École Normale Supérieure
- Lyon – (FRANCE).
Laboratoire de Physique. ENS-Lyon & CNRS
Laboratoire transdisciplinaire Joliot-Curie.


**Dr. Santiago Cuesta-López**

**Advanced Materials, Nuclear Technology & Bio/Nanotechnology**
**Universidad de Burgos, Parque Científico – I+D+I, CITI- Burgos, Spain**

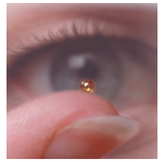

**In collaboration with:**

**DIN/DENIM – (UPM, SPAIN), T.S. Van Erp (UKL – Leuven), Francesco Delogu (UCA-Italy), M.Peyrard (ENS -Lyon), D. Angelov (ENS-Lyon) , A. Wildes (ILL-Grenoble), A.Velazquez-Campoy (Unizar-ARAID), R. Iglesias (Univ. Oviedo), A.Caro (LANL-USA), M. Tolley (RAL & SRFC-UK) … and many more …**

## PROJECTS & ACTIVITIES - I

## MATERIALES PARA INGENIERÍA EN CONDICIONES EXTREMAS

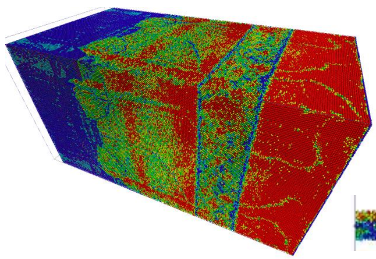
**EU FP7 – HiPER ESFRI. Fp7-infraestructures-2007-1. Grant Agreement No. 211737.**
**Target delivery group. WP11. Responsible of Design Advanced Materials for NF target manufacturing.**

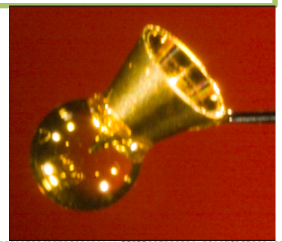


Our role (WP11): Advanced Materials Modelling (15 WPs and 20 international partners):

• Shock propagation and design of advanced materials under extreme pressures. Inertial Fusion Target Manufacturing.

• Reach with simulations the scale of experiments and close interaction.





Stress distribution and evolution of the GB:

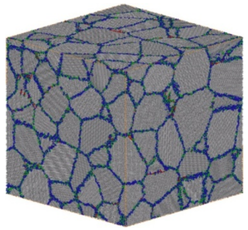
**EU.FP7-NMP-2010-SMALL-4. RADINTERFACES: Radiation damage resistant nanocrystaline materials / Self – healing materials:**

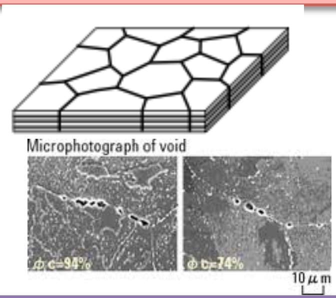
WPs and 10 international partners:

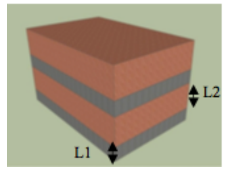
• Design, inclusion and test of new compounds and substitutions.

• Multiscale modelling of multilayered, nanostructured / nanocrystalline samples. Diffusion phenomena. Clustering phenomena.

• Interface between scales and methodologies. Interface Theory – Experiment.



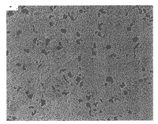


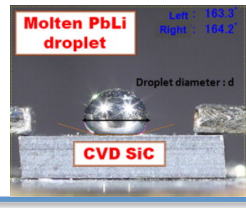




**TECHNOFUSION 2009-2012:**

**Advanced Materials.**

**Design and neutron scattering of liquid metals: structural properties / diffusion/ -> eutectic PbLi (Fusion), eutectic PbBi (SNS )**





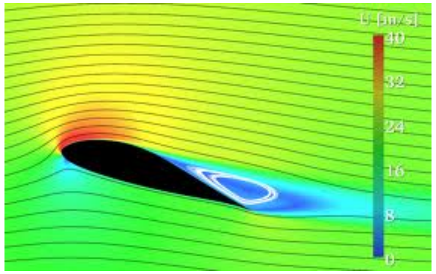



*S.Cuesta-Lopez, PhD. E-mail: scuesta@ubu.es*
*Advanced Materials & Nuclear Technology*

# OTHER PROJECTS & ACTIVITIES

## ADVANCED CFD TECHNOLOGY / MATERIALS SCIENCE / INNOVATION

- Simulation of small prototypes (eolic energy).
- Adapt codes and solutions coming from nuclear fusion to build a CFD tool to simulate wind tunnels.
- Mechanical modelling of materials ( FEM) – Innovation of materials in prototypes



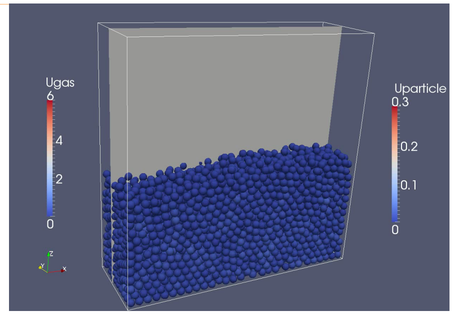

## INNOVATION IN PRECISION MACHINERY INDUSTRY. GENERAL INDUSTRY?

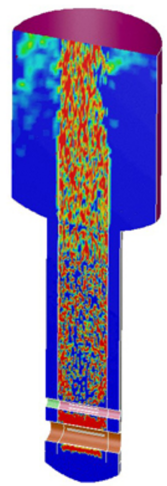
The University of Nottingham

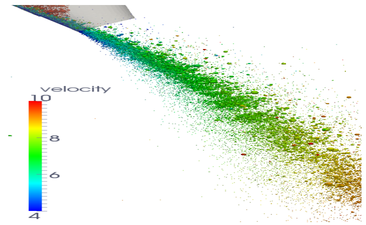
**** IMPROVEMENTS IN CUTTING FLUIDS AND LUBRICATION SYSTEMS.**

Models for two schemes: **Minimal Quantity Lubrication (MQL) / Minimal Quantity Cooling Lubrication (MQCL) vs Cooling Air/Gas and Minimal Quantity Lubrication (CAMQL)**
Simulation of Nanofluids is possible by means of our unique multiscale modelling **technology and CFD – coupled to Discrete Element Methods (DEM) – LIGGGHTS.**

**** DEVELOPMENT / IMPLEMENTATION OF POWERFUL CFD SOLUTIONS COMING FROM BASIC "exoteric" SCIENCE TO SOLVE COMMON INDUSTRIAL PROBLEMS.**



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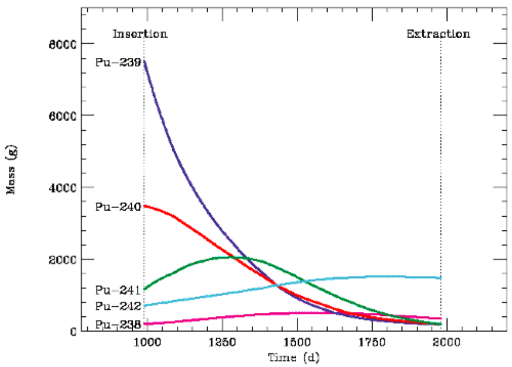
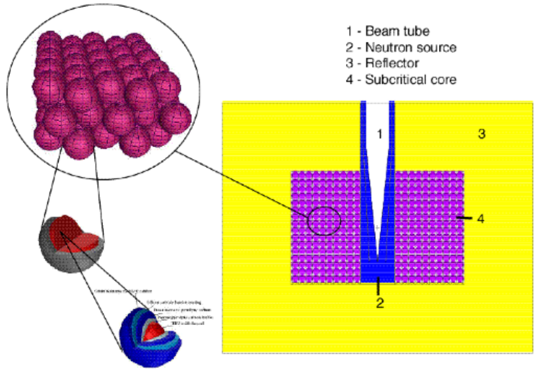
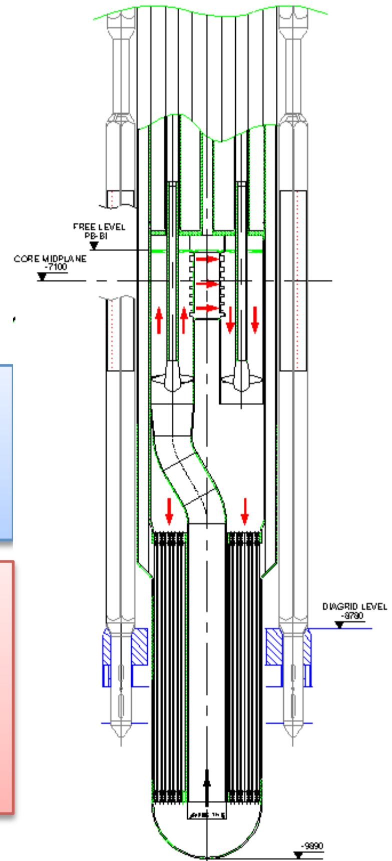
## PROJECTS & ACTIVITIES - II

## TECNOLOGíA NUCLEAR

** European Union 5th Framework Program of EURATOM "Prototype Design Study – Experimental Accelerator Driven System (PDS-XADS)", reference FP5-EAECTP C.* (2001 – 2003)




** FIKW-CT-2001-00179, and European Union 6th Framework Program of EURATOM "Transmutation on Adiabatic Resonance Crossing, TARC"* (2005 – 2006). EURADWASTE.

** Codes for Generation And Transport Of Particles – Radioprotection*

Radiation shielding for a neutron tomography instrument (3 axis diffract.) – Anteproyecto Industrial LA3P Lab - DGA (Spain) – 2002

*** Incorporation of Advanced Materials into modern civil engineering /radiations shielding /** ipm project management



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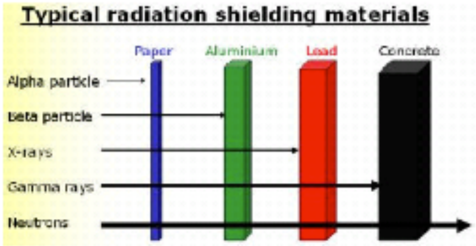
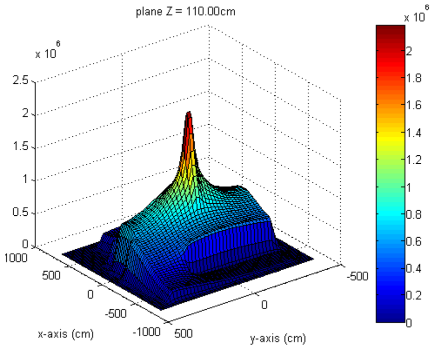
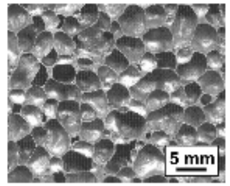
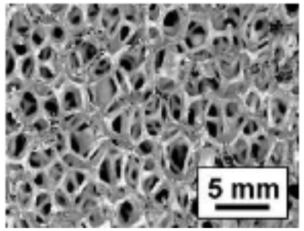
**Materials development: It is critical !**



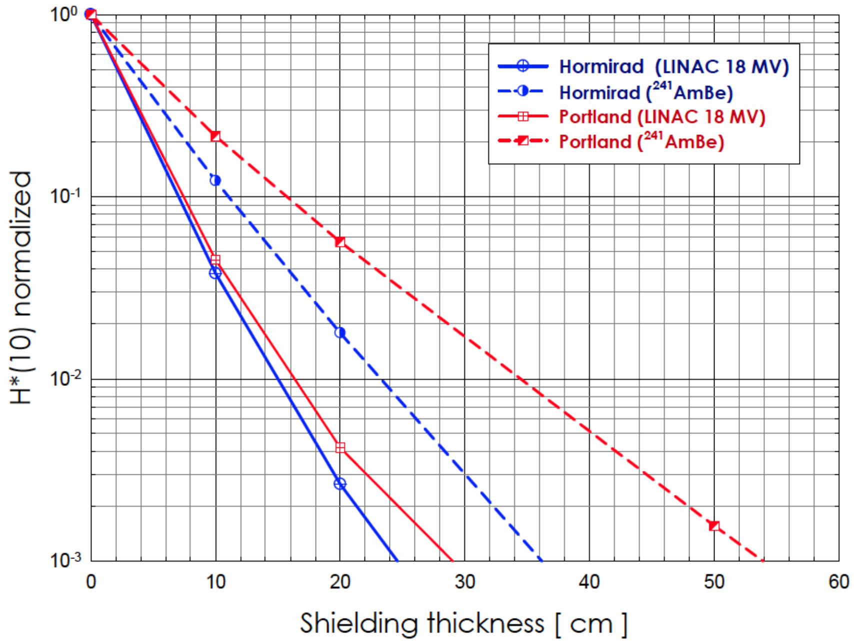




- **Test of commercial building materials.**
- **Introducing new advanced materials in the code ! Mimics + weights !**

| Element | **Hormirad™** density: 3.44 ÷ 4.10 g·cm$^{-3}$ | **Portland concrete** density: 2.30 g·cm$^{-3}$ |
|---|---|---|
| Fe | 60.80% | 1.40% |
| O | 31.26% | 52.91% |
| Ca | 4.36% | 4.40% |
| Si | 1.87% | 33.70% |
| H | 0.44% | 1.00% |
| Mg | 0.39% | 0.20% |
| P | 0.29% | - |
| Ti | 0.19% | - |
| Al | 0.17% | 3.39% |
| K | 0.06% | 1.30% |
| Mn | 0.06% | - |
| V | 0.05% | - |
| C | 0.04% | 0.10% |
| S | 0.01% | - |
| N | 0.003% | - |
| Na | - | 1.60% |



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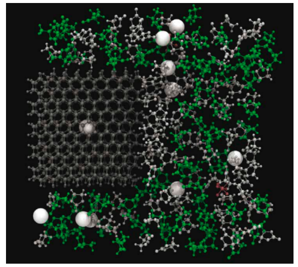
*Advanced Materials & Nuclear Technology*

# PROJECTS & ACTIVITIES – “”In Pause””- Interests

## * JST-Japan / Toyota.Theoretical design of new materials for Ion-Li batteries.

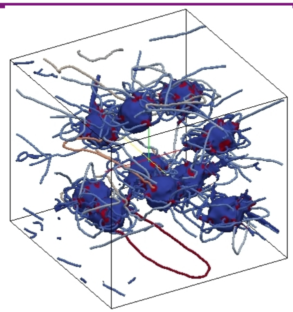
* *Modelling experimental electrolytes and their properties:* PEO, $LiPF_6$ in propylene carbonate (PC) and $LiPF_6$ in a 1:2 (w/w) mixture of ethylene carbonate (EC) and dimethyl carbonate (DMC).

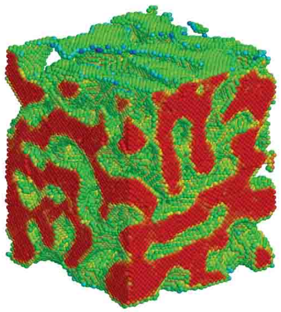
* <u>We will provide clear answers to the following questions:</u>
- Is faster diffusion of Li ions directly related to contributing to faster charging?, How the anode/cathode influence to this diffusion and what is the optimal material in this case? How relevant and limiting is the Li-ion intercalation into the electrode?.
- How is the evolution of the diffusion rate after the inclusion of an additional oscillating electric field?

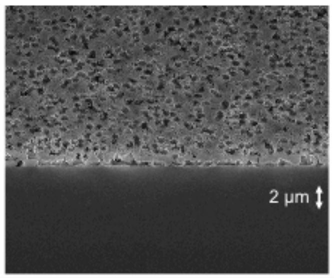


## * Miscellaneous:

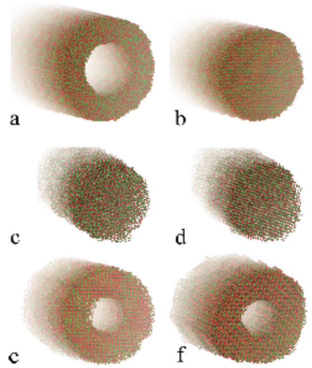
**SELF-HEALING METALLIC FOAMS:**
- Molecular damage repair.
- Surface interactions.
- Tension/Stress response



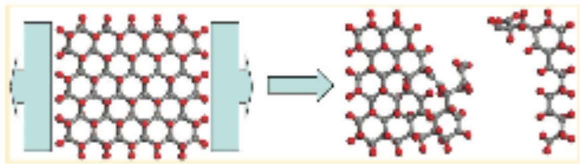





**-> Phase transitions in TiO2 nanotubes/Nanowires. Conduction properties**

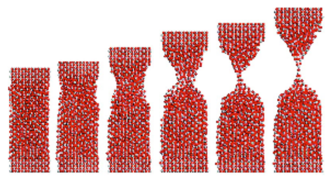



**-> Chemical reactions in graphene/graphane under uniaxial tension**



**-> Mechanical and conduction properties of CNT under torsion**

**-> Tensile properties of TiO2 Nanowires:**



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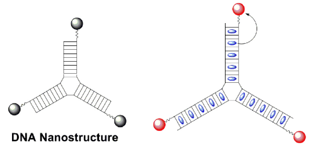
*Advanced Materials & Nuclear Technology*

# Physics of DNA/RNA NANO-assembly.
# Characterizing structural assemblies and scaffolds in μRNA, Hairpins and loop structures.

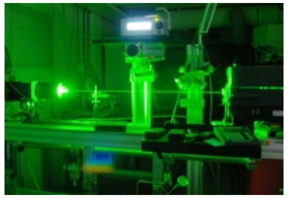
**The specificity of the interactions between complementary base pairs make DNA a useful nano-scale construction material.**

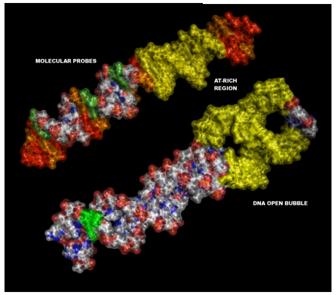
## ** MOLECULAR PROBES CREATED BY HIGH INTENSITY UV LASER IRRADIATION:

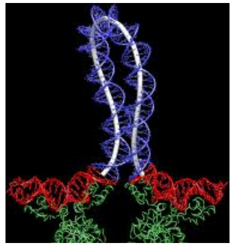



**Obtain instantaneous information without perturbing structure or dynamics.**

S.Cuesta-López, et al" *Nucl. Acids. Research. 39 (12): 5276-5283. (2011).*





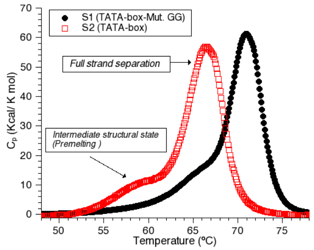



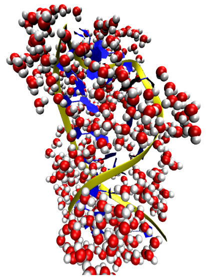
**<u>OUR MAIN GOAL</u>: Adapt to Look into the structural stability and integrity of DNA/RNA "puzzle pieces" – Nanoarchitecture. Stability of association.**

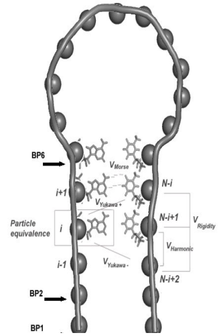
## ** THERMODYNAMICS OF DNA/RNA HAIRPING AND SUPRASTRUCTURES :

Assessing the thermodynamics of Nano-molecular constructions.

