Drug Delivery

DOI: 10.1002/ange.200504599

Laser-Induced Release of Encapsulated Materials inside Living Cells**

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Drug delivery into biological cells is an important and growing area of application.[1] Among other systems, such as gels,[2] polymeric micelles,[3] liposomes,[4a,b] and colloids,[4c] nanoengineered polyelectrolyte multilayer microcapsules[5] offer a unique opportunity to combine surface multifunctionality with design flexibility for the delivery of encapsulated materials into designated compartments and cells.[6] Furthermore, microcapsules can be arranged in arrays for imaging,[7a,b] could be appropriate candidates for a cell-sorting system,[7c,d] and serve as fluorescence markers for the characterization of cells by fluorescence-activated cell sorting (FACS).[7e] The capsules are fabricated using the layer-by-layer (LbL) method[8] by alternately adsorbing oppositely charged polymers on colloidal templates followed by core dissolution. In this regard, proteins and biocompatible polymers have also received increased interest.[9] The main advantage of such a method is the precise control over the chemical composition of the surfaces.

In the area of biomedical applications, polyelectrolyte-multilayer capsules are envisioned for the delivery of encapsulated materials into biological cells.[6] Recently, we have presented the real-time monitoring and remote release of encapsulated materials from polyelectrolyte-multilayer capsules on the single-capsule level.[10] Such an approach[10] is different from the studies reported by other research groups[11] in that it is performed on a single-capsule level, which is the method ideally suited to applications where precise control is necessary. In addition, the distinctive feature reported in reference [10b] is the measurement of the temperature rise induced locally by absorption of laser light by nanoparticles.

In general, nanoparticles[12] are becoming ubiquitous components that link chemistry and physics with biology and biochemistry. They can be embedded in the walls of capsules to provide functionality,[6a] and they are also finding increasing interest for biological imaging.[13] Herein, we show that polyelectrolyte-multilayer capsules containing metallic nanoparticles in their walls can be remotely activated to release encapsulated material inside living cells. Fluorescently labeled polymers were chosen as a model system for encapsulated materials. The remote-release experiments were conducted according to the following scheme. The polyelectrolyte-multilayer shells were doped with metal nanoparticles, which served as absorption centers for energy supplied by a laser beam. These absorption centers cause local heating that disrupts the local polymer matrix and allows the encapsulated material to leave the interior of the capsule.

When using lasers with biological objects, it is important to minimize the absorption of laser light by cells and tissue. This can be accomplished by choosing the laser wavelength in the biologically "friendly" window[14a,b]—the near-infrared (NIR) part of the spectrum. Usually the spectral properties of water[14c] serve as a good criterion, as it constitutes 80–85 % of eukaryotic cells. Indeed, in water the temperature rise in the focus of a laser diode with wavelength 850 nm and operating at optical powers up to 100 mW during less than 1 s exposure time was reported to be under 1 K.[14d] Other important parameters that control the interaction of laser light with the absorption centers are the size of the nanoparticles and their concentration on the microcapsules.[10b] The concentration of metal nanoparticles plays an important role for two reasons: 1) when the distance between the two adjacent nanoparticles is of the order of their size, the thermal effects produced by

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[**] We thank Dr. A. L. Rogach and Dr. A. S. Susha for providing nanoparticles, Annegret Praast for technical assistance, Anne Heilig for AFM measurements, and Rona Pitschke for TEM measurements. We gratefully acknowledge the Volkswagen-Stiftung (I/80-051-054) and the 6th FP EU projects STREP NMP3-CT-2005-516922 "SelectNANO" and STREP 01428 "NANOCAPS". Support by NATO grant no. CLG981299 and the Emmy Noether Program of the Deutsche Forschungsgemeinschaft is also acknowledged.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

adjacent nanoparticles add up; and 2) the interaction of nanoparticles located in close proximity to each other results in an increase of absorption at lower energies or higher wavelengths (causing the so-called red shift) compared to the surface plasmon resonance band of stand-alone nanoparticles.[15] In this regard, spatial arrangement of the nanoparticles is essential[16a–c] and control of their distribution is paramount.[16d]

In the present studies we used silver nanoparticles[10a] for the remote activation of microcapsules, defined as deformation of their shape upon laser illumination, inside the cells. These nanoparticles were chosen because they provide dark contrast in transmission microscopy as a result of their high concentration on the capsules. Most of the silver nanoparticles were larger than 20 nm. These features lead to nonvanishing absorption[10a] in the NIR part of the spectrum as a consequence of dipolar and higher-order multipolar contributions[15b,c] and interaction between the nanoparticles.[15a] This finding is consistent with the visible–NIR spectral characteristics of silver nanoparticles with larger sizes[15b] located in clusters.[15c] Further studies were conducted of the release of encapsulated polymers from microcapsules containing gold and gold sulfide nanoparticles.[17] These nanoparticles absorb in the NIR part of the spectrum,[17] and the nature of the NIR absorption is the subject of continuing research.[17e]

