Water-Soluble Fluorescent Silver Nanoclusters

By *Hangxun Xu* and *Kenneth S. Suslick**

Ag nanoclusters consist of several to roughly a hundred atoms and possess sizes comparable to the Fermi wavelength of electrons; they exhibit molecule-like properties, including discrete electronic transitions and strong fluorescence. These nanoclusters are of significant interest because they provide the bridge between atomic and nanoparticle behavior in noble metals. Since the first observations of photoluminescence from Ag nanoclusters, substantial effort has been made to prepare Ag nanoclusters and explore their potential applications. The synthesis of well-defined Ag nanoclusters, however, is difficult due to aggregation of nanoclusters, especially in aqueous solutions. In this Research News article, we highlight some recent progress on solution-based synthesis of water-soluble Ag nanoclusters using radiolytic, chemical reduction and photochemical approaches to prepare fluorescent Ag nanoclusters.

1. Introduction

Nanoparticles (i.e., diameters greater than ∼2 nm) have been the subject of intense research in the past decades due to their unique size- and shape-dependent catalytic, optical, electrical, magnetic, and chemical properties, which are often distinct from their bulk counterparts.[1–5] When the particle size is further reduced and approaches the Fermi wavelength of electrons (i.e., the electron de Broglie wavelength at the Fermi level: ∼0.5 nm for Au and Ag), the continuous density of states breaks up into discrete energy levels leading to the observation of dramatically different optical, electrical and chemical properties compared to nanoparticles.[6,7] In order to distinguish these ultra-small particles, nanoparticles smaller than 2 nm are usually called nanoclusters. In this size regime, metal nanoclusters become molecular species and size-dependent strong fluorescent emission can often be observed upon photoexcitation in the UV–visible range.[7] In particular, Ag nanoclusters, which show brighter fluorescence than Au nanoclusters in solutions, have received considerable attention in the past few years owing to their great promise in a wide range of applications. Historically, colloidal Ag was the core of most photographic processes. More recently, gas-phase and low-temperature matrix-isolated Ag nanoclusters were the first observed to show discrete absorption and fluorescence.[8] Chemiluminescence due to the formation of excited Ag_2^* and

Ag_3^* clusters during Ag condensation with Ar has also been reported.[9] Silver nanoclusters have also been observed in frozen solutions and zeolites.[10,11] These extreme conditions, however, are not amenable to real world applications.

To explore potential applications of Ag nanoclusters, synthesis of stable, water-soluble individual Ag nanoclusters is essential. The preparation of Ag nanoclusters in aqueous solutions is difficult due to the tendency of Ag nanoclusters to aggregate. The nanoclusters will generally grow continuously to form larger clusters so as to reduce their surface energy until their growth is stopped by pre-formed templates or capping agents. Recently, different synthetic methods have been developed to prepare water-soluble fluorescent Ag nanoclusters. Here, we review several different approaches to the synthesis of fluorescent Ag nanoclusters in aqueous solutions.

2. Radiolytic Approaches to Form Ag Nanoclusters

Radiolytic reduction of Ag ions in aqueous solutions at ambient conditions generally yields Ag nanoparticles in the presence of stabilizers. These Ag nanoparticles exhibit a surface plasmon resonance band in the 380 to 400 nm range. Henglein et al. found that under certain conditions, i.e. in the presence of polyphosphate or polyacrylate in aqueous solution during γ-ray irradiation, clusters of several Ag atoms are formed.[12–15] Ag nanoclusters prepared in this way do not show the surface plasmon band of nanometer-sized metallic Ag particles, but do have absorption bands characteristic of Ag nanoclusters consisting of a few Ag atoms at 275 nm (Ag_4^*), 300 nm (trimer), 330 nm (pentamer), and 345 nm (heptamer).[12] These Ag nanoclusters are short lived and aggregated to form large nanoparticles after several hours. Pulse radiolysis experiments confirmed that Ag nanoclusters formed during the γ-ray irradiation of Ag ions in aqueous solutions without stabilizers.[16] The lifetime of the Ag nanoclusters without any stabilizers, however, was too short to be detected in a conventional radiation-induced reduction synthesis of Ag nanoparticles.

