

DOI: 10.1002/anie.200600214

Light-Regulated Release of DNA and Its Delivery to Nuclei by Means of Photolabile Gold Nanoparticles**

Gang Han, Chang-Cheng You, Byoung-jin Kim,
Rosemary S. Turingan, Neil S. Forbes, Craig T. Martin,
and Vincent M. Rotello*

Gene therapy is one of the most promising prospects in biomedical and bioorganic realms.^[1] The success of gene therapy benefits mainly from having effective gene-delivery vectors for transporting plasmid DNA, small interfering RNA, or antisense oligonucleotides into target cells. The delivery of therapeutic nucleotides using nanomaterials such as polymeric micelles,^[2] dendrimers,^[3] nanorods,^[4] nanotubes,^[5] and nanoparticles^[6] is attracting increasing attention as a consequence of their unique dimensions and properties.^[7–10] Despite much progress, unpacking DNA inside target cells in a spatiotemporally controlled fashion is a major limiting factor in designing these artificial carriers.^[11] Although several intracellular release strategies have been employed, including low pH,^[12] high enzyme concentration,^[13] and redox materials inside the cells,^[14] the use of light as an external stimulus represents a unique site- and time-specific means of unloading DNA. As such, photosensitive synthetic DNA carriers will provide new directions for gene delivery owing to the potential for versatile and facile chemical modifications and the modularity of the carrier–DNA complex.

As a highly orthogonal external stimulus, photochemical processes enjoy wide use in surface patterning,^[15] advanced materials,^[16] biochemistry,^[17] and drug-delivery systems.^[18,19] Light-regulated methods uniquely limit the resultant biological effects to the illuminated areas with temporal control.^[20] For example, biologically active molecules can be modified with photosensitive groups to be essentially bioinert; they can then be reactivated by photointervention.^[21] Such caged compounds have shown tremendous applications in chemical

[*] G. Han, Dr. C.-C. You, R. S. Turingan, Prof. Dr. C. T. Martin, Prof. Dr. V. M. Rotello
Department of Chemistry, University of Massachusetts
710 North Pleasant Street, Amherst, MA 01003 (USA)
Fax: (+1) 413-545-4490
E-mail: Rotello@Chem.umass.edu
B. J. Kim, Prof. Dr. N. S. Forbes
Department of Chemical Engineering, University of Massachusetts
Amherst, MA 01003 (USA)

[**] This research was supported by the NIH (EB 004503(VR) and GM55002 (CTM)). Confocal images were taken at the Central Microscopy Facility at the University of Massachusetts at Amherst, which is supported by a grant from the National Science Foundation (NSF BBS 8714235).



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

biology, such as the control of DNA hybridization,^[22] DNA transcription,^[23] RNA ribosome function,^[24] and modulation of aptamer function.^[25] Recently, the delivery of caged DNA or proteins into cells by microinjection followed by uncaging with light at desired times or locations has become a valuable tool for studying living organisms.^[26] However, construction of the caged genetic materials can be costly and involves complicated handling, purification, and storage. Furthermore, the microinjection approach is limited in allowing high-throughput DNA delivery, as only one cell can be manipulated at a time, and the DNA itself may suffer degradation inside cells. Caged-delivery vectors bearing light-controlled DNA-binding functionality provide a means for overcoming these challenges. Systems of this type can be easily constructed and manipulated in a modular fashion and provide potential protection for DNA. Thus, they offer a simple and novel approach for the functional delivery of DNA in a well-regulated fashion.

Our recent studies demonstrated that cationic gold nanoparticles provide an efficient platform for DNA surface binding. These nanoparticles display a high affinity for electrostatic interactions with DNA, which results in the suppression of DNA transcription by T7 RNA polymerase in vitro^[27] and effective DNA delivery in mammalian cells.^[28] Herein, we construct a positively charged gold nanoparticle bearing a photoactive *o*-nitrobenzyl ester linkage, which allows temporal and spatial release of DNA by light. These cationic photocleavable nanoparticles (NP-PC) initially associate with DNA through charge pairing (Figure 1 a). Near-UV irradiation (> 350 nm) cleaves the nitrobenzyl linkage, releasing the positively charged alkyl amine and leaving behind a negatively charged carboxylate group (NP-TCOOH). This reversal in electrostatics leads to the efficient release of DNA from the nanoparticle, resulting in a high level of recovery of DNA transcription in vitro. Moreover, effective DNA delivery and release in living cells with significant nuclear localization of the DNA were obtained with this system, thus providing an important proof of concept for the development of light-regulated biological-macromolecule and drug-delivery systems.

