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Direct Electronic Control of Biomolecular Systems: Using Nanocrystals as Antennas for Regulation of Biological Activity

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ABSTRACT

We report a means of directly controlling DNA dehybridization by radio frequency magnetic field coupling to a nanometer scale antenna covalently linked to the DNA. The method of control relies on induction heating of an Au nanocrystal, which raises the temperature of a biomolecule to which it is covalently bound, while leaving surrounding molecules relatively unaffected. Because heat dissipation in biomolecules in solution is rapid(<50 picoseconds[1]) this switching is reversible. This technique is specific, reversible, and non-optical. Since it can be used in solution, it has the potential to be extended to systems *in vivo*. The ability to differentially control local temperature forms the basis of control of properties such as hybridization and enzyme activity, and has the potential of controlling many biological processes.

EXPERIMENTAL

Induction heating works by creating alternating eddy currents in a metal. This is achieved by placing the metal in an alternating magnetic field, which generates in the sample eddy currents that are converted to heat by the Joule effect[2]. This technique of heating a conductor by placing it in an alternating magnetic field has generally been used to heat macroscopic samples (length scales ~cm). Here we apply it to metallic nanocrystals (diameter ~ 1nm) in solution. Induction heating is accompanied by a skin depth effect which results from partial cancellation of the magnetic fields. As a result, the majority of the power absorbed by a conductor is concentrated in the skin depth d_0 given by

$$d_0 = \frac{1}{2\pi}\sqrt{\frac{\rho \cdot 10^7}{\mu_r \mu_0 f}} \tag{1}$$

where μ_r= magnetic permeability, μ_0= permeability of free space, ρ=material resistivity, and f=frequency of the alternating magnetic field. The power density is described by

$$P = 4\pi H_e^2 \mu_0 \mu_r f F \frac{d_0}{d} \tag{2}$$

where d= sample diameter, H_e = magnetic field strength, and F is a transmission factor that has a sigmoidal dependence on (d/d_0). This results in optimal power absorption or heating when d/d_0=3.5. To inductively heat a Au nanocrystal with d=1-10nm such that d/d_0=3.5, alternating magnetic fields with f=1GHz (radio frequency range) is required[2].

Alternating magnetic fields were generated by applying an alternating current to a coil. Currents with f=1GHz (radio frequency) were obtained by using an RF signal generator (Hewlett

Packard) in conjunction with a linear amplifier. The power applied to the coil ranged from 0.4W to 4W, though these are considered to upper limits to the exact power inside the coil from losses due to setup architecture.

In order to demonstrate reversible, electronic control, we have constructed a DNA hairpin-loop oligo covalently linked to a nanocrystal antenna. 38mer DNA hairpin/loop oligos (Research Genetics, Huntsville, AL) which are self-complementary at each end for 7 bases[3] (Figure 1a) were covalently linked to a 1.4nm Au nanocrystal functionalized with a single sulfo-N-hydroxy-succinimide (NHS) ester (Nanoprobes, Yaphank, NY) by a primary amine appended to one of the bases on the DNA [4-8]. Because of the constraint of the loop, the DNA rehybridizes on a timescale comparable to dehybridization[9]. The amount of dehybridization was monitored by the hyperchromicity of DNA, which was measured by optical absorbance at 260nm (A_{260}). A solution of the nanocrystal-linked oligos (oligo M) (Figure 1, inset) in 1X phosphate buffered solution (PBS) was put into a radio frequency magnetic field (RFMF) with f=1GHz and power=4W. A_{260} was monitored in a UV/visible spectrophotometer as the RFMF was pulsed at 15s intervals (Figure 1b). As the RFMF was switched on, A_{260} increased from 0.22 to 0.25 OD, indicating that M dehybridized with the RFMF (top curve). When the RFMF was switched off, A_{260} decreased back to its original value. Cycling through the on and off states is completely repeatable. The on/off ratio of A_{260nm} is 1.09, consistent with calculated absorption changes for an oligo of this sequence at this concentration hybridizing and dehybridizing 7 bases. Control solutions with only DNA (oligo N) resulted in no change in the absorbance with RFMF (lower curve). This demonstrates that inductive coupling to a covalently bound metal nanocrystal antenna can reversibly dehybridize DNA on a timescale of at most several seconds.

