nano newsletter

nº 10

December 2007
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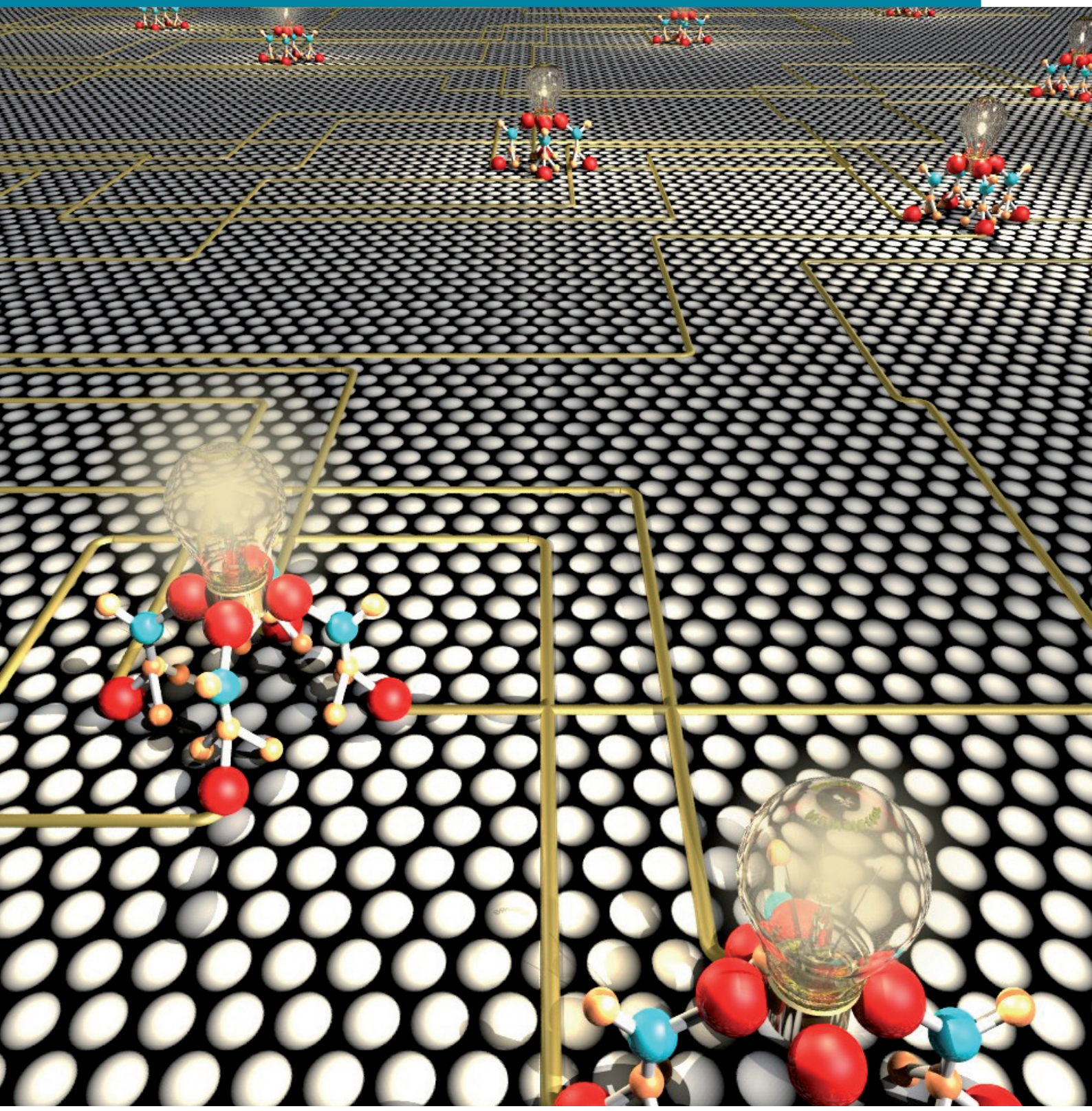

- Towards Self-assembly of Molecular Moulds by Complementary Hydrogen-bonding
- Water as a mediator of protein-nanoparticle interactions: entropy driven protein binding as a paradigm for protein therapeutics in the Biopharma industry?

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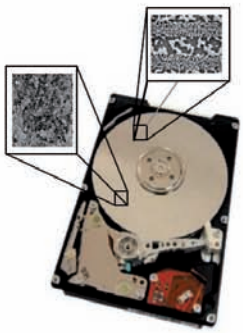
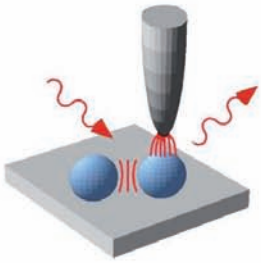


Fabrication and characterization of natural and artificial meta-magnetic materials, multilayers and nanostructures, under supervision of Dr. Andreas Berger.



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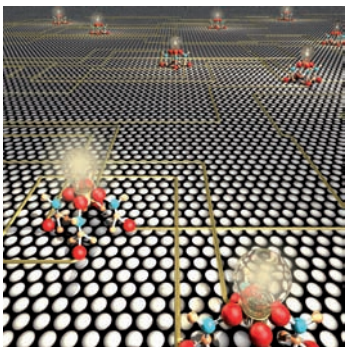

Cover Picture
Vision of a molecular electronics based circuit.

Design: Enrique Sahagun
(MOLE group - Universidad Autonoma de Madrid, Spain)

Depósito Legal: M-43078-2005

Dear Readers:

This E-Nano newsletter issue contains articles dealing with several aspects of the “Molecular Electronics” field, currently very active.

The paper by Wei Xu et al provides a better understanding on how to form metallic nanostructures that may act as nanoscale “connectors” to molecular devices: an interesting bottom-up approach towards this goal being to use organised molecular structures that may act as moulds for subsequent formation of metallic nanostructures.

Another area in which nanotechnology has the potential to make an enormous impact is medicine. Protein therapies are considered to be leading candidates for treating a wide range of diseases from the deficiencies to more well-known examples ranging from cancer, to neurodegeneration and bones diseases among others. The paper by Kenneth A. Dawson et al aims therefore at presenting a general view on how to design nanomaterials for incorporation of protein therapeutics and showing the importance of water as a mediator of the interactions between nanoparticles and proteins.

We would like to thank all the authors who contributed to this issue as well as the European Union (ICT/FET/nanoICT) and the Pico-Inside EU Integrated Project for their close collaboration and partial financial support.

2007 has proved to be another successful year of publishing for the E-Nano newsletter. Therefore, we would like to thank you, our readers, for your interest, support and collaboration and finally wish you a healthy and happy New Year 2008!

Dr. Antonio Correia
E nano newsletter Editor
Phantoms Foundation

EDITORIAL INFORMATION

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Towards Self-assembly of Molecular Moulds by Complementary Hydrogen-bonding

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Keyword: STM, hydrogen bonding, molecular moulding, nanotechnology, supramolecular chemistry

Introduction

A central goal within the PICO-INSIDE integrated project is to form metallic nanostructures that may act as nanoscale "connectors" to molecular devices. An interesting bottom-up approach towards this goal is to use organised molecular structures that may act as moulds for subsequent formation of metallic nanostructures, as shown schematically in **Figure 1(a)**. The idea in molecular moulding is to use the interaction between an organic molecule and metal atoms to form well-defined metallic nanostructures on surfaces. The principle was demonstrated by Rosei et al **[1]** who used a $C_{90}H_{98}$ organic molecule known as the Lander to synthesize metallic nanostructures on a Cu surface. As shown in the **Figure 1(b)**, Lander molecules consist of an aromatic board equipped with bulky moieties that act as legs, lifting the board away from the surface. At room temperature, there is a high mobility of step edge atoms on the Cu surface so that when Lander molecules were deposited, they can trap and stabilize such thermally activated atoms to form a well-defined nanostructure at the step edges. The nanostructure was revealed by scanning tunnelling microscope (STM) manipulation at low temperature. Since the space underneath the molecular board favourably matches the dimension of the double-row nanostructures, the low-coordinated atoms of these metallic protrusions are stabilized by interaction with the pi-system of the aromatic board. In this way the shape of the organic molecule is effectively used as a mould that is replicated in the resulting metallic nanostructure. Molecular moulding effects have also been demonstrated for isolated metal structures underneath molecules adsorbed at terraces **[2]** and for larger molecules **[3]**. To form a moulded 1D metallic wire, prearrangement of the molecular moulds is required. Growth of a 1D molecular structure from Lander-type compounds was previously realized by adsorption onto templates formed on a Cu surface by exposing it to oxygen **[4]**. However, this approach is limited to substrates that display a templating effect. A much more versatile approach that may be extended to other substrates is to organise the molecules through exploitation of specific intermolecular interactions.

Supramolecular chemistry based on noncovalent interactions is a powerful synthetic tool for the preparation of complex molecular architectures **[5,6]**. Especially, hydrogen bonds are useful for controlling molecular self-assembly due to the reversibility, specificity, directionality, and cooperative strength of this class of interactions **[7,8]**. In recent years, a number of studies have characterized hydrogen-bonded structures formed by molecules adsorbed on solid surfaces under vacuum conditions **[9-11]**. Most studies so far have involved only homomolecular interactions, and very few structures based on heteromolecular interactions have been investigated **[12-14]**. To enable the synthesis of self-assembled multicomponent nanostructures on surfaces, suitable protocols of stoichiometry, deposition order, and thermal treatment have to be established and systems with sufficiently high intermolecular binding strengths have to be identified **[15, 16].**

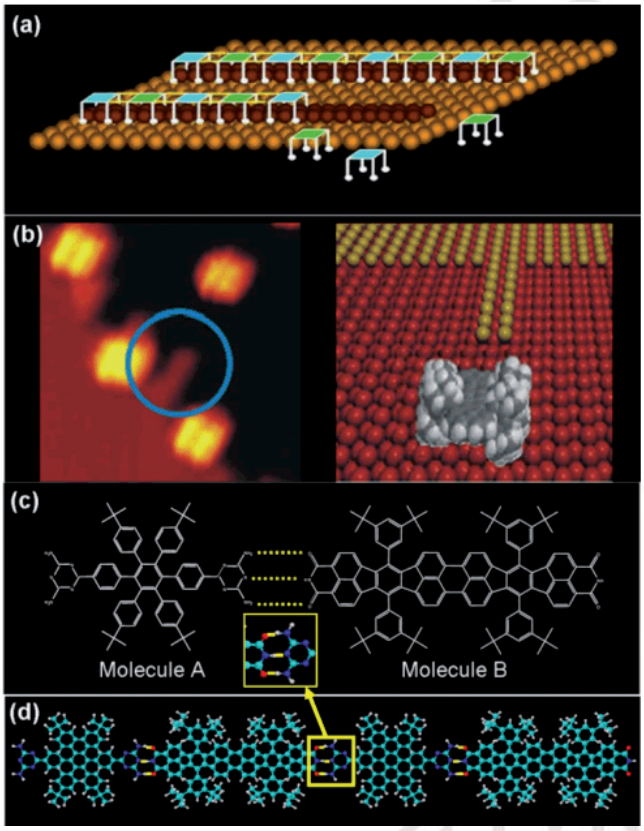

Figure 1: *Concept of self-assembly of molecular mould.* ***(a):*** *Schematic drawing of the growth of one-dimensional metallic wires (red) by the alternating A-B-A-B molecule chains (pale blue and green) with lifting legs,* ***(b):*** *Molecular molding of atomic wires, adapted from* ***[1]****,* ***(c):*** *The interaction of two Lander Molecule A and B by the triple complementary hydrogen bond between the diamino-pyridine and diimide functional groups,* ***(d):*** *A-B-A-B… molecular chain formed by the triple hydrogen bond.(red: O, blue: N, pale blue: C, white: H)*

An extensively studied heteromolecular H-bonding motif results from the interaction between diamino-pyridine and diimide moieties, exhibiting three complementary NH•••O and NH•••N hydrogen bonds **[7,17-19]**. This classic H-bonding interaction has been exploited in the solution phase **[18, 19]**, in the solid state **[7]**, and more recently at interfaces **[12,13]**. Two Lander-type molecules exhibiting this type of complementary interaction have therefore been designed as candidates (see **Figure 1 c and d**) for self-organising molecular moulds. Molecule A, bis(diaminotriazine), ($C_{64}H_{68}N_{10}$), consists of central benzene ring connected with six phenyl group by sigma bond with 4 tert-butyl groups to lift the molecular board and 2 diamino-pyridine groups on both sides. Molecule B is a

functionalized violet-Lander molecule with diimide end-groups on both sides and tert-butyl groups as the 'spacer legs'. These two molecules are expected to create a one-dimensional chain by alternating A-B-A-B molecules via triple complementary hydrogen bonding between the diamino-pyridine and diimide functional groups shown in **Figure 1 (d)**. Once this 'molecular tube' is built up, metal atoms may be trapped inside it to mould an atomic wire.
The scope in the present newsletter presentation is to review recent results **[20]** for a prototypical and simple molecular system exhibiting the same complementary diamino-pyridine and diimide interaction, namely the cyanuric acid/melamine (CA/M) system (see **Figure 2**). Detailed understanding of the interactions and organisational principles in this system are invaluable for guiding efforts formation of self-assembled structures from the more complex Lander-type compounds. In addition preliminary results for the Lander-type compound A on the Au(111) surface are presented.

