INSTITUTO NICOLÁS CABRERA

XX International Summer School "Nicolás Cabrera"

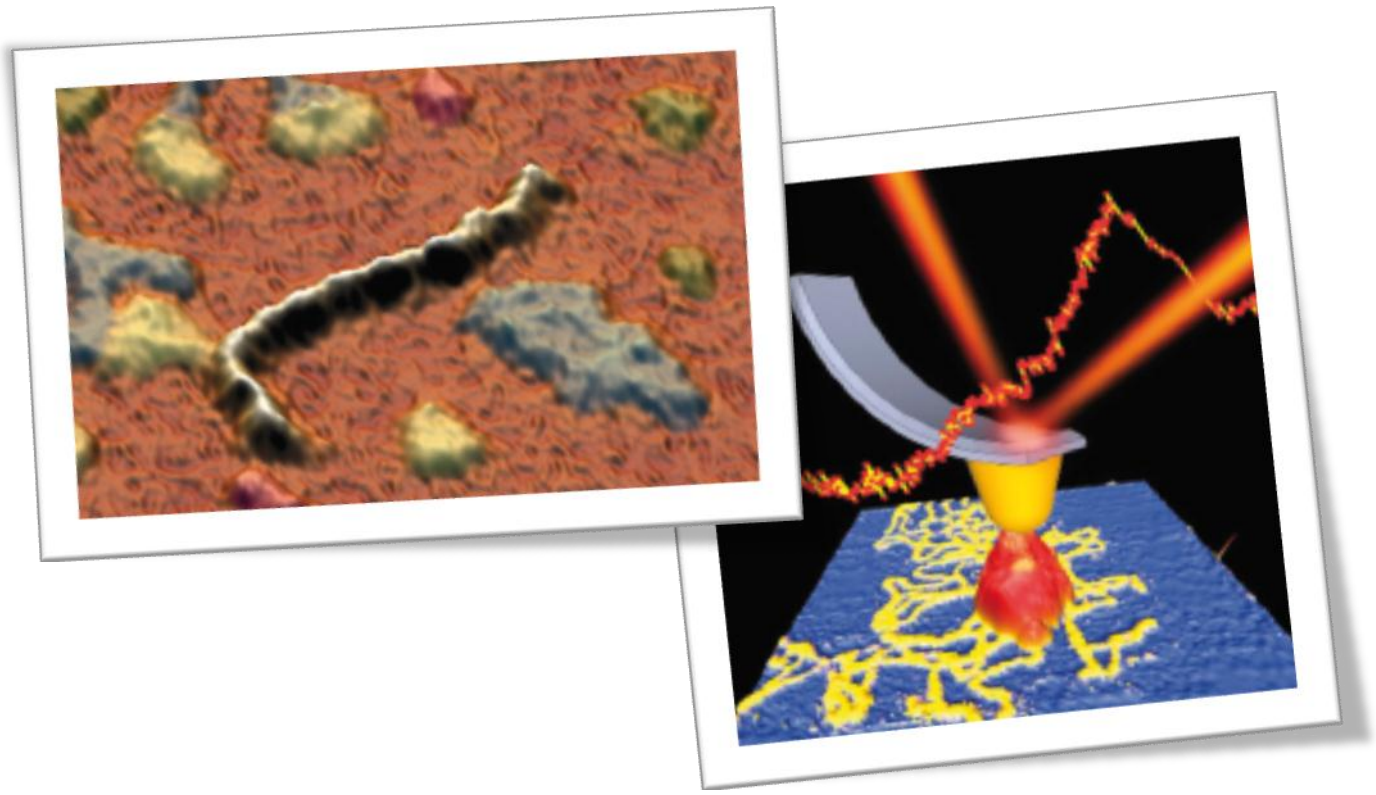
BIOMOLECULES AND SINGLE MOLECULE TECHNIQUES

21 - 26 July, 2013

Residencia "La Cristalera"

Miraflores de la Sierra

Madrid - Spain



UNIVERSIDAD AUTONOMA DE MADRID

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Prof. Nicolás Cabrera

For those of us who had the pleasure of knowing him in his mature years, Nicolás Cabrera comes as close as humanly possible to the ideal figure of a renowned scientist and a perfect gentleman, someone who has a keen insight in the ways of nature and at the same time is fully at ease with his fellow humans, always benevolent and encouraging, yet also reserved and detached in his judgment. Science and academics were in his family line, as he was the son of Blas Cabrera, the leading physicist in Spain between the two world wars. He was given by birth the opportunity to study and work with famous people in France and England, and happily his natural talent was up to the challenge and thrived on it. But he also knew sorrow in his life as an exile from a country torn by civil strife. In his years in North America, there was always an undertone of sadness in his eyes and a longing to return to a free and democratic Spain.

Jack Mitchell recalls that the Cabreras were living in an apartment in Paris when he recruited Nicolás to come to Bristol and work with Neville Mott as a post-doc. In Bristol he produced not only the fundamental theory of crystal growth, but also, with Mott, an important paper on the theory of the oxidation of metals [1]. It was perhaps this work that most impressed Allan Gwathmey of the Chemistry Department at the University of Virginia, and led him to arrange with Jesse Beams, then Chairman of Physics, to offer Cabrera a position, which he accepted in 1952. His scientific and leadership impact at Virginia extended also to Materials Science and other areas of Engineering, as he actively pursued interdisciplinary contacts, and his work transcended narrow boundaries.

My personal recollections of Cabrera date from a splendid spring day in 1962, when I came to give a seminar on dislocation dynamics at the University of Virginia. Those were the happy days in which young physicists did not have to apply for jobs, and I was barely aware of the fact that this was, in effect, an interview. Cabrera, who had just become Chairman, took a genuine interest in what I was doing, asked penetrating questions, and at the same time made me feel completely at ease, no small feat considering that I knew he was an author of the Burton-Cabrera-Frank theory of crystal growth [2], which was already enshrined in textbooks then (and appears even more fundamental today, more than fifty years later). Cabrera was a very effective recruiter at all levels, making effortless use of his natural charm and cultivated European style in personal contacts, and keeping a high awareness of possible candidates through his high-level contacts in the scientific world. Four years later, when I arrived in Charlottesville as the newest faculty member, Cabrera himself came to meet us at the station, which impressed my wife no end.

With Jack Mitchell, Doris Wilsdorf and her husband Heinz (in Materials Science) Virginia was then a leading center of research on dislocations and mechanical properties. Cabrera, however, was always looking for fresh fields. When he called me to his office to tell me of problems he had in mind, I was surprised (but should not have been) that it had nothing to do with crystal growth or dislocations. Instead, he told me of experiments under way in Aerospace Engineering on atom-surface interactions. He thought that atom scattering could be used to detect surface phonons, analogously to neutron scattering in the bulk. The kinematics was right but some approximate cross sections had to be computed to prove that the method was feasible. It sounded complicated enough that we needed a graduate student to help, and as it happened Dick Manson had just asked me for a thesis project. After we read up on scattering theory and surface elastic waves, everything turned out pretty much as Cabrera had anticipated for a simple elastic hard wall [3]. Actual surfaces are much more complicated, as we learned from Frank Goodman, who was working with the Aerospace people. The four of us hammered out a long paper [4], which required some effort to develop a common language, coming as we did from different specialties. Cabrera kept us together, focussing on the physics, and we deferred to him, naturally. This is something I have seen happen repeatedly in collaborations with him. He was a good listener and a natural leader; he did not dominate a discussion but came to the essential point, noting quickly where there was a real disagreement and where it was just a matter of semantics.

In 1967 Cabrera went on leave for a year in Caracas, and then accepted a leadership post at the resurgent Autonomous University of Madrid when Franco was still in power. There were several reasons for these developments. The Physics Department at Virginia had grown and prospered under Cabrera's leadership, not only in Solid State, but also in Nuclear Physics. Unfortunately, some of the senior people had strong and apparently incompatible personalities, although they all had good relations with Cabrera. His patience and diplomatic skills were taxed, especially after some petty problems developed over the management of the nuclear facilities. Scheming and pettiness were so alien to his nature that he was saddened by them, and also felt that he had better ways to spend his time and energy. Although the Cabrera children had grown up as Americans and Blas, then enrolled at Virginia, was clearly on his way to his own physics career in the U.S., nostalgia for Spain was always near the surface, especially for Nicolás's lively wife, Carmen. There were evenings at the Cabreras with singing and guitars, and as the party took wing more and more of the songs were Spanish. But fundamentally it was Nicolás who felt an obligation to return to Spain and rebuild science there.

That it took courage and dedication to do so was apparent to me at the Varenna Summer School on `Dynamic Aspects of Surface Physics' in 1974, organized and directed by Frank Goodman. Although several aspects of surface physics were covered at this school, it was a seminal event in the field of atom-surface interactions, with Boato and Cantini reporting their high-quality diffraction data, Giorgio Benedek lecturing on surface phonons, and J.P. Toennies as one of the `students' absorbing it all and preparing to make the study of surface phonons by He scattering a reality in his laboratory. Cabrera was expected to be one of the main lecturers, but he was able to attend only part of the time, as the situation in Spain had taken a turn for the worse. Incidentally, he was accompanied by Javier Solana, who was then his assistant in

Madrid, before embarking on his still-rising political career. (He had come to Virginia to complete his graduate studies shortly before Cabrera left).

Things were dramatically different in 1980, when I was Cabrera's guest at the Autonomous University of Madrid, in the Institute that now bears his name. He was now Don Nicolás, an almost legendary figure to the young generation of physicists in his own country. Those were heady days for Spain and its reborn democracy. Cabrera's dreams and hopes had come true, and yet he was still striving for improvement, trying to set up his institution in a new, less bureaucratic way. In the midst of all the political excitement and the administrative struggles, he still found time to take out a yellow notepad, legal size, and work out equations and ideas.

Nicolás Cabrera was not driven by ambition or the hunger for recognition; after recognition and power came to him, he was as gentle and considerate as before. He knew adversity and acknowledged that the world is far from perfect, but remained an incurable optimist and was simply convinced that if one does the right thing it will all work out. And in the end it did.

V. Celli

Department of Physics,

University of Virginia

[1] Cabrera N and Mott N F 1999 Rep. Prog. Phys. 12 163
[2]Burton W C, Cabrera N and Frank F C 1951 Trans. Roy. Soc. (London) A243 299
[3]Cabrera N, Celli V and Manson R 1969 Phys. Rev. Letters 22 346
[4]Cabrera N, Celli V, Goodman F O and Manson R 1970 Surf. Sci. 19 67

This text can be found in the web page of the Nicolás Cabrera Institute (www.uam.es/inc). It was written on the occasion of a homage to Prof. Nicolás Cabrera (1913-1989), and published in Journal of Physics Condensed Matter, Volume 14, Issue 24 (2002).

Nicolás Cabrera was an outstanding scientist who strongly influenced the development of Physics in Spain in the last third of the twentieth century. His personal contributions to science are widely known and cited today. As the current head of the Department of Condensed Matter Physics, in the Universidad Autónoma de Madrid, I will refer briefly to his role in the creation of the Physics Department of our University.

As with many other Spanish scientists, writers and artists, Cabrera was exiled as a consequence of the civil war. Before this, the new physics developed mainly in Europe had also permeated Spanish society. This led talented young people to go abroad to learn in the most prestigious laboratories. One of the most prominent of these was Nicolás's father, Blas Cabrera, who was then the leading Spanish figure in the growing international activity on physics. He was the head of a modern and well equipped laboratory of physics and chemistry, close to the students' residence where Dalí, García-Lorca and Buñuel were living then. He made an important experimental contribution to magnetism, and it was on this subject that Nicolás Cabrera performed his first experiments under the supervision of S Velayos.

At that time, scientists such as Santiago Ramón y Cajal, Blas Cabrera and others contributed, through their talent and efforts, to a small `golden age' of Spanish science. Civil war swept away all this blossoming activity. More than thirty years later, Nicolás often dreamt of emulating his father's endeavours for the development of science in Spain.

The opportunity came in the 1960s when he accepted the offer of the Spanish Minister of Education, Villar Palasí to set up the Department of Physics of the new Universidad Autónoma de Madrid. In this he encouraged Cabrera to adopt the same criteria of quality and efficiency that he had used as head of a new department in the University of Virginia. For many young people beginning their careers in physics then, myself included, Cabrera was a mythical figure. His return to Spain with the aim of forming a new and modern Department of Physics was very exciting for us, and we wanted to be part of it. His great talent and personal qualities enabled him to quickly assemble an excellent group of young scientists. He was granted autonomy by the authorities to offer positions according to his personal criteria, fulfilled by considering mainly CVs, recommendation letters and interviews.

The early years were extremely exciting due to the friendly and creative atmosphere promoted by Cabrera. Many reputable scientists visited our Department and gave interesting seminars, so valued by Cabrera as to make attendance quasi-compulsory. It is impossible in a short space to give a full account of all the innovations that he introduced in teaching and research; we felt that our department was creative and competitive, and our scientific standards were soon recognized by the international community.

Although Cabrera dedicated much time to organizational work, he made a great effort to continue the investigations on surface physics previously undertaken in Virginia. An ambitious plan was launched, following his arrival to Madrid, to build a machine to perform experiments on atomic-beam scattering by surfaces. That became the most important experimental challenge of this period. Former colleagues on surface physics, such as Vittorio Celli, spent prolonged periods in our department working in Cabrera's research group. Unfortunately all these exciting activities suffered the suppressions of the University governors and the Ministry of Education during Franco's last years. Several professors were banned from the university for

their political opinions. Others, who came to Spain attracted by Cabrera's project, had to leave the country due to the hostile atmosphere created by the political authorities. It was very sad for us to realize that such attitudes found support among many representing the Spanish academic establishment.

Cabrera's work stood out among the extremely low scientific level of most of the university departments and research centres of physics in Spain at that time. Fortunately, this devastating period concluded with Franco's death, and Cabrera's projects survived. During this dark time Cabrera thought often of going back to the USA, but he stayed, out of consideration for the young people that he had engaged on his personal challenge. He had the opportunity to see his work bear fruit, and that the steps he had taken became an irreversible turning point in the development of physics in Spain.

S Vieira

Department of Condensed Matter Physics,

Universidad Autónoma de Madrid

This text can be found in the web page of the Nicolás Cabrera Institute (www.uam.es/inc). It was written on the occasion of a homage to Prof. Nicolás Cabrera (1913-1989), and published in Journal of Physics Condensed Matter, Volume 14, Issue 24 (2002).

Introduction

School scope and goals

Most of what we know in chemistry and biology has been determined by bulk studies where billions of individual entities (molecules or molecular aggregates) are considered, providing averaged results. The usual approach is to assume that all the molecules are indistinguishable when performing their task, although this is often not the case. Biochemical processes are likely to be highly asynchronous and intermediate states will be poorly populated. Moreover, some physical magnitudes such as forces or intermolecular distances are hardly accessible using bulk techniques. The development of single-molecule approaches opened the possibility of observing individual molecules and to measure properties that otherwise were impossible to obtain from an undifferentiated ensemble. The main goal of this school is to give an introduction to both theoretical and experimental single-molecule approaches to solve a number of biological problems. There will be dedicated sessions to atomic force microscopy, optical and magnetic tweezers, single-molecule fluorescence techniques as well as a full day dedicated to theoretical approaches to biology.Therefore, this summer school is intended for biologists, experimental and theoretical physicists willing to get a dive in the exciting field of single-molecule biophysics.

School Topics:

Atomic force microscopy

Optical and magnetic tweezers

Single-molecule fluorescence techniques

Theory on soft matter.

Single-molecule applications

Organizers

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KEYNOTE SPEAKERS.

Philip Nelson University of Pennsylvania, USA. Pages 22, 46, 53.

Toshio Ando Kanazawa University, Japan. Pages 23, 53, 57.

Erwin Peterman Vrije Universiteit, Amsterdam, The Netherlands. Pages 24, 61,63.

Félix Ritort Universtitat de Barcelona, Spain. Pages 25, 65, 68.

Ulrich Keyser University of Cambridge, UK. Pages 26, 46, 49.

Urs Greber University of Zurich, Switzerland. Pages 27, 62, 64.

Fred MacKintosh Vrije Universiteit, Amsterdam, The Netherlands. Pages 28, 61, 64.

Daniel Müller Eidgenössische Technische Hochschule Zürich, Switzerland. Pages 29, 59.

Ricardo García Instituto de Ciencia de Materiales de Madrid, CSIC, Spain. Pages 30, 71.

Rudolf Podgornik Institut "Jožef Stefan", Slovenija. Pages 31, 66, 69.

Melike Lakadamyali The Institut of Photonic Sciences ICFO, Barcelona, Spain. Pages 32, 66, 69.

Jan Lipfert Delft University of Technology, The Netherlands**.** Pages 33, 47, 49.

Iwan Schaap Georg August Universität, Göttingen, Germany. Pages 35, 53, 59.

INVITED SPEAKERS.

Mauricio García-Mateu Centro de Biología Molecular Severo Ochoa, Madrid, Spain. Pages 34, 56.

Arvind Raman Purdue University, USA. Pages 36, 63.

Wouter Roos Vrije Universiteit, Amsterdam, The Netherlands. Pages 37, 60.

Sergi García-Manyes King´s College London, UK. Pages 38, 72.

Carolina Carrasco Centro Nacional de Biotecnología, CSIC, Madrid, Spain. Pages 39, 51.

Alessandro Podesta Università degli Studi di Milano, Italy. Pages 40, 57.

Francesco Mantegazza Università di Milano-Bicocca, Italy. Pages 41, 51.

Borja Ibarra IMDEA Nanociencia, Madrid, Spain. Pages 42, 48.

Ricardo Arias-González IMDEA Nanociencia, Madrid, Spain. Pages 43, 48.

Table of contents

Scientific program ……..14

The speakers……..21

Oral presentations……..45

Poster presentations……..79

List of participants…….. 111

The organizers…….. 115

Scientific Program

Sunday 21 July 2013

17:00-20:00	Registration and check-in
20:00-21:00	Dinner

Monday 22 July 2013

Morning Session Day 1	
Session Topic:	***Optical and Magnetic Tweezers (I)***
Session Chair:	*Fernando Moreno-Herrero (Centro Nacional de Biotecnología, CSIC, Spain)*
8:40-9:00	Welcome words. The organizers.
9:00-10:00	*Inference in biological physics* Philip Nelson (University of Pennsylvania, USA) Keynote speaker. Page 46
10:00-11:00	*Optical tweezers – Force spectroscopy on single molecules (part I)* Ulrich Keyser (University of Cambridge, UK) Keynote speaker. Page 46.
11:00-11:30	Coffee break
11:30-12:30	*Magnetic tweezers: how do they work, what are they good for, and how can their capabilities be expanded?* Jan Lipfert (Delft University of Technology, The Netherlands) Keynote speaker. Page 47.
12:30-13:00	*Using optical tweezers to study DNA replication dynamics at single-molecule level* Borja Ibarra (IMDEA Nanociencia, Madrid, Spain) Invited speaker. Page 48.
13:00-13:30	*Mechano-chemical characterization of the genetic information carriers* Ricardo Arias-González (IMDEA Nanociencia, Madrid, Spain) Invited speaker. Page 48.
13:30-15:00	Lunch
Afternoon Session Day 1	
Session Topic:	***Optical and Magnetic Tweezers (II)***
Session Chair:	*Pedro J. de Pablo (Universidad Autónoma de Madrid, Spain)*
15:00-16:00	*Optical tweezers – Force spectroscopy on single molecules (part II)* Ulrich Keyser (University of Cambridge, UK)

	Keynote speaker. Page 46.
16:00-17:00	*Applications of magnetic tweezers: Probing the response of double-stranded RNA to force and torque at the single-molecule level* Jan Lipfert (Delft University of Technology, The Netherlands) Keynote speaker. Page 49.
17:00-17:30	Coffee break
17:30-18:00	*Magnetic tweezers measurements of the nanomechanical properties of DNA* Francesco Mantegazza (Università di Milano-Bicocca, Italy) Invited speaker. Page 51.
18:00-18:30	*Modulation of the translocation properties of a model helicase by DNA sequence content within the track* Carolina Carrasco (Centro Nacional de Biotecnología, CSIC, Spain) Invited speaker. Page 51.
18:30-18:45	*Wringing out a ribonucleoprotein complex* Maria Tikhomirova (Westfalen Wilhelms-University Muenster) Selected poster presentation 1. Page 52.
18:45-19:00	*Single-molecule folding mechanisms of the apo- and Mg^{2+}-bound forms of the human neuronal calcium sensor 1 (NCS1)* Mohsin M. Naqvi (University of Modena and Reggio Emilia) Selected poster presentation 2. Page 52.
19:00-20:00	Free time
20:00-21:00	Dinner
21:00-22:00	Posters and beers

Tuesday 23 July 2013

Morning Session Day 2	
Session Topic:	***Atomic Force Microscopy (I)***
Session Chair:	*Pedro J. de Pablo (Universidad Autónoma de Madrid, Spain)*
9:00-10:00	*What happens inside the eye's photoreceptor cells* Philip Nelson (University of Pennsylvania, USA) Keynote speaker. Page 53.
10:00-11:00	*Atomic force microscopy in biology* Iwan Schaap (Georg August Universität Friedrich Hund, Göttingen, Germany) Keynote speaker. Page 53.

11:00-11:30	Coffee break
11:30-12:30	*Development of high-speed atomic force microscopy* Toshio Ando (Kanazawa University, Japan) Keynote speaker. Page 53.
12:30-13:00	*Manipulation and Biological Implications of the Mechanical Properties of Viruses* Mauricio García-Mateu (Centro de Biología Molecular Severo Ochoa, Madrid, Spain) Invited speaker. Page 56.
13:00-13:30	*Adsorption of proteins on nanostructured surfaces: investigating the nanoscale interaction mechanisms by atomic force microscopy* Alessandro Podesta (Università degli Studi di Milano, Italy) Invited speaker. Page 57.
13:30-15:00	Lunch
Afternoon Session Day 2	
Session Topic:	***Atomic Force Microscopy (II)***
Session Chair:	*Fernando Moreno-Herrero (Centro Nacional de Biotecnología, CSIC, Spain)*
15:00-16:00	*Applications of high-speed atomic force microscopy* Toshio Ando (Kanazawa University, Japan) Keynote speaker. Page 57.
16:00-17:00	*Cell mechanics with AFM and optical traps* Iwan Schaap (Georg August Universität Friedrich Hund, Göttingen, Germany) Keynote speaker. Page 59.
17:00-17:30	Coffee break
17:30-18:30	*High-resolution atomic force microscopy and spectroscopy of native membrane proteins* Daniel Müller (Eidgenössische Technische Hochschule Zürich, Switzerland) Keynote speaker. Page 59.
18:30-19:00	*Probing viral material properties by Atomic Force Microscopy* Wouter Roos (Vrije Universiteit, Amsterdam, The Netherlands) Invited speaker. Page 60.
19:00-20:00	Free time
20:00-21:00	Dinner
21:00-22:00	Posters and beers

Wednesday 24 July 2013

Morning Session Day 3	
Session Topic:	***Physics of single-molecule approaches and Fluorescence (I)***
Session Chair:	*Pedro J. de Pablo (Universidad Autónoma de Madrid, Spain)*
9:00-10:00	*Spying on single biomolecules using fluorescence microscopy* Erwin Peterman (Vrije Universiteit, Amsterdam, The Netherlands) Keynote speaker. Page 61.
10:00-11:00	*Elasticity and dynamics of cytoskeletal and extracellular networks (Part I)* Fred MacKintosh (Vrije Universiteit, Amsterdam, The Netherlands) Keynote speaker. Page 61.
11:00-11:30	Coffee break
11:30-12:30	*Through the eyes of a virus – imaging adenovirus entry into cells* Urs Greber (University of Zurich, Switzerland) Keynote speaker. Page 62.
12:30-13:30	*Towards quantitative structure-property relationships of biological samples using dynamic atomic force microscopy* Arvind Raman (Purdue University, USA) Invited speaker. Page 63.
13:30-15:00	Lunch

Afternoon Session Day 3	
Session Topic:	***Physics of single-molecule approaches and Fluorescence (II)***
Session Chair:	*Fernando Moreno-Herrero (Centro Nacional de Biotecnología, CSIC, Spain)*
15:00-16:00	*Intraflagellar transport in the chemosensory cilia of C. elegans tracked and captured with single-molecule fluorescence microscopy* Erwin Peterman (Vrije Universiteit, Amsterdam, The Netherlands) Keynote speaker. Page 63.
16:00-17:00	*Elasticity and dynamics of cytoskeletal and extracellular networks (Part II)* Fred MacKintosh (Vrije Universiteit, Amsterdam, The Netherlands) Keynote speaker. Page 64.
17:00-17:30	Coffee break
17:30-18:30	*Imaging and modeling of virus egress from infected cells* Urs Greber (University of Zurich, Switzerland) Keynote speaker. Page 64.

