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Autoreferat

Structural and thermodynamic aspects of the human transcription factors function. Studies of TFIIB and YY1 proteins in model systems.

Kraków, February 2019

1. Personal data:

1.1. First name and last name:

Andrzej Górecki

1.2. Diplomas and degrees

VI 2006 **doctor of biological sciences** in the field of biochemistry

Faculty of Biochemistry, Biophysics and Biotechnology
Jagiellonian University
Title of the doctoral dissertation: "Kinetic and thermodynamic studies of cAMP interactions with the mutants of *Escherichia coli* CRP receptor protein"
Supervisor: prof. dr hab. Zygmunt Wasylewski

VI 2000 **Master of Science in Technical Physics** in the field of medical physics and dosimetry

Faculty of Physics and Nuclear Technique
AGH University of Science and Technology
Thesis title: "Computer modeling of the spatial structure of macromolecules"
Supervisor: prof. dr hab. Krzysztof Kułakowski

1.3. Information on previous employment in scientific units:

from **January 2009 - adjunct professor**: Department of Physical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University

July VII 2004 - December 2008 - assistant: Department of Physical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University

November 2001 - June 2004 - engineering and technical position: Department of Physical Biochemistry, Institute of Molecular Biology, Faculty of Biology and Earth Sciences of the Jagiellonian University

2. Indication of achievement* resulting from art. 16 sec. 2 of the Act of 14 March 2003 on academic degrees and academic title, and on degrees and title in the field of art (Journal of Laws of 2016, item 882, as amended in Journal of Laws of 2016, item 1311):

The basis for the application to initiate the habilitation proceedings (scientific achievement) is a series of **six thematically related scientific publications**.

2.1. Title of scientific achievement:

Structural and thermodynamic aspects of the human transcription factors function. Studies of TFIIB and YY1 proteins in model systems.

2.2. List of publications included in the scientific achievement

Compiled publications with bibliometric parameters and information on my contribution are presented in the table below.

Table 1. List of the publications constituting the achievement.

Publication	IF from the year of publication	MNiSW points
1. Anna Nieborak, **Andrzj Górecki** (2016) "Significance of the pathogenic mutation T372R in the Yin Yang 1 protein interaction with DNA - thermodynamic studies", FEBS Lett. 2016 Mar;590(6):838-47. My contribution to this work consisted of planning the experiments, assessment of the reliability of the conducted experiments, interpretation of research results, performing the experiments whose the results are given in Figures 2 and S1, analysis of the experimental data whose results are shown in figures 3, 5, S2 and in tables 1 , 2 and 3, preparation of the diagrams presented in Figures 1 and 4. My contribution also included providing funding for the conducted research, writing the manuscript and fulfilling all the duties resulting from the status of the corresponding author. I estimate my percentage share at 70%.	3.623	30

2. **Andrzej Górecki**, Piotr Bonarek, Adam Kazimierz Górka, Małgorzata Figiel, Mateusz, Wilamowski, Marta Dziedzicka-Wasylewska, (2015) „Intrinsic disorder of human Yin Yang 1 protein", Proteins: Structure, Function, and Bioinformatics, 83:1284–1296. My contribution to this work consisted of planning the experiments, assessing the reliability of the conducted experiments, interpreting research results, performing the experiments whose results are shown in Figure 9, analysis of experimental data whose results are shown in figures: 1, 2, 3, 5, 6, 7, 8 and 9 and in table 1. My contribution also included providing partial financing of the research, writing the manuscript and fulfilling all obligations arising from the status of the corresponding author. I estimate my percentage share at 50%.	2.499	30
3. **Andrzej Górecki**, Małgorzata Figiel, Marta Dziedzicka-Wasylewska, (2015) "In vitro fluorescence studies of transcription factor IIB - DNA interaction", Acta Biochim Pol. 62(3):413-21. My contribution to this work consisted of planning the experiments, determining the reliability of the conducted experiments, interpreting research results, performing the experiments whose results are shown in figures 3, 4, analyzing the experimental data whose results are shown in figures: 1, 2, 3, 5, 6, 7 and in Tables 2 and 3. My contribution also included providing funding for the research, writing part of the manuscript and fulfilling all the obligations arising from the status of the corresponding author. I estimate my percentage share at 70%.	1.187	15
4. Golebiowski FM, **Górecki A**, Bonarek P, Rapala-Kozik M, Kozik A, Dziedzicka-Wasylewska M. (2012) An investigation of the affinities, specificity and kinetics involved in the interaction between the Yin Yang 1 transcription factor and DNA. FEBS J. 2012 Sep;279(17):3147-58. My contribution to this work consisted of planning the experiments, determining the reliability of the conducted experiments, interpreting research results, developing methods for analyzing the experimental data whose results are shown in figures 1, 2, 3 and in Tables 2 and 3. My contribution also included providing a partial financing for the research, adjusting the manuscript and fulfilling all the obligations arising from the status of the corresponding author. I estimate my percentage share at 40%.	4.250	30

5. Filip M. Golebiowski, **Andrzej Górecki**, Piotr Bonarek, Marta Dziedzicka-Wasylewska (2011) Efficient overexpression and purification of active full length human transcription factor Yin Yang 1 in Escherichia coli" Protein Expression and Purification, 77(2):198-206. My contribution to this work consisted of planning the experiments, determining the reliability of the experiments, interpreting the research results, developing a method for analyzing the experimental data whose results are shown in figure 5. My contribution also included providing funding for the research, correction of the manuscript and fulfilling all the obligations resulting from the status of the corresponding author. I estimate my percentage share at 40%.	1.587	15
6. Małgorzata Figiel, **Andrzej Górecki** (2017) "Physical interaction of human Yin Yang 1 protein with DNA". Crit Rev Oncog 22(1-2):75–97 My contribution to this work consisted of planning the experiments, determining the reliability of the experiments, interpreting the research results, developing a method for analyzing the experimental data whose results are shown in figure 5. My contribution also included providing funding for the research, correction of the manuscript and fulfilling all the obligations resulting from the status of the corresponding author. I estimate my percentage share at 40%.	0	0