Photolabile gold nanoparticles (NP-PC, Figure 1 b) were prepared by place exchange of 1-pentanethiol-capped 2-nm gold clusters with *o*-nitrobenzyl ester functionalized thiol ligands.^[29] The *o*-nitrobenzyl ester group is a commonly used biocompatible species that is photocleavable. It has long-term stability under ambient light, but can be removed quickly and efficiently by UV light (> 350 nm) with minimal adverse effects on biological systems.^[30] The positively charged dimethylethylammonium functionalities at the periphery of the nanoparticles provide good water solubility and allow interaction with DNA through complementary charges. Furthermore, tetraethylene glycol (TEG) was incorporated as a tether to increase both the biocompatibility and water solubility of the particles.^[31]

A solution of NP-PC was irradiated with UV light ($\lambda = 350$ nm), and the course of the photochemical reaction was monitored by UV/Vis spectroscopy to establish the cleavage of the *o*-nitrobenzyl ester linkage. Irradiation led to a decrease in absorption at 304 nm and an increase at 342 nm

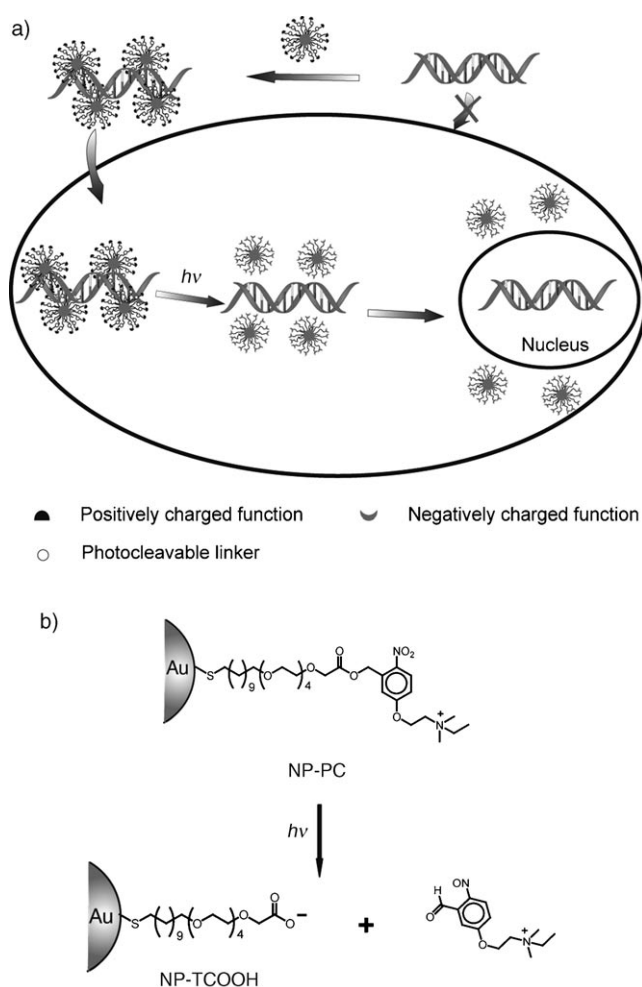


Figure 1. a) Schematic illustration of the release of DNA from the NP-PC–DNA complex upon UV irradiation within the cell; b) schematic presentation of light-induced surface transformation of NP-PC.

over time (Figure 2), thus indicating the breakage of the photolabile ester bond and the generation of *o*-nitrobenzaldehyde concomitant with charge conversion on the NP-PC