Encapsulation of macromolecules can be performed, for example, by pH-controlled[18] swelling and shrinking of capsules[19] or with a matrix polyelectrolyte system.[20] We encapsulated an Alexa Fluor 488 (AF-488) dextran conjugate by a thermal[21] treatment method developed by Köhler et al.[21b] and based on the size reduction of strong polyelectrolyte sodium poly(styrene sulfonate)(PSS)/poly(diallyldimethylammonium chloride) (PDADMAC) microcapsules upon heating. Indeed, temperature was shown to affect the polyelectrolyte multilayers.[22] The heat-induced shrinking of microcapsules with a balanced charge ratio of polyelectrolytes is attributed to the reduced water/polyelectrolyte interface and subsequently lower surface energy.[21b] Therefore, the heat treatment of microcapsules applied in our study was accompanied by a reduction in size from about 4.5 to about 3 μm, which entrapped the dextran.

Figure 1 presents AFM images of a typical dried capsule before and after heat treatment. Upon heat treatment, the thickness of the walls of the capsules increases from about 14 to about 42 nm. Furthermore, the polymers incorporated inside the capsules smooth the surfaces of their walls. Peaks and valleys in the range of 55–120 nm can be seen in the thermally treated capsules without encapsulated polymer (Figure 1 d–f). In contrast, the thermally treated capsules containing encapsulated polymer exhibit a uniform thickness of about 40 nm. The presence of polymers inside the microcapsules leaves the average wall thickness virtually unchanged but alters the texture and reduces the roughness (Figure 1 g–i). Nanoparticles embedded in the walls of the capsules can also be seen after heat treatment (inset to Figure 1 i). The capsules were constructed on silica templates, which have a negligible effect on polyelectrolyte multilayers.[21b]

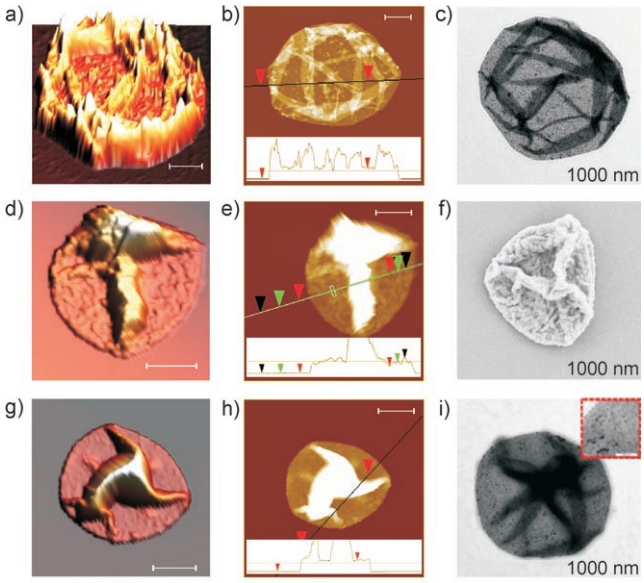

Figure 1. a,b) AFM and c) TEM images of $(PSS/PDADMAC)_4$ polyelectrolyte-multilayer capsules with gold and gold sulfide nanoparticles embedded in their walls before thermal treatment; the height marked by the red arrows in the inset to (b) corresponds to 28 nm. d,e) AFM and f) SEM images of a similar capsule after thermal treatment without encapsulated polymer; the heights marked by the green, red, and black arrows in the inset to (e) are 55, 87, and 120 nm, respectively. g,h) AFM and i) TEM images of a similar capsule after thermal treatment with encapsulated AF-488 dextran. The height marked by the red arrows in the inset to (h) is 82 nm; the inset to (i) shows a magnified area. All values for heights correspond to the double wall thickness. The scale bars in all images correspond to 1 μm.

The mechanical properties of the polyelectrolyte microcapsules[23a–f] and multilayers[23g–j] have been the subject of extensive research. The studies conducted by AFM[23b,c] revealed that forces in the range of hundreds of piconewtons are sufficient to induce buckling of capsules that were not thermally treated. The study of the mechanical properties of thermally treated PSS/PDADMAC capsules at room temperature demonstrated[23e] that after heating for 20 min at 50 °C, the stiffness increased by four times (from ≈220 to ≈870 $pN\,nm^{-1}$) and, even more remarkably, by more than ten times (from ≈220 to ≈2600 $pN\,nm^{-1}$) upon heat treatment at 55 °C. Such an enhancement of the stiffness is attributed to the increase of the wall thickness that accompanies the heat shrinking. The improvement of the mechanical integrity of thermally treated capsules was also consistent with our observations,[6c] as the thickness of the walls has an important influence on the percentage of capsules that are deformed upon ingestion by cells. Capsules with thicker walls are less likely to be deformed, and are thus more suited to the delivery of encapsulated materials.