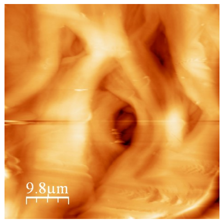
OWN PARTICULAR METHODOLOGY:
COMBINING **Atomistic molecular modelling + Coarse grained modelling + Nano-calorimetry+ Neutron Scattering**

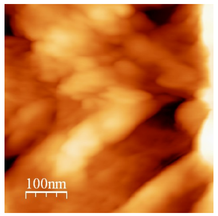


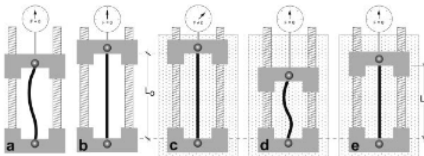





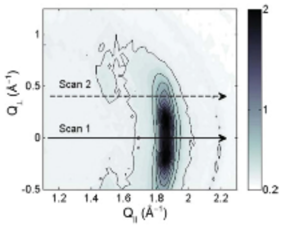

## ** DNA FIBER & FILM STRETCHING: IMPROVING MANUFACTURE FOR POSSIBLE INDUSTRIAL APPLICATIONS:








•**S.Cuesta-López et al. Phys.Rev.Lett.** 106, 048101. (2011)

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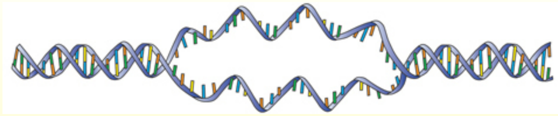
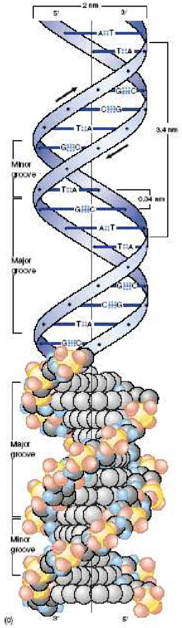
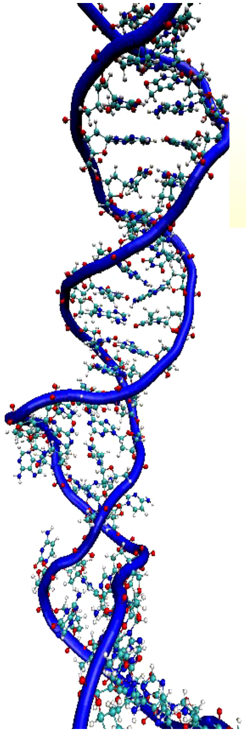
## What are DNA bubbles ?



## Why are they important?

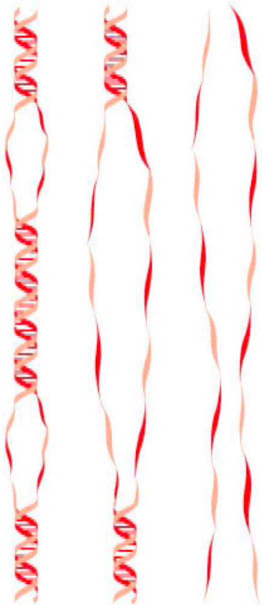


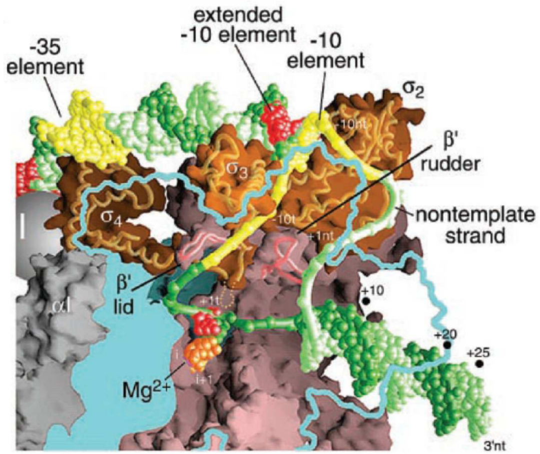


**DNA is not a static entity breaths at different time and length scales !!**

**Protagonists as dynamical objects !!!**

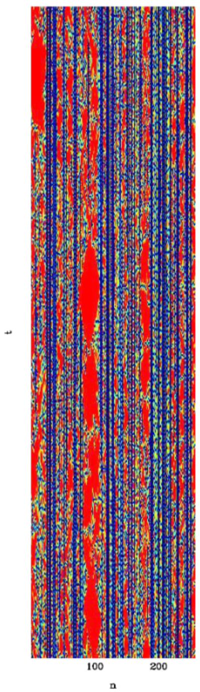
**Key mechanisms in biological processes !!!**

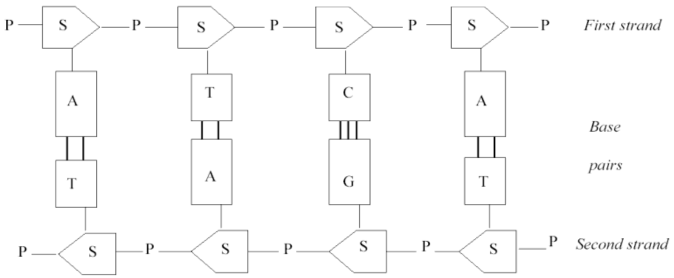


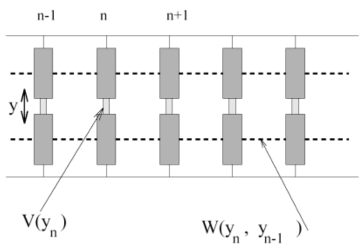


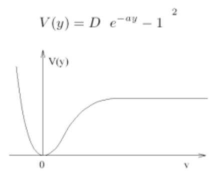

T=350 K

Calc: 1031 Y Min: 0.000 Max: 1.500




**<u>DNA DENATURATION AND DYNAMICS AT THE *MESOSCALE*:</u>**

# A model progressively improved.

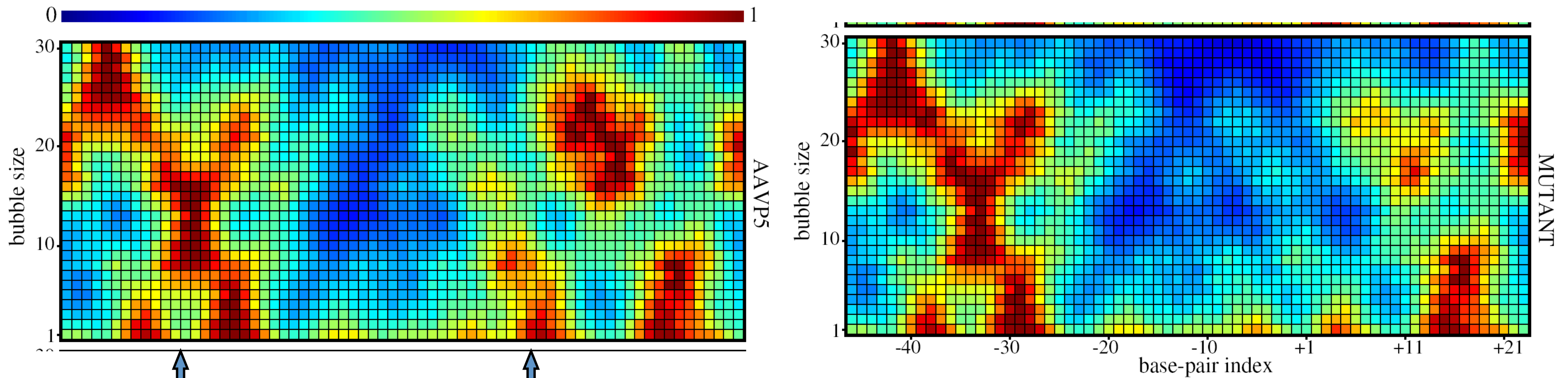
S. Cuesta-López, Theodorakopoulos & Peyrard. In preparation. (2011)
Peyrard & Cuesta-Lopez, J. of Physics Condensed Matter 21, 034103 (2009)
T.S. Van Erp, S. Cuesta-López, et al *Phys. Rev. Lett.*, 95, 218104, (2005). & *Physical Review Letters*. 97, 059802 (2006) & *Physical Review Letters*. 96, (23):239802. (2006).

**ARE WE ABLE TO MAKE A DYNAMICAL MAP OF OUR GENOME?**

**2012-2013**

**Web-server, melting map server**

## The adeno-associated viral (AAV) P5 promoter.




**Yin Yang 1 (YY1) TF at -33**

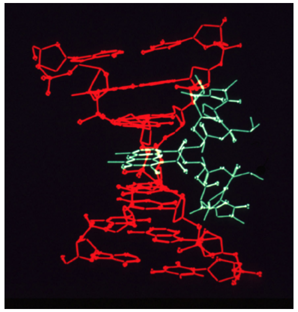
**TSS *(Transcription Starting Site)***

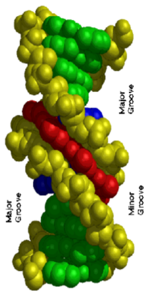
**Mutation - NO TRANSCRIPTION !!**

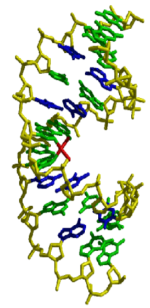
---

**CAN WE PREDICT BETTER BINDING SITES FOR DNA DRUG BINDERS?**

* DNA is not only a chemical entity. Flexibility and opening fluctuations play a biological role.
* Sequence dependence affects stability of potential binders.
* Looking for cavities to protect hydrophobic drugs.
* Looking for "calm places for drugs to live/interact."







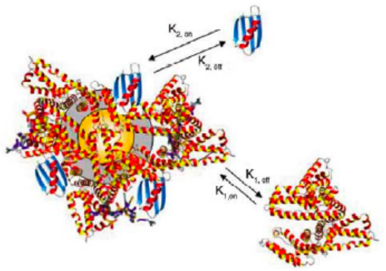
# Physico/chemical basis of Nanoparticle (NP) Biocompatibility and BioToxicity.

## Organic and Inorganic Nanoparticles (NPs) for medicine

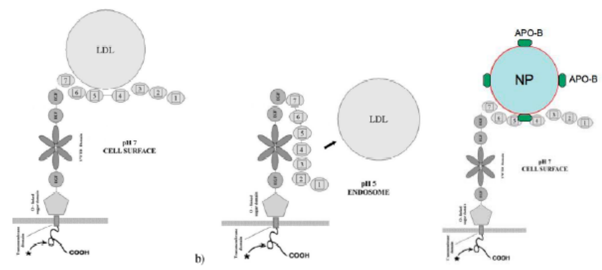
**BIOPHYSICAL BASIS OF NP BIOCOMPATIBILITY/TOXICITY:**

** Interaction of a functionalized Nanoparticle, in physiological conditions, with common plasma proteins.
** Dynamical and collective behavior of an ensemble of nanoparticles inside plasma in physiological conditions.
** Study of different sets of cell receptor efficiencies .

**EXPERIENCE IN MODELLING & WORKING WITH THE LDL-R:**



♦ **Using the LDL-r and EGF-r as routing/functionalization strategies**




**PROPOSE A NEW METHODOLOGY BASED ON OUR LONG EXPERIENCE WITH BIOPHYSICAL TECHNIQUES:**

♦ Strong background in Molecular Modeling. “In silico” modeling of molecular adsorption of proteins onto NP surface.
♦ Combining **AFM, Neutron scattering techniques and Nano-calorimetry (DSC,ITC)**.
♦ Combining both ITC and size exclusion chromatography: Screen an ensemble of plasma proteins against a set of two different NPs coronas:

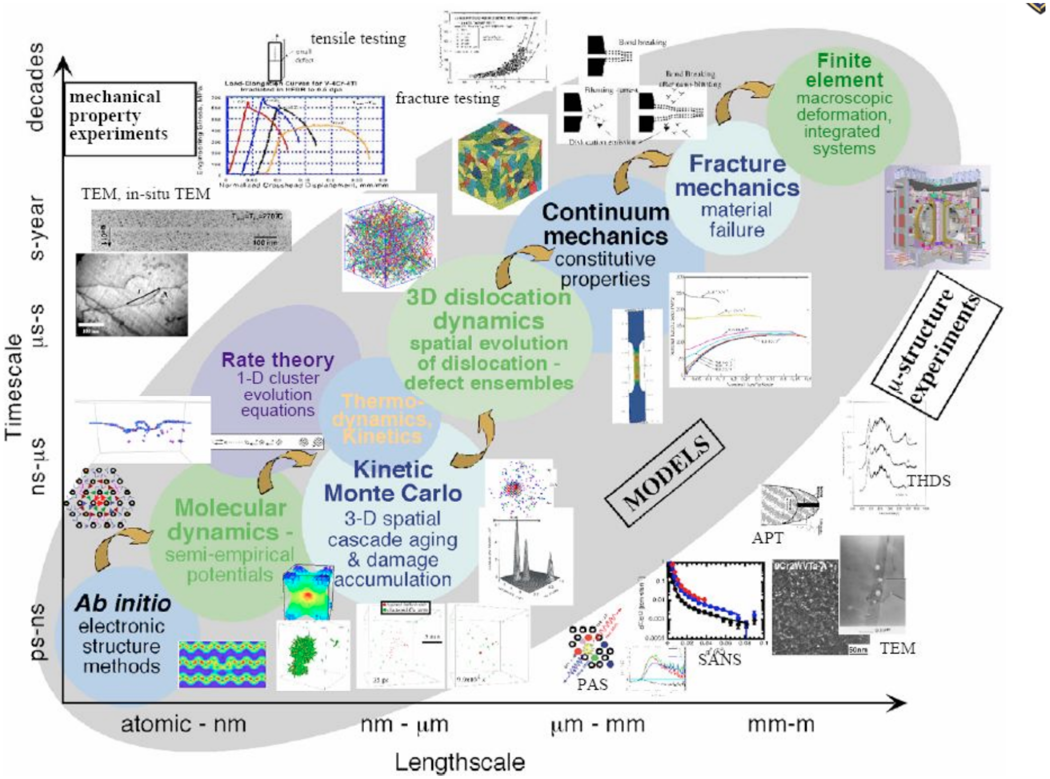
- HSA-NP and IO-NP.Albumin, Apolipoproteins (AI,E), immunoglobulins and fibrinogen.

**Biophysical study of NP biocompatibility:**
**Physiological interactions of NP**

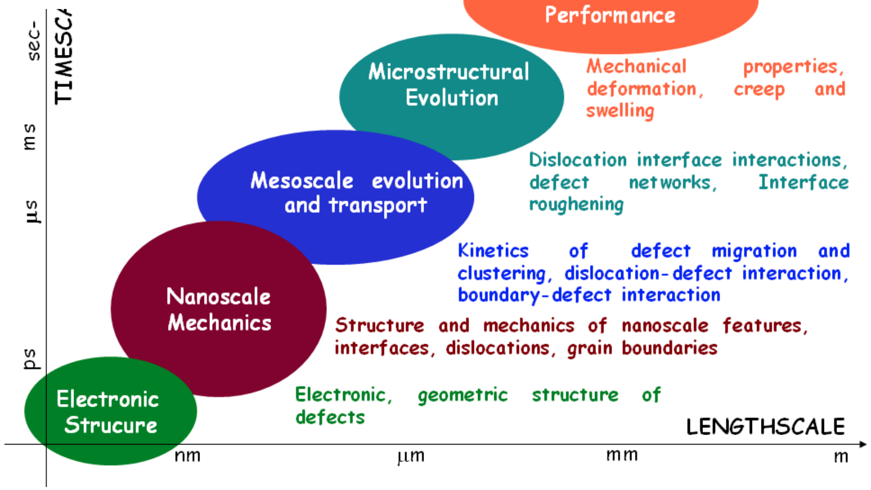
- Interaction of model NPs with common plasma proteins. (ITC & SEC)
- SANS study of the adsorption and interaction of particular plasma proteins with NPs
- Molecular modelling of the adsorption of common proteins onto a NP.

**MAIN GOAL**
Understand, from a physico-chemical point of view, the interactions governing the biocompatibility of functionalized NPs

# MULTI-SCALE MODELLING IN MATERIALS SCIENCE AND TECHNOLOGY




*Multi-Scale Phenomena*



## OUTLINE:

- **Materials under extreme conditions. Atomistic view of shock-wave propagation in matter.**

- We are modeling shock-wave generation and propagation in single crystal materials Fe, Au, Ta, W, and Al by means of different MD methodologies.
-Double layer conformations FeAl, AlCu are also being evaluated. Interest in inertial confinement nuclear fusion – target design.
- New nanostructured materials, like nanocrystaline Fe, Cu, Ni are being tested under high pressure conditions.
- Generation of ultra-hard materials under high pressure.

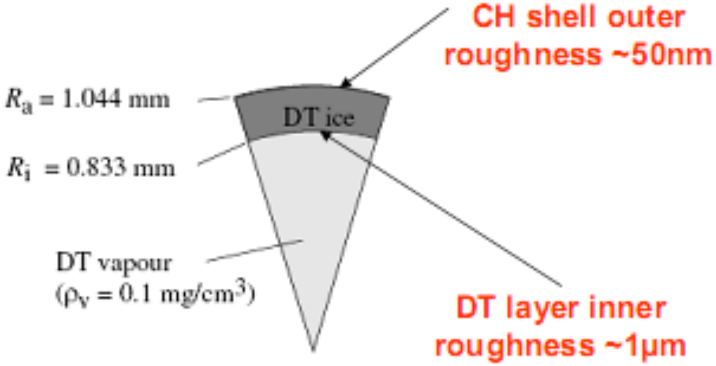
- **Approach to model nano-crash phenomena in materials.**

- Nanoscale studies of debris effects in shielding materials (i.e. first wall in INF).
- Atomistic view of impacts in materials. Interest in space industry.
- Nanofracture & nanoindentation phenomena.

