CHARLES UNIVERSITY

Faculty of Science

Department of Biochemistry

Doctoral study programme: Biochemistry

Summary of the Doctoral thesis

Structural characterization of interaction between transcription factors and DNA

Strukturní charakterizace interakce transkripčních faktorů s DNA

Mgr. Růžena Filandrová

Supervisor: RNDr. Petr Novák, Ph.D.

Consultant: RNDr. Petr Man, Ph.D.

Prague 2021

Abstract

Transcription factors are proteins that mediate gene expression regulation through interactions with DNA and other factors. They allow a cell to respond to various stimuli and play a crucial role in many biological processes such as control of cell cycle progression, differentiation of cells during development or immune response. To understand these processes, the knowledge of the transcription factors 3D structure together with the mechanism of their interaction with DNA is essential. However, some of the typical features of transcription factors, such as is for example the presence of intrinsically unstructured regions, make the 3D structure determination by the commonly used high resolution methods challenging. Therefore, utilization of complementary methods like structural mass spectrometry (MS), which was used in this thesis, might prove to be beneficial to explore the structural basis of the transcription factor-DNA interaction.

In first part of this work, a set of structural mass spectrometry methods with the main focus on hydrogen/deuterium exchange mass spectrometry (HDX-MS) was optimized and tested on two transcription factor-DNA complexes and their DNA binding motifs and proved to be able to provide structural information about regions of transcription factors inaccessible by the classical high-resolution methods as well as about structural dynamics of the transcription factor-DNA complex.

In the other part of the thesis, the structural mass spectrometry methods were, together with other techniques, such as smFRET, gel shift or fluorescence anisotropy, used to investigate whether and how the sequential context of the M-CAT motif and its orientation affects its interaction with the DNA binding domain of transcription factor TEAD1 (TEAD1-DBD). The obtained results have shown that the sequences of the DNA regions flanking the M-CAT motif affect its binding affinity to TEAD1-DBD and moreover, the transcription factor was found to be able to bind also to the inverted 5'-CCTTA-3' M-CAT motif, albeit with lower affinity. This low affinity interaction was then structurally characterized and might present a potential way of regulation of the transcription factor activity. Finally, the binding cooperativity of FOXO4 and TEAD1 transcription factors were studied utilizing oligonucleotides with adjacent response motifs.

1. Introduction

1.1. Transcription Factors

The term “transcription factor” is commonly used for proteins that are both capable of binding a specific DNA sequence and able to activate or repress the initiation or elongation phase of synthesis of RNA from DNA template or, in other words, to regulate transcription[1–3]. With these properties, the transcription factors allow a cell to respond to diverse stimuli by promoting expression of specific set of genes. They play a crucial role in many biological processes such as control of cell cycle progression, differentiation of cells during development, immune response or maintenance of intracellular metabolic balance[4–7]. And finally, the general transcription factors are proteins that form the core initiation complex with RNA Polymerase II which is needed for literally every gene expression event to start[3].

In 2018 there were 1639 known human proteins identified as a transcription factor[2]. Even though their number seems high, the processes they regulate are so complex, that it could never be sufficient if transcription factors worked on their own – and they in fact almost never do so. Transcription factors can bind to DNA in cooperation with each other and also interact with numerous cofactors to fine-tune the gene expression regulation so the cell will be able to produce the proteins it needs in precisely the right moment[2,8]. Since the transcription regulation is such a complicated process, it is no surprise that mutations in transcription factor genes, their binding sites or errors in their regulation by cofactor presence can lead to various diseases. For example, transcription regulators and nucleic acid binders are significantly over-represented in cancer genes and approximately a third of human developmental disorders is caused by mutation in transcription factor genes[2,9,10]. However, the exact way of how the network of transcription factors and cofactors works and how their combinations affect DNA binding and transcription output is not yet properly understood. That is why the interactions of transcription factors with DNA, as is the case in this work, or other proteins is currently a frequently studied topic.

1.1.1. Structure of Transcription Factors

Transcription factors are typically modular in structure, which means they consist of several structural domains with different functions. A typical transcription factor comprise of one or

more DNA binding domains (DBD) that binds a specific DNA sequence and one or more effector domains which might serve either to mediate their transcription regulating function through various mechanisms or to regulate the activity of the transcription factor itself (Fig. 3)[2].

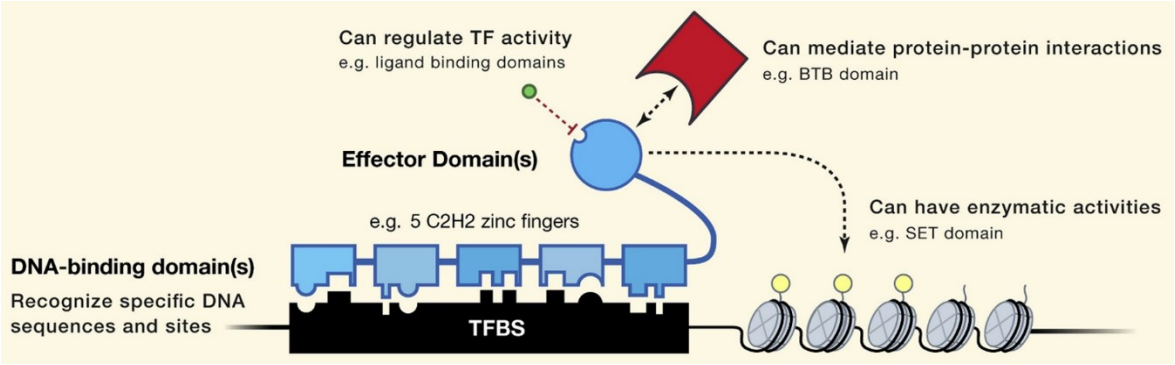


Figure 1: Structure of a typical transcription factor*. A DNA binding domain is used to recognize and bind to a specific DNA sequence whereas an effector domain may use various mechanisms to affect transcription[2].*

The DNA binding domain serves mainly to make a sequence specific contact with DNA, but some of these domains are also able to interact with other proteins and thus bind to DNA as a homo or a heteromultimer[11]. The structure of DNA binding domain is also a parameter according to which can transcription factors be sorted to families of members using the same DNA binding mechanism. Each of the transcription factor families has its own structural motif which they use to bind DNA. The binding is usually a result of a combination of noncovalent interactions between specific (and usually highly conserved) amino acids on the interaction interface (which in many cases includes an α-helix inserted to DNA's major grove) and bases of the DNA response motif or the DNA's sugar-phosphate backbone. Nevertheless, sequence independent noncovalent interactions may also contribute to the binding[3].

Through their effector domains, transcription factors can activate or repress transcription in various points including opening of chromatin, recruitment of the basal transcription machinery or release of RNA Polymerase II from pausing. The effector domain that interacts with components of the preinitiation complex are called transactivation domains. From the point of view of the 3D structure, the transactivation domains often contain unstructured regions than only become structured upon binding of their interaction partner (a general transcription factor or another protein, DNA, or a small molecular ligand) which serves as a template for its shaping. By this mechanism, the unstructured region may even fold differently depending on the type of the ligand or upon being post-translationally

modified[12,13]. Apart from the basal transcription machinery, some effector domains can also interact through specific interfaces with histone modifying enzymes to facilitate or repress access to transcription start sites[14,15].

1.1.2. Low Affinity Binding Sites

As a low affinity binding site is usually considered a sequence that is bound up to 1000-fold more weakly than the optimal DNA response motif but still more strongly than a random DNA sequence[16]. For a long time it was believed, that presence of low affinity binding sites in genome does not have any functional relevance, however, in the last decade a number of studies emerged showing that they might play a very important role in explaining the so called transcription factor specificity paradox[16–19]. This paradox concerns the fact, that eukaryotic transcription factor families usually contain paralogs with very similar DNA binding preferences and yet they affect transcription of different set of genes. Partially it can be explained by cell type specific expression of each paralog but sometimes different paralogs are expressed in the same cell and still they preserve the ability to distinguish between the affected genes[16,17].

There are several possible ways of how a transcription factor can bind to a low affinity site or even prefer it to the optimal one. For instance, binding to DNA in complex with an interaction partner can change transcription factor's structure and thus its binding specificity can be shifted to previously not preferred DNA sequence[17,20]. Moreover, spacing between the interaction partners binding sites can in some cases compensate for poor binding affinity[21]. Another possible affinity modifier are epigenetic modifications of DNA, especially the CpG methylation which can alter the binding affinity even in the paralog specific manner[22]. Furthermore, the intrinsic DNA shape of the binding site may also be the source of different paralog specific binding affinity in spite of the fact, that the final complex structure is the same[23]. And finally, there is the factor of local transcription factor concentration. The binding site occupancy is a result of combination of two factors – the binding site affinity (described by the dissociation constant of protein-DNA complex) and local transcription factor concentration[16]. This provides a handy mechanism for concentration dependant transcription regulation where at low transcription factor concentrations only high affinity sites are occupied and when the concertation rises, the transcription factor starts to bind the low affinity sites as well [24,25].