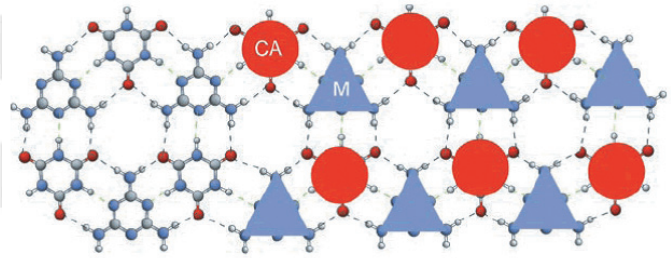

Figure 2: *Schematic representation of the cyanuric acid–melamine- (CA1M1) lattice stabilized by O•••H and N•••H hydrogen bonds. (red: O, blue: N, white: H)*

Experiment

The M-CA experiments were performed in a UHV chamber equipped with standard facilities for sample preparation and characterization. Prior to deposition, the single-crystal Au(111) sample was cleaned by several cycles of 1.5 keV Ar+ sputtering followed by annealing to 770 K for 15 min, resulting in a well-ordered herringbone reconstruction. Powders of M and CA (≥99% and ≥98% purity, respectively; Acros) were thoroughly degassed prior to deposition. Both CA and M were deposited by thermal sublimation from molecular evaporators held at ≈ 356 K.
Simultaneous deposition of CA and M was achieved by heating both evaporators and overlapping their output beam on the surface. Molecules were deposited onto a sample held at room temperature (≈ 300 K). A typical deposition time of 5 min resulted in coverages below half saturation of the first monolayer. After deposition, the sample was transferred in situ to a variable-temperature Aarhus STM. All images presented here were collected in the constant tunnelling current mode and STM measurements were obtained in a temperature range of 100-160 K to thermally stabilize the molecular structures.

Results and Discussion

Adsorption structures formed upon deposition of CA or M individually on Au (111) are shown in **Figure 3 and Figure 4** respectively along with optimized models for the resulting hydrogen-bonded molecular networks. In both cases, large well-ordered islands are observed. The herringbone reconstruction does not appear to change upon molecular adsorption or affect the self-assembly patterns observed in this study. STM images of CA and M, as shown in **Figure 3(a) and Figure 4(a)**, respectively, depicts CA as compact entities with near-circular symmetry, whereas M molecules are resolved with a characteristic three-spoke shape, attributed to the position of the amino groups.
The overlayer formed from pure CA (**Figure 3(a)**) is hexagonally close-packed and can be modeled by the structure shown in **Figure 3(b)**, in which each molecule

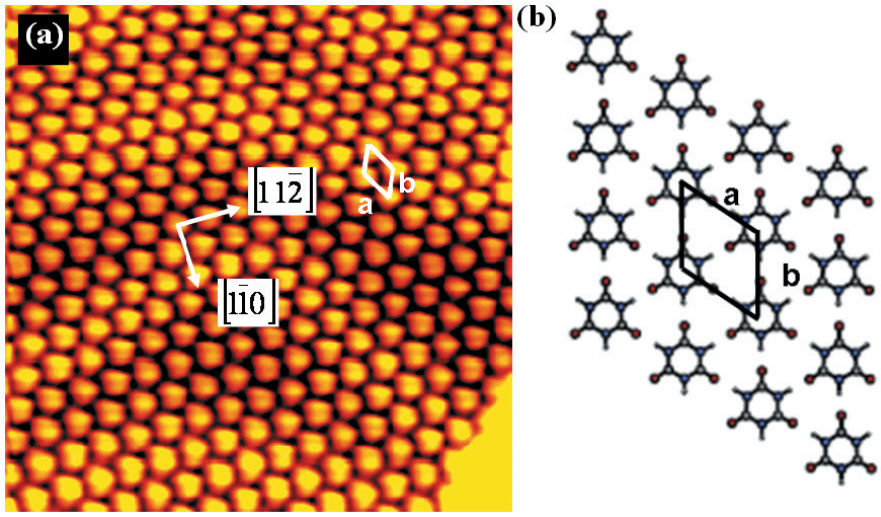

Figure 3: (a)*: A STM image (102 Å x 102 Å) of CA (I_t = 0.72 nA, V_t = 1250 mV) on Au(111) ,* ***(b)*** *Optimized models for the hydrogen-bonded network of CA.*

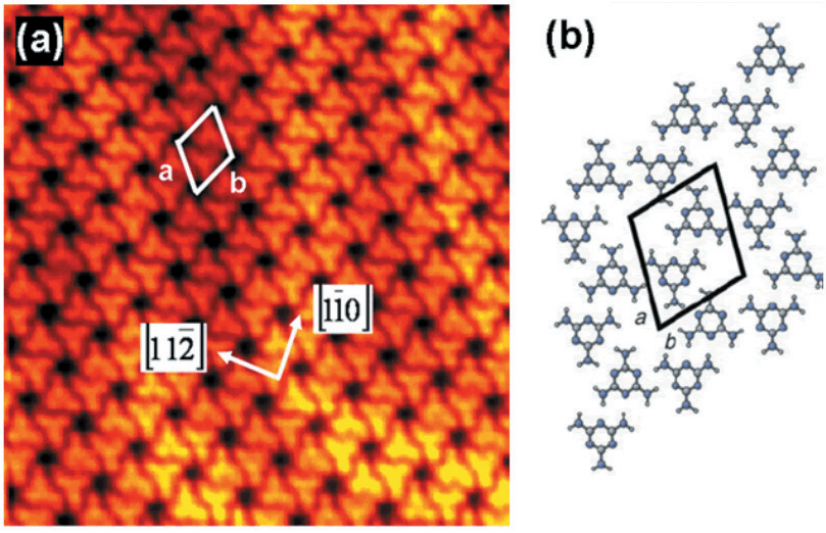

Figure 4: (a): *A STM image (102 Å x 102 Å) of Melamine (I_t = 0.57 nA, V_t = 1486 mV) on Au(111),* ***(b)*** *Optimized models for the hydrogen-bonded network of Melamine.*

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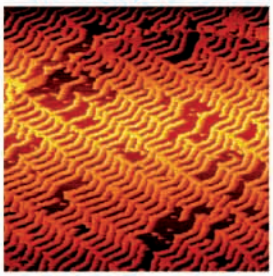
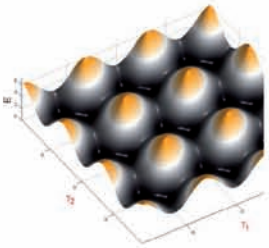
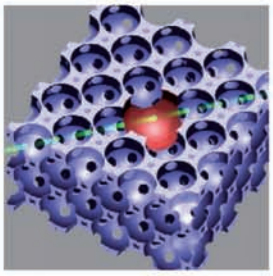
Molecular Nanoscience
Array of PCBM molecules on Au (III)

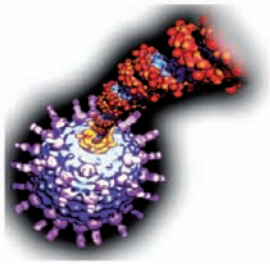




Nanoscience at very low temperatures
Potential landscape of a superconducting qubits

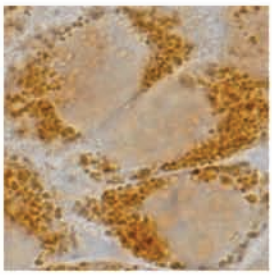
Nanoelectronics Nanophotonics
Photonic crystals





Biomachines and manipulation of biomolecules
Biological molecular motors

Nanomagnetism and the biomedical application of magnetic nanoparticles
Magnetic nanoparticles inside tumoral cells



Nanofabrication and advanced instrumentation
Molecular smile assembled with STM

IMDEA- Nanociencia is a private Foundation created by joint initiative of the regional Government of Madrid and the Ministry of Education of the Government of Spain in February 2007 to manage a new research Institute in Nanoscience and Nanotechnology (IMDEA-Nanociencia), which is located in the campus of the Universidad Autónoma de Madrid, 12 km away from Madrid downtown with an excellent communication by public transportation with the Madrid-Barajas airport (25-30 min) and Madrid downtown (15-20 m).

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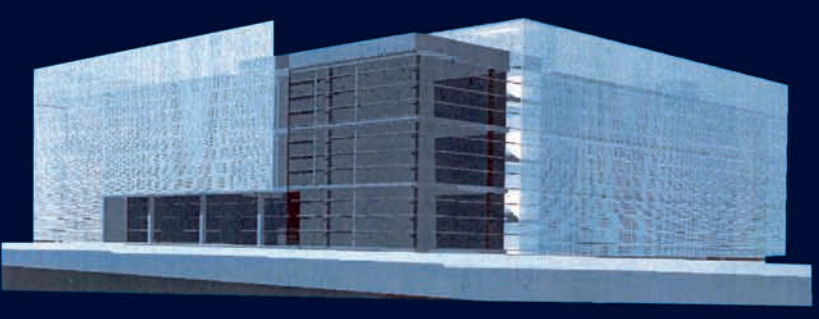

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associates through six H bonds to symmetrically distributed neighbours. In contrast, M forms a more complicated structure in which the spokes of neighbouring molecules interdigitate, leading to the formation of six-membered rings surrounding open pores in the network. From the corresponding optimized model for M (**Figure 4(b)**) the structure is seen to involve double H-bonding interactions to three neighbours surrounding each molecule.

Simultaneous deposition of CA and M leads to a self-assembled network as shown in **Figure 5(a)**. The characteristic circular and three-spoke shape for CA and M is resolved (most clearly seen at the top-left side of the image), showing that the structure is a binary mixed phase involving hetero-molecular H-bonding. Domains of pure CA islands are found to coexist with this mixed phase, most likely due to a slight excess of CA molecules on the surface. Comparison of the area indicated with the hexagon marked "1" in **Figure 5(a)** to the model for the CA_1M_1 structure shown in **Figure 2** reveals that the observed structure corresponds to a motif based on the complementary CA•••M coupling.

Insight into the molecular interaction energies underlying formation of the observed structures were obtained from calculations using the SCC-DFTB method **[20]**. The melamine dimer involves two H bonds between the NH donor and N acceptor groups and the calculated binding energy (E_b) for formation of the dimer from the isolated molecules is 0.34 eV. Similarly, the dimer formed from CA molecules has a binding energy of 0.22 eV. The binding

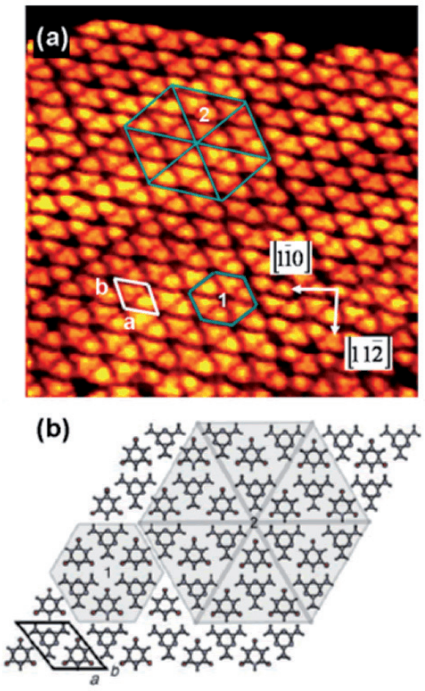

Figure 5: (a): *STM image (102 Å x 104 Å) of a self-assembled network resulting from simultaneous deposition of M and CA on Au (111). The characteristic ball- and three-spoke shapes for CA and M are clearly resolved. The structure is a mixed phase corresponding to the network presented in Fig. 2 (I_t=0.56 nA, V_t=1250 mV).* ***(b)****: Optimized model for CA_1M_1 network. The hexagon marked "2" in (b) shows corresponding areas as a guide to the eye.*

energies for the optimized periodic networks of CA and M shown in **Fig. 3(b)** and **Fig. 4(b)** are 0.65 eV molecule^{-1} and 0.47 eV molecule^{-1}, respectively which fits well with summation of the pairwise interaction energies. In both the M and CA networks, each molecule forms six H bonds, and the stronger binding energy of the CA networks reflects that the NH•••O hydrogen bonding interactions are stronger than the NH•••N bonds, as expected **[8]**. For the complementary M-CA coupling involving three hydrogen bonds, a dimer binding energy of 0.48 eV is found. The corresponding binding energy for the stoichiometric CA_1M_1 network **(Fig. 5)** is 0.82 eV molecule^{-1}, which is slightly more that than obtained by summing the pair-interaction energies showing a slight tendency to additional stabilization in the extended network, which may result from each amino group on the M molecules engaging in two H-bonds to oxygen atoms on adjacent CA molecules.
We finally present preliminary results for the Lander compound A functionalised with melamine-type moieties. A high-resolution STM image of a self-assembled structure formed form this compound on the Au(111) surface is shown in **Figure 6**. Every molecular entity exhibits 4 bright lobes in a rectangular shape (11.0 Å x 6.5 Å), which is attributed to the tert-butyl groups. The diamino-pyridine groups are not visible in this STM image, which may result from decoupling of these groups from the substrate by the spacer legs of the molecule.
Based on the results for melamine described above, a possible structure that might be anticipated for compound A is a zig-zag stripe packing through double hydrogen bonding between neighbouring melamine units. However, this is not borne out in the STM image, which instead displays a crisscross arrangement where each molecule has its axis orthogonal to the axis of the four nearest neighbours in the structure. A tentative molecular model is superimposed on **Fig .6** to indicate the intermolecular interactions. The diamino-pyridine endgroups appear to embed between two tert-butyl groups on a neighbouring molecule. The model suggests there is some steric crowding but a possible tilting/rotation of the melamine group due to its interaction with substrate and neighbouring molecules may help to stabilise the observed structure. The organisation of the shown adsorption phase thus appears to be dominated by Van der Waals closing packing as opposed to specific intermolecular hydrogen-bonding.

