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Perturbed Angular Correlation Spectroscopy for structural and dynamic studies of artificial DNA nanostructures

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Abstract:

We propose structural and dynamical investigations of artificially designed biomolecular nanostructures especially made of DNA building blocks using Perturbed Angular Correlation (PAC) Spectroscopy. Hg(II) has a particular preference for linear coordination geometries, and has the capacity to bridge DNA base pairs. Thus, Hg(II) may be used as a structurally well defined unit, which can control the design of structrure and dynamics of such DNA nanostructures. PAC spectroscopy may provide information on the local structure and dynamics at the Hg(II) binding sites. These experiments will be complemented with other experimental techniques (NMR, UV-Vis, CD, fluorescence, AFM) which provide information on local structure as well as global structure of the biomolecules. We launch a new collaboration that intends performing biophysics experiments at ISOLDE.

Introduction and Motivation:

The extraordinary property of DNA getting smartly self-assembled and creating numerous geometrical patterns in 1-, 2-, and 3 dimensions, has made it a valuable tool to design more varied structures than any other material. Because of its diverse physical, chemical, and biological properties [Zha09, Tat00], DNA self-assembly and self-alignment techniques have presented unprecedented possibilities for bottom-up nanofabrication with a great number of potential applications. These properties have made DNA nanotechnology one of the core research fields in bionanotechnology. For last decade, novel DNA molecules have been designed aiming to construct molecular aggregates with well defined structures and

properties, and new fabrication methods have been deviced in order to overcome obstacles in the construction of functionalized nanostructures with nanometer scale precision to meet the challenges of its potential applications in the field of DNA nanotechnology.

The outcome of the design effort of the structures at nanometer scale depend primarily upon the intermolecular interactions with specificity and controlled geometry. DNA nanotechnology is exceptionally versatile with controlled, programmable and predictable nanostructure fabrication. It employs association of sticky ends of branched DNA motifs for the construction of desired structures [Yan30, Par06]. The branched DNA molecules permit the fabrication of self-assembled geometrical targets with desired topology. Various DNA structures have been assembled using these unique properties. Because of growing demands in various fields of applications, novel, innovative and well characterized DNA structures are required. This can only be achieved in an interdisciplinary approach.

Here we fabricate a new 1D DNA nanostructure; 1D DNA nanotrack, based on two DNA units called as T-motifs with diverse sequence. Each T-tile has one loop strand and six single strands, so total 14 DNA oligomers are assembled to get the 1D DNA nanotrack (Figure 1(a)). Due to the existence of the three crossover junctions in the T motif, the DNA duplexes can be tied up closely to stabilize the nanostructure against the intrinsic curvature stress by nucleotide stacking. This DNA nanotrack unit has a square hole inside the structure and its size is nearly 20 nm × 20 nm and the height is 1.2 nm. Atomic Force Microscopy has been applied in structural characterization of the nanotracks appearing as polymers of the nanotrack unit, and the average length is about 600 nm as shown in the Figure 1(a).

In Figure 1(b), we present a new DNA nanostructure fabrication method called as the Angle Control Scheme (ACS). The main concept of ACS is the following: A full turn of double helix of *B*-form DNA has an angle of 360° with 10.5 nucleotides. By the subtraction or addition of a few nucleotides from a sticky-end or a body of a specific DNA tile such as a Double Crossover (DX), one can control the helical angle change between two adjacent tiles at $34.3° \times N$ where N is number of removed or added nucleotides from the reference of the full turns. To demonstrate the applicability and versatility of ACS, we have fabricated a number of DNA nanostructures such as a 2D Plane Lattice, a 3D Zigzag Lattice, and a 3D Obtruded Lattice using DX building blocks. There has also possible to construct various dimensional DNA nanostructures made of C-motifs as shown in Figure 1(c).

Metal ions display diverse functions in biological systems. Similarly, metal ions may be used to control structure and function of synthetic biomolecules, and thus be a tool in the design of molecules with a desired function. In this project we address a variety of questions concerning both the function of metal ions in natural systems and in synthetic biomolecules.

Studies on metal ion interactions with DNA or RNA molecules have indentified preferred sites of interaction for different classes of metals, determined thermodynamics binding parameters, and elucidated the structures of some complexes [Blo99]. DNA structures were formed by mixing a stoichiometric quantity of each strand in standard physiological $1\times$TAE/Mg^{2+} buffer [Tris-Acetate-EDTA (20 mM Tris (pH 7.6), 2 mM EDTA) with 12.5 mM magnesium acetate]. For the one-pot annealing, equimolar mixtures of strands were cooled slowly from 95 °C to 25 °C (room temperature) by placing the test tube in 2 L of boiled water in a styrofoam box for at least 40 hours to facilitate hybridization. Copper ion modified DNA nano rings can be fabricated by incubation in three different copper ionic solutions, 5, 0.5,

and 0.05 mM. As we can see in Figure 2, structures are still kept its own ring-shape. Similarly, Ag(I) and Hg(II) ions have been shown to bind to DNA and RNA molecules [Joh10], [Mue08], [Joh08].