The mechanism for the formation of Ag nanoclusters under γ-ray irradiation in aqueous solutions is thought to be the reduction of Ag^+ by reactive species from the radiolysis of water, i.e., solvated electrons, e_{aq}^-, OH·, H·, or secondary radicals R· (when radical scavengers like alcohols were also present in the

[*] Prof. K. S. Suslick, H. Xu
School of Chemical Sciences
University of Illinois at Urbana-Champaign
E-mail: ksuslick@illinois.edu

Materials
Views
www.MaterialsViews.com

ADVANCED
MATERIALS
www.advmat.de

RESEARCH NEWS

solution), followed by slow aggregation of isolated Ag atoms.[17] This process can be written in a simplified way:

$$Ag^+ + e_{aq}^- \text{ or } R\cdot \rightarrow Ag^0 \qquad (1)$$

$$n\,Ag^0 \rightarrow Ag_n \text{ (where } Ag_n \leq 2\,nm \text{ for nanoclusters)} \qquad (2)$$

As mentioned above, in order to prevent aggregation of Ag nanoclusters polymers acting as stabilizers need to be added in the solution during the radiolytic reduction of Ag ions.[12]

$$Ag_n + Ag_m \rightarrow Ag_{(n+m)} \text{ (nanoparticles)}$$
$$\text{(where } 2\,nm < Ag_{(n+m)} \leq 100\,nm) \qquad (3)$$
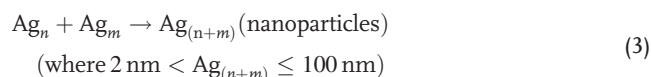
Functional groups with a high affinity for Ag and Ag^+ ensure the binding of polymer chains onto the nanocluster surface while the polymer chains prevent nanoclusters from coalescing with neighboring clusters and thus inhibit at an early stage further growth of nanoclusters through electrostatic repulsion or steric hindrance.

These Ag nanoclusters are highly reactive in solution and very sensitive to UV light. Ag nanoclusters can be easily catalyzed to convert to larger metallic Ag particles under UV light irradiation.[13] When a nucleophilic reagent (e.g., NH_3, SH^- and CN^-) is added to the Ag nanocluster solution, a sharp, strong absorption band corresponding to larger metallic Ag particles appears, and all the characteristic absorption bands of Ag nanoclusters disappear.[18] The great reactivity of Ag nanoclusters towards nucleophilic reagents is ascribed to the fact that practically all the atoms in a nanocluster are ''surface'' atoms and highly reactive.[14]

Although the radiolytic reduction methods have been extensively explored to produce Ag nanoclusters in aqueous solution and are simple and reproducible, the fluorescence from Ag nanoclusters was not originally reported by Henglein et al. Indeed, the fluorescence properties of Ag nanoclusters produced by radiolytic reduction method were only recently reported in 2005 by Treguer et al.[19]

3. Chemical Reduction Preparation of Ag Nanoclusters

In addition to the radiolytic reduction method, Henglein et al. also reported that Ag nanoclusters can be synthesized through the chemical reduction of Ag^+ by $NaBH_4$ in aqueous solution or by bubbling an alkaline Ag^+ solution with H_2 and CO in the presence of polyphosphate.[14] This method, however, simultaneously produces large metallic Ag nanoparticles in the solution as confirmed by absorption spectra; a strong absorption band at 380 nm corresponding to Ag nanoparticles can be observed.

