Publishable Executive Summary

The goal of our program is a nano-technology platform based on a 100 nm size grid of addressable molecular building blocks ("nodes") to constitute a bottom-up modular approach to place functional groups at defined positions in space with sub-nm precision. An almost complete freedom of choice, for grid assembly as well as for positioning of functional groups, is based on a "digital" (in contrast to analog) code for molecular recognition. This is achieved using DNA base-pair recognition. After the two first years of the project we have among other things developed novel three-way DNA monomers that subsequently efficiently can be incorporated into trigonal DNA oligonucleotides using standard solid-phase synthesis. Each of these nodes has three oligonucleotide arms so that a group of six nodes are connected into a hexagon. Also, these trigonal nodes have been functionalised with fluorescent moieties and/or lipid anchoring groups for future functionalisation and attachment/anchoring of the DNA nano-network (see Figure below).

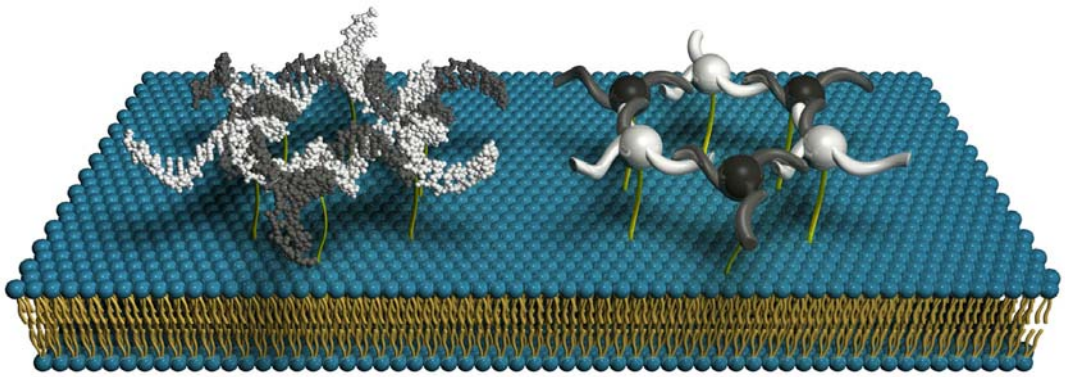

Figure. To the left molecular model and to the right schematic picture of start hexagon consisting of six nodes, each carrying three deoxyribonucleotide arms with mutually complementary sequences of ten bases. Lipid chains, covalently tethered to oligonucleotide, serve the role of anchoring the hexagon to a lipid matrix. The grid will be built up, radially from the start hexagon, by a net of hexagons and nodes, each being uniquely addressable by its base-sequence code.

Self-assembly is frequently applied in nano-science, but then generally refers to the crystal-like structures that result from weak intermolecular bonds between identical molecules, or by co-ordination to heavy-metal surfaces. Those kinds of structures are often periodic but, because of lack of control of individual structure at a molecular level, do not provide possibilities of advanced nano-engineering, bridging the microscopic (atom level) and the macroscopic (lithography level) that is a main objective of our program.

The partners represent expertise in synthetic and supramolecular chemistry and in biophysical, colloidal and photophysical chemistry, needed for the joint venture: 1. Synthetic strategy for node building blocks. 2. Tuning interactions and reaction rates to ascertain correct assembly into grid attached to lipid support. 3. Positioning of functional groups. 4. Analytical tools to follow assembly and verify structures. Later various applied

projects are anticipated to take over, attaching interesting functional groups at pre-determined positions on the grid map, and supramolecular structures extending perpendicular to the grid surface, into the 3rd dimension. The program is thus a novel bottom-up approach to achieve self-organizing nanostructures with high resolution and control of properties and performance.

One main objective is development of a technology for fast, simplistic and general synthesis of large (at least 100 nm size) regular grid structures composed of nodes, each of which is uniquely addressable and is on nm size. The assembly of the grid should be fast and simple, based on hydrogen-bonding and stacking of heterocyclic aromatic molecules (DNA bases) in aqueous solvent. This concept should later be easy to scale up and apply for industrial applications. The precision of positioning of a functional group could be critical for many applications (function of enzyme, sensor etc) and an objective is to reach sub-nm precision (the size of each hexagon, with typically ten nucleo-basepairs at the edge, is 8 nm and each base step 0.3 nm). High structural fidelity and convenient assembly rates are achieved using DNA base-pair recognition and stacking into rigid double-helical structures.

The goals successfully achieved in the second year of the project may be briefly summarized as follows:

- Several branched phosphoramidite monomers have been developed, and functionalized oligonucleotides, containing them as node, been synthesized.

- Systematic studies of oligonucleotide hybridization have provided a platform for how supramolecular double-stranded nets of oligonucleotides may be constructed. This has been studied mainly with FRET, fluorescence and UV melting experiments and gel electrophoresis.

- Studies of oligonucleotide-liposome interactions, with dynamic light scattering, polarized light spectroscopy etc give evidence for complex interactions and aggregation.

