driven by either acidic or basic conditions. The CB-OH form is ultralow fouling (non-sticky) owing to its zwitterionic structure,[4] while the CB-Ring is reactive (sticky) towards nucleophiles (for example amine moieties) owing to the lactone.[14] In this work, we provide the experimental evidence in support of these claims. A simple strategy for applying this novel smart material, using only the material itself, for ligand immobilization with an ultralow fouling background is presented in Scheme 1 (right-hand side). Specifically, CB-OH polymers can first be converted into the sticky state where ligand conjugation occurs by primary amine moieties. Unreacted CB-Ring groups can then be switched back into zwitterionic CB-OH, resulting in a protein-resistant background. Using this strategy, we present a proof-of-concept experiment in which a high throughput antibody array for early cancer diagnostics is realized.

The synthesis of CB-OH initially proceeded by the reaction of sarcosine *tert*-butyl ester with glycidyl methacrylate followed by the addition of methyl iodide to obtain the CB-OH *t*Bu ester. Subsequent treatment with trifluoroacetic acid (TFA) to remove the protecting groups and neutralization using basic ion-exchange resins provided the final product, CB-OH, which was obtained as a white powder after lyophilization (Supporting Information, Figure S1). The reaction details and also the ^{1}H NMR and ^{13}C NMR spectroscopic data for both CB-OH *t*Bu ester and CB-OH are given in the Supporting Information. Ion-trap mass spectrometry (IT-MS) further confirmed the result by giving a m/z value of 246.1 for the protonated form of CB-OH (the calculated molecular weight MW for $C_{11}H_{19}NO_5H^+$ is 246.3).

Based on our hypothesis, the open carboxylate form (CB-OH) should have an equilibrium lactone ring counterpart (CB-Ring) that forms in the presence of acidic media. This was tested by dissolving CB-OH in TFA for 2 h (or overnight in a TFA/acetonitrile mixed solvent at 1:10 v/v). The resulting product, precipitated in diethyl ether, consisted solely of the CB-Ring structure according to ^{1}H NMR and ^{13}C NMR spectra (see the Supporting Information) with a m/z value of 228.1 by IT-MS (calculated MW for $C_{11}H_{18}NO_4^+$: 228.3). Additionally, CB-Ring contained a characteristic ^{1}H NMR peak (m, 1H, $CH_2{=}C(CH_3)COOCH_2C\boldsymbol{H}(O{-})CH_2{-}$) at $\delta = 5.53$ ppm in [D]TFA or $\delta = 5.38$ ppm in D_2O, which was absent in the zwitterionic state, and thus allowed the equilibrium kinetics to be quantified in different deuterated solvent environments (Figure 1). It was found that in an acidic environment (such as [D]TFA), CB-OH had a half-life of about 14 minutes and was fully converted into the CB-Ring structure within 2 hours. Going in the reverse direction using aqueous buffer at pH 7, the CB-Ring structure was quickly hydrolyzed with a half-life of about 4 minutes. Under basic conditions (that is, pH 10) the half-life of CB-Ring became even shorter (<1 minute), with complete conversion into the zwitterionic form in less than 6 minutes. The acidic or basic conditions necessary to drive the equilibrium in the corresponding directions are also indicated in Scheme 1.

The amino reactivity (that is, the sticky characteristic) of the CB-Ring was then studied using a model molecule, benzylamine, in both aqueous and organic environments. The positively charged lactone could be efficiently conjugated to the amine group, and the resulting CB-OH–benzyl conjugate, purified by HPLC, was found to have a m/z of 335.4 by IT-MS (calculated MW for $C_{18}H_{27}N_2O_4^+$: 335.4). A control experi-

