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An Engineered DNA-Binding Protein Self-assembles Metallic Nanostructures

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Biological fabrication routes can provide a way to overcome the limitations presented by current chemistry-based nanoparticle arrangement and assembly methods. Many recent assembly strategies utilize DNA as the templating molecule by patterning gold nanoparticles on DNA through chemical conjugation via, for example, a sulfhydryl bond.[1] Reliance upon this chemistry, however, limits applications because it acts indiscriminately on several different metals and is only useful for some noble-metal nanoparticles. Strategies that covalently link nanoparticles to proteins or DNA risk denaturation or distortion of native protein, distortion of DNA, and/or disruption of the plasmonic or photonic properties unique to nanoparticles.[2] We present a strategy for nanoparticle patterning on DNA that utilizes the biologically based self-assembly properties of DNA-binding proteins to facilitate the targeted immobilization of nanoparticles on DNA. Here we show that a derivative of the DNA-binding protein TraI spontaneously organizes colloidal gold nanoparticles on DNA through an engineered gold-binding peptide motif. This system, based solely on specific, noncovalent, biologically determined interactions, represents significant progress on the route to spontaneously ordered assembly of nanoparticles important for downstream applications in nanoelectronics and photonics.

TraI (192 kDa, 1756 residues) is the *E. coli* F-plasmid-encoded relaxase/helicase that harbors sequence-specific single-stranded-DNA-binding activity (relaxase domain) and nonspecific single and double-stranded-DNA-binding activity (helicase domain).[3] We engineered TraI with a gold-binding motif at an internal permissive site after residue Q369 to direct the assembly of gold nanoparticles (AuNPs) on DNA through noncovalent interactions. Permissive sites, regions of proteins that tolerate a wide variety of amino acid additions without disrupting native protein function, were previously identified through transposon/epitope tag mutagenesis in TraI.[4,5] This study utilizes TraI's nonspecific DNA-binding activity as the first step toward optimization of this biologically based nanoparticle-templating strategy. Because TraI is also capable of sequence-specific DNA binding, this work paves the path to the final step of the biologically based strategy: addressable, targeted immobilization of nanoparticles on DNA.

Inorganic binding peptides identified by several groups[6,7] have demonstrated potential as molecular tools due to their high material affinity and specificity.[8] Proteins non-specifically interact with gold,[9] but our strategy relies on increased gold-binding specificity by including a gold-binding motif. By incorporating a gold-binding peptide while maintaining native DNA-binding function, we create a potent bifunctional molecular building block.

The gold-binding peptide GBP1 (MHGKTQATSGTIQS), selected from a combinatorial peptide-display library,[6] was incorporated into TraI in tandem repeats because previous research found that five (5×) or seven (7×) repeats of GBP1 exhibited a higher affinity for gold particles than a single copy.[6,10] Se quences coding for the gold-binding peptides GBP1-5×, GBP1-7×, and the silica-binding control peptide QBP (LPDWWPPPQLYH)[11] were inserted through molecular cloning techniques into the *traI* gene at a permissive site after codon 369 (Experimental Section); this permissive site lies in a region that codes for a large surface-exposed linker between the protein's functional domains.

The engineered TraI derivatives were tested for maintenance of their in vivo DNA-binding activity by comparison to wild-type TraI in a bacterial-mating assay. *E. coli* F′*ΔtraI* donor strains carrying plasmids with the complementing *traI+*, *traI369GBP1-5×*, *traI369GBP1-7×*, and *traI369QBP* alleles were proficient for F-plasmid transfer to a recipient strain, indicating that the essential DNA-binding activities (relaxase and helicase) of the encoded proteins were intact. The engineered TraI proteins were purified as previously described.[12] Purified proteins exhibited DNA-dependent ATPase activity, indicating that these protein derivatives maintained their DNA-binding activity in vitro (Experimental Section).[13] In our prior study, TraI with a cuprous oxide binding peptide (CN225) incorporated at a different permissive site near its C terminus was shown to precipitate cuprous oxide on DNA.[12] However, the TraI inorganic binding derivatives of this study are the first to utilize internal permissive sites, as opposed to N- or C-terminal sites. Use of an internal permissive site circumvents the risk of degradation of inorganic binding repeats from the end of the protein and the specificity for gold instead of cuprous oxide provides an opportunity for electronic and photonic applications.

