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From Protein Complexes to Synaptic Connections and Cell Communities, Cellular Interactions

This special issue of the JEDLIK LABORATORIES REPORTS contains the works presented on the two conferences: 'From Protein Complexes to Synaptic Connections' and 'Cell Communities, Cellular Interactions' held jointly between 27-29 October 2019 in Esztergom, Hungary.

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Program

From Protein Complexes to Synaptic Connections

Cell Communities, Cellular Interactions

Sunday

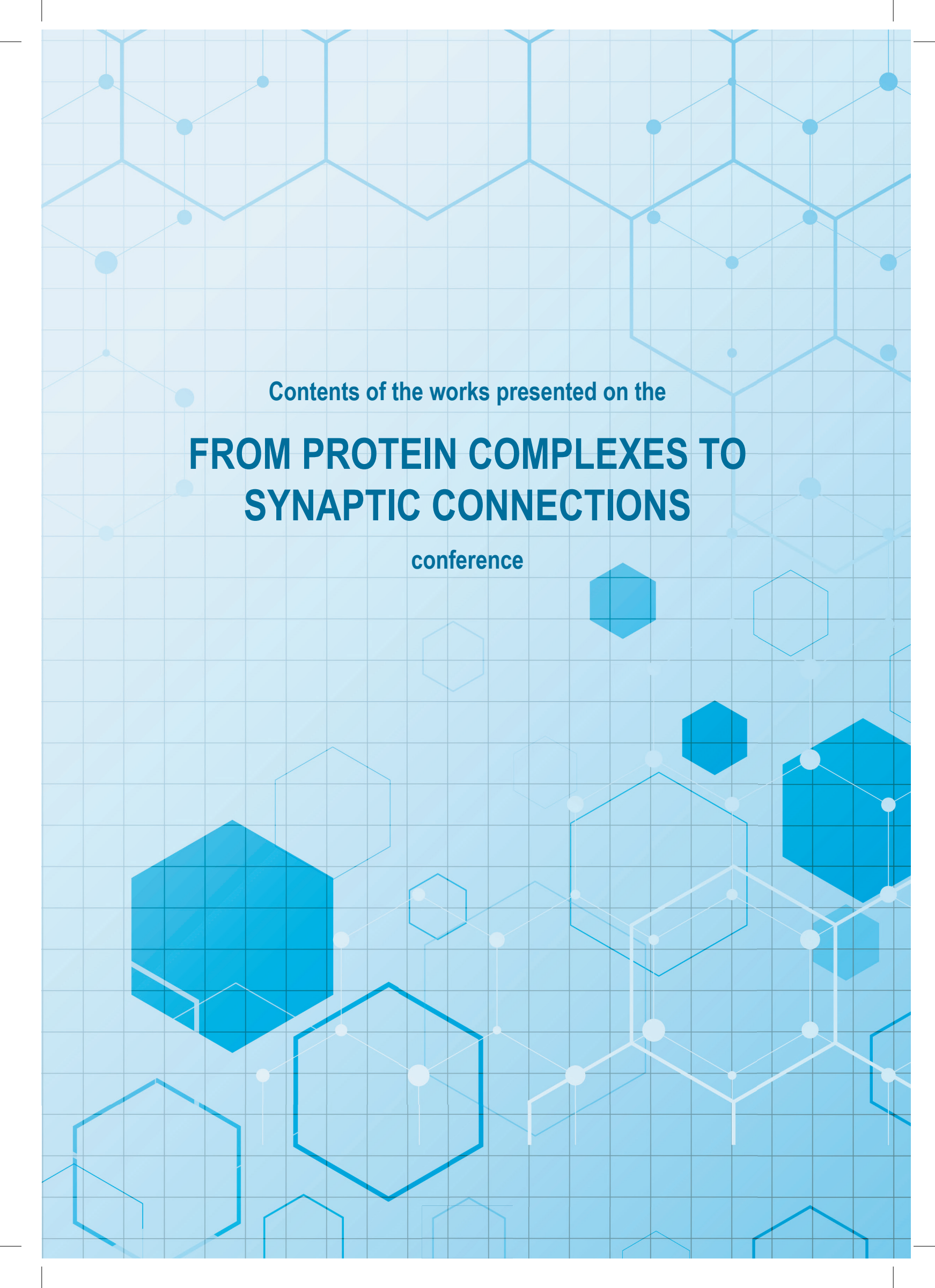
12:00 – 14:00	Registration / lunch
14:00 – 14:30	Opening ceremony
14:30 – 15:15	Seth Grant
15:15 – 16:00	Livia Leoni
16:00 – 18:30	Poster/coffee
19:00 – 22:00	Dinner

Monday

09:00 – 09:45	Alexandre Bonvin	
09:45 – 10:30	Kiran Patil	
10:30 – 11:00	Coffee	
11:00 – 11:20	Tamás BEKE-SOMFAI	Tünde GAIZER
11:20 – 11:40	Bertalan KOVÁCS	János JUHÁSZ
11:40 – 12:00	Tibor PÁLI	Rowan HOWELL
12:15 – 13:00	András PERCZEL	
13:00 – 14:30	Lunch	
14:30 – 15:15	Adrian Tett	
15:30 – 15:50	Balázs Zoltán ZSIDÓ	Gyula BATTÁ
15:50 – 16:10	Rita BÖRZSEI	László GALGÓCZI
16:10 – 16:30	Marcell MISKI	Marie RICCI
16:30 – 16:50	Amelie STEIN	Imola SZÍGYÁRTÓ
16:50 – 17:20	Coffee	
17:20 – 18:05	Kresten Lindorff-Larsen	
18:05 – 18:50	Duccio Cavalieri	
19:00 – 22:00	Dinner	

Tuesday

09:00 – 09:45	Chiara Verpelli	
09:45 – 10:30	Paul Grant	
10:30 – 11:00	Coffee	
11:00 – 11:20	Judit GLAVINICS	Klára KIRSCH
11:20 – 11:40	Mohd Yaqub Mir	László DOBSON
11:40 – 12:00	Eszter NAGY-KANTA	Balázs LIGETI
12:15 – 13:00	László Nagy	
13:00 – 13:45	Christoph Sensen	
13:45 – 14:00	Closing ceremony	
14:00 – 15:30	Lunch	



Contents of the works presented on the

FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

conference

FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

Invited lecture

How protein complexes built the synaptome architecture of the brain

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Keywords: postsynaptic proteome, synaptome architecture

Since Ramon y Cajal's neuroanatomical studies in the 19th century it has been recognised that the hallmark of brain complexity is the vast number of synapses. In the last two decades it has become clear that synapses have a remarkable molecular complexity, with the postsynaptic proteome (PSP) of excitatory synapses containing over 1000 highly conserved proteins in all vertebrates. The PSP is not a soup of proteins but is made from many multiprotein complexes and supercomplexes. Labelling of these multiprotein complexes shows they are differentially distributed into different synapses to produce a remarkable synapse diversity, known as the synaptome. The synapse types are patterned on all scales – from individual dendrites to the brain-wide systems level – revealing a spatial architecture, known as the synaptome architecture. We have recently examined the temporal changes in the mouse synaptome architecture and find there is a temporal program which we call the Lifespan Synaptome Architecture (LSA). The LSA suggests that a genetic program that controls the supramolecular assembly of proteins into complexes also controls the spatio-temporal diversity of synapses across the lifespan. This program is the target of many genetic disorders that change the synaptome in particular parts of the brain at different ages and thereby could produce the behavioural phenotypes associated with neurological and psychiatric diseases.

FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

Invited lecture

Integrative modelling of biomolecular complexes

Alexandre M.J.J. Bonvin

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Keywords: protein-protein docking, HADDOCK

The prediction of the quaternary structure of biomolecular macromolecules is of paramount importance for fundamental understanding of cellular processes and drug design. In the era of integrative structural biology, one way of increasing the accuracy of modelling methods used to predict the structure of biomolecular complexes is to include as much experimental or predictive information as possible in the process.

We have developed for this purpose a versatile information-driven docking approach HADDOCK (<http://haddock.science.uu.nl>) [1]. HADDOCK can integrate information derived from biochemical, biophysical or bioinformatics methods to enhance sampling, scoring, or both [2]. The information that can be integrated is quite diverse with as most recent addition cryo-EM maps [3]. In my talk, I will illustrate HADDOCK's capabilities with various examples and describe some recent developments in the modelling of small molecules [4] to membrane protein complexes [5] and large assemblies..

References

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FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

Invited lecture

Dynamics reveals new aspects of oncogenic KRas

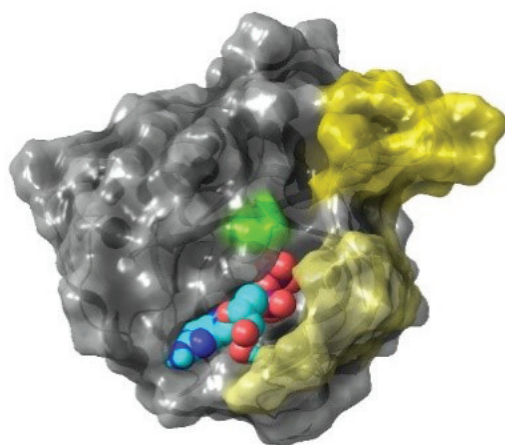
Gyula Pálffy¹, István Vida¹, Dóra K. Menyhárd¹, Zoltán Orgován², György M. Keserű², András Perczel^{1,*}

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Keywords: nuclear magnetic resonance, solution dynamics, chemical shift mapping



The membrane-bound small GTPase, KRAS, is a molecular switch, which alternates between its active (GTP-bound) and inactive (GDP-bound) forms. Its most prevalent oncogenic variants G12C, G12D, and G12V cause cancer, as these mutations froze the active or “on” state modifying signal transduction pathways, cell proliferation, differentiation etc..

The N-term 87 residues (effector lobe) includes the P-loop (10-17), switch I (30-38) and switch II (59-76) regions. The latter two play key role in autohydrolysis and have intrinsic dynamic properties revealed here. The comprehensively dynamical analysis, both fast (ps-ns) and slow (μ s-ms), of KRas and its mutants are to be presented. We found that both Switch regions are indeed dynamic showing Rex term in Switch-I region, while a mutation in the P-loop alters its mobility. In addition, Y32 of Switch-I has a special and opposite mobility, namely it is more rigid in its GTP-bound form. NMR dynamics in conjunction with MD and QM-MM structural information help us to better understand GTP hydrolysis and the mechanism of on→off.

These oncogenic mutants were thought as „undruggable” proteins until recently, when a new binding pocket was discovered and thus, several molecules were probed for KRas-G12C such as ARS-853 and ARS-1620. In the presence of small candidates ¹H-¹⁵N chemical shift mapping help us to screen new and selective candidates.

FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

Invited lecture

Biophysical experiments and biomolecular simulations: A perfect match?

Kresten Lindorff-Larsen

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Keywords: structural ensembles, reweighting, nuclear magnetic resonance

Biological macromolecules are dynamic entities and their ability to adopt alternative conformations can be central to their function. Examples include motions that underlie regulation or ligand binding, membrane proteins that act as efficient and selective transporters across biological membranes, or intrinsically disordered proteins that can adopt their shape to binding partners. I will discuss methods and applications for how simulations and experiments can be used synergistically to study protein and RNA dynamics [1].

Functional protein motions are often described as an exchange between a dominant, ground state structure and one or more minor states. The structural and biophysical properties of these transiently and sparsely populated states are, however, difficult to study, and an atomic-level description of those states is challenging. Using soluble proteins with extensive NMR data available as a test system, we have shown how enhanced sampling simulations can be used to capture accurately complex conformational changes in proteins, and I will discuss such examples [2,3].

Despite recent progress, one may still find that a simulation does not match quantitatively experimental measurements. Then, experiments and simulations may be combined in a very direct fashion to provide a description of the molecular motions that combines the details of atomic simulations with the accuracy afforded by experimental measurements [4,5]. The resulting conformational ensembles may provide novel insight into biomolecular systems that are not obtainable by simulations of experiments alone. I will discuss how this may be achieved, and give examples of the application of such approaches using both NMR and small-angle scattering experiments to refine conformational ensembles of proteins [6–8] and RNA [9,10].

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FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

Invited lecture

*Shank synaptic scaffold proteins: keys for understanding the
pathogenesis of autism and other synaptic disorders*

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Keywords: synapse development, synaptic plasticity, autism spectrum disorder

Shank proteins are multidomain scaffold proteins located at the post synaptic density (PSD) of glutamatergic synapses where they interact with many signaling and scaffolding proteins. Through this interaction Shank proteins regulate synapse development, spine structure and synaptic plasticity. Deletions, duplications, and coding mutations in all the three SHANK genes are present in several patients with syndromic autism spectrum disorder (ADS) and intellectual disability (ID) underlying the importance of Shank proteins in modulating intracellular signaling pathways that are essential for neuronal functions. However, several evidences suggest that mutation of different SHANK genes, in both human and mice, cause different clinical features suggesting that different Shank family members proteins might have distinct functions in modulating synaptic maturation and function. Understand their specific roles is essential to better define glutamatergic synaptic function and to define molecular pathways that can provide new therapeutic targets to treat patients with SHANK mutations. I will discuss the results obtained in our lab characterizing several in vitro and in vivo animal model that are essential to better define how disruption of SHANK scaffolding proteins affects the formation and function of synapses and neural circuits.

FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

Selected talks

Extracellular vesicles as complex model systems for increased understanding on structure and function of membrane active proteins and peptides

Tamás Beke-Somfai¹, Priyanka Singh¹, Imola Cs. Szigyártó¹, Judith Mihály¹, Ferenc Zsila¹, Szilvia Bősze², Zoltán Varga¹

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Keywords: extracellular vesicles, membrane active peptides, polarized light spectroscopy, membrane biophysics

Extracellular vesicles (EVs) have the potential to reform the diagnosis and therapy of various diseases and thus it is an extensively studied direction.¹ However, these species are also in the size range suitable for membrane biophysics, while they preserve the complex composition of a biological membrane. Consequently, they are optimal for monitoring the structure, orientation, and function of biomolecules associated to EVs.

Here we demonstrate that polarized light spectroscopy techniques can provide important information on red blood cell derived EVs (REVs) and molecules inserting into their bilayer. Using a Couette flow cell, and linear dichroism (flow-LD) measurements, EVs can be oriented by shear force, and provide insight to properties of oriented macromolecules in the vesicles.² The Soret-band of the LD spectra demonstrates that hemoglobin molecules are oriented and associated to the lipid bilayer in freshly released REVs. Moreover, three different antimicrobial peptides (AMPs), CM15, melittin and gramicidin were selected and investigated for their interactions with REVs using a diverse set of techniques. The peptide-membrane interactions reveal several novel function of AMPs, including their ability to remove associated proteins from the surface of REVs (Figure 1). These results promote that EVs will provide excellent opportunities to better understand the function and mechanism natural membrane active biomolecules.

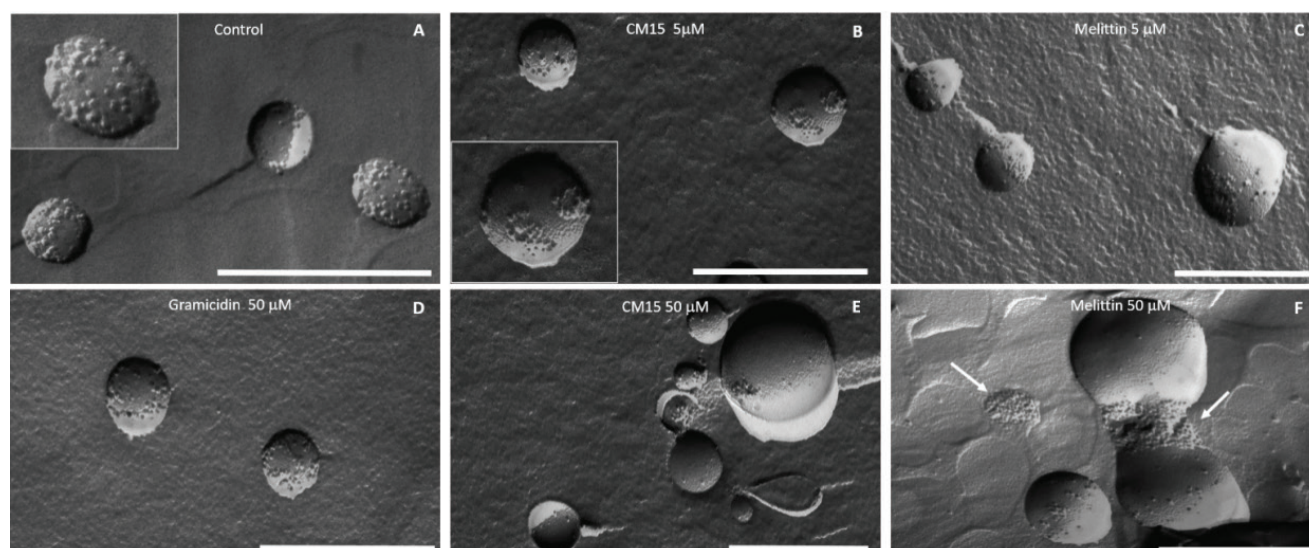


Figure 1. Morphology changes of the isolated REV with the addition of AMPs studied by freeze-fracture transmission electron microscopy. FF-TEM of REV control (A) and REV-AMPs sample measured after addition of 5 and 50 μM peptide concentrations (Melittin (C, F), CM15 (B, E), Gramicidin (D))

Acknowledgments

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FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

Selected talks

Ligand binding regulates supramodular dynamics in PSD-95 PDZ12 tandem

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Keywords: PSD-95, PDZ12 tandem, molecular dynamics simulation, dynamic structural ensembles

Introduction

The postsynaptic density protein-95 (PSD-95) is one of the most studied proteins of nerve cells. [1] It is involved in organizing and regulating the distribution of membrane receptors at the post-synaptic site. NMDA receptor, voltage-gated potassium-channel Kv1.4 and many other membrane proteins were previously identified as its binding partners. However, the underlying mechanism through which PSD-95 selects its interaction partners is still poorly understood. [2]

The two N-terminal PDZ domains of PSD-95 form an independent structural and functional subunit, often referred to as PDZ tandem. Such arrangement of adjacent PDZ domains allows for a synergistic effect, which may range from ultra-weak interdomain interaction (e.g. PSD-95 PDZ12 tandem) to interdomain chaperoning effect, where the only known function of one of the PDZ domains is to regulate the proper folding of the other domain (e.g. PDZ12 and PDZ45 of GRIP1). [3-4]

PDZ domains have traditionally been categorized into 3 classes based on the 4 C-terminal residues (Φ X[S/T] Φ X Φ , Φ X Φ X Φ , and Φ X[D/E] Φ X Φ for classes I, II, and III, respectively, where Φ denotes a hydrophobic residue and X denotes any residue). [5] A growing amount of evidence indicates the importance of the poorly conserved $\beta 2$ - $\beta 3$ loop interacting with upstream ligand residues which might play a decisive role in fine-tuning the ligand specificity [6].

The PDZ12 tandem of the PSD-95 protein was shown to possess a rigid structure in the apo-state, however ligand binding induces considerable interdomain mobility. [7] We presume that regulation of the relative orientation and dynamics of the two PDZ domains might be a key feature in organizing the distribution of the binding partners. In order to elucidate the atomic-level mechanism of the inner motions in the PDZ tandem, we performed all-atom molecular dynamics (MD) simulations, including NMR-derived experimental dynamic parameters (NOEs and S^2 order parameters) as external restraints.

Methods

In total, three structural ensembles were created, one of the free form and two of the complex forms of PDZ12 tandem. Nuclear Overhauser effect (NOE) restraints were applied to all three ensembles, while only one of the two complex ensembles was further restrained with S^2 order parameters. Simulations were done by gromacs 4.5.5 with an in-house extension to handle NOE restraints in a pairwise manner over the replicas as well as to include S^2 order parameter restraints as described previously [8]. The first (representative) model of the solution NMR structure of PDZ12 was used as input structure which was accessed in the Protein Data Bank (PDB code: 2KA9) [7].

For validation of the structural ensembles, backbone RMSD values and experimental parameters were back-calculated from the ensembles. Back-calculated order parameters were computed with the CoNSENsX + web-server. RMSD values were calculated for backbone atoms N, C α , and C. Both order parameters and backbone RMSD were calculated separately for the two domains, after superimposing the structures on residues 1–91 and 96–186 for the full-length PDZ1 and PDZ2 domains, respectively.

Principal component analysis (PCA) and calculation of the overlap between various PCs were carried out by ProDy. When determining intradomain motions, due to their large and in this context functionally irrelevant fluctuations, three residues from the flexible N- and C-terminal regions were excluded from the analysis, considering only the core region of both domains: residues 4–88 (PDZ1) and 99–183 (PDZ2). When determining interdomain motions, all structures were superimposed to a common PDZ1 core region template, and PCA was carried out for the core region of PDZ2 domain, without any further displacement.

Results

The generated structural ensembles correspond well to the experimental data. Backbone RMSDs indicate that both domains in all ensembles have a well-folded, compact structure. Local RMSD values are slightly above average for the N- and C-terminal tails as well as for the β 1 β 2 and β 2 β 3 loops. (Table 1). By applying S² order parameters as restraints, a high correlation can be achieved without compromising chemical shift correlation (Table 1).

Ensemble	No. of conformers	Chemical shift correl.		S ² correl.	PDZ1 RMSD	PDZ2 RMSD
		N	H			
<i>Original PDB</i>	20	0.83	0.41	0.10	0.57 $\pm$ 0.01	0.62 $\pm$ 0.04
<i>Free</i>	1448	0.85	0.55	0.22	0.73 $\pm$ 0.01	0.66 $\pm$ 0.01
<i>Complex</i>	1448	0.85	0.55	0.16	0.75 $\pm$ 0.02	0.58 $\pm$ 0.01
<i>Complex (S² restrained)</i>	1448	0.85	0.55	0.88	0.67 $\pm$ 0.01	0.63 $\pm$ 0.02

Table 1. Summary of the RMSD of the original and generated ensembles and their compliance with the experimental parameters.

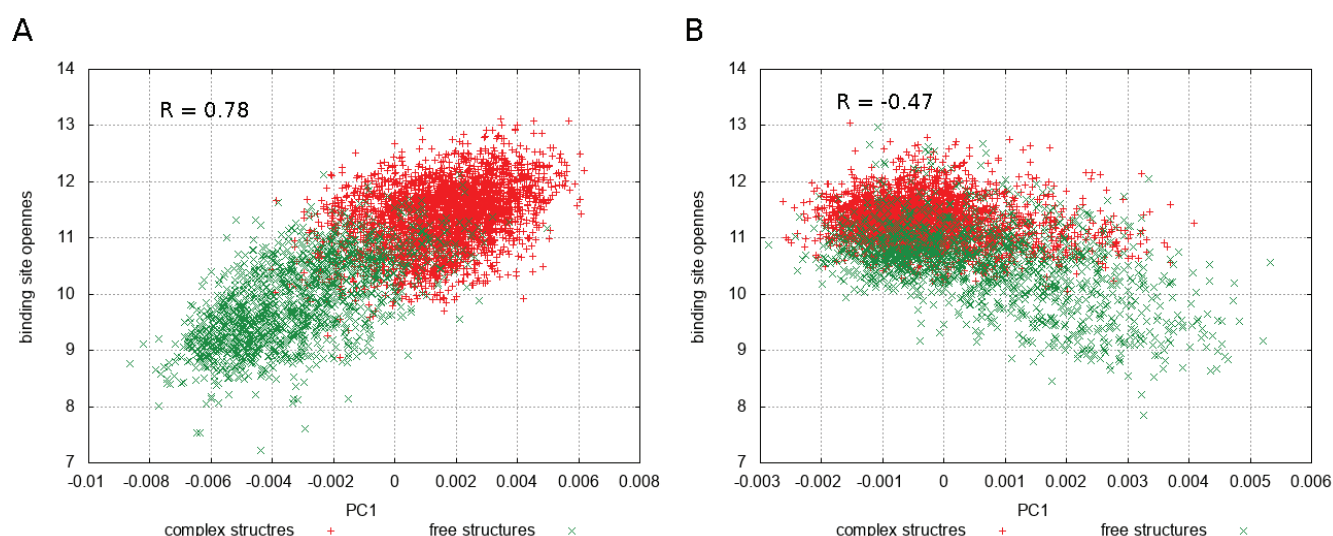


Figure 1: analysis of the intradomain motions. PC1 in PDZ1 (A) and PDZ2 (B) domains, plotted against the opening of the ligand binding site (i.e. distance between C α of PHE17-LEU77 and PHE112-LEU172 in PDZ1 and PDZ2, respectively).

To analyze interdomain motions, principal component analysis (PCA) was carried out on the core region of both PDZ domains to uncover the nature of the intradomain motions (for details, see Methods). The first principal component in PDZ1 correlates well with the binding site openness ($R = 0.78$) (Figure 1A). The free and complex structures cover a different region along PC1 (and therefore along the binding site openness as well), with the free structures being more extended from the closed binding site almost to the widely open conformation, but the complex structures most frequently occurring in the open conformation. The binding site opening

is a motion present both in the free and complex forms of PDZ1, but it is slightly quenched by ligand binding. The same effect is still present in PDZ2, but is far less pronounced than in PDZ1 (Figure 1B). Good correlation can only be achieved by excluding the flexible loops (residues 105–109 and 117–127) from the PCA ($R = -0.47$).

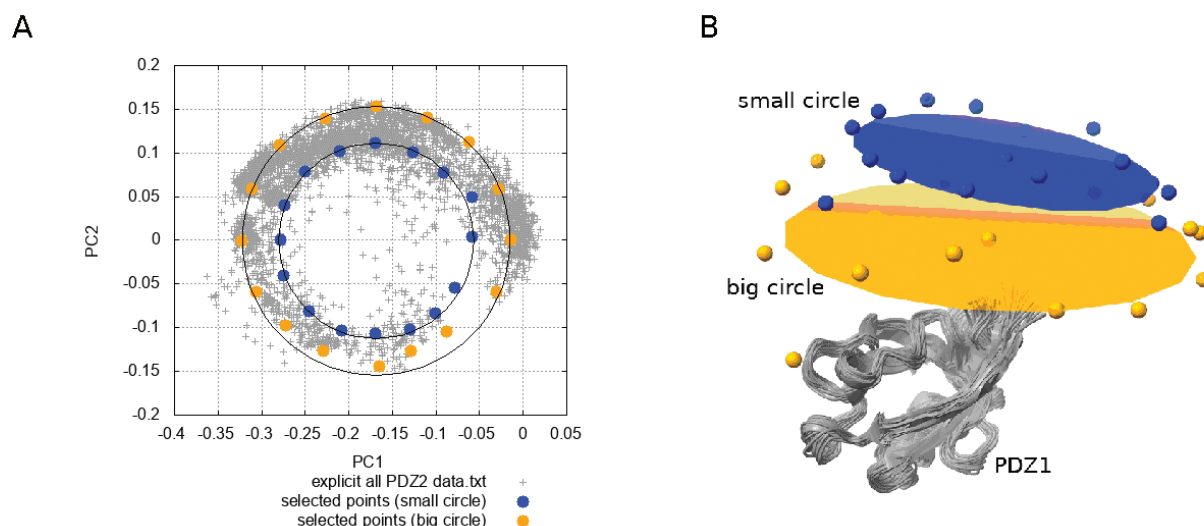


Figure 2: analysis of the interdomain motions. Possible interdomain orientations sampled in our simulations cover a large conformational space (A), which can be best represented as two circles along which the PDZ2 domain moves relative to the PDZ1 domain (B).

