INSTITUTO NICOLÁS CABRERA

XXIII International Summer School 'Nicolás Cabrera'

The Physics of Biological Systems: From Biomolecular Nanomachines to Tissues and Organisms

Miraflores de la Sierra, Madrid, 10-15 July 2016

Fundación BBVA

excelencia UAM CSIC

Scope and Goals

The advances in experimental techniques and the explosion of data are allowing the characterization of biological systems with an unprecedented accuracy, which is turning Biology into a quantitative science. Since Physics is the language of the quantitative, the goal of this Summer School is to explore how physical concepts and tools expand our understanding of the complex functions and organization of living systems. By bringing together leading scientists who work at the interface of both disciplines, the School will offer a comprehensive view of some of the more interesting open problems in Biology and how they can be approached (and experimentally tested) from a physics perspective.
The School is aimed at an interdisciplinary audience (biologists, physicists, mathematicians and engineers) interested in the quantitative description of biological phenomena. Lectures will provide both primers for the non-specialist and updates of the latest advances on the selected topics, with many opportunities of interaction between the School participants.

Topics and speakers:

The School is organized in a series of topics, from the micro to the macro scale, with the unifying description of physical biology. World-leading specialists (both theoreticians and experimentalists) will provide a wide perspective of each field, emphasizing contact points between different disciplines.

The physics of biomolecules and assemblies.

Jose Onuchic (Rice University, USA).

William A Eaton (National Institutes of Health, USA).

Devarajan Thirumalai (University of Texas Austin, USA).

George Lorimer (University of Maryland, USA).

Yann Chemla (University of Illinois at Urbana-Champaign, USA).

Physical principles of biological networks.

Jordi García-Ojalvo (U Pompeu-Fabra, Barcelona, Spain).

Pieter Rein ten Wolde (AMOLF, Amsterdam, The Netherlands).

Jané Kondev (Brandeis University, USA).

Lingchong You (Duke University, USA).

Physics of tissues and organisms.

James Briscoe (The Francis Crick Institute, London, UK).

Alfonso Martínez-Arias (U of Cambridge, UK).

Kirill Korolev (Boston University, USA).

Edo Kussell (New York University, USA).

Thomas Gregor (Princeton University, USA).

History and venue:

The International Summer School "Nicolás Cabrera", funded by the *BBVA Foundation*, deals with current topics in materials science, condensed matter physics, nanoscience and biophysics since 1994. The School is the meeting point for numerous scientists all over the world, who share a few days in Madrid in a particularly pleasant and interacting environment.

The School is organized in the residence "La Cristalera" in Miraflores de la Sierra, a small village in the mountains near Madrid.

Organizers:

Raúl Guantes, *Biodynamics and Computational Biology Lab*, Materials Science Institute 'Nicolás Cabrera', UAM.

David G. Míguez, *Biophysics and Systems Biology Lab*, Materials Science Institute 'Nicolás Cabrera', UAM.

Víctor Muñoz, *National Biotechnology Center*, CEI CSIC-UAM.

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The Physics of Biological Systems: From Biomolecular Nanomachines to Tissues and Organisms

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Scientific program

Monday 11 July

Morning session

8.30-9.00 *Opening*
9.00-10.00 George Lorimer
10.00-11.00 Dave Thirumalai. *RNA and Protein Folding: Spontaneous and Assisted.*
11.00-11.30 *Coffee-break*
11.30-12.30 Pieter R. ten Wolde. *Fundamental Limits to Cellular Sensing.*
12.30-13.30 Lingchong You. *Programming bacteria in time and space.*

Afternon session

15.30-16.30 James Briscoe. *Transcriptional networks and the interpretation of morphogen signaling I.*
16.30-17.30 Thomas Gregor. *Decoding positional information I.*
17.30-18.00 *Coffee-break*
18.00-19.00 Yann Chemla. *"The little engines that could": understanding nature's nanomachines.*

Tuesday 12 July

Morning session

9.00-10.00 Jordi García-Ojalvo. *Dynamics of cell regulation: constraining our conceptual understanding of living processes I.*
10.00-11.00 Jané Kondev. *Building a Proportional Cell: The Physics of Biological Size Control I.*
11.00-11.30 *Coffee-break*
11.30-12.30 Lingchon You. *Probing bacterial responses to antibiotics.*
12.30-13.30 Thomas Gregor. *Decoding positional information II.*

Afternon session

15.30-16.30 James Briscoe. *Transcriptional networks and the interpretation of morphogen signaling II.*
16.30-17.30 Pieter R. ten Wolde . *The Thermodynamics of Biochemical Copying.*
17.30-18.00 *Coffee-break*
18.00-19.00 Dave Thirumalai. *Architectural basis of Motility of Motors.*

19.00-21.00 *Poster session*.

Wednesday 13 July

Morning session

9.00-10.00 George Lorimer.
10.00-11.00 Yann Chemla. *Direct measurement of structure-function relationships with "next generation" single molecule techniques.*
11.00-11.30 *Coffee-break*
11.30-12.30 Jané Kondev. *Building a Proportional Cell: The Physics of Biological Size Control II.*
12.30-13.30 Jordi García-Ojalvo. *Dynamics of cell regulation: constraining our conceptual understanding of living processes II*.

Afternon session

15.30-16.30 Edo Kussell. *Evolutionary phase transitions in random environments*.
16.30-17.00 *Coffee-break.*
17.30- *Visit to the city of Segovia.*

Thursday 14 July

Morning session

9.00-10.00 William Eaton. *Transition Paths in Protein Folding: Experiment, Theory, and Simulations.*
10.00-11.00 Alfonso Martínez-Arias. *Genetically supervised self-organization in ensembles of mouse ES cells I.*
11.00-11.30 *Coffee-break*
11.30-12.30 José Onuchic. *The energy landscape for protein folding and molecular machines.*
12.30-13.30 Edo Kussell. *Natural selection driven by DNA binding proteins shapes genome-wide motif statistics.*

Afternon session

15.30-16.30 Kirill Korolev. *Phase transitions in population dynamics of microbes and cancer:* I. *Evolution in asexual populations and its applications to the tug-of-war between beneficial and deleterious mutations in cancer*
16.30-17.30 José Onuchic. *Relating the energy landscape for protein folding with co-evolutionary information.*
17.30-18.00 *Coffee-break*

Friday 15 July

Morning session

9.00-10.00 Alfonso Martínez-Arias. *Genetically supervised self-organization in ensembles of mouse ES cells II.*
10.00-11.00 Kirill Korolev. *Phase transitions in population dynamics of microbes and cancer:* II. *Demographic fluctuations shape evolution and ecology of microbial colonies.*
11.00-11.30 *Coffee-break*
11.30-12.30 William Eaton. *Sickle Cell Disease: Biological Physics, Molecular Pathology, and Drug Discovery.*

12.30-13.30 *Poster awards. Closing remarks.*

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<tr><td>11:30-12:30</td><td>P. R. ten Wolde</td><td>L. You</td><td>J. Kondev</td><td>J. Onuchic</td><td>W. Eaton</td></tr>
<tr><td>12:30-13:30</td><td>L. You</td><td>T. Gregor</td><td>J. García-Ojalvo</td><td>E. Kussell</td><td>Poster awards. Closing remarks</td></tr>
<tr><td>13:30-15:30</td><td>Lunch</td><td>Lunch</td><td>Lunch</td><td>Lunch</td><td>Lunch</td></tr>
<tr><td>15:30-16:30</td><td>J. Briscoe</td><td>J. Briscoe</td><td>E. Kussell</td><td>K. Korolev</td><td rowspan="8">BUS TO MADRID AND MADRID-AIRPORT</td></tr>
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Dinner in town</td><td>Coffee-break</td></tr>
<tr><td>18:00-19:00</td><td>Y. Chemla</td><td>D. Thirumalai</td><td rowspan="3">Free time</td></tr>
<tr><td>19:00-20:00</td><td rowspan="2">Free time</td><td rowspan="2">Posters and Wine Session</td></tr>
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<tr><td>21:00-22:00</td><td>Dinner</td><td>Dinner</td><td>Dinner</td></tr>
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Abstracts for lectures

Physical principles of biological networks.

DYNAMICS OF CELL REGULATION: CONSTRAINING OUR CONCEPTUAL UNDERSTANDING OF LIVING PROCESSES.

Jordi Garcia-Ojalvo
Universitat Pompeu Fabra

Living systems are intrinsically dynamical. Increasing evidence shows that cells and organisms operate in non-stationary regimes, even when subject to constant environmental conditions. This type of behavior is well established in nonlinear physics, where it takes the form of non-steady attractors such as limit cycles and noise-induced pulses. In my first talk I will discuss the challenges posed by the dynamics of cellular processes, and the benefits that this behavior offers for the determination of the molecular mechanisms of these processes. In the second talk I will present recent results that exemplify these benefits, by discussing our observations of collective oscillations in structured bacterial populations, and their role in the uncovering of their metabolic and electrophysiological underpinnings.

FUNDAMENTAL LIMITS TO CELLULAR SENSING

Pieter Rein ten Wolde
AMOLF

Experiments in recent years have shown that living cells can measure chemical concentrations with extraordinary precision. This raises the question what is the fundamental limit to the precision of chemical sensing. Berg and Purcell already pointed out in the seventies that cells can reduce the sensing error by increasing the number of measurements in two principle ways: by increasing the number of receptors or by taking more measurements per receptor via the mechanism of time integration. In this talk I will first discuss the Berg-Purcell limit, which is the fundamental limit to the sensing precision as set by the number of receptors, the integration time and the receptor correlation time; I will describe how the latter is determined by the concentration, diffusion constant, and the receptor-binding rate of the ligand. I then address the question how the signaling networks downstream of the receptor can integrate out the receptor-ligand binding noise. I will show that these networks can not only reach the Berg-Purcell limit, but even beat it by some 10%. The signaling network not only integrates out receptor-ligand binding noise, but will also inevitably add its own intrinsic noise to the signal. We show that equilibrium networks that are not driven out thermodynamic equilibrium face a trade off between the removal of receptor input noise and intrinsic noise: the sum cannot be decreased below a limit set by the number of receptors. The downstream network, no matter how complicated its architecture is or how many molecules are devoted to making it, cannot improve the sensing precision beyond this bound. The reason is that these equilibrium systems cannot time integrate the receptor state. To break this sensing limit and to enable time integration, the network must be driven out of equilibrium. This does not only require readout molecules to store the history of the receptor state, but also energy to store the past receptor states reliably. This leads to a new design principle, which states that in an optimally designed system all fundamental resources - receptors and their integration time, readout molecules, and energy - are equally limiting, so that no resource is wasted. I show that the *E. coli* chemotaxis system obeys this design principle.

THE THERMODYNAMICS OF BIOCHEMICAL COPYING

Pieter Rein ten Wolde
AMOLF

One of the defining characteristics of life is self-replication. The self-replication of living cells, be it bacterial cells or cells in a developing embryo, is a process in which the cell makes a copy of itself. This copy process at the cellular scale requires copying at the molecular scale. During the cell cycle the DNA needs to be replicated and new proteins have to be made. Also other biological processes such as time integration rely on molecular copying. In this talk I will discuss the thermodynamics of biochemical copying. I will first review Maxwell's demon and Szilard's engine, which illustrate that taking a measurement - a process in which the state of the measured system is copied into that of the measurement device - inevitably requires energy. I then discuss how molecular copying can be rigorously mapped onto a copy protocol as considered in the computational literature, and describe the trade-off between the energetic cost and the precision of copying. I will show that cellular copy protocols can come surprisingly close to, but cannot reach, the fundamental limit of thermodynamically optical protocols.

BUILDING A PROPORTIONAL CELL: THE PHYSICS OF BIOLOGICAL SIZE CONTROL

Jané Kondev
Brandeis University

Since the late 1800s cell experiments have revealed that the size of organelles is controlled by the cell and that their proper functioning requires a specific size. For example, the sizes of mitotic spindles responsible for segregating chromosomes during cell division scale with cell size in developing embryos. Similarly, hair cells of the inner ear carefully control the length of their stereocila, which are micron sized protrusions responsible for detecting sound.
In the first talk I will describe the puzzle of size control in cell biology and the limiting pool mechanism that has been proposed as a solution. The idea of size control by a limiting pool of diffusing components is that a structure grows by accumulating molecular components from solution, until their pool is depleted. I will describe the successes of this simple idea in explaining recent experiments on spindles and nucleoli in developing embryos, and its limitations in controlling the size of multiple structures.
In the second talk I will discuss recent experiments on actin cables and microtubules and the role that molecular motors have in controlling their length. In particular, I will show how directed motion of motors on filaments leads to a feedback mechanism capable of controlling their length without depleting the pool of actin and tubulin monomers.
In light of these findings I will conclude by describing our current understanding of the design principles of size control by cells.

