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NON-GLOBULAR PROTEINS IN MOLECULAR PHYSIOPATHOLOGY

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Abstract book

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INVITED TALKS

S01	Invited talk

Order-Disorder Transitions of Coiled-coil Barrels During Virus Assembly

Alasdair Steven

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Assembly processes, particularly those of larger and more complex viruses, involve conformational changes that include rigid-body domain rotations; transitions induced by limited proteolysis; and order-disorder transitions. This presentation will cover two systems of the latter kind that are unusual in that the ordered state is a multi-chain α-helical coiled coil barrel. (A) In HIV assembly, the Gag polyprotein assembles into the immature particle, to be dissected into its component domains during maturation. The final cleavage takes place between the capsid (CA) and nucleocapsid (NC) domains connected by Spacer 1. Now-persuasive evidence indicates that Sp1 forms a hexameric coiled coil barrel in the immature virion but is disordered when alone (as in the mature virion) or in CA-Sp1 particles assembled in vitro. Certain reagents that bind to and stabilize the barrel, impeding cleavage, inhibit the virus. (B) Bacteriophage P22 capsid has a portal protein at one of its twelve icosahedral vertices that serves as a conduit for DNA to enter during assembly and for ejection proteins and DNA to exit during infection. The 120-residue C-terminal domain of portal forms a 12-membered coiled coil barrel in the crystal structure (PDB: 3LJ5). Cryo-EM and bubblegram imaging of virions show the distal half of the barrel to be disordered and to present binding sites for e-proteins (W. Wu, J.C. Leavitt, N. Cheng, E.B. Gilcrease, T. Motwani, C.M. Teschke, S.R. Casjens & A.C. Steven – Ms. in submission, 2016). During infection, the e-proteins precede the DNA along the portal channel in an at least partly disordered state en route into the host bacterium.

S02	Invited talk

Coiled coils - between structure and unstructure

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Due to the regularity of their interactions, coiled coils are frequently very stable proteins and many have been reported to withstand extreme chemical and thermal conditions, even when they comprise chains of little more than 30 residues. Given this thermodynamic stability, it may come as a surprise that coiled coils are close to the unfolded state; in fact, it is not uncommon for them to be mistaken for natively unstructured polypeptides by disorder prediction programs. This failure cuts both ways: highly charged sequences that are largely devoid of hydrophobic residues and lack sequence repeats indicative of coiled-coil structure are often predicted as coiled coils, even though they are most likely unstructured. We propose that coiled-coil sequences have evolved to resemble unstructured polypeptides because they need to ensure in-register folding of rods that are sometimes many hundreds of residues long. Since packing interactions are structurally the same all along the rod, coiled coils are confronted with many, essentially isoenergetic intermediates that could trap the folding chains out of register if they formed spontaneously. To prevent this, coiled coils have evolved sequences that allow them to be quite stable thermodynamically, once folded, but have kinetic folding barriers that maintain them in an unstructured state until folding has been initiated at a nucleation site and is therefore guaranteed to be in register. This nucleation site can be within the coiled coil (‘trigger sequence’), or in an adjacent domain (such as the foldon of T4 fibritin).

S03	Invited talk

Implications of dark proteome for personalized health

Burkhard Rost

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The objective of our group is to predict aspects of protein function and structure from sequence. The wealth of evolutionary information available through comparing the whole bio-diversity of species makes such an ambitious goal achievable. Our particular niche is the combination of evolutionary information with machine learning. We developed methods to predict from sequence protein interactions (incl. networks), cellular localization, functional classifications and the effects of sequence variants upon molecular function and the organism. In this talk I will focus on protein-protein interactions, present the concept of the Dark Proteome and how protein disorder appears to play a unique role in evolution, and will present some surprising results from effect-prediction methods for the analysis of large populations.

S04	Invited talk

Inferring folding energy landscapes from repeat-protein sequences

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Natural protein molecules fold, move and function according to the information encoded in their energy landscapes. For most architectures, this information is still difficult to deconvolute from the linear sequence of amino acids, as the energy contributions are small, numerous and distant. In contrast, repeat-proteins encode similar structural features in a quasi-linear way, facilitating the description, evaluation and evolution of their energy landscapes. I will present and discuss the application of statistical analysis of genomic data to extract physically meaningful information about repeat-proteins energy landscapes. We evaluated the energetic signatures of natural repeat-proteins and inferred the population of intermediates and folding routes. This knowledge can be used to design sequences with sculpted asperities in the landscape, such that the stability, cooperativity and folding polarization of a repeat-array can be fine-tuned

S05	Invited talk

Floppy but not sloppy: Molecular Plasticity in the Dark Proteome of the Nuclear Transport Machinery

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The mechanisms by which intrinsically disordered proteins (IDPs) engage in rapid and highly selective binding is a subject of considerable interest and represents a central paradigm to nuclear pore complex (NPC) function, where nuclear transport receptors (NTRs) move through the NPC by binding disordered phenylalanine-glycine-rich nucleoporins (FG-Nups). Combining single molecule fluorescence, molecular simulations, NMR and other spectroscopic methods we recently showed that a rapidly fluctuating FG-Nup populates an ensemble of conformations that are prone to bind NTRs with diffusion-limited on-rates, as shown by stopped-flow kinetic measurements. This is achieved using multiple, minimalistic, low affinity binding motifs that are in rapid exchange when engaging with the NTR, allowing the FG-Nup to maintain an unexpectedly high plasticity in its bound state. We propose that these unique physical characteristics enable a rapid and specific transport mechanism in the physiological context, a notion supported by our single molecule in-cell assays on intact NPCs. The ultrafast binding mechanism seems distinct from classical folding upon binding mechanisms and is unexpectedly not driven by electrostatics. However, time resolved emission spectra experiments highlight a central role of protein solvation, which points to a novel pathway on how ultrafast interaction between biomolecules can be mediated. This has dramatic implications beyond the transport field on how transient, yet specific protein-protein interaction networks can be modulated in general in the cell and provides evidence on why IDPs enriched during evolution of more complex organisms.

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S06	Invited talk

From Biological Self-Assembly to Novel Peptide Nanostructures of Unique Mechanical, Optical, Piezoelectric and Semiconductive Properties

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Bio-inspired nanotechnology is a key front in the field of molecular self-assembly of new structures and composite families at the nano-scale. Concept and notions from biological self-assembly could allow the design and fabrication of nanomaterials, while molecular self-assembly paradigm could be applied to biological systems. Our work on the mechanism of aromatic peptide self-assembly, lead to the discovery that the diphenylalanine recognition motif self-assembles into peptide nanotubes with a remarkable persistence length. Other aromatic homodipeptides (including those with non-coded amino acids as DOPA) could self-assemble in nano-spheres, nano-plates, nano-fibrils and hydrogels with nano-scale order. The modification of peptide building blocks with the Fmoc protecting group allows the formation of hydrogels with nano-scale order. We demonstrated that the peptide nanostructures have unique chemical, physical and mechanical properties including ultra-rigidity as aramides, semi-conductive, piezoelectric and non-linear optic properties. We also demonstrated the ability to use these peptide nanostructures as casting mould for the fabrication of metallic nano-wires and coaxial nano-cables. The application of the nanostructures was demonstrated in various fields including electrochemical biosensors, tissue engineering, and molecular imaging. We had developed ways for depositing of the peptide nanostructures and their organization. We had use inkjet technology as well as vapour deposition methods to coat surface and from the peptide "nano-forests". We recently demonstrated that even a single phenylalanine amino-acid can form well-ordered fibrilar assemblies of distinct electron diffraction pattern and toxic properties. The combination of DNA properties and peptide backbone in the form of Peptide Nucleic Acid (PNA) resulted in light emitting assemblies that exhibit both stacking and Watson-Crick base-pairing. We recently extended our studied to single amino acids and metabolites. We established the concept that even these entities can form well-ordered assemblies with amyloid like properties including ultrastructural morphology, toxicity leading to apoptosis, ThT-binding, and Congo-red birefringence.

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S07	Invited talk

The mechanism of aggregation of non-globular proteins

Fabrizio Chiti

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Non-globular proteins have a high propensity to self-assemble into misfolded protein aggregates due to the lack of a globular fold that in folded proteins bury all the hydrophobic amino acid residues in the interior of the protein and engages many amide and carbonyl backbone groups in the formation of hydrogen bonds in β-sheets and α-helices. This high propensity to aggregate is attenuated by well defined traits in the amino acid composition, such as the low number of hydrophobic residues, the high net charge and the high fraction of proline residues.

In spite of these stratagems devised by protein evolution, the aggregation of intrinsically disordered proteins represents a constant challenge in all living organisms and is apparent in well defined pathological conditions such as Alzheimer's disease, Parkinson's disease, frontotemporal dementia, type II diabetes and many others, all associated with the deposition of intrinsically disordered peptides/proteins in amyloid aggregates. I will describe the aggregation process of intrinsically disordered proteins such as the amyloid β peptide and α-synuclein, associated with Alzheimer's and Parkinson's diseases, respectively, describing both the various oligomer intermediates forming during the process and the regions of the sequence promoting aggregation and forming the cross-β core of the resulting amyloid fibrils. The various algorithms predicting such key regions will be presented in a survey and the agreement between the predictions and the experimental data will be presented.

S08	Invited talk

Systematic development of small molecules and characterization of their effect on Aβ42 aggregation at a single microscopic step level

Johnny Habchi

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The conversion of the Aβ peptide into pathogenic aggregates is linked to the onset and progression of Alzheimer's disease. Although this observation has prompted an extensive search for therapeutic agents to modulate the concentration of Aβ or inhibit its aggregation, all clinical trials with these objectives have so far failed, at least in part because of a lack of understanding of the molecular mechanisms underlying the process of aggregation and its inhibition. To address this problem we describe a chemical kinetics approach for rational drug discovery, in which the effects of small molecules on the rates of specific microscopic steps in Aβ aggregation are analyzed quantitatively. By applying this approach we identify and optimize a rich pool of compounds that target specific microscopic steps in Aβ42 aggregation and we validate these small molecules in human cerebrospinal fluid and in a C. *elegans* model of AD. These results suggest that the prevention of the nucleation of Aβ by small compounds could potentially reduce the risk of onset of Alzheimer's disease by acting as "neurostatins", and more generally that our strategy provides a general framework for the rational identification of a range of candidate drugs directed against neurodegenerative disorders.

S09	Invited talk

Engineering folding and function of tandem-repeat proteins

Laura Itzhaki

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Researchers in the field of protein folding and design face significant challenges arising from the two inter-related features of typical that define most globular protein structures, namely topological complexity and cooperativity. In striking contrast to globular proteins, tandem-repeat proteins (such as ankyrin, tetratricopeptide and HEAT repeats) have regular, modular, linearly arrayed structures, which means that the biophysical properties of even very large repeat proteins are highly amenable to dissection and to rational redesign. I will present our work towards understanding the folding and function of repeat proteins and illustrate their potential for exploitation in materials and biomedical applications.

S10	Invited talk

Discovery and Characterisation of Novel Functional Modules in Intrinsically Disordered Regions

Norman E Davey

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Higher eukaryotic proteomes contain extensive intrinsically disordered regions. These regions control the localisation, stability and modification state of proteins. Furthermore, they integrate information encoded in their environment to make regulatory decisions in reaction to cell state changes. The functional role of the vast majority of these regions is unknown. Various estimates have suggested that there may be upwards of one hundred thousand interaction interfaces in these regions. However, to date, only a small portion of the functional elements predicted to reside within these regions have been characterised. The majority of known interfaces in disordered regions belong to a class of compact, degenerate and ex nihilo evolvable interaction modules known as short, linear motifs (SLiMs). We integrate common discriminatory attributes from evolutionary, proteomic and genomic data to discover novel SLiMs, and novel mechanisms regulating the conditional functionality of these motifs. In this talk, we introduce our recent in silico motif discovery work. We provide examples where in silico motif discovery drove the experimental characterisation of novel families of motifs; and the expansion of known families. Finally, we discuss general insights that these analyses give us into the complicated specificity determinants, conditionality and evolutionary plasticity of motif-centric post-translational regulatory networks. The quest to identify the complete repertoire of functional modules in the disordered regions of the human proteome and to understand the pre- and post-translational mechanisms controlling their function is a formidable challenge. However, it is vital that this challenge is successfully tackled if we are to gain a molecular description of the physiological and pathological processes of the cell.

