WP3.5. Engineering of peptide scaffolds disrupting protein-nucleic acid interactions – a case of EST and HIF-1 transcription factors involved in cancer. (Vondrášek)

Research aims

- Bioinformatic analysis of ETS/HIF-1 transcription factors interacting with DNA from PDB and 3D FootPrint databases
- Construction of consensual sequence recognition pattern and identification of DNA binding domain scaffold
- Synthesis of DNA binding scaffold derived from ETS/HIF-1 proteins and structural and biophysical characterization of the interaction
- Design and synthesis of low-molecular peptide ligands based on structural and sequence parameters obtained from ETS/HIF-1 – DNA complexes.

Research plan and methodology

The following combination of methods for the study of protein -- DNA interactions in EST and HIF-1 transcription factors will be used. It should create a single workflow allowing the development of DNA-binding ligand competing with transcription factors on a sound, physical basis. In particular, we aim to utilise and extend the current knowledge about the biophysical characteristics of protein -- DNA interactions. In details we will proceed according the following strategy:

- **Analysis of structural and thermodynamic databases** and selection of candidates for further rational screening. In this bioinformatic part of the project we aim to systematically analyse the 3D structures of ETS/HIF-1 -- DNA complexes deposited into the RSCB PDB database [1] and utilise the binding characteristics of both transcription factors obtainable from the 3D-footprint database [2] and from the literature to be further analyzed and studied using the experimental and theoretical methods. In particular, we aim to map correlations between the amino acid composition of the ETS/HIF-1 interaction interface, the recognised base sequence of the DNA molecule, and the thermodynamic characteristics of the interaction. We aim to focus on covariations involving larger blocks of length from 2 to 5 residues, and couple these statistics to the thermodynamic data available. These peptide residue blocks, as well as the identified proteins and their shorter interfacial fragments will be further candidates for computer simulations and experimental measurements.
- **Validation of computational approaches** to the study of peptide dynamics and protein (peptide) -- DNA interactions. We aim to perform explicit solvent MD simulations of free proteins, peptides and protein/peptide -- DNA complexes in order to assess the validity of the currently used force fields and simulation protocols for studying peptide dynamics, for which no authoritative consensus about the validity of the methods had been established [3]. In addition, we aim to compare the traditional free energy perturbation approach to calculating the binding free energies to the modern non-equilibrium simulation-based methods. Data regarding the performance, accuracy, and convergence characteristics of the latter are currently sought [4,5]
- **Biophysical and structural characterization of the peptides and peptide -- DNA complexes** using ITC and MST methods in combination with Electronic Circular Dichroism (ECD) and NMR methods. We aim to study the fundamental biophysical characteristics of EST/HIF-1 -- DNA interactions using the minimalist approach involving protein peptide fragments derived from binding interfaces. The measured data will also serve as a validation of the values of thermodynamic properties obtained from computer simulations. Last but not least the structural assessment of the studied complexes by NMR method will allow us to verify a relative importance of particular amino acid at the binding interface.
- **Exploration of the stabilisation of the bound state-like conformation** as a means of improving the binding affinity. We aim to explore how the conformational equilibrium of a free peptide can be shifted towards conformations which resemble the conformation of the state with bound DNA [6] and whether this shift affects the DNA-binding affinity of the peptide. In particular, if we could show that the stabilisation of the bound state-like conformations significantly improves the binding of the proteins, then a systematic strategy for improving

binding affinities of biomolecules will be developed. Biochemical linkers introducing cyclisation into the peptide backbone, physical effects, and solution additives will be tested as possible conformation-shifting agens. The perturbed systems will be studied first using computational methods followed by experimental methods.

[1] Bernstein, F.C., Koetzle, T.F., Williams, G.J., Meyer, E.F., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, M., *Eur. J. Biochem*., **1977**, *80*, 319–324.
[2] Contreras-Moreira, B., *Nucleic Acids Res*., **2010**, *38*, D91–D97.
[3] Rauscher, S., Gapsys, V., Gajda, M.J., Zweckstetter, M., de Groot, B.L. and Grubmüller, H., *J. Chem. Theory Comput*., **2015**, *11*, 5513–5524.
[4] Goette, M. and Grubmüller, H., *J. Comput. Chem*., **2009**, *30*, 447–456.
[5] Seeliger, D., Buelens, F.P., Goette, M., de Groot, B.L. and Grubmuller, H., *Nucleic Acids Res*., **2011**, *39*, 8281–8290.
[6] Jen-Jacobson, L., Engler, L.E. and Jacobson, L.A., *Structure*, **2008**, *8*, 1015–1023.

Research schedule

2017-2018

- Bioinformatics analysis of structural and thermodynamic databases for ETS/HIF-1 transcription factors, MD simulations and calculations of binding affinities

2019-2020

- Gibbs free energy change determination using ITC Microcalorimetry, Binding constant determination using Microscale Thermophoresis and VCD Spectroscopy

2017-2020

- ECD Spectroscopy and structural studies using NMR spectroscopy

2020-2022

- Total chemical synthesis of the low molecular molecules/ peptides and shifting the conformational state equilibria.

Publications and patents

Publications (Jimp)

	Jimp
2017	0
2018	2
2019	2
2020	2
2021	2
2022	2
Total	10

Nucleic Acids Research
Chemistry a European Journal
Protein Science
Journal of Chemical Theory and Computation
ACS Chemical Biology
ChemBioChem
Structure
Journal of Physical Chemistry

Cooperation with foreign institutions

- University of Stockholm, DNA-Protein binding assays development
- University of North Carolina, Chapell Hill, Free energy perturbation approach, non-equilibrium thermodynamics