Analysis of the interdomain motions reveals that the relative orientation of the two domains covers a large conformational space. When plotting the resulting first and second principal components, the data points exhibit a circular arrangement (Figure 2A). The data points were sampled along two circles that have a radius 0.8 and 1.1 times that of the best-fitting circle in a way that the nearest data point was chosen to the 16 equidistant points in both circles. Upon visual inspection, it became evident that these data points, which lie along a 2D circle on the PCA plot, represent such PDZ–tandem structures where the PDZ2 domains are arranged along a 3D circle (with the PDZ1 domains being superimposed). Furthermore, the distance of each data point from the center of the circle corresponds to the degree by which the orientation of the two domains deviates from the extended interdomain conformation, that is, the interdomain bending angle. This observation can be best demonstrated by plotting the centers of mass of the PDZ2 domains that were sampled from the two circles: they fit relatively well to two nearly parallel circles (Figure 2B).

Structures in which there were at least 20 interdomain atom–atom contacts between heavy atoms within a 5 Å cutoff range (termed ‘tight structures’) were further analyzed to better understand the effect of interdomain interactions on the supertertiary dynamics. We found that using the polar coordinates, computed with the aid of the best-fitting circle, the tight structures can be clustered in seven clusters by hierarchical clustering algorithm (complete linkage) (Figure 3). The seven representative structures correspond to seven tightly packed interdomain orientations, in which the interdomain interface is formed between different regions.

PDZ12 tandem structures of PSD-95 determined previously by various experimental techniques were checked and compared to our clusters. The FRET structure falls outside of the circle of the interdomain conformational space covered by our simulations. However, reanalysis of the structure revealed that it represents the average of two states, an open- and a closed-like conformation. In general, we found that the open-like and the 3ZRT structures have no interdomain interaction (‘loose structures’), therefore they do not belong to any cluster. But the rest of the structures can clearly be represented by one of our clusters, lending experimental support for the diversity of interdomain orientations proposed based on our simulations.

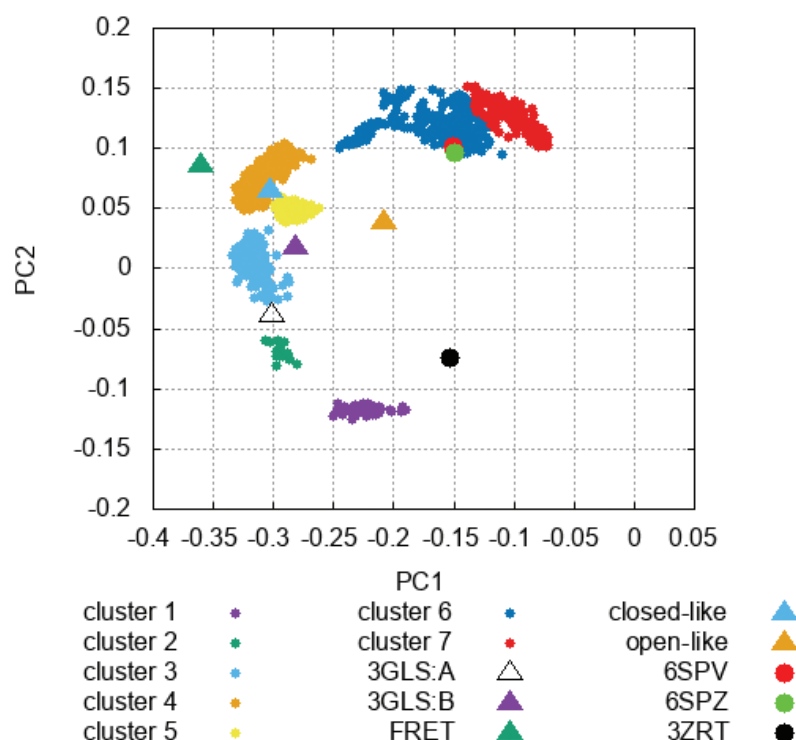


Figure 3: PCA plot of the 7 clusters of the tight structures, in which substantial interdomain interactions can be observed, compared with experimental structures. Experimental structures were determined by X-ray (accessed in PDB, under IDs 3GLS, 6SPV, 6SPZ and 3ZRT) and FRET (including the open-like and closed-like structures).

Finally, by plotting the principal components of intradomain motions and coloring each dot (representing a structure) according to the cluster of interdomain arrangement it belongs to, we demonstrated that intra- and interdomain interactions are not independent from each other. This observation highlights a putative mutual regulative mechanism between ligand binding and supertertiary dynamics.

To conclude, our results strongly suggest that ligand binding regulates interdomain orientation by modulating the probabilities of the formation of domain–domain interactions through different interdomain interfaces. It is also worth speculating that, vice versa, the relative orientation of the two domains in the PDZ12 tandem might have a role in shifting the preference for different binding partners, with the aid of the same β 2- β 3 loop.

Acknowledgments

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FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

Selected talks

Spectroscopy based functional structure biology of biomembranes and membrane proteins

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Keywords: membrane biophysics, biomembrane, membrane protein, structure prediction, structure biology, epr, ftir, spectroscopy

Structural biology of membrane proteins faces the challenges of isolating, solubilising and crystallising proteins (that are natively hosted by the lipid matrix) in a foreign environment [1]. In addition, there is always the intriguing question: to what extent the obtained structures and features of isolated membrane proteins correlate with those under native, in-membrane conditions. Since the native fold of a membrane protein assumes a native environment, namely the lipid bilayer, the sequence-to-fold coding is valid only in the native membranous environment. Therefore, our approach is to measure structural data on the target membrane proteins in their functional, native membrane environment, and combine the so-obtained data with theoretical methods [2,3].

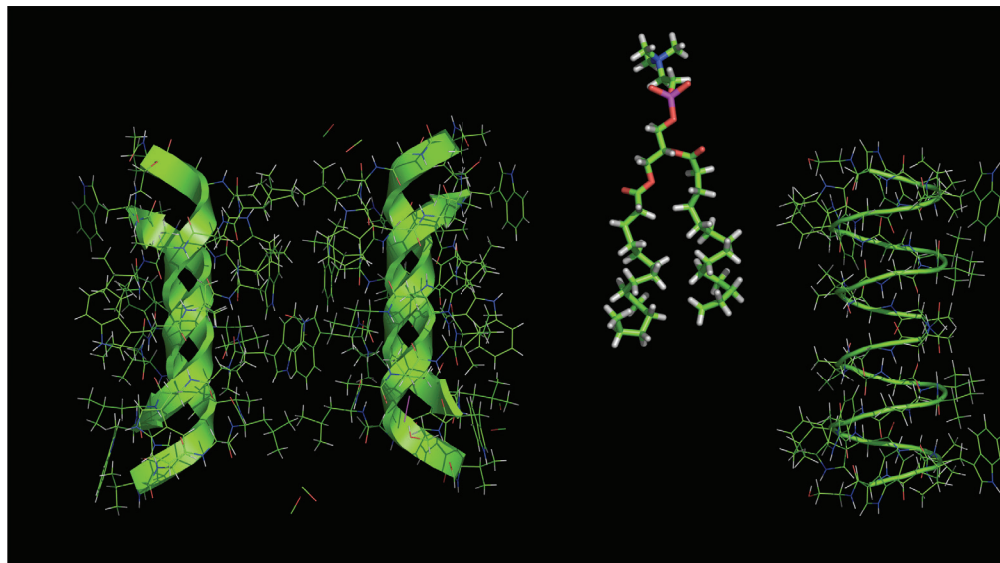


Figure 1. The membranous dimer conformations of gramicidin A, shown together with a phospholipid (based on Kota et al., 2004).

Membrane lipids and proteins are coupled structurally, dynamically and functionally [4]. The protein-lipid interface takes several different forms, all of which are crucial to biology [5]. Activity measurements on purified membrane proteins require that they are inserted and assembled in the bilayer properly. The lipids assure the insulation capacity of the membranes, and their hydrophobic double layer provides the proper conditions for the membrane proteins. Therefore, the role of the lipid-protein interface is crucial in all biomembrane functions [6]. As a consequence, isolated studying isolated and solubilised membrane proteins is both difficult and has limited functional relevance.

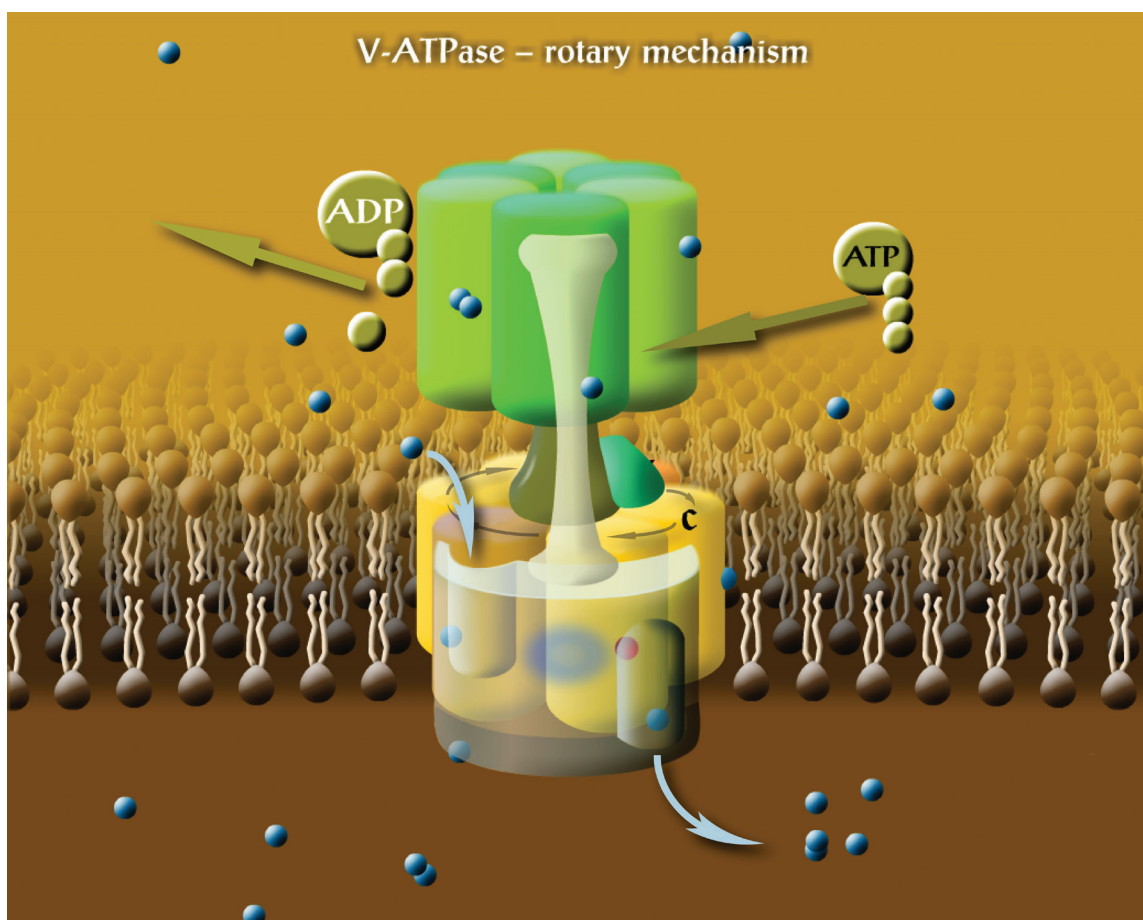


Figure 2. The rotary mechanism of V-ATPase. Each subunit c binds a proton when in contact with lipids. Protons enter and leave the rotor in hydrophilic input and output channels, between subunits c and a (based on Ferencz et al., 2013).

The main projects of my research group are: the proton-transporting membrane protein complex, the vacuolar proton-ATPase (V-ATPase) [7,8]; soluble and trans-membrane alpha-helix and beta-strand polypeptide bundles and proteins [9,10] (Fig. 1); and molecular interactions [11] in native and model biomembranes. These topics are strongly related to each other both thematically and methodically. Our studies aim at structure-function relationships, folding and assembly of membrane proteins and polypeptides and the unique functional and structural role of the protein-solvent interface. Our spectroscopy based, function-driven structure biology approach relies on the techniques of Fourier transform infrared (FTIR), site-specific (spin label) electron paramagnetic resonance (EPR), UV, visible and fluorescence spectroscopies, and high-sensitivity differential scanning calorimetry (DSC). We use both non-invasive and labelling techniques for studying the lipid-protein, or, more generally, the solvent-protein interface: we apply and further develop spin-label EPR [12] and the non-invasive FTIR spectroscopies [8] to study model and biological membranes. These methods have the advantage of displaying separated lipid- and protein-related spectral regions. Thus, both lipids and proteins can be studied individually.

In my talk, specific examples of the application of the above approach will be presented together with a presentation of the latest relevant results on the vacuolar proton-ATPase (V-ATPase) (Fig. 2). V-ATPase is the transport protein complex that is responsible for the acidification in eukaryotic cells, and it is nature's most universal proton pump. It is a membrane-bound molecular rotary engine, which converts the chemical energy from ATP hydrolysis to the rotation of the rotor domain via a torque between specific subunits. Our studies on V-ATPase aim at subunit-subunit and subunit-lipid interactions, the effect of synthetic inhibitors on function and subunit assembly, the uncoupling of passive proton translocation and ATP hydrolysis, and the details of the rotary mechanism.

Acknowledgments

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FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

Selected talks

Calculation of complex structures of protein targets using a fragment blind docking approach

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Keywords: protein, interaction, complex, docking, pharmacodynamics

Determination of protein complex structures and the development of new drugs targeting protein-protein interfaces are great challenges of current drug design. Whereas the number of experimentally determined complexes is constantly increasing in hot fields of signal transduction or epigenetics, the production of a new protein kinase or a histone complex is still time consuming and requires extensive laboratory resources. Besides experimental structure determination, theoretical methods can help to overcome such limitations. Among theoretical approaches, computational docking is the most often used tool for prediction of atomic resolution complex structures [1, 2].

Fast docking methods are based on a compromise between computational cost and accuracy which necessitates some approximations to the detriment of accuracy [3]. To reduce the searching area an estimated knowledge of the binding site is required. To further ease computational time the use of an incomplete hydration of the ligand-target interface [4] and partial or complete neglect of target flexibility during ligand binding is necessary. Stochastic docking algorithms are also used [5] instead of systematic exploration.

However, restricting the search to a given location to reduce computational costs requires previous knowledge and might neglect additional allosteric binding sites [6]. Ignoring hydration is also problematic as some pockets can be occupied by water molecules preventing the binding of a ligand *in vivo* [7]. The limitations caused by ignoring target (protein) flexibility are also well-known [8]. Finally, the stochastic approaches provide dubious results regarding the reproducibility and reliability of the results.

Unravelling the problem inflicted by the first approximation there is a novel method called fragment blind docking (FBD) [9]. FBD is based on Wrap 'n' Shake [3], a recently introduced method, and composed of four consecutive steps, fragmenting, wrapping, linking and welding.

The cumbersome manageability of macromolecules greater than tripeptides proposed the idea of fragmentation which is clearly necessary in the case of protein-ligand complexes such as histones and their writers and readers. To further complicate the challenge of docking the protein-ligand complexes of histones they also have a great post-translational variability and complex structure of histones proposed a term 'histone code'. In the effort of understanding and unraveling this code, computational aid is inevitable to complement biophysical-chemical methods.

It is the best to achieve dipeptides after fragmentation regarding manageability. The second step, Wrapping, covers the surface of the target molecule with the copies of the fragments. Parallel wrapping runs are performed with every dipeptide fragment. The final steps of FBD re-builds the original structure of the ligand polypeptide by pairwise linking of the fragments. This step is performed automatically by an algorithm that probes all possible pairwise combinations and produces the longest peptide from the connected amino acid pairs. Two radicals are formed from the previously blocked N- and C- terminals and if the fragments are in the

correct position to allow the building of an amide bond, it creates a tetrapeptide and so on, ending in a pool of candidate structures. These candidates then undergo the process of welding which involves the systematic rotation of bonds. Then the refinement of the final structure is carried out by energy-minimization.

In this presentation, we feature another approach of FBD, which is still under evaluation and development. This approach combines the first steps of FBD with a homology modeling step for re-construction of the complex structure. We test our new approach by calculation of complexes of histones, protein kinases and somatostatin receptors which are of utmost importance for understanding the pathobiochemistry of various diseases and for the development of new drugs [10,11].

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FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

Selected talks

Calculation of complex structures of somatostatin receptors

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Keywords: SST, protein-ligand complex, somatostatin receptor, homology modelling, drug design

In the last decade, complex mechanisms of pain and the relationships between the sensory nerves, immune system, and vascular system have been thoroughly investigated. However, neuropathic pain due to traumatic, metabolic or toxic injury of the nerves still affects large patient population and there is no effective therapy for these people. The varied classic non-steroidal anti-inflammatory drugs (NSAIDs) are inefficient for neurogenic pain and opioids have an effect only in high doses. Adjuvant analgesics that are commonly used for epilepsy and depression might be effective in certain patients, but due to their severe side-effects, the long-term application is not possible [1].

According to the classic theory of nerve regulation, sensory nerves like capsaicin sensitive neurons, mediate different sensations to the central nervous system from the area of the skin, joints and other internal organs (classic afferent function). Besides their classic sensory/nociceptive functions neuropeptides release from the nerve endings causing vasodilatation, plasma protein extravasation and activation of inflammatory cells on the innervation area, and therefore, they evolve neurogenic inflammation (local efferent function). Furthermore, somatostatin (SST) also known as somatotropin release inhibitory factor gets into the systemic circulation and relieves the pain and neurogenic inflammation in other part of the body (systemic efferent function) [2].

SST effects are mediated via five somatostatin receptor (SSTR) subtypes which comprise two classes on the basis of their phylogeny, structural homologies and pharmacological properties. Receptors (SSTR2, SSTR3 and SSTR5) with high affinity to octreotide, a synthetic SST analogue, were grouped in the first class, whereas the other class includes SSTR1 and SSTR4, displaying low or negligible affinity for the analogue [3]. Our group discovered that among the five inhibitory SST receptors SSTR4 mediates potent analgesic and anti-inflammatory effects without hormone secretion inhibition, therefore, it is a promising novel target for drug development [4].

All five receptors belong to the rhodopsin-like receptor family of G-protein coupled receptors (GPCRs) and possess seven transmembrane domains that provide the characteristic structure of GPCRs. Unfortunately, crystal structures have not been determined experimentally for any SSTR subtypes. However, common structural features, and conserved sequence in transmembrane regions have been identified.

SST is an endogenous cyclic neuropeptide and widely expressed in the body, it occurs in the peripheral, as well as the central nervous system and plays an important role in regulation of the endocrine system, neurotransmission and cell proliferation. It occurs as a tetradecapeptide (SST-14) or an N-terminally extended form (SST-28). Both peptides contain disulphide bridge between cysteines at positions 3 and 14 which stabilizes the structure. Structure-activity relationship studies showed that in the pharmacophore region of SST included the Phe7-Trp8-Lys9-Thr10 tetrapeptide, Trp8 and Lys9 were essential for binding to all SSTR subtypes, whereas Phe6 is specifically important for SSTR4 activation [5,6].

The therapeutic application of native SST is limited due to its diverse biological effects and rapid degradation (<3 min half-life in the circulation), consequently there is a great need to identify metabolically stable, highly potent and receptor selective SST analogues as potential analgesic drug candidates.

Despite the development of numerous SST analogues and recent release of new, potent molecules [7, 8, 9], the molecular structure of SSTRs and the exact binding site or modes of the ligands have not been determined experimentally. However, such information could be essential for the design of potent, receptor-selective drugs. The aim of our research group is the modelling of the structure of SSTRs, identification of the binding sites of the new ligands and the examination of binding mechanisms using target-ligand complexes. Due to the size of the receptors and the complexes, molecular mechanics methodologies are involved in our investigations. Well-established techniques of homology modelling [10, 11] and docking [12, 13] were applied. To answer the challenge of blind docking prediction of the binding mode of SST, our recent techniques Wrap 'n' Shake [14] and fragment blind docking [15] were also adopted for the work. Preliminary findings of our research will be presented in the above-detailed physiological context of SSTRs.

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FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

Selected talks

Integrative modeling of possible layouts of Kir2.1 localized in membranes and connected by PSD-95

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Keywords: Kir2.1, PSD-95, postsynaptic density, protein complex, protein interaction network

The structure of the postsynaptic density is still not known, although it has a major role in information delivery from the postsynaptic receptors to the inner cell and it also influences the location of the receptors which has an effect on neuron polarization. We model the possible structures of the postsynaptic density complexes paying particular attention to the possible PSD-95-organized layout of Kir2.1 receptors in this article. Using the Integrative Modeling Platform we created bead-chain models of the Kir2.1 and PSD-95 based on experimental and bioinformatical data. We created possible layouts (hexagonal) by combinatorial methods based on known or predicted domain-domain (or virtual domain) interactions. The Integrative Modeling Platform simulates those binding by approaching the proteins with Monte Carlo sampling. We calculated how the protein numbers depend on the size of the hexagonal-structured PSD-95-Kir2.1 system. I made several simulations containing two protein Kir2.1 and one PSD-95 in order to estimate the furthest distance the PSD-95 is able to bridge between the two Kir2.1. According to the simulations this distance should be somewhere between 70-75Å.

0.0 About the role of PSD in synaptic transmission.

It was long observed that some potassium channels form clusters on the cell membrane in the postsynaptic side of the neurons [1]. The Kir2.X-type channels are coclustered with PSD-95 [2]. Despite the fact that coclustering is experimentally proven, there is still few information about how they form complexes and how PSD-95 anchors the potassium channels. Our aim is to find possible layouts for potassium (mainly the Kir2.1 receptor) channels mediated by PSD-95 proteins in the postsynaptic density. We make three dimensional models of the protein using the Integrative Modeling Platform IMP. The main goal is to create PDB files of the possible layouts in order to analyze them and to give a direction for the in vitro and in vivo researches.

0.1 The Kir2.1 potassium channel

Kir2.1 is one of the most important potassium ion channels. The channel got its name by its function: inwardly-rectifying potassium channel which means it allows potassium to flow into the intracellular matrix and hardly allows backwards current. The location of Kir2.1 channels can affect how a neuron works since overexpression of Kir2.1 channels can lead to hyperpolarization due to the potassium currents. The protein is a heterotetramer of four 427-residue subunits. According to the prediction of IUPred2A [3] the Kir2.1 is an ordered protein with small disordered C-terminal segments as seen in Figure 1. The C-terminus harbours a PDZ-domain-binding motif with the sequence Glu-Ser-Flu-Ile (residues 423-427), through which Kir2.1 coprecipitates with PSD-95 [2].

0.2 The PSD-95 protein

PSD-95 (postsynaptic density protein) is one of the most abundant postsynaptic scaffold proteins. It plays a major organizing role in the PSD. The proteins of the postsynaptic density do not form a structured network in the absence of PSD-95 [4]. PSD-95 contains three PDZ domains as well as an SH3 and a GK domain. Unlike Kir2.1, the N-termini of PSD-95 is the most disordered region (see Figure 2). This information is functionally meaningful because the N-termini of PSD-95 are subject to palmitoylation. The flexibility of the N-terminus is thought to help anchoring the protein to the membrane.

1.1 Integrative Modeling Platform - IMP

The Integrative Modeling Platform (hereinafter IMP) [5] is a platform for modeling three dimensional structure of large complexes merging several different data, including experimental information about interaction surfaces. The modeling has five stages [6]:

Gathering Data: For modeling it is inevitable to have some data. We used PDB structures for having initial three-dimensional coordinates and we predicted disorder regions using IUPred. We used domain regions predicted by Pfam. These are the main information sources for the bead-chain models. Domain-domain interaction data and expression levels were used to create complexes in a graph representation. Other a priori data can be added.

Representation: The representation of the bead-chain model is a key step. It defines the resolution of the model and the degrees of freedom of each segment. There are three main types of segment. The floppy bodies usually represent the disordered regions. This region is movable, each bead of a floppy body can change its relative coordinate to each other. The resolution is often small which means higher beads in the chain. The rigid bodies usually represent domains. The beads of a rigid body can not change its relative position to each other, they remain in the same conformation. The resolution is often high leading to smaller beads. A super rigid body represents a molecule, it contains rigid bodies connected by floppy bodies. The relative position of the rigid bodies can move through the linkage of floppy bodies.

Give Restraints: Restraints gives biological meaning of the random movements. We used two main restraints: The Exclude Volume restricts two beads taking the same place while the Cross-Links defines and calculates relative distances per residue.

Sampling - Monte Carlo: Running Monte Carlo simulations the best frames (conformations) are sampled which is continually refreshed during the simulation.

Analyses: The structures can be analysed by different methods: filtering by scores, clustering, distances and localization densities.

2. Results and Discussion

2.1 Possible layouts of the Kir2.1 localized in the membrane

Since one PSD-95 has three PDZ domains, the protein may be able to link together two Kir2.1 channels. This possible linkage can be compatible with several different layouts. We manually constructed two of the possible arrangements of the receptors, corresponding to a hexagonal and a rectangular lattice, respectively. We consider the latter one as less likely as the distribution is regarded suboptimal in this case.

2.2 The size of the hexagonal layout determines the amount of proteins needed.

We can consider the layout as a graph where the receptors are the nodes while the linker PSD-95s are the edges. One modeled unit of the hexagonal layout contains seven receptors and eight edges. This can be expanded in all directions gradually. As Figure 5 shows, another unit can be added to the first unit vertically and horizontally as well. Both additional units have common nodes with the first unit so actually fewer proteins were added than the proteins in the original unit. Considering these intersections, the number of proteins needed to establish the layer can be determined by the number of rows and columns. Let n be the number of columns and m be the number of rows. The protein numbers are

$$\begin{aligned} \text{Kir 2.1/nodes} &= 7 + 5(m - 1) + (n - 1)(6 + 4(m - 1)) \\ \text{PSD - 95/edges} &= n(8 + (m - 1)7) \end{aligned}$$

We get these numbers using the Figure 5. The left bottom hexagon contains 7 nodes. Every time we expand it to the right we get 6 more nodes. If we expand the original hexagon upwards, we get five more nodes every time. During every expansion upwards if we want to expand to the right as well we get 4 more nodes than when only one row exists. The same reasoning can be applied to the edges as well.

$$\begin{aligned} \text{Kir 2.1/nodes} &= 4nm + 2n + m \\ \text{PSD - 95/edges} &= n(7m + 1) \end{aligned}$$

We can observe in Figure 6 that after a threshold (N,M) the number of PSD-95 molecules increases at a much higher rate than the number of Kir2.1. This leads to a huge difference needed between the two protein expression levels.

2.2.1 The expression levels of the Kir2.1 and PSD-95 does not directly refute the ratio of the proteins made by the hexagonal layout According to the research: H.sapiens - Frontal cortex, SC(Kim,Nature,2014) [7], at the frontal cortex the abundance of PSD-95 is 35.7 while the abundance of the Kir2.1 is 3.88. The number of Kir2.1 should be around 10% of the number of the PSD-95 at the frontal cortex. At grid size (100,100) this ratio is around 57% (see Figure 6). So the expression levels from the frontal lobe do not directly refute the existence of a hexagonal layout, even a possible grid size can be determined parametrized by n or m at the ratio when the Kir2.1 is 10% of the PSD-95.

$$\begin{aligned} 0.1n(7m + 1) &= 4nm + 2n + m \\ n &= \frac{m}{-3.3m - 1.9} < 0 \end{aligned}$$

Because the ratio is an approximation, the numbers are not always integer but $n, m \in \mathbb{N}^+$. n can not be negative so the ratio from the experiment and the number of proteins in the hexagonal layout can not be fully reconciled. Based on the equations let introduce x as the variable of the ratio:

$$n = \frac{\frac{1}{x}m}{-(\frac{4}{x}-7)m+1-\frac{2}{x}}$$

The counter is always positive so the denominator should be positive. We get that the ratio should be higher than

$$x > \frac{2+4m}{7m+1}$$

Which leads us to the original ratio at $m=100$ it is $x=0.57$. Notwithstanding this is a contradiction, the expression levels alone do not refute the hexagonal layout because there should be more PSD-95 in the system which does not take part in the hexagonal layout and also there can be Kir2.1 outside the hexagonal cluster. It should be further noted that the expression levels of genes does not necessarily correlated well.

