Spetsai Summer School 2017

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Spetsai Summer School 2017

We would like to thank our main sponsors:

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Practical Information

DATES AND VENUE

24 September - 1 October 2017

Spetses Hotel, 180 50 Island of Spetses, Greece.

Website http://www.spetsai.org/

THE ISLAND OF SPETSES

The Island of Spetses is a relatively isolated and car-free island, which lies at the entrance to the Argolic Gulf, 88 kilometers southwest of Athens and 2 kilometers from the mainland of the Peloponnese.
The Spetses Summer School provides an ideal framework for optimal interactions between students and teachers and among students themselves.

Scientific Program

SUNDAY SEPTEMBER 24

Arrival

All day	Registration
Evening	Welcome Reception with light dinner

MONDAY SEPTEMBER 25

Interactions in Cellular Networks: Systems Biology Approaches

09:00-10:00	**Uri Alon: 1.** How to Study Biological Networks and Circuits
10:00-10:15	Coffee Break
10:15-11:15	**Uri Alon:** 2. Evolutionary Tradeoffs and the Geometry of Phenotype Space
11:30-13:00	Poster Session I
13:00-14:15	Lunch
14:30-16:00	Poster Session II
16:00-17:00	**Anne Claude Gavin:** 1. Measuring Protein-Ligand Interactions
17:00-17:15	Break
17:15-18:30	Proposal planning I
18:30	Dinner

TUESDAY SEPTEMBER 26

Studying Proteins and Their Interactions: Systems Biology, Structural Biology and Drug Discovery

09:00-10:00	**Anne Claude Gavin: 2. EMBO Lecture:** Biological Networks in Health and Disease
10:00-10:30	Coffee Break
10:30-11:30	**M. Madan Babu:** 1. Introduction to Unstructured Proteins
11:30-11:45	Break
11:45-12:45	**M. Madan Babu:** 2. Unstructured Proteins: Cellular Complexity and Diseases
13:00-15:00	Lunch and Free Time for Collaborative Project Proposals
15:00-16:00	**Tom Blundell:** 1. Multiprotein Systems in Space and Time: Increasing Complexity to Achieve Selectivity Through Stages, Scaffolds, and Strings
16:00-16:15	Coffee Break
16:15-17:15	**Tom Blundell:** 2. Multiprotein Systes in Space and Time: Targeting Protein-Protein Interfaces Using Fragment-Based Drug Discovery
17:30-18:30	Proposal planning II
18:30	Dinner
21:00-22:00	**Tom Blundell:** Science, Society, and Politics: Experiences of a Permanent Gap-Year Student

WEDNESDAY SEPTEMBER 27

Studying proteins and their interactions: Biological Data Science

09:00-10:00	**Martin Krzywinski:** 1. Essence of Statistics
10:00-10:30	Coffee Break
10:30-11:30	**Martin Krzywinski:** 2. Improving Your Visual Science Communication
11:45-12:45	Panel Discussion: Science Careers
12:45-14:00	Lunch
14:00-18:30	Workshop with Ensemble Kartoshkes: Science performance and communication"
18:30	Dinner

THURSDAY SEPTEMBER 28

Studying Proteins and Their Interactions: Cell Biology and Biophysical Approaches

09:00-10:00	**Stephen Michnick:** 1. Mechanisms and consequences of Macromolecular Phase Separation
10:00-10:30	Coffee Break
10:30-11:30	**Stephen Michnick:** 2. The Phases and Dephases of Endocytosis
11:30-11:45	Break
11:45-12:45	**Lene Oddershede:** 1. Single Molecule Force Spectroscopy
13:00-14:00	Lunch
14:15-15:15	Afternoon Tutorials I
15:30-16:30	**Lene Oddershede:** 2. Cellular Mechanics in Health and Disease
16:30-16:45	Coffee Break
16:45-17:15	**Clemens Kaminski:** 1. A Short History of the Optical Microscope
17:15-17:30	Break
17:30-18:30	**Clemens Kaminski:** 2. Nanoscale Imaging of Neurotoxic Proteins
18:30	Dinner

FRIDAY SEPTEMBER 29

Studying Proteins and their Interactions: Biophysical Approaches and Genetics

09:00-9:30	**Gabriella Kaminski Schierle: 1.** Towards a Molecular Understanding of Protein Misfolding Using an All-Optical Approach
09:30-09:45	Break
9:45-10:45	**Gabriella Kaminski Schierle:** 2. Towards a Molecular Understanding of Protein Misfolding Using an All-Optical Approach
10:45-11:00	Coffee Break
11:00-12:00	**Daniel Otzen:** 1. Protein Stability at the Individual and Global Level
12:00-12:15	Break
12:15-13:00	**Daniel Otzen:** 2. Functional Protein Self-Assembly
13:00-14:15	Lunch
14:15-15:15	Afternoon Tutorials II
15:30-16:30	**Song Yi:** 1. Fundamentals of Machine Learning and Application to Network Analysis
16:30-16:45	Coffee Break
16:45-17:45	**Nidhi Sahni:** 2. Linking Genotype to Phenotype Through Cellular Networks
17:45-18:30	Preparation of oral student proposal presentations and feedback from lecturers on other proposals
17:45-18:30	Dinner

SATURDAY SEPTEMBER 30

Studying Proteins and Their Interactions: Cell Biology and Biophysical Approaches

09:00-10:00	**Carol Robinson:** 1. Mass Spectrometry – From Folding Proteins to Rotating Motors
10:00-10:30	Coffee break
10:30-11:30	**Carol Robinson:** 2. Mass Spectrometry and Its Role in Drug Discovery
11:30-11:45	Break
11:45-12:45	**Carol Robinson:** Women in Science
12:45-15:30	Lunch and Free Time
15:30-17:30	Student Presentations of Collaborative Projects
Evening	Farewell Party

SUNDAY OCTOBER 1

Departure

List of Lecturers and Abstracts

Anne-Claude Gavin, gavin@embl.de
Senior group leader Structural and computational biology Unit
European Molecular Biology Laboratory, Germany

Scientific interests: Protein-protein interaction, lipid metabolism, protein complexes. biological membranes, proteomics

Carol Robinson, cvrpa@chem.ox.ac.uk
Dr Lee's Professor of Chemistry
University of Oxford, United Kingdom

Scientific interests: Mass spectrometry, membrane proteins, lipid complexes, GPCR's

Clemens Kaminski, kaminski.clemens@gmail.com
Professor of Chemical Physics
Dept. of Chemical Engineering and Biotechnology, University of Cambridge

Scientific interests: Development of Optical Microscopy techniques to investigate molecular mechanisms of disease

Daniel Otzen, dao@inano.au.dk
Professor of Nanobiotechnology
Interdisciplianary Nanoscience Center, Aarhus Univeristy

Scientific interests: Protein stability, folding kinetics, misfolding and self-assembly, bacterial amyloid.

Gabriella Kaminski Schierle, gsk20@cam.ac.uk
Lecturer in Molecular Biotechnology
Department of Chemical Engineering and Biotechnology,
University of Cambridge

Scientific interests: Neuroscience, Molecular mechanisms, Alzheimer's, Huntington's, and Parkinson's disease

Lene Oddershede, oddershede@nbi.ku.dk
Professor, StemPhys Center Leader,
Niels Bohr Institute, University of Copenhagen

Scientific interests: Physics of living matter from the single molecule to whole cell level with focus on the dynamics and evolution of stem cells and their relation to cancer.

Martin Krzywinski, martink@bcgsc.ca
Staff Scientist
Canada's Michael Smith Genome Sciences Centre

Scientific interests: Data visualization, information design, science communication, intersection of art and science

M. Madan Babu, madanm@mrc-lmb.cam.ac.uk
Programme Leader,
MRC Lab of Molecular Biology, Cambridge, UK

Scientific interests: Systems Biology, Disordered Proteins, GPCRs, Networks, Data Analysis, Machine Learning, Protein Science, Transcription, Evolution

Nidhi Sahni, NSahni@mdanderson.org
Assistant Professor, The University of Texas MD Anderson Cancer Center

Scientific interests: disease mutations, systems biology, signal transduction, molecular interactions, cancer, immunology

Song Yi, SYi2@mdanderson.org
Faculty Member, The University of Texas MD Anderson Cancer Center

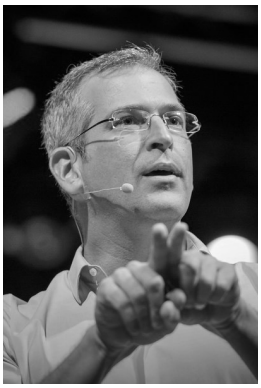
Scientific interests: computational systems biology, bioinformatics, signalling network, disease pathways, immunology, mutations

Stephen Michnick, stephen.michnick@umontreal.ca
Professor of Biochemistry
University of Montreal, Canada

Scientific interests: Biochemical networks, physical basis of cellular molecular organization and mechanical properties.

Tom Blundell, tom@cryst.bioc.cam.ac.uk
Professor Emeritus
University of Cambridge, United Kingdom

Scientific interests: Structural biology of complex multi-protein regulatory systems, Biocomputing and drug discovery.



Uri Alon, urialonw@gmail.com
Professor
Weizmann Institute of Science

Scientific interests (5-10 words): Biological networks and circuits using a combined experimental and theoretical approach

ANNE-CLAUDE GAVIN

Measuring Protein–Ligand Interactions

Eukaryotic cells use membrane-bounded organelles with unique lipid and protein compositions to regulate and spatially organize cellular functions and signalling. As part of this tight control, many proteins are regulated by lipids. In humans, the importance of these regulatory circuits is evident from the variety of disorders arising from altered protein–lipid interactions, which constitute attractive targets for pharmaceutical drug development. However, the full repertoire of interactions remains poorly explored and exploited because their detection is still difficult to achieve on a large and systematic scale. I will present pioneering technologies such as affinity-purification (AP)-lipidomics and the liposome microarray-based assay (LiMA) that will enable protein-lipid interactions to be deciphered on a system level. I will also illustrate the importance of these methods with two pilot studies conducted in yeast and human cell lines. They revealed surprising insights, such as the discovery of a new, conserved family of oxysterol-binding protein (OSBP) with unexpected specificities for phosphatidylserine, an important signaling lipid. The datasets also reveal cooperativity as a key mechanism for membrane recruitment of pleckstrin homology (PH) domains.

CAROL ROBINSON 1

Mass Spectrometry – From Folding Proteins to Rotating Motors

During the early days of electrospray mass spectrometry our attention was drawn to the remarkable observations that showed that individual proteins could retain elements of their folded structure following transition from solution to gas phase [1]. These early discoveries led to the detection of folding ligands within the GroEL molecular chaperone [2] and highlighted the potential for large macromolecular complexes to traverse custom built mass spectrometers[3].

Two decades after the introduction of electrospray for the analysis of proteins the mass spectra of intact ribosomes have become almost routine. These 2.5 MDa particles remain intact during their flight through the mass spectrometer yielding new information about the stoichiometry of subcomplexes and the effects of modifications[4].

Knowledge of the intact mass of a protein or complex is only one part of the mass spectrometry information available however. Data from the disruption of protein complexes is leading to subunit interaction maps and architectural models[5]. Such models are enhanced by coupling with ion mobility mass spectrometry in which the collision cross-section of a protein complex can be defined. In the first demonstration of this method a ring–shaped undecameric protein complex was shown to retain its overall topology following the phase transition from solution to gas phase[6]. Together with chemical crosslinking and hydrogen deuterium labelling techniques new models can be proposed for even the most intransigent protein complexes.

During the last 10 years, with the discovery of experimental conditions that allow mass spectrometry of membrane embedded macromolecular complexes, new information and hypotheses are emerging about the effects of lipid binding[7,8]. These new insights can take the form of lipids involved in mediating drug efflux in drug resistance mechanisms, competing with substrate binding sites in lipid flippase reactions and potentiating oligomeric states in G-protein coupled receptors.

In this lecture, I will trace the developments in mass spectrometry that have made possible the transition from determining the mass of an individual protein to elucidating the structures and dynamics of large assemblies such as rotary ATPases as well as other membrane complexes.

References

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7. Laganowsky, A. et al. Membrane proteins bind lipids selectively to modulate their structure and function. Nature 510, 172-175, doi:10.1038/nature13419 (2014).
8. Gupta, K. et al. The role of interfacial lipids in stabilizing membrane protein oligomers. Nature 541, 421-424, doi:10.1038/nature20820 (2017).

CAROL ROBINSON 2

Mass Spectrometry and Its Role in Drug Discovery

Ever since the first mass spectra of non-covalent complexes, involving proteins and their ligands, the potential to use mass spectrometry for drug discovery has been apparent. Early examples include identifying ligands that stabilise tetrameric transthyretin [1] and crosslink decameric forms of C-reactive protein [2] as a means of combatting amyloidosis and heart disease respectively.

Initial concerns over the nature of interactions have given way to greater understanding of the contributions of different molecular forces to the survival of non-covalent protein ligand complexes in the gas phase. This understanding lays the foundations for current studies that enable binding stoichiometries, K_D values and cooperative binding modes to be assessed [3].

In addition to protein ligand interactions mass spectrometry is readily applied to the study of antibody or nanobody complexes, particularly in defining overall binding stoichiometries [4]. Moreover, through the use of hydrogen exchange labelling techniques, different epitopes can be distinguished for the same antigen.

New developments that enable membrane protein complexes to be transported in 'detergent clouds', releasing naked membrane protein complexes only once in the gas phase, are enabling study of antibiotic targets and uncovering ways in which drugs penetrate the membrane bilayer [5,6]. Particularly exciting is the cooperativity we have uncovered for lipid and drug binding events that mediate conformational changes in drug efflux pumps [7].

One of the problems with drug binding to membrane targets is the potential for off-target binding. This often arises as drugs 'hitchhike' on other transporters and interfere with proper processing mediated by these membrane assemblies in vivo. A case in point is the 'off target' binding of anti-HIV protease drugs to the membrane embedded human metalloprotease ZMPSTE24 [8]. In this case we were able to monitor processing of a peptide, derived from lamin, and to provide the first molecular evidence that drug binding prevented critical processing.

The advent of ion mobility mass spectrometry, with its ability to measure the collision cross section of proteins and their assemblies, has meant that conformational changes upon ligand binding can now be assessed and ligands ranked according to their ability to resist unfolding [9]. Such approaches have enabled us to identify a wide range of lipids that stabilise membrane proteins, including those that are important for structure and function [10]. The critical effects of lipids have prompted our investigations into detergent-free delivery of complexes for mass spectrometry and our interest in obtaining assemblies directly from membranes.

In closing, I will present data on some of our most challenging targets to date – the G-protein coupled receptors, which depend heavily on their lipid environment for structure and function. Overcoming these difficulties was a major challenge but is now enabling drug binding [11] and coupling to be studied in entirely new ways.

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CLEMENS KAMINSKI 1

A Short History of the Optical Microscope

The development of the optical microscope is intricately linked to the history of modern science. Instrument makers in the 17th century perfected the art of making high power lenses [1]. Simultaneously, the wave theory of light propagation progressed at a rapid pace [2]. Anatomists and medics made ground breaking discoveries on matters of life itself [3] and their needs drove theoreticians and experimentalists to some of the greatest scientific discoveries. It may come as a surprise that despite the continuous progress in the field now for over 400 years there is no decline in the pace at which the field is progressing. On the contrary, several Nobel prizes were awarded over the last 10 years in the field of optical microscopy [4]. Today we can see things much smaller than the wavelength of light and we can do this inside living cells with molecular sensitivity and specificity, a concept unthinkable only 15 years or so ago. In this talk I will take you through the history of the light microscope from the days of Robert Hooke to modern instruments, and give you an introduction to the physical principles behind state of the art techniques. Finally, I will show some examples of our own work from efforts to unravel molecular mechanisms of human diseases.