S11	Invited talk

AlphaRep: a library of artificial repeat proteins as an efficient source of specific binding proteins

Philippe Minard

Institute of Integrative and Cellular Biology, Université Paris Sud, France

Our general objective is to create new proteins binding specifically to predefined protein targets. The strategy is to create a large library based on a scaffold protein, from which binders for any protein target can be selected by genetic sorting methods such as Phage Display, or Protein Complementation Assay. The key feature of a protein scaffold is to accommodate a highly diverse binding surface while maintaining its stability and on this respect protein structures made from repeats are very well adapted. We have developed a library of artificial repeat proteins named alphaReps. The sequence design was inspired from a family of natural HEAT-like repeats originally found in thermophilic microorganisms. Methods to synthetize high quality libraries were developed and a large, diverse library was assembled. Tight and specific binders for a range of predefined protein targets have been selected from this library. Potential applications of this approach will be presented.

S12	Invited talk

Recognising intrinsically disordered regions in protein family databases

Rob Finn

European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SD, UK.

The InterPro consortium protein family domain databases have historically focused on evolutionarily conserved, globular domains. However, a survey of Pfam sequence coverage has shown that a large fraction of unannotated residues in the human proteome are intrinsically disordered. Such regions play varied and important roles in protein biology and are essential to annotate, yet are underrepresented in many prominent resources. While some efforts have been made to model disordered regions using profile hidden Markov models, these have proved somewhat unsuccessful and remain under-represented within Pfam. To improve the situation, the InterPro database in collaboration with the MobiDB resource, have been investigating how intrinsically disordered regions can be systematically calculated as part of the monthly InterPro data releases on UniProt. Initial results are extremely encouraging, adding to the wealth of annotations within InterPro, with the long range predictions frequently explaining the absence of a protein family upon a sequence.

S13	Invited talk

Conformations and molecular functions encoded by sequence patterns of non-globular proteins

Rohit V. Pappu

Washington University in St. Louis, St Louis, USA

Previous studies from our lab have shown that conformations of disordered proteins are determined by a combination of amino acid composition and the patterning of oppositely charged residues. In this talk, I will present results from recent studies, which showcase how charge patterns in linear sequences determine the functions of disordered regions that are tethered to ordered domains. I will also present surprising results that showcase the impact of sequence-patterns on pKa shifts that control disorder-to-order transitions such as coil-to-rod transitions. These findings have important implications for molecular evolution and the synergies between disordered regions and ordered domains.

S14	Invited talk

Three complementary mechanisms of quality control ensure RNP granule functionality and dynamics

Simon Alberti

Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Liquid-liquid phase separation can create functionally distinct reaction compartments consisting of proteins and RNAs, which have major roles in cellular dynamics and physiology. RNA-binding proteins (RBPs) with domains of low sequence complexity (LC) are the key players that mediate this process of phase separation in cells. Recent data suggests that aberrant phase transitions of these proteins may be closely tied or even causative to the pathogenesis associated with diseases such as ALS. Thus, understanding how physiological phase transitions give rise to dysfunctional RNPs and eventually pathological RBP-containing aggregates will be key to understanding a range of neurodegenerative diseases.

Here, we study several human LC domain-containing RBPs that are associated with age-related neurodegenerative diseases such as ALS. We show that in vivo these proteins phase separate to form liquid, membrane-less compartments. Using an in vitro "aging" assay, we demonstrate that reconstituted RNP compartments of these RBPs have a strong drive to convert with time from a liquid to an aggregated, pathological state. We identify three mechanisms that regulate the conversion of these RNPs into pathological aggregates: First, we find that heterogeneity in RNP granule composition prevents liquid-to-solid transitions, due to mutual interactions of RBPs, which frustrate nucleation events that are necessary for aggregation. Second, we show that a dedicated chaperone surveillance machinery prevents intra-granular interactions between RBPs and misfolded proteins, which have a tendency to accumulate in RNP granules because of their hydrophobic properties and affect RNP granule dynamics and material properties. And third, we uncover that aberrant RNP granules are selectively recognized by protein quality control machinery to be targeted to the aggresome and subsequently degraded by autophagy.

These findings suggest that nature has come up with three independent mechanisms of regulating aggregation-prone RBPs in RNP granules: 1) RNP granule-intrinsic mechanisms that control the conformational properties of RBPs through RNP granule composition, 2) a specialized chaperone surveillance machinery that keeps RNP granules free of aberrant protein conformations, and 3) a disposal machinery that selectively eliminates irretrievable, aberrant RNP granules in toto. We hypothesize that all three pathways weaken with increasing age and that genetic mutations in pathway components accelerate the manifestation of a disease state.

S15	Invited talk

Understanding the mechanisms of folding and binding of intrinsically disordered proteins

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Intrinsically disordered proteins often become structured upon interacting with their partners. The mechanism of this "folding upon binding" process, however, has not been fully characterised yet. In this talk, I will describe our work on the characterization of the folding mechanism of intrinsically disordered systems, in comparison to that of globular protein. This analysis will be based on our recent studies, which allowed us to investigate the role of the frustration arising from the competition between function, misfolding, and aggregation in a globular domain.

Whilst the folding of globular, minimally frustrated proteins, is strongly biased towards the native conformation via a robust nucleation-process, in the case of intrinsically disordered proteins, folding occurs by heterogeneous nucleation. We suggest that this templated folding mechanism may enable intrinsically disordered proteins to achieve specific and reliable binding with multiple partners while avoiding aberrant interactions.

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SELECTED TALKS

T01	Intrinsic disorder

High GC Content Causes Young Proteins to be Intrinsically Disordered

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The de novo creation mechanism of orphan proteins involves creation of short ORFs from noncoding regions. A small fraction of these ORFS can then become fixed in the population. However, de novo created proteins need to, at the bare minimum, not cause harm to the organism, i.e. they do evolve under some selective constraints. It has been proposed that in order not to aggregate these proteins must either fold properly or be intrinsically disordered and indeed, it has been shown that Drosophila orphan proteins are more disordered than ancient proteins. However, the opposite trends exist in yeast. To the best of our knowledge no one has proposed a valid explanation for this difference. To solve this riddle we study the relationship between intrinsic disorder and protein age in 400 eukaryotic species. We found that on average there are no large differences between proteins of different age, with the exception that younger proteins are shorter. However, when we take the GC content into account we find that this can explain the differences between yeast and drosophila. A young protein with high GC content exhibits a large degree of disordered and coiled residues, but a low fraction of helices and sheets. The reason is that GC content is correlated with codons coding for disorder promoting amino acids so that at high GC content, a higher fraction of disorder promoting residues can be expected for randomly generated proteins. The opposite is true for helix and sheet promoting residues. This lead us to speculate that structural features are not a strongly determining factor for de novo creation. Instead de novo created proteins strongly resemble randomly generated proteins given a particular GC level. Later during evolution the memory of their creation is gradually lost and the dependency on GC content is weakened.

T02	Intrinsic disorder

Preliminary characterization of the Henipavirus V protein and its interaction with the cellular protein DDB1

Edoardo Salladini, Vincent Delauzun and Sonia Longhi

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The Nipah and Hendra viruses (NiV and HeV) have been recently identified within the Paramyxoviridae family as zoonotic agents responsible for severe encephalites in humans, and have been grouped within the Henipavirus genus(1). The susceptibility of humans, their high pathogenicity, the wide host range and interspecies transmission led to the classification of HeV and NiV as biosecurity level 4 (BSL-4) pathogens and as potential bio-terrorism agents. To date no therapeutics agents, nor vaccines are available to fight against these severe pathogens. The genome of Henipaviruses encodes the V protein via a mechanism of co-transcriptional editing whereby a non-templated guanosine is added at the editing site of the P messenger. As a result, the P and V proteins are identical for the first 404 (HeV) or 406 (NiV) residues(2, 3). The region shared by the P and V proteins, referred to as PNT, is intrinsically disordered(4). By contrast, the C-terminal region of the V protein is predicted to be structured and to adopt a Zn-finger conformation(5).

In paramyxoviruses, the V protein plays a key role in the evasion of the interferon (IFN) response, via both an antagonist activity of IFN signaling and inhibition of IFN induction(6, 7). In henipaviruses, the V protein has an antagonist activity of IFN signaling and inhibits the translocation of STAT proteins in the nucleus. STAT proteins (STAT1 and STAT2) are key signal transducers in the IFN pathway of antiviral response. Once imported in the nucleus, STAT proteins interact where they interact with IRF-9 to form the ISGF3 complex that activates the transcription of IFN-stimulated genes leading to the synthesis of proteins (such as PKR, 2'5' OAS, Mx ..) that inhibit viral replication(6).

Several paramyxoviruses are able to hijack the cellular ubiquitin ligase E3 complex to promote the rapid degradation of STAT proteins. This activity relies on the ability of the V protein to bind to DDB1 and to recruit STAT proteins onto the ubiquitin ligase E3 complex. DDB1, a highly conserved 127 kDa cellular protein, is indeed a component of the ubiquitin ligase E3 complex. The interaction between the V protein and DDB1 is therefore a promising target for antiviral strategies as its inhibition is expected to lead to reduction/abrogation of the ability of these viruses to escape the innate immune response.

As a first step towards the characterization and the inhibition of the Henipavirus V-DDB1 interaction, we have undertaken the cloning, expression and purification of the Henipavirus V protein. We herein report the bacterial expression, purification and preliminary characterization of

the V protein from both NiV and HeV. Spectroscopic and hydrodynamic data show that they are mostly disordered. Concomitantly, we have expressed and purified DDB1 from insect cells. Interaction experiments are in progress.

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T03	Intrinsic disorder

To unravel at the atom-level intrinsically disordered proteins and the effects induced by cancer alterations integrating enhanced sampling simulations and biophysical techniques.

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Despite substantial progress in the development of protein force fields for molecular dynamics simulations, applications to intrinsically disordered proteins have revealed that most force fields give rise to an unrealistic level of compaction of the molecule under investigation [1,2]. Recently, two independent potential solutions to this problem have been suggested in the form of force field modifications that either involve using existing force fields with a new water model [3] or re-balancing the Lennard-Jones interactions between protein and water molecules in existing force fields [4]. These new 'IDP' force fields hold promise for providing a much more accurate description of the experimentally-observed compactness of IDPs, for example by small-angle-X-ray scattering, and at the same time take advantage of the developments in accuracy in protein force fields. They could be also used in principle to study the role of disordered tails or linkers in mainly folded proteins [3,5]. We have thus employed some of these new force fields in a metadynamics-based approach to sample the conformational landscape of several intrinsically disordered regions of proteins belonging to transcriptional co-activators, inhibitors of cell cycle or apoptosis, ubiquitin-binding proteins, and the intracellular domains of membrane receptors and transporters. We also assess the impact of cancer-related mutations and phosphorylation on the conformational ensemble of the protein. We validated the simulated ensembles using NMR data and analyzed them to identify regions with increased propensity to form transient secondary structure and tertiary contact as well as important hotspots for recruitment of different biological partners.

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T04	Intrinsic disorder

Modulation of neuronal tau protein interaction with protein partners

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Aggregates of the neuronal Tau protein are found inside neurons of Alzheimer's disease patients. Development of the disease is accompanied by increased, abnormal phosphorylation of Tau. Tau is a paradigm for the growing class of intrinsically disordered proteins, often found associated with pathologies such as neurodegenerative diseases, hence increasing the interest to understand the molecular parameters underlying their functions. In the course of our molecular investigation of Tau functions and dysfunctions in the disease, nuclear magnetic resonance (NMR) spectroscopy is used to identify the multiple phosphorylations of Tau and to characterize Tau interactions with its molecular partners. These tasks remain challenging due to Tau highly dynamical character and its 80 Ser/Thr residues, potential sites of phosphorylation that can be combined to give a multiphosphorylated Tau, leading to a very complex regulation of Tau interactions. It has proven crucial to identify phosphorylation sites to be able to link specific phosphorylations with structural or functional modifications. Functional aspects include the regulation by Tau phosphorylation of both interaction of Tau with protein partners and aggregation. The phosphorylated Tau samples characterized by NMR can be further used to decipher phospho-dependent interactions, for example with 14-3-3 proteins, and developed protein-protein interaction inhibitors.