2.3 One PSD-95 molecule can bridge 70-75Å between Kir2.1 molecules.

At a distance around 70-75Å high oscillation can be observed between the two binding sites (see Figure 4). The PSD-95 molecule behaves like a pendulum. The frequency of this oscillation depends on the distance and can determine how strong the bridge between the two receptors. This oscillation weakens as the receptors are getting closer to each other. The first distance the oscillation is almost negligible is around less than 35Å. Scrutinizing the PDZ domains as a cylinder we get that the average length of a PDZ domain is 31Å and the radius is 7Å. This can be one of the limitations why 35Å-higher distances of the potassium channels makes the PSD-95 commute between them (see Figure 8).

Acknowledgments

I wish to thank various people for their contribution to this project; Zoltán Gáspári and Attila Csikász-Nagy for their contributions and supervisions. My grateful thanks are also extended to Kornél Komjáti for his help learning the basics of IMP. The authors acknowledge the support of the National Research, Development and Innovation Office – NKFIH through grant no. NN124363. The research was funded by the European Union and co-financed by the European Social Fund under grant number EFOP-3.6.2-162017-00013. Molecular graphics and analyses performed with UCSFChimera, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH P41-GM103311

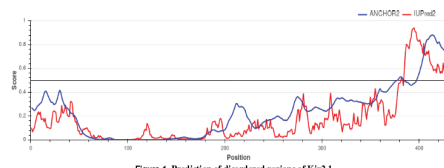


Figure 1. Prediction of disordered regions of Kir2.1

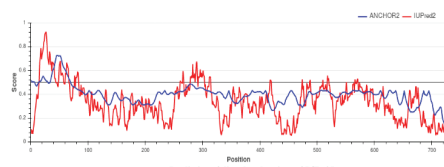


Figure 2. Prediction of disordered regions of PSD-95

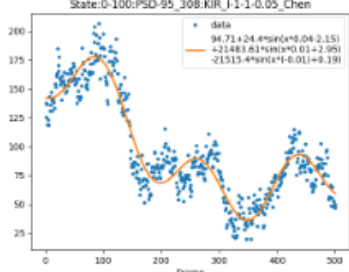


Figure 9. The distance between PDZ1 and Kir2.1 (Kir2.1-Kir2.1 distance:36.65Å):

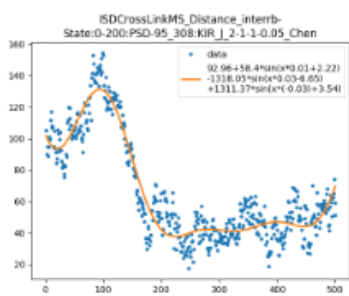


Figure 10. The distance between PDZ2 and Kir2.1 (Kir2.1-Kir2.1 distance:36.65Å): The first distance where oscillation observed. The PSD-95 is always closer to one of the Kir2.1 than the other.

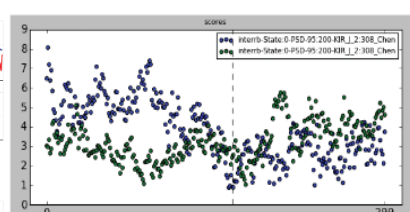


Figure 4. The score of bindings: The scores indicate how favorable a given binding interaction is in a certain frame of the simulation. The lower the score the tighter the interaction is. The blue and green dots refer to the PDZ1 and PDZ2 binding scores, respectively. The dashed vertical line indicates the frame shown in Figure 3 was taken. There are two inner oscillations. At the beginning one PDZ domain (green) reaches the desired target while the other PDZ domain moves apart. After a while both PDZ domains approach their target and start to oscillate between the two Kir2.1 subunits. At a certain frequency this oscillation can be considered as a dynamic bridge between the receptors that might play a role in the communication between the two receptors.

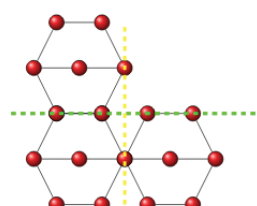


Figure 5. Hexagonal layout of Kir2.1 organised by PSD-95

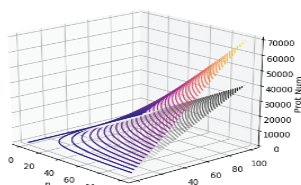


Figure 6. The number of proteins: The black shows the number of nodes (Kir2.1) while the coloured mesh shows the number of edges (PSD-95) based on an $n \times m$ grid.

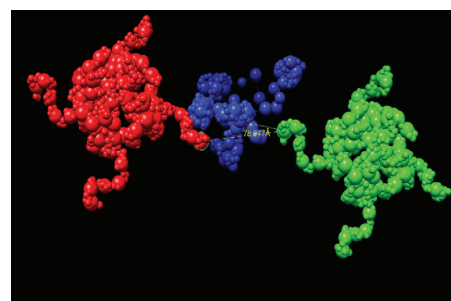


Figure 3. The structure where the distance between the potassium channels is 78.94Å:

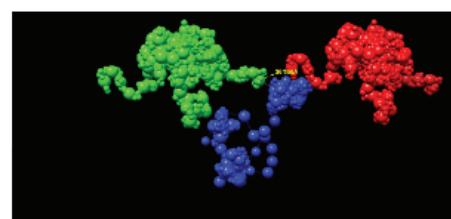


Figure 7. The structure where the distance between the potassium channels is 36.65Å: The first distance where oscillation observed. The PSD-95 is always closer to one of the Kir2.1 than the other.

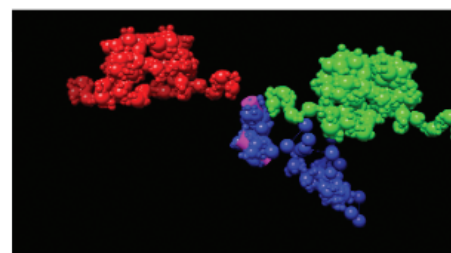


Figure 8. The PDZ domains as cylinders where the distance between the potassium channels is 36.65Å: The blue protein is the PSD-95 while red and green are the Kir2.1 channels. The purple is the averaged cylinder of the PDZ domains. The length of the cylinder:31Å, radius of the cylinder:7Å.

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FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

Selected talks

Towards improved biophysical models of protein folding to identify disease-causing mutations

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Keywords: protein stability, genetic variation

The rapid decrease in DNA sequencing cost is revolutionizing medicine and science. In medicine, genome sequencing has revealed millions of missense variants that change protein sequences at a single site, yet we only understand the molecular and phenotypic consequences of a small fraction of these. Within protein science and biotechnology, high-throughput multiplexed assays enable us to probe the effects of thousands of variants in a single experiment.

We have used computational and experimental approaches to determine the consequences of missense variants in proteins, with the aim of using such models both for diagnosing genetic diseases, and for providing mechanistic insight into disease. In particular, we have focused on the effects of individual amino acid changes on protein folding and stability, linking biophysical calculations with protein degradation and abundance in cells. By examining a range of proteins and diseases we have found that loss of stability is a common driver for genetic diseases, and that predictions of changes in thermodynamic protein stability are useful to assess the pathogenicity of genetic variation. I will discuss these ideas using recent examples from our laboratories.

At the same time, our work has also revealed areas where our understanding and ability to predict the effect of amino acid changes is still imperfect. I will discuss how we are using sequence analyses and high-throughput experiments to understand the origin of these effects, thus paving the way for more accurate biophysical models for use in personalized medicine.

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FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

Selected talks

Nanoscale Bayesian clustering-based characterization of the presynaptic molecular architecture at GABAergic synapses

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Keywords: synapse, CB1, STORM, bassoon

The endocannabinoid system is a retrograde pathway that has a major role in the regulation of synaptic strength in the central nervous system. The CB1 cannabinoid receptor which is located on the axon terminals of many neuron types can efficiently suppress anterograde neurotransmission. Characteristically high levels of CB1 expression can be observed in certain subsets of GABAergic inhibitory interneurons. Although the dysfunction of CB1 is linked to multiple neurological diseases such as epilepsy, little is known about the principles that control the quantity and the localization of CB1 receptors in the axon terminals of distinct neuronal types. Previous results of our group showed that the boutons of different CB1-positive interneuron types (targeting either the perisomatic or the dendritic region of principal cells) in the mouse hippocampus contain CB1 and the presynaptic active zone protein bassoon in a different ratio. The CB1/bassoon ratio varied according to the cannabinoid sensitivity of the synapses of interneuron subtypes, indicating that this ratio might be an important property determining the efficacy of endocannabinoid signaling.

CB1 containing interneurons can be further classified by the presence or absence of the vesicular glutamate transporter 3 (vGluT3) protein. Based on previous measurements, in CB1+/vGluT3+ cells CB1 is present in a higher abundance compared to CB1+/vGluT3– cells. Thus, the goal of this study is to compare the ratio of CB1 and bassoon in the two interneuron types. We utilized immunostaining against bassoon, and performed correlated confocal and STORM super-resolution microscopy to investigate the distribution of bassoon in the axon terminals of individual cells. For the objective quantification of the distribution we used a novel clustering method based on Bayesian statistics. We have incorporated the algorithm in the software that is developed by our group for correlated confocal and super-resolution microscopy.

We found no difference in the quantity of bassoon, the number of bassoon clusters, and the size of clusters between the boutons of CB1+/vGluT3+ and CB1+/vGluT3– perisomatic interneurons. Since CB1 density was higher in the former cell type, our results suggest that endocannabinoid signaling might be more efficient in the synapses of vGluT3-expressing interneurons.

FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

Selected talks

Structural correlates of modular organization of signal transmission in the primate somatosensory cortex

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Keywords: axonal patches, bouton density, axonal convergence

Introduction: Axonal connections of column size cortical regions exhibit patchy distribution all over the primate cerebral cortex. The axonal patches represent specific target sites as e.g. columns of similar orientation preference in the visual cortex. However, axonal connections are also densely distributed outside of the patches without any apparent grouping. Instead, the out-patch axons exhibit a radial spreading from the origin towards probably distant target sites including the patches [1] [2]. Interestingly, both patch and out-patch axons form axon terminal-like structures suggesting their role in synaptic transmission.

Aim: The aim of our study is to understand whether axons play a similar role in the propagation of activity and dissemination of information within and outside of the patches. To answer this question morphological properties of reconstructed axons within and outside of the patches were compared for intra- and inter-areal connections in the somatosensory cortex of squirrel monkeys.

Methods: Distal finger pad representations of six squirrel monkeys were injected with BDA via iontophoresis in area 3b (3 cases) and area 1 (3 cases) [1] [2]. Aldehyde fixation was made by transcardial perfusion after 10-20 days survival. Series of 50 µm thick horizontal, vibratome sections were collected at regular intervals from flattened cortex. Standard ABC protocol was used to visualize BDA labeling with nickel intensified DAB as the chromogen. Sections were then osmicated and flat embedded in Durcupan. Intra and Inter areal axons of areas 3b and 1 were studied. Axonal patches were mapped and outlined by using Neurolucida. Reconstruction of BDA labelled axonal segments was also carried out with Neurolucida at high magnification within and outside of the axonal patches. Tortuosity, Inter-varicosity distance, Density of varicosity and Thickness of the individual axonal segments within and out of patches were compared. Convergence of the axonal segments was also studied in two domains of axonal termination. Their abundance was also quantified within and outside of the patches.

Summary: Our findings suggest that axons have similar tortuosity but different bouton density within and outside of the patches. Specifically, intrinsic connections exhibit higher bouton density within than outside of the patches. Moreover, the Inter-varicosity distance, the length of axonal segment between two boutons is significantly higher for out-patch axons than patch axons. Regarding convergence, we compared distances from a bouton to its nearest neighbors belonging to the same or different axons. We found that this bouton convergence is significantly higher in-patch than out-patch. Finally, our results also show that the out-patch long-range axons are significantly thicker than in-patch axons which may lead to faster signal transmission along the out-patch axons.

Conclusion: The increased bouton density accompanied by extensive axonal convergence (cris-cross) could result in a highly efficient way of signal transmission in terminal arborization patches of the cerebral cortex. In

contrast, the long range thick axons outside the patches could provide input to extra classical receptive field and form the structural correlate of cortical plasticity.

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FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS

Selected talks

Functional and structural investigation of an intrinsically disordered segment of the post-synaptic scaffold protein GKAP

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Keywords: DLGAP1, protein structure, protein NMR, post-synaptic density

Introduction

Molecular mechanisms underlying memory and learning are remarkably complex, and still many questions are to be answered regarding the details of neuronal signaling and synaptic plasticity. PSD is an electrodense entity located in the synaptic junction of glutamatergic synapses, in the dendritic spine of post-synaptic neurons. It is a dynamic network of many interacting proteins maintaining, regulating, modulating a large number of complex interactions involved in processes like glutamatergic signaling, dendritic development and synaptic plasticity. Any disturbances in this complex web might cause a cascade of consequences, thus induce a high range of molecular and structural abnormalities in an affected neuron [1, 2, 3].

Guanylate kinase associated protein (GKAP, or DLGAP1 for Discs large-associated protein 1) is an essential component of PSD. It has an essential role in synaptic scaling by regulating the turnover of glutamate receptors in response to synaptic activity. It is linked to several psychological and neurological disorders, such as autism spectrum disease, obsessive-compulsive disorder, schizophrenia and Alzheimer's disease [4, 1].

The human GKAP (DLGAP1) protein shares 98% identity at the amino acid level to rat GKAP (Genbank accession numbers U67987 and U67988) [5]. Human GKAP isoform 1 is 977, while rat isoform 1 is 992 amino acids long. GKAP1a isoform (today rat GKAP isoform 3) was described for the first time in the literature, which is a 666-amino acid long transcription variant of the protein, missing the first 300 residues of isoform 1.

GKAP has three known domains. On the N terminal, a GK-binding region (GKAP-GBR) consisting of five 14-amino acid long repeat sequences binds to the GK region of the PSD-95 protein [6]. PSD-95 is the most common element of the PSD, it binds directly to NMDA receptors. The dynein binding region (GKAP-DLC) is located downstream of the repeats. It binds to the light chain of the dynein motor protein, this way it controls microtubule dynamics and play a fundamental role in centrosome positioning and cell polarity [7]. The GH1 (GKAP homology domain 1) domain is located at the C terminal and is highly conserved in the DLGAP family. This region is mainly globular, it consists of 4 alpha helices [8]. Through a few amino acids located at the C-terminal GKAP can also bind to the important PSD protein Shank [4].

Based on *in silico* predictions, most part of GKAP is intrinsically disordered, except for the helical regions of the GH1 domain. The tertiary, 3D structure and the dynamics of these disordered domains is yet to be characterized.

Intrinsically disordered proteins or regions do not adopt a single well-defined structure, rather exhibit large conformational variability and can only be represented by a conformational ensemble. The most suitable tool to characterize the structure and dynamics of disordered proteins is NMR spectroscopy [9, 10]. This technique is unique in providing residue, or even atomic level information on structural preferences and internal dynamics of intrinsically disordered proteins. Multidimensional NMR measurements require non-radiant isotope-labeled (^{15}N and ^{13}C), high purity and concentrated protein samples. Measured spectra then can serve as basis for ensemble-based protein structure models, which are decisive to understand the biological function of these proteins.

Materials and Methods

My aim is to explore the structure and dynamics of the GKAP protein focusing on the not-yet-described disordered regions, the GBR and DLC domains. I was working with cDNA of the rat GKAP1a gene. I used a pEV plasmid construct (see Fig. 1) including a 6x His tag, TEV protease cleavage site and the fragment corresponding to the shortest sufficient binding site of dynein (GKAP-DLC domain, 329-384 residues in rat GKAP1a). For functional assays I also produced a plasmid carrying the insert of the dynein light chain (DYNLL2).

I plan to investigate the relevance of the number of repeat sequences in the GBR domain, therefore I designed three constructs to cover this region: one containing the first three repeats (GBR 1-3), one containing the last two repeats (GBR 4-5), and one containing all five (GBR 1-5). I cloned these constructs to a pEV, and later to a pGEX plasmid vector for both His-tagged and GST-tagged purification.

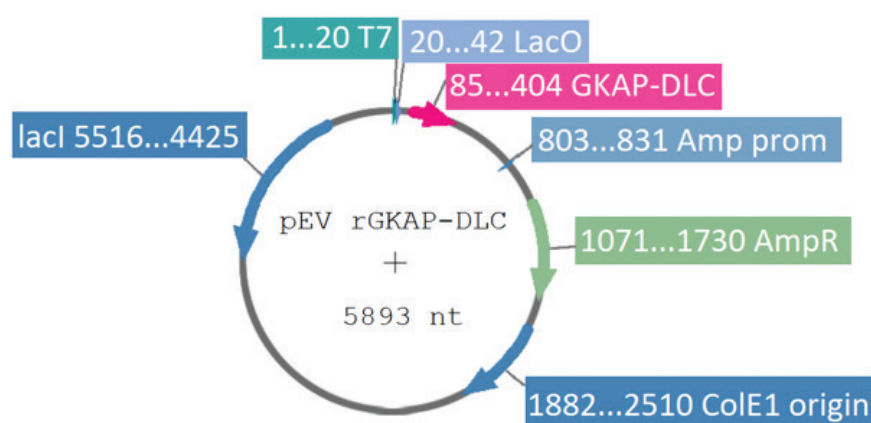


Figure 1: GKAP-DLC construct inserted to pEV vector

E. coli bacteria cells were transformed, DH5 α for plasmid production and BL21 for protein expression. The former was induced with chloramphenicol treatment, the latter with IPTG. After plasmid isolation control enzymatic digestion and DNA sequencing was performed to confirm the validity of the sequences inserted. After protein expression induction cells were stored at -20°C until exploration and protein purification. For isotope-labeled protein production cells were grown in minimal media enriched in ^{15}N and/or ^{13}C labeled nitrogen and carbon source. In case of DYNLL2 and GKAP-DLC, cell exploration was accomplished with sonication. For protein purification, immobilized metal ion affinity chromatography (IMAC, using the 6x His-tag attached to the proteins and Ni^{+} charged NTA resins) and size-exclusion chromatography (SEC) was performed using an FPLC device.

Sample concentration was calculated based on Nanodrop absorbance detection. Purity, size and structural control measurements included SDS-PAGE, mass spectrometry, and electronic circular dichroism (ECD) spectroscopy. Protein-protein interactions were investigated with gel filtration, native PAGE, pull-down assay, isothermal titration calorimetry (ITC), Trp fluorescence measurements and with titration NMR spectroscopy. NMR measurements were executed at 25°C .

Results

The purification protocol of DYNLL2 and GKAP-DLC was optimized. GKAP-DLC was expressed in inclusion bodies in the bacteria, and IMAC was possible in denaturing conditions only, presumably due to the disorder in this region. Renaturation was carried out during the elution from the Ni^{+} charged resins during IMAC. Based

on the ECD spectroscopy, the molecular ellipticity of DYNLL2 mostly fits the typical curve of alpha helical structure, while the ellipticity of GKAP-DLC shows features commonly observed in disordered proteins.

To validate the functionality of the dynein-binding region, GKAP-DLC and DYNLL2 protein-protein interaction assays have been performed. Pull-down assay, isothermal titration calorimetry and ECD spectroscopy measurement confirmed the interaction between the two proteins.

NMR measurements of ^{15}N single-, and ^{13}C double labeled GKAP-DLC domains have been performed. The HSQC spectrum also confirms this region to be disordered. Addition of DYNLL2 causes deletion of some peaks and shift of others (see Fig. 2). Assignment of the measured 2D and 3D spectra is underway.

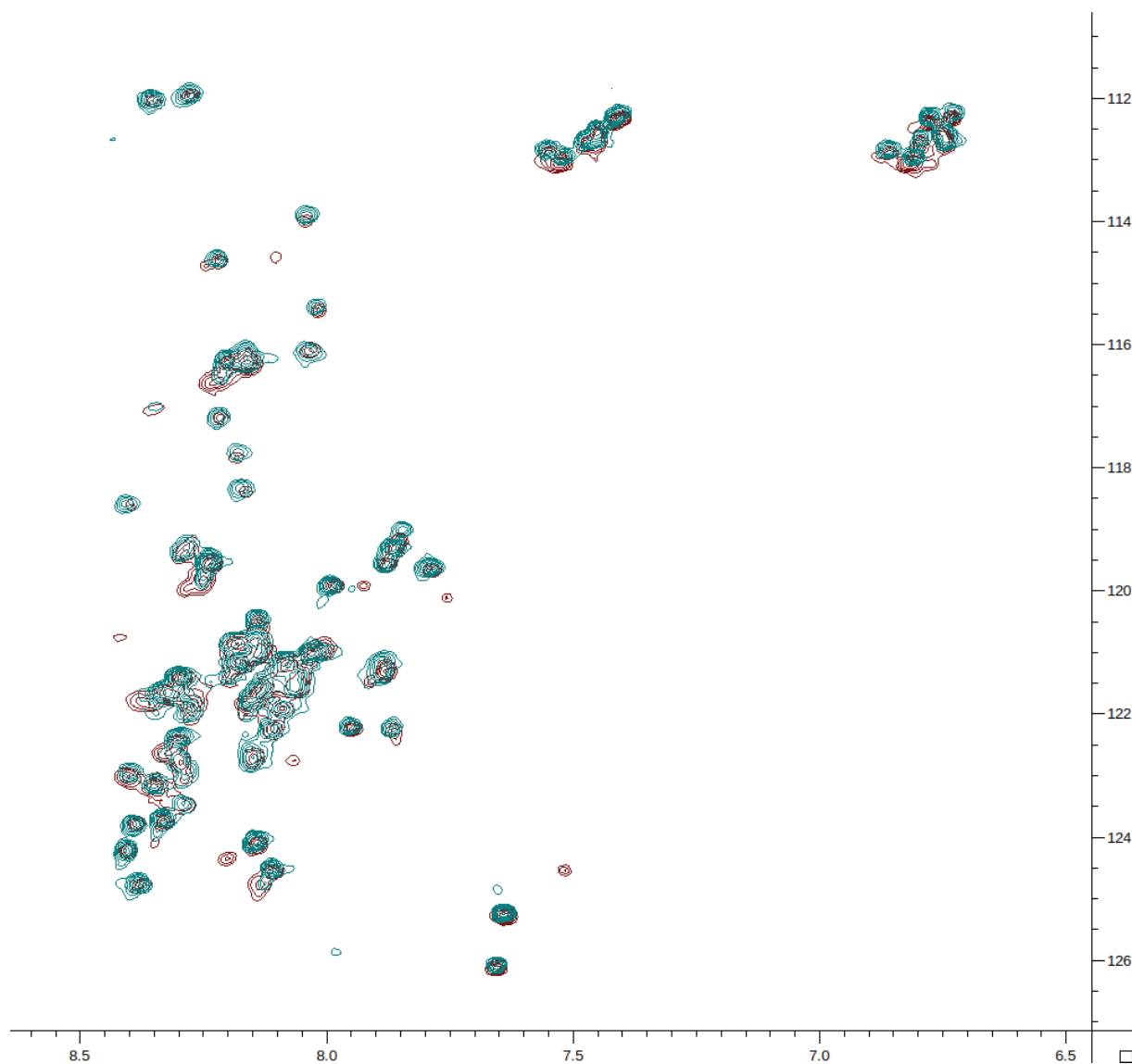


Figure 2: NMR HSQC spectra of ^{15}N labeled GKAP-DYL alone (blue) and in complex with unlabeled DYNLL2 (red). Addition of DYNLL2 causes deletion of some peaks and shift of others.

Discussion

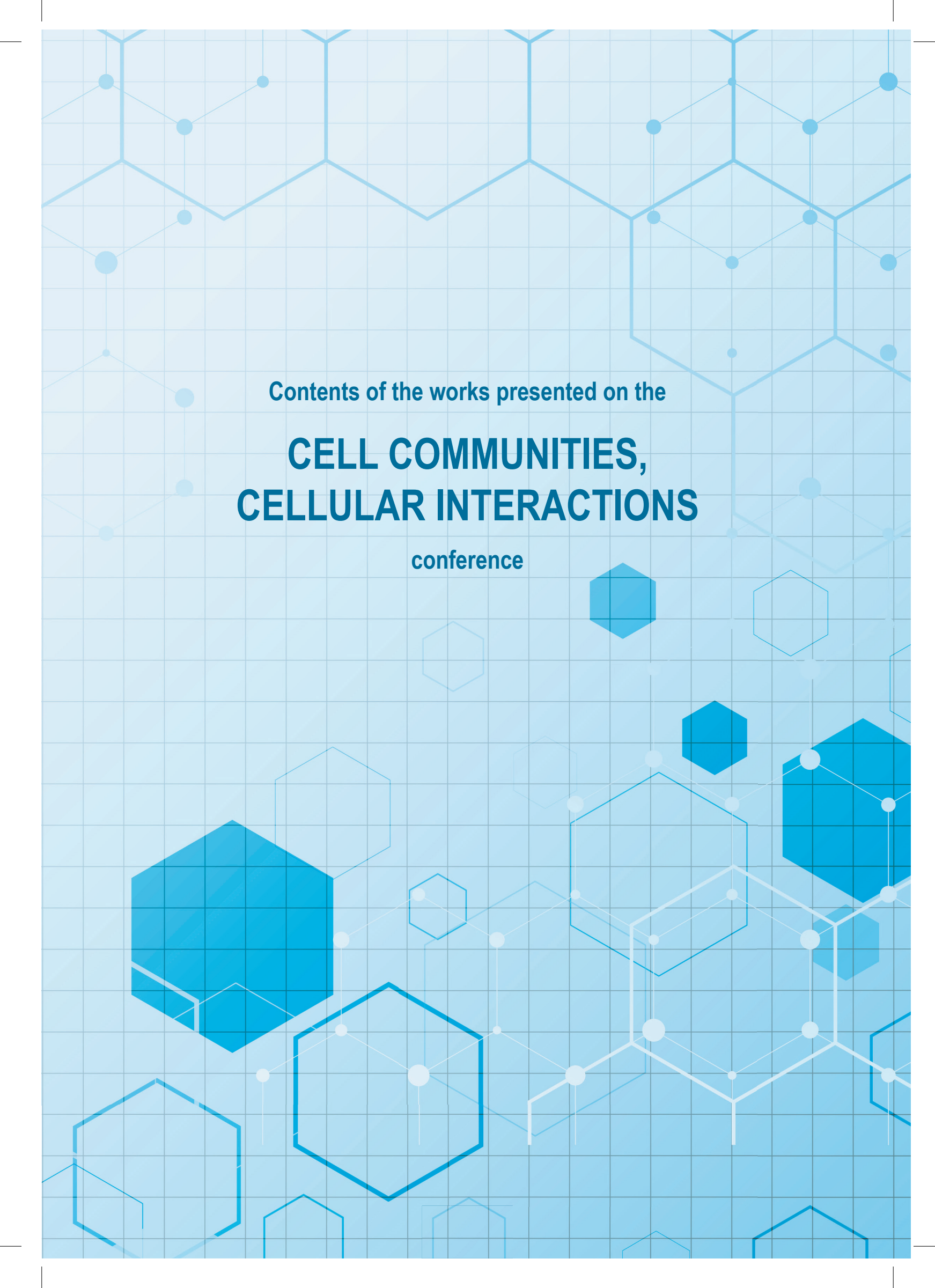
The importance of scaffold proteins in PSD is unquestionable. Many details of the molecular mechanisms underlying the organization of this large protein matrix are yet to be described. These discoveries might have relevance in a large number of neurological diseases and their therapies. GKAP is one of the essential members of these proteins, one of the most difficult to examine due to its disorder. NMR spectroscopy is the most powerful and robust technique to investigate the structure and dynamics of disordered proteins, therefore it is applicable in the examination of the GKAP protein by itself and bound to its interaction partners. So far, our results are in agreement with the corresponding literature. We plan to focus on the detailed structural and functional characterization of GKAP.