The chemical reduction of Ag^+ ions with a highly reductive agent is usually very quick. Polymers with poor affinity for Ag or Ag^+ were not able to prevent Ag nanoclusters from aggregating in the solution under a strong reducing agent, thus large Ag nanoparticles are formed in the reduction process. Dickson and coworkers found that DNA can be used as a template to produce

stable, water-soluble, nanoparticle-free, and fluorescent Ag nanoclusters with $NaBH_4$ as the reducing agent.[20] Ag^+ ions have a strong interaction with single-stranded DNA and, upon addition of $NaBH_4$, small Ag nanoclusters with 2–4 atoms are formed. Mass spectral analysis indicated that small Ag nanoclusters are bonded to the DNA template. ^1H NMR spectra revealed that the Ag nanoclusters cause significantly greater shifts for cytosine compared to other bases, indicating that Ag has a high affinity for cytosine bases on single-stranded DNA. Motivated by this observation, single-stranded DNA consisting of 12 cytosine bases was used to prepare strongly emissive, near-IR emitting Ag nanoclusters (Figure 1).[21,22] Ag nanoclusters prepared in this way have a fluorescence quantum yield of 17%. These Ag nanoclusters show excellent photostability, high quantum efficiency, and essentially no photoblinking on experimentally relevant time scales (0.1 to >1000 ms).

One problem that still remains, however, is that multiple emitting (red and blue/green) species are formed when the
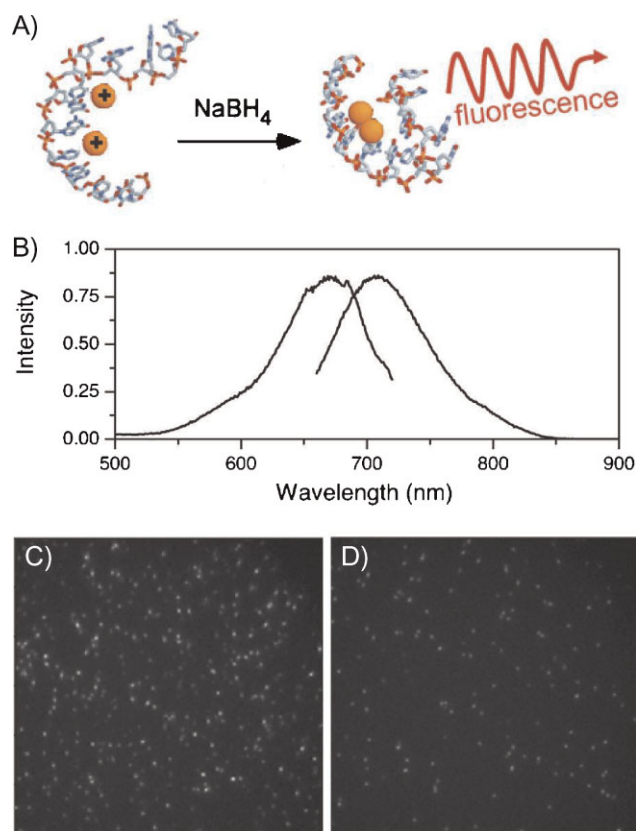

Figure 1. Near-IR-emitting Ag nanoclusters bound to an oligo-DNA. A) Schematic of the IR-emitting Cytosine$_{12}$-Ag$_n$ formation. After complexation of Cytosine$_{12}$ DNA with silver cations, the mixture is reduced with $NaBH_4$ and the near-IR-emitting Ag nanocluster is formed. B) Normalized excitation and emission spectra of the Ag nanoclusters. C) Image of single IR-emitting Cytosine$_{12}$-Ag$_n$ moleculs is given in a poly(vinyl alcohol) (PVA) film. D) For comparison, the fluorescent image of single Cy5.29 molecules (a common fluorophore) in a PVA film. The image dimensions are $40\,\mu m \times 40\,\mu m$, and imaging conditions of (C) and (D) are identical. Reproduced with permission from [21]. Copyright 2007, National Academy of Sciences.

