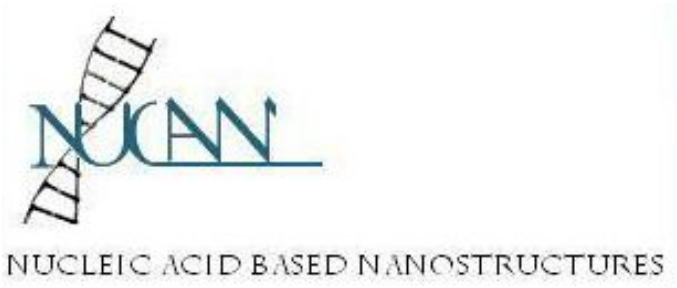


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**NUCAN**

**Nucleic Acid Based Nanostructures**

Instrument: Specific targeted research project STRP

Thematic priority: 3 - NMP

# Publishable Final Activity Report

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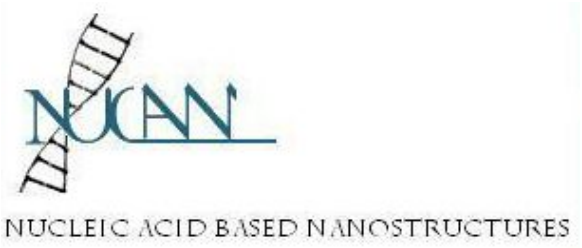


# Nucan: Nucleic Acid Based Nanostructures

**Contractors**

1. Fraunhofer Institute for Biomedical Engineering (FhG)
2. University of Copenhagen (UKBH)
3. University of Dortmund (UniDo)
4. CEA Saclay (CEA)
5. Institute for Physical High Technology (IPHT)
6. University of Newcastle (UNew)
7. University of Bologna (UniBo)
8. Karolinska Institute Novum (CRC-KI)
9. Alphacontec Berlin (ALPH)
10. Nanotec Electronica Madrid (Nanotec)

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## 1.Project execution

Objectives

The central objective of the NUCAN project has been the development of the technology for the handling and construction of nanometre sized basic building blocks and of **n**ucleic **a**cid **b**ased **nano**structures (*Nabnanos*) built from these as a basis technology for the production of nanostructures for various applications (Fig. 1).

Getting smaller is a goal in technological areas as different as electronics and pharmaceutics, in order to increase computing power or to advance drug screening systems, respectively. Therefore nanotechnology is recognised as an upcoming technology in a multitude of fields.

The bio-molecular approach is of special interest because of the biotechnological promise of cheap production facilities using bioreactors and because of the highly evolved capabilities of bio-molecules. Moreover, optimisation strategies are inherent to nucleic acids and may be transferred to artificial systems, as has been demonstrated for aptamers and ribozymes in recent years.

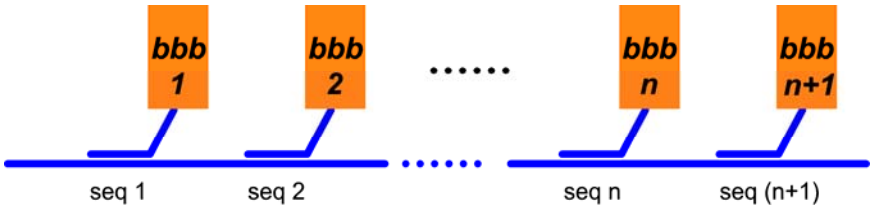


Fig. 1. Basic structure of *Nabnanos* (**n**ucleic **a**cid **b**ased **nano**structures). Basic building blocks ("*bbb*") of, possibly, different type 1, 2, ..., n couple to a nucleic acid strand at specific sites (sequences seq 1...seq n) according to their base sequence.

This overall aim has been targeted by the following steps:

1. Construct basic building blocks from DNA, PNA (peptide nucleic acid), proteins, nanotubes and metallic nanoparticles.
2. Find rules how to design and construct basic building blocks.
3. Form nucleic acid based nanostructures (*Nabnanos*) by directed self-assembly of the basic building blocks.
4. Guide the development by well defined applications.
5. Show the usefulness of *Nabnanos* in the context of three different areas of research and development, namely in:

   Nano- and bio-electronics (sub-micro- and molecular electronics )

Bioanalytics (e.g. surface bound ligands for single molecules and single cells)
Pharmascreening (drug target screening)

A resulting nanometre scale "toolbox" has been envisaged consisting of basic building blocks and of protocols for their assembly. Its applicability was to be demonstrated in such diverse fields like electronics, bioanalytics and drug screening.