References:

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4. The Nobel Prize in Chemistry 2014, Eric Betzig, Stefan W. Hell, William E. Moerner (http://tinyurl.com/yatefykm)

CLEMENS KAMINSKI 2

Nanoscale Imaging of Neurotoxic Proteins

The self-assembly of proteins into ordered macromolecular units is fundamental to a variety of diseases. For example, in Alzheimer's Disease (AD) and Parkinson's Disease (PD), proteins that are usually harmless are found to adopt aberrant shapes; one says they 'misfold'. In the misfolded state the proteins are prone to aggregate into highly ordered, toxic structures, called protein amyloids and these make up the insoluble deposits found in the brains of patients suffering from these devastating disorders. A key requirement to gain insights into molecular mechanisms of disease and to progress in the search for therapeutic intervention is a capability to image the protein assembly process in situ i.e. in cellular models of disease.

In this talk I will give an overview of research to gain insight on the aggregation state neurotoxic proteins in vitro (1, 2), in cells (3, 4, 5) and in live model organisms (5). In particular, we wish to understand how these and similar proteins nucleate to form toxic structures and to correlate such information with phenotypes of disease (4). I will show how direct stochastic optical reconstruction microscopy, dSTORM, and multiparametric imaging techniques, such as spectral and lifetime imaging, are capable of tracking amyloidogenesis in vitro, and in vivo, and how we can correlate the appearance of certain aggregate species with toxic phenotypes of relevance to PD and AD (6-8).

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5. Kaminski Schierle, et al, ChemPhysChem (2011)
6. Michel, et al, JBC (2014)
7. Pinotsi, et al, PNAS (2016)
8. Murakami, et al, Neuron (2015)

DANIEL OTZEN 1

Protein Stability at the Individual and Global Level

Proteins are the most versatile components in the cell and key to life. Critical to their function is their thermodynamic stability (robustness or resistance to unfolding) and the strength of their interactions with (i.e. affinities to) other proteins - collectively abbreviated PS-SPI. Protein stability determines how close in energy the proteins are to losing their native structure and thus their biological function; in turn, that biological function is normally associated with their ability to bind other (folded) proteins. The stabilities of individual proteins [1-3]and protein complexes [4] have been studied in vitro for decades, largely by spectroscopy and calorimetry. Yet these studies ignore the influence of other cell components and the environment in general. We need to understand the biological function of human proteins by determining their physical interactions, how stability influences interactions and vice versa and how these aspects in turn influence human health and disease. For this we need innovative technologies that allow systematic proteomic identification and investigation of the many functionally diverse protein complexes present in a typical human cell, i.e. a quantitative proteome-level understanding of PS-SPI. This remains a challenge but we there are exciting developments taking place these years and my talk will provide some impressions of these efforts. Currently there are no cell-wide methods that accurately measure affinity [15], and measurement of individual interactions in vivo require either purification of components or laborious labelling strategies [6]. Peptide arrays provide some information but need to be followed up by direct measurements [7]. Current approaches to analyze protein stability at the proteomic level rely on irreversible aggregation [8], biological degradation [9] or susceptibility to protease attacks [10] and do not provide true (thermodynamic) stability. I wil also discuss new approaches that combine electrophoresis with mass spectrometry to potentially provide more direct thermodynamic stabilities.

References:

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DANIEL OTZEN 2

Functional Protein Self-Assembly

Although amyloid is often associated with debilitating diseases such as Alzheimer's, Huntington's and Parkinson's, there is a growing number of examples of beneficial use of amyloid in all walks of life. In the bacterial world alone, a significant proportion of sampled species appear to produce functional bacterial amyloid (FuBA) [1], which may serve multiple purposes such as surface adhesion, biofilm formation, enhanced surface hydrophobicity, extracellular casing or strengthening of the cell wall [2, 3] and even bacterial warfare. FuBA proteins are generally extremely robust and can resist boiling SDS solutions, though they can still be degraded by proteases. They are evolutionarily optimized to form amyloid structures and thus represent an excellent example of "organized complexity". As monomers, they assume a random coil structure in solution but show an extremely high propensity to self-assemble and form amyloid under a wide range of conditions. Biogenesis is under tight spatio-temporal control, thanks to a simple but efficient secretion system which in E. coli, Pseudomonas and other well-studied bacteria includes a major amyloid component that is kept unfolded in the periplasm thanks to chaperones, threaded through the outer membrane via a pore protein [4] and anchored to the cell surface through a nucleator and possibly other helper proteins. In these systems, amyloid formation is promoted through imperfect repeats which appear to form stacked β-hairpin structures [5], but other evolutionarily unrelated proteins either have no or only partially conserved repeats [6] or simply consist of small peptides with multiple structural roles. This makes bioinformatics analysis challenging, though the sophisticated amyloid prediction tools developed from research in pathological amyloid, e.g. TANGO [7] and AGGRESCAN [8], together with the steady increase in identification of amyloid will strengthen genomic data mining. Functional amyloid represents an intriguing source of robust yet biodegradable materials with new properties, when combining the optimized self-assembly properties of the amyloid component with e.g. peptides with different binding properties or surface-reactive protein binders [9]. Sophisticated patterns can also be obtained by co-incubating bacteria producing different types of amyloid, while amyloid inclusion bodies [10] may lead to slow-release nanopills.

References

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GABRIELLA KAMINSKI SCHIERLE

Towards a Molecular Understanding of Protein Misfolding Using an all Optical Approach

Proteins have an intrinsic propensity to self-assemble into aggregates. The process is initiated by the formation of an aggregation-nucleus from the native monomers. Usually aggregation corresponds to the association of proteins, whose polypeptide chain is folded into a structural state that is non-functional; one says the protein is in a 'misfolded state'. Aggregation thus propagates the misfolded protein state and this phenomenon is at the heart of a number of so-called 'protein misfolding diseases'(1). A key problem in misfolding diseases is that normal protein homeostasis is disrupted in the cell, as aggregation lowers the propensity of the protein pool to remain soluble. Small oligomeric species may furthermore elicit toxicity directly, through interference with vital metabolic processes in the cell. These problems are at the heart of major neurodegenerative diseases, such as Alzheimer's Disease (AD) and Parkinson's Disease (PD), in which usually harmless, soluble proteins self-assemble into highly regular, fibrillar aggregate structures called amyloids(2). In AD and PD, amyloids are formed from intrinsically disordered proteins (IDPs), i.e. those that normally lack defined secondary and tertiary structures under physiological conditions. In response to changes in the molecular environment, or due to point mutations in their peptide sequence, IDPs partially fold and adopt metastable β-sheet conformations. These partially folded states self-assemble into oligomers, which then gradually convert into stable, β-sheet rich conformations, thus forming aggregation nuclei, from which elongation into the fibrillar amyloid state then proceeds through consecutive monomer addition(3). In both AD and PD, aggregation appears to be initiated in certain, well defined regions in the brain, before the gradual spreading of aggregated species takes place throughout the entire brain. In the final stages of the disease, a significant fraction of the brain mass of affected patients is replaced by insoluble solid plaques, which are predominantly composed of fibrillar amyloids of the proteins tau and amyloid β (Aβ) in Alzheimer's, and the protein α-synuclein in the case of Parkinson's Disease.

Thus, understanding the formation and structural characteristics of amyloid fibrils is vital for the development of therapeutics for many neurodegenerative disorders. We have developed a fluorescence-lifetime based aggregation sensor which can readily be applied in vitro and in vivo, permitting the screening of small molecule drugs against protein aggregation(4–6). In order to characterise amyloid fibril growth, we apply super-resolution imaging and demonstrate that there is heterogeneity in the growth rates of individual amyloid fibrils which can be attributed to structural polymorphism(7, 8). We also show that Tau and heparin form composite fibrils and thus structurally prevent seeding.

In order to understand the normal physiological and pathological function of alpha-synuclein, a protein linked to Parkinson's disease, we have applied a combination of optical and NMR spectroscopy techniques. We have discovered that alpha-synuclein acts as a calcium sensor at the pre-synapse. In particular, we show that a sub-group of alpha-synuclein-positive vesicles at the pre-synapse is specifically responsive to changes in calcium concentrations. This is in contrast to VAMP-2 positive vesicles which do not form localised clusters at the pre-synapse upon calcium exposure. NMR data indicate that this calcium sensing capacity of alpha-synuclein is mediated via the negatively charged C-terminus, which, upon calcium binding, directly interacts with synaptic vesicles. We further demonstrate that the alpha-synuclein calcium sensor needs to be finely tuned as increased levels of calcium over a prolonged time in the presence of alpha-synuclein can directly result in pathology. Similarly, using a cell model of Parkinson's disease, toxicity can either be prevented by a decrease in the level of alpha-synuclein or by isradipine, a voltage-gated calcium channel inhibitor.

In summary, by applying primarily optical techniques we have shed light on amyloid protein function, aggregation kinetics and structural characteristics which together may help to develop therapeutic strategies against various forms of neurodegenerative diseases.

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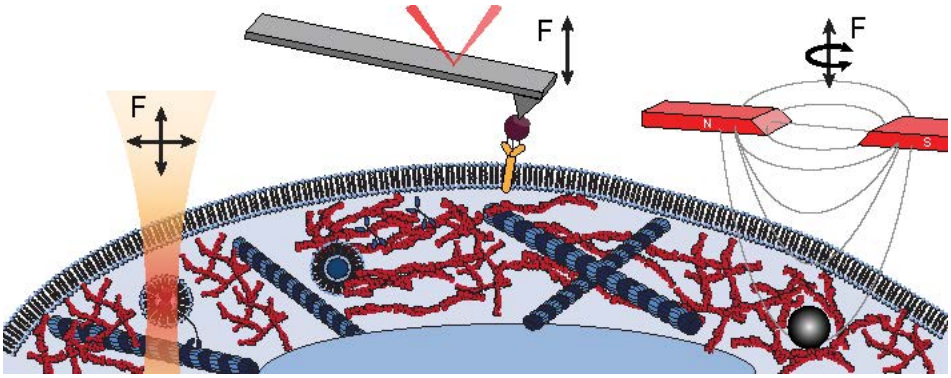
LENE ODDERSHEDE 1

Single Molecule Force Microscopy

The bio-molecule is among the most important building blocks of biological systems and a full understanding of its function forms the scaffold for describing the mechanisms of higher order structures as organelles and cells. Force is a fundamental regulatory mechanism of bio-molecular interactions driving many cellular processes. The forces on a molecular scale are exactly in the range that can be manipulated and probed with single molecule force spectroscopy [1]. Significant progress has been made by performing force spectroscopy on single molecule in an in vitro environment [2]. The in vitro setting also has the advantage that the effect of a single parameter can be investigated in a systematic manner. However, the natural environment of a bio-molecule is inside a living cell, hence, this is the most relevant environment for probing their function. In vivo single molecule force spectroscopy is currently being realized, however, challenged by the complexity of the cell [3].

The development of different modalities of force spectroscopies, and their successful use, is an excellent example of how collaboration across different disciplines, in this case physics and biology, has fostered the development of novel techniques to uncover exciting science. This presentation will present the most commonly used single molecule force spectroscopy techniques, namely optical tweezers, magnetic tweezers, and atomic force microscopy. The strengths and limitations of each of these wide-spread and successful techniques will be described and related to in vivo usage. As optical tweezers is the only tool capable of manipulation and measuring inside a living organism without perturbing the membrane and without the need for external labelling, most emphasis will be put on this technique and there will be a hands-on simulation session during the presentation. Also, novel techniques such as acoustic-based force spectroscopy [4] will be presented. As an alternative to techniques that measure mechanical forces directly, there exists fluorophores whose emission is correlated with the force applied to them [5], these types of techniques will also be presented. With all these force-measuring techniques, the scene is set for quantifying and understanding the role of cellular mechanics in health and disease.



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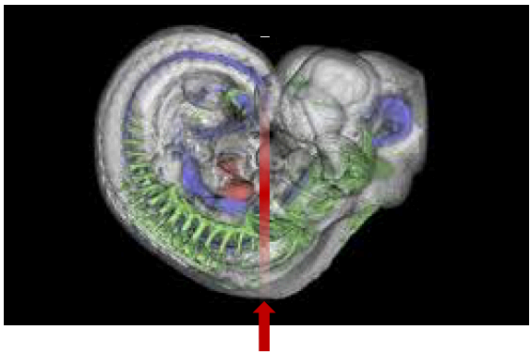
LENE ODDERSHEDE

Cellular Mechanism in Development and Disease

To understand the basics of life, it is essential to study the mechanics and function of single molecules. Action-reaction mechanisms at the single molecule level provide information that can be up-scaled to explain functional properties of organelles, the entire cell, and even of the whole organism. Also, as many diseases originate from failure at the single molecule level, for instance by the malfunction of a misfolded protein, a deeper understanding of action-reaction mechanisms might help deciphering the origin of the disease. Certain diseases are known to cause changes in the physical and biomechanical properties of cells [1]. These include cancer, malaria, and sickle cell anemia among others [2,3]. Typically, physical changes of the cells' mechanical properties can result in several fold increases or decreases in cell membrane stiffness or cytoplasmic visco-elasticity and it has been shown that the aggressiveness of cancer cells correlates with the forces exerted by the individual cell [4]. Such changes of biomechanical properties are significant and can result in severe pathology and eventual catastrophic breakdown of the bodily functions. The significant changes in biomechanical properties of cells related to onset of diseases may serve as a way to quickly diagnose the disease.

It is becoming more and more evident that biomechanics is not only crucial for diseased cells, it is also one of the factors governing the development of living organisms and it has been shown, using embryonic stem cells, that early in development local small forces may have important roles in development [5,6]. Also, the differentiating cells themselves change their mechanical properties significantly as they specialize [7] and this property can also serve as a bio-marker for determining the state of a given cell.

In this presentation, we will focus on the role of biomechanics in disease and during development. Research into biomechanics can give us an in-depth knowledge of the mechanisms underlying differentiation, development and disease progression, and can also serve as a powerful tool for detection and diagnosis. Through the methods outlined in the previous talk, it is now possible to perform mechanical manipulation inside whole living organisms, e.g., optical manipulation inside developing zebrafish embryos and AFM manipulation inside the brains of developing larvae [8], thus providing important information regarding the role of biomechanics during disease and development.



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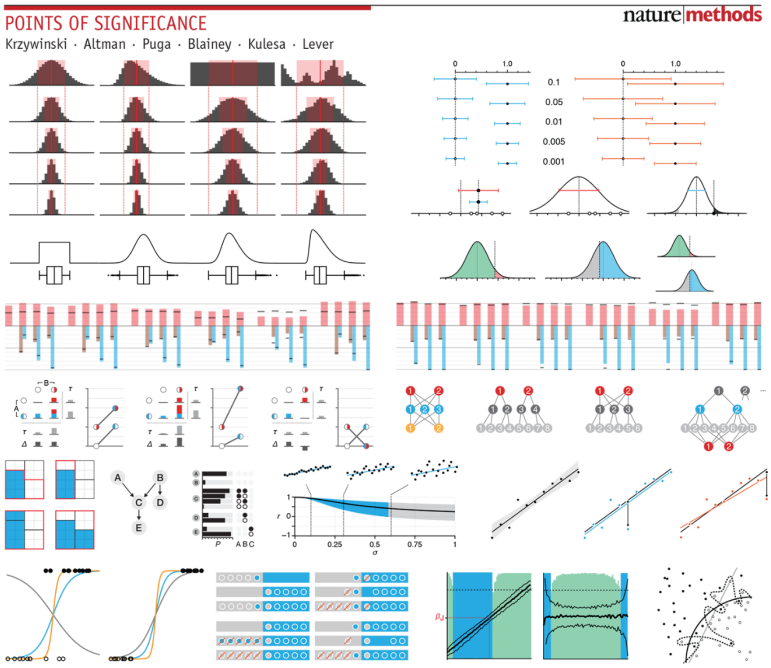
MARTIN KRZYWINSKI 1

Essence of Statistics

When you repeat an experiment, you almost never get the same result. Instead, observations span a range of values because of biological variability and limits in your equipment. You should then ask: “If results are different each time, how do I know whether a measurement is compatible with my hypothesis?”