cytosine-rich oligonucleotide is used as a template. Petty and coworkers found that there are base sequence specific influences on Ag nanocluster formation and stabilization.[23] They employed three different oligonucleotides, dT_{12}, $dT_4C_4T_4$ and $dC_4T_4C_4$, where T = thymine and C = cytosine, to study whether specific emissive Ag nanoclusters and DNA conjugates can be selected. Both absorption and fluorescence spectral results indicated that thymine-rich oligonucleotides direct formation of Ag nanoclusters that show only blue/green-emission, whereas cytosine-rich oligonucleotides form both red- and blue/green-emitting Ag nanoclusters. Gwinn et al. studied the sequence-dependent fluorescence from Ag nanoclusters with different sequences and secondary structures of the bases that comprise the DNA strand.[24] Oligo-DNA sequences present a large parameter space for preparing new water-soluble, fluorescent Ag nanoclusters; the fluorescence intensities and maximum emission peaks can be controlled by the DNA sequences and their secondary structure, as observed using six 19-base DNA oligomers (Figure 2).[24] The DNA loop-dependent fluorescence was also observed when Ag^+ ions on DNA hairpins with identical stems and loops of 3 to 12 cytosines were reduced with $NaBH_4$.[25] Ag nanoclusters synthesized with the smallest hairpin (which had 3 cytosines in the loop) only shows one emission peak, while other sequences show multiple emission peaks. Based on the fluorescence wavelengths and chemical stability, they can distinguish 4 different types of fluorescent Ag nanoclusters, possibly consisting of different numbers of Ag atoms. For the DNA hairpin with 9 cytosines in the loop, they discovered red emission from Ag nanoclusters with 13 Ag atoms and green emission from Ag nanoclusters with 11 Ag atoms.[25] Their findings may ultimately lead to precise control the fluorescent properties of Ag nanoclusters through choice of DNA sequence and structure. These DNA-templated Ag nanoclusters show strong fluorescence with high quantum yields and have been successfully applied as fluorophores in single molecule microscopy, biolabeling, and single molecule optoelectronics.[21,26–31]

Other non-DNA templated Ag nanoclusters have also been synthesized recently by chemical reduction. A multistage "cyclic reduction under oxidative conditions" (CROC) approach has been applied to prepare Ag nanoclusters with water-soluble chiral thiols (e.g., captopril ((2S)-1-[(2S)-2-methyl-3- sulfanylpropanoyl]-pyrrolidine-2-carboxylic acid), glutathione (GSH = γ-Glu-Cys-Gly), and cysteine) as strong protecting and stabilizing ligands.[31] $NaBH_4$ was used as the reducing agent and hydrogen peroxide was used as an oxidizing agent. Ag nanoclusters consisting of 22–28 atoms can be prepared in this way as evidenced by mass spectroscopy analysis.[32] A protein stabilizer, the enzyme bovine pancreatic α–chymotrypsin (CHT), was also used to prepare fluorescent Ag nanoclusters of 1 nm average diameter.[33] Ag_n nanoclusters (n < 10) were also obtained in microemulsions using $NaH_2PO_2 \cdot H_2O$ as a mild reducing agent in a kinetically-controlled manner.[34]

