AQS3 SEE CHANGE IN PROTEIN CHARACTERIZATION

Patrick King

Specion/Redshift

Infra-Red (IR) analysis has been long accepted as a powerful tool in protein characterization, particularly in the Amide I band (~1600 - 1700 cm-1), which gives detailed secondary-structural information that can be critical in determining protein structure-activity relationships, stability, batch-to-batch comparisons and in formulation studies as a few examples. Technologies traditionally used for secondary structure analysis, such as benchtop Fourier Transform IR (FTIR) or Circular Dichroism (CD), suffer from a number of issues that have prevented their routine use in this area, preventing this application from reaching its full potential. These include concentration and buffer restrictions, incompatibility with a range of excipients, a lack of automation, low spectral reproducibility and for FTIR, water subtraction problems.

Microfluidic Modulation Spectroscopy (MMS) is a new key technology that was brought to market in 2019 by RedShift Bioanalytics. It focuses on the IR Amide I region to produce exceptionally high data quality and reproducibility that aim to solve the aforementioned issues encountered with traditional technologies. It is fully automated, running samples from 24- and 96-well plates, compatible with a very broad concentration range (0.1 to >200 mg/ml), and is also compatible with a wide range of complex buffer systems and excipients, including those that absorb in the amide I region, surfactants and organic solvents. The platform includes a powerful software package that facilitates data analysis, and can be included in the automation procedure. This presentation highlights the technical benefits of MMS and its application in the protein structural workflow, giving relevant application examples.

Thursday, March 19, Session II

L5

STRUCTURAL ALPHABET FOR STRUCTURAL ANALYSIS OF NUCLEIC ACIDS

Bohdan Schneider and Jiří Černý

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A newly developed structural alphabet [1] is the first alphabet universally applicable to both types of nucleic acids, RNA and DNA. In the talk, I will demonstrate some of the applications of the alphabet. It can be used to statistically weight sequence preferences of different conformational forms and to discover RNA and DNA structural motifs. The alphabet is based on 96 dinucleotide conformational classes, NtC, that were identified by analysis of structures of ~60 thousand RNA and ~60 thousand DNA steps. The resulting automated assignment of the conformational classes to any nucleic acid structure is available at the website dnatco.org [2] and is an extension of our previously published algorithm assigning only DNA structures [3].

1. Božíková et al., to be published 2020.
2. Černý et al., Nucleic Acids Research 2016, 44, W284.
3. Schneider et al., Acta Cryst. D 2018, 74, 52-64.

L6

PHYLOGENETICALLY DIVERGED STRUCTURAL MOTIFS IN TELOMERIC DNA

M. Gajarský[1,2], M. Lenarčič Živković[3], K. Beková[1], J. Plavec[3], L. Trantírek[1]

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Telomeres, specialized nucleoprotein complexes, cap and protect the physical ends of chromosomes and are essential for genome stability. Telomeric DNA buffers the end replication problem caused by the DNA polymerases inability to copy the very end of the molecule.[1] In vast majority of somatic cells this chromosome shortening inevitably leads to cell death, however, in germ, stem, and cancer cells it is countered by telomerase, or ALT pathway (Alternative Lengthening of Telomeres).[2,3]

Full understanding of biological role and functioning of telomeres is therefore a crucial part of cancer and ageing research. Our focus is on structural behaviour of telomeric DNA. Its typical features in all eukaryotic kingdoms are single stranded, guanine rich 3'-overhang, conserved repeats of primary sequence ($T_{2\text{-}4}AG_{2\text{-}3}$) and inherent ability to adopt a non-canonical DNA structure, G-quadruplex.[4]

Our study focuses on the aberrant examples in phylogenetic tree, in hope to better understand conserved characteristics crucial for proper functioning of telomeres. Despite apparent strong conservation of DNA primary sequence in telomeric repeats and capacity of G-quadruplex formation present in almost all species (see Figure 1), our data suggest that the crucial characteristic of telomeric DNA that is preserved throughout evolution is not the primary sequence, nor the G-quadruplex folding, but the ability to form various, stable, non-canonical structures in general. Considering abundance of unusual non-canonical motifs recently discovered on telomeric sequences, telomeres are also an extremely useful source of information about structural behaviour of the DNA molecule for future biotechnological, drug-target, or aptamer research.

