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Enzymatic Manipulation of DNA−Nanomaterial Constructs

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Biomolecules provide a dramatically enhanced set of structurally diverse tools for the assembly of unique bionanoconstructs.[1] These next-generation materials can form the basis of novel device technologies by utilizing the highly convergent, self-assembling capability of biopolymers to direct the formation of three-dimensional constructs.[2−3] Studies on DNA interactions with Au colloids have illustrated that biofunction is only partially maintained.[4−8] In fact, inhibition of bioactivity has been observed in the presence of Au colloids.[4−6] Biocompatibility between inorganic nanomaterials and biological scaffolding is crucial to the development of biomaterials.

The retention of DNA function in the presence of nanomaterials is largely unexplored.[9] The impact of nanomaterials in bioconstructs can be analyzed by analysis of the biofunction of site-specific DNA binding proteins in the presence of DNA−nanocrystal conjugates. Since naturally occurring proteins are extremely sensitive to the conformational integrity of the DNA, the DNA conformation and enzyme activity in the presence of 1.4-nm Au allows direct analysis of biofunction. Proteins such as bacterial DNA methyltransferases (M.*Eco*RI and M.*Hha*I) or restriction endonuclease, R.*Eco*RI, are known to bind and produce specific conformational changes in DNA. M.*Eco*RI recognizes the GAATTC sequence, methylating the second adenine by bending the DNA approximately 55−59° and flipping the target adenine out of the DNA duplex.[10] Methyl transfer to the extra-helical adenine requires the cofactor S-adenosylmethionine. M.*Hha*I on the other hand, flips out its target base, 5′-C of GCGC, but does not bend the target DNA.[11] Using highly selective bacterial proteins that induce sequence-specific conformational perturbations within DNA, we observe the absolute maintenance of bifunction for biomaterials composed of duplex DNA appended with 1.4-nm Au particles. Our results provide a foundation for interfacing more complex and diverse protein−DNA systems.

The studied 40-mer duplex DNA with an incorporated basepair target site is Z-CTAAGGCACACGACATATGCGCGAATTCT-CACTATCAC, where Z represents a hexane thiol modification of the 5′-phosphate backbone (Figure 1). Au appended DNA was prepared by treatment of the 40-mer duplex DNA with 2 equiv of Nanogold (1.4 nm)[12] to produce freely soluble Au−DNA constructs with single Au nanomaterials appended at the 5′ ends that can be imaged by TEM (Figure 2).

The Au−DNA 40-mer nanoconstruct in the absence of protein produces a Au separation distance distribution of 12 ± 2 nm, in good agreement with the calculated persistence length of a native 40-mer strand of duplex DNA (Figure 1a).[13] The 12-nm spacing was verified over several serial dilutions of the Au−DNA constructs (Supporting Information, Figure 1). There is no evidence for nonspecific nanomaterial interactions with the DNA backbone.

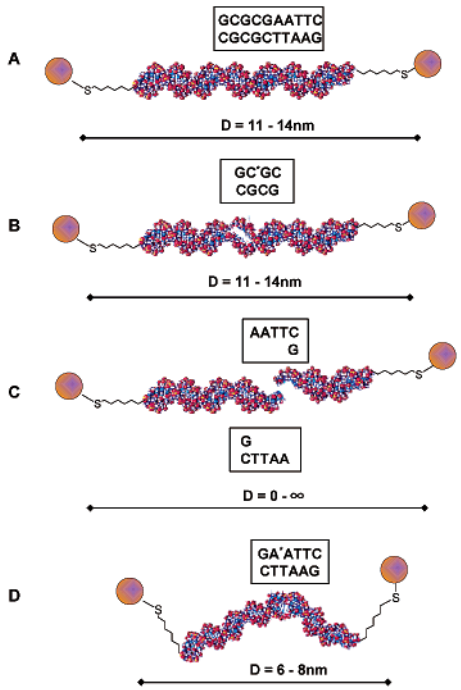

Figure 1. Representation of (A) (ds) DNA−nanomaterial conjugate with the *Hha*I and *Eco*RI recognition site, (B) the conjugate with the binding of the M.*Hha*I enzyme (Note: little perturbation of the length of the DNA upon enzyme binding), (C) the conjugate afte the cutting of the DNA at the R.*Eco*RI recognition site, (D) the conjugate with a 59 °C bend due to the binding of M.*Eco*RI enzyme.

Addition of M.*Hha*I does not modulate the separation distance (12 ± 2 nm) (Figure 2). However, upon addition of M.*Eco*RI to a solution of the nanoconstruct, the binding of the protein produces a drastic decrease in the Au−Au separation distance (6−8 ± 2 nm) (Figure 3). The measured distances correlate with the expected topological changes induced by the binding of M.*Hha*I and M.*Eco*RI.[9] The methylation activities of M.*Eco*RI and M.*Hha*I with the Au nanocrystal conjugates are 91 and 93%, respectively, when compared to the unappended DNA, suggesting biofunction of the DNA−enzyme complex is maintained.[14,15]