1.2.TEAD Family of Transcription Factors

Transcriptional enhancer associated (or activator for both possibilities are used in the literature) domain (TEAD) is a family of transcription factors that share a highly evolutionary conserved DNA binding domain – the TEA domain[26]. In mammals, the TEAD family consists of four members named Tead1 to Tead4[27]. All four members of the family share the same domain structure and express a high degree of homology, especially in the DNA binding domain and YAP binding domain regions[28]. However, the TEAD isoforms differ in their tissue and stage of development expression patterns[29,30].

Gene inactivation studies in mice also provided an insight into function of each TEAD isoform. Tead1 as a crucial regulator of cardiac muscle differentiation and growth and also revealed its importance for maintaining normal adult heart function[31–33]. Tead2 was shown to be responsible for neural tube closure [34] and, in cooperation with Tead1 and Tead4, for heart development[32,33]. Silencing Tead4 demonstrated its activity in primary myoblast differentiation[35], whereas no functional information has thus far emerged for Tead3.

Since the deregulation of cell proliferation, growth, differentiation or apoptosis are well known properties of tumorigenesis and given the involvement of TEAD proteins in all of these processes, it is no surprise, that the TEAD proteins have been heavily studied in this context[27,29]. TEAD proteins were found to upregulate expression of numerous genes connected with cell proliferation such as are *CYR61* and *CTGF*[36,37], anti-apoptotic genes *AXL, Livin or Survivin*[38,39]*,* genes known to be oncogenes or even tumor markers (*MYC, Mesothelin*)[40,41] or genes encoding glucose transporters *GLUT1* and *GLUT3* which are needed by quickly growing cells to satisfy their energy needs[42,43]. On top of that, increased TEAD activity was observed in multiple types of solid tumors[44–47] and in some of them it was also identified as a marker of poor prognosis[27,44]. Therefore, given the involvement of TEADs in cancer development, it is clear, that their activity in organism must be strictly regulated to prevent unchecked cell proliferation.

Soon after the initial discovery of the first TEAD protein, an unusual property typical for this family of transcription factors was found. Although they possess the ability to bind DNA, they are not able to activate transcription on their own and can only do that through interaction with other proteins – their coactivators[48,49]. This provides a handy mechanism through which can the activity of TEAD proteins be regulated in reaction to different signals and conditions. Among the identified coactivators, YES-associated protein (YAP) and its

paralog TAZ (transcriptional coactivator with PDZ-binding motif) are the two most well-established[50,51]. They both form nuclear complexes with all TEAD proteins and together they are the main effectors of the Hippo signalling pathway which plays a major role in organ size control, cell proliferation and tumorigenesis[37,52]. Apart from the Hippo pathway, TEAD proteins might also be regulated by other (Hippo independent) coactivators or by posttranslational modifications[53–55].

1.2.1. Structure of TEAD proteins

As was already mentioned, all the four mammalian TEAD proteins share the same domain architecture. They consist of two main structural domains (DNA and YAP binding) which are both highly conserved plus two, more variable, unstructured regions. One of the variable regions is present on the very N-terminus and is followed by a DNA binding domain which is then connected by a hydrophobic region rich in proline to a C-terminal transactivation (or YAP/TAZ binding) domain. Although no high-resolution structure is yet available for the full-length protein, structures of the individual domains have been separately solved for some members of the family[38,56].

1.2.1.1. DNA binding (TEA) domain

The first high-resolution structure of a DNA-free TEA domain (TEAD-DBD) in solution was solved for human TEAD1 by using NMR. It was found to be a folded globular protein consisting of three α-helices (H1, H2, and H3) connected by two loops (long L1 and a shorter L2). (Figure 2A) [57].

The NMR study provided the first insight to the position of the protein-DNA interaction interface as well. Helix H3 and the L2 loop immediately preceding it were identified as the DNA recognition region[57]. This knowledge was later expanded by two studies that used X-ray crystallography to solve the structure of TEAD-DBD. First, a crystal structure of TEAD1-DBD missing the longer L1 loop has found the L1 loop to also be involved in DNA binding[58]. Next, a crystallographic structure was published for the whole human TEAD4-DBD in complex with DNA which confirmed, that both previously identified regions (helix H3 and L1 loop) form the DNA recognition interface. In the TEAD4-DBD·DNA complex, helix H3 is inserted into DNA's major grove where it is held by specific non-covalent interactions (mainly hydrogen and salt bridges) between H3 residues and bases of the DNA

recognition motif while the L1 loop makes a sequence independent contact with the minor grove where it stabilizes the complex mostly by hydrophobic packing. The main structural difference between the free and bound forms of TEAD-DBD is found in the H3 helix, which is in the bound state prolonged and rotated 30° relative to helices H1 and H2 to better fit in the DNA's major grove (Figure 2B)[59].

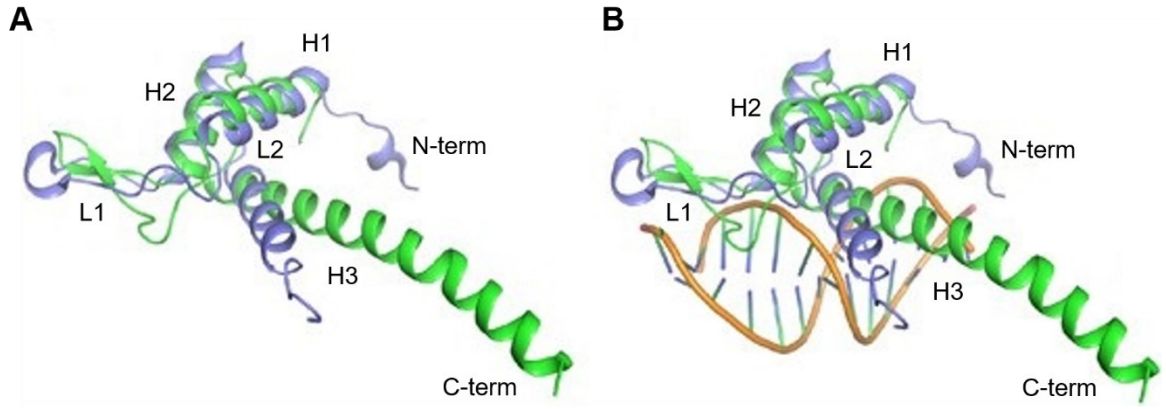


Figure 2: Structural superposition of TEAD-DBD in apo state (A) and in complex with DNA (B) *Comparison of apo state TEAD1-DBD solved by NMR (blue) and crystallographic structure of DNA-complexed TEAD4-DBD (green). Helix H3 and loop L1 were identified as the DNA recognition regions. Helix H3 is prolonged and 30° rotated in the bound form. Adapted from*[59].

1.3. Methods for Structural Characterization of Transcription Factors

To structurally characterize a complex formed between a transcription factor and its DNA response motif a number of biophysical methods can be used. The most commonly utilized are the well-established high-resolution techniques of X-ray crystallography and nuclear magnetic resonance (NMR) with the more recent addition of cryo-electron microscopy (CryoEM). While the obvious advantage of these techniques lies in the ability to solve a protein structure at atomic resolution, they all have their limitations in the experimental conditions that need to be met. To overcome these limitations, the results received by usage of different high-resolution techniques can be combined and further expanded by lower resolution methods such as small-angle X-ray scattering (SAXS), fluorescence resonance energy transfer (FRET) or structural mass spectrometry (structural MS) which is utilized in this thesis[60].

2. Aims of the Thesis

The aims of this thesis were to contribute to the development of a set of structural mass spectrometry methods for characterization of transcription factor complexes with their cognate response motifs and to apply these methods to structurally characterize the interaction between the DNA binding domains of FOXO4 and TEAD1 proteins and their DNA response motifs.

The specific goals were:

- To prepare and characterize a panel of HDX-MS compatible proteases to be used to improve sequence coverage of complex protein systems – namely integral membrane proteins and protein-DNA complexes
- To explore the potential of native MS for quick evaluation of transcription factor-DNA complex formation and their sorting according to binding affinity
- To find out whether and how the sequential context of the M-CAT motif affects its interaction with TEAD1-DBD
- To explain the different affinities of M-CAT motif and its inverted version to TEAD1-DBD

3. Results and Discussion

The aim of the first part of this thesis was to optimize a set of structural mass spectrometry methods to be used for characterization of transcription factor-DNA complexes and subsequently to test them on a model system consisting of FOXO4 transcription factor and its DNA response motif. The second, and main, part was then focused on structural characterization of TEAD1 interaction with its M-CAT response motif in different sequential contexts using not only the previously optimized methods, but also other techniques such as smFRET or fluorescence anisotropy binding assay.