Statistics helps us answer this question by quantitatively modelling the role of chance in your experiment and representing data not as precise measurements but as estimates with error. The practical application of this theoretical framework is to associate uncertainty to the outcome of experiments and to assign confidence levels to statements that generalize beyond observations. In other words, in Huxley's quote from my other abstract, "The great tragedy of Science—the slaying of a beautiful hypothesis by an ugly fact," it helps you to measure this "ugliness".

Although many fundamental concepts in statistics can be understood intuitively, you must recognize the limits of your intuition when thinking about chance and probability. The Monty Hall problem is a classic example of how the wrong answer can appear too quickly and too credibly before your eyes. When a solution was published in Parade magazine, thousands of readers (many with PhDs) wrote in that the answer was wrong—they trusted their intuition more than their calculation. Comments varied from “You made a mistake, but look at the positive side. If all those PhDs were wrong, the country would be in some very serious trouble” to “I must admit I doubted you until my fifth-grade math class proved you right”.

In this talk I will share with you my experience of explaining the essence of statistical thinking accumulated over the course of writing over 30 Points of Significance columns, which appear in Nature Methods. The columns are aimed at helping you move beyond an intuitive understanding of fundamental statistics relevant to your work. I’ll use examples from both descriptive and inferential statistics, such as sampling, testing, P-values and confidence intervals, replication, classification and model assessment. I’ll also point out what we can learn from Bayesian approaches.

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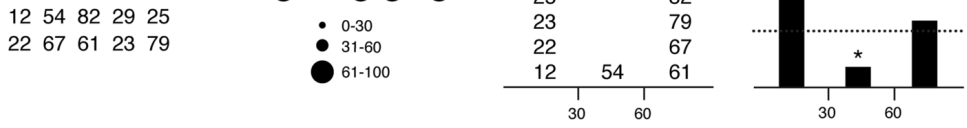
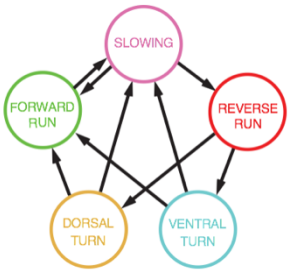
MARTIN KRZYWINSKI 2

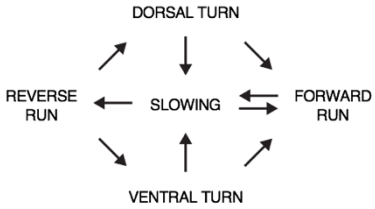
Improving Your Visual Science Communication

Well-designed figures can illustrate complex concepts and patterns that may be difficult to express concisely in words. Figures that are clear, concise and attractive are effective–they form a strong connection with the reader and communicate with immediacy. These qualities can be achieved by employing principles of graphic design, which are based on our understanding of how we perceive, interpret and organize visual information.

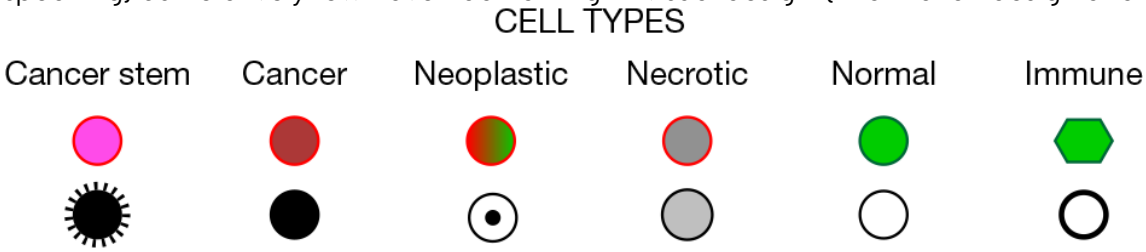


Because figures often act as a first explanations, it's critical to distinguish essentials from details and merely interesting tangents. While everything may indeed be important, initially some things are more important than others. Classifying aspects of the science this way always feels risky—how do I know that I know enough to justify leaving things out?





We all use words to communicate information–our ability to do so is extremely sophisticated. We have large vocabularies, understand a variety of verbal and written styles and effortlessly parse errors in real time. But when we need to present complex information visually, we may find ourselves at a 'loss for words', graphically speaking.
Do images and graphics possess the same qualities as the spoken or written word? Can they be concise and articulate? Are there rules and guidelines for visual vocabulary and grammar? How can we focus the viewer's attention to emphasize a point? Can we modulate the tone and volume of visual communication? These and other questions are broadly addressed through design, which is the conscious application of visual and organizational principles to communication. All of us have already been schooled in 'written design' (grammar) and most of us have had some experience with 'verbal design' (public speaking) but relatively few have had training in 'visual design' (information design and visualization).



This talk distills core concepts of information design into practical guidelines for creating scientific figures. I will explain how to generate visual vocabularies with graphical elements, combine them into phrases, and package them into a complete figure, the visual equivalent of a paragraph. We show how effective designs for this composition are underpinned by conclusions from studies in visual perception and awareness.
Don't forget: an exhaustive explanation is an exhausting one. Aim to leave your readers energized and motivated to continue to conversation, which should flow naturally beyond the scope of the design. They can always ask for more but they cannot ask for less.
Participants are encouraged to send me (martink@bcgsc.ca) one of their particularly vexing figures (EPS, PDF or AI) for redesign and inclusion in the talk or to work on together during the week.

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M. MADAM BABU 1

Our understanding of protein function has been predominated by the view that proteins need to adopt a defined three-dimensional structure to be able to carry out their function. Indeed, crystal structures of numerous proteins have been instrumental in establishing the structure–function paradigm. For example, the structures of numerous enzymes have highlighted the need for particular chemical groups to be positioned in spatial proximity in order to catalyse specific reactions. While this concept is absolutely fundamental for a large number of proteins, research over the last decade has identified segments in proteins that do not adopt a specific three-dimensional structure but are nevertheless important for function. This suggests that conformational flexibility and heterogeneity might be important for certain types of protein function. Such segments, usually referred to as intrinsically disordered regions (IDRs), may either fold upon binding to the right interaction partner, or may still remain disordered in a complex.

While there was scepticism from many researchers about the existence and the functional relevance of IDRs, exciting developments over the last decade have unambiguously established the functional contribution of disordered segments. Importantly, IDRs are prominent in the proteomes of all studied organisms. In the first lecture, I will provide a general introduction to intrinsically disordered regions and highlight the benefits and potential danger of having such segments in proteins. I will then discuss how altered expression of proteins containing IDRs can result in cancer and protein aggregation diseases. In the second lecture, I will highlight the general principles by which such segments mediate interactions and discuss how regulatory mechanisms such as alternative splicing and asymmetric mRNA localisation make use of disordered regions to increase cellular complexity. I will also discuss how gene fusions involving disordered proteins can cause disease such as cancer.

Introduction to Unstructured Proteins

Proteins containing intrinsically disordered regions (IDPs) are enriched in signalling and regulatory functions because disordered segments permit interaction with several proteins and hence the re-use of the same protein in multiple pathways. Understanding IDP regulation is important because altered expression of IDPs is associated with many diseases such as neurodegeneration and cancer. Recent studies show that IDPs are tightly regulated and that dosage-sensitive genes encode proteins with disordered segments. The tight regulation of IDPs may contribute to signalling fidelity by ensuring that IDPs are available in appropriate amounts and not present longer than needed. The altered availability of IDPs may result in sequestration of proteins through non-functional interactions involving disordered segments (i.e., molecular titration), thereby causing an imbalance in signalling pathways. In this lecture, I will first provide a general introduction to intrinsically disordered regions, discuss the regulation of IDPs, address implications for signalling, disease and drug development, and outline open questions in the field.

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M. MADAN BABU 2

Unstructured Proteins: Cellular Complexity and Diseases

In the second talk, I will discuss how cellular systems employ various regulatory mechanisms and disordered regions to enhance the functional versatility of proteins and increase organismal complexity. Specifically, I will discuss how alternative splicing of disordered regions that contain interaction motifs and post-translation modification sites can rewire protein interaction networks in space and time. I will also discuss how asymmetric localisation of transcripts that code for proteins with disordered regions can increase signaling fidelity and cellular regulation. Finally, I will present results from a recent work wherein we described how gene fusions involving disordered proteins tend to constitutively rewire protein interactions networks and cause diseases such as cancer. In this lecture, I will discuss work from our group and that of others and will present implications for human disease and the emergence of complexity in cellular systems.

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NIDHI SAHNI

Linking Genotype to Phenotype Through Cellular Networks

In the past decade, genome and exome sequencing projects have identified thousands of genetic variants in patients across a large number of Mendelian disorders, complex traits and cancer types. However, the explosion of genomic information has left many fundamental questions regarding genotype-phenotype relationships unresolved. One critical challenge is to distinguish causal disease mutations from non-pathogenic polymorphisms. Even when causal mutations are identified, the functional consequence of such mutations is often elusive. As a systems biologist and experimentalist, my work has focused on methods to seek a systems-level understanding of the underlying genetic and epigenetic aberrations in disease heterogeneity and immunity. The ultimate goal is to address fundamental problems in the modern era of personalized or precision medicine. In this lecture, I go over the methods and results of our recent high-throughput genome-scale studies. The complexity of genotype-phenotype relationships has been underappreciated. The absence of efficient high-throughput analyses of disease variants has therefore created a bottleneck in understanding the underlying disease mechanisms. We show that genes and gene products do not function in isolation but interact with each other as components of complex interactome networks of various macromolecules (DNA, RNA or proteins) and metabolites. In this lecture, I describe a systematic approach to investigate genetic variant-specific effects on molecular interactions at large scale across diverse human diseases. Remarkably, in comparison to non-disease polymorphisms, disease mutations are more likely to associate with interaction perturbations. I will also present the extent to which and how single-nucleotide disease mutations cause protein interaction alterations. We have been able to find different mutations of the same gene give rise to different interaction profiles, accounting for distinct disease outcomes. In collaboration with Dr. Song Yi, we recently characterized molecular interaction changes caused by patient-specific mutations, and revealed an interaction perturbation landscape of human disease. Together, we provide unprecedented evidence for widespread interaction-specific perturbations across a broad spectrum of human Mendelian disorders. Our approach is insightful in prioritizing disease-causing variants, and uncovering patient mutation-specific disease mechanisms at a base-pair resolution, a critical step towards personalized precision medicine. The studies presented here elucidate, from a unique network perturbation perspective, a systems-level explanation of human disease.

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SONG YI

Fundamentals of Machine Learning and Application to Network Analysis

We have now entered a big data era, surrounded by all sorts of information. For instance, a deluge of genetic information has been identified by next-generation sequencing to be associated with various diseases. However, it remains difficult to elucidate disease mechanisms from such big data. One of the most common tasks performed by data scientists to solve this problem is machine learning. We leverage on the advances in systems biology, functional genomics and bioinformatics, and seek to explore the functional and contextual effects of genotypic variants derived from patients. As a computational and systems biologist, my research interests include cancer signaling, immunology and evolution, synthetic lethality and drug resistance, and multi-omics network biology. I collaborate with experimentalists to identify principles or rules in biological systems, and then apply them to identify novel targets in a predicted manner. Our ultimate goal is to integrate computational algorithms and experimental data to build a quantitative and systematic understanding of molecular signaling and interactome networks in human health and disease. In this lecture, I introduce the basic idea of teaching a computer to learn concepts, and provide an overview of machine learning techniques to explore, analyze, and leverage data. In the following lecture, Dr. Nidhi Sahni outlines experimental platforms and results. Machine learning techniques have recently been applied to identify key features predictive of essential lncRNAs, and applied to figure out large numbers of genetic interactions and gene functions. It becomes increasingly clear that, biological networks and cellular systems underlie most genotype-to-phenotype relationships, and perturbations of such networks lead to human disease. In this lecture, I also describe systems-level algorithms and frameworks we have developed to address fundamental biological questions in disease. Our work contextualizes and prioritizes many candidates identified by systematic cancer screens, illustrating how our interactome dataset can be used as a scaffold to formulate novel hypotheses of mechanistic insights. By establishing a functional variomics platform, we stratify disease-causing mutations at a single base-pair resolution, and elucidate the mechanism of disease mutation-associated network alterations. We also use the machine learning greedy algorithm to detect recurrent mutations that could explain the vast majority of splicing alterations in cancer. Furthermore, integration of different types of molecular interactions uncovers interaction perturbations at a better resolution, expanding our ability to understand complex biological systems. Together, our results on perturbed interaction partners hint at a potential to design patient-specific precision medicine, and have major ramifications for our understanding of individualized therapy in disease.

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STEPHEN MICHNICK 1

Mechanisms and Consequences of Macromolecular Phase Separation

Over a century ago, colloidal phase separation of matter into non-membranous bodies was recognized as a fundamental organizing principal of cell "protoplasm." Recent insights into the molecular properties of such phase-separated bodies present challenges to our understanding of cellular protein interaction networks, as well as opportunities for interpreting and understanding of native and pathological genetic and molecular interactions. In this lecture, I will briefly review examples of and discuss physical principles of phase-separated cellular bodies. I will discuss some of the main methodologies that are being used to characterize phase-separated biopolymers in the cell including active and passive rheology to study mechanical properties of droplets and how these reflect their internal organization. Finally, I will reflect on how knowledge of the principles of macromolecular phase separation may direct future research on their functions.

STEPHEN MICHNICK 2

The Phases and Dephases of Endocytosis

Hydrodynamic and mechanical forces determine cell shape and movement. A key process in morphogenesis is clathrin-mediated endocytosis (CME). We are exploring evidence that the energy required for the early stages of CME is mediated by liquid-liquid phase separation from the cytoplasm of low complexity prion-domain-containing proteins. The energy needed to form a nascent endosome is generated by viscoelastic properties of the droplet and interactions of the droplet with the cytosol and membrane. I will illustrate how a combination of biophysics and bioinformatics approaches revealed this model and could be used to predict other forms of large-scale organization of matter in living cells and how these organizations integrate cellular processes.

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TOM BLUNDELL 1

Multiprotein Systems in Space and Time: Increasing Complexity to Achieve Selectivity Through Stages, Scaffolds, and Strings.

I have argued over the years that **cell regulatory systems must be complex if they are to achieve high signal to noise**. The cell membrane, the cytoplasm and nucleus provide very crowded environments where interactions would be frequent and diffusion of molecules impeded. Binary interactions would occur opportunistically giving rise to noise in the system, but cooperative formation of multiprotein systems would be less likely to form by chance, especially if they have many components and ordered-assembly mechanisms. Such arguments are consistent with the ever-increasing complexity that continues to emerge from studies of molecular mechanisms of cell regulation and signalling[1,2].

I will discuss these ideas of complexity with respect to several different systems, beginning with early ideas of receptor recognition by insulin (largely preformed structure) and glucagon (concerted folding and binding). I will discuss complexity of signalling systems involving secondary receptors, such as heparan sulphate, and focus on multicomponent signalling complexes involving growth factors and cell surface receptors such as MET and FGFR where there is strong evidence for receptor clustering[3].

I will then discuss the relevance of complexity and colocalisation to DNA double-strand-break repair through Non-Homologous End Joining (NHEJ) and through Homologous Recombination. The NHEJ process comprises synapsis, end processing and ligation. A significant question is: how are components coordinated in space and time throughout synapsis, end processing and ligation? We have observed 3 different mechanisms that ensure appropriate spatial co-localisation of components[2].

The first is **a stage where the main actors gather and engage**. In NHEJ this comprises a Ku heterodimer, interacting with DNA broken ends and binding a variety of players including DNA-PKcs, XLF and PAXX. A further stage, DNA-PKcs, interacts with Ku heterodimer, DNA, BRCA1 and Artemis, often depending on post-translational modification and binding of other factors[4,5,6].

The second is **a scaffold that is constructed quickly and disassembled easily** to facilitate cell responses. In NHEJ a scaffold is assembled from protein filaments of XLF/XRCC4 to bridge Ku heterodimers, and regulated by LigIV, to which XRCC4 tails are tightly attached, so bringing it close to broken ends.

The third is **a string that ties components together**. An example of this is the intrinsically disordered polypeptide of the 300-residue C-terminal-extension of Artemis. Foldable regions of Artemis C-terminus interact with DNA-PKcs and LigIV, apparently functioning to keep components close by, making them available but not obstructing the DNA repair process. Other strings include BRCA1 and APLF.

