Workshop

Reverse mathematical methods for reconstructing molecular dynamics in single cell

Centro Di Recerca Mathematica Ennio de Giorgi, Pisa, Italy

October 15th-19th

Organizing Committee:
David Holcman, Ecole Normale Supérieure, France
Khanh Dao Duc, University of Berkeley, USA
Natahanaël Hozé, Institut Pasteur, France

Partially sponsored by ***the Biophysical Society***

SCIENTIFIC PROGRAM

Monday 15: ***Session on transport and cell structure***

9:00-9:15: Introductory Remarks

9:15-10:15: E. Peterman (*A single-molecule view on intracellular transport in vitro and in vivo*)
10:15-11:00: E. Koslover (*Drift, Wander, or Stick: Disentangling Modes of Organelle Motion in Living Cells*)

11:00-11:30: Coffee Break

11:30-12:15: M. Betterton (*Modeling spindle assembly and chromosome alignment in mitosis*)
12:15-13:00: M. Evans (*Antimicrobial resistance: modelling the efficacy of antibiotic treatment*)

13:00-14:30: Lunch and coffee

14:30-15:15: D. Sullivan (*Proteome-scale cell cycle dependent dynamics from asynchronous fluorescent microscopy images*)
15:15-16:00: R. Murphy (*Integrating Information from Diverse Microscope Images: Learning and Using Generative Models of Cell Organization*)

Tuesday 16: ***Session on Image analysis (time series analysis)/ Single Cell Data***

8:45-9:45: D. Holcman (*Single Particle Tracking techniques*)
9:45-10:30: E. Avezov (*Single particle tracking reveals nanofluidic properties of the Endoplasmic Reticulum*)

10:30-10:45: Coffee Break

10:45-11:30: P. Parutto (*Characterization of the dynamics of the endoplasmic reticulum luminal protein calreticulin and the CaV2.1 calcium channels at synapses*)
11:30-12:15: C. Kervrann (*Computational methods for fluorescence microscopy and intracellular dynamics analysis*)
12:15-13:00: O. Gandrillon (*A probabilistic view on GRN inference from single cell data*)

13:00-14:30: Lunch and coffee

14:30-15:15: G. Huber *(Topological Structures in the Endoplasmic Reticulum: Structure, Function and Formation*)
15:15-16:00: D. Coombs (*Multistate modelling and parameter inference for particle tracking analysis and super-resolution imaging of immune cell surface receptors)*

Wednesday 17: ***Session on image analysis (single molecule imaging, SPT, FRET)***

9:00-9:45: C. Vestergaard (*Mapping spatio-temporal dynamics of single biomolecules at the whole-cell scale*)
9:45-10:30: A. Amitai (*Diffusion of nuclear proteins and its link to 3d chromatin organization*)

10:30-10:45: Coffee Break

10:45-11:30: S. Pressé (*Novel Statistical Tools for Single Molecule Imaging: A foray into Bayesian Nonparametrics*)
11:30-12:15: S. Sonneveld (*Single molecule imaging of mRNA translation regulation in living cells*)
12:15-13:00: E. Lerner *(Solving structures of highly dynamic highly heterogeneous conformations of the bacterial transcription initiation complex)*

13:00-14:30: Lunch and coffee

14:30-15:15: K. Dao Duc (*Evolution and functions of the ribosome exit tunnel*)
15:15-15:35: R. Cardim Falcao (*Multi-state Diffusion Analysis with Measurement Errors*)
15:35-16:05: M. Lu (*Direct in-cell observation of structural progression of amyloid-β Arctic mutant aggregation*)

19:00-21:00: Workshop Dinner

Thursday 18: ***Session on Gene expression (stochastic modeling and analysis) 1***

8:45-9:45: D. Erdmann-Pham *(Hydrodynamic Limit of the inhomogeneous l-TASEP with open boundaries)*
9:45-10:30: L. Ciandrini (*Ribosome economics*)

10:30-10:45: Coffee Break

10:45-11:30: T. Tuller *(Modeling and analyzing the flow of molecular machines in gene expression)*
11:30-12:15: J. Chubb (*Origins and implications of transcription bursts*)
12:15-13:00: R. Yvinec (*Stochastic single gene expression model: analytical results on bursting models*)