1. Greider, C. W.; Blackburn, E. H. *Sci. Am.* 1996, **274** (2), 92–97.
2. Greider, C. W.; Blackburn, E. H. *Nature* 1989, **337** (6205), 331.
3. Bryan, T. M.; Englezou, A.; Gupta, J.; Bacchetti, S.; Reddel, R. R. *EMBO J.* 1995, **14** (17), 4240–4248.
4. Tran, P. L. T.; Mergny, J.-L.; Alberti, P. *Nucleic Acids Res.* 2011, **39** (8), 3282–3294

This research was supported by the grant from Czech Science Foundation (19-26041X), by the grant from Ministry of Health of the Czech Republic (NV19-08-00450), and by the project SYMBIT (CZ.02.1.01/0.0/0.0/15_003/0000477) funded by the European Regional Development Fund and Ministry of Education, Youth, and Sports (MEYS) of the Czech Republic. MEYS is also acknowledged for its support of access to research infrastructure (CEITEC 2020 LQ1601; CIISB-LM2018127).

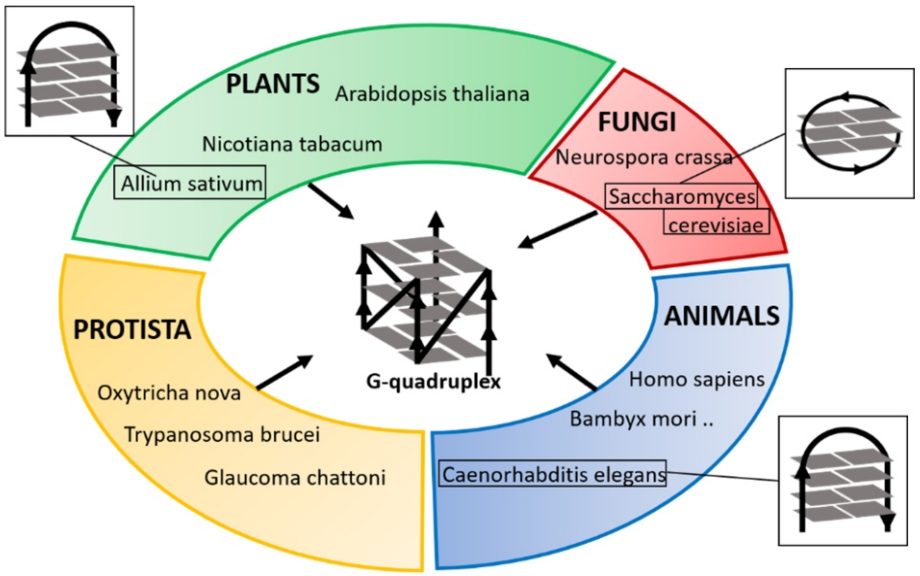

Figure 1. Non canonical DNA structures in telomeres of eukaryotic kingdoms.

L7

FORMATION PROPENSITY OF PSEUDO-CIRCULAR G-HAIRPINS

Beková Kateřina[1,*], Lenarčič Živković Martina[1,3], Gajarský Martin[1], Stadlbauer Petr[2], Šponer Jiří[2], Vicherek Lukáš[1], Fiala Radovan[1], Rosenberg Ivan[3], Petrová Magdaléna[3], Plavec Janez[3], Trantírek Lukáš [1]

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Recently, Gajarsky *et al*. [1] showed that 11-nt long sequence, 5´- d(GTGTGGGTGTG)-3´ (SC11) from telomeric DNA of *Saccharomyces cerevisiae*[3], adopts novel type of mixed parallel/antiparallel foldback DNA structure stabilized by G:G base pairs, referred to as pseudo-circular G-hairpin (PGH).

Here, we analyzed the PGH structure and its primary sequence to identify the minimal sequence requirements for PGH formation. We discovered more than ten SC11 sequence variants that are able to form PGHs. Our bioinformatics analysis revealed that the sequences with PGH-formation potential are both abundant and nonrandomly distributed in metazoan genomes. These sequences are overrepresented at evolutionarily conserved regulatory genomic foci, particularly in gene introns, which suggests their active biological role(s). Most importantly, by determining the high-resolution structure of extended SC11 sequence, we show for the first time that pseudo-circular G-hairpins might exist in at least two distinct topological forms. The structure of the novel topological form reveals unprecedented atomistic details of the junction between the protruding single stranded DNA and the pseudo-circular element of the PGH. Altogether, our data provide novel insight into the principles of the folding of G-rich oligonucleotides that could be applied to the prediction of natural and/or the design of artificial DNA recognition elements.