PROGRAMMING BACTERIA IN TIME AND SPACE

Lingchong You
Duke University

Microbes are by far the most dominant forms of life on earth. In every imaginable habitat, they form complex communities that carry out diverse functions. Members of each microbial community may compete for resources, collaborate to process the resources, or to cope with stress. They communicate with each other by producing and responding to signaling molecules. And they innovate by exchanging genetic materials. These interactions raise fundamental questions

regarding the evolutionary and ecological forces that shape microbial consortia. Our lab has adopted a combination of quantitative biology and synthetic biology to explore these questions. We

engineer gene circuits to program dynamics of one or more bacterial populations, and use them to examine questions in cellular signal processing, evolution, ecology, and development.

PROBING BACTERIAL RESPONSES TO ANTIBIOTICS

Lingchong You
Duke University

As the first line of defense against bacterial infections, antibiotics have been hailed as the single most significant therapeutic discovery in medicine in the 20th century. However, they are falling victim to their own success. Decades of overuse and misuse are causing a major crisis: bacteria have developed resistance against every existing antibiotic and they are doing so at an alarming rate, considering the timescale at which new antibiotics can be developed. To help to combat bacterial infections, it is important to develop a quantitative understanding of response to antibiotics by individual bacterial cells and bacterial populations. In this talk, I will discuss our recent and ongoing efforts along this line, with a particular focus of the collective dynamics by bacterial populations.

Physics of tissues and organisms.

TRANSCRIPTIONAL NETWORKS AND THE INTERPRETATION OF MORPHOGEN SIGNALING

James Briscoe
The Francis Crick Institute

Tissue development relies on the spatially and temporally organised allocation of cell identity, with each cell adopting an identity appropriate for its position within the tissue. In many cases, transcriptional networks controlled by extrinsic signals determine these cellular decisions. A mechanistic understanding of pattern formation and cell fate decisions therefore requires insight into how the regulatory interactions between transcription factors and external signals determines the switches in gene expression that generate the cell identity. One common strategy of pattern formation relies on positional information provided by secreted signalling molecules -- morphogens -- emanating from localized sources within, or adjacent to, the developing tissue. The spread of a morphogen from its source creates a spatial gradient in the tissue. Cells are sensitive to the level of the morphogen and convert the continuous input into a set of discrete gene expression profiles at different distances from the morphogen source. An example of this is the development of the vertebrate neural tube. Distinct neuronal subtypes are generated in a precise spatial order from progenitor cells arrayed along the dorsal-ventral axis of the neural tube. Underpinning this organization is a complex network of extrinsic and intrinsic factors. Particularly well understood is the mechanism that determines the generation of different neuronal subtypes in ventral regions of the spinal cord. In this region of the nervous system, the secreted protein Sonic Hedgehog (Shh) acts in graded fashion to organize the pattern of neurogenesis. This is a dynamic process in which exposure to Shh generates progenitors with successively more ventral identities. A gene regulatory network composed of transcription factors regulated by Shh signaling play an essential role in determining the graded response of cells. Thus the accurate patterning of the neural tube and the specification of neuronal subtype identity in this region relies on the continuous processing and constant refinement of the cellular response to graded Shh signaling. Quantitative

data and dynamical systems modeling is beginning to provide a mechanistic understanding of how this is achieved and offering fresh insight into the problem of embryonic pattern formation.

DECODING POSITIONAL INFORMATION

Thomas Gregor
Princeton University

Cells in a developing embryo are thought to encode their position and thus their identities through the concentrations of various morphogen molecules. It is further accepted that these morphogens are organized in a sequential genetic cascade in which positional identities are progressively and gradually refined. Here we show that in the early fly embryo cell identities are already encoded in the earliest morphologic markers, the maternal gradients, and manifest themselves at the level of the gap genes. We combine measurements on the mean and (co)variance of concentration across multiple embryos to build an optimal dictionary for decoding positional information, which explicitly determines precise and unambiguous estimates of position along the anterior-posterior axis. Tests of this dictionary in mutant embryos agree quantitatively with experiment. Our results challenge the traditional view of a refinement process involving a complex gene cascade and argue for a system operating at sufficient initial precision that specifies all cell types instantaneously.

GENETICALLY SUPERVISED SELF ORGANIZATION IN ENSEMBLES OF MOUSE ES CELLS

Alfonso Martínez Arias
University of Cambridge

Embryonic Stem (ES) cells are clonal derivatives from the blastocysts of mammalian embryos which have the potential to give rise to all lineages of the embryo and the ability to self renew this ability. Mouse ES cells have proven an excellent system in which to study developmental events, in particular the mechanisms of cell fate decisions. However, while it is easy to coax them into elements of different tissues in culture, they do not organize themselves as they do in embryos. Non adherent culture leads them to form aggregates in which some cell types appear in clusters but there is no overall organization.

Recently we have developed a non adherent culture system in which small aggregates of mouse ES cells undergo symmetry breaking, polarized gene expression and growth and gastrulation like movements in vitro. Using a variety of reporter ES cell lines and comparison with the embryo we observe organization of Wnt, Nodal and FGF signaling that mirrors events in the embryo and have shown that they can develop in culture until an equivalent of embryonic day 9 (E9). Analysis of pattern formation in these aggregates suggests that they do not develop through standard self organization but that there is a strong genetic components to what otherwise would be simple thermodynamically driven process. These issues will be discussed in course of the two talks.

An important value of this organoid system is the ability to generate spatially organized niches and we have used this system to study the emergence and dynamics of a stem/progenitor population that gives rise to the spinal cord and the paraxial mesoderm.

PHASE TRANSITIONS IN POPULATIONS DYNAMICS OF MICROBES AND CANCER

Kirill Korolev
Boston University

Any living system is subject to the laws of evolution from single cells, to tissues, organs, and communities. I will introduce the main concepts in population genetics from the point of view of statistical physics and show how the ideas from physical kinetics, phase transitions, and Langevin equations contribute to our understanding of evolutionary dynamics. We will see how simple models predicted tumor suppressor genes at least a decade before their discovery and how many paradoxes in cancer research can be resolved by considering mutations deleterious to tumor cells. We will then focus on population dynamics in spatial populations and learn about competition and cooperation in microbial colonies. In particular, I will discuss the evolution of cross feeding and other types of microbial mutualism.

I. EVOLUTION IN ASEXUAL POPULATIONS AND ITS APPLICATIONS TO THE TUG-OF-WAR BETWEEN BENEFICIAL AND DELETERIOUS MUTATIONS IN CANCER

I will begin with a review the fundamental results in population genetics drawing on the ideas from nonequilibrium statistical physics. We will then use these results to understand the classical view of cancer progression. At the end, I will briefly talk about our work on the role of mutations damaging to the tumor and the resulting barrier to cancer progression.

II. DEMOGRAPHIC FLUCTUATIONS SHAPE EVOLUTION AND ECOLOGY OF MICROBIAL COLONIES

I will describe generic differences between population dynamics in spatial and well-mixed populations primarily focusing on demographic fluctuations (genetic drift). First, we will consider evolution via neutral, beneficial, and deleterious mutations and see how these ideas can be tested in experiments with microbes. Then, we will discuss interactions between microbial species, and I will show how they lead to spatial pattern formation and phase transitions. Finally, we will apply these ideas to understand the evolution of microbial cross-feeding.

EVOLUTIONARY PHASE TRANSITIONS IN RANDOM ENVIRONMENTS

Edo Kussell
New York University

Bacteria can use memory mechanisms to increase long term growth rates in rapidly changing environments. In this talk, I will discuss experiments that show the existence of phenotypic memory in *E.coli* during metabolic transitions. I will present theoretical results on the optimization of memory levels, which we obtained by a new analytical method for calculations of long-term growth rates in randomly changing environments. I describe several distinct evolutionary phase transitions associated with memory optimization, and discuss the structure of the resulting phase diagram. The results have implications for the evolution of gene regulation and motivate further experiments.

NATURAL SELECTION DRIVEN BY DNA BINDING PROTEINS SHAPES GENOME-WIDE MOTIF STATISTICS

Edo Kussell
New York University

Ectopic DNA binding by transcription factors and other DNA binding proteins can be detrimental to cellular functions and ultimately to organismal fitness. The frequency of protein-DNA binding at non-functional sites depends on the global composition of a genome with respect to all possible short motifs, or k-mer words. To determine whether weak yet ubiquitous protein-DNA interactions could exert significant evolutionary pressures on genomes, we correlate in vitro measurements of binding strengths on all 8-mer words from a large collection of transcription factors, in several different species, against their relative genomic frequencies. Our analysis reveals a clear signal of purifying selection to reduce the large number of weak binding sites genome-wide. This evolutionary process, which we call global selection, has a detectable hallmark in that similar words experience similar evolutionary pressure, a consequence of the biophysics of protein-DNA binding. By analyzing a large collection of genomes, we show that global selection exists in all domains of life, and operates through tiny selective steps, maintaining genomic binding landscapes over long evolutionary timescales.

The physics of biomolecules and assemblies.

THE ENERGY LANDSCAPE FOR PROTEIN FOLDING AND BIOMOLECULAR MACHINES

José Onuchic
Rice University

It is amazing how cells have created a number of molecular machines specialized for undertaking tasks needed to control and maintain cellular functions with exquisite precision. Due to fact that biomolecules fluctuate via thermal motion and their dynamics is diffusive, biological machines are fundamentally different from those experienced by conventional heat engines or machines in the macroscopic world. One of the key features of biological machines is the conformational changes triggered by the thermal noise under weak environmental perturbation. Therefore we can explain how they behave using ideas borrowed from the energy landscape theory of protein folding and polymer dynamics. This "new view" allows us to envisage the dynamics of molecular motors from the structural perspective and it provides the means to make several quantitative predictions that can be tested by experiments. For the kinesin motor, a prototype of the biological machines in the cell, molecular simulations of an explicit kinesin and microtubule structures show that fluctuations and flexibility inherent to the structure leads to versatile adaptation of the molecular structure, allosteric communication controlled by internal mechanics, and large amplitude stepping motion harnessing the thermal fluctuation.

RELATING THE ENERGY LANDSCAPE FOR PROTEIN FOLDING WITH CO-EVOLUTIONARY INFORMATION

José Onuchic
Rice University

Understanding protein folding and function is one of the most important problems in biological research.

Energy landscape theory and the folding funnel concept have provided a framework to investigate the mechanisms associated to these processes. Since protein energy landscapes are in most cases minimally frustrated, structure based models (SMBs) have successfully determined the geometrical features associated with folding and functional transitions. Structural information, however, is limited with respect to different functional configurations. This is a major limitation for SBMs. Alternatively statistical methods to study amino acid co-evolution provide information on residue–residue interactions useful for the study of structure and function. Here, we show how the combination of these two methods gives rise to a novel way to investigate the mechanisms associated with folding and function.

We will also show how this combined approach can be used to develop a procedure to predict the association of protein structures into homodimers. Coevolutionary contacts extracted from Direct Coupling Analysis (DCA) in combination with SBMs guide the simulations of dimerization. Identification of dimerization contacts using DCA is more challenging than intradomain contacts since direct couplings are mixed with monomeric contacts. Therefore a systematic way to extract dimerization signals has been elusive. We provided evidence that the prediction of homodimeric complexes is possible with high accuracy for all the cases we studied which have rich sequence information. For the most accurate conformations of the structurally diverse dimeric complexes studied, the mean and interfacial RMSDs are 1.95 Å and 1.44 Å, respectively. This methodology is also able to identify distinct dimerization conformations as for the case of the family of response regulators, which dimerize upon activation.