13:00-14:30: Lunch and coffee

14:30-15:15: R. Kulkarni (*Modeling fluctuations and large deviations in stochastic gene expression*)
15:15-16:00: O. Carja *(Evolution under uncertainty: an adaptive role of stochastic phenotypic variation)*

Friday 19: ***Session on Gene expression 2***

9:00-9:45: E. Levine (TBA)
9:45-10:30: S. Choubey (*Decoding the mechanisms of transcriptional dynamics from nascent RNA measurements)*
10:30-10:45: Coffee Break
10:45-11:30: J. Szavits-Nossan (*Mathematical modelling of mRNA translation: old questions and new insights*)

Abstract List
(in alphabetical order of author)

Assaf Amitai
MIT, Cambridge, USA

Diffusion of nuclear proteins and its link to 3d chromatin organization

The interaction of proteins with chromatin regulates many cellular functions. Most DNA-binding proteins interact both non-specifically and transiently with many chromatin sites, as well as specifically and more stably with cognate binding sites. These interactions and chromatin structure are important in governing protein dynamics. These questions can be addressed theoretically using diffusion models. I will show how that the dynamics of proteins is determined by the 3d organization of chromatin in the nucleus. The time to find a chromatin target depends on chromatin organization around it, which determines the local association and disassociation rates. Hence, the problem of facilitated search by a protein can be mapped to a continuous-time Markov chain.

I the second half of the talk I will discuss how protein dynamics can be analyzed to reveal new features of chromatin and protein organization (In collaboration with Anders Hansen & Xavier Darzacq, UC Berkeley). CTCF, a zinc-finger DNA binding protein, functions as a master regulator of 3D genome organization by regulating chromatin looping. Using single-particle tracking (SPT) of endogenously Halo-tagged CTCF in live mammalian cells we discovered that CTCF exhibits a highly unusual, anomalous motion. CTCF proteins generally exist in either a chromatin-associated (bound) or diffusing state (free). We developed a computational framework to study specifically the diffusing subpopulation and found CTCF behavior to be highly anisotropic. Surprisingly, this anisotropy was localized to “domains”, suggesting transient trapping of CTCF in these domains. We found that certain mutations changed the characteristic of CTCF dynamics, allowing it to perform isotropic, Brownian-like motion. Micro-C analysis revealed the importance of these domains in maintaining chromatin loops. In summary, I will present our unpublished discovery of a novel mechanism of nuclear diffusion and a surprising link to CTCF-mediated nuclear organization.

Edward Avezov
University of Cambridge, UK Dimentia Institute Cambridge

Spatio-temporal dynamics of Endoplasmic Reticulum resolved by single particle tracking reveals a flow mediated luminal transport

The Endoplasmic Reticulum (ER), a network of membranous sheets and pipes, supports functions encompassing biogenesis of secretory proteins and delivery of functional solutes throughout the cell periphery. Molecular mobility through the ER network enables these functionalities. The diffusion-driven molecular motion (traditionally presumed by default), alone is not sufficient to explain the kinetics of luminal transport across supramicron distances. Understanding the ER structure-function relationship is

critical in light of mutations in ER morphology regulating proteins that give rise to neurodegenerative disorders. Applying super-resolution microscopy and stochastic analysis of single particle trajectories of ER luminal proteins revealed that the topological organization of the ER correlates with distinct trafficking modes of its luminal content: with a dominant diffusive component in tubular junctions and a fast flow component in tubules. Particle trajectory orientations resolved over time revealed an alternating current of the ER contents, whilst fast ER nanoscopy identified energy-dependent tubule contraction events at specific points as a plausible mechanism for generating active ER luminal flow. The discovery of active flow in the ER has implications for timely ER content distribution throughout the cell, particularly important for cells with expensive ER-containing projections e.g. neurons, sanctioning efforts to understand the ER transport through mathematical modeling and biophysical analysis.