1. Gajarský, M., Živkovič, M. L., Stadlbauer, P., Pagano, B., Fiala, R., Amato, J., ... & Trantiřek, L. (2017). Structure of a stable G-Hairpin. *Journal of the American Chemical Society*, *139*(10), 3591-3594.

This project was supported by grants from the Czech Science Foundation (19-26041X), project SYMBIT (CZ.02. 1.01/0.0/0.0/15_003/0000477) funded by the Europe-an Regional Development Fund and Ministry of Education, Youth, and Sports (MEYS) of the Czech Republic and by the grant from Ministry of Health of the Czech Republic (NV19-08-00450). This project was also supported by the project MSCAfellow2@MUNI (CZ.02.2.69/0.0/ 0.0/18_ 070/0009846) funded by MEYS. MEYS is also acknowledged for their support of access to research infrastructure (CEITEC 2020 LQ1601; CIISB-LM2018127; Czech-BioImaging LM2015062; EATRIS-CZ LM20150 64). This project was supported by NCBR, Faculty of Science, Masaryk University. Computational resources were supplied by the project "e-Infrastruktura CZ" (e-INFRA LM2018140) provided within the program Projects of Large Research, Development and Innovations Infrastructures.

L8

NACHRDB: SOLVING THE PUZZLE OF STRUCTURE-FUNCTION RELATIONSHIPS IN NICOTINIC ACETYLCHOLINE RECEPTORS (NACHRS)

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The nicotinic acetylcholine receptor (nAChR) is an evolutionary ancient large (~300 kDa) allosteric membrane protein mediating the synaptic transmission [1]. This prototypic member of the family of pentameric ligand-gated ion-channels is involved in many physiological processes (from learning to motor control), neurological diseases (Alzheimer's and Parkinson's diseases, schizophrenia, epilepsy), and addictions (alcohol, tobacco) [2]. Since its biochemical isolation in 1970, extensive studies generated huge amounts of structural-functional data. However, the cumulative knowledge on nAChRs, spanning ~50 years of research, is not systematically accessible. The wide variety in receptor types, residue numbering schemes, and methods used, together with diverse terminology, the absence of

