MINUTES

4th META Meeting

held at Centre NAST, University of Rome Tor Vergata (I)
9th December 2012

META Project Workpackage I
DNA motherboards

After the seminar talk given by Prof. Tibor Hianik at the Centre NAST of the Tor Vergata University the European participants to workpackage META team gathers for the 4th META meeting.
Present at the meeting were:

Prof. Silvia Licoccia,	META coordinator
Prof. Tibor Hianik,	Responsible for Comenius University Bratislava unit
Dr. Lucia Mosiello	NAST – ENEA unit, wpI
Prof. Mariano Venanzi	NAST-ToV Chemistry Dept.
Dr. Piero Morales	NAST ENEA unit, wpI coordinator
Dr. Stefano Vespucci	NAST-ENEA Unit

Scheduled subjects of the meeting were

1) Organization of experimental work at ORNL:
   a) Participation of Bratislava researchers to: a1) the petide adhesion computer simulation work and: a2) the lithography- fabrication and characterization work
   b) Transfer of the necessary funds to Bratislava administration

2) Overview and scientific discussion on the current state of the project
3) Plan of activities and collaboration within european partners of WorkPackage I
4) Transfer of information to the ORNL partner

Discussion:

1) Organization of work at ORNL

1a) Prof. Hianik agrees on developing a plan of secondments of Bratislava researchers at ORNL on the two mentioned research lines a1 and a2. Piero Morales underlines the necessity of intensive formation periods of researchers on lithography and SPM facilities, prior to their ORNL secondment in this line of nanofabrication activity. Strict contact of Bratislava theorists possibly interested in the peptide adhesion simulation work with the persons following this research in Rome (dr. Massimo Celino, dr. Giulio Gianese, dr. Caterina Arcangeli) is also necessary. Silvia Licoccia and Piero Morales will provide a sheet of the planned presence of Rome researchers at ORNL, once approved by the american partners, detailing the length of secondments and the aim of each person's activity. On this basis further secondments of Bratislava researchers will be proposed to the ORNL partner. It is discussed and agreed that secondments will be distributed on the four years time, starting with few young researchers.

1b) Prof. Hianik will provide Silvia Licoccia with a check of the Bratislava unit bank coordinates for the first financial transfer. To avoid bureaucratic problems possibly arising from unspent finance the money transfer will be proportional to the planned secondments.

2) Overview of the state of the project

Piero Morales illustrates progresses in: a) assemblage of DNA nanogrids; b) peptides adhesion on Cr, TiO2 and ZnO surfaces. Problems arising in both subjects are also fully discussed. In particular:

2a) Evidence of assemblages of partial square double stranded DNA architectures is already obtained from AFM characterization of samples incubated on mica for variable times and then briefly rinsed and dried. AFM scanning is performed on dry samples. Quality of images is generally very low except for some areas, and anyway lower than that of published work obtained in liquid.

One major problem evidenced by AFM analysis is that architectures at the level of 20 nm sided squares tend to coalesce into lumps that adsorb rarely on the surface. When they do, the observed topography is rather messy, due to superposition of many squares with random orientation. Outside such messy areas the specimen is basically flat (RMS in the angstrom range) with minor contamination. Adsorbed lumps are rare, and thus difficult to locate.

Tibor Hianik suggests saturation of the mica specimen with Mg+ ions to neutralize negatively charged mica surface, prior to incubation with DNA structures. Neutral or positively charged specimen surface will facilitate adsorption of negatively charged DNA. On the other hand such saturation should already occur due to the magnesium ions charged TAE Mg buffer solution in which architectures selfassemblage is performed. AFM scans in water can be performed at Bratislava, or with the available facilities at Engineering Sapienza University in Rome and ORNL.

2b) Experiments carried out at the NAST laboratories, both at ENEA and Tor Vergata, supply rather uncertain results on the specificity of adhesion onto the selected materials. Although there is reasonable adhesion of these peptides, specificity is not very high, almost certainly insufficient for the project purposes. In some cases AFM shows a reasonable specific adhesion of the chromium specific peptide onto a chromium surface with respect to the titania specific peptide, but fluorescence of the TAMRA chromophore tagget onto peptides shows almost no specificity with respect to most surfaces.