4. Photoreduction Synthesis of Ag Nanoclusters

The first example of strong, size-dependent fluorescence from Ag nanoclusters prepared by photoirradiation was reported by Dickson and co-workers in 2001 in AgO films.[35] The observed
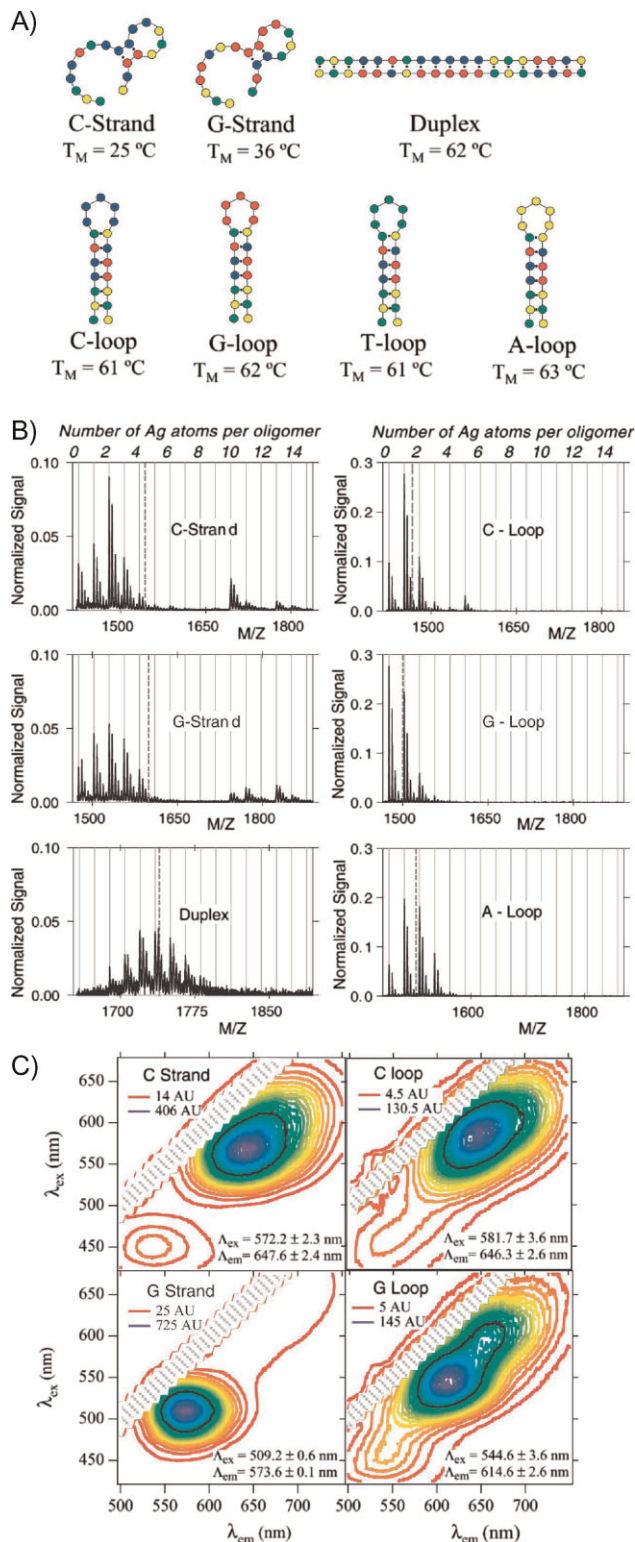

Figure 2. A) Cartoons of the 19-base DNA oligomers used by Gwinn et al.[24] Blue = cytosine (C), green = thymine (T), red = guanine (G), and yellow = adenine (A). B) Mass spectra of the DNA-Ag solutions, for DNA sequences as labeled. C) Contour maps of fluorescence emission vs. excitation from the DNA-Ag solutions. The black contour lies at the half maximum intensity. Reproduced with permission from [24].
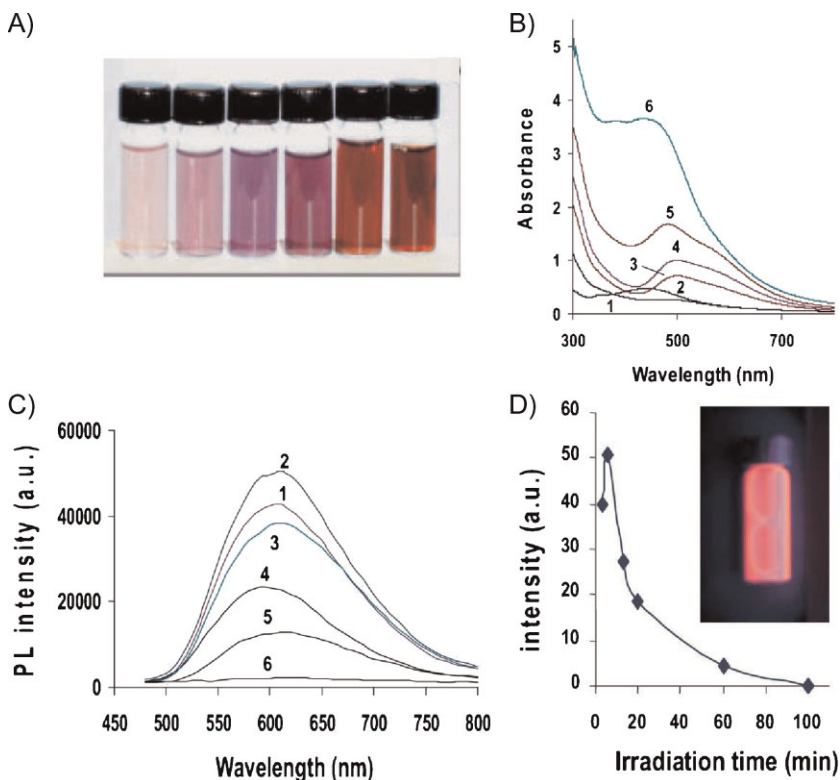
Materials
Views
www.MaterialsViews.com