Meredith Betterton
University of Colorado Boulder, USA

Modeling spindle assembly and chromosome alignment in mitosis

Mitosis ensures the proper segregation of chromosomes into daughter cells, which is accomplished by the mitotic spindle. During fission-yeast mitosis, chromosomes establish bi-orientation as the bipolar spindle assembles, meaning that sister kinetochores become attached to microtubules whose growth was initiated by sister poles. This process must also correct erroneous attachments made by the kinetochores during the attachment process. Our goal is to build a 3D computational model of spindle assembly based on a realistic description of microtubule, kinetochore, and chromosome dynamics, in order to interrogate the role of specific mechanisms in these chromosome bi-orientation and error correction processes. We have added chromosomes to our previous computational model of spindle assembly, which included microtubules, a spherical nuclear envelope, motor proteins, crosslinking proteins, and spindle pole bodies (centrosomes). In the new model, each chromosome is represented by a pair of sister chromatids, and sister kinetochores are represented as chromatid-associated polygons. In preliminary work, we have explored the mechanical properties of kinetochores and their interactions with microtubules that achieve amphitelic spindle attachments at high frequency. A plausible set of minimal assumptions yields simulations that generate chromosome attachment errors, but resolve them, much as normal chromosomes do.

Rebecca Cardim Falcao
University of British Columbia, Canada

Multi-state Diffusion Analysis with Measurement Errors

Single particle tracking is a powerful tool to study the mobility of molecules in the cell membrane.The most common approaches in analyzing these kind of data are mean square displacement and analyses with one or more hidden Markov states. However, in most

experiments, positional measurements contain systematic and random errors, and to achieve proper fits, we must take these errors into account. In this work, we develop a hidden Markov model with multiple diffusive states. Our goal is to estimate the diffusion coefficients and transition probabilities between the different states, incorporating uncertainty due to measurement error in a rational way. We test our methods using simulated data and present results using particle tracks obtained from surface receptor molecules on B cells.

Oana Carja
Stanford/CZ Biohub, San Francisco, USA

Evolution under uncertainty: an adaptive role of stochastic phenotypic variation

Molecular processes are fundamentally stochastic. Randomness is the rule in transcription, translation, cell-to-cell variation in protein levels, and heterogeneity in interactions. One common assumption is that such phenotypic variation is simply noise, and scientists often appeal to the statistics of large numbers when developing deterministic theories, ignoring any potentially adaptive role of epigenetic stochasticity. Yet evidence is accumulating that epigenetic variance constitutes an evolutionary driving force across diverse biological processes, including the persistence of pathogens under drug pressure or the development of cancerous neoplasms. All these systems are fundamentally characterized by high levels of environmental change and uncertainty: either persistent, global, temporal fluctuations in selection pressure, or local, micro-environmental and spatially-defined selective forces. In this talk, I will explore epigenetic strategies for fluctuating selection and the fundamental differences between local triggers and global spread in shaping molecular responses to environmental stochasticity.

Sandeep Choubey
Max Planck Institute for the Physics of Complex Systems, Germany

Decoding the mechanisms of transcriptional dynamics from nascent RNA measurements

Cells constantly face environmental challenges and deal with them by changing their gene expression patterns. They make decisions regarding which genes to express and which genes not to express based on intra-cellular and environmental cues. These decisions are often made by regulating the process of transcription. While the identities of the different molecules that take part in regulating transcription have been determined for a number of different genes, their dynamics inside the cell are still poorly understood. The development of novel single-cell sequencing and imaging techniques, as well as a better exploitation of currently available single-molecule imaging techniques, provides an avenue to interrogate the process of transcription and its dynamics in cells by quantifying the number of RNA polymerases engaged in the transcription of a gene (or equivalently the number of nascent RNAs) at a given moment in time as well as a function of time. In

this talk, I will demonstrate that measurements of the cell-to-cell variability in the number of nascent RNAs provide a probe for deciphering mechanisms of transcription initiation in cells. We propose a simple kinetic model of transcription initiation and elongation from which we calculate nascent RNA copy-number fluctuations. By testing this theory against published nascent RNA data, we gain new mechanistic insights. Our analytical framework can be used to extract quantitative information about dynamics of transcription from single-cell sequencing data, as well as from single-molecule imaging and electron micrographs of fixed cells, and provides the mathematical means to exploit the quantitative power of these technologies.