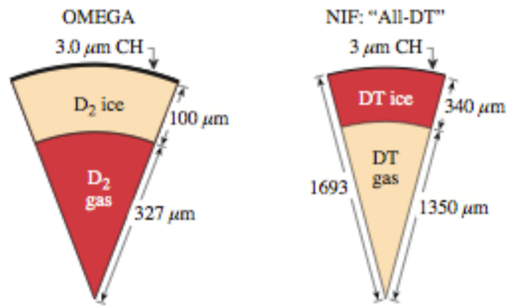
- **FP-7. RADINTERFACES.**

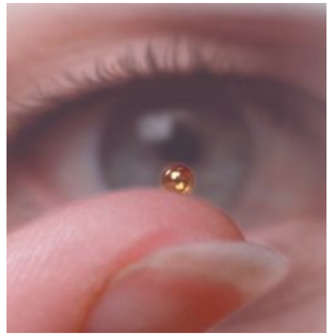
- Radiation Damage in Nanostructured multilayered materials. Critical for nuclear industry development in next decade.

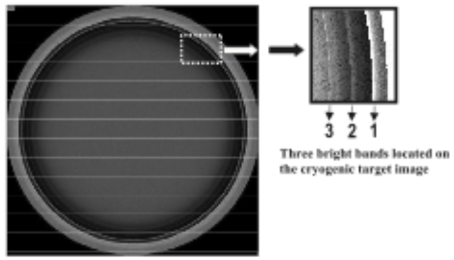
- **Shock wave propagation is the key process in the implosion of the fuel capsule and ignition :**

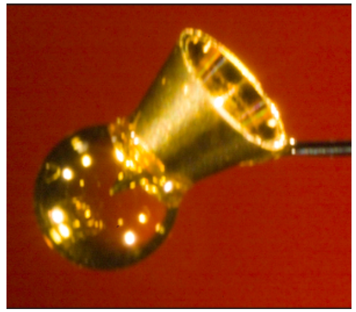


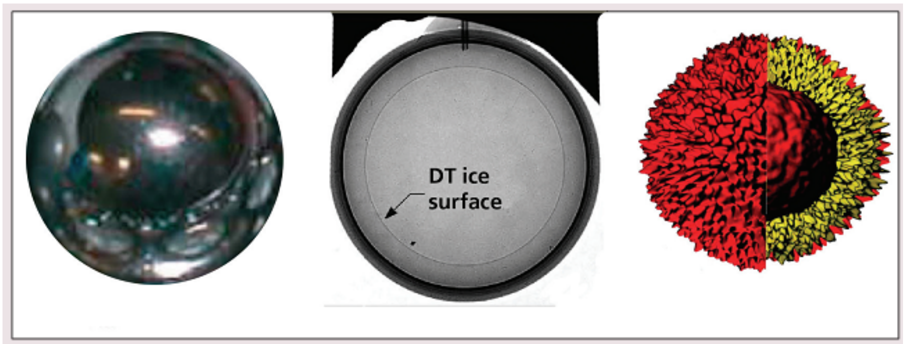

Shell cross-section





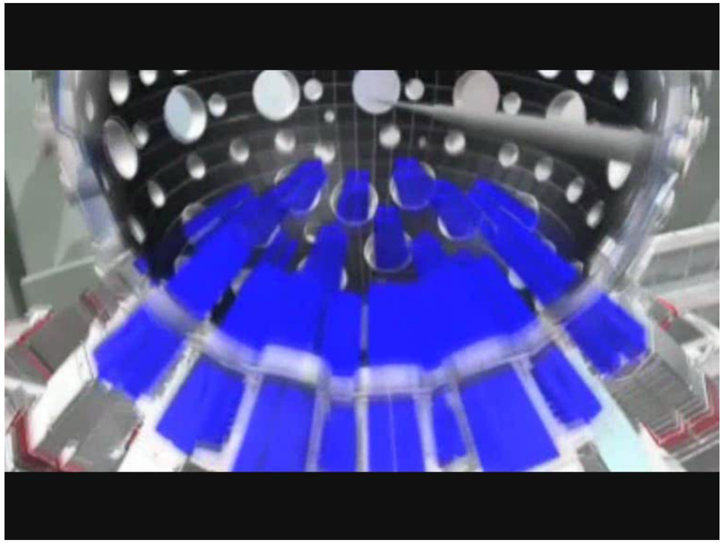





WHY SHOCKWAVES? :

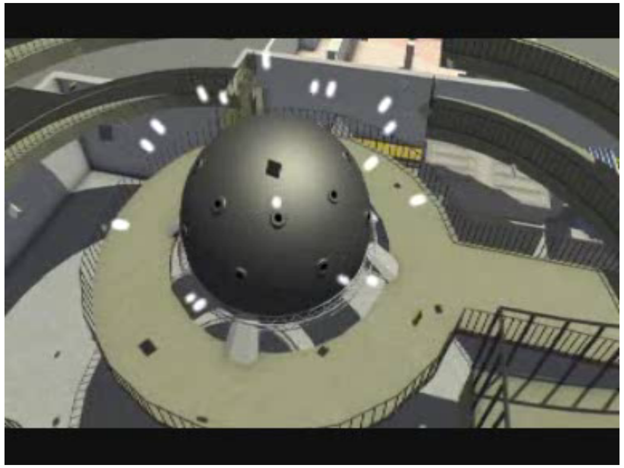
ADVANCED MATERIALS & TARGET DESIGN

*Indirect drive* approach:
First at NIF



***Fast Ignition*** **is a promising approach.**

→ Target is the KEY !!



**Two-step ignition offers lower driver energies with the possibility of higher gain.**

# *"In silico"* shock generation:

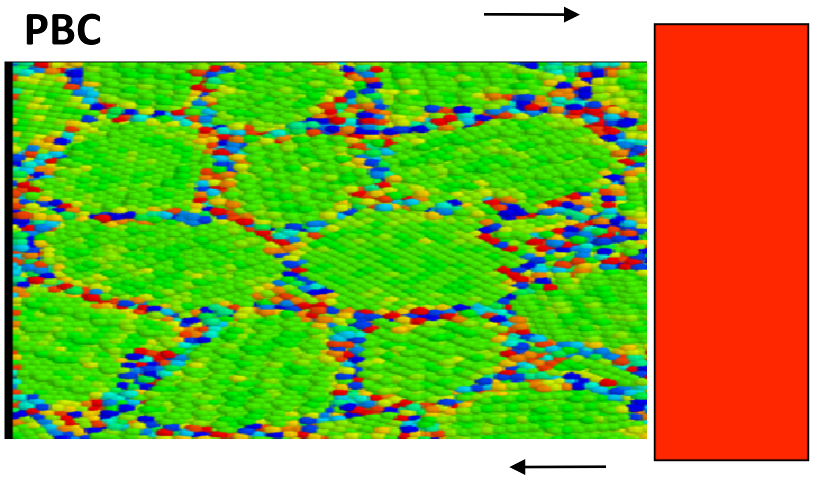
- **We use EAM and MEAM potentials to describe atom interactions.**
- **High Performance Computing at the atomistic scale with own and public codes (LAMMPS).**
- **Momentum mirror method. Shock. Adiabatic NEMD:**

System is launched towards a static mirror that reflects every particle .
In other words, the sample is slammed up against a specularly reflecting wall with velocity Up. As a result a shockwave is propagated in the other sense at velocity Us-Up.

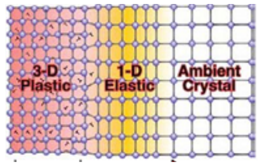
**Kai Kadau et al., Science, 296, 1681 (2002).**
**Brad Lee Holian et al., Science 280 2085 (1998).**

**Up**

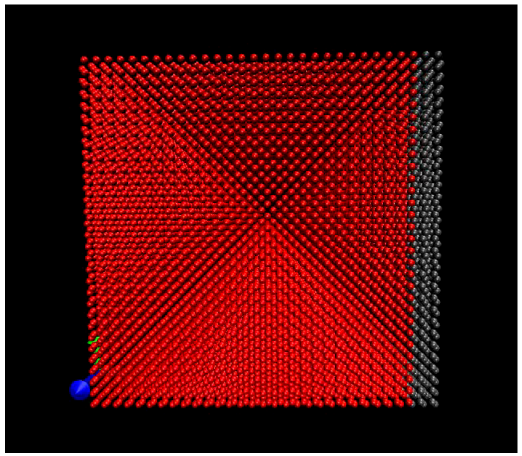
**PBC**



**Us - Up**

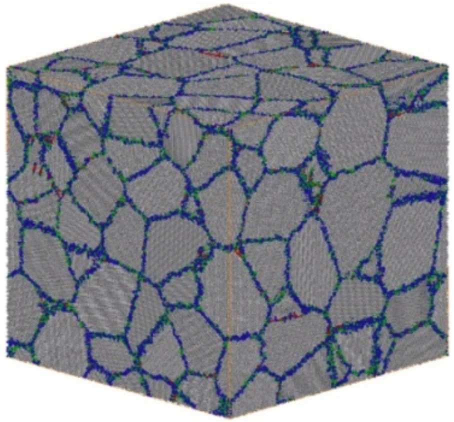


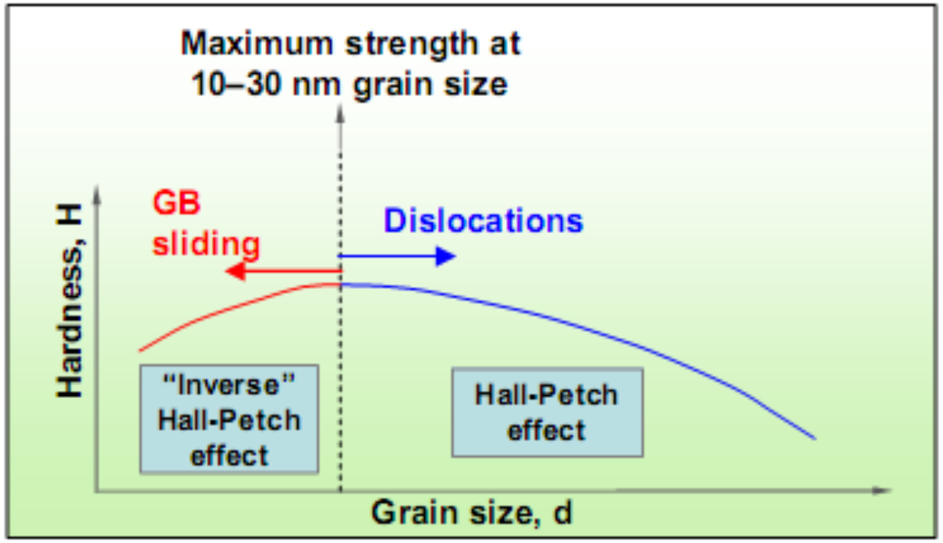

**Material Layer acting as mirror**



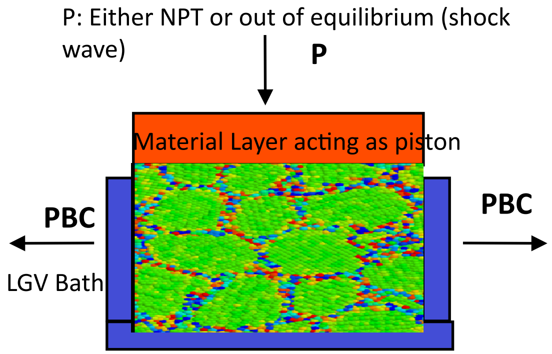
## ULTRA-HARD NANOSTRUCTURED MATERIALS:

**OUR GOAL:** **Create/design new very strong materials suitable for multiple applications in addition to IF targets !**





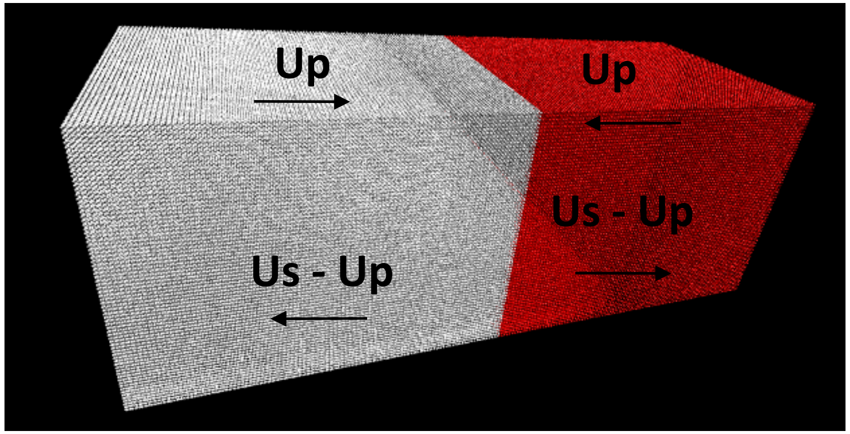

**NEMD coupled to Dissipative Brownian Dynamics:**

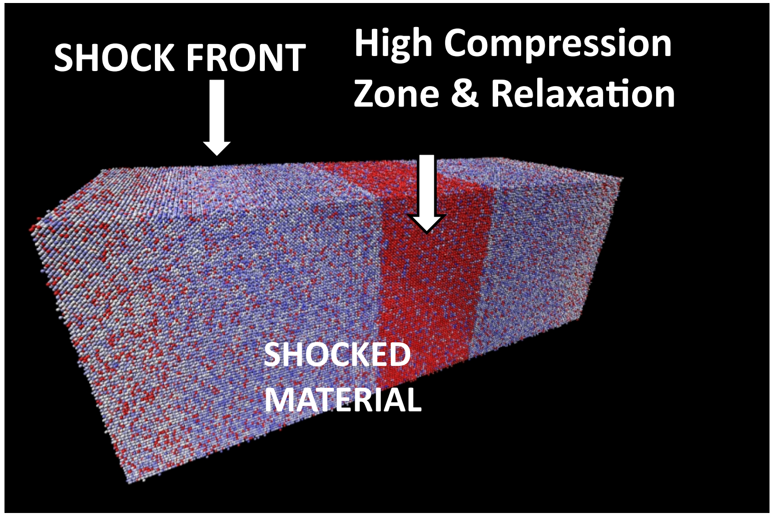



- We have developed different simulation procedures in Cu, Fe, and W based nanocrystalline cells in order to produce unique results predicting the generation of shocked nanocrystalline structures exhibiting ultra-hard properties. We evaluate their viability as future engineering materials.

## • Double Impact Shock:

Two blocks of material are launched towards each other with velocity Up. As a result a shockwave is propagated on each sense at velocity Us-Up.

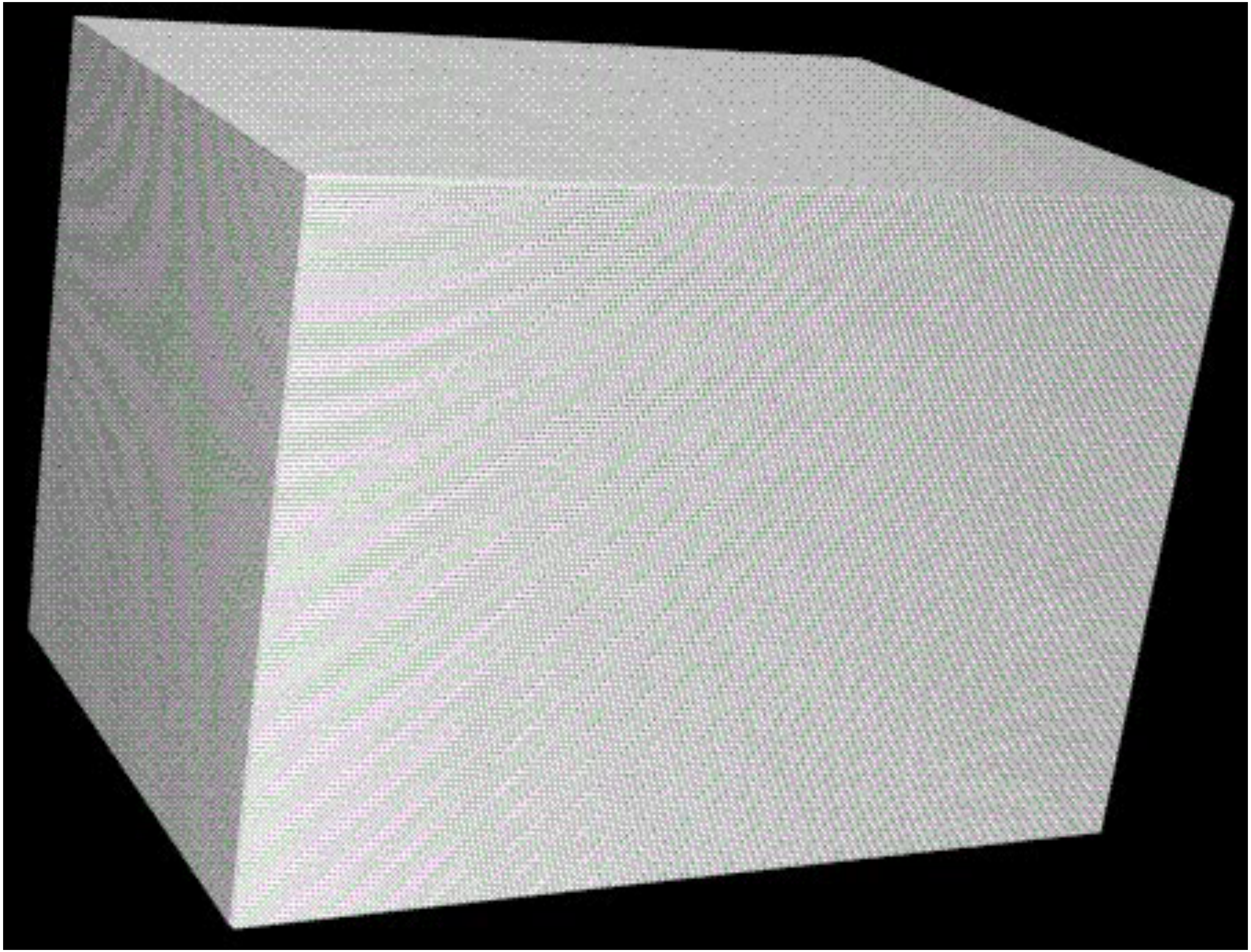



• We are applying this method to EAM potentials for Ta and Be (under test) .