All encapsulation and release experiments were performed with the AF-488 dextran conjugate because it is significantly brighter and more photostable than other green fluorophores,[24a,b] which is a required condition for experiments under physiological conditions. The pH stability of encapsulated AF-488 dextran at different pH values was

investigated in comparison to that of fluorescein isothiocyanate (FITC) dextran (see Supporting Information), which is a commonly used, strongly pH-dependent, fluorescently labeled polymer that exists in four forms in solution.[24c–f] At $pH > 5$, both phenol and carboxylic groups of the FITC dye molecules are ionized, whereas at $pH < 5$ the majority of them are in their neutral or cationic, predominantly nonfluorescent, state. This results in decreasing fluorescence at lower pH values (see Supporting Information). A sharp contrast between the pH stability of AF-488 and FITC dextran is observed. Therefore, AF-488 can be used in experiments where stability is required, whereas FITC is intended for pH and other sensors. In addition, experiments were performed to determine both the mechanical integrity of capsules without nanoparticles and the photostability of encapsulated AF-488 dextran.

In fluorescent dyes the excitation from the ground state to the first singlet state S_1 dominates the absorption processes,[25] while higher-order photon excitation may influence the signal only at high photon fluxes (typically with femtosecond lasers).[25b] The laser wavelength (830 nm) is located outside the 450–510 nm absorption band of AF-488 dextran (see Supporting Information). Notwithstanding this fact, a control experiment was conducted in which microcapsules without embedded nanoparticles were exposed to laser light with intensities and conditions similar to those used in the release studies (see Supporting Information). It served to test both the photostability (or exclude the possibility of photobleaching) and the mechanical integrity of microcapsules filled with AF-488 dextran but without nanoparticles in their walls upon laser excitation. Illumination was performed by a laser operating in the continuous wave (CW) mode at 830 nm with an incident intensity of 50 mW. Notably, although the laser operated in a CW mode, the shutter of the laser was opened for a short pulse (on the order of seconds or less) during the illumination. No fluorescence intensity changes were observed before and after illumination. In addition to test the photostability, this experiment also provides evidence that a capsule without nanoparticles is not deformed upon illumination with laser light. Note that the laser beam was directed from the top, thus pushing the capsule against the cover slide as a result of the radiation pressure of light[26] so that the capsule remained in the focus. Further studies were conducted with capsules containing nanoparticles in their walls.

Before the release studies, we performed experiments on the remote activation of capsules inside living cells. For this purpose, we fed living cells with capsules containing silver nanoparticles in their walls. These capsules were prepared according to the method described previously;[10a] they had no encapsulated material inside and were chosen for dark contrast in transmission. An ingested capsule was illuminated with a CW laser beam directed from the bottom and operating at 830 nm with a power of 50 mW. Figure 2 demonstrates that a capsule can be opened or activated remotely inside a cell. The rupture of the capsule (Figure 2c) demonstrates that a laser–nanoparticle interaction through thermal processes[10b] is responsible for its activation. Other processes, for example transport of protons or electron

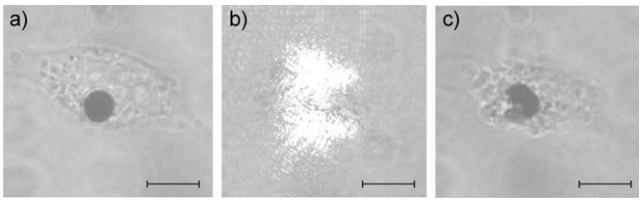

Figure 2. Remote activation of a capsule containing silver nanoparticles in its walls. The capsule was ingested by a living MDA-MB-435S cancer cell. The images show the cell before (a), during (b), and after (c) illumination with a laser. The scale bars correspond to 10 μm.

redistribution around the nanoparticles,[27] do not determine the activation of and eventual release from microcapsules, because the polyelectrolyte multilayers were shown to be permeable for protons[24c] and the local redistribution of electrons cannot cause the rupture of the capsules. In the next step, release experiments were conducted with AF-488 dextran-filled capsules containing gold and gold sulfide nanoparticles in the walls.

Figure 3 demonstrates the release of encapsulated AF-488-labeled dextran inside a living cell upon laser illumination. The fluorescence image of the capsules is presented in

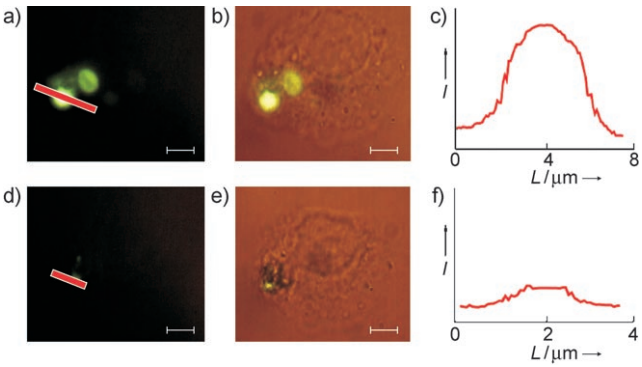

Figure 3. Sequence of images showing the release of fluorescent AF-488 dextran inside a living MDA-MB-435S cell. a) Fluorescence image of a filled capsule; b) superimposed fluorescence and transmission images of the capsule inside the same cell; c) fluorescence intensity *I* profile plotted along the length *L* of the red line in (a). d)–f) Similar data after exposure of the cell to a laser beam. The scale bars in all images correspond to 5 μm.