Jonathan Chubb
University College, London (UCL), England

Origin and implications of transcription bursts

Abstract: TBA

Luca Ciandrini
Université de Montpellier, France

Ribosome economics

Abstract: TBA

Daniel Coombs
University of British Columbia, Canada

Multistate modelling and parameter inference for particle tracking analysis and super-resolution imaging of immune cell surface receptors

T and B cells of the immune system detect and respond to foreign invaders using surface bound receptor molecules. These molecules can be labelled and their motion tracked on an individual basis on live cells, or their positions can be determined en masse and at extraordinary resolution on fixed cells. I will present recent work on quantitative methods for studying single receptor motion. I will also talk about our efforts to assess and quantify receptor clustering, based on super-resolution microscopy experiments.

Khanh Dao Duc
UC Berkeley, USA

Evolution and function of the ribosome exit tunnel

Recent developments in sequencing and electron microscopy have allowed to study the traffic of ribosomes during translation and the details of their molecular structure. By applying various theoretical, statistical and computational approaches to ribosome profiling data and cryo EM structures, I will show how a sub-compartment of the ribosome -the ribosome exit tunnel- plays key roles in regulating the translation dynamics and other major co-translational processes, e.g. protein folding or antibiotic resistance.

Dan Erdmann-Pham
UC Berkeley, USA

Hydrodynamic Limit of the inhomogeneous l-TASEP with open boundaries

The open-boundary totally asymmetric simple exclusion process (TASEP) with extended particles is an interacting particle system on the lattice of length N, where particles of size l are injected at the left, move (under particle exclusion) to the right at site-specific rates, and leave the lattice with at characteristic rates at the end.

Its versatility and tractability have made the TASEP an attractive stochastic process in various communities: While the homogeneous and near-homogeneous 1−TASEP have been explored by mathematicians and physicists in the context of KPZ universality and non-equilibrium phase transitions, more complex versions of it have found application in modeling processes like molecular transport, gene expression and traffic dynamics.

Despite much progress, when particles are of extended size $l > 1$ and move at site-dependent rates (as in most biological processes), theoretically analyzing the behavior of the system and the associated phase diagram has remained elusive. Here we present such an analysis in the hydrodynamic limit. Upon deriving the general PDE satisfied by the density of particles, we characterize the associated phase diagram and show that its boundaries are determined by four quantities only; namely the particle size l, and the first, the last, and the minimum hopping rates along the lattice. For each region of the phase diagram, our closed-form formulas for the flux and site-specific particle density agree well with Monte Carlo simulations.

We will apply our theoretical work on a data set of ribosome profiles measured during protein synthesis, illustrating how the underlying biological mechanisms optimize for production rates and costs by judicious choice of relevant parameters.

Martin Evans
University of Edinburgh, Scotland

Antimicrobial resistance: modelling the efficacy of antibiotic treatment

In order to combat antibiotic resistance it is important to have a basic understanding of

how antibiotics work. In this talk I will describe how coarse-grained statistical physics style models can be used to understand the dynamics of ribosome-targetting antibiotics. Our model reveals a complex interplay exists between physiology and antibiotic action and predicts an emergent classification into bactericidal or bacteriostatic antibiotics which respectively kill microbes or inhibit their growth.The model reveals that growth-dependent susceptibility of bacteria is controlled by a single parameter characterizing the reversibility of antibiotic transport and binding. Remarkably these predictions are quantitatively supported by experimental results.

Reference: Growth-dependent bacterial susceptibility to ribosome-targeting antibiotics, Philip Greulich, Matthew Scott, Martin R. Evans, Rosalind J. Allen, Molecular Systems Biology 11:796 (2015)

Olivier Gandrillon
Ecole Normale Supérieure de Lyon, France

A probabilistic view on GRN inference from single cell data

Abstract: TBA

Gregory Huber
CZ Biohub, USA

Topological Structures in the Endoplasmic Reticulum: Structure, Function and Formation

The endoplasmic reticulum (ER) has long been considered an exceedingly important and complex organelle in eukaryotes. It is a membrane structure, part folded lamellae, part tubular network, that both envelopes the nucleus and threads its way outward, all the way to the cell's periphery. Recently, refined imaging of the ER has recently revealed beautiful and subtle geometrical forms – reminiscent of minimal surfaces and Riemann sheets – that play a crucial role in the organelle's global structure. I'll review the discovery and physics of Terasaki ramps and discuss their relation to cell-biological questions. Rather than being a footnote in a textbook on differential geometry, these structures suggest answers to a number of the ER's structure-function problems.