• **In depth comparison of bcc and fcc materials. Full simulations with W, Au, Ni, Cu and Fe .**

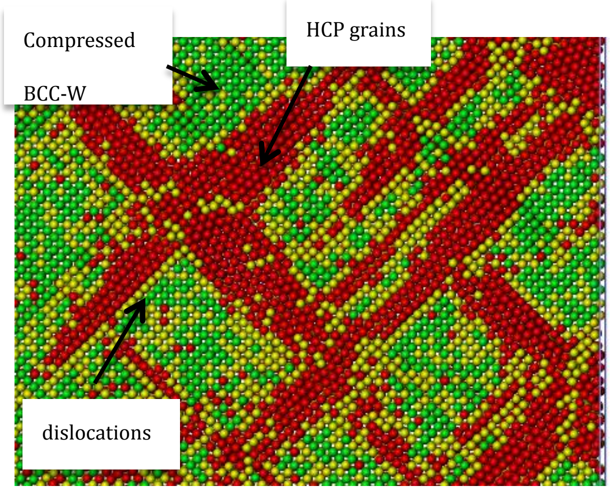
**Playing with the PBC in the shock propagation direction we are also studying shock wave instabilities & interferences.**



## First results on shockwave generation and propagation on W, and Ta:

- We have carried out shocks in Fe, W and Ta from low strengths Up=100 m/s up to high compression rates Up=2500 m/s.

- Above a critical shock strength, the material reacts nucleating many small close-packed grains in the shock-compressed body-centered cubic crystal that grow on a picosecond time scale to form larger, energetically favored grains.




Zoom into a sample of W well behind the shockfront after 5ps of MD and a shock strength of $U_p$= 1000 m/s. The atoms are colored according to their centro-symmetry parameter: red (packed hcp grains), green (uniaxially shocked bcc), and yellow (dislocations).

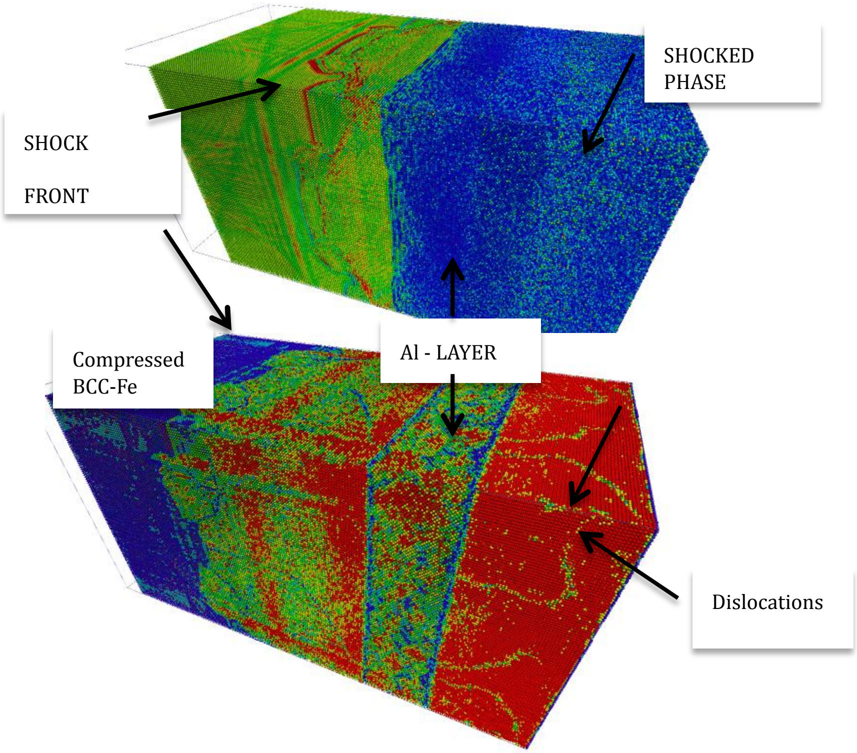
$$P = \sum_{i=1}^{6} |\vec{R}_i + \vec{R}_{i+6}|^2$$

- The instantaneous grain size of the transformed material in the overdriven region but below the melting transition, is much larger on average in the case of tungsten and tantalum with respect to shocks of homologue strength in iron.

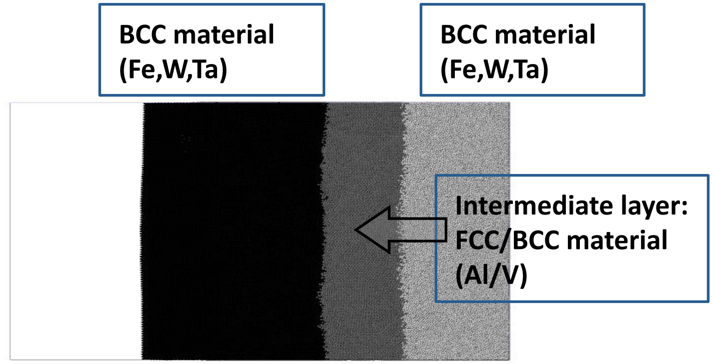
- A detailed characterization of the nucleation phenomena and the hcp grain size distribution is in progress.

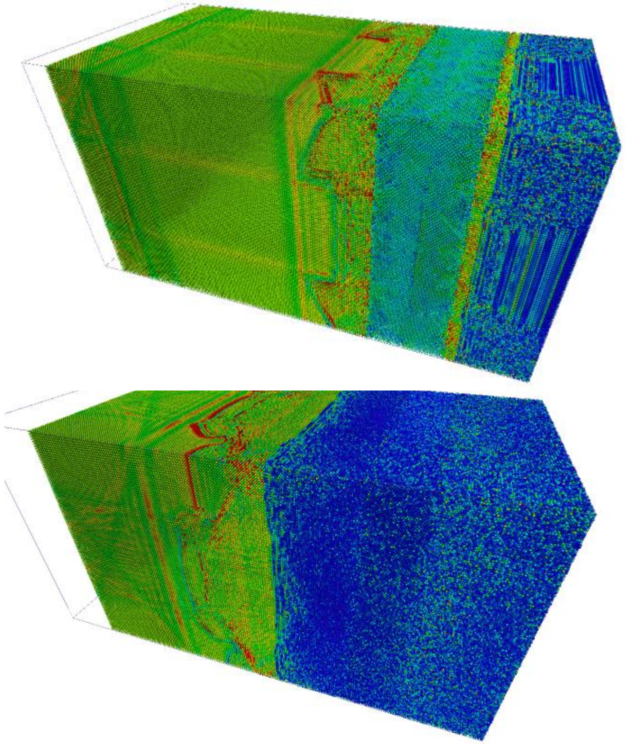

- **S.Cuesta-Lopez, J.M. Perlado, Fusion Science and Technology, In press (2010/2011).**
- **S.Cuesta-Lopez, J.M. Perlado, Phys. Rev.B, Under Review (2011).**

## Shocks in a double layer conformation:




(top) Final state in a three layered material Fe(bcc)-Al(fcc)-Fe(bcc) obtained after 6ps of propagation of a shock induced [001] wave. Shock pressure of about 50 GPa. (bottom) structural analysis of a shock propagation (Vs = 500 m/s) in the same piece of material. Atoms are coloured according to a combination of structural analysis: coordination, common-neighbour and centro-symetry parameter calculation. Red atoms are organised in an induced HCP lattice. A population of dislocations where generated in the relaxed zone of the shock (right from the Al layer). They have been identified and represented as yellow-green loops. Green atoms are uniaxially compressed lattice atoms.

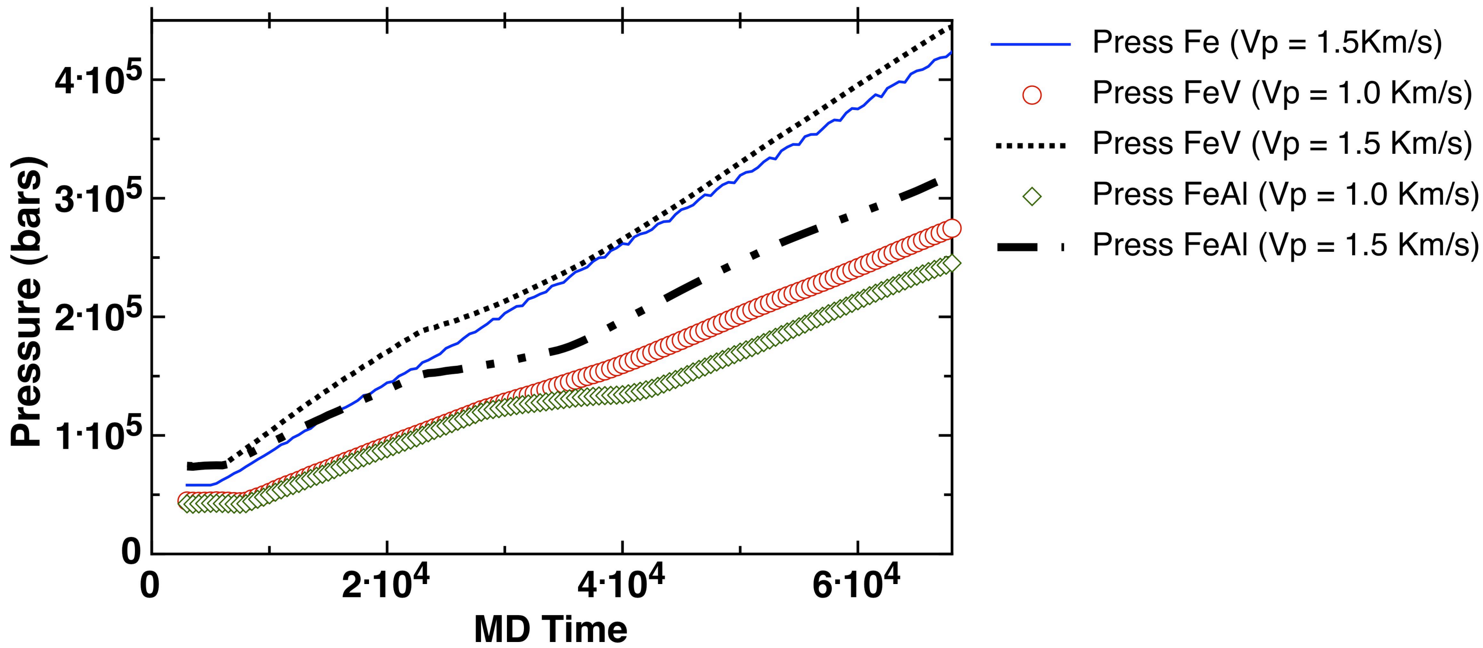


Colours show the propagation of thermal instabilities originated by the border between bcc/fcc materials.

Final states at 2 and 4 ps in a three layered material Fe(bcc)-Al(fcc)-Fe(bcc. Propagation of a shock induced [001] wave of Vp= 1.5Km/s

# Double layer conformations: bcc vs fcc materials and shock pressure transmission:

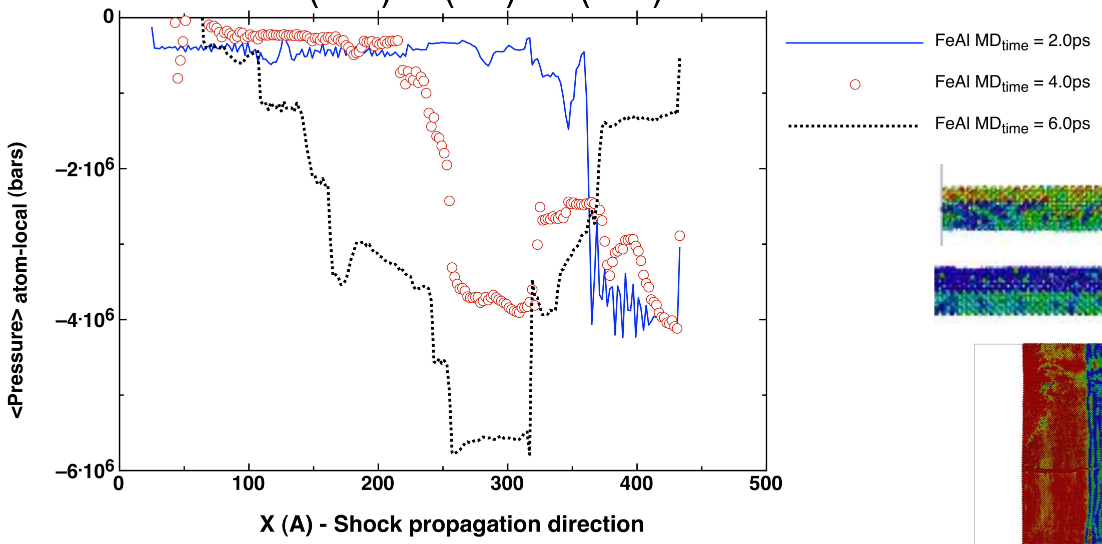



- Pressure transmission seems to be more efficient in double layered samples, provided the grain boundary formed is of the type bcc/bcc.

- Note the effect of blockade in the transmission generated when the shock front reaches the interface between bcc/fcc materials.


- **S.Cuesta-Lopez, J.M. Perlado, Fusion Science and Technology, In press (2010/2011).**

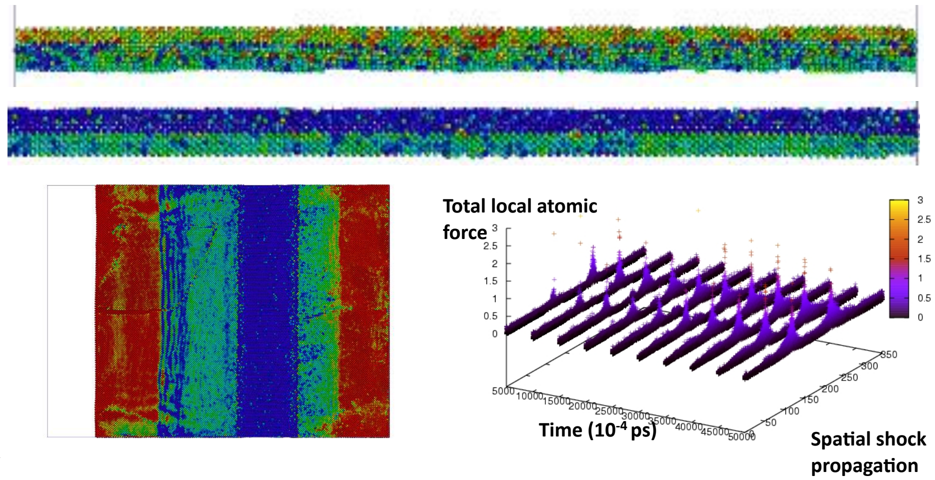
## Fe(bcc)-Al(fcc)-Fe(bcc).



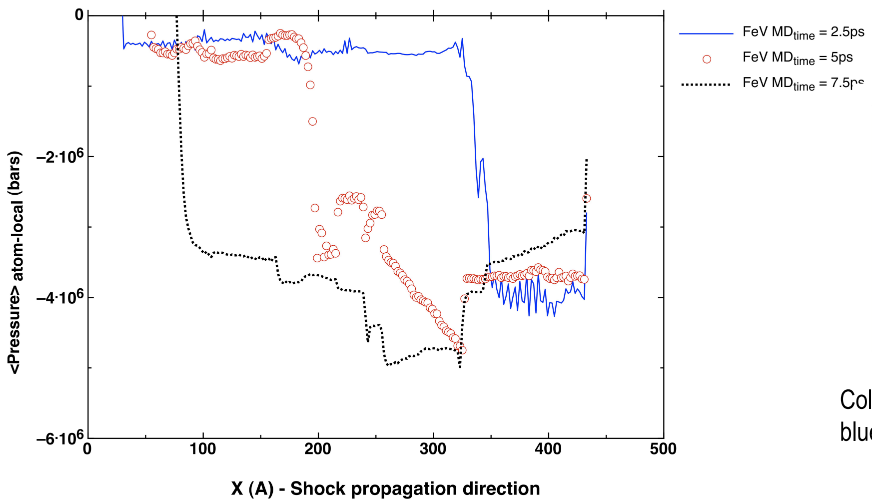

Average local pressure (local) per atom (stress tensor) at different cuts in the shock propagation direction. Three layered material Fe(bcc)-Al(fcc)-Fe(bcc). Propagation of a shock induced [001] wave of Vp= 1.0Km/s

## Stress distribution and evolution of the GB:

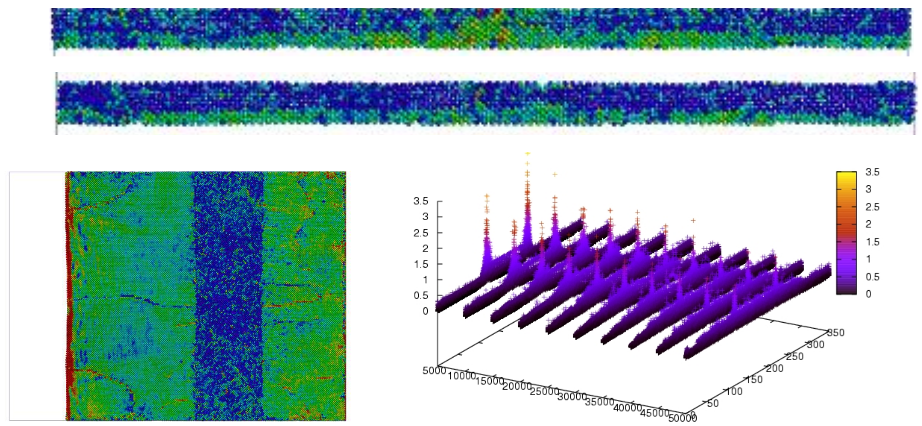
### Fe(bcc)-Al(fcc)-Fe(bcc).




## Fe(bcc)-V(bcc)-Fe(bcc).

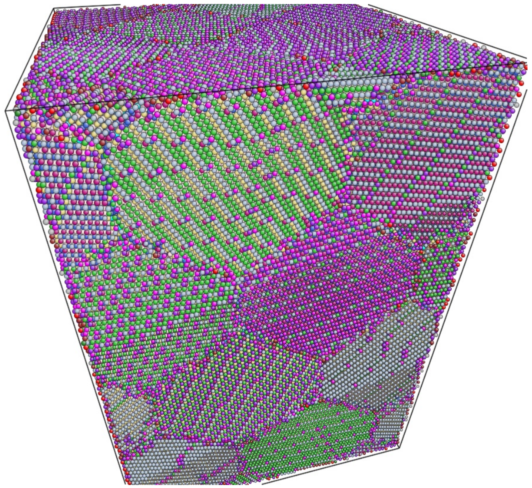


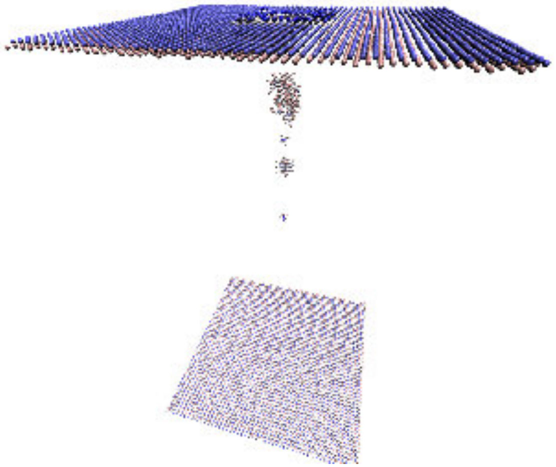

### Fe(bcc)-V(bcc)-Fe(bcc).

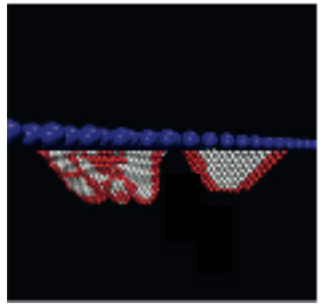


Color scale showing local pressure (local) per atom (stress tensor), compression. Red -1.0e6 to blue -5.0e6 bars.