3.1. Improving Sequence Coverage and Resolution in HDX-MS

To achieve a good spatial resolution in HDX-MS, the proteolytic digestion is the key factor, that needs to be optimized. The standardly used protease, porcine pepsin, does not always provide optimal digestion profile needed to achieve the best possible resolution and therefore other proteases were developed to be used instead or in combination with it[61]. Therefore, a set of alternative proteases compatible with the HDX-MS conditions was either recombinantly produced (Nepenthesins I and II and Rhizopuspepsin) or bought (Aspergillopepsin – protease type XIII) and immobilized on POROS-20 AL resin. The protease columns were then used to improve sequence coverage and resolution in HDX-MS analysis of integral membrane proteins and transcription factors and the results were included in **Publication I** and **Publication II and III**, respectively.

3.1.1. Publication I

In this study, the digestion of four integral membrane proteins (Cl−/H+ exchange transporter, leucine transporter, dopamine transporter, and serotonin transporter) by porcine pepsin and three alternative aspartic proteases either in-solution or immobilized on-column in HDX-MS compatible conditions was compared.

Digestion with immobilized proteases resulted in higher sequence coverage in almost all protease-membrane protein combinations. This is probably due to elevated pressure and temperature, which can destabilize the detergent micelles and make the transmembrane parts of the proteins accessible while the higher local protease/protein ratio that can be achieved on column can also play its role[62]. Out of the proteases in solution, only Nepenthesin II provided sequence coverages at least close to those of immobilized proteases.

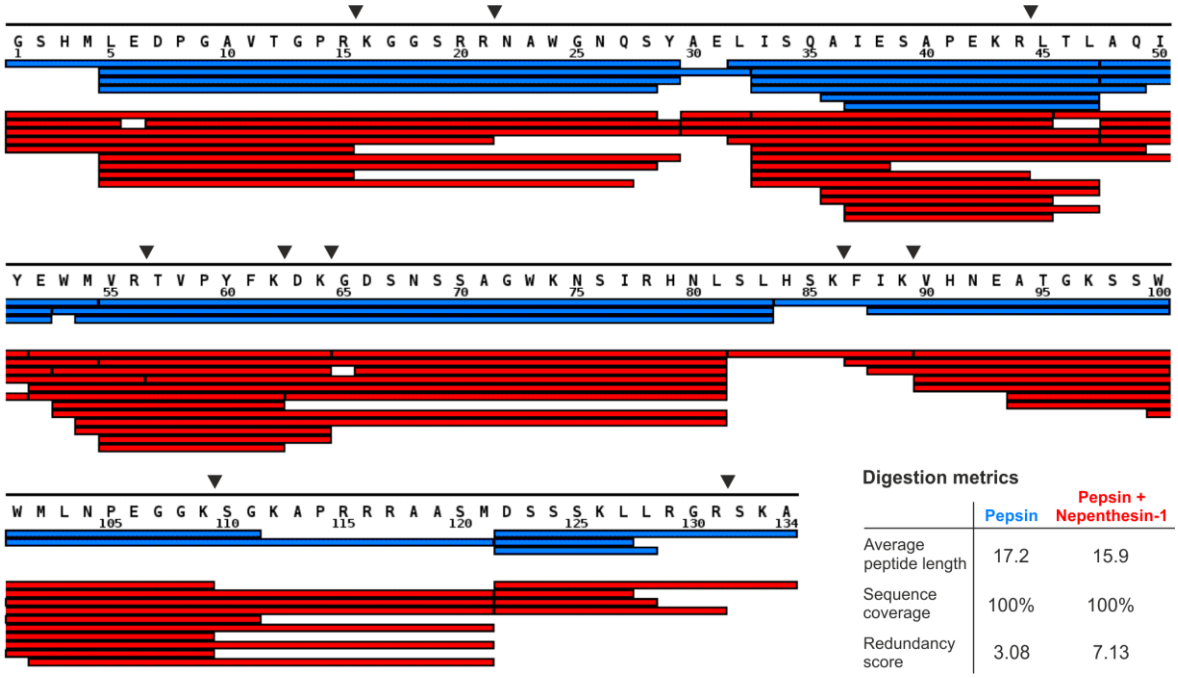
Interestingly, the choice of the best protease for digestion of membrane proteins was found to be highly protein specific. The highest sequence coverages were achieved by either immobilized pepsin for the Cl−/H+ exchange transporter and leucine transporter or by immobilized Rhizopuspepsin for dopamine transporter, and serotonin transporter. The number of identified peptides also differed considerably. This is particularly interesting considering the fact, that the leucine, dopamine and serotonin transporters are structurally related and share a similar fold and hydrophobic properties of the transmembrane helices. Thus, the results have shown, that screening of a set of available proteases, immobilized on column if possible, is always beneficial even for closely structurally related integral membrane proteins.

3.1.2. Publication II

In this publication, an optimized protocol for performing HDX-MS experiments on transcription factor-DNA complexes was presented. Similar to the integral membrane proteins, it was observed, that protein-DNA systems can behave surprisingly differently even when the proteins as well as DNAs used are similar in size. Out of the two system for which the protocol was optimized, the FOXO4-DBD/DAF16 serves as an example where short dsDNA (13 bp) did not interfere significantly with peptide recovery, and therefore only digestion conditions were optimized to reach good HDX spatial resolution. On the other hand, the 15 bp long dsDNA used in the TEAD1-DBD/M-CAT system had a strong impact on peptide recovery and therefore, the quench and chromatography conditions needed to be adjusted as well.

As for the digestion optimalization, all the protease columns were tested both alone and in pairs in serial configuration. The sequence coverage maps of the best conditions compared with the standardly used pepsin are shown in Figure 3. While pepsin provided a full sequence coverage in both systems, the number of identified peptides and their redundancy significantly improved upon the use of alternative proteases. In both examples shown here, the advantage of alternative proteases for the digestion of DNA binding proteins (which are rich in basic residues) lied in their ability to cleave after the Lys and Arg residues giving rise to additional peptides and finally resulting in better spatial resolution. Nevertheless, the choice of the optimal protease for the digestion step out of those able to cleave after basic residues, again, proved to be highly protein dependent.

A



B

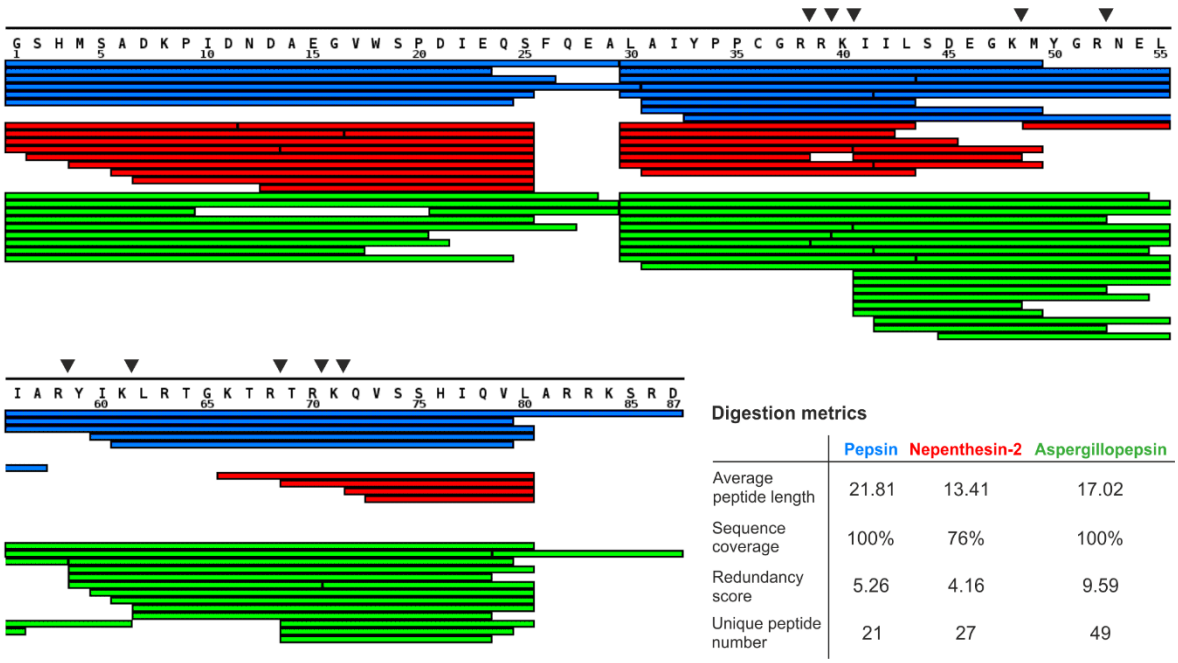


Figure 3: Sequence coverage maps of the two studied transcription factors. *(A) FOXO4-DBD where initial proteolysis by pepsin (blue) was replaced by combined digestion with pepsin followed by nepenthesin-1 (red) after optimization (b) TEAD1-DBD. Pepsin (blue) provided full sequence coverage but lower spatial resolution (longer average peptide length). Nepenthesin- 2 (red) led to over-digestion, which is indicated by gaps in the sequence and the short length of detected peptides. The green bars show the final conditions where aspergillopepsin (protease type XIII) was utilized and 2M urea was added to quench buffer to prevent precipitation. Arrowheads above the sequence indicate the cleavage sites after basic residues that were introduced by the alternative proteases.*