Charles Kervrann
Inria Rennes, France

Computational methods for fluorescence microscopy and intracellular dynamics analysis

During the past two decades, biological imaging has undergone a revolution in the development of new microscopy techniques that allow visualization of tissues, cells, proteins and macromolecular structures at all levels of resolution. Thanks to recent advances in optics, digital sensors and labeling probes, one can now visualize sub-cellular components and organelles at the scale of a few dozens nanometers to several hundreds of nanometers. As a result, fluorescent microscopy and multimodal imaging has become the workhorse of modern biology. All these technological advances in microscopy, created new challenges for researchers in quantitative image processing and analysis. Therefore, dedicated efforts are necessary to develop and integrate cutting-edge approaches in image processing and optical technologies to push the limits of the instrumentation and to analyze the large amount of data being produced.

In this talk, we present image processing methods, mathematical models, and algorithms to build an integrated imaging approach that bridges the resolution gaps between the molecule and the whole cell, in space and time. The presented statical methods are dedicated to the analysis of proteins in motion inside the cell, with a special focus on Rab protein trafficking observed in time-lapse confocal microscopy or total internal reflection fluorescence microscopy. Nevertheless, the proposed image processing methods and algorithms are flexible in most cases, with a minimal number of control parameters to be tuned. They can be applied to a large range of problems in cell imaging and can be integrated in generic image-based workflows, including for high content screening applications.

Elena Koslover
University of California San Diego, USA

Drift, Wander, or Stick: Disentangling Modes of Organelle Motion in Living Cells

Eukaryotic cells utilize a variety of transport modes to distribute organelles within their cytoplasm. Individual organelles can be carried processively by molecular motors, exhibit random walk motion akin to diffusion, drift via advection in a flowing cytoplasmic fluid, or be tethered to stationary cellular structures. The efficiency and relative contributions of these different modes of transport can be estimated theoretically through a combination of stochastic simulations and physical modeling of the underlying fluid flows and forces that drive each mode. However generating such quantitative models requires the ability to categorize the motion of organelles observed in vivo and to accurately extract the parameters describing their motion. We will discuss how organelle trajectories extracted from fluorescent video microscopy data can be analyzed to gain quantitative insight into the sources of organelle motion.

In particular, we will demonstrate how to disentangle diffusive motion from slowly varying advective drift of lysosomal organelles in deforming motile neutrophils, using a wavelet-based methodology. A substantial contribution of deformation-driven cytoplasmic flow to the mixing of organelles in such cells is demonstrated using a simplified physical model parameterized from in vivo data. Additionally, we will show that diffusion, directed active transport, and tethering to intracellular structures all

contribute to the dispersion of peroxisomes in fungal hyphae, and that much of the seemingly diffusive motion can be accounted for by hydrodynamic entrainment from passing motor-driven organelles. A method for distinguishing tethered from diffusive motion in short particle trajectories will be discussed. Our results highlight the importance of combining together diverse transport mechanisms for distributing organelles through eukaryotic cells, while establishing new approaches for identifying and quantifying the mechanisms at play in experimentally observed organelle trajectories.

Rahul Kulkarni
University of Massachusetts - Boston, USA

Modeling fluctuations and large deviations in stochastic gene expression

In several biological systems, phenotypic variations are seen even among genetically identical cells in homogeneous environments. Recent research indicates that such `non-genetic individuality' can be driven by rare events arising from the intrinsic stochasticity of gene expression. Characterizing the fluctuations that give rise to such rare events motivates the analysis of large deviations in stochastic models of gene expression.

In this talk, I will discuss analytical approaches developed by my group for general stochastic models of gene expression. We combine approaches from queueing theory and non-equilibrium statistical mechanics to characterize large deviations and driven processes for general models of gene expression. Modeling gene expression as a Batch Markovian Arrival Process (BMAP), we derive exact analytical results quantifying large deviations of time-integrated random variables such as promoter activity fluctuations. The results obtained can be used to quantify the likelihood of large deviations, to characterize system fluctuations conditional on rare events and to identify combinations of model parameters that can give rise to dynamical phase transitions in system dynamics. We apply the formalism developed to gain insights into the impact of post-transcriptional regulation on fluctuations and rare events in stochastic models of gene expression.