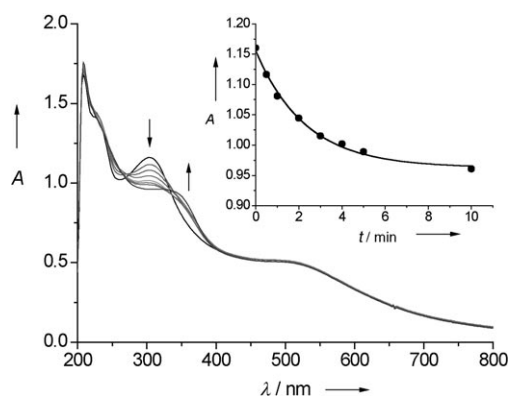


Figure 2. UV/Vis spectral changes of NP-PC (1.0 μm) upon irradiation with UV light ($\lambda = 350$ nm). Inset: The plot of absorbance at 304 nm against irradiation time shows that the photochemical reaction approached maximum conversion within 10 min.

monolayer. The photochemical conversion on the monolayer of the gold particles approached maximum within 10 min.

An ethidium bromide (EtdBr) fluorescence assay was used to follow the interaction between NP-PC and DNA. EtdBr is weakly fluorescent in aqueous solution but exhibits strong fluorescence upon intercalation into the DNA duplex.^[32] It is expected that the complementary electrostatic interaction of NP-PC with DNA would block the hydrophobic grooves of DNA and thus diminish the EtdBr–DNA interaction, which should be reflected by fluorescence changes in EtdBr. Indeed, drastic fluorescence quenching was observed when NP-PC was added to a solution of DNA and EtdBr, suggesting that EtdBr is expelled from the DNA duplex, and its intrinsic fluorescence is quenched by the bulk water molecules.^[25,28,33] Because anionic gold nanoparticles cannot bind with negatively charged DNA molecules, the absorption effect of the gold core on fluorescence quenching was subtracted with NP-TCOOH as a reference. The corrected fluorescence titration curve shows that the fluorescence quenching due to complex formation is dependent on nanoparticle concentration, and a plateau is reached at a NP-PC/37-mer DNA ratio of 6:1 (see Supporting Information), indicating a binding stoichiometry of 6:1.

DNA transcription by T7 RNA polymerase was studied *in vitro* to determine the impact of complex formation on DNA function and the functional efficiency of DNA release.^[34] Premixed (5 min) 37-mer DNA/NP-PC (1:6) solutions were exposed to UV light ($\lambda = 350$ nm) for varying periods of time followed by a T7 RNA polymerase transcription assay.^[27] The product 20-mer RNA transcripts were subjected to gel electrophoresis for quantitative determination of the extent of reaction, with the transcription level obtained in the absence of nanoparticles set to 100%. Before UV irradiation, the transcription was less than 5% of the control (Figure 3). Upon irradiation, however, the transcription level increased significantly, reaching a maximum of about 75% recovery in 8 min (Figure 3). A control experiment showed that DNA alone irradiated by UV light for 10 min has essentially identical levels of transcription to that

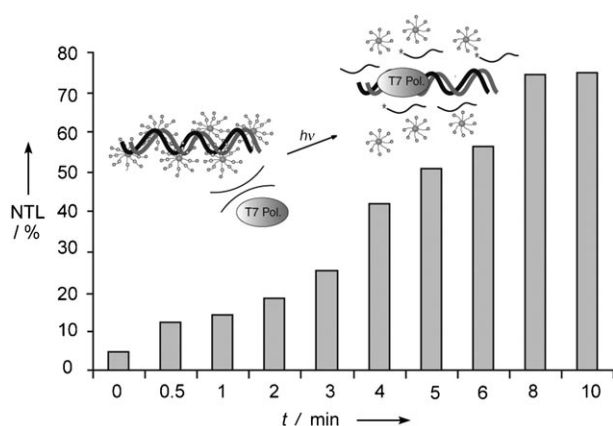


Figure 3. Normalized transcription level (NTL) of 20-mer RNA products upon irradiation at 350 nm for different times, showing the efficient transcription restoration by T7 RNA polymerase (T7 Pol.). Inset: Proposed mechanism of light-triggered transcription recovery.