Localized surface plasmon resonance (LSPR) analysis was used to measure the binding of the protein derivatives to AuNPs. Noble metal nanoparticles exhibit characteristic UV–visible absorption bands that result from their LSPR spectra. The peak extinction wavelength, λ_{max}, of the LSPR spectrum

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depends on the size, shape, and interparticle spacing of the nanoparticle as well as its dielectric properties and those of the local environment.[14] Shifts in LSPR absorbance peaks are commonly used as a measure of adherence of molecules to noble metal nanoparticles.[15] Charge-transfer interactions at nanoparticle surfaces and local refractive index changes enable the transduction of macromolecular binding events into optical signals based on changes in the LSPR extinction.[14] The citrate-capped 15 nm AuNPs (1.4×10^{12} particles mL^{-1}, Ted Pella) used in this study exhibit a characteristic LSPR peak at 520 nm, which shifts when protein binds to the nanoparticles. The affinity of the engineered TraI derivatives for colloidal AuNPs was tested by analyzing the LSPR shifts of gold particles upon exposure to protein.

Determination of peak maxima shifts at several different protein concentrations indicates that TraI369GBP1-5× and TraI369GBP1-7× bind or cap 15 nm gold nanoparticles better than TraI369QBP or bovine serum albumin (BSA) controls (Figure 1 A). Peak shifts were measured at protein concentrations from 0.01–0.5 μM. Maximal shift was observed at 0.1 μM, with the TraI-GBP1 derivatives showing at least 2–3 nm greater shifts than the controls (Figure 1 B). Because TraI369GBP1-5× and TraI369GBP1-7× exhibited similar maximal LSPR shifts (Figure 1 B), we continued only with TraI369GBP1-7× in subsequent analyses.

A salt precipitation test provided further evidence that TraI369GBP1-7× binds colloidal gold particles. High ionic concentrations (e.g., high NaCl) cause gold nanoparticles to agglomerate and precipitate, as evidenced by a large shift and peak broadening in the LSPR absorbance maximum. However, if the AuNPs are capped by a protein, as shown in the schematic in Figure 2, they will remain stable at high salt concentrations and the LSPR maximum will not dramatically shift or broaden. We tested this ability of TraI derivatives to stabilize AuNP LSPR peaks in 1.5 M NaCl at a range of protein concentrations (0.01 to 0.1 μM). TraI369GBP1-7× stabilized AuNPs in the presence of NaCl to concentrations as low as 0.05 μM (Figure 2). In contrast, the lowest concentration of TraI369QBP that stabilized AuNPs was 0.1 μM; this indicates that TraI369GBP1-7× provided better capping and prevented AuNP agglomeration due to salt interactions. Enhanced stability of TraI369GBP1-7×-capped gold particles as well as higher overall LSPR shifts suggest that the number of TraI369GBP1-7× molecules on the surface of nanoparticles is higher than in the case of the control protein.

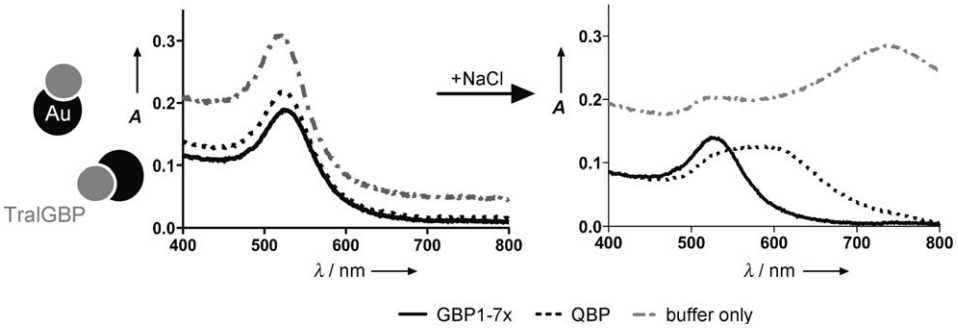

Figure 2. LSPR analysis of stabilization of 15 nm AuNPs with 0.05 μM TraI369 derivatives before (left) and after (right) 1.5 M NaCl exposure. Buffer only is AuNPs with the addition of 50 mM TrisCl pH 7.6, 50 mM KCl buffer without protein.