3.1.3. Structural Characterization of the FOXO4-DBD/DAF16 Model System (Publication III)

The optimized protocol for performing HDX-MS experiments on transcription factor-DNA complexes was, together with chemical cross-linking and homology modelling, utilized for structural characterization of a model complex consisting of the DNA binding domain of human transcription factor FOXO4 (FOXO4-DBD) and its cognate Daf-16 family member-binding element (DAF16). The obtained 3D structure model of the complex was finally compared to the available high-resolution structures.

The HDX-MS results revealed two main regions to be affected by DNA binding. First of them was interestingly not supposed to make direct contact with the duplex DNA according to the crystallographic structure[63]. Therefore, the decrease in deuteration could not be attributed simply to a protection effect but more likely to indirect conformational effects induced by binding. On the other hand, the second region with significant decrease in deuteration in the bound form was helix H3, which the crystal structure places directly in the major groove of the DNA duplex[63] making direct steric protection induced by bound DNA the best explanation for the observed exchange reduction. These observations were further confirmed by utilizing transplatin to cross-link the protein with its DNA ligand and by quantitative chemical protein-protein cross-linking.

The obtained 3D structures closely matched the previously published NMR and X-ray structures[63,64], thus confirming that the utilized combination of structural mass spectrometry methods could effectively guide model-building operations to obtain information about regions inaccessible by the classical high-resolution methods. The conformational changes upon DNA binding which have been observed in the regions not in direct contact with DNA might suggest an adaptive binding mechanism, where conformational changes may be necessary to establish specific substrate-ligand interactions.

3.2. Structural Characterization of TEAD1 Recognition of Genomic DNA

All human TEAD transcription factors bind the core 5'-ATTCC-3' consensus binding motif, which has been broadly referred to as "M-CAT". However, searching the JASPAR database has shown, that the flanking sequences around this motif can vary depending on the TEAD family member[65]. Furthermore, we have noticed, that the regulatory regions of human genes that were previously identified to be regulated by TEAD transcription factors are abundant

not only in the M-CAT motifs but also in their inverted variant (5'-CCTTA-3').

3.2.1. Publication IV

The focus of this study was to investigate whether and how the sequential context of the M-CAT motif affects its binding properties towards the isolated TEAD1-DBD as well as to explore the possibility of TEAD1-DBD binding to the inverted 5'-CCTTA-3' motif.

A series of double stranded DNA constructs that placed either the 5'-ATTCC-3' core of the consensus M-CAT motif or its inverted 5'-CCTTA-3' version in different sequence contexts (further referred to as M-CATs) was prepared and the binding properties of their complexes with TEAD1-DBD were initially compared by using native nanoelectrospray ionization MS (nESI-MS) and a fluorescence anisotropy-based binding assay (Figure 4). The complex formation was confirmed for all the tested M-CATs including those containing the inverted motif. Nevertheless, the results have shown that the M-CAT motif orientation strongly affects the binding affinity of the dsDNA constructs to TEAD1-DBD (the inverted M-CAT had a 10x higher K_D than the classical one), whereas the sequence of the strand surrounding the M-CAT motif has much lower, albeit still significant, influence.

As a next step, quantitative chemical cross-linking with MS detection and HDX-MS were utilized to reveal the spatial arrangement of free TEAD1-DBD versus one bound to the differently oriented M-CATs in solution as well as to identify their interaction interface. Similarly to the FOXO4-DBD/DAF16 complex, the DNA binding affected not the H3 helix and L1 loop that were previously identified as the binding interface, but also other regions of the protein that were not in direct contact with DNA in the published high-resolution structures[57,59]. And again, this effect might be attributed to a potential loss of flexibility and subsequent stabilization of the TEAD1-DBD structure in a more fixed conformation upon DNA binding which is in agreement with what was already suggested for the C- terminal helix[59]. Nevertheless, neither the quantitative cross-linking, nor HDX-MS results have revealed any significant differences apart from those that could be attributed to different dissociation constants between the two motif orientations as the same regions were affected by DNA binding and the only difference was the degree of the protection effect.

While the information obtained so far did not reveal any significant difference in the mechanisms used by TEAD1-DBD to recognize the different M-CAT orientations, a possibility remained that the protein may bind the inverted M-CATs by using the same

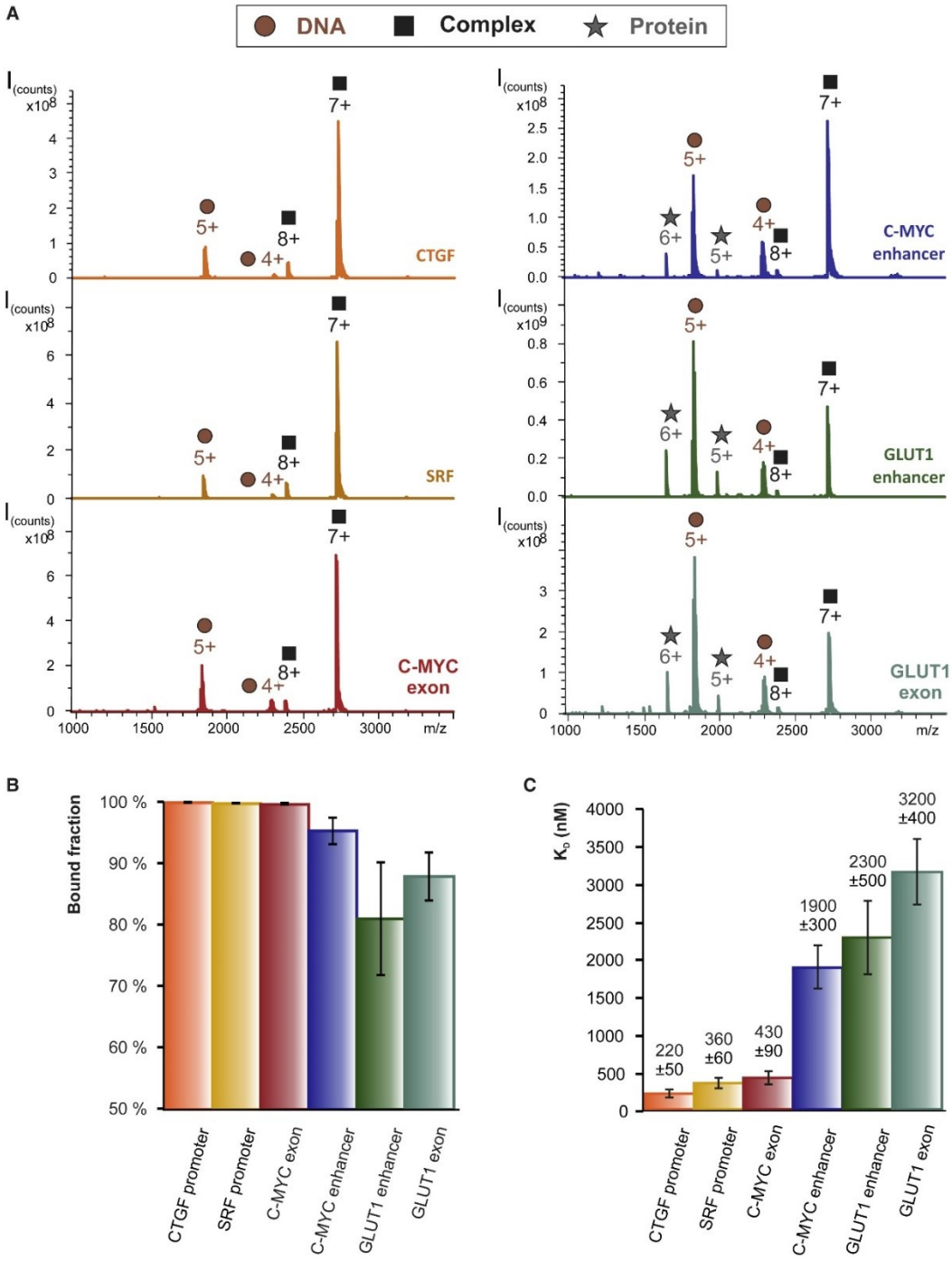


Figure 4: (A) Native ESI-MS spectra of complexes of TEAD1-DBD with each M-CAT *in the study, which revealed the ratios of free versus bound components. The most intense charge states of free protein (gray star), free DNA (brown circle), and the complex (black square) are highlighted* ***(B) Percentage of bound protein*** *derived from the signal intensities in the nESI-MS spectra.* ***(C) Comparison of dissociation constants (K_D) of selected TEAD1-DBD/M-CAT complexes determined by fluorescence anisotropy binding assay.*** *Complexes containing M-CATs with binding motifs in the 5' to 3' orientation (i.e., SRF promoter, CTGF promoter, and C-MYC exon) had approximately 10 times higher K_D than those with the inverted motif.*