We suggest that such “stages”, “scaffolds” and “strings” have complementary roles, often binding to the same partners but operating in different ways over space and time. I will discuss these mechanisms and the nature of the cooperative interactions that give rise to efficiency and selectivity in NHEJ.

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TOM BLUNDELL 2

Multiprotein Systems in Space and Time: Targeting Protein-Protein Interfaces Using Fragment-Based Drug Discovery.

Further to my discussion of the necessary complexity **cell regulatory systems** I will discuss the increasing interest **in targeting protein-protein interfaces of multiprotein assemblies** in the design of chemical tools and therapeutic agents[1].

Evidence is accumulating that such an approach will offer greater opportunities in improving specificity and selectivity compared to targeting active sites of proteases, protein kinases and other enzymes involved in post-translational modification. I will illustrate this with fragment-based drug discovery[2] targeting homologous recombination, the other mechanism of double-strand-break repair. We have produced nanomolar inhibitors of the Rad51-BRCA2 system[3,4].

Incidentally **fragment-based discovery is an example of decreasing complexity** – using small fragments of molecular weight <300 daltons – to increase promiscuity and thus use a smaller library that still targets complex and even "undruggable" sites[5].

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TOM BLUNDELL 3

Science, Society, and Politics: Experiences of a Permanent Gap-Year Student.

Working with Nobel Laureate Dorothy Hodgkin in the 1960s and early 1970s introduced me to the idea that science transcends national boundaries as well as race, religion, gender and age. Her team was multinational and I soon found myself interacting closely with the Indian, Chinese, African and South American members of her group and their friends who came to visit, as well as enjoying the company of European, US, Australian and New Zealand researchers who were the majority of scientists at that time. Discussions developed about politics with the Liang Dong-Cai who soon returned to China for the cultural revolution, with M Vijayan who had been brought up in Kerala left-wing politics, and with black colleagues who came from Ghana and from South Africa to Oxford in the period of Apartheid. I went to US in 1964 and 69 to find out about race issues in the South – to join the student protest movement and in 1969 to give talks about insulin.

The situation in Oxford City was challenged by increasing racial diversity as West Indians and Bengalis were recruited to Pressed Steel and Morris Motors. I found myself pulled into city politics and elected a City Councillor at the end of the Wilson Government, with new pressures of pedestrianizing the City Centre and making North Oxford a Conservation Area, all of this competing with our work on insulin in the day time with some nights on the English Electric KDF9 computer and my modern jazz group in the evening.

One day in 1972 I left it all to travel across Siberia on the steam train to Khabarovsk on the Chinese border, to live in Tokyo learning language and culture, to visit Taiwan to find out about Chinese opera and then to India to study carnatic music and learn to play the veena. Of course science and scientists were always around as Dorothy's group members everywhere. It was later in the 70s before I managed to get to Beijing and Shanghai to listen to Maoist versions of Chinese Opera and to catch up with Dong Cai who had led the Chinese Insulin work in Beijing!

In the 80s, I married Dr Bancinyane Lynn Sibanda in Zimbabwe; her extended family had helped organise ZAPU, the freedom movement led by Joshua Nkomo. Later Lynn and I somehow got into and toured South Africa, expecting to be arrested but making friends with members of all races in their segregated universities. And so, it has continued over the following 30 years with new excitements, such as walks over the Andes, participation in the Rio Carnival and boating up the Rio Negro in the upper Amazon after discovering the Opera House in Manaus did not have performances every night.

More and more I realized that scientists could not only enjoy themselves but also contribute to our health, wealth and environment. I chaired the Royal Commission on the Environment, co-writing "Energy and Climate Change" presented to the Queen in 2000, I had earlier surprisingly been invited to be advisor to Prime Minister Margaret Thatcher in Number 10 in 1988 -1990 and I co-founded Astex Therapeutics in 1999, which attained a value of $886 million 2013 and now has several oncology drugs in advanced clinical trials and a drug for breast cancer, Kisqali, jointly with Novartis, gaining FDA and European marketing approval this year.

A Cambridge city newspaper once called me a Marxist Entrepreneur, but I feel like a Permanent Gap-Year Student!

URI ALON 1

How to Study Biological Networks and Circuits

To understand biological networks, our lab has defined **network motifs**: basic interaction patterns that recur throughout biological networks, much more often than in random networks. The same small set of network motifs appears to serve as the building blocks of transcription networks from bacteria to mammals. Specific network motifs are also found in signal transduction networks, neuronal networks and other biological and non-biological networks.
We experimentally studied the function of each network motif in the transcription network of E. coli. Each network motif can serve as an elementary circuit with a defined function: filters, pulse generators, response accelerators, temporal-pattern-generators and more. Evolution seems to have converged on the same motifs again and again, perhaps because they are the simplest and most robust circuits that perform these information-processing functions.
To carry out these experiments we developed automated systems for measuring the behavior of gene circuits in living cells. We developed a library of 2000 E. coli strains in which green fluorescent protein reports for the activity of the vast majority of the organisms' promoters [Zaslaver 2006] . Using robotic liquid handling, we obtain accurate dynamics of E. coli promoters under desired conditions.

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URI ALON 2

Evolutionary Tradeoffs and the Geometry of Phenotype Space

Organisms, tissues and molecules often need to perform multiple tasks. But usually no phenotype can be optimal at all tasks at once. This leads to a fundamental tradeoff. We study this using the concept of Pareto optimality from engineering and economics. Tradeoffs lead to an unexpected simplicity in the range of optimal phenotypes- they fall on low dimensional shapes in trait space such as lines, triangles and tetrahedrons. At the vertices of these polygons are phenotypes that specialize at a single task. We demonstrate this using data from animal and fossil morphology, bacterial gene expression and other biological systems.

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List of Participants and Posters

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Rational Design of Therapeutic Antibodies and the Development of New HTP Screening Assays

Owing their high affinity, specificity and inherently low toxicity, antibody-based drugs are the fastest growing class of therapeutics on the biopharmaceutical market, targeting chronic inflammation, cancer and infectious diseases. However, the success for some of these drugs is relative due to high costs of development and administration.
Methods to develop novel antibodies commonly result in large variant libraries (>10.000) that require extensive and time-consuming experimental characterisation. In particular, insufficient solubility represents a major bottleneck for drug formulation and subcutaneous administration, where highly concentrated antibody solutions (≥100 mg/ml) are a necessity to achieve therapeutically relevant dosing in a small injection volume (< 1.5 ml). The high cost of failing at a late development stage has led to considerable computational efforts to predict drug developability on the basis of the antibody sequence motifs.
Here, we quantitatively validate solubility predictions of an antibody library (15 mAbs) obtained with the in silico tool CamSol using a myriad of biophysical developability assays. We show that the biophysical assays conform well to the solubility predictions, suggesting that the selection of soluble lead candidates is possible based on the amino acid sequence. Thus, early variant library screening potentially increases the chance of a successful, effective drug launch and significantly reduces costs.

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Novel Factors of Arabidopsis thaliana NMD mRNA Surveillance Pathway

Nonsense-mediated mRNA decay (NMD) is a conserved mRNA surveillance mechanism to prevent the production of potentially harmful proteins by eliminating aberrant mRNAs carrying premature translation termination codons (PTC). The key NMD effectors, ATP-dependent RNA helicase UPF1 together with UPF2 and UPF3, form a core of the NMD complex in all eukaryotes. In addition, in vertebrates this process involves SMG1, SMG5-9, the ribosome, the exon-exon junction complex (EJC) and eukaryotic release factors ERF1-ERF3A. Recent studies revealed a whole set of new additional and auxiliary NMD components, including RNA helicases, subunits of the eukaryotic initiation factor, transcription-export (TREX) complex, various signaling proteins and nucleus-associated RNA-binding proteins.
NMD, as been well described in yeast, fruit flies and humans, but it still remains poorly characterized in plants. To identify new plant NMD factors we have analyzed UPF-interacting proteins by affinity purification using a transgenic Arabidopsis line expressing tagged UPF1. Besides UPF2-UPF3 and SMG7 we have retrieved ribosomal and RNA-binding proteins, splicing factors, RNA helicases, eukaryotic initiation factor 3/4, and proteins involved in nuclear transport and proteolysis. We have focused on three families of RNA helicases, splicing factors and TPR proteins of unknown function. To investigate their involvement in NMD we have applied the VIGS assay (Virus-Induced Gene Silencing), which allows for quick and efficient testing of many potential NMD factors using transient transfection of N.benthamiana *leaves.This approach was followed by analyses of endogenous NMD substrates in Arabidopsis T-DNA insertions mutant of factors under study. We will present results of these proteomic and functional analyses.*

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A Tale of Two Proteins: CcdA and CcdB Ensure Ratio-Dependent Transcription Regulation in a Toxin-Antitoxin System

Bacterial persister cells play an important role in several chronic infectious diseases. Persisters are tolerant to several classes of antibiotics because they are in a dormant, non-dividing state. This dormancy can be caused by toxin-antitoxin systems, small genetic elements, widespread on bacterial genomes, with intricate auto-regulatory features. In this poster, we present the structures of the molecular complexes and the interactions that drive the transcriptional regulation of the ccdAB toxin-antitoxin system, found on the F plasmid of Escherichia coli. Electrophoretic mobility shift assays show that the antitoxin CcdA binds only weakly to the operator and has low specificity for individual binding sites. Affinity and specificity are increased by the toxin CcdB, which serves as a spacer between CcdA dimers to create a multivalent repressor with equally spaced DNA binding sites. This results in a unique extended complex of alternating CcdA and CcdB dimers that spirals along the ccdAB operator, which was visualized using transmission electron microscopy. The multivalency of operator binding sites induces a sharp on-off response for transcription, regulated by the toxin:antitoxin ratio. Mathematical modeling further shows that the ratio at which this switch occurs can be modulated by non-specific interactions with the excess of chromosomal DNA. Altogether, we unraveled the molecular mechanisms governing the ratio-dependent transcriptional regulation in the ccdAB toxin-antitoxin system.

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The Resurrection of a Dead Enzyme

The genome of B.anthracis contains ten putative polysaccharide deacetylase (PDA) genes, with high sequence homologies. This family of enzymes catalyses the removal of an acetyl group from the peptidoglycan layer that surrounds the gram-positive bacteria, rendering the cell invisible to the host's immune system. Crystallographic studies complemented by mass-spectrometry and mutagenesis, reveal an unusual hydroxylation which targets the α-carbon of a conserved Proline, in the active site of the NodB catalytic domain. The presence of this post-translational modification correlates with the activity of the de-N-acetylation reaction, suggesting a contribution to catalysis via a maturation step. One of the PDA's namely BA3943, has lost its ability to perform catalysis, due to the absence of key residues. Mass spectrometry and enzymatic activity experiments on BA3943, confirmed the absence of both hydroxylation and de-N-acetylation reactions. Structure solution at 1.5A (unpublished), allowed for the careful re-construction of the active site, to the extent that it would be able to perform catalysis. Enzymatic activity experiments confirmed the resurrection of the enzyme's activity, and masspec analysis reported an increase in the α-carbon hydroxylation level, consistent with previous results. Further experiments are being performed, to elucidate the exact mechanism by which this hydroxylation event contributes to the proposed model of catalysis. To conclude, the role of this dead enzyme inside the cell remains a mystery, with few data supporting its prominence as a sporulation agent.

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Engineering Specific Antibodies for the Usutu Virus

The rapid rise of the previously obscure flavivirus Zika virus (ZIKV) that has caused millions of infected individuals globally shows the strong potential of other presently unknown pathogens to become a major threat. Putative emerging pathogens could be other poorly characterised or even unclassified Flaviviruses.
Usutu Virus (USUV) - presently insufficiently described - and West Nile Virus (WNV) are mosquito-borne flaviviruses belonging to the family Flaviviridae. Their envelope (E) protein structures mainly determine the antigenic properties of flaviviruses. Both, the WNV and the closely related USUV have an E protein possessing 3 domains (DI, DII and DIII), with WNV being effectively neutralised by the mouse monoclonal antibody E16 binding to DIII. Cross-reactions observed in serological assays between USUV and WNV show that these viruses share antigenic features, especially in their E proteins.
The aim of this study is to obtain a deeper knowledge of the region corresponding to the WNV E16 epitope in USUV CAR-1969 in general to diagnose and potentially treat USUV infections and distinguish them from WNV infections. We have already established a robust protocol for the expression and purification of the DIII of several USUV and WNV strains which was the first step in solving the crystal structure of DIII of USUV CAR-1969. Furthermore, a second goal is to increase the binding of humanised E16 antibody to CAR-1969 and the wildtype USUV based on the existing E16 antibody for WNV while circumventing cross-reactions.

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Unbiased Identification of Human TXNRD1 and PARP10 Interactors and Substrates by Thermal Proteome Profiling

Thermal proteome profiling (TPP) has literally added a new "stability" dimension to proteomics. The underlying principle is changes in the stability of a protein upon binding to a small molecule, which is manifested by a shift in protein melting curves over an optimized temperature range. We hypothesized that some protein-protein interactions (PPIs) and post-translational modifications (PTMs) might also be able to alter the thermal stability of proteins. As such, we have applied TPP to identify human thioredoxin reductase 1 (TXNRD1) and poly [ADP-ribose] polymerase 10 (PARP10) interactors as well as their substrates in the presence of enzyme cofactors. Proof of principle experiments showed that TPP can indeed detect a fraction of known PPIs for ubiquitin as well as known substrates for TXNRD1. We further continued to identify unique interactors and substrates for PARP10. A number of these putative interactors were confirmed using pulldown and proximity ligation techniques. Furthermore, mass spectrometry confirmed the ADP-ribosylation of several substrate candidates for PARP10. Taken together, TPP can be used as a complementary or exploratory tool for studying PPIs and specific PTMs.

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Mixed Lineage Leukemia (MLL) Proteins Target Regulated Genes Using PHD and CXXC Domains

SET/MLL proteins Lysine (**K**) **M**ethyl**t**ransferases (KMT2) are the core proteins of the multi-subunit COMPASS complexes, which catalyze H3K4 methylation and therefore favor an active chromatin state. Humans have seven KMT2 paralogues (KMT2A-G). The best studied human KMT2 protein is KMT2A (known as MLL1) due to its involvement in the pathogenesis of mixed lineage leukemia (MLL). KMT2A and KMT2B within the hCOMPASS-like complex have the ability to di- and tri-methylate H3K4 localized mostly on gene promoters. In contrast, KMT2C and KMT2D within the hCOMPASS-like complex mono-methylate H3K4 and target primarily cell type-specific distal enhancer regions of the activated genes. KMT2 proteins consist of several domains, including a SET (methyltransferase catalytic domain) and multiple PHD (histone marks reader) domains that are mostly arranged in triples. In addition, KMT2A and KMT2B possess single CXXC domains, which bind CpG islands in promoter sequences.
So far, the detailed recruitment mechanism of MLL-containing complexes to targeted genes has not been described. To address this question, we investigate biochemically the specificity of selected triple PHD domains of KMT2 proteins. Histone peptide array experiments showed that selected triple PHD domains are bound to enhancer-associated histone marks, such as specific multiply acetylated lysines. ITC experiments confirmed preference of the triple PHD domains for an acetylated over the non-acetylated histone peptide. HDX MS experiments are consistent with predicted PHD domain structure, but show no signal for binding. In addition to PHD domains, we are characterizing the specificity of the KMT2A CXXC domain for DNA containing CpG dinucleotides with modified cytosines.

