Biomimicry

DOI: 10.1002/anie.200903010

Biomimetic Encapsulation of Individual Cells with Silica**

*Sung Ho Yang, Kyung-Bok Lee, Bokyung Kong, Jin-Hyun Kim, Hak-Sung Kim, and Insung S. Choi**

Cells, such as yeast, bacteria, and mammalian cells, as well as proteins have been immobilized in inorganic silica matrices for applications in biochemical sensors and reactors.[1] It is of scientific interest and technological importance that such immobilization procedures were found to enhance enzymatic activity and cell viability compared with intact, native forms. The previous examples, however, have dealt only with the entrapment of a collection of cells, rather than individual cells, in the silica matrix, although manipulation of individual cells is thought to be more beneficial in the development of biosensor circuits, lab-on-a-chip systems, and bioreactors, as well as for fundamental studies in cell biology.

Certain unicellular organisms, such as diatoms, radiolaria, and synurophytes, as well as multicellular sponges, are encased with silica that has exquisite hierarchical structures and superior mechanical properties,[2] while most cells in nature do not have siliceous shells and are exposed directly to the outer environments. It would, therefore, expand the areas of living-cell-based applications to coat individual native cells with silica by mimicking nature's selection of silica and enhance cell viability against harsh conditions. Besides the aforementioned applications, single-cell encapsulation within silica shells is of fundamental interest. For example, silica-encapsulated cells are not biogenetic species. These artificially designed cells, which structurally resemble diatoms, would be useful models for studying cellular metabolism at the single-cell level. In spite of the considerable interest, silica encapsulation of individual cells has not been realized to date. Herein, we use biomimetic silicification under physiologically mild conditions to encapsulate individual cells with silica.

Biomimetic silicification, inspired by biosilicification found in nature, does not require harsh reaction conditions that would do harm to proteins and cells but proceeds under mild conditions (i.e., ambient pressure, room temperature or below, and near neutral pH values).[2] In addition to silaffins—catalytic peptides extracted from diatoms—and their derivatives, synthetic polyamines have been utilized for biomimetic formation of silica nanoparticles[3] and thin films[4] by us and others. We also have recently reported that layer-by-layer (LbL) self-assembly[5] could be combined with biomimetic silicification to form silica thin films on a solid substrate under mild conditions.[6]

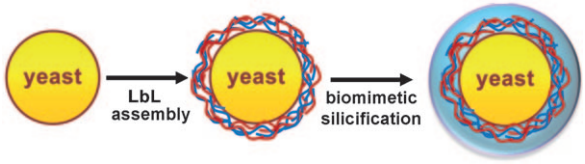
Herein, the LbL self-assembly of polyelectrolytes was chosen as a method for introducing catalytic templates for biomimetic silicification on the surface of living cells after considering that all processes should be mild enough to maintain the viability of the cells and that the LbL process had been utilized for the encapsulation of individual living cells within polyelectrolyte multilayers.[7] For example, the LbL process has been successfully utilized for coating individual yeast cells with calcium phosphate.[7b] Biomimetic silicification would be advantageous over the formation of calcium phosphate in single-cell encapsulation, because silicification can be designed to occur only at the surface with a proper choice of catalytic templates.[4,6] Among the cationic polyelectrolytes, poly(diallyldimethylammonium chloride) (PDADMA, M_w: 100000–200000) was selected as a catalytic template for biomimetic silicification,[6,8] because synthetic polymers containing quaternary amines were found to be chemically catalytic for biomimetic silica formation under physiologically mild conditions.[4a,b,9] We envisioned that the two consecutive, biocompatible processes (LbL and biomimetic silicification) would yield the encapsulation of individual, living cells within silica shells, without disturbance of cells (Scheme 1).

PDADMA and the anionic polyelectrolyte sodium polystyrene sulfonate (PSS, M_w: 70000) were alternately deposited onto the surface of *Saccharomyces cerevisiae* (*S. cerevisiae*; baker's yeast) according to our previous report.[6] Briefly, the yeast cells were immersed alternately in aqueous 0.5 M NaCl and solutions of PDADMA (5 mg mL^{-1}) and PSS