Figure 3a, while Figure 3b shows the superimposed fluorescence and transmission signals from the same cell and the same capsules before illumination by laser light. The capsule appears filled (Figure 3c) before illumination. Similar images of the same capsule after illumination (Figure 3d–f) show that, although there is some leftover fluorescence in the walls of the capsules, most polymer molecules had left the interior of the capsule. The leftover fluorescence traces in the walls of the capsule are consistent with earlier reported experiments.[10b] Notably, the rise in temperature during laser illumination of capsules with embedded nanoparticles is several degrees and it is concentrated in the vicinity of the capsules.[10b] In our experiments, the cells adhered to the substrate both before and after the release of the encapsu-

lated polymer, which indicates that this method is feasible for the delivery of encapsulated materials into cells.

In the activation and release experiments, contrary to the photostability tests, the laser beam was directed from the bottom onto the chamber containing the living cells because of its design. In such a geometry, capsules not taken up by cells are pushed up by the laser beam and away from the field of view as a result of the radiation pressure of light.[26] This effect is demonstrated in Figure 4, which shows that capsules

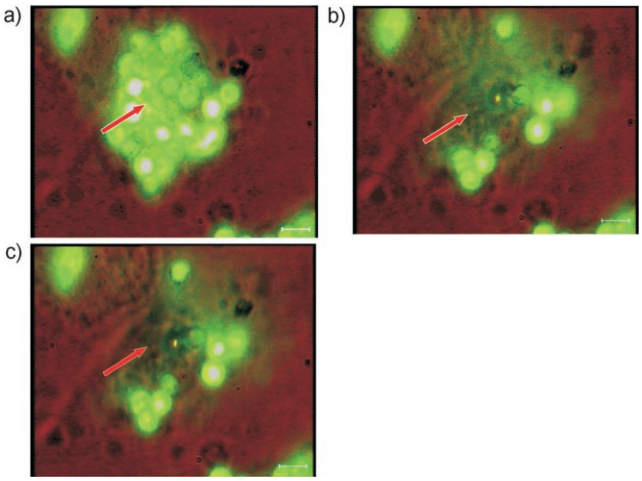

Figure 4. Fluorescence images demonstrating the lifting up of non-internalized capsules located on top of a living MDA-MB-435S cell above and away from the imaging plane or the focus a) before, b) during, and c) after the laser beam illuminated the chamber from the bottom. The capsules were lifted up with a laser power of 50 mW. The red arrows indicate the locations of the capsules that were lifted up. The scale bars in all images correspond to 5 μm.

situated on top of a cell are pushed out of the field of view by the laser. Figure 4a shows that an agglomerate of capsules is located in the field of view slightly above the cell, whose contours can also be seen. Figure 4b shows the same cell and the same agglomerate of capsules during laser illumination, while Figure 4c presents the same cell after liftoff of the capsules. The agglomerate of the capsules is not a heavy aggregate, as part of it can still be seen in Figure 4c. Besides, the cell itself did not undergo changes, which is consistent with the data reported for the temperature rise during laser illumination.[14d] These experiments with "flying capsules" demonstrate that the release of encapsulated material can be carried out only from capsules internalized by the cells; capsules merely adherent to the outer membrane were lifted up and away from the imaging plane.

In conclusion, the release of encapsulated material from polyelectrolyte-multilayer capsules has been demonstrated inside living cells. Metal nanoparticles were incorporated inside the walls of the capsules, and served as energy-absorbing centers for illumination by laser light. AF-488 dextran was successfully incorporated into the capsules using a novel heat-shrinking method. The capsules obtained by such a method exhibit improved mechanical stability—properties important for the delivery of encapsulated material. Upon illumination by laser light, the encapsulated dextran leaves the interior of a capsule inside a living cancer cell. Capsules not internalized by the cells are pushed up by the laser and move away from the field of view upon laser illumination from the bottom. The study presented herein serves as a significant step toward the use of polyelectrolyte-multilayer capsules for the delivery of medicine into biological cells, and is, therefore, relevant to research on drug delivery.[1] The presented method is different from previous, albeit also important, studies in that it is conducted on an individual-capsule level and offers an improved degree of control and monitoring.

Experimental Section

Polyelectrolyte-multilayer capsules were prepared according to the previously described method.[5,21] Silica particles (SiO_2, 4.55 μm; Microparticles GmbH, Berlin, Germany) were alternately coated with four double layers of PDADMAC ($M_w \approx 200$–350 kDa; Sigma–Aldrich, Munich, Germany) and PSS ($M_w = 70$ kDa; Sigma–Aldrich). FITC dextran (Sigma–Aldrich) was used in pH stability tests. All chemicals were used without further purification. The water used in all experiments was prepared in a three-stage Millipore Milli-Q Plus 185 purification system and had a resistivity higher than 18.2 MΩ cm.

