Gold Nanoparticles

DOI: 10.1002/anie.200701060

A Facile Preparative Method for Aggregation-Free Gold Nanoparticles Using Poly(styrene-*block*-cysteine)**

Sinoj Abraham, Il Kim, and Carl A. Batt**

Recently, the synthesis and stabilization of nanoparticles with potentially valuable properties by using various preparative methods have been reported.[1] Stabilization of quantum dots requires a dynamic interdisciplinary effort spanning the fields of chemistry, materials science, biology, and electronic engineering.[2] The possible application of gold nanoparticles (AuNPs) in a diverse arena is the continued research motivation for enhancing their functional properties.[3,4] A wide range of encapsulation protocols have so far been utilized for preparing aggregation-free AuNPs.[5] However, the major challenge that still exists is the reliability and robustness of these methods to control the material properties, especially the quantum confinement effects in their dispersed state. Initially, small molecules, such as alkanethiols, were used as surface stabilizers and later interest shifted towards polymers, for example, thiol-derivatized oligosaccharides,[6] DNA,[7] or dendrimers.[8] The utilization of block-copolymer micelles for the synthesis and stabilization of AuNPs was broached in the last decade with amphiphilic poly(styrene-*block*-ethylene oxide)[9] and poly(styrene-*block*-vinyl pyridine).[10] Later, many similar studies were reported with different polymer systems,[11] especially poly(styrene-*block*-acrylic acid).[12]

The studies on amphiphilic block copolymers as surface stabilizers still continue as a prominent area of research because of their readily tunable properties, such as critical micelle concentration and kinetic stability.[13] Recent advances in controlled radical polymerization (CRP) furthered the scope of block copolymers in diverse fields of research.[14,15] Synthetic polymers conjugated with polypeptides lead to a promising class of block copolymers widely termed "molecular chimeras".[16–19] Our interest in block copolymers and their self-assembly, ability to combine various synthesis methods, and wide application areas led us to consider "molecular chimeras" as possible surface stabilizers of AuNPs. Polymers with thiol functionality received great interest as a result of the special properties imparted by thiol groups. For instance, they can self-assemble into monolayers on gold substrates;[20] however, the synthesis of polymers bearing thiol groups involves tedious chemical routes.[21] Herein, we report the synthesis, self-assembly, and induced assembly of a new class of amphiphilic poly(styrene-*block*-cysteine) copolymers and demonstrate their ability to conjugate with AuNPs, thus inhibiting particle aggregation.

The polystyrene was initially prepared by atom-transfer radical polymerization (ATRP)[15] and was further modified at the chain ends for the synthesis of polypeptide blocks.[18] *S*-Carbobenzoxy-*N*-carboxy-L-cysteine anhydride, the monomer for the extended ring-opening polymerization (ROP), was freshly synthesized with a reversible masking at the thiol moieties. The ^{1}H NMR spectrum of the poly(styrene-*block*-cysteine) ($PS_{100}PCys_{20}$) conjugate is depicted in Figure 1

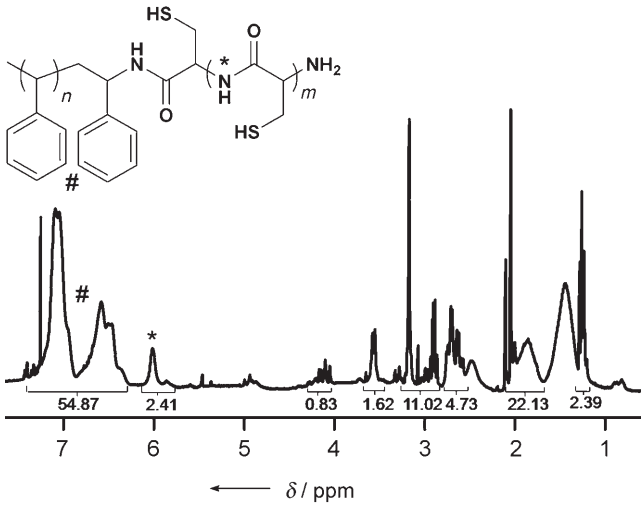

Figure 1. ^{1}H NMR spectrum of poly(styrene-*block*-cysteine) in $CDCl_3$.