[*] S. H. Yang, B. Kong, Prof. Dr. I. S. Choi
Department of Chemistry, KAIST
Molecular-Level Interface Research Center
Daejeon 305-701 (Korea)
Fax: (+82) 42-350-2810
E-mail: ischoi@kaist.ac.kr
Homepage: http://cisgroup.kaist.ac.kr
Prof. Dr. I. S. Choi
Department of Bio and Brain Engineering, KAIST
Daejeon, 305-701 (Korea)
Dr. K.-B. Lee
Biotechnology Fusion Research Team
Korea Basic Science Institute (KBSI), Daejeon 305-333 (Korea)
J.-H. Kim, Prof. Dr. H.-S. Kim
Department of Biological Science, KAIST
Daejeon 305-701 (Korea)
Prof. Dr. H.-S. Kim
Graduate School of Nanoscience and Technology (WCU), KAIST
Daejeon, 305-701 (Korea)

[**] This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2008-313-C00496) and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0083525). We thank M. S. Hyun and M. H. Kim at the National Nanofab Center for the SEM and EDX analyses and Dr. H. S. Kweon and H. J. Cho at KBSI for the TEM analyses. We also thank Y. M. Lee at KAIST and Y. J. Shim at Chungnam National University for the cell culture.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200903010.



Scheme 1. Procedure for silica encapsulation of individual yeast cells. Alternate layer-by-layer (LbL) deposition of polycations and polyanions onto the surface of yeast preceded biomimetic silica formation.

(5 mg mL^{-1}) for 5 min each, which led to the coating of individual yeast cells with PDADMA/PSS multilayers (11/10) (11/10 means 11 layers of PDADMA and 10 layers of PSS). The LbL process was started with positively charged PDADMA, on the basis of reports that the surface of yeast cells was negatively charged,[7d,e] to achieve attractive electrostatic interactions between the cell surface and the polyelectrolyte and was also finished with PDADMA for providing catalytic interactions with negatively charged silicic acid derivatives at the interface. The 50 mM silicic acid solution containing the PDADMA/PSS multilayer-coated yeast was then stirred at room temperature for 30 min, leading to formation of silica-encapsulated yeast (yeast@SiO_2).

Scanning electron microscopy (SEM) was used to characterize the morphology of native yeast, multilayer-coated yeast, and yeast@SiO_2, after 24 h of drying at room temperature (Figure 1 a–f). The SEM micrographs clearly confirmed the single-cell encapsulation of yeast cells within silica shells. The maintenance of the original shape was noteworthy: while native yeast was noticeably shrunk because of dehydration, yeast@SiO_2 maintained its original shape. The multilayer-coated yeast also showed some ability to resist dehydration, but this effect was not as pronounced as for yeast@SiO_2. The high-magnification micrographs (insets of Figure 1 b, d, f) showed that the surface became rougher after biomimetic silicification. The surface was composed of silica nanoparticles that had been observed in previous studies of biomimetic silicification in solution and at surfaces.[4,6] The presence of silica was confirmed by the line-scan analysis of energy-dispersive X-ray (EDX) spectroscopy (Figure 1 g, h). The Si element line profile of yeast@SiO_2 showed that the surface of yeast was covered with silica. The presence of silica shells was further confirmed by transmission electron microscopy (TEM) with microtomous slices of yeast@SiO_2 (Figure 1 i). The thickness of the silica shell was measured to be more than 50 nm, which was expected on the basis of previous reports on the thicknesses of polyelectrolyte multilayers formed on living or fixed cells and colloidal particles[7c,10] and on the biomimetic silicification.[4,6] We also visualized many individual yeast@SiO_2 cells in aqueous solution by staining silica shells with tetracycline (see the Supporting Information, Figure 1). The staining also indicated that each yeast cell was encapsulated by silica.