ADVANCED
MATERIALS
www.advmat.de

RESEARCH NEWS

A)

B)

C)

D)

Figure 3. Ag nanoclusters stabilized in microgel dispersions. A) Photograph and B) UV spectra of poly(N-isopropylacrylamide-acrylic acid-2-hydroxyethyl acrylate) microgel dispersions formed after mixing with Ag^+ ions and UV-irradiation (365 nm at pH 8.14) for different time intervals: **1**, 3 min.; **2**, 6 min.; **3**, 13 min.; **4**, 20 min.; **5**, 40 min.; **6**, 100 min. (A) shows the same times of irradiation from left to right. Evolution of C) photoluminescence spectra and D) photoluminescence intensity ($\lambda_{ex} = 450$ nm) of Ag nanoclusters photogenerated in microgels vs. duration of irradiation. The inset in (D) shows a photoluminescent hybrid microgel obtained after 10 min UV irradiation. Reproduced with permission from [37].

fluorescent Ag nanoclusters consist of 2–8 Ag atoms. In the first report of stable, fluorescent, and water-soluble Ag nanoclusters,[36] Zheng and Dickson employed a similar photoreduction approach to produce fluorescent water-soluble Ag nanoclusters in dendrimers. Ag nanoclusters prepared in this way have much narrower and more stable emission spectra than do individual Ag nanoclusters on AgO films. Chemical reduction of Ag^+ ions in dendrimers with $NaBH_4$ yields Ag nanoparticles (3–7 nm), but with essentially no fluorescence. Kumacheva et al. used polymer microgels (poly(N-isopropylacrylamide-acrylic acid-2-hydroxyethyl acrylate)) as a template to produce fluorescent Ag nanoclusters under UV irradiation (Figure 3).[37] The optical properties and photoluminescence of Ag nanoclusters formed in the interior of polymer microgels can be finely controlled by UV-irradiation time. In addition, since these microgels incorporated pH and temperature sensitive polymers, the fluorescent microgels exhibit responses to external stimuli (i.e., pH and temperature). Poly(styrene sulfonate) capsules, multiarm star polyglycerol-b-polyacrylic acid (PG-b-PAA) copolymers, and organic fluorophore thioflavin (ThT) have also been used as templates to synthesize water-soluble fluorescent Ag nanoclusters under UV irradiation.[30,38,39]

While more complex stabilizers (e.g., dendrimers, polymer capsules, polymer microgels, and multiarm star polymers) have proved useful, even simple, inexpensive, commercially-available polyelectrolytes (such as poly(methacrylic acid) (PMAA) and poly(acrylic acid) (PAA)) can be used to prepare water-soluble fluorescent Ag nanoclusters under photoreduction.[40] Ag nanoclusters prepared in this simple approach have good photostability and excitation-dependent emission. These Ag nanoclusters have pH-dependent, solvatochromic and solvato-fluorochromic properties and are also electrochemiluminescent.[41] These polyelectrolyte-templated Ag nanoclusters have been successfully applied as fluorescent sensors for sensitive detection of Cu^{2+} and cysteine in aqueous solutions.[42,43]