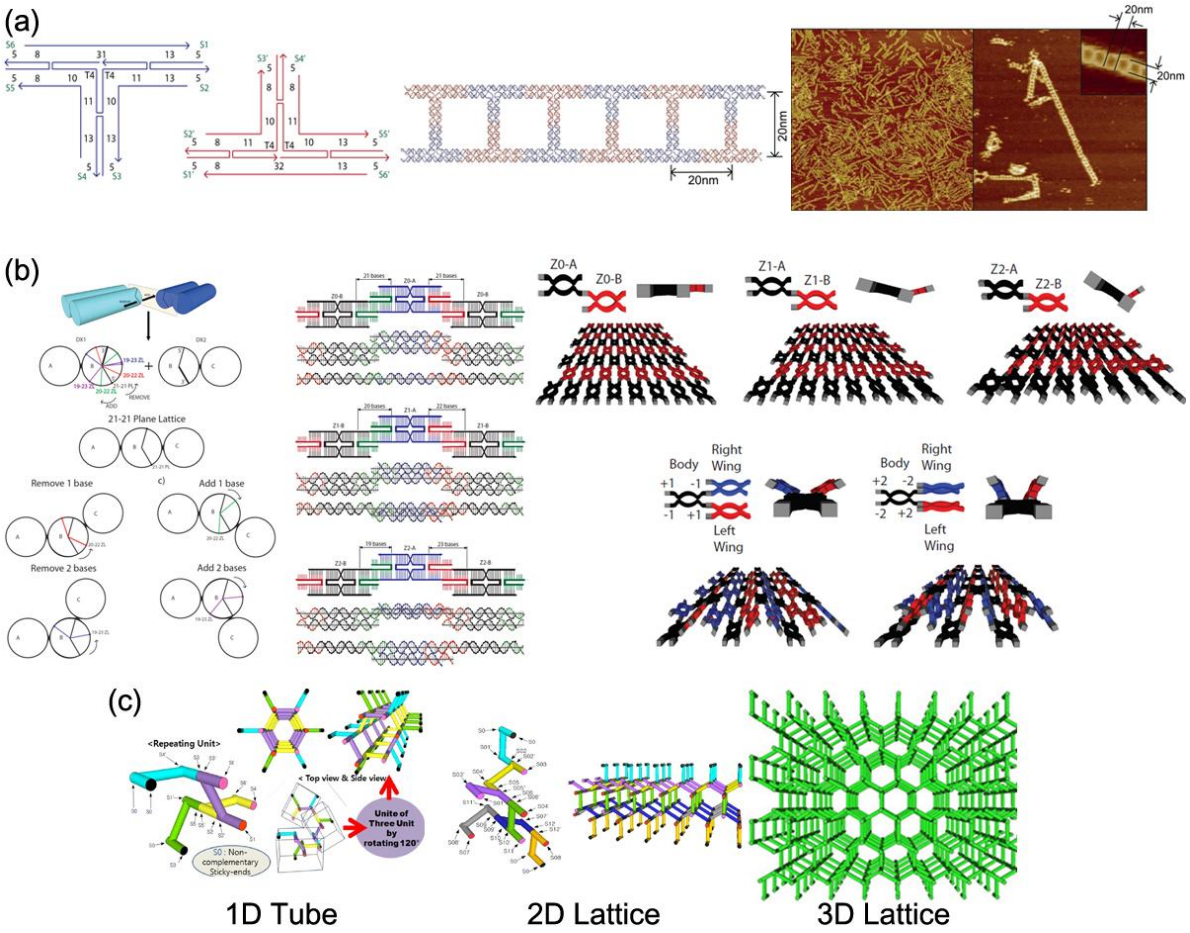

Figure 1. (a) The 1D DNA track nanostructure; schematics of 1D Nanotrack T-shape building blocks, cartoon of a 1D Nanotrack, AFM data of Nanotrack; image sizes are 3μm×3μm (left) and 1μm×1μm (right), inset:100nm×100nm. (b) (Left) A cartoon of the angle control scheme, (middle) Top view and front of two adjacent double crossover tiles, (right) the cartoons of 2D plane, 3D zigzag and obtrude lattices. (c) Various dimensional DNA nanostructures made of C-motifs.