A complete new direction of research has been the application of DCA to construct sequence-dependent statistical potentials for proteins. We take advantage from the sequence diversity in a protein family to infer the underlying Potts model Hamiltonian that governs the correlated mutations observed in sequence data. A number of recent studies have demonstrated that experimentally measured mutational changes in protein stability correlate well with mutational changes in the inferred DCA-potential. This quantitative agreement appears to hold for a diverse range of proteins that have both abundant sequence data and experimental mutational changes in stability.

"THE LITTLE ENGINES THAT COULD": UNDERSTANDING NATURE'S NANOMACHINES

Yann Chemla
University of Illinois at Urbana-Champaign

Through decades of research, we have come to appreciate the living cell as far more complex than a well-mixed container of molecules that encounter by diffusion. The cell can be viewed instead as a highly organized "factory" of biomolecular nanomachines, proteins that carry out essential, specialized mechanical tasks like moving cargo around the cell, manipulating the cell's genome, or propelling the entire cell. In the first lecture, I will discuss important questions and challenges to deciphering the mechanisms by which these nanometer-size engines operate. I will also describe how the emergence of single-molecule techniques (e.g. fluorescence microscopy, optical and magnetic tweezers, etc.) have revolutionized our understanding of these systems.

DIRECT MEASUREMENT OF STRUCTURE-FUNCTION RELATIONSHIPS WITH "NEXT GENERATION" SINGLE MOLECULE TECHNIQUES

Yann Chemla
University of Illinois at Urbana-Champaign

In the second lecture, I will focus on our recent work developing the "next generation" of single-molecule techniques: hybrid instruments that allow simultaneous detection of multiple, orthogonal variables at high resolution. I will discuss several applications of these techniques, in particular recent measurements of a DNA repair helicase, *E. coli* UvrD, that reveal directly how protein stoichiometry and conformation regulate the unwinding of DNA. Using an instrument that combines optical tweezers with single-molecule fluorescence microscopy, we record UvrD unwinding activity with a resolution of a few ångströms (via optical tweezers) simultaneously with helicase stoichiometry and conformation (via fluorescence). Our measurements settle a longstanding debate over which UvrD conformational states are required for unwinding. I will discuss the biological implications of our findings.

RNA AND PROTEIN FOLDING: SPONTANEOUS AND ASSISTED

Devarajan Thirumalai
University of Texas Austin

ARCHITECTURAL BASIS OF MOTILITY OF MOTORS

Devarajan Thirumalai
University of Texas Austin

PROTEIN FOLDING TRANSITION PATHS: SINGLE MOLECULE EXPERIMENTS, THEORY AND ALL-ATOM MD SIMULATIONS

William A Eaton
National Institutes of Health

The transition path is the tiny fraction of an equilibrium, single-molecule trajectory when the free energy barrier separating states is crossed and contains all of the information about the kinetic mechanism. Transition path distributions have been being predicted for protein folding from all-atom molecular dynamics (MD) simulations of the D.E. Shaw group "Anton", using a special-purpose supercomputer, and by simulating the master equation of an Ising-like theoretical model of V. Muñoz and coworkers. However, transition-paths have never been observed experimentally for any molecular system in solution. Because the duration of a protein folding transition path is so short (less than ~10 microseconds), determining just the average transition path time in single molecule experiments is challenging. In this presentation, I will motivate single molecule these measurements by describing the MD simulations and theoretical model, and then show how we are determining average transition path times for proteins from a photon-by-photon analysis of single molecule FRET experiments. Insight into the Kramers description of the kinetics and dynamics of folding as diffusion along a 1D reaction coordinate is obtained from comparing the experimental results with the MD simulations. These single-molecule experimental results are just the first step toward the goal of measuring intra-molecular distances during individual transition paths. Such measurements would provide the most demanding test of folding mechanisms predicted by both simulations and theoretical models.

BIOPHYSICS AND THERAPY OF SICKLE CELL ANEMIA: THE PARADIGM OF PROTEIN AGGREGATION DISEASES

William A Eaton
National Institutes of Health

The molecular pathogenesis of sickle cell anemia is better understood than any other protein aggregation disease. Like amyloid formation, there is a delay period prior to fiber formation, which allows the vast majority of red cells to escape the narrow vessels of the tissues before fiber formation stiffens the cells and causes vaso-occlusion. However, unlike amyloid formation, there is an enormous sensitivity of the kinetics to solution conditions, with the delay time inversely proportional to up to the 40^{th} power of the initial sickle deoxyhemoglobin concentration and a nucleation rate proportional to up to the 80^{th} power. All current strategies by both the pharmaceutical industry and academic laboratories take advantage of this kinetic sensitivity. These include promoting fetal hemoglobin synthesis to dilute sickle hemoglobin (the mechanism of action of hydroxyurea, the only approved drug for sickle cell disease, which ameliorates the course of the disease in 50-60% of patients) and shifting the allosteric equilibrium between the R (oxy) and T (deoxy) quaternary conformations toward R, which does not aggregate. In this presentation I will discuss the detailed biophysical basis of these strategies, as well as our own current research on drug screening using intact sickle cells. Drugs already approved for treating other human diseases that have sufficient anti-sickling activity in our highly-sensitive kinetic assay are expected to be rapidly approved for clinical trials at NIH.

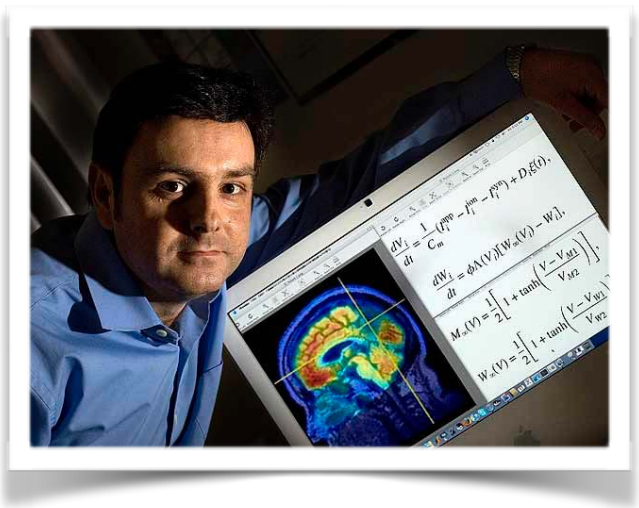
Invited Speakers:

Jordi García-Ojalvo

Professor of Systems Biology, Dynamical Systems Biology lab (Universitat Pompeu-Fabra, Barcelona, Spain).

Awards:

- *Premi Icrea Acadèmia 2014 INSTITUCIÓ CATALANA RECERCA I EST.AV. (ICREA). 2015*
- *ICREA Academia Award. ICREA. 2010*
- *Catalan Government for the Promotion of University Research Award. 2003*



Overview:

Nature is dynamic. From celestial bodies orbiting around each other and emitting radiation in a pulsed manner to the ever changing climate and geology of our own planet, and all the way to living systems, which exhibit all kinds of rhythmic behavior, natural phenomena are governed by the laws of dynamical systems. Nonlinearities in the interactions between the system components and in the response to external perturbations, together with random fluctuations both intrinsic and extrinsic to the process being studied, complicate our understanding of the dynamic world. The goal of our lab is to study and characterize the dynamical behavior of living systems, and use this knowledge to understand how these systems operate and self-organize in complex yet well-coordinated processes.

The phenomena that we study include synchronization, noise-induced phenomena, pattern formation, excitability, and limit-cycle behavior, among others. The living systems whose behavior we examine include bacteria, stem cells, the immune system, and the brain. In these systems we explore processes such as gene regulation, intra- and inter-cellular signaling, cortical oscillations, and information processing.

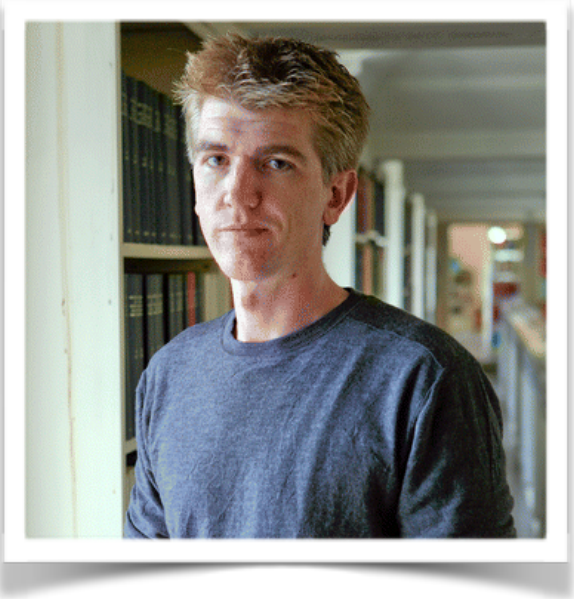
James Briscoe

Group Leader at MRC National Institute for Medical Research
Developmental Dynamics Laboratory (The Francis Crick Institute, London, UK)

Awards:

- *EMBO Gold Medal (2008)*

Overview:



We study how the central nervous system (CNS) is formed in embryos. Despite its complexity, the CNS is assembled in a remarkably reproducible and reliable manner. This precision is necessary for the wiring of nerves into the functional neural circuits that gives the CNS its function. Our research focuses on the spinal cord, which is the part of the CNS that contains the nerves that allow us to sense our environment and respond to it by moving muscles.

The embryonic development of the neural tube provides an example of one of the fundamental questions in biology: how do complex tissues of multicellular organisms develop in a precise and reproducible manner from initially indistinguishable cells? In most tissues, including the neural tube, signals - termed morphogens - act as positional cues to control cell fate specification by regulating the transcriptional programme of responding cells. How do cells receive and interpret these signals? Which genes respond to these signals and how do these coordinate the growth, patterning and morphological elaboration of the neural tube? What is the underlying logic of the transcriptional network and how does this control the spatial and temporal dynamics of pattern formation? To address these questions, we are taking an interdisciplinary approach involving biologists, physicists and computer scientists to investigate these questions.
Our focus is on the signalling mechanisms and transcriptional programme that pattern the neural tube. In ventral regions of the caudal neural tube, the secreted molecule Sonic Hedgehog (Shh) forms an extracellular gradient that governs pattern formation and tissue growth. Using a range of molecular, imaging and modelling approaches that combine single cell resolution dynamic assays of morphogen signaling, cell fate specification, gene regulation and growth we are examining how the gradient of Shh signalling is perceived and interpreted by cells to control gene expression and cell behaviour.

Thomas Gregor

Associate Professor, Department of Physics and Lewis-Sigler Institute for Integrative Genomics
Laboratory for the Physics of Life
Princeton University, USA.

Awards:

- *NSF MRSEC seed award*
- *2010 Searle Scholar*
- *Scialog Fellow (Moore Foundation), 2014–2016*

Overview:
The Laboratory for the Physics of Life at Princeton University studies the basic physical principles that govern the existence of multicellular life. A core focus of the lab is to understand biological development–the complex process through which an organism grows from a single cell into a differentiated, multicellular organism–from a physics perspective. As such, we formulate and experimentally validate quantitative models that describe how individual cells interact and organize in order to generate complex life forms.

Our main interests lie in:

- multicellular pattern formation
- transcriptional regulation in the context of development
- molecular limits to biochemical sensing
- emergence of collective behaviors in multicellular systems.

We work with a variety of organisms in the lab, mainly the common fruit fly, Drosophila melanogaster, and the social amoeba Dictyostelium discoideum.
Our research is primarily experimental, but with a strong theoretical influence. On the experimental side, we approach life or living matter the same way that other physicists look at the stars or study the properties of inert matter: push our ability to make measurements until we understand our errors. To what extend can we trust our measurements, which part of our error is due to the experiment, and which part is due to the seemingly messy underlying biological processes? We are building state-of-the-art microscopes and microfluidics devices, and make heavy use of tools from molecular biology and genetics. On the theoretical side, we design analytical and numerical models, largely drawn from statistical mechanics ideas, to test and guide our experiments.
The lab is part of the Physics Department, of the Lewis-Sigler Institute for Integrative Genomics, and of the Molecular Biology Department. Our research is highly interdisciplinary, working with students from many departments across campus, including physics, biology, computer science, engineering and applied mathematics.

Alfonso Martínez-Arias
Professor of Developmental Mechanics,
Department of Genetics (University of Cambridge, UK).