For activation studies, silver-containing microcapsules were prepared according to the method described earlier.[10a] For release studies, gold and gold sulfide[17] nanoparticles were deposited in the layers according to the method described earlier.[10b] After deposition of eight polyelectrolyte monolayers, silica cores were dissolved in 0.1M HF.[21b] Alexa Fluor 488 dextran conjugate (AF-488 dextran, $M_w = 10$ kDa; Invitrogen, Karlsruhe, Germany) was encapsulated in $(PDADMAC/PSS)_4$ capsules according to the thermal treatment method.[21b,c]

The optical setup used in the experiments was similar to that described previously.[10] The laser was operated in CW mode, and the shutter was opened during illumination for brief pulses of the order of seconds or less. MDA-MB-435S cancer cell lines were used in the experiments; they were seeded on the substrate overnight, then approximately 30 capsules per cell were added and the experiments were carried out after incubation for 4 h as previously reported.[6c]

Received: December 27, 2005
Revised: April 13, 2006
Published online: June 22, 2006

Keywords: drug delivery · multilayers · nanoparticles · photoactivation · polymers

[1] a) R. Langer, *Nature* **1998**, *392*, 5–10; b) M. Ferrari, *Nat. Rev. Cancer* **2005**, *5*, 161–171; c) H. Ai, S. A. Jones, M. M. de Villiers, Y. M. Lvov, *J. Controlled Release* **2003**, *86*, 59–68.

[2] a) A. P. Nowak, V. Breedveld, L. Pakstis, B. Ozbas, D. J. Pine, D. Pochan, T. J. Deming, *Nature* **2002**, *417*, 424–428; b) M. Das, S. Mardyani, W. C. W. Chan, E. Kumacheva, *Adv. Mater.* **2006**, *18*, 80–83.

[3] a) G. S. Kwon, T. Okano, *Adv. Drug Delivery Rev.* **1996**, *21*, 107–116; b) C. Allen, A. Eisenberg, J. Mrsic, *Drug Delivery* **2000**, *7*, 139–145; c) R. Duncan, *Nat. Rev. Drug Discovery* **2003**, *2*, 347–360.

[4] a) B. Chaize, J. P. Colletier, M. Winterhalter, D. Fournier, *Artif. Cells Blood Substitutes Biotechnol.* **2004**, *32*, 67–75; b) M. Michel, M. Winterhalter, L. Darbois, J. Hemmerle, J.-C. Voegel, P. Shaaf, V. Ball, *Langmuir* **2004**, *20*, 6127–6133; c) S. Faraassen,

J. Vörös, G. Csucs, M. Textor, H. P. Merkle, E. Walter, *Pharm. Res.* **2003**, *20*, 237 – 246.

[5] a) E. Donath, G. B. Sukhorukov, F. Caruso, S. A. Davies, H. Möhwald, *Angew. Chem.* **1998**, *110*, 2324 – 2327; *Angew. Chem. Int. Ed.* **1998**, *37*, 2202 – 2205; b) C. Peyratout, L. Dähne, *Angew. Chem.* **2004**, *116*, 3850 – 3873; *Angew. Chem. Int. Ed.* **2004**, *43*, 3762 – 3783.

[6] a) G. B. Sukhorukov, A. L. Rogach, B. Zebli, T. Liedl, A. G. Skirtach, K. Köhler, A. A. Antipov, N. Gaponik, A. S. Susha, M. Winterhalter, W. Parak, *Small* **2005**, *1*, 194 – 200; b) B. G. De Geest, C. Dégunat, G. B. Sukhorukov, K. Braeckmans, S. C. De Smedt, J. Demeester, *Adv. Mater.* **2005**, *17*, 2357 – 2361; c) A. Muñoz Javier, O. Kreft, A. Piera Alberola, C. Kirchner, B. Zebli, A. S. Susha, E. Horn, S. Kempner, A. G. Skirtach, A. L. Rogach, J. Rädler, G. B. Sukhorukov, M. Benoit, W. J. Parak, *Small* **2006**, *2*, 394 – 400; d) M. Fischlechner, L. Toellner, P. Messner, R. Grabherr, E. Donath, *Angew. Chem.* **2006**, *118*, 798 – 803; *Angew. Chem. Int. Ed.* **2006**, *45*, 784 – 789; e) B. G. De Geest, R. E. Vandenbroucke, A. M. Guenther, G. B. Sukhorukov, W. E. Hennink, N. N. Sanders, J. Demeester, S. C. De Smedt, *Adv. Mater.* **2006**, *18*, 1005 – 1009.

[7] a) M. Nolte, A. Fery, *Langmuir* **2004**, *20*, 2995 – 2998; b) B. Wang, Q. Zhao, F. Wang, C. Gao, *Angew. Chem.* **2006**, *118*, 1590 – 1593; *Angew. Chem. Int. Ed.* **2006**, *45*, 1560 – 1563; c) S. C. Grover, A. G. Skirtach, R. C. Gauthier, C. P. Grover, *J. Biomed. Opt.* **2001**, *6*, 14 – 22; d) P. S. Dittrich, P. Schwille, *Anal. Chem.* **2003**, *75*, 5767 – 5774; e) S. Hiller, A. Schnackel, E. Donath, *Cytometry A* **2005**, *64*, 115 – 127.