2.3. Discussing the scientific goal of the work and the results achieved and their possible use.

2.3.1. Introduction

Each living cell has the potential to produce all the possible proteins encoded by its genome. However, in particular cells of the organism only those proteins are present that are needed at a given moment of the cell's life and in a quantity appropriate for the current demand. The number of individual proteins is very precisely controlled, especially at the stage of their production, or more precisely at the transcription stage, i.e. the production of mRNA molecules encoding the sequences of individual proteins. The most important elements controlling the nascence of appropriate proteins at the right time are transcription factors - proteins that precisely control the process of transcription initiation. The first group of these

proteins are General Transcription Factors (GTF), whose primary role is to identify the start site of transcription on the DNA and initiate transcription. These proteins form a Preinitiation Complex (PIC), which leads to the recruitment of RNA polymerase and, consequently, to the initiation of the transcription process. However, a special role in the protein expression process is served by specific transcription factors (STF), which control the dynamics of the process of transcription initiation. They may stimulate the formation of the preinitiation complex, or obstruct the formation of its active form, acting, respectively, as activators or repressors. The activity of specific transcription factors, in contrast to general transcription factors, is precisely regulated by various factors present in the cell, consequently leading to the production of proteins in accordance with the current demand of the cell.

Most of the transcription factors (also called trans factors), both general and specific, have the ability to interact with DNA in a sequence-specific manner. For these proteins, a so-called consensus sequence can be determined, for which the interaction is stronger than for the other sequences. The place on the genome in which the recognizable sequence occurs is referred to as the cis element. The interaction of the trans factors with the cis elements is most often characterized by a bimolecular equilibrium constant (affinity), less frequently by other thermodynamic parameters (standard: enthalpy and entropy of reaction), or kinetic parameters such as association and dissociation rate constants. Affinity describes the efficiency of complex formation depending on the concentration of component elements, while specificity specifies the preferences in the cis elements in relation to random sequences/sites. On the other hand, kinetic parameters describe the reaction rates that determine the cell's ability to modulate the activity of trans elements.

The activity of specific transcription factors can be regulated by the presence of low molecular weight specific ligands whose binding alter the structure of the transcription factor, consequently leading to a change in its affinity to DNA, or a change in other parameters described above. However, very often, not small-molecule ligands but other proteins (called cofactors), interact with the trans factors, affecting their activity. In this case, the structure of the trans factor remains unchanged, but cofactor binding modifies the availability of trans factor's surfaces capable of interacting with DNA or other protein factors. Covering the interface of the interaction will lead to a decrease in affinity, while the appearance of an additional interface of interaction may lead to an increase in affinity or a change in specificity.

Although the presented scheme is very simplified, taking into account the huge number of different cis and trans-regulatory elements, and their numerous mutual interactions, the gene transcription regulation scheme emerging from the description above may seem very complicated. Indeed, the level of complexity must be large, since the system must regulate the expression of genes to develop many different cells specific for particular tissues, based on the same set of available genes. This process must be also dynamic to adopt to versatile conditions or to the process of organism development.

In order to understand the process of gene transcription regulation, it is necessary to learn the parameters described above in relation to individual cis and trans regulatory elements. In my current research I focused mainly on two trans regulatory elements, human transcription factors: general - TFIIB and specific - YY1.

2.3.1.1. Transcription factor IIB

The TFIIB protein, being a general transcription factor, participates in the initiation of gene transcription by RNA II polymerase. IIB transcription factors are highly evolutionarily conserved. There is a large sequence similarity between all *Eucaryotes* [1], as well as to the TFB transcription factor [2] from *Archeae*, and the σRNA polymerase subunit from *Procaryota* [3]. Human TFIIB is a 35 kDa monomer [4] and consists of two functional domains. The C-terminal domain, also called core domain, contains two non-ideal repeats of 75 amino acid residues. These subdomains interact with two DNA sequences referred to as BREu and BREd (Upstream & Downstream TFIIB Recognition Element). These sequences flank the TATA box sequence upstream and downstream, respectively. Binding of TBP to the TATA box leads to symmetric bending of the DNA, allowing the interaction of both cis-regulatory elements with the TFIIB subdomains. In this case, TFIIB and TBP contact directly, which is also accompanied by a change in relative arrangement of both TFIIB subdomains [4]. The interaction of these subdomains with DNA is not identical, which provides appropriate directionality in the arrangement of the N-terminal TFIIB domain relative to the TBP-TATA complex and thus determines the appropriate direction of transcription initiation. The TFIIB interaction with specific DNA sequences also occurs in the absence of TBP, which indicates direct binding between these elements. The N-terminal domain contains a single C3H zinc finger that interacts with the RNAP II and TFIIF polymerase [5], and a conserved alkaline charge cluster domain [6]. The intramolecular interaction of N- and C-terminal domains results in a "closed" conformation [6]. The breaking of this interaction leads to the formation of an "open" conformation, which is necessary for the association of TFIIB with the remaining components of the pre-initiation complex [1, 6]. The close-to-open transition is facilitated if TFIIB autoacetylation occurs in the presence of acetyl coenzyme A. It is believed that the association of TFIIB with the TFIID-TFIIA-DNA complex is the limiting step of the entire transcription initiation process. Therefore, the level of gene expression may be increased as a result of the action of protein factors binding the TFIIB protein and recognizing DNA sequences located in the vicinity of the TATA box [7]. Another mechanism of action of transcriptional activators may be the weakening of the intramolecular interaction between the N-terminal and C-terminal domains of TFIIB, favoring the "open" conformation, consequently facilitating the interaction of TFIIB with other components of the pre-initiation complex [8].

It has been shown that TFIIB together with TBP and RNA polymerase II form the minimal complex that is necessary for the initiation of transcription at a basic level and in particular conditions is sufficient for the process.