Acknowledgements

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Contents of the works presented on the

CELL COMMUNITIES, CELLULAR INTERACTIONS

conference

CELL COMMUNITIES, CELLULAR INTERACTIONS

Invited lecture

Yeasts and insects as a model to study the evolution of multicellular interaction networks and the holobiont theory of evolution

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The microbiome is adding a new dimension to the understanding of evolutionary theory. According to the holobiont theory of evolution selection acts on the genes of the host and on the microorganisms that he carries. In this perspective multicellularity can arise both from cooperation of genetically identical cells and genetically heterogeneous cells. Since several of the commensals we carry have a life cycle outside the host, this consideration requires to rethink commensalism in a much wider sense, expanding the limits of the host. Insects provide several examples of the degree at which the host coevolves with the microbiome. Recently there is increasing evidence indicating that fungal ecology includes the insect gut. During their evolution, invertebrates developed defense mechanisms against parasites and pathogens, improving their immune system. Although they lack an acquired immune component, as occurs in vertebrates thus relying only on an innate component, insects have expanded it upon traits of plasticity and adaptation against pathogens in the form of immune priming. The interaction between insects and fungi thus provides a useful example on how microorganisms can invade the host and pass from a passenger state to a commensal state, establishing a network of mutualistic interactions. In the attempt to address the mutual advantages of these dynamic interactions we will discuss how *Saccharomyces cerevisiae* strains can protect against bacterial infections both invertebrates and mammals by eliciting trained immunity. We will thus show how the presence of fungi can shape the bacterial insect microbiome, thus underlining the ecological relevance of this interaction. In this perspective we will rethink how multicellular communities of genetically very different cells can be modelled and seen as a network of competitive and cooperative interactions, where the whole is made of interchangeable and disposable parts that can be acquired or lost, through the interaction with the environment.

CELL COMMUNITIES, CELLULAR INTERACTIONS

Invited lecture

Uncharted putative G-protein coupled receptor diversity in fungi

Laszlo G. Nagy, Csenge Foldi, Zsolt Merenyi

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Fungi represent one of the most important kingdoms with a large diversity of complexity levels (from unicellular yeasts to complex multicellular mushrooms), lifestyles and nutritional modes, and include some of the most devastating plant and animal pathogens. Sensing environmental cues is a key process in multicellular organisms (e.g. for cell-cell communication), and as such its sophistication has been instrumental in the evolution of multicellularity. While there is evidence for the expansion of cell surface receptor families in most multicellular lineages, known receptor families show no signs of expansion in multicellular fungi. This suggests that the evolution of multicellularity in fungi either did not follow this principle or that hitherto undiscovered receptor families should exist. This predisposition and preliminary comparative genomic data led us to a systematic inventory of 7-transmembrane domain proteins with a GPCR-like architecture in the fungal kingdom. Analysis of >400 complete fungal genomes revealed 0 to 537 (mean 82) GPCR-like proteins that lack clear GPCR domain signatures but show some homology to canonical GPCR genes, considered hereafter as putative G-protein coupled receptors (pGPCR). We investigate the function of identified pGPCRs in multicellular development and fungal pathogenicity by mining transcriptomic data, gene knockouts and biochemical assays. Tests of the correlation between cellular complexity and pGPCR repertoires suggest functional links between multicellularity in several fungal groups. There are 5-10 times more pGPCRs than canonical GPCRs in fungal genomes, providing a large pool of uncharacterized fungal proteins that may also be key targets in the development of antifungal drugs.

CELL COMMUNITIES, CELLULAR INTERACTIONS

Invited lecture

How metabolites and xenobiotics shape microbial communities

Kiran Raosaheb Patil

European Molecular Biology Laboratory, Heidelberg, Germany

Increasing evidences support the prominence of metabolic cross-feeding in determining structure and dynamics of microbial communities. I will present the latest work from my lab in using genome-scale metabolic models and metabolomics to uncover specific metabolic interactions in complex microbial communities. I will also discuss how xenobiotics (e.g. drugs) change bacterial phenotypes uncovering a complex yet fascinating dimension to our understanding of life in communities.

CELL COMMUNITIES, CELLULAR INTERACTIONS

Invited lecture

High-resolution metagenomic strategies to explore the hidden diversity in human microbiomes

Adrian Tett

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It is now established that the microbial communities that inhabit humans (the microbiome) play an integral role in health and disease. Yet, much of the true diversity of these microbial systems are either poorly characterised or largely unexplored. Metagenomics offers the opportunity to characterise this diversity including at a sub-species (strain-level) resolution. I will present the challenges, and advancements in computational approaches to exploit this potential. In particular, I will exemplify their use to uncover functional strain-level features pertinent to disease and identify and track individual strains. I will also demonstrate that publicly available metagenomes can be utilised to explore the genetic and population structures of common but poorly characterised members of the microbiome. Focussing on *Prevotella copri*, for which there exists only a few isolate genomes, I will demonstrate how metagenomics has provided new insights into this enigmatic gut microbe. I will show that *P. copri* is not a monotypic species as previously assumed but rather four distinct sub-types. All four types have been a long-standing feature of the human ancestral microbiome, pre-dating human migration out of Africa, but have been rapidly lost in Westernised populations as a consequence of industrialisation over the past few hundred years. Thus, I will demonstrate that metagenomics is not only of epidemiological and clinical relevance but also a tool for asking fundamental questions regarding the true taxonomic diversity of these complex microbial communities.

CELL COMMUNITIES, CELLULAR INTERACTIONS

Invited lecture

*Untangling and exploiting the quorum sensing signaling network of *Pseudomonas aeruginosa**

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Quorum sensing (QS) is a widespread bacterial communication system based on the secretion and perception of specific signal molecules allowing single bacterial cells to coordinate gene expression in response to cell-density and environmental cues.

The Gram-negative bacterium *Pseudomonas aeruginosa* causes various types of severe infections in hospitalized patients and lethal lung infections in cystic fibrosis patients, and it is included in the multidrug resistant ESKAPE group of pathogens.

P. aeruginosa has become a major model organism for studies concerning QS, with the first paper published in 1994 and an average of about 300 publications per year from 2013 to date. The interest of the scientific community about *P. aeruginosa* QS can be explained by the fascinating complexity of this communication system and by its potential as a drug target. In fact, *P. aeruginosa* QS is based on multiple signal molecules and cognate receptors intertwined in the global cell regulatory network. This confers an amazing phenotypic plasticity allowing adaptation to a variety of challenging environmental niches and reflecting *P. aeruginosa* ability to produce a variety of infection types.

This talk will retrace the research about *P. aeruginosa* QS carried out by our group along almost two decades, from the characterization of basic molecular mechanisms contributing to the QS regulatory network and phenotypic plasticity, to the development of synthetic biology tools for anti-virulence drug discovery and liposome-based therapies. By giving solid examples of successes and bottlenecks, this excursus will hopefully help understand why, despite the efforts of the scientific community, the knowledge about *P. aeruginosa* QS is still elusive, and no antivirulence drug targeting QS has entered into clinical trials so far.

CELL COMMUNITIES, CELLULAR INTERACTIONS

Invited lecture

Interpretation of morphogen gradients by a synthetic bistable circuit

Paul Grant

Microsoft Research Cambridge, UK

The formation of pattern by the creation and interpretation of morphogen gradients is one of the most fundamental developmental mechanisms by which organisms build themselves. There have been many attempts at understanding these mechanisms on a quantitative level but they have all been hampered by the need to study them within the context of a complex evolved organism. Very simple molecular mechanisms are capable of producing complex emergent behaviours but that simplicity is obscured by being embedded within transcriptional networks onto which the tweaks and filigrees of millions of years of evolution have accreted. We have developed a system that allows us to rebuild these developmental mechanisms in isolation in *E. coli*, build complete mathematical models, and parameterise those models against data to make quantitative predictions about complex multicellular behaviours. We have engineered an optimized double receiver circuit that orthogonally responds to 3O-C6-HSL or 3O-C12-HSL quorum sensing molecules with different outputs and used this receiver as the basis for a number of patterning circuits. By engineering mutual inhibition between the two signalling systems, we have built a circuit that responds to one signal or the other but not both. This double exclusive reporter recapitulates the mutual inhibition mechanism that is proposed to be central to producing mutually exclusive domains of gene expression with sharp boundaries in the early *Drosophila* embryo and the mammalian neural tube. By studying this mechanism in isolation we show that such a topology can indeed be bistable and, due to hysteresis, produce stable boundaries of gene expression despite changing morphogen gradients. In addition, by adding signal production as well as mutually exclusive reception, we can create two domains of gene expression with a self-organizing boundary in response to a single morphogen gradient.

CELL COMMUNITIES, CELLULAR INTERACTIONS

Invited lecture

*Cell-to-cell communication via the blood stream:
Circulating nucleic acids as molecular disease markers*

Christoph W. Sensen

Institute of Computational Biotechnology, Graz University of Technology, Graz, Austria

The presence of nucleic acids in the cell-free fraction of human blood was first described more than 70 years ago. Using next-generation DNA sequencing and subsequent Bioinformatics analysis, it is now possible to characterize the fragments in detail and determine their role(s), as well as their diagnostic potential for mammalian diseases.

CELL COMMUNITIES, CELLULAR INTERACTIONS

Selected talks

Measuring yeast growth for modeling cell-cell interactions

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Keywords: colony morphology; modelling; communication; systems biology; synthetic biology.

Microbial communities play an essential role in nature, health and biotechnology. Despite their importance our understanding of the cells' communication among them and the behavior of the population is limited. Since controlling these communities could give rise to many useful applications, from decreasing environmental pollution to improving human health, it would be desirable to increase our understanding and ability to predict the behavior of these communities in different environment or composition [1]. Our research group chose to combine mathematical modeling and directed experiments to improve understanding and predictability of microbial communities.

S. cerevisiae, an important research and industrial microorganism, was selected to conduct our wet lab experiments and an agent-based model is under development to run simulations of colonies of a single or mixed culture. The great diversity of the natural strains of *S. cerevisiae* gained more focus recently. Sequencing data of more than 1000 strains are now available [2]. Interactions between natural strains have been shown previously [3]. We would like to extend this knowledge with an approach of combining modeling and high-throughput experiments. Experimental methods are being tested and developed that will support parametrization of the purposefully developed agent-based models. Both solid and liquid phase media are involved in our research to enable characterization of the potential interactions between local and global as were demonstrated before by Kerr *et al.* [4].

High-throughput methodology based on 96-well plates have been well established for liquid cultures, however most researchers only look at OD600 (optical density at 600 nm) [5]. That is useful for determining total population size however does not allow the differentiation between species in co-cultures. As for our purposes this would be indispensable, green and red fluorescence has been added to differentiate between strains. This allows us to generate growth curves for the total population as well as for 2 sub-populations real time for overnight or longer experiments as well. Other approaches were considered as rt-PCR, cell counting or cell plating based methods, these however are not providing 24 hours growth curve and difficult to manage in large scale.

First liquid culture experiments aimed to determine relationship between OD and fluorescent signal in mono-cultures. In case of the GFP signal, the growth curve showed similar characteristics to that observed with OD based measurements. Exponential phase was observable reliably however a decrease in the fluorescent intensity was present after the culture reached stationary phase in all cases. Exact reason behind this needs more investigation. mCherry showed slower dynamics on the timescale of the experiment, this is probably due to different protein folding dynamics. Correlation between OD and fluorescence after logarithmic transformation was found appropriate during exponential growth phase but not reliable after saturation. Quantitative methods

are being developed to describe growth of single strains within the co-culture but only qualitative comparisons were made so far.

Co-culture experiments were also carried out with the primary aim to develop protocol for qualitative measurement. Due to the differences in fluorescent intensity between strains and experiments nonfluorescent strains were added as controls both in mono- and co-cultures. Control was composed by exchanging the competing partner by a nonfluorescent of the same strain. We assume, this way no competition arises within the control population and ratio of the fluorescent strain is maintained as initial. While we are working on better standardization and finding quantitative measures, these controls allow for qualitative comparison of potentially interacting strains. Multiple combinations of strains have been tested and a promising pair (w303 and Y55) was chosen for initial co-culture experiments. A change in the fraction of strains was observed in the competitive set-up of w303 and Y55.

Growth experiments are also carried out on solid media. Multiple challenges need mitigation while testing different methods. The quantification will be used to determine the parameters of the model, hence needs to be quantitative and high-throughput as well as needs to have a good space and time resolution. First cell counting based method was tested. This gives the most relevant result that is easy to fit to model outcome; however the experiments are laborious and do not provide a good temporal resolution. An image analysis-based method is also being evaluated. This provides a better time resolution, however the morphological differences between strains need to be taken into consideration. Image analysis-based method is still preferred as moving towards high-throughput experiments. The same fluorescently labelled strains are used on solid media as well and are visualized with fluorescent imaging. A representative image is shown on Figure 1 A-B, while picture C is showing a simulation of two non-interacting strains performed with our model.

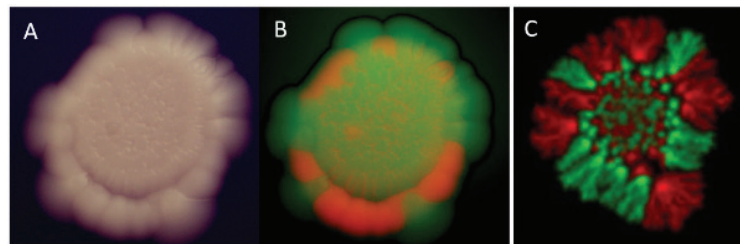


Figure 1 Co-culture of *Sgu165* (GFP, 20% initial ratio) and *Y55* (mCherry, 80% initial ratio) picture taken with Olympus MVX10 bright field (A) and with fluorescent green and red filters(B). Similar co-culture simulated by our model (C).

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CELL COMMUNITIES, CELLULAR INTERACTIONS

Selected talks

Agent-based simulation framework for predicting cell-cell interactions in yeast colony formation

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Keywords: agent-based modelling; cell-cell communication; colony morphology; systems biology; synthetic biology.

Saccharomyces cerevisiae plays an important role in agriculture, industry and biotechnology. Yeast strains are capable of forming colonies with different morphologies in solid media depending on their genetics and the properties of their environment, for example the nutrient source or the density of the culture media [1]. Moreover, cell-cell interactions also play a crucial role in colony growth and in the formation of colony shapes [2]. Some of these interactions are known, for instance yeast cells can promote the growth of each other or can produce toxic compounds against the members of other strains in mixed colonies [3]. We are studying these interaction between natural yeast strains from different habitats via both *in vitro* methods including fluorescence labels and microscopy and *in silico* methods including parameter fitting and agent-based modelling.

Agent-based modelling is applying small decision making particles, so called agents. Agents are capable of sensing and reacting to their environment and their inner state via a set of rules [4]. The behaviour of many agents based on their instructions can result in emergent properties, more complex community level phenomena than the sum of the local rules [5]. They are widespread in various fields, for example communication systems, robot technology, economics or ecology, but they are also used in studying microbial communities [6]. Here, relatively simple agents make the behaviour rules straightforward to define, but high numbers of particles can turn computations challenging. Agent-based methods represent a bottom-up approach to describe complex systems from the actions of their components, in contrast to system level differential equations.

Our model is a hybrid model. The yeast cells are described as agents while nutrients and other chemical compound are interpreted via differential equation as diffusing materials in a computational grid. The life circle of the agents is the following. The agents take up some nutrient in each simulation step, process a portion of it for their metabolism and store the rest as energy. After reaching a certain energy threshold, the cells divide and new agents form in the vicinity of the “mother agents”. In addition, agents can enter into a generally less active, stationary state in case of insufficient amount of nutrient, imitating the G0 stage of yeast cells. Those agents die, whose energy level reaches a minimal threshold, and their remaining energy is fed back to the nutrient field. Agents can also produce other diffusive materials against each other or promoting the coexistence with others allowing to define different interaction networks. Different types of agents with different parameters, representing different yeast strains, can be defined in the model. Finally, both budding and filamentous cell division is implemented in our model.

The tests with these division strategies showed that filamentous growth produces less dense but more extensive colonies, a structure that is preferable in nutrient poor environment. Nutrient level has a profound effect on colony shape and structure even in budding yeast communities. The general layout of these *in silico*

colonies is similar to those that one can produce *in vitro* on solid media. Namely, it is an expanding, more or less circular plate with active cells near the nutrient rich edge and G0 cells in the central region from where the cells already consumed the resources. High nutrient levels enable higher cell densities, higher cell numbers and more regular colony shapes, while low initial nutrient levels produce more irregular shape colonies with earlier switch into G0 state.

Then we studied the effects of different initial colony sizes and densities on colony growth. Colonies with bigger initial radius formed larger colonies with higher cell numbers than smaller ones, even if they were denser, indicating the initial colony size has more effect on grow than the initial cell number. These results agrees with our findings form *in vitro* experiments. The minor importance of initial cell density can be explained with the observation that only the cells in the edge of the starting colony have chance to contribute in colony expansion, because the daughters of inner cells cannot reach the untouched nutrient rich regions. On the other hand, higher initial colony radius indicates higher actively growing surface.

Co-cultures of different *in silico* strains were tested as well. When strains were mixed in the simulation framework, a competition for the nutrient arose. Strains with higher fitness, with faster growth, caused by for example different nutrient uptake or metabolism, could maintain higher population numbers (Figure 1.A). While strains with equal growth rates developed sub populations with similar sizes depending on the initial layout of the cells (Figure 1.B). These sub colonies are having radial growth patterns stating from the region of the initial colony which have been showed experimentally [7]. Figure 1.C shows the scenario of toxin production. The toxin was produced by one of the strains, accumulated in the environment and made the growth of the other stain slower by increasing its energy consumption (indicating the toxin sensitive cells need to spend extra energy in order to tolerate the toxin). The toxin also effects the G0 cell in this scenario, resulting in their earlier death. Nevertheless the toxin producers cannot populate the released areas because they are already out of nutrient.

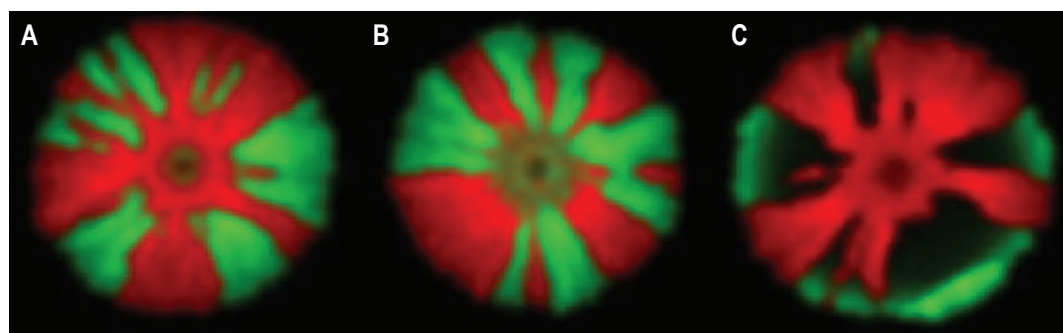


Figure 1: Different interaction schemes of two strains with different fluorescence labels in the agent-based model. Final colony shapes after 1000 iteration. A) Red strain grows faster than the green strain, and it is able to reach double size population after 1000 iterations. B) Strains with equal fitness maintain similar size colonies. C) Red strain produces a toxin against the green one. Accumulating toxin level makes the growth of the green strain slower and let the initially slower red strain to control the size of its competitor.

The future direction of our work is improving positive and negative cell-cell interaction scenarios and characterising the growth and behaviour of more strains with high-throughput techniques and fit the parameters of the model based on these experiments. The defined computational strains can help predicting more complex interaction patterns and guide the experimental work. We are also working on the tree dimensional extension of the model to capture colony morphology and structure more preciously. We hope that our computational framework will contribute to understand more details about cell-cell interactions and guide the design of complex, multi-strain colonies which can make biotechnology industry and agriculture more effective by advising strain compositions capable of specific tasks.

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The research was funded through the project Thematic Research Cooperation Establishing Innovative Informatic and Info-communication Solutions, which has been supported by the European Union and co-financed by the European Social Fund under grant number EFOP-3.6.2-162017-00013. DP was supported by the ÚNKP-19-1-I-PPKE-44 and NM was supported by the ÚNKP-19-1-I-PPKE-70 New National Excellence Program of the Ministry of Human Capacities.

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CELL COMMUNITIES, CELLULAR INTERACTIONS

Selected talks

A compartmental logical model of the Mitotic Exit Network

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Keywords: Logical model, Cell Cycle, Spatial, Compartmental

Introduction

Identifying the relationship between genotype and phenotype was identified as a key challenge for theoretical biology by Sydney Brenner in [1]. Approaches to the problem involve a network representing genetic and physical interactions between genes and a mathematical model operating on this network to identify the dynamical features of the network, to predict phenotypic output. Quantitative models such as systems of Ordinary Differential Equations have been widely used to understand biochemical systems however the size of such models is limited by the large numbers of parameters required. The behaviour of these models can depend critically on the parameter values chosen and these parameters can generally not be measured directly and so depend on model-fitting approaches. Therefore, attention has been given to qualitative modelling formalisms which are likely to scale more effectively to the genome scale.

Generalized logical models are a form of qualitative modelling which has proved scalable, with a near genome-scale model of the cell division cycle of *S. cerevisiae* having been recently published [2]. Logical models generally assume that gene products are well-mixed throughout the cell, however in many cases the precise spatial distribution of proteins is key to their function. In the ODE framework, spatial effects have been incorporated through compartmental models (see for example [3]) however no equivalent currently exists for logical models. Here we propose a framework for compartmental logical modelling and use it to explore a network of proteins controlling exit from mitosis in *S. cerevisiae*.

Progression through the cell cycle in yeast is specified by the activity of Cyclin-Dependent Kinase (CDK), which reaches its highest point in mitosis. Exit from mitosis is controlled by the release of Cdc14, a phosphatase that reverses CDK phosphorylation, leading to cyclin destruction, reverting the cell to its G1 state (reviewed in [4]). Cdc14 is kept sequestered in the nucleolus throughout the majority of the cell cycle but is released in two steps by the interlinked FEAR (CdcFourteen Early Anaphase Release) network and the MEN (Mitotic Exit Network). These proteins take in information about cell cycle stage and alignment of the mitotic spindle to ensure the correct timing of mitotic exit, leading to faithful chromosome segregation. The FEAR network acts in the nucleus, and the early anaphase release of Cdc14 is largely limited to the nucleus. The MEN functions in the cytoplasm and MEN components localise to the yeast centrosome - the Spindle Pole Body (SPB) (reviewed in [5]). Activation of the MEN required for full Cdc14 release into the cytoplasm. The distinct localizations of MEN and FEAR proteins have led to researchers to probe the effects of altering protein localisation through either protein fusions [6], the GFP-nanobody binding system [7] or mislocalising mutations [8]. These experiments have shown that protein localisation is key to the control of mitotic exit, with SPB localisation an essential requirement for the function of many MEN proteins. Control of mitotic exit is therefore a good system to test a compartmental logical model.

Methods

A compartmental logical model is essentially a two-level logical model (reviewed in [9]) that keeps track of both protein localization and activity. For each compartment of the model, each protein has a Boolean variable representing whether the protein is present in this compartment and, if the protein is present, whether it is active. A compartmental logical model is a *generalized* logical model as for each protein in the network, there are $2 \times I$ nodes in the network, where I is the number of compartments. A compartmental logical model can be built by the super-position of a localization network, describing how the localization of proteins is controlled, with an activity network describing the regulation of their activity (outlined in Figure 1A). The localization of proteins can depend on the presence of nearby proteins (sometimes in neighbouring compartments) or the activity of proteins within the same compartment. The activity of proteins depends purely on the activities of proteins within the same compartment. Some compartments may have specific rules to govern the regulation of proteins there. For example, in our model of the MEN, phosphorylation of Dbf2 by Cdc15 can only occur at the SPB, as a consequence of enzymatic funnelling. A compartmental logical model built according to this description will be much larger than a single-compartment model, if the activity network contains N nodes, then the compartmental logical model will have $2 \times I \times N$ nodes.

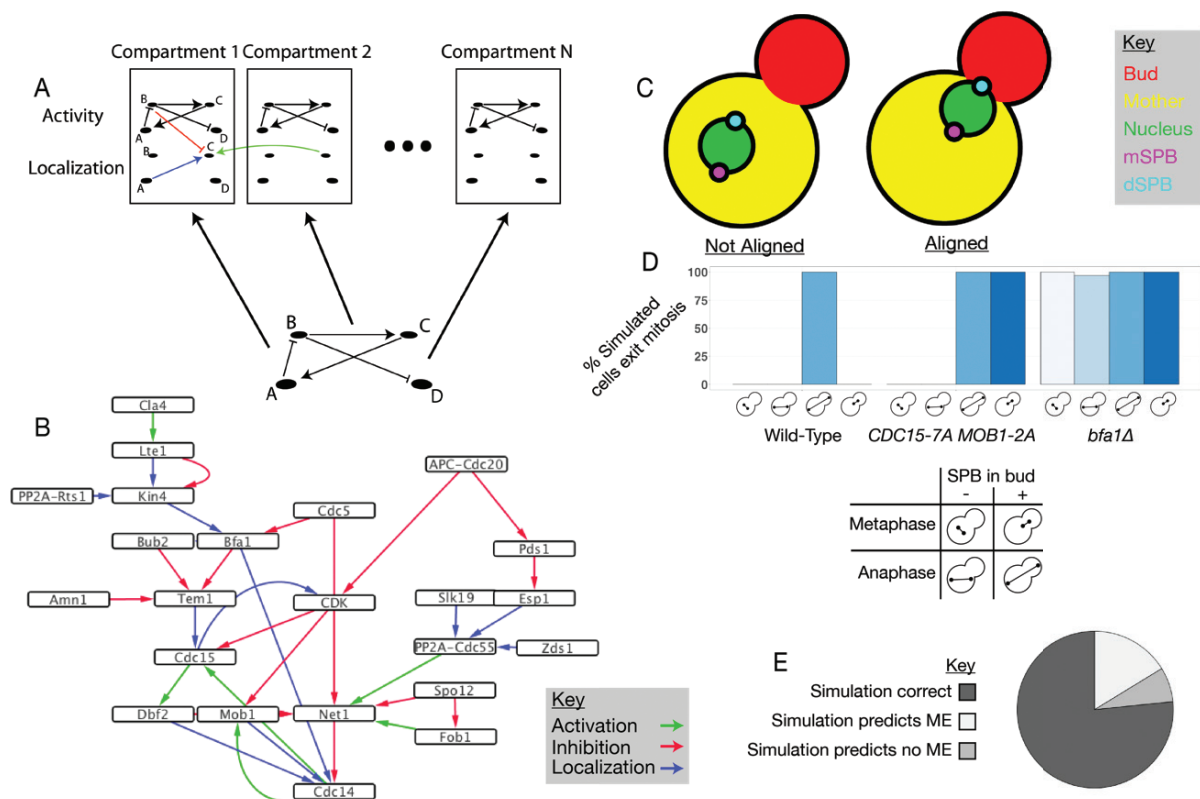


Figure 1: A: Conceptual diagram showing how a logical network can be expanded into a compartmental logical model with N compartments. B: Influence diagram of the MEN and FEAR networks. C: Diagram of compartments used in the model of mitotic exit. The daughter-bound SPB changes from exchanging with the mother cytoplasm to the bud cytoplasm upon alignment of the mitotic spindle, leading to movement of an SPB into the bud. D: Simulation results for Wild-Type, CDC15-7A, MOB1-2A and bfa1Δ cells in the four combinations of cell cycle stage and spindle alignment. For each set of conditions, 100 simulations were run from realistic initial conditions until steady state was reached using an asynchronous update scheme on uniform randomly chosen nodes. The percentage of simulations resulting in the “Mitotic Exit” node being activated was recorded. E: Percentages of mutant phenotypes correctly predicted by the model. 154 mutant phenotypes were simulated and the state of the “Mitotic Exit” node was compared to the literature phenotype.