[8] a) G. Decher, J. D. Hong, J. Schmitt, *Thin Solid Films* **1992**, *210*, 831 – 835; b) Y. Lvov, G. Decher, H. Möhwald, *Langmuir* **1993**, *9*, 481 – 486; c) G. Decher, *Science* **1997**, *277*, 1232 – 1237; d) P. Bertrand, A. Jonas, A. Laschewsky, R. Legras, *Macromol. Rapid Commun.* **2000**, *21*, 319 – 348; e) M. Schönhoff, *J. Phys. Condens. Matter* **2003**, *15*, R1781 – R1808; f) D. M. Delongchamp, P. T. Hammond, *Chem. Mater.* **2003**, *15*, 1165 – 1173; g) E. Blomberg, E. Poptoshev, P. M. Claesson, F. Caruso, *Langmuir* **2004**, *20*, 5432 – 5438; h) S. A. Sukhishvili, *Curr. Opin. Colloid Interface Sci.* **2005**, *10*, 37 – 44; i) C. Jiang, V. V. Tsukruk, *Adv. Mater.* **2006**, *18*, 829 – 840.

[9] a) Y. Lvov, K. Ariga, I. Ichinose, T. Kunitake, *J. Am. Chem. Soc.* **1995**, *117*, 6117 – 6123; b) C. Delgado, G. E. Francis, D. Fisher, *Crit. Rev. Ther. Drug Carrier Syst.* **1992**, *9*, 249 – 304; c) J. Rieger, K. V. Bernaerts, F. E. Du Prez, R. Jerome, C. Jerome, *Macromolecules* **2004**, *37*, 9738 – 9745.

[10] a) A. G. Skirtach, A. A. Antipov, D. G. Shchukin, G. B. Sukhorukov, *Langmuir* **2004**, *20*, 6988 – 6992; b) A. G. Skirtach, C. Dejugnat, D. Braun, A. S. Susha, A. L. Rogach, W. J. Parak, H. Möhwald, G. B. Sukhorukov, *Nano Lett.* **2005**, *5*, 1371 – 1377.

[11] a) S. Sershen, S. L. Westcott, N. J. Halas, J. L. West, *J. Biomed. Mater. Res.* **2000**, *51*, 293 – 298; b) B. Radt, T. A. Smith, F. Caruso, *Adv. Mater.* **2004**, *16*, 2184 – 2189; c) A. S. Angelatos, B. Radt, F. Caruso, *J. Phys. Chem. B* **2005**, *109*, 3071 – 3076; d) X. F. Yuan, K. Fischer, W. Schartl, *Langmuir* **2005**, *21*, 9374 – 9380.

[12] a) C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, *Nature* **1996**, *382*, 607 – 609; b) A. P. Alivisatos, K. P. Johnsson, X. G. Peng, T. E. Wilson, C. J. Loweth, M. P. Bruchez, P. G. Schultz, *Nature* **1996**, *382*, 609 – 611; c) D. I. Gittins, F. Caruso, *Angew. Chem.* **2001**, *113*, 3089 – 3092; *Angew. Chem. Int. Ed.* **2001**, *40*, 3001 – 3004; d) M. El-Sayed, *Acc. Chem. Res.* **2001**, *34*, 257 – 264; e) W. J. Parak, T. Pellegrino, C. M. Micheel, D. Gerion, S. C. Williams, A. P. Alivisatos, *Nano Lett.* **2003**, *3*, 33 – 36; f) L. Manna, D. J. Milliron, A. Meisel, F. C. Scher, A. P. Alivisatos, *Nat. Mater.* **2003**, *2*, 382 – 385; g) M. K. Corbierre, N. S. Cameron, M. Sutton, S. G. J. Mochrie, L. B. Lurio, A. Ruhm, R. B. Lennox, *J. Am. Chem. Soc.* **2004**, *126*, 2867 – 2873; h) E. Katz, I. Willner, *Angew. Chem.* **2004**, *116*, 6166 – 6235; *Angew. Chem. Int. Ed.* **2004**, *43*, 6042 – 6108; i) J. Lee, A. O. Govorov, N. A. Kotov, *Angew. Chem.* **2005**, *117*, 7605 – 7608; *Angew. Chem. Int. Ed.* **2005**, *44*, 7439 – 7442; j) W. J. Parak, T. Pellegrino, C. Planck, *Nanotechnology* **2005**, *16*, R9 – R25; k) C. J. Murphy, N. R. Jana, *Adv. Mater.* **2002**, *14*, 80 – 82; l) D. V. Guzatov, A. A. Oraevsky, A. N. Oraevsky, *Quantum Electron.* **2003**, *33*, 817 – 822; m) M. C. Daniel, C. Astruc, *Chem. Rev.* **2004**, *104*, 293 – 346.

[13] a) J. A. Copland, M. Eghtedari, V. L. Popov, N. Kotov, N. Mamedova, M. Motamedi, A. A. Oraevsky, *Mol. Imaging Biol.* **2004**, *6*, 341 – 349; b) K. Sokolov, M. Follen, J. Aaron, I. Pavlova, A. Malpica, R. Lotan, R. Richards-Kortum, *Cancer Res.* **2003**, *63*, 1999 – 2004; c) M. Eghtedari, J. A. Copland, N. A. Kotov, A. A. Oraevsky, M. Motamedi, *Lasers Surg. Med.* **2004**, *164* Suppl. S16; d) D. Pissuwan, S. M. Valenzuela, M. B. Cortie, *Trends Biotechnol.* **2006**, *24*, 62 – 67.