18:30-24:00	Visit to the city of Segovia. Dinner in town.

Thursday 25 July 2013

Morning Session Day 4	
Session Topic:	***Physics of single-molecule approaches and Fluorescence (III)***
Session Chair:	*Pedro J. de Pablo (Universidad Autónoma de Madrid, Spain)*
9:00-10:00	*Nonequilibrium work relations: basic concepts and derivations* Félix Ritort (Universitat de Barcelona, Spain) Keynote speaker. Page 65.
10:00-11:00	*Super-resolution fluorescence microscopy: concepts and technical developments* Melike Lakadamyali (The Institut of Photonic Sciences ICFO, Barcelona, Spain) Keynote speaker. Page 66.
11:00-11:30	Coffee break
11:30-12:30	*Order and energetics of nucleic acids in viral capsids (part I)* Rudolf Podgornik (Institut "Jožef Stefan", Slovenija) Keynote speaker. Page 66.
12:30-12:45	*Using Highly Inclined, Laminated Optical sheet (HILO) and off-focus imaging for 3D tracking of single molecules in bacteria* Matteo Prayer Galletti (University of Florence) Selected poster presentation 3. Page 67.
12:45-13:00	*Signal-Driven Tethering System based on DNA-Origami linked to Lipid Bilayers* Alexander Ohmann (Technische Universität Dresden) Selected poster presentation 4. Page 67.
13:00-13:30	Free time
13:30-15:00	Lunch
Afternoon Session Day 4	
Session Topic:	***Physics of single-molecule approaches and Fluorescence (IV)***
Session Chair:	*Fernando Moreno-Herrero (Centro Nacional de Biotecnología, CSIC, Spain)*
15:00-16:00	*Extended fluctuation relations applied to free energy recovery of kinetic structures* Félix Ritort (Universitat de Barcelona, Spain) Keynote speaker. Page 68.
16:00-17:00	*Super-resolution fluorescence microscopy: biological applications* Melike Lakadamyali (The Institut of Photonic Sciences ICFO, Barcelona, Spain) Keynote speaker. Page 69.

17:00-17:30	Coffee break
17:30-18:30	*Order and energetics of nucleic acids in viral capsids (part II)* Rudolf Podgornik (Institut "Jožef Stefan", Slovenija) Keynote speaker. Page 69.
18:30-18:45	*Mechanical Unfolding of the One-State Downhill Folding Protein BBL* Jörg Schönfelder (*Centro Nacional de Biotecnologia, CSIC, and IMDEA nanociencia)* Selected poster presentation 5. Page 70.
18:45-19:00	*Monitoring dynamics of human adenovirus disassembly induced by mechanical fatigue* Alvaro Ortega-Esteban (Universidad Autónoma de Madrid) Selected poster presentation 6. Page 71.
19:00-20:00	Free time
20:00-21:00	Dinner
21:00-22:00	Posters and beers

Friday 26 July 2013

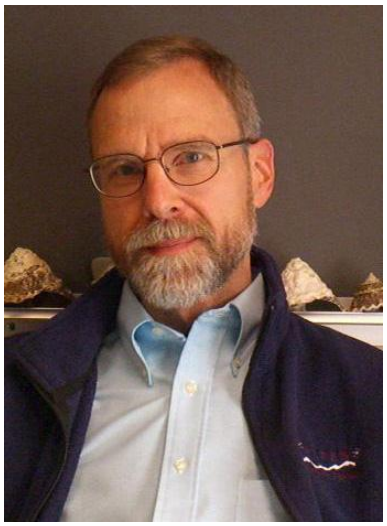
Morning Session Day 5	
Session Topic:	***Applied single-molecule techniques***
Session Chair:	*Pedro J. de Pablo (Universidad Autónoma de Madrid, Spain)*
9:30-10:30	*Fundamental aspects of high resolution imaging and quantitative mapping of biomolecules by dynamic force microscopy* Ricardo García (Instituto de Ciencia de Materiales de Madrid, CSIC, Spain) Keynote speaker. Page 71.
10:30-11:00	*The nanomechanics of individual biomolecules* Sergi García-Manyes (King´s College London, UK) Invited speaker. Page 72.
11:00-11:30	Coffee break
11:30-11:45	*A temperature-controlled Magnetic Tweezers to investigate DNA-translocating proteins* Benjamin Gollnick (Centro Nacional de Biotecnología, CSIC, Spain) Selected poster presentation 7. Page 73.
11:45-12:00	*Mechanical stability and reversible fracture of vault particles* Aida Llauró (Universidad Autónoma de Madrid. Spain) Selected poster presentation 8. Page 74.

12:00-12:15	*(Un) Folding of Engrailed Homeodomain by Multiple Spectroscopic Probes: From Equilibrium to Ultrafast Temperature Jump Kinetics* Nagalakshmi T Sooriyanarayanan (Centro Nacional de Biotecnología, CSIC, Spain) Selected poster presentation 9. Page 75.
12:15-12:30	*Energy transfer studies in binary dye solution mixture of Coumarin 540 and Rhodamine B and its lifetime calculations* Amitansu Pattanaik (University of Delhi, India) Selected poster presentation 10. Page 76.
12:30-13:00	Poster awards. Closing remarks. The organizers.
13:30-14:30	Lunch
15:00	Bus to Barajas airport.

THE SPEAKERS

KEYNOTE SPEAKER: Prof. Philip Nelson

University of Pennsylvania, USA



Professor Philip Nelson studied Physics at Harvard University, where he obtained his PhD degree in 1984. He was Assistant Professor at Boston University (1988), and since 1998 he is Professor of Physics in Pennsylvania University. He has also been a visiting Professor in France (Service de Physique Theorique, CEN Saclay, 1994) and in Israel (Weizmann Institute of Science, 2000).

Professor Philip Nelson features for both research and teaching skills. On one hand, his research interests widespread from Biological Physics to Condensed Matter Physics, passing through the application of theoretical methods in theoretical physics. Specifically, in Biological Physics he is interested in biopolymers; molecular motors; self-assembly; DNA topology and elasticity; stochastic modeling; Bayesian inference and computational neuroscience. He has co-authored about 85 peer reviewed journal publications. On the teaching side, he is the author of a renowned biophysics text book entitled “Biological Physics: Energy, Information, Life (W. H. Freeman and Co., 2004); updated in 2008 and translated into Chinese, Spanish, and Portuguese. Among others, Nelson is founder member of the Nano-Bio Interface Center, Penn (NSF NSEC). He is fellow of the American Physical Society (2003): “for contributions to the understanding of soft biomaterials, quantum fields, and superstrings, using geometrical and topological methods.” In 2001, he received the Ira Abrams Award, highest distinguished teaching honor of Penn’s School of Arts and Sciences. He also received the Emily Gray Prize of the Biophysical Society in 2009 “for far reaching and significant contributions to the teaching of biophysics, developing innovative educational materials, and fostering an environment exceptionally conducive to education in Biological Physics”.

KEYNOTE SPEAKER: Prof. Toshio Ando

Kanazawa University, Japan

Professor Toshio Ando is a biophysicist specialized in the development and use of measurement techniques for understanding the functional mechanism of proteins. Toshio was born in Tokyo and received his B.E. in applied physics and his D.S. in physics from Waseda University. After working at UC San Francisco as a postdoctoral fellow and then as Assistant Research Biophysicist from 1980 to 1986, he joined the faculty at Kanazawa University where he is Professor of Physics and Biophysics, and Director of The Bio-AFM Frontier Research Center. In the last two decades he has been developing high-speed atomic force microscopy (HS-AFM) techniques to directly visualize protein molecules in action at high spatial-temporal resolution. Through extensive efforts, HS-AFM is now highly advanced for practical use. The exquisite dynamic images filmed in recent studies by his group have been continuously demonstrating that this new microscopy is a powerful tool capable of revealing the dynamic process and structure dynamics of biological molecules in stunning detail. For this achievement, Professor Ando and colleagues received a number of awards, including Nikkei BP Technology Prize (2003), Nanoprobe Technology Prize of JSPS (2004, 2010), Hokkoku Culture Prize (2005), Distinguished Service Award of the President of Science Council of Japan (2007), Sakaki Prize of JSPS (2008), Award of the Surface Science Society of Japan (2010), Yamazaki-Teiichi Prize from Foundation for Promotion of Material Science and Technology of Japan (2010), Uchida Prize Medal from Foundation for Promotion of Cardiovascular Research (2012), and recently the NBIC Research Excellence Award from University of Pennsylvania (2012).

KEYNOTE SPEAKER: Prof. Erwin Peterman

Vrije Universiteit, Amsterdam, The Netherlands

Prof. dr. ir. Erwin J.G. Peterman is full professor Physics of Living Systems, University Research Chair at the physics department of VU University Amsterdam. He has obtained his M.Sc. degree at Wageningen University, Molecular Sciences (1993) and his Ph.D. degree at VU University Amsterdam, biophysics of photosynthesis (promotor: R. van Grondelle, 1998). After postdocs at University of California San Diego and Stanford University (advisor: W.E. Moerner), he returned to VU University, first as postdoc (advisor: C.F. Schmidt), later assistant, associate and currently full professor (2013).

His group focuses on single-molecule biophysics and develops fluorescence microscopy and optical tweezers tools for the study of DNA-protein interactions and motor proteins. Recently, this latter line has been expanded in the direction of imaging transport in living cells and animals, in order to unravel the molecular basis of these crucial cellular mechanisms and their involvement in disease. He has coordinated a NWO Groot grant (2008) that has led to the Center for Advanced Laser-based Microscopy at LaserLaB Amsterdam. He is recipient of a VIDI (2002) and a VICI grant (2011).

Peterman teaches several courses on the B.Sc. and M.Sc. level in the realm of biophysics. Since 2010 his is director of the B.Sc. and M.Sc. programs Medical Natural Sciences. Recently, in October 2012, he was appointed Professor in the University Research Chair Programme at VU University Amsterdam. Prof. Peterman has published his work in top journals including Nature, Nature Phyiscs, and The Proceedings of the National Academy of Sciences of the USA.

KEYNOTE SPEAKER: Prof. Félix Ritort

Universitat de Barcelona, Spain

Dr. Felix Ritort carried out his PhD during the years 1989-1991 in theoretical physics in the area of statistical physics of disordered systems under the supervision of G. Parisi in Rome. During the years 1992-2002 he made several postdoctoral stays and contributions to the field of spin glasses, glassy systems and nonequilibrium systems in general. In 2002 he felt much attracted by single-molecule biophysics and the possibility of manipulating individual DNA and RNA molecules to study energy fluctuations and kinetics in real time. In 2002-2003 he spent two years at the Bustamante lab in UC Berkeley as visiting professor to acquire experimental skills to learn about single-molecule manipulation and optical tweezers. During this sabbatical period Dr. Ritort made several contributions to the field of non-equilibrium thermodynamics of small systems and demonstrated the usefulness of fluctuation relations (Crooks relation and Jarzynski equality) to extract free energies of formation of molecular folders such DNA, RNA and proteins. These contributions boosted what is now recognized an emergent field of research, the so-called stochastic thermodynamics. His current experimental and theoretical research is now focused on exploring the many aspects related to the energetics and non-equilibrium behavior of individual molecules pulled under mechanical forces. Ritort's group is recognized worldwide as leader in applying the finest and most powerful theoretically inspired methods to extract accurate quantitative information about thermodynamics and kinetics of molecular interactions. Current lines of research in Ritort's lab include the free energy recovery of molecular structures using fluctuation theorems and a full characterization of nucleic acid thermodynamics from unzipping experiments. Finally his team has recently contributed to develop a new dual-trap counter-propagating setup for pulling dumbbells that is endowed with direct force measurement.

Dr. Ritort work has received over 2300 citations from papers published after 2002. He has an H index=32 and more than 60 invited talks since 2002 at international workshops and conferences. Dr. Ritort has been awarded several prices for his research: the *Distinció de la Generalitat de Catalunya* in 2001 for his theoretical research during the years 1991-2000; *Icrea Academia Price 2008* for his research as scholar at the University of Barcelona; *Premio Bruker 2013* from the *Sociedad de Biofísica de España* for his contributions to molecular biophysics research in Spain.

KEYNOTE SPEAKER: Prof. Ulrich Keyser

University of Cambridge, UK

Ulrich F. Keyser, 38, studied physics at the Technical University of Braunschweig and Leibniz University Hannover, Germany. He graduated in 1999 followed by his PhD degree in condensed matter physics also from Hannover in 2002. During his postdoctoral time at Delft University of Technology, NL, he pioneered the combination of optical tweezers and solid-state nanopores. Since 2007 he has been leading an 'Emmy Noether' research group funded by the Deutsche Forschungsgemeinschaft, and serving as university lecturer at the University of Cambridge, UK. His main research focus is single-molecule biophysics with special emphasis on membrane transport processes and the development of novel technologies. Since 2010 he holds on ERC starting grant with the focus on passive membrane transport of small organic molecules. Recently, he developed a fast particle tracking based on CMOS cameras that allows for real-time particle tracking with up to 10,000 frames per second. At the moment his group at the University of Cambridge consists of 4 postdoctoral researchers and 6 PhD students.

KEYNOTE SPEAKER: Prof. Urs Greber

University of Zurich, Switzerland

Urs Greber research is focused on adenoviruses and rhinoviruses causing human respiratory disease. His goal is to elucidate how viruses take control over membrane and lipid functions and dynamics, cytoplasmic transport processes and metabolism to support their gene expressions, progeny formation and ultimately transmission between cells. His final objective is to give new insights into molecular mechanisms underlying cell functions and infection processes, and provide a basis for viral applications in clinical research and biotechnology. He is coauthor of more than 60 peer reviewed publications in journals such as Cell, Virology, and Cell Host and Microbe. He developed a patent of antivirals by inhibiting a host cell kinase, involved in mediating viral infection. Urs Greber obtained his PhD in 1984 at the Department of Biotechnology of the ETH (Zurich). He occupied postdoctoral positions at the Research Institute of Scripps Clinic (1991) and Yale University School of Medicine (1993). He gained a Swiss Talent for Academic Research & Teaching fellow (1995). After being Associate Professor of Cell Biology at the Institute of Molecular Life Sciences of the University of Zürich (2003), he became Full Professor of Molecular Cell Biology. In the same year he was elected member of the European Molecular Biology Organizaation (EMBO). On the other hand, he is Co-founder and consultant of the biotech company 3-V Biosciences Inc, Menlo Park, CA, USA, which is devoted to develop antiviral drugs. He also has participated in science divulgations programs of the Swiss National TV.

KEYNOTE SPEAKER: Prof. Fred Mackintosh

Vrije Universiteit, Amsterdam, The Netherlands

Fred Mackintosh is Full Professor of Theoretical Physics at the Vrije Universiteit (Amsterdam) since 2001. Fred did his BS in Mathematics and Physics at University of Washington (1984) and obtained a PhD in Physics at Princeton University (1989). Mackintosh was the head of the Theoretical Physics dept. at the Vrije Universiteit between 2003 and 2008 and participated at the review committee of The Human Frontiers Science Programme (2010-2011). Currently, he is member of the editorial board of Physical Review Letters and Fellow of the American Physical Society.

MacKintosh's research focuses on the fundamental material properties of biological and soft matter systems, with emphasis on cell mechanics and the rheology and mechanics of biopolymer networks. Key achievements include: the development of commonly used models of elasticity and dynamics of biopolymer gels, combined experimental and theoretical advances in microrheology and non-equilibrium, motor-activated gels and *active diffusion* in cells, as well as the identification of affine to non-affine transitions and critical behavior in fiber networks.

KEYNOTE SPEAKER: Prof. Daniel Müller

Eidgenössische Technische Hochschule Zürich, Switzerland

Prof. Müller was born on March 22, 1965 in Bad Wimpfen, Germany and studied physics at the University of Technology of Berlin and the Hahn-Meitner-Institute in Berlin, Germany. After finishing his studies he started his Ph.D. in Biophysics at the Forschungszentrum Jülich, Germany, with Georg Büldt and at the Biozentrum Basel, Switzerland with Andreas Engel. In 1997 he finished his Ph.D. and received the Prize for the best Ph.D. thesis in Life Sciences of the University of Basel. In 2000 Daniel Müller received his habilitation 'venia legendi' in Biophysics from the University of Basel. In 2000 Daniel Müller continued his career as a group leader at the newly founded Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden, Germany. In 2002 Daniel Müller accepted a full professorship of Cellular Machines at the Biotechnology Center of the University of Technology, Dresden. He acted as a director of the Center from 2003-2005. In 2006 Müller co-funded one of the largest Bionanotechnology Spin-Offs in Germany. The company developing and manufacturing the world's first robot that fully automatically conducts single-molecule experiments was sold in 2008. In December 2009 Daniel Müller accepted the Chair of Bionanotechnology at the ETHZ Department of Biosystems Science and Engineering (D-BSSE) in Basel.

The research group of Daniel Müller develops bionanotechnological methods that allow quantifying inter- and intramolecular interactions of biological processes. Currently these methods allow to image cells at nanometer resolution, to quantify and localize cellular interactions at molecular resolution and to observe how individual receptors of living cells communicate. Furthermore, it became possible to quantify and structurally localize interactions that fold, stabilize and control the functional state of single membrane proteins in their native environment.

KEYNOTE SPEAKER: Prof. Ricardo García

Instituto de Ciencias de Materiales de Madrid, Spain

Ricardo García (Arganza, Spain) did his Master in Physics at the Universidad de Valladolid (1984) and a Ph.D in Physics at the Universidad Autónoma de Madrid (1984-1990). Then he moved to the University of New Mexico and Oregon (USA) as a postdoctoral researcher under the supervision of Prof. Carlos Bustamante. He is permanent staff at the Spanish National Research Council (CSIC) since 1994 and full CSIC Professor since 2004.

Garcia applies a combined theoretical and experimental approach to develop multipurpose tools for quantitative analysis and manipulation of molecules, materials and devices in the *1* to *100* nm length scale. A key feature of RG's approach is that nanoscale control and device performance should be compatible with operation in technological relevant environments (air or liquids). He has contributed to the development, understanding and optimization of amplitude modulation AFM (tapping mode AFM). In particular, he participates in the development of multifrequency AFM as a unifying scheme for topography and quantitative mapping of material properties with sub-1 nm resolution. He has also contributed to the emergence and optimization of a versatile nanolithography for the fabrication of nano-scale devices based on the spatial confinement of chemical reactions (AFM oxidation nanolithography).

RG is author or co-author of 117 publications in international peer review journals and 15 book chapters. He has published in a wide range of journals such as Science, Cell, Nature Materials, Nature Nanotech., Nano Lett. , Adv. Mater. or Phys. Rev. Lett. RG has an *h-index* of 38. The total number of citations is above 5400 which corresponds to 46 cites/article. RG is the author of a book entitled Amplitude modulation AFM, Wiley-VCH (2010). RG has received several awards and honors such as being fellow of the American Physical Society (2007) and the Technological Innovation Prize from Fundación Madrid+d, Madrid Regional Government. He has co-authored eight patents on nanotechnology instruments and methods and four of them are currently being commercialized by Asylum Research (USA) and Scriba Nanotecnologie (Italy). RG has given 87 invited/keynote presentations in international conferences and workshops.

KEYNOTE SPEAKER: Prof. Rudolf Podgornik

Institut "Jozef Stefan", Slovenija

Rudolf Podgornik is a Slovenian physicist. His field of research is the physics of soft matter, the physics of coulomb fluids and macromolecular interactions, the Lifshitz theory of dispersion interaction, the physics of membranes, polymers and polyelectrolytes and especially the physics of DNA as well as the physics of viruses.

Podgornik discovered the line hexatic phase in the phase diagram of the concentrated DNA solutions. The line hexatic mesophase appears to be the preferred packing form of DNA in bacteriophages. He is the author of more than a 150 scientific papers and a coeditor of a book on "Electrostatic Effects in Soft Matter" (Proceedings of the NATO Advanced Study Institute, Les Houches, France, 1–13 October 2000, Series: NATO Science Series II: Mathematics, Physics and Chemistry, Vol. 46), together with Christian Holm and Patrick Kekicheff. Together with D. Harries, J. DeRouchey, H. H. Strey, and V. A. Parsegian, he coauthored the chapter "Interactions in Macromolecular Complexes Used as Nonviral Vectors for Gene Delivery", in the leading textbook of gene therapy: "Gene Therapy: Therapeutic Mechanisms and Strategies", N. Smyth – Templeton, Marcel Dekker, New York (2008), Third Edition. He is also a co-editor of the book "Electrostatics of Soft and Disordered Matter" to be published in 2013.

Rudolf Podgornik is the head of the research program Biophysics of polymers, membranes, gels, colloids and cells, financially supported by the Slovene Agency for Research and Development. He is a member of the Theoretical Physics Department at the Jozef Stefan Institute in Ljubljana. For many years he was an adjunct scientist at the National Institutes of Health, Bethesda, MD and teaches at the Physics Department, Faculty of Mathematics and Physics, University of Ljubljana and at the Medical Faculty, University of Ljubljana. Since 2011 he is also adjunct professor at the Physics Department, University of Massachusetts, Amherst. He is a coeditor-in-chief of the Journal of Biological Physics, edited by Springer. In 1999 he was awarded the highest prize for scientific excellence in Slovenia, the Zois Award. In 2008 he was a co-recipient of the Martin Hirschorn IAC Prize. This award, made possible by the generosity of Martin Hirschorn, is given once every two years and is funded by the INCE Foundation.

KEYNOTE SPEAKER: Dr. Melike Lakadamyali

The Institute of Photonic Sciences ICFO, Barcelona, Spain

Dr. Melike Lakadamyali obtained her B.S. in 2001 in Physics from the University of Texas at Austin and her PhD in 2006 in Physics from Harvard University. During her PhD, she developed fluorescence microscopy methods for imaging influenza virus infection under the supervision of Prof. Xiaowei Zhuang. After her PhD, she joined the neuroscience lab of Prof. Jeff Lichtman at the Center for Brain Science at Harvard University, where she worked on imaging neuronal connectivity with super-resolution microscopy. As of September 2010 she is a Fundacio Cellex Barcelona NEST fellow and the Group Leader of the Advanced Fluorescence Imaging and Biophysics group at the Institute of Photonic Sciences (ICFO), Barcelona, Spain.

KEYNOTE SPEAKER: Dr. Jan Lipfert

Delft University of Technology, The Netherlands

Jan Lipfert studied physics and economics in Heidelberg, Germany, unsure whether to apply quantitative reasoning to society or to the natural sciences. After completing his undergraduate, he spent a year in Uppsala, Sweden, where he obtained a Masters degree and became fascinated by applying quantitative methods to biological problems. As a Fulbright fellow, he pursued a year of graduate studies at the University of Illinois at Urbana-Champaign, USA, where obtained another Masters degree, taking advanced courses in biological, mathematical, and condensed matter physics. For his Ph.D., he decided to continue west to Stanford University. At Stanford, he worked with Sebastian Doniach and Daniel Herschlag (and many others), using a combination of computer simulations, bench top biochemistry and small-angle X-ray scattering to study the structure, dynamics, and interactions of (membrane) proteins and RNAs. For his post doc, Jan decided to return to Europe and join the lab of Nynke Dekker at Delft University of Technology in the Netherlands. As a post doc and Veni fellow of the Dutch Organization for Scientific Research (NWO) at Delft, he developed novel magnetic tweezers instruments for single-molecule studies of torque and twist and applied them to investigate the properties and dynamics of DNA, RNA, and nucleo-protein filaments. Jan will move to Munich and start his own lab at the Ludwig-Maximilian-University in summer 2013.

KEYNOTE SPEAKER: Prof. Mauricio García-Mateu

Centro de Biología Molecular Severo Ochoa, Madrid, Spain

Mauricio G. Mateu, a professor at the Universidad Autónoma de Madrid and Group Leader at the Centro de Biología Molecular "Severo Ochoa" (CBMSO) in Madrid, Spain, received a Ph.D. in Chemistry (Biochemistry and Molecular Biology) in 1986. His scientific training involved long-term research in three top laboratories, respectively led by Prof. Severo Ochoa and Prof. Esteban Domingo at the CBMSO and by Prof. Sir Alan R. Fersht at the Centre for Protein Engineering, Medical Research Centre, Cambridge, UK. As an expert biochemist and molecular biologist and virologist, he has been working on the relationships between structure and function of viruses for over 25 years. His group is currently involved in the study of the assembly, disassembly, stability and dynamics of viruses and viral particles. He is an author of over 120 scientific publications, having published over 90 original research articles in a wide range of international peer-reviewed journals, including high-impact factor ones such as EMBO J., Nature Structural Biology, PNAS, etc. His recent discoveries together with their collaborators include: i) the elucidation of molecular determinants of assembly, thermal stability and mechanical properties of model virus capsids (foot-and-mouth disease virus, the minute virus of mice and the human immunodeficiency virus); ii) the development of compounds able to inhibit viral capsid assembly and HIV infection; and iii) the engineering of viral particles of improved thermal or mechanical stability for the development of vaccines or nanotechnological applications. He has also recently edited an integrated textbook on the Structure and Physics of Viruses (Springer SBM, 2013). His h-index=33 is among the highest among spanish virologists, and his articles have received over 3600 citations.