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Importin Beta has Global Roles in Control of Mitotic Cell Division via Distinct Partners and Molecular Mechanisms

Importin beta is the major vector for protein import in interphase nuclei and a RAN GTPase effector. After nuclear envelope breakdown, when nuclear transport ceases, importin bet regulates several steps of mitosis. Given its many pleiotropic roles, it is not surprising that importin beta is increasingly found to be overexpressed in many cancer types displaying high genetic instability. Selective inhibitors are being developed, yet the precise mechanisms through which importin beta contributes to cell transformation in cells in which it is overexpressed are still incompletely understood.
Importin beta structure enables it to interact with many partners, making it difficult to precisely pinpoint its targets in distinct pathways. Moreover, a broad array of importin beta-mitotic interactors is only starting to be dissected. In this work, we have sought to dissect importin beta-dependent functions in mitosis and gain new information on downstream molecular pathways that it regulates. To that aim, we have generated stable cell lines that can be induced to overexpress importin beta, either wild-type or in mutant forms defective for specific domains, and have asked how they affect mitosis using multiple approaches, including interactomics, time-lapse imaging and functional assays.
We find that elevated expression of importin beta alters mitotic microtubule (MT) functions via at least two pathways: one pathway regulates factors implicated in SUMO conjugation of mitotic proteins; an independent pathway is operated via HURP, a candidate target in MT stabilization. Defective MT functions induced by importin beta deregulation ultimately hinder mitotic progression and chromosome segregation, increasing genetic instability in daughter cells. These results contribute to clarify the mechanisms through which high importin beta influences transmission of the genetic integrity in mitosis.

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Use of Proximity Dependent Biotinylation to Determine Novel Effectors of Necroptotic Cell Death

Necroptosis is a form of programmed cell death associated with the pathology of various inflammatory, autoimmune, and neurodegenerative diseases, as well as with ischaemia-reperfusion injuries. It is initiated when certain cell surface receptors are activated to induce cell death under caspase-compromised conditions. This leads to a chain of molecular events, resulting in destabilisation of cellular membranes and release of intracellular contents. The most downstream known essential effector of necroptosis is a protein called mixed lineage kinase domain-like (MLKL). During necroptosis, MLKL is phosphorylated and translocates to membranes: two events that are critical for cell death to occur. However there is still much we don't understand about what occurs downstream of MLKL activation, including whether any other proteins are involved in this process.
Due to difficulties in identifying interactors of MLKL using conventional immunoprecipitation I am using BioID, a proximity-dependent biotinylation technique that can be used in living cells. By fusing a biotin ligase to a protein of interest, this technique enables enrichment of weak and transient protein interactions, as well as stable interactions that may be disrupted using other methods. Proteins can then be identified using mass spectrometry, and further investigated as to their importance during necroptosis. So far, I have shown that a biotin ligase can be fused to MLKL without disrupting cell death pathways, and that biotinylated proteins can successfully be isolated for downstream analysis. I hope this technique will yield novel interactors to help us better understand necroptosis and how to limit its contribution to disease states.

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Dynamics of Cancerous Tissue Correlates with Invasiveness

One of the primary threats concerning cancer cells is their ability to spread and create new tumors. It is therefore of interest to characterize differences between cells with the ability to invade, versus cells that are confined to the primary tumor. Approaching this from a material science perspective, we analyzed tissue dynamics using particle image velocimetry (1). This optical technique is non-intrusive and allows visualization of flow dynamics of confluent monolayers. Localized clockwise and anti-clockwise vorticity patterns were observed around dividing cells and the overall magnitude of the dynamics were found to correlate with invasive potential of the cell lines. In both the human and murine breast cancer cells investigated, the invasive phenotypes had significantly higher velocities and flow dynamics than their non-invasive counterparts. This collective motion of cells was reproduced by a continuum-scale model of the mechanical interaction between the cells, the Coulomb-like friction with the substrate, and the self-propelling forces of the cells. From the model, we extracted the force exerted by a dividing cell onto its surroundings, and found that this was significantly larger for invasive than non-invasive cell lines. The strong correlation suggests that mechanical signals in breast cancers induce adjacent cells to yield the way for invading neighbors. Therefore, the dynamics could be a reliable parameter for judging aggressiveness, independent of aberrant biochemical signaling, cell shape, cell-adhesion markers, and tissue connectiveness.

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Understanding the Extent of TRAF RING Hetero-Dimerization

TNF Receptor Associated Factors (TRAFs) are a family of seven adaptor proteins that regulate both cell survival and apoptosis in many immune response pathways. Dysregulation or mutation of TRAFs has been linked to development of B cell lymphomas and several inflammatory disorders[1]. TRAFs act as scaffolds to recruit downstream substrates and are also thought to modulate signal transduction via ubiquitylation. Ubiquitylation is one of the most abundant post-translational modification and is regulated by a cascade of enzymes E1, E2 and E3s. E3 ubiquitin ligases confer substrate specificity, thereby playing a significant role during cell signalling.
TRAFs are defined by the presence of a C-terminal coiled coil domain as well as a TRAF-C domain. These domains form a stable trimer and the TRAF-C domain interacts with the cytoplasmic domains of various receptor peptides. At the N-terminus most TRAFs have a RING domain followed by four-five Zn finger domains and these are important for ubiquitylation. Structural and biochemical studies have shown that dimerization of the RING domain is crucial for the E3 ligase activity of TRAF6[2]. Although both the trimeric receptor binding domain, and dimeric E3 ligase activity have been well studied, it is uncertain how these TRAF-C/CC trimers and RING dimers assemble/associate during signal transduction.
Surface conservation mapping of the RING domains from TRAFs that co-localize during signalling shows a highly conserved RING dimer interface indicating the possibility of TRAF RING heterodimer formation. We have investigated the importance of RING dimerization for ubiquitylation and whether RING heterodimers can form between co-localized TRAFs. Our studies suggest a novel molecular mechanism mediating ubiquitin transfer by dimeric TRAF RING domain. In addition, our in vitro *biochemical assays show that TRAF RING heterodimers can form. This is an exciting observation that suggests a higher level of complexity in TRAF-mediated signalling is possible.*

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A Comparative Structural Studies on Heterotrimeric G Proteins Gamma Subunits: A. thaliana and O.sativa Examples

In plants, heterotrimeric G proteins are involved in the transmission of signals that activate several signaling pathways including those regulating seed germination and size, growth, differentiation, and responses to biotic and abiotic stress. Although plant G proteins have been identified at the genomic level and several studies in vivo functional studies have been carried out, there is no data available on the biochemical and structural characterization of the plant Gβ and Gγ subunits. On the other hand, recently there is increased interest in the plant Gγ subunits due to the discovery of several classes and also their direct involvement in plant stress responses.
The presented study involves computational analyses for modeling the structures RGG1 (Rice G-protein Gamma Subunit-1) and AGG1 (Arabidopsis G-protein Gamma Subunit-1) and the biochemical and biophysical characterization of the two purified proteins. Homology modeling yielded an extended helical structure for both RGG1 and AGG1 proteins. For experimental characterization studies, RGG1, RGG2 (Rice G-protein Gamma Subunit-2) and additionally AGG1 were expressed in E.coli and purified from bacteria for dynamic light scattering (DLS) and circular dichroism spectroscopy (CD) analyses. Stability of structures was investigated in thermal denaturation experiments by CD. Comparative results will be presented and discussed in the context of plant gamma subunit function(s).

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Microstuctural Analysis of the Protein-DNA Interactions at the Emergence of the Spontaneous Point Mutations

An intriguing theme of the origin of the spontaneous point mutations in DNA has excited researchers' mind during several decades suggesting mispairs as their source, that, however, has not been authentically established to date.
By combining Watson-Crick "tautomeric hypothesis" consisting in the formation of the mispairs by the participation of the mutagenic tautomers of the DNA bases and the approach consisting in the formation of the wobble base pairs involving only DNA bases in the canonical tautomeric form, we succeed to establish a novel scenario of the microstructural mechanisms of the incorporation and replication errors arising at the DNA replication. It was discovered a novel mechanism of the mutual tautomeric transformations of the DNA base pairs with Watson-Crick and wobble architecture as the key to understanding of the intimate mechanisms of the spontaneous point mutations. Moreover, it was outlined a complete set of the 12 incorrect DNA base pairs causing spontaneous point mutations - A·C/C*·A, G*·T/T·G*, G·A_{syn}, A*·G^*_{syn}, A*·A_{syn}, G·G^*_{syn}, C·T/T·C, C*·C/C·C* and T*·T/T·T* - and mechanisms of their acquisition of the enzymatically-competent conformation directly in the recognition pocket of the high-fidelity replicative DNA-polymerase, which ultimately guarantees their successful chemical incorporation into the structure of the synthesized DNA double helix.*

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Characterization of Oxidized Alpha Synuclein Oligomers

Alpha synuclein (aSN) is a 14.5 kDa protein involved in Parkinson's Disease (PD). aSN can be divided into three regions: The N-terminal, the hydrophobic Non-Abeta-Component region, and an unstructured C-terminal. aSN self-associates into several different types oligomers. The off-pathway oligomer has been extensively characterized and is thought to be the cytotoxic aSN species [1]. Oxidation of aSN induces the formation of highly stable oligomers [2] and is thought to be relevant to the progression of PD.
Copper ions are used to catalyze the oxidation of aSN and investigate the oligomerization under oxidizing conditions. We followed the early and late stages of aggregation under oxidation using fluorescence spectroscopy techniques, size-exclusion chromatography, circular dichroism and mass spectrometry.
Incubating monomeric aSN with $CuCl_2$ and H_2O_2 oxidizes all four methionine residues in aSN and forms dityrosines within 5 minutes. Oxidation of aSN accelerates the self-assembly of aSN monomers along with a C-terminal fragmentation of aSN (aa. 104-140). The oxidation induced oligomers also decreases the interaction with negatively charged lipid vesicles consisting of dioleylphosphatidylglycerol.

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Alpha-Synuclein Oligomers

Oligomeric αSN is considered to be the potential toxic species responsible for the neurotoxicity of Parkinson's disease. This is based on several in vitro observations that amyloid oligomers are able to permeabilize membranes by pore formation, and it has also been confirmed in vivo. These findings make αSN oligomers interesting to study in regards to prevent Parkinson's disease.
A characteristic of Parkinson's disease is the accumulation of Lewy bodies within nerve cells, and the simultaneous death of nerve cells. The main component of Lewy bodies is the protein α-synuclein (αSN). αSN is able to self-associate into oligomers and amyloid fibrils. Mutations, environmental changes and post-translational modification can stimulate this aggregation process. Oxidative stress is thought to be a mechanism to cellular dysfunction Parkinson's disease and the amount of reactive oxygen species is found increased in Parkinson's disease. Oxidation of lipids in the membrane can release different degradation products, among these the most reactive are the aldehydes. Aldehydes such as 4-hydroxynonenal (HNE) can be generated in the oxidation breakdown of polyunsaturated fatty acids and its presence has shown to increase the oligomer formation of αSN.
We use different biophysical techniques and cell assays to study how HNE modifies αSN and to study the toxicity of HNE modified αSN oligomers. We would like to use in-cell NMR to study the oligomer inside mammalian cells.

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How Does Annexins React to Physical Changes in the Membrane?

In this study, we are working with the concept of optical tweezers, used as a non invasive method for investigations of biological systems. By using optical tweezers, membranes can be manipulated e.g., by pulling tethers. To visualize the work, scanning confocal microscopy is used. The work is focused on the vitally important functions of cellular membrane repair mechanisms. These membrane repair mechanisms are thought to involve cascades of events such as recruiting some necessary biomolecules for patching holes. The cell membrane is made up of many dierent types of lipids and proteins.
When cells are exposed to stress and physical obstacles, their membranes tend to break and holes start to form. This work concentrates on the interactions between the protein family annexins and the cellular membranes. Many of these proteins are known for protein-protein and membrane-protein interactions. The expectation is that the research, which is investigating the mechanical parts of biophysics, helps to expand current knowledge within protein dynamics. This can be interesting in e.g., cancer research. It can also help with expanding the knowledge of the principles behind interactions between dierent biomolecules and how human diseases are implicated by this. For this work, an investigation of how annexin A5 crystallizes and how these crystals are prone to lipid rearrangment is performed.

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Mutational Study of Exposed Hydrophobic Groups in Aβ42 Fibril-Catalyzed Nucleation

Alzheimer's disease is a neurodegenerative disease. The amyloid peptide Aβ42 is involved in the pathology of the AD. Aβ42 is unfolded in its monomeric form, but has a high propensity to self-assemble into highly ordered highly ordered amyloid fibrils. This process of Aβ42 self-assembly from monomeric to fibrillar aggregates involves a series of microscopic steps. The aggregation process involves the formation of oligomers, which are believed to be the neurotoxic species. However, it is not clear which residues are involved in the formation of Aβ42 oligomers. The Aβ42 fibril structure solved by solid-state NMR shows the presence of two hydrophobic patches on the fibril surface. It can be reasoned that these hydrophobic patches are the regions where the oligomer formation is initiated. We replaced these hydrophobic residues with serine to see whether the secondary nucleation and hence the oligomer formation is affected in absence of the hydrophobic residues on the fibril surface. We created serine mutants for the four hydrophobic residues on the fibril surface: V18S, A21S, V40S, and A42S, two double-serine mutants for the two hydrophobic patches: V18S+A21S and V40S+A42S, and one mutant where all the four hydrophobic residues were replaced with serine. The aggregation kinetics was then studied for all these serine mutants using Thioflavin T fluorescence assay. We performed aggregation assay and studied the kinetics for all the above stated serine mutants of Aβ42. We observed that the aggregation for all the mutants was dominated by secondary nucleation. Hence it can be inferred that the hydrophobic patches on the fibril surface are not the site of initiation of secondary nucleation.

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SAXS and NMR Studies of Fibrillating α-synuclein and its Interaction With Phospholipd Bilayers as a Model for Parkinson's Disease Pathogenesis

A broad range of diseases are associated with aggregation of proteins into amyloid deposits. Amyloid deposits have been linked to the cause of several neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (PD). A major hallmark of PD is the presence of Lewy bodies whose primary constituent is α-Synuclein (αsn). Although αsn is an intrinsically disordered protein alone, its interactions with other constituents, lipids in particular, may induce an α-helical secondary structure leading to potential functional alterations of the protein. Several other secondary and tertiary structural conformations may however be relevant for these protein:lipid interactions.
In this project, we aim to understand how co-structural dynamics of αsn and membrane constituents define and/or alter the biological function by using multidisciplinary approaches particularly by integration of several structural methods for in vitro characterization and cell biology techniques for functional characterization. Structural and functional aspects of αsn will be studied on with regard to membrane interactions and with a goal of achieving an understanding of how the protein can be therapeutically targeted. Small angle X-ray scattering (SAXS) and Nuclear magnetic resonance spectroscopy (NMR) were already employed monitoring αsn:lipid interactions and the structures of co-aggregates with the achievement of raising substantial questions to guide the project.
Additionally, several assays were carried out by Plate Reader in order to investigate the fibrillation kinetics of αsn in different conditions. The ultimate goal of the project is to enable rational design of therapeutics targeting the fuzzy interactions between αsn and lipid bilayer environment.

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Prevalance of Vitamin D Deficiency in Turkey

AIM and BACKGROUND: In the last few years, more attention has been given to Vitamin D deficiency and several studies demonstrated that Vitamin D deficiency is prevalant worldwide. Moreover, there is a strong relationship between low circulating concantrations of vitamin D and many common disease including skeletal and non-skeletal disease. Synthesis of vitamin D3 is induced by UV light in skin and Cholecalciferol converted to 25-hydroxyvitamin D [25-(OH)D]or calcidiol by 25α-hydroxylase in the liver and subsequently in the kidney into its' active metabolite 1,25-dihydroxyvitamin D by the 1α-hydroxylase, an enzime which is stimulated by parathyroid hormone. Multiple factors like ethnicity, age, sex, diseases, and medication influence vitamin D concentrations. Vitamin D deficiency *is defined as a serum concentration of ≤20 ng/mL. This study aimed to determine prevalance of vitamin D deficiency among adulta in Konya, Turkey.*
MATERIALS and METHODS: The 25(OH) vitD_3 test results of 3179 patients who applied to Selcuk University Medical School Hospital for routine control between the years of 2016 August and 2017 August were collected retrospectively. Vitamin D levels were measured by a choromotographic method(LC/MS/MS) with API3200. Statistical analysis was performed with IBM SPSS v21.
RESULTS AND CONCLUSIONS: Vitamin D levels of 453 (14.2%) patients were <5 ng/mL. Vitamin D levels of 1062 (33.4%) patients were <10 ng/mL. Vitamin D levels of 2001 (62.9%) patients were <20 ng/mL. Vitamin D deficiency seems to be very common in Turkey despite high levels of sunshine and UV radiation throughout the year. It is also surprising that vitamin D deficiency is prevalant, despite the common use of vitamin D consisting drugs. We think that there is a strong relationship with clothing and vitamin D deficiency.