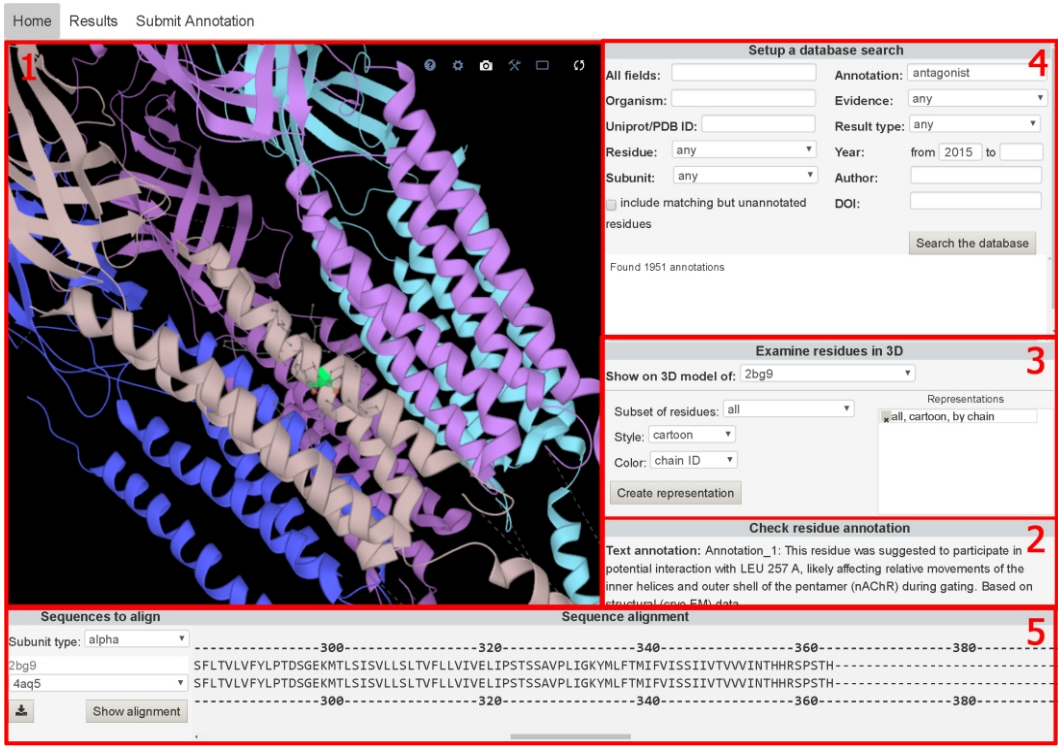

Figure 1. The NAChRDB workspace. The Home tab contains: (1) an interactive 3D visualization of nAChR model, (2) annotation records for a selected residue, (3) 3D visualisation settings, (4) a search section for querying structural, functional, and literature-related information; (5) a sequence alignment viewer. The Results tab (B) provides the search results in the form of an interactive sorted table. All results are available for download in. csv and. json format. The Submit tab (C) provides an opportunity for the users to report annotation information, contributing to NAChRDB data maintenance.

comprehensive structural annotation, and the scattered nature of the existing findings make it harder to summarize the current knowledge and apply it efficiently to promote further discoveries. There is no single resource providing an access to and visualization of such diverse, complex, and extensive information. To fill this gap, we developed NACHRDB(https://crocodile.ncbr.muni.cz/Apps/NAChRDB/) – a web-accessible manually curated database which not only provides intuitive and fast access to relevant structural-functional data on nAChRs, but also facilitates its interpretation by integrating the residue-level annotations with interactive and highly responsive visualization of sequence and 3D structure [3]. Besides, NACHRDB can provide the users with a prediction of residues potentially relevant for the allosteric regulation of nAChRs, based on the analysis of partial atomic charges profile. We believe that NACHRDB not only can guide the further studies in the field of nAChRs, helping the researchers to detect hitherto unknown association between structure and function of nAChRs, but also serve as a key starting point in unification of the state-of-art knowledge in the broad field of ion channels.

1. Changeux J-P. The nicotinic acetylcholine receptor: a typical “allosteric machine”. Philos Trans R Soc Lond B Biol Sci. 2018;373. doi:10.1098/rstb.2017.0174.

2. Albuquerque EX, Pereira EFR, Alkondon M, Rogers SW. Mammalian Nicotinic Acetylcholine Receptors: From Structure to Function. Physiol Rev. 2009;89: 73–120. oi:10.1152/physrev.00015.2008.

3. Chareshneu A, Pant P, Ramos RJT, Gökbel T, Ionescu C-M, Koča J. NAChRDB: A Web Resource of Structure-Function Annotations to Unravel the Allostery of Nicotinic Acetylcholine Receptors. bioRxiv. Cold Spring Harbor Laboratory; 2020; 2020.01.08.898171. doi:10.1101/2020.01.08.898171

We would like to acknowledge Dr. David Sehnal for his help with the implementation of 3D viewer based on the LiteMol suite.

L9

LARGE-SCALE ATOMISTIC SIMULATIONS OF THE RIBOSOME AND ITS EXIT TUNNEL

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A vast majority of proteins in living organisms are synthesized by ribosomes. The ribosome is a biomolecular complex of ribosomal RNA and a few dozen proteins. The ribosome consists of two subunits, which have distinct roles. While the small 30S subunit reads the genetic information stored in messenger RNA, the large 50S subunit catalyzes peptide bond formation. The catalytic center is located deep in the 50S subunit, so the nascent peptide chain leaves the ribosome through a 10-nm long tunnel. A growing body of evidence has been gathered suggesting that the exit tunnel is not a passive environment but a functional part of the ribosome. For instance, certain nascent peptide sequences interact with the tunnel walls and cause ribosomal stalling. The translational arrest is also involved in the action of a large class of antibiotics.

Traditional biophysical techniques such as X-ray crystallography or cryo-electron microscopy have difficulties in resolving the tunnel content due to the high intrinsic flexibility of the nascent peptide. All-atom computer simulations can provide insights into the protein synthesis on ribosomes at molecular details and complement the structural information by dynamics and energetic data [1].

This contribution gives a brief overview of our results related to the ribosome exit tunnel. In particular, we describe a stalling peptide called VemP, which forms a compact secondary structure within the ribosome tunnel and causes translational arrest. We show that various amino acids of VemP play various roles in sequence sensitivity of translational stalling previously reported by biochemical experiments. While the amino acids near the tunnel constriction act as an anchor which likely slows down the translation, the C-terminal directly inhibits the ribosome catalytic center. Further through bioinformatic analyses, we explain how the tunnel walls are modulated by the presence of a nascent peptide or an antibiotic.