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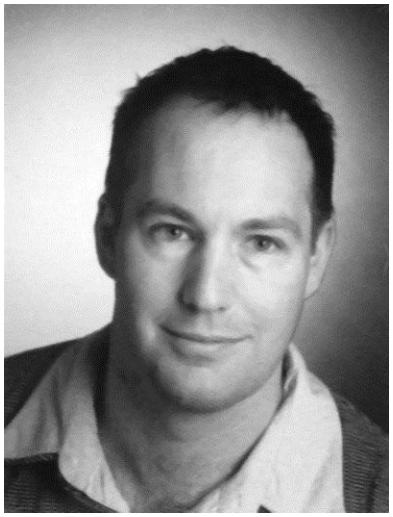
KEYNOTE SPEAKER: Dr. Iwan A.T. Schaap

Georg August Universität, Göttingen, Germany



Iwan Schaap studied molecular biology and virology at the Universiteit Utrecht in the Netherlands. In 2001 he moved for his PhD studies to the group of Prof. C. F. Schmidt at the Vrije Universiteit Amsterdam, the Netherlands. Here, in the faculty of physics, he used atomic force microscopy and finite element analysis to study the nano-mechanical properties of biological samples including microtubules, DNA tetrahedra and viruses. In 2006 he took up a post-doctoral research fellow position at the National Institute of Medical Research in London, UK, to investigate, still with AFM, the structure of malarial actin and the mechanics of Influenza viruses. Since 2008, Iwan Schaap is a research group leader at the Georg-August Universität, Göttingen, Germany. The focus of his research is on the understanding of the relationship between structure and function of biological materials. Over the last years he has shifted his attention from single proteins and viruses to whole cells. Currently he applies AFM in combination with optical trapping to learn how mechanics control the differentiation and adaptation of cells.

INVITED SPEAKER: Prof. Arvind Raman

Purdue University, USA

Dr. Arvind Raman is a Professor of Mechanical Engineering and University Faculty Scholar at Purdue University with interests in applied nonlinear dynamics, nanomechanics, and fluid-structure interactions. His group has significantly advanced the understanding of complex dynamics in nanotechnology applications such as Atomic Force Microscopy (AFM) and micro- and nano-electromechanical systems (MEMS/NEMS), in gyroscopic systems for data storage and manufacturing, in electronics cooling, and in biomechanics. He has mentored fifteen PhD students, co-authored more than a hundred peer-reviewed journal articles, held visiting positions at the Universidad Autónoma de Madrid (Spain), University of Oxford (UK), and Darmstadt University of Technology (Germany), and secured funding from the NSF, NIH, NASA, NNSA, and several national and international industrial sponsors. He is an ASME fellow and recipient of the Gustus Larson Memorial Award from the ASME, Keeley fellowship (Wadham College, Oxford), College of Engineering outstanding young investigator award, and the NSF CAREER award. At Purdue he has pioneered the use of cyber-infrastructure in the AFM community for research and education through advanced simulation tools and online classes which are used by thousands around the world and led College of Engineering strategic initiatives for global engagement in Latin America.

INVITED SPEAKER: Dr. Wouter Roos

Vrije Universiteit, Amsterdam, The Netherlands

Following his physics undergraduate studies at the University of Amsterdam and University College Dublin, Wouter Roos moved to Heidelberg for his Ph. D research. There he developed microstructered surfaces for the study of cellular and cytoskeleton mechanics. Roos continued this work during his post-doctoral research at the Max-Planck-Institute for Metals Research in Stuttgart and the Institut Curie in Paris. Returning to Amsterdam he changed topics - while remaining in the biomechanics field - and he focused on the material properties of viral nanoparticles. He is now assistant professor at the Physics and Astronomy department of the Vrije Universiteit Amsterdam where he is leading the Bio-AFM group.

Recent research highlights:

- J. Snijder, C. Uetrecht, R. Rose, R. Sanchez, G. Marti, J. Agirre, D. M. Guérin, G. J. Wuite, A. J. R. Heck, W. H. Roos *Probing the biophysical interplay between a viral genome and its capsid* **Nature Chemistry** (2013) *in press*
- W. H. Roos, I. Gertsman, E. R. May, C. L. Brooks III, J. E. Johnson, G. J. L. Wuite *Mechanics of bacteriophage maturation* **Proc. Natl. Acad. Sci. U. S. A.** (2012) Vol. 109, 2342-2347
- M. Baclayon, G. K. Shoemaker, C. Uetrecht, S. Crawford, M. Estes, B. Prasad, A. J. Heck, G. J. Wuite, W. H. Roos *Pre-stress strenghtens the shell of Norwalk Virus Nanoparticles* **Nano Letters** (2011) Vol. 11, 4865-4869
- W. H. Roos, R. Bruinsma, G. J. L. Wuite *Physical Virology* **Nature Physics** (2010) Vol. 6, 733-743

INVITED SPEAKER: Dr. Sergi García-Manyes

King´s College, London, UK

Dr Sergi Garcia-Manyes graduated in Chemistry (BSc) from the University of Barcelona in 2000 with Honors. He got his MSc in Analytical Chemistry in 2001 and obtained his PhD in Physical Chemistry from the same University in 2005 under the supervision of Prof. Fausto Sanz. His PhD work focused on the study of the nanomechanical properties of well-defined surfaces using SPM techniques, mainly AFM. These studies gained insight into the basic elastic and plastic deformation properties of a wide variety of substrates, spanning from ionic crystals to HOPG and lipid bilayers, at the nanoscale. During his PhD studies, he undertook short research visits to the Materials Science Division of the Lawrence Berkeley National Lab (California) in the group of Prof. Miquel Salmeron and to the Biology Department of Columbia University (New York), in the group of Prof. Julio Fernandez.

Short after completing his PhD, Sergi joined the group of Prof. Julio Fernandez at Columbia University, first as a postdoctoral researcher and then as a research associate. During the period 2005-2012, he used the newly developed single molecule force-clamp technique to study the conformational dynamics of single proteins during their individual folding pathways and to elucidate the effect of a mechanical force on the outcome of a chemical reaction, at the single bond level. His research has been published in several papers in the leading journals of the field.

From 2012, Sergi is a tenured Lecturer in Physics in the Department of Physics of King's College London and a group leader in Structural Biology in the Randall Division of Cell and Molecular Biophysics. His multidisciplinary research group is composed of a mixture of talented PhD students and postdoctoral researchers, the aim of which is to understand how mechanical force affects the mechanical stability of cellular membranes and also to elucidate how individual proteins equilibrate under the effect of a calibrated mechanical force. Sergi was recipient of the 2012 award of the Spanish Biophysical Society. He is currently funded by the European Union through two grants from the Marie Curie Action, by the BBSRC council, by the Royal Society and by the British Heart Foundation. He was recently awarded the prestigious EPSRC Early Career Fellowship.

INVITED SPEAKER: Dr. Carolina Carrasco

Centro Nacional de Biotecnología, CSIC, Madrid, Spain

Dr. Carolina Carrasco graduated in Optics and Optometry at the University of Granada in 1996 and in Physics at the University of Granada in 2000. She got the postgraduate degree Master of Advanced Studies at the University of Granada in 2002. Because of her interest in the field of single molecule biophysics, in 2002 she moved to Madrid to obtain her PhD in Physics at the Universidad Autónoma de Madrid under the supervision of Dr. Pedro José de Pablo. During that time she extensively used Atomic Force Microscopy to study the mechanical response of single viruses upon deformation. In 2006 she discovered how the viral genome is used as a structural element in the Parvovirus (C. Carrasco et at., *PNAS*, 103, 13706-13711, 2006), and in 2008 she showed that it is possible to modify the elasticity of a viral particle by protein engineering (C. Carrasco et al., *PNAS*, 105, 4150-4155, 2008). During her D. Phil., she enjoyed a five months internship at the University of Amsterdam under the supervision of Prof. C. F. Schmidt where she employed her experience with Atomic Force Microscopy in liquid to study the elasticity of microtubules as well as the dynamics of single kinesin proteins. Carolina´s PhD work (2002-2008) was awarded by the Universidad Autónoma de Madrid with marks of "*Cum Laude*" and the "*Ph.D. Extraordinary Prize*". She enjoyed of one year postdoctoral contract (2008-2009) at the Materials Science Institute of Madrid (ICMM-CNB) under the supervision of Dr. P. A. Serena where they showed true high resolution at single molecule level of viruses in physiological conditions (*PLoS One*, 7, e30204-e30204, 2012). Since November 2009, she has extended her expertise to the Magnetic Tweezers technique in "The Molecular Biophysics of DNA Repair Nanomachines Lab" led by Dr. Fernando Moreno-Herrero at the National Centre of Biotechnology (CNB-CSIC) at Madrid. Her research is focused on understanding DNA translocation and unwinding by helicase-nuclease motor proteins at the single-molecule level. Understanding the molecular mechanisms of nanomachines is important for molecular biology and medicine because they are involved in cellular repair pathways of which defects are associated with human disease. In June 2012, she was awarded a *Juan de la Cierva* fellowship by the Spanish Ministry of Science after a national high-level competitive process. She has participated as speaker in international congress as Gordon Research Conferences, European Biophysics Congress and Biophysical Society Meeting. Carolina has published her work in high-profile journals such as PNAS, Biophysical Journal, JACS, PLoS One, and J Struct Biol.

INVITED SPEAKER: Dr. Alessandro Podestà

Università degli Studi di Milano, Italy

Alessandro Podestà (AP) is assistant professor at the Department of Physics of the University of Milano, where he got the MS in Physics in 1998 and the PhD in Materials Science in 2002; his main research interests pertain to the study and characterization of physical and chemical properties of interfaces at the nanometer scale using scanning probe techniques based on Atomic Force Microscopy (AFM). In particular, AP studies the interfacial properties of thin nanostructured films and the interaction of biological entities (DNA, DNA-protein complexes, and cells) with relevant biocompatible (nanostructured) surfaces. AP is the scientist in charge of the AFM group of the Molecular Beam and Nanocrystalline Materials Laboratory and of the Interdisciplinar Centre for Nanostructured Materials and Interfaces (CIMAINA) of the University of Milano. He works in an interdisciplinary research environment, collaborating with physicists, biologists, chemists, engineers. AP is author of more than 50 publications on peer-reviewed journals and his H-index (WoS) is 17. AP participates to several funded research projects, in some cases as Principal Investigator or local coordinator. AP is involved in educational activities, including university courses and classes of general physics and experimental nanotechnology, as well in science communication and dissemination activities; he is tutor of BC, MS, and PhD students.

INVITED SPEAKER: Prof. Francesco Mantegazza

Università di Milano-Bicocca, Italy

Dr. Francesco Mantegazza (Milano, Italy, April 1964) graduated in Physics and received his PhD in Experimental Physics at the University of Milano under the supervision of Prof. V. Degiorgio (Univ. Pavia, Italy).

The research activity of F. Mantegazza has been mainly experimental and always inherent to the fields of soft matter and biophysics. At the beginning of his career, F. Mantegazza studied the properties of transport and equilibrium of colloids (micelles, DNA, macromolecules, microemulsions, viruses, proteins, polyelectrolytes, fractal aggregates). Such complex fluid systems were studied by using several techniques, including electric birefringence, static and dynamic light scattering, optical turbidity, optical microscopy, dielectric spectroscopy, and electrophoresis. In particular F. Mantegazza studied the Stretched-Exponential decay of the electric birefringence in micellar solutions, polymers, microemulsions, and fractal aggregates. In collaboration with D. A. Saville (Princeton, NJ, USA) and A. Delgado (Granada, Spain), F. Mantegazza showed the presence of a fundamental mechanism of polarization in colloidal suspensions, called Maxwell-Wagner, active in the regime of MHz. Later on, in collaboration with T. Bellini (Univ. Milano, Italy) and N. Clark (Univ. Boulder, CO, USA) F. Mantegazza studied the optical and dielectric properties of disordered systems of thermotropic liquid crystals mixed with nanoparticles. In the frame of his biophysical activity, few years ago F. Mantegazza developed a new experimental light scattering technique for the study of the interactions between biological macromolecules. Such technique allows to obtain the values of the association constants for interactions between protein and protein, glycolipid and protein, enzyme and substrate, antigen and antibody, DNA and protein.

Recently F. Mantegazza developed a Magnetic Tweezers system for the study of DNA nanomechanics. By using this technique F. Mantegazza studied the activity of antitumor drugs, which exert their activity by strongly binding on the DNA molecules. Such results were confirmed by AFM and single molecule fluorescence experiments performed in his lab. Furthermore by using Magnetic Tweezers techniques, F. Mantegazza analysed the onset of DNA instability under specific external applied force and imposed twist values, which was revealed by an increase in the temporal fluctuations in the DNA extension. These fluctuations occur in the presence of a continuous interval of equilibrium states, ranging from a plectonemic state to a state characterized by denaturation bubbles.

INVITED SPEAKER: Dr. Borja Ibarra

IMDEA Nanociencia, Madrid, Spain

Dr. Borja Ibarra got his PhD in Molecular Biology at Universidad Autónoma de Madrid. As a graduate student he studied the molecular and structural basis of the molecular motors involved in DNA packaging into virus particles. As a postdoctoral fellow at Carlos Bustamante laboratory, Dept. of Physics UC Berkeley, he used optical tweezers assays to manipulate and measure in real time the DNA replication activity of single polymerase-DNA complexes. He demonstrated for the first time that mechanical tension applied to the DNA modulates the proofreading activity of the protein in a similar manner to the incorporation of a mismatched nucleotide. Back in Spain (2007) he worked as a research associate at the CNB (Spanish National Center of Biotechnology), where he used for the first time in Spain a state-of-the-art dual beam optical tweezers apparatus to measure the mechanical work developed by a DNA polymerase during DNA replication and unwinding activities. In 2010 he started his own laboratory at IMDEA Nanoscience which is focused on mechanistic aspects of molecular motors with special emphasis on enzymes involved in DNA metabolism.

Selected publications:

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INVITED SPEAKER: Dr. Ricardo Arias-González

IMDEA Nanociencia (Madrid, Spain)

Dr. Arias-Gonzalez received both his master degree in theoretical physics in 1997 and his Ph.D. in 2002 from Complutense University in Madrid (Spain). During his Ph.D. research in the Materials Science Institute of Madrid (CSIC, Spain) and short stays in NIST (Washington, D.C., USA), EMBL-Heidelberg (Germany) and École Centrale Paris (France), he used theory and simulations to understand the electromagnetic field in nanoparticles. He moved to U.C. Berkeley (USA) for his postdoctoral training in single-molecule biophysics in 2003, where he studied DNA and developed temperature control methods for laser tweezers systems. In 2006, he joined the National Center of Biotechnology in Madrid (CSIC, Spain), where he established the *Optical Nanomanipulation Lab*, an experimental environment for single-molecule manipulation. He performed mechano-chemical experiments with optical and magnetic tweezers, structural experiments with AFM and theoretical analysis to understand the conformational states of double-stranded nucleic acids, DNA replication and information processing and electrophysiology of single centrosomes. In 2008, he joined IMDEA Nanoscience, where, as part of a collaborative effort, he also developed a parallel research line on functional, biocompatible nanoparticles.

Selected publications:

- E. Herrero-Galán, M.E. Fuentes-Pérez, C. Carrasco, J.M. Valpuesta, J.L. Carrascosa, F. Moreno-Herrero and J.R. Arias-González, Mechanical Identities of RNA and DNA Double Helices Unveiled at the Single-Molecule Level, *J. Am. Chem. Soc.* 135, 122-131 (2013).
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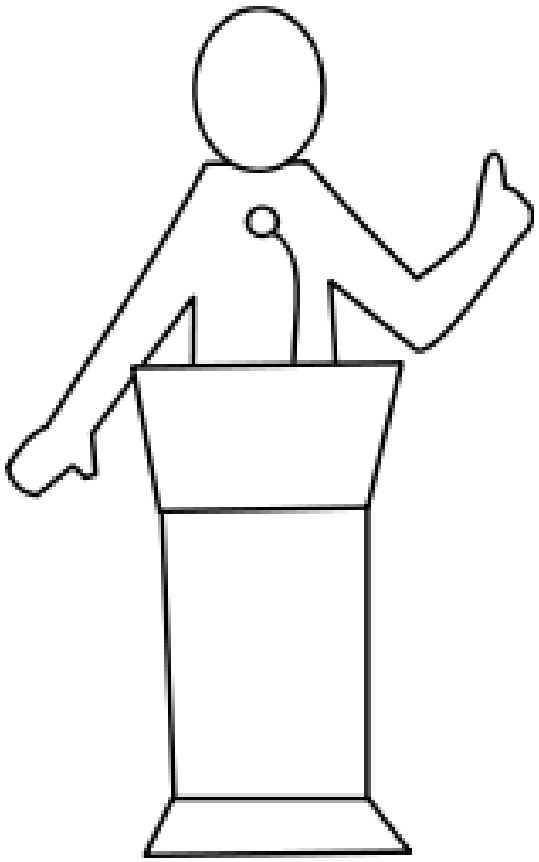

ABSTRACTS. ORAL PRESENTATIONS

Inference in biological physics

Philip Nelson

University of Pennsylvania, USA

I'll discuss two recent experiments, in which knowing a little bit about probability goes a long way in helping us to extract the meaning from experiments. The first involves change point detection in single-molecule studies of molecular motors. The second involves simultaneous activity measurements on intact mammalian retina.

Optical tweezers – Force spectroscopy on single molecules

Ulrich Keyser

University of Cambridge, UK

Light microscopy is one of the most versatile and successful measurement techniques providing accurate spatial and temporal information of particle movements. On the molecular level, optical tweezers offer the opportunity to characterize and monitor mechanical properties of single DNA or protein molecules in aqueous environments. Only a few decades after their introduction, optical tweezers are now a widely used tool in physics and biology. Optical tweezers can exert picoNewton forces on micron-sized objects like colloidal particles while measuring the resulting displacements with sub-nanometre accuracy.

In the first part of the lecture course I will introduce the underlying physics of optical trapping that allows mechanical manipulation of dielectric particles with photons. Starting from a discussion of the optical forces in the ray optics regime, the lecture will also present how optical tweezers can be accurately calibrated. Two key techniques for realising force measurements that rely on following the motion of the trapped particle, namely photo diodes and video-based particle tracking, will be discussed as well.

Magnetic tweezers: how do they work, what are they good for, and how can their capabilities be expanded?

Jan Lipfert

Delft University of Technology, The Netherlands

Magnetic tweezers (MT) are a powerful tool to manipulate single DNA or RNA molecules and to study nucleic acid-protein interactions in real time. In typical MT set up, DNA or RNA molecules are tethered between a flow cell surface and micrometer-sized magnetic beads.

Permanent or electromagnets are used to apply both forces and torques to the tethered molecule. In this talk, I will first discuss the basic principles of MT. In particular, I will describe how the forces exerted in the MT can be calibrated from thermal fluctuations (1,2). In addition, the applied forces can be understood and calculated from first principles, by computing the magnetic fields and taking into account the induced magnetization (3). Furthermore, I will mention our recent efforts towards a first-principles, quantitative understanding of how MT apply torque.

While conventional MT can apply and measure forces, they only apply torque, but do not track the rotation of the bead or measure torque directly. In a second part, I will discuss our recent work developing extensions of MT that overcome these limitations. Magnetic torque tweezers (MTT) enable the direct measurement of torque, based on angular tracking and a novel magnetic geometry providing a weak rotational clamp (4). A second scheme, termed freely-orbiting magnetic tweezers (FOMT), takes the approach to the limit where the rotation of the bead is not at all constrained by the magnets (5). FOMT therefore enable sensitive detection of changes in the twist of nucleic acids and permit to probe torsional properties from equilibrium fluctuations.

Finally, I will discuss applications of MT, in particular highlighting recent results obtain with the MTT and FOMT approaches on the properties of double-stranded DNA as well as the assembly of filaments of repair proteins on DNA (4-6).

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Using optical tweezers to study DNA replication dynamics at single-molecule level

Borja Ibarra

IMDEA Nanociencia, Madrid 28049, Spain.

It has been over two decades since methods of single molecule manipulation were first introduced in biochemical research. Since then, the application of these methods to an expanding variety of biological problems has grown rapidly, from mechanical properties of nucleic acid to mainly all processes involved in nucleic acid metabolism (replication, transcription, translation, recombination, repair, packaging, etc), protein folding and molecular motion of a great variety of enzymes (such as kinesin, dynein, myosin, etc). The ability to analyze biological systems at single molecule level opens avenues of investigation that are not possible using more 'classical' techniques that measure aggregate properties of molecular populations.

Optical Force Microscopy or optical tweezers forms much of the backbone of the field of research devoted to study (and manipulate) biology at single molecule level. This technique provides access to two unprecedented possibilities: 1) follow the activity of a single motor protein in real time and 2) measure the mechanical force exerted by the protein during its biochemical cycle and apply external mechanical force on the protein. This information is crucial to quantify the real time kinetics and the mechano-chemical processes of a biological reaction. In the talk I will describe the series of optical tweezers-based assays we have employed in our laboratory to study the dynamics of an essential biological reaction, DNA replication.

Mechano-chemical characterization of the genetic information carriers

J. Ricardo Arias-González

IMDEA Nanociencia, Madrid 28049, Spain.

Nucleic acids are the genetic information carriers; linear polymers with writing and reading directionality that are common to all living beings on Earth. Their alphabet is a set of four symbols, different from the binary system used in computer and communication science to date. Information is stored and transmitted by the arrangement of complementary data lists into double-helix, long molecules that are subjected to thermal fluctuations in solution.

The structure and conformation of these molecular substrates, on the one hand, are known by molecular biologists since the 1950s. They are, on the other, soft, flexible platforms that

interact with proteins, being these interactions at the core of information processing in cells. In this talk, we will introduce the mechano-chemical characterization of nucleic acids, an analysis that has been made possible only recently thanks to the single-molecule approach and the development of force-measuring optical and magnetic tweezers and single-molecule microscopy with AFM. The material properties of nucleic acids are deeply rooted in their distinct structures, and these in turn are related to their stereochemical identities. This integral view, obtained from chemical, structural and physical approaches, is ultimately essential to explain their cellular role.

The dynamic behavior and self-assembly capacity of nucleic acids are not only important from a biological point of view but also in nanotechnology, to produce scaffolding structures, for selectivity and recognition –important to biosensor development–, high-density and long-term archiving, and in nanomedicine.

Optical tweezers – Force spectroscopy on single molecules

Ulrich Keyser

University of Cambridge, UK

Light microscopy is one of the most versatile and successful measurement techniques providing accurate spatial and temporal information of particle movements. On the molecular level, optical tweezers offer the opportunity to characterize and monitor mechanical properties of single DNA or protein molecules in aqueous environments. Only a few decades after their introduction, optical tweezers are now a widely used tool in physics and biology. Optical tweezers can exert picoNewton forces on micron-sized objects like colloidal particles while measuring the resulting displacements with sub-nanometre accuracy.

In the second part, I will discuss several examples how optical tweezers can be used to investigate the mechanical properties of single biopolymers like DNA that are held in confinement and under tension. One striking example of the versatility of the technique is the insertion and control of single DNA molecules into nanopores.

Applications of magnetic tweezers: Probing the response of double-stranded RNA to force and torque at the single-molecule level

Jan Lipfert

Delft University of Technology, The Netherlands

Double-stranded RNA (dsRNA) plays a number of roles in biological processes in which it often encounters mechanical strain; examples include the packaging of double-stranded RNA (dsRNA) viral genomes into capsids, deformations of the ribosome during translation, and more generally conformational changes of functional RNAs while folding or due to interactions

with proteins. While the response of dsDNA to applied forces and torques has been measured with exquisite precision, much less is known about dsRNA. We have developed a “polymerase-stall” labeling method that allows us to generate fully double-stranded RNA constructs carrying multiple biotin and digoxigenin labels at opposite ends. Using the functionalized dsRNA constructs in a range of a complementary magnetic tweezers assays (1-3), we have probed the elastic properties of dsRNA and, in addition, determined force and torque induced structural transitions that go beyond linear response behavior. From the force-extension response, we have determined the bending persistence length and the stretch (or Young’s) modulus of dsRNA and find values in agreement with previous measurements (4,5) and overall similar to dsDNA. Employing our novel magnetic torque tweezers assays, we have probed the torsional response of dsRNA and again find a behavior that is generally similar to dsDNA. Surprisingly, measurements of the twist-stretch coupling reveal a striking difference between dsRNA and dsDNA. While DNA lengthens when overwound, RNA shortens. In addition, we have studied the dynamics of the buckling transition under positive twist (6) and discovered that the characteristic time scale of the transition is about two orders of magnitude slower for RNA than for DNA. We expect that these measurements of the fundamental properties of dsRNA can help refine our models for twist-storing polymers and inform quantitative models of RNA function *in vivo.*

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Magnetic tweezers measurements of the nanomechanical properties of DNA

Francesco Mantegazza

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Magnetic Tweezers (MT) are an innovative single molecule method which allows to manipulate a single macromolecule (tipically DNA) connected to a micron-sized bead. By means of macroscopic magnetic fields, MT can apply to the DNA a controlled torque and force in the range of femto/pico Newton. The possible MT manipulations include stretching and torsion of the DNA molecule, giving detailed information about the nano-mechanical properties of DNA.