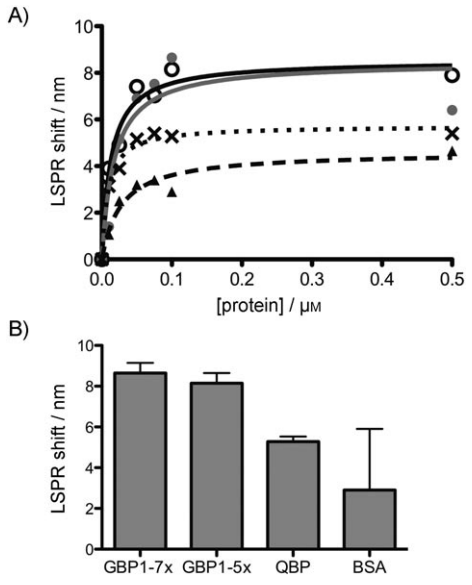

Figure 1. AuNP LSPR peak-shift analysis for several TraI369-binding derivatives. A) Best fit line ($y=B_{max}*x/(K_d+x)$) for protein binding to AuNPs as indicated by LSPR shift. Legend indicates TraI protein containing GBP1-7× (●), GBP1-5× (○), or QBP (×) sequences. BSA (▲) is a nonspecific binding protein control. Each concentration of all proteins was analyzed a minimum of $n=4$. B) LSPR peak shifts at 0.1 μM protein concentration. Error bars represent one standard deviation of the measured shifts.

In contrast, we determined if TraI369GBP1-7× could simultaneously bind AuNPs and DNA (Figure 3 schematic). Previous research on the sequence-specific dsDNA-binding *lac* repressor protein indicated that this protein can bind to gold through its charged DNA-binding domain.[16] If the TraIGBP1-7× derivative was binding gold mainly through its DNA-binding domains, upon DNA addition we expect GBP1-7× and QBP TraI derivatives to behave similarly in gold plus high salt concentrations. We assayed the nonspecific ssDNA-binding activity of TraI369GBP1-7×'s helicase domain by mixing the protein with boiled salmon sperm DNA (Experimental Section). The binding derivatives were assayed at a higher concentration (0.1 μM) than shown in Figure 2 because at this concentration both proteins stabilized AuNPs without added DNA. Therefore, at 0.1 μM the effect of DNA–protein interactions on the AuNP capping ability of tested proteins could be measured. The proteins were mixed with ssDNA, added to gold, and exposed to 1.5 M NaCl. DNA-bound TraI369GBP1-7× continued to stabilize

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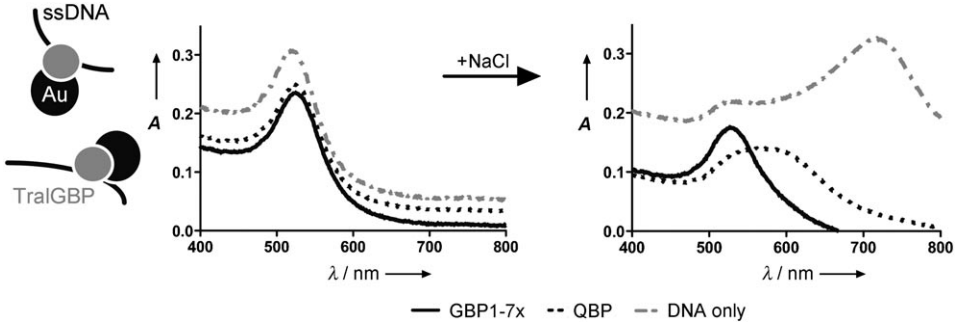

Figure 3. LSPR analysis of stabilization of 15 nm AuNPs with 0.1 μM TraI369 derivatives with ssDNA (0.02 mg mL^{-1}) before (left) and after (right) 1.5 M NaCl exposure. DNA only is a control with DNA in buffer added to AuNPs.

AuNPs whereas DNA-bound TraI369QBP did not (Figure 3). We conclude from these data that TraI369GBP1-7× is a bifunctional protein that has specific interactions with AuNPs through its GBP1 motif and simultaneously binds ssDNA.