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T05	Intrinsic disorder

Linear motif combination and conservation of flexibility determines the displacement ability of an intrinsically disordered viral oncoprotein

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In the present work, we have explored the biophysical basis for efficient disruption of host protein-protein interactions by a viral oncoprotein. We studied the interaction between the intrinsically disordered E1A protein from human adenovirus and the retinoblastoma (Rb) cellular tumor suppressor. This interaction is mediated by two highly conserved linear motifs present within E1A, which are joined by a 70-residue linker region. Each of these motifs binds to a distinct and highly conserved surface on the RbAB domain. The E1A region harboring both motifs was found to bind with 1:1 stoichiometry and extremely high (KD = 24 pM) affinity to Rb. Even when the individual viral motifs establish lower affinity interactions (KD = 140 nM) compared to the cellular E2F counterpart (KD = 12 nM) the arrangement of two motifs joined by a disordered linker explains the effectiveness of the E1A protein, where one motif (LxCxE) acts as a docking site, increasing the effective concentration of the motif that competitively displaces E2F. A simple model derived from polymer physics predicts the compound affinity with excellent accuracy, indicating that the linker region joining both E1A motifs behaves as a flexible polymer of optimal length. Sequence conservation and affinity prediction analyses reveal that disordered nature and optimal length are highly conserved within the E1A protein family. These evidences suggest that the effectiveness of viral proteins in disrupting the cellular network relies on both the presence of distinct motif combinations as well as on conservation of flexibility within specific viral protein regions.

T06	Intrinsic disorder

Disentangling polydispersity for the PCNA-p15 interaction: A disordered, transient and multivalent macromolecular assembly

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Summary:

The interactions of Intrinsically Disordered Proteins with globular partners govern a multitude of biological processes. In the majority of cases these interactions are of low to moderate affinity, thus forming transient complexes that are difficult to characterize at a structural level due to their inherent polydispersity. We have tackled this challenge while studying the interaction of the intrinsically disordered p15 with ring-shaped trimeric PCNA by combining small-angle X-ray scattering (SAXS) with molecular modeling strategies. SAXS data measured at different relative concentrations of both proteins code for the structure and conformational fluctuations of the five species that co-exist in solution. By using explicit ensemble descriptions for the five individual species, built using integrative approaches and molecular dynamics simulations, we collectively interpreted the SAXS profiles as population-weighted thermodynamic mixtures. The introduction of explicit description of protein and hydration shell dynamics have revealed crucial to precisely describe the SAXS profile of PCNA and its complexes with p15. The analysis demonstrates that the N-terminus of p15 penetrates the ring and emerges on the backside of PCNA, providing the structural bases of previous biochemical and biophysical observations.

This study shows the power of ensemble-based approaches to decode structural, dynamic and thermodynamic information from SAXS data. This strategy paves the way to decipher the structural bases of flexible, transient and multivalent macromolecular assemblies involved in pivotal biological processes such as cell signaling and regulation.

T07	Intrinsic disorder

Modelling Intrinsically Disordered Proteins. Challenges when comparing to experiments.

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Modelling Intrinsically Disordered Proteins (IDPs) requires generating an adequate ensemble – from a good force field and enough sampling – and an accurate predictor to obtain experimental data from the generated ensemble.

Several works have addressed the (in)adequacy of present-day force fields to describe intrinsically disordered protein ensembles. We will show how the Maximum Entropy principle comes to our rescue to reweigh ensemble structures and how this method compares to a Bayesian approach. We will apply this to Residual Dipolar Couplings data and Chemical Shifts, in a study of an IDP binding process.

We will also explore the accuracy of the predictors of experimental data, and how they can distort our interpretation of the structural ensemble. In particular, the lack of information of errors in SAXS predictors considerably diminishes our ability to distinguish between different structures.

T08	Intrinsic disorder

Intrinsic disorder is at hart of the ratio-dependent autoregulation in two-gene bacterial operon

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Ratio-dependent regulation is observed when two signals non-independently affect expression of a gene. It has been observed in the regulation of bacterial and yeast metabolic genes and in the regulation of toxin-antitoxin modules. Toxin-antitoxin modules are bacterial stress-response elements implicated in the bacterial persistence and interesting model systems of autoregulated bacterial operons. The autorepression of the operon depends on the molar ratio its two gene products (toxin and antitoxin) by a mechanism termed conditional cooperativity. The molecular details and prevalence of such mechanism in toxin-antitoxin modules are still unclear. Here, we studied the regulatory mechanism of the higBA2 module form the human pathogen Vibrio Cholerae. By combining techniques of structural biology, global thermodynamic analysis and computational modeling we uncovered a novel molecular mechanism of ratio-dependent regulation in the higBA2 module. Antitoxin N-terminal antitoxin domain is intrinsically disordered and interacts with two targets: with the operator and with toxin. This competitive interactions of the intrinsically disordered domain introduce negative cooperativity into regulatory circuit and provide basis of the functionally important ratio-dependent regulation.

T09	Protein repeats

Designed proteins scaffolds to stabilize nanoclusters: sensing and imaging appliactions.

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Nobel metal nanomaterials with interesting size-dependent electrical, optical, magnetic, and chemical properties have been intensively pursued. Especially, metal nanoclusters (NCs) have attracted special attention in recent years due to their unique optical, electrical, and chemical properties, which are very much different from those of the bulk metal and metallic nanoparticles. These metal NCs can be stabilized by different molecules including dendrimers, small molecules, DNA, and proteins. Protein-stabilized AuNCs offer unique properties, however up to now protein-based nanocluster synthesis has been done using commercially available globular proteins, which properties are not easily tuned, therefore limiting their applicability in different research areas.

In this work, we explore the potential of designed repeat proteins as templates for nanocluster synthesis and stabilization. Designed repeat proteins are an interesting template due to their modular structure which allows for the engineering of their function and stability, adding advantages for their application in different fields compared to other proteins. For example, for biosensing and molecular imaging, the recognition properties of the repeat protein scaffolds can be tuned, giving rise to repeat proteins with the same structure but with different binding activity by introducing few variations in the primary sequence. In particular, we focused on the designed consensus tetratricopeptide repeat proteins (CTPRs), and their application as building blocks in order to generate functional hybrid structures.

Here, we show two examples of the use of CTPR proteins as scaffolds to template fluorescent nanoclusters and the proof of concept applications in sensing and imaging. (1) We show the ability of CTPR to encapsulate and stabilize fluorescent gold nanoclusters. Since the structural and functional integrity of the protein template is critical for applications, protocols that retain the protein structure and function have been developed. Finally, a CTPR module with specific binding capabilities has been successfully used to stabilize nanoclusters and tested as a sensor. (2) In a second generation of designs, specific metal coordination sites have been introduced into the CTPR

proteins. We have explored the potential of those designed proteins as templates for the synthesis and stabilization of Cu nanoclusters. We studied the effect of the position of the specific metal-binding site in the protein structure on the formation and stabilization of metal NCs and the final properties of the resulting clusters. In addition, we have evaluated the potential application of these CTPR-NCs complexes in the field of cellular imaging as non specific cellular marker or specific cellular marker using an CTPR proteins which include in their structure a localization peptides, such as a nuclear localization signal.

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T10	Protein repeats

A Census of Tandem Repeats in Proteomes

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The dramatic growth of proteomic data has led to increasing efforts to make sense of millions of protein sequences by systematic large scale structural and functional annotation of the proteomes. Over the last few decades, numerous studies demonstrated the abundance and functional importance of periodic protein sequences representing arrays of repeats that are directly adjacent to each other (so called tandem repeats or TRs). Here we present the results of a comprehensive up-to-date survey of these TRs in protein sequences. The TR census was made possible by development of a Meta-Repeat-Finder pipeline integrating several known TR detection methods. The need for such a pipeline derives from the fact that depending on the size and character of the repeats, some methods perform better than others, but no single best approach exists to cover the whole range of repeats. To obtain the most complete set of TRs, we devoted considerable efforts to select the most effective combination of TR detection programs and to develop the scoring procedure able to distinct between genuine TRs and non-TR regions (1-3). As a result, our analysis provided a global view on TRs in proteins. It allowed to correct or refine previously published conclusions and to discover new relationships between different categories of the TRs. In particular, it was shown that the TRs occur in more than half of all proteins independent of the kingdoms of life (eukarya, archaea, bacteria, viruses). This result shows that the TR abundance in proteins was previously greatly underestimated. The Gene Ontology analysis revealed that the preferential molecular function of the TR containing proteins is described as "binding". The Meta-Repeat-Finder and TR database developed in this work will be publicly available to detect TRs in a given protein or to perform a large-scale and cross-species comparative analysis of TRs with queries defined by the users.

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T11	Protein repeats

Novel repeat protein families: design, assembly and polymerization

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The complexity of protein interactions found in Nature limits the possibility to understand what is the range of properties (e.g. thermodynamic stability, solubility) that proteins can achieve and how this translate into emerging properties through their interactions. Computational design is a promising tool to create simplified and controlled model systems able to investigate sequence-structure relationships and support specific interactions.

We computationally designed new proteins using modular repeating units as building block, and we explored the range of achievable geometries. Building blocks formed by two helices of variable length were designed and validate sequence using the Rosetta macromolecular modeling suite. Sampling of backbone conformations and side chain identities was performed simultaneously in all repeat units, reducing dramatically the complexity of the search space [1]. Out of 83 proteins selected for experimental characterization, 54% possessed the expected secondary structure, were stable at more than 95°C and 3M guanidine hydrochloride (some above 7M), were monomeric in solution with the structure confirmed by small angle X-ray scattering. 15 crystal structures confirmed the accuracy of the designs [2].

The designs described above can be used as scaffolds for protein, nucleic acid, small molecule binders and enzymes, and new structures can be designed for specific applications. The stability and modular structure of designed repeat proteins allowed to design self-assembling systems with defined cyclic symmetry and develop a strategy for the production of protein fibers with controllable and homogeneous length.

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T12	Protein repeats

Tandem Repeat proteins at a glance: functions, diseases and role in protein-protein interaction network.

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Motivation

Tandem repeat (TR) protein structures are abundant in nature and widespread across all types of organisms. Their periodic sequence folds into a modular and elongated architecture. They play a role in a number of different pathways, suggesting their association to a large number of diseases. A possible explanation of the evolutionary framework that lead to TR proteins widespreadness and importance is given by the properties of their modular structure, ideal for highly specialized and rapidly evolving binding functions. The present study shows an assessment of TR proteins function and role in the protein-protein interaction (ppi) network. In addition, it presents the first analysis of the relationship between repeat proteins and diseases from OMIM (Online Mendelian Inheritance in Man).

Methods

The dataset of human proteins containing repeat regions is collected from RepeatsDB. RepeatsDB provides a resource for structurally validated TR proteins, extracted from the PDB and classified. The background for enrichment calculation is given by SwissProt and PDB data banks, and three additional sets of disease-related classes were identified to compare against human TR proteins, i.e. kinases, homeobox proteins and ion channels. The GO annotation of all proteins was collected and processed to define enriched functions in the TR dataset. A non-redundant set of protein-protein interactions is retrieved from the IMEx consortium, the general features of the network (degree, connectivity, neighbor degree) were analyzed and compared to the TR dataset features. The significance of the differences was assessed. The disease annotation is extracted by the field "diseases" of the Uniprot description. The number of proteins associated to at least one OMIM disease ID was divided by the total number of proteins, obtaining the fraction of disease-associated proteins in each dataset.

Results

The results show that TR regions are ubiquitous in organism, cellular location, biological role and functional pathways. In addition, they are characterized by a higher number of interactors than the protein-protein interaction network average. They are also significantly associated to diseases. This is is agreement with the observation that typical disease genes are intermediate between hubs of core biological functions, whose disruption causes lethality, and genes for which high haplotype diversity and mutation rates are advantageous. The results highlight the importance of TR protein recognition, classification and study for a better understanding of the cell machinery and disease insurgency, as well as opening up a promising field in biomolecular engineering.