interacting region, but in an actual orientation of the entire protein rotated by 180°. This hypothesis was tested by using molecular docking experiments. Figure 5 then displays the

structures of the two complexes that manifested the most stable interactions which corresponded to the initial TEAD1-DBD/M-CAT complex containing the classical M-CAT followed by a sequence, with the M-CAT motif modeled in the complementary strand which is equivalent to a 180° rotation of the entire TEAD1-DBD (Fig. 5B). The simulations also revealed that the shapes of the interacting grooves were very similar in both motif orientations which suggests a binding mechanism where TEAD1-DBD at first recognizes the overall shape of the major groove, which is similar in both orientations, and then the specific amino acid-nucleotide interactions, whose number and strength differ depending on the motif orientation, stabilize the complex. The results obtained by molecular docking simulations together with the idea of 180° rotated binding orientation were subsequently experimentally confirmed by a single-molecule Förster resonance energy transfer (smFRET) study (Fig. 5C).

.Finally, the ability of TEAD1 to bind to the low affinity inverted M-CATs in vivo was confirmed by a ChIP-qPCR study. Taken together, the presence of M-CAT sites with widely different affinities in the human genome may provide the basis for possible regulatory mechanisms relying on the actual concentration of a certain transcription factor in the proximity to a gene regulatory region as was already reported for some other transcription factors[24,66].

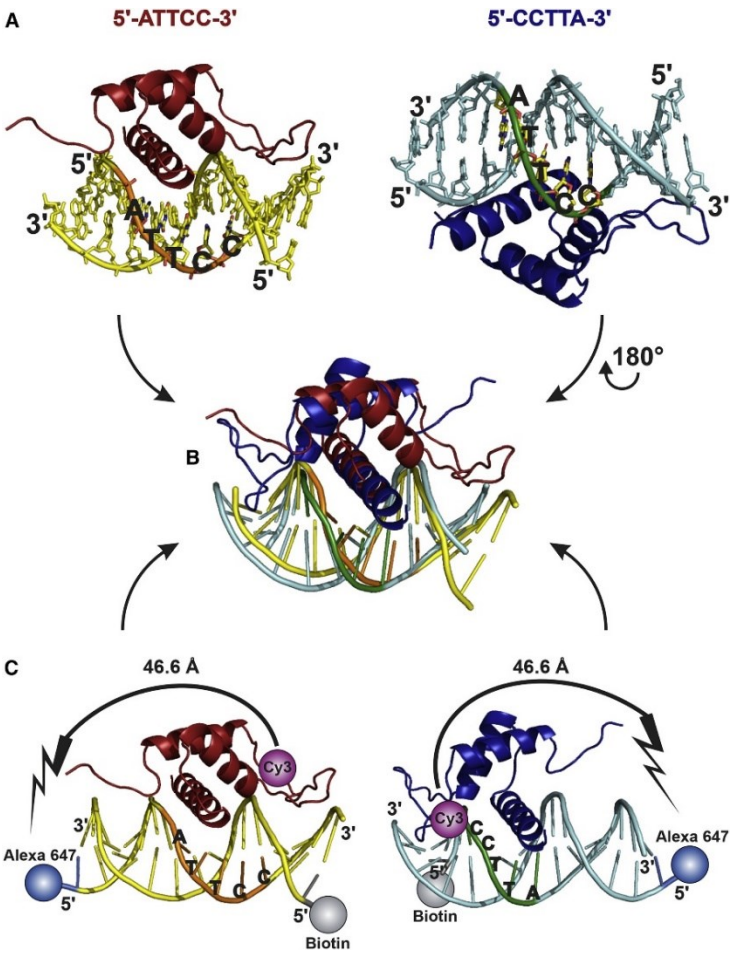


Figure 5: (A) TEAD1-DBD/M-CAT models used for MD simulations *showing the relative position and orientation of the C-MYC enhancer 5'-CCTTA-3' and the C-MYC exon 5'-ATTCC-3' DNA sequences with respect to the TEAD1-DBD.* ***(B) Structure superposition of DNA constructs*** *corresponding to the most stable interactions according to ΔG calculations* ***(C) smFRET study****. DNA and protein were labelled with donor and acceptor fluorophores whose distance (and thus FRET effectivity) depended on the respective orientations of the binding partners.*

3.2.2. Effect of the flanking sequences around the M-CAT motif

In **Publication IV** we have found that the DNA sequence surrounding the M-CAT motif has a significant influence on the affinity of the dsDNA construct to TEAD1-DBD. To examine this effect more closely, a series of dsDNA constructs, which all contained the core ATTCC binding motif either in the classical 5'-3' or in the inverted 3'-5' orientation, was prepared. What these constructs differed in was the identity of the two bases on the 5' side of the M-CAT core motif and one base on its 3' side.

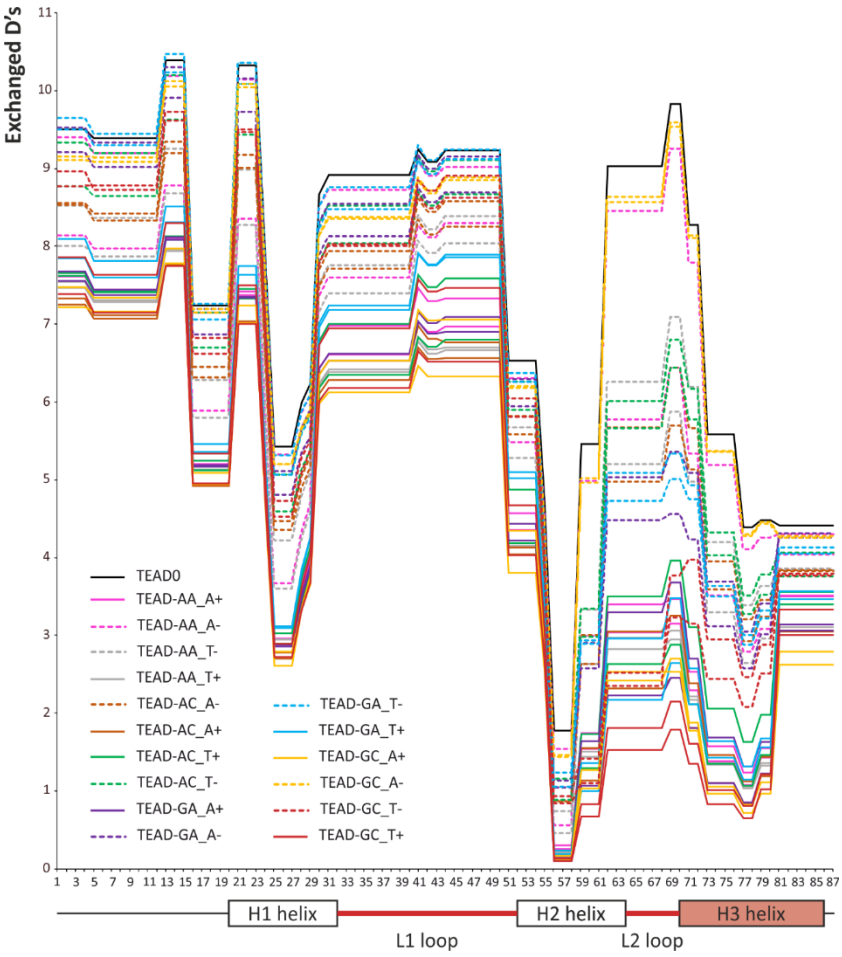


Figure 6: Comparison of HDX-MS results *depicted as number of exchanged deuterium atoms along the sequence for the series of M-CATs differing in the identity of bases flanking the core binding motif (shown as XX_X in the labels where the underscore signifies the position of the core motif). Regions previously identified as responsible for DNA binding are highlighted in red in the structure scheme under the picture. Inverted binding motives are shown as dotted lines and labelled by a minus symbol. The strongest binders exchange less deuterium atoms and therefore are positioned in the bottom of the chart while the weakest binders show a similar pattern as the sample where no DNA was present (TEAD0 - black line).*

After an initial screening by native MS performed to evaluate the complex formation and to sort the series of M-CATs according to their affinity to TEAD1-DBD, the HDX-MS was employed to further explore the binding interface. The results (Fig. 6) have shown that, similarily to the six M-CATs examined in **Publication IV**, the highest differences in deuteration rates could be observed in helix H3 and the L2 loop preceding it, thus suggesting, that all M-CATs bind to the same

region of TEAD1-DBD. Nevertheless, the fact that the degree of protection from deuteraion strongly depended on the affinity of each M-CAT to the protein allowed identification of the strongest binders (5'-GCATTCC(T/A)-3') as well as the weakest binder (3'-GCATTCCA-5'). Interestingly, the sequence identified as the strongest binder differs from the most abundant sequence of a TEAD binding site deposited in the JASPAR database suggesting that the higher affinity might potentially compensate for the lower abundance of this motif in the human genome[65].

