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DNA Nanoparticle Formation By Novel Polyamine Analogues: Ionic, Structural And Temperature Effects

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Nonviral gene delivery vehicles are under development to facilitate gene therapy of different diseases. A prerequisite for gene therapy is the condensation of DNA into nanometric particles for its efficient uptake into the target cells. Natural polyamines that are abundant in the living cells aid in the packaging of cellular DNA and are also know to condense DNA into compact particles in vitro. In order to understand the structural effects of polyamines in DNA nanoparticle formation and develop novel polyamines for gene therapy, we synthesized a series of spermine analogues of the general formula, $NH_2(CH_2)_nNH(CH_2)_nNH(CH_2)_nNH(CH_2)_nNH_2$ and $NH_2(CH_2)_nNH(CH_2)_nNH(CH_2)_nNH(CH_2)_nNH_2$, n = 3 or 4 and studied their effectiveness in condensing λ phage DNA using static and dynamic light scattering methods. The EC_{50} (polyamine concentration at midpoint of DNA condensation) values for condensation was lowest for hexamines (0.25 μM) and highest for spermine (2.4 μM) in the presence of 10 mM Na^+. Dynamic light scattering measurements indicate that polyamines condense DNA into compact particles with hydrodynamic radii in the range of 40 to 70 nm. Effects of temperature on the hydrodynamic radii of these particles were also measured at different temperatures between 20 to 70°C. For both spermine and a pentamine, the hydrodynamic radius remained relatively stable until 50°C and increased significantly with temperature. In contrast, the hexavalent polyamine analogue, 3-4-3-4-3 exhibited a gradual increase in the hydrodynamic radii from 56 to 79 nm as the temperature increased from 20 to 70°C. The different effects of these polyamines on the hydrodynamic radius of condensed DNA suggest different modes of polyamine binding with DNA. Studies on temperature effects might help to develop optimal methods for storing DNA nanoparticles as pharmaceutical agents.

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Stretching DNA in Nanochannels

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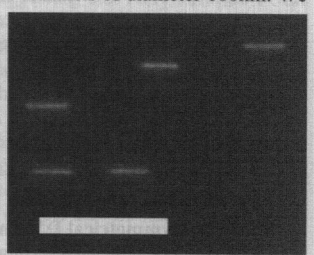
We have stretched DNA molecules by confining them in channels with diameters close to the persistence length (~65nm) of the DNA. Nanoimprinting lithography was used to make the nanofluidic channels of diameter 100nm. We applied an electric field over the device to introduce the fluorescently stained DNA into the nanofluidic channels and observed the DNA in an epi-fluorescence microscope. An image of a few stretched lambda phage DNA molecules is shown in the figure below. The scale bar is 25microns.



The technique gives us the possibility for direct visualization and linear analysis of DNA. We will present results showing the potential for measurements of size distributions of DNA samples significantly faster and with significantly less sample than necessary for standard gel-based techniques. We will discuss possible future applications and challenges lying ahead.

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Development of DNA Dumbbell as a Molecular Wire

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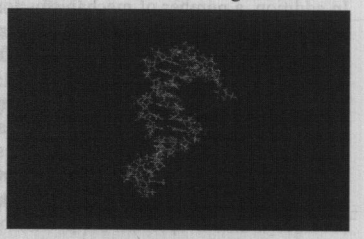
DNA dumbbells with the Drew-Dickerson stem sequence [d(CCAACGTTGG)] and four thymines (T_4) in single-stranded loops were developed as a novel molecular wire construct. Modified bases were introduced to interior loop positions to enable directed covalent attachment of gold and titanium dioxide nanoparticles. Carboxy-dT modifiers were synthesized into the hairpin at loop positions 2 and 3. Dithiol phosphoramidite (DTPA) modifiers were synthesized into the complementary hairpins also at loop positions 2 and 3. DNA hairpins with complementary 4 base-pair overlap were mixed in equimolar concentration, annealed and sealed with T_4 ligase. Circular dichroism spectra were collected from 20° to 55°C in 5°C increments from 205 to 320 nm with a JASCO J-810 spectrophotometer. The utility of these DNA dumbbells as molecular wires was developed through directed covalent attachment of metal nanoparticles with unique electronic signals. To this end, the DTPA modifiers were covalently attached to a gold nanoparticle. The carboxy-dT modifiers enabled the covalent attachment of a TiO_2 via a dopamine linker. DNA dumbbells provide a novel construct for development of molecular wires.



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Self Assembled DNA Monolayers as a Mechanism for Redox Probe Control

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The Watson-Crick complementary of DNA has proven to be a robust and versatile mechanism for engineering both static and dynamic structures on the nanometre scale. Chemical modification of DNA strands with thiol residues is an efficient method of forming DNA monolayers on gold surfaces. Together, these offer the potential for DNA modulated surface electrochemical processes. We have constructed several different double stranded DNA monolayers on gold surfaces, consisting of a thiolated DNA strand and its ferrocene-tagged complement. The degree of complementarity between these two strands has been altered to give the ferrocene tag a range of flexibility near the electrode surface. When held rigidly near the gold surface by the DNA construct, the ferrocene exhibits a electrochemical response consistent with a surface-confined system. When given a sufficient single stranded DNA tether, the ferrocene exhibits a diffusive electrochemical response. This demonstrates the use of DNA to modulate the electrochemical response of a redox probe near an electrode surface. We are now working on producing a similar system with a biologically significant electro-active molecule.

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Biosensors for aqueous toxicants: a novel DNA-based system compared with *C. dubia* and submitochondrial particle (SMP) assays

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Water pollution is emerging as a major global problem as the world's fresh water supplies become progressively contaminated. Mercury is a one of the major toxicants present in waste water but other heavy metal ions as well as herbicides and insecticides contribute to the problem. Current methods for detecting these toxicants are complex (usually laboratory based), slow and expensive. Here we report a relatively inexpensive, portable and quick method for detecting heavy metal ions and other toxicants using a fluorescent probe bound to DNA. Concentrations of toxicants at or above the maximum levels permitted by EPA authorities dissociate the DNA from its ligand which is observed as a loss of fluorescence from the sample. We compare this method with two established methods based on a submitochondrial particle assay and with a bioassy using a freshwater organism (*C. dubia*). Given the diversity of the three assays, they exhibit remarkably comparable results.

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