Awards: Waddington Medal 2012

Overview:
A common interest of the people in our lab is how cells make decisions about their fates during development. In particular we are interested in the actual nature of the decision event i.e. what do we mean by 'a decision' in the context of cells and development, and what are the molecular processes associated with this event.
For many years we studied this process in Drosophila where genetic analysis allowed the identification of genes associated with this process. Exhaustive studies in many systems by us and others revealed that development is associated with a sequence of changes in state that take a cell from a naïve state to a differentiated situation. The states are associated with cell type specific gene regulatory networks (GRN), usually driven by transcription factors, and a few signalling pathways which seem to be involved in many processes irrespective of specific GRNs.

Understanding the process of cell fate decisions could thus be formulated as a problem of unwrapping the impact that sequential interactions between Signalling Pathways and Transcriptional networks have on the phenotypes of cell populations. Analysis and consideration of these processes in Drosophila led us to the propose that all cell fate decision processes are characterized by an intermediate, the transition state, which is a consequence of the dynamics of the process. A change of cell state or fate (from A to B) can be divided into two steps: from the original state (A) to the transition state (A/B) and from this to the new state (B) (Fig. 1). A transition state is characterized by coexpression of genes from both states (hence the A/B identity) and is probabilistic i.e from here a cell can either move to a new state or return to the state of origin, and At the level of a population of cells, this probabilistic nature of the transition state is the source of a regulative mechanism: not all cells that can go from A to B will adopt the B state and the transition state provides a source of variation for selection.

Our work uses a combination of modern genetics and cell biology with live imaging, quantification and modeling.

Pieter Rein ten Wolde

Biochemical Networks (AMOLF, Amsterdam, The Netherlands)

- Fellow of the American Physics Society

Overview:
Biochemical networks are the central processing units of life. They can perform a variety of computational tasks analogous to electronic circuits. Their design principles, however, are markedly different: in a biochemical network,computations are performed by molecules that chemically and physically interact with each other. The aim of the Biochemical Networks group and group leader Pieter Rein ten Wolde is to unravel their design principles using a combination of database analyses, theory and computer simulation.

Biochemical networks not only operate in time, but also in space. Arguably the best example is embryonic development where spatial gradients in the concentrations of morphogens – substances governing the patterning of tissue – encode positional information for differentiating cells. Also within cells, compartmentalization, scaffolding and localized interactions are actively exploited to enhance the regulatory function of biochemical networks. In this research line we aim to elucidate the spatial design principles of biochemical networks.

Among the most fascinating time devices in biology are circadian clocks, which are found in organisms ranging from cyanobacteria and fungi, to plants, insects and animals. These clocks are biochemical oscillators that allow organisms to coordinate their metabolic and behavioural activities with the 24-hour cycle of day and night. Remarkably, these clocks can maintain stable rhythms for months or even years in the absence of any daily cue, such as light/dark or temperature cycles, from the environment. In multicellular organisms, the robustness might be explained by intercellular interactions, but it is now known that even unicellular organisms can have very stable rhythms. This robustness is surprising, given the fact that experiments in recent years have vividly demonstrated that protein synthesis, which is required to sustain the clock, is highly stochastic. Clearly, the clock is designed in such a way that it has become resilient to the intrinsic stochasticity of the underlying biochemical reactions. We aim to elucidate the design principles that underlie the robustness of circadian clocks, with the cyanobacterium S. elongatus as a model system.

Jané Kondev

Professor of Physics, Physical biology of the cell (Brandeis University, USA)

Awards:

- *Cottrell Scholar Award: "Theoretical studies of Dense Polymer Systems" (2002).*
- *Research Innovation Award: "Geometry of Two-Dimensional Turbulence" (2001).*
- *NSF CAREER Award: "Geometrical Approaches to Strongly Correlated Matter" (2000)*

Overview:

Jané Kondev is a theoretical physicist who works primarily on problems in molecular and cell biology. Research in the Kondev group is driven by quantitative experiments on single molecules and single cells, which are typically performed in biology labs the group collaborates with. The goal is to provide a mathematical framework that can explain the available quantitative data, and makes testable predictions that can guide new experiments. Members of the group are inspired by the new vistas that open up when life's processes are described in the language of theoretical physics, thus providing new insights about outstanding problems in biology.

The goal of his group is to uncover mathematical laws that govern the living world. The 21st century has seen an explosion of quantitative data in Biology and one of the great challenges is to develop equally quantitative models that are capable of explaining all this data; for such models to be useful they must also provide experimentally testable predictions. If physics is the science that seeks to describe Nature using the language of mathematics then physical biology is that part of physics that concerns itself with the living world.

Kirill Korolev

Assistant Professor of Physics and Bioinformatics
Department of Physics and Program in Bioinformatics at Boston University, USA.

Overview:
Many phenomena are complex and unintuitive. I study them through the lens of simple mathematical models that capture only the most essential aspects of the problem, but still make good quantitative predictions. My work focuses on questions in biology and physics related to population dynamics. One area of my research is ecology and evolution of interacting species ranging from a two-species population of cooperators and defectors to multi-species microbial communities comprising the human microbiome.

I also work on evolutionary dynamics of cancer progression and adaptation in populations undergoing a geographic expansion. Other research interests include switching between alternative states of an ecosystem and fundamental questions in evolution like the role of horizontal gene transfer, epigenetics, and genetic architecture. This type of work often draws on the ideas and methods from statistical physics and stochastic processes, and involves both analytical and computational analysis.

Edo Kussell

Associate Professor
Quantitative Biology & Biological Physics (New York University, USA).

Overview:
We combine theoretical biophysical approaches with experiments and bioinformatics to explore systems that exhibit complex, population-level phenomena. Current experiments in the lab focus on population dynamics of microorganisms in fluctuating conditions.

Theoretical work is focused on statistical mechanical descriptions of population- and evolutionary-dynamics. Several projects address the evolution of stochastic switches in bacteria, as well as bacterial genome evolution. We also collaborate with other labs to understand stochastic gene expression in higher organisms, including plants and flies.

Stochastic switches are genetic circuits that allow cells to maintain or switch their phenotypic state spontaneously, without directly sensing their environment. Surprisingly, this behavior can be advantageous over sensor-mediated responses in certain environments. For example, if environmental changes are rare, or if certain changes are particularly catastrophic, stochastic switching can be selected over sensing. The cost of sensing and response (or responsive switching) is an important parameter that determines which adaptive mode is preferred.

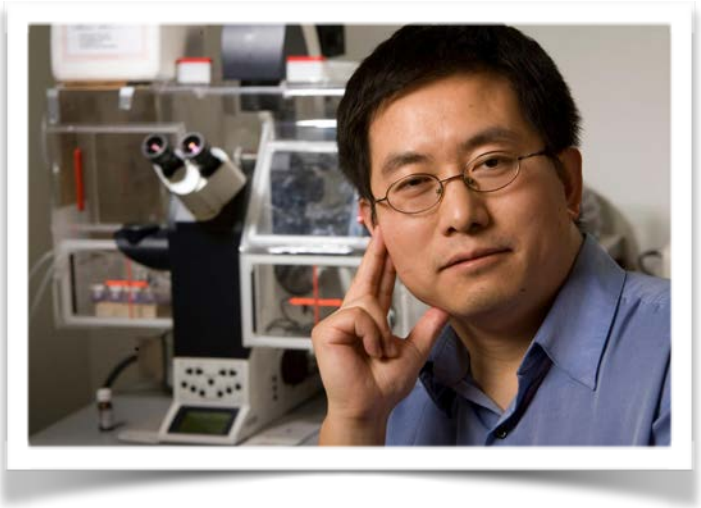
Pathogenic bacteria and fungi exhibit a multitude of stochastic switching mechanisms – often known as phase variation mechanisms – many of which are critical for pathogenicity and evasion of the host's immune response. Stochastic switches are also intimately involved in antibiotic persistence.

Lingchong You

Laboratory of Biological Networks (Duke University, USA).

Awards: Fellowships for Science and Engineering. David and Lucile Packard Foundation. 2006

Overview:



Our research is at the nexus of biology, engineering, and medicine. We combine mathematical modeling and experiments to analyze dynamics of cell signaling processes, including cell cycle regulation, bacterial response to antibiotics, and cell-cell communication. These studies will allow us to gain insights into the design laws of natural biological systems. Based on insights learnt from natural systems, we construct synthetic gene circuits with well-defined functions. In addition to generating useful systems, implementation of such synthetic gene circuits will also enable us to reduce biological complexity and to probe biological design strategies in a well-defined framework. Our research is primarily in two related directions.

- Bacterial Population Dynamics These projects focus on engineering synthetic gene circuits that can precisely program bacterial growth, death, and aggregation in complex environments. Projects in this direction have implications for developing new technologies for gene and drug delivery, designing effective treatment strategies against bacterial infections, and for green fabrication of new materials.

- Mammalian Cell Cycle Regulation These projects aim to define a quantitative framework to analyze and perturb mammalian cell cycle regulation. Their outcome will provide insight into development of novel strategies to reprogram and interfere with cell cycle regulation for cancer therapy.

Despite their apparent diversity, all our projects are united by a common theme as illustrated in the figure. In each case, we examine the dynamics of a specific network of interacting genes, chemicals, or cell populations. We ask what dynamics can result from the network, how the dynamics can be modulated by perturbing different network nodes, how the dynamics account for the biological function of the network, or how the dynamics can serve as foundation for practical applications. The modeling or experimental approaches we take are determined by the specific questions we ask.

José Onuchic

Department of Chemistry (Rice University, USA).

Awards:

- *Fellow of the American Physical Society in 1995*
- *Fellow of the American Academy of Arts & Sciences 2009*
- *Fellow of the Biophysical Society in 2012.*
- *Member of the U. S. National Academy of Sciences 2006*
- *Member of Brazilian Academy of Sciences 2009*

Overview:

José has led the biological physics community as it attempts to devise an integrated picture of a variety of model biochemical and biological systems. His research has expanded across the scales of molecular-level interactions to cellular systems to organized multi-cellular structures. At Rice he is moving this view towards medical applications focusing on cancer. In protein folding, he has introduced the concept of protein folding funnels as a mechanism for the folding of proteins. Convergent kinetic pathways, or folding funnels, guide folding to a unique, stable, native conformation. Energy landscape theory and the funnel concept provide the theoretical framework needed to pose and to address the questions of protein folding and function mechanisms. He also works on theory of chemical reactions in condensed matter with emphasis on biological electron transfer. He is also interested in stochastic effects in gene networks with connections to bacteria decision-making and cancer.

William A Eaton

NIH Distinguished Investigator
Chief, Laboratory of Chemical Physics
Section Chief, Biophysical Chemistry Section, Laboratory of Chemical Physics (National Institutes of Health, USA).

Awards:

- *Member, National Academy of Sciences*
- *Fellow, American Academy of Arts and Sciences*
- *Foreign Member, Accademia Nazionale dei Lincei, Rome*
- *Max Delbruck Prize in Biological Physics, American Physical Society*
- *Founders Award, Biophysical Society*
- *Hans Neurath Award, Protein Society*
- *Humboldt Research Award for Senior Scientists*

Overview:
The purpose of our research is to understand the physics of protein folding and to discover a drug for sickle cell disease.

Our basic research is concerned with fundamental aspects of the mechanism of protein folding. A series of novel techniques have been developed to study the dynamics of fast processes in protein folding. These include the use of nanosecond pulsed lasers to trigger and monitor the folding reaction, as well as single molecule fluorescence measurements. Simple theoretical models are used to interpret the experimental results and expose the basic underlying physics of these processes. The experimental results and theoretical modeling are providing critical benchmarks for the construction of a detailed picture of the sequence of events as a protein forms its native conformation from the random structures of the unfolded polypeptide chain.

A highly sensitive and pathophysiologically-relevant kinetic assay has been developed to screen compounds for ant-sickling activity. The assay uses laser photolysis to induce sickling and automated image analysis to detect the formation of sickle fibers in individual red cells. As a strategy for the most rapid path to bringing a drug to market, the first phase of the screen is to test all U.S. Food and Drug Administration-approved drugs.

Understanding the physics of protein folding is essential for understanding protein mis-folding, the cause of many human diseases, including Alzheimer's disease, type II diabetes, and Parkinson's disease. Hydroxyurea is the only drug that is currently used to treat sickle cell disease, and helps, but does not cure, only about 50 percent of patients. Additional drugs are critically needed.