1. L. Bock, M. H. Kolář, H. Grubmüller, Curr. Opin. Struct. Biol., **49**, (2018), 27.

A part of the simulation results was obtained through Open Calls of IT4Innovations Ostrava in projects OPEN-12-9 and OPEN-15-49.

CL1

WHEN PROTEIN STABILITY MATTERS

Piotr Wardega

NanoTemper Technologies spzoo

Prometheus characterizes thermal and chemical unfolding under native conditions using nanoDSF technology. Our technology is completely label-free; it precisely measures the intrinsic fluorescence of a protein while it is being subjected to either chemical, time-resolved or most commonly, thermal denaturation.

The fluorescence of tryptophan in a protein is strongly dependent on its close surroundings. By following changes in fluorescence various stability describing parameters can be assessed in a truly label-free manner. The dual-UV technology developed by NanoTemper allows for rapid signal detection, providing an unmatched scanning speed as well as data points density. This approach yields an ultra-high resolution unfolding curves which translate to very accurate detection of even minute unfolding signals, allowing for better evaluation and understanding of studied protein's structure. Furthermore, since no fluorophores , for protein labeling, are required as in historical DSF, sample solutions are analyzed independent of buffer nature or compositions.

NanoDSF uses high quality glass capillaries to provide— impressive sample saving- only microliters are required, -wide range of concentrations which are quickly

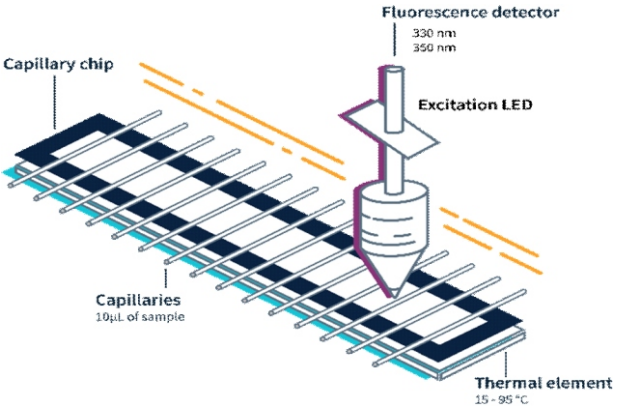
measured in solution, and hard-to-measure viscous samples are examined with no problem.

Experiments performed with Prometheus platform deliver T_m, T_{onset} and T_{agg} , C_m, G and G as well as time resolved parameters in isothermal conditions.

Our Prometheus allows for parallel measurement of protein aggregation propensity of tested samples, providing valuable information on their colloidal state.

All above make nanoDSF, and specifically Prometheus platform, the method of choice for easy, fast and precise analysis of protein folding and stability.

On the practical note; Prometheus does not require maintenance, flushing, equilibration or any other between-experiments interventions, making it money saving and multi users- friendly, as samples solutions do not come in direct contact with the instrument.



To discuss your specific research interest we invite you to visit our company booth during the meeting.

Friday, March 20, Session III

L10

MODULATING FOXO3 TRANSCRIPTIONAL ACTIVITY BY SMALL, DBD-BINDING MOLECULES

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FOXO3 is a member of FOXO Transcription Factor family. FOXO proteins share a conserved DNA-binding motif called winged-helix DNA-binding domain (DBD). FOXO3 recognizes specific DNA sequence. Thourgh interaction with target DNA it modulates various biological processes, such as cell death, cell-cycle arrest, DNA repair and energy homeostasis [1] . Due to its ability to induce cell cycle arrest it is considered a tumor suppressor. However, in certain cases, it has been shown that FOXO3 promotes tumor development and angiogenesis via maintaining cancer cell energy homeostasis. FOXO3 can also enhance tumor cell resistence to chemoterapeutic agents [2]. Therefore, targeting of FOXO3 transcriptional activities by specific inhibitors can help to prevent drug resistance in cancer therapy.

A pharmacophore screening identified a small molecule compound that physically interacts with the FOXO3-DBD and modulates the FOXO3 transcriptional program in human cells. The mode of interaction between this compound and the FOXO3-DBD was characterized by

Figure 1 – Graphical scheme of abstract