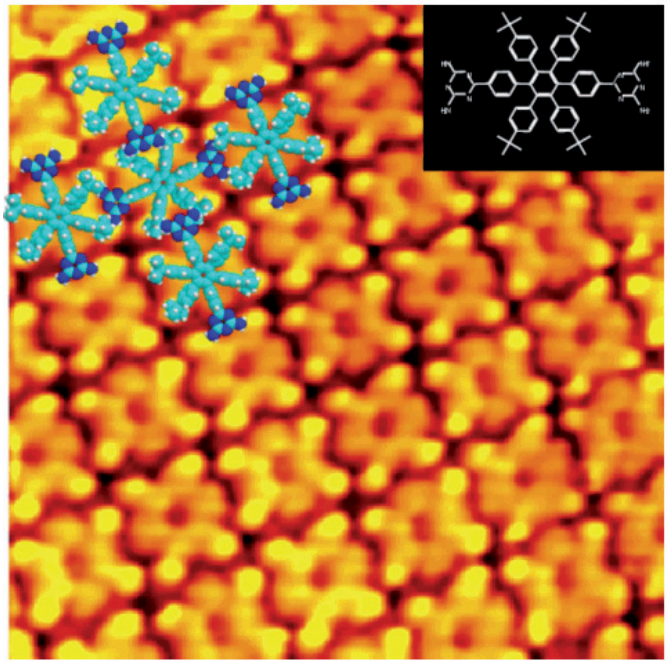

Figure 6: *High resolution STM image (80 Å x 80 Å) of a self-assembled structure formed from compound A on Au(111). The corresponding structural formula is given in the inset. Its full-space models are superimposed, showing that the diamino-pyridine endgroups interdigitate between two tert-butyl groups of nearest-neigh-bouring molecules. (I_t = 0.84 nA, V_t = 1250 mV)*

Summary and Outlook

In summary, we have reviewed recent results for the adsorption and co-adsorption of Melamine and Cyanyric Acid on the Au(111) surface. Detailed insight into the structures formed by this model system and the associated hierarchy of homo- and heteromolecular interaction strengths, are important to guide future efforts towards using this important complementary hydrogen-bonding motif in self-assembly of more advanced surface nanostructures. As and example, two custom designed Lander type molecules exhibiting similar complementary diamino-pyridine and diimide groups were discussed. Preliminary results for one of these compounds shows a structure which appears to be dominated by steric effects and non-specific interactions as opposed to H-bonded motifs anticipated from the results for pure Melamine. Further experiments are underway in appropriate co-adsorption systems that utilize the complementary triple-hydrogen bonding interaction where the higher interaction strength is anticipated to lead to unidirectional molecular arrangements relevant for experiments into molecular moulding of metallic wires.

Acknowledgement

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Spanish Pavilion at nanotech 2008 – World's Largest Nanotechnology Exhibition

The Phantoms Foundation and The Spanish Institute for Foreign Trade (ICEX) have promoted the first Spanish Pavilion at nanotech2008 (Japan), as an initiative under the program **España, Technology for Life**.

This program, carried out by ICEX, focuses in the promotion in foreign markets of Spain's more Innovative and leading Industrial Technologies and products. The Spanish participation will group 12 Companies and Research Centres providing an outlook of the most innovative projects and products in various fields of Nanoscience & Nanotechnology.

The Spanish Pavilion will therefore promote the "Spanish Nanoscience and Technological Offer", allowing to:

1. Represent the Scientific, Technological and Innovative agents of the country as a whole.
2. Foster relationships with other nanotech2008 participants.
3. Promote Spanish culture of innovation.
4. Better integrate the Spanish "Science-Technology-Company-Society" system in Europe and Far East.
5. Generate and develop scientific and technological knowledge
6. Improve competitiveness and contribute to the economic and social development of Spain.

The **Spanish Institute for Foreign Trade (ICEX)** ("Instituto Español de Comercio Exterior") is the Spanish Government agency serving Spanish companies to promote their exports and facilitate their international expansion.

It is part of the Spanish Ministry of Industry, Tourism and Trade ("Ministerio de Industria, Turismo y Comercio"). To meet its objectives, ICEX is assisted by the network of Spanish Embassy's Economic and Commercial Offices and, within Spain, by the Regional and Territorial Offices. The Institute acts in the following areas:

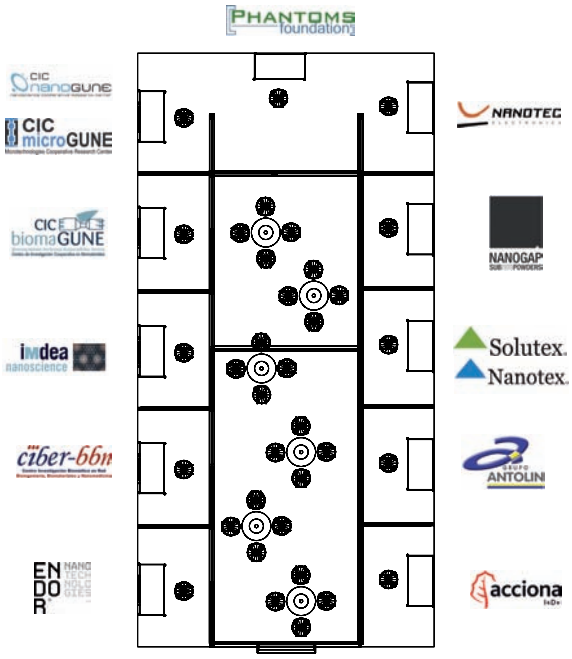
(1) It designs and carries out commercial promotion and investment programs in foreign markets;

(2) It prepares and provides information regarding international markets and the Spanish products offered; and

(3) It promotes the teaching of technical skills to business people and the training of professionals in foreign trade. See: "Master in International Corporate Management".

The **Phantoms Foundation** (Madrid, Spain) focuses its activities on Nanoscience, Nanotechnology and Emerging Nanoelectronics and is now a key actor in structuring and fostering European Excellence and enhancing collaborations in these fields. This non-profit Association is also playing an important role as a dissemination platform in national and 6th-7th framework programs European funded projects to spread excellence among a wider audience and help in forming new networks.

In particular, the Phantoms Foundation agglutinates and coordinates the efforts made in the field of Nanoscience and Nanotechnology by Spanish groups from universities, research institutes and companies through several initiatives such as scientific events, networking and participation to International Exhibition events such as nanotech2008.



Spanish Pavilion scheme: participating companies & Research Centres

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2nd EU/FET Cluster Meeting

Nanoelectronics represent a strategic technology considering the wide range of possible applications. These include telecommunications, automotive, multimedia, consumer goods and medical systems.
Many of the potential emerging nanoelectronic applications still require substantial work in order to be transformed into marketable technology. A concerted effort must therefore be made at the European level to both understand and commercialise molecular and atomic scale technology in order to maintain a competitive advantage for Europe and keep Europe at the forefront of the next nanoelectronics revolution, a revolution beyond nanotechnology.
In order for the field of emerging nanoelectronics to continue growing exponentially worldwide and therefore lead to new commercial applications and to change the micro and nanoelectronics paradigm, it is necessary to educate new researchers who can work across traditional disciplines. High-level dissemination activities such as the **2nd EU/FET-Cluster meeting** (Las Palmas de Gran Canaria (The Canary Island, Spain): November 13-16, 2007) will help to establish a critical mass of R&D at a European level and to stimulate development of an interdisciplinary community of researchers.
During this meeting, an open plenary session (1 day) and restricted EU project reviews took place.
The 1 day plenary session program was composed of EU representatives presentations, proactive action related talks (invited) and short contributions on IST/FET FP6 scientific research highlights and future FP7 nanoICT funded projects.
FP6 projects
"E-Nano" - ULTRAGAN, D-DOT FET, NANOSPIN and SUBTLE
"Bio" - GOLDEN BRAIN and DNA NANODEVICES
"Single Atom/Single Molecule" - PICO-INSIDE
"Nanowires" - NODE
"CNTs" - CANDICE
FP7 projects
nanoICT Coordination Action
"Single Atom/Single Molecule" - MOLOC, AFSID, SINGLE and CHIMONO
"Switches and Interconnects" - CATHERINE, GRAND, NABAB and VIACARBON
This meeting was organised by the Phantoms Foundation, the EU/FET NanoICT proactive initiative and the CEMES/CNRS (in collaboration with the Pico-Inside IP project). It brought together about 150 scientists primarily associated with FP6 and FP7 projects.
Invited talks and the Working Session: "2020 and Beyond – Long-Term Challenges for the FET-Nano Community" served as a starting point to encourage long term visionary views from the audience. A summary of the invited talk is presented below.

More info available at:
http://www.phantomsnet.net/Picoinside/EUFET/index.php

Quantum Transport in Carbon Nanotubes : effects of doping, defects and physisorbed molecules

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Their unusual electronic and structural physical properties promote carbon nanotubes as promising candidates for a wide range of nanoscience and nanotechnology applications. Not only can nanotubes be metallic, but they are mechanically very stable and strong, and their carrier mobility is equivalent to that of good metals, suggesting that they would make ideal interconnects in nanosized devices. Further, the intrinsic semiconducting character of other tubes, as controlled by their topology, allows us to build logic devices at the nanometer scale, as already demonstrated in many laboratories.
The tremendous importance of the transport properties of nanotubes **[1]**, both from a fundamental and technological point of view, justifies wealth of work and theories developed to deal with 1D systems involving a confined electron gas. The purpose of the present research is to define the electronic and transport properties of nanotubes in relation with their atomic structures. Since quantum effects are prominent in nanotube physics, the electronic quantum transport has been investigated using both the Landauer-Buttiker and the Kubo-Greenwood formalisms, allowing to extract generic properties such as quantum conductance, conduction mechanisms, mean-free-paths... Within both frameworks, the well-known ballistic properties of armchair metallic nanotubes have been reproduced. However, defects, doping and chemical functionalisation can alter this ideal situation. For example, even a small amount of boron or nitrogen dopants can drastically modify the electronic transport properties of the tube, which is certainly a key effect for future nanoelectronics. Indeed, the energy-dependent quantum transport properties of chemically doped carbon nanotubes have been investigated **[2]**. By combining first-principles methods to tight-binding approaches, the mean-free path and the length-dependent conductance scalings have been derived by simultaneously taking into account the chemical nature of impurities together with their random distribution over micrometer length scale. Our calculations allow

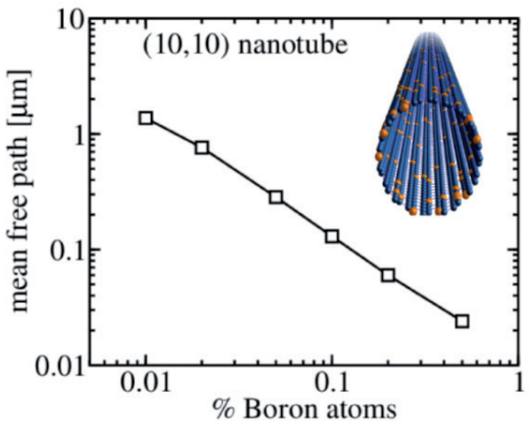

Figure 1: *Electronic mean-free path in randomly B-doped armchair carbon nanotubes as illustrated in the inset.*

direct comparison with experiments and demonstrate that a small amount of dopants (<0.5%) can drastically modify the electronic transport properties of the tube.

From a physical point of view, the relevant information is that a low density of dopants yields diffusive regimes, with a mean-free path (λ_e) decreasing linearly with dopant concentration following the Fermi golden rule **(Figure 1)**. Moreover, our calculations suggest mean-free paths in the order of 175–275 nm for boron-doped nanotubes with diameters in the range 17–27 nm and 1.0% of doping, in very good agreement with experimental data **[2]**.

The chemical sensitivity of the electronic transport in carbon nanotubes under the physisorption of molecular species or covalent functionalisation is also an important issue as for example in sensing applications or to disperse uniformly these long 1D systems into a solvent or a host material. The π-stacking interaction between various planar organic molecules (such as benzene, azulene, pyrene and DDQ), and carbon nanotubes has been investigated within the framework of *ab initio* calculations **[3-4]**. The adsorption of these molecules on the sidewall of the cylindrical carbon structure induces a small binding energy compared to conventional covalent functionalisation. Such a weak interaction is found to be only physisorption and

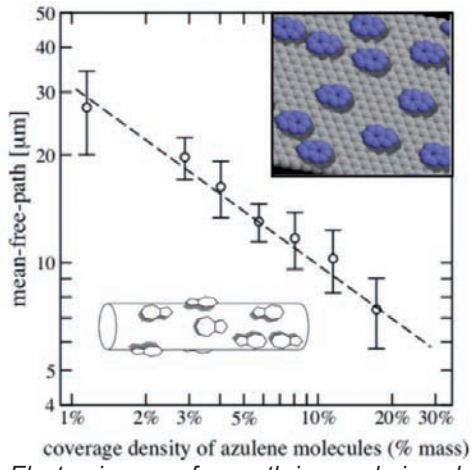

Figure 2: *Electronic mean-free path in armchair carbon nanotubes randomly decorated with physisorbed azulene molecules as illustrated in the two insets.*

leads to minor and predictable modifications of the electronic structure. These changes in the electronic behavior of the host carbon nanotube are ruled by the relative positions of the molecular levels of the isolated molecule and both the valence and conduction bands of the perfect tube. However, although the π-stacking interaction is weak, molecular physisorption could have an impact on the electronic transport properties of the carbon nanotubes. A *tight-binding* scheme, parametrised with first-principles calculations, is able to tackle with the complex electronic transport properties of chemically grafted conducting nanotubes with a random coverage of physisorbed molecules. Our calculations demonstrate that the impact of physisorption on the transport regime critically depends on the HOMO-LUMO gap position of the attached molecules with respect to the Fermi level of the nanotube **[5]**. Benzene molecules yield vanishing modulations of the intrinsic conductance, whereas azulene molecules (with HOMO-LUMO gap of about ~2.07 eV) produce substantial elastic backscattering in the nanotube, resulting in mean free paths on the order of a few micrometers for large coverage. Consequently, our simulations demonstrate clearly that the nature of the attached molecules will strongly affect the transport properties of the decorated tubes. In addition, the electronic mean free path exhibits a downscaling law with a lower dependence on the coverage density of grafted molecules than for conventional substitutional doping or homogeneous disorder **(see Figure 2)**. Indeed, in contrast, the effect of substitutional chemical impurities investigated in **[2]**, was shown to produce a linear decreasing of λ_e with the impurity density. Different from substitutional impurities, in the case of physisorption, the underlying carbon lattice is only disturbed as a second-order process, and as confirmed by our numerical results, corresponding backscattering behavior turns out to be much weaker **[5]**. Such result could have important consequences on the understanding of the (bio)sensing capability of molecular objects such as nanotubes.