without UV irradiation, which suggests that DNA function is retained in the presence of UV light. Such a high level of restoration of DNA-transcription ability indicates that the DNA–NP-PC complexes are efficiently dissociated upon irradiation. As discussed previously, the cationic NP-PC electrostatically associates with DNA to afford DNA–nanoparticle supramolecular complexes. Therefore, the initially observed transcription inhibition is ascribed to competition between the nanoparticles and the RNA polymerase for binding to the DNA. Upon UV irradiation, the cationic NP-PC is converted into the anionic NP-TCOOH, which is no longer able to associate with DNA molecules, thus releasing the DNA for transcription. This result not only provides evidence for functional DNA release *in vitro* through the light regulation of our photolabile nanoparticles, but also demonstrates the feasibility of NP-PC as a novel photocontrolled bioactive system.

Next, light-triggered DNA delivery and release were investigated in living cells by fluorescence microscopy. Gold nanoparticles are reported to quench conjugated fluorophores effectively through energy transfer.^[35] Once detached from the nanoparticles, the fluorophores “light up” as the energy-transfer pathway is removed. Herein, as a model system, we used fluorescein (FAM)-labeled 37-mer DNA (F-DNA), which bears the same sequence as that used in the T7 RNA polymerase assay, to detect DNA delivery and release *in vivo*. On the basis of the 6:1 binding stoichiometry, NP-PC–F-DNA complexes were prepared at a slight molar excess of 10:1 for optimal cellular internalization.^[24,36] Mouse embryonic fibroblast cells were incubated with NP-PC–F-DNA for 6 h in 96-well plates and then washed with phosphate-buffered saline (PBS) to remove the uninternalized nanoparticles. The cells were irradiated for 2 h with a hand-held low-power UV lamp, and fluorescence-microscopy images were taken before and after irradiation. Significant fluorescence with UV irradiation was observed inside most cells (Figure 4, right), whereas the control experiment without irradiation did not show observable fluorescence (Figure 4, left). No fluorescence was observed in cells incubated with merely F-DNA, thus indicating that the cellular uptake of naked DNA is inefficient. Furthermore, untreated cells with and without NP-PC incubation also showed no observable fluorescence after irradiation; autofluorescence from nanoparticles or cells is therefore precluded.^[29] Taken together, the results clearly show that cationic NP-PC can effectively carry DNA into the cells and that the DNA is successfully unloaded

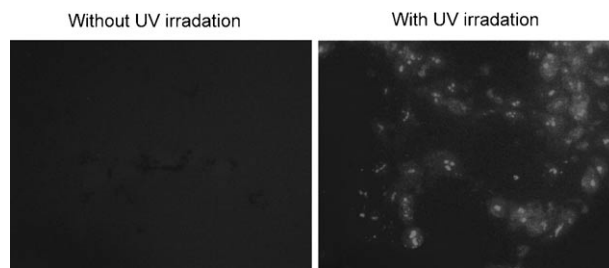


Figure 4. Representative fluorescence-microscopy images showing the phototriggered DNA release from the NP-PC–F-DNA complex.

from gold nanoparticles with the intervention of UV light, thus demonstrating that light provides effective spatiotemporal control.

Effective accumulation of DNA in the nucleus after dissociation from transporting vectors is an essential step in gene delivery, as gene expression takes place inside the cell nucleus.^[37] To evaluate the subcellular distribution of the released DNA, specifically with respect to its nuclear localization within cells after UV irradiation, the nuclear stain 4',6-diamidino-2-phenylindole (DAPI) was used. A sample of the NP-PC-F-DNA complex (10:1) was incubated with mouse embryonic fibroblast cells for 6 h in glass-bottomed dishes and washed with PBS buffer. After UV irradiation for 2 h, fluorescence-microscopy images were taken immediately. Although some F-DNA molecules were found in the cytoplasm, significant nuclear localization of the DNA is clearly demonstrated by colocalization of F-DNA (red) and DAPI (yellow) (Figure 5a). In accord with the fluorescence-microscopy results, confocal microscopic studies further confirmed that the unloaded F-DNA accumulated inside the nucleus (Figure 5b).