Results

We built a compartmental model of mitotic exit using a combination of expertise-based and model fitting approaches, (see Figure 1B&C). The FEAR network, which acts within a single compartment, was well suited to model fitting, so we used the CellNOptR package [10], which employs a genetic algorithm to fit a Boolean model to mutant phenotypes, to train the activity network for FEAR components. As the MEN acts in many different compartments, we used an expertise-based approach to create a model based on the consensus view of MEN activity. At first, we intended to create a Boolean model equivalent, in which each protein has a single level of

activity and localization. However, we quickly realised that a single level of activity would not be sufficient to model some aspects of regulation. For example, CDK activity partially decreases from a peak in metaphase to an intermediate level in anaphase before falling to its G1 level in telophase. Therefore, CDK and Cdc14 were modelled to have three states: high, low and off.

While building the model, we tested various theories about how temporal information on cell cycle stage can be integrated into the decision-making process. The MEN can be thought of as a coincidence detector, waiting for both temporal and spatial signals before allowing mitotic exit to occur. However, there is still contention over how exactly MEN activity is restricted to anaphase. Two kinds of mutants show MEN activity prior to anaphase: deletion of either member of the Bub2-Bfa1 complex [12] and mutations of the CDK phospho-sites *CDC15-7A*, *MOB1-2A* [11]. We found that the phospho-mimetic mutant's behaviour was captured by the model, with exit from mitosis not restricted to anaphase but still initiated by entry of the SPB into the bud. However, the behaviour of *bfa1Δ* did not match experiments, as cells simulated under this condition did not exit from mitosis (see Table 1).

Model	Phospho-Mutant Phenotype	<i>bfa1Δ</i>
Original	✓	✗
Cdc5 translocation	✗	✓
Multi-level MEN	✓	✓

Table 1: Behaviour of different models of temporal regulation with respect to the *bfa1Δ* and *CDC15-7A*, *MOB1-2A* mutants. The experimentally-observed behaviour of *bfa1Δ* cells is to release Cdc14 independently of spatial or temporal signals, while the *CDC15-7A*, *MOB1-2A* mutant is insensitive to temporal but not spatial signals.

An alternative explanation of the restriction of mitotic exit to anaphase is that the Bub2-Bfa1 complex is the recipient of a temporal signal that prevents its inhibition in metaphase. One plausible mechanism for this signal is the translocation of Cdc5 across the SPB from the nuclear-facing side to the cytoplasmic-facing side in anaphase [13]. This model successfully captures the expected behaviour of *bfa1Δ*, however it can no longer capture the behaviour of *CDC15-7A*, *MOB1-2A*, which now behaves like the wild-type model (see Table 1).

The failure of both these models suggests the model is limited by the inclusion of a single level of activity. One solution may be that *bfa1Δ* does not represent the physiological OFF-state of Bfa1. This can be modelled by the introduction of multilevel logic for Bub2, Bfa1, Tem1, Cdc15 and Dbf2. In this framework, Bub2 and Bfa1 may vary in activity between low and high levels to control the physiological variation in Tem1 activity. However, when either is fully deleted, Tem1 becomes hyperactivated and this state is passed through Cdc15 to Dbf2. This multi-level logical model is capable of capturing both the behaviour of *bfa1Δ* and *CDC15-7A*, *MOB1-2A* (see Table 1 and Figure 1D)

To test whether the compartmental logical model of the MEN could capture the behaviour of known mutants, we simulated 154 mutant phenotypes recorded from the literature, comprising deletions, overexpressions, hyperactive and localisation mutants. The model shows good fit to experimental data from the literature, fitting 77% of 154 mutant phenotypes simulated (Figure 1E). Note that these phenotypes were not used in the training of the FEAR network.

Discussion

In conclusion, we present a compartmental framework for logical modelling and apply it to a network controlling mitotic exit in budding yeast. This work extends the logical formalism to model systems where proteins are regulated through their localisation and we hope this framework can be used as a starting point for other researchers to build compartmental logical models. In building the model, we were able to distinguish between a number of models for how cell cycle stage feeds into regulation of mitotic exit. We found the compartmental logical modelling framework could accurately capture mutant phenotypes relating to mitotic exit, including mutants which are specifically altered in protein localization. Furthermore, the compartmental model has allowed us to make predictions about mutant phenotypes including perturbations which are not currently possible using established genetic tools. For example, MEN components generally localise to the daughter-directed SPB,

however our model predicts that a mutant of Cdc15 that localised constitutively to the mother-directed SPB would be able to initiate mitotic exit from there, and would be insensitive to spindle alignment defects. We intend to develop experimental methods to test these hypotheses in living cells.

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CELL COMMUNITIES, CELLULAR INTERACTIONS

Selected talks

Structure, dynamics and unfolding of antimicrobial disulfide proteins by NMR

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Keywords: structural ensemble, thermal unfolding, CEST

Small antimicrobial disulfide proteins like PAF [1,2] are generally believed to exist as rock hard entities, though they have many charged residues. We have shown [3,4] by thermal unfolding, ¹⁵N-relaxation, ¹⁵N chemical exchange saturation transfer (CEST) and ensemble molecular dynamics that this is not the case. Even under the conditions of maximum protein stability, NMR „invisible” protein states may persist due to conformational and dynamical heterogeneity. The hidden nature of these states in everyday NMR experiments may be explained by their low populations and/or fading effects due to exchange between two or more states in the intermediate time scale (ms-us) regime. These reversible „unfolded” states can be biologically relevant, e.g. connected to disulfide shuffling. NMR monitoring of DMSO induced chemical unfolding is now supported with MD calculations and DSC microcalorimetry. A benefit of less structured intermediates could be more efficient conformational-selection type recognition upon molecular interactions. In fact, there must not be a sharp boarder between the globular and the „disordered” proteins (IDPs) world. Practical consequences may have impact on the validation of MD simulations or even protein concentration measurements. Here we summarize our experiences with proteins AFP, PAF, PAFB, PAFC, NFAP and NFAP2.

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CELL COMMUNITIES, CELLULAR INTERACTIONS

Selected talks

NFAP2: A promising antifungal protein in the treatment of Candida infections

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Keywords: sequence motif, cytotoxicity, cationic antifungal protein

In the consequence of the emerging number of human mycoses caused by (multi)drug-resistant *Candida* strains, there is a substantial demand to develop new mono- or polytherapeutic antifungal agents with low risk for resistance development. Our recent findings indicate that NFAP2, a low molecular weight, cysteine-rich and cationic antifungal protein isolated from the supernatant of the filamentous ascomycete, *Neosartorya (Aspergillus) fischeri* NRRL 181 is a promising candidate to develop new therapeutic strategies to treat *Candida* infections [1].

NFAP2 and its putative homologs represent a distinct group of cysteine-rich antifungal proteins from filamentous ascomycetes with a unique feature to inhibit the growth of clinically relevant yeasts due to a prompt pore formation ability in fungal cell membrane [1]. NFAP2 proved to be effective against both planktonic, and sessile biofilm cells of a fluconazole (FLC)-resistant *Candida albicans* strain, and was able to interact synergistically with FLC, the first-line *Candida* therapeutic agent and significantly decreased its effective in vitro and in vivo concentrations [2,3].

From economic view, one of the limiting factors for future application of antifungal proteins and peptides as therapeutic agents is the low-yield production by the native producer. To meet this requirement, we developed a *Penicillium chrysogenum*-based expression system for optimal bulk production of correctly processed and folded NFAP2. The applied *P. chrysogenum* strain is fermentable and generally recognized as safe (GRAS) organism by the US Food and Drug Administration. Beside the recombinant production, we proved that the method of native chemical ligation of peptide fragments using solid-phase fluorenylmethyloxycarbonyl chemistry combined with an additional oxidation step represents a fast and reliable method to synthesize functionally active and correctly folded NFAP2 [2].

The applied protein synthesis method allowed the functional mapping of NFAP2 to reveal peptide motifs responsible for antifungal activity. Antifungal susceptibility testing with different synthetic peptide fragments of NFAP2 combined with in silico structural analyses indicated that the highly flexible, mid-N-terminal part of the protein influences the antifungal activity that rather depends on the net charge and hydrophilicity of this region than on the primary structure [2].

Electronic circular dichroism (ECD) spectroscopy investigations indicated that NFAP2 is a β -pleated protein and the structure is stabilized by intermolecular disulphide bonds between cysteine residues [2]; furthermore, that conformational change is not required for the antifungal activity [3]. Thermal unfolding experiments followed by ECD revealed that the native fold remain intact up to approximately 70 °C and the thermal denaturation is reversible [2]. Preliminary nucleic magnetic resonance (NMR) investigations of ^{15}N -, ^{13}C -labelled NFAP2 evidenced the results from in silico structural investigations and ECD spectroscopy, viz. the β -pleated structure is stabilized by intramolecular disulphide bridges and NFAP2 has a very flexible, long N-terminal loop region.

NFAP2 did not exert cytotoxic effects on primary human keratinocytes and dermal fibroblasts in vitro thus its potential therapeutic application was suggested. We provided for the first time information about the in vivo antifungal efficacy of a cysteine-rich antifungal protein as topical agent for the treatment of mucosal fungal infection caused by a drug-resistant strain. An in vivo murine vulvovaginitis model experiment demonstrated that NFAP2 significantly decreases the number of FLC-resistant *C. albicans* cells in infected vaginal tissue, and combined application with FLC enhances the efficacy. Histological examinations showed neither morphological alterations, nor pathological reactions of the vagina and vulva after NFAP2 treatment [3].

All the above detailed results suggest that NFAP2 is a potential therapeutic candidate to overcome drug resistance of *Candida* species.

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CELL COMMUNITIES, CELLULAR INTERACTIONS

Selected talks

Anionic Food dyes modulate Mechanism of Action of a Salivary Bactericidal Peptide by forming Aggregates with Enhanced Antimicrobial Activity

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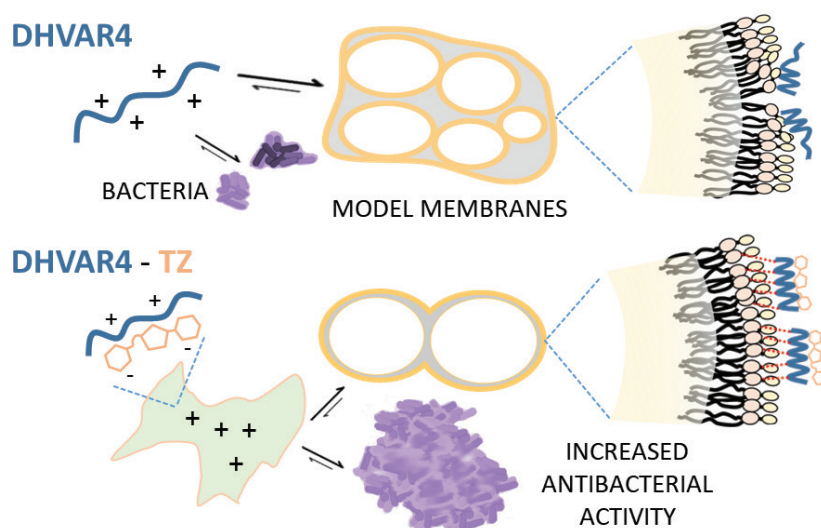
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Keywords: Antimicrobial peptides, food colors, membrane biophysics, aggregation, molecular mechanism

Due to the rapid increase of resistant bacterial infections, novel solutions for antibacterial treatments are highly desired. Natural antimicrobial peptides (AMPs), and sequences derived from them may offer several beneficial properties, such as little to no resistance developed against them during their million years presence [1]. Majority of AMPs are polycationic belonging to the innate immune system of all multicellular organisms. Their positive charge, short sequence and amphipathic nature provide specificity to biomembranes of pathogen microbes. Although some have intracellular targets, the action mechanism of most AMPs is based on the disruption of cell membranes. However, despite the intensive research in this field, several challenging questions related to their *in vivo* interactions, mechanistic function, and often observed lower activity remain unanswered. Related, it is also hard to track how diet and additional external effects may manipulate *in vivo* function of these species, limiting their rational design.

Here we demonstrate that the human salivary AMP-derived DHVAR4 forms complexes with commonly used anionic food colors, such as tartrazine (TZ), which progresses into formation of cationic aggregates. The process leads to increased toxic activity against both Gram-positive and Gram-negative bacteria, but not to mammalian cells. In contrast to synergistic effects observed for mixed antibacterial compounds, this is a new mechanism, where TZ acts on the AMP in principle as a non-covalent stapling agent, increasing its helicity when on a lipid bilayer. This results in an altered action mechanism on the head-group region in lipid bilayers, manifesting in more controlled bilayer fusion mechanism and bacterial agglutination. The sequence similarity of cationic AMPs and the conformation-inducing effect observed recently [2][3] suggests that there may be a general mechanism where *in vivo* bioactivity of host defense peptides could be manipulated in a controlled manner by other compounds present at infection points. This offers a key strategic concept for future design of antimicrobials.



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CELL COMMUNITIES, CELLULAR INTERACTIONS

Selected talks

Sequences optimization of β^3 -peptide foldamers for biomedical application

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Keywords: β -amino acids, foldamers, spectroscopy, lipid bio-physics, oligomers

Foldamers are important class of synthetic macromolecules capable to mimic the structural behaviour of biomolecules as well as are highly resistant to proteolytic degradation by peptidase. In the last two decades the rapidly growing scientific research in foldamers contribute to a broad range of application in nanotechnology, medical field, biopolymer surface recognition, catalysis, biotechnology ^[1].

Short, acyclic β -peptide sequences (named as RS set) were synthesized using solid phase peptide synthesis (SPPS) and Fmoc strategy, in a continuous-flow apparatus ^[2]. Hydrophilic and lipophilic behaviours were investigated using a combination of Attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR), NMR (including ROESY, TOCSY and DOSY spectra), circular dichroism (CD) and fluorescence spectroscopy, differential scanning calorimetry (DSC) and molecular dynamics simulations (MD). The morphology was established by using transmission electron microscopy (TEM), and the size of particles was measured by dynamic light scattering (DLS).

Several compounds were simultaneously water soluble and showed affinity for forming small oligomers. Experimental and simulation results indicate the presence of oligomeric bundles in water for RS3bK. The two nearly parallel layers are stabilized by inter- and intramolecular hydrogen bonds (Figure 1). Cluster analysis also indicates a zig-zag type conformation with some fluctuations due to the flexible acyclic residues. Similar sheet structures of β -peptides were earlier predicted by ab initio quantum chemical investigations ^[3,4,5]. The MD results showed the presence of hydrogen bonds between Glu and Lys residues suggesting the formation of salt bridges as supported also by FTIR.

To determine the membrane activity of RS foldamers we carried out several biophysical studies using uni- and multilamellar liposomes mimicking mammalian and bacterial biomembranes. All designed sequences show membrane activity, situated on the surface of the lipid bilayer perturbing the primary hydration shell of the lipid molecules or below the head group region of the bilayer as supported by perturbation of the CH₂ stretching band of lipid acyl chains. When comparing the water soluble RS foldamers, the highest change in peptide secondary structure was presented by RS3bK after the interaction with the lipid bilayer. The relative amount of random coil structure in liposomal system decrease and a more ordered structure with increase of intermolecular H-bonding content were observed.

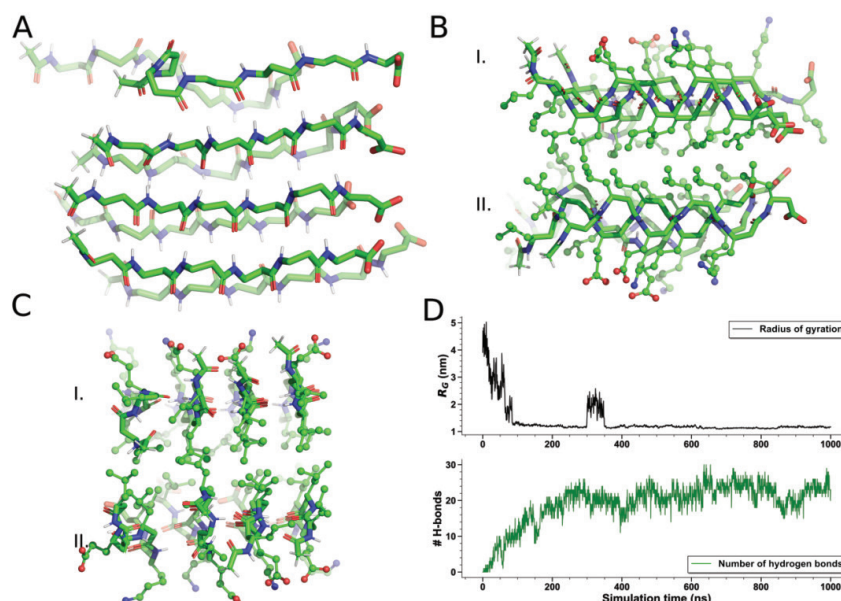


Figure 1. Self-assembly of RS3bK molecules into octameric bundles in water (MD result)

To demonstrate potential towards practical applications, the water soluble scaffold is also able to readily host fully hydrophobic small molecules such as pyrene. UV-Vis measurements showed that pyrene molecule is inserted into the foldamer assembly similarly as inserts into lipid bilayers. It is expected that by modification of the external and internal side, targeting and guest molecules preference could be modulated for diverse applicability.

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CELL COMMUNITIES, CELLULAR INTERACTIONS

Selected talks

Phospho-switches in the MAP kinase pathway - a case study of ATF2

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Keywords: MAP kinase, docking motif, transactivation domain, phospho-switch

The transactivation ability of AP-1 transcription factors (e.g. c-Jun, ATF and c-Fos) are regulated by phospho-switches. Phosphorylation of transactivation domains (TAD) by mitogen-activated protein kinases (MAPK) is a downstream response to growth factors, cytokines or stress. Since the discovery of MAP kinases about 30 years ago - an extensive list of substrates and partners has been uncovered for JNK, p38 and ERK. Now we know a great detail about their biological roles and regulation, but less effort has been invested in the exploration of exact molecular mechanisms.

Our aim was investigate the structural background of the recruitment of ATF2 by its well-known paralogue activators - JNK and p38. For studying those interactions we used combined structural biology and in vitro cell-based techniques.

Although ATF2 has been described in the 1990s as one of the first JNK substrates after JUN itself, we still know very little about how it is able to recruit JNK. Early experiments pinpointed the existence of docking motif at the N-terminus [1], and later on the structure of a short, 11 amino acid ATF2 peptide bound to JNK has been determined by crystallography [2] despite of the very weak affinity. However, we observed that ATF2 contain an evolutionary conserved C2H2 type Zn-finger which overlaps with the basic region of the D-motif. In one of our first experiments, we performed a pull-down assay to more accurately map the docking element located on the N-terminus of ATF2. We found that the minimal interaction site between ATF2 and JNK is more than just a D-motif: it also encompasses the Zn-finger domain (19-58). Removal of Zn²⁺ ions by treating the protein with EDTA greatly diminished ATF2 binding to JNK1. Similar results were obtained by fluorescence polarization measurements and isothermic calorimetry titrations (ITC) using recombinant ATF2 fragments and JNK1. EDTA treatment decreased the binding affinity of ATF2 towards JNK1 by a magnitude from 5 μ M to 50 μ M, and this decrease was almost completely due to the increase in the entropic term. Thus it makes sense to assume a „rigidifying” role of the Zn-finger to help ATF2 fitting into the D-site of JNK1. Mutational analysis of the Zn-finger pointed out that a surface lysine (Lys⁴⁸) located on the Zn-finger of ATF2 is also essential for its interaction towards JNK1. To gain more precise insight into the coordination of ATF2 residues by JNK1, we performed NMR HSQC titration with JNK and ¹⁵N labeled ATF2 construct (19-78) containing TPTP phosphorylation sites as well. In addition to the expected changes in the ATF2 D-motif, the intensity of most Zn-finger residues also similarly changed, but the C-terminal phosphorylation sites remained unbound.

Obtaining a preliminary model of the JNK-ATF2 complex we used HADDOCK 2.2 docking software [3]. The input model for JNK were generated from 2XS0 and manually created ensemble were used for ATF2. Unbound NMR models of the ATF2 Zn-finger (1BHI) were fused with three different D-peptide - JNK models representing the hydrophobic region in all possible orientation (model from the incomplete ATF2-JNK3 (4H36) crystal structure, homology models from the NFAT4-JNK1 and MKK7-JNK1 structures). In the ensemble we retained only 25 models that did not lead to extensive clashes with the surface of JNK1 (using the hydrophobic region as a pivot point). For the HADDOCK run unambiguous distance restraints were defined according to classical

docking motif contact points in the hydrophobic pocket, and for maintaining the Zn^{2+} tetrahedron structure. The lowest energy structure were selected and used for mutational validation of the possible contact points.

Based on our HADDOCK model, we designed a double mutant, which can bind to JNK hundred fold stronger (50 nM) compared to wild type ($\sim 5 \mu\text{M}$). With this double mutant we were able to crystallize the JNK1-ATF2(19-58) complex, and solved the crystal structure by molecular replacement at 2.7 Å resolution. The crystal structure showed a good agreement to the HADDOCK model (the RMSD for all main ATF2 chain C α atoms, using JNK as the reference, was 0.9 Å) and provided the first experimental structure for the correct, biologically relevant JNK1-ATF2 complex. We successfully validated the major contact points of the JNK-ATF2 interaction with YFP fragment complementation assay, luciferase based dynamic complementation assay and GAL4 transactivation assay in HEK-293T cells.

The minimal JNK binding region (19-58) showed no detectable binding towards p38 therefore we used the TAD(19-100) of ATF2 for mapping the p38 binding motif. In luciferase fragment complementation assay we stimulated the p38 and JNK pathway with anisomycin and observed that the interaction of ATF2-p38 is phosphorylation dependent in contrast to the static signal from ATF2-JNK interaction. After mapping we could determine an evolutionary conserved motif – the putative F-site – responsible for this effect. We were able to determine the binding affinity of phosphorylated p38 and ATF2 TAD with ITC ($\sim 90 \mu\text{M}$), which in the same range of K_m derived from kinetic measurements in previous studies [4].

Besides the canonical TPTP phosphorylation site there is a SP site located at the N-terminus of the F-site and can be phosphorylated only by JNK [5]. This phosphorylation motif is conserved in *Vertebrates*, but a non-phosphorylatable NP mutant exists in lower animals. With ITC measurement we found that phosphorylation of the SP site impair the ATF2 binding to pp-p38. This phenomena suggests that phosphorylation capability of p38 is highly depends on the JNK activity. In contrast two lower animals where JNK and p38 can act together, dissection of the two pathway may occur in *Vertebrates*. We are still investigating the co-regulation mechanism in cell-based assays.

According to our results we can say, that ATF2 recruit its upstream activators with different and specific elements. ATF2 transactivation activity depends on its phosphorylation state and temporal regulation of it seem to be a complex mechanism involving the two kinases. Fine-tuning of the transcription also guided by the heterodimerization partners of ATF2, which are mostly MAP kinase substrates as well. This research may contribute to better understand the combinatorial events in transcription.

Acknowledgments

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CELL COMMUNITIES, CELLULAR INTERACTIONS

Selected talks

Interplay between folding and binding modulates protein sequences, structures, functions and regulation

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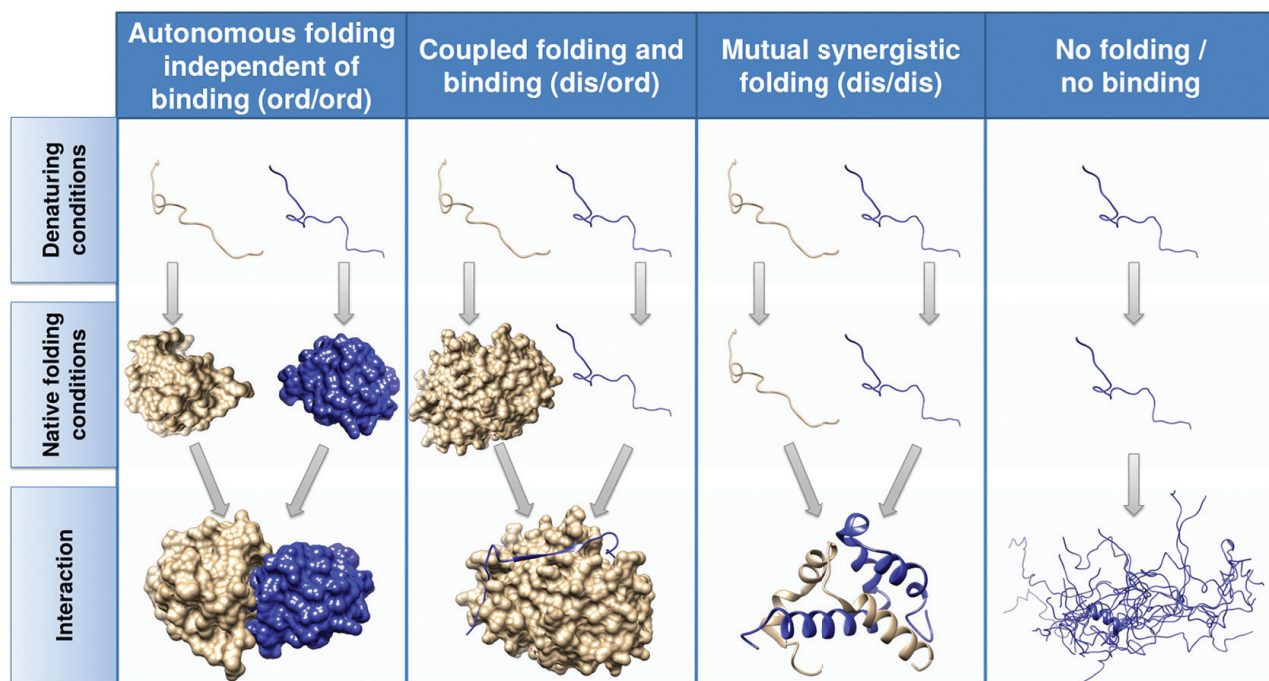
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Keywords: intrinsically disordered proteins, protein-protein interaction, coupled folding and binding, mutual synergistic folding, cell regulation

Intrinsically Disordered Proteins (IDPs) mediate highly diversified and crucial functions in living cells. While lacking a stable structure, a remarkable proportion of IDPs are capable to fold via interactions with (most commonly protein) partners. Protein disorder has been recognized as a major determinant in several properties of proteins; yet the way the binding process is reflected in these features in general lacks this detail of description. Assuming the classic binary classification of proteins, each partner in an interaction can be either ordered or disordered. Thus, there are three possible scenarios of the interplay between binding and folding: autonomous folding and independent binding (where all interacting partners are ordered), coupled folding and binding (where an IDP binds ordered partners) and mutual synergistic folding (involving exclusively IDPs). Recent advances in database development enabled us to identify a large amount of bound structures from all three classes, opening ways to assess the nature of these interactions through the sequence-structure-function paradigm. Since folding and binding share a similar biophysical background, these interactions can be described by the same approach, showing how the formation of structure from proteins with different structural states is mirrored at different levels, highlighting fundamentally different properties in terms of protein sequence, structure, function and regulation, depending on the structural properties of the interacting partners. We found that strikingly, the properties of interactors in terms of sequence and adopted structure are defined not only by the intrinsic structural state of the protein itself but also to a comparable extent by the structural state of the binding partner. The three different types of interactions are also regulated through divergent molecular tactics of post-translational modifications. This not only widens the range of biologically relevant sequence and structure spaces defined by ordered proteins but also presents distinct molecular mechanisms compatible with specific biological processes, separately for each interaction type. The distinct attributes of different binding modes identified in this study can help to understand how various types of interactions serve as building blocks for the assembly of tightly regulated and highly intertwined regulatory networks. The appreciation of this interplay between folding and binding is the basis for the successful charting of unknown territories in the protein interactome, the understanding of how different binding modes assemble regulatory networks, and the development of future pharmaceutical applications.



CELL COMMUNITIES, CELLULAR INTERACTIONS

Selected talks

Exploring the quorum sensing systems in bacterial genomes

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Keywords: bacterial cell-cell signalling; quorum sensing; operons; gene topology

Quorum sensing (QS) is an essential form of bacterial cell-cell communication. Despite the rapidly growing genomic information about bacterial communities, microbiomes and genomes, our knowledge on functions and regulation of quorum sensing systems is still scarce. In order to explore the true prevalence of QS systems our research group is developing an automated bioinformatic pipeline to identify them and their characteristic gene topologies in bacterial genome databases.