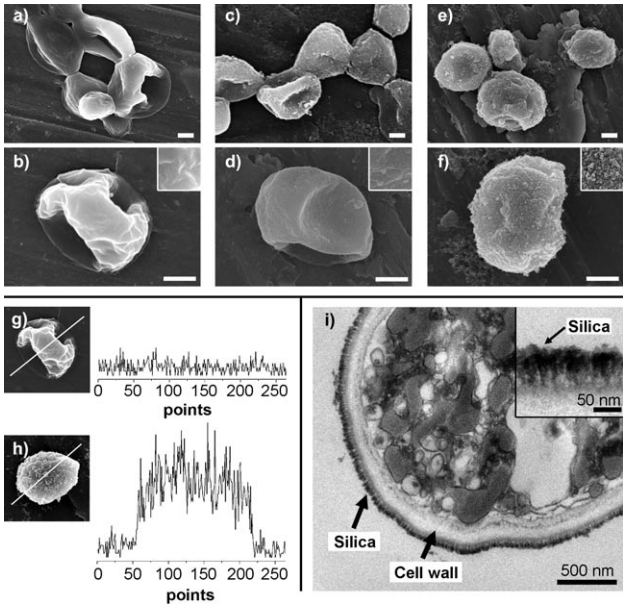


Figure 1. SEM micrographs of a, b) native yeast, c, d) PDADMA/PSS-multilayer (11/10)-coated yeast, and e, f) yeast@SiO_2 at different magnifications. The scale bars are 1 μm. Inset figures in (b), (d), and (f) show the surface morphologies of yeast cells at each step. Part (e) clearly shows that yeast cells were encapsulated separately and individually within a silica shell that was composed of silica nanoparticles, as shown in the inset of (f). g,h) EDX spectroscopy line profiles for silicon of native yeast (g) and yeast@SiO_2 (h), thus confirming the presence of the silica shell. i) TEM micrographs of microtome-sliced yeast@SiO_2 also confirm the presence of silica shells, and the magnified micrograph (inset) shows that the thickness of the silica shell is above 50 nm.

Long-term viability of yeast@SiO_2 was investigated with native yeast cells as a comparison (Figure 2). Both native yeast and yeast@SiO_2 were stored in pure water without any nutrients at 4 °C for 30 days. On day 1, most of the native yeast cells (ca. 97 %) were alive, but the viability of yeast@SiO_2 was found to be about 77 %, probably because of chemical stress in the course of LbL assembly and physical stress from centrifugation. On day 30, although the cells in both samples had maintained their original shapes, the cell

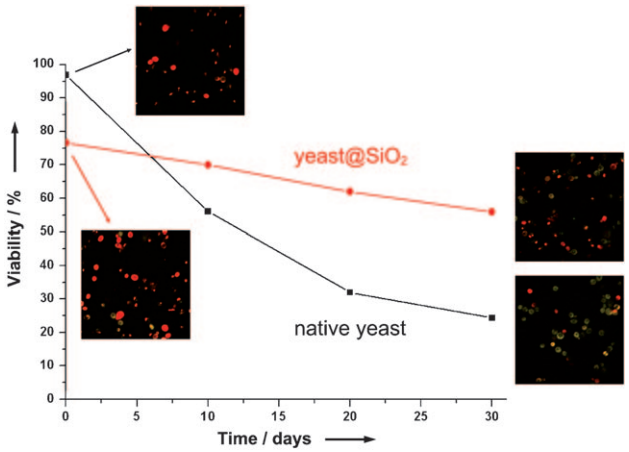


Figure 2. Viability of native yeast and yeast@SiO_2. A fluorescent probe, FUN 1 cell stain from Invitrogen, was used for the viability test. FUN 1 cell stain determined the metabolic activity of yeast; the conversion from original yellow-green to red-orange required both membrane integrity and metabolic activity of yeast cells. Yeast cells in red were considered alive, and the ones in green and yellow-green were considered dead. At least 300 yeast cells were counted for calculating the cell viability.

viability was profoundly different. The cell viability of yeast@SiO_2 was about 56%, but only 24% of native yeast cells were alive. The observed long-term viability was in good agreement with reports on the entrapment of a collection of yeast cells[1a] or bacteria[1c] in a silica matrix, in which the cell viability was about 55% after 30 days. When we consider the number of live cells on day 1 as a reference, the enhancement of the cell viability was about threefold. As we expected, the biomimetic silicification involving biocompatible processes increased the long-term viability of individual yeast cells, probably by retaining intact cytoplasmic environments through the stabilization of cellular membranes under physicochemical pressure and by protecting the cellular structure from dehydration.

After confirming the cell viability of yeast@SiO_2, we also investigated whether yeast cells were capable of dividing under culture conditions after silica encapsulation. The native yeast and yeast@SiO_2 were incubated in yeast mold (YM) broth at 30°C, and cell growth was monitored by measuring the absorbance at 600 nm (Figure 3). In the case of native