Figure 1. Induction heating of nanocrystals linked to DNA and evidence dehybridization. Left, Sequence of the DNA hairpin molecule dehybridized by induction heating of a Au nanocrystal. The molecule is self-complementary at the ends for 7 bases and has a primary amine in the loop region to which the nanocrystal is covalently linked. Right, Absorbance at 260nm (A_{260}) of a solution of DNA hairpin oligos linked to 1.4nm Au nanocrystals (oligo M) as a function of RFMF switching (solid squares). The arrows indicate when the RFMF is on and off. Empty circles: the response of the same DNA hairpin oligo (N) with no nanocrystal attached in the RFMF. The concentrations of M and N are 1×10^{-6}M.

To determine the effective temperature that inductive coupling to the nanocrystal antenna produces in its local environment, we designed a two-phase system in which an oligo is dehybridized from a solid support into solution (Figure 2a). Oligo X is a 12mer that has a FAM on the 5' end and a 1.4nm Au nanocrystal on the 3' end (T_m = 40°C). It was labeled with the nanocrystal as described in the previous section. Oligo Y is a 68mer that has a biotin on the 5' end, which is complementary to the 12mer. X and Y were hybridized, and the X-Y hybrid was incubated with streptavidin agarose beads to allow the biotin on the Y to bind to the streptavidin. The agarose beads comprise the solid phase. Samples were washed several times with 1X PBS to remove free molecules in the supernatant. When X is dehybridized from Y, it diffuses from the solid phase to the supernatant. The amount of dehybridization is proportional to the amount of X in the supernatant, which was measured by fluorescence spectroscopy. One sample was exposed to the RFMF and its supernatant removed. Its fluorescence spectrum was compared to supernatants of identically prepared samples thermally heated at specific temperatures. The supernatant fluorescence spectra (Figure 2b) show FAM intensity increasing with temperature, indicative of increased amounts of X dehybridized. The sample exposed to RFMF (solid line) has intensity in between the 30°C and 50°C samples. The integrated peak intensity of the spectra (Figure 2c) as a function of incubation temperature (squares) can be fit to a sigmoidal (solid line), corresponding to a thermal denaturation curve. From the intensity of the RFMF sample (dashed line) the extrapolated temperature is 35°C, indicating the effective temperature that the effect if the RFMF of the nanocrystal produces on X is ~13°C above ambient. This change in temperature is sufficient for control of many biological processes.