The conclusions drawn from the data in Figure 2 and 3 are further supported by experiments assaying protein stabilization of AuNPs in low-percentage agarose gels. In particular, these studies again show that TraI369GBP1-7×, but not TraI369QBP, stabilizes AuNPs in the presence of ssDNA when excess protein, DNA, and nanoparticle-capping ligand (citrate) are removed from the reaction (Figure S1 in the Supporting Information). The protein-induced stability of AuNPs in the absence of excess capping ligand suggest that these particles would be particularly suited for biological applications or for building nanostructures by using biological interactions.[17]

Given that the TraI369GBP1-7× derivative interacts with AuNPs and DNA in solution, we tested the ability of this protein to organize these particles on a solid surface by using transmission electron microscopy (TEM). Protein and DNA were mixed and then added to 15 nm AuNPs. TEM imaging of gold–protein conjugates assembled on ssDNA shows that TraI369GBP1-7× decorates ssDNA to produce metallic self-assembled nanostructures (Figure 4). Analysis of similar mixtures of TraI369QBP with ssDNA and AuNPs did not show any protein–DNA complexes associated with gold.

Our results demonstrated that the engineered TraI bifunctional protein arranged several gold nanoparticles along the ssDNA molecule, packing them tightly together (Figure 4). Additional results indicate that a range of AuNP packing is attainable in this system and that dsDNA can also serve as the organizing substrate (Figures S2 and S3); this suggests a versatile role for this engineered TraI in self-assembling nanostructures. One of the major challenges in nanophotonics is to control proximity among nanoparticles.[18] Biological molecules have the potential for precise spatial and temporal control. Our results are unique in demonstrating this potential by using purified biological building blocks to mediate the organization of nanoparticles on DNA. We have shown DNA-binding proteins can be engineered with inorganic binding peptides to control targeted immobilization of nanoparticles on a DNA template.

This proof-of-concept experiment is strong evidence that noncovalent, biological interactions could be one way of implementing targeted nanoparticle immobilization. The self-assembly principles demonstrated in this work will be fundamental to constructing higher-ordered hybrid nanoarchitectures through DNA–protein–nanoparticle interactions. These interactions are complex but addressable through sequence design, both in the proteins and the DNA.

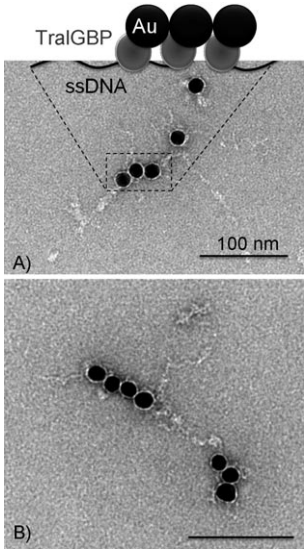

Figure 4. TEM analysis of TraI369GBP1-7× (0.1 μM) with ssDNA (0.1 mg mL^{-1} boiled 1 kb NEB DNA ladder)

Experimental Section

Genetic engineering of inorganic binding peptide sequences into *tral*: To insert the QBP silica-binding peptide into the TraI permissive site after residue Q369, the QBP-coding region was PCR amplified from the replicative form M13 phage DNA encoding the QBP sequence.[11] Primers with BglII restriction sites (5′-ACT TTC AAC AGT TTC GGC CAG ATC TCC ACC-3′ and 5′-TTC GCA ATT CCT TTA GAT CTA CCT TTC TAT TCT CAC TCT-3′) were purchased from IDT (San Diego, CA, USA) and resuspended in 10 mM Tris-HCl to 100 μM. Five PCR reactions containing 20 μM of each primer, 2 mM dNTPs, 10× Pfu buffer, Pfu turbo (Stratagene), and template DNA were run under the following cycling conditions: 94 °C 2 min, (95° 30 s, 55° 60 s, 72° 30 s) x30, 72° 10 min, 4° hold. PCR reactions were pooled and purified (Quiquick Kit, Qiagen). Concentrated PCR product was digested with BglII (NEB) to create sticky DNA ends. p99*tral*::Q369 plasmid DNA[5] was linearized with BamHI (NEB) within the i31 insertion sequence. Digested PCR DNA was mixed at a 3:1 or 15:1 molar excess with the linearized plasmid followed by overnight ligation (NEB T4 DNA ligase) at 16 °C. The ligation mixture was subjected to BamHI digestion to eliminate religated p99*tral*::Q369, and the DNA was transformed into competent *E. coli* DH5α. After overnight incubation on LB agar plates supplemented with ampicillin (100 μg mL^{-1}), colonies were picked in pools for colony PCR. Colonies were suspended in H_2O (20 μL),