Work performed

Throughout the funding period all ten contractors have been involved. Seven meetings were organised at Potsdam/Berlin, Saclay/Paris, Madrid, Stockholm and Jena. Two international symposia were arranged by IPHT. The second one "DNA-Based Nanodevices 2008" served to present the project's final results to the public and found wide coverage by the local press.
At the first meeting the work on the design of DNA and PNA sequences was coordinated and then carried out in dedicated workgroups for each envisaged task. A first set of basic building blocks was defined and optimised both *in silico* and *in vitro* applying specially developed sequence design software and microarray technology. A carrier DNA strand of 103 nucleotides and 4 DNA oligomers of 22 bases length complementary to distinct sites of the carrier strand were designed and synthesized. Sequences were optimised for high and homogeneous hybridisation efficiency, minimal tendency to form single-stranded secondary structures and minimised cross-hybridisation. This was done with respect to DNA-protein conjugates as well as to PNA clamps. These oligomers served for the preparation of chemically active DNA-protein conjugates with, e.g., streptavidin and Cyp119. The latter is a P-450 type redox enzyme from the archeon *Sulfolobus acidocaldarius*, which was cloned and overexpressed. After conjugation to DNA it still showed activity even after heating to 90°C. This demonstrates that self-assembled DNA-protein nanostructures with enzymatic activity can be employed also at elevated temperatures. For high-throughput *in vivo* studies the design was varied to shorter oligomers in order to reduce costs. The 103mer also carries a 15mer binding site for PNA clamps. The 88mer DNA (without the PNA binding site) was circularised and coupled to 1, 2, 3, and 4 of the above mentioned 22mer oligonucleotides, respectively, each carrying a streptavidin molecule allowing universal combination with any biotinylated structures.

For nanoelectronics nucleic acid based nanostructures have been assembled using metallic nanoparticles and single walled carbon nanotubes (SWNT). Gold and silver nanobeads of various sizes were coupled to DNA oligomers by thiol-gold and by avidin-biotin chemistry. Subsequent hybridisation of complementary oligomers resulted in the self-assembly of metallic Ag-Ag, Au-Au and Ag-Au nanostructures. Also gold-silver core-shell nanoparticles with tailored optical properties were assembled by DNA-DNA interactions. Their optical and self-assembly characteristics were investigated.

Coupling of DNA to carbon nanotubes was achieved both by covalent chemistry and by biotin-streptavidin coupling. By the action of strong acids nanotubes were purified and carboxylic defects were introduced to which DNA was covalently coupled. In order to favour binding to the ends of the nanotubes, their side walls were protected by surfactants. Linear nanostructures of two nanotubes being connected at their ends by DNA hybridisation were constructed. Also branched 3-pad junctions were prepared and investigated that connect three nanotubes at a defined distance, a prerequisite for the production of self-assembled transistors.

In order to produce larger, regular constructs from these *Nabnanos*, and to bridge the gap between the nanoscale and the macroscopical world, longer single stranded DNA was produced by rolling circle amplification. Serving as an immobilised support for *Nabnanos* it can be more easily addressed by readily available DNA oligomers as compared to double-stranded DNA. By enzymatical synthesis ssDNA molecules of 20 µm length and more were produced, each consisting of several hundred repeats of the mentioned 103 base sequence. These strands are surface bound at one end and are aligned *in situ* in parallel to allow extensive regular structures made of *Nabnanos*. Synthesis and alignment can be monitored in real-time by fluorescence microscopy in a dedicated fluid chamber. Chemical surface modifications and protocols were optimised with respect to adhesion of both educts and product, to synthesis efficiency and to fluorescence background. Similarly, protocols for chemical surface modifications have been developed in order to contact *Nabnanos* to surfaces like electrodes, silicon and glass.

To confer a higher mechanical stiffness to DNA scaffolds complex branched structures were designed. They consist of DNA tiles being composed of and mutually connected by a set of different oligonucleotides which form parallelograms. These parallelograms were made to self-assemble to rail-like straight very rigid rods of more than 200 nm length. By variation of experimental conditions molecular kinks were introduced that allowed the synthesis of quasi-circular *Nabnanos*. Molecular models and simulations were developed to understand the mechanisms of supramolecular self-assembly.

Single and double stranded DNA was oriented, aligned and immobilised by fluid flow and by dielectrophoresis using alternating electric fields. Metallic nanoparticles were assembled dielectrophoretically into longer chains and to produce nanogaps.

For the construction of a DNA nanogrid that is stabilised by PNA clamps more than 20 different PNAs were synthesised and PNA-DNA triplex formation was studied systematically. A DNA cloverleaf nanostructure was prepared from a circular DNA and four biotinylated PNA clamps that folds into the target structure upon binding of avidin. Based on the SNAP-tag concept a binding reaction of PNA to hAGT was designed that allows specific coupling of a given PNA to a protein by common protein engineering. This allows the building of two- and three-dimensional protein structures via conjugation to dsDNA.

