PDNA as building blocks for membrane-guided self-assemblies

D. Pompon1 and A. Laisné
Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, UPR2167, Avenue de la Terrasse, 91190 Gif-sur-Yvette, France

Abstract
Different semi-synthetic PDNAs (protein–DNA complexes), which encompass a protein core engineered from the cytochrome $b_5$ scaffold, an embedded tuneable redox cofactor, a synthetic linker and a large oligonucleotide, were designed, synthesized and purified to homogeneity. These building blocks can be reversibly attached to Ni-DOGS {1,2-dioleoyl-sn-glycero-3-[N(5-amino-1-carboxypentyl)iminodiacetic acid]succinyl}-doped supported membranes through a metal chelate bridge with the protein part and be polymerized in a fully controllable manner using a solid-phase synthesis strategy and a stepwise addition of suitable complementary oligonucleotides. The resulting structures could recreate a large range of regular distribution of patterned redox and absorbing centres separated by fully tuneable distances and geometry. Kinetic parameters for the self-assembly of building blocks were determined using SPRI (surface plasmon resonance imagery). Structures of resulting nano-objects were characterized using gel electrophoresis and single molecule approaches following decoration of assemblies with quantum dots.

Objectives
Construction of optically and electronically active nano-objects featuring highly tuneable sizes and shapes in the 10–500 nm range is a challenge in the development of the use of biological structures as building blocks for functional nano-structured devices. An additional constraint for practical use is the requirement that nano-objects can be easily incorporated into inorganic nano-structured material such as silicon-based devices, supporting electrical or optical signal transduction. The present study describes the construction of PDNA (protein–DNA complex)-based nanostructures possessing such properties. Objects were designed to be associated to metal and dielectric containing support surfaces constituting an anisotropic environment controlling positioning and orientation of the self-assembled biological structures.

Building blocks structure and construction
PDNAs are molecular building blocks that encompass a stable globular protein core embedding a tuneable redox cofactor derived from haemin, a 39 bp oligonucleotide that holds the specific recognition properties and a membrane-association domain derived from modified phospholipid. The different parts were linked together, in a unique way, using synthetic spacers as described in Figure 1. Constructed PDNAs are fully defined objects at the molecular level. Their three-dimensional structures were computed using the experimental NMR-based three-dimensional structure for the cytochrome $b_5$ protein part and Molecular Dynamics for model refinement. Structure of the DNA part following hybridization was computed assuming the B-form of the double-stranded DNA. Shortly, the hydrophobic membrane anchor of native human cytochrome $b_5$ was removed by genetic engineering to obtain a soluble form. A tag with the -NGHHHHH-CO$_2$H sequence was added to the truncated C-terminus of the recombinant protein and a unique cysteine residue was introduced by directed mutagenesis on a surface loop [1]. The thiol-extremity was coupled through a synthetic bifunctional linker with different types of oligonucleotides bearing an amino-group-terminal extension at their 3’-end [2]. Resulting PDNAs lacking the synthetic lipid extension were synthesized and purified to homogeneity at milligram levels [3] and their homogeneity carefully checked by gel DNA and protein electrophoresis and by HPLC coupled with MS. Natural haemin cofactor can be easily replaced by analogues with modified porphyrin rings or metal to modulate redox and optical properties. The lipid part was finally associated to the construct through a nickel-chelate involving the His$_4$ part of the protein and the amino-triacetate part of the modified phospholipid. In such a PDNA, the lipid–protein link can be cleaved by nickel chelation with histidine or EDTA and the nucleic acid–protein link by reduction with DTT (dithiothreitol).