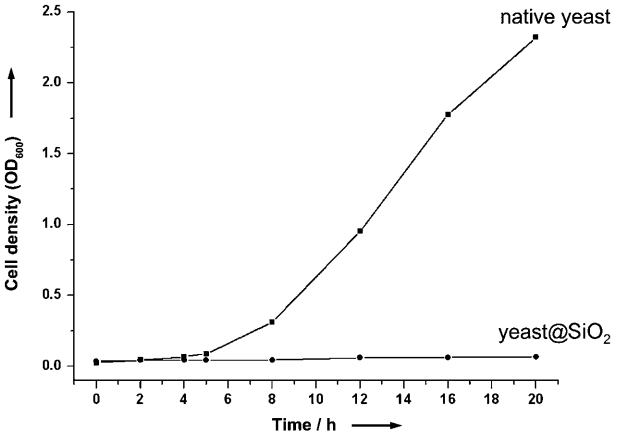


Figure 3. Growth curve of native yeast and yeast@SiO_2. The information on cell density was obtained from absorbance measurements at 600 nm (OD_{600}). OD_λ is defined as $-\log_{10}(I/I_0)$, where I_0 is the intensity of the incident light beam and I is the intensity of the transmitted light beam.

yeast cells, there was a lag phase for the first approximately 4 h; immediately after this lag time came the exponential growth phase (■ in Figure 3). In stark contrast, the growth curve of yeast@SiO_2 remained in the lag phase until 20 h (● in Figure 3), thus implying that the silica shell prevented yeast@SiO_2 from dividing and let it remain in the resting phase (G0). In addition to the observation that silica-encapsulated yeast cells were metabolically active, this result showed that cell division could be controlled in a designed way by biomimetic silicification.

To demonstrate the applicability of our method to other living cells, the LbL process and biomimetic silicification were also applied to *Escherichia coli* (*E. coli*) and *Bacillus atrophaeus* spores. *E. coli* was coated with PDADMA/PSS mutilayers (6/5), and the subsequent biomimetic silicification led to the formation of *E. coli*@SiO_2. *E. coli*@SiO_2 maintained its original shape after 24 h of drying, and the silica encapsulation of individual *E. coli* cells was confirmed by EDX spectroscopy line-scan analysis (See the Supporting Information, Figure 2a,b). The viability of *E. coli*@SiO_2 was tested with a LIVE/DEAD BacLight bacterial viability kit (Invitrogen), which indicated that most of the *E. coli*@SiO_2 cells did not survive. A further experiment showed that the LbL process affected the viability of *E. coli*, because most native *E. coli* cells were found to be dead after the first step of the LbL process (deposition of a single PDADMA layer). The low viability of *E. coli*, therefore, could be explained by the antibacterial properties of quaternary amines in PDADMA.[11]

Among living cells, the silica encapsulation of individual microbial spores, in particular, would be of technological importance, because robust spores were thought to be strong candidates for the stable sensing element of cell-based biosensors and would need to be surface-functionalized for such applications.[12] In light of this background, we attempted to encapsulate individual *Bacillus atrophaeus* spores with silica as a representative example (see the Supporting Information, Figure 2c,d). The morphological change of the spore surface, along with the EDX line profile for Si, confirmed the successful encapsulation of individual spores with silica.

In summary, we demonstrated a method for encapsulating individual cells, such as yeast, *E. coli*, and microbial spores, within a silica shell, by combining two biocompatible processes, LbL and biomimetic silicification. Apart from the intriguing appearance, the individual encapsulation with silica has several advantages. 1) The silica encapsulation was found to greatly enhance cell viability by protecting the cell from harsh environments. The silica shell should improve the mechanical strength and chemical stability of the native cell membrane. 2) The silica shell can be further functionalized by silica chemistry. The introduction of functional groups onto the cell surface has generally involved complicated chemical and biological processes, and the reported methods were limited because the chemical treatment is usually harmful to cells.[13] Well-established silica chemistry, in combination with biomimetic silica encapsulation, would be a simple but versatile approach to surface functionalization of cells. 3) The silica-encapsulated cells could be used as a useful model for biological studies. For example, cell-to-cell communication can be studied by adjusting the permeability of signaling molecules with the silica encapsulation. Compared with cells in the silica matrix that have physical and mechanical restrictions,[1] individually encapsulated cells are free from those restrictions, and therefore individual cells can be manipulated at will for biological studies.

The field of interfacing individual living cells with chemical (organic or inorganic) entities is still in its infancy. Appropriate methods should maintain the functional and structural integrity of the cells to be modified after modifications of cell surfaces. We believe that the work demonstrated herein suggests a simple but widely applicable method for providing bio-nanointerfaces of cells.