## - Atomistic view of Nanocrashes, nanoindentation and impact phenomena:







**- We have created "in silico" nanocrystaline samples modelling shielding and first wall materials. Grain sizes around 10-30nm. We compare to single crystal.**

**- We are investigating different debris sizes (always < 50 nm) and velocities spectrum.**

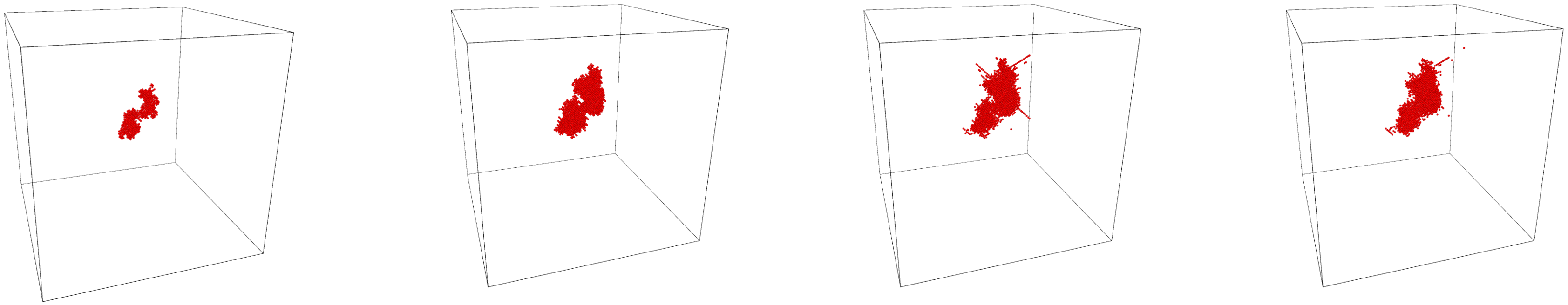
**-Grain boundaries seem to manage better both dislocations and atomic damage …**

**SCIENTIFIC ACTIVITY - 5**

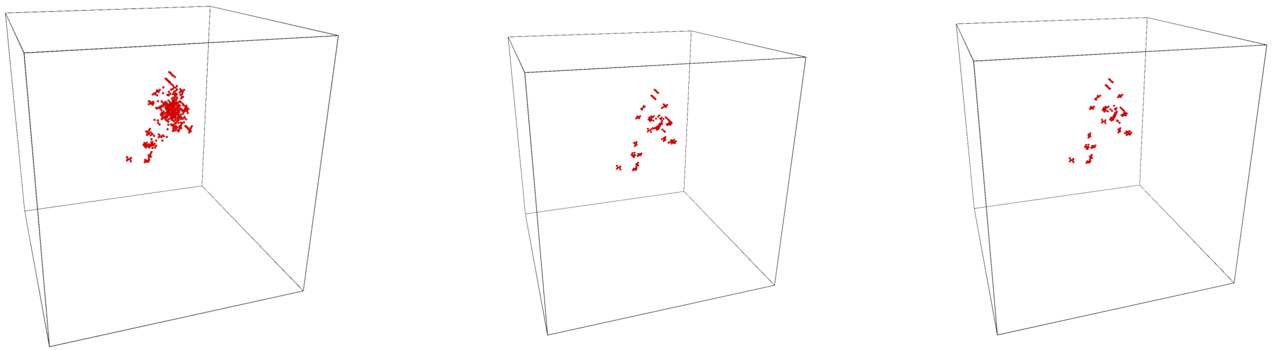
**W subcascade phenomena. Characterization. Law extrapolation.**

W-0K- 10KeV

TIME



0.2 ps



20 ps



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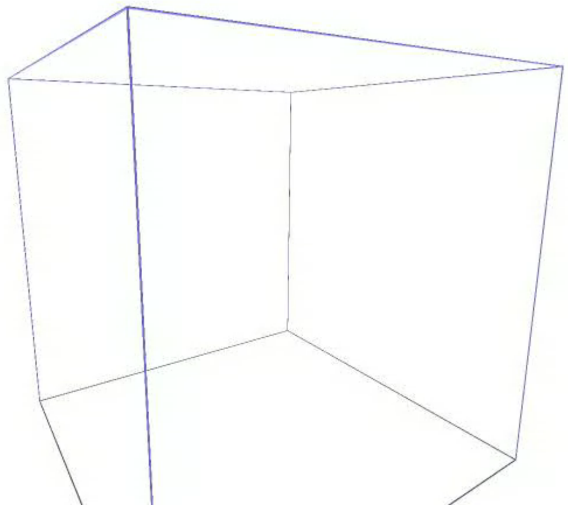
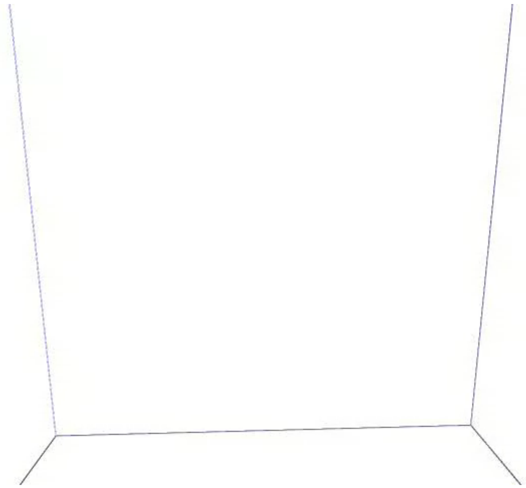
## SCIENTIFIC ACTIVITY - I

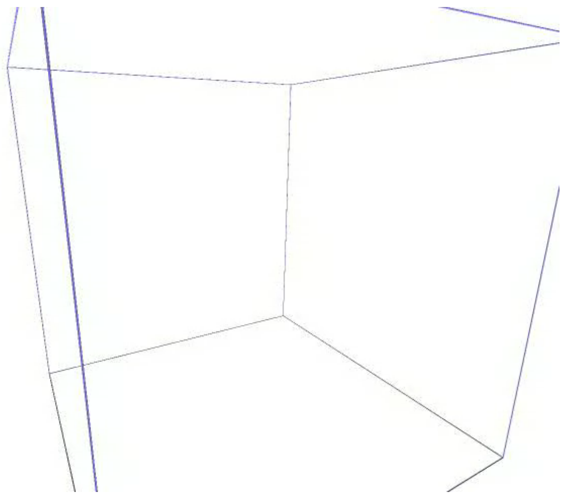
## TOWARDS A RAD. DAMAGE DATA BASE IN Cu, Nb, W

Cu-0K- 0.1KeV



Nb-0K- 1KeV





**A visual data-base is also being produced**

**Our database is comparing the following potentials:**
**Cu: Caro et al 2011. S.M. Foiles, Phys. Rev. B 32, 7685 (1985), Mishin Cu EAM1 PRB (2001)63:224106,**
**W: Zhou W Acta mater(2001)49:4005**
**Nb: LANL- Caro et al.-2011.**
**M. R. Fellinger, H. Park, J. et al. Phys. Rev. B 81, 144119 (2010).**
**Force-matched EAM potential for niobium; implemented by M. R. Fellinger**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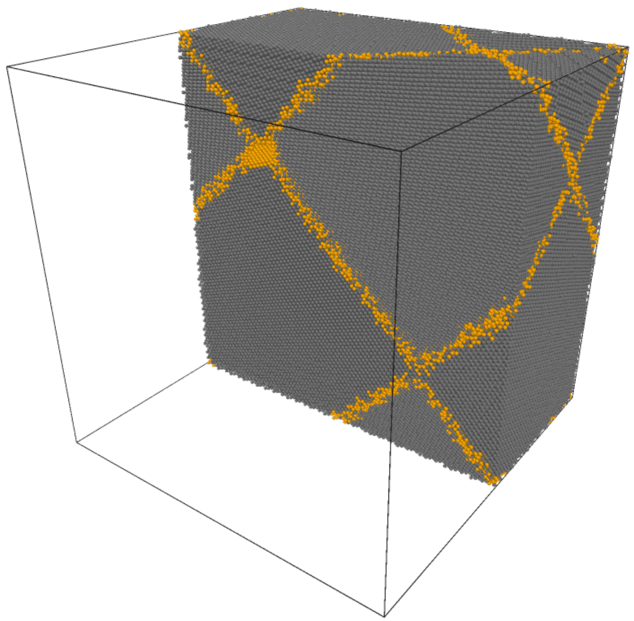
## SCIENTIFIC ACTIVITY - 2

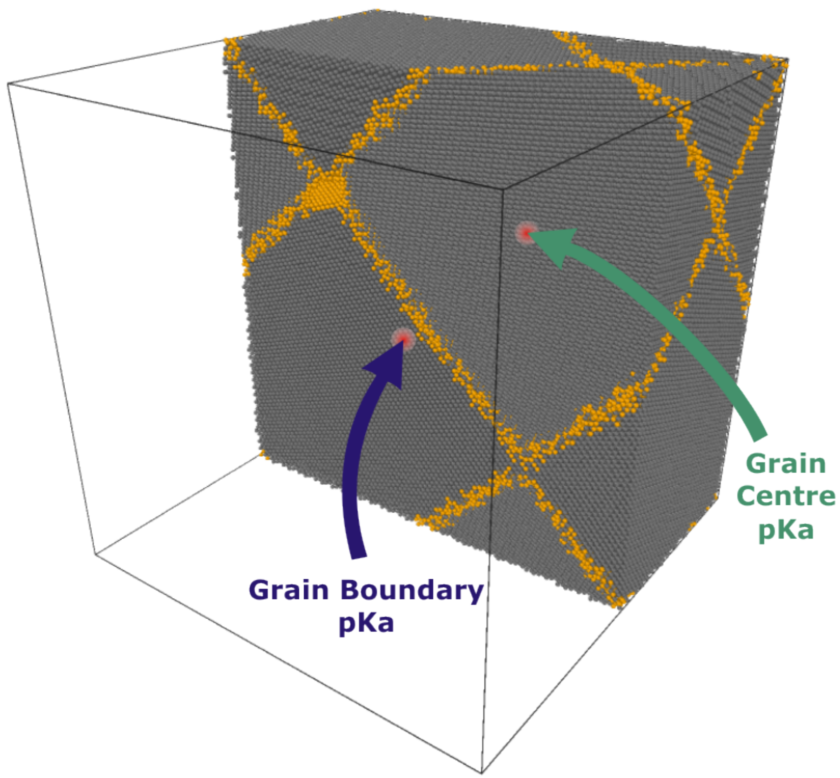
## PRODUCTION OF NANOCRYSTALLINE SAMPLES CASCADE SIMULATIONS

* We have developed a methodology for NC-samples production and GB relaxation.
* Samples range in the size of 1M to 5M atoms
* Simulations at 10KeV / 30 KeV (two reg.) are being produced for NC-Cu,Nb,W. (T dependence).

Note ! Important initial step for He bubbles.

Three different samples have been already produced and relaxed.

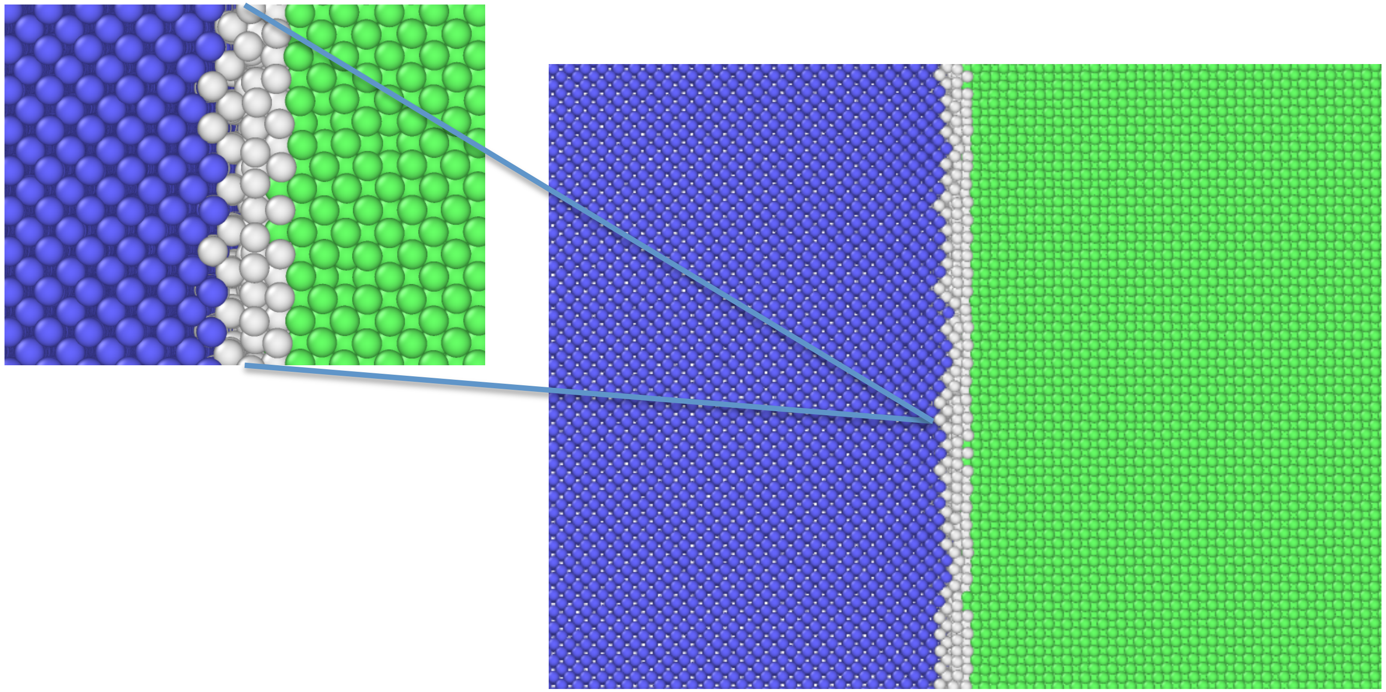


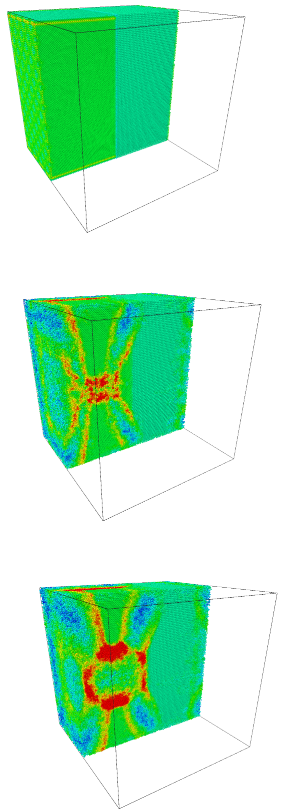


## SCIENTIFIC ACTIVITY - 3

**CuNb – Interface Generation / Relaxation**
**Cascade simulations directed towards interface.**

* We have developed a methodology for NbCu-samples production and interface adaptive relaxation.
* We have discovered problems in the CuNb potential (LANL).
* Simple minimization of the interfaces is not enough.
* Samples range in the size of 1M to 10M atoms
* Simulations of cascades at 10KeV / 30 KeV are being produced for CuNb interfaces.
But need check of potentials. (T dependence).



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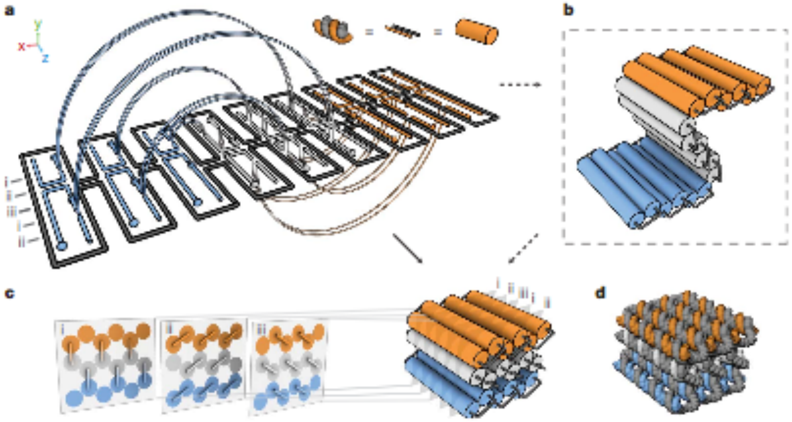
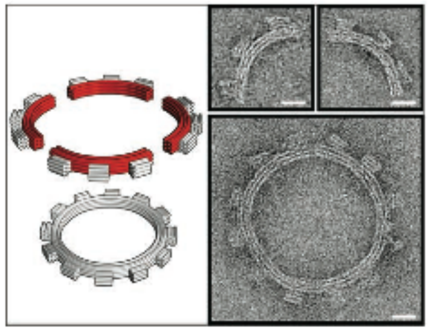
## An example: DNA as a “nanotech – tool”

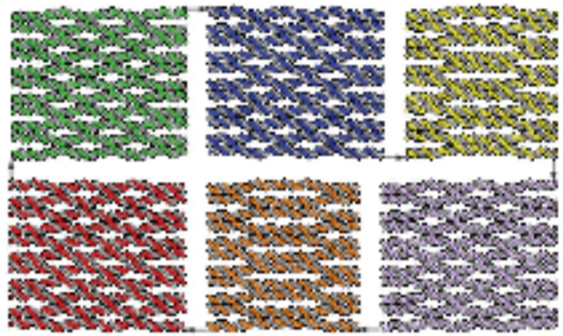
**The specificity of the interactions between complementary base pairs make DNA a useful construction material.**