At last, like in most materials, the presence of defects in carbon nanotube has been demonstrated experimentally **[6,7]**. These defects may take different forms: vacancy, adatom, “Stone-Wales” defect, 5/7 pair, atom in substitution, and are known to modify the electronic properties of carbon nanotubes **[8]**. It is crucial to understand the properties of these defects in order to conquer their detrimental effects, but also because controlled defect introduction may be used to tune nanotube properties in a desired direction. Consequently, the modifications induced by those defects in the electronic properties of the carbon hexagonal network have been investigated using first-principles calculations **[9]**. One of the most frequently observed defect is the mono-vacancy which consists in a single carbon atom removed from the graphene sheet. In this specific case, the remaining hole undergoes a Jahn-Teller distortion upon relaxation, where two of the atoms near the vacancy move closer, forming a pentagonlike structure while the third atom is displaced by 0.43 Å out of the plane, as illustrated in **Figure 3a page 14**, and observed experimentally **(Figure 3b)**. This vacancy defect

has a significant influence on the electronic structure of honeycomb lattices. Indeed, due to the presence of a vacancy in the supercell, new flat bands (low electronic group velocity) arise near the Fermi level (E_F), predominantly resulting from the atoms located around the corresponding vacancy. All these electronic states of the single vacancies in graphene are strongly localized and yield typical defect states with sharp peaks located around E_F. Computed constant-current STM images of this vacancy defect have been calculated within a *tight-binding* approach in order to facilitate the interpretation of STM images of defected carbon nanostructures **[9]**. Such a STM image is illustrated in **Figure 3c** and does not reveal any threefold symmetry due to the atomic reconstruction mentioned above. The largest protrusion is located on the sole two-coordinated atom located in front of the pentagon with a long C–C bond produced by the reconstruction. The trigonal symmetry of the image, as observed experimentally **(Figure 3e)** can be restored by invoking a dynamical Jahn-Teller effect, where the pentagon rotates by ±2π/3. This dynamic switching between degenerate structures is activated by a barrier of 0.13 eV. The image would then be an average of three structures equivalent to that of the nonreconstructed one rotated by ±2π/3, as represented in **Figure 3d.**

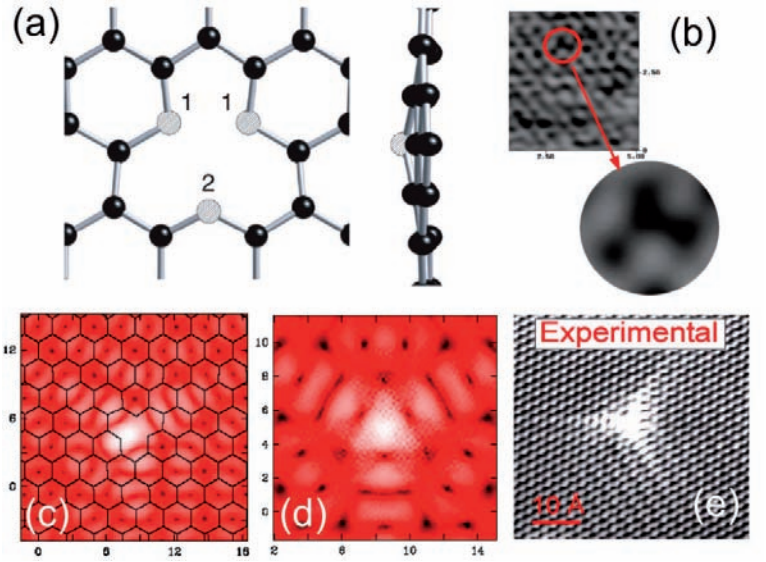

Figure 3: *The vacancy defect : **(a)** atomic struture (top (left) and side (right) views); **(b)** vacancy observed in air-plasma oxydized HOPG graphite; **(c)** calculated STM curent map at constant height (0.5nm) above a vacancy in a single graphene layer ; **(d)** average image for the three equivalent structures as represented in (c) (see text) in order to compare with the experimental STM image of a vacancy in graphene **(e)**.*

At last, these vacancy defects should also play a key role in the chemical reactivity of carbon nanotubes. Indeed, the modulation of the conductance due to specific molecules adsorbed at the defected sites should be detectable thus suggesting nanotubes as potential candidates for sensing applications. These vacancies should also be considered as magnetic impurities in nanotubes **[10]**. Consequently, spin-dependent transport properties need to be investigated in these defected carbon nanostructures, thus opening the way for spintronic applications.

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Scanning Probe Microscopy of Adsorbates on Insulating Films: First Steps towards a Modular Molecular Logic

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Ultrathin insulating films on metal substrates are unique systems to use the scanning tunneling microscope to study the electronic properties of single atoms and molecules, which are electronically decoupled from the metallic substrate.

Individual gold atoms on an ultrathin insulating sodium chloride film supported by a copper surface exhibit two different charge states, which are stabilized by the large ionic polarizability of the film **[1]**. The charge state and associated physical and chemical properties such as diffusion can be controlled by adding or removing a single electron to or from the adatom with a scanning tunneling microscope tip. The simple physical mechanism behind the charge bistability in this case suggests that this is a common phenomenon for adsorbates on polar insulating films. For example in the particular case of Ag adatoms even three different charge states could be observed **[2]**. In the case of molecules on ultrathin NaCl films the electronic decoupling allows the direct imaging of the unperturbed molecular orbitals. This will be shown for individual pentacene molecules **[3]**. Scanning tunneling spectroscopy of these double-barrier tunneling-junctions reveals strong electron-phonon coupling to NaCl phonons. Using atomic/molecular manipulation a covalent bond between an individual pentacene molecule and a gold atom can be formed. This bond formation is reversible and different structural isomers can be produced. Direct imaging of the orbital hybridization upon bond formation provides insight into the energetic shifts and occupation of the molecular resonances.

Molecular switches will be an essential part of possible future molecular devices. The bistability in the position of the two hydrogens in the inner cavity of single free-base naphthalocyanine molecules **(Figure 1)** constitutes a two-level system that can be manipulated and probed by low-temperature scanning tunnelling microscopy. When adsorbed on an ultrathin insulating film, the molecules can be switched in a controlled fashion between the two states by excitation induced by the inelastic tunnelling current. The tautomerization reaction can be probed by resonant tunnelling through the molecular orbitals **(Figure 2)**. Coupling of the switching process such that charge injection in one molecule induced tautomerization in an adjacent molecule will be shown.

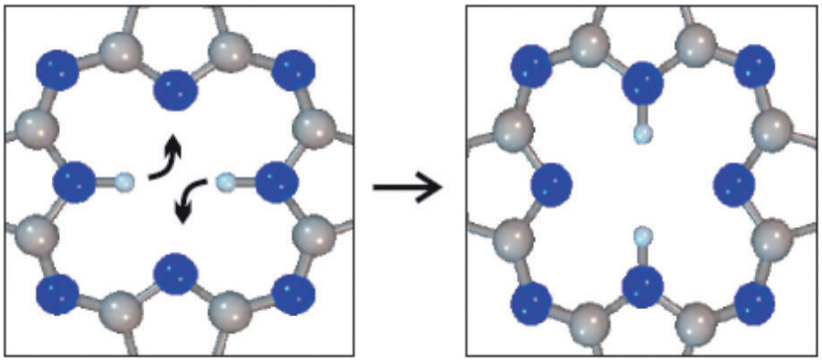

***Figure 1:** Model of hydrogen tautomerization in the inner cavity of a naphthalocyanine molecule*

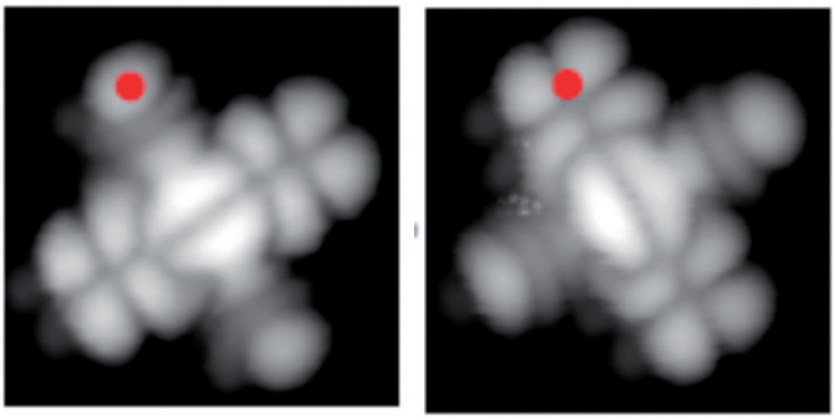

***Figure 2:** Switching between the two configurations as imaged directly by the orientation of the LUMO of the naphthalocyanine molecule.*

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Atomistic understanding of transport through a single dopant atom

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Isolated donors in silicon have received renewed attention in the last decade due to their potential use in quantum electronics **[1-4]**. The donors form 3D Coulomb (thus truly atomistic) potentials in the silicon lattice that can bind up to two electrons **[5]**. In the majority of proposals for quantum electronics, isolated donors act as the binding sites for the information-carrying electrons. The ability to perform (quantum) operations is crucially provided by one (or more) gate electrodes around the donor site. Although many proposals are based on the functionality of isolated single donors, experimental access to such systems has proven to be difficult **[6-8]**.

In this talk, we will discuss resonant tunneling spectroscopy measurements on the eigenlevels of single As donors in a three terminal configuration, i.e. a gated donor which is a basic element for quantum electronics. The donors are incorporated in the channel of (p-type) prototype transistors called FinFETs. The local electric field due to the built-in voltage between the channel and the gate electrode forms a triangular potential at the interface. The measured eigenlevels are shown to consist of levels associated with the donors Coulomb potential, levels associated with the triangular well and hybridized combinations of the two. The theoretical framework in which we describe this system is based on a tight binding approximation. The correspondence between the transport measurements, the theoretical model and the local environment of the donor provides a robust atomic understanding of actual gated donors.

The FinFET consist of a (p-type) silicon nanowire between source and drain, with a gate electrode deposited on three sides **(See Figure 1a)**. The samples in this research have a gate length of 60 nm. Due to the relatively increased capacitance between the gate eletrode and the corner regions of the nanowire, the later experiences a reduced potential. This so-called corner effect confines the source/drain-current to a narrow region at the very edges **[9]** which contains only a few As donor atoms. In about one out of seven devices the distinctive resonances of the D^0 and D^- charge states of a single As donor can be observed in the transport measurements **[7]**. These donors are (thus) located close to the gate interface. Here, we will focus on the eigenlevels of the D^0 (single electron) charge state.

The eigenlevels of the gated As donor are determined from its measured stability diagram, i.e. a plot of the differential source/drain conductance (dI/dV) as a function of bias voltage (VB) and gate voltage (VG), see **Figure 1b**. The total electric transport increases as an excited eigenlevel enters the bias window defined by source/drain, giving the stability diagram its characteristic pattern **[10]** indicated by the dashed black lines.

Six separate samples all showing at least one characteristic pair of D^0 and D^- charge states in the transport measurements where found. The eigenlevels are heavily influenced by the electric field from the nearby gate electrode. The electric field is induced by the built-in voltage between gate and channel and can be estimated to be at around 21 MV/m by electrostatic modelling of the FinFET

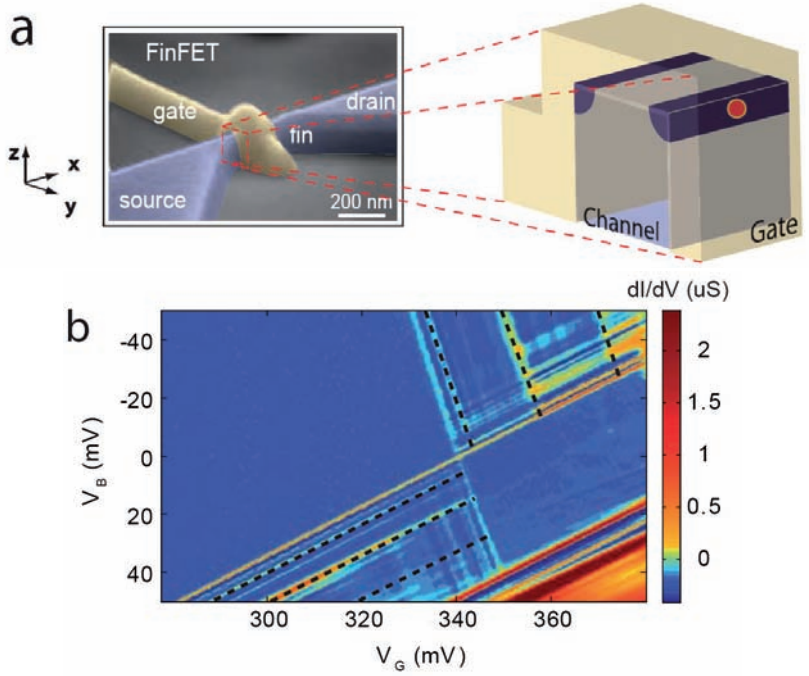

Figure 1: a) *Colored Scanning Electron Micrograph of a FinFET device. Blow-up schematically shows channel/gate with current-carrying region (dark-blue) and donor atom (red dot).* ***b)*** *Stability diagram of a typical D^0 charge state. The dashed black lines indicate the presence of excited eigenlevels.*
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device. This is quite comparable to the Bohr field of the donor, ~30 MV/m.