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The Structural Basis for GHR Recognition by JAK2

Cytokine receptors play a pivotal role in many cellular processes, including metabolism control, neural stem cell activation, inflammatory responses, bone development, as well as blood cell and immune cell development and growth. GHR is an archetypical member of the class I cytokine receptor family. This family does not possess intrinsic kinase activity, however they bind JAK kinases via a conserved intracellular proline rich box1 motif and a less conserved box2 sequence consisting of acidic and aromatic residues. The JAKs are multi-domain proteins possessing an N-terminal FERM (band 4.1, ezrin, radixin, moesin) domain followed by SH2, pseudokinase, and kinase domains. The FERM domain is required for receptor binding and the SH2 domain is also proposed to be involved in receptor binding. Activation of the receptor results in JAK phosphorylation followed by further phosphorylation of multiple tyrosine residues on the intracellular domain of the receptor and subsequent phosphorylation of STATs. We sought to determine the structure of Box1-2 of GHR bound to FERM-SH2 domains of JAK2. We have optimised a recombinant protein expression method for the protein complex resulting in high purity monodispersed sample. Identity and proper folding of the purified protein was validated using mass spectrometry and circular dichroism spectroscopy, respectively. Protein crystals identified from screening crystallisation conditions are being analysed to determine structural basis of JAK2 interaction with GHR. In addition, Box1-2 peptide sequences from other class I cytokine receptors will be assessed for their binding kinetics to JAK.

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Dual Mechanisms of LYN Kinase Dysregulation Drive Aggressive Behaviour in Triple Negative/Basal-like Breast Cancer Cells

High expression of the SRC-family kinase LYN has been previously reported in triple-negative/basal-like breast cancer (TN/BLBC) and its cell-of-origin, the c-KIT-positive mammary luminal progenitor. Here we demonstrate that LYN is a downstream effector of c-KIT in normal mammary epithelial cells. However, in Brca1-mutant TN/BLBC, LYN activity is activated independently of c-KIT by the Prolyl Isomerase PIN1, which is normally transcriptionally repressed by BRCA1. LYN activation, in turn, protects Brca1-null cells from death upon genotoxic stress. Furthermore, we show that in TN/BLBC a distinct LYN splice variant is up-regulated, driving migration and invasion. Thus, we have identified dual molecular mechanisms – changes in splice isoform ratios and uncoupling from upstream signals – which drive the critical activity of LYN in this aggressive breast cancer subtype.

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Investigating TGFβ Regulated Cell Proliferation Decisions in Single Cells

The transforming growth factor-β (TGF-β) signaling is one of the most important signaling events as it regulates many cellular responses including cell proliferation, migration, and death. It is well known that TGF-β has different roles in the regulation of cell proliferation depending on the specific cellular context. Although the main components of the TGF-β pathway have been extensively studied, it remains unclear how cells decode and integrate TGF-β signal into cell fate decisions in single cells. We plan to combine mathematical modeling and quantitative experiments to analyze how the cells read TGF-β activation and translate it into cell proliferation fate decision at single cell level. We will perform live-cell imaging experiments to trace the correlations between Smads signaling dynamics and cell proliferation in individual cells with different TGF-β stimulations using microfluidics system. Mathematical models will be developed to explore how Smads signaling dynamics decide cell proliferation responses. In this project, we aim at getting a better understanding on how single cells decipher Smads signaling dynamics, which may provide new insights for controlling cell proliferation with the manipulation of TGF-β signaling.

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Profiling the Peptide Binding of Sorcin Using a Phage Peptidome Representing the Intrinsically Disordered Regions of the Human Proteome

Sorcin (Soluble Resistance-related Calcium binding protelN) is a penta-EF hand protein found highly expressed in several human tumors and overexpressed in chemoresistant cell lines. Sorcin confers MultiDrug Resistance (MDR) when overexpressed, and it is considered as a marker of MDR [1],[2]. Although Sorcin is an interesting potential cancer target, its binding partners upon calcium binding are poorly known.
Proteomic Peptide Phage Display (ProP-PD) is a novel high-throughput method used to characterize protein-protein interactions (PPIs) [3]. In ProP-PD peptides derived from the human proteome are displayed on the major coat protein (pVIII) of the M13 phage, which results in a multivalent display [4]. ProP-PD differs from combinatorial phage display because the library is specifically designed on the human proteome. In this study, we used a ProP-PD library of 16-mer representing the internal disordered regions of the human proteome. Such regions are enriched in Short Linear Motifs (SLiMs), which are involved in crucial interactions for cell signalling. Phage display selection process was performed against immobilized Sorcin, in order to gain information about its preferred binding motifs. In a previous combinatorial peptide phage display selection against Sorcin we found an enrichment in peptides containing ϕϕxP and/or ϕE/D motifs suggesting that two binding sites may be exposed for interactions upon calcium binding [5]. Our preliminary data show that:

- The dataset of selected peptides is enriched in calcium binding proteins
- In line with our previous study, the dataset contains peptides with ϕϕxP and/or ϕE/D motifs
- These residues appear to be conserved

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Using Antibiotic Resistance to Select and Evolve Aggregation-Resistant Protein Therapeutics

The production and formulation of biopharmaceuticals can be hindered by protein aggregation which can occur at every stage of the manufacturing process, ultimately jeopardising the successful development of promising candidates from becoming the next blockbuster biologic. Investigating protein aggregation and stability can be laborious, due to the difficulties in expression and purification for in vitro *analysis. To address this, we have developed an* in vivo *platform to characterise the aggregation propensity of biopharmaceuticals that circumvents the need for recombinant expression and downstream analysis. The system, based on a split β-lactamase enzyme assay, enables the identification of aggregation-prone sequences inserted between the two enzyme domains, via the survival rates of the bacteria in which they are expressed.*
Applying the system to therapeutic proteins with known drug development issues, the antibody MEDI-1912 and granulocyte-colony stimulating factor (G-CSF), we demonstrate the system's ability to distinguish between aggregation and non-aggregation prone sequences, offering a powerful tool for assessing protein aggregation and stability earlier in the industrial pipeline. Furthermore, by developing a directed evolution methodology we show that this system can be used as a novel strategy to modulate the aggregation propensity of protein scaffolds enabling the identification of evolved variants with reduced aggregation propensity.

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Study on the Amyloid Fibrils Based on AFM-Based Technologies

More than 50 neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, have been discovered nowadays and becoming more and more common among human beings. Each of the disorders is related to a specific protein or peptide, which suffers a conformational and structural transformation from native soluble monomers to toxic oligomers, then protofibrils and eventually form fibrillar protein aggregates.
We are using the Atomic Force Microscope (AFM) based technologies to discover the molecular structure transformation and pathway of fibrillation of these proteins and peptides, including the topographical transformation, stiffness, and secondary structural transformation during protein aggregation. AFM is capable to provide morphological information of sample and statistical analysis like height distribution, surface profile and roughness, which enable us to distinguish the structure and monitor the morphological transformation. AFM-infrared nanospectroscopy (AFM-IR) is performed for chemical analysis to determine secondary structure of the proteins at nanoscale. An absorption spectrum as a function of wavenumber at a certain location and an absorption map at a certain wavenumber with high spatial resolution (approximately 50 nm lateral resolution) are recorded, to understand the conformation of different secondary structures in a single specimen and the distribution of a certain secondary structure, respectively. Quantitative imaging (QI)-AFM is able to measure the mechanical property (stiffness) of the specimen at the nanoscale and distinguish individual protein aggregates according to their stiffness.

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Effect of Quercetin on the Glutathione-S-Transferases, RLIP76, MRP1, and MRP4 Functioning in Human Peripheral Blood Cells

OBJECTIVES: to investigate the effect of flavonol quercetin on the activity of the cytosolic enzyme glutathione-S-transferase (GST); on the functioning of proteins associated with multidrug resistance: Ral-interacting protein 1 (RLIP76) and the multidrug resistance protein 1 and 4 (MRP1, MRP4).
METHODS: Erythrocytes isolated from the human blood of healthy donors were used as subjects of the study. MRP1 transport activity was estimated by residual retention of fluorescent dye calcein (CAL) in cells; MRP1 and RLIP76 transport activity – by the degree of export of glutathione conjugates with 1-chloro-2,4-dinitrobenzene (DNP-SG); MRP1 and MRP4 functioning – by the kinetics of the export of glutathione conjugates with monochlorbimane (biman-SG). Cytosolic GST type π activity and reduced glutathione (GSH) concentration were assessed by spectrometric standard protocols.
RESULTS: It was shown that the transport of DNP-SG and biman-SG conjugates from human erythrocytes preincubated with quercetin in micromolar range of concentrations was reduced correspondingly at 35-45% and 20-30% in comparison with intact cells (control), but the intensity of CAL fluorescence in red blood cells treated with current flavonol did not change. At the same time, it was detected quercetin-mediated decreasing (at 20-30%) of GSTπ activity in human erythrocytes. This fact completely negates the effect of quercetin on the transport of biman-SG conjugates and partly on the export of DNP-SG conjugates from erythrocytes because it is known that formation of both types of conjugates occurs with the GSTπ participation. Nevertheless, GSH concentration was decreased at 10-20% after erythrocytes incubation with quercetin and the values of these changes are in good agreement with value ranges of rise of RLIP76 activity.
CONCLUSION: Thus, effect of quercetin in micromolar range of concentrations on human erythrocytes leads to a shift in the balance of "prooxidants-antioxidants" in favor of the former. Along with this, it is occurred activation only of the protein-transporter RLIP 76, but not MRP1 and MRP4 – the main function of which to participate in maintenance of oxidative homeostasis in human erythrocytes.

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Sequence Determinants of Secondary Nucleation

Amyloid fibrils are formed by ordered aggregation of polypeptides and proteins. Many amyloidogenic proteins are associated with neurodegenerative diseases including Alzheimer's disease. The mechanism of formation of fibrils varies between different amyloid peptides that exist in vivo*, still seems to contain a small set of microscopic steps: primary nucleation and elongation and possibly also fragmentation or surface catalyzed secondary nucleation. To understand the sequence determinants of the mechanistic details, a set of eight charge mutants of Aβ M (1-40) that differ in aggregation kinetics were studied. Self and cross seeding of mutant and wild type peptides were performed using ThT as reporter of the aggregation kinetics. We identify that mutations produced different behaviors. We identify a set of different seeding effects:*

- *The identity of the seed determines the effect*
- *The identity of the monomer determines the effect*
- *The identity of none is critical*

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Imaging Stem Cell Transcription Dynamics

In blastocyst development, the activation of Erk through Fgf signaling has been related to the separation of epiblast and primitive endoderm states in embryonic stem cells (ESC). This differentiation event is characterized by the up- and down-regulation of mutually exclusive transcription factors resulting in two distinct cell fates.
In this project, we will study transcription dynamics during this differentiation event, in particular the role and dynamics of enhancer-promoter looping and the associated regulation of epiblast and primitive endoderm specific gene transcription. By combining an Erk activation system in ESC with single molecule localization microscopy, we aim to localize and track single gene promoter, enhancer, and associated transcription factors with high resolution in live stem cell nuclei as they differentiate. To allow the detection of single molecules inside live cells, we have installed and modified an inverted TIRF microscope to allow stochastic activation and localization of photo-switchable fluorescent protein. It is our aim to implement different DNA and mRNA targeting techniques with fluorescent probes in order to not only localize genes and enhancer looping, but also transcription activation and bursting.

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Development of a Surface Plasmon Resonance Assay to Measure the Affinity of Coagulation Factor Va to Membranes

While great strides have been made in developing methods for studying transmembrane proteins, tools for investigating protein-membrane interaction are limited. A promising tool for investigation of such interactions is the nanodisc. The nanodisc is a uniform membrane disc consisting of lipids, and other membrane components, encapsulated by a membrane scaffolding protein, thereby providing a uniform mock membrane surface. The uniformity of the nanodisc makes it an ideal tool for structural studies. Hence, we plan on using nanodiscs to study the structure of membrane bound coagulation factor Va (FVa). Mutations in FV can both lead to excessive bleeding and excessive clotting, and the activity of FVa is dependent on binding to a membrane surface. Using hydrogen deuterium exchange measured by mass spectrometry we will determine the changes in dynamics of the amide backbone hydrogens upon membrane binding. This together with structural information gained by electron microscopy may provide valuable insight into the role of membrane interaction for the function of FVa. As part of the studies, and to guide the structural work, we need to determine the affinity of FVa to the nanodisc. For this we are using surface plasmon resonance (SPR) as the main technology. Here I will present the challenges in making an SPR assay capable of determining the affinity and binding kinetics of FVa to the nanodiscs.

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Metastasis - A Journey Of Force

The metastatic cascade is a physically highly demanding process. On the one hand cancer cells have to resist mechanical forces as compression, tension and high shear stresses in the blood stream. On the other hand they have to generate and apply forces on the environment while squeezing through the dense network of extracellular matrix or the endothelial lining of blood vessels. In addition, the ability to adapt to different physical conditions is essential for a successful metastatic colonization, as secondary tumor sites often exhibit vastly different mechanical conditions than the organ of tumor origin.
Here, we investigated mechanical changes of tumor cells during the process of invasion. By combining micro-rheology measurements with a 3D spheroid invasion assay we could reveal significant changes of the intracellular visco-elasticity during cancer cell invasion.
Furthermore, we probed the adaptability of cancer cell lines of different invasive potential to changes in their microenvironment. Particle tracking microscopy in cells cultures in collagen matrices of variant stiffness revealed striking differences in the intracellular responses to changes in the microenvironment. Interestingly, a correlation between the magnitude of intracellular plasticity and the invasive potential was detected.
Further investigations of the mechanical interaction between cancer cells and their microenvironment as well as the mechano-sensing machinery will give deeper insights in the contribution of physics on cancer progression.

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The Effect of Functional Amyloids in Parkinson's Disease

Aggregation of α-synuclein (α-SN) is one of the key hallmarks of Parkinson's disease (PD) neuropathology. Studies suggest that α-SN nucleation and aggregation may originate in peripheral tissues such as enteric neurons in the gut and the olfactory system and aggregated α-SN can be retrogradely transported via the vagal nerve from the intestine wall to the brain. Furthermore, the risk of PD in patients who has undergone vagotomy is decreased compared to a matched general population.
Comparing microbiome composition of PD transgenic rats and their healthy controls showed a decrease in the bacterial family Prevotellaceae*, in correspondence with previous results. To look for functional bacterial amyloid (FuBA) proteins in these microbiome samples I am using a method with formic acid treatment followed by LC-MS/MS which has shown able to identify amyloid proteins in complex samples.*
A different method to investigate the effect of FuBAs in PD is using transgenic α-SN overexpressing C. elegans*. Feeding these worms with FuBA-producing bacteria have shown to affect the degree of α-SN aggregation – however, our preliminary results show that feeding the worms with* V. brasiliensis*, which shows extensive amyloid formation, actually decreases α-SN aggregation.*

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Clathrin Light Chains Modulate the Biophysical Properties and Function of Clathrin

Three clathrin light and heavy chains (CLCs and CHCs) form a so-called triskelion, which assembles into membrane-associated, macromolecular lattices to sequester cargo into transport vesicles.
Highly conserved differences between CLCs, tissue-specific splicing and expression of the two CLC genes point towards distinction functions. We hypothesize that CLCs alter the biophysical properties of clathrin to accommodate specialized function in various tissues.
We found that CLC isoforms segregate on a clathrin triskelion, but that these triskelia mix within assemblies. Characterizing clathrin of defined CLC composition, we observed stability of the clathrin triskelion to vary with CLC isoform, while neuronal splice inserts decreased stability respectively. Neuronal variants further showed reduced ability to deform membranes and increased clathrin cage disassembly rates.
Our findings revealed specific CLC composition and their influence on biophysical properties of clathrin. Neuronal variants in particular altered stability and tensile strength of the clathrin lattice and its component triskelion. These "weaker" lattices may be preferred in a presynaptic setting to facilitate rapid uncoating and tightly controlled packaging of synaptic vesicles by clathrin adaptors and other factors.