Eitan Lerner
Hebrew University, Israel

Solving structures of highly dynamic highly heterogeneous conformations of the bacterial transcription initiation complex

In order to study biomolecular mechanisms and rationally design small molecule inhibitorsactivators, it is important to study biological molecules through their structure at atomic resolution. Classical structural biology techniques have allowed the reconstruction of the 3D structure of many important biological molecules at atomic resolution level. However, in order to function properly, biological molecules are able to intrinsically and dynamically interconvert between many different structural forms and topologies at a multitude of rates. One out of a myriad of examples is the case of the bacterial RNAP polymerase complex with a DNA promoter sequence and its ability to initiate DNA

transcription not from a specific site on DNA but rather from many different possible ones. There is currently only one crystal structure of the bacterial transcription initiation complex at this stage of DNA transcription, however, single-molecule Forster resonance energy transfer (smFRET) measurements have shown the complex at this stage dynamically interconverts between two conformational states. In this talk I have shown how the transcription initiation complex dynamically interconvert (in microseconds) between two conformational states, where one is characterized by the existing crystal structure, whereas the other represents an unsolved structure. I will show how by combining structural simulations constrained by spatial constraints derived from multiple single-molecule fluorescence-based measurements, I solve the ensemble structures that have the highest likelihood to represent the second unresolved conformational state of the bacterial transcription initiation complex at atomic resolution. This talk showcases how molecular biophysics and modeling can be combined to resolve realistic representations of dynamic conformations.

Meng Lu
University of Cambridge, England

Direct in-cell observation of structural progression of amyloid-β Arctic mutant aggregation

Hereditary Aβ mutations, such as the Arctic Glu22-to-Gly (E22G) mutation, lead to increased intracellular accumulation of β-amyloid and disease onset at a young age (1, 2). It remains largely unknown, how the Arctic mutation leads to aggressive protein aggregation and increased toxicity. Here, we constructed stable cell models expressing wild-type (WT) and E22G Aβ42 fused to mCherry to study the aggregation kinetics of the Arctic Aβ mutant and its heterogeneous structural forms. Arctic mutant peptides assemble to form fibrils at a much faster rate than WT peptides and rapidly accumulate to form fibril bundles or clusters and later aggresomes. All aggregate species, as revealed by fluorescence-lifetime imaging (FLIM) (3) and 3D Structural Illumination Microscopy (SIM), display a lowered fluorescence lifetime and highly compact structures with a strong affinity among individual fibrils. The aggregates formed by Arctic mutant Aβ are also more resistant to intracellular degradation than their wild-type counterparts.

Robert Murphy
Carneggie Mellon University, USA

Integrating Information from Diverse Microscope Images: Learning and Using Generative Models of Cell Organization

Systems biology efforts require accurate, cell-type specific information about the shape and distributions of subcellular structures and the distributions of proteins and other macromolecules in order to be able to capture and simulate cellular spatiotemporal dynamics. We have developed tools to build *generative* models of cell organization

directly from microscope images of many cells. Our open source system, CellOrganizer (http://CellOrganizer.org), contains components that can build probabilistic generative models of cell, nucleus, organelle and protein distributions. The models also capture heterogeneity within cell populations. A critical challenge in constructing these models is to be able to learn the dependence of these distributions upon each other, i.e., the spatial relationships between different components. As these relationships are learned, generative models can be created from images of different proteins and organelles and then combined to create synthetic cells having many more components than can be imaged together. An important use for these models is to generate synthetic cell shapes and organelle distributions that can be used as geometries for cell simulations. This permits a structured exploration of the dependencies of cellular biochemistry upon cell morphology and organization.