(details in the Supporting Information). Initially, the self-assembly of this amphiphilic block copolymer was studied by preparing a stable micelle solution in DMF/H_2O. An illustration of the molecular self-assembly process is shown in Figure 2.

A solution of the polymer in DMF (20 mg mL^{-1}) was mixed with the same amount of degassed water and DMF and

[*] S. Abraham, Prof. I. Kim
Department of Polymer Science and Engineering
Pusan National University
Busan 609735 (Korea)
Fax: (+82) 51-513-7720
E-mail: ilkim@pusan.ac.kr
Homepage: http://mslab.polymer.pusan.ac.kr
Prof. C. A. Batt
Department of Food Science
Cornell University
Ithaca, NY 14853 (USA)
E-mail: cab10@cornell.edu

[**] This work was supported by the Korea Research Foundation (KRF-D00422) and the National Core Research Center Program from MOST/KOSEF (R15–2006-022-01001-0). I.K. thanks the LG Yonam Foundation for sabbatical support and the Center for Ultramicrochemical Process Systems. C.A.B. thanks the Ludwig Institute for Cancer Research for its support.

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

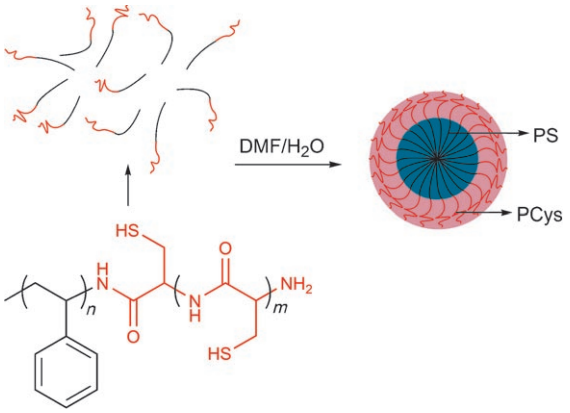

Figure 2. Steps in the self-assembly of poly(styrene-*block*-cysteine) ($PS_{100}PCys_{20}$) conjugated block copolymer.

heated at 110 °C for 2 h and then cooled slowly at a rate of 1 K min^{-1} in an automated thermal cycler. This process facilitates the controlled precipitation of polymer, thus inducing molecular self-assembly and resulting in a stable micelle solution. The micelle solution was then dialyzed against deionized water and trace amounts of precipitated polymer were removed by centrifugation. To understand the mode of micellization, an NMR-scale reaction was carried out and real-time spectra were recorded at various temperatures (Figure 3). The resonance intensity of aromatic protons of polystyrene decreases with decreasing solution temperature,

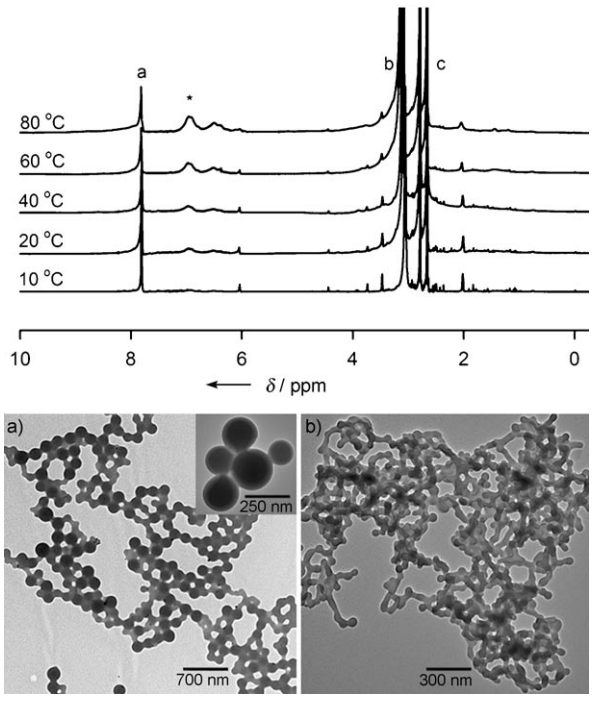

Figure 3. Top: NMR spectra of the block copolymer solution monitored at various temperatures, which indicate the mode of micellization. Peaks a–c correspond to solvents and that marked (*) corresponds to the aromatic protons of polystyrene. Bottom: TEM images of a) spherical micelles and b) rodlike micelles.

which implies micelle formation with polystyrene as the core and polycysteine as the corona. Spherical micelles with diameters of 150 to 300 nm are clearly visible in the TEM images (Figure 3 a).