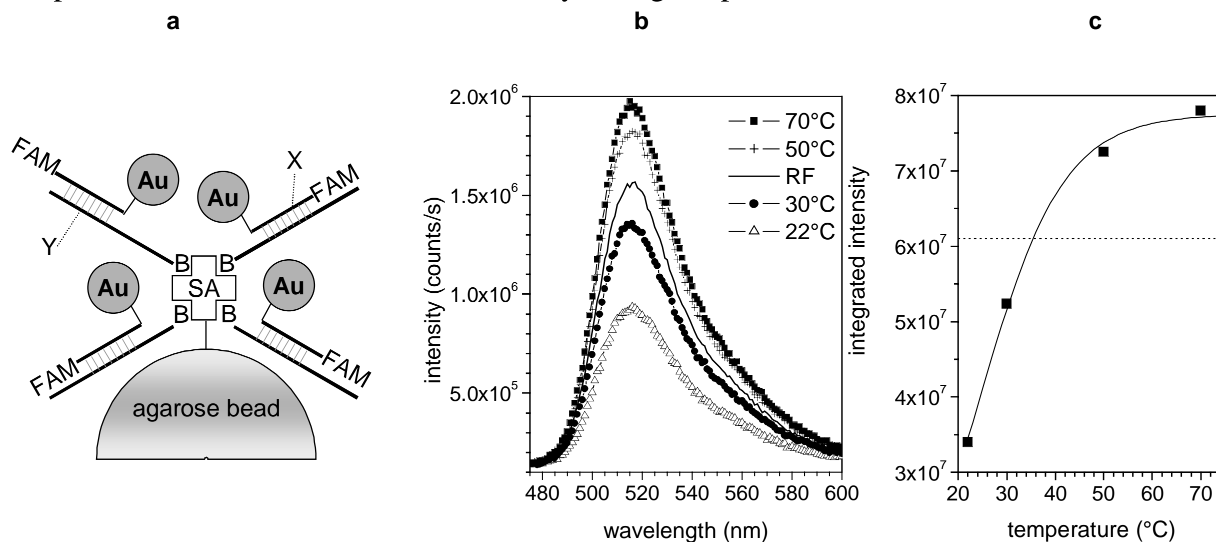

Figure 2. Determination of effective temperature from induction heating of DNA linked to a Au nanocrystal. a, The two-phase system used. If the X-Y complex is dehybridized, X is no longer bound to the solid support (agarose bead) and can diffuse into the supernatant. b, Fluorescence spectra of the supernatant of the system shown in the inset. Each sample is subject to heat of a fixed temperature (22°C, 30°C, 50°C, 70°C) or a RFMF (solid line). The fluorescence intensity is greater for samples subject to higher temperatures. c, Integrated peak intensity of the supernatant fluorescence spectra shown in Figure 2a (squares) and a sigmoidal fit (solid line), and the intensity of the sample exposed to RFMF (dotted line).

One important property of this technique is the ability to address molecules with an antenna selectively while having a lesser effect on molecules which are not bound to a nanocrystal antenna. In order to demonstrate selective dehybridization we used a two-phase system in which X was mixed with Z, an oligo which had no nanocrystal attached (Figure 3a). Z is identical in sequence to X but has tetramethylrhodamine (TMR, Molecular Probes, Eugene, OR) on the 3' end, which emits at a wavelength distinct from FAM (λ_{max} = 563nm). The two-phase system is comprised of tetrameric avidin acrylic beads with both X-Y and Z-Y hybrids on the surface in approximately equimolar amounts. One sample was exposed to the RFMF and the supernatant was compared to a thermally heated sample by fluorescence spectroscopy. The 70°C sample (Figure 3b, right panel) has peaks at both 515nm and 563nm, indicating dehybridization of both X and Z. The difference of the spectra before and after RFMF (Figure 3, left) shows increased intensity at 515 nm due to dehybridized X but a negligible change in intensity at 563nm. The percentage of X in the supernatant is ~80% for the RFMF sample, while for the 70°C sample it is ~55%. The slight amount of Z that was dehybridized in the RFMF sample is most likely a consequence of the proximity of the two types of molecules associated with the tetrameric avidin (where the intermolecular separation is expected to be >10 nm). This confirms that Z is relatively unaffected by induction heating of a nanocrystal in its proximity. Figure 3c shows the A_{260} with the RFMF on and off for a fixed concentration of M as N was added. A_{260} on and off values increase linearly, but the difference (ΔA_{260}) remains constant. Samples of increasing concentration of M show ΔA_{260} increasing with concentration (not shown). These experiments indicate that the induction heating of M is sufficiently localized such that surrounding molecules are not affected.