2.3.1.2. Transcription factor YY1

The Yin Yang 1 (YY1) protein is a specific transcription factor present in all tissues. It is highly evolutionary conserved in all mammals and many vertebrates, and its analogues can be found in such a phylogenetically distant organism as the fruit fly [9]. YY1 plays a key role in a number of physiological processes such as embryogenesis, cell cycle control, proliferation or cell differentiation. By controlling cell growth and differentiation, YY1 is also important in a number of pathological processes, in particular in carcinogenesis. YY1 also participates in the control of snRNA transcription [10] and viral genes expression [11].

It is estimated that about 10% of all the promoter regions in the human genome contain sequences potentially recognized by YY1, however not all of them participate in the regulation of transcription. It has been shown that YY1 controls the expression of hundreds of genes, acting either as an activator, repressor, or transcription initiator. The fact that this protein can act as both a positive and a negative regulator of gene expression process reflected its name, derived from the ancient Chinese philosophy, in which yin and yang terms mean two opposite but complementary forces. YY1 interacts with over 100 different proteins, including many of transcription factors. The binding partners are believed to affect the type of YY1 activity. Several schemes of YY1 functioning have been distinguished. The simplest model of repression assumes the binding of YY1 to the promoter region, leading to the displacement of other activatory transcription factors. The YY1 repressor function may also result from its interaction with an activator, where YY1 may either directly block its activatory region or recruit co-repressor factors. The last two processes may occur either with or without YY1-DNA interaction. As an activator, YY1 can stimulate the binding of basic transcription factors to the promoter region, or additionally, lead to the recruitment of coactivators. YY1 also has the ability to recruit histone acetylases and deacetylases, leading to chromatin remodeling and, consequently, influencing the expression of particular genes [12].

Despite the very important role of YY1 protein in the transcription regulation and the detailed knowledge of its functioning, our insight to the structural aspects of the protein is very limited. Due to sequence homology, in particular the presence of four C2H2 zinc fingers, YY1 belongs to the family of GLI-Krüppel proteins [12]. YY1 consists of 414 amino acid residues and its molecular mass is 44.7 kDa. Specific interaction with DNA interaction was mapped to the C-terminal part of the protein (DBD) that contains the aforementioned zinc fingers. This structure recognizes a specific sequence, usually referred to as (C/g/a)(G/t)(C/t/a)CATN(T/a)(T/g/c) [13]. The second and third zinc finger are mostly responsible for the interaction with this

sequence. The N-terminal part of the YY1 protein (NTF) is responsible for interactions with other proteins. The structure of human YY1 was determined at atomic resolution only for the C-terminal fragment containing four C2H2 zinc fingers, during interaction with DNA duplex [14] and for a short fragment located in the central part of the protein forming an anti-parallel beta sheet structure when interacting with MBTD1 protein [15]. Within NTF, several regions can be distinguished: a region containing acidic residues (amino acids 43-53), a region rich in glycine residues, a sequence of 11 repeated histidine residues (amino acids 70-80), as well as a region of repeating lysines and glycines (amino acids 154-201) [16]. It is recognized that DBD is mainly responsible for the repression of transcription, whereas NTF appears to be a potential activator of transcription [16, 17].

2.3.2. The genesis of publications included in the scientific achievement.

For many years the relation between structure and function of transcription factors was the major interest of the research group of professor Zygmunt Wasylewski, a long-term head of the Department of Physical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology (FBBB) of the Jagiellonian University. The research was conducted *in vitro* and included structural, kinetic and thermodynamic aspects of transcription factors interaction with small molecule ligands and nucleic acids. The group of explored proteins included prokaryotic transcription factors such as: tryptophan repressor, tetracycline repressor and CRP receptor protein. I focused on the latter protein in the beginning of my laboratory work, after joining the team of Professor Wasylewski in 2001. I studied single-point mutants of CRP and the obtained results allowed me to prepare a doctoral dissertation in the field of in biochemistry entitled "Kinetic and thermodynamic studies of cAMP interactions with the mutants of *Escherichia coli* CRP receptor protein", defended in 2006.

After my PhD defense, my scientific interests remained strongly related to the transcription regulation process, however, I concentrated on eukaryotic proteins involved in this process. I started to work on human TFIIB and YY1 transcription factors. In 2006 I became the principal investigator of the research project P01 2006 31 No. 3128 entitled "Studies of molecular mechanisms of interaction of human Yin Yang 1 protein, regulating the processes of gene transcription, with TFIIB protein and DNA", financed by the Polish Committee for Scientific Research (Pol: KBN, Komitet Badań Naukowych).

The experience that I have gained in the preparation of prokaryotic proteins could be directly applied to TFIIB protein. Therefore, it was relatively easy to prepare the active protein. Using fluorescence spectroscopy methods, we investigated TFIIB interaction with the promoter DNA sequences. These studies are described in the publication: **Andrzej Górecki, Małgorzata Figiel, Marta Dziedzicka-Wasylewska,**

(2015) "In vitro fluorescence studies of transcription factor IIB - DNA interaction", Acta Biochim Pol. 62(3):413-21.

However, it was much more challenging to transfer the previously gained experience into effective preparation of YY1 protein. Optimization of the protein expression and purification processes together with the activity tests are described in the publication: **Filip M. Golebiowski, Andrzej Górecki, Piotr Bonarek, Marta Dziedzicka-Wasylewska (2011) Efficient overexpression and purification of active full length human transcription factor Yin Yang 1 in Escherichia coli" Protein Expression and Purification, 77(2):198-206.**

The reason for the difficulties encountered during optimizing YY1 preparation arised mostly from the structure of the protein itself, in particular from the lack of its stable structure. The results of the research have led us to the clear conclusion that the N-terminal fragment of YY1 is intrinsically disordered, therefore we classified this protein into the group Intrinsically Disordered Proteins (IDPs). Our results were published in the paper: **Andrzej Górecki, Piotr Bonarek, Adam Kazimierz Górka, Małgorzata Figiel, Mateusz, Wilamowski, Marta Dziedzicka-Wasylewska, (2015) „Intrinsic disorder of human Yin Yang 1 protein", Proteins: Structure, Function, and Bioinformatics, 83:1284–1296.**