T13	Protein repeats

Collective Dynamics and Modulation of Function

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Biomolecules are complex molecular machines that visit many conformational and dynamic states to perform their functions. As much as the stable conformational states, the transition pathways in between are of interest to comprehend the molecular functional mechanism. Intrinsic dynamic modes driven Langevin Dynamics simulations are able to disclose plausible conformational pathways between two given states and underlying dynamical network of mechanistically key sites from globular to nonglobular proteins. As an exemplary case study, pore forming conformational pathways of bacterial proteins are explored to map the dynamics of the conformational transition from an inactive monomer to the one in transmembrane assemblies of the attack mechanism to the host. Understanding pore-forming process and the conformational control in that may contribute to the design of strategies for the prevention of this process by pathogenic miucroorganisms.

T14	Aggregation

Structural Studies of Amyloid-like Protein Fibrillation

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Protein amyloid fibrillation is associated with a number of grave diseases, most notably the neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (1). Protein and peptide fibrillation also constitutes a major challenge in the biopharmaceutical industry, where fibrillation must be avoided to ensure product safety (2). The structural investigation of protein fibrillation is however inherently challenging, since a number of structural species co-exist during the fibrillation reaction. These species cover a wide range of sizes (nm to µm) and exist in different volume fractions over time, in an equilibrium that is highly sensitive to the experimental conditions. Isolation of individual species is thus not possible.

At the same time, it is important to investigate the structural species formed during the fibrillation pathway. Not only are these key to understanding the molecular principles behind the process, but also accumulating evidence links such intermediate species to cytotoxic activity, central to the progressive, degenerative diseases. We use small angle X-ray scattering (SAXS) as a central method to investigate the fibrillation reaction. Formation of α-synuclein (aSN) fibrils is associated with Parkinson's disease. We have previously characterized the low-resolution structure of intermediately formed aSN oligomers (3) and reveal that these oligomers are building blocks of the fibril structure (4). We have recently demonstrated, that early amyloidogenic aSN species can disrupt lipid model systems, and that lipid:protein co-aggregates in a non-amyloid state are formed in this context (5) while the effect on lipid membranes varies depending on the lipid composition (6). Among our most recent (unpublished) data are the SAXS and CD-based investigations of the stability of mature fibrils, providing evidence of a highly dynamic equilibrium of different structural species. This is in accordance with results published from a related amyloid system (transthyretin, (7)). While the methodology behind the data analysis from such complex systems has been well elaborated both by us (8), and others (e.g. 9), the need for a robust, objective and (semi-)automated analysis system is evident, and our latest efforts in this direction will be presented. We apply the newly developed software to the analysis of a familial mutant of aSN, revealing the occurrence of intermediate species, which have a different nature than those previously characterized (unpublished results).

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T15	Aggregation

Inhibiting activity of fullerenol C60(OH)16 on Aβ1-40 amyloid aggregation - in vitro and in silico study

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Self-assembly of Aβ peptides and their accumulation in deposits in human brain is characteristic sign in Alzheimer's disease (AD). There is no cure for AD, but one of the promising approaches for AD treatment is an inhibition of amyloid aggregation [1].

We have studied the ability of fullerenol - water soluble derivative of fullerene C60 - to inhibit process of Aβ1-40 amyloid fibrillization using in vitro and in silico methods. Fullerenol C60(OH)16 was prepared by Solvent-Free Reaction [2]. The inhibiting activity of fullerenol was investigated at different Aβ1-40:fullerenol ratios = 100:1, 20:1, 1:1 and 1:20 using Thioflavin T (ThT) assay and atomic force microscopy (AFM). The data have shown that fullerenol is able to reduce formation of amyloid fibrils. The highest inhibitory activity (~70%) was observed for highest fullerenol concentration (ratio 1:20). The IC50 value is in low range (μg/ml) suggesting that fullerenol interferes with Aβ1-40 aggregation at stoichiometric concentrations. The in silico data obtained by MM/PBSA methods have supported experimental outcome. It was demonstrated that fullerenol tightly binds to monomer Aβ1-40 and the polar negatively charged amino acids play key role in this binding. The electrostatic interactions dominantly contribute to the binding propensity via interactions of oxygen atoms from COO - groups from polar negatively charged amino acids with OH groups of fullerenol. This interaction stabilizes contact with either D23 or K28 of the salt bridge. The salt bridge becomes flexible upon fullerenol binding leading to inhibition of Aβ aggregation. In order to assess the biocompatibility of fullerenol we have measured its effect on viability of human neuroblastoma cell line SH-SY5Y by WST-1 assay. We have found that fullerenol was non toxic for these cells at concentrations which are able to effectively inhibit Aβ1-40 peptide amyloid fibrillization during 72h of exposure.

Our results suggest that fullerenol C60(OH)16 represents promising candidate of therapeutics for Alzheimer's disease.

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T16	Aggregation

Molecular evolution of short cyclic peptides that rescue the misfolding, aggregation, and the associated pathogenic effects of proteins linked to protein misfolding diseases

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It has now been widely recognized that many serious human diseases, such as Alzheimer's disease, Parkinson's disease, familial forms of amyotrophic lateral sclerosis, type 2 diabetes, certain forms of cancer etc., are initiated by a common mechanism: the misfolding of specific proteins, which results in the formation of pathogenic protein oligomers and higher order aggregates. Compounds with the ability to specifically bind to these proteins and rescue their misfolding, constitute potential therapeutic agents against this type of disorders. In this work, we describe the application of molecular evolution approaches for the discovery of molecules with the ability to rescue the misfolding and aggregation of proteins (MisPs) associated with protein misfolding diseases (PMDs). In order to achieve this, Escherichia coli cells are first engineered genetically so as to biosynthesize very large combinatorial libraries of random cyclic oligopeptides. Then, the same cells are modified further so that the rare clones producing sequences with the ability to correct the folding of the target MisPs can be rapidly identified by high-throughput genetic screening. Lead sequences identified in this manner are subsequently subjected to more detailed evaluation by biochemical and biophysical methods of protein analysis, and their ability to inhibit MisP-induced pathogenicity is tested using appropriate human cell assays and in vivo models of the PMD of interest. The cyclic peptides capable of rescuing the misfolding of the target MisP and of antagonizing its associated pathogenicity constitute lead molecules against the specific PMD. We will describe our efforts to identify molecules that rescue the misfolding and aggregation of the amyloid β (Aβ) peptide and of certain misfolded pathogenic variants of human Cu/Zn superoxide dismutase 1 (SOD1), with the aim of developing potentially therapeutic compounds against Alzheimer's disease and familial forms of amyotrophic lateral sclerosis, respectively.

T17	Aggregation

S100 proteins as novel modifiers of proteostasis in health and disease

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S100 proteins are vertebrate-specific signaling molecules which have a dynamic cellular range of concentrations and oligomeric states, and are involved in diverse cellular processes. Structurally they are typically homodimers, containing two EF-hand type Ca2+ binding sites and, in some cases, additionally regulatory Zn2+ and Cu2+ binding sites [1]. S100 proteins are involved in aspects of regulation of proliferation, differentiation, apoptosis, Ca2+ homeostasis, energy metabolism, inflammation and migration/invasion through interactions with a variety of target proteins including enzymes, cytoskeletal subunits, receptors, transcription factors an nucleic acids. Many S100 proteins exert extracellular regulatory effects and expression of a particular S100 protein can be induced upon pathological circumstances in a cell type that does not express it in normal physiological conditions. Thus, extracellular S100 proteins exert regulatory activities over a vast number of cells and are engaged in multiple cellular processes such as innate and adaptive immune responses, cell migration and chemotaxis, tissue development and repair, tumor cell invasion and neuroinflammation [2].

Age-related neurodegenerative diseases, such as Alzheimer's disease (AD), are associated with loss of neurons structure or function and a decay in brain and cognitive functions. Nowadays, there are approximately 50 neurodisorders, in which dysregulated protein expression is a common feature, correlate with the misfolding of normally soluble proteins and their subsequent conversion into aggregates [3]. Previous work from our laboratory suggests that S100s influence proteostasis across these disease-states. One such finding relates to the observation that S100 proteins are aggregation prone proteins that form amyloid oligomers and therefore will influence downstream signaling and overall cell proteostasis. Another important point is that S100 proteins forms amyloids structures in pro-inflammatory environments and that mechanism is facilitated by metal ions, like calcium, cooper or zinc [4]. Preliminary results suggest that the S100A8 and A9 regulation mechanisms occur through protein-protein interactions under mediation of metal ion binding, influencing their aggregation. Further, it is also known that S100A8 diffuse aggregates precede the formation of plaques in AD mice models [5]. Yet, the specific mechanism and global processes through which S100 aggregation occurs and the manner in which it can induce pathogenic behavior remain unclear.

In this project, we will undertake a systems approach combining molecular and cellular studies in neurodegeneration models, interactome and proteomics analysis, chemical biology peptidomics

and bioimaging to uncover the mechanisms underlying alterations in protein homeostasis networks, resulting from the altered S100 functions. With this approach we seek to explore new roles of S100 proteins as novel modifiers of proteostasis networks in disease.

Acknowledgements

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T18	Aggregation

The mechanism underlying amyloid polymorphism is opened for Alzheimer's disease Aβ peptide

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It has been demonstrated using Aβ40 and Aβ42 recombinant and synthetic peptides that their fibrils are formed of complete oligomer ring structures. Such ring structures have a diameter of about 8-9 nm, the oligomer height of about 2-4 nm and the internal diameter of the ring of about 3-4 nm. Oligomers associate in a fibril in such a way that they interact with each other, overlapping slightly. There are differences in the packing of oligomers in fibrils of recombinant and synthetic Aβ peptides. The principal difference is in the degree of orderliness of ring-like oligomers that leads to generation of morphologically different fibrils. Most ordered association of ring-like structured oligomers is observed for a recombinant Aβ40 peptide. Less ordered fibrils are observed with the synthetic Aβ42 peptide. Fragments of fibrils the most protected from the action of proteases have been determined by tandem mass spectrometry. The C-terminus is not accessible to proteases for both Aβ(1-40) and Aβ(1-42) fibrils. It was shown that unlike Aβ40, fibrils of Aβ42 are more protected, showing less ordered organization compared to that of Aβ40 fibrils. Thus, the tandem mass spectrometry data agree with the electron microscopy data and structural models presented here. X-ray data support our model. Using structural model for Aβ(1-40) fibrils (2m4j.ent) we constructed the possible model for Aβ(1-42).

T19	Aggregation

Rational design of binding proteins targeting specific epitopes within intrinsically disordered proteins

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Protein aggregation is closely related to many human diseases, such as Alzheimer's and Parkinson's disease, and it is also a major bottleneck for the development of protein drugs. I will present a computational strategy for the modular design of soluble binding proteins, including antibodies, designed to inhibit the disease-related aggregation of their target proteins, as well as showing some experimental validations and applications. Specifically I will first introduce the CamSol method of predicting solubility changes upon mutations and designing protein variants with improved solubility, which we have used to optimise the solubility of domain antibodies. I will then describe a method for the de novo design of protein-protein interactions, to target disordered proteins or disordered regions. We applied this method to design domain antibodies and molecular chaperones binding to specific target linear epitopes and able to inhibit the disease-related aggregation of their target proteins.

T20	Aggregation

Repositioning Tolcapone as a potent inhibitor of transthyretin amyloidogenesis and its associated cellular toxicity

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Background:

Transthyretin (TTR) is a plasma homotetrameric protein implicated in fatal systemic amyloidoses. TTR tetramer dissociation precedes pathological TTR aggregation. Native state stabilizers are promising drugs to treat the TTR amyloidoses.

Material and Methods:

Here, we used biophysical, cell biology and in vivo studies to repurpose Tolcapone, an FDA-approved molecule for Parkinson's disease, as a very potent TTR aggregation inhibitor.

Results and Discussion:

Tolcapone binds specifically to TTR in human plasma, stabilizes the native tetramer in vivo in mice and humans and inhibits TTR cytotoxicity. The crystal structures of Tolcapone bound to wild type TTR and to the V122I cardiomyopathy-associated variant explain why this molecule is a better amyloid inhibitor than Tafamidis, so far the only drug in the market to treat the TTR amyloidoses.

Conclusions:

Overall, Tolcapone, already in clinical trials, is a strong candidate for therapeutic intervention in these diseases, including those ocurring in the central nervous system, for which no small molecule approach exist.

POSTER PRESENTATIONS

P01	NGP classification and coordination

Methodology for pairwise comparison of low-complexity regions in sequence and structure databases.