It is possible to use MT in elucidating the binding mechanism and effects of small DNA ligands and in measuring the DNA stability against mechanical DNA denaturation at different environmental conditions. Here we present a brief description of the fundamentals of the method, as well as a short summary of some recent results.

Modulation of the translocation properties of a model helicase by DNA sequence content within the track

Carolina Carrasco

Centro Nacional de Biotecnología, CSIC, Spain.

Unrepaired DNA breaks can lead to genomic instability or cell death. For repair by the ubiquitous homologous recombination pathway, broken ends are first processed to produce a 3′-ssDNA overhang. In *Bacillus subtilis*, this reaction is catalysed by AddAB helicase-nuclease complexes; motor proteins that unwind the DNA duplex and degrade the nascent single-strands in a manner regulated by specific single-stranded DNA sequences called Chi recombination hotspots. We have used Magnetic Tweezers to investigate the real-time dynamics of AddAB translocation on dsDNA and the effect of Chi and Chi-like sequences on this process. AddAB translocation traces showed a complex appearance with variable velocities between 200-400 bp/s at room temperature. We found that AddAB was prone to stochastic pausing in areas which contained many Chi-like sequences. Experiments using an AddAB mutant that is unable to recognize Chi strongly suggest that this pausing is due to transient recognition of Chi-like sequences. Experiments using substrates containing *bona fide* Chi sequences showed that AddAB also pauses at Chi, but these events are not exponentially distributed, suggesting a multistep process. We propose a model for the recognition of Chi and Chi-like sequences to explain the origins of this pausing behavior during failed or successful hotspot recognition.

Selected Poster Presentation 1

Wringing out a ribonucleoprotein complex

Maria Tikhomirova[1], Tomas Sinkunas[2], Peter Daldrop[1], Mark. D. Szczelkun[3], Virginijus Siksnys[2], Ralf Seidel[1]

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[3]DNA-Protein Interactions Unit, School of Biochemistry, Medical Sciences Building, University of Bristol, Bristol BS8 1TD, UK.

Selected Poster Presentation 2

Single-molecule folding mechanisms of the apo- and Mg^{2+}-bound forms of the human neuronal calcium sensor 1 (NCS1)

Mohsin M. Naqvi[1], Mariela R. Otazo[2,3], Pétur O. Heidarsson[4], Birthe B. Kragelund[4], and Ciro Cecconi[2]

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[3] Center of Applied Technologies and Nuclear Development (CEADEN), Department of Physics, calle 30, No. 502, Miramar, La Habana, Cuba.

[4] Structural Biology and NMR Laboratory, Department of Biology, University of Copenhagen, Ole Maaløes vej 5, 2200 Copenhagen N, Denmark.

Neuronal calcium sensors (NCS) are responsible primarily for sensing changes in neuronal Ca^{2+} concentration and their members are linked to a number of disorders such as schizophrenia and autism, and to cognitive abilities such as learning and memory. NCS1 is the primordial member of the NCS family and is reported to bind an array of interaction partners, resulting in a broad interactomes. Here we used optical tweezers to study the folding mechanism of NCS1 at the single molecule level. Specifically we characterized the behavior of NCS1 in the presence of Mg^{2+} and of no divalent ions (apo form). In the former case, NCS1 unfolds and refolds through an intermediate structure stabilized by Mg^{2+} binding. In the second case, the apo form unfolds and refolds in a two state manner, displaying a molten globule-like behavior. Through constant-force experiments and hidden Markov model analysis, the free energy landscape of NCS1 with and without Mg^{2+} has been reconstructed. The results of these studies have been compared with those obtained in the presence of Ca^{2+} in order to shed light on the mechanisms by which changes in calcium and magnesium concentrations inside a cell affect the folding process and, ultimately, the function of NCS1.

What happens inside the eye's photoreceptor cells

Philip Nelson

University of Pennsylvania, USA

The human eye is a fantastically sensitive instrument, capable of registering the absorption of single photons, and yet generating very low noise. The story of how these facts were discovered, and of the biochemical cascade that implements this performance, is a great example of biophysical analysis.

Atomic force microscopy in biology

Iwan A. T. Schaap

Georg August Universität, Germany

AFM employs a sharp probe to mechanically scan a sample and generates a 3D topography of its surface. Because of its high resolution and the ability to work in liquid, the technique is gaining popularity to study the dynamics of bio-molecules. However, the forces exerted by the AFM probe can easily disturb, or even destroy, the soft and fragile biological samples. I will discuss how the imaging forces affect the resolution and how they can be controlled to obtain representative images. Furthermore I will show how the probe-sample interaction can be used for the controlled deformation of samples to study their mechanical properties.

Development of high-speed atomic force microscopy

Toshio Ando

Kanazawa University, Japan

Atomic force microscopy (AFM) was originally invented to visualize atoms on solid surfaces. In biological sciences, this microscopy is now an indispensable tool in the analyses of the structure and mechanical properties of biological specimens at the single molecule level. However, the AFM's slow imaging rate has reduced its full usefulness; not only the inefficiency in imaging studies but also the infeasibility of analyzing dynamic events occurring in the sample. An AFM image is constructed by the acquisition of sample height information at many points over the sample surface, and its acquisition at each point spends a certain amount of time due to feedback delay. Therefore, it takes at least 30 s (usually minutes) to capture an image even for a small area of the sample. Moreover, the scanner easily vibrates when scanned fast. However, these speed deteriorating issues that have long been adhered to AFM are not irremovable in principle.

There are three factors to develop for the materialization of tapping-mode high-speed AFM (HS-AFM) for biological research: (i) increasing the feedback bandwidth by reducing the time delays of all devices contained in the feedback loop, (ii) minimizing mechanical vibrations of the scanner, and (iii) achieving a low-invasive performance. The factor (ii) can contribute to increasing the speed performance but the factor (iii) appears to be contradictory to it (see Ref. 1 for detailed technical developments).

How is the highest possible imaging rate determined? Supposing that the X-Z profile of the sample surface has a sinusoidal shape with a periodicity λ and the X-scanner is moved at velocity V_s, the sample height right under the cantilever tip changes with time at frequency $f = V_s/\lambda$ (Fig. 1). Therefore, the Z-scanner operated by feedback control moves in the direction opposite to the sample height at frequency f. When the feedback loop has a time delay τ_0, its phase delay θ relative to the sample height change is $\theta = 2\pi f\tau_0$. Because of this phase delay, the sample surface does not look perfectly flat when viewed from the cantilever. Therefore, an excessive force is exerted to the uphill region of the sample from the cantilever tip, whereas at the downhill region a force smaller than the aimed strength is exerted. Both the excessive and smaller forces are problem. When the excessive force is too excessive, the sample is damaged. When the smaller force is zero, the tip is completely detached from the sample surface (i.e., parachuting occurs), resulting in the loss of sample height information. When the maximum possible phase delay is θmax at which these problems are not caused, the maximum possible Vs (Vs ^{max}) is given by Vs $^{max} = \lambda\theta max/(2\pi\tau_0)$. Thus, under the condition (scan range in the X-direction, W; number of scan lines, N), the maximum possible imaging rate R_{max} (frames/s) is given by $R_{max} = \lambda\theta_{max}/(4\pi NW\tau_0)$. The feedback bandwidth f_B is usually defined by a feedback frequency at which π/4 phase delay occurs; $2\pi f_B\tau_0 = \pi/4$. So, finally, R_{max} is expressed as

$$R_{\max} = 2\lambda\theta_{\max} f_B/(\pi NW) \quad (1)$$

Eq. 1 indicates that endeavors have to be made not only for increasing fB but also for increasing θmax. To carry out low-invasive imaging, the set point of cantilever oscillation amplitude As should be set close to the free oscillation ampliltude A0. However, under this condition, parachuting often occurs. Once occurred, it takes a long time for the tip to land on the surface again because the saturated error signal ($A_0 - A_s$) is very small. Therefore, making the high-speed performance compatible with the low-invasive one is

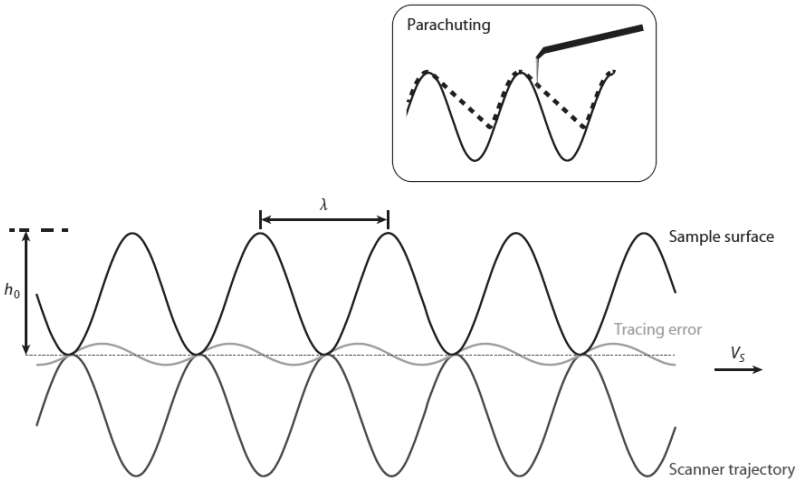

Fig. 1: Scanner movement tracing sinusoidally shaped sample surface with periodicity λ and amplitude $h0/2$, when no parachuting occurs. The inset shows a trajectory of the bottom swing position of the tip (*broken line*) relative to the sample surface when parachuting occurs.

the issue most difficult to solve. We solved this issue by developing a new feedback control technique designated as “dynamic PID control”.[2]

The main time-delaying factors are the mechanical devices, i.e., cantilever and scanner. To increase the response speed of cantilevers, we developed small cantilevers with length 6–10 μm, width 2 μm, and thickness 90–130 nm (Fig. 2).[3,4] Their mechanical properties are as follows; bending-mode resonant frequency f_c, 600 kHz–1.2 MHz in water; spring constant k_c, 0.1–0.2 N/m; quality factor in water Q_c, ~2. Therefore, the time $\tau_c = Q_c/(\pi f_c)$ in which a cantilever responds to a brief step-wise force is 0.53–1.1 μs. The high resonant frequency of the Z-scanner (170–370 kHz) was simply achieved by the use of small piezoactuators. However, the response speed of the Z-scanner is low because of its large Q-factor Q_z. There is a method to reduce the Q-factor (see the modified motion equation, Eq. 2).

$$m(d^2z/dt^2) + \gamma(dz/dt) + kz = F(t) - \gamma'(dz/dt) \quad (2)$$

The last term in the right-hand side increases the coefficient of friction from γ to γ + γ’, which reduces the Q-factor. What Eq. 2 indicates is as follows; when the displacement of the Z-scanner is measured and its minus derivative with an appropriate gain is added to the input of the Z-piezodoriver, the Q-factor is reduced and hence Z-scanner’s vibrations are eliminated. However, it is quite difficult to measure the Z-scanner’s displacement. We solved this problem by using a mock Z-scanner constructed with a LRC circuit which is characterized with a transfer function similar to that of the real Z-scanner. Instead of monitoring the Z-scanner’s displacement, the output from the mock Z-scanner is used to perform Q-control (Fig. 3).[5]

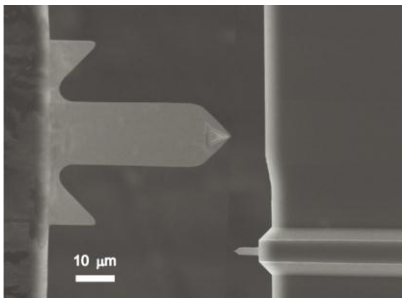

Fig.2: SEM images of (upper left) a conventional cantilever and (lower right) a small cantilever.

From these technical developments, the feedback bandwidth reached ~110 kHz. Importantly, fragile biological samples are not disturbed by the tip-sample interaction.1 For example, under the realistic condition for imaging biological molecules (θmax = π/9, λ = 10 nm, W = 150 nm, and N = 100), imaging can be carried out at 16.3 frames/s. This HS-AFM has recently been used for studying various dynamic events of proteins in action.[6]

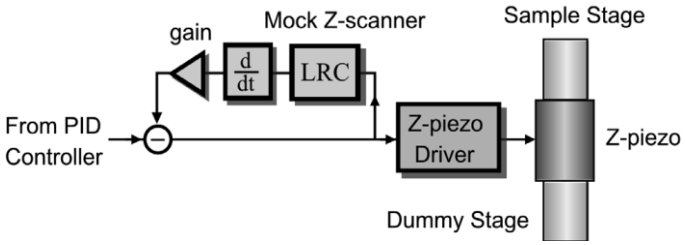

Fig.3: Active Q-control technique with the use of a mock Z-scanner.

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Manipulation and Biological Implications of the Mechanical Properties of Viruses

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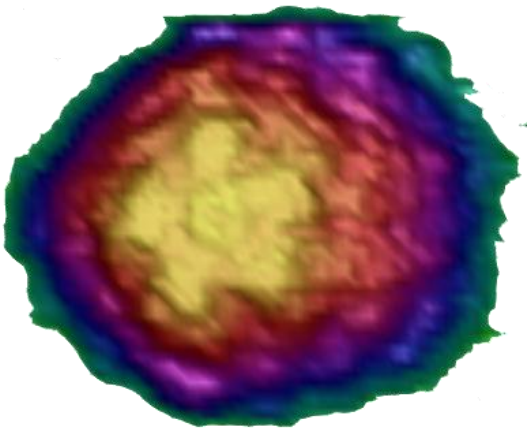

We use atomic force microscopy and protein engineering techniques to study and manipulate the mechanical properties of small viruses. We aim at understanding the molecular determinants that underlie the physical properties of viruses, and also at the design of viral particles with improved thermal and/or mechanical resistance for bio/nanotechnological applications. In collaboration with Dr. P.J.de PabloÂ´s and J.GÃ³mez-HerreroÂ´s groups (Dept. of Physics of the Condensed Matter, UAM) we found that, in the minute virus of mice (MVM), segments of the viral DNA bound to specific sites at the capsid inner wall act like molecular buttresses that decrease the mechanical elasticity of most regions in the viral particle. However, the regions around channels involved in biologically relevant molecular translocation events are kept free from bound DNA, and remain as elastic as in the empty capsid. Our recent studies indicate that this anisotropic distribution of mechanical stiffness may be a biological adaptation to prevent MVM inactivation without impairing infection. We have also mechanically disassembled single MVM particles using AFM, and experimentally identified theoretically predicted assembly/disassembly intermediates. From a nanotechnological perspective, our studies have led to the engineering of the mechanically stiffer viral capsids known to date. The figure shows an AFM image of minute virus of mice.

Adsorption of proteins on nanostructured surfaces: investigating the nanoscale interaction mechanisms by atomic force microscopy

Alessandro Podestà

Università degli Studi di Milano, Italy.

Nanoscale morphology plays an important role in protein adsorption processes on nanostructured biocompatible surfaces (such as titania or zirconia); the charging behavior of these surfaces in acqueous electrolytes may also have important consequences in protein-surface interactions. Here we report on recent studies of surface morphology and of the charging behaviour of cluster-assembled titania surfaces carried out primarily by Atomic Force Microscopy (AFM). AFM turned out to be a very powerful and versatile tool for the characterization of diverse physico-chemical interfacial properties of nanostructured titania films; the relevance of our findings to the understanding of protein adsorption processes is discussed.

Applications of high-speed atomic force microscopy

Toshio Ando

Kanazawa University, Japan

Proteins are dynamic in nature. The molecules are fluctuating, changing their structures, binding to and dissociating from the interaction partners, and traversing a range of energy and chemical states. Most, if not all, of these dynamics and their statistical distributions are hidden in traditional ensemble-averaged measurements. To overcome this problem, single molecule biophysics was created about two decades ago, exploiting the advancements of lasers, fluorescence microscopy, optical spectroscopy, and optical tweezers.[1] Since then, our understanding of the functional mechanism of proteins has become incomparable to the past. Moreover, super-resolution techniques surpassing the diffraction limit was recently added to fluorescence microscopy.[3] However, protein molecules themselves are invisible in these single-molecule measurements, even with super-resolution. The structure of proteins has been studied by X-ray crystallography, electron microscopy, and NMR. These techniques have to date revealed detailed three-dimensional structures of over 60000 proteins, and the list is continuing to grow. However, these techniques basically operate based

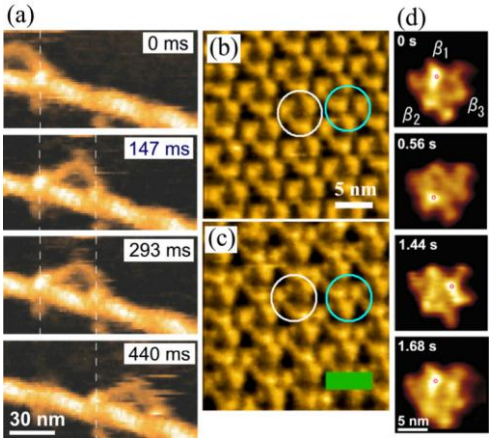

Fig. 1: Dynamic processes of proteins in action captured by high-speed AFM. (a) Walking myosin V, (b, c) Bacteriorhodopsin under (b) dark and (c) illuminated conditions, (d) rotary propagation of conformational change in rotorless F1-ATPase.

on ensembleaveraging, and more seriously, obtained structures are merely limited to static snapshots. Thus, the simultaneous and direct observation of structure and dynamics of single proteins molecules has long been infeasible.

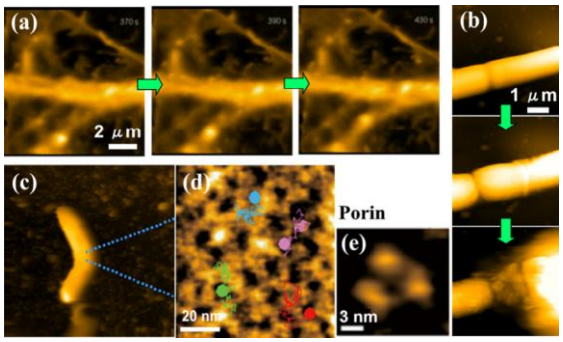

Fig. 2: Dynamic processes of cells captured by high-speed AFM. (a) Hippocampus neuron, (b) Bacillus treated with lysozyme, (c) Magnetic bacterium (MB), (d) Protein diffusion on MB surface, (e) porin trimer isolated from the outer surface of MB.

To materialize this long-quested dream, high-speed atomic force microscopy (HS-AFM) has been developed and now comes of age. The latest HS-AFM can capture dynamic images of biomolecules at sub-100 ms temporal and sub-molecular resolution. Importantly, the function of fragile molecules is not disturbed by the interaction with a cantilever tip. In fact, e.g., walking myosin V on actin filaments,[4] photo-activated structural changes in bacteriorhodpsin,[5] and rotary propagation of conformational changes in rotorless F1-ATPase[6] have successfully been captured on video (Fig. 1). The high-resolution movies not only provide corroborative 'visual evidence' for previously speculated or demonstrated molecular behaviors but also reveal more detailed behaviors of the molecules, leading to a comprehensive understanding of how they function. Nevertheless, current HS-AFM has the following limitations: (i) The scan range of the high-speed scanner is limited to 1 µm, 4 µm, and 1 µm in the X-, Y-, and Z-directions, respectively, to achieve high-resonant frequencies for fast scan, and (ii) because the cantilever tip makes contact with the sample, very soft surfaces such as the membranes of live eukaryotic cells are largely deformed by the contact, which disables the visualization of molecules on such soft surfaces. Recently, the first problem was removed by a modified inversion-based feed forward control technique. Scanning over ~50 × 50 µm^2 and line scan at ~1 kHz is now possible without production of vibrations. As a result, large samples such as a live eukaryotic cell can now be imaged within ~60 s when a relatively small number of scan lines (~200) are used, as shown in Fig. 2a and b. After taking a whole topography image of a large biological sample, we can image molecules in a local area of interest on the sample surface when the surface is relatively rigid. For example, it is possible to observe dynamics of molecules on the surfaces of live bacterial cells, intracellular organelles (such as mitochondria and nuclei), and small structures such as neuronal spines. In fact, in situ visualization of porin trimers moving on the outer surface of a live magnetic bacterium was recently accomplished (Fig. 2c, d, and e).[7] Such in situ dynamic molecular imaging will have a great impact on cell biology because dynamic molecular processes occurring on these surfaces are largely unknown.

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Cell mechanics with AFM and optical traps

Iwan A. T. Schaap

Georg August Universität, Germany

Measurements of the elastic properties of cells are widely used as an indicator for cellular changes during differentiation, upon drug treatment, or resulting from the interaction with the supporting matrix. Cell mechanics are routinely quantified by AFM indentation experiments while applying nano-Newton forces. Because the resulting deformations are in the micrometer range, the measurements will be affected by the finite thickness of the cell, viscous effects and even cell damage induced by the experiment itself.

We use a combination of AFM and optical trapping to study the non-linearity of the cellular response by applying forces between 10 and 1000 pN. At forces of up to 30 pN, the response is elastic and largely determined by the presence of the actin cortex. At higher forces, viscous effects that follow a weak power law, start to dominate the response of the cell.

I will show examples of this combined approach, which enables the separation of the cell's viscous and elastic components, to study the interaction of cells with its substrate and to follow cell differentiation.

High-resolution atomic force microscopy and spectroscopy of native membrane proteins

Daniel Müller

Eidgenössische Technische Hochschule Zürich, Switzerland

Membranes confining cells and cellular compartments are essential for life. Membrane proteins are molecular machines that equip cell membranes with highly sophisticated functionality. Examples of such functions are signaling, ion pumping, energy conversion, molecular transport, specific ligand binding, cell adhesion and protein trafficking. However, it is not well understood how most membrane proteins work and how the living cell regulates their

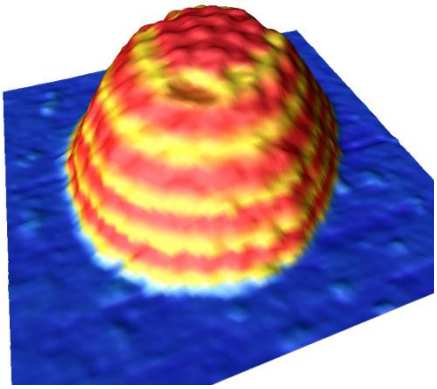
function. We review how atomic force microscopy (AFM) can be applied for structural and functional investigations of native membrane proteins. High-resolution time-lapse AFM imaging records membrane proteins at work, their oligomeric state and their dynamic assembly. The AFM stylus resembles a multifunctional toolbox that allows the measurement of several chemical and physical parameters at the nanoscale. In the single-molecule force spectroscopy (SMFS) mode, AFM quantifies and localizes interactions in membrane proteins that stabilize their folding and modulate their functional state. Dynamic SMFS discloses fascinating insights into the free energy landscape of membrane proteins. Single-cell force spectroscopy quantifies the interactions of live cells with their environment to single-receptor resolution. In the future, technological progress in AFM-based approaches will enable us to study the physical nature of biological interactions in more detail and decipher how cells control basic processes.

Probing viral material properties by Atomic Force Microscopy

Wouter H. Roos

Vrije Universiteit

Force spectroscopy experiments are turning into a standard tool to study the material properties of macromolecular assemblies such as viral shells (capsids). Here I present AFM nanoindentation studies on the mechanical properties of various viral capsids. Combining these measurements with high resolution imaging of the viral nanoparticles before and after indentation gives a complete picture of the response of such nanocontainers to mechanical stress. By presenting examples of recent work from my lab I will illustrate how diverse the properties of viruses can be. For instance a variety of viral nanoparticles turn out to possess pre-stressed shells. This had already been predicted 10 years ago by applying continuum elastic theory, but until recently it was impossible to test this prediction experimentally. We have now been able to show that this macroscopic theory holds at the nanoscale by devising a novel combined experimental and simulation approach to tackle the problem of how to test pre-stress in nanometer sized complexes. We also studied genome uncoating of human pathogens such as adenovirus. I will show how by using different host cell molecules, such as integrin and defensin, we for the first time reveal a direct link between mechanical stability and genome uncoating. Finally, I present data on the influence of pH on genome-capsid interactions of an insect virus. It turns out that the genome has a dual role. At neutral pH it stabilizes the densely packed virion and, upon pH increase, it triggers uncoating by relaxing the stabilizing interactions with the capsid. All in all these experiments provide striking examples of the ingenious ways in which nature designed a versatile set of protein nanocontainers.