Self-assembly of building blocks into nano-objects
PDNAs building blocks can be assembled into large nano-objects using overlapping complementary oligonucleotides (Figure 2). Three self-assembly strategies were developed. The first was used to construct extended building blocks composed of two complementary oligonucleotides overlapping the 5’- and 3’-end of the PDNA respectively. This gives rise...
to molecular blocks encompassing a PDNA and two single-stranded DNA extensions that can be used as recognition elements to build extended structures. These simple objects were built by stoichiometric hybridization of the three complementary components in solution followed by purification of the formed trimer. Quality of constructs was checked by gel electrophoresis. For construction of larger objects, simple mixing of solutions of the different building blocks generally led to poor results because of the multiplicity of possible combinations and the sequence ambiguity when several competing hybridizations can occur. A more efficient approach was a solid-phase synthesis strategy consisting of linking a biotinylated priming sequence to a solid support, which can be a gold surface covered with a polymer such as dextran or micro-beads modified by covalently bound streptavidin. This primer is used to initiate a multistep sequential elongation of the construct using a suitable washing step between each addition of a new PDNA or oligonucleotide block. Use of a magnetic-bead support is convenient but does not allow monitoring of individual hybridization steps. Variable hybridization rates of building blocks very rapidly led in such a case to uncontrolled elongation generating a high proportion of wrong structures. In contrast, running the construction on a suitable porous polymer attached to a gold surface allowed real-time monitoring of reactions by SPR (surface plasmon resonance imagery). Use of a cleavable linker between the primer and the matrix allowed recovery of full assemblies. The third approach consisted of directly building, step by step, the objects on to a supported membrane, with or without a primer sequence attached to specific surface nano-structures. In such a case, PDNAs are bound to the membrane through their Ni-DOGS (1,2-dioleoyl-sn-glycero-3-[N(5-amino-l-carboxypentyl)iminodiacetic acid]succinyl) lipid extension. This last approach is more direct but elongation efficiency and thus quality of final constructs are highly dependent on the microrugosity and homogeneity of the support. Different building modes can be also combined; for example, building blocks lacking the lipid part can be pre-assembled in
solution into nano-objects before binding and elongation in the membrane-bound mode.

**Characterization of nano-objects**

Developed nano-objects can be characterized at different levels. Use of SPRI monitoring during the object construction is a particularly useful tool, permitting both quantification of building-block addition and kinetic analysis of the different steps. The size of final constructs can be evaluated following electrophoresis on agarose gel as for regular DNA. To allow accurate evaluation of sizes, the link between the DNA and the protein/lipid part has to be cleaved prior to analysis with a reducing agent such as DTT or mercaptoethanol. Large objects including five or more building blocks were found to be relatively fragile and could be stabilized before electrophoresis by enzymatic ligation. Additional characterization of assemblies can be performed at a single-molecule level by decoration of the nano-object extremities with complementary oligonucleotides linked to QDs (quantum dots). QDs with different colours can be used at extremities. Reading was easily performed using epifluorescence microscopy with a colour video camera or better by visualization of an individual object using CM (confocal microscopy). Single-molecule emission spectra can be recorded and used for identification and the size of large construct evaluated when exceeding 150 nm. The structure of individual objects can be also monitored by AFM (atomic force microscopy) following binding on Ni-NTA (Ni$^{2+}$-nitriolriacetate)-modified thermal silica held on silicon.

**Concluding remarks**

PDNAs are very versatile molecular building blocks with tuneable electron transfer, absorption and fluorescence properties. Redox PDNAs can be assembled in a fully controllable manner in nanometric objects of variable geometries with fully tuneable sizes ranging from ten to several hundreds of nanometres. The unique approach of sequential assembly on a supported membrane allows controlled building of large objects without substrate defect interference. Both global (gel electrophoresis, SPRI) and single-molecule (CM and AFM) real-time characterizations of assemblies have been successfully developed.

We thank Dr Gilles Truan (Centre National de la Recherche Scientifique, UPR2167) for the help in molecular modelling and communication of human cytochrome *b$_5$* co-ordinates. The present study is part of the NANOBIOFORME project supported by the French ‘Agence National de la Recherche’ PNANO (Programme National en Nanosciences et Nanotechnologies).

**References**


Received 2 January 2007