5. Conclusions and Outlook

The synthesis and application of water-soluble fluorescent Ag nanoclusters is an emerging research area. We have reviewed various synthetic schemes for the preparation of water-soluble and highly fluorescent Ag nanoclusters. These emissive Ag nanoclusters are very stable and their chemical and physical properties can be controlled by varying the synthetic conditions and the stabilizing polymeric ligation. Fluorescent Ag nanoclusters have already found wide applications in bioimaging, single molecule microscopy, and chemical sensing.[21,26–30,42–43]

While substantial progress has been achieved in the preparation of Ag nanoclusters, more efficient and precise synthetic methods are still needed. Control over the specific number of silver atoms and charge on each nanocluster, for example, remains problematic. The synthesis of specific Ag nanoclusters and the correlation of their chemical and physical properties with their size are unsolved challenges. In addition, the catalytic activity of these ultra-small Ag nanoclusters has not yet been explored, although larger Ag nanoparticles have been shown to be catalytically active for a variety of oxidation reactions.[44–46]

[1] G. Hodes, *Adv. Mater.* **2007**, *19*, 639.

[2] J. Park, J. Joo, S. G. Kwon, Y. Jang, T. Hyeon, *Angew. Chem. Int. Ed.* **2007**, *46*, 4630.

[3] M. A. El-Sayed, *Acc. Chem. Res.* **2004**, *37*, 326.

[4] Y. N. Xia, Y. J. Xiong, B. Lim, S. E. Skrabalak, *Angew. Chem. Int. Ed.* **2009**, *48*, 60.

[5] C. J. Murphy, A. M. Gole, J. W. Stone, P. N. Sisco, A. M. Alkilany, E. C. Goldsmith, S. C. Baxter, *Acc. Chem. Res.* **2008**, *41*, 1721.

[6] J. P. Wilcoxon, B. L. Abrams, *Chem. Soc. Rev.* **2006**, *35*, 1162.

[7] J. Zheng, P. R. Nicovich, R. M. Dickson, *Annu. Rev. Phys. Chem.* **2007**, *58*, 409.

[8] S. Fedrigo, W. Harbich, J. Buttet, *J. Chem. Phys.* **1993**, *99*, 5712.

[9] W. Schulze, I. Rabin, G. Ertl, *ChemPhysChem* **2004**, *5*, 403.

[10] G. A. Ozin, F. Hugues, *J. Phys. Chem.* **1983**, *87*, 94.

[11] A. D. Stevens, M. C. R. Symons, *J. Chem. Soc. Farad. T. 1* **1989**, *85*, 1439.

[12] A. Henglein, *Chem. Phys. Lett.* **1989**, *154*, 473.

[13] T. Linnert, P. Mulvaney, A. Henglein, H. Weller, *J. Am. Chem. Soc.* **1990**, *112*, 4657.

[14] A. Henglein, *J. Phys. Chem.* **1993**, *97*, 5457.

[15] B. G. Ershov, A. Henglein, *J. Phys. Chem. B* **1998**, *102*, 10663.

[16] P. Mulvaney, A. Henglein, *Chem. Phys. Lett.* **1990**, *168*, 391.

[17] M. Mostafavi, N. Keghouche, M. O. Delcourt, J. Belloni, *Chem. Phys. Lett.* **1990**, *167*, 193.

[18] A. Henglein, P. Mulvaney, T. Linnert, *Faraday. Discuss.* **1991**, *92*, 31.

[19] M. Treguer, F. Rocco, G. Lelong, A. Le Nestour, T. Cardinal, A. Maali, B. Lounis, *Solid State Sci.* **2005**, *7*, 812.

[20] J. T. Petty, J. Zheng, N. V. Hud, R. M. Dickson, *J. Am. Chem. Soc.* **2004**, *126*, 5207.

[21] T. Vosch, Y. Antoku, J. C. Hsiang, C. I. Richards, J. I. Gonzalez, R. M. Dickson, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 12616.

[22] C. M. Ritchie, K. R. Johnsen, J. R. Kiser, Y. Antoku, R. M. Dickson, J. T. Petty, *J. Phys. Chem. C* **2007**, *111*, 175.