In summary, we have developed a photolabile gold nanoparticle that provides effective light-regulated control over DNA–nanoparticle interactions. We have demonstrated that light is effective in triggering DNA release from gold clusters both in vitro and in vivo. These cationic nanoparticles bind with DNA through complementary surface electrostatic interactions; upon UV irradiation, the electrostatic nature of the surface of the nanoparticles is converted, thus resulting in effective DNA release. We have demonstrated that such release leads to a high level of DNA-transcription recovery in vitro. Most importantly, effective DNA delivery and release were also observed in cells, with significant nuclear localization of the DNA molecules. This light-mediated release strategy paves a simple and unique way for delivering therapeutic materials into cells in a spatiotemporally controlled fashion. Furthermore, versatile surface modification of gold nanoparticles combined with the controlled interactions of biomolecules could be used to enhance the transfection of genetic materials as well as protein and drug delivery.

Experimental Section

All chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA) unless otherwise noted. Two complementary 37-mer unlabeled DNA single strands **S1** (5'-TAATACGACTCACTATAGGGAGAC-CACAACGGTTTCC-3') and **S2** (3'-ATTATGCTGAGTGATATCCCTCTGGTGTGGCCAAAGG-5') were synthesized and purified according to the method reported previously.^[30] The FAM-labeled DNA single strand **S3** (5'-(FAM)TAATACGACTCACTATAGGGAGACCACAACGGTTTCC(FAM)-3') was purchased from Integrated DNA Technologies, Inc (Coralville, IA, USA). Concentrations of single-stranded DNA stock solutions in tris-EDTA (TE) buffer (pH 7.8, tris = tris(hydroxymethyl)aminomethane (10 mM), EDTA = ethylenediaminetetraacetate (1 mM)) were calculated using the weighted sums of the different measured molar-extinction coefficients for each base at 253, 259, and 267 nm. **S2** was annealed with **S1** and **S3** separately by combining equivalent molar amounts of the individual sequences (final concentration 50 μ M), heating to 90 °C for 5 min, and slowly cooling to room temperature. DNA was stored at –20 °C. The detailed experimental procedure for the synthesis of