Experimental Section

Biomimetic silica encapsulation: Aqueous NaCl solution (0.5 M) was used for preparing poly(diallyldimethylammonium chloride) (PDADMA, M_w: 100000–200000, 20 wt% in H_2O, Aldrich) and sodium polystyrene sulfonate (PSS, average M_w: ca. 70000, powder, Aldrich) solutions. The final concentration of the solutions was 5 mg mL^{-1}. The cells were alternately immersed in the PDADMA solution and the PSS solution for 5 min for each step. After the LbL process, multilayer-coated cells were placed in the 50 mM silicic acid solution, which had been independently prepared by stirring an HCl solution (0.1 mM) of tetramethyl orthosilicate (TMOS, 100 mM) at room temperature for 20 min and adding the resulting solution to aqueous sodium phosphate buffer (100 mM, pH 5.5) with 1:1 (v/v) ratio. After 30 min, the substrate was removed and washed with 0.5 M aqueous NaCl solution.

Characterization: Scanning electron microscopy (SEM) imaging and energy-dispersive X-ray (EDX) spectroscopy elemental analysis were performed with a Sirion FEI XL FEG/SFEG microscope (FEI Co., The Netherlands) with an accelerating voltage of 10 kV after sputter-coating with platinum. Viability of yeast and *E. coli* was measured with FUN 1 cell stain (Invitrogen) and LIVE/DEAD BacLight bacterial viability kit (Invitrogen), respectively. Silica shells were stained with tetracycline hydrochloride (cell culture tested, Sigma). The stained cells were observed with a LSM 510 META microscope (Carl Zeiss, Germany). Transmission electron microscopy (TEM) imaging was performed using a Tecnai-G2 Spirit Twin instrument (FEI Co., The Netherlands). Specimens were fixed with glutaraldehyde and OsO_4 and then dehydrated in ethanol. The fixed samples were embedded in Epon 812/Araldite M resin. Thin sections (ca. 80 nm) were cut by using ULTRACUT UCT ultramicrotome (Leica, Austria) and stained with uranyl acetate and lead citrate.

Received: June 4, 2009
Revised: August 28, 2009
Published online: October 23, 2009

Keywords: biomimetic synthesis · cell modification · encapsulation · self-assembly · silicates

[1] a) H. K. Baca, C. Ashley, E. Carnes, D. Lopez, J. Flemming, D. Dunphy, S. Singh, Z. Chen, N. Liu, H. Fan, G. P. López, S. M. Brozik, M. Werner-Washburne, C. J. Brinker, *Science* **2006**, *313*, 337; b) H. R. Luckarift, J. C. Spain, R. R. Naik, M. O. Stone, *Nat. Biotechnol.* **2004**, *22*, 211; c) N. Nassif, O. M. M. Bouvet, M. N. Rager, C. Roux, T. Coradin, J. Livage, *Nat. Mater.* **2002**, *1*, 42.

[2] a) D. Losic, J. G. Mitchell, N. H. Voelcker, *Adv. Mater.* **2009**, *21*, 2947; b) M. B. Dickerson, K. H. Sandhage, R. R. Naik, *Chem. Rev.* **2008**, *108*, 4935; c) M. Sumper, E. Brunner, *Adv. Funct. Mater.* **2006**, *16*, 17; d) C. Sanchez, H. Arribart, M. Madeleine, G. Guille, *Nat. Mater.* **2005**, *4*, 277; e) N. Kröger, S. Lorenz, E. Brunner, M. Sumper, *Science* **2002**, *298*, 584; f) M. Sumper, *Science* **2002**, *295*, 2430.

[3] a) E. G. Bellomo, T. J. Deming, *J. Am. Chem. Soc.* **2006**, *128*, 2276; b) M. M. Tomczak, D. D. Glawe, L. F. Drummy, C. G. Lawrence, M. O. Stone, C. C. Perry, D. J. Pochan, T. J. Deming, R. R. Naik, *J. Am. Chem. Soc.* **2005**, *127*, 12577; c) J. J. Yuan, R. H. Jin, *Adv. Mater.* **2005**, *17*, 885; d) E. Brunner, K. Lutz, M. Sumper, *Phys. Chem. Chem. Phys.* **2004**, *6*, 854; e) M. R. Knecht, D. W. Wright, *Langmuir* **2004**, *20*, 4728; f) M. R. Knecht, D. W. Wright, *Chem. Commun.* **2003**, 3038; g) S. V. Patwardhan, S. Clarson, *J. Mater. Sci. Eng.* **2003**, *23*, 495.

