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thus being an emerging target for immunotherapy applications. It has unusual substrate selectivity, preferring longer peptides to shorter ones and its trimming rates are influenced by sequence, although no clear preference motifs have yet to be identified, hindering efforts to predict the enzyme's complex effects on the cellular immunopeptidome. To help understand the mechanism of substrate selection by ERAP1 we set out to crystallize the enzyme in complex with substrate analogues. We used x-ray crystallography to solve two structures of ERAP1 at 1.62 A and 1.68 A with bound two substrate analogues, one 15mer and one 10mer, designed based on ERAP1-sensitive antigenic peptide precursors. The N-terminus of both peptides is found bound in the catalytic site resembling the transition-state intermediate formed during catalysis. Both peptides extend away from the catalytic site, along the internal cavity of the enzyme, making a series of atomic interactions that can influence selectivity. While both peptides extend along the base of domain II towards the domain II/domain IV junction, the 15mer diverges through the central region of the cavity and has its C-terminus stabilized in a pocket of domain IV by Tyr684, Lys685 and Arg807 in a manner reminiscent of carboxypeptidase recognition, while its middle portion is disordered. Analysis of the crystal structures suggests that the mechanism that underlies the unique specificity of ERAP1 revolves around sequence-dependent opportunistic binding in combination with specific C-terminal recognition for longer peptides. Our results provide a framework for understanding how ERAP1 influences the cellular immunopeptidome and adaptive immunity.

P-27-056
How folding and binding intertwine during protein complex formation provides an additional layer of functional regulation

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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. 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 (sequence, structure, function and regulation). High-level cellular processes not only utilize an interconnected network of all three basic interaction modes, but in extreme cases the same disordered region can be involved in different interaction modes. This creates competing interactions, comprising a switching mechanism that chooses between radically different outputs of the same regulatory subnetwork.

P-27-057
Structural and functional analysis of *Candida glabrata* Rpn4-like protein

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Candida glabrata causes 15% of all candidiasis cases. In difference of other pathogenic fungi *C. glabrata* is opportunistic pathogen and closely related to baker's yeast Saccharomyces cerevisiae. One of its unique features is the marked tolerance to oxidative stress and azole antifungals. In baker's yeast Rpn4 transcriptional factor provides tolerance to different kinds of stress including heat shock, DNA damage and oxidative stress. *RPN4* gene deletion leads to sensitivity to fluconazole and amphotericin antifungals. *C. glabrata* has the ortholog of ScRpn4, coding by *CAGL0K01727 g* gene. It's amino acid sequence close to ScRpn4 on 48% but the only similar domain is its DNA-binding region. We cloned this gene and characterized its activity in *S. cerevisiae* background. We have shown that CgRpn4 restores wild phenotype in rpn4-d genetic background in multiple stress conditions. Also we dissected its N-terminal region and found out that similar to ScRpn4 it contains transactivational domain. Unique short N-terminal sequence enriched in glutamine and aspartate residues also involved in its ability to activate proteasomal genes. In presence of oxidative agent 4-NQO CgRpn4 activates directly or indirectly genes involved in oxidative stress response including YAP1 and thioredoxin. In *C. glabrata* cells CgRpn4 expression is induced by heat, methane methylsulfonate and 4-NQO. Potential targets of CgRpn4 which include genes coding proteasome subunits and YAP1 are also induced in these conditions. We propose that in *C. glabrata* cells CgRpn4 is involved in stress response and possibly participates in its pathogenic properties. This work was supported by Russian foundation of basic research grant No. 18-34-00704.

P-27-058
Biochemical and biophysical properties of the human nucleoporin aggregates

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Amyloids are unbranched protein fibrils with a characteristic spatial structure and unusual features including high resistance to detergent or protease treatment. Numerous investigations of amyloids are in the top of interest due to increasing incidence of amyloid-associated disorders, for instance Alzheimer's disease, Parkinson's disease, type II diabetes etc. Previously we analyzed a set of human proteins, which physically interact with huntingtin protein (the expansion of repeats in the corresponding gene is linked with Huntington's disease development) according to BioGRID database, to the ability to form amyloid aggregates, and found one perspective candidate nucleoporin Nupl1 (Nup53). In this work we have shown that the protein forms amyloid-like aggregates in yeast and bacteria cells. For further investigation of amyloid properties of Nupl1 we obtained aggregates of the purified protein *in vitro* and investigated their properties. This analysis revealed that Nupl1 can form fibrils, which are resistant to proteases and detergents, and stained with amyloid specific dyes (Congo Red and Thioflavin T). Taking together these results allow us to suppose that Nupl1 is a new human

amyloid. The research was supported by the Russian Science Foundation (17-74-10159) and research resource center "Molecular and Cell Technologies" of Saint-Petersburg State University.