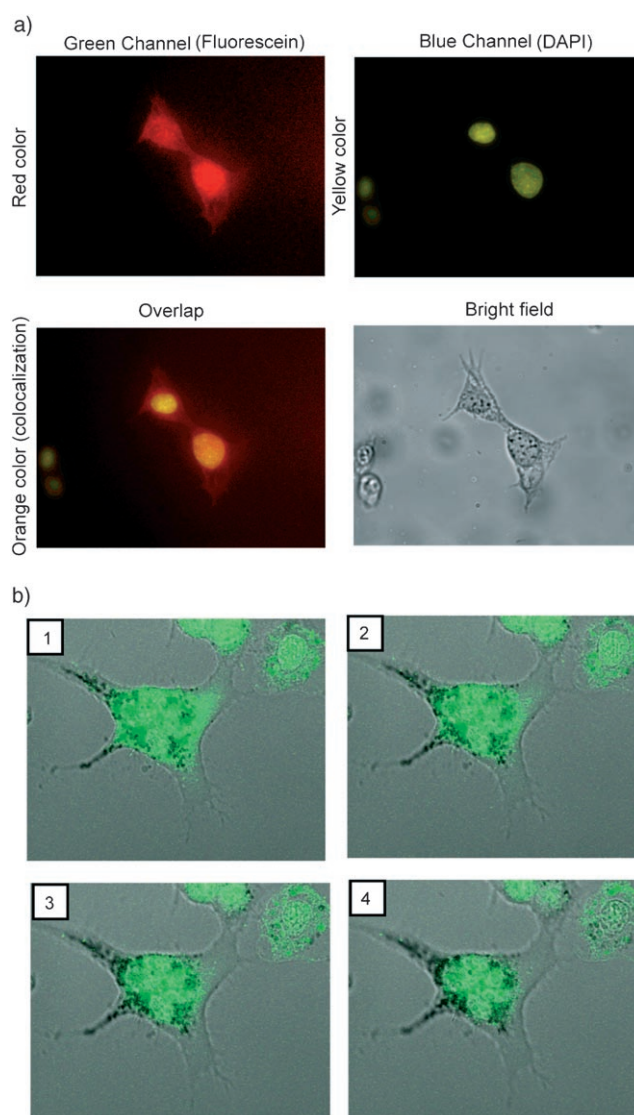


Figure 5. a) Fluorescence- and bright-field-microscopy images illustrating nuclear localization of DNA released from the NP-PC–DNA complex by photo-triggering. For an improved observation of overlapping F-DNA and nuclei stained with DAPI, we colored the green (fluorescein) and blue (DAPI) channels red and yellow, respectively. b) Confocal-microscopy images showing that the photoreleased DNA effectively accumulates inside the nucleus of cells. Panels 1–4 show four consecutive slices of the middle sections of z-series confocal images (interval = 1.0 μ m).

the photolabile thiol ligand and the corresponding NP-PC is described in the Supporting Information.

UV: Samples were irradiated in quartz cuvettes using a Rayonet photochemical reactor (Southern N.E. Ultraviolet Co., Middletown, CT, USA) at the wavelength of 350 nm. UV/Vis spectra of NP-PC were recorded with a HP 8452A spectrophotometer.

EtdBr: This assay was modified from a reported procedure.^[33] Samples were prepared in PBS buffer (pH 7.8, potassium phosphate (20 mM), sodium chloride (100 mM)) unless otherwise noted. Fluorescence spectra were recorded in a conventional quartz cuvette (10 \times 10 \times 35 mm³) on a Shimadzu RF-5301 PC spectrofluorometer with excitation at 545 nm. Both the excitation and emission slit widths were 5 nm. During the titration, a mixture (2 mL) of 37-mer DNA (0.1 μ M) and EtBr (1 μ M) was placed in the cuvette, and the initial

emission spectrum was recorded. Aliquots (10 μ L) of a solution of 37-mer DNA (0.1 μ M), EtBr (1 μ M), and NP-PC (3.5 μ M) or NP-TCOOH (3.5 μ M, as control) were subsequently added to the solution in the cuvette. After each addition, a fluorescence spectrum was recorded. The normalized fluorescence intensities calibrated by respective controls at a selected wavelength (589 nm) were plotted against the ratio [NP-PC]/[DNA] (see Supporting Information).

T7 RNA Polymerase: This assay was modified according to that previously described.^[34] The assay was carried out in PBS buffer. To mediate the reversal of inhibition of transcription, DNA/NP-PC (1:6) solutions were premixed for 5 min and then irradiated at 350 nm for different times (0, 0.5, 1, 2, 3, 4, 5, 6, 8, and 10 min) in a Rayonet photochemical reactor. DNA solutions irradiated for 0 and 10 min served as controls. T7 RNA polymerase (enzyme/DNA = 5:1, [DNA] = 0.1 μ M) and excess nucleotide triphosphates were then added to the mixture. The nucleotide triphosphates included ³²P-labeled guanosine 5'-triphosphate (GTP) for isotopic detection. The enzymatic reaction proceeded for 5 min at 37°C before being quenched with EDTA (15 mM) in formamide (95%). The 20-mer RNA transcripts were resolved by electrophoresis in a polyacrylamide (20%) and urea (7 M) gel (Supporting Information) and visualized and quantified with a Storm 840 phosphorimager to determine the extent of reaction. Transcription levels obtained in the absence of NP-PC were set to 100%.

Cell culture: Mouse embryonic fibroblast cell lines were gifts from Dr. R. Johnson, University of California, San Diego. Cells were grown in a cell-culture flask in high-glucose Dulbecco's Modified Eagle Medium (DMEM; glucose (4.5 g L⁻¹)) containing 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.4, 25 mM) supplemented with fetal bovine serum (FBS; 10%). Cultures were maintained at 37°C under a humidified condition with CO₂ (5%).