The expertise to produce active recombinant YY1 and its fragments made it possible to study their interaction with DNA. Several promoter sequences were selected for the research, for which YY1 was shown to regulate their expression. The obtained results have been described in the publication: **Golebiowski FM, Górecki A, Bonarek P, Rapala-Kozik M, Kozik A, Dziedzicka-Wasylewska M. (2012) An investigation of the affinities, specificity and kinetics involved in the interaction between the Yin Yang 1 transcription factor and DNA. FEBS J. 2012 Sep;279(17):3147-58.**

Establishing a system suitable for studying the interaction of YY1 with DNA, it was possible to determine how the interaction is affected by the pathogenic mutation of YY1 that correlates with pancreatic cancer. The results of these investigations have been described in the publication: **Anna Nieborak, Andrzej Górecki (2016) Significance of the pathogenic mutation T372R in the Yin Yang 1 protein interaction with DNA - thermodynamic studies, FEBS Lett. 2016 Mar;590(6):838-47.**

The results of the two abovementioned publications showed that the interaction between YY1 and DNA is relatively weak and non-specific. This unexpected conclusion led me to analyze the literature data for all the cases, for which the physical interaction between the YY1 protein and DNA was demonstrated. The review was published as: **Małgorzata Figiel, Andrzej Górecki (2017) "Physical interaction of human Yin Yang 1 protein with DNA". CritRevOncog 22(1-2):75–97.**

The described analysis of the literature data suggests that one of the reasons why YY1 protein can effectively act as a transcription factor, despite the weak affinity and low specificity for DNA, is the occurrence of tandem repeats of YY1 sites. This conclusion is the basis of my current scientific research.

The described scientific works are the basis for the presented habilitation achievement.

2.3.3. Exposition of the main results of the habilitation work.

Very often, transcription factors are considered as quite passive elements of the system of protein expression regulation - characterized by a stable spatial structure. Most often, conformational changes are observed for specific transcription factors as a result of ligand binding. The significance of these changes is perceived in a binary manner: the protein has or has not the ability to bind to DNA. However, the research conducted at the Department of Biochemical Physics of FBBB, based on the prokaryotic CRP transcription factor, showed that the way of regulation may have a much more subtle nature. Based on the fluorescence quenching method, it was shown that CRP adopts slightly different spatial conformations upon interaction with different promoter sequences. The observed changes are located close to the activator region and have been characterized by different fluorescence quenching coefficients. Since this parameter describes the availability of a small-molecule quencher to a fluorophore located at the described location, the changes that occur can translate as a different availability for RNA polymerase. Thus, the ability to activate transcription depends not only on the affinity of the cis- and trans-regulatory elements, but also on the sequence of the cis element [18]. This conclusion is limited to the CRP protein for which the experiments were performed. However, the dynamic nature of protein structure, observed in many other experiments, may indicate its more general character.

TFIIB protein appears to be an ideal transcription factor to examine the general character of the hypothesis presented above. Its monomeric structure and occurrence of a single tryptophan residue (W52) allows to perform experiments analogous to those described above for CRP protein, without the need to modify TFIIB's fluorescent properties. For this purpose, appropriate genetic constructs have been prepared that allow the expression of TFIIB protein in a prokaryotic system and gave the possibility of easy purification, thanks to the attached histidine tag. The fermentation and purification process was carried out on a chromatographic resin with immobilized nickel ions, yielding - after optimization - a preparation characterized by purity suitable for spectroscopic experiments. The fluorescence quenching experiments performed with potassium iodide showed that TFIIB possesses two classes of tryptophan residues - characterized by significantly different quenching parameters, despite the occurrence of a single tryptophan residue in the protein sequence. This observation indicates the presence of two

different conformations of TFIIB, which is consistent with the equilibrium of "open" and "closed" conformations described in the literature. In the experiments we also used two short - twenty-nucleotide - DNA duplexes encoding sequences recognized by TFIIB: AdML and AdE4, which are fragments of the adenovirus major late promoter and the adenovirus E4 protein promoter. The presence of these oligonucleotides has statistically significantly affected the quenching parameters. In particular, the equilibrium between the two forms of TFIIB has been disturbed - in a manner characteristic for each sequence. According to the postulate found in the literature, this equilibrium is important for TFIIB protein activity [19] Therefore, the interaction with different DNA sequences may lead to different activity of TFIIB. In order to monitor the conformational changes in other regions of TFIIB, its fluorescent properties have been modified. First, attempts to create a model system for this purpose were based on introducing point mutations, leading to the removal of the tryptophan residue from its native site and introducing it to a different location. However, this proved to be impossible. Replacing the tryptophan residue with the phenylalanine residue, which is perceived to be the least invasive, has influenced the secondary structure of the protein, thus the obtained protein could not become a model for studying the behavior of the native TFIIB. Similarly, the use of other substitutions found in the literature (Y, V, L) led to changes in the secondary structure or lack of protein expression. The analysis of homologous sequences for the human TFIIB protein showed that the W52 residue is strongly evolutionarily conserved, suggesting its structurally important function. Due to the inability to change the location of the tryptophan residue, fluorescence labelling was applied to modify the spectroscopic properties of TFIIB. MIANS dye was used, which has the possibility of chemically modifying free cysteine residues. Analysis of the available spatial structure of TFIIB allowed us to indicate three potential labeling sites: C181, C194, C223. These residues are not evolutionarily conserved, therefore a series of mutations of these cysteine residues have been introduced in order to make the fluorescence labeling more specific. The introduced mutations did not affect the secondary structures of the obtained mutants, so they could be used for further experiments. Fluorescent labeling of the obtained mutants allowed us to obtain proteins preferentially labeled at various locations. The fluorescence intensity of the obtained preparations strongly depended on the presence and sequence of the DNA, thus it was shown that conformational changes occurring under the influence of the interaction with the promoter region are global and may affect the interaction with many elements of the transcription regulation system. The obtained results are presented in the publication: **Andrzej Górecki, Małgorzata Figiel, Marta Dziedzicka-Wasylewska, (2015) "In vitro fluorescence studies of transcription factor IIB - DNA interaction", Acta Biochim Pol. 62(3):413-21.**