Quorum sensing is an autocrine signalling mechanism whereby the signal is produced by a cell and sensed by both the same cell and its target cells. It allows the signal producing bacteria to sense the proximity of each other by the environmental concentration of a QS signal molecule. When its concentration reaches a certain level, specific cellular functions will be activated in each susceptible cell resulting in synchronized behaviour in population scale [1]. This synchronized behaviour then allows a bacterial community to carry out tasks that a single cell could not. These tasks, for example forming biofilm, initialising swarming, maintaining genetic competence, producing extracellular enzymes for processing nutrient sources or toxins for pathogenicity requires a lot of energy, therefore they need precise regulation.

The best known and most studied QS systems are those which use acyl-homoserine lactone (AHL) signals. They were first described in the context of regulating bioluminescence of *Vibrio fischeri* [2]. The system contains two proteins a signal synthase, called LuxI, and response regulator, called LuxR which has a signal binding and a DNA binding domain. LuxI has a continuous baseline AHL signal production. AHL molecules can move in and out of the cells free via diffusion and in the presence of high AHL concentration the signal activates LuxR. Active LuxR then binds to the DNA and increases the production of LuxI, LuxR and other Lux genes responsible for bioluminescence [1]. AHL systems are widespread in Gram-negative bacteria they are extensively studied [3]. Many luxR and luxI homologues were found in different species with different gene topologies [4].

Many Gram-positive species are also capable of quorum sensing; however, they predominantly use small, sometimes post-translationally modified or circularised peptide signals which cannot cross their cell wall via diffusion. These bacteria use active transport for pumping out the signal. The return can be either via active transport, such as in short hydrophobic peptide (SHP) signalling [5], or by a more complex two-component system, for example in the ComQXPA operon of *B. subtilis*. Here the ComX signal (modified by ComQ after translation) first binds to the extracellular part of a transmembrane receptor ComP, and after that its intracellular part, a histidine kinase phosphorylates transcription factor ComA. ComA becomes activate upon phosphorylation and mediates the upregulation of the QS system and bacterial competence genes by binding their promoter [6]. Some Gram-negative species, for example *Xanthomonas* apply a related mechanism wherein small signal molecules, for example diffusible signalling factors (DSF) leave the cell by diffusion and bind to transmembrane receptors of a two-component system [7].

With the advent of next generation sequencing, the number of known bacterial genomes is dramatically increasing. One would expect a similar increase in the number of known QS systems, but this does not appear to be the case. First, a large proportion of genes in the known genomes is not annotated, since the QS genes are not part of the core genome, they most often belong to what is termed “shell genome”, which remains largely unannotated in current sequence databases.

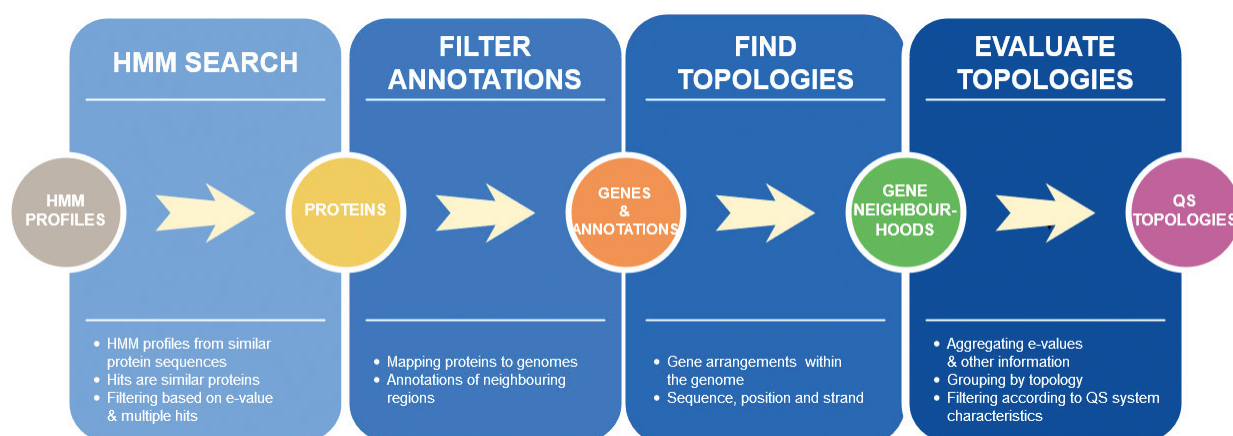


Figure1: Schematic outline of the automated annotation pipeline for QS genes.

We are developing an automated annotation pipeline to identify and classify different QS systems in genome databases such as NCBI refseq (Figure 1). We are using Hidden Markov Models (HMM) to construct sequence profiles [8] for each protein of a given QS system using its known examples from sequence databases. The databases then are searched with these profiles and the filtered hits are organised into operon candidates. Then different topologies can be defined based on the order and orientation of the QS genes. Bacterial genes are often ordered into conserved operons, therefore the operon topologies and their chromosomal position (gene neighbourhood) are worth to be compared with the phylogenetic trees of the species having the system.

A census of AHL genes identified 27 topologies that differed not only in the mutual orientation of the QS genes but also in the genes found in between the synthase and the sensor-regulator genes [9]. The interspersed genes included negative regulatory TFs some of which were shown to play a stabilizing role. Others were involved in DNA mobilization, signal degradation, etc. so one could suppose that the interspersed genes are tightly co-regulated with the QS genes and are part of the target genes to be activated at higher cell densities. A further, interesting type of AHL topologies are solo sensor regulator genes [10][11]. These genes may respond to the same signal as the canonical QS system found in the same cell, in this case they can provide a link between the QS system and target genes. Clearly, the signal concentration within the cell is in equilibrium with the environment, but it can regulate a variety of solo sensor-regulator genes thereby switching on or off a variety of genes. (Alternatively, solos may respond to other signals coming from the environment.

A typical Gram-positive QS system is ComQXPA known primarily in Bacilli group. It consists of 4 tandemly oriented genes with little variation among various occurrences. A systematic survey revealed however that there are characteristic overlap patterns which form characteristic topology groups whose members cluster together by protein sequence [12].

Most *in vitro* studies of quorum sensing are limited to species with medical or agricultural importance or to the ones that are easy to culture under laboratory conditions, while these are only representing a small portion of microbial diversity. In order to get a broader view about the channels of bacterial cell-cell communication and the distribution of these channels, the “bacterial languages and dialects” we are planning to scan the growing genome databases with the profiles of other, less known QS system as well. The results could provide information not just about the communication mechanisms of the species, but also about their possible interactions with other cell or their evolution. For example, exploring the same QS system among different species living in the same microbial community indicates the opportunity of inter-species communication or eavesdropping inside the microbiome. Or the origin of a system that was found in a species, but not in its close relatives, could have been a horizontal gene transfer event, if other members of its natural microbiome possess those genes. Unlocking more and more details of bacterial cell-cell communication can help us to understand the structure

and dynamics of microbial communities and brings us closer to the rationally designed synthetic microbiomes capable of specific medical, biotechnological or industrial tasks.

Acknowledgments

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POSTER PRESENTATIONS
for the two conferences

POSTER SESSION

DMSO-induced unfolding of the small antifungal disulfide protein PAF and variants

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Keywords: structural ensemble, thermal unfolding, CEST

In the last decades cysteine-rich, cationic antifungal miniproteins were discovered that show remarkable stability against stress (temperature, pH, chemicals and even proteases). They have rigid β -barrel structures stabilized by 3-4 disulfide bridges and are potential drug candidates. Folding-unfolding studies of these proteins may help to understand their mode of action. Earlier we have studied [1] the cold and heat unfolding of PAF using NMR, that supported its dynamical heterogeneity. Here we applied dimethyl-sulfoxide (DMSO) as chemical denaturant to induce reversible unfolding of native PAF, PAF^{D19S} inactive [2] and PAF^{F31N}, PAF^{D55N} active variants. To monitor chemical unfolding both NMR and DSC microcalorimetry were used. T_m melting points and ΔH unfolding enthalpies were determined from DSC thermograms as a function of DMSO concentration, and the linear decrease of T_m corroborated unfolding. The inactive variant exhibited smaller stability, resulting in lower melting point. From the fitting of the intensities of NMR ^1H - ^{15}N HSQC cross-peaks we derived the free enthalpy of unfolding as $\Delta G = -23$ kJ/M and ca. 50% of PAF was still folded at ~ 70 v/v % DMSO, at 37°C. At 50 v/v % DMSO concentration ^{15}N NMR relaxation analysis yielded PAF order parameters $S^2 = 0.8$ - 0.9 range – except flexible loop regions- which means that the observable signals belong to a relatively rigid form of PAF. Indeed, the NMR structures of PAF and PAF^{D19S} in DMSO are very similar to the known aqueous solution structures, the fold is conserved. However the β -strands changed at both termini, suggesting that the unfolding starts around these regions, similarly to thermal unfolding experiments.

Acknowledgments

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POSTER SESSION

Effects of DNA damage on the cell cycle through the circadian clock

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Keywords: chronotherapy, DNA damage, circadian clock, cell cycle, in-silico experiments, Matlab, *Neurospora crassa*, phase-response curve

Most cancer treatment induces DNA damage to eliminate highly proliferative cells. This process, however, also affects healthy cells creating numerous side effects ranging from infertility to heart problems. Cells respond differently to DNA damage depending on where they are in their cell cycle at the time of the treatment. Intriguingly, circadian rhythms provide temporal information to the cell cycle machinery to control the timing of cell divisions. Personalized chronotherapy based on this: the time of the treatment based on the patient's natural daily rhythm.

To understand the impact of DNA damage in altering the timing of cell divisions via circadian rhythms, we built a mathematical model and performed in-silico experiments for the proposed system that integrates circadian rhythms, cell cycle, and DNA damage response in *Neurospora crassa*.

One part of the model is the main mechanism of the circadian rhythm in *Neurospora*, which is a negative regulatory feedback loop, because FRQ protein inhibits its own transcription factor, White Collar Complex. The other part of the model based on the interactions of CLB1 (CycLinB), because it takes the main role in the coupling between the 2 cycles. STK-29 (homologue of mammalian Wee1) inhibits CLB1 in the cell cycle before mitosis and its concentration is influenced by White Collar Complex, the core molecular clockwork. When we do not introduce any coupling between the cell cycle and circadian rhythm, the duration of a single cell cycle is about 3 hours. But when the circadian rhythm influences the cell cycle, the duration of each cell cycle is different: their length depends on the circadian time. The reason is that WCC stops the cell cycle through STK-29, right before mitosis. Meaning that if the concentration of WCC is higher, the duration of the cell cycle is longer. This happens in the middle of the subjective days of our simulations.

We composed a unified code for the analysis in the Matlab programming language to simplify examinations. We simulated the perturbation of the circadian clock and the cell cycle upon DNA damage and analysed the consequences of this perturbation on the following cycles using phase-response curves. We can observe that in the subjective day the phase shift is near to 0, but in the subjective night, high phase advance appears.

We analysed the behaviour of the cell cycle, where the strength of connection between the cell cycle and circadian rhythm was noisy. We wanted to examine the number of nuclei in mitosis after perturbation in the subjective day and night. According to the results, perturbation upon DNA-damage in the subjective day did not lead to relevant changes in the cell cycle. But, when the effect of the perturbation upon DNA-damage was in the subjective night we got other conclusions. Immediately after the disturbance we observed a perturbed system when cell cycles are not synchronised. After some days, the amplitude of the oscillations resets, but a high phase-advance appears.

In summary we can see, that it matters, when perturbation upon DNA-damage occurs: it can result in high shifts in the timing of the cell cycle or have almost no effect. This consequence may have relevance to cancer

treatment as well. Depending on the timing of the induction of DNA-damage, the healthy cells reacts differently: they can continue their normal division or they can be perturbed as the side-effect of the treatment.

In the future, we would like to add to the model the direct effect of the DNA-damage on the cell cycle as well, to be able to analyse the general effect of the DNA-damage on the cell cycle.

Acknowledgments

The research was funded through the project Thematic Research Cooperation Establishing Innovative Informatic and Info-communication Solutions, which has been supported by the European Union and co-financed by the European Social Fund under grant number EFOP-3.6.2-162017-00013.

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POSTER SESSION

Biological noise can be reduced by coupled feed forward loops

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Abstract: Cellular networks and their diversity play an important role in biology. Furthermore, Cellular noise leads to probabilistic behavior in nature. The underlying mechanistic details of these networks can be captured by mathematical models and their computational simulations. Network motifs, like feed forward loops were shown to hold special dynamical characteristics. The special features of various versions of feed forward loops (coherent or incoherent type) were reported to provide sign sensitive delays or accelerations of signals by the network. Here we are investigating the effects of cellular noise on coupled feed forward loops. Our results reveal that special couplings of feed forward loops can lead to noise reduction.

Keywords: Cellular noise; computational simulation; mathematical modelling; feed forward loops [3].

Because of external signal the cells respond differently, although being in a homogeneous medium. We are interested here to characterize the decision-making process by coupled feed forward loops. We are looking for the noise reduction tendency of our models.

We have already learned that low number of chemical species present in a cell causes a large fluctuation in the system due to inherent intrinsic noise, and this noise is inversely proportional to the square root of the number of molecules [1].

$$\text{noise} \sim 1/\sqrt{N}, \text{ where } N \text{ is number of molecules}$$

We are investigating the input-output relationship of topological models of coupled feed-forward loops. Earlier it has been reported that in a signaling cascade due to the presence of micro-RNA [4] leads to the reduction of noise. It was also observed that when a system is subjected to an annihilation module, which is composed by the two species that are co-expressed followed by joint degradation, then it can function as a low-pass filter and reduce the noise. The noise reducing capacity of these modules come at a price of some delay in the response time of the output of the network. A combination of the annihilation module and linear filter turns the system into an incoherent feed forward loop, which can perform efficient noise reduction without introducing any delay to the response [2].

Inspired by this efficiency of coupled modules in noise reduction we decided to investigate how combinations of various type of feed-forward loops can function as noise reducing networks. The proposed models are represented as follows:

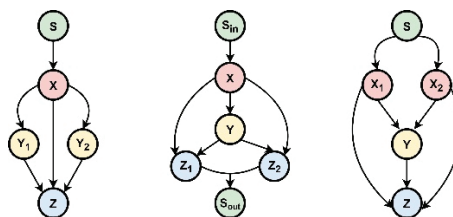


Fig1- Diagram of coupled feed-forward loops with multiple intermediates(left), Diagram of coupled feed forward loop with multiple outputs(middle), Diagram of coupled feed forward loop with multiple inputs(right). The interactions can be positive or negative leading to various combination of coherent and incoherent feed forward loop.

The system equations for multi input coupled feed-forward loops for the purely coherent type1 case can be represented as:

$$\frac{dx_a}{dt} = K_{px} \cdot K \cdot x_i + K_a \cdot S \cdot x_i - K_{dx} \cdot x_a - K_{ppx} \cdot PP \cdot x_a$$

Where $x_i = x_{tot} - x_a$, K_{px} is the phosphorylation and K_{ppx} is the dephosphorylation rate. K and PP are kinase and phosphatase respectively.

$$\frac{dy_{1a}}{dt} = K_{py1} \cdot K \cdot y_{1i} + K_1 \cdot x_a - K_{dy1} \cdot y_{1a} - K_{ppy1} \cdot PP \cdot y_{1a}$$

$$\frac{dy_{2a}}{dt} = K_{py2} \cdot K \cdot y_{2i} + K_1 \cdot x_a - K_{dy2} \cdot y_{2a} - K_{ppy2} \cdot PP \cdot y_{2a}$$

Where $y_{1i,2i} = y_{tot} - y_{1a,2a}$, $K_{py1,2}$ is the phosphorylation and $K_{ppy1,2}$ is the dephosphorylation rate.

$$\frac{dz_a}{dt} = K_{pz} \cdot K \cdot z_i + K_2 \cdot K_3 \cdot K_4 \cdot x_a \cdot y_{1a} \cdot y_{2a} - K_{dz} \cdot z_a - K_{ppz} \cdot PP \cdot z_a$$

Where $z_i = z_{tot} - z_a$, K_{pz} is the phosphorylation and K_{ppz} is the dephosphorylation rate. All the reactions are written for the AND configuration. For OR configuration it will be sum of $(K_2 \cdot x_a + K_3 \cdot y_{1a} + K_4 \cdot y_{2a})$ instead of $(K_2 \cdot K_3 \cdot K_4 \cdot x_a \cdot y_{1a} \cdot y_{2a})$. Here we are considering the post translational modifications for the molecules, so that they can attain the active state from the inactive state via an intermediate by phosphorylation-dephosphorylation process. This multisite phosphorylation adds nonlinear transition between active -inactive forms while keeping all reactions with mass-action kinetics[5]. Performing linear noise approximation, we get the idea how perfectly our model reduces noise.

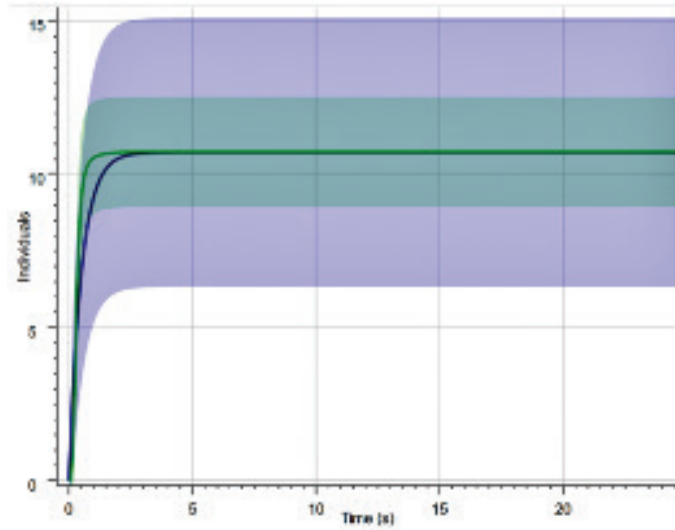


Fig2- We have plotted the input signal(blue) with the output signal (green) for the multi input feed forward loop purely coherent type1 model, considering active inactive state of species.

We performed the Gillespie simulation for coupled feed forward loop with multiple intermediates coherent type-1 in OR configuration and coupled feed forward loops with multiple input coherent type-1 in OR configuration. We can find that the noise reduction capacity for coupled feed forward loop with multiple input coherent type-1 is more compared to the other model. When we calculate the Fano factor(which is the measure of dispersion of any probability distribution) for this model we get the lower value compared to the coupled feed forward loop with multiple intermediates coherent type-1 in OR configuration model. The Fano factor for coupled feed forward loops with multiple input coherent type-1 in two different steady states are 0.26 (in lower steady states) and 0.13 (in higher steady states), whereas the Fano factor for coupled feed forward loop with multiple intermediate coherent type-1 in two different steady states are 0.38 (in lower steady states) and 0.16 (in higher steady states).

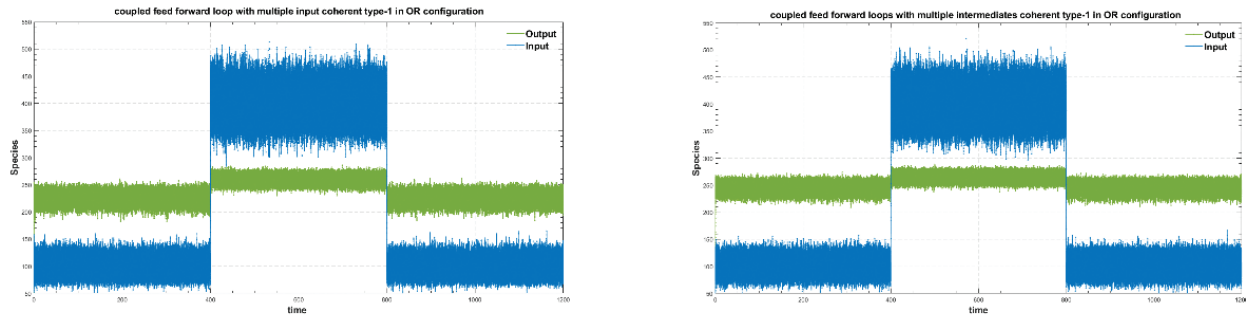


Fig3- We have plotted the input signal(blue) with the output signal (green) for the coupled feed forward loops with multiple input coherent type-1 in OR configuration model (Left) and coupled feed forward loop with multiple intermediates coherent type-1 in OR configuration model (Right).

We have compared this result with simple feed forward loop[3] model in OR configuration. The Fano factors for simple feed forward loop in OR configuration in two different steady states are 1.3 (in lower steady states) and 0.98 (in higher steady states). Both of our model works well in reducing noise compared to simple feed forward loop.

Both models can perform the noise reduction task, but they don't follow the fold change trends with the input. It means that for quantitative changes for the output to the input doesn't bear a constant ratio throughout the time course of simulations and ideally this should be 1. In future we would like test various model architectures to find which one can perform the noise reduction as well as follow the fold change trends of the input signal.

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POSTER SESSION

Continuous-flow solid phase peptide synthesis of foldamer oligomers and its biophysical characterizations with model membranes

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Keywords: foldamer, β -aminoacid, lipid membrane.

The β -peptides¹ are the closest homologues of the natural α -peptides, but the insertion of a methylene group between the peptide bonds enhances the freedom of conformation of these systems and allows numerous programmable secondary structures to exist.

By forming distinct architectural conformations², peptide foldamers studies may contribute to understand protein folding in biomedical applications and nanotechnologies.

We have synthesized β -leucine rich heterochiral oligomers containing different charged amino acids by continuous-flow solid phase peptide synthesis³ (CF-SPPS). Peptide purity was determined by reverse-phase HPLC equipped with a Thermo LCQ Fleet mass spectrometer. Peptide1 and Peptide2 showed membrane activity with model membranes DOPC (1,2-dioleoylglycerophosphocholine) and DOPC-DOPG (1,2-dioleoylglycerophosphocholine - 1,2-dioleoylphosphatidylglycerol) and will be used as scaffold to make other foldamers by changing the nature of amino acid and later on, the length.

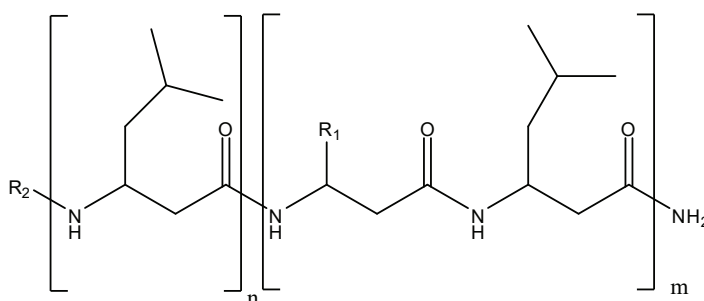


Figure 1 : Peptide1 and Peptide2 structure family
(R_1 = charged amino acid, R_2 = aliphatic chain $n = 0-1$ and $m = 0-2$)

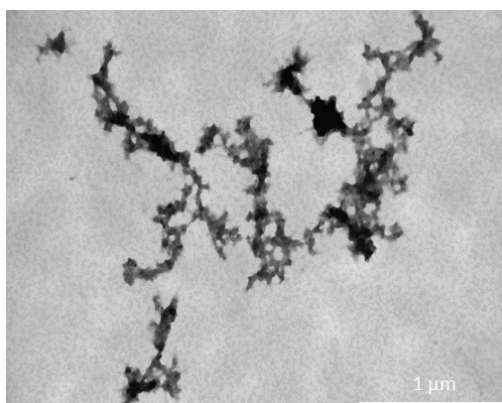


Figure 2 : TEM image of RS3b2K (x60000)

These foldamers show self-assembling patterns (figure 2). In addition, the interaction between these foldamers and lipid model membranes will be characterized by a set of different biophysical techniques such as circular dichroism (CD), dynamic light scattering (DLS), Fourier-transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM).

Here, we will focus on the analogue containing 2 lysines as charged amino acids to understand the behavior of the foldamer with lipid membranes and also to improve the water solubility.

Acknowledgments

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POSTER SESSION

Membrane-active peptide assemblies induced by hemin and its metabolites

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Keywords: antimicrobial peptide, peptide assembly, hemin, conformational change

Basic amphipathic membrane-active peptides target bacteria *via* various mechanisms, the key factor in their action involves attraction to the negatively charged bacterial membrane surface accompanied by peptide assembly coupled with disorder-to-order structural transition. We have recently reported that similar peptide sequestration and conformational change occurred in the presence of small molecules of synthetic and natural origin, among them the endogenous compound hemin and its metabolites interacting with model peptides melittin and its hybrid CM15 [1], and the human antimicrobial and immunomodulatory peptide LL-37 [2]. Extending the investigation to various cationic membrane-active peptides with proven antimicrobial and/or anti-cancer activity to address small molecule effects in membrane environment, our *in vitro* binding assays demonstrated that alteration in peptide structure and activity is dependent mainly on peptide selectivity toward charged lipids, and bile pigment affinity and efficiency to induce peptide folding and aggregation. Morphology of peptide-hemin assemblies was found similar to those detected for other peptide complexes with various partners pointing to a general driving force of aggregation primarily controlled electrostatically by charge neutralization effects. Our results with hemin and its metabolites are in line with previous studies in our group on peptide-lipid-small molecule systems suggesting that small molecule secondary structure regulators can modulate peptide function under *in vivo* conditions providing complex molecular environment.

Acknowledgments

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POSTER SESSION

Quest for Biomarkers in Microbiome Data

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Keywords: microbiomes, metagenome, biomarkers

Microorganisms can be found in almost every environment from deep-sea to high mountains.

Microbiomes are important not only because they have huge metabolic potential due to their extremely large gene pool and heterogeneity, but they form communities and they form an ecosystem with host organisms like a human.

It was found in many studies that human microbiome and specific species have been associated with various diseases like Type 2 diabetes [1] and liver cirrhosis [2]. The advantage of looking at the microbiome as a potential biomarker is it is being non-invasive for certain diseases while providing a cost efficient solution for information about the state of the host.

In this preliminary study, we used data of 4 large-scale microbiome studies of hundreds of individuals such as the Human Microbiome Project (phase 1) with various health-conditions (diabetes (type 2), obesity, healthy, etc.). The studies include both participants living in non-western countries and participants from western countries such as USA, UK, Canada. At first, we were interested in phage composition in the gut, thus the non-faecal samples were discarded from the analysis.

Overall, the largest and one of the first projects was the Human Microbiome Project (first phase) with the goal of identifying and characterizing human microbial flora on various body sites, incorporating mostly healthy individuals. The other projects studied the possible relationships between diseases and microbiome.

Microbiomes are compositional data [3] by their natural and most common interpretation, which is ignored in most of the studies, potentially introducing many false positive identifications.

In this study we applied compositional data analysis methods for the robust identification of potential biomarkers. The results presented here provide a promising base for further studies.

Acknowledgements

The project is funded by Hungarian government grants OTKA 120650 (Microbiome bioinformatics: computer analysis and modelling of complex microbial communities). The research was also funded through the project Thematic Research Cooperation Establishing Innovative Informatics and Info-communication Solutions, which has been supported by the European Union and co-financed by the European Social Fund under grant number EFOP-3.6.2-162017-00013.

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POSTER SESSION

How disease-causing mutations affect different structural elements of PSD proteins

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Keywords: postsynaptic density, disease-causing mutation

The postsynaptic density (PSD) is a specialized part of the postsynaptic neuron, and its primary function is to transduce the information between pre - and postsynaptic cells in the central nervous system. PSD is a complex, continuously and dynamically changing network of more than a thousand proteins with different functions [1]. The delicate balance of structured and disordered protein segments contribute to the maintenance of protein-protein interactions. The binding of different partners are also highly depending on the spatiotemporal control of the cell, often regulated through different posttranslational modifications (PTMs), such as ubiquitination and phosphorylation [2].