Further studies should look at making the connections among protein and mis-folding folding theory, experiments, and computer simulations. They should also look at the development of additional drugs for sickle cell disease.

Devarajan Thirumalai

Department of Chemistry (University of Texas Austin, USA).

Awards:

- *National Science Talent Research Fellow, India*
- *Camille and Henry Dreyfus Foundation: Distinguished New Faculty Award*
- *Presidential Young Investigator Award*
- *Outstanding Junior Faculty Award, University of Maryland*
- *Camille and Henry Dreyfus Foundation, Teacher Scholar Award*
- *Distinguished Faculty Research Fellowship Award, University of Maryland*
- *Humboldt Research Award for Senior U.S. Scientists 2009*
- *Elected to Royal Society of Chemistry 2009*

Overview:

A grand challenge in molecular biology is to understand how biomolecules (e.g., proteins and RNA) reach their functional native states starting from an astronomical number of random coil conformations. The understanding of how these processes occur impacts the design of biomolecules of specified topology, and can offer critical clues to understanding diseases caused by conformational defects such as prions.

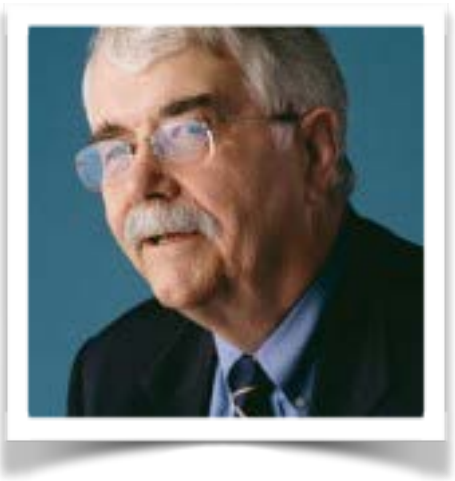
Our research group is primarily focused on discovering general principles that govern the folding of biomolecules. We use a combination of theoretical and computational strategies to answer several questions. In particular, we are interested in:

- Folding mechanisms of large protein and RNA molecules
- Causes of misfolded conformations in proteins
- Variations in the folding mechanisms of proteins in response to changes in environment
- Role of chaperones in aiding protein folding
- Molecular basis for the function of molecular machines

Another area of interest is in the study of changes in DNA shapes due to stretching and condensation. Currently, it is possible to manipulate single DNA molecules using optical tweezers. Such experiments provide direct measurements of the extension of DNA subject to force. The reversible stretching and condensation of DNA, which are important steps in the replication process, is being studied using statistical mechanical models. Such studies will provide detailed, microscopic description of the collapse of DNA and other synthetic polyelectrolytes and polyampholytes.

George Lorimer

Distinguished University Professor, Chemistry & Biochemistry,
Institute for Physical Science and Technology
University of Maryland, USA



Awards:

- *Alexander von Humboldt Foundation*
- *Royal Society of London, Fellow*
- *National Academy of Sciences, Member*

Overview:
Solving the mysteries and broadening understanding of how proteins assemble themselves and the serious consequences that occur if proteins do not fold correctly, leading to the discovery of novel proteins to combat disease.

The folding of proteins from the huge ensemble of conformers that characterize the unfolded state to the small number of conformers associated with the biologically active native state is an exergonic process. From a statistical mechanical viewpoint, the unfolded polypeptide chains follow multiple trajectories to the native state. However, the landscape may be rough and pitted with "false minima" in which the chain's progress to the native state is "frustrated". As an important early event in evolution Nature developed the chaperonin nano-machine (exemplified by the E. coli proteins GroEL and GroES) which couples the hydrolysis of ATP to the unfolding of "frustrated" polypeptide chains, providing them with renewed opportunities to partition stochastically to the native state (the global free energy minimum) or to non-native false minima.

Yann Chemla

University of Illinois at Urbana-Champaign, USA.

Awards:

- Donald Biggar Willett Scholars
- CAREER Award at the Scientific Interface from the Burroughs-Wellcome Fund
- Sloan Foundation Fellowship in 2010,
- Beckman Fellow at the U. of I.'s Center for Advanced Study in 2012/13.

Overview:

The Chemla lab studies mechanical processes in biology. Our interests range from how proteins interact with DNA - bending, wrapping, or translocating along the molecule - to how cells swim and process information from the environment. We use state-of-the-art biophysical techniques such as optical tweezers and fluorescence to detect such processes at the level of a single molecule or cell. These techniques are extremely powerful since they are not subject to the averaging artifacts of traditional ensemble biochemical methods.

Students in the Chemla group work on all facets of research: design and construction of instrumentation, development of biological systems, and quantitative analysis and modeling of collected data.

Abstracts

AFM study of ParB-DNA interactions

A. Martin-Gonzalez[1], G. Fisher[2], J. Madariaga[1], C. L. Pastrana[1] , M.S. Dillingham[2], F. Moreno-Herrero[1].
1 Dept. of Macromolecular Structures, National Center of Biotechnology, CNB-CSIC, Madrid, Spain
2 School of Biochemistry, Medical Science Building, University of Bristol, Bristol, United Kingdom.
E-mail: alejandro.martin@cnb.csic.es

Body of abstract

The ParABS system is in charge of the condensation and re-organization of the chromosome during its segregation in bacteria. It comprises the ParB protein, which specifically binds to the centromere-like DNA sequence *parS*, and ParA, an ATPase that binds DNA non-specifically. Previous studies have shown that ParB not only binds *parS* specifically, but it also binds DNA non-specifically promoting the formation of more complex structures that result in DNA condensation [1] [2]. However, the domains of the protein involved in the non-specific binding are not clearly identified. As a consequence, the process of ParB-mediated DNA condensation and its role during chromosome segregation is not fully understood. We have used Atomic Force Microscopy and different mutations of the ParB protein to characterize the mechanism of DNA of condensation. We employed mutants that impair *parS*-binding (R149G) and ParB dimerisation (R80A) and studied the effect of these mutations in DNA condensation. The R149G mutant preserved condensation. The R80A mutant was not able to fully condense the DNA but it interacted with the DNA forming network-like structures. Our results suggest that the specific and non-specific binding modes of ParB are carried out by different regions of the protein, which can be related to different dimerisation domains. Experiments with other single-molecule techniques supporting our findings will be presented and discussed.

This work was supported by a fellowship from the Spanish ministry of Economy and Competitiveness REF(BES-2015-071244).

References

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[2] Taylor, J.A., et al., *Specific and non-specific interactions of ParB with DNA: implications for chromosome segregation.* Nucleic Acids Res, 2015. **43**(2): p. 719-31.

Understanding the mechanisms involved in ultrasound-mediated drug delivery using microbubbles

Inés Beekers[1,2], Shreyas B. Raghunathan[3], Tom van Rooij[2], Verya Daeichin[2], Nico de Jong[2,3], Klazina Kooiman[2], Sebastiaan J. Trietsch[1]
[1]Mimetas B.V., Leiden, the Netherlands
. [2]Department of Biomedical Engineering, Thorax Center, Erasmus MC, Rotterdam, the Netherlands.
E-mail author1@e-mail.address

Ultrasound contrast agents are encapsulated micro-sized gas bubbles that are clinically used to enhance contrast in ultrasound imaging. Additionally, these microbubbles alsohave a therapeutic potential since ultrasound insonification of microbubbles can locally increase endothelial cell membrane permeability (sonoporation), open cell-cell contacts and stimulate endocytosis. Since the responsible mechanisms still remain unknown, further investigation is needed to understand the relationship between the microbubble oscillation and the effects on the cells.
To study these underlying mechanisms, the ideal *in vitro* endothelial cell model includes 3D cell culture, flow, and co-culture. Therefore, we propose to use the elaborate microfluidic channel structure of the OrganoPlate™ (Mimetas BV) [1]. The oscillations of a microbubble submitted to an ultrasound pressure wave can be characterized while monitoring thedrug uptake by endothelial cells grown in the microfluidics. Figure 1 shows a sketch of the ideal *in vitro*3D model for studying the mechanisms involved in ultrasound-mediated drug delivery.
This preliminary study has been performed to study the feasibility of using OrganoPlates™ for *in vitro* ultrasound-mediated drug delivery research, as they have never been used for studies involving ultrasound. The acoustic pressure propagation through an OrganoPlate™ was modeled with a finite element tool (PZFlex[2]). The homogeneous transmission into the OrganoPlate™ demonstrates its potential to create an ideal *in vitro* microfluidics model for studying the biophysical mechanisms involved in ultrasound-mediated drug delivery.

References
[1] Trietsch et al, Lab Chip, 2013; [2] Vaughn et al, PZFlex, 2001.

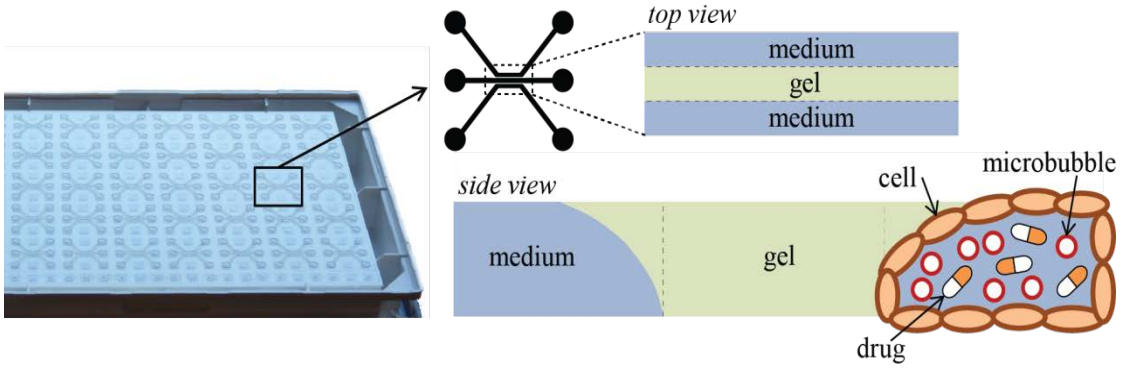

Figure 1. Bottom of the OrganoPlate™ [1] revealing the microchannel structure of three adjacent lanes. The bottom right illustration shows the desired *in vitro* setup with endothelial cells, flowing microbubbles and therapeutic agent.

The impact of the pathway topology and non-linearities in cancer treatments

Doldán-Martelli, Victoria[1], Míguez Gómez, David[1]
[1]Insituto Nicolas Cabrera, Facultad de Ciencias, Módulo 08, 4th floor,
Universidad Autónoma de Madrid, 28049 Cantoblanco, Madrid
E-mail: victoria.doldan@uam.es

Abstract
The network topology of signaling pathways is essential to understand the cellular response to drug treatments [1]. In cancer cells, many mutations deregulate the normal behavior of proliferative pathways and different small molecule inhibitors have been developed for cancer treatment.

Nonetheless, their effect can suffer modifications due to the topology of the targeted network: non-linear interactions and feedback-loops motifs can create bistable responses leading to sensitization or habituation, which affects the drug efficiency in periodic or combinatorial treatments.

Here we design a semi-automatic method to create generic signaling networks, using classical ODE models based on Michaelis-Menten equations [2]. By allowing non-linear interactions between the components of those networks, we can test potential input signals using inhibitory drugs against different elements of the pathway. The analysis of treatment effect show the impact of non-linearities in the dynamics of the drug response and the importance of the pathway architecture when designing new treatments to inhibit non-linear pathways.

References
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[2] Ma W. *et al.* Defining network topologies that can achieve *biochemical adaptation.* Cell. 2009 Aug 21;138(4):760-73.