AFM image of a viral nanoparticle

Spying on single biomolecules using fluorescence microscopy

Erwin Peterman

Vrije Universiteit, Amsterdam, The Netherlands

Observing, tracking and measuring individual biomolecules has provided a whole new view on function and mechanism of the molecules of life. In this lecture I will provide an introduction to one of the key single-molecule methods, single-molecule fluorescence microscopy. I will discuss the physical background of the method, instrumentation required, methods to fluorescently label the biomolecules of interest and parameters that can be obtained using this technology, in vivo and in vitro.

Elasticity and dynamics of cytoskeletal and extracellular networks

F.C. MacKintosh

Vrije Universiteit, The Netherlands

Much like the bones in our bodies, the cytoskeleton consisting of filamentous proteins largely determines the mechanical response and stability of cells. We review recent experimental and theoretical efforts to uncover the basic mechanisms of elasticity and dynamics of cytoskeletal networks and related extracellular matrices that also consist of protein fibers. These efforts have identified a number of key differences relative to most synthetic polymer systems, including a highly non-linear elastic response. Unlike passive materials, however, living cells are kept far out of equilibrium by metabolic processes and energy-consuming molecular motors that generate forces to drive the machinery behind various cellular processes. We also describe recent advances both in theoretical modeling of such networks, as well as experiments on reconstituted in vitro acto-myosin networks and living cells. We show how such internal force generation by motors can lead to dramatic mechanical effects, including strong mechanical stiffening. Furthermore, stochastic motor activity can give rise to diffusive-like motion in elastic networks. This can account for both probe particle motion and microtubule fluctuations observed in living cells. We also show how the collective activity of myosin motors generically organizes actin filaments into contractile structures, in a multistage non-equilibrium process. This can be understood in terms of the highly asymmetric load response of actin filaments: they can support large tensions, but they buckle easily under piconewton compressive load

Through the eyes of a virus – imaging adenovirus entry into cells

Urs F. Greber

Institute of Molecular Life Sciences, University of Zurich, Switzerland

Viruses are a serious threat to humans and livestock with devastating impact around the world. There are no adequate therapies available against most viral diseases, largely because viruses provide few targets for therapeutic attack. Furthermore, viral targets, i.e. their genes, mutate rapidly, which gives rise to resistance against drugs and vaccines. A key strategy to interfere with viral disease is to target the host. But this requires detailed knowledge of cell and infection biology, and highly advanced imaging methods of cells and viruses. Bio-imaging of molecules, organelles and cells in living organisms is rapidly changing the way we think about how cells and organisms function in healthy states, and how they respond to infections. It ranges from super-resolution methods in light microscopy and cryo-electron tomography, and more traditional structural methods including x-ray crystallography and molecular EM, to advanced live cell imaging at subcellular and cellular resolution for small- and large-animal studies. Bio-imaging will have broad implications on mechanism-based pharmacology, structure-based vaccine design, and new therapeutic approaches to virus disease.

Viruses carry genetic information between cells and individuals. They are built from nucleic acids, proteins and sometimes sugars and lipids. They traffic in and between cells, penetrate membranes, and release their genome and turn on the viral gene expression program. Upon entry into cells, they unfold uncharted levels of complexity. To understand the underlying mechanisms and develop new anti-viral strategies, detailed molecular and cell biological experimentation is required involving a whole range of methods. In this lecture I will discuss how human adenoviruses enter, uncoat and deliver their DNA genome into the nucleus.

Human adenoviruses are small DNA-tumor viruses replicating in the nucleus. Their structure is related to non-enveloped PRD bacteriophages. They comprise a large family of agents infecting the respiratory, gastrointestinal and urinary tracts, or cause epidemic kerato-conjunctivitis. Recently, the first zoonotic transmission of an adenovirus has been reported from monkeys to humans. This virus caused a deadly epidemic in the monkey colony and severe respiratory disease in humans that were in close contact to the monkeys.

Here, I will describe how adenoviruses use mechanical cues from the cell to trigger initial steps of their uncoating at the plasma membrane, and the final disruption of the capsid at the nuclear pore complex. I will also discuss how we use continuous wave stimulated emission depletion (cw-STED) microscopy to analyse the import of incoming single viral DNA-genomes into the nucleus. Low abundance of signals favors the use of STED as opposed to photo-activated localization microscopy (PALM) or structured illumination microscopy (SIM). We expect that super-resolution microscopy will complement cell and systems biology studies of virus entry, and may give new insights for the development of synthetic nano-machines with optimized delivery properties to diseased cells or tissues.

Towards quantitative structure-property relationships of biological samples using dynamic atomic force microscopy"

Arvind Raman

Purdue University, USA

AFM methods to map the quantitative material properties of biological samples can be divided into quasi-static methods where the cantilever bending is proportional to the force at the tip and dynamic methods where the local properties are extracted from the cantilever oscillation. This presentation will focus on challenges and recent advances towards quantitative mapping of local properties of biological samples in liquids. We will cover the basics of cantilever excitation in liquids, to force spectroscopy, and finally to recent methods for transduction of higher harmonics of tip-sample forces in order to extract quantitative properties on viruses, bacteria and live cells.

Intraflagellar transport in the chemosensory cilia of C. elegans tracked and captured with single-molecule fluorescence microscopy

Erwin Peterman

Vrije Universiteit, Amsterdam, The Netherlands

Cilia are protrusions present in most eukaryotic cells, with essential functions in motility and sensing. Development and maintenance of these microtubule-based organelles is crucially dependent on a specific transport process called intraflagellar transport (IFT). In the chemosensory cilia of C. elegans, two kinesin-2-family motors, heterotrimeric kinesin-II and homodimeric OSM-3-kinesin, act together in order to establish anterograde transport. Using quantitative fluorescence microscopy we show that kinesin-II gradually undocks from IFT trains that are initially formed out of tens of both motors allowing the OSM-3-kinesin train to reach terminal velocity already at the middle segment. The precise mechanism of how IFT trains are relayed between the two types of motors is, however, unknown and its unraveling requires assessing the dynamics of individual motor proteins. To this end we employed single-particle tracking in combination with photoactivated localization microscopy (sptPALM). We are able to follow single motor proteins deep inside the living organism and observe rich motor dynamics, such as transitions between processive walks, back-stepping, pausing and diffusion. Building superresolution images from single molecule localizations allows us to resolve the ultrastructure of cilia, onto which we can map single-motor trajectories. Our findings are the outset for a single-molecule view on IFT.

Elasticity and dynamics of cytoskeletal and extracellular networks

F.C. MacKintosh

Vrije Universiteit, The Netherlands

Much like the bones in our bodies, the cytoskeleton consisting of filamentous proteins largely determines the mechanical response and stability of cells. We review recent experimental and theoretical efforts to uncover the basic mechanisms of elasticity and dynamics of cytoskeletal networks and related extracellular matrices that also consist of protein fibers. These efforts have identified a number of key differences relative to most synthetic polymer systems, including a highly non-linear elastic response. Unlike passive materials, however, living cells are kept far out of equilibrium by metabolic processes and energy-consuming molecular motors that generate forces to drive the machinery behind various cellular processes. We also describe recent advances both in theoretical modeling of such networks, as well as experiments on reconstituted in vitro acto-myosin networks and living cells. We show how such internal force generation by motors can lead to dramatic mechanical effects, including strong mechanical stiffening. Furthermore, stochastic motor activity can give rise to diffusive-like motion in elastic networks. This can account for both probe particle motion and microtubule fluctuations observed in living cells. We also show how the collective activity of myosin motors generically organizes actin filaments into contractile structures, in a multistage non-equilibrium process. This can be understood in terms of the highly asymmetric load response of actin filaments: they can support large tensions, but they buckle easily under piconewton compressive load.

Imaging and modeling of virus egress from infected cells

Urs F. Greber

Institute of Molecular Life Sciences, University of Zurich, Switzerland

Advanced bio-imaging offers major opportunities for collaboration between chemical biologists, cell biologists, computational scientists, and infectious disease specialists. Proceeding from molecules to organelles, cells, tissues, mice, nonhuman primates and eventually humans will lead to substantial translational advances for the benefit of human health, including attenuation of virus infection. Viruses spread between cells, tissues and organisms by cell-free and cell-cell transmissions. Both mechanisms enhance disease but it is difficult to distinguish between them. We have investigated how human adenoviruses, which cause respiratory disease or epidemic kerato-conjunctivitis are spreading from cell to cell. Wet lab experiments, and a recently developed multi-scale computational model for virus spread in 2-dimensional cell cultures have accurately modeled the spreading behavior of adenovirus in cultured cells. The basis of the model are experimentally determined parameters, such as the diffusion constant of adenovirus in the extracellular medium, the probability of infection depending on local virus concentration, the probability and the average time for an infected cell to lyse, and the probability of cell death for uninfected cells. We expect that results from

these and related studies will provide valuable novel insights for the field of oncolytic viruses, and suggest targets for combinatorial oncolytic strategies fertilizing other fields of life sciences that use imaging and simulation.

Nonequilibrium work relations: basic concepts and derivations

Felix Ritort

Universitat de Barcelona, Spain

The recent advent of micromanipulation tools allow scientists to monitor and follow molecular processes one molecule at a time. Single molecule experiments make possible to measure energies as small as 1kcal/mol, opening a new field to explore energy exchange processes and non-equilibrium phenomena in small biological systems. Fluctuation theorems establish relations governing energy exchange processes in systems in contact with thermal sources, providing new methodologies to obtain equilibrium information from non-equilibrium experiments [1,2]. In these lectures I will present the basic concepts and applications underlying fluctuation relations. In the first lecture I will discuss how small systems must be approached from a statistical physics viewpoint and I will derive the nonequilibrium work equality by Jarzynski and the fluctuation relation by Crooks. Applications to recover free energy differences in DNA and RNA structures from single molecule experiments will be presented. In the second lecture I will derive an extended fluctuation relation [3] applicable to recover free energy differences of kinetic molecular structures such as intermediate states, misfolded states and affinity measurements of intermolecular binding [4]. Finally I will also briefly show how the extended fluctuation relation can be applied in mechanical unzipping experiments [5,6]. to recover base pairing free energies in RNA, essential to improve free energy prediction of RNA secondary structures.

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Lecture 1.

Super-resolution fluorescence microscopy: concepts and technical developments

Melike Lakadamyali

The Institute of Photonic Sciences ICFO, Barcelona, Spain

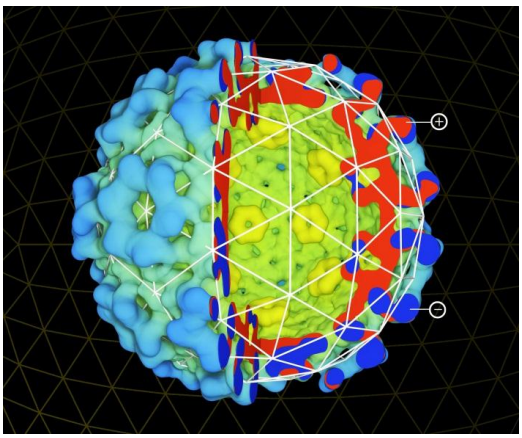
The past decade has seen a revolution in far-field optical microscopy. A number of methods have been developed that overcome the diffraction limit of spatial resolution. In this lecture I will overview the super-resolution techniques based on patterned illumination (Stimulated Emission Depletion Microscopy - STED) and those based on single molecule detection and localization (Stochastic Optical Reconstruction Microscopy - STORM and (Fluorescence) Photoactivation localization Microscopy - PALM and fPALM) along with technical developments that extend these techniques into multi-color imaging and 3D imaging.

Order and energetics of nucleic acids in viral capsids I

Rudolf Podgornik

Institut "Jozef Stefan", Slovenija

I will present some recent advances in our understanding of the packing of DNA and RNA in viral capsids. I will describe the ordering of dsDNA in bacteriophage capsids and various attempts at its description in the framework of polymer physics and liquid crystal physics. I will specifically provide a detailed framework for the understanding of the DNA osmotic pressure in the capsids. I will also present a different type of ordering for the ssRNA viruses. I will conclude by describing the electrostatic energies and the corresponding osmotic pressures in ssRNA viruses and dsDNA bacteriophages. The two types of viruses differ crucially in the spatial distribution of their genome charge which leads to essential differences in their free energies, depending on the capsid size and total charge in a quite different fashion. Differences in the free energies are trailed by the corresponding characteristics and variations in the osmotic pressure between the inside of the virus and the external bathing solution. I will also show on what level do the base-sequence effects become important for the energetics of nucleic acid packing in capsids.



Selected Poster Presentation 3

Using Highly Inclined, Laminated Optical sheet (HILO) and off-focus imaging for 3D tracking of single molecules in bacteria

Matteo Prayer Galletti, Lucia Gardini, Francesco S. Pavone

LENS - European Laboratory for Non-Linear Spectroscopy / University of Florence

Bacteria are generally small enough to allow, in principle, fast and uniform distribution of any diffusible molecule by simple thermal diffusion. Together with this observation, the lack of defined compartments within bacterial cells has led to the common view of these cells as spatially undifferentiated. Of course, this could be an oversimplification and still raises the question of how these organisms keep their molecular pathways straight by simple diffusion. Recently, single molecule and super-resolution methods have become amenable for studying the dynamics of expression and localization of fluorescently-labeled proteins in living bacteria. These technologies thus offer great potential for revealing novel aspects of prokaryotic subcellular organization, dynamics and functions. In this work we aim at implementing single-molecule detection, localization and 3D tracking. To obtain this, we present a custom made optical setup able to perform Fluorescent Imaging with One Nanometer Accuracy (FIONA). The apparatus exploits the out-of-focus properties of the Point Spread Function (PSF) to measure the depth of the focal plane, coupled with a feedback system that avoids stage drifting and allows auto-focusing. We also employ HILO instead of simple epifluorescence, which raises the signal-to-noise ratio up to eight-fold. The HILO sheet always passes through the center of the specimen plane, which allows for optical sectioning. The apparatus allows us to measure the distance between particles in the z axis, and can be used in a epifluorescence, HILO or TIRF configuration. We propose to use this apparatus to gain insight into the spatial organization and diffusion of selected molecules and cellular components within bacteria.

Selected Poster Presentation 4

Signal-Driven Tethering System based on DNA-Origami linked to Lipid Bilayers

A. Ohmann[1,2], A. Szuba[1,2], G.S. Cañón Bermúdez[1,2], V. Natarajan[1,2], T. Schlichthärle[1,2], M. Vahdatzadeh[1,2], P. Vasudevan[1,2], K.E. Viacava Romo[1,2], M.S. Grieb[1], F. Moreno-Herrero[3], M.E. Fuentes-Perez[3], A. Czogalla[2], S. Diez[1], M. Schlierf[1], R. Seidel[2]

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[3] Department of Macromolecular Structure, Centro Nacional de Biotecnologia, CSIC, Darwin 3, 28049 Cantoblanco, Madrid, Spain

Considerable work has been put into building DNA-origami structures for a variety of applications and into linking oligonucleotides to lipid membranes. By bringing these systems together, we devised a novel biological tethering system based on a controllable DNA origami

box coupled to a vesicle. The system comprises of a hexagonal DNA origami box that is attached to a lipid vesicle by cholesterol-modified oligonucleotidic “anchor strands”. Opening of the DNA-origami box can be controlled by the binding of specific ligands to aptamer locks. Upon opening, several single-stranded DNA “catcher strands” are exposed. These strands are complementary to “receiver strands” linked to target species present in solution. Consequently, these target species bind to the DNA-origami box only in the presence of a signal establishing a signal-driven tethering system. Our system may be used as a signal-driven targeted drug delivery system in which drugs or compounds encapsulated in vesicles are delivered to specific targets. Other potential applications include vesicle fusion by membrane destabilization, using the system to “fish” for a specific target in solution and forming highly ordered vesicle networks which may be extended to artificial tissue.

Extended fluctuation relations applied to free energy recovery of kinetic structures

Felix Ritort

Universitat de Barcelona, Spain

The recent advent of micromanipulation tools allow scientists to monitor and follow molecular processes one molecule at a time. Single molecule experiments make possible to measure energies as small as 1kcal/mol, opening a new field to explore energy exchange processes and non-equilibrium phenomena in small biological systems. Fluctuation theorems establish relations governing energy exchange processes in systems in contact with thermal sources, providing new methodologies to obtain equilibrium information from non-equilibrium experiments [1,2]. In these lectures I will present the basic concepts and applications underlying fluctuation relations. In the first lecture I will discuss how small systems must be approached from a statistical physics viewpoint and I will derive the nonequilibrium work equality by Jarzynski and the fluctuation relation by Crooks. Applications to recover free energy differences in DNA and RNA structures from single molecule experiments will be presented. In the second lecture I will derive an extended fluctuation relation [3] applicable to recover free energy differences of kinetic molecular structures such as intermediate states, misfolded states and affinity measurements of intermolecular binding [4]. Finally I will also briefly show how the extended fluctuation relation can be applied in mechanical unzipping experiments [5,6]. to recover base pairing free energies in RNA, essential to improve free energy prediction of RNA secondary structures.

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Super-resolution fluorescence microscopy: biological applications

Melike Lakadamyali

The Institute of Photonic Sciences ICFO, Barcelona, Spain

In this lecture I will give a few specific examples of biological problems that can now be studied at an unprecedented level of detail by using the novel super-resolution imaging methods. In particular I will focus on applications to cargo transport, protein nano-organization and quantitative imaging.

Order and energetics of nucleic acids in viral capsids II

Rudolf Podgornik

Institut "Jozef Stefan", Slovenija

I will present some recent advances in our understanding of the packing of DNA and RNA in viral capsids. I will describe the ordering of dsDNA in bacteriophage capsids and various attempts at its description in the framework of polymer physics and liquid crystal physics. I will specifically provide a detailed framework for the understanding of the DNA osmotic pressure in the capsids. I will also present a different type of ordering for the ssRNA viruses. I will conclude by describing the electrostatic energies and the corresponding osmotic pressures in ssRNA viruses and dsDNA bacteriophages. The two types of viruses differ crucially in the spatial distribution of their genome charge which leads to essential differences in their free energies, depending on the capsid size and total charge in a quite different fashion. Differences in the free energies are trailed by the corresponding characteristics and variations in the osmotic pressure between the inside of the virus and the external bathing solution. I will also show on what level do the base-sequence effects become important for the energetics of nucleic acid packing in capsids.

Selected Poster Presentation 5

Mechanical Unfolding of the One-State Downhill Folding Protein BBL

Jörg Schönfelder [a,b] and Victor Muñoz [a,b,c]

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Downhill protein folding describes a scenario in which the free energy barrier separating the folded and unfolded state of a protein is missing or marginal (< 3 RT). Furthermore downhill folding proteins are supposed to populate transiently a continuum of intermediate structures before reaching the folded or native state (One-State Folding).

This scenario should allow a direct observation of all intermediate states along the folding pathway and may endow the protein with remarkable mechanical-elastic properties.[1]

The small alpha-helical 40 residue protein domain BBL has been identified as a one-state downhill folder that folds in a microsecond timescale according to a battery of thermodynamic, kinetic and single molecule approaches.[2] According to its physiological role BBL is present as the peripheral subunit binding domain (PSBD) of the 2-oxogluterate dehydrogenase (OGDC) multienzyme complex of Escherichia coli. Therein it is described to act as a swinging arm that channels substrates between subunits of the complex.[3]

Our approach is to probe the mechanical properties of BBL both experimentally using the Atomic Force Microscope (AFM) in the field of Single Molecule Force Spectroscopy (SMFS) and computationally using Steered Molecule Dynamics (SMD) Simulation with NAMD. With this approach we try to get new insights into the behavior of a one-state downhill folding protein as it offers the tracking of the whole unfolding/folding process from denatured to folded state.

After building several polyprotein constructs using biomolecular techniques we were able to detect the unfolding patterns of individual BBL domains using a PicoForce AFM (VEECO). Furthermore recent results reveal a remarkably high unfolding force for such a small alpha-helical protein (50–100pN) and they show a high variability in the detected unfolding pathways of the BBL domain. In parallel, we have performed SMD simulations of the mechanical unfolding of the BBL domain (using NAMD and VMD [4]), where we fail to detect the high mechanical resistance that has been observed experimentally.

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Selected Poster Presentation 6

Monitoring dynamics of human adenovirus disassembly induced by mechanical fatigue

A. Ortega-Esteban [1], A. J. Pérez-Berná [2], R. Menéndez-Conejero [2], S. J. Flint [3], C. San Martín [2] and P. J. de Pablo [1]

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The standard pathway for virus infection of eukaryotic cells requires disassembly of the viral shell to facilitate release of the viral genome into the host cell. Here we use mechanical fatigue, well below rupture strength, to induce stepwise disruption of individual human adenovirus particles under physiological conditions, and simultaneously monitor disassembly in real time. Our data show the sequence of dismantling events in individual mature (infectious) and immature (noninfectious) virions, starting with consecutive release of vertex structures followed by capsid cracking and core exposure. Further, our experiments demonstrate that vertex resilience depends inextricably on maturation, and establish the relevance of penton vacancies as seeding loci for virus shell disruption. The mechanical fatigue disruption route recapitulates the adenovirus disassembly pathway in vivo, as well as the stability differences between mature and immature virions. (1)

1. Ortega-Esteban, A., Pérez-Berná, A. J., Menéndez-Conejero, R., Flint, S. J., San Martín, C. and de Pablo, P. J. (2013) Monitoring dynamics of human adenovirus disassembly induced by mechanical fatigue. *Sci. Rep.* **3**, 1434.

Fundamental aspects of high resolution imaging and quantitative mapping of biomolecules by dynamic force microscopy

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This lecture will highlight some aspects that need to be considered when aiming to achieve high resolution images and maps of mechanical properties of biomolecules. The aspects to be discussed apply to both contact and dynamic AFM experiments, though more emphasis is placed in the second. Specifically, the lecture will examine the relationship between lateral resolution, noise, forces and biomolecule deformation. It will be explained why high resolution imaging is hard to achieve on soft materials. The lecture will include some practical considerations about the operational parameters to achieve the best compromise between resolution and sample deformation.

To perform simultaneous imaging and force spectroscopy mapping is always a goal in microscopy. The lecture will also discuss the potential of multifrequency AFM methods to records images and maps of nanomechanical properties of soft matter. Contact mechanics provide the basis to transform measurements into elastic and rheological properties of nanoscale systems. A guide to contact mechanics models from the perspective of a force microscopist will be presented. The lecture will be complemented by some examples of mapping three-dimensional protein-water interfaces.

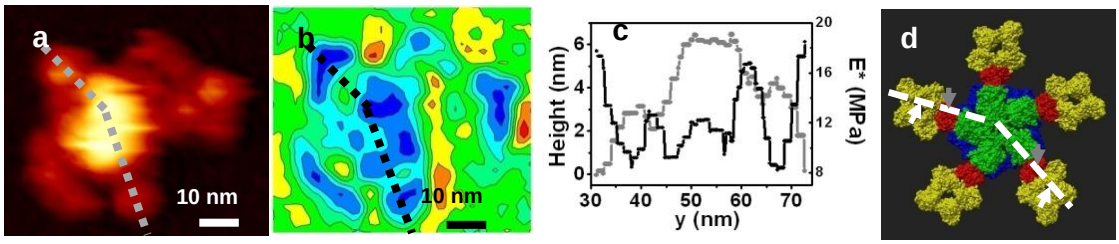

Figure. Multifrequency AFM map of the topography and flexibility of an antibody in buffer. **a**, Topography. **b**, Effective elastic modulus. **c**, numerical values along the marked line. **d**, Model of an IgM antibody

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H. V. Guzman, A. P. Perrino, and R. Garcia, Peak Forces in High-Resolution Imaging of Soft Matter in Liquid, *ACS Nano* **7**, 3198–3204 (2013)

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D. Martinez-Martin, E.T. Herruzo, C. Dietz, J. Gomez-Herrero, and R. Garcia, Noninvasive protein structural flexibility mapping by bimodal dynamic force microscopy, *Phys. Rev. Lett.* **106**, 198101 (2011).