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Not Your Typical TPR Protein – RNA-Binding Proteins with Helical Repeats

We investigate the structure and function of two families of RNA-binding proteins with helical repeats. One group is the Interferon induced protein with tetratricopeptide repeat (IFIT) family, which are antiviral effectors that sequester viral RNA and prevent translation of viral proteins, as part of the vertebrate innate immune response. Although the crystal structures of some IFITs are known, it is not clear how they cooperate in multi-IFIT complexes to counter viral infection. We study the RNA preference of IFITs when present in a larger assembly, and we are exploiting their unique properties for use in diagnostics and biotechnology applications.
The second group of our interest are the Fas-activated serine threonine kinase domain (FASTKD) proteins, which were misannotated as kinases, but seem to bind and regulate the processing of mitochondrial transcripts in animals. FASTKD proteins contain predicted helical repeats and a putative PD(D/E)XK nuclease domain overlapping with another small domain called RNA-binding domain abundant in Apicomplexans (RAP). The structure of any of the FASTKD proteins is not known, and no reliable structural homologues could be identified. We are using structural biology methods and RNA-binding assays, together with a phylogenetic analysis of protein with RAP domains, to determine the structure and function of FASTKD proteins.

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S100 Proteins as Novel Modifiers of Proteostasis in Pathophysiological States

Decay in brain and cognitive functions are some characteristics of age related neurodegenerative diseases, such as Alzheimer's disease (AD), which are correlated with different protein expression patterns within cells and activation of inflammatory pathways. A consequence of the dysregulated expression is the misfolding of normally soluble proteins and their subsequent conversion into toxic amyloid aggregates [1] which is accompanied by upregulation of pro-inflammatory cytokines such as S100 proteins. S100s are small (12kDa) Ca^{2+}-binding signaling proteins which occur mostly as homodimers. Ca^{2+} binding occurs at two EF-hand motifs and some homologues contain additional regulatory Zn^{2+}/Cu^{2+} binding sites [2].
S100 proteins are involved in numerous intra and extracellular pathophysiological processes and some neuronal S100s are consistently altered in neurodegeneration, including AD. Among these are S100A8, S100A9 and the heterodimer S100A8/A9 (calprotectin, CP) which seem to undergo self-assembly upon zinc and calcium binding, a process which is likely mechanistically linked to the cross-beta forming propensity which we have recently elucidated [3,4].
In this work, we report an investigation dealing with our hypothesis that zinc and calcium-binding to neuronal S100s promote the formation of protein deposits in the AD brain and that this is related to changes in zinc homeostatic levels, which are known to be altered in ageing and neurodegeneration. For the purpose, we analysed brain sections from APP23 AD mice models and different ages (3 and 15months), using immunohistochemical analysis and fluorescence microscopy in combination with biochemical assays. We have analysed the occurrence of S100A8 and S100A9 protein assemblies in correlation to brain zinc levels and the presence of amyloid plaques. Here we present our preliminary results that pave the way to explore new roles for S100s as modifiers of Alzheimer's Disease.

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Phenylalanine Hydroxylase Regulation in Health and Disease

The hepatic enzyme phenylalanine hydroxylase (PAH) catalyses the rate-limting step in degradation of phenylalanine (L-Phe) and ensures that intermediates for neurotransmitter synthesis are made and that L-Phe does not reach the brain in toxic amounts. A dysfunctional PAH leads to the disease phenylketonuria (PKU) with irreversible brain damage if the patient does not follow a life-long protein-restricted diet. As most dysfunctional PAH proteins are misfolded we are searching for pharmacological chaperones – small molecules that bind PAH to help it fold and maintain the correct structure - that can alleviate the diet.
Human PAH is composed of four identical subunits with three domains. This complex structure includes flexible hinges between the domains. This facilitates a large conformational change that is needed for substrate activation, and for it to be almost inactive between protein meals to ensure that sufficient L-Phe is available for protein synthesis. However, the important flexibility deters crystallization of the full-length human PAH and there is no crystal structure of a complete mammalian PAH in complex with L-Phe. The activated structure is thus not known and the basis for the regulatory mechanism that allows switching between the inactive and active form is not completely elucidated.
We are using structural techniques such as SAXS and X-ray crystallography in combination with binding studies and molecular dynamics simulations to characterize the regulatory substrate binding event in PAH and the effect of potential pharmacological chaperones.

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Molecular Mechanism of Aβ42 Peptide-Fibril Adsorption From Atomistic Simulations

Alzheimer's Disease is quickly becoming the most pressing medical issue facing a globally ageing population, due in large part to a lack of understanding of the fundamental physics and chemistry responsible for the pathogenesis. The disease is associated with the aggregation of soluble amyloid-β (Aβ) protein into fibrils and has been found experimentally to demonstrate a nucleation-elongation polymerisation mechanism, in which the rate-determining association of few protein monomers into a growth-competent nucleus is followed by fast incorporation of additional soluble subunits into insoluble quaternary complexes. The reaction cascade is strongly accelerated by secondary nucleation, in which the surfaces of existing fibrils drive the formation of further oligomers and fibrils. This autocatalysis is the positive feedback mechanism by which Alzheimer's Disease becomes fatal, and as such is critical to understand in order to be able to design therapeutics. Here we use long all-atom, explicit-solvent molecular dynamics simulations with a novel enhanced sampling scheme, based on scaling solvation free energy, to probe the interaction of a free $A\beta_{42}$ monomer with an $A\beta_{42}$ fibril. In doing so we identify possible binding epitopes on both species, probe the thermodynamics of adsorption and propose an overall enthalpically driven mechanism of fibril-catalysed peptide collapse that is consistent with experimental findings. Our work is important methodologically as we improve on the accuracy and sampling of peptide interaction simulations, as well as biophysically and biochemically as we provide residue-level resolution of a medically salient macromolecular interaction.

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Evidence for Self-Replication of Alzheimer-Associated Aβ42 Amyloid Along the Sides of Fibrils

A key molecular process implicated in the pathology of Alzheimer's disease is the aggregation of amyloid β peptide (Aβ) into amyloid fibrils via smaller intermediates. The current consensus in the field is that monomers and fibrils are relatively inert, whereas smaller aggregates are the neurotoxic species. The majority of these toxic species seem to be formed in solutions composed of both monomeric and fibrillar species through the process of secondary nucleation at the fibril surface. Despite the critical importance of secondary nucleation for spreading of pathology and generation of toxic oligomers, direct structural data revealing the molecular details of the process have been sorely lacking, leaving open a number of important questions regarding the structure, size, and activity of the detaching species, and the location of catalytic sites on the fibrils.
Here we used NMR and optical spectroscopy to identify conditions that enable the capture of transient species during the aggregation of Aβ. Cryo-EM images show that new aggregates protrude from the surface of the progenitor fibril. These protrusions are structurally distinct from the well-ordered fibrils observed at the end of the aggregation process. The data provide direct evidence that self-replication through secondary nucleation occurs along the sides of fibrils.

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Structural Studies of the Yeast Ergosterol Transporter NCR1

My PhD project aims to improve our understanding of sterol homeostasis by studying some of the key proteins related to transport using xray crystallography and cryo-EM. In yeast, as in animals, sterols are enriched at the trans-golgi-network and then sorted throughout the cell via vesicles. The membrane protein NCR1*, part of the Niemann-Pick C1 (*NPC1*)-family, is proposed to be a key component of this pathway in fungi.* NCR1 *is thought to be working in tandem with the soluble* NCR2 *in order to embed ergosterol stored in the yeast lysosome-like vacuole into the membrane. The process is conserved from* Saccharomyces cerevisiae *to humans. For instance, yeast* NCR1 *can substitute for the human homolog* NPC1 *in fibroblasts, suggesting that* NCR1 *can transport both cholesterol as well as ergosterol. Linked to cholesterol in humans is the fatal lysosome storage disease Niemann-Pick type C, where cholesterol is accumulated in the lysosomes that causes progressive neurological deterioration and premature death. A fundamental mechanistic understanding of the metabolic pathway of cholesterol is essential to figure out how to counteract the detrimental effects of aberrant accumulation of cholesterol on the human organism. Yeast, being easy to cultivate and manipulate genetically, is thus a perfect model-organism to elucidate the complex pathways of sterol transport through means that would be extremely difficult to employ in human cell lines.*

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A New Sight to Interaction Between b-Lactoglobulin and AITC

Different perspective towards interaction between β-Lactoglobulin and AITC β-Lactoglobulin (bLg) is a globular milk protein considering as a major component of the whey with ~ %60 ratio. It belongs to the lipocalin family which allows binding of bLg to the wide range of hydrophobic ligands. More importantly, bLg can act as a transporter by the means of this property. A monomer bLg has two disulphide bonds and one free thiol group which play essential role to form tertiary structure of bLg. Therefore, this infrastructure of bLg reacts to pH change in a very different and specific way. In this study, the interaction between bLg and allylisothiocyanate (AITC) which is well-known anticarcinogenic and antimflamatuar organosulfur compounds presenting in cruciferous vegetables (Brassicaceae) is studied. The interaction is examined against varied pH (3-8.5) and characterized by Isothermal Titration Calorimetry (ITC) and Circular Dichroism (CD) devices. According to ITC results, AITC and bLg complex showed the 3 site of sequential binding model (KNF model) and high affinity at a level of 104-105 M-1 association constant. Additionally, AITC bound exothermic and endothermic at different pH which means ligand binding sites of bLg changes its conformation at different pH. The results showed that characteristic properties of the interaction between bLg AITC makes the bLg accompanying AITC promising anticarcinogen compound to develop for treatment of cancer on the cell basis.

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Obtaining and Characterization of Bifunctional scfv-Fusion Protein for Targeted Delivery of Therapeutic Drugs

The development of methods for the utilization of natural and recombinant proteins as therapeutic agents is one of the main directions in modern medicine. However, due to the high clearance rate of the majority of the cytokines and hormones, they are administered in doses that are several times higher than therapeutic doses in order to achieve the necessary concentration in target tissues and organs. Today, the search continues for an effective method of delivery of therapeutic proteins that enables the use of agent in the minimum effective dose, provides its targeted delivery into damaged tissue and ensures its prolonged action.

The aim of our research is the creation of bifunctional ligand for targeted delivery of therapeutic proteins by affinity binding to extracellular matrix. The model of chimeric ligand, consisting of ScFv against human growth hormone (HGH) and heparin binding site (HBS) of human FGF-2 was developed.

The plasmid vector pET24-ScFv-HBS was transformed into E.coli cells strain BL21 (DE3). After induction of protein expression target protein was accumulated in the form of inclusion bodies. The inclusion bodies were solubilized in buffer solution containing 7M GuHCl. Purification and renaturation of ScFv-HBS was performed using Ni-NTA column with gradient decreasing concentration of chaotropic agent. The evaluation of the bifunctional activity of the obtained chimeric protein after renaturation was performed by ELISA (by binding ScFv to HGH and HBS to heparin). Thus, chimeric protein ScFv-HBS, as a perspective ligand for the delivery of HGH into mammalian tissues, was obtained in soluble and functional active form.

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Spontaneous Reverse Movement of mRNA-Bound tRNAs Through the Prokaryotic Ribosome

The problem of spreading of bacterial resistance to known antibiotics makes the studies of molecular mechanisms of inhibition important field of modern biomedicine. Different stages of the protein biosynthesis process are the common targets of many famous antibiotics. Here we present studies of specific inhibitor, Madumycin II, belonging to the class of macrocyclic antibiotics Streptogramins A. Antibiotics of this class acts as inhibitors of protein biosynthesis and their target is the peptidyl transferase center (PTC), located on the 50S bacterial ribosome subunit. Due to the ability of madumycin II to bind to PTC, leading to the structural changes in 23S rRNA near PTC, PTC becomes catalytically inactive [1]. The staying of madumycin II on the ribosome prevents the correct positioning of CCA-ends of tRNAs, located in the A and P ribosome sites.
The experimental system we use to elucidate the details of inhibition is based on pure, reconstituted translation system in vitro, consisting on Escherichia coli 70S ribosomal functional complexes (initiation complexes, programmed with MFT-encoding mRNA, containing initiator fMet-tRNA^{fMet} in the P site), and pre-formed ternary complex [^{14}C]-Phe-tRNA^{Phe}·EF-Tu·GTP in the presence of Madumycin II. Madumycin II binds to the initiation complex and inhibits the formation of the first peptide bond, but does not influence on the formation of the second peptide bond. The efficiency of the drug is practically independent of the size of the amino acid, attached to the A-site tRNA.

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Autocatalytic Amplification of Alzheimer-Associated Aβ42 Peptide Aggregation in Human CSF Through Secondary Nucleation

The aggregation of amyloid β peptide (Aβ) has been inferred from experiments in a pure buffer system to involve a double nucleation mechanism, with primary nucleation of monomers in solution and secondary nucleation of monomers on fibril surface [1]. The fibril surface may therefore serve as a catalyst for secondary nucleation leading to rapid amplification of the number of aggregates, underlying the autocatalytic nature of the process.
We ask whether secondary nucleation occurs also in a body fluid - cerebrospinal fluid (CSF). Aggregation of recombinant Aβ42 into fibrils in human CSF was monitored using ThT fluorescence assay, in 20 mM Hepes/NaOH, 140 mM NaCl, 1 mM $CaCl_2$, pH 8.0, with addition of 10 μM ThT, at 37°C. Experiments were initiated at constant CSF concentration (0, 5, 15 or 66%) at a range of Aβ42 monomer concentrations (0.8-10 μM). Finally, reactions were started at constant CSF and Aβ42 monomer concentrations, but supplemented at time zero with preformed fibrils – seeds – at a range of concentrations (0-33%), to confirm the secondary nucleation mechanism.
We find a pronounced retardation of Aβ42 aggregation in CSF - significant already at low (1%) CSF - compared to physiological salt buffer, pH 8.0, in accordance with earlier reports [2]. Seeding trials resulted in shorter lag-times when adding higher concentrations of seeds, indicating that secondary nucleation occurs also in the body fluid investigated.

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Understanding Intracellular Communication Through Metabolite-Induced Thermal Stability of Proteins

The reductionist approach in biology has helped in understanding how proteins interact with different molecules (proteins, metabolites, nucleic acid etc.) in isolation. The present challenge is in expanding our knowledge of molecular recognition to a system-wide scale. The present study aims to elucidate transient protein-metabolite interactions in cells using the decreased conformational entropy or increased thermodynamic stability of proteins upon ligand (metabolite) binding. We have extended two-dimensional thermal proteome profiling approach to study ATP and GTP induced stabilities changes in proteins using cell lysates in the presence and absence of endogenous metabolites. We observed thermal shifts in more than 600 proteins in a dose-dependent manner up on ATP addition in both lysate types. Among the proteins that show altered thermal stability close to 50% are known ATP binders and close to 25% are proteins with no prior evidence of interaction with ATP. The newly identified potential ATP binders seem to enrich for proteins in physical proximity to known ATP binders or proteins existing in complexes, suggesting that ATP may influence the stability of protein complexes in cellular systems. Furthermore, this study has also identified new transmembrane protein targets that may be regulated by these nucleotides. Thus, thermal proteome profiling seems to be an important tool to identify and characterize global footprints of weak protein-metabolite interactions and provide system-wide insights on the role of metabolites in regulation of cellular networks.