The eigenlevels of a gated As donor were calculated in a tight-binding approximation as a function of local electric field (F) and distance to the gate interface (d). **Figure 2a** shows the eigenenergies as a function of field for d = 4.3 nm as an example. Three electric field regimes can be distinguished. At the low field limit (F ~ 0 mV/m) we obtain the spectrum of a bulk As donor. In the high field limit (F ~ 40 MV/m) the electron is pulled into the triangular well at the interface and the donor is ionized. In the cross-over regime (F ~ 20 MV/m) the electron is delocalized over the donor and triangular well potential. Strong tunnelling interaction between the two sites causes hybridization of levels characterized by the anti-crossing behaviour of spectral lines. The ground state is an hybridized anti-bonding state of well-like and donor-like parts, see **Figure 2b**.

The correspondence we find between the measured eigenlevels in the six samples and the tight-binding approximation shows we have robust model for As donor states in a silicon three-terminal geometry. Furthermore, the model is able to predict the (independently determined) local environment of each donor, giving us confidence that we have an atomic understanding of these single gated donors.

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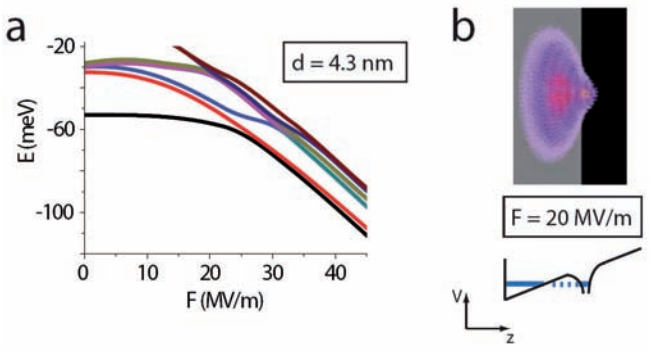

Figure 2: a) *Eigenenergies (E) of an As donor 4.3 nm be-low a SiO_2 interface as a function of electric field (F) calculated in a tight-binding approximation.* ***b)*** *Wavefunction density of the ground state of an As donor at d = 4.3 nm and F = 20 MV/m. The gray plane represents the SiO_2 interface. The ground state is a hybrid combination of donor-like and well-like states. Full color images available at: http://www.phantomsnet.net/Foundation/newsletters.php*

http://www.m4nano.com

"Modelling for Nanotechnology" (**M4nano**) is a WEB-based initiative leaded by four Spanish Institutions: **Phantoms Foundation**, **Parque Científico de Madrid (PCM)**, **Universidad Autónoma de Madrid (UAM)** and **Universidad Complutense de Madrid** to maintain a systematic flow of information among research groups and therefore avoid that research efforts in Nanomodelling remain fragmented.

Networking is vital to any scientist and even more so for persons working in the inter-disciplinary field of Nanotechnology and in particular Nanomodelling. To fulfil this necessity, **M4nano** will provide a comprehensive guide -"who's who"- of groups working in Modelling at the nanoscale listing their accomplishments, background, infrastructures, projects and publications. In this way, **M4Nano** completes the aim of both raising awareness of scientists in Nanotechnology modelling issues and aiding them in developing beneficial collaborations and employment opportunities.

Emerging research areas such as Molecular Electronics, Biotechnology, Nanophotonics, Nanofluidics or Quantum Computing could lead in the mid-term future to possible elements of nano-based devices. Modelling behaviour of these possible nanodevices is therefore becoming more and more important and should allow to: (i) Visualise what happens inside a device (ii) Optimise the devices under study (iii) Improve understanding of device properties (physical, chemical, etc.).

M4nano in close collaboration with other European Research Institutions deeply involved in "modelling at the nanoscale" will develop tools such as a user's database, a forum to stimulate discussions about the future of Nanocomputing, a source of documents (courses, seminars, etc.) on modelling issues, etc. and in the mid-term future implement a computational HUB, repository of simulation codes useful for modelling and design of nanoscale electronic devices .

Information spreading will also be enhanced using mailing list alerts, press releases and flyers. Collaborations with similar initiatives such as the **NanoHub** (USA) or **Icode** (Italy) will also be set-up.

For more Information, contact:
Dr. Antonio Correia (antonio@phantomsnet.net)

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NANO Vacancies - http://www.phantomsnet.net/Resources/jobs.php

PostDoctoral Position (Instituto de Ciencia de Materiales de Aragón, Spain): *"Molecular Approach to Nanomagnets and Multifunctional Materials (MAGMANet)"*

Carry out experiments of specific heat in the region of very low temperatures under orientable magnetic fields. Fabrication and use of highly-sensitive calorimeters of reduced sizes down to the micron and submicron scale. Investigate quantum phenomena, like quantum tunnelling, quantum coherence, and quantum phase transitions in arrays of molecular nanomagnets.

The deadline for submitting applications is March 03, 2008
Possible candidates can get further information from: Fernando Luis-Vitalla. E-mail: **fluis@unizar.es**

PostDoctoral Position (IBEC - Institut de Bioenginyeria de Catalunya, Spain): *"Near-Field Optics & SPM"*

The BioNanoPhotonics group at IBEC focuses its research activities in the field of single (bio)molecule detection and analysis working at the intersection between Physics and Biology (**http://www.nanobiolab.pcb.ub.es**). The group is currently offering one research position at PostDoc level. The successful candidate will work on the development and application of advanced near-field optical tools for the investigation of molecular processes at the single molecule level and in the living cell.

The deadline for submitting applications is February 17, 2008
Applicants should send a covering letter stating their suitability for and reasons of interest in the position, together with a full CV, including the list of published work and contact details of referees by email at **jobs@ibec.pcb.ub.es** (Ref.: Single Molecule).

PostDoctoral Position (Institut d'Imagerie Biomédicale - CEA, France): *"Design, synthesize and functionalize novel polymeric nano-sized lipidic rings ("nanorings") to explore their in vivo distribution."*

The objective of this project is to design, synthesize and functionalize novel polymeric nanosized lipidic rings ("nanorings") to explore their in vivo distribution. Nanorings are promising candidates (i) for the cargoeing of substances of biological interest such as drugs or contrast agents and (ii) as escort agents to address these compounds to specific targets via the coupling of specific ligands on their surface.

The deadline for submitting applications is February 17, 2008
For more information please contact Bertrand Tavitian (**bertrand.tavitian@cea.fr**).

PhD Position (Institut d'Imagerie Biomédicale - CEA, France): *"Design, synthesize and functionalize novel polymeric nano-sized lipidic rings ("nanorings") to explore their in vivo distribution."*

The objective of this project is to design, synthesize and functionalize novel polymeric nanosized lipidic rings ("nanorings") to explore their in vivo distribution. Nanorings are promising candidates (i) for the cargoeing of substances of biological interest such as drugs or contrast agents and (ii) as escort agents to address these compounds to specific targets via the coupling of specific ligands on their surface.

The deadline for submitting applications is February 17, 2008
For more information please contact Bertrand Tavitian (**bertrand.tavitian@cea.fr**).

PhD Position (DRFMC-CEA-GRENOBLE, France): *"Non-contact atomic force microscopy investigations of self-organized pi-conjugated molecular wires"*

Our group is now mastering the self-organization and NC-AFM imaging with sub-molecular resolution of oligomer-based molecular wires on conducting surfaces. To realize a molecular wire based device, the next step is to achieve similar levels of molecular structuration and of near-field imaging resolution on insulating surfaces.

NANO Vacancies - http://www.phantomsnet.net/Resources/jobs.php

The deadline for submitting applications is March 15, 2008
Applications (to be sent at **benjamin.grevin@cea.fr**) should include a detailed curriculum vitae and a letter in which the applicant state her/his interests and experiences relevant to this project.

PhD Position (UPV/EHU, Spain): *"Problems related to the characterization of the electronic properties of complex systems"*

The research projects will directly address fundamental problems related to the characterization of the electronic properties of complex systems. Topics to be addressed are:

* Theoretical description of charge transport in molecular systems
* Quantum dots and nanotubes for quantum information applications.
* Nanostructured materials for photovoltaic devices

We consider applications until the position is filled. Please send your application as soon as possible to Angel Rubio (**Angel.Rubio@ehu.es**).

PostDoctoral Positions (UPV/EHU, Spain): *"Two postdoc positions on applications to complex systems with biological interest"*

Postdoc position on applications to complex systems with biological interest: our purpose is to understand the specific process of light emission by the chromophore. This will allow us to extract information about its “in-vivo” conformation and how its environment, inside the protein, leads to changes in the emission properties (color and intensity). The latter problem suggests the investigation of new ideas beyond present TDDFT/QM/MM implementations. Our last objective is to pave the way for a completely new “divide and conquer” strategy, with the aim of incorporating the dynamical coupling between the optically active part of the protein, the protein itself, and the surroundings (solvent, substratum, etc.)

We consider applications until the positions are filled. Please send your application as soon as possible to Angel Rubio (**Angel.Rubio@ehu.es**).

PostDoctoral Position (Academy of Sciences of the Czech Republic, Czech Republic): *"Chemistry of Functional Molecules Group: Organic synthesis of helical aromatics for an application in molecular electronics"*

A post-doctoral position is available to perform a complex synthesis of various helical aromatics in their racemic and nonracemic forms. The target compounds will be subjected to physicochemical studies being focused on the conductance aspects and employing AFM/STM. The present position is funded by the European Integrated Project "Pico-Inside" (**www.picoinside.org**).

Applications including her/his CV, list of publications and the names of two referees should be sent by e-mail to: **stara@uochb.cas.cz**

PhD Position (Université de Bordeaux, France): *"QM/MM treatment of localized excited states in organic-inorganic hybrid materials and application to photo sensitizers grafted on silica."*

The position is based in Pau, with travel to Bordeaux and short internships with developers of the component codes in Daresbury, UK and San Sebastian in Spain. Candidates should have a Masters degree or equivalent in scientic computing and quantum and classical molecular modelling.

Interested candidates are encouraged to contact I. Baraille **(isabelle.baraille@univ-pau.fr)** and provide vitae, list of publications and letters of recommendation.

NANO Conferences http://www.phantomsnet.net/Resources/cc.php

(February 2008)

4th International Conference on Industrial Dimensional Metrology (Metromeet2008).
21-22, February 2008. Bilbao (Spain)
http://www.metromeet.org
NanoMetrology & Standards

Nano Tech 2008 - Nano Bio 2008.
13-15 February, 2008. Tokyo (Japan)
http://www.ics-inc.co.jp/nanotech/en/index.html
Nanotechnologies

Women in Nano Winter School.
07-09 February, 2008. Kranjska Gora (Slovenia)
http://nano.ijs.si/womeninnano_winterschool.html
Nanotechnologies

Hot Topics in Molecular Imaging 2008 - TOPIM'08.
04-08 February, 2008. Les Houches (France)
http://www.e-smi.eu
Nanotechnologies, Molecular Imaging

(March 2008)

NanoFair2008 - New Ideas for Industry.
11-12 March, 2008. Dresden (Germany)
http://www.vdi-wissensforum.de/index.php?id=769
Nanoelectronics, NanoMaterials, Nanotechnologies

NANO Conferences http://www.phantomsnet.net/Resources/cc.php

Porous Semiconductors - Science and Technology.
10-14 March, 2008. Mallorca (Spain)
http://www.mtm.upv.es/psst2008/START/BeginFlash.asp?nocache=125517
NanoPhotonics & Nano-Optoelectronics, NanoChemistry, NanoSensors & NanoDevices

NanoECO. Nanoparticles in the Environment-Implications and Applications.
02-07 March, 2008. Monte Verità (Switzerland)
http://www.empa.ch
NanoMaterials, NanoFabrication, Nanotechnologies

(April 2008)

Nanospain2008.
14-18 April, 2008. Braga (Portugal)
http://www.nanospainconf.org
Nanoelectronics, NanoBiotechnology, Nanotechnologies

International Meeting on the Chemistry of Nanotubes : Science and Applications.
06-09 April, 2008. Zaragoza, (Spain)
http://www.icb.csic.es/fileadmin/grupos/G-CNN/CHEMONTUBES2008/chemontubes_announcement.pdf
NanoTubes, NanoChemistry

NanoEnergy2008.
01-03 April, 2008. Paris (France)
http://www.upperside.fr/nanoenergy2008/nanoenergy2008intro.htm
Energy

NANO News - http://www.phantomsnet.net/Resources/news.php

Insulated nanowires bring molecular machines one step closer (January 11, 2008)
http://www.nanowerk.com/news/newsid=4014.php
In a development that brings superdense memory devices and molecule-sized machines a step closer to reality, scientists at Japan's Institute of Physical and Chemical Research (RIKEN) have succeeded in creating 1-nanometer-thick electric wires with a layer of insulation.
Keywords: Molecular Electronics

Complete list of FP7 nanotechnology calls for proposal (January 10, 2008)
http://www.nanowerk.com/news/newsid=4001.php
Cordis has published an overview list of activities in the Seventh Framework Program (FP7) with calls for proposals in 2008 of direct relevance to nanotechnology.
Keywords: Scientific Policy

First projects launched under FP7 now profiled on CORDIS (January 01, 2008)
http://www.nanowerk.com/news/newsid=3865.php
Information on the first projects accepted for funding under FP7 has now also been published on CORDIS.
Keywords: Scientific Policy

Flat nanotubes (January 11, 2008)
http://www.nanowerk.com/spotlight/spotid=4012.php
Researchers have synthesized novel, unconventional nanotubes that are distinctly different from any previously reported nano- and microtubes. These tubes display flattened and thin belt- or ribbon-like morphologies.
Keywords: Carbon Nanotubes

Nanotechnology innovation may revolutionize gene detection in a single cell (January 10, 2008)
http://www.physorg.com/news119196747.html
Scientists at Arizona State University's Biodesign Institute have developed the world's first gene detection platform made up entirely from self-assembled DNA nanostructures.
Keywords: Nanobiotechnology, Self-Assembly

EU projects minimize size of semiconductor chips (January 09, 2008)
hhttp://www.nanowerk.com/news/newsid=3979.php
Two EU-funded projects have been pushing the limits of chip miniaturization, trying to make complementary metal-oxide semiconductor chips (CMOS) even smaller than they already are. While the NanoCMOS project, which was completed in 2006, helped develop 45 nanometer (nm) node semiconductors, its follow-up project NANOPULL is aiming at 32nm and ultimately 22nm features.
Keywords: Nanoelectronics, Nanofabrication

Berkeley Researchers Make Thermoelectric Breakthrough in Silicon Nanowires (January 09, 2008)
http://www.lbl.gov/Science-Articles/Archive/MSD-silicon-nanowires.html
Energy now lost as heat during the production of electricity could be harnessed through the use of silicon nanowires synthesized via a technique developed by researchers with the U.S
Keywords: Nanomaterials, Nanofabrication

Carbon Nanotubes could lead to new bottom-up-engineered nanostructures and devices (January 07, 2008)
http://www.physorg.com/news118920838.html
Scientists have determined how to connect carbon nanotubes together like water pipes.
Keywords: Carbon Nanotubes, Nanosensors & Nanodevices

US National Nanotechnology Initiative releases new strategic plan (January 02, 2008)
http://www.nanowerk.com/news/newsid=3878.php
The 2007 NNI Strategic Plan describes the vision, goals, and priorities of the NNI to ensure that the United States derives growing economic benefits and improved quality of life for its citizens and remains a global leader in nanotechnology R&D in the years to come.
Keywords: Scientific Policy

Water as a mediator of protein-nanoparticle interactions: entropy driven protein binding as a paradigm for protein therapeutics in the Biopharma industry?