Images: All fluorescence images were obtained with an Olympus X71 inverted microscope with excitation at 470 nm and emission at 525 nm for the green (fluorescein) channel, and with excitation at 360 nm and emission at 460 nm for the blue (DAPI) channel. Confocal images were obtained with a Zeiss LSM510 microscope equipped with a 40 \times objective lens. An argon laser (488 nm) was used to provide the excitation for fluorescein-labeled DNA.

Plates (96-well): Mouse embryonic fibroblast cells (12000 cells/well) were incubated with the NP-PC-F-DNA complex (10:1, [F-DNA] = 0.2 μ M) for 6 h on the culture plates followed by washing with PBS buffer. Fluorescence-microscopy images were taken (magnification 20 \times , exposure time 500 ms) before and after 2 h of irradiation at 365 nm using a hand-held low-power Spectroline ENF-240C UV lamp.

Glass-bottomed dishes: Mouse embryonic fibroblast cells (3000 cells/dish) were plated overnight, then incubated with the NP-PC-F-DNA complex (10:1, [F-DNA] = 0.2 μ M) for 6 h on the dishes followed by washing with PBS buffer. After 2 h of irradiation with a hand-held UV lamp, fluorescence-microscopy images were taken (magnification 40 \times , exposure time 1000 ms). The confocal-microscopy image was taken by using the parameters noted above.

