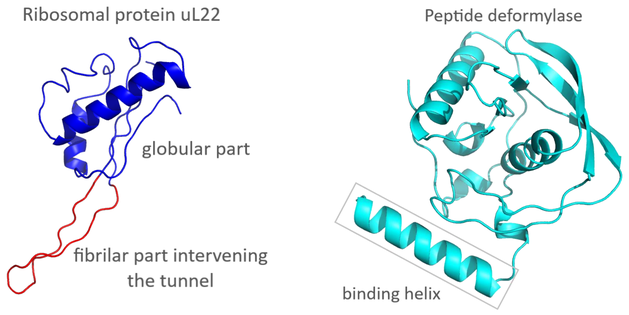
Structural and Simulation Analysis of Ribosomal Tunnel Proteins

Michaela Černeková, Hugo McGrath and Michal H. Kolář

Department of Physical Chemistry, University of Chemistry and Technology in Prague

MOTIVATION

Ribosomes contain exit tunnel through which nascent protein leaves the particle. Inside the exit tunnel, there is a constricted part formed by protein uL22 and uL4 (in Bacteria) The protein uL22 contains, besides the loop inside the tunnel, also globular part at the surface of the ribosome interacting with non-ribosomal protein, peptide deformylase (PDF). Atomistic computer simulations represent a convenient tool to study ribosome structure and dynamics [Bock et al. 2018]. Here we use them to address a question, whether behaviour of the ribosomal protein uL22 inside the tunnel can be allosterically affected by interactions with PDF. To this aim, we performed multiple simulations of three ribosomal systems and analyzed their trajectories on the submicrosecond time scale.



METHODS

A. Model of the Ribosome with PDF

- using Protein Data Bank structures 4V5B and 2AI8
  - system “NONE” - the ribosomal part only
  - system “HELIX” - the ribosomal part and the binding helix of PDF
  - system “WHOLE” - the ribosomal part with whole PDF
- cutting out the models and preparation for simulations

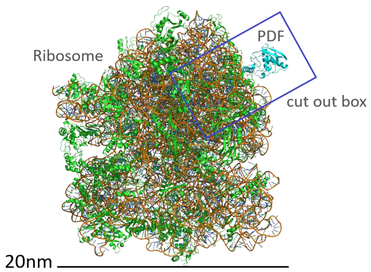
B. Molecular Dynamics Simulations

- for each system, 8 independent MD simulations were done:
  - force fields: ff10 and ff12SB, SPC/E, Joung&Cheatham
  - Gromacs 2019, GPU accelerated
  - 8x330 ns/traj per each system

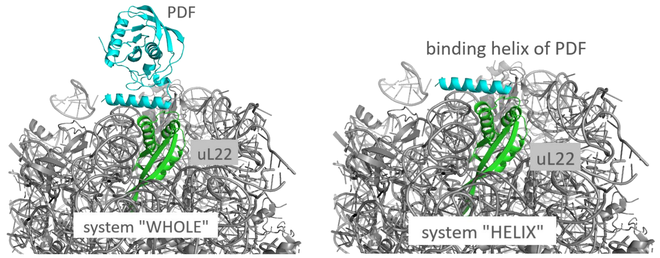
C. Analysis of Protein Data Bank Structures of the Protein uL22

- extracting the protein from the ribosomal structures and generating one multistate model
- principal component analysis (PCA) of the C-alpha Cartesian coordinates
- sequence conservation analysis using Clustal Omega webserver

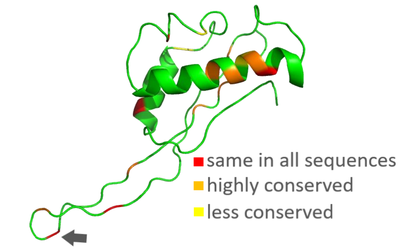
RESULTS



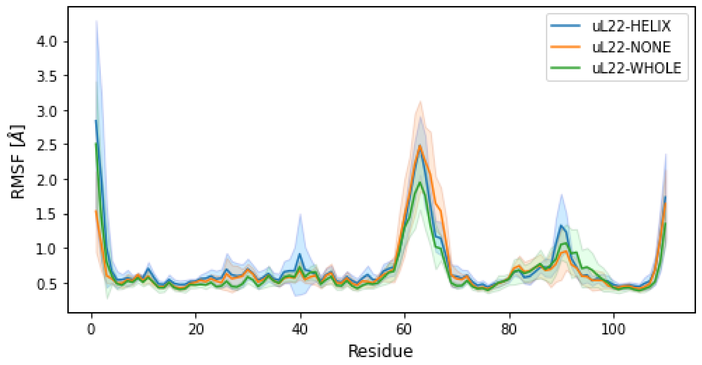
Representation of cut out box.



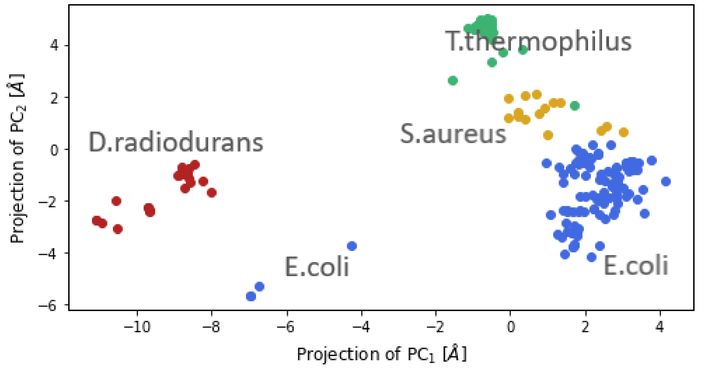
Comparison of the system containing whole PDF with the system containing just binding helix of PDF.



Some of the most conserved amino acids are placed in the loop intervening the tunnel (pointing arrow).



Root mean square fluctuations of uL22 backbone averaged over 8 independent trajectories.
Shaded areas represent standard errors of the mean.
The most flexible part, besides the termini, is the loop at the surface of the ribosome (around residue 60) and the loop intervening the tunnel (around residue 90).



Principal component analysis of uL22 from Protein Data Bank. 202 structures were analysed and we can see clusters of 4 different types of Bacteria. One of the E.coli structures outside their cluster is 4V5B.

CONCLUSION

- challenging molecular dynamics simulations were done with the unique model
- parts of the protein uL22 seem to be sensitive to presence of PDF
- sequence conservation analysis showed some amino acids in the tunnel as highly conserved

OUTLOOK

- advanced analysis (PCA, distance matrix)
- biased simulations

REFERENCES

GROMACS: M.J. Abraham, D. van der Spoel, E. Lindahl, B. Hess, and the GROMACS development team, GROMACS User Manual version 2019, http://www.gromacs.org.

4V5B: Bingel-Erlenmeyer, Rouven, et al. "A peptide deformylase–ribosome complex reveals mechanism of nascent chain processing." Nature 452.7183 (2008): 108-111.

2AI8: Smith, Kathrine J., et al. "Structural variation and inhibitor binding in polypeptide deformylase from four different bacterial species." Protein Science 12.2 (2003): 349-360.

Bock, Lars V., Michal H. Kolář, and Helmut Grubmüller. "Molecular simulations of the ribosome and associated translation factors." Current opinion in structural biology 49 (2018): 27-35.

ACKNOWLEDGEMENT

The project is supported by the Czech funding agency (GJ19-06479Y). MČ acknowledges a funding by the Internal Grant Agency of UCT Prague.