Currently, the actual dissociation constants of each complex are being measured to complement the obtained information and a publication concerning the influence of the flanking sequences around the core M-CAT motif on its interaction with TEAD1-DBD is being prepared.

4. Summary

The aims of this thesis were to contribute to development of a set of structural mass spectrometry methods for characterization of transcription factor complexes with their cognate response motifs and to apply these methods to structurally characterize the interaction between the DNA binding domains of FOXO4 and TEAD1 proteins and their DNA response motifs. The following results were obtained and included in the four attached publications:

- A set of alternative HDX-MS compatible immobilized proteases needed to improve sequence coverage and resolution was prepared, characterized and their usability was tested on two protein systems
- The choice of the best protease for digestion of a givens system in HDX-MS is highly protein specific even in case of proteins sharing a similar fold
- Addition of denaturant urea to quench buffer as well as including washing steps between analyzed samples and screening for the best option from a set of alternative proteases is beneficial to obtain best possible resolution from HDX-MS analysis of transcription factor-DNA complexes
- The combination of structural mass spectrometry methods can effectively guide model-building operations to obtain information about regions inaccessible by the classical high-resolution methods as well as about structural dynamics of the transcription factor-DNA complex
- A significant loss of flexibility upon DNA binding was observed in both FOXO4/DAF16 and TEAD1/M-CAT complexes
- TEAD1-DBD is able to bind to the inverted 5'-CCTTA-3' M-CAT motif with lower affinity both in vitro and in vivo
- TEAD1-DBD binds to the inverted motif in a 180° rotated orientation
- Native nESI-MS was found to be a quick method for confirmation of transcription factor-DNA complex formation with the ability to differentiate between strong and week binders with very low sample consumption
- Sequences of the regions flanking the M-CAT motif affect its binding affinity to TEAD1-DBD as well with 5'-GCATTCC(T/A)-3' being the strongest binder
- TEAD1-DBD and FOXO4-DBD are able to bind together to one oligonuceotide containing both response motifs

References:

1. Fulton, D. L., Sundararajan, S., Badis, G., Hughes, T. R., Wasserman, W. W., Roach, J. C. & Sladek, R. TFCat: the curated catalog of mouse and human transcription factors. *Genome Biol.* **10,** R29 (2009).
2. Lambert, S. A., Jolma, A., Campitelli, L. F., Das, P. K., Yin, Y., Albu, M., Chen, X., Taipale, J., Hughes, T. R. & Weirauch, M. T. The Human Transcription Factors. *Cell* **172,** 650–665 (2018).
3. Lodish, H., Berk, A., Kaiser, C. A., Krieger, M., Bretscher, A., Ploegh, H., Amon, A. & Scott, M. P. *Molecular Cell Biology*. (W.H. Freeman and Company, 2013).
4. Vaquerizas, J. M., Kummerfeld, S. K., Teichmann, S. A. & Luscombe, N. M. A census of human transcription factors: function, expression and evolution. *Nat. Rev. Genet.* **10,** 252–63 (2009).
5. Accili, D. & Arden, K. C. FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. *Cell* **117,** 421–426 (2004).
6. De Pooter, R. F. & Kee, B. L. E proteins and the regulation of early lymphocyte development. *Immunol. Rev.* **238,** 93–109 (2010).
7. Lania, L., Majello, B. & Napolitano, G. Transcriptional control by cell-cycle regulators: A review. *J. Cell. Physiol.* **179,** 134–141 (1999).
8. Reiter, F., Wienerroither, S. & Stark, A. Combinatorial function of transcription factors and cofactors. *Curr. Opin. Genet. Dev.* **43,** 73–81 (2017).
9. Boyadjiev, S. & Jabs, E. Online Mendelian Inheritance in Man (OMIM) as a knowledgebase for human developmental disorders. *Clin. Genet.* **57,** 253–266 (2000).
10. Furney, S. J., Higgins, D. G., Ouzounis, C. A. & López-Bigas, N. Structural and functional properties of genes involved in human cancer. *BMC Genomics* **7,** 1–11 (2006).
11. Eguchi, A., Lee, G. O., Wan, F., Erwin, G. S. & Ansari, A. Z. Controlling gene networks and cell fate with precision-targeted DNA-binding proteins and small-molecule-based genome readers. *Biochem. J.* **462,** 397–413 (2014).
12. Garza, A. S., Ahmad, N. & Kumar, R. Role of intrinsically disordered protein regions/domains in transcriptional regulation. *Life Sci.* **84,** 189–193 (2009).
13. Minezaki, Y., Homma, K., Kinjo, A. R. & Nishikawa, K. Human transcription factors contain a high fraction of intrinsically disordered regions essential for transcriptional regulation. *J. Mol. Biol.* **359,** 1137–49 (2006).
14. Frietze, S. & Farnham, P. J. Transcription factor effector domains. *Subcell. Biochem.* **52,** 261–77 (2011).
15. Fry, C. J., Pearson, A., Malinowski, E., Bartley, S. M., Greenblatt, J. & Farnhamt, P. J. Activation of the murine dihydrofolate reductase promoter by E2F1: A requirement for CBP recruitment. *J. Biol. Chem.* **274,** 15883–15891 (1999).
16. Kribelbauer, J. F., Rastogi, C., Bussemaker, H. J. & Mann, R. S. Low-Affinity Binding Sites and the Transcription Factor Specificity Paradox in Eukaryotes. *Annu. Rev. Cell Dev. Biol.* **35,** 357–379 (2019).
17. Slattery, M., Riley, T., Liu, P., Abe, N., Gomez-Alcala, P., Dror, I., Zhou, T., Rohs, R., Honig, B., Bussemaker, H. J. & Mann, R. S. Cofactor Binding Evokes Latent Differences in DNA Binding Specificity between Hox Proteins. *Cell* **147,** 1270–1282 (2011).
18. Ramos, A. I. & Barolo, S. Low-affinity transcription factor binding sites shape morphogen

responses and enhancer evolution. *Philos. Trans. R. Soc. B Biol. Sci.* **368,** (2013).

19. Wang, J., Malecka, A., Trøen, G. & Delabie, J. Comprehensive genome-wide transcription factor analysis reveals that a combination of high affinity and low affinity DNA binding is needed for human gene regulation. *BMC Genomics* **16 Suppl 7,** S12 (2015).

20. Nagy, G. & Nagy, L. Motif grammar: The basis of the language of gene expression. *Comput. Struct. Biotechnol. J.* **18,** 2026–2032 (2020).

21. Farley, E. K., Olson, K. M., Zhang, W., Rokhsar, D. S. & Levine, M. S. Syntax compensates for poor binding sites to encode tissue specificity of developmental enhancers. *Proc. Natl. Acad. Sci.* **113,** 6508–6513 (2016).

22. Kribelbauer, J. F., Laptenko, O., Chen, S., Martini, G. D., Freed-Pastor, W. A., Prives, C., Mann, R. S. & Bussemaker, H. J. Quantitative Analysis of the DNA Methylation Sensitivity of Transcription Factor Complexes. *Cell Rep.* **19,** 2383–2395 (2017).

23. Zeiske, T., Baburajendran, N., Kaczynska, A., Brasch, J., Palmer, A. G., Shapiro, L., Honig, B. & Mann, R. S. Intrinsic DNA Shape Accounts for Affinity Differences between Hox-Cofactor Binding Sites. *Cell Rep.* **24,** 2221–2230 (2018).

24. Lorenzin, F., Benary, U., Baluapuri, A., Walz, S., Jung, L. A., von Eyss, B., Kisker, C., Wolf, J., Eilers, M. & Wolf, E. Different promoter affinities account for specificity in MYC-dependent gene regulation. *Elife* **5,** 1–35 (2016).