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boiled for 5 min, then spun down to pellet cell debris. The supernatant was used as the DNA template for screening PCR reactions with 20 μM primers (5′-TTG CCT GAT TGG TGG CCT CC-3′ and 5′-CTG TCA GCA GGT CCA CCA TGC-3′), 2 mM dNTPs, 10× Taq buffer, and Taq DNA polymerase (NEB). The reaction conditions were 94 °C 2 min, (95° 30 s, 55° 60 s, 72° 60 s) ×30, 72° 10 min, 4° hold. Candidate colonies were re-streaked for single colonies and plasmids were purified (Qiagen). Candidate plasmids were sequenced with primer 5′-CCA GAA CGG TCG GCA TAC TGC-3′ to verify the correct insert.

The procedures described above were also followed to insert the coding sequences for GBP1-5× and GBP1-7× into p99*traI*::Q369. GBP1-5× was amplified from plasmid pSB3057 and GBP1-7× from plasmid pSB3053, alkaline phosphatase expression vectors described previously.[6] The amplification primers used for both were 5′-GCC CGG ACA CCA GAT CTG CCT ATG C-3′ and 5′-CCA GAA CCT CGA GGG AGA TCT TCA TCG ATG CTC TGG-3′ Plasmids containing the correct QBP, GBP1-5×, and GBP1-7× inserts, were selected for subsequent work. The corresponding proteins were designated as TraI369QBP3, TraI369GBP1-5× and TraI369GBP1-7×. The three mutant proteins were compared to wild-type TraI in a conjugation assay. An XK1502 F′Δ*traI* donor strain was proficient for plasmid transfer to a recipient strain in the presence of the pTrc99A plasmid derivatives carrying the *traI*+, *traIQ369QBP3, traIQ369GBP1-5*× , and *traIQ369GBP1-7*× alleles, indicating that the essential biological activities (helicase and relaxase) of the encoded proteins were intact. The mating efficiencies for cells expressing the three TraI derivatives were between 15 and 230 times the mating efficiency for cells expressing wild-type TraI, consistent with previous permissive site analysis.[5]

Protein purification: *E. coli* MC4100-derived strain BN20 (Δ(*lac pro*)X111 *araD rpsL rpoB malG*(Am) *malT*(Con) *zhe*::Tn10 *thi recA*::*catA*)[19] was used as a background strain for the induction and purification of TraI protein derivatives. BN20 carrying the plasmid p99*traI*::Q369GBP1-5×, p99*traI*::Q369GBP1-7× or p99*traI*::Q369QBP was grown in LB medium (500 mL) supplemented with ampicillin (100 μg mL^{-1}) at 30 °C to an OD_{600} of 0.3–0.4. The cells were induced with IPTG (0.5 mM) for 2 h. The cells were then collected by centrifugation and the dry cell pellet was frozen at −80 °C until use. The cell pellet was homogenized in sucrose buffer (5 mL 50 mM Tris–HCl, pH 7.6, 10 mM EDTA, 20% sucrose). Lysozyme was added to a final concentration of 0.1 mg mL^{-1} and PMSF added to 40 μg mL^{-1} and the cell suspension left on ice 30 min. The cells were freeze-thawed on dry ice three times to aid in lysis. $MgCl_2$ was then added to a final concentration of 20 mM followed by addition of DNaseI (~25 μg). The suspension was incubated at room temperature until the viscosity decreased. The insoluble membrane-containing fraction was separated from the cytoplasmic fraction by centrifugation at 10 000 *g* for 20 min at 4 °C. TraI-rich, precipitated fractions were recovered from the soluble cellular extract fraction by ammonium sulfate precipitation at 30–40% saturation, resuspended into buffer A (5 mL 20 mM Tris–HCl, pH 7.6, 0.1 mM EDTA, 10% glycerol), and dialyzed overnight against the same buffer (1 L). Proteins were loaded on a DEAE Sephacel column (Sigma) equilibrated in buffer A and developed with a NaCl step gradient (100–300 mM NaCl in buffer A). Fractions eluting at ~200 mM NaCl were pooled and dialyzed against buffer A. The protein was concentrated with Amicon protein columns (100 000 MWCO) and buffer A was exchanged for 50 mM Tris–HCl pH 7.6, 50 mM KCl. Protein was stored at −80 °C until use. Protein concentrations were determined by using the Bio-Rad dye-binding protein assay kit. ATPase assays, described previously,[13] confirmed purified proteins maintained helicase activity. TraI369GBP1-7× and TraI369QBP3 exhibited DNA-dependent ATPase activity, with specific activities of 29.16 U μg^{-1} and 83.16 U μg^{-1}, respectively. One unit (U) is defined as 1 nmol of ATP hydrolyzed in 20 min at 25 °C.