Force And Twist Dependence Of Repc Nicking Activity On Torsionally-Constrained Dna Molecules

Cesar L. Pastrana[1], Carolina Carrasco[1], Parvez Akhtar[2], Sanford H. Leuba[3], Saleem A. Khan[2], and Fernando Moreno-Herrero[1]
[1]Department of Macromolecular Structures, Centro Nacional de Biotecnologia, CSIC, Darwin 3, 28049 Cantoblanco, Madrid, Spain
[2]Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, 450 Technology Drive, Pittsburgh, PA 15219, USA
[3]Department of Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA
E-mail author1@e-mail.address

Many bacterial plasmids replicate by an asymmetric rolling-circle mechanism that requires sequence-specific recognition for initiation, nicking of one of the template DNA strands, and unwinding of the duplex prior to subsequent DNA synthesis. Nicking is performed by a replication-initiation protein (Rep) that directly binds to the plasmid double-stranded origin and remains covalently bound to its substrate 5'-end via a phosphotyrosine linkage. It has been proposed that the inverted DNA sequences at the nick site form a cruciform structure that facilitates DNA cleavage. However, the role of Rep proteins in the formation of this cruciform and the implication for its nicking and religation functions is unclear. Here, we have used magnetic tweezers to directly measure the DNA nicking and religation activities of RepC, the replication initiator protein of plasmid pT181, in plasmid sized and torsionally-constrained linear DNA molecules. Nicking by RepC occurred only in negatively supercoiled DNA and was force- and twist-dependent. Comparison with a type IB topoisomerase in similar experiments highlighted a relatively inefficient religation activity of RepC. Based on the structural modeling of RepC and on our experimental evidence, we propose a model where RepC nicking activity is passive and dependent upon the supercoiling degree of the DNA substrate.

Bifurcation analysis of pattern formation in filamentous cyanobacteria

Katherine Gonzales-Moreno, Javier Muñoz-García, Saúl Ares

[1]Departamento de Matemáticas and Grupo Interdisciplinar de Sistemas Complejos (GISC), Universidad Carlos III de Madrid, 28911 Leganés, Spain

Cyanobacteria are model organisms for studying oxygenic photosynthesis.The genus *Anabaena* lives in colonies in the form of one-dimensional filaments. Under nitrogen deprivation conditions, some cells in the filament differentiate into a specialized cell type called heterocyst, that fixes environmental nitrogen into chemical forms usable for the colony. This differentiation is tightly regulated both spatially and temporally, such that roughly 1 out of 10 cells differentiate into heterocysts, forming a quasi-regular pattern in the filament [1-4].

Here we make a bifurcation analysis to better understand heterocyst pattern formation.For this study we use a model based on a system of differential equations for each cell, with variables that represent the concentration of two major genes identified in the process, the activator *hetR* and the inhibitor *patS*. This model is based on the one presented in Ref. [5].The methodology was to simulate the model's differential equations fort he concentrations of molecular species in a finite set of cells.Using the software package *XPPAUT* we made a bifurcation analysis for the different parameters of the model, finding the kind of instabilities through which the pattern appears.

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[2] Thiel T (2004) in *Genetics and Regulation of Nitrogen Fixing Bacteria*, eds Klipp W, Masepohl B, Gallon J, Newton W (Kluwer Academic Publishers), pp 73-110.

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Engineering DNA self-assembled nanomotors

Ibon Santiago[1] and Andrew J. Turberfield[1]
[1] Department of Physics, University of Oxford, Oxford, United Kingdom
ibon.santiago@physics.ox.ac.uk

Anisotropic chemical catalysis supports the creation of local concentration gradients around a structure leading to its self-propulsion [1]. However, the construction of small synthetic catalytic motors presents experimental challenges at the nanoscale, where it is hard to create such geometric anisotropy using traditional methods.

With the tools of DNA nanotechnology we can self-assemble nanostructures from bottom-up with designed morphology [2]. We present DNA self-assembled nanostructures which we functionalise site-specifically with catalysts at desired loci in a Janus-type fashion. These bio-conjugated catalysts are either metallic nanoparticles or enzymes capable of decomposing hydrogen peroxide, such as platinum and catalase respectively. Our aim is to create a rationally designed molecular swimmer.

We also show an experimental platform to study active matter at the nanoscale, where we make use of fluorescence correlation spectroscopy and light scattering methods to measure the diffusion of fluorescent DNA nano devices and their catalysts in the presence of fuel.

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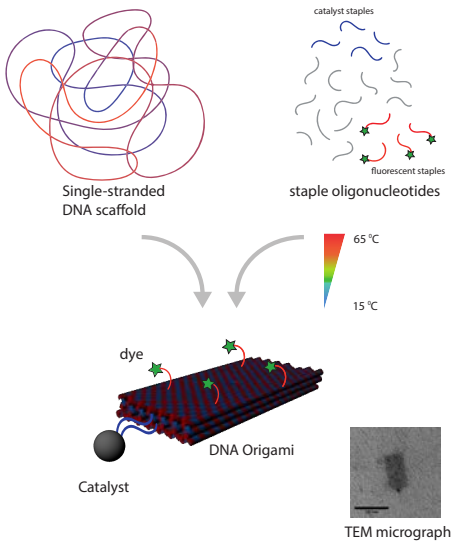

Figure 2 Scheme of DNA nanomotor assembly. A 3D DNA Origami block structure binds site-specifically to catalysts

Development and characterization of a hybrid apparatus combining Magnetic Tweezers and TIRF microscopy to study single DNA-protein interactions

Julene Madariaga-Marcos[1], Mark S. Dillingham[2] and Fernando Moreno-Herrero[1]
[1] Department of Macromolecular Structures, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas, Madrid, Spain
[2] DNA:Protein Interactions Unit, School of Biochemistry, University of Bristol, Bristol, United Kingdom.
jmadariaga@cnb.csic.es

Atomic Force Microscopy (AFM), Magnetic Tweezers (MT) and Optical Tweezers (OT) are single-molecule techniques that can be employed to study DNA-protein interactions. There is an increasing interest in combining these single-molecule devices with fluorescence because mechanical measurements could eventually be correlated with the presence of a protein. For instance, other groups have combined AFM with STED super-resolution imaging. In our group, we have developed a hybrid setup incorporating total internal reflection fluorescence (TIRF) microscopy to our MT. This is a powerful approach because it allows us to correlate biological activity with precise positioning and stoichiometries (directly observed by fluorescence) of the proteins. We have implemented an objective-type TIRF, where the excitation beam is directed to the sample surface and the fluorescence emission is collected by the same objective. This implementation allows facile switching between TIRF and epi-illumination, if desired. The setup is combined with a new multistream laminar flow microfluidics device, which permits to control in a precise way the addition of proteins and reagents of interest. We have characterized the apparatus performing several proof of concept experiments, and we have also started testing it with proteins, such as AddAB, a bacterial helicase-nuclease complex involved in DNA repair, and ParB, a bacterial centromere binding protein involved in DNA condensation.

Single-molecule level study of the mechanism of human mitochondrial DNA replication.

Kateryna M. Lemishko,[1,2] Borja Ibarra[1], Laurie S. Kaguni [3]

[1] IMDEA-Nanoscience Institute, Faraday, 9, Ciudad Universitaria de Cantoblanco, 28049, Madrid, Spain;

[2] Spanish National Center for Biotechnology (CSIC), Darwin, 3, Ciudad Universitaria de Cantoblanco, 28049, Madrid, Spain
[3]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824
E-mail: kateryna.lemishko@imdea.org

Mitochondria are eukaryotic organelles, responsible for cellular energy generation. Besides their role in energy production, mitochondria are essential for cellular activity regulation, e.g. cell signaling and cell death. Mitochondria have their own DNA (mtDNA) and, in general, human cells contain thousands of mtDNA copies. A reduction in the number of mitochondrial DNA molecules or accumulation of mtDNA mutations may cause so-called 'mitochondrial diseases' that, in humans, affect tissues highly dependent on mitochondrial metabolism, such as brain, heart , liver, skeletal muscles and kidney tissues [1]. Therefore, mitochondrial dysfunction and, partly, mitochondrial diseases occurrence, in some measure, depend on effectiveness and accuracy of mtDNA replication.

The mitochondrial DNA replication machinery is much simpler than its nuclear DNA equivalents. The 'minimal replisome', that is capable of processive DNA synthesis, can be reconstituted *in vitro* with just three proteins: the TWINKLE DNA helicase, the single-stranded DNA binding protein (mtSSB) and the mitochondrial DNA polymerase γ [2].

The mechanism of human mtDNA replication has not yet been fully characterized. It is unclear how the proteins, involved in mtDNA replication, act at the replication fork. In present work, we aimed to develop hairpin-based DNA systems that would allow us to detect and characterize at the single-molecule level activities of the proteins participating in human mtDNA replication, employing optical tweezers approach.

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Formation and maintenance of nitrogen fixing cell patterns in filamentous cyanobacteria

Javier Muñoz-García[1], Saúl Ares[1]
[1]Departamento de Matemáticas and Grupo Interdisciplinar de Sistemas Complejos (GISC), Universidad Carlos III de Madrid, 28911 Leganés, Spain
saul@math.uc3m.es

Cyanobacteria forming one-dimensional filaments are paradigmatic model organisms of the transition between unicellular and multicellular living forms. Under nitrogen limiting conditions, in filaments of the genus Anabaena some cells differentiate into heterocysts, which lose the possibility to divide but are able to fix environmental nitrogen for the colony. These heterocysts form a quasi-regular pattern in the filament, representing a prototype of patterning and morphogenesis in prokaryotes. Recent years have seen advances in the identification of the molecular mechanism regulating this pattern [1-4]. We use this data to build a theory on heterocyst pattern formation, for which both genetic regulation and the effects of cell division and filament growth are key components [5]. The theory is based on the interplay of three generic mechanisms: local autoactivation, early long range inhibition, and late long range inhibition. These mechanisms can be identified with the dynamics of hetR, patS and hetN expression. Our theory reproduces quantitatively the experimental dynamics of pattern formation and maintenance for wild type and mutants. We find that hetN alone is not enough to play the role as the late inhibitory mechanism: a second mechanism, hypothetically the products of nitrogen fixation supplied by heterocysts, must also play a role in late long range inhibition. The preponderance of even intervals between heterocysts arises naturally as a result of the interplay between the timescales of genetic regulation and cell division. We also find that a purely stochastic initiation of the pattern, without a two-stage process [1], is enough to reproduce experimental observations.

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A model for non-additive input integration predicts cooperative and emergent responses in Notch intercellular signalling systems

Juan Camilo Luna-Escalante[1 *], Pau Formosa-Jordan[2] and Marta Ibañes[1]
[1]Department of Condensed Matter, Faculty of Physics, University of Barcelona, Barcelona, Spain
[2]Sainsbury Laboratory, Universityof Cambridge, Cambridge, United Kingdom
* jcluna@ecm.ub.edu

Cell-to-cell communication between adjacent cells can be mediated by proteins anchored in the plasmic membrane. This mechanism is most relevant during Metazoan development, where coordinated cell proliferation and differentiation take place. Notch signalling is known to drive coordinated cell differentiation in cell arrays, such that a tissue exhibits periodic salt-and-pepper patterns, where cells adopt one of two possible states, or wave-like propagation of differentiation, where cells transit from one state to another. Notch signalling is activated by the interaction between membrane ligands and Notch receptor, such that the ligands in neighbouring cells serve as input for Notch signal activity of each cell. The study of Notch signalling has been typically restricted to the case in which the input is associated to one type of ligand only. In this work, we have addressed Notch signalling when two different types of ligands serve as input, by using a computational-theoretical approach. In this scenario, inputs can be integrated additively (i.e., ligands use independent resources to trigger Notch signal) or non-additively (i.e., ligands share resources to trigger Notch signal). Our results show that the outcome of Notch signalling critically depends on the type of input integration. When the integration is non-additive, the outcome can be either driven cooperatively by both ligands or can be an emergent state, which is not expected from the individual role of each ligand when acting in isolation. Overall, this type of input integration constitutes a dynamical mechanism to achieve robustness and to provide evolvability.

toyLIFE: a simple model to understand the complexities of the genotype-phenotype map

Pablo Catalán[1,2], Clemente F. Arias[1], Jose A. Cuesta[1,2,3], Susanna Manrubia[1,4]
[1]Grupo Interdisciplinar de Sistemas Complejos (GISC)
[2]Departamento de Matemáticas, Universidad Carlos III de Madrid, Spain
[3]Institute for Biocomputation and Physics of Complex Systems, Zaragoza, Spain
[4]National Biotechnology Centre (CSIC), c/ Darwin 3, 28049 Madrid, Spain.
E-mail pcatalan@math.uc3m.es

Our understanding of biological evolution is in constant change, due to the continuous arrival of new sets of data, mostly a consequence of next generation sequencing. One big aspect of this growing understanding is the realization that the relationship between genotypes and the phenotypes that are developed from them is highly complex. This complexity has evolutionary consequences that we need to include in our models of evolutionary dynamics.