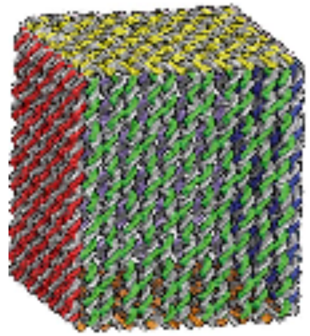
**Present and future ->**

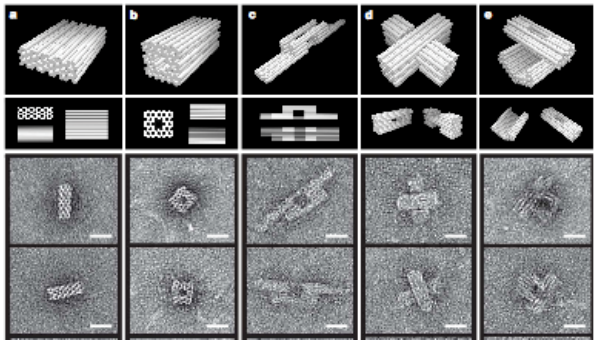
**DNA “origami”**

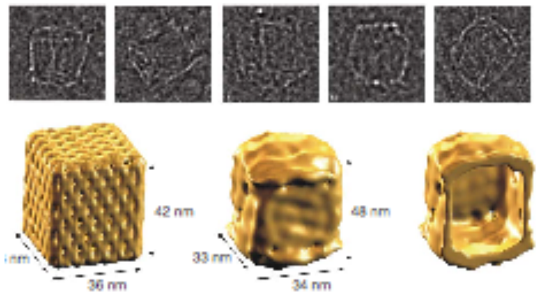


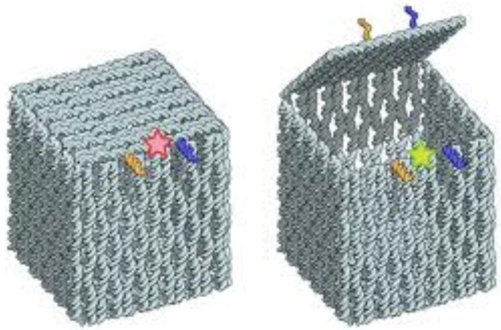








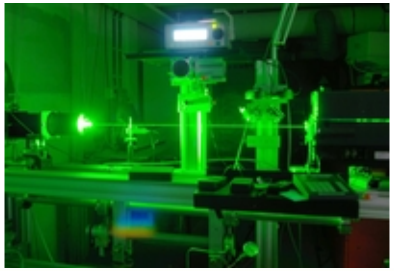


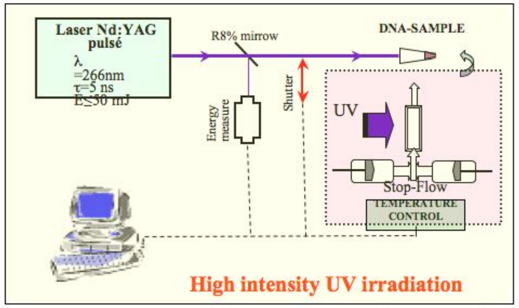



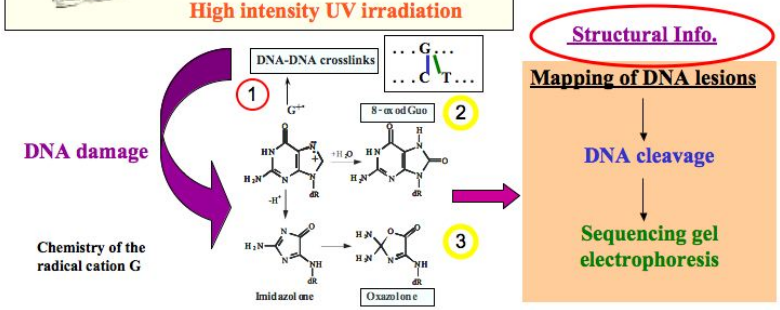
Everything is based on physicochemical interactions at molecular level

# MOLECULAR PROBES CREATED BY HIGH INTENSITY UV LASER IRRADIATION

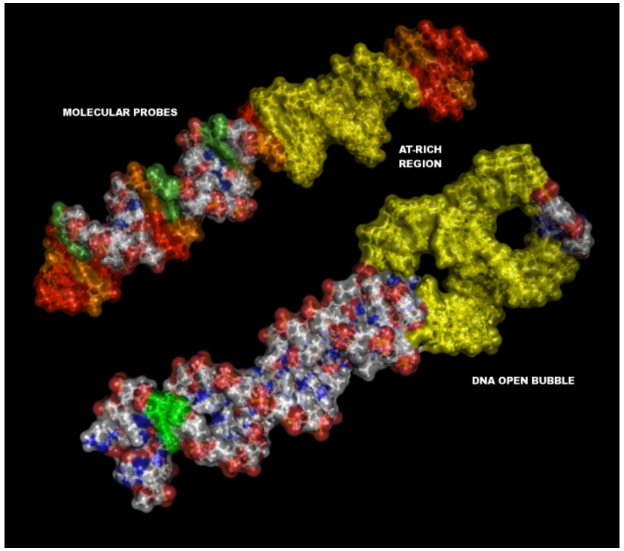
Based on the work of Dimitar Angelov et al.

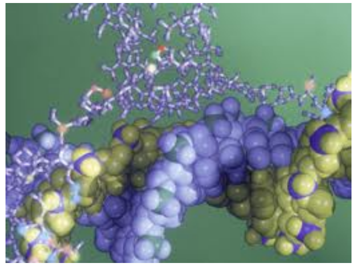


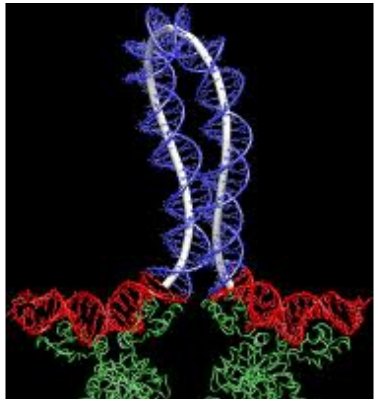



* Obtain instantaneous information without perturbing structure or dynamics.

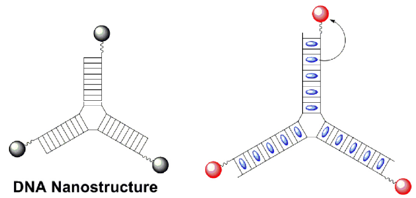




* Physics of DNA/RNA assembly.
Characterizing structural assemblies in μRNA, Hairpins and loop structures.

* Structural stability and integrity of DNA/RNA "puzzle pieces" - Nanoarchitecture.

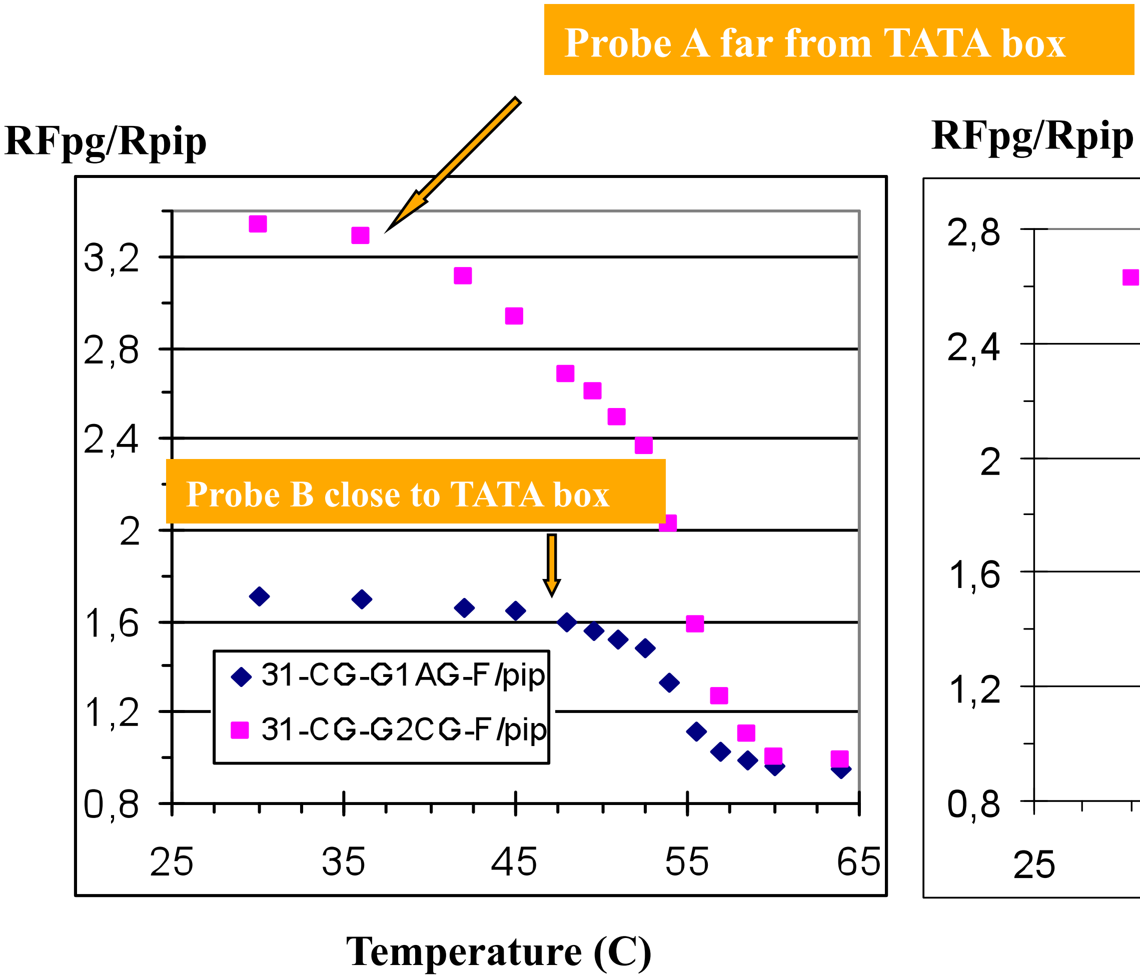


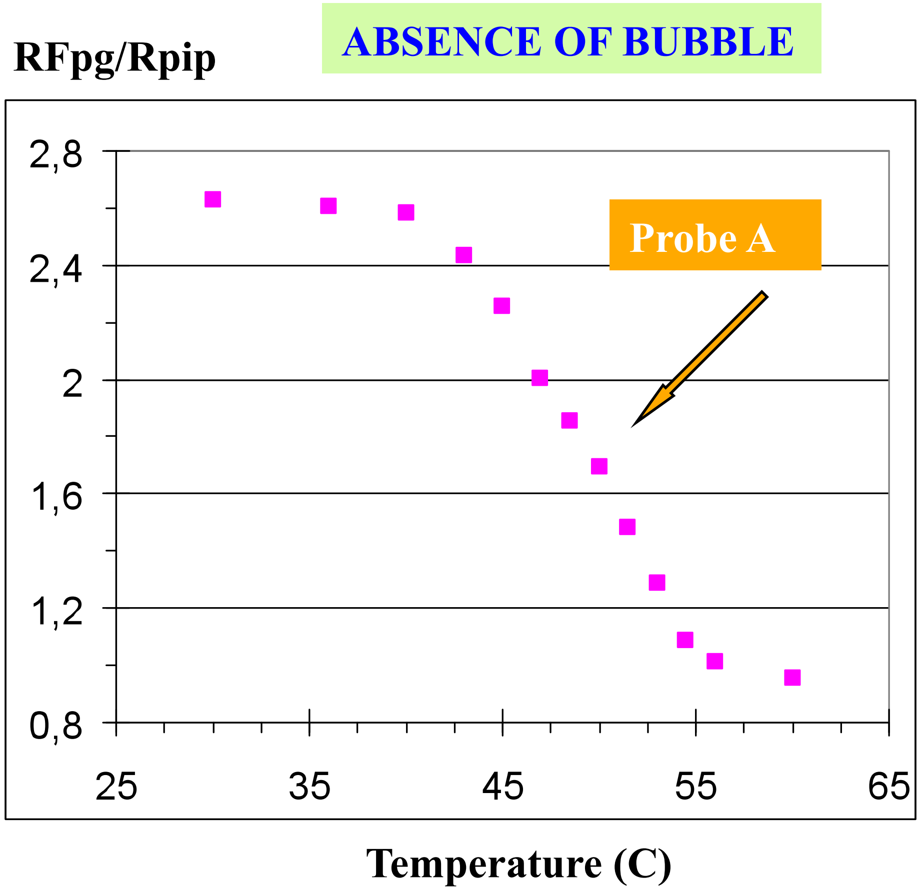

**DNA FLUCTUATIONS PROPAGATE IN GENE PROMOTERS !!! FIRST EVIDENCE !!!**

*** WE HAVE DISCOVERED THIS EFFECT IN DIFFERENT GENES OF BACTERIA LIKE YERSINIA PESTIS, HELICOBACTER PYLORI AND MANY MORE !!!!

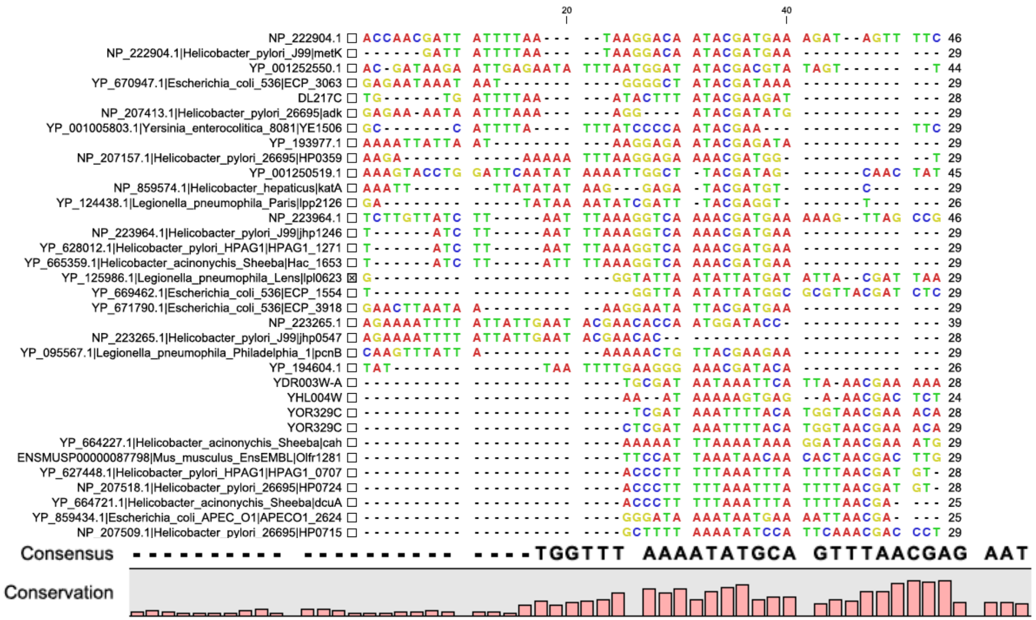
S.Cuesta-Lopez et al. *Nucl. Acids. Research*. *NAR-02191-F-2010.DOI.GKR096*. (2011)
S.Cuesta-Lopez et al. *Eur. Phys. Letters* (2009)

The effect disappears when we have shorter oligos with NO AT-rich prone bubble region