However, the large size of these micelles is not in proportion to the molecular weight of the block copolymer.[21] The formation of multicompartment micelles could be a reason for this observation. The thiol moieties of the hydrophilic corona can facilitate intermicelle conjugation, thus leading to the organization of independent micelles to form large multicompartment micelles. Furthermore, large aggregations of micelles were also observed in the TEM images, but these aggregates were unlikely to correlate with the actual state in solution, as the samples on TEM grids were allowed to dry. On increasing the concentration of the block copolymer in the stock solution to 40 mg mL^{-1}, rodlike aggregates of the micelles were formed (Figure 3 b). Thus, the individual micelles can reduce their contact area with the solvent mixture and can exist in the lowest-energy state. Aggregates of four to six micelles are also formed in solution, as observed in the light-scattering spectra at low temperature (Figure 4).

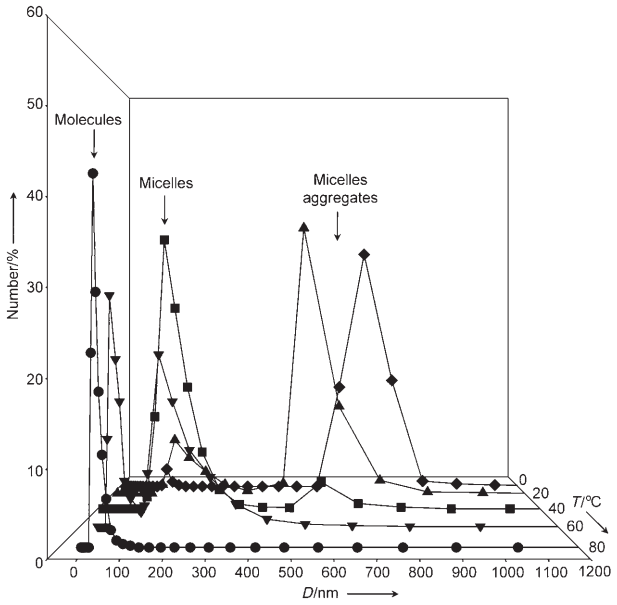

Figure 4. Hydrodynamic diameter of micelles by light-scattering spectroscopy. Number/% is the percentage number of molecules or micelles present in the micellar solution at the particular temperature. At any temperature, there are molecules, micelles, and micellar aggregates in the solution.

This micellar solution of $PS_{100}PCys_{20}$ was stirred with a 20-nm gold colloidal solution (7×10^{11} particles mL^{-1}) for about 2 h. The solution was then centrifuged at 10000 rpm for 15 min to remove excess reactants, free micelles, and free AuNPs. The TEM image of these hybrid micelles distinctly shows conjugated AuNPs on the surface of the micelles (see the Supporting Information). The thiol groups at the surface of micelles render this S–Au conjugation, which also indicates the orientation of the molecular self-assembly with the polystyrene core and polycysteine corona.

Efforts have been made to encapsulate individual AuNPs in the core of micelles by conjugating them with polymer molecules prior to micellization.[9–12] The thiol moieties, which are the main driving force of polymer self-assembly, as well as gold conjugation make this a unique system. The high affinity of the thiol groups for gold induces the organization of polymer molecules in the reverse fashion of their self-assembly described above. Encapsulation using this polymeric system does not require any initial surface modification of AuNPs with common aliphatic ligands or thiolipids. The procedure is similar to the micellization protocol described above with additionally the presence of gold colloid solution (20 nm). A plausible scheme of AuNP functionalization is depicted in Figure 5. The strong interaction of thiol groups

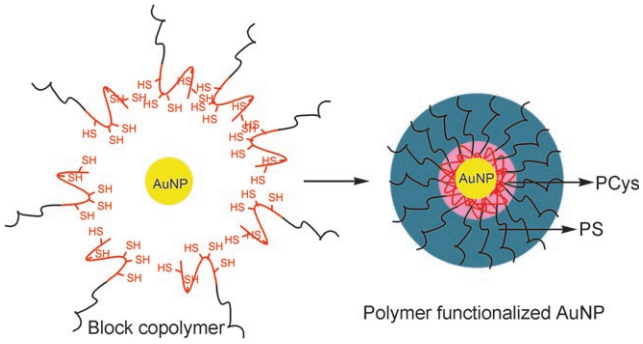

Figure 5. Encapsulation by the block copolymer coats the AuNPs with a hydrophobic outer shell.

with the AuNPs overcomes the tendency of polymer molecules to self-assemble and results in gold–polymer conjugates.

