Fuzziness in complexes of viral proteins (OTKA NN 106562**)**

Monika Fuxreiter

Summary

The project aimed at investigating the functional, structural and evolutionary reasons of fuzziness in viral complexes. Using experimental and computational techniques, we provided detailed insights into the biological roles and molecular mechanisms of the Measles virus nucleoprotein N_{TAIL} and compared it to the homologous N_{TAIL} segments of Hendra and Nipah virus nucleocapsids. We performed in silico evolution experiments to probe the selection pressure on the fuzziness of N_{TAIL}. We assembled an experimental database of fuzzy complexes, including various viral assemblies and characterized the binding mechanisms, focusing on the role of the fuzzy regions. We used the database as a training set to develop a predictor for intrinsically disordered regions, which do not tend to fold in complexes, i.e. remain fuzzy. We designed Measles N_{TAIL} variants with varying degree of fuzziness and probed their binding properties in vitro.

In addition to investigating specific model systems, we also carried out large-scale studies on viral motifs, using the eukaryotic linear motif and viral databases, to compare the properties of virus-host and host-host protein interactions. We found that fuzziness is a key feature in viral protein interactions, in contrast to the host proteins, where binding elements prefer to be more structured and less flexible. We also studied viral motif interactions with the Mediator, a transcriptional coactivator and related it to development of various diseases. Finally, to understand the regulation of viral capsid formation, we analyzed higher-order assemblies and laid down fundamental principles for self-organization of biological matter and pointed out the role of fuzziness (it was published in Cell).

We published three high-impact papers (IF 45.6, 45.6 and 32.85) and one more is under review. Four additional papers (with IF > 3) were published. The PI gave 16 lectures in international conferences on the theme, students and postdocs presented 3 posters in international meetings.

Detailed description of the results, based on the Aims as specified in the proposal

Aim 1 *Description of the conformational ensemble and binding mechanisms in the nucleoprotein- phosphoprotein complexes in Measles, Nipah and Hendra viruses.*

i) In collaboration with Sonia Longhi's lab, we carried out a series of deletion experiments of the fuzzy Measles virus (MeV) N_{TAIL} to analyze the relationship between the length, dynamic properties and binding affinity to the MeV phosphoprotein XD. The results revealed that interactions improve with shortening of the N-terminal fuzzy region, supporting the excluded volume model of binding. Intriguingly, replacement of MeV N_{TAIL} fuzzy region by an artificial intrinsically disordered (ID) sequence resulted in similar trends, suggesting a sequence-independent inhibitory effect of the fuzzy region. Similar observations were also obtained with another MeV N_{TAIL} binding partner hsp70 as well as for the

homologous N_{TAIL}/XD pairs from the Nipah and Hendra viruses, supporting the generality of the model.

Publication: Gruet A et al. (2015) Fuzzy regions in an intrinsically disordered protein impair protein-protein interactions. *FEBS J.* 283, 576-594.

Presentations: IDPbyNMR conference (Grossetto, Italy, 2014), Non-globular proteins meeting (Porto, Portugal, 2015).

ii) We assembled an experimental database of fuzzy complexes (FuzDB, protdyn/database.org) including many viral assemblies. At present 108 entries with *structural evidence* demonstrating the structural multiplicity or dynamic disorder in the bound form of the protein and *biochemical, functional evidence* supporting that the heterogeneous region impacts the formation, regulation or activity or the complex are included. The FuzDB also analyzes the structural data to provide detailed binding mechanisms. A general model of viral protein interactions have been provided, elaborating the roles of the fuzzy regions.

Publications:
Sharma R, Raduly Z, Miskei M, Fuxreiter M (2015) Fuzzy complexes: Specific binding without complete folding. *FEBS Lett*. 589, 2533-42.

M. Miskei, Cs Antal, M. Fuxreiter FuzDB: database of fuzzy complexes, a tool to develop stochastic structure-function relationships for protein complexes and higher-order assemblies, *Nucleic Acids Research*, under review

Presentations

ASBMB conference (Boston, USA 2015), IUBMB-37th FEBS meeting (Sevilla, Spain, 2012), Lorne Conference on Protein Structure and Function (Lorne, Australia, 2013), Max Perutz Laboratories (Bécs, Austria, 2016), Biozentrum, Bázel (Switzerland, 2015)

Aim 2 *Designing N_{TAIL} constructs in Nipah and Hendra viruses with varying degree of fuzziness and probe their binding efficiency.*

i) We performed bioinformatic analysis on Measles, Hendra and Nipah virus N_{TAIL} to identify intrinsically disordered binding (IDB) sites, which are likely engaged in partner interactions and also adopt a well-defined structure upon binding. The predicted IDBs were in accord with experimentally characterized regions. We analyzed different parameter sets and wide range of conditions to distinguish between ID regions that fold and those, which remain disordered in the bound form. We developed a simple predictor for these three viral systems, to identify fuzzy regions with quantitative scores to characterize fuzziness (per residue). This specialized predictor has been employed to i) design variants with varying degree of fuzziness/dynamics ii) to assess selection pressure on fuzziness. The computational results indicated an evolutionary pressure to maintain fuzziness of the segments, which flank the interaction sites (denoted as Boxes in the MeV literature). This is in accord with the experimental results on truncated variants.