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Abstract
The role of water as a mediator of protein-nanoparticle interactions has largely been overlooked to date. Many of the techniques available depend on there being a significant conformational change upon interaction in order for there to be a measurable output. We argue that in analogue with many well-known assembly reactions in biology, protein-nanoparticle association may be entropy driven and governed by the release of water and/or counter ions. We report examples where proteins seem to bind to surfaces with minimal change to their conformation and in an entropy-driven process, whereby the binding of the protein at the surface releases large numbers of water molecules, thereby increasing the entropy of the system overall. As this binding mechanism is completely non-perturbing to the protein, it can easily be overlooked, but it has enormous potential for the design of protein therapeutics, where the proteins can be delivered via the nanoparticles without loss of their functionality as a result of the binding process.

Introduction
Protein therapies are considered to be leading candidates for treating a wide range of diseases from deficiencies (such as Pompe disease, a glycogen storage disease resulting from a deficiency of acidalpha glucosidase) **[1]**, to more well-known examples ranging from cancer, to neurodegeneration, bone diseases and many others **[2, 3]**. The advantages of protein therapies include low dosage, high specificity and the fact that is now possible to produce proteins in large quantities, and of high quality **[4]**. However for some of these examples, there are considerable challenges to be overcome before some protein therapies will become a reality. Interestingly enough, as the challenge of making sufficient quantities of protein (by recombinant techniques) diminishes, a primary challenge that arises is the delivery of enough of the protein to the right location. This is sometimes attempted by encapsulation of proteins in order to protect them from enzymatic degradation *in vivo*, as well as other targeting machinery. However, less well understood is the challenge of ensuring that the encapsulation process itself does not irreversibly denature or deactivate the protein. Nanoparticles are likely to be a versatile route to protein delivery, as they are small enough to access locations relevant for protein therapeutics, such as the brain (likely accessible for particles somewhat less than *circa* 30 nm), and sub-cellular locations (access to the cell is feasible for particles less than *circa* 100 nm) including the nucleus (likely accessible *circa* 35 nm).
Nanoparticle delivery systems can improve the stability of therapeutic agents against enzymatic degradation **[5, 6]**, and modulate the release rate to achieve the desired therapeutic levels in target tissues the required duration for optimal therapeutic efficacy **[7]**.There is also the intriguing (though as yet speculative) possibility that they could circumvent the multi-drug resistance that has arisen towards many anticancer drugs **[8]**. Maintenance of protein activity (and thereby conformational stability) remains one of the most important challenges to widespread and successful use of biodegradable drug delivery formulations, such as nano- or micro-particulate carriers. Successful nanoparticle delivery systems require a high loading capacity to reduce the quantity of the carrier required for administration. This suggests that either some sort of shell architecture or bulk incorporation would be preferred over surface carriage (the latter has numerous other limitations for many treatments). Shell architectures are notoriously difficult to regulate, and there are indications that a bulk loading of the protein drug would be useful. Here however, one necessarily has the issue of extensive contact between the protein and encapsulating material. The consequences of this are largely unknown but some possibilities may be inferred from the analogy of protein associations with surfaces **[9-15]**. In particular, it is known that surface adsorption may alter the conformation of the protein, and indirectly, the protein functionality. These conformational changes adsorption vary greatly depending on the nature of the surface **[16-18]**, and the protein stability **[19, 20]**. The protein may be bound in a preferred orientation **[21, 22]** and the degree of denaturation depends on the foreign surface composition. Some proteins are bound in native-like fashion **[23]**, some experience increased domain motions, others undergo denaturation of the tertiary structure, and in some case even the secondary structure is lost **[24, 25]**. Nanoparticles may induce quite different structural effects than “flat” surfaces **[25]**. An important issue is also whether these effects are reversible or irreversible, as a reversible process will allow released protein to regain its native structure and be fully functional. In this paper we wish to address the general question of which materials might be suitable as nanoparticle carriers of drugs. We emphasize that our discussion is of a more strategic nature, but here we address only one particular issue, that of protein stability in intimate contact with the carrier. We will not enter into the details of biocompatibility, biodegradability and clearance pathways, all of which are also central to any application of this type. We emphasize an approach to materials design that will lead to association of the protein molecules to the carrier material, without loss of functionality. We will employ the idea that high affinity between particles and protein can arise in many different ways through different combinations of non-covalent intermolecular interactions. Thus high affinity may arise both through interactions that involve conformational changes of the protein or through interactions involving a largely unaffected protein structure. There may be an interesting paradigm around delivery vehicles for protein therapies could be built. In order for binding of proteins to a surface to occur spontaneous-

ly, the overall free energy of the system must be lowered, according to Gibbs law of free energy:

$$\Delta_{ads} G = \Delta_{ads} H - T\Delta_{ads} S < 0$$

where Δ_{ads} is the net change of the thermodynamic parameters G (Gibbs free energy), H (enthalpy) and S (entropy) upon binding, and T is the absolute temperature. The main contributions to the energy and entropy of protein binding have been classified by Norde as follows **[26]**:

(a) electrostatic interactions between proteins and charged surfaces enabling charge neutralisation with release of small counterions;

(b) dispersion interactions, which are always attractive but which decrease with increasing distance between protein and surface;

(c) dehydration of the surface and parts of the protein molecule gives an increase in entropy which governs association

(d) structural rearrangements of the proteins which can contribute to both enthalpy and entropy by decreasing the ordered secondary structure and increasing the intra-molecule hydrogen bonding, depending on the polarity of the surface and the rigidity of the protein.

Of these, dehydration and structural rearrangements are considered to be the dominant contributions determining adsorption processes. It has to be noted here that of course structural rearrangement always goes against binding. If the bound conformation had lower free energy than the free conformation, the protein would already be there in its free state. However, part of the available free energy of binding can be used by a system to drive a conformational change upon binding, case the binding affinity is lower compared to of the interacting partners being pre-structured in or close to the bound conformation also in their free states. Hydrogen bonding contributes little to association reactions. This is because all hydrogen bonding donors and acceptors are commonly satisfied both in the bound and free states, either by hydrogen bonding to water or to other particle or protein groups, and hydrogen bonds with different partners is close to isoenergetic.

Clearly, for applications involving protein therapeutics, irreversible structural rearrangement of the proteins is highly undesirable, as the functionality of proteins is so closely dependent on the three dimensional structure **[27, 28]**. In spite of this, considerable research efforts are being directed towards the binding of proteins to oppositely charged surfaces, as a method of achieving high loading capacities. A preliminary screening of these papers suggests that in many cases the authors do not study the conformational changes of the proteins in the bound state. In other cases protein activity is studied upon release, but here the examples are often restricted to small proteins with reversible folding such that refolding occurs spontaneously after release. We also note that such studies are performed immediately upon loading, not taking into account changes that occur to the protein conformation after long-term adsorption at the surface **[29, 30]**. A fruitful scientific approach would be to rely only on the entropic contribution when water is released upon protein binding to the carrier, and to seek to eliminate the enthalpy contributions (and protein conformational changes) entirely. The concept is simple. A large protein molecule that competes effectively with water on the carrier material will, when it binds, release many tens of water molecules, and thereby many degrees of freedom, with the loss of only the degrees of freedom attributable to the single protein molecule. This is an enormous force for protein binding, without any effect on the enthalpic balance, and therefore without any effect on the activity of the protein.

As an example of our ideas, we discuss the use of novel temperature-responsive nanoparticles as carriers for protein therapeutics. There is nothing exceptional about these materials, from a more general point of view, and we introduce them only for the purpose of illustration. Thus, we present a set of tailored copolymer nanoparticles based on N-isopropylacrylamide and N-*tert*-butylacrylamide that allows us to systematically investigate how the size and composition (hydrophobicity) of the particles affects their interaction with proteins, and the nature of the binding process. Using isothermal titration calorimetry (ITC) we assess the enthalpy (and indirectly the entropy) changes upon binding as a function of the nanoparticle hydrophobicity, and find that for these nanoparticles the binding of many different proteins (including fibrinogen, lysosyme, ovalbumin and human carbonic anhydrase II) is driven by an increase of entropy, with almost no enthalpy change. On the contrary, the binding of human serum albumin is enthalpy driven. Circular dichroism experiments confirm that the binding of these proteins to the nanoparticles does not induce any significant conformational changes to the proteins. Thus, we suggest that release of structured water from the nanoparticle surface is the driving force for the protein binding to the surfaces, without the need for structural rearrangement of the protein.

Experimental Details

Nanoparticles.

N-*iso*propylacrylamide-co-N-*tert*-butylacrylamide (NIPAM:BAM) copolymer particles of 70 and 200 nm diameter and with three different ratios of the co-monomers (85:15, 65:35 and 50:50 NIPAM:BAM) were synthesized in SDS micelles as described previously **[31]**. The procedure for the synthesis was as follows: 2.8g monomers (in the appropriate wt/wt ratio), and 0.28g crosslinker (N,N-methylenebisacrylamide) was dissolved in 190mL MilliQ water with either 0.8g SDS (for the 70nm particles) or 0.32g SDS (for the 200nm particles) and degassed by bubbling with N_2 for 30 minutes. Polymerisation was induced by adding 0.095g ammonium persulfate initiator in 10 mL MilliQ water and heating at 70 °C for 4 hours. Particles were extensively dialysed against MilliQ water for several weeks, changing the water daily, until no traces of monomers, crosslinker, initiator or SDS could be detected by proton NMR (spectra were acquired in D_2O using a 500 MHz Varian Inova spectrometer). Particles were freeze-dried and stored in the fridge until used.

Proteins.

A range of different proteins were selected for this study, to cover a range of parameters such as protein size, protein charge, protein fold (representatives of several diffe-

rent fold-classes are used) as well as proteins that are major components of human plasma and/or implicated in cell-material interactions. The proteins are summarised in **Table 1.**

Name	ca.Mw / kDa	Fold type	Special properties	P	B
Human serum albumin	66	α	Plasma protein Fatty acid binding	X	
Fibrinogen	340	β	Plasma protein. Surface active	X	
Ovalbumin	~45	α /β	Serpin-like		S
Carbonic anhydrase II	30	β barrel	Zinc binding. Enzyme.	X	
Calmodulin	16	α EF-hand	Key intracellular signalling protein	X	
α -lactalbumin	14	α /β	Most common protein in human milk Forms HAMLET with oleic acid	X	
Lysozyme	14	α /β	Cell-lysing. Basic	X	
PGB1	6	β grasp	IgG binding	X	

Table 1: *P = purified in our lab. B = bought or obtained from other lab. S= Sigma.*

Human serum albumin from Sigma (A3782, fatty acid free, 99% pure) was purified from dimer and contaminating proteins using gel filtration on a 200 x 3.4 cm Sephadex G50 column in ammonium acetate buffer, pH 6.5 (75 mg protein in 20 ml buffer was applied at a time). Fractions containing HSA monomer were pooled, lyophilized and desalted by gel filtration on a G25 Sephadex superfine column in Millipore water. Analytical gel filtration showed that after this procedure more than 99% of the protein was in the monomeric form.

Human fibrinogen was purchased from Sigma and purified by gel filtration.

Ovalbumin was purchased from Sigma.

Human carbonic anhydrase II ("pseudo wildtype" with the C206S mutation) was expressed from the pCApwt plasmid (a kind gift from Professor Bengt-Harald Jonsson, Linköping University) in *E. coli* Bl21 De3 PlysS Star. The cells were harvested by centrifugation at 6000 g for 5 minutes and the pellet was frozen. The protein was purified using ion exchange chromatography and gel filtration as follows. The cell pellet was suspended in buffer A (10 mM Tris/HCl, pH 7.5 with 1 mM EDTA; total volume 200 ml for pellet from a 5.4 litre culture), sonicated on ice, poured into an equal volume of boiling buffer A, heated to 85 ºC, and then rapidly cooled on ice. Precipitated *E. coli* protein were removed by centrifugation at 15000 rpm for 15 minutes, and the supernatant was pumped on a (3.4 x 25 cm) DEAE cellulose anion exchange column that was eluted using a NaCl gradient from 0 to 0.2 M in buffer A. Carbonic anhydrase II fractions were lyophlized, dissolved in 25 ml H_2O, and applied to a 3.4 x 180 cm sephadex G50 superfine gel filtration column. The column was packed and operated in 50 mM ammonium acetate buffer, pH 6.5. The pure carbonic anhydrase II fractions were pooled and dialyzed against Millipore water.

