

## P5 – High Speed AFM Characterization of the Dynamic of Synthetic Proteo-Nucleic Complexes

Aude LAISNE<sup>1</sup>, Eric LESNIEWSKA<sup>2</sup> and Denis POMPON<sup>1</sup>

<sup>1</sup> Centre de Génétique Moléculaire of CNRS, 91190 Gif-sur-Yvette, France.

<sup>2</sup> Institut Carnot de Bourgogne, UMR CNRS 5209, 21000 Dijon, France.

Synthetic proteo-nucleic structures (PDNA) encompassing a ss-DNA sequence covalently attached to a cytochrome b5-derived protein domain through a synthetic linker were designed. PDNAs can bind to natural or supported membranes through an histidine-tag-nickel-NTA modified phospholipids. Alternatively the structures can be attached, while keeping free lateral diffusion capabilities, by interaction of the protein his-tag with a substrate supported field of nickel ions. Floating individual tiles can be in turn self-assembled together in the presence of half-complementary ss-DNA, to form surface constrained chains of protein domains linked by ds-stranded DNA segments.

A combination of experimental (single molecule confocal microscopy, surface plasmon resonance imagery, static atomic force microscopy) and numeric simulation approaches were used to characterize factors controlling self-assembly on the surface. Dynamic of interaction between PDNA assemblies was investigated using high speed AFM in solution and evidenced unusual modes of lateral diffusion of the structures on mica surface and transient formation of linear and cycling complexes involving base pairing between very short DNA stretches. Dynamic AFM thus constitutes a very promising tool for the characterization of the time resolved aspects of the self-assembly of biomimetic structures.

# High speed AFM characterization of the dynamic of synthetic proteo-nucleic complexes

Aude LAISNE<sup>1</sup>, Eric LESNIEWSKA<sup>2</sup> and Denis POMPON<sup>1</sup>

<sup>1</sup> Centre de Génétique Moléculaire de CNRS - F91190 Gif-sur-Yvette, France <sup>2</sup> Institut Carnot Bourgogne - University of Bourgogne - F21000 Dijon, France.

**Context:** Proteo-nucleic complexes are major components of biological systems. However mechanisms governing self-assembly of such building blocks to constitute large functional superstructures remain poorly characterized. Particularly very little information is available on the dynamic of the process and on the effect of micro-environment resulting from protein and surface interfaces on DNA-DNA self-recognition by base pairing.

- Objectives:**
- > To design fully defined artificial proteo-nucleic self-assemblies usable as model.
  - > To compare mechanisms of self-assembly by base-pairing in solution (3D) and on surface constrained (2D) environments featuring different geometries.
  - > To model the self-assembly processes by combining macroscopic data and statistical description of single molecule population.

**Schematic for PDNA building blocks**

**Structure of b5-based PDNA building block**

**Simple assembly of secondary building blocks through hybridization of PDNAs with complementary ss-DNAs**

**Self-assembly of PDNA tiles**

Two types of PDNA tiles and over-lapping ss-DNA were hybridized leading to proteo-nucleic polymer formation. Polymer distribution was analyzed by gel electrophoresis and pattern simulated for different experimental conditions

EXPERIMENTAL (left) and SIMULATED (right) gel electrophoresis patterns of proteo-nucleic polymers

Left, experimental size distribution of DNA (1 & 2) and PDNA (3) polymers. Effect of polymerization time is visualized (30s curve 1 and 15min curve 2)

Right, simulation of experimental distributions using rate constants determined by rapid kinetic analysis of hypochromic effects and automatically generated models.

Automated model generation, solver and realistic electrophoresis gel simulation

**Self-assembly on supported membrane**

Supported membrane was built by vesicle fusion on SAM covered gold layer (1). PDNA were attached by interaction of the protein domain HIS-tag with NTA-Ni-modified lipids (2). Self-assembly of PDNA was performed by hybridization with over-lapping ss-DNA (3). The reaction was monitored by surface plasmon resonance imagery (right panel).

For AFM imagery purpose, the gold supported membrane was replaced by a nickel ion field supported on cleaved mica. Protein HIS-tag domains interact directly with ion field while preserving lateral mobility of constructs.

**Real time AFM analysis of the dynamic of interactions between PDNA constructs diffusing on ion field**

Three examples of randomly picked interactions between single molecules of PDNA constructs.

Interactions by short base-pairing between the free ss-DNA extremities were visualized by formation of transient complex and cycling species.

Dynamic analysis of a population of transiently interacting PDNA constructs. Left, static image of the population. Right, superposition of two frames (red and green) at 100 ms interval showing immobile (white) and moving (red and green) segments.

High-speed AFM 0.1sec between 2 images

Observations were performed in tapping mode in liquid. Small cantilevers with a spring constant of 0.1-0.2 N/m, a resonant frequency of 0.8-1.2 MHz and a quality factor of ~2 in buffer solution were used ( $A_z$  was set to 4-5 nm with feedback  $-0.9 A_z$ )

**Single molecule characterization of PDNA nanostructure**

Each extremity of the assembly was decorated with a different quantum dot (red and green). Left, schematic. Right, a mixed population of individual (green and red) and assembled modules (yellow) was visualized using high resolution confocal microscopy to monitor single molecule light emission.

A single molecule emission spectra of red, green and yellow spots (left) and time dependence of the light emission (right) illustrating synchronized blinking

**Conclusions & Perspectives**

A method to build proteo-nucleic polymers associated to surface by methods preserving their lateral mobility was devised. High speed AFM and confocal microscopy allow to access single molecule behavior in population. They are complementary with macroscopic tools like surface plasmon resonance imagery for the characterization of mechanisms and dynamic of self-assemblies.