Advancements in sequencing technologies resulted in not only the determination of the human genome but also the observation of naturally occurring variations. The phenotypic effect of mutations is on a wide-range spectrum from neutral polymorphisms through disease-causing germline mutations (DCMs) with serious consequences to the quality of life to often lethal somatic mutations. Most mutations are non-synonym single nucleotide variations (SNVs), resulting in an amino acid change on the protein level. Such changes can affect important functional and structural segments of the proteins, perturb their structure and the dynamics, and their outcome often alters different biological pathways required to maintain the synaptic plasticity [3].

Despite our increasingly growing knowledge about inherited SNVs and their outcome, molecular-level understanding how these changes impair protein function greatly lacks behind, especially in the case of PSD proteins giving place to an extremely high amount of PPIs. By investigating those missense mutations that result in the loss of function structurally essential elements can be recognized, giving hints on how diseases emerge.

We used Uniprot [4] as the primary database for both the mutations and the protein sequences. The list of proteins harboring the PSD was obtained from SynaptomeDB [5] and Genes2Cogniton [6]. Structural segments of proteins were determined using webserver, publicly available databases, and a pipeline developed in our research group.

Altogether we investigated more than 2100 germline DMs and more than 1800 polymorphisms in the proteins of PSD. We found DCMs are somewhat more abundant in the PSD compared to the human proteome. We also found that the abundance of DMs in different structural regions differs between PSD and the proteome.

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POSTER SESSION

An improved multi-well plate assay for stress resistance characterization of wild yeast

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Keywords: Yeast, Growth rate, Cultivation, Optimization, Method, Multi-well plate, Stress resistance,

S. Cerevisiae is the most important model organism to understand evolutionary adaptation to man-made environments. To reveal the molecular mechanisms behind the stress resistance evolution against industrially relevant inhibitors, we have to determine the growth rate of the genetically diverse yeast strains in the presence of different stress compounds.

However, cultivation of non-lab yeasts in multi-well plates remains challenging. Non-lab yeasts have versatile physiological response to stress and they often attach to the well-plates or to each other. Such cell behaviour results in an inhomogeneous cell suspension and biased optical density readout during the growth rate determination assay. Therefore, the growth curves of “sticky” genotypes don’t reflect the real cell density change, which decreases the reliability of the final growth rate results. To overcome this issue, we started to develop a modified plate assay.

For testing different measurement settings, we designed a 96 well plate layout with 22 different yeast strains representing the non-lab yeast populations. The evaluation of growth data was based on the CV of calculated strain-specific growth rates. A strain was considered measurable if the growth rate CV was lower than 20% under the given condition. We tested several conditions which might reduce the cell suspension inhomogeneity in the presence and absence of stress compounds, such as different media volume, different surface-treated 96 well plates, shaking profile and intensity, etc.

In the optimized growth rate determination assay we cultivated the cells cultures in 100 µl medium in cell repellent 96 well plates. We apply 500 cpm (medium shaking) as reader settings. With the initial settings we were able to measure 6 genotypes out of the 22 selected ones, while with using the improved method we were able to determine the growth rate of 16 strains out of the 22 genotypes tested in total.

Here we developed a high-throughput method to measure the fitness of the non-lab yeasts avoiding cell attachment during liquid cultivation.

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POSTER SESSION

Identifying ComQXPA bacterial quorum sensing system candidates in reference sequence databases using Hidden Markov Models

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Keywords: cell-cell signalling, quorum sensing, bacterial communication, gene topology, operons, Hidden Markov Model

Introduction

Quorum sensing is a cell-cell communication mechanism the goal of which is to regulate and synchronize cell density dependent behaviours of bacterial cells, such as virulence, bioluminescence, biofilm formation and many more. There are various reasons for studying these systems: for example, as pathogens use quorum sensing for regulating toxin production, development of possible compounds blocking these communication pathways are promising complementary treatments to antibiotics. Genes participating in quorum sensing signalling have specific, conserved topologies within the bacterial chromosome. They are usually located near each other in an operon under common regulation. [1]

Why computational methods?

Due to the properties mentioned above quorum sensing systems are ideal subjects to automated database screening procedures. While the rapidly growing number of publicly available annotated (and unannotated) sequence data (e.g. NCBI RefSeq) can be challenging as a result of its size (since big data requires different approaches), it offers the possibility of a comprehensive census of quorum sensing systems in bacterial genomes. Importance of this is further emphasized by the fact that just a small fraction of bacterial species can be studied under laboratory conditions.

Our approach

Our research group is developing a bioinformatic pipeline suitable for identifying quorum sensing systems in bacterial genomes. The basis of the pipeline is database searching with Hidden Markov Models (HMM). HMM search is a probabilistic method widely used in bioinformatics research. From many similar sequences of a protein coded by quorum sensing genes of different bacterial species an HMM profile can be built with which proteins resembling to the original sequences can be found [2]. After identifying the proteins, the corresponding genes can be found and then mapped to genomes. By examining the neighbourhood of these genes topologies can be found to which labels can be assigned. Then the results can be grouped together and evaluated based on different statistics. One of the main advantages of this method is that unusual topologies are also identifiable, even those that are not described in literature. Besides these it is a very flexible process with many adjustable parameters.

Identifying comqxpA system candidates

One of the quorum sensing systems which regulates competence of a Gram-positive organism, *Bacillus subtilis* is called *comQXPA*. It consists of four genes encoding four proteins: the precursor of the signal peptide ComX which is cleaved by ComQ, the signal binding histidine-kinase ComP and the transcription regulator, ComA. The genes of this system are located on the same strand of the DNA next to each other in a particular,

highly conserved order [3]. With the help of the pipeline developed by our group the goal of the current study was to find the occurrences of this system in bacterial genomes – those which are well-known as well as those not mentioned in literature before. The inputs of the program were HMM profiles for the entire proteins and their characteristic domains, a fresh version of the NCBI Reference Sequence database and a configuration file containing additional information about the system (e.g. characteristic gene lengths).

Since *B. subtilis* and its quorum sensing systems are well-studied, previous research with HMM models of these system exist and can be used for validating the results of the pipeline. In a previous work by Dogsa et. al [4] 60 comQXPA systems were identified with similar methods from which we found all that were possible to. Some contigs were removed from the database and in case of some others, the annotations changed, for example some what was once considered a gene, is annotated now as a pseudogene. In addition to the known ones we found new operon candidates not mentioned in literature before. Due to the exponentially growing sequence databases providing more and more data each year and our improved pipeline becoming more complex, 531 system candidates were identified in total. By examining these putative operons we discovered that 97% of the topologies can be separated into two groups: one of which has an overlap between the *comX* and *comQ* genes and those that do not have any overlap between their genes.

Our conclusion is that although our findings need further study, it is likely that the competence quorum sensing system of *B. subtilis* is more widespread than previously were thought based on laboratory experiments. Our long-term goal is a comprehensive study of all well known quorum sensing systems in available genomic data.

Acknowledgments

The project is funded by Hungarian government grants OTKA 120650 (Microbiome bioinformatics: computer analysis and modelling of complex microbial communities).

The research was also funded through the project Thematic Research Cooperation Establishing Innovative Informatic and Info-communication Solutions, which has been supported by the European Union and co-financed by the European Social Fund under grant number EFOP-3.6.2-162017-00013.

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POSTER SESSION

Computational analysis of structural changes in the antimicrobial peptide CM15 upon complex formation with drug molecule suramin

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Keywords: computational chemistry, molecular dynamics, antimicrobial peptides, CM15, suramin

Dynamic increase of resistant bacterial infectious diseases continuously requires development of novel compounds against them. Understand the molecular level mechanism of natural host-defense peptides or antimicrobial peptides (AMPs) is a crucial step towards rational design and development of new compounds. A particular set of these peptides have disordered structure, the ordering of which may modify their antimicrobial properties. Recent experiments demonstrate that such conformational transitions of AMPs could be mediated by the presence of small organic compounds [1, 2], such as approved drug molecules. However, the molecular mechanisms underlying these structural changes are unclear. To resolve it, we apply molecular docking and molecular dynamics-based approaches to rigorously analyze the interactions between the drug suramin and the AMP CM15, a synthetic unstructured hybrid peptide. We characterize the energetic properties of putative CM15–suramin complexes revealing particular impacts of CM15 residues as well as the parts of suramin on these interactions [3]. We find that α -helical content of the peptide is increased in the presence of suramin, which is in agreement with the experimental data [2].

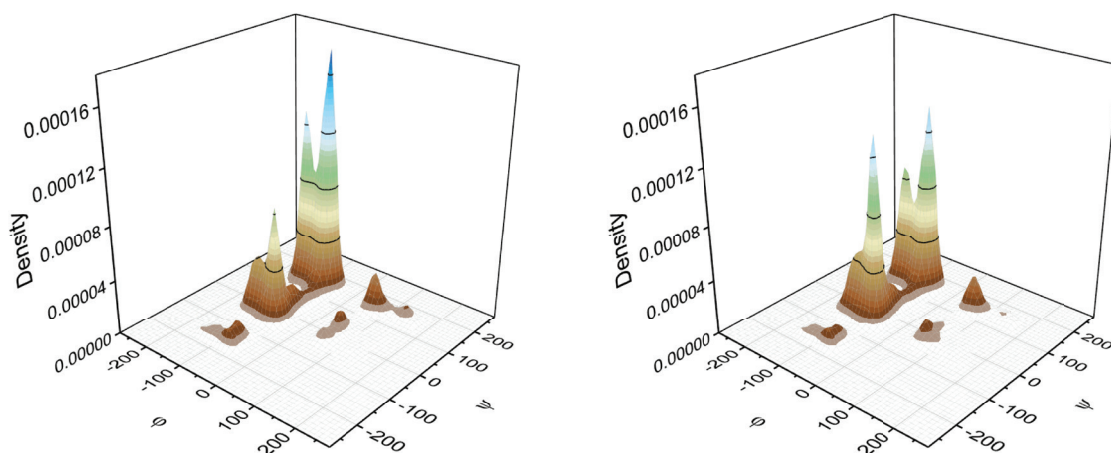


Figure 1. Normalized distribution of the dihedral angles of CM15 without suramin (left) and with suramin (right) proving suramin's ability to increase helicity of CM15

Kinetics analysis from canonical molecular dynamics and metadynamics simulations suggest that the effect of suramin does not promote the formation of α -helix but rather results from its ability to stabilize the α -helical population in the conformational pool of the peptide. To further analyze the system, we also carry out simulations in membrane environment which is considered to be more relevant in terms of the function of AMPs. Results suggest competitive interaction between the three component system leading to aggregation formation

between CM15 and suramin, and embedding of suramin into the membrane. Potentially, understanding the physico-chemical basis underlying the interactions between drug molecules and disordered AMPs both in aqueous phase and membrane environment will prove useful in strategies for antimicrobial compound development. Further on, the given computational protocol for the analysis of such flexible systems provide a basis for future theoretical investigation of similar biomolecular complexes.

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POSTER SESSION

Predicting off-target effects of kinase inhibitors using network propagation on protein-protein interaction networks

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Keywords: microbiomes, metagenome, biomarkers

Combination therapies proved to be efficient in the treatment of complex multigene diseases (cancer, hypertension or diabetes)[1]. However, most combinations were found in tedious empirical ways. Now there is a growing interest for finding interacting drug partners using in silico methods. One potential avenue is the network propagation hypothesis [3,4]: genes or proteins, including those affected by pharmacons, are interlinked by an interaction network, which is perturbed by the action of drugs. Perturbations can propagate (diffuse) via the links of this network; combination effects will result in unexpected accumulation of perturbations at various points of the network. We introduced the concept of Target Overlap Score (TOS) (gross effect of drug combination, the portion of jointly perturbed genes giving a simple [0,1] measure), which uses weighted Jaccard distance to score the drug-drug or drug-disease interactions.

One of the key limitations of the method is that it cannot distinguish between synergistic and antagonistic drug combinations, thus it is difficult to introduce experimental data into the system.

In this work we plan to integrate experimental data available in public databases into network based prediction of drug interactions. The idea is to use a generalized framework of network interactions in which both pharmacon target genes and disease-affected genes are symbolically represented as off-target gene neighborhoods. Known off-target genes can also be added as separate neighborhoods.

In the previous studies the neighborhoods were computationally generated by calculating the diffusion of perturbation around a known target or off-target gene. Here we use experimentally determined neighborhoods extracted from a database of gene expression data, mainly microarray experiments. It is also possible to combine the two principles (i.e. using calculated and experimentally derived neighborhoods) by calculating the diffusion neighborhood around experimentally validated off target genes.

Acknowledgements

The research was funded through the project Thematic Research Cooperation Establishing Innovative Informatics and Info-communication Solutions, which has been supported by the European Union and co-financed by the European Social Fund under grant number EFOP-3.6.2-162017-00013 and ED_17-1-2017-0009 (within the National Bionics Program).

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POSTER SESSION

Continuous-Flow Solid-Phase Synthesis of Photoswitchable Foldamers

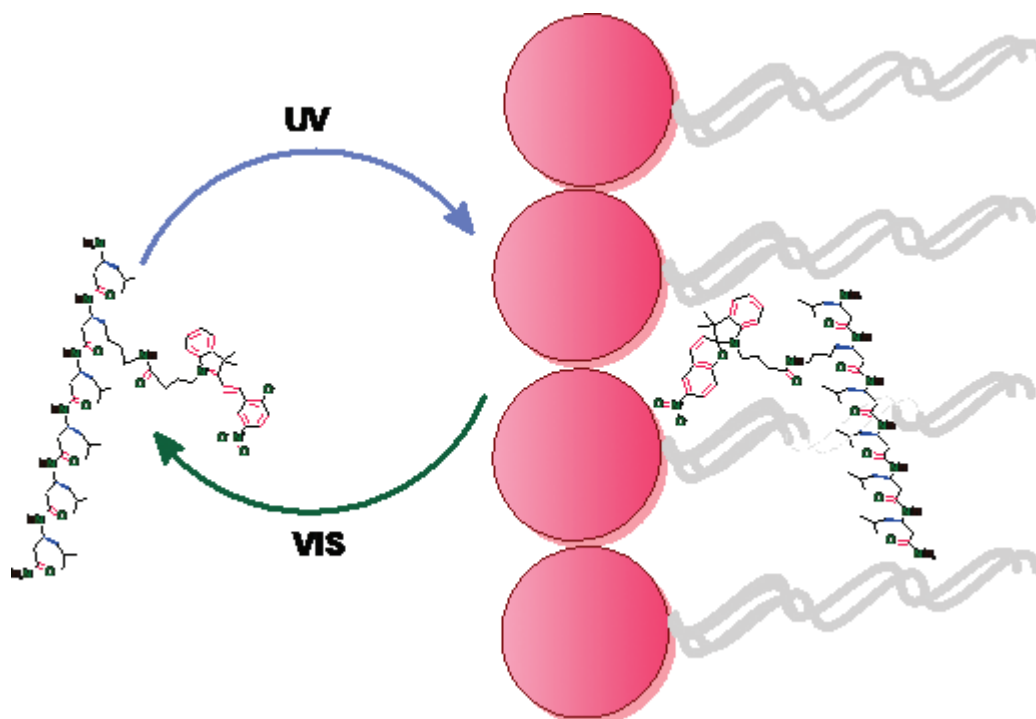
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Keywords: Photochromic switches, Spiropyran, Foldamer, Continuous flow solid phase synthesis.

In recent years the use of light as a stimulus to perform biomolecular interactions has increased^{1,2}. Research on photochromic switches has amplified by its significant application in photopharmacology. Accordingly, research on photochromic switches has been amplified by its significant potential for applications in photopharmacology. Photochromic molecules have an improved lifetime of photoactivated states and are sensitive to a wide range of wavelengths of light. These photoisomers can be used as linkers to attain photoswitchable property and can also adopt predictable conformations. Azobenzenes, diarylethenes and spiropyran derivatives are some of the well-known photoisomers that can be used as photoswitches. New peptide linkers can be synthesized by using photoisomerisable molecules. Photoswitchable peptides can be activated by light either irreversibly or in a reversible fashion. The properties, function and the biological activities of the modified peptides can be transformed by light monitoring. The helicity of the peptides controlled by using UV or visible light is ideal to study the conformational change in the biological system.



Solid phase synthesis using a continuous-flow (CF) reactor becomes increasingly popular among synthetic techniques³. Its effective mixing of substrates, faster heat and mass transfer, and shorter reaction times makes synthesis of peptide derived biomolecules relatively efficient. Using CF reactor solid phase peptide synthesis can be done without difficulty. The main objective of our research is to use continuous flow solid phase technique to synthesize foldamers with spiropyran derivatives in the sequence and to carry out its biophysical characterization. In the initial stage of our work, we have been able to synthesize a selected foldamer

sequence effectively but without spiropyran moiety. The challenge is to optimize the synthetic conditions to stop spiropyran degradation upon coupling it to the foldamer sequence. Photochromes such as azobenzene or Iminothioindoxyl will also be considered for incorporation with foldamer sequence in the future to explore their photo-switch property, conformational change inducing effect, and to investigate the foldamer-photoswitch construct via biophysical characterization.

Acknowledgments

This work was funded by the Momentum Programme (LP2016-2) of the Hungarian Academy of Sciences, the National Competitiveness and Excellence Program (NVKP_16-1-2016-0007) and the GINOP grant (BION-ANO_GINOP-2.3.2-15-2016-00017).

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POSTER SESSION

Bioinformatic Analyses of Proteins Coordinating G2 Size Control in Fission Yeast

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Keywords: G2 size control, phylogenetic analysis, fission yeast, Wee1, Cdc25

G2 phase size control is based on the inhibition of a cyclin / cyclin-dependent kinase (Cdk) heterodimer called MPF (M-phase promoting factor), until a critical cell size is reached. In the G2 phase of the cell cycle, the Tyr-15 amino acid of the regulatory Cdk1 subunit of MPF is phosphorylated by the Wee1 kinase, generating the low-activity form of MPF, called pre-MPF. In late G2 phase, the inhibiting phosphate group is removed by the Cdc25 phosphatase, producing the high-activity MPF form [1]. Cdc25 protein is a dual-specificity phosphatase with an increasing concentration during G2 phase. The unicellular eukaryote fission yeast *Schizosaccharomyces pombe* has cylindrically shaped cells, growing exclusively at the tips with a constant diameter during the mitotic cycle. In this organism, G2 size control is achieved by monitoring cell length via interactions of Pom1, Nif1, Cdr1 and Cdr2 proteins, regulating MPF via the Wee1 kinase [2]. Cdr1 and Cdr2 (together with some further proteins) form large protein complexes in the middle of the cylindrical cells. These ones are called the medial cortical nodes, which can attract and inhibit the Wee1 kinase [3]. Pom1 and Nif1 proteins form spatial gradients, decreasing from the end of the cylindrical cell to the middle. While the cell size is small, the inhibitor concentration is large enough even at the medial cortical nodes to keep the Cdr2 proteins inactive (by Pom1) and Cdr1 proteins inactive (by Nif1), so that they are unable to phosphorylate Wee1. Therefore MPF is kept in its inactive preMPF form and the cell remains in G2 phase. However, as the cell size increases, the concentration of Pom1 and Nif1 decreases in the middle of the cell, so that Cdr2 and Cdr1 become active to phosphorylate and inactivate Wee1, thereby facilitating formation of the active form of MPF, leading to mitotic onset [4].

Here, a search for homologues of these key proteins was performed in the genomes of several eukaryotic model organisms to analyze the evolution of G2 size control. Both the known upstream pathways regulating Wee1 protein (Pom1 → Cdr2, and Nif1 → Cdr1) have been found to be characteristic only in fission yeasts. This is a valuable, but not surprising result, since cylindrical cell shape is very rare in the universe of unicellular eukaryotes. Mik1, a backup copy of Wee1 kinase probably appeared in the common ancestor of the fission yeasts. The duplication resulting in Wee1A and Wee1B isoforms probably happened in a common ancestor of higher animals, while the dual-specificity Myt1 protein (found only in animals) could be a variant between an ancient serine / threonine kinase and the Wee1 tyrosine kinase. Probably both the ancestors of plants and that of fungi may have lost the *myt1* gene. In fission yeasts, Pyp3 is a backup phosphatase of Cdc25, also activating MPF in late G2. Interestingly, we have found that the small lbp1 phosphatase appeared to be a closer homologue of Cdc25, although its function is different. Moreover, Cdc25 homologues identified in plants were found to be more closely related to lbp1 rather than to Cdc25 of fission yeast. In the Cdc25-like proteins, a novel conserved region was found with the consensus sequence LxxG(Y/F) [5].

Acknowledgments

This work was supported by the National Research, Development and Innovation Office of Hungary (NKP-2018-1.2.1-NKP-2018-00005), and the BME-Biotechnology FIKP grant of EMMI (BME FIKP-BIO).

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POSTER SESSION

Identification of transcriptomic changes during brain aging and neurodegeneration using interactome-based Support Vector Machine model

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Keywords: aging, brain, transcriptome, interactome, systems biology, machine learning

Gene expression profiles (both transcriptome and proteome) vary greatly during aging and are associated with cognitive decline and neurodegenerative diseases. High-throughput measurement of the proteome is still technically challenging but transcriptomics can be used to infer proteomics changes. Here, we study human brain aging, Alzheimer's disease (AD) and Age-related Macular Degeneration (AMD) using RNA-seq data available via the GTEx Consortium, Mayo Clinic and Gene Expression Omnibus (GEO). We hypothesized that analyzing gene expression changes in context of the protein-protein interactome (PPI) will lead to more conclusive biological findings than single gene analysis. First, an unweighted and undirected graph was constructed based on PPI catalogs optimized for high reliability (PICKLE, OmniPath). Second, condition-specific weights were assigned to this graph as follows. For each PPI link, a Support Vector Machine (SVM) model was fitted to predict group affiliation (young/control versus old/pathological) of the samples using gene expression data corresponding to the linked proteins. Matthews correlation coefficient between prediction and ground truth was used to assign link weights. Next, in the resulting weighted graph, proteins with significantly elevated eigenvector centrality were selected to test for overrepresentation analysis of Reactome terms.

Within repeated measurements in the GTEx data, our novel approach increased the reproducibility of top gene sets from 9-21% to 36-48%. Furthermore, inclusion of PPI information to the model turned out to be essential to produce Reactome term enrichments in a robust manner.

Neocortical and cerebellar aging showed extensive overlaps with AD and AMD, suggesting the instrumental roles of toll-like receptor, interleukin and EPO signaling together with TGF- β in all (physiological and pathological) forms of neurodegeneration/aging. Alternations of glutamatergic neurotransmission, hemostasis and insulin secretion were more characteristic of physiological brain aging whereas perturbations of adaptive immunity, cell cycle, circadian clock and DNA repair were ranked higher in pathologies. Alzheimer's Disease shared the involvement of senescence, stress response and of inflammasomes with brain aging. The intersection of AMD- and aging-related Reactome term sets contained, among others, axon guidance, apoptosis, VEGF- and PDGF pathways. Overall, those results demonstrate the benefits of our novel integrative approach and emphasize key neuro-immunological and vascular aspects of brain aging and neurodegeneration.

Data analyzed, with sources:

Human brain aging – GTEx Consortium – <https://gtexportal.org>

Alzheimer's Disease – Mayo Clinic – <https://www.synapse.org/#!/Synapse:syn5550404>

Age-Related Macular Degeneration – EyeGEx Database – <https://gtexportal.org>

PPI – OmniPath – <http://omnipathdb.org>

PPI – PICKLE – <http://www.pickle.gr/>

Acknowledgments

Supported by the Hungarian Brain Research Program Grant, No. 2017-1.2.1-NKP-2017-00002

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Milman, Nir Barzilai, Tony Wyss-Coray
Nature Medicine, manuscript accepted for publication.
Preprint: <https://www.biorxiv.org/content/10.1101/751115v1.full>

POSTER SESSION

Cellular Interactions in Yeast Colonies

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Keywords: yeast, *Saccharomyces cerevisiae*, colony growth, cellular communication

After the more thorough understanding of the intracellular processes, nowadays more and more researchers are interested in understanding the interactions occurring between cells. During our experiments, we focus on examining the interactions that arise, in our case, between yeast cells. This is important because in their natural environment yeast strains grow in mixed colonies, while most laboratory experiments of microbial colony growth are focusing on single strains. The most common interaction that occurs in every microbial colony is the competition for nutrients, but besides these microbes can develop various strategies to survive, like cooperating with each other or producing toxins against each other. Synthetic metabolic cooperation has been shown between engineered *Saccharomyces cerevisiae* strains [1], however less is known about natural strains. Rivero et al. [2] identified both cooperation and cheater control mechanism between wild strains of budding yeasts experimentally and these interactions also depend on culturing conditions. In order to get a bigger picture about cell-cell interactions and their molecular background, we examine these interactions between different natural strains of the budding yeast *Saccharomyces cerevisiae*.

Because our experiments are focused on examining the different strains in mixed colonies, the strains must be differentiated somehow. For this, the strains were labelled with two fluorescent proteins EGFP and mCherry, and we also used unlabelled strains as controls. First the unique growth characteristic of each strain was determined. During the experiments the strains were grown individually in SD liquid media with glucose as the carbon source, and they were kept on 29°C in a 96 well plate. A plate reader was used to follow the growth of both labelled and unlabelled strains by measuring optical density and fluorescence. With these individual measurements we also wanted to find out how optical density and fluorescence correlate to each other. By analysing our results, it was found that during the exponential growth phase there is a linear correlation between fluorescence and optical density, so cell number can be determined by fluorescence measurements.

After the determination of the growth characteristic of each strain, the mixed colony measurements were performed. During our experiments we used the Y55 and W303 strains of the budding yeast *Saccharomyces cerevisiae*. Initially we used haploid *a* mating type strains for the experiments, but to check our result we worked with the haploid α types too. Both strains were labelled with mCherry and EGFP fluorescent proteins. The differently labelled strains were mixed together in a 50 -50% ratio in all possible combinations. The mixed colonies made of unlabelled and labelled cells of the same strains were used as controls. The growth of these colonies was also measured by a plate reader the same way as the individual strains and 5 parallel measurements were made at the same time for every mixed colony. Here we mainly focused on the fluorescence measurements to distinguish the different strains in the same colony. From the experimental results the growth curves of the colonies were determined by analysing the obtained data. From these growth curves we draw a conclusion about the interactions occurring between the two strains. We found so far uncharacterised interactions between the investigated strains. The Y55 strain grows slower in the presence of W303 cells, so W303 has a negative effect on the growth of Y55. Furthermore, when grown together with Y55, W303 grows faster than alone. Both the haploid *a* and haploid α mating type strains show this interaction. These results suggest, that W303 wins the competition in this mixed co-culture, but these observations require more detailed validation.

Our main goal, and future plan is to determine the cause of this interaction, whether it is just that W303 is a better competitor for the nutrients, or that it may produce some kind of toxin against Y55. It is also important for us to determine how such interactions between different strains influence the future of mixed colonies. We also started working with mixed strains on solid media to see if the same interactions occur as in the liquid cultures. It is also planned to run similar measurements with different strains on a high-throughput automatized pipeline.

Along with the laboratory experiments an agent-based model is being developed to simulate the growth of single and mixed colonies. Our laboratory experiments are also used to provide better parameters for the model and the model will be used to predict the behaviours of more complex co-culturing experiments.

Acknowledgements

The research was funded by the project Thematic Research Cooperation Establishing Innovative Informatic and Info-communication Solutions, which has been supported by the European Union and co-financed by the European Social Fund under grant number EFOP-3.6.2-162017-00013.