Human calmodulin was expressed from a Pet3a plasmid in *E. coli* Bl21 De3 PlysS Star, and purified using heat treatment, DEAE cellulose anion exchange chromatography, hydrophobic interaction chromatography on a phenyl sepharose column, gel filtration DEAE sephacel anion exchange chromatography **[32]**.

Human α-lactalbumin was purified from human milk using ammonium sulphate precipitation, hydrophobic interaction chromatography on a phenyl sepharose column, and dialysed **[33]**.

Lysozyme from chicken egg white from Sigma purified using ion exchange chromatography and gel filtration.

Protein G B1 was expressed in *E. coli* Bl21 De3 PlysS Star from a synthetic gene cloned into PetSac plasmid, and purifed using anion exchange chromatography (DEAE ce-llulose column) and gel filtration (3.4 x 180 cm sephadex G50 superfine column) **[34]**.

The purity of proteins was confirmed by SDS PAGE and agarose gel electrophoresis, isoelectric focussing and ^{1}H NMR spectroscopy.

Particle handling.

Due to the inverse solubility of polyNIPAM, particle solutions were prepared by dissolving the particles on ice to ensure good solubility of the particles (i.e. to ensure that the solutions are below the lower critical solution temperature of the particles).

Thiol-linked nanoparticles.

NIPAM:BAM:acrylic acid copolymer nanoparticles were synthesized as above, with the addition of appropriate amounts of acrylic acid to obtain particles with on average less than one carboxyl group on the particle surface. Acrylic acid was distilled under reduced pressure before use to remove stabilizers. A stock solution of 1 mg/ml acrylic acid was prepared, and 10 μL (70nm particles) or 1.4 μL (200nm particles) of this solution was added to the monomer solution. Reaction proceeded at 70 °C for 4 hours followed by dialysis against MilliQ water for a couple of weeks. The covalent attachment of homocysteine to the acrylic acid groups involves the formation of amide bonds between the primary amino group of the amino acid and carboxylic acid **[35]**. Briefly, 50 mL of the particle solution (after dialysis) was adjusted to pH 5 by small amounts of 5 M NaOH. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride was added to a final concentration of 150 mM to activate the carboxylic acid moieties. After 1 hour of incubation with stirring at 4 °C, 0.4 g homocysteine was added and the pH was readjusted to 5. The reaction mixture was incubated for 5 h at room temperature under stirring, dialysed extensively against MilliQ water to ensure that no residual chemicals remained, and freeze-dried.

Conjugation of nanoparticles to gold surfaces for SPR studies.

The SIA Au kit (BIAcore AB, Uppsala) was used for sensor chip preparation. Thiol-linked nanoparticles were dissolved at 0.2 mg/ml in 20 mM sodium phosphate buffer, 100 mM NaCl, pH 7.5) on ice and 120 μl was applied to a 10 x 10 mm gold surface for four hours or over night, before the surface was rinsed with H_2O, dried and assembled in a sensorchip cassette. The change in response units after coupling of the nanoparticles to gold reveals the amount of immobilized nanoparticles. Densely packed layers of 70 and 200 nm particles yields 35 kRU and 100 kRU, respectively, and the increase in response obtained in separate coupling trials ranged from 20 to 50 % of these numbers, indicating efficient coupling of the particles.

Isothermal titration calorimetry.

Individual proteins were titrated from stock solutions (concentration varied from protein to protein, but were typically in the range 1 mM) into nanoparticle solution in 10 mM Hepes/NaOH buffer, pH 7.5 with 150 mM NaCl and 1 mM EDTA at 5ºC. The reaction cell contained 9 nM of the 70 nm or 0.4 nM of the 200 nm particles composed of 85:15 or 50:50 NIPAM:BAM. The first protein injection was 1 μl followed by a series of 5 μl injections. The protein concentration in the stock solutions were determined by amino acid analysis after acid hydrolysis (analysis purchased from BMC Uppsala). Data were fitted using equation 1 which assumes a simple 1:1 binding isotherm with affinity, stoichiometry and ΔH as variable parameters, yielding the change in heat content from injection i-1 to i as

$$\Delta Q(i) = Q(i) - Q(i-1) + \frac{dV_i}{V_0}\left[\frac{Q(i)+Q(i-1)}{2}\right] \qquad (1)$$

Each heat of injection is (equation 2)

$$Q = \frac{V_{cell}[np]_{tot} N\Delta H^o}{2}\left(1 + \frac{[HSA]_{tot}}{N[np]_{tot}} + \frac{1}{K_A N[np]_{tot}} - \sqrt{\left(1 + \frac{[HSA]_{tot}}{N[np]_{tot}} + \frac{1}{K_A N[np]_{tot}}\right)^2 - \frac{4[HSA]_{tot}}{N[np]_{tot}}}\right) \qquad (2)$$

where N is the number of moles of adsorbed HSA per particle, ΔH^o is the enthalpy change upon binding (J/mol HSA), V_{cell} is the cell volume and K_A is the equilibrium association constant.

Surface Plasmon Resonance (SPR) experiments.

SPR studies of protein associating to and dissociating from nanoparticles were performed using a BIAcore 3000 instrument (BIAcore AB, Uppsala). The flow buffer contained 10 mM Tris/HCl pH 7.4 with 3 mM EDTA, 150 mM NaCl and 0.005% Tween20, and was filtered (0.2 μm filter) and degassed for at least 30 minutes. Each sensorchip surface with attached particles was washed for at least 5 hours at a flow rate of 50-100 μl/min and then equilibrated at 10 μl/min for at least 30 minutes or until the baseline was stable. Single proteins or full plasma were alkylated with iodoacetamide to eliminate free thiol groups to avoid covalent coupling to the gold, using 25 mM iodoacetamide in 25 mM ammonium hydrogen carbonate buffer followed by gel filtration on a Nap-10 column (Amersham Biosciences) to remove excess reagent. The proteins were diluted in the flow buffer and injected over 30 minutes to study the association kinetics. After 30 minutes, buffer was flown over the sensorchip surface for 10-24 hours at 10 μl/min.

Association and dissociation data were fitted using equations 3 and 4, respectively

$$R(t) = C1\,(k^{1on}/(k^{1on}+k^{1off}))\,(1-\exp(-(k^{1on}+k^{1off})t)) + C2\,(k^{2on}/(k^{2on}+k^{2off}))\,(1-\exp(-(k^{2on}+k^{2off})t)) \qquad (3)$$

$$R(t) = A1\exp(-k^{1off}t) + A2\exp(-k^{2off}t) \qquad (4)$$

Data for single proteins were fitted assuming single processes, i.e. C2 and A2 were set to zero.

Circular Dichroism.

$$K_A = \frac{[np \bullet HSA]}{[np][HSA]}$$

CD spectra were recorded using a Jasco J-720 spectropolarimeter, thermostated at 10 °C. Spectra in the near-UV region (320-250 nm) used 0.25 mg/ml protein diluted in physiological buffer in a 10 mm quartz cell. Protein spectra were determined in the absence and presence of 10 mg/ml particles of 70 nm diameter with 50:50, 65:35 or 85:15 NIPAM:BAM.

Results

We have recently reported a detailed study of the binding of human serum albumin (HSA) to nanoparticles of increasing hydrophobicity and size, and found that the association reaction was exothermic for all levels of nanoparticle hydrophobicity and all nanoparticle sizes **[23].** Strikingly, a much larger number of injection was needed to reach saturation for the more hydrophobic particles compared to the more hydrophilic ones, implying a higher surface coverage for the more hydrophobic particles. Furthermore, the signal for several of the more hydrophilic particles (75:25, 85:15 or 100:0 NIPAM:BAM particles) was only slightly higher than in the blank titration (HSA into water) indicating that adsorption to these particles occurs with a smaller enthalpy change. The affinity does not vary drastically over the hydrophobicity range but a small trend towards higher affinity for the more hydrophilic particles was observed. The enthalpy change was negative in all cases, and was more negative for the most hydrophobic particles **[23]**. A typical ITC binding curve for HSA titrated into copolymer nanoparticles is shown in **Figure 1 page 28**, illustrating the exothermic nature of the binding. In this case the entropy change upon binding was unfavourable and the binding process is enthalpy driven **[23]**.

Having shown that HSA binds to the copolymer nanoparticles, we were then interested to determine if the binding

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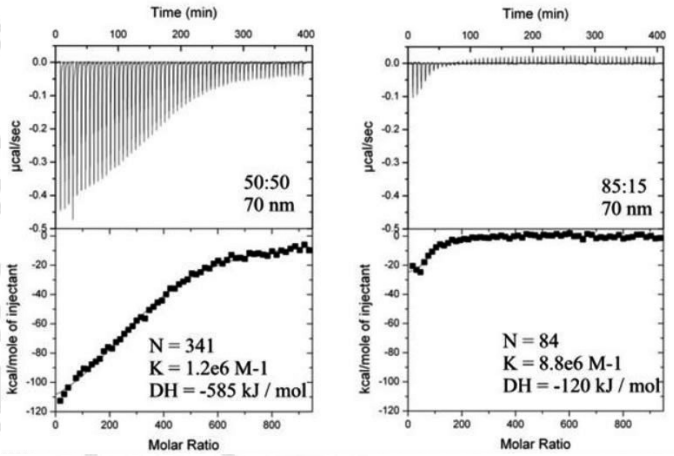

Figure 1: *Raw ITC data for HSA titrated into a 9.3 nM solution of 70 nm 50:50 and 15:85 particles, and binding isotherm of HSA to copolymer particles.*

affected the conformation of the proteins. To this end, near-UV spectra of HSA were determined in the absence and presence of 70nm copolymer nanoparticles of increasing hydrophobicity. Circular dichroism (CD) spectroscopy measures differences in the absorption of left-handed polarized light verses right-handed polarized light which arise due to structural asymmetry. Thus it can be used to detect structural differences between the protein in solution (i.e. in its native state) and protein bound to a particle. Near-UV (350–250 nm) CD measurements give information on the structural confinement of aromatic residues and SS-bonds and may thereby report on changes in the tertiary structure. From **Figure 2**, it is clear that adsorption of HSA to the copolymer nanoparticles does not induce very major structural re-arrangements, although the degree of conformational change increases somewhat on the more hydrophobic (50:50) nanoparticles. While local or global unfolding would lead to a decrease in near-UV CD signal, the near-UV CD signal of HSA increases upon binding to the particles, suggesting if anything a slight increase in the rigidity of the native structure. Like all spectroscopies, CD reports on the average properties of the system, so great care was taken in these measurements to ensure that protein and nanoparticle concentrations were such that all of the HSA was bound to the nanoparticles (based on the binding stoichiometry determined by ITC).

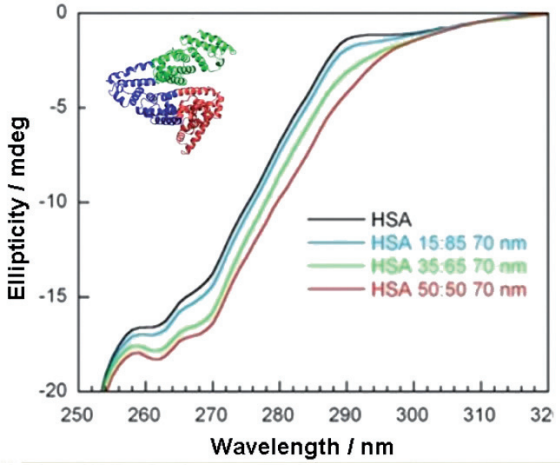

Figure 2: *Circular dichroism data for HSA with 70 nm copolymer particles of increasing hydrophobicity, indicating that as the particle surface hydrophobicity increases (going from 15-50% of the hydrophobic comonomer) the conformation of HSA changes slightly, due to increasing structural confinement of aromatic residues and SS-bonds by the hydrophobic surfaces.*

Further evidence that binding of HSA to the copolymer nanoparticles does not result in significant conformational changes can be obtained from limited proteolysis experiments. In these experiments, the fragmentation or digestion pattern of HSA in the native state is compared to the digestion pattern of HSA bound to the nanoparticles. Because the protease used (trypsin) is rather large, and because the adsorbed proteins are expected to be close together on the surface, we anticipate that only cleavage sites at the outermost layer of the adsorbed protein will be accessible to limited proteolysis for steric reasons. Preliminary studies (unpublished data) suggest that there are no new enzymatically accessible sites (not present in the native configurations) introduced by adsorption of HSA to the particles. Instead, we simply see an increase in the rates of digestion. This suggests that diffusion of the enzyme from one HSA to the next is facilitated by them being bound closely together at the surface. Having confirmed that binding of HSA to copolymer nanoparticles is enthalpy driven and occurs without major structural arrangements, we were interested to see if this was a general phenomenon of protein binding to the copolymer nanoparticles. To this end, several other proteins with different physical properties, such as size, charge and fold class as listed in Table 1, were also studied by ITC and CD to determine the driving force for binding and the effect of binding on the protein conformation. The proteins studied include α-lactalbumin, calmodulin, lysozyme, fibrinogen, Protein G B1 domain, ovalbumin and human carbonic anhydrase II, and for each of these proteins, no signal could be observed by ITC. Data for two of these proteins (α-lactalbumin and human carbonic anhydrase II) are shown in **Figure 3** as representative data. The reason for this lack of signal could be that adsorption occurs without an enthalpy change (that is, the binding is entirely entropy driven due to the release of water), or these proteins may not bind to the particles. One way to distinguish binding with certainty is surface plasmon resonance, which determines changes in the surface thickness upon binding of proteins. In these experiments, the same set of proteins were injected one-by-one over 70 nm 50:50 particles that had been linked via a thiol group to gold. The SPR curves are shown in **Figure 4 page 29** clear that α-lactalbumin,