P-27-059
Amyloidogenic interactions in regulation of autophagy

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Golgi-Associated plant Pathogenesis Related-protein 1 (GAPR-1) functions as a negative regulator of autophagy. The molecular mechanism of this regulation involves retention of Beclin 1, a major autophagy-related protein, at the Golgi. A Beclin 1-derived peptide that corresponds to the potential binding interface efficiently induces autophagy by competing with GAPR-1/Beclin 1 interaction. However, so far a direct interaction between GAPR-1 and Beclin 1 could not be observed. Therefore we hypothesized that GAPR-1/Beclin 1 interactions are based on oligomeric and/or amyloidogenic properties of both proteins. In this study, humanized yeast model system is used to study the amyloidogenic propensity of GAPR-1 and Beclin 1 and to investigate GAPR-1/Beclin 1 interactions. Protein segregation into Fluorescent Foci (FF) in the yeast cytosol has been shown to correlate with the propensity of a protein to form amyloid. Overexpression of GAPR-1 and Beclin 1 resulted in formation of FF in cytosol over time. Interestingly, in co-expression experiments the formation of Beclin 1 FF was inhibited and the number of GAPR-1 FF per cell were reduced, suggesting that the two proteins interact. These effects were efficiently reversed when mutant GAPR-1 or Beclin 1 lacking the suggested binding sites were used. Direct interaction between both proteins was confirmed by bimolecular fluorescence complementation analysis and by Beclin 1 re-localization to the GAPR-1 positive structures using plasma membrane-targeted GAPR-1. Finally, in a proof-of-principle experiment we show that Beclin 1 peptide can efficiently reverse the formation of FF in co-expression experiments, suggesting that GAPR-1/Beclin 1 interaction was interfered by the peptide. Together our results suggest that amyloidogenic interactions are involved in regulation of autophagy by regulating the interaction between GAPR-1 and Beclin 1.

P-27-060
Biophysical study of interaction between the scaffolding protein 14-3-3 and phosphorylated CaMKK2

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Calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2) is a member of the Ca2+/calmodulin-dependent kinase family involved in adiposity regulation, glucose homeostasis and cancer. CaMKK2 is an upstream activator of CaMKI, CaMKIV and the AMP-activated protein kinase (AMPK), and the AMPK:CaMKK2 complex has been shown to regulate energy balance by acting in the hypothalamus. The CaMKK2 is regulated through phosphorylation by various kinases including the cAMP-dependent protein kinase (PKA) and AMPK. It has been suggested that the phosphorylation of residues S100 and S511 by PKA creates two 14-3-3 binding motifs, however the role of 14-3-3 protein in the regulation of CaMKK2 is unclear. The goal of this study was the preparation of CaMKK2 stoichiometrically phosphorylated at S100 and S511 and the biophysical characterization of its complex 14-3-3 using analytical ultracentrifugation and small angle X-ray scattering (SAXS). Recombinant CaMKK2 was prepared as kinase dead mutant containing only two PKA phosphorylation sites Ser100 and Ser511 (CaMKK2 D330A, T145A, S495A). CaMKK2 was expressed in E. coli cells and purified using nickel chelating chromatography and size exclusion chromatography. Phosphorylation of CaMKK2 at S100 S511 by PKA was optimized to achieve stoichiometric phosphorylation at both sites and the result of phosphorylation reaction was monitored using phos-tag SDS-PAGE and mass spectrometry. Next, the interaction between phosphorylated CaMKK2 and 14-3-3 protein was investigated using sedimentation velocity analytical ultracentrifugation. These measurements revealed 1:2 molar stoichiometry between CaMKK2 and 14-3-3 and the binding affinity in low micromolar range. Data obtained from SAXS measurements were used for an ab initio reconstruction of the CaMKK2:14-3-3 complex. This work was supported by the Czech Science Foundation (project 19-00121S).

P-27-061
Investigation of DNA-binding properties of the UxuR and ExuR proteins, regulators of hexuronate metabolism in gammaproteobacteria

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Besides glycolysis that converts glucose into two molecules of pyruvate relieving free energy in the form of ATP and NADH, gammaproteobacteria can get energy from several alternative metabolic pathways induced in stress conditions. For example, *Escherichia coli* (E. coli) can use the hexuronates D-glucuronate and D-fructuronate as the sole carbon sources. These sugars are metabolized by the Ashwell pathway, which generates intermediates that are converted to pyruvate via the Entner–Doudoroff pathway. The hexuronate metabolism in *E. coli* is regulated by two related transcription factors from the GntR family, UxuR and ExuR, which have 46% identity. Using various genomic approaches the binding sites of ExuR and UxuR proteins on *E. coli* chromosomal DNA have been determined and a number of targets for the proteins, including autoregulation sites, have been identified. Bioinformatics analysis of the obtained results allowed to determine DNA consensuses which are specifically recognized by ExuR and UxuR. To measure the binding constants of selected oligonucleotides to the proteins the surface plasmon resonance has been chosen. The affinity of the UxuR protein for DNA fragments containing the region that is supposed to be specifically recognized by the UxuR protein is in the nanomolar range. The ExuR protein forms a less stable complex with a DNA fragment containing the putative region specifically recognized by the ExuR protein. A DNA fragment containing a nucleotide sequence that can be recognized by both proteins binds to proteins with different affinities. These results allow us to obtain stable DNA-protein complexes suitable for crystallization. This work was supported by Russian Scientific Foundation 18-14-00322.

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