While methylation assays provide insight into biofunction, a more sensitive measure of bioactivity in the M.*Eco*RI DNA−protein complex is the dissociation constant K_d. The value of K_d can be extracted from the concentration-dependent histograms in Figure 3, where a bimodal distribution arises, corresponding to the calculated distance for the free 40-base pair oligomers at 12 nm, and the enzyme−DNA complex at 6−8 nm.[16] Increasing the concentration from 50 to 85 nM results in a shift in the histogram population without a shift in the center of mass positions. This suggests the bimodal distribution arises predominately from the binding dissociation constant and not from DNA cleavage or

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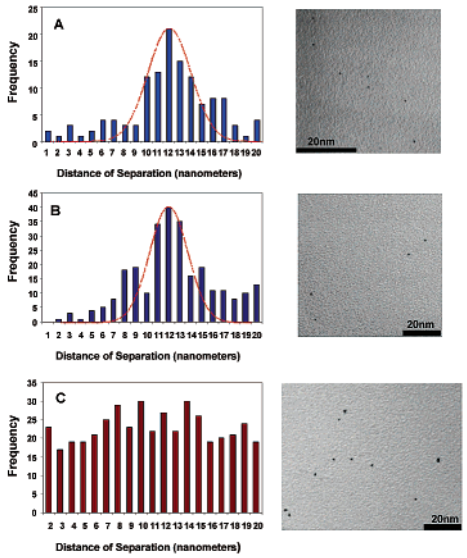

Figure 2. (A) Histogram of distance of separation based on TEM of Au nanocrystals attached by (ds) DNA. (B) Same as A with the addition of 85 nM M.*Hha*I. (C) Same as A with the addition of R.*Eco*RI. Overlay represents the Gaussian fit to the frequency data. TEM scale bar = 20 nm.

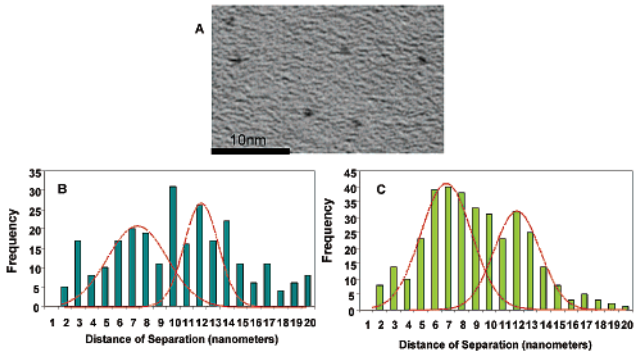

Figure 3. (A) TEM of DNA−Au conjugates with M.*Eco*RI (85 nM). (B) Au nanocrystals 1.4 nm attached by (ds)DNA with 50 nM M.*Eco*RI. (C) Au nanocrystals 1.4 nm attached by (ds)DNA with 85 nm M.*Eco*RI. TEM scale bar = 10 nm.

anomalies in the protein−DNA interaction. The measured K_d for the nanoconstruct is ∼44 nM (±2.2 nM) in good agreement with the dissociation constant (K_d = 43 nM).[17] The measured distance for the DNA−enzyme complex corresponds to a conformational change in the DNA arising from a 55 to 59° bend, which correlates with DNA−M.*Eco*RI topology measured by AFM and gel-shift techniques.[9] The observation of the same binding affinities and bending angles confirms biofunction is maintained in these constructs.

Treatment with the endonuclease R.*Eco*RI catalyzes the double-stranded DNA cleavage at the same site recognized by M.*Eco*RI (Figure 1C). This results in a random separation distance for the Au nanocrystals (Figure 3C) with no obvious grouping, confirming the TEM histograms arise from specific protein−DNA interactions, not TEM-induced anomalies.

Our studies into biofunction demonstrate for the first time direct evidence of the lack of conformational affects of nanomaterials on bioactivity. Statistical analyses of a large set of constructs suggest that DNA modified with 1.4-nm Au nanomaterials does not disrupt the activity of the native proteins. The elucidation of compatibility and activity between biological polymers and nanomaterials shows that conformational modulation of DNA nanomaterial constructs may allow the design, preparation, and manipulation of broad architectures for nanoelectronics or nanosensors.

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Supporting Information Available: Large-field TEM images and an illustrative description of how distances between gold dots were measured (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Methylation assays were done over a period of 20 min with 440 nM DNA, 4 μM M.*Eco*RI, or 3 μM M.*Hha*I and 60 μM ^{3}H AdoMet under standard conditions. Enzymes were dialyzed against argon-sparged 50 mM PBS. All activity measurements were corrected by subtraction of the activity from a sample lacking enzyme and represent single turnover conditions.
(16) The dissociation constant for the M.*Eco*RI−DNA complex (K_d) was determined using the following expression: K_d = [Free Enz][Au−DNA]/[Enz*Au−DNA Complex]. Since the amount of enzyme added is in a 100-fold excess, the amount of free enzyme ([Free Enz]) for this calculation is assumed to be approximately equivalent to the original enzyme concentration $[Enz]_i$. The ratio of the concentration of free DNA to the Enz*Au−DNA complex can be estimated from the area under the curve associated with the 12-nm separation distance (free) and the area under the curve associated with the 7-nm separation distance (enzyme−DNA complex) measured in Figure 3. The $[DNA]_i$ was measured by absorption spectroscopy ($[DNA]_i$ = 220 pM) prior to the addition of enzyme.
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