[14] a) A. Roggan, M. Friebel, K. Dorschel, A. Hahn, G. Müller, *J. Biomed. Opt.* **1999**, *4*, 36 – 46; b) A. N. Yaroslavsky, I. V. Yaroslavsky, T. Goldbach, H. J. Schwarzmaier, *Proc. SPIE-Int. Soc. Opt. Eng.* **1996**, *2678*, 314 – 324; c) G. M. Hale, M. R. Querry, *Appl. Opt.* **1973**, *12*, 555 – 563; d) A. Schönle, S. W. Hell, *Opt. Lett.* **1998**, *23*, 325 – 327.

[15] a) U. Kreibig in *Physics and Chemistry of Finite Systems: From Clusters to Crystals* (Eds.: P. Jenna, S. N. Khanna, B. K. Rao), Kluwer Academic Publishers, London, **1991**; b) U. Kreibig, B. Schmitz, H. D. Breuer, *Phys. Rev. B* **1987**, *36*, 5027 – 5030; c) T. Kahlau, M. Quinten, U. Kreibig, *Appl. Phys. A* **1996**, *62*, 19 – 27.

[16] a) L. Lu, R. Capek, A. Kornowski, N. Gaponik, A. Eychmüller, *Angew. Chem.* **2005**, *117*, 6151 – 6155; *Angew. Chem. Int. Ed.* **2005**, *44*, 5997 – 6001; b) M. Morikawa, N. Kimizuka, *Chem. Commun.* **2005**, *38*, 4866 – 4868; c) G. Zhang, D. Y. Wang, H. Möhwald, *Angew. Chem.* **2005**, *117*, 7945 – 7948; *Angew. Chem. Int. Ed.* **2005**, *44*, 7767 – 7770; d) A. G. Skirtach, C. Déjugnat, D. Braun, A. S. Susha, A. L. Rogach, G. B. Sukhorukov, unpublished results.

[17] a) H. S. Zhou, I. Honma, H. Komiyama, J. W. Haus, *Phys. Rev. B* **1994**, *50*, 12052 – 12056; b) R. D. Averitt, D. Sarkar, N. J. Halas, *Phys. Rev. Lett.* **1997**, *78*, 4217 – 4220; c) T. Norman, Jr., C. D. Grant, D. Magana, J. Z. Zhang, J. Liu, D. Cao, F. Bridges, A. van Buuren, *J. Phys. Chem. B* **2002**, *106*, 7005 – 7012; d) G. Raschke, S. Brogl, A. S. Susha, A. L. Rogach, T. A. Klar, J. Feldman, B. Fieres, N. Petkov, T. Bein, A. Nichtl, K. Kürzinger, *Nano Lett.* **2004**, *4*, 1853 – 1857; e) J. Z. Zhang, A. M. Schwartzberg, T. Norman, Jr., C. D. Grant, J. Liu, F. Bridges, T. Van Buuren, *Nano Lett.* **2005**, *5*, 809 – 810.

[18] a) D. Yoo, S. S. Shiratori, M. Rubner, *Macromolecules* **1998**, *31*, 4309- 4318; b) J. D. Mendelsohn, C. J. Barrett, V. V. Chan, A. J. Pal, A. M. Mayes, M. F. Rubner, *Langmuir* **2000**, *16*, 5017 – 5023; c) S. Sukhishvili, S. Granick, *J. Am. Chem. Soc.* **2000**, *122*, 9550 – 9551; d) H. H. Rmaile, J. B. Schlenoff, *Langmuir* **2002**, *18*, 8263 – 8265; e) L. Richert, F. Boulmedias, P. Lavalle, J. Mutterer, E. Ferreux, G. Decher, P. Schaaf, J.-C. Voegel, C. Picart, *Biomacromolecules* **2004**, *5*, 284 – 294; f) S. E. Burke, C. J. Barrett, *Macromolecules* **2004**, *37*, 5375 – 5384.

[19] a) A. A. Antipov, G. B. Sukhorukov, S. Leporatti, I. L. Radtchenko, E. Donath, H. Möhwald, *Colloids Surf. A* **2002**, *535*, 198 – 200; b) T. Mauser, C. D. Déjugnat, G. B. Sukhorukov, *Macromol. Rapid Commun.* **2004**, *25*, 1781 – 1785; c) C. D. Déjugnat, D. Halozan, G. B. Sukhorukov, *Macromol. Rapid Commun.* **2005**, *26*, 961 – 967; d) Z. An, H. Möhwald, J. Li, *Biomacromolecules* **2006**, *7*, 580 – 585.

[20] a) D. V. Volodkin, A. I. Petrov, M. Prevot, G. B. Sukhorukov, *Langmuir* **2004**, *20*, 3398 – 3406; b) A. I. Petrov, D. V. Volodkin, G. B. Sukhorukov, *Biotechnol. Prog.* **2005**, *21*, 918 – 925.