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POSTER SESSION

Modulating Active Structure and Function of Cationic Antimicrobial Peptide CM15 with the Drug Suramin: Towards in Vivo Complexity

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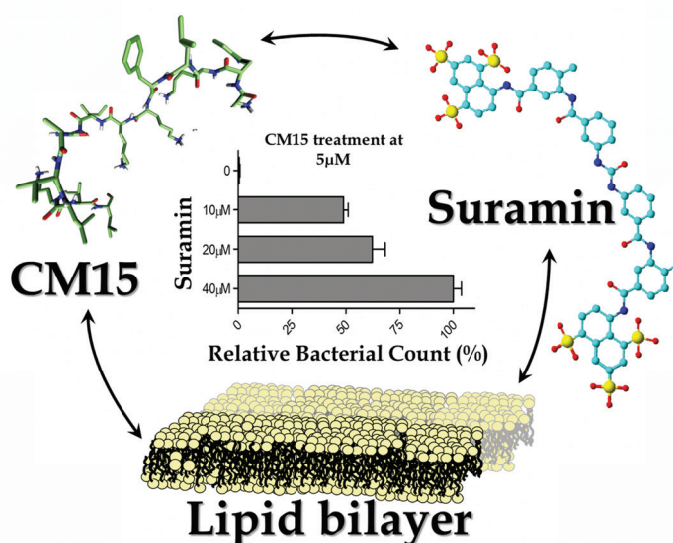
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Keywords: Antimicrobial peptides, circular dichroism, folding, IR spectroscopy.

Antimicrobial peptides (AMPs) kill bacteria by targeting their membranes via various mechanisms involving peptide assembly often coupled with disorder-to-order structural transition [1]. However, similar conformational changes were recently reported in our research group for several AMPs, where small organic molecules of both endogenous and exogenous origin induced folded peptide conformation. Thus, the local complex molecular environment in vivo can significantly affect the function of AMPs and natural host defense peptides; nonetheless, this area is hardly explored. To address the relevance of such interactions on structure and function, herein we tested the effect of the therapeutic drug suramin, a symmetrical, hexasulfonated naphthylurea, on the membrane activity and antibacterial efficiency of a potent hybrid AMP, CM15 (KWKLFKKIGAVLKVL). Results provided insight into a dynamic system where peptide interaction with lipid bilayers is interfered with the competitive binding of CM15 to suramin, resulting in an equilibrium dependent on peptide-to-drug ratio and vesicle surface charge.

In vitro bacterial tests show that when CM15-suramin complex formation dominates over membrane binding, antimicrobial activity is abolished [2]. Based on this case study, it is proposed that small molecule secondary structure regulators can modify AMP function, which should be considered and could potentially be exploited in future development of AMP-based antimicrobial agents.



Acknowledgments

This work was supported through grants provided by the Momentum Program (LP2016-2), the National Competitiveness and Excellence Program (NVKP_16-1-2016-0007) and GINOP (BIONANO_GINOP-2.3.2-15-2016-00017). We also thank the National Research Development and Innovation Office, Hungary (grants OTKA 104275, 115431, 124077).

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POSTER SESSION

Characterization of the interaction between Shank-PDZ and GKAP-GH1 domains

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Keywords: post-synaptic density, Shank, GKAP, interaction

The post-synaptic density

Shank proteins (SH3 and multiple ankyrin repeat domains protein) and GKAP (guanylate kinase-associated protein, also known as DLGAP1) are both abundant components of the post-synaptic density (PSD), a tightly-packed protein complex found in the dendritic region of some types of neurons. The PSD is most commonly observed in excitatory synapses and its function had been connected to processes such as building memories, studying and development of behaviour. Understanding the PSD is therefore a significant step towards deducing the workings of the brain, and also possibly provides several new drug targets to improve yet poorly understood neurological disorders. The level of complexity of the PSD, however, does not allow for viewing it as a single unit, and requires the detailed mapping of the underlying mechanisms. The PSD contains hundreds of different proteins, most of which contain several domains, and the number of interactions those domains are capable of vary. The PSD can be characterized as a network, but a highly complex one, with not nearly enough experimental data available yet to build a sufficient model from. [1]

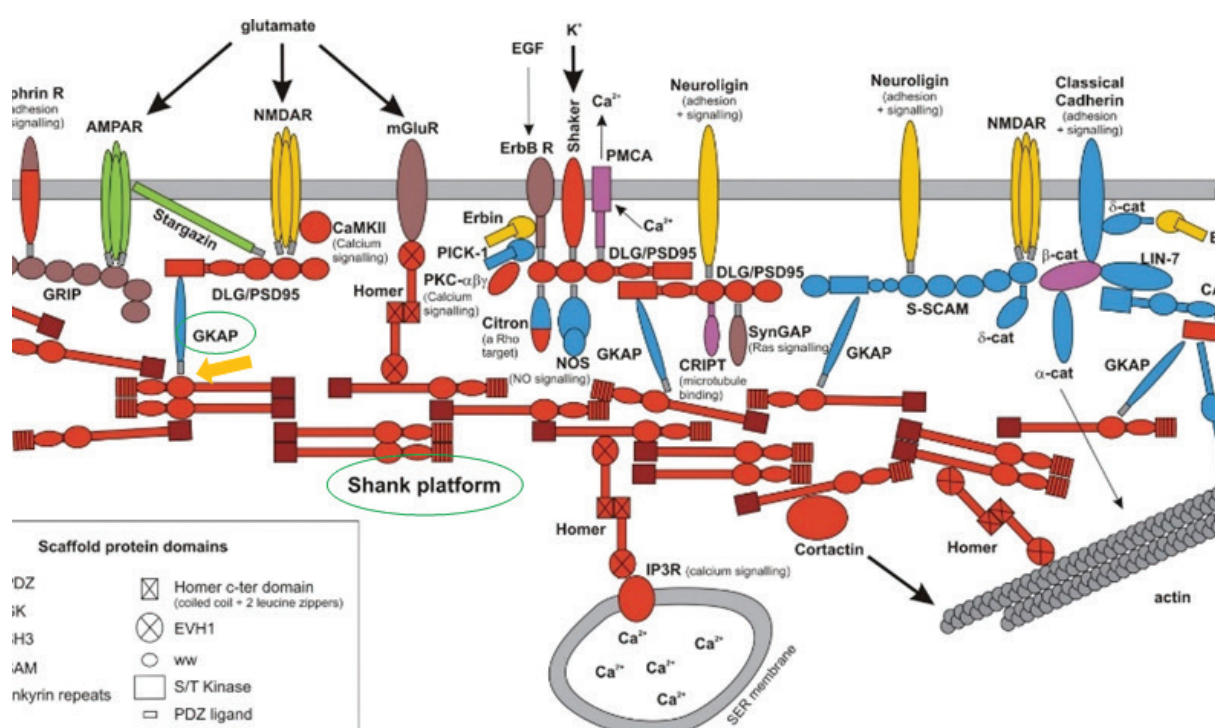


Figure 1. Graphic representation of the postsynaptic density. The interaction in question is marked with a yellow arrow, the two proteins discussed in this paper are circled with green. [2]

Aims

Our group follows a bottom-up approach, in which we first characterize several known domain-domain interactions first by replicating existing results and then create models based on and supported by our own experimental data. The long-term goal of the project is to obtain NMR-based structure models that can be used for molecular dynamics simulations, to build whole-protein models from individual domain data, use bioinformatics to simulate changes of intramolecular behaviour affected by interaction, and generally gain a better understanding of the dynamic behaviour of the PSD.

The domains Shank-PDZ and GKAP-GH1

Shank and GKAP proteins are both characterized as scaffold proteins lacking enzymatic activity, yet playing a role in information processing via building interactions and also contributing to significant morphological changes within the dendrite. Both proteins have multi-domain structures with alternating globular and disordered regions and in some cases, semi-disordered segments. Members of the Shank protein family (Shank1, 2 and 3) all have a characteristic domain composition (from which the protein family's name is derived from), most of which are globular and some proved stable in separated form, while disordered regions can act as linkers or contain binding sites (eg. Homer binding site in Pro-rich region). [3] [4] GKAP is a protein consisting of mostly disordered segments with few globular regions. The overall structure of the full-length protein is not yet fully modelled. [5]

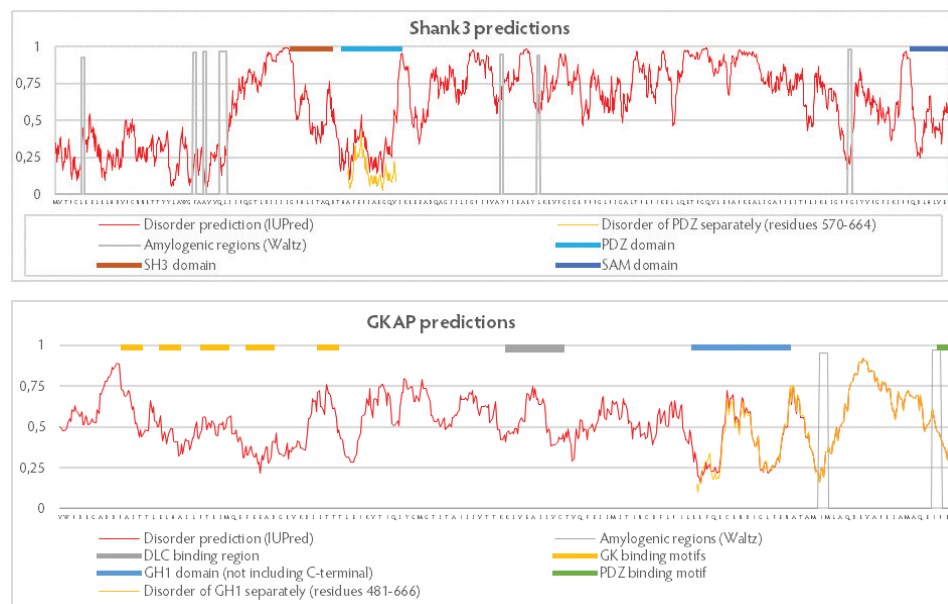


Figure 2. Structure predictions regarding disorder and aggregation probabilities of *Rattus norvegicus* Shank3 (isoform 1) and GKAP (isoform 3) respectively. Sequence segments forming globular domains of significance are also marked.

The particular domains chosen for this study were the PDZ domain of the Shank family and the C-terminal GH1 domain of GKAP, which form the interaction between the two proteins.

Shank-PDZ

The Shank PDZ domains are highly conserved both in the members of the protein family and through species, however they also show significant differences from other known PDZ domains. The Shank PDZ domains are highly promiscuous, known to bind about a dozen different partners using the same binding pocket, however show a uniquely high binding affinity to the C-terminal motif of the GKAP protein. X-ray crystallographic structures of the domain exist, however show contradicting conformations, and NMR-based structures are yet to be solved. [6] [7] [8] [9]

GKAP-GH1

The GH1 domain possesses a semi-globular structure with alternating alpha-helices separated by disordered loops. The exact function of this domain (besides its ability to bind Shank-PDZ domains) remains elusive.

Similarly to Shank-PDZ domains, GH1 is highly conserved among species. An X-ray crystallographic structure of the domain is available, however was heavily altered to achieve the stability required for the measurement. NMR measurements obtained in solution could provide a much better basis for modelling. [5]

This interaction is unique and a good candidate for our experiments for multiple reasons. Via GKAP, Shank is suggested to be in connection with PSD-95 and NMDARs, while on “the other side” interacts with the cytoskeleton, which has straightforward implications for the connection between synapse and cell morphology. [10] Disordered segments are suggested to play a critical role in forming the interaction, and understanding their dynamics might provide an explanation for the much higher binding affinity of GH1 to this PDZ compared to other Shank-PDZ interactions. [8]

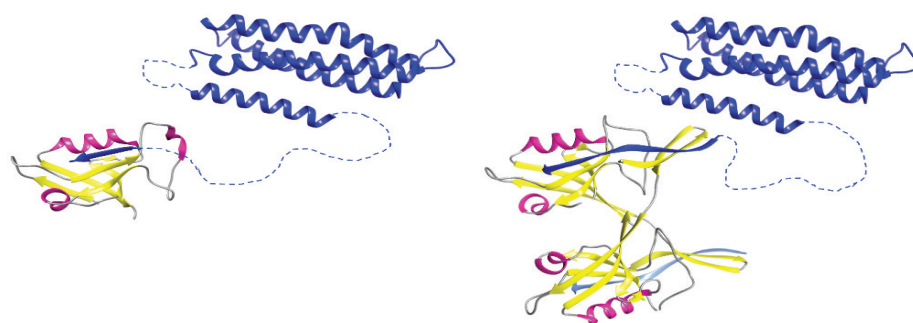


Figure 3. Schematic representations of possible binding conformations of a Shank-PDZ and GKAP-GH1 domain. A. PDZ (yellow-pink) binds GH1 (blue) as a monomer. B. PDZ extended towards N-terminal (yellow-pink) binds longer segment of GH1 (blue) as a dimer. Structures under the PDB IDs 5OVC, 5IZU and parts of 4R0Y were used for illustration purposes. Disordered region of GH1 are drawn with segmented lines.

Experimental results

Recently we have successfully completed the optimization of the protein production and purification process in case of a Shank1-PDZ construct and a GKAP-GH1 (extended to the C-terminal). Further experiments on a second, longer Shank3-PDZ construct (lengthened towards the N-terminal akin to the structure under 5IZU in the PDB [8]) are in progress.

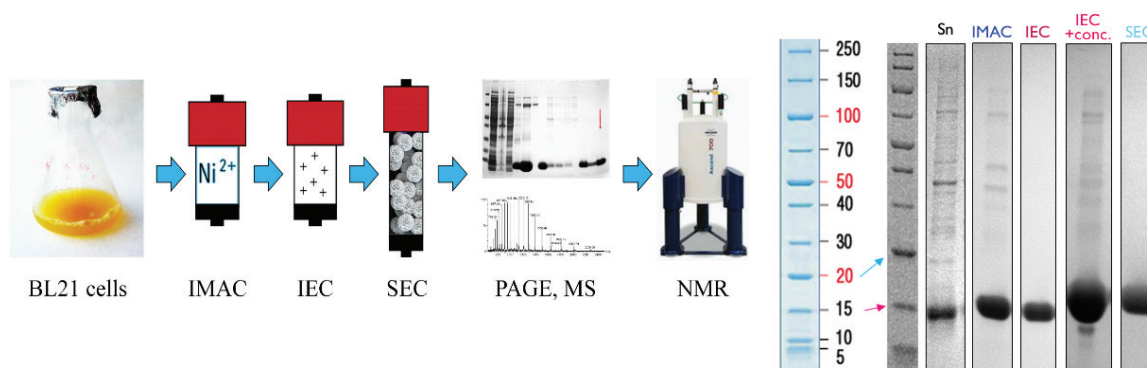


Figure 4. A. Summary of the purification protocol. BL21 *E. coli* cells were used for protein production either in LB broth or M9 minimal media. Cells were sonicated and centrifuged and supernatant was purified using IMAC (separating 6-His labelled molecules), IEC (separating molecules with charge) and SEC (size exclusion) columns. Quality of samples was checked with multiple different methods including SDS-PAGE and MS. Samples of good quality were sent for NMR experiments to the University of Debrecen. B. Montage demonstrating the results of each purification step (on the construct Shank1-PDZ).

NMR and ECD spectra

Unlabelled samples both in case of Shank1-PDZ and GH1 proved suitable for experiments, and preliminary ECD and NOESY NMR spectra were obtained. The results are in alignment with the current knowledge on the domains: both show a well-folded structure and GH1 consists of mostly alpha-helices while Shank-PDZ predominantly forms beta-sheets. Shank1-PDZ retained its stability in isotope-labelled form (^{15}N and ^{13}C non-radioactive isotopes) and therefore was suitable for measuring 2- and 3-dimensional NMR spectra. The HSQC

spectrum further proves that the structure is well-folded as the signals show high dispersion and the pattern is characteristic of a globular protein. The number of the peaks is in agreement with the expectations and resonance assignment is in progress.

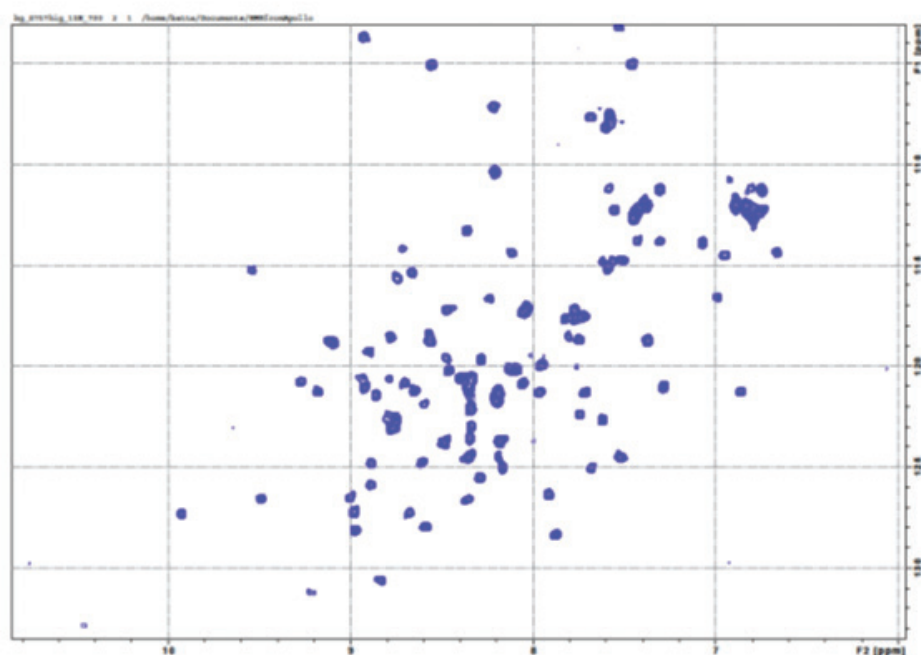


Figure 5. Preliminary HSQC spectrum of the construct Shank1-PDZ.

Interaction experiments

Multiple experiments confirmed the binding of the pairs, in case of both Shank-PDZ constructs. Native PAGE allowed us to observe successful binding both in case of the Shank1-PDZ and Shank3-PDZ constructs with GH1. Mixing all three together resulted in a pattern that suggests stronger binding affinity of Shank3-PDZ. Size-exclusion chromatography experiments suggest a larger molecule in the mixed sample that is not observable in the individual controls. A pull-down assay experiment was carried out in which one of the constructs was labelled with a 6xHis tag, while the other had it removed with TEV protease. Only the construct with the 6xHis can bind the IMAC resin, therefore observing neither proteins in the wash fractions and both in the elutions confirms binding. A preliminary ITC experiment was done with all three protein constructs. Only the measurement with Shank1-PDZ appeared successful. Further experiments with this method are required to obtain data that can support future bioinformatics simulations.

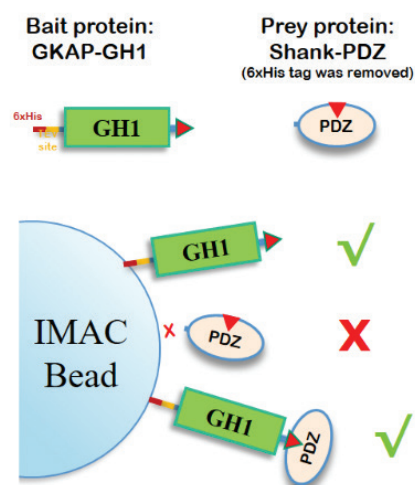


Figure 6. Schematic representation of the pull-down experiment.

Conclusions

Experiments observing the interactions proved promising results and confirmed that the domain constructs are functional. Measurements indicate folded structure. These two observations suggest that the designed domain constructs behave akin to physiological behaviour. Further experiments to measure 3D NMR spectra of all constructs are still required, and NMR measurements of the constructs are among future plans.

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POSTER SESSION

Extracellular vesicles as a complex model membrane reveals new functions of antimicrobial peptides

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Keywords: Extracellular vesicles (EV), Linear dichroism (LD), Circular dichroism (CD), freeze-fracture transmission electron microscopy (FF-TEM) and Liposome.

Extracellular vesicles (EV) have a phospholipid bilayer membrane similar to the cell membrane, with diameters ranging from 40 nm to 5 μ m. They are released by various cell types and have been found in cell culture media, as well as body fluids such as breast milk, urine, amniotic fluids, saliva and blood. EVs are in the focus of scientific interest showing a great potential to revolutionize the diagnosis and therapy of various diseases. However, their size range makes them suitable for membrane biophysics, while they preserve the complex composition of a biological membrane. To test whether new information could be obtained on membrane active biomolecules, we employed antimicrobial peptides and investigated their interactions with EVs.

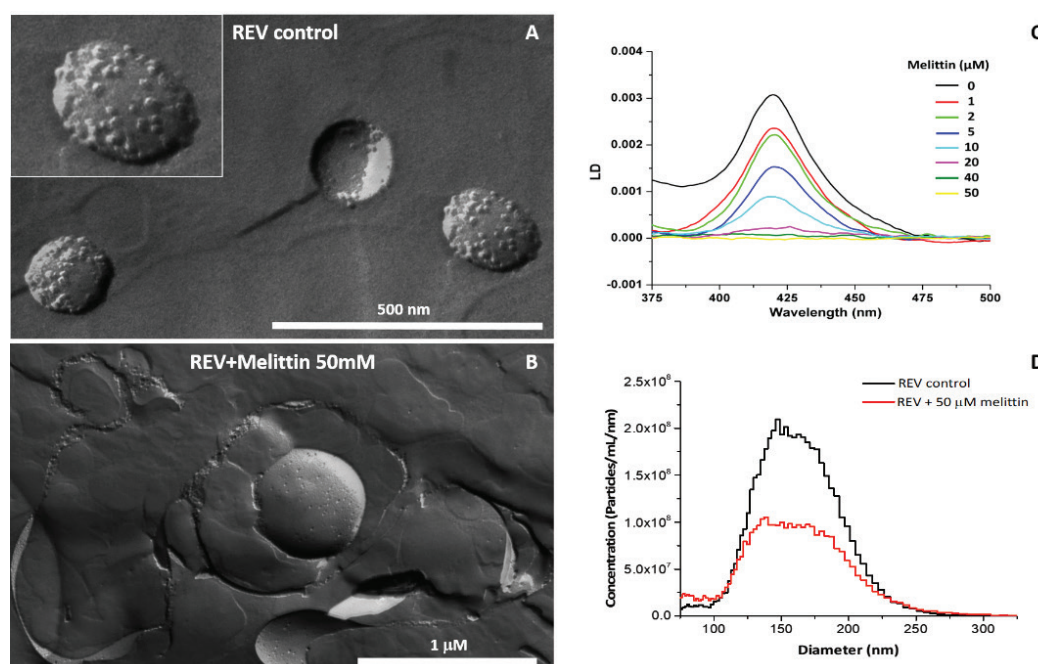


Figure 1: FF-TEM image of freeze-fracture replicas of the isolated EV subpopulations derived from RBCs cells (A) and with the addition of Melittin (B), LD signal changes at the Soret band of REV sample with the successive addition of Melittin (C) and size distributions of the control REV and REV with 50 μ M melittin samples (D).

We demonstrate that red blood cell-derived EVs (REVs) can be used as model membrane systems giving the opportunities to better understand the function and mechanism of natural membrane-active biomolecules. REVs were isolated using a standard protocol and purified by size-exclusion chromatography. We selected three different antimicrobial peptides (AMPs), CM-15, gramicidin and melittin and investigated their interactions

with REVs using different biophysical characterization methods: attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR), linear dichroism (LD)¹ and circular dichroism (CD). Particles size and morphology were characterized by dynamic light scattering (DLS), microfluidic resistive pulse sequencing (MRPS) and freeze-fracture transmission electron microscopy (FF-TEM). The peptide-membrane interactions reveal several novel functions of AMPs, including their affinity to remove adsorbed proteins from the vesicle surface. This is a feature which so far has not been observed for these AMPs, which could not be seen using simple artificial lipid vesicles. Based on results we can conclude that REVs as complex membrane systems provide a better alternative to conventional model membranes to study AMP-membrane interactions.

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CONTENTS

Program	5
FROM PROTEIN COMPLEXES TO SYNAPTIC CONNECTIONS	7
Invited lecture	9
How protein complexes built the synaptome architecture of the brain	9
Integrative modelling of biomolecular complexes	10
Dynamics reveals new aspects of oncogenic KRas	11
Biophysical experiments and biomolecular simulations: A perfect match?	12
Shank synaptic scaffold proteins: keys for understanding the pathogenesis of autism and other synaptic disorders	14
Selected talks	15
Extracellular vesicles as complex model systems for increased understanding on structure and function of membrane active proteins and peptides	15
Ligand binding regulates supramodular dynamics in PSD-95 PDZ12 tandem	17
Spectroscopy based functional structure biology of biomembranes and membrane proteins	21
Calculation of complex structures of protein targets using a fragment blind docking approach	24
Calculation of complex structures of somatostatin receptors	26
Integrative modeling of possible layouts of Kir2.1 localized in membranes and connected by PSD-95	29
Towards improved biophysical models of protein folding to identify disease-causing mutations	33
Nanoscale Bayesian clustering-based characterization of the presynaptic molecular architecture at GABAergic synapses	34
Structural correlates of modular organization of signal transmission in the primate somatosensory cortex	35
Functional and structural investigation of an intrinsically disordered segment of the post-synaptic scaffold protein GKAP	37
CELL COMMUNITIES, CELLULAR INTERACTIONS	41
Invited lecture	43
Yeasts and insects as a model to study the evolution of multicellular interaction networks and the holobiont theory of evolution	43
Uncharted putative G-protein coupled receptor diversity in fungi	44
How metabolites and xenobiotics shape microbial communities	45
High-resolution metagenomic strategies to explore the hidden diversity in human microbiomes	46
Untangling and exploiting the quorum sensing signaling network of <i>Pseudomonas aeruginosa</i>	47
Interpretation of morphogen gradients by a synthetic bistable circuit	48
Cell-to-cell communication via the blood stream: Circulating nucleic acids as molecular disease markers	49
Selected talks	50
Measuring yeast growth for modeling cell-cell interactions	50
Agent-based simulation framework for predicting cell-cell interactions in yeast colony formation	52
A compartmental logical model of the Mitotic Exit Network	55
Structure, dynamics and unfolding of antimicrobial disulfide proteins by NMR	59
NFAP2: A promising antifungal protein in the treatment of <i>Candida</i> infections	60

Anionic Food dyes modulate Mechanism of Action of a Salivary Bactericidal Peptide by forming Aggregates with Enhanced Antimicrobial Activity	62
Sequences optimization of β^3 -peptide foldamers for biomedical application	64
Phospho-switches in the MAP kinase pathway - a case study of ATF2	66
Interplay between folding and binding modulates protein sequences, structures, functions and regulation	68
Exploring the quorum sensing systems in bacterial genomes	70
POSTER SESSION	73
DMSO-induced unfolding of the small antifungal disulfide protein PAF and variants	75
Effects of DNA damage on the cell cycle through the circadian clock	76
Biological noise can be reduced by coupled feed forward loops	78
Continuous-flow solid phase peptide synthesis of foldamer oligomers and its biophysical characterizations with model membranes	81
Membrane-active peptide assemblies induced by hemin and its metabolites	83
Quest for Biomarkers in Microbiome Data	84
How disease-causing mutations affect different structural elements of PSD proteins	85
An improved multi-well plate assay for stress resistance characterization of wild yeast	87
Identifying ComQXPA bacterial quorum sensing system candidates in reference sequence databases using Hidden Markov Models	88
Computational analysis of structural changes in the antimicrobial peptide CM15 upon complex formation with drug molecule suramin	90
Predicting off-target effects of kinase inhibitors using network propagation on protein-protein interaction networks	92
Continuous-Flow Solid-Phase Synthesis of Photoswitchable Foldamers	94
Bioinformatic Analyses of Proteins Coordinating G2 Size Control in Fission Yeast	96
Identification of transcriptomic changes during brain aging and neurodegeneration using interactome-based Support Vector Machine model	98
Cellular Interactions in Yeast Colonies	100
Modulating Active Structure and Function of Cationic Antimicrobial Peptide CM15 with the Drug Suramin: Towards in Vivo Complexity	102
Characterization of the interaction between Shank-PDZ and GKAP-GH1 domains	104
Extracellular vesicles as a complex model membrane reveals new functions of antimicrobial peptides	109
REGISTERED PARTICIPANTS LIST	111