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Regions of protein sequences with biased amino acid composition, also called Low-Complexity Regions (LCRs), are abundant among proteins. Although numerous studies have shed light on their important role in the regulation of biological processes, LCRs are notably difficult to compare due to their significant divergence across protein families and their divergent evolution history.

In this publication we present a methodology for the comparison of LCRs obtained from proteins deposited in both the UniProt (The UniProt Consortium) and the PDB (RCSB Protein Data Bank). The first step of the computational procedure consists of the extraction of the protein sequences from UniProt (22,737,842 proteins) and PDB (326,859 extracted proteins). Using the SEG algorithm (Wootton and Federhen, 1993) 168,148 and 556 LCRs were obtained from UniProt and PDB respectively. These data were further enriched with the correspondent protein family information obtained from Uniprot and PFAM (Finn and Tate, 2008). For each dataset we performed the pairwise comparison of all the possible LCR combinations belonging to different proteins families. As metric of sequence similarity we used the biopython pairwise2 algorithm and the CDHIT-2D method (Cock and Antao, 2009; Li and Godzik, 2006). Finally the results were score sorted and expert interpreted.

Although we mainly found (1) linkers, (2) identical/similar LCRs from the same domain in 2 different proteins and (3) tags artificially added to proteins, some case studies of interest were also collected and analyzed.

P02	NGP classification and coordination

Structural characteristics of late embryogenesis abundant proteins in the leaves of resurrection plant Ramonda serbica

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Resurrection plants (desiccation tolerant) are useful models for unraveling protective mechanisms that keep proteins and membranes in intact state in absence of water and protect them against oxidative injury upon rehydration while enzymes are not fully active. Late embryogenesis abundant proteins (LEAPs) are involved in protection against desiccation-induced aggregation of sensitive reporter proteins, yet their role in antioxidative protection has to be elucidated. LEAPs are mostly consisted of hydrophilic amino acids that ensure their random conformation in water solution, which turns to helix-coiled structure during dehydration.

Here, we analyzed proteome profiles in desiccated and hydrated leaves of Ramonda serbica, endemic and tertiary relict resurrection plant. In desiccated leaves three enzymes involved in phenolic/flavonoid metabolism (CHI, chorismate synthase andflavonol sulphotransferase) were induced in comparison to hydrated leaves. Several new isoforms of antioxidative enzymes (superoxide dismutase, ascorbate peroxidase and monodehydroascorbate reductase) were induced in desiccated leaves.

One LEAP (RsLEA1, homologue to LEAP D-29 OS=Gossypium hirsutum) was identified in both leaf conditions, while only in dehydrated leaves additional LEAP (RsLEA2, homologue to 1 OS=Oryza sativa subsp. indica) was detected. Protein RsLEA1 was composed of 302 amino acids (AAs), and RsLEA2 of 333 AAs, with similar distribution of basic, acidic and polar uncharged residues (~20 %, ~20% and 22%, respectively). Hydropathy analysis showed that the predicted RsLEA1,2 were both hydrophilic proteins. Higher abundance of Cys residues in RsLEAP2 may contribute to higher tolerance to oxidative stress during desiccation and revival. Predicted secondary structure of both LEAPs in R. serbica consisted only from helical structures (three α-helices structure in RsLEA1, and four in RsLEA2). The three-dimensional structure of LEAPs in R. serbica is a key to understand their function and regulation of their intrinsic structural disorder-to-order transition, as well as to identify their target molecules in the cell. Up-regulated flavonoids under desiccation might have a significant role in interactions between LEAPs and target molecules. Flavonoid-LEAP complexes (particularly stable with Lys) might be responsible for antioxidative protection of proteins, together with induced antioxidative enzymes.

P03	Intrinsic disorder

Mechanism of molecular recognition between an elongated repeat protein and its intrinsically disordered binding partner.

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Cancer is the one of the leading causes of death world-wide, with colorectal cancer being one of the top five most common. One mechanism underlying colorectal cancer is disruption of the canonical WNT signalling pathway, leading to a dysregulation of cell cycle promotor genes, including cyclin D1 and c-myc, and thereby an increased rate of cell division.

Beta-catenin is an Armadillo-repeat protein that acts as the signal transducing molecule in the canonical WNT signalling pathway. In healthy cells, beta-catenin is constantly being expressed but is sequestered and destroyed by the beta-catenin destruction complex. When a WNT signal is received, the destruction complex is inhibited, allowing beta-catenin to accumulate in the cytosol where it then migrates to the nucleus and forms complexes with members of the Tcf/LEF family of transcription factors to promote the cell cycle. Failures in this pathway generally lead to increased cytosolic beta-catenin and its subsequent migration to the nucleus resulting in over-expression of cell cycle promotors and uncontrolled proliferation.

Beta-catenin is, therefore, an important target for the development of new anti-cancer drugs. However it has remained elusive due to its fulfilling two main roles within the cell. As well as being involved the canonical WNT signalling pathway, it is also crucial for cell-to-cell adhesion at Adherins junctions where it binds to intracellular domain of the transmembrane protein E-cadherin. Disruption of this interaction is pro-metastatic and is one of the contributors to the lethality of colorectal cancer.

Beta-catenin's binding partners (E-cadherin, Tcf/LEF family transcription factors, as well as axin and APC in the destruction complex) all interact via intrinsically disorder domains binding to the groove of the superhelix formed by beta-catenin's Armadillo repeats, forming an extended contact surface with many shared contacts between binding partners. For this reason, it has proven difficult to achieve specificity towards any given binding partner.

To aid in our understanding of this disease I have conducted a study of the mechanism of beta-catenin binding to its partner Tcf4, the member of the Tcf/LEF family that is most abundant in humans. The results suggest that structural polymorphism is a major contributor to this interaction, and they identify a multi-step pathway of binding; our hope is that this information may be useful in designing more effective drugs for treating this form of cancer.

P04	Intrinsic disorder

Revealing the Conformational Ensemble of WIP with NMR

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WIP (WASP-interacting protein) is an intrinsically disordered protein (IDP) that regulates actin polymerization and is involved in cell motility and invasion in the context of the immune response, cancer metastasis, and many other biological processes. Linked to signaling pathways (1) (2) and exemplifying the one-to-many binding mode typical of IDPs (3), WIP contains multiple binding sites for protein-protein interactions.

Solution NMR is an excellent approach for probing transiently and partially-formed structure of IDPs in binding and folding events, due to its ability to characterize the conformational ensemble of IDPs. The NMR tool-box includes measurements of chemical shifts, J-couplings, residual dipolar couplings, temperature effects, relaxation rates and solvent exposure, all of which report on the structural propensities of the studied polypeptide in an ensemble-averaged manner. Our research focuses on 3 different WIP segments,

(i) the actin-binding N-terminal WH2 domain (residues 2-65),

(ii) the WASP-binding C-terminal domain (residues 442-503),

(iii) the cortactin-binding part of the proline-rich central region (residues 122-205)

In the case of the N- and C-terminal WIP peptides, our results concurred in identifying WIP segments exhibiting a propensity for partial secondary structure in their conformational ensemble. In particular, measurements of residual dipolar couplings (RDCs) were useful in establishing these findings. Notably, the conformational ensemble of both WIP domains echoes the eventual conformation of WIP in its actin- or WASP-bound state. Importantly, in both cases a previously unrecognized segment in each domain was determined to contribute to the binding affinity (4, 5). Initial characterization of the cortactin-binding WIP segment is currently underway with the aim of precisely defining the binding site. Overall these results demonstrate the potential impact of high-resolution NMR upon the field of biologically active unstructured proteins.

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P05	Intrinsic disorder

Towards a better understanding of the molecular mechanisms by which fuzzy regions affect the folding rate of adjacent molecular recognition elements

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In spite of the partial disorder-to-order transition that intrinsically disordered proteins often undergo upon binding to their partners, a considerable amount of residual disorder may be retained in the bound form, thus resulting in a fuzzy complex[1]. The complex between the intrinsically disordered C-terminal domain of the measles virus (MeV) nucleoprotein (NTAIL) and the C-terminal X domain (XD) of the viral phosphoprotein is an illustrative example of fuzziness[2]. Indeed, although binding to XD triggers alpha-helical folding within a MoRE encompassing residues 486-502[3], the NTAIL region preceding this MoRE remains conspicuously disordered [4, 5]. Fuzzy regions flanking molecular recognition elements (MoREs) may enable partner fishing through non-specific, transient contacts, thereby facilitating binding, but may also disfavor binding through various mechanisms. In a previous study we have shown that shortening the N-terminal fuzzy region results in increased binding towards XD [6]. Kinetics studies that made use of a previously described single-site Trp XD variant[7] and either a peptide mimicking the MoRE or full-length NTAIL, provided support for a scenario where the fuzzy appendage would reduce the rate of folding of NTAIL[6]. The underlying molecular mechanisms remain however to be elucidated. In particular, it remains to be established whether this rate enhancement effect varies linearly with increasing truncation, and whether this effect is sequence-specific. To address these issues, and in view of an in-depth kinetic characterization, we are currently generating a series of truncated MeV NTAIL constructs whose sequence is either that of wt NTAIL or that of a previously reported artificial NTAIL variant[6].

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P06	Intrinsic disorder

Plasmid vs. chromosome-encoded proteins structural disorder: case study of prokaryotes

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We analyzed 139 Archaeal and 2554 Bacterial proteomes (http://www.ncbi.nlm.nih.gov/) for intrinsically disordered protein (IDP) content of their chromosomes and plasmids and its relationship with functional categories of proteins and other genome and proteome characteristics – genome size, proteome size, average protein length and %GC. We have used three methods (PONDR® VSL2b (Peng et al, 2006; Obradovic et al, 2005), IsUnstruct (Lobanov et al, 2013), IUpred-L (Dosztanyi et al., 2005a, 2005b)) and three measures (the averaged fraction of disordered residues by proteins in a proteome, the fraction of AA residues in long (>30 AA) disordered regions, the percentage of proteins (in a proteome) with at least one long (>30 AA) disordered region).

For all the three predictors and all the three measures, Bacteria have significantly more IDPs than Archaea (in range between 10% to 24% and 6% to 20% on average respectively, for the percentage of disordered AA and different predictors; Student's T test for equality of means, p-value < 0.01; similarly for other measures). These results are in accordance with previously published (Oates et al, 2013; Pavlović-Lažetić et al, 2011).

Plasmid coded proteins contain significantly more IDPs than chromosome (or whole genome) coded proteins in both prokaryote superkingdoms (e.g. for Bacteria, 16%-33% in plasmid coded proteins vs. 10%-24% in chromosome coded proteins, depending on a predictor). These findings are proved to be statistically significant by using Mann-Whitney nonparametric and Student T test (p-value < 0.00001).

Chromosome and plasmid coded proteins that belong to Cp and Isp COG groups have significantly higher IDP content than Me group, as expected (Pavlovic-Lazetic et al, 2011). Plasmid coded proteins, in comparison with chromosomal ones, have significantly higher IDP content in the N.C. COG group only (and poorly characterized implying high content of non orthologous and/or "novel" proteins).

Correlation analysis between IDP content and genome/proteome characteristics shows that statistically significant positive correlation exists between average protein length of complete proteomes and IDP content of complete proteomes as well as chromosomal (and in smaller extent plasmid) part separately and that it is highest for high average protein length proteomes. Similar

holds for %GC, proteome and genome size, except that plasmid coded proteins follow this pattern for largest genomes and proteomes only.

Data obtained from such analysis may help in understanding mechanisms of evolution, as well as in further developments in synthetic biology.

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P07	Intrinsic disorder

Analyses of present proteins with reduced number of amino acids reveal the importance of disordered regions, conformational diversity and cavities in protein function.

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Background

Despite considerable effort devoted to studying how life originated on Earth, there is still no accepted theory of how life could have emerged. Underlying this question is how principal macromolecular components of living systems, for example proteins and nucleic acids, could have also originated. In reference to proteins, the conventional view is that they have evolved by means of gene duplication, exon shuffling and random mutations from a small set of "starter sequences" ((McLachlan, 1972) and (Patthy, 1991)). An alternative theory stands that proteins have originated from random sequences of amino acids(for example Shakhnovich & Gutin, 1990). This theory is supported by Miller's experiment(Miller, 1953) and his demonstration that amino acids could be easily synthetized in the putative early conditions of Earth. Assuming the existence of a system to join amino acids, White and Jacobs suggested a protein synthetic "big bang" of random sequences where a portion of them could have folded to form proto-proteins(White & Jacobs, 1990). In this work we studied actual proteins/domains with reduced number of amino acids in their composition. Our main hypothesis is that, at the early stages of proteins origin, the number of amino acids available could have been limiting. This hypothesis is also supported by different theories explaining how the different amino acids composing actual proteins were gradually incorporated during evolution(Brooks, Fresco, Lesk, & Singh, 2002). Using these proteins, we studied different structural/dynamical properties such as: conformational diversity, presence of disorder, tunnels, cavities and pockets.