Received: January 18, 2006

Published online: March 30, 2006

Keywords: DNA · gold · nanoparticles · photochemistry · transcription

- [5] W. Wu, S. Wieckowski, G. Pastorin, M. Benincasa, C. Klumpp, J. P. Briand, R. Gennaro, M. Prato, A. Bianco, *Angew. Chem.* **2005**, *117*, 6516–6520; *Angew. Chem. Int. Ed.* **2005**, *44*, 6358–6362.
- [6] D. Luo, W. M. Saltzman, *Nat. Biotechnol.* **2000**, *18*, 893–895.
- [7] E. Katz, I. Willner, *Angew. Chem.* **2004**, *116*, 6166–6235; *Angew. Chem. Int. Ed.* **2004**, *43*, 6042–6108.
- [8] C. M. Niemeyer, *Angew. Chem.* **2001**, *113*, 4254–4287; *Angew. Chem. Int. Ed.* **2001**, *40*, 4128–4158.
- [9] L. Pasquato, P. Pengo, P. Scrimin, *J. Mater. Chem.* **2004**, *14*, 3481–3487.
- [10] A. Verma, V. M. Rotello, *Chem. Commun.* **2005**, 303–312.
- [11] D. Luo, *MRS Bull.* **2005**, *30*, 654–658.
- [12] W. J. Li, Z. H. Huang, J. A. MacKay, S. Grube, F. C. Szoka, *J. Gene Med.* **2005**, *7*, 67–79.
- [13] C. A. H. Prata, Y. X. Zhao, P. Barthelmy, Y. G. Li, D. Luo, T. J. McIntosh, S. J. Lee, M. W. Grinstaff, *J. Am. Chem. Soc.* **2004**, *126*, 12196–12197.
- [14] S. Giri, B. G. Trewyn, M. P. Stellmaker, V. S. Y. Lin, *Angew. Chem.* **2005**, *117*, 5166–5172; *Angew. Chem. Int. Ed.* **2005**, *44*, 5038–5044.
- [15] a) D. Ryan, B. A. Parviz, V. Linder, V. Semetey, S. K. Sia, J. Su, M. Mrksich, G. M. Whitesides, *Langmuir* **2004**, *20*, 9080–9088; b) A. del Campo, D. Boos, H. W. Spiess, U. Jonas, *Angew. Chem.* **2005**, *117*, 4785–4791; *Angew. Chem. Int. Ed.* **2005**, *44*, 4707–4712.
- [16] K. N. Plunkett, A. Mohraz, R. T. Haasch, J. A. Lewis, J. S. Moore, *J. Am. Chem. Soc.* **2005**, *127*, 14574–14575.
- [17] H. Bayley, *Nat. Chem. Biol.* **2006**, *2*, 11–13.
- [18] N. Nishiyama, A. Iriyama, W. D. Jang, K. Miyata, K. Itaka, Y. Inoue, H. Takahashi, Y. Yanagi, Y. Tamaki, H. Koyama, K. Kataoka, *Nat. Mater.* **2005**, *4*, 934–941.
- [19] N. K. Mal, M. Fujiwara, Y. Tanaka, *Nature* **2003**, *421*, 350–353.
- [20] K. Berg, P. K. Selbo, L. Prasmickaite, A. Hogset, *Curr. Opin. Mol. Ther.* **2004**, *6*, 279–287.
- [21] H. Ando, T. Furuta, R. Y. Tsien, H. Okamoto, *Nat. Genet.* **2001**, *28*, 317–325.
- [22] B. Ghosh, F. R. Haselton, K. R. Gee, W. T. Monroe, *Photochem. Photobiol.* **2005**, *81*, 953–959.
- [23] L. Krock, A. Heckel, *Angew. Chem.* **2005**, *117*, 475–477; *Angew. Chem. Int. Ed.* **2005**, *44*, 471–473.
- [24] S. G. Chaulyk, A. M. MacMillan, *Nucleic Acids Res.* **1998**, *26*, 3173–3178.
- [25] A. Heckel, G. Mayer, *J. Am. Chem. Soc.* **2005**, *127*, 822–823.
- [26] J. P. Pellois, T. W. Muir, *Angew. Chem.* **2005**, *117*, 5859–5863; *Angew. Chem. Int. Ed.* **2005**, *44*, 5713–5717.
- [27] C. M. McIntosh, E. A. Esposito, A. K. Boal, J. M. Simard, C. T. Martin, V. M. Rotello, *J. Am. Chem. Soc.* **2001**, *123*, 7626–7629.
- [28] K. K. Sandhu, C. M. McIntosh, J. M. Simard, S. W. Smith, V. M. Rotello, *Bioconjugate Chem.* **2002**, *13*, 3–6.
- [29] See Supporting Information for details.
- [30] D. M. Rothman, M. E. Vazquez, E. M. Vogel, B. Imperiali, *J. Org. Chem.* **2003**, *68*, 6795–6798.
- [31] R. Hong, N. O. Fischer, A. Verma, C. M. Goodman, T. Emrick, V. M. Rotello, *J. Am. Chem. Soc.* **2004**, *126*, 739–743.
- [32] J. Olmsted III, D. R. Kearns, *Biochemistry* **1977**, *16*, 3647–3654.
- [33] D. L. Boger, B. E. Fink, S. R. Brunette, W. C. Tse, M. P. Hedrick, *J. Am. Chem. Soc.* **2001**, *123*, 5878–5891.
- [34] P. Gong, E. A. Esposito, C. T. Martin, *J. Biol. Chem.* **2004**, *279*, 44277–44285.
- [35] C. H. Fan, S. Wang, J. W. Hong, G. C. Bazan, K. W. Plaxco, A. J. Heeger, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 6297–6301.
- [36] H.-T. Song, J.-S. Choi, Y.-M. Huh, S. Kim, Y.-W. Jun, J.-S. Suh, J. Cheon, *J. Am. Chem. Soc.* **2005**, *127*, 9992–9993.
- [37] a) O. Zelphati, F. C. Szoka, *Pharm. Res.* **1996**, *13*, 1367–1372; b) O. Zelphati, F. C. Szoka, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 11493–11498.

- [1] W. F. Anderson, *Science* **1992**, *256*, 808–813.
- [2] D. W. Pack, A. S. Hoffman, S. Pun, P. S. Stayton, *Nat. Rev. Drug Discovery* **2005**, *4*, 581–593.
- [3] U. Boas, P. M. H. Heegaard, *Chem. Soc. Rev.* **2004**, *33*, 43–63.
- [4] A. K. Salem, P. C. Searson, K. W. Leong, *Nat. Mater.* **2003**, *2*, 668–671.