The nanomechanics of individual biomolecules

Sergi Garcia-Manyes

King's College London, UK

Understanding the molecular mechanisms that confer mechanical stability to well-defined biological systems is a major challenge in modern physics, chemistry and biology. However, the molecular mechanisms by which mechanical force distributes across different length-scales, spanning the cellular level down to the single bond, are largely unknown. Using single molecule force-clamp spectroscopy AFM, we unveil the effect of a mechanical force on the free-energy landscape governing three distinct biologically relevant force-activated processes; the rupture of cell membranes, the (un)folding pathways of individual proteins and the effect of a mechanical force on the outcome of a chemical reaction, occurring at the single bond level. We first study how a constant pushing force affects the rupture kinetics of a stack of lipid bilayers, and find that the overall mechanical stability of the lipid bilayer results from a

complex and fine mechanochemical balance, where the chemical composition of both the headgroup and tail has a crucial effect. Furthermore, force clamp spectroscopy allows us to monitor for the first time, with exquisite sub-Ångström sensitivity, the conformational dynamics of a single refolding protein during its individual folding trajectory from highly extended states. Contrary to previous belief, our experiments demonstrate that the acquisition of the protein's native conformation occurs after dynamic maturation of an ensemble of collapsed states that are mechanically labile and structurally heterogeneous. These results support the validity of statistical mechanics models in describing the folding of a small protein on biological timescales. Finally, using a combination of protein engineering techniques with single molecule force-clamp spectroscopy we examined the influence of force on the rate at which a protein disulfide bond is reduced by nucleophiles in a bimolecular substitution reaction (S_N2). Our experiments directly identify a reactivity switch occurring at ~500 pN, resulting from a force-induced conformational change in the ground state of the disulfide bond. The single protein data is providing a new view that will help guide the development of theories on the statistical dynamics of folding and *ab-initio* studies of a chemical reaction while placed under a stretching force; of common occurrence in nature.

Selected Poster Presentation 7

A temperature-controlled Magnetic Tweezers to investigate DNA-translocating proteins

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Single-molecule experiments with DNA-translocating proteins are highly sensitive to the surrounding thermal energy and thus require a well-defined temperature inside the sample chamber. For high-numerical-aperture oil-immersion microscopes, different methods for temperature control exist (1). Even if only heating above room temperature is needed, these approaches cannot always be easily adapted to techniques like Magnetic Tweezers because they may for example restrict the space available on top of the sample cell (2), thus limiting the maximum force that can be applied in a permanent-magnet-based system.

Here, we present a simple modification of a Magnetic Tweezers setup (3) that does not affect the function of its essential components. Inspired by the approach described in (4), we combine resistive foil heaters, thin-film platinum resistance temperature detectors and a software-based PID feedback for thermal control of both the microscope objective and the baseplate supporting the sample chamber. This configuration enables us to carry out single-molecule experiments at temperatures in solution of up to 40 °C and with a precision of 0.1 °C.

We have employed our temperature-controlled Magnetic Tweezers to compare the translocation activity of the bacterial DNA helicase-nuclease complex AddAB (5, 6) at different thermal settings with values obtained from bulk measurements. These proof-of-principle

experiments (7) provide the basis for using our experimental setup to study the mechanism of recombination hotspot scanning of AddAB (8) at physiological conditions.

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Selected Poster Presentation 8

Mechanical stability and reversible fracture of vault particles

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Vaults are the largest ribonucleoprotein particles found in eukaryotic cells, with an unclear cellular function and promising applications as drug delivery containers. In this paper we study the local stiffness of individual vaults and probe their structural stability with Atomic Force Microscopy (AFM) under physiological conditions. Our data show that the barrel, the central part of the vault, governs both the stiffness and mechanical strength of these particles. In addition, we provoke single protein fractures in the barrel shell and monitor their temporal evolution. Our high-resolution AFM topographies show that these fractures occur along the contacts between two major vault proteins and disappear over time, thus removing any mark of the previous rupture. This unprecedented systematic self-healing mechanism, which may enable these particles to reversibly adapt to certain geometric constraints, might help vaults safely pass through the nuclear pore complex.

Selected Poster Presentation 9

(Un) Folding of Engrailed Homeodomain by Multiple Spectroscopic Probes: From Equilibrium to Ultrafast Temperature Jump Kinetics

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Many single-domain proteins fold in milliseconds or longer. However, the advent of fast folding kinetic techniques has permitted to identify many other proteins that fold in the order of (few) microseconds and thus very closely to the folding speed limit[1]. This suggests that the proteins that fold in microsecond timescale either cross a marginal single free energy barrier, multiple very small barriers (multi-state), or no barrier at all (one-state downhill)[2]. This results in the potential observation of broad complex unfolding transitions in these ultrafast folding proteins (in contrast to simple two-state behavior). Engrailed homeodomain (EnHD), a 61-residue α-helical protein with a simple helix-turn-helix topology folds in microseconds and exhibits an apparently complex (un) folding process[3, 4]. The observed complexity in the (un) folding behavior of EnHD rules out a simple two-state model and thus, makes this protein an interesting system to apply a large-scale multiprobe approach that combines equilibrium, fast folding and Single Molecule FRET measurements.

In this work, we present the results that combine 'equilibrium thermal unfolding' monitored by Differential Scanning Calorimetry (DSC), far UV Circular Dichroism (fCD), near UV Circular Dichroism (nCD), Steady-State Fluorescence and FT-Infrared (FTIR) with the 'ultrafast kinetics' studied by nanosecond Fluorescence and Infrared Temperature Jump kinetic measurements with spectral resolution. A comparison of equilibrium thermal unfolding of EnHD, by multiple probes, reveals heterogeneity in unfolding behaviors. The thermal unfolding by FT-Infrared spectroscopy exhibits "Wavelength-dependency" in the unfolding. The fluorescence signal of engrailed upon thermal unfolding is affected by (i) Contact quenching in the core of the native structure; (ii) Solvent effects, resulting in large spectra shift of about 27nm upon unfolding; (iii) Forster Resonance Energy Transfer from a tyrosine as donor to a tryptophan, which are placed at a distance of ~12 Å in the native structure.

Infrared Relaxation monitored at two wave numbers 1646 cm^{-1} and 1636 cm^{-1} yield single- exponential decays in tens of microseconds. Global SVD Analysis of Fluorescence decay vs. Temperature resolves the 3 components observed in equilibrium. Double exponential fits for the three components together yield a slow rate, that matches that of Infrared, with an additional fixed fast phase of ~ 0.8 μs. However, these decays can also be nicely fit to a stretched exponential. A comparison of 3 amplitudes obtained for the three components from Fluorescence kinetics with that of the 2 amplitudes obtained for the two frequencies from IR kinetics for the major folding phase clearly implicate probe dependency

and complex unfolding.

In order to uniformly describe the complex behavior observed with both equilibrium and kinetics, we describe an approach to fit all the experimental unfolding curves and decays, in a sequential way from equilibrium to kinetics, to a simple One Dimensional Free Energy Surface (1D-FES) Model[5, 2], in which the order parameter is described as a function of nativeness (n). This model yields a barrier height of 1.28 kJ/mol (DSC Heat Capacity Data) at Tm (~326K), which is ~ 0.47 RT near Tm (<3RT), falling within the 'downhill folding' regime.

In Summary, probe-dependent amplitudes or unfolding transitions, fast kinetics and an estimate of the barrier height obtained from DSC indicate complex folding mechanism of engrailed homeodomain.

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Selected Poster Presentation 10

Energy transfer studies in binary dye solution mixture of Coumarin 540 and Rhodamine B and its lifetime calculations

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The effect of acceptor concentration on the energy transfer in the case of binary dye mixture [Coumarin 540 (donor) + Rhodamine B (acceptor)] has been studied[1]. The concentration of Coumarin 540 (donor) is kept constant at 10^{-5} M while the concentration of Rhodamine B (acceptor) is varied. The energy transfer study is done at constant excitation wavelength 463 nm of Coumarin 540 so that it does not get practically absorbed by Rhodamine B and the energy emitted by Coumarin 540 is only absorbed (accepted) by the acceptor dye to get its characteristic emissions. The excitation and emission curves were obtained by the Spectrophoto-fluorometer at constant concentration (10^{-5} M) of donor dye (Coumarin 540). The Stern Volmer plot, plotted for varying concentrations of the acceptor dye (Rhodamine B), gives the value of energy transfer rate constant k_T ($k_{D\rightarrow A}$) to be 3.3475 M^{-1}. The data obtained from the Time Resolved Fluorescence Spectrophotometer was analyzed for the determination of lifetimes at various emission peaks. The experimentally calculated value of lifetime at 427 nm is 3.02 ns which is found to be in good agreement with the theoretical value. The detailed results will be discussed in the presentation.

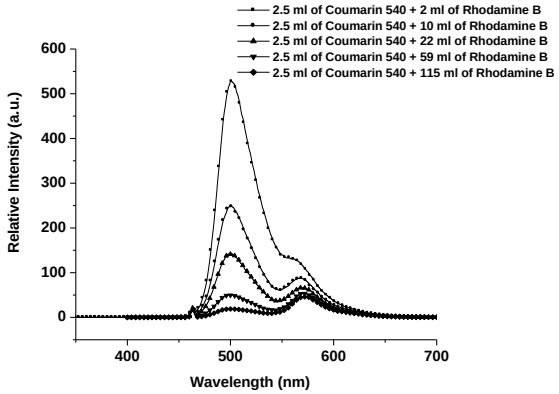

*Figure 1.*Fluorescence Emission spectra of binary dye mixture of Coumarin 540 and Rhodamine B in ethanol. The *concentration of* Coumarin 540 is fixed at 10^{-5} M while concentration of Rhodamine B (acceptor*) is varied.*

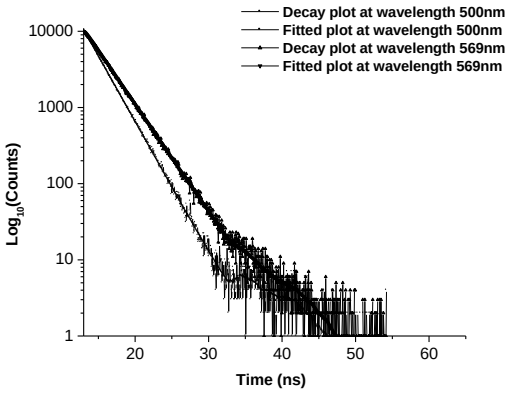

Figure 2. Experimental Lifetime Decay and Fitted plots of binary dye mixture of Coumarin 540 and Rhodamine B in ethanol at various wavelengths.

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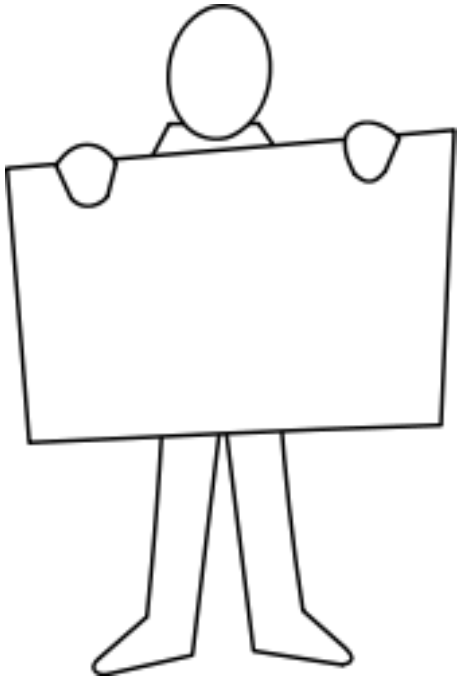

ABSTRACTS. POSTER PRESENTATIONS

Adio P1 81
Alemany P2 82
Assenza P3 82
Barroso P4 83
Cartagena P5 83
Castellanos P6 85
Collado-González P7 86
Dumitru P8 87
Fuentes-Perez P9 88
Gollnick P10 89
Hernando-Pérez P11 90
Herrero-Galán P12 91
Javani P13 92
Kulkarni P14 92
Llauró P15 93
Martínez P16 94
Marti-Prieto P17 94
Medina P18 95
Mohammed Khaled P19 96
Naqvi P20 97
Noculak P21 97
Ohmann P22 98
Ortega-Esteban P23 99
Pastrana P24 100
Pattanaik P25 101
Pattanaik P26 102
Platen P27 103
Prayer P28 103
Ramos P29 104
Rico P30 105
Schönfelder P31 105
Shokri P32 106
Sooriyanarayanan P33 107
Tikhomirova P34 108
van Santen P35 109
Zuttion P36 110

P1

A transient intermediate of EF-G dependent translocation identified by single molecule FRET

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Translocation of tRNAs is catalyzed by an enzyme called elongation factor G. EF-G mediated translocation is fast. For that reason it is difficult to study tRNA trajectories though the ribosome as they are affected by numerous structural rearrangements not only of the translation factor but of the whole ribosomal machinery.

We monitor tRNA movement through the ribosome in real time by employing a single molecule fluorescence approach to study the pathway individual ribosomes follow during translocation. We use a TIRF microscope with dual color detection to track FRET signals between ribosomes that are donor-labeled on ribosomal protein L11 and carry acceptor-labeled tRNA molecules. To slow down translocation to a rate amenable by our TIRF microscope, we engineered a set of mutant EF-G variants and use antibiotics to stall translocation at specific intermediates.

Our experiments reveal an intermediate state of translocation that has not been observed before. Mutants of EF-G that do not support translocation are stalled in the intermediate state before they dissociate from the ribosome. For translocation competent mutants of EF-G it is consumed in the course of translocation to form the post translocation state. Once the ribosome arrived in the post state the intermediate state cannot be induced by reverse translocation. This leads to the suggestion that translocation is driven to completion by an irreversible step taking place before the tRNAs have arrived in their post translocation positions, which most likely involves a conformational change of both EF-G and the ribosome.

P2

Exploring the free-energy landscape of nucleic acids

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The unzipping of nucleic acids is crucial to access the genetic information, essential for living organisms. Interestingly, the sequence of nucleic acids provides to themselves specific elastic and energetic properties that, in turn, determine their unfolding and refolding kinetics. Therefore, a proper characterization of the free-energy landscape of nucleic acids constitutes a major area of interest. Force-spectroscopy techniques are ideally suited to characterize the kinetics of biomolecules under mechanical stress. In this work, we carry out pulling experiments with optical tweezers to characterize different DNA hairpins and unveil their free-energy landscape using the Kramers theory [1]. We study the unfolding and folding kinetics under force, and how the force-dependent position of the transition state correlates with the molecular plasticity. Finally, we investigate the presence of intermediate states along the unfolding pathway and their effect on the unfolding kinetics of DNA molecules.

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P3

Universality of Behaviour in the Mesoscale Properties of Amyloid Fibrils

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Under abnormal circumstances, normally soluble proteins may aggregate and form highly stable filamentous structures, known as *amyloid fibrils*. Such structures play a central role in neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases, to cite but a few examples (1). Because of the heterogeneity in sequence of the several proteins able to undergo a disorder-leading fibrillation process, it has been proposed that such ability is a general property of polypeptides, and systematic *in vitro* experiments have shown that even disease-unrelated proteins form fibrils and probed their structural features (1). Here, we

introduce an original Coarse-Grained model to describe the mesoscale structure of amyloid fibrils. Our model gives parameter-free laws for the periodicity, depending on the geometrical arrangement of protofilaments. These predictions are validated experimentally on multistranded betalactoglobulin fibrils, providing quantitative agreement with the AFM data.

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P4

Assessment of Silver Nanoparticles Penetration in Latex Gloves using AFM.

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Metal nanoparticles (MNPs) have generated great interest because its optical, electrical, catalytic and bactericides properties. These properties depend on the size, shape and dispersion of MNPs, which can be controlled since its synthesis, and can be observed by different techniques. Atomic Force Microscopy (AFM) is now a powerful tool that allows the visualization of particles with nanometer-scale resolution. Despite the extended used of MNPs across the world, the policies ruling its manipulation and the safety measures are not clear enough, due to a lacking of a systematic study about how this systems react with the existent protection media. In this work we use AFM to assess the penetration of silver nanoparticles in the latex gloves used in labs, via the study of the gloves' surface before and after exposure to silver nanoparticles. The sizes of the silver nanoparticles were between 5 and 20 nm with aggregates from 1-4 μm [1].

P5

Quantitative multi-harmonic AFM methods for in vitro high resolution local material property mapping of live cells and viruses

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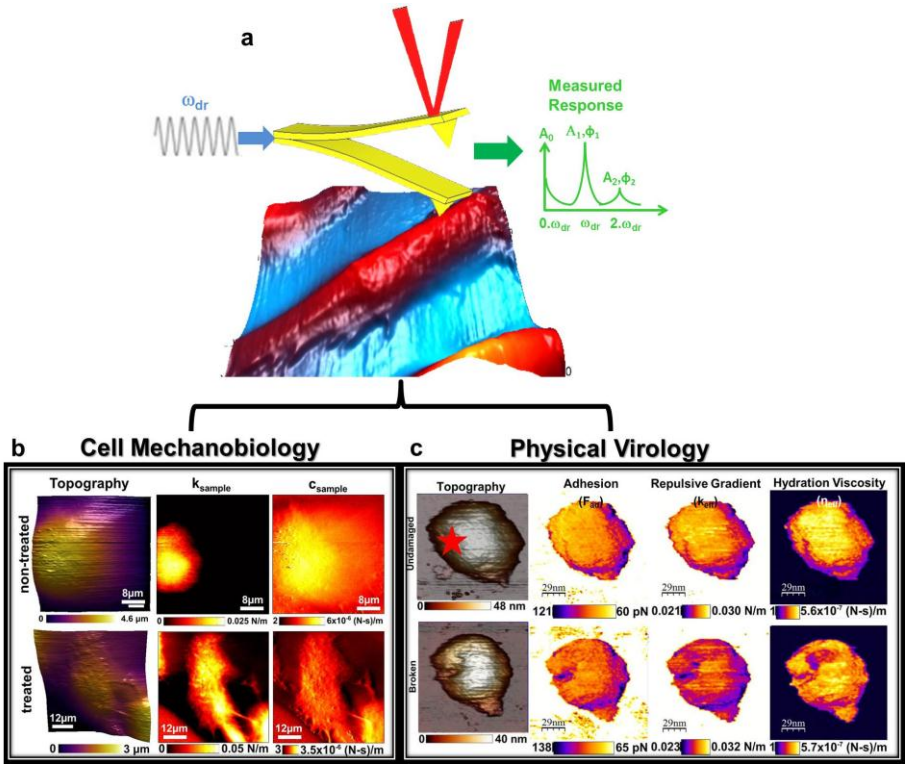
Understanding the relationships between the local material properties of live cells and viruses in physiological conditions with their molecular structure and biological functions is of great significance in mechanobiology, bionanotechnology, and nanomedicine. Recently [1, 2]

we have proposed a novel method based on an adaptation of standard tapping mode atomic force microscopy (AFM) where the 0^{th}, 1^{st}, and 2^{nd} harmonics of vibration (multi-harmonic observables) of Lorentz force excited microcantilevers are simultaneously acquired during a scan. Once an appropriate tip-sample interaction model is identified with the unknown material properties, the recorded multi-harmonic observables are used to quantitatively map those material properties over the sample at the same resolution and imaging throughput (pixel/min) as standard tapping mode AFM. This enables a 2-5 order improvement in imaging throughput for quantitative material property mapping compared to say the traditional force-volume method.

Here we present the method and its application to high resolution mapping of rheological properties of live fibroblasts and human breast cancer cells with and without the expression of a tumor suppressant. We also present results on the high resolution maps of local electrostatic repulsion force gradient, hydration layer viscosity, and adhesion on intact and partially disrupted bacteriophage ϕ29 virions. We demonstrate via these examples how mapping the local material properties of live cells and viruses before and after a change is induced can provide insight into their molecular structure and biological function.



a) Schematic of multi-harmonic AFM with a live fibroblast in physiological condition, showing that the tip-sample force nonlinearity leads to anharmonics nω of the drive frequency. b) Application to cancer cell mechanobiology, tracking induced variations in local materials properties when expressing Syk protein tyrosine kinase on human breast carcinoma cells. Topography image of a breast cancer cells with and without active Syk tumor suppressant and their corresponding material property maps, effective spring constant k_{sample} (N/m), and damping c_{sample} (N-s/m). c) Application to physical virology, AFM-induced partial disruption of a bacteriophage ϕ29 mature virion. Topography images of an intact and disrupted ϕ29 virion

along with their corresponding material property maps, adhesion force F_{ad} (pN), electrostatic repulsive force gradient k_{eff} (N/m), and the intrinsic hydration layer viscosity η_{eff} (N-s/m). Images pixels; 256X256.

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P6

Conformational mechanisms of sliding and homing-to-target in protein-DNA binding

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Proteins that bind specific DNA sequences need to find a short target within the enormous pool of binding sites provided by genomic DNA. The way they solve the problem is by binding non-specifically to DNA and sliding while bound. The sliding mechanism for DNA binding has been studied theoretically, computationally and experimentally using single-molecule methods (1-3). However, very little is known about DNA sliding from the protein viewpoint. For this reason, we aims at investigating whether DNA sliding and homing-to-target in transcription factors involve conformational rheostat mechanisms (defined as conversion of stochastic conformational fluctuations of downhill folding protein modules onto gradual responses to specific signals).

We will use the DNA-binding engrailed homeodomain (engHD), a ~60-residue helix bundle downhill module that binds with extremely high affinity (Kd =8pM) to the DNA sequence TAATTA (4-6). We have already cloned, expressed, purified and studied the folding of this domain, and started to studying DNA binding and conformational dynamics using SMF methods. Our purpose is, using a proper signal readout, be able to detect binding to a battery of DNA sequences that will differ in their binding affinity proportionally to their divergence from TAATTA (especially the last two bases). If binding to different sequences involves different engHD conformational ensembles (e.g. increasingly compact the tighter the binding), the readout of the sensor could discriminate between alternative sequences in real-time (seconds).

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P7

Resveratrol and its antifungal and antioxidant activity against Candida albicans

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The putative candicidal activity of resveratrol is currently a matter of controversy. The antifungal activity as well as the antioxidant response of resveratrol against *Candida albicans*, have been tested in a set of strains with a well-established genetic background. Viability of *C. albicans* CAI-4 strain was not affected by the addition of resveratrol at concentrations commonly employed on antifungal activity tests -10-40 μg/ml- nevertheless a clear decrease in viability -53.3%- could be appreciated with a tenfold increase -400 μg/ml-. Similar results where obtained when we studied *C. albicans tps1Δ/tps1Δ* strain which has its trehalose-6-phosphate synthase disrupted therefore it is sensitive to oxidative stress. Catalase and the intracellular amount of trehalose are specific markers of oxidative stress in yeasts. No changes in those markers were measured in *C. albicans* CAI-4 strain nor intracellular trehalose amount in *C. albicans tps1Δ/tps1Δ* strain but an increase in the catalase activity could be measured in mutant deficient in trehalose synthesis. This fact can be explained by the adaptive tolerance proposed by our group. Surprisingly, resveratrol at the lower concentration used -40 μg/ml- decreased the activity of catalase enzyme in presence of H2O2 instead of increased it. It has been proposed that resveratrol can exert its biological activity by modulating sirtuins so that future works will include the study of the way in which resveratrol can modify the relationships between sirtuins and different transcriptional factors as p53 or FOXO in order to know how viability and antioxidant response can be regulated.

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P8

Nanomechanical analysis of poly(lactic-co-glycolic acid) as a biodegradable scaffold

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Poly(lactic-*co*-glycolic) acid(PLGA) has been successfully used as a biodegradable polymer because it undergoes hydrolysis in the body to produce the original monomers, lactic acid and glycolic acid.We have carried out a multiscale analysis of proteins on biodegradable scaffolds (poly(lactic-*co*-glycolic acid)) and active semiconductor surfaces (pentacene) by using advanced force microscopy methods (AFM) (1).This study involved the adsorption of antibodies (pentameric immunoglobulinM) onto these surfaces.

Nanomechanical measurements of the biodegradable scaffold have also been employed upon exposure to water, cell culture medium and neural progenitor stem cells. The estimation of Young´s modulus has been performed by using different models (Sneddon, Derjaguin-Muller-Toporov (2)). Force-distance curves have been recorded, processed and analyzed for the estimation of Young´s modulus. Multifrequency AFM (bimodal) has been used to map simultaneously several mechanical properties with nanoscale spatial resolution on PLGA. Bimodal AFM has been applied to measure the topography, flexibility and viscosity of antibodies (pentameric IgM) deposited on PLGA (3).

Additionally, we have used a focused ion beam to pattern PLGA deposited on silicon with the purpose of studying the competition between swelling and degradation of the scaffold. This is accomplished by following the changes in volume of the PLGA patterns as a function of the time immersed in water at the same time that nanomechanical measurements are performed.