To explore the conformational diversity of these proteins with reduced number of amino acids we used CoDNas (database of protein conformational diversity). Tunnels were estimated using MOLE, pockets and cavities using Fpocket and the presence of disorder was estimated counting missing residues (more than 5 consecutive missing residues excluding amino and carboxyl ends). As the number of amino acids in a protein is correlated with protein length, we considered only the proteins showing a number of amino acids which are not correlated with length as derived from bootstrapping analysis.

Using bootstrapping analysis, we considered proteins with less than 17 amino acids as those with a reduced number of amino acids. The total number of proteins in this set is 603 with an

average number of residues of 16. We found that these proteins are in average shorter than those containing more than 17 amino acids (average 59 and 253 respectively). They also have more disordered regions (average 6.67 and 2.33), 80% of them lack tunnels and they show more important cavities when the percentage of cavity volume in reference to conformer volume was estimated (5.26 and 2.54 % in average).

Our conclusions are that it is possible, using reduced alphabets of amino acids (as it is expected to be the case in the origin of early proteins), to obtain proteins with the ability to fold and function. It is interesting to note that these proteins have higher conformational diversity and higher levels of disordered regions than those proteins with 20 amino acids. The correlation of the presence of disorder and a higher conformational diversity is according to recent results for proteins with normal number of residues(Zea et al., 2016). The higher conformational diversity, absence of tunnels and proportionally larger cavities indicate that these proteins could have functional bias when compared with current protein.

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P08	Intrinsic disorder

Helicity and Tetramerization in the KcsA Cytoplasmic Domain

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The intracellular C-terminal domain (CTD) of KcsA, a bacterial homotetrameric potassium channel, is a 40-residue long segment which natively adopts a helical bundle conformation with four-fold symmetry. A hallmark of KcsA behavior is a pH-induced conformational change which leads to opening of the channel at acidic pH, but crystal structures of full-length KcsA failed to observe a pH-effect upon the CTD. We approached this question by studying the behavior of soluble peptides corresponding to residues 128-160 of the CTD (CTD34) using nuclear magnetic resonance (NMR), sedimentation equilibrium (SE) and molecular dynamics. CTD34 is monomeric with a helical tendency at low concentrations and tetrameric at high concentrations, with a KD of 2.0 x 10(-11) M(3) at neutral pH. It also undergoes pH-dependent dissociation, due to the loss of an inter-helical hydrogen bond between residues H145 and R147. Alanine scanning mutagenesis along the CTD34 sequence showed single-residue contributions to tetramer stability in the 0.5-3.5 kcal/mol range and a correlation between helicity and tetramerization. To extend the significance of these findings to the cellular environment we embedded full-length KcsA in lipoprotein nanodiscs (LPNs), membrane-like particles that circumvent the interference of free detergent/phospholipid with inter-subunit interactions. In this system we have verified the pH-based gating and the contribution of various residues to tetrameric stability. We conclude that tetramerization follows the accepted paradigm of protein folding promoted first by hydrophobic and then electrostatic interactions, with helical CTD34 serving as a template for folding.

P09	Intrinsic disorder

Robotics-inspired algorithms and high-performance computing to explore the energy landscape of intrinsically disordered proteins

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Exploring the energy landscape of highly-flexible biomolecules is a hard task for computational methods because of their huge conformational variability.

To solve this problem, we have recently developed an algorithm, called T-RRT, that combines ideas of robot motion planning algorithms and methods in statistical physics. It explores the conformational space of a molecular system by incrementally growing a search tree that is intrinsically biased towards unexplored regions. A stochastic transition tests favours the exploration of low-energy areas, and it involves a self-additive parameter that helps the exploration to overcome local minima traps. Our first works demonstrated the ability of the method to efficiently find diverse conformational transition pathways for small and medium-size peptides. In order to study larger systems, such as IDPs, we have developed an hybrid parallel implementation of a multiple-tree variant of the T-RRT algorithm. It combines shared-memory and distributed-memory programming paradigms with the aim of better exploiting the architecture of current computer clusters. In this talk, we will remind the principle of the (Multi-)T-RRT algorithm and we will provide explanations about its parallel implementation, showing super-linear speedup.

P10	Intrinsic disorder

High binding affinity of α-helix-forming MoRFs as a functional necessity

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For a widespread group of intrinsically disordered proteins, partner recognition is mediated by segments termed MoRFs (molecular recognition features) that have been found to fold into a defined structure upon binding with specific binding partners (1-3). In spite of large conformational entropy loss upon folding some MoRFs can bind their partners with extraordinarily high affinity (4). The probability of finding them in the family of intrinsically disordered binding proteins, however, appears to be very small (5).

Here we examine the functional reason for the extraordinarily high affinity of an α-helix-forming MoRF CcdA for its partner CcdB from E. coli toxin-antitoxin module ccd. — is it a necessity or simply the product of chance? We will demonstrate by thermodynamic analysis and structural characterization of association of wild type CcdA and CcdB and their mutants and of binding of these proteins with other proteins and DNA within the "ccd interactome" why the unusual combination of intrinsic disorder and high binding affinity is not a coincidence but rather nature's solution to an unusual functional problem.

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P11	Intrinsic disorder

Fluorimetric detection of Staphylococcus aureus in biological fluids by peptide-aptamer-based device.

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Staphylococcus aureus causes a wide spectrum of diseases and there is a huge demand for its point-of-care detection. Using aptamers - DNA sequences acting as artificial recognition elements - known to interact with S. aureus, we developed a device where the detection of S. aureus is based on the displacement by bacteria of a fluorescein-labeled peptide that selectively recognizes the aptamer. The displacement of the peptide is associated with a detectable change in the fluorescence of the labeled peptide. For the design of specific peptides we exploited the energy-based amino acid-base recognition code, previously obtained for several protein-DNA complexes, to mutate Lambda-Cro DNA binding domain for the specific recognition of a DNA segment of the aptamer. Nanostructured zirconium dioxide supports have been used for immobilizing the aptamers.

This approach can be extended to other biological agents for which selective aptamers have been identified, allowing applications in clinical and environmental monitoring.

P12	Intrinsic disorder

Partially disordered delta subunit of RNA polymerase from Bacillus subtilis studied by NMR

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Delta subunit of RNA polymerase, found in Gram-positive bacteria only, contains a disordered domain of a highly repetitive sequence, rich in acidic residues. The delta subunit is not essential for survival in pure cultures, but it is important for bacteria competing with other species in the natural environment. The protein makes RNA polymerase sensitive to initiating NTPs, but the molecular details of its action are not known. In order to get insight into the biophysical properties of the delta subunit, we combined advanced NMR techniques (multidimensional spectroscopy utilizing non-uniform sampling, measurement of residual dipolar couplings, paramagnetic relaxation enhancement, and 15N auto- and cross-correlated relaxation) with site-directed mutagenesis, small-angle X-ray scattering and computational approaches. The results revealed the role of electrostatic interactions in the behavior of the disordered domain and described its dynamical properties with the resolution of individual residues.

P13	Intrinsic disorder

Cross validation of molecular dynamics simulations of an intrinsically disordered protein using NMR data

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Molecular dynamics simulations on intrinsically disordered proteins are an emerging field. Indeed, the majority of force fields available before 2014 were designed for folded proteins and to study protein folding. This leads to overly compact structures in simulations run even of short peptides, which are known to be intrinsically disordered. By balancing solvent-solvent and protein-solvent energies in existing force fields, the overly compact structure sampling can be circumvented. The new approach has been tested on a well characterized IDP, i.e. the intracellular domain of the human prolactin receptor isoform SF1b, for which we measured both NMR chemical and shifts and the Radius of Hydration (Rh). This allows us to compare the accuracy of these newly developed force fields for unfolded proteins with state-of-the-art force fields for folded proteins in reproducing experimentally-derived structural parameters.

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P14	Intrinsic disorder

Metadynamics to investigate the phosphorylation-mediated regulatory mechanisms in the disordered tail of Na+/H+ exchanger isoform 1 and implications for cancer progression.

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Disordered regions are common in many proteins involved in regulatory functions, scaffolding activities and cell signaling since their heterogeneous conformational ensembles allow the interaction with multiple and different partners, hence integrating diverse cellular pathways (1). Intrinsically disordered proteins (IDPs) are also tightly regulated by post-translational modifications (PTMs) that can exert different effects, such as disorder to order transitions and local (de)stabilization of secondary structure elements. The PTM-induced conformational changes in IDPs can in turn affect the recruitment of binding partners and modulate different signaling events. Understand the molecular mechanisms of the regulation mediated by disordered regions and their modification by PTMs is of primary importance since alterations in IDPs are associated with changes in signaling pathways in several diseases, such as cancer. We integrated enhanced molecular simulations based on metadynamics sampling with NMR measurements to investigate the effects induced upon recently characterized phosphorylations (2) on a functionally important disordered region in the intracellular tail of the Na+/H+ exchanger isoform 1 (NHE1). Indeed, it has been suggested that deregulation of NHE1 in breast cancer is a preponderant factor leading to cancer cell migration and metastasis (3). Moreover many cancer-related mutations identified upon genomic profiling of cancer patients are localized in the disordered domains of NHE1. We identified a structural motif common to different NHE1 disordered domains and other disordered proteins for helix stabilization induced by phosphorylation, where electrostatic interactions with upstream charge residues play a major role.

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P15	Intrinsic disorder

Structural and functional similarities between wt delta133p53beta isoform and the mutant p53 protein in promotion of cancer

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The p53 protein is a well described anti-oncogene. It is a 393 aa, multi-domain protein with 3 pronounced regions: N-terminally, the non-structured transactivation domain (≈100 aa);, the central globular DNA-binding domain (≈200 aa) and the C-terminal oligomerisation domain (≈100 aa). In a large proportion of cancers, p53 is inactivated by missense mutations in the DNA binding domain. These mutations abolishes protein functions by two different modes. The first type of mutations occurs in α-helices responsible for DNA binding. These "contact" mutations produce a dominant negative protein unable to bind DNA. The second type of mutations occurs in residues responsible for keeping the structure of the DNA binding domain. These "structural mutants" results in a significantly modified conformation of this domain. Interestingly, structural mutations frequently causing gain of new functions and tumours carrying them have very aggressive features. On the other hand, tumours without p53 mutations could also have very aggressive phenotypes, indicating additional mechanics involved in cancer progression. Recently it was demonstrated that the TP53 gene, apart from the canonical p53 protein, codes for 12 protein isoforms. Some of them, like Δ133p53β, are particularly up regulated during cancer progression. This isoform is depleted of the transactivation domain and the first 33 aa of the DNA binding domain and has shorter and different C-terminal region with respect to canonical p53. It was shown that the Δ133p53β isoform is involved in promotion of the cancer stem cell phenotype and induction of invasion of metastatic cells. These observations demonstrated functional similarity between wt Δ133p53β and p53 structural mutants. The scope of this study was to evaluate structural features of the Δ133p53β isoform.

Using the co-immunoprecipitation approach with p53 antibodies able to distinguish between wt and mutant structures, we demonstrated that wt Δ133p53β behaves identically to the structural p53 mutants. The antibody used for this analysis has an epitope in position 213-217 of the p53 protein, which is deeply buried in core of the DNA binding domain. This epitope in Δ133p53β is accessible for the antibody indicating that this region was subjected to significant rearrangement in respect to the wt p53 protein. Our in silico structural prediction supports this conclusion suggesting that the absence of the N-terminal 33 aa region destabilize the remaining part of the DNA-binding domain. In addition in silico prediction demonstrated a long non-structured C-terminal region. All together our experimental and in silico results demonstrates that the wt Δ133p53β form, similarly to the

p53 structural mutants, destabilizes and unfolds the 3D structure causing a gain of new functions. These modifications of the wt Δ133p53β structural properties in comparison with wt p53 can explain its roles in the promotion of cancer progression.