25. Zheng, Y. & Levens, D. Tuning the MYC response. *Elife* **5,** 2015–2017 (2016).

26. Jacquemin, P., Hwang, J. J., Martial, J. A., Dollé, P. & Davidson, I. A novel family of developmentally regulated mammalian transcription factors containing the TEA/ATTS DNA binding domain. *J. Biol. Chem.* **271,** 21775–85 (1996).

27. Huh, H., Kim, D., Jeong, H.-S. & Park, H. Regulation of TEAD Transcription Factors in Cancer Biology. *Cells* **8,** 600 (2019).

28. Santucci, M., Vignudelli, T., Ferrari, S., Mor, M., Scalvini, L., Bolognesi, M. L., Uliassi, E. & Costi, M. P. The Hippo Pathway and YAP/TAZ–TEAD Protein–Protein Interaction as Targets for Regenerative Medicine and Cancer Treatment. *J. Med. Chem.* **58,** 4857–4873 (2015).

29. Pobbati, A. V. & Hong, W. Emerging roles of TEAD transcription factors and its coactivators in cancers. *Cancer Biol. Ther.* **14,** 390–8 (2013).

30. Kaneko, K. J. & DePamphilis, M. L. Regulation of gene expression at the beginning of mammalian development and the TEAD family of transcription factors. *Dev. Genet.* **22,** 43–55 (1998).

31. Chen, Z., Friedrich, G. A. & Soriano, P. Transcriptional enhancer factor 1 disruption by a retroviral gene trap leads to heart defects and embryonic lethality in mice. *Genes Dev.* **8,** 2293–2301 (1994).

32. Liu, R., Lee, J., Kim, B. S., Wang, Q., Buxton, S. K., Balasubramanyam, N., Kim, J. J., Dong, J., Zhang, A., Li, S., Gupte, A. A., Hamilton, D. J., Martin, J. F., Rodney, G. G., Coarfa, C., Wehrens, X. H., Yechoor, V. K. & Moulik, M. Tead1 is required for maintaining adult cardiomyocyte function, and its loss results in lethal dilated cardiomyopathy. *JCI insight* **2,** (2017).

33. Sawada, A., Kiyonari, H., Ukita, K., Nishioka, N., Imuta, Y. & Sasaki, H. Redundant roles of Tead1 and Tead2 in notochord development and the regulation of cell proliferation and survival. *Mol. Cell. Biol.* **28,** 3177–89 (2008).

34. Kaneko, K. J., Kohn, M. J., Liu, C. & DePamphilis, M. L. Transcription factor TEAD2 is

involved in neural tube closure. *Genesis* **45,** 577–87 (2007).

35. Joshi, S., Davidson, G., Le Gras, S., Watanabe, S., Braun, T., Mengus, G. & Davidson, I. TEAD transcription factors are required for normal primary myoblast differentiation in vitro and muscle regeneration in vivo. *PLOS Genet.* **13,** e1006600 (2017).

36. Zhang, H., Pasolli, H. A. & Fuchs, E. Yes-associated protein (YAP) transcriptional coactivator functions in balancing growth and differentiation in skin. *Proc Natl Acad Sci U S A* **108,** 2270–2275 (2011).

37. Zhao, B., Ye, X., Yu, J., Li, L., Li, W., Li, S., Yu, J., Lin, J. D., Wang, C.-Y., Chinnaiyan, A. M., Lai, Z.-C. & Guan, K.-L. TEAD mediates YAP-dependent gene induction and growth control. *Genes Dev.* **22,** 1962–71 (2008).

38. Landin-Malt, A., Benhaddou, A., Zider, A. & Flagiello, D. An evolutionary, structural and functional overview of the mammalian TEAD1 and TEAD2 transcription factors. *Gene* **591,** 292–303 (2016).

39. Landin Malt, A., Cagliero, J., Legent, K., Silber, J., Zider, A. & Flagiello, D. Alteration of TEAD1 expression levels confers apoptotic resistance through the transcriptional up-regulation of Livin. *PLoS One* **7,** publikováno online (2012).

40. Hucl, T., Brody, J. R., Gallmeier, E., Iacobuzio-Donahue, C. A., Farrance, I. K. & Kern, S. E. High cancer-specific expression of mesothelin (MSLN) is attributable to an upstream enhancer containing a transcription enhancer factor dependent MCAT motif. *Cancer Res.* **67,** 9055–65 (2007).

41. Schütte, U., Bisht, S., Heukamp, L. C., Kebschull, M., Florin, A., Haarmann, J., Hoffmann, P., Bendas, G., Buettner, R., Brossart, P. & Feldmann, G. Hippo signaling mediates proliferation, invasiveness, and metastatic potential of clear cell renal cell carcinoma. *Transl. Oncol.* **7,** 309–21 (2014).

42. Wang, W., Xiao, Z.-D., Li, X., Aziz, K. E., Gan, B., Johnson, R. L. & Chen, J. AMPK modulates Hippo pathway activity to regulate energy homeostasis. *Nat. Cell Biol.* **17,** 490–9 (2015).

43. Vališ, K., Talacko, P., Grobárová, V., Černý, J. & Novák, P. Shikonin regulates C-MYC and GLUT1 expression through the MST1-YAP1-TEAD1 axis. *Exp. Cell Res.* **349,** 273–281 (2016).

44. Knight, J. F., Shepherd, C. J., Rizzo, S., Brewer, D., Jhavar, S., Dodson, A. R., Cooper, C. S., Eeles, R., Falconer, A., Kovacs, G., Garrett, M. D., Norman, A. R., Shipley, J. & Hudson, D. L. TEAD1 and c-Cbl are novel prostate basal cell markers that correlate with poor clinical outcome in prostate cancer. *Br. J. Cancer* **99,** 1849–58 (2008).

45. Liu, Y., Wang, G., Yang, Y., Mei, Z., Liang, Z., Cui, A., Wu, T., Liu, C.-Y. & Cui, L. Increased TEAD4 expression and nuclear localization in colorectal cancer promote epithelial–mesenchymal transition and metastasis in a YAP-independent manner. *Oncogene* **35,** 2789–2800 (2016).

46. Wang, C., Nie, Z., Zhou, Z., Zhang, H., Liu, R., Wu, J., Qin, J., Ma, Y., Chen, L., Li, S., Chen, W., Li, F., Shi, P., Wu, Y., Shen, J. & Chen, C. The interplay between TEAD4 and KLF5 promotes breast cancer partially through inhibiting the transcription of p27 Kip1. *Oncotarget* **6,** 17685–17697 (2015).

47. Zhou, Y., Huang, T., Zhang, J., Wong, C. C., Zhang, B., Dong, Y., Wu, F., Tong, J. H. M., Wu, W. K. K., Cheng, A. S. L., Yu, J., Kang, W. & To, K. F. TEAD1/4 exerts oncogenic role and is negatively regulated by miR-4269 in gastric tumorigenesis. *Oncogene* **36,** 6518–6530 (2017).

48. Xiao, J. H., Davidson, I., Matthes, H., Garnier, J. M. & Chambon, P. Cloning, expression, and transcriptional properties of the human enhancer factor TEF-1. *Cell* **65,** 551–568 (1991).

49. Lin, K. C., Park, H. W. & Guan, K. L. Regulation of the Hippo Pathway Transcription Factor TEAD. *Trends Biochem. Sci.* **42,** 862–872 (2017).

50. Vassilev, A., Kaneko, K. J., Shu, H., Zhao, Y. & DePamphilis, M. L. TEAD/TEF transcription factors utilize the activation domain of YAP65, a Src/Yes-associated protein localized in the cytoplasm. *Genes Dev.* **15,** 1229–41 (2001).

51. Mahoney, W. M., Hong, J.-H., Yaffe, M. B. & Farrance, I. K. G. The transcriptional co-activator TAZ interacts differentially with transcriptional enhancer factor-1 (TEF-1) family members. *Biochem. J.* **388,** 217–25 (2005).

52. Zhao, B., Wei, X., Li, W., Udan, R. S., Yang, Q., Kim, J., Xie, J., Ikenoue, T., Yu, J., Li, L., Zheng, P., Ye, K., Chinnaiyan, A., Halder, G., Lai, Z. & Guan, K. Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes Dev.* **21,** 2747–61 (2007).

53. Jiang, S. W., Dong, M., Trujillo, M. A., Miller, L. J. & Eberhardt, N. L. DNA Binding of TEA/ATTS Domain Factors Is Regulated by Protein Kinase C Phosphorylation in Human Choriocarcinoma Cells. *J. Biol. Chem.* **276,** 23464–23470 (2001).