- Spreading/mixing behaviour of lipid-anchored DNA oligomers in lipid layer has been possible to follow with fluorescence microscope techniques.

- Studies of lipid-modified DNA oligomers-lipid membrane interactions. Proof of insertion of lipid-modified DNA oligomers into membranes of different lipid compositions.

- Development of chemical ligation methods to produce covalently linked ring structures from the non-covalently linked hexagons, allowing structure verification by MS techniques, AFM etc has been developed. These new cross-linked hexagons may in the future serve as the nano-construct building-blocks rather than adding each oligonucleotide every time a network is constructed (*i.e.*

pre-assembly of different hexagons that are then cross-linked, stored and subsequently used when needed).

- A hexagon made up of linear as well as three-way oligonucleotides has been attached to a lipid bilayer. Still more proof is needed for publication of these results.

- Lipid ODNs have non-covalently been attached to a hydrophobic surface at very high immobilisation densities.

The synthetic protocols developed in Southampton allow high-scale production of tripod nodes, and also inclusion of functional moieties such as fluorescent artificial cytosine analogues, tC and tCO, developed and studied in Gothenburg. As a first model system, the hybridization of "normal" unfunctionalised oligonucleotides, with inserted mismatch sites to provide flexibility at hexagon corners, has been studied in Gothenburg and the spectroscopic and hydrodynamic characterization tools refined to this end. Although preliminary AFM results, as well as electrophoresis and FRET results, support that hexagons (in contrast to linear or branched structures) can be made, both AFM and various forms of EM show that imaging the hybridization, as well as control of hybridization itself, are more demanding and ambiguous tasks than was originally anticipated. Additional approaches including covalent hexagon closure and new contrast methods are therefore desirable to develop and test. Parallel studies of lipid-nucleic acid interactions and also other supramolecular approaches have as planned been performed and developed in Florence and Strasbourg, and manipulation technology for large lipid vesicles and for controlled lipid mixing on microfabricated surfaces have been set up in Gothenburg.

The goals of addressability and uniqueness of the positions in the grid can be considered at various levels. With a grid of size 14,000 square nm, which is a reasonable goal, one has in principle around 10,000 addressable base positions, which corresponds to nm precision. With each arm of a node having 10 nucleobases, defining 4^{10} = 1,048,576 code possibilities it should thus in principle be possible to make the grid unique thermodynamically. That would require the synthesis of a stock having several hundred different node species. Therefore, we also try to exploit kinetic principles so that after building a net surrounding a core hexagon, one could “re-use” codes at the perimeter in order to reduce the number of nodes required in stock (an ambiguity of coinciding codes will have to be handled, for example, by higher order “tagging”). Also, we have started the development of different techniques for covalently locking substructures of the DNA-nanonetwork. We think that this will become more and more important and maybe even necessary as the number of hexagons of the nanostructure keeps increasing.

One goal of the project is that each grid will have a unique internal structure, to be used as a Lego system for nano-science Aufbau applications, such as for building microscopic molecular motors or a set of reaction sites for sequential catalysis of a biochemical reaction (artificial enzyme). Another objective is to develop a strategy for parallel mass-production of identical grids. This objective is achieved by the use of lipid vesicles, each

being an islet covered in an identical way by a single grid. Experiments with lipids carrying a positive net charge in mixture with normal zwitter-ionic lipids have been performed, to give lipid vesicles with variable surface charge density. In this way we have found it possible to immobilize DNA without using any anchoring lipid chains. A longer-term objective is to combine different vesicles, carrying different grids, into supra-vesicle structures, whereby recognition between vesicles as well as total function of structure are provided by grid-grid interactions. An even longer-term objective is the transformation of these meso-structures by solidification into robust, permanent structures.

Looking ahead, the strategic impact of a positive outcome of the project can hardly be exaggerated as it may lead to a completely new way of thinking, with emerging technologies that could become bases for a new nanotechnological industry. Several inventions that can be expected to result in connection to the development of our grid technology, such as node production technology, grid build-up technology and grid addressing technology can then be anticipated to lead to new companies in a near future. Furthermore, in the wake of this novel nanotechnology, we may envisage a number of additional powerful inventions with interesting industrial applications, such as biosensor technologies, including single-molecule DNA sequencing devices and electro- and photo-chemical detectors, surface-catalytic technologies, including cascade organic-chemical reactors for high-yield production of pharmaceutical compounds, as well as construction of artificial enzymes and of micro-robotic devices and so on. With success, this phase will add by the formation of further a considerable number of prolific companies.

The new way of thinking can also lead to an industry that differs significantly from the classical industry, as we know it today: a molecular-mechanical industry. The products, whether they be chemical micro-reactors, chip biosensors or electromagnetic micro-machines, may be micro-fabricated in a mode characterized by high efficiency and without any environmental pollution. Hence, the AMNA technologies may represent a real breakthrough that will promote transformation of the European industry to a high-added value organization with many benefits from occupational point of view.

(AMNA webpage: http://www.phc.chalmers.se/english/res_AMNA.html)