P16	Intrinsic disorder

Crystallization of DC8E8 antibody tetratope on the molecule of intrinsically disordered protein tau

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Accumulation of intrinsically disordered protein tau in the form of insoluble aggregates is a common feature of neurodegenerative tauopathies. Tau protein is engaged in the regulation of microtubular dynamics in the neurons, however, under pathological conditions tau interacts with itself, escapes disordered state and forms toxic oligomers and aggregated filaments. Monoclonal antibody DC8E8 is able to inhibit tau-tau interaction [1, 2] and therefore it holds promise for the immunotherapy of Alzheimer's disease. Minimal epitope of DC8E8 represents amino acid motif HXPGGG that is present in each of the four microtubule binding repeat regions (MTBRRs) of tau. Unravelling the unique mode of recognition of DC8E8 can aid to reveal hindered structural features of tau protein and their implication for tau protein biology.

In the presented study we have crystallized the Fab fragment of DC8E8 antibody with 12-mer and 18-mer peptides covering its epitopes from all four MTBRRs of tau, where the antibody Fab fragment serves as a crystallization chaperon for the disordered tau peptide. The biophysical measurements have shown that the antibody has the highest affinity against the second MTBRR. The antibody has the lowest affinity for the fourth MTBRR that is more than two orders of magnitude lower than its affinity for MTBRR2. The obtained crystals will, after the successful structure solution, help to unravel the molecular basis for the affinity difference between these highly homological intrinsically disordered epitopes and the basis of the anti-aggregatory action of DC8E8 monoclonal antibody.

Acknowledgement:

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P17	Intrinsic disorder

Intrinsic disorder and complex formation in myelin proteins

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Myelin is a crucial structure for ensuring rapid nerve impulse transmission in vertebrates. The myelin sheath is a differentiated plasma membrane of a glial cell, wrapped dozens of times around a neuronal axon, and its biochemical composition is unique. The compact multilayered myelin proteolipid membrane contains specific proteins and very little aqueous solvent. Our work focuses on the fine details of myelin protein structure, their interactions with lipid membranes, and the mechanisms, through which they induce membrane multilayer formation. Myelin proteins present large degrees of intrinsic disorder, and they have in general little homology to other proteins. However, myelin proteins specifically interact with binding partners, including membranes and other proteins, and the disordered domains of these proteins play central roles in the interactions. I will present specific examples of myelin intrinsically disordered proteins and their specific high-affinity interactions. The data are crucial to understand the process of myelination as well as demyelinating neurodegenerative diseases, such as multiple sclerosis.

P18	Intrinsic disorder

Regulation of protein disorder by local structural propensities

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Introduction: Disordered proteins (IDPs) exist as a conformational ensemble of interconverting conformers, void of bulky elements of secondary structure elements, e.g. alpha helices or beta sheets. Specific regions of IDP may adopt temporarily and spatially confined secondary structure conformation, measured as a propensity by spectroscopic techniques like NMR. On the other hand, X-ray crystallography is able to report about snapshots of IDPs local conformations as imprinted in the crystal structure of IDPs complexes with (mostly) globular partners. It is not clear what the proportion of crystallographically detected conformation is in the conformational ensemble of IDP in solution.

Materials and Methods: We have determined X-ray structure of short IDP tau peptide in the complex with monoclonal antibody specific to tau N-terminal region. Observed structural features were corroborated by mutational study, biophysical characterization of complex formation and molecular modelling.

Results: High resolution data revealed strikingly strong interaction within the tau polypeptide, which was further examined for its effect on the kinetic and thermodynamic of tau protein binding with antibody. Observed parameters were mechanistically assessed in the course of a microsecond molecular modelling simulation.

Conclusion: Local and global conformation of IDP tau chain may be efficiently regulated by a local propensities to stable non-bulky conformations.

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P19	Intrinsic disorder

Dimerization inhibition of Maf transcription factors by designed peptides

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Transcription factors belonging to the Maf family bind DNA target sequences as homo- or hetero-dimers, exploiting a leucine zipper (LZ) motif for protein-protein interactions. Their DNA-binding domain contains a highly conserved extended homology region (EHR) that allows to recognize longer DNA sequences than other LZ transcription factors.

Overexpression of Mafs is observed in about half of the cases of multiple myeloma, an hematological malignant disorder characterized by the clonal proliferation of plasma cells in the bone marrow and presently lacking a targeted pharmacological approach. This motivated us to undertake a peptide inhibitor strategy. The LZ domain of c-Maf, one of large Maf oncogenes, was produced by solid phase peptide synthesis. Structural features and dimerization properties were characterized by circular dichroism spectroscopy and MALDI-TOF mass spectrometry, highlighting a tight coupling between dimerization and secondary structure content (eukaryotes transcription factors are reported to possess extended disordered regions).

Peptidic c-Maf dimerization inhibitors were rationally designed and synthesized. The most successful compounds were shown to bind to c-Maf LZ monomers with dissociation constants in the low micromolar range, promoting folding of their partially disordered structure and efficiently inhibiting dimerization. These promising results endorse the peptide inhibitor approach for interfering with the function of c-Maf and, more generally, of intrinsically disordered proteins, often considered undruggable targets.

P20	Protein repeats

The Effect of Loop Insertions on the Folding of Tandem-Repeat Proteins

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Multi-sequence alignment and the identification of conserved residues have allowed the design of highly stable consensus repeat proteins. The folding of repeat proteins has been shown to follow a 1-D Ising model, wherein fixed values for intra- and inter-repeat stability combined with the number of repeats are used to define the free energy of unfolding. Nevertheless, some questions remained unanswered, and we are using consensus-designed tetratricopeptide repeats (CTPRs) to address them: How are CTPR proteins able to remain soluble and stable without capping repeats? What role does the loop between adjacent repeats play in this folding paradigm? How do the loops contribute to inter-repeat stability? Here we explore the role of loops in the thermodynamics and kinetics of repeat protein folding by inserting variable-length unstructured loops at single or multiple sites along the repeat array. In particular we seek to understand how long loops are accommodated in the current folding models and to what extent the repeat protein is able to close these loops efficiently and still fold correctly. We also want to assess the feasibility of inserting functional peptide/protein motifs in between the stable inter-repeat interfaces so that we can expand the scope for exploiting repeat proteins as building blocks in biomaterials and the controlled geometric arrangement of function within them. Our preliminary results show that CTPR proteins with multiple loop insertions are stable and correctly folded. However, we find that stability no longer increases with the number of repeats, in contrast to what is observed in normal CTPR proteins and in striking deviation from the repeat protein folding paradigm. Further analysis is required to understand this breakdown in folding cooperativity and the implications for protein design.

P21	Protein repeats

Collective Dynamics Underlying Gating Mechanism of Repeat Protein FimD

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FimD is a repeat protein whose function is important for the virulence of E. coli since it serves as a catalyzer and a foundation of the pilus assembly. It has a β-barrel structure located on the outer membrane that is blocked by a plug domain (PD, I256-V315) inside, in case of inactivity. This structure corresponds to the closed conformational state. With the shift of the PD to the periplasm, FimD assumes the open conformational state leading to the pilus formation, where α-helix (N443-T448), β-turn (Y222-N232) and P-linkers (S240-V255 and P316-T329) were known to be functional.

In this study, conformational transition pathways between the open and closed states of FimD were explored using Langevin Dynamics (LD) simulations guided by intrinsic dynamic modes of Anisotropic Network Model (ANM). Starting with an RMSD (root mean square deviation) of 17.5 Å between the two conformations, the target conformation was approached within 4-5 Å RMSD in the open to closed transition. Certain collective ANM modes were identified to be instrumental in this transition and the underlying dynamical network of hinge residues were seen significantly to reside in or neighbor α-helix, β-turn, P-linkers and PD, and additionally overlapped known mutations leading to an extreme open conformation and/or pilus deficiency. Supporting the significance of the latter modes of motion, their restriction led to changes in the dynamic behavior and blocked the transition at an intermediate conformation. The functional mechanism disclosed here could be plausible for further validation by experimental studies on FimD.

P22	Protein repeats

CTPR proteins: nanotubes design and nanostructured films

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Nowadays state-of-the-art in biotechnology is capable of manipulating the physico-chemical properties at molecular scale. Bottom-up design of complex functional nanostructures is of the great interest because it allows to synthesize organized structures using the intrinsic self-assembly properties of simple components. In this sense, repeat proteins are useful tools for this task due to their modular and hierarchical structure that can be the basis to construct complex supramolecular assembly (as protein nanotubes and films). In addition, their modularity provides the possibility of introducing reactivities at define positions for precise patterning of other molecules that can furnish specific and complexes properties (such as gold nanoparticles).

In our approach, we use consensus tetratricopeptide repeat (CTPR) proteins based on the TPR structural motif, a 34 amino acids and helix-turn-helix structure for their application as building blocks in order to generate biomaterials and functional nanostructures.

Here we report the ability to design CTPR-based nanotubes through rational design, by the introduction of novel interacting interfaces is possible to promote the interaction between two CTPR superhelical structures into a new nanotube structure. We envision that these structures can be very attractive in different applications, including the synthesis of nanowires or the encapsulation of drugs.

Additionally, we have already described the formation of self-assembled CTPR protein films. We demonstrated that within the films the proteins units retain their structure and function and can induce order into other elements generating functional films. However, those films lacked stability in aqueous solution which a key requirement for their application. In the present work we show preliminary results on the crosslinking of protein films, which allowed for an increased stability in water.

P23	Protein repeats

Aggregatibacter Adhesins and their interaction with implant materials

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The lack of knowledge about bacterial adhesion mechanisms hinders the development of novel antibacterial surfaces. Consequences are failing implants, inefficient electrodes, and biofouling of off-shore materials. The current, slow approach to this problem is trial-and-error testing of potentially beneficial surface modifications. We are currently following a bottom-up approach, starting with molecular biology studies of repetitive bacterial adhesion factors, and of their interaction with surfaces. We develop bacteria-surface adhesion assays to obtain new knowledge on adhesin function. Secondly, we try to translate the results into novel surface modifications of dental implants to test the theories, leading to more effective antibacterial surfaces, and the improvement of the adhesion assays. We have established links between biology and materials science, fostering interdisciplinary research collaboration. The aim are implant surfaces that allow for tissue integration, but at the same time inhibit bacterial adhesion.

P24	Protein repeats

Sequence pattern representation in protein protein interactions models

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Protein-protein interaction maps provide a valuable framework for a better understanding of the functional organization of the proteome. Sequence-based methods have shown that the genome-wide sequence information alone may be sufficient to identify novel PPIs. We aimed to understand how sequence pattern information relates to proteins affinities for protein protein interactions and developed models that use two forms of sequence-order representation: pseudo amino acid composition and informational spectra. Models were intended to describe transcriptional regulation, a process that controls the transcriptional program of the entire cell mostly via PPI. They are based on random forest algorithm and sequences were encoded by physicochemical features that determine short-range and long-range interaction properties. The predictive capabilities were assessed on independent test sets and models' performances were compared to state of the art methods that rely on another commonly used sequence information, signature products. Furthermore, we analysed evolutionary relatedness of models in transcriptional regulation and evaluated how efficiently model trained on human protein interactions predict interactions in other species. These analyses revealed strengths and weaknesses of pattern based models and how this approach may contribute to systems-level understanding of gene regulatory processes.

P25	Protein repeats

Coevolution of the repeated glutamine and proline codons in the mammalian Huntington disease gene

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Background: The tandem single-amino-acid repeats (homopeptides) are very common in mammalian proteins1. The human Huntington disease (HD) gene contains a CAG microsatellite encoding a polyglutamine tract, followed by cryptically simple regions encoding a proline-rich tract2. Expansion of the CAG repeats are associated with Huntington disease3. The HD gene is evolutionary conserved between Drosophila and humans4, but repeated CAG codons are seen only in the vertebrates, while repeated CGG codons are present only in the mammals. The aim of the study was to improve understanding of the repeated codons evolution in the HD gene.