[4] a) S. H. Yang, J. H. Park, W. K. Cho, H.-S. Lee, I. S. Choi, *Small* **2009**, *5*, 1947; b) W. K. Cho, S. M. Kang, D. J. Kim, S. H. Yang, I. S. Choi, *Langmuir* **2006**, *22*, 11208; c) D. J. Kim, K.-B. Lee, T. G. Lee, H. K. Shon, W.-J. Kim, H.-j. Paik, I. S. Choi, *Small* **2005**, *1*, 992; d) D. J. Kim, K. B. Lee, Y. S. Chi, W. J. Kim, H.-j. Paik, I. S. Choi, *Langmuir* **2004**, *20*, 7904.

[5] a) P. J. Yoo, K. T. Nam, A. M. Belcher, P. T. Hammond, *Nano Lett.* **2008**, *8*, 1081; b) S. Kidambi, I. Lee, C. Chan, *Adv. Funct. Mater.* **2008**, *18*, 294; c) P. T. Hammond, *Adv. Mater.* **2004**, *16*, 1271, and references therein; d) I. Lee, *Chem. Mater.* **2003**, *15*, 4583.

[6] S. H. Yang, I. S. Choi, *Chem. Asian J.* **2009**, *4*, 382.

[7] a) S. S. Balkundi, N. G. Veerabadran, D. M. Eby, G. R. Johnson, Y. M. Lvov, *Langmuir* **2009**, DOI: 10.1021/la900971h; b) B. Wang, P. Liu, W. Jiang, H. Pan, X. Xu, R. Tang, *Angew. Chem.* **2008**, *120*, 3616; *Angew. Chem. Int. Ed.* **2008**, *47*, 3560; c) A. L. Hillberg, M. Tabrizian, *Biomacromolecules* **2006**, *7*, 2742; d) S. Krol, M. Nolte, A. Diaspro, D. Mazza, R. Magrassi, A. Gliozzi, A. Fery, *Langmuir* **2005**, *21*, 705; e) A. Diaspro, D. Silvano, S. Krol, O. Cavalleri, A. Gliozzi, *Langmuir* **2002**, *18*, 5047.

[8] N. Laugel, J. Hemmerle, C. Porcel, J.-C. Voegel, P. Schaaf, V. Ball, *Langmuir* **2007**, *23*, 3706.

[9] a) J. J. Yuan, O. O. Mykhaylyk, A. J. Ryan, S. P. Armes, *J. Am. Chem. Soc.* **2007**, *129*, 1717; b) Y. Jia, G. M. Gray, J. N. Hay, Y. Li, G.-F. Unali, F. L. Baines, S. P. Armes, *J. Mater. Chem.* **2005**, *15*, 2202; c) G. M. Gray, J. N. Hay, *Mater. Res. Soc. Symp. Proc.* **2003**, *775*, 179.

[10] a) R. Georgieva, S. Moya, E. Donath, H. Bäumler, *Langmuir* **2004**, *20*, 1895; b) E. Donath, G. B. Sukhorukov, F. Caruso, S. A. Davis, H. Möhwald, *Angew. Chem.* **1998**, *110*, 2323; *Angew. Chem. Int. Ed.* **1998**, *37*, 2201.

[11] a) S. B. Lee, R. R. Koepsel, S. W. Morley, K. Matyjaszewski, Y. J. Sun, A. J. Russell, *Biomacromolecules* **2004**, *5*, 877; b) Z. Jia, D. Shen, W. Xu, *Carbohydr. Res.* **2001**, *333*, 1.

[12] a) K.-B. Lee, Y. H. Jung, Z.-W. Lee, S. H. Kim, I. S. Choi, *Biomaterials* **2007**, *28*, 5594; b) T. J. Park, K.-B. Lee, S. J. Lee, J. P. Park, Z.-W. Lee, S. Y. Lee. I. S. Choi, *J. Am. Chem. Soc.* **2004**, *126*, 10512.

[13] a) S. T. Laughlin, J. M. Baskin, S. L. Amacher, C. R. Bertozzi, *Science* **2009**, *320*, 664; b) J. A. Codelli, J. M. Baskin, N. J. Agard, C. R. Bertozzi, *J. Am. Chem. Soc.* **2008**, *130*, 11486; c) E. Saxon, C. R. Bertozzi, *Science* **2000**, *287*, 2007; d) L. K. Mahal, K. J. Yarema, C. R. Bertozzi, *Science* **1997**, *276*, 1125.