Pierre Parutto
Ecole Normale Supérieure, Paris, France

***Characterization of the dynamics of the endoplasmic reticulum luminal protein calreticulin and the CaV2.1 calcium channels at synapses*.**

Recent developments of super-resolution microscopy techniques allows to image and track different types of individual molecules in living cells with resolutions of tens of nanometers. The theoretical analysis of such trajectories, for characterizing the type of motion and its characteristics, has however not been much developed, a large part of the conducted analyzes remaining static or based on mean squared displacement. Our group previously developed a methodology to analyze trajectories based on the Smoluchowski limit of the Langevin Equation, characterizing local estimators for untangling deterministic forces acting on the molecules from thermal fluctuations. These estimators however rely on large number of redundant trajectories that cannot be attained when only few molecules of a type are used locally by the cell or the molecules exhibit very heterogeneous motion or the geometry of the domain imposes strong constraints on their motion. I will present recent advances of the group where we characterized. 1. a flow motion for the luminal Endoplasmic Reticulum (ER) protein calreticulin made possible by previously reconstructing the ER network from the trajectories and 2. transient arrests of the voltage dependent calcium channels CaV2.1 at active zones of hippocampal synapses and the quantification of confinement times.

E. Peterman
VU University Amsterdam, Netherlands

A single-molecule view on intracellular transport in vitro and in vivo

Our cells and in particular our neurons are too large for thermal-driven diffusion to be an effective means of transport of proteins, organelles and other cargoes. Instead, eukaryotic organisms have evolved motor proteins that drive unidirectional motion over relatively long distances while consuming chemical energy in the form of ATP. Over the last two

decades, important insights into the molecular mechanisms of several key motor proteins of the kinesin, myosin and dynein families have been obtained, among others by the application of advanced single-molecule methods. Over the last five years in my laboratory, the focus has shifted from studying the behavior of single motor proteins, working on their own *in vitro*, to studying motor proteins in their cellular environment. In our cells, motor proteins do not work on their own: cargoes are often transported by multiple motors, of the same type, but often also of opposite directionality. In addition, our cells are a very crowded environment, with many proteins bound to the motors' tracks, which might hamper their motion and could lead to 'traffic jams'.

To study these kinds of problems *in vivo*, we have focused on a particular transport mechanism, intraflagellar transport (IFT), which takes place in cilia and flagella and is essential for the assembly and maintenance of these organelles. As a model system we use IFT in the chemosensory cilia of the nematode *C. elegans*. In these organisms, IFT is driven by groups of tens of three different motor proteins: 2 kinesin-2's (the slow kinesin-II and the fast OSM-3) that drive transport of cargo trains from base to tip of the cilium, and IFT dynein that drives transport back to the base. In order to visualize IFT components with fluorescence microscopy, we generate mutant-nematodes expressing fluorescent versions of the IFT component of interest. Our fluorescence and image analysis approaches allow us to visualize, track and quantify trains of IFT components moving together as well as individual motor or IFT proteins. Together, bulk and single-molecule data provide new, deep insights into the mechanisms of motor cooperation.

To get more insight into the effect of crowding at high motor densities on kinesin motility properties, we performed *in vitro* assays. We developed new analysis techniques based on image correlation to extract motor density, velocity and run length in an automated way. This approach allowed us to elucidate the physical nature of crowding and test TASEP model predictions. Using the kinesin motor proteins kinesin-1 and OSM-3, we identified crowding effects in qualitative agreement with TASEP (totally asymmetric simple exclusion process) predictions, and we achieved excellent quantitative agreement by extending the model with motor-specific interaction ranges and crowding-dependent detachment probabilities. These results confirm the applicability of basic nonequilibrium models to the intracellular transport and highlight motor-specific strategies to deal with crowding.

Steve Pressé
Arizona State University, USA

Novel Statistical Tools for Single Molecule Imaging: A foray into Bayesian Nonparametrics

One route to modeling biophysical dynamics involves the bottom-up, molecular simulation, approach. In this approach, approximate classical potentials are used to simulate short time local motions in order draw insight on dynamics at longer time and larger length scales. Here we take a different route. Instead we present a top-bottom

approach to building models of single molecule dynamics. The approach we present exploits a novel branch of Statistics – called Bayesian nonparametrics (BNPs) – first proposed in 1973 and now widely used in data science as the important conceptual advances of BNPs have become computational feasible in the last decade. BNPs are new to the physical sciences. They use flexible (nonparametric) model structures to efficiently learn models from complex data sets. Here we will show how BNPs can be adapted to address important questions in biophysics directly from the data which is often limited by factors such as finite photon budgets as well as other fluorophore and data collection artifacts. More specifically, we will show that BNPs hold promise by allowing complex spectroscopic time traces (e.g. smFRET, photon arrivals) or images (e.g. single particle tracking) to be analyzed and turned into principled models of single molecule motion – from diffusion to conformational dynamics and beyond.