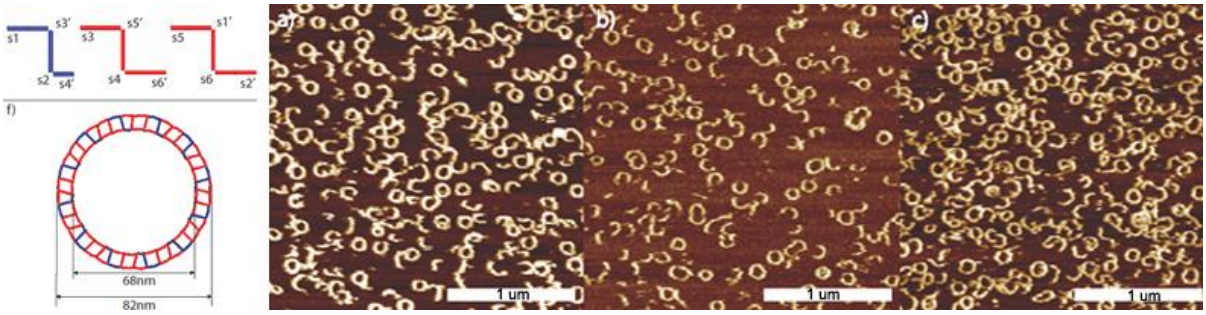

Figure 2. (Left) A schematic for three Z-shape unit building blocks and a DNA ring structure. DNA nano ring with 82 nm (outer) and 68 nm (inner diameter). AFM images of copper-modified DNA nano rings. DNA nano ring solution is incubated in three different copper ionic concentrations. (Right) AFM images of DNA nano rings incubated in three different copper ionic concentrations, 5, 0.5, 0.05 mM respectively.

The novel key element in design efforts in this project, is to add Hg(II) to the DNA molecules, and exploit the Hg(II) ions particular preferences for linear X-Hg-X structures to generate completely novel DNA nanostructures potentially with novel functionalities. Thus, the design will reflect a balance between the intermolecular interactions present in the classical DNA self-assembly and the coordination chemistry displayed by the Hg(II) ions. In order to test how this balance may be shifted, a variety of well characterized DNA oligomers will be investigated with both sub- and superstoichiometric concentrations of Hg(II) added, to elucidate the impact on structure and dynamics of the biomolecular nanostructures.
The project involves other experimental techniques such as NMR, UV-Vis, fluorescence, and CD spectroscopies and AFM, providing complementary data, as well as interpretation of the experimental data by quantum mechanical calculations of spectroscopic properties.

Experimental Considerations:

The experiments will follow the techniques developed by the groups involved in experiments IS448 and IS488 over the last number of years. This will involve the use of the “ice finger” which is attached to the end of the GLM beamline. Using this, ions of ^{199}Hg are collected into metal-free water. This will then be transported to building 115 where, upon defrosting, the experiments described above can take place.

It will be necessary to carry out these runs in parallel with the experiments of IS488 so that transfer of expertise can be obtained. As the half-life of ^{199}Hg is only 42 mins, it is quite possible to accommodate this into their running time.

The samples will be DNA structures which will be prepared in Korea. They exist either in a liquid buffer phase or in a dry phase. These are sufficiently robust to be transported via standard shipping prior to experiments.

Measurements are made with the 6-detector PAC machines which are located in building 115. Currently there are two of these machines, but more will arrive during early 2011 (at least 2) so there won’t be a bottleneck for the actual measuring.

Beam Request:

We request 6 shifts of 199Hg to be delivered over the next two years. This is available from a Pb target equipped with a VADIS ion source. Typical intensities are ~ 2e8 ions s^{-1}. If the test runs in 2011 produce promising results, perhaps the remaining 2012 shifts would be dropped in lieu of submission of a full proposal.