LSPR: 15 nm citrate-capped colloidal gold nanoparticles (95 μL; Ted Pella, Prod. #15704–20) were mixed with protein stock solution (5 μL in 50 mM Tris-HCl pH 7.6, 50 mM KCl). For experiments with DNA, salmon sperm DNA was boiled for 10 min then placed on ice to obtain ssDNA. ssDNA (1 μL, 0.1 mg mL^{-1}) was added to stock protein (5 μL). This mixture was then added to gold nanoparticle stock solution (94 μL). Protein and gold or protein, DNA, and gold solutions were incubated for 2 h at 25 °C with gentle shaking. Absorbance spectra were recorded from 400–800 nm with a 96-well TECAN plate reader (TECAN, San Jose, CA). For experiments with added salt, NaCl stock solution (5 M, 40 μL) was then added to the assays and absorbance spectra were recorded.

TEM: Negatively stained samples were prepared as described previously.[20] Briefly, sample was prepared by mixing protein (5 μL) with DNA (1 μL) at room temperature for at least 10 min. AuNPs (94 μl) were then added to this mixture. A drop (2 μL) of the sample was spotted onto a freshly glow discharged (negative charge), carbon-coated copper mesh grid. Excess protein sample was blotted off with filter paper, and washed three times with H_2O before staining with uranyl formate (0.25%) and air-drying. The grid was inserted into a 100 kV TEM (FEI Morgagni) and images were recorded at nominal magnification of 14 000× at the specimen level using a bottom mount 4k×2k Gatan charge-coupled device camera.

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[1] a) G. Hermanson, *Bioconjugate Techniques*, Academic Press, Boston, **1996**; b) T. Nishinaka, A. Takano, Y. Doi, M. Hashimoto, A. Nakamura, Y. Matsushita, J. Kumaki, E. Yashima, *J. Am. Chem. Soc.* **2005**, *127*, 8120–8125; c) J. Zhang, Y. Liu, Y. Ke, H. Yan, *Nano Lett.* **2006**, *6*, 248–251.

[2] J. Irrgang, J. Ksienczyk, V. Lapiene, C. Niemeyer, *ChemPhysChem* **2009**, *10*, 1483–1491.

[3] a) P. K. Dash, B. A. Traxler, M. M. Panicker, D. D. Hackney, E. G. Minkley, Jr., *Mol. Microbiol.* **1992**, *6*, 1163–1172; b) L. M. Street, M. J. Harley, J. C. Stern, C. Larkin, S. L. Williams, D. L. Miller, J. A. Dohm, M. E. Rodgers, J. F. Schildbach, *Biochim. Biophys. Acta, Protein Proteom.* **2003**, *1646*, 86–99.

[4] B. Traxler, E. Gachelet in *Methods in Enzymology, Vol. 421: Sets of Transposon-Generated Sequence-Tagged Mutants for Structure–Function Analysis and Engineering* (Eds.: K. Hughes, S. Maloy), Elsevier, San Francisco, **2007**.

[5] R. J. Haft, G. Palacios, T. Nguyen, M. Mally, E. G. Gachelet, E. L. Zechner, B. Traxler, *J. Bacteriol.* **2006**, *188*, 6346–6353.