Stijn Sonneveld
Hubrecht University, Netherlands

Single molecule imaging of mRNA translation regulation in living cells

Regulation of gene expression is essential for all living cells, and the cell uses a multitude of pathways to ensure proper control of gene expression. One central process in gene expression is mRNA translation; the decoding of mRNA into a protein. A better understanding of translation itself and of translational control requires a method that can visualize translation of single mRNAs in living cells.
Here, we report a microscopy-based method that enables visualization of single mRNAs undergoing hundreds of rounds of translation in live cells. This allows us to quantitatively measure initiation and elongation rates of ribosomes on single mRNAs for the first time, revealing a remarkable amount of heterogeneity in their translation dynamics. We further applied this translation imaging method to study gene expression control by small RNAs, which regulate the expression of 1000s of target genes through their association with the effector protein Argonaute (Ago). Ago/small RNA binding to mRNAs results in endonucleolytic cleavage and/or translational repression of the target mRNA. Using our imaging method we directly observed the dynamics of target repression by Ago, which revealed that translation of target mRNA potently stimulates target silencing. Taken together, we present here a powerful tool for observing translation of single mRNAs, and for studying translational regulation by small RNAs.

Devin Sullivan
KTH Royal Institute of Technology, Sweden

Proteome-scale cell cycle dependent dynamics from asynchronous fluorescent microscopy images

One of the most crucial and complex systems within the cell is the process of growth and division known as the cell cycle. Due to its central role in cellular function, disorders in this highly regulated system are often the cause of diseases including cancers, neurodegenerative disease and aging. Understanding the proteins involved in this process can aid in treating these diseases. In this work we have developed a novel model of determining continuous cell cycle position of fluorescent microscopy images of asynchronous cells. We utilize this model to construct continuous pseudo-time models of dynamic responses in protein expression over the cell cycle to understand and predict potential protein-protein interactions.

Juraj Szavits-Nossan
University of Edinburg, Scotland

Mathematical modelling of mRNA translation: old questions and new insights

Abstract: Understanding how codon choice affects mRNA translation is one of the long-standing questions in molecular biology. Recent advances in next-generation sequencing allow us to monitor translation at a single-nucleotide resolution and mathematical modelling is now required to interpret the new data. A standard mathematical model for mRNA translation -- the totally asymmetric simple exclusion process (TASEP) -- has been known for almost fifty years, yet its steady-state solution is still unknown. I will present a power series method for solving the TASEP in the regime of small initiation rate and discuss how the solution can be used to get new insights on translational efficiency in the cell.

Tamir Tuller
Tel Aviv University (TAU), Israel

Modeling and analyzing the flow of molecular machines in gene expression

Gene expression is a fundamental cellular process by which proteins are synthesized based on the information encoded in the genetic material. During this process macromolecules such as ribosomes or RNA polymerases scan the genetic material in a sequential manner.

I will describe various computationalmathematical models for the flow of such macromolecules. These models are can be simulated efficiently and some of them amenable to rigorous mathematical analysis.

I demonstrate how these models can be used to predict various aspects related to the expression levels of genes and to study important biological phenomena such as competition for finite resources, the evolution of genomes, and intracellular traffic jams.

These models can also can be used for optimization of gene expression for various biotechnological objectives.

C. Vestergaard
Institut Pasteur, Paris, France

Mapping spatio-temporal dynamics of single biomolecules at the whole-cell scale

Abstract: TBA

Romain Yvinec
INRA Tours, France

Stochastic single gene expression model: analytical results on bursting models

In this talk, I will present stochastic models of self-regulating gene expression. I will present adiabatic limiting procedure that allows to reduce the dimension of the models (Yvinec et al. (2014)). Some of the limiting models obtained are piecewise deterministic Markov models (PDMP), and faithfully reproduce the so-called bursting phenomena. I will show that these reduced models can be studied in details: asymptotic convergence, bifurcation, switching times, and eventually parameter estimation through inverse problem (Mackey et al. (2011, 2013)). I will explain how such study may have implication on the understanding on gene regulation mechanism.

References

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