This polymer–AuNP conjugate was subjected to TEM analysis. The polymer shells appear white in contrast to the background, where the residue solution dried during the preparation of TEM samples. Figure 6 shows a representative TEM image. It is clear that AuNPs (20 nm) are covered by a polymer shell with a total diameter of about 100 nm and uniform thickness. Most hybrid conjugates appear to be well-defined and contain singly occupied AuNPs. The ^{1}H NMR spectrum of the sample is consistent with polymer adsorbed on the AuNPs. Compared to the free polymer molecules, the resonances are significantly broadened. The absence of sharp resonances showed that the sample contained negligible amounts of free thiols (see the Supporting Information). These AuNP–polymer conjugates are stable without any additional cross-linking, but there are free amino groups available for cross-linking by simple carbodiimide chemistry.

Repeated centrifugation did not result in deformation or fusion of the spherical conjugates and no ellipsoidal deformations were observed in the TEM images. Possible in situ reinforcement of the polymer shell can also take place by inter/intramolecular coupling between the thiol groups. The UV/Vis absorption band corresponding to the surface plasmon resonance (SPR) energy of this polymer–AuNP conjugate is red-shifted (534 nm) compared to that of free AuNPs (522 nm) of the same diameter.[22] This observation is consistent with the decrease in SPR energy with increase in

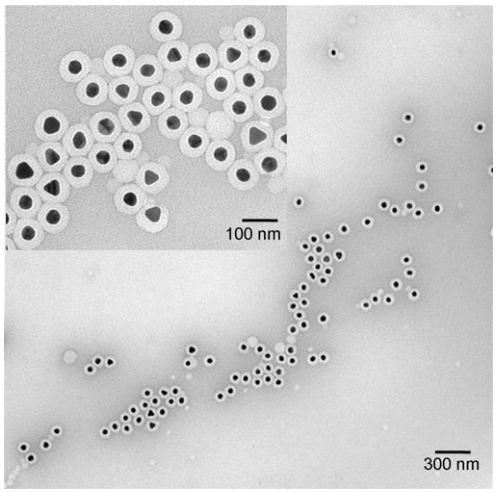

Figure 6. TEM images of block copolymer–AuNP conjugate.

the refractive index of the surrounding medium.[12c,23] Moreover, the UV absorption of these nanoparticle conjugates after storage over a prolonged time resembled that of the newly synthesized material (see the Supporting Information). From all these experimental observations, it can be concluded that the AuNPs are resistant to aggregation upon conjugation with this class of poly(styrene-*block*-cysteine) polymers.

In summary, a new class of "molecular chimeras" was successfully utilized to stabilize nanoparticles. The self-assembly of one such block copolymer was studied in the presence and absence of AuNPs. The polymer assembly was reversed in the presence of AuNPs. Only a few polymer systems have been developed for the stabilization of nanoparticles to date, and the successful use of this new class of block copolymers as surface stabilizers with a new preparative protocol should find wide application.

Experimental Section

Encapsulation of AuNPs: A solution of AuNPs (20 mL; 20 nm in diameter, 7.0×10^{11} particles mL^{-1}) was concentrated to 250 μL by centrifugation at 14000 rpm for 30 min. The concentrated solution was diluted with DMF (750 μL), and this solution (50 μL) was mixed with polymer stock solution (5 μL), DMF (25 μL), and air-free water (25 μL). Up to 96 batches at a time could be treated in the thermal cycler (Bio-Rad). The mixture was heated to 110 °C for 2 h, and then cooled at a rate of 1 °C min^{-1}. To remove excess reactants and free micelles, a 5-ml batch of this solution was diluted with water (2 mL) and centrifuged at 10000 rpm for 30 min. This step was repeated twice more and a concentrated polymer–gold conjugate solution was obtained.