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MLKL is Required for RIP1 Regulated Inflammatory Demyelination in EAE Mouse Model

Experimental autoimmune encephalomyelitis(EAE) has long served as an animal model for human Multiple sclerosis (MS), a demyelination disease in which the insulating covers, myelin structure, and nerve cells in the brain and spinal cord are damaged. Recently we found the RIP1 inhibitor 1165 could significantly reduce the disease symptoms in C57 WT mice challenged by EAE, especially the inflammatory reflex induced by infiltrated leukocytes such as macrophage. Surprisingly, inhibition of RIP1 could also block the function of MLKL, the crucial protein of necroptosis, expressed as disease on set and translocated from the cytoplasm of myelin producing cell in CNS, oligodendrocyte to its myelin containing membrane structure. Mlkl$^{-/-}$ mice also showed a decreased clinical score and delayed demyelination process comparing to WT and these mice still kept the normal inflammatory reflex, indicating the function of MLKL in EAE induced demyelination is the downstream of RIP1 regulated inflammatory.

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Robust Regulation of Hepatic Pericentral Amination by Glutamate Dehydrogenase Kinetics

Impaired glutamate dehydrogenase (GDH) sensitivity to its inhibitors causes excessive insulin secretion by pancreatic beta-cells and defective ammonia metabolism in the liver. These symptoms are commonly associated with hyperinsulinism/hyperammonemia syndrome (HI/HA), which causes recurrent hypoglycaemia in early infancy. Hepatic localization of GDH amination and deamination activities linked with the urea cycle is known to be involved in ammonia metabolism and detoxification. Although deamination activities of hepatic GDH in the periportal zones of liver lobules and its connection to the urea cycle have been exhaustively investigated, physiological roles of GDH amination activity observed at pericentral zones have often been overlooked. Using kinetic modelling approaches, here we report a new role for hepatic GDH amination kinetics in maintaining ammonia homeostasis under an excessintrahepatocyte input of ammonium. We have shown that α-ketoglutarate substrate inhibition kinetics of GDH, which include both random and obligatory ordered association/dissociation reactions, robustly control the ratio between glutamate and ammonium under a wide range of intracellular substrate variation. Dysregulation of this activity under pericentral nitrogen insufficiency contributes to the breaking down of ammonia homeostasis and thereby can significantly affect HI/HA syndrome.

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Structural Basis of Quiescence and Activation of the Translocase Motor SecA: A Paradigm for the Regulation of Molecular Motors

More than a third of the proteins of any cell are exported from the cytoplasm, where they are synthesized, to cellular membranes or across them. To understand how exported proteins find the way to their final destination we focus on the ubiquitous and essential Sec pathway that takes proteins across the eukaryotic ER or the plasma membrane of Bacteria and Archaea, using E.coli as a model system. The Sec translocase is a membrane localized multi-subunit protein complex consisting of the SecYEG membrane channel and the cytosolic ATPase molecule SecA. SecA interacts with cytosolic preproteins and translocates them across the SecYEG channel by coupling ATP hydrolysis to translocation work. SecA undergoes multiple finely regulated conformational changes during its functional cycle. Energetic coupling to work is based on transformation of SecA from a quiescent to an activated state by a tightly regulated mechanism that remains elusive. In this study, we reveal a nexus of checkpoints that keep cytosolic SecA conformationally and catalytically auto-inhibited. Using HDX-MS analysis and mutants with defined catalytic responses, we determined the complete structural dynamics profile of SecA and present how these change in subsequent activation steps that include binding to nucleotide. A core checkpoint lies at the interface of the ATPase motor and the preprotein binding region and is strategically located to control cross-communication with the SecYEG channel and couple the release of auto-inhibition elements to result in translocase channel activation. This mechanism has widespread implications for non-folded protein-interacting motors, translocases and chaperones.

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Characterization of the phospho-proteomics of cellular binding partners of the HIV tether LEDGF/p75

Lens Epithelium Derived Growth Factor/p75 (LEDGF/p75) is a transcriptional co-activator tethering proteins and protein complexes to active chromatin. The C-terminus of LEDGF/p75 comprises the Integrase Binding Domain or the IBD that facilitates its tethering activity. This domain is seized by the Human Immunodeficiency Virus (HIV) to allow the integration of the virus into active chromatin. Our lab developed the first-in-class small molecules, termed LEDGINs, inhibiting the LEDGF/p75 HIV interaction which are currently in early clinical development (Christ et. al., 2010). In addition to HIV integrase, LEDGF/p75 also tethers several disease-specific and cell cycle-oriented factors in a similar fashion. Using NMR spectroscopy and protein interaction assays, we defined the interaction profile of LEDGF/p75 with its cellular binding partners MLL, JPO2, IWS1, MED1 and PogZ. Association of LEDGF/p75 with JPO2 (involved in Medulloblastoma) and MLL (mixed-lineage leukemia) makes it an interesting target of study from a therapeutic perspective. These data show that a very short intrinsically disordered but conserved IBD-binding motif (IBM), present in these unrelated proteins, is responsible for bringing about the interaction with LEDGF/p75. We also show that this interaction can be modulated by post-translational modifications, specifically CK2-driven phosphorylation. The importance of these phospho-sites were verified in vitro through mutational analysis. Based on IBM conservation, we validated the effect of phosphorylation on LEDGF/p75 and its cellular binding partners.

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NMR studies of the structure and interactions of small heat shock protein Hsp21

Small heat shock proteins (sHsps) are ATP-independent chaperones present in both pro- and eukaryotic organism s. They are characterised by the conserved α-crystallin domain (ACD), the C-terminal region (CTR), and the N-terminal region (NTR). There are not many crystal structures of sHsps available, and there are especially few that include the generally flexible terminal regions.
We have looked at Hsp21, a 21kDa sHsp present in the chloroplast of Arabidopsis thaliana. It forms a dodecameric structure of 240kDa.
In Rutsdottir et al. 2017, cross-linking mass spectrometry, small angle x-ray scattering (SAXS) and homology modelling was used to create a structural model of Hsp21. The best fitted model was found when treating the approximately 80 amino acid long NTR region as highly flexible and facing outwards into the solution.
We have verified this model using nuclear magnetic resonance spectroscopy (NMR). The visible NMR signals from Hsp21 appears to originate from slightly more than 20 amino acids arranged in a pattern consistent with intrinsically disordered proteins (IDPs). We currently have assigned NMR signals from 17 amino acids, all of which are located in the NTR.
We are currently performing additional NMR studies in order to further characterize the structure of the NTR. We are also aiming to study Hsp21 client interactions, by building on previous studies using SAXS and mass spectrometry.

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Interaction of kringle-containing plasminogen fragments with carboxy-terminal fibrin D-D domains

BACKGROUND: Plasminogen kringle domains ensure the proenzyme interactions with fibrin clot. Plasminogen interaction with specific binding sites in fibrin C-terminal D-domains initiates the proenzyme activation and subsequent clot lysis. In this study, we investigated the interaction of plasminogen kringle-containing fragments K1-3 and K5 with fibrin fragment DD.
OBSERVATIONS: Fragments K1-3 and K5 have high affinity to fragment DD (K_d is 0.02 and 0.054 μM). The level of Glu-plasminogen binding to fragment DD is decreased by 50-60% in the presence of K1-3 and K5. The binding levels of K1-3 and K5 with immobilized fragment DD before and after C-terminal lysine residues removal indicate that K5 interaction is independent and K1-3 is partly dependent from C-terminal lysines.
K1-3 interacts with complex of DD-immobilized K5 as well as K5 with complex of DD-immobilized K1-3. The plasminogen fragments do not displace each other from binding sites located in fibrin fragment DD, but can compete for the interaction. Thus, plasminogen kringles bind to different sites in peripheral fibrin domains.
Analysis of K1-3 and K5 binding to fibrin fragment DD with reduced disulfide bonds showed the interaction of both plasminogen fragments with γ-γ-chains of fragment DD. Thus, widely known plasminogen-binding site Aα 148-160 in fibrin D-domains is not a single binding sequence of fibrin peripheral domains or plasminogen-binding site is not linear.
CONCLUSIONS: Fibrin fragment DD contains different binding sites for plasminogen kringle fragments K1-3 and K5, which can be located close to each other. Possible plasminogen kringle-binding sites are located in α- and γ-chains.

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Probing protein tumbling inside human cells via in-cell NMR

The intracellular environment comprises a plethora of molecules, whose composition is under constant change. How do proteins exert their functionality in this crowded environment and what structural challenges do they face? Scientists have long tried to answer these questions by studying protein functionality and structural behavior in vitro. These simplified systems come with the weakness of not reflecting the full molecular complexity of the cytoplasm, and consequently one may ask; to what extent do in vitro data have in vivo relevance? To address this issue, we have developed novel methods where NMR is used to analyze the structural and dynamic properties of proteins inside live cells.
Our results indicate a strong correlation between the net charge of proteins and their mobility in the cytoplasm. We note how human proteins go from being soluble to becoming “sticky” when transferred from human cells to E. coli. However, this stickiness can be counteracted by introducing one or several surface mutations and therewith adapt the protein to its new environment. Tiny differences between homologous proteins indicate a structural optimization on a genetic level which appear to have developed faster than the basic cellular functionality. An investigation of the molecular rules behind this optimization is expected to provide fundamental knowledge of, not only how the evolution of protein properties is governed, but also of the underlying causes of protein diseases such as ALS; where single mutations can induce inexplicable aggregation, massive proteome collapse and neuronal death.

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The effect of amyloid formation on the pKa values of α-synuclein

α-synuclein is a 140 residue long amyloidogenic protein. The formation of α-synuclein amyloid fibrils and their accumulation into Lewy bodies is associated with Parkinson´s disease. The last 40 residues of α-synuclein - referred to as the C-terminal tail - are of interest in this project. The tail is highly acidic, consisting of 15 acidic groups, and has been found to be unstructured in both monomeric and fibrillar form. We examined the effect of amyloid formation on the pKa values of the acidic residues in the C-terminal tail of α-synuclein. A significant increase in pH, from 5.5 to 6.5, was detected during fibril formation of α-synuclein. The increase in pH suggests that the affinity of α-synuclein for protons increases during fibril formation, thus indicating an upshift in the average pKa value of the acidic residues. Calculations of the average pKa value of α-synuclein showed a pH-shift from 4.3 to 5.4 during fibril formation. The pH increase during fibrillation was also measured for an α-synuclein mutant, in which five acidic residues in the C-terminal tail had been substituted for five non-charged polar residues. The increase in pH during fibrillation was less for the mutant (from pH 6.4 to 6.9). The calculated shift in the average pKa value of the mutant during fibril formation was 4.2 to 4.6. These results indicate that the upshift of pKa values of the acidic residues is linked to the close proximity between the acidic residues of the tails within the fibrils.

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Modulation of amyloid formation in Parkinson's disease by fish allergen

The aggregation process of α-synuclein into amyloids is thought to be the main reason for neuronal cell loss in Parkinson's disease. No cure for the disease currently exists and symptoms can only be treated short term; therefore a better understanding of what causes or prevents Parkinson's disease is needed. The Mediterranean diet, in which fish is a significant constituent, has been negatively correlated to both Parkinson's disease and Alzheimer's disease to a similar extent. Fish has also been correlated to a reduced incidence of Alzheimer's disease. A calcium binding protein found in fish, known as β-parvalbumin (β-PV), has been shown to be very resistant against degradation under a simulated gastrointestinal environment, and it can be found in the bloodstream following a diet consisting of fish. Mammals barely express β-PV but instead the ortholog α-PV (approximately 70 % sequence similarity between Pacific cod β-PV and human α-PV) is expressed in both muscles and certain interneurons. Furthermore, α-synuclein has been shown to colocalize with neuronal α-PV in PV-positive interneurons. We aim to study if the fish allergen, β-PV, can affect the aggregation of α-synuclein. It has been shown that β-PV adopts an amyloid conformation in the digestive tract and it is suggested that the protein is taken up in an aggregated form. Thus, this interaction could be of physiological relevance.

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Protein complexes in human milk

Human milk is an important source of nutrition for infants. It contains a wide array of proteins, peptides and other components promoting infant development and protecting from viral and bacterial infections. It is known that milk proteins can associate with each other to form complexes. It has been proposed that most biological processes are performed by protein complexes. Hence identification and characterization of milk protein complexes is important to understand the role of milk for a newborn.
In this work, we have investigated some protein complexes from human milk. Using gel filtration and affinity chromatography, cross-linking with glutaraldehyde following by SDS-PAGE and MALDI mass-spectrometry we have established stoichiometry of lactoferrin (LF) complexes and immunoglobulin G (IgG)-LF complexes. The complexes are stable in the presence of low concentrations of NaCl and dissociate under 0,6 M NaCl. It is known that LF has a large number of biological activities and functions. Since LF is found not only in human milk, but also in other biological liquids such as epithelial secretions, barrier body fluids, and also in the secondary granules of leukocytes, we propose that investigation of LF complexes and complexes LF with IgG in milk is important for understanding of the LF role in different biological liquids.

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Search into unevolved protein space

The protein sequences found in nature represent a tiny fraction of the potential sequences that could be constructed from the 20-amino-acid alphabet. To help define the properties that shaped proteins to stand out from the space of possible alternatives, we conducted a systematic computational and experimental exploration of random (unevolved) sequences in comparison with biological proteins. In our study, combinations of secondary structure, disorder, and aggregation predictions are accompanied by experimental characterization of selected proteins.
We found that the overall secondary structure and physicochemical properties of random and biological sequences are very similar. Moreover, random sequences can be well-tolerated by living cells. Contrary to early hypotheses about the toxicity of random and disordered proteins, we found that random sequences with high disorder have low aggregation propensity (unlike random sequences with high structural content) and were particularly well-tolerated. This direct structure content/aggregation propensity dependence differentiates random and biological proteins.
Our study indicates that while random sequences can be both structured and disordered, the properties of the latter make them better suited as progenitors (in both in vivo *and* in vitro *settings) for further evolution of complex, soluble, three-dimensional scaffolds that can perform specific biochemical tasks.*

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The use of BioID to identify potential BAG6 substrates

Failure of protein quality control pathways can lead to harmful accumulation of aggregation-prone proteins, as occurs in many human diseases. Defining the range of quality control systems that cells use to handle such aggregation-prone proteins is vital for understanding the molecular basis of these diseases and the development of new treatments. BAG6 is a key component of cellular protein quality control systems, which through its ability to bind hydrophobic sequences plays a critical role in the cellular handling of aggregation-prone proteins containing exposed hydrophobic domains. BAG6 contributes to at least two distinct quality control pathways: ER-associated degradation which removes misfolded proteins from the ER, and cytosolic quality control system that degrades mislocalised membrane proteins. Even though BAG6 is now firmly implicated in handling of different types of hydrophobic domains in the cytosol, there is little understanding on the range of cellular substrates of BAG6. To better understand BAG6 substrate specificity determinant, an unbiased proteomic approach BioID was used to identify endogenous protein interactions based on proximity in living cells. BAG6 with a biotin ligase fused at the C-terminus conjugated biotin moiety on proteins coming into close contact and biotin-labelled proteins were isolated and analysed with mass spectrometry. Biotinylation was also performed under conditions where degradation is blocked with proteasome inhibitor, which would be predicted to stabilise some rapidly turnover proteins and labile interactions.

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Using optogenetics to study the dynamics of TGF-β signaling pathway

Transforming Growth Factor β (TGF-β) signaling pathway plays significant roles in regulating many cellular processes, which include cell proliferation, differentiation and migrations. Although the principal components of TGF-β signaling have been identified and explored in decades, it is still very difficult to study the wring and dynamics of the network under different pattern of stimulations. In this project, we are trying to disentangle this classic signaling pathway by applying the new optogenetics tool, by which light is used to control cellular behavior precisely in time and space. We are engineering optoTGFBRs by introducing light-sensitive protein pairs (PhyB/PIF; CIBN/CYR2) that can interact with each other rapidly and reversibly upon different light wavelength. TGF-β signaling pathway can therefore be trigged ON and OFF at the single cell level by manipulating the illumination pattern. In addition, a fluorescent protein labeled Smad will be expressing in the stable cell line, which plays as an indicator of the TGF-β signaling pathway activation. By simultaneously monitoring the translocation of the receptors and Smad proteins using live cell imaging, and in combination with mathematical models, the dynamics of TGF-β signaling pathway in single cells could then be quantitatively characterized. Compared with classical chemical-inducing activation, optoTGFBRs could be used to study various aspects of TGF-β signaling pathways in vitro/in vivo with higher spatiotemporal resolution.