Materials and methods: DNA was extracted from hair or bucal swabs obtained from 18 mammalian species, mostly from the Belgrade Zoo. PCR amplification of the HD repeated codons was performed using primers designed to amplify the human sequence3. PCR products were analyzed at 6% denaturing polyacrilamide gels stained by silver. Bands with profile characteristic for microsatellite loci were eluted, reamplified, and prepared for sequencing. Direct sequencing of PCR products was performed using the BDT v.1.1. kit, and analyzed on an ABI 310 Genetic Analyzer using the Sequencing Analysis Programs (Applied Biosystems, CA, USA). Sequence alignment was done by BLAST.

Results: The alignment of the nucleotide and supposed amino-acid sequences of analyzed mammalian species, and mouse, rat and non-mammalian species (Ensembl database) indicated that there are evolutionary conserved amino-acids, and a QPn tract is periodically repeated. These led us to propose a model predicting the coevolution of repeated glutamine and proline codons in the mammalian HD gene. The coevolution probably resulted from an interplay between mutational processes (replication slippage and synonymous and nonsynonymous point mutations, and selection (purifying selection and selection on the reading frames) in which tandemly repeated codons could be accumulated. The balance between these processes differs between two regions: replication slippage and strong purifying selection probably were the main force for the evolution of the repeated glutamine codons, while replication slippage, synonymous and non-synonymous substitutions, and weaker purifying selection drove evolution of repeated proline codons.

Conclusion: Analyzes of nucleotide and amino-acid sequences of the repeated codons in the HD gene showed that polyglutamine and polyproline coding regions could coevolved during the mammalian evolution by an interplay between mutational and selection processes, creating two homopeptide regions. These rapid changes during evolution in an ancient gene, such as the HD

gene, could assign huntingtin protein with new functions through interaction with new partner proteins or modifying protein-protein interaction. Our results suppor an idea that evolution of the homopeptide regions might make a significant contribution to the reorganization of protein-protein interaction network over evolutionary time1.

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P26	Aggregation

Identification of novel human prion-like proteins and characterization of their amyloid cores

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Prions are infectious proteins able to change from a soluble and often disordered state into an amyloid state which is self-propagating, infectious and transmissible. They are involved both in functional roles and disease and their evolutionary conserved properties highlight prion impact in life. Proteins associated to classical amyloid diseases such as Alzheimer's and Parkinson's were shown to act as prion-like proteins bearing self-propagating properties across cells. Thus, human prion-like proteins exist and there could be more of them without being identified yet. Their identification and analysis is crucial to understand their biological function and their implications for human health and disease. We have exploited three computational algorithms, PAPA, pWALTZ and PrionScan, to detect potential human prion-like proteins and to define its aggregation core. We analyzed the complete human proteome and studied the interaction network and association to disease of the putative prions. Then, we examined experimentally the amyloid potential of twelve human short regions predicted to nucleate prion formation direct by standard techniques such as amyloid-dyes binding, Transmission Electrom Microscopy (TEM), Circular Dichronism (CD) and Fourier Transform InfraRed (FT-IR). The results show that all selected regions are able to form amyloid structures in vitro. These data illustrate the efficiency of our prediction strategy and points that human prion-like proteins could be more abundant than previously assumed.

P27	Aggregation

Development of a dataset of non-aggregative protein sequences from NMR resolved structures to test aggregation prediction algorithms

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In recent years proteins with the potential to form amyloids have gained increasing interest, mainly due to their role in a number of diseases, most notably Alzheimer's, Parkinson's, Huntington's, type II diabetes and transmittable prion disease. Amyloidogenicity of a protein is inherently determined by the amino acid sequence. Thus, a number of computational methods to predict amyloidogenecity and in a broader sense protein aggregation, based on the analysis of amino acid sequence, have been developed (Ahmed and Kajava, 2013). To test these methods several datasets containing both aggregative and soluble peptides have been constructed. However, most of the peptides in these databases are very short (~6 amino acids), with not more than 1000 peptides in any given dataset. Hence, there is a lack of experimental data, which can be used to benchmark computational prediction algorithms.

In this context, we turned our attention to the PDB, with about 9000 protein structures determined by NMR spectroscopy. One of the major conditions for NMR experiments is that the proteins are soluble at very high concentrations (~1 mM). Moreover, a large portion of these proteins have their structures flanked by large (~30 amino acids) unfolded terminal regions (see e.g. MobiDB (Potenza, et al., 2015)). In general, the unfolded regions have the highest propensity for aggregation, if that they have amyloid forming regions. As we know that the unfolded regions, from NMR-studied proteins do not form aggregates, even at high concentrations, this further suggests that they can be used as a set of non-aggregative sequences. The dataset and the results of the benchmarking of amyloid propensity prediction methods on this dataset will be presented.

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P28	Aggregation

Discovering new putative prions in Plasmodium falciparum: A computational and experimental analysis

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Prion-like proteins can switch between a soluble intrinsically disordered conformation and a highly ordered amyloid assembly. This conformational promiscuity is encoded in specific sequence regions, known as prion domains (PrDs). Prions are best known as the causative factors of neurological diseases in mammals. However, its is getting wither accepted that at least in some species prion proteins can be biologically important, specially to adapt the organism to different environments. Prion domains are disordered regions characterised by a special amino acid composition, principally repeats of Qs and Ns. The proteome analysis of Plasmodium falciparum has shown an especial enrichment in Ns (31% of the whole proteome) and this property has been associated with the capacity to evade the immunity response. Moreover, the putative prion proteins of P. falciparum may have unknown functions and may affect the aggregation of host proteins. However currently it has not yet been experimentally demonstrated that these can behave as prions. In the present work, we performed a stringent computational analysis of P. falciparum proteome and validated the prion potential of four candidate proteins the protein kinase PK4, the asparagine-rich antigen Pfa35-2, the putative translation initiation factor IF-2 and the putative transport protein SEC24. In the light of the obtained results, we provide here strong evidence that the detected PrD in P. falciparum contain a short amyloid segment with the ability to potentially nucleate the PrD assembly and, consequently, the protein conformational switch. This can have implications in human health and the understanding of P. falciparum biology.

P29	Aggregation

Structure of the acridine derivates affects amyloid aggregation of Aβ peptide

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The presence of amyloid aggregates in different tissues has toxic consequence to various cell types leading to their dysfunction or death [1]. Nowadays, the precise mechanisms of toxicity are not fully elucidated; however, based on previous studies, the most promising therapeutic approaches seem to be targeting a protein stability and misfolding, direct inhibition of the self-assembly process or clearance of amyloid aggregates [2]. In the past few years, a range of low molecular weight compounds have been selected to actively inhibit amyloid aggregation and to promote the disaggregation of amyloid filaments in in vitro systems, including cell cultures. The advantage of the low molecular weight compounds is ability to cross the blood brain barrier easily and avoid immunological response [3].

We have investigated the ability of structurally distinct acridine derivatives to prevent the formation of Aβ amyloid aggregates in vitro. Studied acridines consist of the planar tricyclic core, aliphatic linker with different length terminated with side group. Fluorescence spectroscopy and atomic force microscopy have shown that impact of the studied small molecules on Aβ peptide amyloid fibrillization strongly depends on the structure of acridines. However, the presence of the side group attached to the acridine core through linker caused significant increase in the inhibiting activity. Interestingly, the inhibition efficiency characterized by the half-maximal inhibition concentration IC50 depended on the length of the linker. The shortest linker (2 or 3 carbons) exhibited the lowest inhibitory activities.

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P30	Aggregation

Insoluble off-pathway aggregates as crowding agents during protein fibrillization

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The screening of drugs candidates for the treatment of amyloidosis and neurodegenerative diseases frequently involves in vitro measurements of amyloid fibril formation. Macromolecular crowding and off-pathway aggregation (OPA) are, by different reasons, two important phenomena affecting the scalability of amyloid inhibitors and their successful application in vivo. On the one hand, the cellular milieu is crowded with macromolecules that drastically increase the effective (thermodynamic) concentration of the amyloidogenic protein. On the other, off-pathway aggregates, rather than amyloid fibrils, are increasingly appointed as the causative agents of toxicity. The present contribution reveals that insoluble off-pathway aggregates of hen egg-white lysozyme (HEWL) are a peculiar type of crowding agents that, unlike classical macromolecular crowders, decrease the thermodynamic concentration of protein. Illustrating this effect, OPA is shown to resume after lowering the fraction of insoluble aggregates at constant soluble HEWL concentration. Protein depletion and thioflavin-T fluorescence progress curves indicate that OPA rebirth is not accompanied by additional amyloid fibril formation. The crystallization-like model extended to account for OPA and time-dependent activity coefficients is able to fit multiple kinetic results using a single set of three parameters describing amyloid nucleation, autocatalytic growth and off-pathway nucleation. The list of fitted results notably includes the cases of aggregation rebirth and all types of progress curves measured for different HEWL concentrations. The quantitative challenges posed by macromolecular crowding and OPA find here a unified response with broader implications for the efficient design of on- and off-pathway inhibitors.

P31	Aggregation

Towards the design of aggregation-resistant proteins

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Protein aggregation is a widespread phenomenon linked to the onset of an increasing number of human degenerative disorders. It also represents a major limitation for the production and marketing of protein-based therapeutics Therefore, tools to accurately predict aggregation propensities and ultimately design aggregation-resistant proteins are receiving increasing interest. One of the main limitations to undertake these tasks is that most available algorithms focus on linear sequences and not on protein structures. To overcome this constraint we have developed AGGRESCAN 3D (A3D), a novel in house web server for the prediction of exposed aggregation-prone residues on the 3D structures of globular proteins. As a proof of principle of the potency of such approach, we describe here the re-design of the solubility of GFP and an SH3 domain by rationally engineering point mutations at their surfaces. An excellent correlation between predicted and measured solubility was observed, thus validating the methodology for the further design of soluble protein-based therapeutics.

P32	Aggregation

Differences in activation energies of amyloid-like fibril elongation

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Prion protein can adopt a pathogenic conformation, which is the basis for proteinaceous infectivity. The infectivity is based on self-replication of this pathogenic prion structure. One of possible mechanisms for such replication is the elongation of amyloid-like fibrils. We measured elongation kinetics of prion amyloid-like fibrils at different conditions. Our data show that both increases in temperature and GuHCl concentration help to unfold monomeric protein and thus accelerate elongation. Once the monomers are unfolded, further increases in temperature raise the rate of elongation, whereas the addition of GuHCl decreases it. We demonstrated a possible way to determine different activation energies of amyloid-like fibril elongation by using folded and unfolded protein molecules. Further studies of amyloid-like fibril elongation using two polymorphs of sheep prion protein revealed that change of a single amino acid in the prion protein sequence can result in dramatic differences in activation energy of fibril elongation.

P33	Aggregation

In Silico and in Vitro Study of Binding Affinity of Tripeptides to Amyloid β Fibrils

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Self-assembly of Aβ peptides into amyloid aggregates has been suggested as the major cause of Alzheimer's disease (AD). AD is a frequent type of dementia, and the number of cases is substantially growing with population age. Nowadays, there is no medication for AD, but experimental data indicate that reversion of the process of amyloid aggregation reduces the symptoms of disease.

In this work, all combinations of tripeptides (8 000) were studied for their ability to destroy Aβ1-40 fibrils by in silico methods. The docking method and the more sophisticated MM-PBSA (molecular mechanics Poisson− Boltzmann surface area) method were employed to calculate the binding affinity and mode of tripeptides to Aβ1-40 fibrils. The ability of four tripeptides identified as the best binders and four tripeptides with weak or no binding affinity to depolymerize Aβ fibrils was also investigated experimentally using atomic force microscopy and Thioflavin T fluorescence assay.

It was shown that tripeptides prefer to bind to hydrophobic regions of 6Aβ9−40 fibrils. Tripeptides WWW, WWP, WPW and PWW were found to be the most potent binders. In vitro experiments showed that these tight-binding tripeptides have significant depolymerizing activities and their depolymerizing constants (DC50 values) determined from dose−response curves were in low micromolar range. The ability of non-binding (GAM, AAM) and weak-binding (IVL and VLA) tripeptides to destroy Aβ1-40 fibrils was negligible. In vitro data of tripeptide depolymerizing activities support the predictions obtained by molecular docking and all-atom simulation methods.

Our results suggest that presence of multiple complexes of heterocycles forming by tryptophan and proline residues in tripeptides is crucial for their tight binding to Aβ fibrils as well as for extensive fibril depolymerization. We recommend PWW for further studies as it has the lowest experimental binding constant.

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