The second human transcription factor I have studied is YY1 protein. Its function is thoroughly described, but its structural properties are resolved only to a small extent. No systematic research was available on YY1 interaction with cis-regulatory elements and trans factors. Lack of details describing these processes

precludes a precise description of the regulation of transcription by YY1 protein. This was the reason I tried to create a model system to study such interactions. The first stage of work performed under my supervision was to obtain the YY1 recombinant protein in a bacterial expression system. A very important stage of the research was to obtain samples of high purity and high activity. Plasmids carrying the native nucleotide sequence of human *yy1* gene were prepared encoding additional histidine tags at the N- or C-terminus of the protein. We also prepared the plasmid for the expression of the YY1 fragment encoding four zinc fingers (DBD) separately. In addition, we prepared a construct encoding the native amino acid sequence of YY1 with an N-terminal histidine tag, but with a nucleotide sequence optimized for expression in *E. coli*. All the plasmids were prepared for expression in the pET system. Three *E. coli* strains were used for the expression of the recombinant proteins: Rosetta2 (DE3), containing a plasmid enriching the pool of tRNAs for rare codons; BL21 (DE3) Star with inactivated RNAse E, thus prolonging the half-life of mRNAs; and base strain BL21 (DE3). The level of expression of the recombinant proteins did not depend on the strain, nor on the presence of rare codons in the construct, but on the location of the attached histidine tag. YY1 constructs were only expressed if they carried a N-terminal tag, but the lack of a tag in the DBD construct did not hinder its expression. The probable reason for the problems with the expression of YY1 variants were secondary structures on the 5' end of the native mRNA that were located differently in the variants with the N-terminal tag and were absent from the zinc finger coding construct. Optimization of the preparation of YY1 variants led to the development of a purification procedure carried out under denaturing conditions in 6M guanidine hydrochloride. The use of a single step column chromatography based on a NiNTA resin allowed to obtain an 80% pure preparation. The material was subjected to reduction, and then a number of protein refolding conditions were examined, to finally develop the optimal composition of the dialysis buffers. In order to further improve the purity of the preparation and to select the correctly folded protein molecules, column chromatography was used based on immobilized DNA duplexes with the sequence specific for YY1. The obtained material was characterized by high purity (up to 96%) and activity significantly higher than the preparation prior to the last chromatographic step. The sample obtained in this scheme can be stored at -80°C for several months. Optimization of the protein expression and purification process, and activity tests are included in the publication: **Filip M. Golebiowski, Andrzej Górecki, Piotr Bonarek, Marta Dziedzicka-Wasylewska (2011) Efficient overexpression and purification of active full length human transcription factor Yin Yang 1 in Escherichia coli" Protein Expression and Purification, 77(2):198-206,** and in the doctoral dissertation of Filip Gołębiowski defended in 2012.

Having obtained recombinant YY1 protein in the amount and purity suitable for physicochemical experiments, I focused on the study of its structural parameters . Three variants of the YY1 protein were used in the experiments: the full-length protein, the C-terminal fragment (DBD), and the N-terminal fragment (NTF). All three

proteins were prepared with no additional tags. The success in efficient production of full-length protein indicates the correctness of the hypothesis described above, according to which the basic problem in the expression of YY1 in bacteria is the occurrence of secondary structures in the native mRNA encoding this protein. The use of a sequence optimized for expression in *E. coli* made it possible to bypass this difficulty. Our proteolytic experiments showed that both YY1 and NTF, in contrast to DBD, are characterized by high proteolytic susceptibility. This is characteristic for proteins with a flexible spatial structure, which is typical for intrinsically disordered proteins (IDP). Analysis of the amino acid composition for the content of polar and hydrophobic residues indicated that YY1 protein may belong to the IDP family of proteins. Insufficient number of hydrophobic residues, in particular within NTF, results in the inability to form a hydrophobic core present in globular proteins, consequently leading to an unstable spatial structure. In addition to the excessive proteolytic susceptibility, disordered proteins have a much larger hydrodynamic radii that do globular proteins of the same at mass. The conducted size exclusion chromatography experiments allowed us to determine the hydrodynamic radii and finally classify fragments of YY1 to particular structural groups of proteins. The hydrodynamic radius obtained for DBD allowed to classify it as a globular protein or a molten globule, while NTF was classified as a random coil. On the other hand, the full-length protein has a more compact structure and can be classified as a pre-molten globule. In denaturing conditions, the hydrodynamic radii of all three proteins increased, which indicates the presence of some stable spatial structures. The full-length protein has a smaller hydrodynamic radius than its N-terminal fragment alone, which may indicate the existence of interaction between N and C- terminal fragments of YY1. The interaction is also confirmed by the results of circular dichroism measurements, showing different numbers of amino acid residues forming particular secondary structures of YY1 in relation to the sum of these structures calculated for DBD and NTF. Analysis of the content of secondary structures confirms the high content of random structures in NTF, reaching as much as 61%. NTF contains also a number of stable structures, in agreement with the changes observed under denaturing conditions in relation to native conditions, i.e. hydrodynamic radius increase, shift in the emission spectrum of tryptophan residues, and change in the shape of CD spectra . The latter change is typical for IDPs, occurring without a clear cooperative denaturing transition that would be characteristic for globular proteins. The bioinformatic analysis of the YY1 sequence based on the predictors of secondary structures and unstructured regions allows to confirm that YY1 belongs to the group of IDPs. Indication of NTF as the region containing the largest content of random structures allows to understand the role attributed to this fragment in the literature, since this fragment is treated as the main regulatory element of YY1, responsible for most interactions with the partner proteins. IDPs, having an extended spatial structure, have a much larger surface than do globular proteins of the same mass, which potentially allows them to interact with many partners. This observation explains the possibility of more than 100 different molecular partners identified for