Preparation of TEM samples: TEM grids were treated with an oxygen plasma (from a Harrick plasma cleaner/sterilizer) for 20 s to render their surface hydrophilic. $(NH_4)_2MoO_4$ (2 μL; 2% aqueous solution by weight), water (5 μL), and micelles or polymer–gold conjugate (3 μL) were mixed on the surface of a plastic Petri dish to form a small bead. A TEM grid was then floated on top of the bead with the hydrophilic face contacting the solution. The TEM grid was carefully removed with a pair of tweezers, wicked with a filter paper to remove excess liquid, and then dried in air for 1 min.

The synthesis of the polymers and their intermediates and other micellization procedures are available as Supporting Information.

Received: March 10, 2007
Revised: May 2, 2007
Published online: June 28, 2007

Keywords: block copolymers · gold · micelles · nanoparticles · peptides

[1] a) M. C. Daniel, D. Astruc, *Chem. Rev.* **2004**, *104*, 293 – 346; b) C. Burda, X. Chen, R. Narayanan, M. A. El-Sayed, *Chem. Rev.* **2005**, *105*, 1025 – 1102; c) B. P. Khanal, E. R. Zubarev, *Angew. Chem.* **2007**, *119*, 2245 – 2248; *Angew. Chem. Int. Ed.* **2007**, *46*, 2195 – 2198.

[2] a) W. P. McConnell, J. P. Novak, L. C. Brousseau, R. R. Tenent, D. L. Feldheim, *J. Phys. Chem. B* **2000**, *104*, 8925 – 8930; b) J. H. Fendler, *Nanoparticles and Nanostructured Films*, Wiley-VCH, Weinheim, **1998**.

[3] R. Sato, H. Ahmed, D. Brown, B. F. G. Johnson, *J. Appl. Phys.* **1997**, *82*, 696 – 701.

[4] R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger, C. A. Mirkin, *Science* **1997**, *277*, 1078 – 1081.

[5] a) X. H. Gao, Y. Y. Cui, R. M. Levenson, L. W. K. Chung, S. M. Nie, *Nat. Biotechnol.* **2004**, *22*, 969 – 976; b) B. Dubertret, P. Skourides, D. J. Norris, V. Noireaux, A. H. Brivanlou, A. Libchaber, *Science* **2002**, *298*, 1759 – 1762; c) W. E. Doering, S. M. Nie, *Anal. Chem.* **2003**, *75*, 6171 – 6176; d) N. Liu, B. S. Prall, V. I. Klimov, *J. Am. Chem. Soc.* **1997**, *119*, 11653 – 11659.

[6] J. de la Fuente, A. G. Barrientos, T. C. Rojas, J. Rojo, J. Canada, A. Fernandez, S. Penades, *Angew. Chem.* **2001**, *113*, 2317 – 2321; *Angew. Chem. Int. Ed.* **2001**, *40*, 2257 – 2261.

[7] a) Y. W. Cao, R. Jin, C. A. Mirkin, *J. Am. Chem. Soc.* **2001**, *123*, 7961 – 7962; b) Z. Li, R. Jin, C. A. Mirkin, R. L. Letsinger, *Nucleic Acids Res.* **2002**, *30*, 1558 – 1562.

[8] a) J. W. Worden, Q. Dai, Q. Huo, *Chem. Commun.* **2006**, 1536 – 1538; b) R. C. Hedden, B. J. Bauer, A. P. Smith, F. Gröhn, E. Amis, *Polymer* **2002**, *43*, 5473 – 5481.

[9] J. P. Spatz, A. Roescher, M. Möller, *Adv. Mater.* **1996**, *8*, 337 – 340.

[10] a) J. P. Spatz, A. Roescher, S. Sheiko, G. Krausch, M. Möller, *Adv. Mater.* **1995**, *7*, 731 – 735; b) M. Antonietti, E. Wenz, L. Bronstein, M. Seregina, *Adv. Mater.* **1995**, *7*, 1000 – 1005; c) J. P. Spatz, S. Mössmer, M. Möller, *Chem. Eur. J.* **1996**, *2*, 1552 – 1555; d) J. P. Spatz, T. Herzog, S. Mössmer, P. Ziemann, M. Möller, *Adv. Mater.* **1999**, *11*, 149 – 153; e) J. P. Spatz, S. Mössmer, C. Hartmann, M. Möller, T. Herzog, M. Krieger, H. G. Boyen, P. Ziemann, B. Kabius, *Langmuir* **2000**, *16*, 407 – 415.