MSc thesis: The role of fuzziness in complexes of viral proteins.

ii) We designed MeV, Hendra and Nipah MeV N_{TAIL} variants with varying degree of fuzziness/dynamics. Modifications either destabilized the predicted interaction sites or stabilized the flanking fuzzy regions. Results obtained on deletion variants with distinct dynamic properties have been published (*FEBS J.* 283, 576-594) in collboration with Sonia Loghi's lab. Testing of the variants with modified dynamics is still ongoing in our lab.

Presentations: FEBS3+ conference (Portoroz, Slovenia, 2015), Protein Society meeting (Barcelona, Spain, 2015), Japanese Protein Society meeting (Tokushima, Japan, 2015), ASBMB conference (Boston, USA 2015).

iii) We dissected the FuzDB database into a training set and a test set, and initiated development of a general predictor for fuzziness. We analyzed distinct physico-chemical parameters for folding and fuzzy regions and derived three features, which could distinguish between the two ID region types. We also assembled a larger database from PDB with evidence for disorder in both monomeric and complex forms, to test the general fuzzy predictor.

Poster: Gordon Research Conference on Intrinsically Disordered Proteins (Les Diablerets, Switzerland, June 26- July 1, 2016)

Aim 3 *Studying how small-molecule inhibitors bind to N_{TAIL} of the Nipah and Hendra viruses and how they impact fuzziness.*

i) Using our computational tools, we localized those mutations, which significantly affected the dynamic properties of MeV N_{TAIL}. The resulted consensus disorder and fuzziness profiles were employed as a selection criteria to conduct in silico evolution simulations. The function representing the selection criteria had been calibrated previously using the experimental results on variants with enhaced/decreased binding affinities (see Aim 1). Mutations to increase or decrease the binding affinity of MeV N_{TAIL} for XD were predicted. HTS assay had been established in Sonia Longhi's lab to probe the impact of these positions on inhibition of the interaction and virus replication.

Publication: Thangaraju K et al. (2016) Amino Acids

ii) We analyzed dynamic viral interaction sites in the Mediator with the goal to develop small molecule inhibitors. Pathogenic viruses (e.g., E1A, herpes simplex VP16, Kaposi's sarcoma associated virus) attack gene-specific regulatory sites in Mediator and reprogram the host cell transcription machinery. We performed a high-throughput bioinformatic study on Mediator sequences to analyze the structural features of the viral target sites and compared them to experimental data. In most cases, we found that these regulatory sites are located either in fuzzy regions, or in IDBs, which were flanked by fuzzy regions. We hypothesize that modulating the dynamic properties of the fuzzy regions could alter their binding preferences to unfavor the viral sequences. We also provided specific target sites for drug development.

Publication: Fuxreiter M, Tóth-Petróczy A, Kraut DA, Matouschek AT, Lim RY, Xue B, Kurgan L, Uversky VN. (2014) Disordered proteinaceous machines *Chem Rev.* 114, 6806-6843

Presentations: EMBO Practical Course on Biomolecular Simulations (Paris, 2015)

Aim 4 *Studying viral proteins, which mimic the motif-mediated protein-protein interactions of the host and characterize structural features of the flanking regions. Probing potential regulatory pathways involving viral proteins with fuzzy regions.*

Viral proteins have been proposed to hijack the eukaryotic regulatory system by mimicking linear protein interaction motifs. We aimed at understanding why viral motifs are more efficient than the host motifs, given the same interaction site. We performed bioinformatic analysis using the viral motifs of the eukaryotic linear motif database (ELM) as well as those on the Mentha database. We compared the structural and dynamic properties of the viral motifs to those of the host proteins. We also generated a subset of motifs, which target the very same domain based on experimental evidence. We found that the properties of the viral and host motifs are largely different, and the human motifs tend to be less structured than their viral counterparts. Comparing the motif properties to those of the flanking sequences, a marked difference was observed. Host motifs were more stable than their embedding environment, and likely serve as molecular recognition elements. Viral motifs in contrast, are more flexible/dynamic than the flanking regions and do not tend to fold upon binding. We found that fuzziness is a key feature of viral motif binding, that results in improvement of the thermodynamic parameters of binding as well as imparts promiscuity for many different targets.

Publication**:** Duro N, Miskei M, Fuxreiter M (2015) Fuzziness endows viral motif mimicry. *Mol Biosyst.*, 11, 2821-29.

Presentations: EMBO ESF conference on PPIs (2013), Hiroshima University (Hiroshima, Japan, 2015), Protein Society meeting (Barcelona, Spain)

Poster: Gordon Research Conference on Intrinsically Disordered Proteins (Les Diablerets, Switzerland, June 26- July 1, 2016)

General aspects/conclusions

Viral capsids are assembled by a self-organization process. These higher-order structures were observed to undergo biologically relevant phase transitions, and become a hot topic during the grant period. We performed theoretical analysis to understand the physical properties of the higher-order assemblies, and elucidate the basis of organization, as well as the regulation of their formation. Applying the fuzzy concept, and using polymer physics approaches, we developed a general, theoretical framework for higher-order assemblies.

Publications:

Wu, H, Fuxreiter M (2016) The Structure and Dynamics of Higher-Order Assemblies: Amyloids, Signalosomes, and Granules. *Cell* 165, 1055-1066.

van der Lee R, et. al. (2014) Classification of Intrinsically Disordered Regions and Proteins. *Chem Rev.* 114, 6589-6631

Presentations: Protein Society meeting (Barcelona, Spain), ASBMB conference (Boston, USA 2015)