For the preparation of nearly all of the aforementioned structures numerous biochemical protocols were developed and optimised in the project's course. For this purpose several physical, chemical and biological characterisation methods were applied, that often had to be specially adapted. These were, e.g., microarray technology as well as fluorescence, electron and scanning force microscopy. A novel method was established to measure local DNA concentrations electronically without labelling. Atomic force microscopy (AFM) found widespread use, since it offers the necessary resolution without the need for any chemical modification of the *Nabnanos* for contrast enhancement or staining. For the measurement of intramolecular and intermolecular forces a dedicated AFM instrument was developed, i.e. both hardware and software. It is capable to maintain a constant interaction force between the AFM tip and a molecule that bridges the substrate surface and the probe in the so-called force clamp mode. Both for the characterisation of *Nabnanos* and for writing nanosized structures functionalisation of AFM tips was advanced. By AFM-based molecular ink lithography oligonucleotides were directly deposited onto glass surfaces in 200 nm sized spots that can be arbitrarily positioned and serve as anchoring points for Nabnanos. A commercial software package for instrument control, data acquisition and data presentation of AFM experiments was enhanced. It now allows force spectroscopical imaging to obtain adhesion and stiffness maps. The developed graphical user interface makes it possible to easily acquire force-versus-distance curves for each point of a complete AFM image scan. Several tools have been implemented to cope with the resulting huge amount of data. A survey on immobilisation techniques compatible with AFM force measurements has been performed.

As a basis for nanoelectronic circuit connections electrically conductive nanowires were produces by several methods. Polypyrrole nanowires of about 1 nm diametre and 10 µm length were synthesised with DNA as a template and contacted to electrodes. Measurements

on single wires revealed a conductivity value close to that of bulk polypyrrole powder. Also polypyrrole three-wire branches could be produced. Thicker ropes of up to 20 nm thickness were prepared by self-association of these nanowires. Metallic nanowires in the same size range were prepared by decoration of DNA by gold clusters and were electrically characterised. From DNA that had been preloaded with silver ions wires of around 1.7 nm diametre were produced by UV light exposure. A further metallisation protocol was developed employing precipitation of palladium oxide onto DNA followed by a reduction step. This led to homogeneous, continuous nanowires of 20 to 25 nm thickness that can withstand currents of more than 10 µA. Their conductivity amounts to about a tenth of bulk palladium. This makes palladium nanowires three orders of magnitude more conductive than polypyrrol nanowires.

These palladium nanowires were employed to contact the above mentioned nanostructures consisting of carbon nanotubes and DNA. Both metallic and semiconducting nanotubes were studied at room temperature in air. The current-voltage characteristic showed the typical behaviour of a carbon nanotube field effect transistor (CNTFET) with only minor variations in performance between devices. These properties are on equal footing with the best performances of CNTFETs of comparable geometry fabricated by standard top-down lithographical methods, without DNA scaffolds, reported in the literature. For a similar nanodevice with selectively metallised DNA-nanotube hybrids single electron transistor (SET) properties could be demonstrated at 10 K. This is the first time that SET performance was reported for a DNA-nanotube based device.

Further nanowires were produced by DNA templated growth of CdS with about 10 µm length and diameters between 8 nm and 15 nm. Electrical transport measurements on single nanowires revealed the formation of continuous wires with non-linear current-voltage characteristics. Quantum confinement was demonstrated by photoluminescence.

For the application to high throughput screening of medical samples two of the streptavidin conjugated 22mer oligonucleotides were coupled to a set of 22 ligands in all possible pairwise combinations. Each of these 484 pairs was coupled to a fluorescently labelled 44mer oligonucleotide. These nanostructures were tested on 6 different human, adherent cell lines in triplicate, which resulted, together with controls, in more than 10 000 transfections to be performed and measured. *Nabnanos* were found to be stable in the culture media and to be able to pass the cell membrane. The method's usefulness to identify functional, combinatorial ligands has been demonstrated and 8 ligand pairs showing combinatorial effects could be identified. It was found that the ligands' relative position on the scaffold DNA is of importance and that their effect is cargo dependent. This application of *Nabnanos* has the potential

to have a significant impact on drug delivery. The prospect of systemic delivery combined with highly specific targeting of cell-types will enable re-formulation of a plethora of existing drugs that today have serious side effects due to non-specific uptake.

## 2. Dissemination and use: Publishable results

Publishable results have been disseminated by peer reviewed publications in international journals and by lectures given on conferences before an international audience:

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