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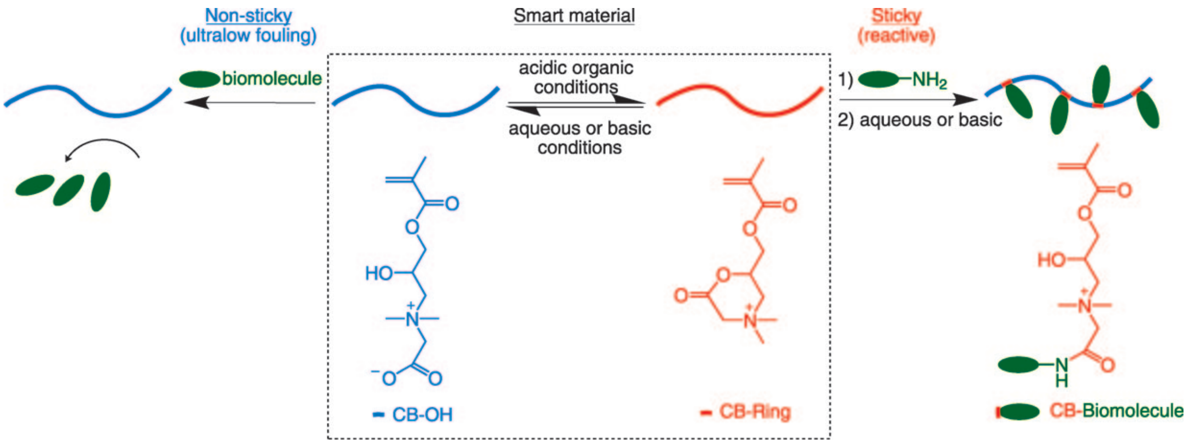
[**] This work was supported by the Defense Threat Reduction Agency (HDTRA1-10-1-0074) and the Office of Naval Research (N000140910137 and N000140711036).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201100004.



Scheme 1. The switchable properties of a material based on unique equilibrium states. CB-OH (blue) illustrates the "non-sticky" state, which resists biomolecular adsorption (green). CB-Ring (red) represents the "sticky" state, which covalently binds to amine-containing biomolecules. Unreacted CB-Ring groups can then be converted back into an ultralow fouling background (blue). Switching between CB-OH and CB-Ring occurs only by the addition or removal of one water molecule from the material.

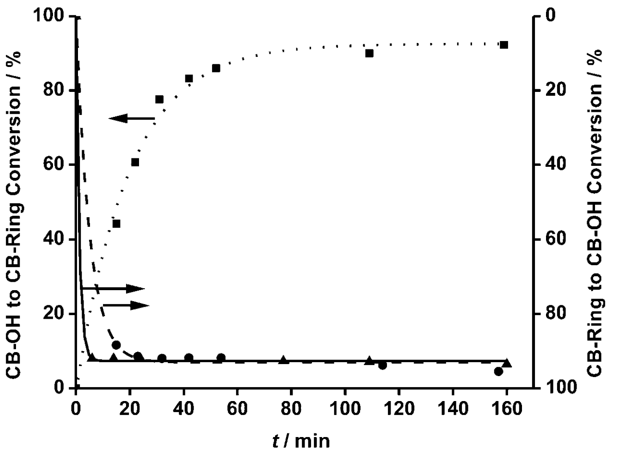


Figure 1. The equilibrium kinetics between CB-OH and CB-Ring structures in three deuterated solvent environments. These include an acidic solvent ([D]TFA, •••••) along with pH 7.3 (-----) and pH 10 (——) buffers made from 200 mM Na_2CO_3 in D_2O titrated with DCl. Conversion was calculated by ^1H NMR spectroscopy using a ratio of the characteristic peak for CB-Ring (1 H, $CH_2{=}C(CH_3)COOCH_2C\mathit{H}(O{-})CH_2{-}$) and a common peak of both structures (6 H, $-CH_2N(C\mathit{H}_3)_2CH_2-$). For further experimental details, see the Supporting Information.

ment using the non-sticky zwitterionic form (CB-OH) as the starting material resulted in no conjugation as determined by both HPLC and IT-MS. As shown in Figure 1, the hydrolysis rate of CB-Ring strongly depends upon the condition used. It was thus necessary to quantify the conjugating efficiency under different environments. Owing to the basic character of benzylamine, any initial CB-Ring could undergo only conjugation or hydrolysis. By measuring the final concentration of CB-OH by HPLC and comparing it to the control, a 5 molar excess of benzylamine with a 30 minute reaction time resulted in efficiencies of 60 % in pure water and 83 % in acetonitrile. It is likely that the aqueous conjugating efficiency was lower owing to the competition between aminolysis (to form the conjugate) and hydrolysis (to form CB-OH).