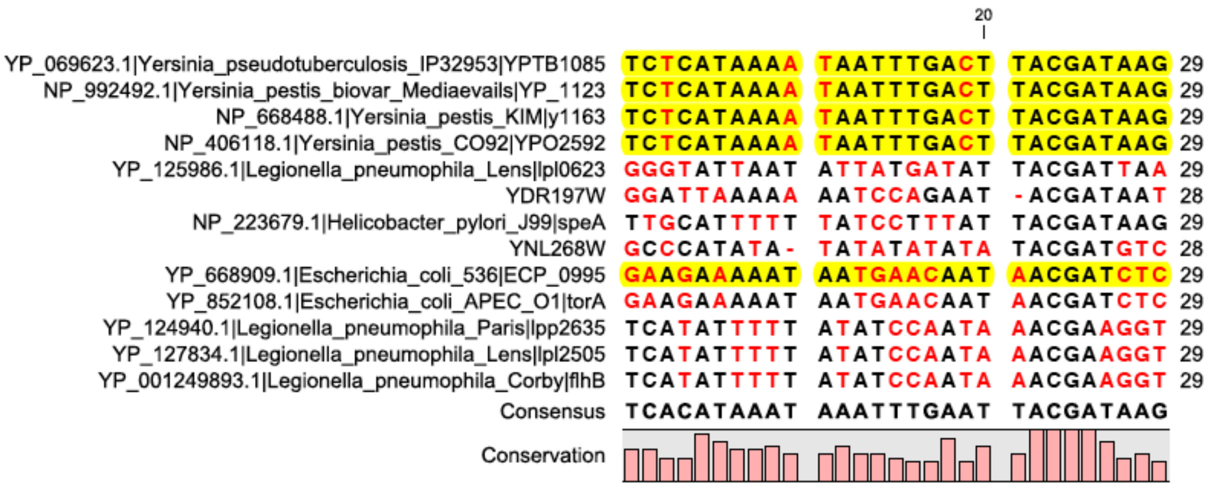


## $-45$ to $+20$ around the transcription-starting site $(+1)$




**Looking for a very restrictive pattern:**




$$(w)_m(N)_p wwACGA$$

$$9 \leq m \leq 12$$

$$4 \leq p \leq 6$$

## EXACT PRESENCE OF OUR SEQUENCES IN IDENTIFIED GENES

S4- AAAAATAATGAACAATAACGAT

|NC_000913.2| gb|U00096.2| Escherichia coli str. K-12 substr. MG1655 chromosome. Position: 1058472 to 1058493
|AC_000091.1| dbj|AP009048.1| Escherichia coli str. K12 substr. W3110 DNA. Position: 1059671 to 1059692
|NC_012947.1| gb|CP001665.1| Escherichia coli 'BL21-Gold(DE3)pLysS AG'. Position: 2718848 to 2718827
|NC_012967.1| gb|CP000819.1| Escherichia coli B str. REL606. Position: 1075539 to 1075560
|NC_011748.1| emb|CU928145.2| Escherichia coli 55989 chromosome. Position: 1167916 to 1167937
|NC_011740.1| emb|CU928158.2| Escherichia fergusonii ATCC 35469 chromosome. Position:1233703 to 1233724
|NC_011751.1| emb|CU928163.2| Escherichia coli UMN026 chromosome. Position: 1232381 to 1232402
|NC_007946.1| gb|CP000243.1| Escherichia coli UTI89, Position: 1047532 to 1047553
|NC_008563.1| gb|CP000468.1| Escherichia coli APEC O1, Position: 1046489 to 1046510
|NC_009800.1| gb|CP000802.1| Escherichia coli HS, Position: 1120085 to 1120106
|NC_009801.1| gb|CP000800.1| Escherichia coli E24377A, Position: 1137132 to 1137153
|NC_010468.1| gb|CP000946.1| Escherichia coli ATCC 8739 Position: 2850666 to 2850645
|NC_010473.1| gb|CP000948.1| Escherichia coli str. K12 substr. DH10B, Position: 1112400 to 1112421
|NC_011415.1| dbj|AP009240.1| Escherichia coli SE11 DNA, Position: 1142585 to 1142606
|NC_011741.1| emb|CU928160.2| Escherichia coli IAI1 chromosome, Position: 1110913 to 1110934
|NC_011750.1| emb|CU928164.2| Escherichia coli IAI39 chromosome, Position: 2204306 to 2204285
|NC_013361.1| dbj|AP010953.1| Escherichia coli O26:H11 str. 11368 DNA, Position: 1550618 to 1550639
|NC_013353.1| dbj|AP010958.1| Escherichia coli O103:H2 str. 12009 DNA, Position: 1144584 to 1144605
|NC_013364.1| dbj|AP010960.1| Escherichia coli O111:H- str. 11128 DNA, Position: 1175204 to 1175225
|NC_011745.1| emb|CU928162.2| Escherichia coli ED1a chromosome, Position: 1093419 to 1093440
|NC_004337.1| gb|AE005674.1| Shigella flexneri 2a str. 301, Position: 1046838 to 1046859
|NC_004741.1| gb|AE014073.1| Shigella flexneri 2a str. 2457T, Position: 1051070 to 1051091
|NC_010498.1| gb|CP000970.1| Escherichia coli SMS-3-5, Position: 2137381 to 2137360
|NC_013941.1| gb|CP001846.1| Escherichia coli O55:H7 str. CB9615, chromosome Position: 1284960 to 1284981
|NC_004431.1| gb|AE014075.1| Escherichia coli CFT073, Position: 1093989 to 1094010
|NC_007384.1| gb|CP000038.1| Shigella sonnei Ss046, Position: 1078887 to 1078908
|NC_007613.1| gb|CP000036.1| Shigella boydii Sb227, Position: 2221893 to 2221872
|NC_011742.1| emb|CU928161.2| Escherichia coli S88 chromosome, Position: 1050077 to 1050098
|NC_002695.1| dbj|BA000007.2| Escherichia coli O157:H7 str. Sakai DNA, Position: 1237602 to 1237623
|NC_007606.1| gb|CP000034.1| Shigella dysenteriae Sd197, Position: 920445 to 920466
|NC_008253.1| gb|CP000247.1| Escherichia coli 536, Position: 1053574 to 1053595
|NC_008258.1| gb|CP000266.1| Shigella flexneri 5 str. 8401, Position: 1055416 to 1055437
|NC_010658.1| gb|CP001063.1| Shigella boydii CDC 3083-94, Position: 2106513 to 2106492
|NC_011353.1| gb|CP001164.1| Escherichia coli O157:H7 str. EC4115, Position:1242635 to 1242656
|NC_011601.1| emb|FM180568.1| Escherichia coli 0127:H6 E2348/69, strain E2348/69. Position: 1092006 to 1092027
|NC_013008.1| gb|CP001368.1| Escherichia coli O157:H7 str. TW14359, Position: 1242921 to 1242942

## EXACT PRESENCE OF OUR SEQUENCES IN IDENTIFIED GENES

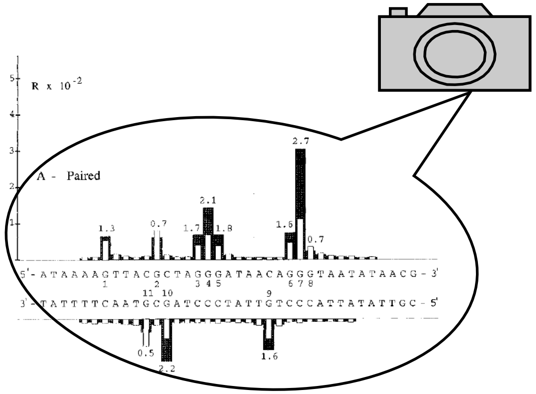
**S5-** ATAAAATAATTTGACTTACGATAA

|NC_014029.1| gb|CP001593.1| Yersinia pestis Z176003 chromosome.Position: 2971517 to 2971540
|NC_010634.1| gb|CP001048.1| Yersinia pseudotuberculosis PB1/+ chrom. Position: 1286406 to 1286429
|NC_010465.1| gb|CP000950.1| Yersinia pseudotuberculosis YPIII chrom. Position: 3334594 to 3334571
|NC_010159.1| gb|CP000901.1| Yersinia pestis Angola. Position: 1927953 to 1927930
|NC_009708.1| gb|CP000720.1| Yersinia pseudotuberculosis IP 31758. Position: 3332366 to 3332343
|NC_009381.1| gb|CP000668.1| Yersinia pestis Pestoides F.Position: 3013580 to 3013557
|NC_008150.1| gb|CP000308.1| Yersinia pestis Antiqua. Position: 2795943 to 2795920
|NC_008149.1| gb|CP000305.1| Yersinia pestis Nepal516. Position: 1240988 to 1241011
|NC_006155.1| emb|BX936398.1| Yersinia pseudotuberculosis IP32953. Position: 1303904 to 1303927
|NC_005810.1| gb|AE017042.1| Yersinia pestis biovar Microtus str. 91001. Position: 1204012 to 1203989
|NC_003143.1|emb|AL590842.1| Yersinia pestis CO92.Position: 2914936 to 2914959
|NC_004088.1|gb|AE009952.1| Yersinia pestis KIM 10 chromosome. Position: 1311087 to 1311110

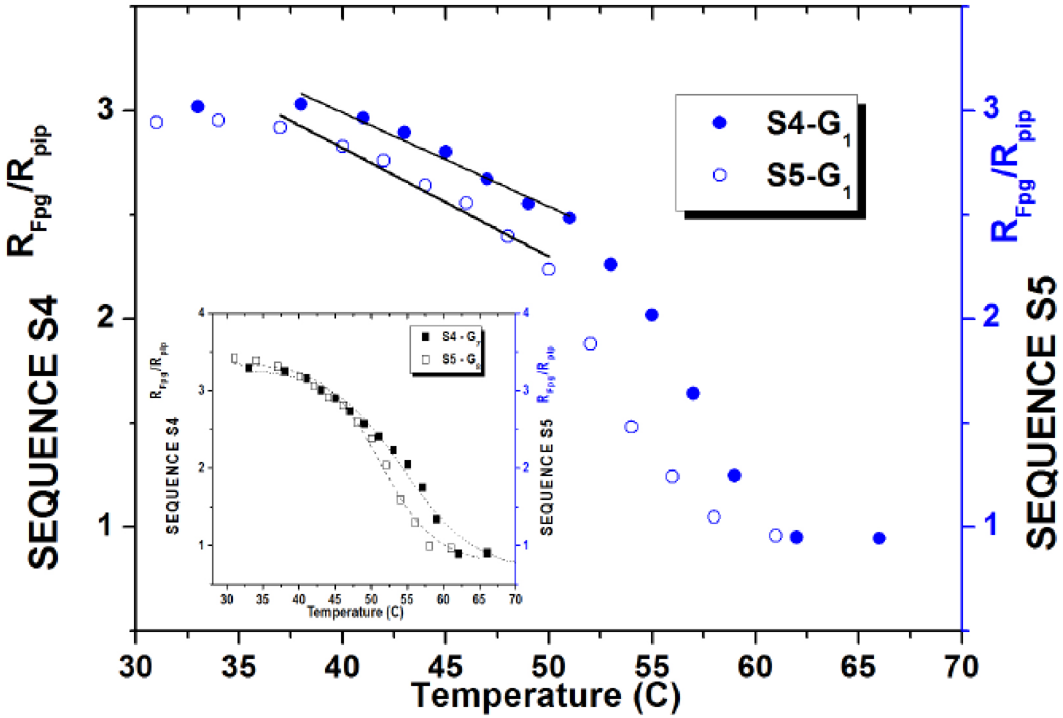
## IMPORTANT BIOLOGICAL IMPLICATIONS AT THE LEVEL PROTEIN-DNA INT. AND GENE SILENCING !!!

### Critical implications in:

- Fundamental biology: protein-DNA interaction.
- Drug binding.
- Structural effects in DNA design for assemblies and sensors.




Structural information for the stability of all the GC pairs present in our sequence is available at one shot !!



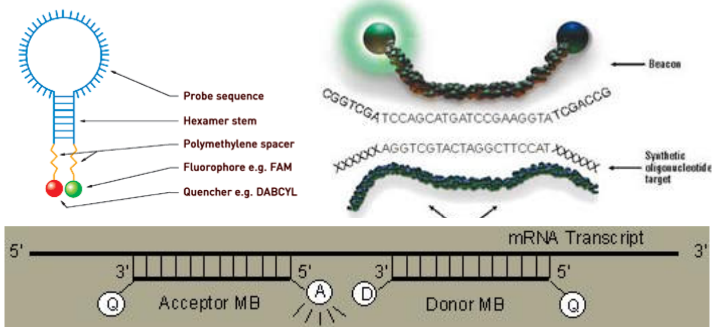

S4: 5' ggcgtAAAAATAAT **G**$_7$ AACAATAAC **G**$_1$ ATCTtccgg 3

S5: 5' ggcgtATAAAATAATTT **G**$_8$ ACTTAC **G**$_1$ ATAAtccgg 3

*** The effect is confirmed in two promoter regions of well known bacteria !!! :

*Escherichia Coli* (ECP-0995)
*Yersinia Pestis CO92* (YPO2592)

# Learning from nature: DNA/RNA hairpins as molecular probes.
# Biophysics of *"Molecular Beacons"*. Biosensors.

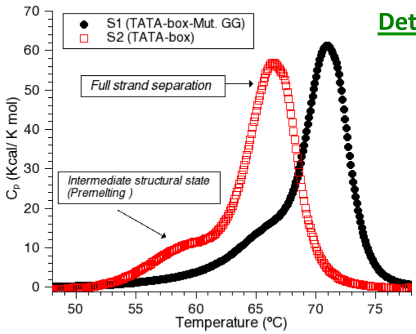



Bacterial activity in food ? Food technology development? Burgos ??

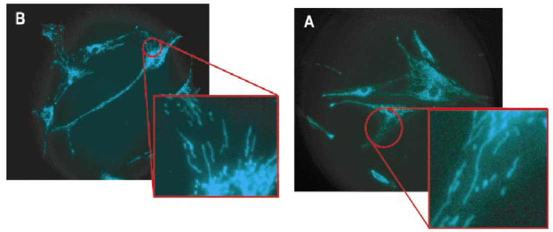
Fluorescence resonance energy transfer (FRET)

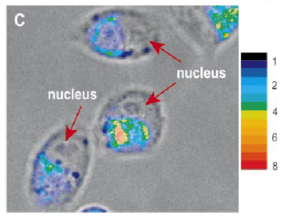
IN VIVO detection of gene expression

Molecular & cellular sensors. Early Cancer detection.




Detecting pre-melting in genes (particular gene expression):

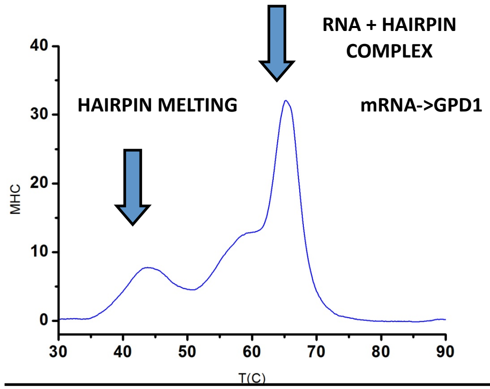



THERMODYNAMICS OF DNA HAIRPINS: SENSOR + TARGET BINDING. FIRST TESTS !

**Nano-Calorimetry & Biomolecular interactions**

Fast and simple way to test designs ! Thermodynamics + mol. Affinity -> DSC+ITC !




Detecting structural assemblies in $\mu$RNA, Hairpins and loop structures

Work of Philip J. Santangelo et al
Nucleic Acids Research, 2004, Vol. 32, No. 6 e57

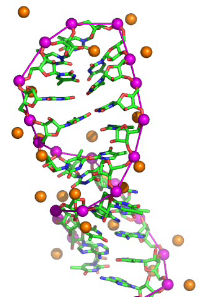
# THEORETICAL & COMPUTATIONAL DESIGN OF DNA/RNA HAIRPINS

## Effective design process of hairpin structures for technological applications:

Molecular Tests in solution

Final Design and synthesis

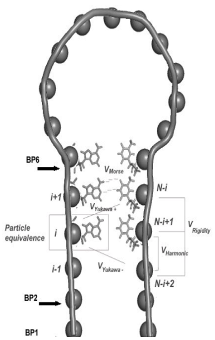
**T-12HP Loop**
**10 ns MD explicit solvent.**
**10.000 $H_20$ Molecules !!**



Coarse-grained models

DNA stability/folding
Nearest-neighbor model

All atom MD modelling



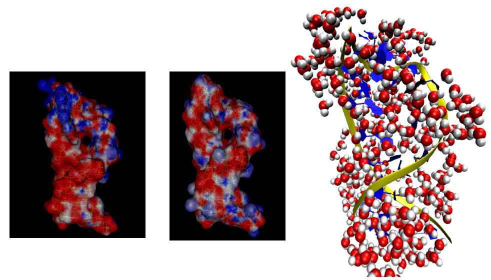

**Based on Peyrard-Bishop model**

**S.Cuesta-Lopez et al Eur. Phys. J. E 16, 235 246 (2005)**

**Table 1** Nearest-neighbor thermodynamic parameters for Watson-Crick base pairs.

| Sequence | $\Delta H$ (ref. [9]) (kcal/mol) | $\Delta S$ (ref. [9]) (eu) | $\Delta G$ (ref. [9]) (kcal/mol) | $\Delta G$ (ref. [8]) (kcal/mol) |
|---|---|---|---|---|
| AA/TT | -8.3 ± 1.1 | -23.3 ± 3.1 | -1.04 ± 0.07 | -1.00 ± 0.01 |
| AT/TA | -8.5 ± 0.8 | -24.6 ± 2.4 | -0.91 ± 0.04 | -0.88 ± 0.04 |
| AC/TG | -9.5 ± 1.1 | -26.0 ± 2.7 | -1.49 ± 0.09 | -1.44 ± 0.04 |
| AG/TC | -8.2 ± 0.9 | -22.3 ± 2.7 | -1.33 ± 0.06 | -1.28 ± 0.03 |
| TA/AT | -6.5 ± 0.6 | -19.0 ± 1.7 | -0.60 ± 0.04 | -0.58 ± 0.06 |
| TC/AG | -8.7 ± 0.8 | -23.6 ± 2.4 | -1.35 ± 0.03 | -1.30 ± 0.03 |
| TG/AC | -8.9 ± 0.4 | -24.3 ± 1.3 | -1.51 ± 0.05 | -1.45 ± 0.06 |
| CC/GG | -8.8 ± 0.6 | -21.7 ± 1.7 | -1.90 ± 0.04 | -1.84 ± 0.04 |
| CG/GC | -11.4 ± 0.9 | -29.7 ± 2.5 | -2.25 ± 0.06 | -2.17 ± 0.05 |
| GC/CG | -10.1 ± 1.0 | -25.4 ± 2.6 | -2.28 ± 0.06 | -2.24 ± 0.03 |
| init_AT | 4.4 ± 1.4 | 10.7 ± 3.9 | 1.19 ± 0.09 | 1.03 ± 0.05 |
| init_GC | 1.7 ± 0.5 | 2.4 ± 1.3 | 1.12 ± 0.03 | 0.98 ± 0.05 |
| symmetry | 0 ± - | -1.4 ± - | 0.4 ± - | 0.4 ± - |

**MFOLD server**



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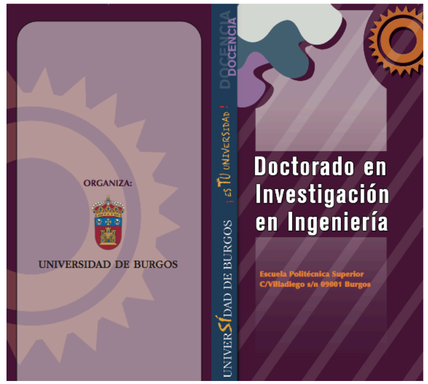
- Lyon – (FRANCE).
Laboratoire de Physique. ENS-Lyon & CNRS
Laboratoire transdisciplinaire Joliot-Curie.