[23] B. Sengupta, C. M. Ritchie, J. G. Buckman, K. R. Johnsen, P. M. Goodwin, J. T. Petty, *J. Phys. Chem. C* **2008**, *112*, 18776.

[24] E. G. Gwinn, P. O'Neill, A. J. Guerrero, D. Bouwmeester, D. K. Fygenson, *Adv. Mater.* **2008**, *20*, 279.

[25] P. R. O'Neill, L. R. Velazquez, D. G. Dunn, E. G. Gwinn, D. K. Fygenson, *J. Phys. Chem. C* **2009**, *113*, 4229.

[26] C. I. Richards, S. Choi, J. C. Hsiang, Y. Antoku, T. Vosch, A. Bongiorno, Y. L. Tzeng, R. M. Dickson, *J. Am. Chem. Soc.* **2008**, *130*, 5038.

[27] J. H. Yu, S. M. Choi, C. I. Richards, Y. Antoku, R. M. Dickson, *Photochem. Photobiol.* **2008**, *84*, 1435.

[28] J. H. Yu, S. Choi, R. M. Dickson, *Angew. Chem. Int. Ed.* **2009**, *48*, 318.

[29] T. H. Lee, J. I. Gonzalez, J. Zheng, R. M. Dickson, *Acc. Chem. Res.* **2005**, *38*, 534.

[30] N. Makarava, A. Parfenov, I. V. Baskakov, *Biophys, J* **2005**, *89*, 572.

[31] W. W. Guo, J. P. Yuan, E. K. Wang, *Chem. Commun.* **2009**, 3395.

[32] N. Cathcart, P. Mistry, C. Makra, B. Pietrobon, N. Coombs, M. Kelokhani-Niaraki, V. Kitaev, *Langmuir* **2009**, *25*, 5840.

[33] S. S. Narayanan, S. K. Pal, *J. Phys. Chem. C* **2008**, *112*, 4874.

[34] A. Ledo-Suarez, J. Rivas, C. F. Rodriguez-Abreu, M. J. Rodriguez, E. Pastor, A. Hernandez-Creus, S. B. Oseroff, M. A. Lopez-Quintela, *Angew. Chem. Int. Ed.* **2007**, *46*, 8823.

[35] L. A. Peyser, A. E. Vinson, A. P. Bartko, R. M. Dickson, *Science* **2001**, *291*, 103.

[36] J. Zheng, R. M. Dickson, *J. Am. Chem. Soc.* **2002**, *124*, 13982.

[37] J. G. Zhang, S. Q. Xu, E. Kumacheva, *Adv. Mater.* **2005**, *17*, 2336.

[38] Z. Shen, H. W. Duan, H. Frey, *Adv. Mater.* **2007**, *19*, 349.

[39] D. G. Shchukin, I. L. Radtchenko, G. B. Sukhorukov, *ChemPhysChem* **2003**, *4*, 1101.

[40] L. Shang, S. J. Dong, *Chem. Commun.* **2008**, 1088.

[41] I. Diez, M. Pusa, S. Kulmala, H. Jiang, A. Walther, A. S. Goldmann, A. H. E. Muller, O. Ikkala, R. H. A. Ras, *Angew. Chem. Int. Ed.* **2009**, *48*, 2122.

[42] L. Shang, S. J. Dong, *Biosens. Bioelectron.* **2009**, *24*, 1569.

[43] L. Shang, S. J. Dong, *J. Mater. Chem.* **2008**, *18*, 4636.

[44] K. Watanabe, D. Menzel, N. Nilius, H. J. Freund, *Chem. Rev.* **2006**, *106*, 4301.

[45] Y. Y. Chen, C. Wang, H. Y. Liu, J. S. Qiu, X. H. Bao, *Chem. Commun.* **2005**, 5298.

[46] T. Mitsudome, S. Arita, H. Mori, T. Mizugaki, K. Jitsukawa, K. Kaneda, *Angew. Chem. Int. Ed.* **2008**, *47*, 138.