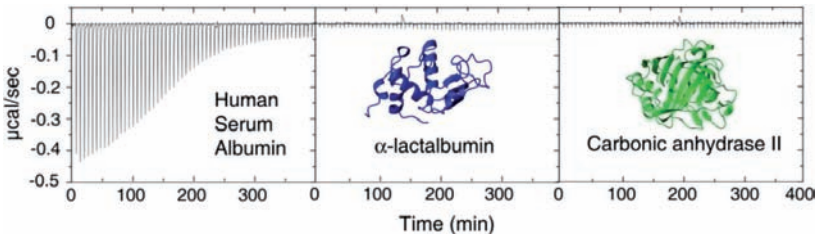

Figure 3: *Raw ITC data for different proteins titrated into a 9.3 nM solution of 70 nm 50:50 particles.* ***a)*** *human serum albumin;* ***b)*** *α-lactalbumin and* ***c)*** *human carbonic anhydrase II. All panels are plotted on the same scale and the proteins are injected at approximately the same molar concentrations.*

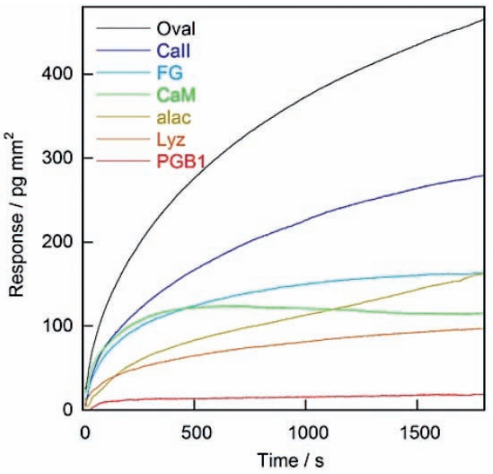

Figure 4: *SPR data for ovalbumin (black), carbonic anhydrase II (blue), fibrinogen (light blue), calmodulin (green), α-lactalbumin (brown), lysozyme (orange) and the protein G B1 domain (red) injected over 70 nm 50:50 NIPAM:BAM particles linked via a thiol group to gold. Each protein was dissolved at 5-20 μM in 10 mM Hepes/KOH, 0.15 M NaCl, pH 7.4 with 3 mM EDTA and 0.005 Tween20. The same buffer was used as the running buffer.*

calmodulin, lysozyme, fibrinogen, ovalbumin and human carbonic anhydrase II all interact with the 70 nm 50:50 particles. Only Protein G B1 domain was found by SPR not to bind to the 50:50 BAM:NIPAM copolymer particles. Thus we can conclude that PGB1 associates weakly or not at all with the particles, while for the majority of the proteins tested, association with the particles is an entropy-driven process, which explains why no signal was observed by ITC.

Having determined that the majority of the proteins in Table 1 bind to the copolymer nanoparticles, we could also confirm that the binding did not alter the protein conformation by CD spectroscopy. Again, protein and nanoparticle concentrations were controlled to ensure that all protein was bound to the nanoparticles to ensure that the signal was not averaged over bound and unbound protein states. Here, even less effect of the copolymers on the protein conformations was observed, even for the most hydrophobic copolymer particles (50:50 BAM:NIPAM) where the signals overlaid, as shown in **Figure 5 page 30**.

Discussion

In aqueous solution, native globular proteins are folded with their hydrophobic groups clustered in the core away from solvent. Billions of years of evolution of the amino acid sequence has selected hydrophobic residues with shape complementarity to allow for close packing of the cores **[37-39]**. Proteins are nevertheless marginally stable as the beneficial interactions that govern the native structure are counterbalanced by a large entropic loss on going from a large ensemble of unfolded states to a more restricted set of conformations, as well as by repulsive electrostatic interactions present in the native state **[40]**. The fact that surfaces exposed to physiological solutions are immediately coated by a layer of adsorbed proteins **[36, 41, 42]** implies that the binding is a favourable process, resulting either in a reduced enthalpy, or an increase in entropy due to increased conformational freedom of the protein or release of structured water. Dehydration of parts of the protein molecule is often a major factor in protein binding. In the free state, polar and non-polar groups at the protein and particle surface are solvated by counter ions and water molecules, and a fraction of these ions and water molecules are released upon association between protein and particle giving a favourable entropic contribution to the free energy of association **[43]**. However, as we have shown here, the binding of a range of different proteins to the copolymer nanoparticles does not result in major structural rearrangements or loss of conformation, so it is unlikely that the binding results in release of water from the sites of interaction on protein and particle. Furthermore we have also shown that the enthalpy of binding of some of these systems is very small, so it is clear that the entropic gain comes from release of water either from the protein, or polymer, or more likely from both.

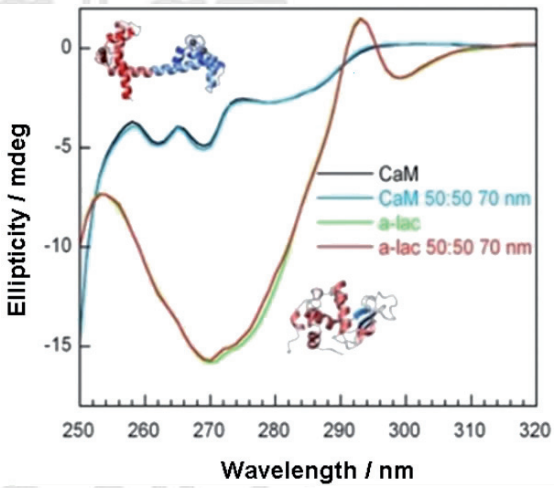

Figure 5: *CD data for α-lactalbumin and calmodulin with 70 nm 50:50 BAM:NIPAM copolymer particles (the most hydrophobic particles used in this study). No evidence of conformational change is observed.*

The copolymer nanoparticles used in this study are composed of N-isopropylacrylamide (NIPAM) and N-tert-butylacrylamide. Polymers composed of N-isopropylacrylamide show interesting phase behavior in aqueous solution. At low temperatures they display excellent solubility and exist as expanded coils. Raising the temperature gradually causes the polymer chains to contract slightly until the temperature reaches a critical temperature, called the lower critical solution temperature (LCST) at which point the polymer chains undergoe a transition to a collapsed globular state. The collapse transition results from the fact that with increasing temperature the isopropyl groups become increasingly hydrophobic and eventually the entropy-driven hydrophobic effect overcomes the energy-driven hydrogen bonding between the amide groups and the surrounding water, causing the side chains to become attractive to each other and contract **[44-46]**. This behavior is reminiscent of the thermal denaturation of proteins in aqueous medium **[47]**. Such materials therefore are of interest because their degree of hydrophobicity (and the amount of structured water associated to them) can be exquisitely controlled by temperature changes, or by co-polymer composition.

Many assembly reactions in biology are entropy driven. There may be no or even an unfavorable enthalpy of association that is overcome by a large and favorable increase in entropy due to desolvation of the interacting units. Probably the most well-known example is the protein folding reaction. In the unfolded state of the protein, exposed hydrophobic (apolar) groups restrict the configurational freedom of water molecules in a least the first layer around these groups. The lack of hydrogen bonding capacity of the apolar groups increased ordering of these water molecules in an ice-like so-called clathrate structure. A major driving force for folding is release of clathrate water into bulk upon burial of the apolar groups in the well-packed hydrophobic core of the native state. This gives a large and favorable gain in entropy. Likewise, many protein-ligand interactions are entropy driven. For example binding of metal ions like Ca^{2+} is often associated with none or

even an unfavorable enthalpy change. However, the free metal ion is coordinated by several hydration water molecules that are released into bulk when the ion binds to the protein giving a large favorable entropic contribution to the free energy of binding **[48]**. Other well-known examples are found among DNA binding proteins. Association of some proteins with DNA is entropy driven due to the release upon binding of counter ions that are associated with the free DNA **[49]**.

It is thus reasonable to expect that some protein-nanoparticle interactions are entropy driven and governed by the release of water and/or counter ions from particle and protein. To find such cases it is necessary to study, a given protein-nanoparticle interaction by a combination of techniques, e.g. calorimetry to measure the enthalpy on association (or lack of), spectroscopy to study the conformational changes (or lack of), and surface plasmon resonance that can monitor binding even in the absence of enthalpic or conformational change. Our hypothesis is that interaction of proteins with the nanoparticles results in release of large amounts of the water from the surface of the proteins, as well as the structured water around the *iso*-propyl and *tert*-butyl side chains of the polymer. This is depicted schematically in **Figure 6**. As it happens, nanoparticles based on N-isopropylacrylamide have the additional advantage of offering control of the release rate of the loaded therapeutic molecules, as a result of the fact that the degree of swelling (the LCST) of the nanoparticles can be easily controlled by the ratio of the comonomers **[50, 51]**. Indeed, such polymers and particles have been used for controlled delivery of therapeutic molecules by ourselves **[6, 51]**, and others for several years now, including for protein delivery **[52-54]**. However, we emphasize the ideas underlying this paper are aimed at a more general view on how to design nanomaterials for incorporation of protein therapeutics.

The entropy increase that results from displacement of water from proteins upon binding to (certain) nanoparticles may also be seen as analogous to that which results from the displacement of small counterions from micelles by polyions, which is a very well understood phenomenon in colloid science **[55, 56]**. In the case of the surfactant micelles, the hydrophobic tails cause the surfactant molecules to aggregate in order to reduce their exposure to aqueous solution, which then results in the surfactant counterions being forced into much closer contact than would normally be the case, due to their natural repulsion for one another. This balance between hydrophobicity and charge repulsion determine the size of surfactant micelles **[57]**. Introduction of a charged polymer (a polyelectrolyte or polyion) to a surfactant solution results in immediate formation of a mixed surfactant-polyion complex, as the loss of entropy of the polyion by binding to the surfactant micelle is more than compensated by the huge increase in entropy due to the release of many small counterions **[55, 56]**.

We also make a general point about the importance of multiple techniques in this whole field of bionanomaterial interactions. Without the SPR data, we may have concluded that no binding of these proteins occurs. To our knowledge there is no previous data in the literature regarding entropy-driven binding of proteins to nanoparticles without a consequent dramatic alteration of the protein conformation. This may be a consequence of the fact that many of the studies in the literature focus on highly charged nanoparticles, as these are considered more useful for protein therapeutics on account of the fact that they potentially allow higher loading. On the other hand, it is possible that this type of entropy-driven binding has occurred in other systems, but that it has not been noted because of the lack of an enthalpic or protein structural signal. This is an interesting paradox, for it is precisely the absence of these effects that we now highlight as a useful way forward in protein carrier therapeutics.

Clearly, in an emerging field such as that of bionanointeractions, where so little is known and where many surprises are in store for us, the use of several complementary techniques will be required in order to really understand the behaviour of complex systems.

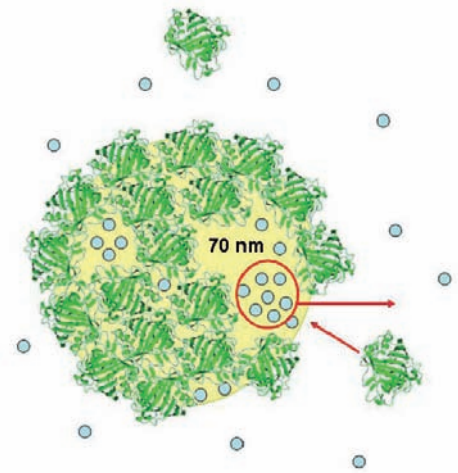

Figure 6: *Schematic illustration of the release of surface-bound water from the nanoparticles upon binding of proteins. Particle and proteins are drawn to scale, water molecules are not to scale. Binding of a single protein molecule to the particle surface will release many water molecules, increasing the overall system entropy.*

Conclusions

The importance of water as a mediator of the interactions between nanoparticles and proteins is elucidated, and a novel approach to the design of carriers for protein therapeutics, based on the entropic release of surface water is described. For protein therapeutics, it is important that the functionality of the protein is not compromised as a result of conformational changes induced by interaction with the surface. Water-mediated protein binding is an entropic process, which does not affect the enthalpic balance of the protein's conformation, and as such offers a unique approach to loading of nanoparticles with proteins whilst maintaining their native conformation.

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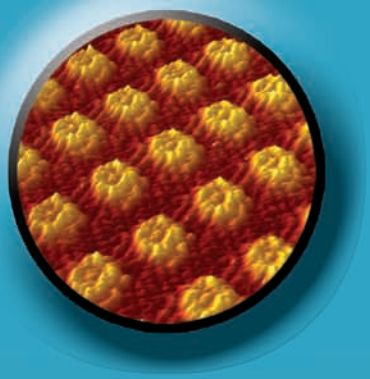
SURFACE ANALYSIS

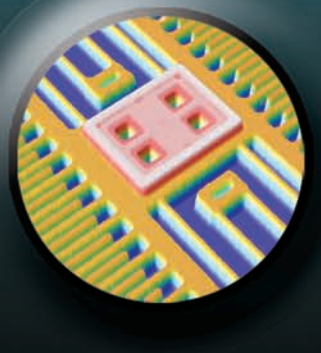


AFM / STM

Materials, polymers, biology, electrochemistry...

SNOM Microscopy

Reflection, transmission, Apertureless...
SECM, Deep Trench, nanopipette probes...



3D Optical Profilometry

New confocal technology...
Interferometry (PSI/VSI)...

And also...

Mechanical profilometry, nano-indentation...

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