The reason for such disappointing results could be related to the presence of the bulky TAMRA chromophore heavily conditioning the peptide conformation in a different way with respect to the experiments reported in the literature, where the peptide was part of the protein of a bacteriophage virus. Also, TAMRA fluorescence quenching on the different surfaces is another source of uncertainty of the measurements. No indication from the simulation work in this respect is available yet, so we are considering the possibility of using a small fraction of the expensive oligo-peptides complexes that we have purchased in view of the final nanogrid assemblages to check, by AFM topography or phase contrast, specific adhesion of the actual complex to be used. Also available is a oligo-cysteine complex for thiol binding onto a gold adhesion nanopad.

Lucia Mosiello reports of a commercially available Zinc specific aptamer that could be used at one corner of the DNA grid construction, together with one thiol at the opposite

corner. In case of unsuccessful use of the chosen metal-specific peptides, the metal aptamer binding is considered as a viable alternative. Lucia Mosiello and Tibor Hianik will investigate further along this line.

3) Plan of activities and collaboration

Following suggestions from the ORNL partners, first attempts of ordered addressing on a high number of devices should be made on test devices of relaxed size, easily fabricated by lithographic techniques. Biochemists at ENEA are investigating the possibility of building linear double strands of DNA functionalised a with sulfur group at one end and with a metal specific peptide at the other end. Such linear strands should be as short and rigid as possible and at the same time long enough to not pose significant problems in the lithographic fabrication of the adhesion metallic nanopads. A compromise DNA length of about 100 nm, implying a separation between pads of the order of 80 nm with a pad size of about 20-30 nm is believed possible. This relaxed size should allow a high number of devices to be made under computer control, with no need of manual alignment of the electron beam for each device. A high number of devices will be informative on the statistics of adhesion of the DNA strands on the two pads. It will also make it easier to experiment on aptamer insertion into the linear strand and on protein binding onto the aptamer. The thrombin aptamer already investigated both by Hianik and by the USA group that first developed the hierarchical assembly of DNA nanogrids, should be the first to be inserted, so that a statistically determined yield of the whole selfassemblage process (DNA single strand + aptamer+ nanopads+ thrombine) can be obtained by topographic observation of the devices. Other aptamers, including possible ones for metals will be experimented. T. Hianik and L. Mosiello will propose a selection of possible suitable aptamers
E-beam nanofabrication of such relaxed size metal pads is already been attempted exploiting the e-beam lithography facility of CNR-IFN in Rome. When achieved in a reliable way, stepwise selfassemblage of the bio-organic part of the device will be measured at Bratislava by quartz crystal microbalance and AFM methods.

A workshop on the project progresses to be held in Bratislava will be organized in the spring 2012.

5) Transfer of information to ORNL

It is essential that ORNL researchers are kept informed of the progresses of META and can share the plan of secondments of EU researchers at ORNL. Piero Morales will ask Mike Simonson if he continues in his role of coordination of the USA partner and if so will report to him on the state of WPI. It was intended from the beginning that a website of the project be developed. PM proposes to supply all the technical information to be displayed on the website on WPI and made available in detail to the USA partners on an intranet section.

Actions

S. Licoccia and P. Morales

1) Supply sheet of information on steps to be taken for secondments at ORNL (within one week);

2) Supply temporary list of NAST associated researchers seconded to ORNL, with dates of arrival and return and precise aim of work at ORNL (within one week)
3) Investigate feasibility of website

T. Hianik
1) Supply S. Licoccia with bank coordinates for payments to Comenius University (beginning 2012)
2) Supply partners with plan of secondments of Bratislava researchers (beginning 2012)
3) Investigate Zn specific aptamer feasibility (ASAP, with Lucia Mosiello)
4) Supply Bratislava theorists interested in peptides simulation with references to NAST-ENEA persons and work already done (with Piero Morales)

P. Morales
1) Test of adhesion of oligo-peptide conjugates on gold, Cr, TiO2, ZnO. AFM imaging on micropatterned surfaces both in topography and phase contrast. Within end of Feb 2012
2) Feasibility of 100 nm long DNA strands functionalized with cysteine on one side and sticky end on the other side (peptide or aptamer) (with biologists/biochemists at Casaccia). Within January 2012
3) e-beam fabrication of series of couples of 30 nm pads, 80 nm apart each side (with CNR IFN(with M. Caruso and A. Gerardino)). Within Feb 2012.
4) Mg++ saturation of mica specimens, AFM imaging of selfassembled DNA architectures adsorbed on mica, in water (with Engineering University Sapienza Roma, prof. M.S. Sarto)
5) End of DNA architectures selfassemblages and characterizations (March 2012)

9 December 2012