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P9

Replication initiation proteins studied with Atomic Force Microscopy

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DNA replication is a fundamental cellular process whose mechanism is still not well understood. Replication requires a specific DNA region, known as the origin of replication (Ori), as well as specific proteins, called replication initiation proteins (Rep). Both DNA and proteins form the replication initiation complex. The origin of replication in plasmids and phage DNA contains some conserved elements. These include specific binding sites (iterons) for Rep proteins, DnaA boxes for DnaA proteins and an AT-rich region where DNA melting occurs. In this work, we used the Atomic Force Microscope (AFM) to study the binding of Rep proteins to the origin of replication in the broad-host-range plasmid RK2 (1). The origin of replication in RK2 plasmid is called OriV. It possesses 5 iterons where the replication initiation protein TrfA binds, four DnaA boxes for DnaA proteins and four 13-meres in the AT rich region (2). Using the AFM, we were able to capture the binding of TrfA to the iterons region. Interestingly, while bound to the iterons, TrfA also interacts with a ssDNA oligonucleotide containing the sequence of one of the strands of the AT rich region. Moreover, the TrfA-ssDNA interaction is dependent on the sequence of the oligonucleotide. Our AFM approach was also applied to RepE protein, a replication initiation protein from plasmid F. Notably, we found that binding of RepE was also favored by the equivalent ssDNA oligonucleotide of the AT-rich region of plasmid F. These findings enable to create a general model in which firstly, Rep proteins induces the melting of the AT-rich region and secondly, specific interaction of Rep protein with one of the melted ssDNA occur.

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P10 (selected for oral presentation)

A temperature-controlled Magnetic Tweezers to investigate DNA-translocating proteins

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Single-molecule experiments with DNA-translocating proteins are highly sensitive to the surrounding thermal energy and thus require a well-defined temperature inside the sample chamber. For high-numerical-aperture oil-immersion microscopes, different methods for temperature control exist (1). Even if only heating above room temperature is needed, these approaches cannot always be easily adapted to techniques like Magnetic Tweezers because they may for example restrict the space available on top of the sample cell (2), thus limiting the maximum force that can be applied in a permanent-magnet-based system.

Here, we present a simple modification of a Magnetic Tweezers setup (3) that does not affect the function of its essential components. Inspired by the approach described in (4), we combine resistive foil heaters, thin-film platinum resistance temperature detectors and a software-based PID feedback for thermal control of both the microscope objective and the baseplate supporting the sample chamber. This configuration enables us to carry out single-molecule experiments at temperatures in solution of up to 40 °C and with a precision of 0.1 °C.

We have employed our temperature-controlled Magnetic Tweezers to compare the translocation activity of the bacterial DNA helicase-nuclease complex AddAB (5, 6) at different thermal settings with values obtained from bulk measurements. These proof-of-principle experiments (7) provide the basis for using our experimental setup to study the mechanism of recombination hotspot scanning of AddAB (8) at physiological conditions.

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P11

Direct measurement of phi29 phage stiffness provides evidence of internal pressure

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It has been proposed that the phage φ29 dsDNA translocation to the host is initiated by a push mechanism followed by a pulling activity performed by the bacteria proteinaceous machinery [1]. The push mechanism would be triggered by the elastic energy provided by the internal pressure built up during the DNA packing process [2]. So far, the direct demonstration of presence of internal pressure inside phage φ29 has remained elusive. By using atomic force microscopy (AFM) nanoindentation experiments, we directly probe the stiffness of individual full and empty viral particles of phage ϕ29 [3] under different buffer conditions. We have found that full DNA particles are stiffer than empty ones. To decipher the physical origin of such mechanical reinforcement [4] we performed further experiments in the presence of the trivalent counter ion spermidine, that induces DNA condensation even inside viruses[5]. Our real time experiments show that spermidine soften the full virion to empty particles values in a reversible way. These experimental findings provide evidence of the existence of internal pressure in φ29 virion. A finite element analysis of the experiments reveals an estimate of the pressure of 40 atm inside the capsid, which is similar to theoretical predictions[6].

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P12

Mechanical Identities of RNA and DNA Double Helices Unveiled at the Single-Molecule Level

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Double-stranded (ds) RNA is the genetic material of a variety of viruses and has been recently recognized as a relevant molecule in cells for its regulatory role. Despite that the elastic response of dsDNA has been thoroughly characterized in recent years in single-molecule stretching experiments, an equivalent study with dsRNA is still lacking. Here, we have engineered long dsRNA molecules for their individual characterization contrasting information with dsDNA molecules of the same sequence. It is known that dsRNA is an A-form molecule unlike dsDNA, which exhibits B form in physiological conditions. These structural types are distinguished at the single-molecule level with atomic force microscopy (AFM) and are the basis to understand their different elastic response. Force–extension curves of dsRNA with optical and magnetic tweezers manifest two main regimes of elasticity, an entropic regime whose end is marked by the A-form contour length and an intrinsic regime that ends in a low-cooperative overstretching transition in which the molecule extends to 1.7 times its A-form contour-length. DsRNA does not switch between the A and B conformations in the presence of force. Finally, dsRNA presents both a lower stretch modulus and overstretching transition force than dsDNA, whereas the electrostatic and intrinsic contributions to the persistence length are larger (1).

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P13

Pseudomonas veronii and Yersinia rohdei isolated from Antarctic ice-melting waters are able to produce at 4°C silver nanoparticles (AgNPs) with antibacterial activity

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Several psychrophilic bacteria were isolated from the ice-melting waters of the St George island (Antartica) and tested for AgNPs production. Broths from liquid cultures in nutritive medium of *Pseudomonas veronii* and *Yersinia rohdei* isolates were able to produce these nanoparticles at 30° and 4°C. The AgNPs were characterized through the recording of their UV spectra, Electron microscopy, X-Ray Difraction, and Dynamic Light Scattering analysis. The antibacterial activity of the nanoparticles were tested against two Gram negative, *Escherichia coli* and *Klebsiella pneumoniae*, and one Gram positive, *Staphilococcus epidermidis*, bacteria. Both were active against the three test bacteria, however they showed a higher activity against the Gram positive than against the two Gram negative, that were equally inhibited. The kinetics of nanoparticles synthesis was studied up to two months showing that after synthesis for about 20 days, those prepared at 30°C started to decrease in absorbance but for the samples at 4°C their absorbances were still increasing. The stability of the nanoparticles was tested by continuous irradiation and incubation at the temperature of synthesis up to 10 months. After this time, eventhough the shape of the UV-espectra changed producing a decrease of absorbance at the λ_{max} and an increase at lower and higher wavelengths, the AgNPs synthesized at 4°C still showed antimicrobial properties, but not those kept at 30°C.

P14

Imaging single DNA molecules dispersed on the mica substrates using non-contact atomic force microscopy

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We used non-contact mode atomic force microscopy to image single DNA molecules deposited on the atomically flat mica substrates. Various DNA molecules, viz., Salmon sperm DNA (500 µm), λ-DNA (48 kbp ≈ 16 µm) and PCR amplified DNA (150 bp ≈ 51 nm) were used to study their potential as biomolecular templates. Since DNA and freshly cleaved mica substrates both are negatively charged, the mica were silanized before the deposition of DNA. The attractiveness of DNA to silanized mica is very high which requires low concentration of DNA

solutions to prepare samples for AFM. For the Salmon sperm DNA (100 μg/ml solution) we observed networks of entangled DNA molecules covering the mica surface, forming stacked monolayers of DNA, and with further lowering the concentrations to 10 to 0.1 μg/ml, we could image the single DNA strands. The height profile of the single molecules was typically 3 nm, whereas the inherent broadening in the AFM imaging leads to the apparent width of the single DNA upto 20 nm. We also incubated mica substrates in few mM solution of $MgCl_2$ and the deposition on these substrates shows relatively less agglomeration of DNA molecules even with 100 μg/ml solutions. For commercial λ-DNA and PCR amplified DNA, we observed monodispersed molecules on the silanized mica and particularly in the case of PCR amplified DNA, the length of the molecules (around 50 nm) measured in AFM matches well with its Gel picture. Single DNA templates are proposed for several devices applications, for example, forming metallic nanowires to be used as interconnects. We achieved the metallization of DNA molecules using an external reducing agent for the reduction of the silver ions (ex-situ) as well as the localized aldehyde groups on the DNA acting as a reducing agent (aldehyde derivatization). The metallized nanowires show the enhanced dimensions of 2-3 nm compared to single DNA height in the AFM images. The metallization process is well controlled in the aldehyde derivatization for achieving uniform coating of DNA templates with silver clusters. Additional evidences were provided by TEM and SEM images which also show the formation of silver clusters along the length of the DNA.

P15 (selected for oral presentation)

Mechanical stability and reversible fracture of vault particles

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Vaults are the largest ribonucleoprotein particles found in eukaryotic cells, with an unclear cellular function and promising applications as drug delivery containers. In this paper we study the local stiffness of individual vaults and probe their structural stability with Atomic Force Microscopy (AFM) under physiological conditions. Our data show that the barrel, the central part of the vault, governs both the stiffness and mechanical strength of these particles. In addition, we provoke single protein fractures in the barrel shell and monitor their temporal evolution. Our high-resolution AFM topographies show that these fractures occur along the contacts between two major vault proteins and disappear over time, thus removing any mark of the previous rupture. This unprecedented systematic self-healing mechanism, which may enable these particles to reversibly adapt to certain geometric constraints, might help vaults safely pass through the nuclear pore complex.

P16

A new bio-quantum model for repair of DNA damage

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The mechanisms by which eukaryotic cells perceive and recognize breaks in DNA strands have not yet been elucidated. The rapid induction of ATM kinase activity immediately after exposure to ionizing radiation suggests that radiation acts at a previous stage of signal transduction. Former studies suggest that activation of ATM does not need a direct link with DNA strands damages. But some questions remained open about recognize the breaks, the factors for the high response rate and extent of the ATM and others. In this work we propose an original model for the signaling mechanism of the ATM kinase, as well as for the recognition of DSBs embedded in a genome of billions of base pairs. It is envisaged that this also could give satisfactory answers to some of the key issues mentioned above. It will be proposed and worked out a model for signaling between DNA and repair proteins, based upon already established concepts related to the existence of solitons in macromolecules and cells. It will be incorporated to the model a quantum-coherent effect known as free water dipole laser, which is expected to explain how the ATM (a "molecular motor") delineates its navigation route toward the DNA damaged site. Preliminary experimental evidences obtained by our group, as the likely electric character of DNA-repair mechanism, will be reinterpreted.

P17

Comparing optical and magnetic tweezers for studying the RecQ helicase

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Helicases are molecular motors that use the energy from ATP hydrolysis to translocate and unwind dsDNA, providing the ssDNA substrate needed in many cellular processes such as DNA replication, recombination and repair. Among DNA helicases, the RecQ family (conserved from bacteria to human) is essential for the maintenance of the genomic integrity. In this work we use optical and magnetic tweezers to study the E. Coli RecQ helicase. These single-molecule techniques allow to manipulate a DNA substrate (DNA hairpin) and follow in real-time the activity of a single RecQ motor. Mechanical force is applied at the ends of the DNA molecule, assisting the unwinding catalyzed by the helicase.

We measure the unwinding velocity under different applied forces and different ATP conditions. Interestingly, we find that the unwinding rate depends only weakly on the force applied, revealing that RecQ behaves as an active helicase. Whereas magnetic tweezers allow us to perform several experiments simultaneously, optical tweezers offer data with a higher time and spatial resolution from which we are able to observe pauses and backward steps of the motor.

The quantitative understanding of the differences between both methods is a fundamental step towards the combined use of these techniques for future projects.

P18

Study of peculiar DNA fragmentation patterns induced by gammas and electrons – statistical approaches at the nanoscale.

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Breaks in DNA, resulting in fragmented parts, can be produced by ionizing radiation which, in turn, is the starting point in the search for novel physical aspects of DNA strands. Double strand breaks in particular cause disruption of the DNA strand, splitting it into several fragments. Double-stranded pBS plasmid DNA was recently irradiated at this Laboratory with gammas at doses ranging from 1 to 12 kGy and electrons beams from 1 to 10 kGy. Fragment-size distributions were determined by Atomic Force Microscopy. The fragment distributions from irradiation with gammas revealed discrete-like patterns at all doses, suggesting that these patterns are modulated by the base pair composition of the plasmid. Irradiation with electron beams, at very high dose rates, generated continuous distributions of highly shattered DNA fragments. In order to understand the physical aspects underlying these observations a new simple mechanical model for this molecule is proposed. In this model, a Morse-like potential is used to describe the DNA-radiation interaction. Two power laws, used to fit results of the model, suggest, firstly, that distribution of fragments size is selectively non-extensive at low dose, and, secondly, that a transition phase is present in the DNA fragment distribution pattern. Fragment- sizes at high doses are stochastically distributed, indicating that breaking probabilities are nearly the same for all sites of the DNA strands.

P19

Investigation of MCM-DNA interaction using Atomic Force Microscopy

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Basic cellular processes as DNA replication are crucial to cell life. Understanding at the molecular level the mechanisms that govern DNA replication in proliferating cells is fundamental to understand disease connected to genomic instabilities, as genetic disease and cancer. The replication process in eukaryotes begins at specific, multiple sites on the DNA called origins of replication, by proteins called replication initiators: a pre-replication protein complex is recruited onto the DNA double helix (at the origins of replication), to separate the strands and initiate replication forks. A key component of the fork is the replicative helicase, responsible for the opening of DNA. In eukaryotes it is composed by the MCM (mini chromosome manteinance) complex, six homologous MCM proteins known as MCM2-MCM7, which are organized to form an hexamer.

The purpose of this study was to use Atomic Force Microscopy (AFM) to investigate the interaction between the MCM complex and dsDNA. When the protein is working as an helicase, to operate the DNA unwinding, MCM is supposed to “load” on DNA forming a ring around one strand and unzipping the DNA by moving along. Before “loading”, MCM is supposed to interact with DNA less tightly, with the DNA wrapping around the protein. In this novel, pre-activation conformation MCM is said to be “associated” with DNA (1). We exploited the excellent imaging capabilities of AFM to investigate the topological aspects of such MCM-DNA interactions.

The DNA molecules were deposited onto a suitable substrate prior imaging. We used freshly cleaved mica as a substrate. In order to overcome the repulsive electrostatic interaction between the negatively charged mica and the negatively charged DNA backbone, we used two strategies: in the first, freshly cleaved mica with a buffer solution containing divalent ions (e.g. Mg++) was employed; in the other case, mica was treated with poly-L-ornithine, a positively charged synthetic aminoacid. We demonstrated that in the first case the dsDNA molecules were equilibrating on the surface, while in the case of poly-L-ornithine treated mica, the amino group in the polymer were pinning the DNA molecules from the solution, preventing their equilibration. We then tested DNA interaction with MCM helicase as a function of DNA length (250 and 1000 base-pair (bp) long dsDNA, corresponding to about 85 nm and 340 nm contour length respectively) on freshly cleaved mica (with a MgCl2 buffer solution). We observed that for the long 1000bp DNA fragments the interaction was stable while for short, 250 bp long fragments the interaction with MCM was weak and not stable. We also observed that at room temperature the MCM protein is often “loaded” to the 1000bp dsDNA, for different concentration of the MCM complex.

1. Costa A. and Onesti S. ,2008. The MCM complex: (just) a replicative helicase? Biochem Soc Trans 36:136–140.

P20 (selected for oral presentation)

Single-molecule folding mechanisms of the apo- and Mg^{2+}-bound forms of the human neuronal calcium sensor 1 (NCS1)

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Neuronal calcium sensors (NCS) are responsible primarily for sensing changes in neuronal Ca^{2+} concentration and their members are linked to a number of disorders such as schizophrenia and autism, and to cognitive abilities such as learning and memory. NCS1 is the primordial member of the NCS family and is reported to bind an array of interaction partners, resulting in a broad interactomes. Here we used optical tweezers to study the folding mechanism of NCS1 at the single molecule level. Specifically we characterized the behavior of NCS1 in the presence of Mg^{2+} and of no divalent ions (apo form). In the former case, NCS1 unfolds and refolds through an intermediate structure stabilized by Mg^{2+} binding. In the second case, the apo form unfolds and refolds in a two state manner, displaying a molten globule-like behavior. Through constant-force experiments and hidden Markov model analysis, the free energy landscape of NCS1 with and without Mg^{2+} has been reconstructed. The results of these studies have been compared with those obtained in the presence of Ca^{2+} in order to shed light on the mechanisms by which changes in calcium and magnesium concentrations inside a cell affect the folding process and, ultimately, the function of NCS1.

P21

Influence of lanthanides concentration on upconversion process in $NaGdF_4$ nanocrystals

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Among the various types of fluorescent nanoparticles that are currently used in bio-imaging, lanthanide-doped upconverting nanoparticles (UCNPs) are attracting considerable

attention. Low photodamage of biological specimens, deep tissue penetration and lack of autofluorescence make them excellent optical markers.

The NIR-to-visible upconversion based on sequential energy transfers between lanthanide dopants involving their real metastable-excited states with lifetimes as long as several milliseconds is a process orders of magnitude more efficient than the 2-photon absorption process typically used in multiphoton microscopy.

Additionally lack of photobleaching and photoblinking make UCNPs ideally suited for single-molecule imaging experiments.

Fluoride host matrix is widely used because of the low-phonon energy and highly efficient conversion of NIR to higher energies. Promising alternative is $NaGdF_4$ matrix, where yttrium ions are replaced by gadolinium ions. This replacement can even more decrease phonon energy in system (1) and opens new attractive application paths for nanocrystals as multifunctional optical and magnetic markers.

In this work, set of $NaGdF_4:Yb^{3+}/Tm^{3+}$ samples with different lanthanide molar ratio has been synthesized under identical synthesis conditions using co-thermolysis method. In order to characterize obtained nanocrystals both optical (PL, PLE, absorbance, emission decay time) and structural (XRD, HRTEM, DLS, FTIR, Raman) measurements have been carried out.

(1) M. Banski, A. Podhorodecki, J. Misiewicz, M. Afzaal, P. O Brien, *J. Mater. Chem.C* **1**, 801, (2013).

P22 (selected for oral presentation)

Signal-Driven Tethering System based on DNA-Origami linked to Lipid Bilayers

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Considerable work has been put into building DNA-origami structures for a variety of applications and into linking oligonucleotides to lipid membranes. By bringing these systems together, we devised a novel biological tethering system based on a controllable DNA origami box coupled to a vesicle. The system comprises of a hexagonal DNA origami box that is attached to a lipid vesicle by cholesterol-modified oligonucleotidic “anchor strands”. Opening of the DNA-origami box can be controlled by the binding of specific ligands to aptamer locks. Upon opening, several single-stranded DNA “catcher strands” are exposed. These strands are

complementary to “receiver strands” linked to target species present in solution. Consequently, these target species bind to the DNA-origami box only in the presence of a signal establishing a signal-driven tethering system. Our system may be used as a signal-driven targeted drug delivery system in which drugs or compounds encapsulated in vesicles are delivered to specific targets. Other potential applications include vesicle fusion by membrane destabilization, using the system to “fish” for a specific target in solution and forming highly ordered vesicle networks which may be extended to artificial tissue.

P23 (selected for oral presentation)

Monitoring dynamics of human adenovirus disassembly induced by mechanical fatigue

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The standard pathway for virus infection of eukaryotic cells requires disassembly of the viral shell to facilitate release of the viral genome into the host cell. Here we use mechanical fatigue, well below rupture strength, to induce stepwise disruption of individual human adenovirus particles under physiological conditions, and simultaneously monitor disassembly in real time. Our data show the sequence of dismantling events in individual mature (infectious) and immature (noninfectious) virions, starting with consecutive release of vertex structures followed by capsid cracking and core exposure. Further, our experiments demonstrate that vertex resilience depends inextricably on maturation, and establish the relevance of penton vacancies as seeding loci for virus shell disruption. The mechanical fatigue disruption route recapitulates the adenovirus disassembly pathway in vivo, as well as the stability differences between mature and immature virions. (1)

1. Ortega-Esteban, A., Pérez-Berná, A. J., Menéndez-Conejero, R., Flint, S. J., San Martín, C. and de Pablo, P. J. (2013) Monitoring dynamics of human adenovirus disassembly induced by mechanical fatigue. *Sci. Rep.* **3**, 1434.

P24

Condensation of DNA mediated by the bacterial centromere binding protein Spo0J/ParB

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The condensation and dynamic re-organization of the chromosome is crucial to the cell cycle of all living organisms. In B. subtilis, the centromere binding protein Spo0J/ParB has recently been implicated in the recruitment of condensins at parS sequences close to the origin of replication (1, 2). We have studied the binding of Spo0J to DNA molecules using magnetic tweezers. At reduced forces, we observe a progressive condensation of the tethered DNA molecule in high Spo0J concentrations. The condensation phenomenon was reproduced in torsionally and non-torsionally constrained DNA, and in molecules both with and without parS sites, pointing to a non-specific binding mode of Spo0J at high concentrations in agreement with bulk studies. Experiments with competitor DNA containing the parS sequence partially inhibited condensation, revealed specificity for parS, and confirmed that the observed condensation was Spo0J-mediated. Our data lead us to propose a model in which condensation is induced by interactions between neighbouring Spo0J proteins due to the Brownian motion of the DNA tether. Computer simulations based on this model qualitatively reproduce our magnetic tweezers results.

1. Gruber S., and Errington, J. (2009) Recruitment of condensing to replication origin regions by ParB/Spo0J promotes chromosome segregation in *B.subtilis*. *Cell*, **137**: 685-696.

2. Sullivan, N.L., Marquis, K.A. and Rudner, D.Z. (2009) Recruitment of SMC by ParB-*parS* organizes the origin region and promotes efficient chromosome segregation. Cell, **137**: 697-707.

P25 (selected for oral presentation)

Energy transfer studies in binary dye solution mixture of Coumarin 540 and Rhodamine B and its lifetime calculations

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The effect of acceptor concentration on the energy transfer in the case of binary dye mixture [Coumarin 540 (donor) + Rhodamine B (acceptor)] has been studied[1]. The concentration of Coumarin 540 (donor) is kept constant at 10^{-5} M while the concentration of Rhodamine B (acceptor) is varied. The energy transfer study is done at constant excitation wavelength 463 nm of Coumarin 540 so that it does not get practically absorbed by Rhodamine B and the energy emitted by Coumarin 540 is only absorbed (accepted) by the acceptor dye to get its characteristic emissions. The excitation and emission curves were obtained by the Spectrophoto-fluorometer at constant concentration (10^{-5} M) of donor dye (Coumarin 540). The Stern Volmer plot, plotted for varying concentrations of the acceptor dye (Rhodamine B), gives the value of energy transfer rate constant k_T ($k_{D\rightarrow A}$) to be 3.3475 M^{-1}. The data obtained from the Time Resolved Fluorescence Spectrophotometer was analyzed for the determination of lifetimes at various emission peaks. The experimentally calculated value of lifetime at 427 nm is 3.02 ns which is found to be in good agreement with the theoretical value. The detailed results will be discussed in the presentation.

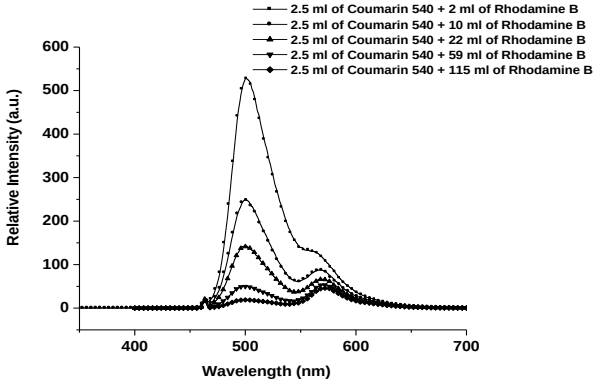

Figure 1.Fluorescence Emission spectra of binary dye mixture of Coumarin 540 and Rhodamine B in ethanol. The concentration of Coumarin 540 is fixed at 10^{-5} M while concentration of Rhodamine B (acceptor) *is varied.*

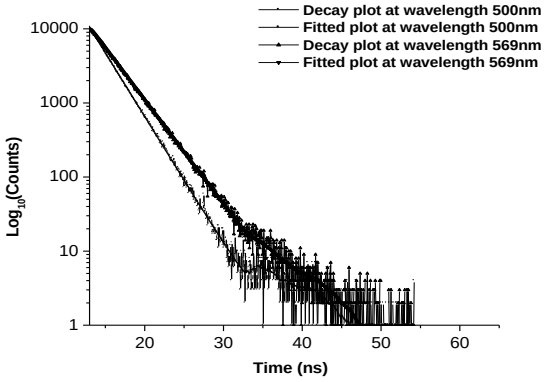

Figure 2.Experimental Lifetime Decay and Fitted plots of binary dye mixture of Coumarin 540 and Rhodamine B in ethanol at various wavelengths.