[6] S. Brown, *Nat. Biotechnol.* **1997**, *15*, 269–272.

[7] a) R. R. Naik, L. L. Brott, S. J. Clarson, M. O. Stone, *J. Nanosci. Nanotechnol.* **2002**, *2*, 95–100; b) K. T. Nam, D. W. Kim, P. J. Yoo, C. Y. Chiang, N. Meethong, P. T. Hammond, Y. M. Chiang, A. M. Belcher, *Science* **2006**,

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312, 885 – 888; c) M. Sarikaya, C. Tamerler, A. K. Jen, K. Schulten, F. Baneyx, *Nat. Mater.* **2003**, *2*, 577 – 585; d) C. Mao, C. E. Flynn, A. Hayhurst, R. Sweeney, J. Qi, G. Georgiou, B. Iverson, A. M. Belcher, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 6946 – 6951.

[8] a) A. Sengupta, C. K. Thai, M. S. Sastry, J. F. Matthaei, D. T. Schwartz, E. J. Davis, F. Baneyx, *Langmuir* **2008**, *24*, 2000 – 2008; b) S. R. Whaley, D. S. English, E. L. Hu, P. F. Barbara, A. M. Belcher, *Nature* **2000**, *405*, 665 – 668; c) R. G. Woodbury, C. Wendin, J. Clendenning, J. Melendez, J. Elkind, D. Bartholomew, S. Brown, C. E. Furlong, *Biosens. Bioelectron.* **1998**, *13*, 1117 – 1126; d) J. Wei, T. Kacar, C. Tamerler, M. Sarikaya, D. Ginger, *Small* **2009**, *5*, 689 – 693.

[9] J. M. Guisan, *Immobilization of Enzymes and Cells*, 2nd ed., Humana, Totowa, **2006**.

[10] a) S. Brown, M. Sarikaya, E. Johnson, *J. Mol. Biol.* **2000**, *299*, 725 – 735; b) C. Tamerler, E. E. Oren, M. Duman, E. Venkatasubramanian, M. Sarikaya, *Langmuir* **2006**, *22*, 7712 – 7718; c) M. Hnilova, E. E. Oren, U. O. S. Seker, B. R. Wilson, S. Collino, J. S. Evans, C. Tamerler, M. Sarikaya, *Langmuir* **2008**, *24*, 12440 – 12445.

[11] E. E. Oren, C. Tamerler, D. Sahin, M. Hnilova, U. O. S. Seker, M. Sarikaya, R. Samudrala, *Bioinformatics* **2007**, *23*, 2816 – 2822.

[12] a) H. X. Dai, W. S. Choe, C. K. Thai, M. Sarikaya, B. A. Traxler, F. Baneyx, D. T. Schwartz, *J. Am. Chem. Soc.* **2005**, *127*, 15637 – 15643.

[13] B. A. Traxler, E. G. Minkley, *J. Mol. Biol.* **1988**, *204*, 205 – 209.

[14] A. Haes, R. Van Duyne, *J. Am. Chem. Soc.* **2002**, *124*, 10596 – 10604.

[15] a) M. Iosin, F. Toderas, P. L. Baldeck, S. Astilean, *J. Mol. Struct.* **2009**, *924–926*, 196 – 200; b) J. A. Tullman, W. F. Finney, Y. J. Lin, S. W. Bishnoi, *Plasmonics* **2007**, *2*, 119 – 127.

[16] M. K. Calabretta, K. S. Matthews, V. L. Colvin, *Bioconjugate Chem.* **2006**, *17*, 1156 – 1161.

[17] R. Levy, N. T. K. Thanh, R. C. Doty, I. Hussain, R. J. Nichols, D. J. Schiffrin, M. Brust, D. G. Fernig, *J. Am. Chem. Soc.* **2004**, *126*, 10076 – 10084.

[18] Y. Chen, K. Munechika, D. Ginger, *MRS Bull.* **2008**, 536 – 542.

[19] B. D. Nelson, B. Traxler, *J. Bacteriol.* **1998**, *180*, 2507 – 2514.

[20] M. Ohi, Y. Li, Y. Cheng, T. Walz, *Biol. Proced. Online* **2004**, *6*, 23 – 34.

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