**Dr. Santiago Cuesta-López (UBU)**
**Dra. Lorena Romero Santacreu (UBU/UV)**
**Advanced Materials, Nuclear Technology & Nanotechnology**
**Universidad de Burgos, Parque Científico – I+D+I, CITI- Burgos, Spain**

**In collaboration with:**

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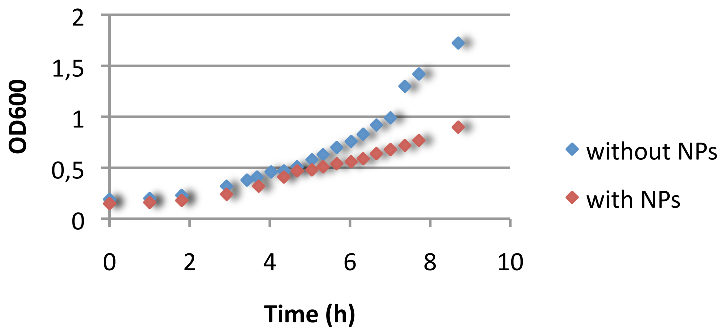
# Evaluation of the toxicity of NANOMATERIALS & Nanoparticles (NP)

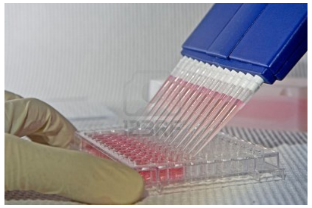
**METHODOLOGIES TO TEST THE CITOTOXICITY OF NANOMATERIALS:**

**1-** **RAPID SCREENING, USING CULTURES OF THE EUKARYOTIC MODEL ORGANISM *Saccharomyces cerevisiae* WITH DIFFERENT CONCENTRATIONS OF NPS/NANOMAT.**

Evaluation of the cell growth with NPs by spectrophotometry




RAPID SCREENING, USING CULTURES OF YEAST WITH DIFFERENT CONCENTRATIONS OF NP

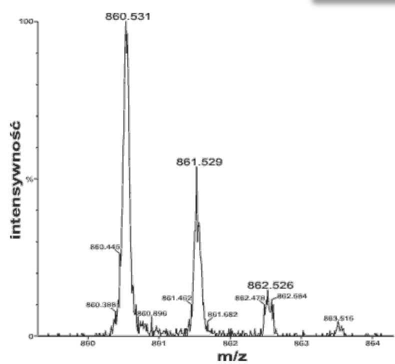


Using microplates we can quickly compare among different NPs. **This screening can be a previous, cheap and quick way to reject toxic NPs.**

**Homologue fast assays are possible in mammalian cells. See 2-**

**UPTAKE AND ACUMULATION OF NPs INTO THE CELL**

Mass spectrometry: Extract of cells cultivated with NPs




NP intake and internalization into cells will be quantified by means of mass spectrometry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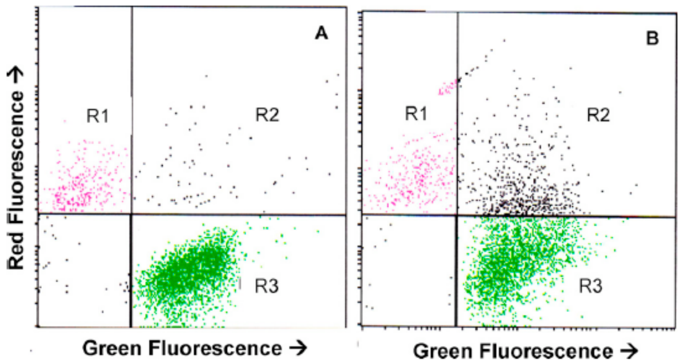
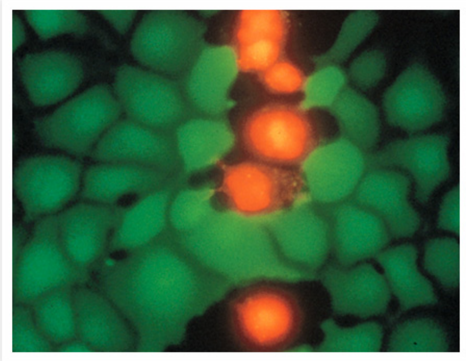
*S.Cuesta-Lopez, PhD. E-mail: scuesta@ubu.es*
*Advanced Materials & Bio/Nano-Physics*

## 2- IMPACT OF NPS ON CELL SUBCELLULAR COMPARTMENTS
## (applicable in bacteria, yeast or other eukaryotic organisms like mammalial/human cells)

### Viability assays using LIVE/DEAD assays to determine damage of the cell membrane using flow cytometry

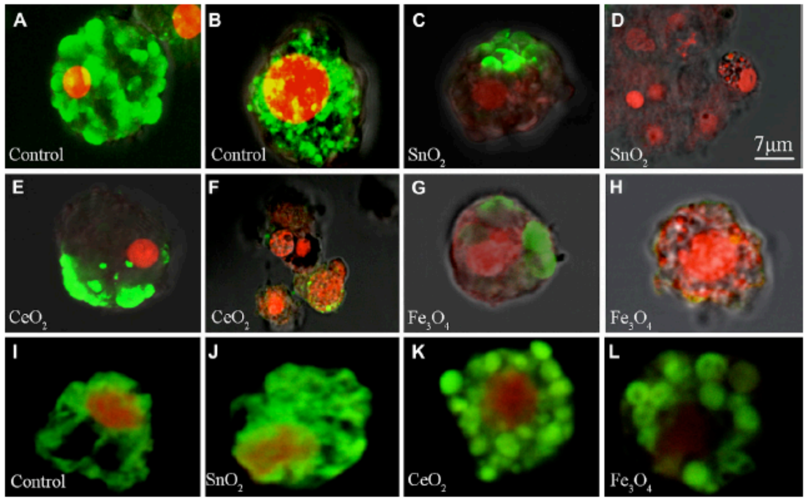
This makes it possible to distinguish live cells from dead cells



The viability assays can be evaluated using flow citometry, fluorescence microscopy or fluorometry using microplates to compare among many samples.

### Evaluation by microscopy of cell stained to visualize the cell subcellular compartments




To determine the damage of the cell membrane and the organelles integrity
To determine the subcellular localization of NPs accumulation

The figure shows the Impact of different NPs on endoplasmic reticule (A to H) and lysosomes (I to L) stained (green fluorescence). Nuclei are counterstained in red fluorescence.



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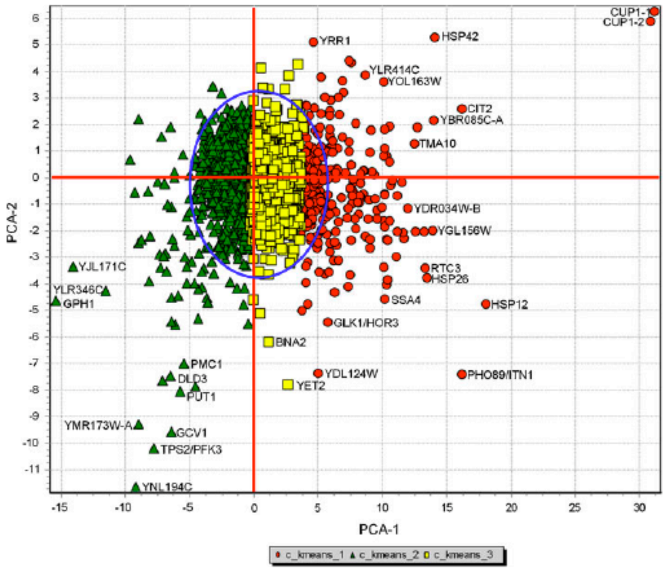
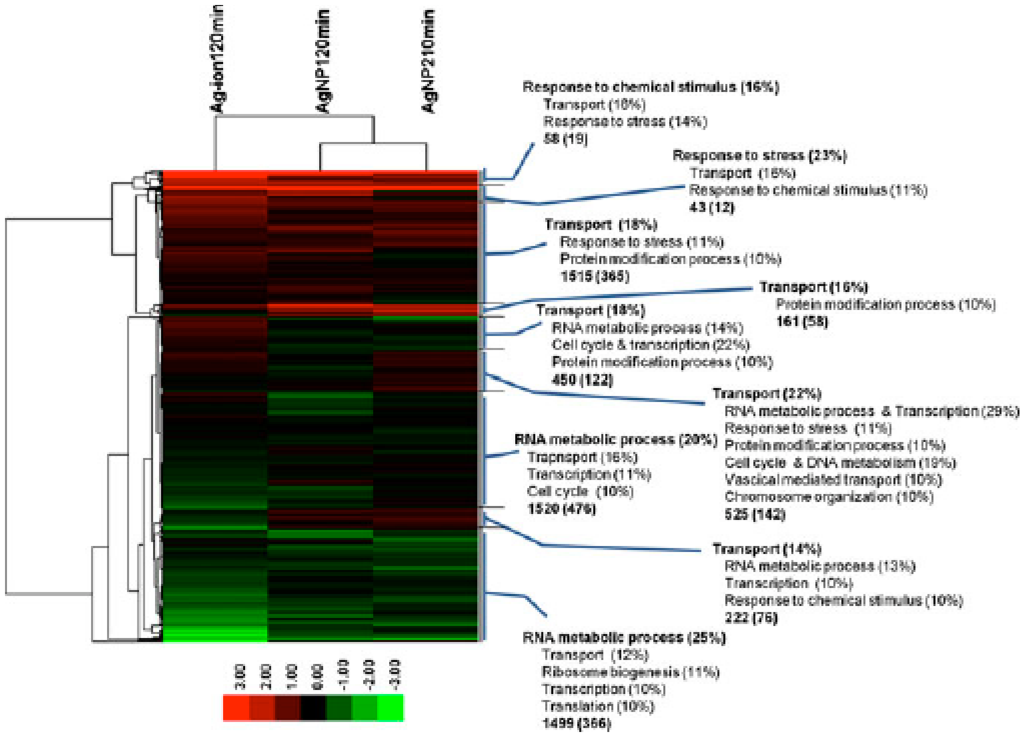
## 3- PROPOSAL OF GENOMIC STUDIES IN CELLS, REVEALING CHANGES IN GENE EXPRESSION AFTER EXPOSURE TO NPs

Genomic studies analyze the gene expression of the whole genome and with this methodology we can compare the gene expression between two condition (absence or presence of NPs).




Principal component analysis The colors indicate the differentially expressed genes affected by NPs.




Hierarchical clustering analysis of gene expression data. Red color indicates overexpression and green underexpression. This analysis tell us the cellular process and functions that are affected by the presence of NPs.

- Identify expression changes in <u>genes related with human diseases like cancer</u>. This changes may be not detected in toxicity assays.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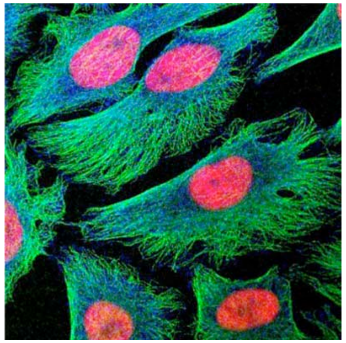
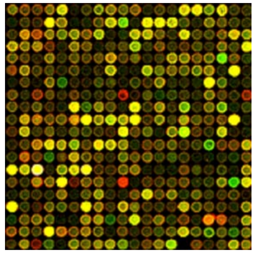
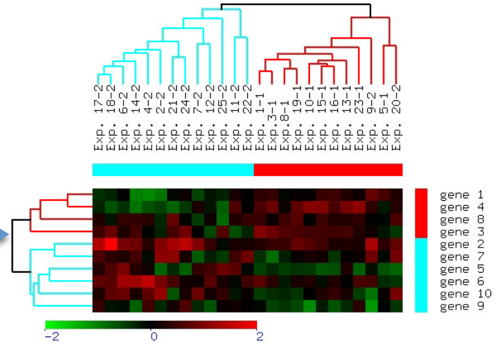
4-

## WE PROPOSE A COMPREHENSIVE STUDY TO KNOW THE RESPONSE TO NPS IN HUMAN BRONCHIAL EPITHELIAL CELLS

GENOMIC ANALYSIS TO DETECT TRANSCRIPTIONAL RESPONSE AND TO KNOW THE KIND OF DAMAGE (oxidative toxicity, cell wall damage, …)








Evaluation of in the global gene response using microarrays to detect changes in the gene expression of BEAS-2B cells exposed to NPs.

Bioinformatics analysis of results: Normalization, significance analysis of microarrays, Principal Component Analysis, clustering

•Identify patterns of behavior (groups of genes affected specifically) **to build up and patent future genomic detection kits of citotoxicity.**

**Points 3 & 4 have been identified as a nice base to explore in a EU-Project**

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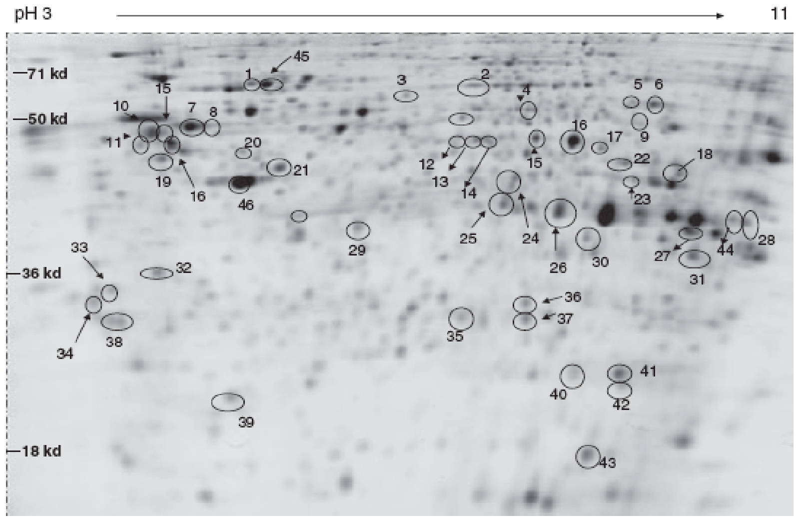
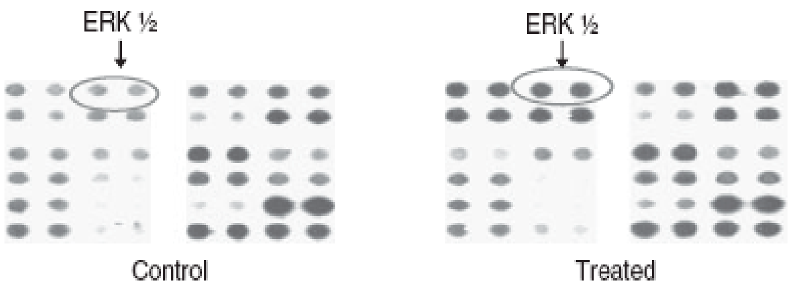
*Advanced Materials & Bio/Nano-Physics*

5- PROTEOMIC ANALYSIS TO DETECT POTENTIAL TOXICITY AND DETOXIFICATION PATHWAYS




Total of proteins are separated by isoelectric point and mass on 2D gels gel electrophoresis. By comparison between 2D gels of protein extracts of cells in presence and absence of NPs we can know the proteins differential expressed. Mass spectrometry proteins **can identify these proteins and tell us the toxicity pathways and networks that are associated with exposure to engineered nanomaterials, like oxidative stress pathway and others related to cancer and inflammatory diseases.**




This array is a rapid and sensitive to detect the specific phosphorilation and activation of the most important human protein kinases. Kinases are known to regulate the majority of cellular pathways, especially those involved in signal transduction, so we can identify the upstream kinase signaling pathways that are activated in response to nanomaterials by comparison with a control (absence of NPs).

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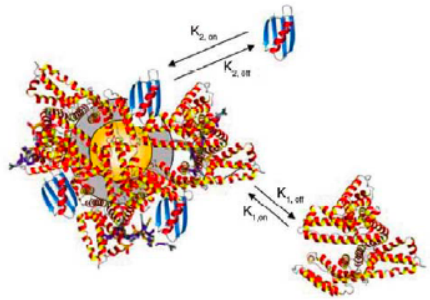
# OTHER STUDIES: Complementary – basic biophysics

## Measure of toxicity by studying nanoparticle interaction with plasma proteins

### BIOPHYSICAL BASIS OF NP TOXICITY:

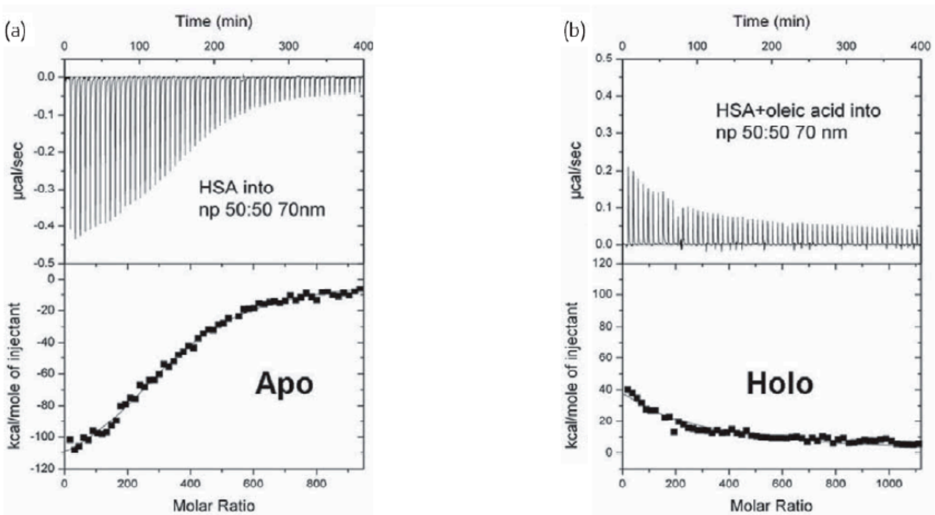
** Interaction of a Nanoparticle, in physiological conditions, with common plasma proteins.



### PROPOSE A NEW METHODOLOGY BASED ON OUR LONG EXPERIENCE WITH BIOPHYSICAL AND MOLECULAR BIOLOGY TECHNIQUES:

♦ Combining both **Nano-calorimetry** ITC and protein electrophoresis to screen an ensemble of plasma proteins against NPs:

### Protein-nanoparticle affinity: Isothermal titration calorimetry (ITC)




Protein-protein interactions and other molecule reactions can be studied through ITC. We propose this technique to study nanoparticle-plasma proteins interactions



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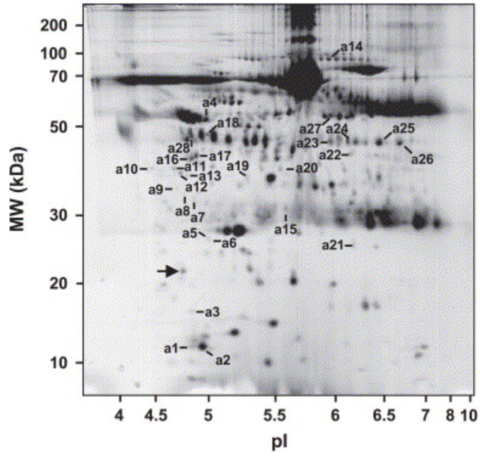
**PROTEIN ELECTROPHORESIS. We propose two different experiments to show quantitatively the ability of nanoparticles to selectively adsorb plasma proteins.**

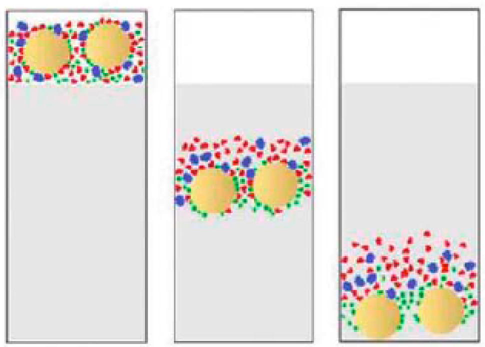
## Incubation NPs-proteins and 2D gel electrophoresis

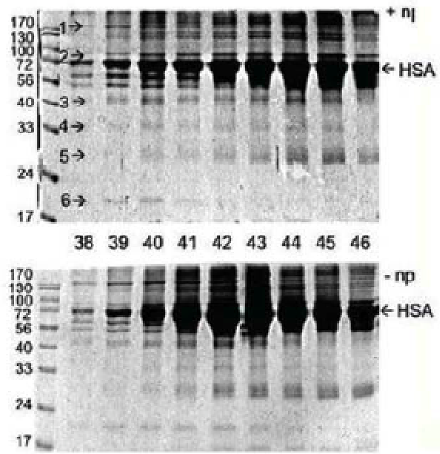
Incubation of NPs with human plasma, after separation by centrifugation and washing, the adsorbed proteins will be eluted from the particle surface using a protein-solubilizing solution and analyzed by 2D gel electrophoresis. Mass spectrometry proteins **can identify proteins that selectively interact with NPs.**




## Size-exclusion chromatography study of nanoparticle-protein interactions

The elution time of different plasma proteins is shifted depending on their affinity for the nanoparticle surface. Each fraction collected from the size-exclusion column contains many different proteins, which can be further separated and identified by gel electrophoresis using denaturing acrylamide gels.



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Thank you !

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Questions?