[11] K. J. Watson, J. Z. Sonbinh, T. Nguyen, C. A. Mirkin, *J. Am. Chem. Soc.* **1999**, *121*, 462 – 463.

[12] a) Y. Kang, T. A. Taton, *Macromolecules* **2005**, *38*, 6115 – 6121; b) Y. Kang, K. J. Erickson, T. A. Taton, *J. Am. Chem. Soc.* **2005**, *127*, 13800 – 13801; c) Y. Kang, T. A. Taton, *Angew. Chem.* **2005**, *117*, 413 – 416; *Angew. Chem. Int. Ed.* **2005**, *44*, 409 – 412; d) E. R. Zubarev, J. Xu, A. Sayyad, J. D. Gibson, *J. Am. Chem. Soc.* **2006**, *128*, 4958 – 4959.

[13] G. Riess, *Prog. Polym. Sci.* **2003**, *28*, 1107 – 1170.

[14] a) O. W. Webster, *Science* **1991**, *251*, 887 – 892; b) J. S. Wang, K. Matyjaszewski, *J. Am. Chem. Soc.* **1997**, *119*, 674 – 680; c) M. Kato, M. Kamigaito, M. Sawamoto, T. Higashimura, *Macromolecules* **1995**, *28*, 1721 – 1723; d) K. A. Davis, K. Matyjaszewski, *Macromolecules* **2000**, *33*, 4039 – 4047.

[15] S. Abraham, C. S. Ha, I. Kim, *J. Polym. Sci. Part A* **2005**, *43*, 6367 – 6378.

[16] a) H. Schlaad, M. Antonietti, *Eur. Phys. J. E* **2003**, *10*, 17 – 23; b) Y. Bae, S. Fukushima, A. Harada, K. Kataoka, *Angew. Chem.* **2003**, *115*, 4788 – 4791; *Angew. Chem. Int. Ed.* **2003**, *42*, 4640 – 4643.

[17] a) H. A. Klok, J. F. Langenwalter, S. Lecommandoux, *Macromolecules* **2000**, *33*, 7819 – 7826; b) S. Lecommandoux, M. F. Achard, J. F. Langenwalter, H. A. Klok, *Macromolecules* **2001**, *34*, 9100 – 9111.

[18] a) T. J. Deming, *Nature* **1997**, *390*, 386 – 389; b) T. J. Deming, *J. Am. Chem. Soc.* **1997**, *119*, 2759 – 2760; c) S. A. Curtin, T. J. Deming, *J. Am. Chem. Soc.* **1999**, *121*, 7427 – 7428; d) K. R. Brzezinska, S. A. Curtin, T. J. Deming, *Macromolecules* **2002**, *35*, 2970 – 2976; e) S. Abraham, C. S. Ha, I. Kim, *J. Polym. Sci. Part A* **2006**, *44*, 2774 – 2783.

[19] a) B. A. Cornell, *Opt. Biosens.* **2002**, 457 – 495; b) R. G. Nuzzo, D. L. Allara, *J. Am. Chem. Soc.* **1983**, *105*, 4481 – 4483.

[20] a) G. Carrot, J. Hilborn, J. L. Hedrick, M. Trollsås, *Macromolecules* **1999**, *32*, 5171 – 5173; b) M. Trollsås, C. J. Hawker, J. L. Hedrick, G. Carrot, J. Hilborn, *Macromolecules* **1998**, *31*, 5960 – 5963; c) J. Lang, R. Zana, R. Bauer, H. Hoffmann, W. Ulbricht, *J. Phys. Chem.* **1975**, *79*, 276 – 283.

[21] S. Förster, M. Zisenis, E. Wenz, M. Antonietti, *J. Chem. Phys.* **1996**, *104*, 9956 – 9970.

[22] S. Link, M. A. El-Sayed, *J. Phys. Chem. B* **1999**, *103*, 4212 – 4217.

[23] a) T. R. Jensen, M. L. Duval, K. L. Kelly, A. A. Lazarides, G. C. Schatz, R. P. Van Duyne, *J. Phys. Chem. B* **1999**, *103*, 9846 – 9853; b) A. C. Templeton, J. J. Pietron, R. W. Murray, P. Mulvaney, *J. Phys. Chem. B* **2000**, *104*, 564 – 570.