YY1. The above-mentioned results have been described in the publication: **Andrzej Górecki, Piotr Bonarek, Adam Kazimierz Górka, Małgorzata Figiel, Mateusz, Wilamowski, Marta Dziedzicka-Wasylewska, (2015) „Intrinsic disorder of human Yin Yang 1 protein", Proteins: Structure, Function, and Bioinformatics, 83:1284–1296.**

Since I had recombinant YY1 protein and its fragments characterized by high purity and high activity, it was possible to develop a model system for studying the interaction of YY1 with promoter regions. The measurements were based on fluorescently-labeled duplexes at the 5' end of one of the DNA strands. Such dyes show high mobility – mostly due to the elasticity of DNA. When interacting with a protein, the DNA structure stiffens and a decrease in the mobility of the dye is observed. This process can be accurately monitored by measuring fluorescence anisotropy of the dye. When measured as a function of the protein concentration, it yields a binding curve, and thus a bimolecular equilibrium constant can be determined. The sequences of five promoter regions were selected, based on the important role of YY1 in the regulation of their expression, supplemented with a non-specific sequence. The obtained results showed that the interaction with the promoter sequences is quite weak - characterized by a sub- or a few-micromolar equilibrium constant as well as low specificity. Interaction with a specific sequence is on average only a few dozen times stronger than with a non-specific sequence. The reported YY1-DNA interaction experiments were also performed at various salt concentrations and a strong dependence was demonstrated, which allowed to estimate the amount of ionic bonds that are formed during the interaction. No differences were found for the individual sequences, which indicates an analogous mode of interaction in all the examined cases. Kinetics of the YY1-DNA interaction were also measured based on the SPR technique. In this case, streptavidin-modified DNA were used to immobilize the DNA. The obtained association rate constants for DBD were about 10^6 $M^{-1}s^{-1}$, while dissociation rate constants about 0.2 s^{-1}, mostly independently of the selected sequences. The results obtained for the full-length protein could not be analyzed reliably, which indicates a more complex interaction process. The obtained results have been described in the publication: **Golebiowski FM, Górecki A, Bonarek P, Rapala-Kozik M, Kozik A, Dziedzicka-Wasylewska M. (2012) An investigation of the affinities, specificity and kinetics involved in the interaction between the Yin Yang 1 transcription factor and DNA. FEBS J. 2012 Sep;279(17):3147-58**, and in the doctoral dissertation of Filip Gołębiowski defended in 2012.

The established system suitable for studying the interaction between YY1 and DNA made it possible to determine the effect of mutations present in YY1 on the binding of promoter sites. In this context, I have decided to investigate the significance of the T372R mutation correlated with the occurrence of non-hereditary insulinoma. This mutation is localized in the third zinc finger of YY1, in the DNA-recognizing helix, at a site canonically responsible for the specific interaction with

DNA. Due to the angled arrangement of the helix to the DNA axis, the amino acid residues located in position 372 in the helix must have correspondingly long side chains to be capable to interact with DNA. However, the threonine residue natively present at this site in YY1 has too short side chain to interact with DNA. Consequently, the DNA sequence recognized by native YY1 lacks specificity in the position potentially recognized by the residue 372. In contrast, the arginine residue introduced at this site is suitable for specific interaction with DNA, both because of its size and its chemical nature. Due to the potentially very significant effect of this mutation on the interaction with promoter regions, I decided to investigate its effect on the interaction of YY1 with DNA. For this purpose, genetic constructs of YY1 and DBD carrying the mutation T372R were prepared. An identical fermentation and purification system was used as described before. The preparation did not differ from the previous ones, and the analysis of the secondary structure content, based on measurements of circular dichroism, showed that the proteins carrying the mutations have a secondary structure indistinguishable from the wild types. An interaction analysis was performed for four selected promoter sequences based on measurements of fluorescence anisotropy change and compared to the results obtained for the native proteins. The presence of a positively charged amino acid residue in a site predestined for interaction with DNA should result in increased affinity. This is indeed the case in two out of four studied sequences, for which the affinity increases several times upon introducing the mutation. The interaction with the other two promoter sequences is weakened. Bearing in mind the specific nature of the interaction of the residue located in the position 372 with DNA, the obtained result becomes understandable. In addition, performing experiments based on full-length proteins and their variants truncated to the DNA-binding domain, allowed to determine the importance of the N-terminal fragment of YY1 for this affinity. The NTF fragment does not directly participate in the interaction with DNA, however, it turns out to affect the interaction parameters. This is particularly evident in the case of standard enthalpy and entropy analysis, which were determined based on anisotropy measurements taken at different temperatures. The obtained results lead to the conclusion that the N-terminal fragment of YY1, not participating in direct interaction with DNA, affects the affinity of YY1 to DNA in a significantly sequence-dependent manner. In addition, the observed change in the sequence recognized by the mutant protein [20] indicates the enhancement of the interaction of the first zinc finger with DNA at the expense of the fourth, while the mutation is present in the third zinc finger. This allows to conclude that the molecular recognition of DNA by YY1 is non-additive. The results of these experiments have been described in the publication: **Anna Nieborak, Andrzej Górecki (2016) "Significance of the pathogenic mutation T372R in the Yin Yang 1 protein interaction with DNA - thermodynamic studies", FEBS Lett. 2016 Mar;590(6):838-47.**