To further support our findings about this new switchable material, IT-MS was used to study the fragmentation products of isolated CB-OH, CB-Ring, and CB-OH-benzyl compounds. It was observed that both CB-OH and the conjugate shared the same ionized fragment (m/z 143.1), which was due to the similarity of their molecular structures (Supporting Information, Figure S2). CB-Ring had a completely different fragmentation pattern, owing to its unique ring structure, thus resulting in two characteristic fragments with m/z values of 200.0 and 84.2 (Supporting Information, Figure S2). Furthermore, a major difference could also be observed when comparing the peak intensities for each starting material with their fragments. It was observed that nearly 100 % of both CB-OH and the conjugate could be fragmented compared to only 0.3 % for CB-Ring owing to the difficulty in fragmenting ring-structure molecules.

Previous zwitterionic materials have been found to be ultralow fouling (non-sticky) by effectively resisting protein binding from undiluted human plasma and serum, along with adhesion from cells, bacteria, and other organisms.[4,15] The term ultralow fouling has been used to further stratify low-fouling materials into those which allow less than 5 $ng\,cm^{-2}$ of fibrinogen adsorption thereby effectively inhibiting platelet adhesion that is necessary for blood compatibility.[16] While the introduction of an OH group into zwitterionic carboxybetaine led to the creation of CB-OH, the effect of this moiety on the non-fouling properties should be negligible. This was tested by using thin films of CB-OH (ca. 20 nm) formed by surface-initiated atom-transfer radical polymerization (SI-ATRP) from thiol initiators on gold substrates. SPR biosensors were then used to quantify nonspecific protein binding. Single protein solutions of fibrinogen and lysozyme in phosphate buffer solution (PBS; 1 $mg\,mL^{-1}$) as well as undiluted human plasma were flowed over the CB-OH surface. Undetectable adsorption ($< 0.3\ ng\,cm^{-2}$) was observed for the single proteins, whereas $(3.1 \pm 1.0)\ ng\,cm^{-2}$ of protein fouling was detected for human plasma (see sensorgrams in the Supporting Information, Figure S3). These results revealed that CB-OH was ultralow-fouling to all solutions analyzed (that is, less than 5 ng cm^{-2} of bound protein) and that this important property was maintained despite modifying the original carboxybetaine compound. As a reference, a monolayer of protein binding results in a sensor response of 100–500 ng cm^{-2}.[17]

As a proof-of-concept experiment, we then illustrated the simple manipulation strategy for which the non-sticky and sticky characteristics of this new switchable material could be used to create a protein array. Using the same CB-OH substrates formed by SI-ATRP as above, a 10 minute TFA treatment was used to form a sufficient amount of CB-Ring structures necessary for antibody immobilization. Two antibodies (anti-hCG and anti-Salm) were then contact-printed onto the amine-reactive surface in pH 10 buffer, resulting in a 6 × 12 array (Figure 2 a). Our control study showed immobilization in basic buffer (pH 10) was more efficient than slightly acidic buffer (pH 6) owing to more deprotonated amines under basic conditions (Supporting Information, Figure S4). Subsequent hydrolysis of unreacted CB-Ring components using pH 9 buffer for 60 minutes switched the unspotted background from sticky into ultralow-fouling. Using an SPR imaging biosensor, significant specific detection of hCG was observed for anti-hCG spots while a zero response was observed for both the control antibody and the background (see Figure 2 b).

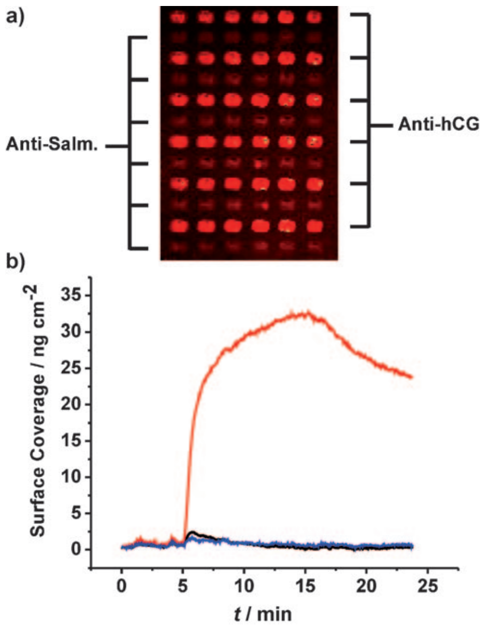


Figure 2. a) A 6 × 12 protein array using thin polymer substrates of the new smart material. Anti-hCG and anti-Salm (both red) were printed as indicated. The background (black) was ultralow fouling. b) The specific detection of hCG was observed for only anti-hCG (red); no response was observed for either the background (black) or the Anti-Salm control antibody (blue).