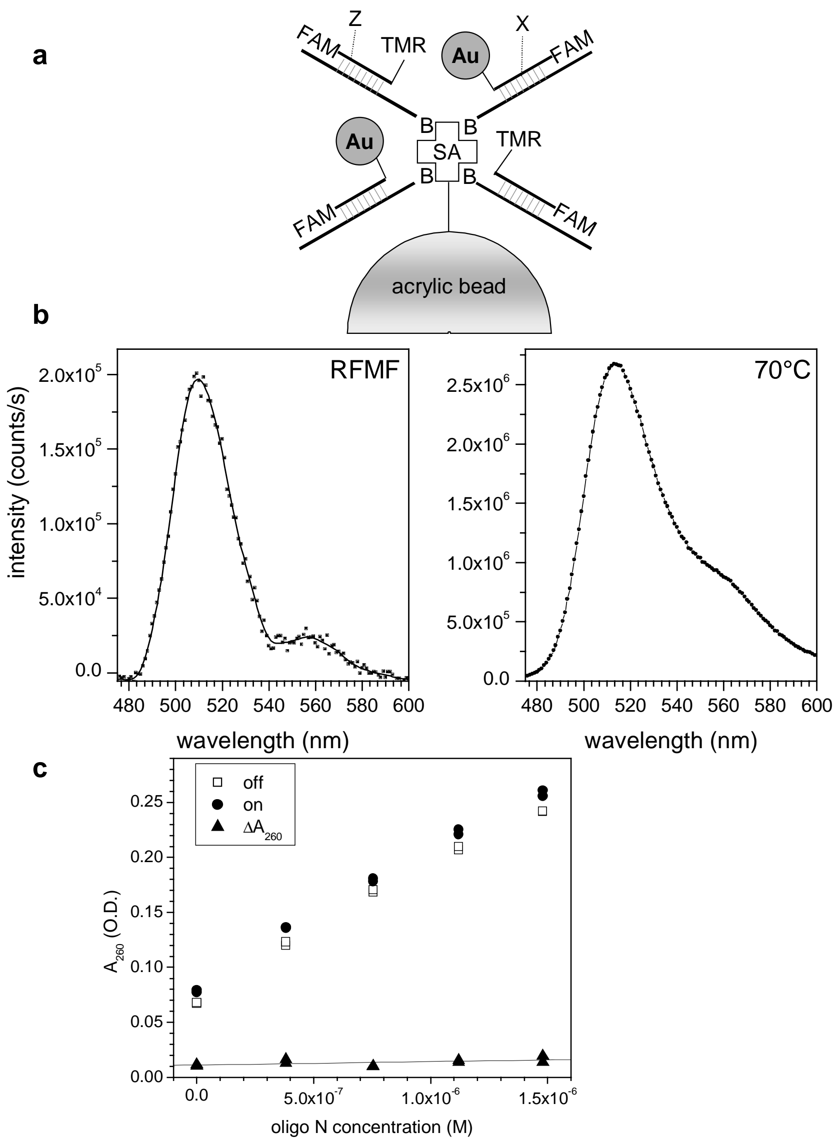

Figure 3. Systems to test selectivity. a, Two-phase system used to test selective dehybridization. b, Difference fluorescence spectra (after dehybridization by heat/RFMF minus before dehybridization) are scaled to the same intensity. (left) Sample that has been exposed to the RFMF. The spectrum shows a peak at 515nm due to FAM, which is present on both X and Z, and a negligible peak at 563nm, which is present only on Z. (right) Spectrum of sample that has been isothermally heated to 70°C.

CONCLUSION

We have demonstrated the use of induction heating of covalently bound nanocrystal antennas to reversibly dehybridize double stranded DNA on the time scale of seconds. Induction heating is sufficiently localized to permit selective dehybridization. This technique permits control of biomolecules in a switch-like manner in solution. Manipulation of DNA by itself is interesting as it has been shown recently that it has potential as an actuator[10] and can be used to perform computational operations [11-13]. Because nanocrystals can be covalently attached

to proteins as well as nucleic acids, this opens the possibility of switching more complex processes such as enzymatic activity [14], biomolecular assembly [15], and gene and protein expression. Furthermore, due to the spatial localization of denaturation that results from this technique, portions of proteins of nucleic acids can be controlled while the rest of the molecule and neighboring species would remain unaffected. Because the addressing is not optical, this technology could be useful in systems that exist in highly scattering medium such as living tissue.

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