The results of the two abovementioned publications indicated a relatively weak and non-specific interaction between YY1 and DNA. This character of interaction is unexpected, due to the need for precise identification of cis-regulatory sites by trans

factors. This unexpected conclusion prompted me to analyze the literature data for all the known cases of the physical interaction between YY1 and DNA. In order to adequately describe the nature of these interactions, the first part of the publication describes in detail the interaction of zinc fingers with DNA, based on the known spatial structure and available sequences recognized by YY1. This is presented in the context of canonical zinc fingers, paying particular attention to the interaction of a specific and non-specific nature. In the following section, we focus on the sequences recognized by YY1. For this purpose, data was collected regarding all the promoter regions described in literature. Due to many mechanisms of gene transcription regulation by YY1, in some of which YY1 does not interact directly with DNA, the first stage of literature analysis was to select only those cases for which a direct interaction was confirmed. The presented data set indicates potential cis-regulatory sites specific for YY1. This set was used to present the preferences of interaction between YY1 and DNA in the form of a sequence logos. Thanks to the collection of all available literature results, our analyzes are much more representative than those presented earlier in the literature. In particular, the sequence logos of sequences preferred by YY1 that acts either as an activator or a repressor, are indistinguishable. Meanwhile, the previously presented analyzes of two research groups based on a limited pool of data indicated the existence of such a difference, but were incompatible with each other. The lack of difference in the activator and repressor sequence recognized by YY1 leads to the conclusion that the role of molecular partners in the regulation of transcription is even more important than previously thought. The rest of the review focuses on the collection of thermodynamic data characterizing the interaction of YY1 with DNA. In the vast majority of cases, the only available information characterizing the thermodynamic interaction is the equilibrium constant. The available literature data were cataloged and compiled with the sequences on which the individual studies were based and the conditions under which the experiments were carried out. In most cases, certain equilibrium constants are of the order of μM, however, the results of some experiments indicate a much stronger interaction, characterized by nM constant equilibrium. There was no significant difference in the buffer composition, the method of sample preparation or the use of a full or truncated version of the YY1 protein that could justify the difference in the specific affinity. The factor that distinguished the stronger-bound sequences was the occurrence of repeats of the sites potentially interacting with YY1. This intriguing conclusion has no equivalent in the analyzes available in other works. At the invitation of Professor Guangchao Sui - an expert dealing with the YY1 protein and its importance in the process of cancer progression - the analyzes described above were published in a special issue of Critical Review in Oncogenesis entitled "YY1 and Metastasis" at work: **Małgorzata Figiel, Andrzej Górecki (2017) "Physical interaction of human Yin Yang 1 protein with DNA". CritRevOncog 22(1-2):75–97**

The review of the literature data and the conclusions presented above turned out to be very inspiring for me. In particular, the occurrence of multiplied YY1-specific

cis-regulatory sites prompted me to undertake efforts to clarify their significance. My current work is focused on these aspects. In my opinion, a comprehensive study of this phenomenon is the key to understanding the complex mechanism of gene expression regulation by YY1.

2.3.4. The possible use of the results of the work.

The above-mentioned series of the publications concerned human transcription factors. The research was based on recombinant proteins obtained in the bacterial system.

An important stage of the conducted research was the development of an efficient expression system and a procedure for protein purification, yielding preparations of high purity and high activity. In particular, for YY1 we managed to develop a system of purification for proteins that do not contain any tags, which is both desirable and currently quite rare. Similar procedures have not been previously described in the literature for YY1. Therefore a detailed description of the preparation included in the presented publications allows for their use by other researchers interested in the production of YY1 protein variants. The presented procedures can also be applied to other proteins, in particular to YY1 retroposon - YY2 protein, present in all placenta. One can also try to use the developed procedures to purify other proteins containing zinc fingers.

An equally important stage of the research work was the development of a measuring system suitable for studying the thermodynamic parameters of the interaction of YY1 with DNA. As it has already been shown in the presented series of publications, its application is also possible for a modified research system, as was the case with DNA binding by YY1 protein carrying the T372R mutation that correlates with pancreatic cancer. In this way, one can evaluate the significance of other mutations identified in the investigated proteins. Similarly, the research system can be enriched with molecular partners of YY1. This is particularly important, since it was shown that cofactors significantly influence the regulation of transcription by YY1.

As I have already mentioned, what is the most intriguing for me is the importance of the tandem repeats of the sequences potentially recognized by YY1 in promoter regions. The developed methodology can be used to examine them thoroughly. In my opinion, this system can also be considered as a model for studying various forms of cooperativity occurring in the process of protein-DNA interaction.

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3. Other scientific and research achievements.

As a student of medical physics - an interdisciplinary field that combined classical physics with biophysics and broadly understood biological and medical knowledge - I had the opportunity to participate in biochemistry classes concerning protein issues. These macromolecules seemed to me easily described using the methods of physics, and at the same time their complexity made it impossible to predict their structure and to capture their functioning. I also had the feeling that this complexity is the basis of all biological processes and the phenomenon of life. This amazing observation directed my interests towards biochemistry. Therefore, having the opportunity, I gladly attended the molecular modeling course of biological macroparticles, and later completed student internships at the Department of Medical Informatics and Telemedicine of Collegium Medicum of the Jagiellonian University headed by prof. Irena Roterman-Konieczny. During the internship, I was involved in a project on molecular modeling of dye molecules interacting with proteins, and I began

to use Insight II software to model the structure of a Congo red molecule. This work resulted in my first scientific publication: M. Skowronek, I. Roterman, L. Konieczny, B. Stopa, J. Rybarska, B. Piekarska, **A. Górecki**, M. Król (2000) "The conformational characteristics of Congo red, Evans blue and Trypan blue" Computers and Chemistry. 24: 429-450. IF: 0,926

After finishing master studies, in 2001 I started working at the Department of Physical Biochemistry, Institute of Molecular Biology, Faculty of Biology and Earth Sciences of the Jagiellonian University, headed by prof. dr hab. Zygmunt Wasylewski. Initially, as an engineering and technical worker, I joined the research team focused on the relationship between structure and function of the protein. It was the first time that I was able to work in a biochemical laboratory and to use the techniques of protein science. Since 2004, after changing the name of the unit, I worked as an assistant at the Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University. Back then, the area of interest of our research group covered prokaryotic transcription factors, such as: tetracycline repressor (tetR) and CRP protein (Cyclic AMP Receptor Protein) from *Escherichia coli* and bacterial RNA polymerase (RNAP). The research was performed with the use of recombinant proteins obtained in the bacterial expression system and purified by various methods of liquid chromatography. The Department widely used a variety of stationary and time-resolved fluorescence techniques, circular dichroism, calorimetric techniques (DSC and ITC), and kinetics studies based on the stopped flow technique. Therefore I had an excellent opportunity to learn the methodology of laboratory work and explore the details of the used techniques.