The study of the actual genotype-phenotype map is, unfortunately, out of our possibilities so far. As a consequence, many theoretical models have been proposed to study some of its general properties. Among these we find RNA and protein folding, gene regulatory networks and also metabolic networks.

However, all of these models omit two important aspects of this map, namely the existence of different levels of expression and the bidirectional interaction between those levels. In a cell, genes are translated into proteins that interact with each other to form protein complexes and regulate the expression of other genes, and that also process the information captured from the outside. All of these levels interact in non-trivial ways, so that mutations in a gene can affect all of the levels and yet leave the function of the cell intact.

We present toyLIFE, a multi-level model for the genotype-phenotype map. toyLIFE contains genes, proteins and metabiolites, that interact through the laws of a simplified chemistry, forming complex regulatory and metabolic networks. We will show how the simple rules of toyLIFE already give rise to very complex behaviors, and that they also capture many important aspects of the genotype-phenotype map already shown in previous models.

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A mathematical model of tissue growth based on progenitor-to-differentiated state transition.

Máximo Sánchez-Aragón1, Arianne Ramaekers2, Isabel Almudí1, Carlos Martín-Blanco1, Bassem Hassan2, Fernando Casares1
1 - Andalusian Centre for Developmental Biology (CABD-CSIC) Seville, Spain
2 - VIB Center for the Biology of Disease KU Leuven, Belgium

Developing tissues are composed of cells that undergo an irreversible transition of gene regulatory states, from unspecified progenitors to precursors of final functional fates. This process is marked by a change in the cells' proliferation potential which is tightly controlled by transcription factors belonging to specific gene regulatory networks (GRN), therefore determining the size of the mature organ.

Here we present a simple mathematical analysis of tissue growth that allows to predict final organ size based on the speed of the cell state transition and on the cell proliferation rate. The model also predicts properties of the system, such as a) sensitivity to initial conditions of the parameters and b) the parameter subspace that ensures the successful termination without overgrowth. We tested the model by predicting the changes in parameters that would explain the size difference of the eyes of two *Drosophila* species, its predictions on the natural eye variations that occur between two species of *Drosophila*, *D. melanogaster* and *D. pseudoobscura*. Even though the initial size of the eye primordia in these two species differ, the model predicts that these size differences alone cannot explain the final sizes of the eyes without the readjustment of differentiation rates, something that we verified experimentally. This would indicate that quantitative differences in pathways involved in controlling the differentiation rate, such as those of Dpp/BMP2 or Hh would be involved in these inter-specific eye size differences. With this framework at hand we expect to identify quantitative effects on cellular parameters for several loss-of-function mutants affecting eye size, and from other dipteran species with varying eye sizes in the hope to restrict the scope of solutions for molecular models of gene regulation.

Single-molecule mechanical characterization of the HmtSSB binding properties to ssDNA

Fernando Cerrón[1,2], Jose A. Morín[3], Javier Jarillo[2], Laurie S. Kaguni[4], Grzegorz L. Cieselki[4], Francisco J. Cao[2], Borja Ibarra[1].

[1]Imdea Nanociencia, Faraday 9, Ciudad Universitaria de Cantoblanco, 28049, Madrid, Spain.
[2]Departamento de Fisica Atomica, Molecular y Nuclear, Universidad Complutense, 28040, Madrid, Spain.
[3]Max Planck Instutite of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307, Dresden, Germany.
[4]Department of Biochemistry and Molecular Biology and Center for Mitochondrial Science and Medicine, Michigan State University, East Lansing, MI, USA.

fercerron@gmail.com

The human mitochondrial single-stranded DNA binding protein (HmtSSB) binds single-stranded DNA (ssDNA) with high affinity and defines the nucleoprotein substrate upon which DNA replication, repair and replication restart processes must act. It, therefore, plays a central role in the mitochondrial genome (mtDNA) maintenance, being indispensable for cell survival. However, little is known about the real time kinetics of HmtSSB binding to the DNA and the energetics and structure of these, highly dynamic nucleoprotein complexes. Using optical tweezers we have developed a single molecule manipulation assay to address these questions. Fits to the obtained nucleoprotein force-extension curves with a novel polymer physics model revealed that HmtSSB proteins bind to the ssDNA in different modes depending on the ionic strength and the protein concentration. Each mode organizes a defined number of nucleotides and presents a particular association energy. Interestingly, our data also show that co-replicational binding of the HmtSSB proteins to DNA favors only the most stable mode of interaction.

Mitochondria and the non-genetic origins of cell-to-cell variability: More is different

Raúl Guantes[1]*, Juan Díaz-Colunga[2] and Francisco J. Iborra[2]*
[1]Department of Condensed Matter Physics, Materials Science Institute 'Nicolás Cabrera' and Institute of Condensed Matter Physics (IFIMAC), Universidad Autónoma de Madrid, Campus de Cantoblanco, Madrid, Spain
[2]Centro Nacional de Biotecnología, CSIC, Campus de Cantoblanco, Madrid, Spain
*Corresponding authors: Raúl Guantes (raul.guantes@uam.es) and Francisco J. Iborra (fjiborra@cnb.csic.es)

Gene expression activity is heterogeneous in a population of isogenic cells. Identifying the molecular basis of this variability will improve our understanding of phenomena like tumor resistance to drugs, virus infection, or cell fate choice[1-5]. The complexity of the molecular steps and machines involved in transcription and translation could introduce sources of randomness at many levels, but a common constraint to most of these processes is its energy dependence. In eukaryotic cells, most of this energy is provided by mitochondria. A clonal population of cells may show a large variability in the number and functionality of mitochondria. Here, we discuss how differences in the mitochondrial content of each cell contribute to heterogeneity in gene products. Changes in the amount of mitochondria can also entail drastic alterations of a cell's gene expression program, which ultimately leads to phenotypic diversity.

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Intranasal insulin treatment attenuates methamphetamine-induced neuroinflammation and cognitive impairment

Elmira Beirami[1], Seyedeh Masoumeh Seyedhoseini[2], Shahrbanoo Oryan[1], Abolhasan Ahmadiani[2], Leila Dargahi[3]

1- Faculty of Biological Sciences, Kharazmi University, Tehran, Iran

2- Neuroscience Research Center, Shahid beheshti University of Medical Sciences, Tehran, Iran

3- NeuroBiology Research Center, Shahid beheshti University of Medical Sciences, Tehran, Iran

Elmira.beirami@gmail.com

Introduction: Methamphetamine(MA) is a well-known psychostimulant drug, the abuse of which is a serious worldwide public health issue. In addition to its addictive effect, repeated or neurotoxic-(i.e.,high dose) MA exposure induces alterations in glial cell functions that contribute to a complex cascade of events, leading to neuroinflammation, neuronal damage and behavioral impairments[1]. Studies in the last decade have demonstrated that insulin signaling plays an important role in various brain functions. Experiments administrating intranasal insulin to young and aged adults have shown that insulin improves learning and memory.Also in the periphery, insulin can decrease inflammation induced by lipopolysaccharide(LPS); but, whether insulin can reduce inflammation within the brain is unknown[2]. In present study, we investigated whether intranasal insulin treatment can reverse MA-induced behavioral changes and neuroinflammation.

Methods: Male Wistar rats,weighting 250-300g, were intraperitoneally treated with escalating doses of MA;1-10mg/kg twice a day for10 days. MA-treated animals as well as corresponding controls, treated with vehicle, were subjected to Y-maze and Novel Object Recognition(NOR) tests one week after discontinuation of drug treatment. In another two groups MA-and vehicle-treated animals received daily intranasal insulin (0.5IU) for 7 days and then were evaluated for behavioral changes. After behavioral tests brains were removed for immunohistochemistry processes.

Results: Repeated escalating doses of MA impaired the spontaneous alteration and recognition performance in Y maze and impaired short- and long-term memory for object recognition in NOR test while, the locomotors activity remained unchanged. Also immunohistological analysis revealed that MA induced the expression of neuroinflammatory marker(GFAP)in the rat hippocampus. Intranasal insulin treatment though didn't affect these parameters in control rats, significantly attenuated the MA-induced neuroinflammation and cognitive impairment.

Conclusion: These findings in parallel to introducing insulin as a potential therapeutic agent for the treatment of MA aversive symptoms, notifies the probable involvement of insulin signaling impairments in MA-induced neural damage.

Keywords: Methamphetamine(MA); insulin; neuroinflammation; recognition memory

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New approach for the description of retinal progenitor cell behavior on retinal differentiation

Mario Ledesma Terrón [1,3*], Florencia Cavodeassi[2] and David Gómez Míguez[1,3]
[1] Universidad Autónoma de Madrid.
[2] Centro de Biología Molecular Severo Ochoa (CBMSO). Development and differentiation department (CSIC-UAM).
[3] Depto. de Física de la Materia Condensada, Instituto Nicolás Cabrera and IFIMAC, Universidad Autónoma de Madrid (UAM).
*e-mail address: mario.ledesma@estudiante.uam.es

The progenitor cells have the ability to divide in new progenitor cells and differentiated cells. The progenitor cells ensure the tissue growth because they are divided increasing the number of cells, while differentiated cells are the responsible of tissue differentiation since they play the specific functions in adult tissue. Thereby, the study of progenitor cell division is elementary to understand the formation of the adult tissue due to the production of progenitor and differentiated cells depend of progenitor cell division. The retinal differentiation is a widely used model to study the physiological implications of progenitor cell division on neural development. In the neural retina, the progenitor cells give rise to seven cellular types, at the same time the retinal size increases. Thus, the study of retinal progenitor cells, in terms of cell cycle length and rates of progenitor and differentiated cells production, is elementary to understand the mechanisms responsible of the fine control between retinal growth and retinal differentiation, along an established interval of time during retinal development. Using theoretical equations, based on a previously reported stochastic model published for our group, we have analyzed the behavior of progenitor cells in the 3D developing retina of zebrafish (Danio rario) embryos during the retinal differentiation.

Amyloid aggregation insights from a folded state

Francisco Ramos-Martín[1], Milagros Castellanos[2] , Víctor Muñoz [3]

[1 ,2, 3] Department of Macromolecular Structures, National Biotechnology Center, Consejo Superior de Investigaciones Científicas, Darwin 3, Campus de Cantoblanco, 28049 Madrid, Spain
[2,3] Nanobiosystems Programme, IMDEA Nanosciences, Faraday 9, Ciudad Universitaria Cantoblanco, 28049 Madrid, Spain
[3] Department of Bioengineering, School of Engineering, University of California, Merced, California 95343, USA
E-mail framos@cnb.csic.es

Proteins that are able to form amyloid aggregates are involved in several pathologies and disorders, including Parkinson's and Alzheimer's, among others. The processes that makes a protein to fold improperly and lead to the formation of this aggregates are still not deeply known. This is neither known for the cases of proteins that are initially folded properly but, after an unfolding process, they undergo some unfolding-refolding processes that make them to follow an aggregation pathway leading to the formation of amyloids at the end [1, 2, 4].

Recent evidences suggest that some structural changes affecting the monomeric forms of this proteins at the start of the aggregation cascade are essential to start this "abnormal" pathway [3].

To study more deeply this processes at the start of the aggregation process, we have performed several circular dichroism and fluorescence studies and, subsequently, we have relied on our AFM setup. With that one we are able to get images of the formed aggregates in the conditions we are working on and, additionally, we can perform several AFM-SMFS experiments. That allow us to study the mechanical properties of the monomers of the studied proteins and to follow the changes they suffer at several times depending on the progress of their aggregation process. This way we focus at the initial stages while they are under aggregation promoting conditions.

To this end, we are focusing currently in the N47A mutant of alpha-spectrin protein that is known to form amyloid aggregates after suffering a clear unfolding process.

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Coarse-grained simulation of actin self-assembling.