Isotope	$T_{1/2}$	Target	Yield (ions/μC)	Ion source	Shipping?
^{199}Hg	42 min	Pb	2×10^{8}	HP (VADIS)	No

References:

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Appendix

Description of the proposed experiment

The experimental setup comprises: *(name the fixed-ISOLDE installations, as well as flexible elements of the experiment)*

Part of the Choose an item.	Availability	Design and manufacturing
SSP-GLM	☒ Existing	☒ To be used without any modification
Chemical Facilities in building 115	☒ Existing	☒ To be used without any modification ☐ To be modified
	☐ New	☐ Standard equipment supplied by a manufacturer ☐ CERN/collaboration responsible for the design and/or manufacturing
PAC setups in building 115	☒ Existing	☒ To be used without any modification ☐ To be modified
	☐ New	☐ Standard equipment supplied by a manufacturer ☐ CERN/collaboration responsible for the design and/or manufacturing

Hazards generated by the experiment

(if using fixed installation) Hazards named in the document relevant for the fixed [COLLAPS, CRIS, ISOLTRAP, MINIBALL + only CD, MINIBALL + T-REX, NICOLE, SSP-GLM chamber, SSP-GHM chamber, or WITCH] installation.

Additional hazards:

Hazards			
	SSP-GLM	*Chemical facilities*	*PAC setups in building 115*
Thermodynamic and fluidic			
Pressure	[volume][l]	N/A	N/A
Vacuum	<10-6 bar	N/A	N/A
Temperature		n/A	-20 -> Room Temp
Heat transfer	N/A	N/A	N/A

Thermal properties of materials	N/A	N/A	N/A
Cryogenic fluid	LN2 for “ice finger”	N/A	N/A
Electrical and electromagnetic			
Electricity	[voltage] [V], [current][A]	230V single phase	230V single phase
Static electricity		N/A	N/A
Magnetic field	[magnetic field] [T]	N/A	N/A
Batteries	☐	N/A	N/A
Capacitors	☐	N/A	N/A
Ionizing radiation			
Target material	Sn (HP –Vadis ion source)		
Beam particle type (e, p, ions, etc)	199m Hg ions		
Beam intensity			
Beam energy	60kV (desired)		
Cooling liquids	[liquid]		
Gases	[gas]	Ar	
Calibration sources:	^{111m}Hg		
• Open source	☒		
• Sealed source	☐ [ISO standard]		
• Isotope	^{199m}Hg		
• Activity	~ 1MBq		Used for tuning detectors. Usually collected into foil.
Use of activated material:			
• Description	Removal from chamber, transported to building 115 in standard Pb Castle shielding.		Placed in PAC machines in building 115 for measurement.
• Dose rate on contact and in 10 cm distance	LA = 100MBq		
• Isotope	^{199m}Hg		
• Activity	Max. 2.5MBq (per sample)		
Non-ionizing radiation			
Laser	N/A	N/A	N/A
UV light	N/A	N/A	N/A
Microwaves (300MHz-30 GHz)	N/A	N/A	N/A
Radiofrequency (1-300MHz)	N/A	N/A	N/A
Chemical			
Toxic		Hg Salt (acutely toxic)	
Harmful		Hg Salt	
CMR (carcinogens, mutagens and substances toxic to reproduction)			
Corrosive			
Irritant	[chemical agent], [quantity]		
Flammable			
Oxidizing	[chemical agent], [quantity]		
Explosiveness	[chemical agent], [quantity]		
Asphyxiant	[chemical agent], [quantity]		
Dangerous for the environment	[chemical agent], [quantity]	Hg Salt	
Mechanical			
Physical impact or mechanical energy (moving parts)	N/A	N/A	N/A

Mechanical properties (Sharp, rough, slippery)	N/A	N/A	N/A
Vibration	N/A	N/A	N/A
Vehicles and Means of Transport	N/A	N/A	N/A
Noise			
Frequency	N/A	N/A	N/A
Intensity	N/A	N/A	N/A
Physical			
Confined spaces	N/A	N/A	N/A
High workplaces	N/A	N/A	N/A
Access to high workplaces	N/A	N/A	N/A
Obstructions in passageways	N/A	N/A	N/A
Manual handling	N/A	N/A	N/A
Poor ergonomics	N/A	N/A	N/A

0.1 Hazard identification

The main hazards associated with this are listed in the chemical section above, Hg Salt, which is added to solutions is acutely toxic and needs to be handled with care. This is always done in the fume cupboard in building 115 with a pair of scientists charged with preparing these solutions. They are properly dressed and protected (breathing masks, multiple pairs of gloves, taped sleeves etc), and as they are specialists in heavy metal toxicity they are used to dealing with the dangers of this material.

Other hazards are less grave. The principal other chemical used is non-toxic:
1xTAE/Mg^{2+} buffer [Tris-Acetate-EDTA (20 mM Tris (pH 7.6), 2 mM EDTA) with 12.5 mM magnesium acetate].

3.2 Average electrical power requirements (excluding fixed ISOLDE-installation mentioned above): *(make a rough estimate of the total power consumption of the additional equipment used in the experiment)*

The power requirements are nothing out of the ordinary