1. Energy transfer studies in binary dye solution mixtures:Acriflavine + Rhodamine 6G and Acriflavine + Rhodamine B; P.D.Sahare, Vijay K. Sharma, D. Mohan, A.A. Rupasov, Spectrochim Acta A Mol Biomol Spectrosc. 2008 Apr;69(4):1257-64

P26

Energy transfer studies in ternary dye solution mixture of Stilbene 420, Coumarine 540 & Rhodamine B and Its lifetime calculations

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The energy transfer in the case of ternary dye mixture [Stilbene 420 (donor) + Coumarin 540 (intermediator) + Rhodamine B (acceptor)] has been studied at various acceptor concentrations. The concentrations of the other two dyes, Stilbene 420 and Coumarin 540, are kept constant at 5×10^{-5} M and 10^{-5} M respectively. The energy transfer study is done at constant excitation wavelength 381 nm of Stilbene 420 so that it does not get practically absorbed by Coumarin 540 or Rhodamine B and the energy emitted by Stilbene 420 is only absorbed (accepted) by the intermediate dye and then transferred to the acceptor dye to get their respective characteristic emissions. The excitation and emission curves were obtained by the Cary Eclipse Spectro- photo-fluorometer at constant concentration (5×10^{-5} M) of donor dye (Stilbene 420).The Stern Volmer plot, plotted for varying concentrations of the acceptor dye (Rhodamine B), gives the value of energy transfer rate constant k_T ($k_{D\rightarrow A}$) to be 0.02887 M^{-1}. The data obtained from Time Resolved Fluorescence Spectrophotometer was analyzed for the determination of lifetimes at various emission peaks. The experimentally calculated value of lifetime at 501 nm is found to be 2.62 ns which is found to be in good agreement with the theoretical value. The results will be discussed in detailed in the presentation.

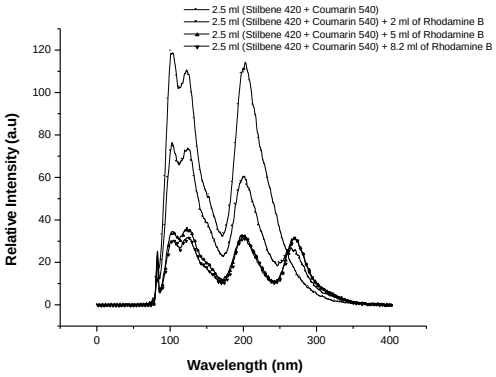

Figure 1.Fluorescence Emission spectra of ternary dye mixture of Stilbene 420, Coumarin 540 & Rhodamine B in ethanol.

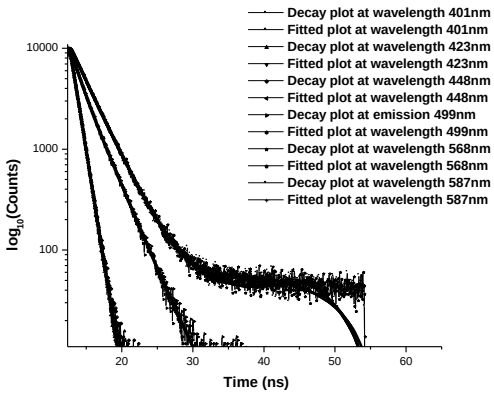

Figure 2.Experimental Lifetime Decay and Fitted plots of ternary dye mixture of Stilbene 420, Coumarin 540 & Rhodamine B in ethanol at various wavelengths.

1. Energy transfer studies in binary dye solution mixtures:Acriflavine + Rhodamine 6G and Acriflavine + Rhodamine B; P.D.Sahare, Vijay K. Sharma, D. Mohan, A.A. Rupasov, Spectrochim Acta A Mol Biomol Spectrosc. 2008 Apr;69(4):1257-64

P27

Investigation of enzyme complex dynamics via atomic force microscopy

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Due to its ability to operate in liquid atomic force microscopy (AFM) is a powerful tool to study biological samples and over the last decades many exciting results have been reported. However, these studies usually represent only a fraction of all available biological specimens. Namely, those samples that are stiff enough to withstand the forces applied by the AFM probe (e.g. DNA, proteins, viruses). Here, we present our strategy to extend the use of AFM for samples that are much softer As a model system we choose the pyruvate dehydrogenase multienzyme complex (PDHc). The PDHc represents a key-complex which links glycolysis to the citric acid cycle by converting central metabolite pyruvate into acetyl-CoA. The core of the human PDHc consists of 60 dihydrolipoamide acetyltransferase enzymes (E2), which assemble into a 50 nm diameter dodecahedral structure. A key trait vital to PDHc function is the flexibility of the N-terminal ”swinging lipoyl domain” of E2, which is capable of reaching the active sites of all proximal enzyme components. Although low resolution structural information exists, the underlying dynamics of catalysis, in particular substrate channeling is not understood. To find the most suitable techniques to study this soft complex we are testing different imaging techniques (amplitude and frequency modulation as well as jumping mode), and high speed force mapping. We will present our first results on the PDHc.

P28 (selected for oral presentation)

Using Highly Inclined, Laminated Optical sheet (HILO) and off-focus imaging for 3D tracking of single molecules in bacteria

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Bacteria are generally small enough to allow, in principle, fast and uniform distribution of any diffusible molecule by simple thermal diffusion. Together with this observation, the lack of defined compartments within bacterial cells has led to the common view of these cells as spatially undifferentiated. Of course, this could be an oversimplification and still raises the question of how these organisms keep their molecular pathways straight by simple diffusion. Recently, single molecule and super-resolution methods have become amenable for studying the dynamics of expression and localization of fluorescently-labeled proteins in living bacteria. These technologies thus offer great potential for revealing novel aspects of prokaryotic subcellular organization, dynamics and functions. In this work we aim at implementing single-molecule detection, localization and 3D tracking. To obtain this, we present a custom made optical setup able to perform Fluorescent Imaging with

One Nanometer Accuracy (FIONA). The apparatus exploits the out-of-focus properties of the Point Spread Function (PSF) to measure the depth of the focal plane, coupled with a feedback system that avoids stage drifting and allows auto-focusing. We also employ HILO instead of simple epifluorescence, which raises the signal-to-noise ratio up to eight-fold. The HILO sheet always passes through the center of the specimen plane, which allows for optical sectioning. The apparatus allows us to measure the distance between particles in the z axis, and can be used in a epifluorescence, HILO or TIRF configuration. We propose to use this apparatus to gain insight into the spatial organization and diffusion of selected molecules and cellular components within bacteria.

P29

DNA-SWCNTs wrapping characterization by AFM

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The combination of biosensors with the new advances in nanotechnology is playing an important role in the rapid evolution of nanobiotechnology, the wide applications of DNA sensing makes these molecules one of the most important bioreceptor in this direction. As a consequence in recent years DNA biosensors have attracted a great deal of attention due to their rapid response, low cost, high selectivity and miniaturization. However, there are some challenges in order to improve their properties such as conductivity and sensitivity.

Regarding enhance electrical detection for biological events, carbon nanotubes (CNTs) have been proposed for this objective. These graphene based nanotubes has extraordinary thermal conductivity, mechanical and electrical properties. Moreover, their structure with high surface-volume ratio, increase the biosensors area and so the bioreceptors attached on it, improving the device sensitivity. In fact several applications based on the functionalization of ss-DNA with CNTs have been reported, in order to improve the detection and sensing efficiency of the biosensors. The majorities of functionalization methods are based on the covalent interaction between DNA and CNTs, complicating the fabrication of biosensors and reduces the electrical properties of the CNT. Efforts are focused in the simplification of this method.

In this way, our aim is to establish a simple DNA-CNT no-covalent functionalization. For this purpose the wrapping abilities of single stranded DNA with single-wall carbon nanotubes (SWCNTs), was used, with the subsequent immobilization of these DNA–CNT complexes by the self-assembly monolayer (SAM) technique on Au surface. Surface Plasmon Resonance (SPR) instrument is needed in order to monitor all the immobilization on the biosensor and two microscopy techniques; Atomic Force Microscope (AFM) and Transmission Electron Microscope (TEM), were used for the complex characterization. Finally we study the possibility of using ssDNS-SWCNTs as a biosensor for selective recognition of complementary DNA. [1].

1. Fernando C. Moraes, Tiago A. Silva, Ivana Csesarino, Serguio A.S. Machado (2012). Effect of the surface organization with carbon nanotubes on the electrochemical detection of bispehnol A. (2013) 14-18.

P30

An experimental study of dsDNA thermodynamics using optical tweezers with force feedback

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Optical tweezers are useful for studying the thermodynamics of dsDNA folding. Feedback-based force clamps are often used to maintain a constant (or controlled) load. This is interesting both to simplify the interpretation of biophysical experiments, avoiding the complications generated by a changing load, and to study the thermodynamics of small systems under different control parameters. We present two different types of feedback, based on active and passive mechanisms, which are adapted to two different experimental situations: pulling and hopping experiments. We present and discuss the results of these experiments. To conclude we discuss possible future improvements.

P31 (selected for oral presentation)

Mechanical Unfolding of the One-State Downhill Folding Protein BBL

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Downhill protein folding describes a scenario in which the free energy barrier separating the folded and unfolded state of a protein is missing or marginal (< 3 RT). Furthermore downhill folding proteins are supposed to populate transiently a continuum of intermediate structures before reaching the folded or native state (One-State Folding).

This scenario should allow a direct observation of all intermediate states along the folding pathway and may endow the protein with remarkable mechanical-elastic properties.[1]

The small alpha-helical 40 residue protein domain BBL has been identified as a one-state downhill folder that folds in a microsecond timescale according to a battery of thermodynamic, kinetic and single molecule approaches.[2] According to its physiological role BBL is present as the peripheral subunit binding domain (PSBD) of the 2-oxogluterate dehydrogenase (OGDC) multienzyme complex of Escherichia coli. Therein it is described to act as a swinging arm that channels substrates between subunits of the complex.[3]

Our approach is to probe the mechanical properties of BBL both experimentally using the Atomic Force Microscope (AFM) in the field of Single Molecule Force Spectroscopy (SMFS) and computationally using Steered Molecule Dynamics (SMD) Simulation with NAMD. With this approach we try to get new insights into the behavior of a one-state downhill folding protein as it offers the tracking of the whole unfolding/folding process from denatured to folded state.

After building several polyprotein constructs using biomolecular techniques we were able to detect the unfolding patterns of individual BBL domains using a PicoForce AFM (VEECO). Furthermore recent results reveal a remarkably high unfolding force for such a small alpha-helical protein (50–100pN) and they show a high variability in the detected unfolding pathways of the BBL domain. In parallel, we have performed SMD simulations of the mechanical unfolding of the BBL domain (using NAMD and VMD [4]), where we fail to detect the high mechanical resistance that has been observed experimentally.

1. V.Muñoz . Annu.Rev.Biophys.Biomol.Struct. 2**007**, 36, 395-412

2 . M.M. Garcia-Mira, M.Sadqui, N. Fisher, J.M. Sanchez Ruiz & V.Muñoz. Science 2002, 298, 2191-2195

3. Perham, RN. Annu Rev Biochem, **2000**, 69, 961-1004

4. Hui Lu et al; Cell Biochemistry and Biophysics **2009,** Volume 55, Number 3, 141-152

P32

Using biomolecule to design a novel method for rapid identification of Salmonella spp. in dairy products

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The aim of this study is to use P-CEIA (Polymixin-Coated Polyester Cloth in EIA) method for rapid isolation of salmonella in dairy products. While other methods such as bacterial culturing and ELISA (ab-EIA) are time consuming and expensive, P-CEIA has been utilized for this study. Different dilutions of S.typhimorium Ra-30 in Buffered peptone water (BPW, pH=7.4) and also ten samples (milk and butterfat) have been studied by two P-CEIA and ab-EIA methods. Analyzing of findings indicates that by using the standard strains of S.typhimorium Ra-30, sensitivity for P-CEIA method was 10^6 cfu/mL while ab-EIA was estimated 10^5 cfu/mL. This sensitivity was balanced after heat treatment by using the sodium deoxy-cholate as a detergent five and three positive results were allocated to unknown milk and butter fat samples which were tested with Ab-EIA and also six and three positive results were allocated to unknown milk and butterfat samples which were tested with P-CEIA. In addition to the P-CEIA method requires 14 hour process time less than ab-EIA method. Analyzing of findings indicates that P-CEIA method is more privileged cause of its firm-inexpensive and stable process for Salmonella detection in dairy products.

P33 (selected for oral presentation)

(Un) Folding of Engrailed Homeodomain by Multiple Spectroscopic Probes: From Equilibrium to Ultrafast Temperature Jump Kinetics

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Many single-domain proteins fold in milliseconds or longer. However, the advent of fast folding kinetic techniques has permitted to identify many other proteins that fold in the order of (few) microseconds and thus very closely to the folding speed limit[1]. This suggests that the proteins that fold in microsecond timescale either cross a marginal single free energy barrier, multiple very small barriers (multi-state), or no barrier at all (one-state downhill)[2]. This results in the potential observation of broad complex unfolding transitions in these ultrafast folding proteins (in contrast to simple two-state behavior). Engrailed homeodomain (EnHD), a 61-residue α-helical protein with a simple helix-turn-helix topology folds in microseconds and exhibits an apparently complex (un) folding process[3, 4]. The observed complexity in the (un) folding behavior of EnHD rules out a simple two-state model and thus, makes this protein an interesting system to apply a large-scale multiprobe approach that combines equilibrium, fast folding and Single Molecule FRET measurements.

In this work, we present the results that combine 'equilibrium thermal unfolding' monitored by Differential Scanning Calorimetry (DSC), far UV Circular Dichroism (fCD), near UV Circular Dichroism (nCD), Steady-State Fluorescence and FT-Infrared (FTIR) with the 'ultrafast kinetics' studied by nanosecond Fluorescence and Infrared Temperature Jump kinetic measurements with spectral resolution. A comparison of equilibrium thermal unfolding of EnHD, by multiple probes, reveals heterogeneity in unfolding behaviors. The thermal unfolding by FT-Infrared spectroscopy exhibits "Wavelength-dependency" in the unfolding. The fluorescence signal of engrailed upon thermal unfolding is affected by (i) Contact quenching in the core of the native structure; (ii) Solvent effects, resulting in large spectra shift of about 27nm upon unfolding; (iii) Forster Resonance Energy Transfer from a tyrosine as donor to a tryptophan, which are placed at a distance of ~12 Å in the native structure.

Infrared Relaxation monitored at two wave numbers 1646 cm^{-1} and 1636 cm^{-1} yield single- exponential decays in tens of microseconds. Global SVD Analysis of Fluorescence decay vs. Temperature resolves the 3 components observed in equilibrium. Double exponential fits for the three components together yield a slow rate, that matches that of Infrared, with an additional fixed fast phase of ~ 0.8 μs. However, these decays can also be nicely fit to a stretched exponential. A comparison of 3 amplitudes obtained for the three components from Fluorescence kinetics with that of the 2 amplitudes obtained for the two

frequencies from IR kinetics for the major folding phase clearly implicate probe dependency and complex unfolding.

In order to uniformly describe the complex behavior observed with both equilibrium and kinetics, we describe an approach to fit all the experimental unfolding curves and decays, in a sequential way from equilibrium to kinetics, to a simple One Dimensional Free Energy Surface (1D-FES) Model[5, 2], in which the order parameter is described as a function of nativeness (n). This model yields a barrier height of 1.28 kJ/mol (DSC Heat Capacity Data) at Tm (~326K), which is ~ 0.47 RT near Tm (<3RT), falling within the 'downhill folding' regime.

In Summary, probe-dependent amplitudes or unfolding transitions, fast kinetics and an estimate of the barrier height obtained from DSC indicate complex folding mechanism of engrailed homeodomain.

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P34 (selected for oral presentation)

Wringing out a ribonucleoprotein complex

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P35

Increased sensitivity of antigen-experienced T cells through the enrichment of oligomeric TCR complexes

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Although memory T cells respond more vigorously to stimulation and they are more sensitive to low doses of antigen than naive T cells, the molecular basis of this increased sensitivity remains unclear. We have shown[1] that the TCR exists as different sized nanoclusters on the surface of resting T cells and that larger nanoclusters are preferentially activated in response to low antigen doses. Through biochemistry and electron microscopy we show[2] that previously stimulated and memory T cells have more and larger TCR nanoclusters at the cell surface than their naive counterparts. Reconstitution of cells and mice with a point mutant of the CD3ζ subunit which impairs TCR nanocluster formation, demonstrates that the increased size of TCR nanocluster is directly responsible for the increased sensitivity of antigen-experienced T cells. Thus, we propose that an "avidity maturation" mechanism underlies T cell antigenic memory.

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P36

Towards a study of DNA helicase mechanics with Optical Tweezers

Benjamin Gollnick[1], Francesca Zuttion[1], Mark S. Dillingham[2], and Fernando Moreno-Herrero[1]

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Damage along double-stranded DNA filaments occurs frequently in all organisms and can lead to cell death. Fortunately, there exist different mechanisms for efficient DNA repair, one being homologous recombination, which involves a particular class of enzymes called helicases. These are molecular motors that move along double-stranded DNA, unwinding the two strands by using the energy of ATP. A bacterial model helicase involved in the initial steps of homologous DNA break-repair is the *B. subtilis* AddAB helicase-nuclease complex (1).

At the single-molecule level, Atomic Force Microscopy and Magnetic Tweezers have been used in our group to shed light on the function of this complex (2, 3). However, with these techniques it is not easily possible to probe the mechanics associated with AddAB activity in an intermediate force range between 5 and 40 pN.

In this work, we aim to directly measure the forces involved in the translocation process of AddAB with a custom-built single-beam Optical Tweezers apparatus adapted from (4). As our experimental design requires the protein to be attached to a streptavidin-coated microsphere via a single biotin linker, in a previous step we have successfully characterized the strength of the streptavidin-biotin interaction (5) using double-stranded DNA molecules with a single biotin at one end. Our results confirm that at a loading rate of about 80 pN/s (corresponding to a pulling speed of 500 nm/s and a trap stiffness of 160 pN/µm in the enthalpic region of the DNA force-extension curve), the mean rupture force is around 50 pN, which we expect to be significantly larger than the DNA-binding force of the AddAB protein complex. As a consequence, we should be able to use our customised Optical Tweezers with a single biotin tag to measure the stalling force of AddAB, which must be larger than 4 pN according to Magnetic Tweezers experiments (3).

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THE ORGANIZERS

ORGANIZER: Dr. Fernando Moreno-Herrero

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Dr. Moreno-Herrero (Oviedo, Spain, March 1976), graduated in Physics by the University of Oviedo in 1998. Intrigued by the physical mechanisms of proteins and their interactions with nucleic acids, Fernando moved to Madrid to undertake a D. Phil. in Biophysics using the Atomic Force Microscope under the supervision of Prof. A. M. Baró. During his D. Phil., Fernando enjoyed three research internships for a total of eight months at the University of California at Berkeley under the supervision of Prof. C. Bustamante. Fernando´s PhD work (1998-2003) was awarded by the Universidad Autónoma de Madrid "*Ph.D. Extraordinary Prize*". On September 2003, Dr. Moreno-Herrero moved to The Netherlands to carry on postdoctoral research in single molecule biophysics at the Delft University of Technology under the supervision of Prof. C. Dekker. During the postdoctoral stage with Prof. Dekker, Fernando focussed on liquid AFM imaging and Magnetic Tweezers to study DNA-repair proteins interactions and the mechanical properties of nucleic acids. In December 2006, the PI started his own independent research line supported by the Spanish *Ramón y Cajal* program at the Fundació Privada Institut Catalá de Nanotecnologia (ICN) in Bellaterra, Barcelona. In December 2009, the PI secured a permanent position at the National Centre of Biotechnology, Madrid (CNB); a research institute of the Spanish National Research Council (CSIC). Since 2009, funded by a European Research Council Starting Grant, Fernando leads the group of Single-Molecule Biophysics of DNA-repair Nanomachines at the CNB. Current research combines development of novel fast AFM technologies and Optical and Magnetic Tweezers machines with the aim to characterize and monitor the real-time dynamics of DNA repair processes at the single-molecule level. Recent work on the AddAB-type helicase-nuclease includes the elucidation of a novel DNA break processing structure namely a DNA loop. Also he has developed novel protocols to determine protein stoichiometry from AFM images of SMC proteins. Finally, the mechanical properties of DNA and RNA and the role of mechanics in protein-NA interactions are also of his interest.

Fernando has published his work in top multidisciplinary journals such as Nature, Nature Nanotechnology, Molecular Cell, PNAS, PRL, JACS, Nano Letters, and Nucleic Acids Research among others. Fernando´s work accumulates over 1150 citations and the impact of his research was recently recognized by the Izasa-Werfen Prize of the Spanish Society for Biochemistry and Molecular Biology.

ORGANIZER: Dr. Pedro J. de Pablo

Universidad Autónoma de Madrid, Spain

Pedro José de Pablo Gómez, was born in Madrid (Spain) an studied Physics at Universidad Autónoma de Madrid. He performed the PhD at the same university, and obtained the Extraordinary PhD prize in 2000, as one of the best thesis of the University. He enjoyed several internships at the department of Physics of Purdue University (USA). In September 2001 he moved to The Netherlands to carry on postdoctoral research on single-molecule biophysics at the Vrije Universiteit under the supervision of Prof. Christoph Schmidt. In September 2003 P. J. de Pablo started his own independent group at Universidad Autónoma de Madrid supported by the Spanish *Ramón y Cajal*. In 2011 he gained a position of Associate Professor at the Universidad Autónoma de Madrid**.** Pedro is currently teaching General Physics for Biology, Thermodynamics and he is also involved in the UAM Biophysics Master as experimental techniques teacher.

Pedro's research has multidisciplinary character, since he has been involved in investigating a wide range of phenomena, including from electromigration and electronic transport in mesoscopic structures, such as carbon nanotubes and DNA, to the study of molecular motors and viruses with Atomic Force Microscopy. He has developed new AFM methods such as *jumping mode* and non-contact electrostatic methods based on non intrusive dynamic AFM modes. During the postdoctoral stage with Prof. Christoph Schmidt at Vrije Universiteit Pedro introduced Atomic Force Microscopy, adapting this technique in two different projects. The first one regarded on following the activity of single molecular motors, such as kinesin on microtubules, with AFM working in liquid milieu. The second project dealt with the mechanical properties of two molecular aggregates such as microtubules and phage ϕ29 in vitro conditions. The publication in PNAS about the ϕ29 bacteriophage, where Pedro is coauthor, is a seminal work that initiated a new research line all-over the world consisting on investigating the mechanical properties of viruses and their implication in the viral biophysics. Up to date, Pedro has a total of 56 peer-reviewed scientific publications in international peer-reviewed journals, including Nature Materials, PNAS, Physical Review Letters and Biophysical Journal, accumulating about 2000 citations.

Timetable	Monday	Tuesday	Wednesday	Thursday	Friday
	22 July	23 July	24 July	25 July	26 July
8:30 - 9:00					
9:00 - 9:30	**Philip Nelson**	**Philip Nelson**	**Erwin Peterman**	**Félix Ritort**	
9:30 - 10:00					**Ricardo García**
10:00 - 10:30	**Ulrich Keyser**	**Iwan Schaap**	**Fred MacKintosh**	**Melike Lakadamyali**	
10:30 - 11:00					**Sergi García-Manyés**
11:00 - 11:30	*Cofee Break*	*Cofee Break*	*Cofee Break*	*Cofee Break*	*Cofee Break*
11:30 - 12:00	**Jan Lipfert**	**Toshio Ando**	**Urs Greber**	**Rudolf Podgornik**	**Short talks (4 posters)**
12:00 - 12:30					
12:30 - 13:00	**Borja Ibarra**	**Mauricio García-Ma**	**Arvind Raman**	*Free time*	**Poster awards. Concluding remarks**
13:00 - 13:30	**Ricardo Arias-González**	**Alessandro Podesta**			
13:30 - 14:00	*Lunch*	*Lunch*	*Lunch*	*Lunch*	*Lunch*
14:00 - 14:30					
14:30 - 15:00					
15:00 - 15:30	**Ulrich Keyser**	**Toshio Ando**	**Erwin Peterman**	**Félix Ritort**	Bus to Barajas airport
15:30 - 16:00					
16:00 - 16:30	**Jan Lipfert**	**Iwan Schaap**	**Fred MacKintosh**	**Melike Lakadamyali**	
16:30 - 17:00					
17:00 - 17:30	*Cofee Break*	*Cofee Break*	*Cofee Break*	*Cofee Break*	
17:30 - 18:00	**Francesco Mantegazza**	**Daniel Müller**	**Urs Greber**	**Rudolf Podgornik**	
18:00 - 18:30	**Carolina Carrasco**				
18:30 - 19:00	**Short talks (2 posters)**	**Wouter Roos**	*Visit to Segovia and dinner*	**Short talks (2 posters)**	
19:00 - 19:30	*Free time*	*Free time*		*Free time*	
19:30 - 20:00					
20:00 - 20:30	*Dinner*	*Dinner*		*Dinner*	
20:30 - 21:00					
21:00 - 21:30	*Posters and beers*	*Posters and beers*		*Posters and beers*	
21:30 - 22:00					