Pau Casanova[1], David G. Miguez[1,2]& Pedro Tarazona[1,3]
[1]Instituto de Ciencia de Materiales Nicolas Cabrera
Condensed Matter Physics Center (IFIMAC),
Universidad Autonoma de Madrid,
28049 Madrid, Spain
[2]Departamento de Física de la Materia Condensada
[3]Departamento de Física Teórica de la Materia Condensada
E-mail: pau.casanova25@gmail.com

Abstract:

We have developed a 2D Montecarlo coarse-grained simulation that mimics the self-assembling of the actin to study the effect of the actin bundling crosslinkers (named ligases during this work). Our approach in this simulation is to consider the direct formation of the bundles without a previous phase of actin single chains. During this study we only have considered two proteins, the actin and the ligases. The interactions of that proteins are actin-actin (with a preferred direction of growing) and the ligase-actin interaction to form the bundle structures connecting two actin chains.

The results of several simulations show a change of phase from the diffused actin and ligase molecules to the bundle structure that depends both on the concentration of actin and ligase of the system.

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Dynamic Analyses of the Hedgehog Signalling Pathway

Zahra Qureshi[1] and Jamil Ahmad[1*]
[1]Research Centre for Modelling and Simulation (RCMS), National University of Sciences and Technology (NUST), Islamabad, 44000, Pakistan.
xahra0101@gmail.com, jamil.ahmad@rcms.nust.edu.pk

In the machinery of any living organism, biological regulatory networks play an active and crucial role, such that a commensurate understanding of their underlying dynamics across different clusters of genes becomes necessary, especially towards perfecting drug therapies to combat cancer. This study focuses on the underlying dynamics of the evolutionarily conserved Hedgehog (Hh) Pathway, which functions as a bi-stable genetic switch with the evolutionary adapted function of controlling the growth, survival and fate of cells across the entire body structure and its transcription factor, Gli, has the dual function of regulating its own expression and taking on the role of an antagonist. However, this pathway when deregulated or mutated contributes to the onset of tumorigenesis, accounting for one-third of all known cancers. The dynamic interplay between its distinct cell fates is unfolded using the Discrete Modelling Formalism of Renè Thomas and Linear Hybrid Modelling. The Discrete Model incorporates a Java based tool, GenoTech, to generate the entire state space using an asynchronous update. Logical parameters are inferred using the Computational Tree Logic (CTL) based model-checker, SMBioNet and the tool Cytoscape assesses the effectiveness of the several resulting cyclic trajectories. Similarly, the Linear-Hybrid modelling is performed using the Parametric Bio-Linear Hybrid Automaton and the tool, HyTech, synthesises delay constraints to capture the phenomenon that wires the system into following a specific cycle or path. Our results not only fit observed data very well but also set out to reveal insights about the nature and forte of crucial interactions, instrumental in maintaining homeostatic and normal behaviour of the pathway. This study makes some new predictions, suggesting that it is the cooperative interactions of SMO and GLI^A coupled with the workings of the GLI auto-regulatory motif that are primarily responsible for the onset of advanced malignant stages, thereby concluding that the GLI auto-regulatory motif plays an extremely crucial role in defining system dynamics when subjected to varying threshold levels and is responsible for an irreversible genetic switch. However, by deterring its autonomous regulatory influence on itself with the assistance of its repressor counterpart GLI^R, the irreversible genetic-switch recuperates, this delicate balance between GLI^R and GLI^A prevents the system from entering into an intense self-replicating perplexity.

Tissue growth and curvature – A quantification of 3D tissue arrangement on surfaces with controlled curvature.

Cornelius Müller[1], Sebastian Ehrig[1], John W. C. Dunlop[1]
[1]Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, Am Mühlenberg 1, 14476 Potsdam, Germany
cornelius.mueller@mpikg.mpg.de

Although the spatial arrangement of cells and extracellular matrix (ECM) components in a tissue is of fundamental importance on biological tissue function, very little is known about how long range order develops during growth. One physical cue that seems to play a role in the three-dimensional tissue formation is the geometry, and in particular the surface curvature of the substrate upon which a tissue is growing. To investigate the role of the substrate geometry on tissue growth we produce surfaces with controlled mean-curvatures and use these surfaces as scaffolds for tissue growth by an osteoblast cell line *in-vitro*. During these cell culture experiments, it is possible to follow cell alignment and total tissue volume which can then be compared to theoretical models for tissue formation and organization. Data thus obtained allows us to test whether tissue growth occurs according tothe Laplace-Young-law, and suggests that at least *in-vitro* tissue behaves much the same as a viscous liquid. A further observation is that we see a strong collective alignment of actin filaments and collagen fibres in particular directions on the surface, indicating a link between the alignment of cells and ECM to the underlying surface curvature. In particular we observe that the zero curvature geodesic lines on the surface of our scaffolds follow a similar path as the actin fibres. In addition we see a correlation between the degree of ordering within the orientation distribution and the values of principal curvatures of the surface, which can be modelled by a simple description of the bending of stiff rods on curved surfaces. In this manner, a deeper understanding of how geometry controls tissue organization and the formation of biological material becomes possible through the interaction of physics and biology, and hopefully will give new insight into the development of tissues during morphogenesis.

Ultrasensitive detection of prostate specific antigen (PSA) by an hybrid mechanical and optoplasmonic nanosensor

Sergio García, Priscila M. Kosaka, Montserrat Calleja and Javier Tamayo
Bionanomechanics Lab. Instituto de Microelectrónica de Madrid (IMM-CSIC) Tres Cantos, Madrid
E-mail author: sergio.garcia@csic.es

Last year the first draft of the human proteome was presented providing 84% of the human proteins. This map will be rapidly refined and an accurate picture of the cancer proteome is expected for the next years. Discovery and detection of these proteins will revolutionize cancer treatment and will allow non-invasive early cancer detection. However, these proteins are shed to the bloodstream in ultralow concentrations and there is no technology today capable of 'detecting' the ultralow concentrations of these proteins in a 'haystack' of plasma proteins (>10 000 different proteins), some of them at concentrations 12 orders of magnitude higher. In this work we detected ultra-low concentrations of prostate specific antigen (PSA), which is currently in clinical use for the diagnosis, monitoring and prognosis of prostate cancer using a new technology that merges nanomechanics and optoplasmonics [1]. Merging mechanical and optical transduction schemes in the same platform provides remarkably superior performance and higher reliability than devices based on a single transduction scheme. A sandwich assay that involves the recognition of PSA first by a surface-anchored antibody and second by an antibody free in solution that recognizes a free region of the captured biomarker. This second antibody is tethered to a gold nanoparticle that acts as mass and plasmonic label. A detection limit of 1×10^{-16} g ml^{-1} in serum is achieved, which is at least seven orders of magnitude better than in routine clinical practice. With simple commercially available cantilevers and unsophisticated instrumentation, the presented technique enables the detection of ultralow concentrations of cancer biomarkers in blood. The use of two different transduction mechanisms in a single platform enables to determine the presence of a protein with extremely high statistical significance. All this attributes bring this hybrid mechanical and optoplasmonic device closer to the implementation in the clinical practice, as it fulfills low cost, simplicity and reduced rates of false positives and negatives [2].

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Novel scenarios of protein-protein binding can occur in the absence of folding free energy barriers.

Rajendra Sharma[1], James Fraser [2], David De Sancho [3], Victor Muñoz[4]
[1] National Biotechnology Center, CSIC, Madrid 28049, Spain
[2]University of California San Francisco, San Francisco, CA 94158, United States
[3]CIC nanoGUNE, San Sebastian 20018, Spain
[4] National Biotechnology Center, CSIC, Madrid 28049, Spain
E-mail author1:rsharma.cnb.csic.es

Abstract
Downhill protein folding is a key prediction of energy landscape theory[1], whereby some proteins are able to populate a single state over all ranges of conditions. Extensive experimental work[2,3] on ultrafast folding proteins has confirmed this theoretical prediction. However, so far the biological implications of the existence of this regime have remained unexplored. One possibility is that these proteins act as rheostats instead of switches, allowing for finer regulation of cellular pathways. This hypothesis was originally proposed for the pyruvate dehydrogenase complex[2], whose peripheral subunit-binding domain (PSBD) binds to the E1 and E3 enzymes. Using an ensemble method to analyze X-ray diffraction patterns[4], we find that the PSBD-E1 complex is compatible with conformations where the PSBD is unstructured, a possibility enabled by the lack of free energy barriers between the folded and unfolded states. Using extensive topology based-coarse grained simulations of two models of PSBD –two-state and downhill– we find that lowering in the free energy barrier for folding results in folding coupled to binding, allowing for a more diffuse complex, as suggested by X-ray crystallography ensembles. This scenario is reminiscent of the "fly-casting" mechanism for intrinsically disordered proteins.

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AFM slow indentations and activity of breast cancer cells

Marina P. López Yubero[1], Mario Encinar[1], Alicia Calzado-Martín[1], Montserrat Calleja[1], Javier Tamayo[1].
[1]Instituto de Microelectrónica de Madrid (IMM-CSIC). c/Isaac Newton,8. Tres Cantos, Madrid, Spain.
E-mail marinap.lopez@csic.es

Abstract
Cell mechanics involves a wide variety of complex phenomena and are known to be related to the health and function of cells [1]. They show viscoelasticity, dynamical and structural heterogeneity, and are driven by active non-thermal processes. Here we explore the cell mechanical response by performing indentations with a spherical probe at different loading rates on three different breast cancer cell lines: MCF10A (healthy), MCF7 (tumorigenic, noninvasive) and MDAMB231 (tumorigenic/invasive). In order to evaluate the influence of metabolic activity of those cells, indentations are performed in two different scenarios: normal conditions, full of nutrients, and in ATP-depleted conditions. One universal feature seems to be a weak dependence of cell mechanical properties on the perturbation frequency; and this feature can be analyzed in the theoretical framework of soft glassy materials. Finally, this work reveals important differences between the Young's modulus of the different cell lines and its dependency on the frequency, in both normal and depleted conditions.

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Instrumentation and data treatment for a nanomechanical and nanooptical sensor for cancer detection

Javier Escobar, Priscila M. Kosaka, Montserrat Calleja and Javier Tamayo
Bionanomechanics Lab. – Instituto de Microelectrónica de Madrid (CNM-CSIC)
Tres Cantos, Spain
E-mail javier.escobar@csic.es

Blood contains a treasure trove of protein biomarkers, most of them still to discover, which will be crucial for early detection of disease and for personalized medicine. We use a sandwich assay that involves the recognition of a protein cancer biomarker first by a surface-anchored antibody and second by an antibody free in solution that recognizes a free region of the captured biomarker. This second antibody is tethered to a gold nanoparticle that acts as mass and plasmonic label. The double signature is detected by means of a silicon cantilever that serves as mechanical resonator for 'weighing' the mass of the captured nanoparticles; and as optical cavity due to the two reflective opposite surfaces, that boosts the plasmonic signal from the nanoparticles [1].

Merging mechanical and optical transduction schemes in the same platform provides remarkably superior performance and higher reliability than devices based on a single transduction scheme. Our work is focused on the development of instrumentation for a hybrid nanomechanical and optoplasmonic nanosensor [2]. The measurement system must be capable to perform the sample measurement and data treatment in an automatized manner and it must also fulfill low cost and simplicity to can be implemented in clinical practices. We have developed an automatized system to characterize and measure plasmonic signals from microcantilevers. A script written in Matlab process all the data in an automatized way and the data treatment can be performed in minutes. Moreover, due to the measurement and data automatization, the probability of a human error that can result in a false positive or false negative test result is considerably diminished.

References

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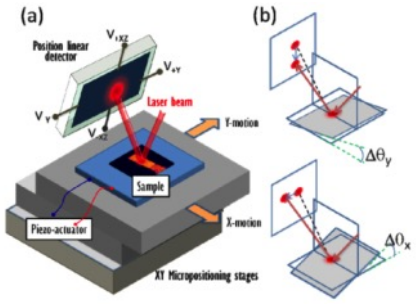

Fig. 1 Scaning laser bean deflection system for nanomechanical characterization of the hybryd nanosensor

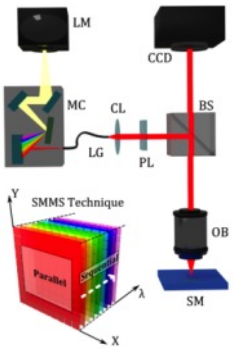

Fig 2. SMMS technique used in detection of optoplasmonic scattering of gold nanoparticles