[21] a) K. Köhler, D. Shchukin, H. Möhwald, G. B. Sukhorukov, *Macromolecules* **2004**, *37*, 9546 – 9550; b) K. Köhler, D. G. Shchukin, H. Möhwald, G. B. Sukhorukov, *J. Phys. Chem. B*

2005, *109*, 18250 – 18259; c) K. Köhler, G. B. Sukhorukov, unpublished results.

[22] a) V. A. Izumrudov, H. O. Ortiz, A. B. Zezin, V. A. Kabanov, *Macromol. Chem. Phys.* **1998**, *199*, 1057 – 1062; b) S. Leporatti, C. Gao, A. Voight, E. Donath, H. Möhwald, *Eur. Phys. J. E* **2001**, *5*, 13 – 20; c) C. Gao, S. Leporatti, S. Moya, E. Donath, H. Möhwald, *Chem. Eur. J.* **2003**, *9*, 915 – 920; d) J. F. Quinn, F. Caruso, *Langmuir* **2004**, *20*, 20 – 22; e) M. Salomaki, I. A. Vinokurov, J. Kankare, *Langmuir* **2005**, *21*, 11232 – 11240.

[23] a) C. Gao, S. Leporatti, S. Moya, E. Donath, H. Möhwald, *Langmuir* **2001**, *17*, 3491 – 3495; b) C. Y. Gao, S. Moya, E. Donath, H. Möhwald, *Macromol. Chem. Phys.* **2002**, *203*, 953 – 960; c) F. Dubreuil, N. Elsner, A. Fery, *Eur. Phys. J. E* **2003**, *12*, 215 – 221; d) A. Fery, F. Dubreuil, H. Möhwald, *New J. Phys.* **2004**, *6*, 1 – 13; e) R. Müller, K. Köhler, R. Weinkamer, G. Sukhorukov, A. Fery, *Macromolecules* **2005**, *38*, 9766 – 9771; f) V. V. Lulevich, S. Nordschild, O. I. Vinogradova, *Macromolecules* **2004**, *37*, 7736 – 7741; g) O. Mermut, J. Lefebvre, D. G. Gray, C. J. Barrett, *Macromolecules* **2003**, *36*, 8819 – 8824; h) D. Collin, P. Lavalle, J. M. Garza, J.-C. Voegel, P. Schaaf, P. Martinoty, *Macromolecules* **2004**, *37*, 10195 – 10198; i) A. J. Nolte, M. F. Rubner, R. E. Cohen, *Macromolecules* **2005**, *38*, 5367 – 5370; j) J. A. Jaber, J. B. Schlenoff, *J. Am. Chem. Soc.* **2006**, *128*, 2940 – 2947.

[24] a) R. P. Haugland in *Handbook of Fluorescent Probes and Research Chemicals*, 7th ed., Molecular Probes, Eugene, OR, **1999**; b) N. Panchuk-Voloshina, R. P. Haugland, J. Bishop-Stewart, M. K. Bhalat, P. J. Millard, F. Mao, W. Leung, R. P. Haugland, *J. Histochem. Cytochem.* **1999**, *47*, 1179 – 1188; c) R. von Klitzing, H. Möhwald, *Langmuir* **1995**, *11*, 3554 – 3559; d) N. Klonis, W. H. Sawye, *J. Fluoresc.* **1996**, *6*, 147 – 157; e) R. Sjöback, J. Nygren, M. Kibuta, *Spectrochim. Acta A* **1995**, *51*, L7 – L21; f) M. M. Marin, L. Lindqvist, *J. Lumin.* **1975**, *10*, 381 – 390.

[25] a) P. S. Dittrich, P. Schwille, *Appl. Phys. B* **2001**, *73*, 829 – 837; b) C. Eggeling, A. Volkmer, C. A. M. Seidel, *ChemPhysChem* **2005**, *6*, 791 – 804.

[26] a) P. Lebedew, *Ann. Phys.* **1901**, *6*, 433 – 458; b) A. Ashkin, *Biophys. J.* **1992**, *61*, 569 – 582; c) R. C. Gauthier, *Appl. Phys. Lett.* **1995** *67*, 2269 – 2271; d) S. C. Grover, R. C. Gauthier, A. G. Skirtach, *Opt. Express* **2000**, *7*, 533 – 539.

[27] a) R. E. Holmin, R. F. Ismagilov, R. Haag, V. Mujica, M. A. Ratner, M. A. Rampi, G. M. Whitesides, *Angew. Chem.* **2001**, *113*, 2378 – 2382; *Angew. Chem. Int. Ed.* **2001**, *40*, 2316 – 2325; b) J. Zhang, R. M. Lahtinen, K. Kontturi, P. R. Unwin, D. J. Schiffrin, *Chem. Commun.* **2001**, *18*, 1818 – 1819; c) J. J. Zhao, C. R. Bradbury, S. Huclova, I. Potapova, M. Carrara, D. J. Fermin, *J. Phys. Chem. B* **2005**, *109*, 22985 – 22994.