54. Gupta, M. P., Kogut, P. & Gupta, M. Protein kinase-A dependent phosphorylation of transcription enhancer factor-1 represses its DNA-binding activity but enhances its gene activation ability. *Nucleic Acids Res.* **28,** 3168–3177 (2000).

55. Noland, C. L., Gierke, S., Schnier, P. D., Murray, J., Sandoval, W. N., Sagolla, M., Dey, A., Hannoush, R. N., Fairbrother, W. J. & Cunningham, C. N. Palmitoylation of TEAD Transcription Factors Is Required for Their Stability and Function in Hippo Pathway Signaling. *Structure* **24,** 179–186 (2016).

56. Gibault, F., Sturbaut, M., Bailly, F., Melnyk, P. & Cotelle, P. Targeting Transcriptional Enhanced Associate Domains (TEADs). *J. Med. Chem.* **61,** 5057–5072 (2018).

57. Anbanandam, A., Albarado, D. C., Nguyen, C. T., Halder, G., Gao, X. & Veeraraghavan, S. Insights into transcription enhancer factor 1 (TEF-1) activity from the solution structure of the TEA domain. *Proc. Natl. Acad. Sci. U. S. A.* **103,** 17225–30 (2006).

58. Lee, D.-S., Vonrhein, C., Albarado, D., Raman, C. S. & Veeraraghavan, S. A Potential Structural Switch for Regulating DNA-Binding by TEAD Transcription Factors. *J. Mol. Biol.* 1–12 (2016). doi:10.1016/j.jmb.2016.03.008

59. Shi, Z., He, F., Chen, M., Hua, L., Wang, W., Jiao, S. & Zhou, Z. DNA-binding mechanism of the Hippo pathway transcription factor TEAD4. *Oncogene* **36,** 4362–4369 (2017).

60. Dimitrova-Paternoga, L., Jagtap, P. K. A., Chen, P. C. & Hennig, J. Integrative Structural Biology of Protein-RNA Complexes. *Structure* **28,** 6–28 (2020).

61. Cravello, L., Lascoux, D. & Forest, E. Use of different proteases working in acidic conditions to improve sequence coverage and resolution in hydrogen/deuterium exchange of large proteins. *Rapid Commun. Mass Spectrom.* **17,** 2387–2393 (2003).

62. Ahn, J., Jung, M. C., Wyndham, K., Yu, Y. Q. & Engen, J. R. Pepsin Immobilized on High-Strength Hybrid Particles for Continuous Flow Online Digestion at 10 000 psi. *Anal. Chem.* **84,** 7256–7262 (2012).

63. Boura, E., Rezabkova, L., Brynda, J., Obsilova, V. & Obsil, T. Structure of the human FOXO4-DBD-DNA complex at 1.9 Å resolution reveals new details of FOXO binding to

the DNA. *Acta Crystallogr. Sect. D Biol. Crystallogr.* **66,** 1351–1357 (2010).

64. Boura, E., Silhan, J., Herman, P., Vecer, J., Sulc, M., Teisinger, J., Obsilova, V. & Obsil, T. Both the N-terminal loop and wing W2 of the forkhead domain of transcription factor Foxo4 are important for DNA binding. *J. Biol. Chem.* **282,** 8265–8275 (2007).

65. Khan, A., Fornes, O., Stigliani, A., Gheorghe, M., Castro-Mondragon, J. A., Van Der Lee, R., Bessy, A., Chèneby, J., Kulkarni, S. R., Tan, G., Baranasic, D., Arenillas, D. J., Sandelin, A., Vandepoele, K., Lenhard, B., Ballester, B., Wasserman, W. W., Parcy, F. & Mathelier, A. JASPAR 2018: Update of the open-access database of transcription factor binding profiles and its web framework. *Nucleic Acids Res.* **46,** D260–D266 (2018).

66. Ridinger-Saison, M., Boeva, V., Rimmelé, P., Kulakovskiy, I., Gallais, I., Levavasseur, B., Paccard, C., Legoix-Né, P., Morlé, F., Nicolas, A., Hupé, P., Barillot, E., Moreau-Gachelin, F. & Guillouf, C. Spi-1/PU.1 activates transcription through clustered DNA occupancy in erythroleukemia. *Nucleic Acids Res.* **40,** 8927–8941 (2012).

List of publications:

1) Möller, I. R., Slivacka, M., Hausner, J., Nielsen, A. K., Pospíšilová, E., Merkle, P. S., **Lišková, R**., Polák, M., Loland, C. J., Kádek, A., Man, P., & Rand, K. D. (2019). Improving the Sequence Coverage of Integral Membrane Proteins during Hydrogen/Deuterium Exchange Mass Spectrometry Experiments. *Analytical Chemistry*, *91*(17), 10970–10978.

2) **Filandrova, R**., Kavan, D., Kadek, A., Novak, P., & Man, P. (2021). Studying Protein–DNA Interactions by Hydrogen/Deuterium Exchange Mass Spectrometry. In *Multiprotein Complexes: Methods and Protocols (Methods in Molecular Biology (2247*)), 193–219, Humana Press Inc.

3) Slavata, L., Chmelík, J., Kavan, D., **Filandrová, R**., Fiala, J., Rosůlek, M., Mrázek, H., Kukačka, Z., Vališ, K., Man, P., Miller, M., McIntyre, W., Fabris, D., & Novák, P. (2019). Ms-based approaches enable the structural characterization of transcription factor/DNA response element complex. *Biomolecules*, *9*(10), 1–21

4) **Filandrová, R**., Vališ, K., Černý, J., Chmelík, J., Slavata, L., Fiala, J., Rosůlek, M., Kavan, D., Man, P., Chum, T., Cebecauer, M., Fabris, D., & Novák, P. (2021). Motif orientation matters: structural characterization of TEAD1 recognition of genomic DNA. *Structure*, 29, 1–12

Růžena Filandrová

PhD Student

PERSONAL

Birthday	:	27th February 1992
Nationality	:	Czech
Languages	:	Czech - native speaker English - advanced (C1) French - basic
Relationship	:	Married
Hobbies	:	LARP and Reenactment, Cycling, Cats

SKILLS AND EXPERIENCE

Mass Spectrometry:

ESI/nESI-FT-ICR, MALDI-TOF
LC-MS, LC-MS/MS (HPLC, UPLC)
Hydrogen/Deuterium Exchange
Chemical Cross-linking
FPOP

Other scientific techniques:

Recombinant protein production in *E.Coli*, Western Blotting, Immobilization of proteins on resins, Protease activity assay, Fluorescence anisotropy K_D assay

Software and other skills:

Bruker DataAnalysis, ProteinScape
StavroX
PyMOL
CorelDRAW
MS Office

CONTACT

+420 604 444 611
filandrova.ruzena@gmail.com
Kysucká 13, 196 00, Prague, Czechia

PROFILE

I am currently a PhD student working in laboratory of Petr Novák (http://peterslab.org/) which is mainly focused on combining mass spectrometry with structural biology. I started here with my Master's thesis project where I optimized recombinant preparation of TEAD1 transcription factor. In my PhD project I continued working with this protein and I have studied its interactions with DNA by using structural mass spectrometry techniques such as H/D exchange and chemical cross-linking. I have also used native MS and fluorescence anisotropy to determine K_D of TEAD1 complexes with different DNA oligonucleotides. Besides my PhD project I have also worked with acidic proteases which I immobilized on resins and tested for use in H/D exchange to improve structural resolution. In the future I hope to continue my carreer in science by joining a lab where I can utilize and further expand my experience in structural mass spectrometry as a postdoc.

EDUCATION

2003 - 2011: High School
Gymnázium Českolipská, Prague, Czechia
2011 - 2014: Bachelor's Degree in Clinical and Toxicological Analysis
Faculty of Science, Charles University, Prague, Czechia
2014 - 2016: Masters's Degree in Clinical and Toxicological Analysis
Faculty of Science, Charles University, Prague, Czechia
2016 - now: PhD in Biochemistry
Faculty of Science, Charles University, Prague, Czechia

NON-SCIENTIFIC WORK EXPERIENCE AND CERTIFICATES

2018 - Obtained Cambridge English FCE, Grade A (CEFR Level C1)
2015 - now - Organizing festivals and games for 200-1000 participants
2013 - 2016 - Receptionist
2010 - 2014 - Leader of Scout troop

ACADEMIC ACHIEVEMENTS AND EXPERIENCE

Publications: 4

Grants: 2018 - 2020 financial support from Charles University Grant Agency

Conference talks: 2

Conference poster presentations: 9 including 3 short poster talks

Teaching: five semesters of practical course in biochemistry, advisor of two bachelor theses

REFERENCE

Petr Novák
Head of Laboratory and PhD Advisor
pnovak@biomed.cas.cz

Petr Man
Senior Scientist
pman@biomed.cas.cz