It should be noted that many smart materials enable switching between hydrophobic and hydrophilic properties. Typical mechanisms for control include changes in light, pH, electric potential, temperature, and redox reactions.[18,19] However, none of these switchable materials have achieved control over the two extreme properties obtained herein. In this work, the non-sticky (resistance to protein adsorption from 100 % blood plasma) and sticky (permanent covalent coupling) incompatible properties were controlled using one single material.

Received: January 2, 2011
Revised: February 6, 2011

Keywords: antifouling · nucleophiles · protein immobilization · switchable materials · zwitterions

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[1] B. D. Ratner, S. J. Bryant, *Annu. Rev. Biomed. Eng.* **2004**, *6*, 41.
[2] R. Langer, *Science* **2001**, *293*, 58.
[3] K. L. Prime, G. M. Whitesides, *Science* **1991**, *252*, 1164.
[4] S. Jiang, Z. Cao, *Adv. Mater.* **2010**, *22*, 920.
[5] S. L. McArthur, K. M. McLean, P. Kingshott, H. A. W. St John, R. C. Chatelier, H. J. Griesser, *Colloids Surf. B* **2000**, *17*, 37.
[6] J. M. Harris, R. B. Chess, *Nat. Rev. Drug Discovery* **2003**, *2*, 214.
[7] J. E. Raynor, T. A. Petrie, A. J. Garcia, D. M. Collard, *Adv. Mater.* **2007**, *19*, 1724.
[8] M. K. Yu, Y. Y. Jeong, J. Park, S. Park, J. W. Kim, J. J. Min, K. Kim, S. Jon, *Angew. Chem.* **2008**, *120*, 5442; *Angew. Chem. Int. Ed.* **2008**, *47*, 5362.
[9] A. Lewis, Y. Q. Tang, S. Brocchini, J. W. Choi, A. Godwin, *Bioconjugate Chem.* **2008**, *19*, 2144.
[10] N. Chiba, M. Ueda, T. Shimada, H. Jinno, J. Watanabe, K. Ishihara, M. Kitajima, *Eur. Surg. Res.* **2007**, *39*, 23.
[11] Z. H. Mi, T. G. Burke, *Biochemistry* **1994**, *33*, 10325.
[12] V. R. Caiolfa, M. Zamai, A. Fiorino, E. Frigerio, C. Pellizzoni, R. d'Argy, A. Ghiglieri, M. G. Castelli, M. Farao, E. Pesenti, M. Gigli, F. Angelucci, A. Suarato, *J. Controlled Release* **2000**, *65*, 105.
[13] J. W. Singer, R. Bhatt, J. Tulinsky, K. R. Buhler, E. Heasley, P. Klein, P. de Vries, *J. Controlled Release* **2001**, *74*, 243.
[14] W. M. Liu, D. D. Xu, O. Repic, T. J. Blacklock, *Tetrahedron Lett.* **2001**, *42*, 2439.
[15] Z. Zhang, J. A. Finlay, L. F. Wang, Y. Gao, J. A. Callow, M. E. Callow, S. Y. Jiang, *Langmuir* **2009**, *25*, 13516.
[16] W. B. Tsai, J. M. Grunkemeier, T. A. Horbett, *J. Biomed. Mater. Res.* **1999**, *44*, 130.
[17] 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**.
[18] Y. Liu, L. Mu, B. H. Liu, J. L. Kong, *Chem. Eur. J.* **2005**, *11*, 2622.
[19] M. Riskin, B. Basnar, V. I. Chegel, E. Katz, I. Willner, F. Shi, X. Zhang, *J. Am. Chem. Soc.* **2006**, *128*, 1253.