My scientific interests were focused on the kinetic, thermodynamic and structural aspects of protein-ligand interactions. Initially, I joined in the study of the kinetics of CRP protein interaction with a small molecule ligand - cyclic AMP, for which I received funding from the Commission for Scientific Research (KBN) in the form of an own grant numbered: 3 P04A 075 22, entitled "Study of the effect of point mutations on the kinetics of cAMP interaction with activator protein CRP". I was the principal investigator in this grant in years 2002 - 2003. I was also involved in the study of kinetics of the interaction of tetracycline repressor (tetR) with tetracycline in a kinetic approach. My research conducted during this period was publish in three papers dealing with kinetics and the conformational changes of CRP and tetR.

S. Kedracka-Krok, **A. Górecki**, P. Bonarek, Z. Wasylewski, (2005) Kinetic and thermodynamic studies of tet repressor-tetracycline interaction. Biochemistry. 44: 1037-46. IF: 3,633; MNiSW points: 25

A. Polit, P. Bonarek, B. Kępys, S. Kędracka-Krok, **A. Górecki**, Z. Wasylewski, (2003) Kinetic studies of cAMP-induced allosteric changes in T127I, S128A and T127I/S128A mutants of cAMP receptor protein from Escherichia coli. J. Biol. Chem. 278: 43020-43026. IF: 6,482; MNiSW points: 35

E. Fic, **A. Górecki**, Z. Wasylewski, (2007) Fluorescence quenching studies of conformational changes induced by cAMP and DNA binding to heterodimer of cyclic AMP receptor protein from Escherichia coli. Protein J. 26: 457-66. IF: 0.962; MNiSW points: 15

Kinetic studies of mutants of the CRP protein have been supplemented with thermodynamic measurements with the use of isothermal titration calorimetry technique (ITC) and measurements of structural parameters based on circular dichroism and time-resolved fluorescence anisotropy techniques. The set of obtained results allowed us to propose a signal transduction path occurring in the CRP protein after binding cAMP. These studies were described in the doctoral dissertation entitled "Kinetic and thermodynamic studies of cAMP interactions with CRP receptor protein mutants from *Escherichia coli*", which I prepared under the supervision of prof. dr hab. Zygmunt Wasylewski and defended in June of 2006 in front of the Council of the Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University, obtaining the degree of doctor of biological sciences in the field of biochemistry. Some of the obtained results have been described in the work:

Górecki A, Kępys B, Bonarek P, Wasylewski Z (2009) Kinetic studies of cAMP-induced propagation of the allosteric signal in the cAMP receptor protein from Escherichia coli with the use of site-directed mutagenesis. Int J of Biol Macromol, 44: 262–270, IF: 2,366; MNiSW points: 25

The summary of all the research on the CRP protein performed by the group of prof. Wasylewski was described in the publication:

E. Fic, P. Bonarek, **A. Gorecki**, S. Kedracka-Krok, J. Mikolajczak, A. Polit, M. Tworzydlo, M. Dziedzicka-Wasylewska, Z. Wasylewski (2009) "cAMP receptor protein (CRP) from Escherichia coli as a model of signal transduction in proteins – a review" J Mol Microbiol Biotechnol, 2009;17(1):1-11, IF: 2,286; MNiSW points: 20

After the defense of my doctoral thesis, I started the research project entitled: "Research on molecular mechanisms of interactions of human Yin Yang 1 protein, regulating gene transcription processes, with TFIIB protein and DNA". This grant was written by prof. Zygmunt Wasylewski and the funding was obtained thanks to his efforts. The sudden and unexpected death of Professor Wasylewski made it impossible to start this project under his supervision. At that time, I was entrusted to work on this project as its principal investigator. Thus, I started leading a research group, whose interests focused on eukaryotic transcription factors: TFIIB and YY1. The research has resulted in a series of publications that form the core of my habilitation work described above. In addition, the obtained results were used to prepare two doctoral dissertations. For one of them, by Filip Gołębiowski, entitled: "Analysis of the interaction of the human Yin Yang 1 transcription factor with DNA", I was a scientific supervisor, and in the second one, by Adam Górka, entitled "Analysis

of intramolecular interactions between the human Yin Yang 1 transcription factor domains, in vitro", I was co-supervisor. Part of the obtained results is published:

Adam Kazimierz Górka, **Andrzej Górecki**, Marta Dziedzicka-Wasylewska, (2018) "Site-directed fluorescence labeling of intrinsically disordered region of human transcription factor YY1. The inhibitory effect of zinc ions." Protein science 27(2):390-401, IF: 2,41; MNiSW points: 25

Due to my experience in applications of stationary and time-resolved fluorescence spectroscopy, I was also involved in the studies of G Protein-Coupled Receptors (GPCRs). The outcome of the research has been a cycle of publications concerning the dimerization of these receptors.

S. Łukasiewicz, E. Błasiak, A. Faron-Górecka, A. Polit, M. Tworzydło, **A. Górecki**, Z. Wasylewski, M. Dziedzicka-Wasylewska, (2007) Fluorescence studies of homooligomerization of adenosine A2A and serotonin 5-HT1A receptors reveal the specificity of receptor interactions in the plasma membrane. Pharmacol Rep. 59: 379-92, IF: 2,29; MNiSW points: 25

Faron-Górecka **A, Górecki** A, Kuśmider M, Wasylewski Z, Dziedzicka-Wasylewska M, (2008) The role of D(1)-D(2) receptor hetero-dimerization in the mechanism of action of clozapine. Eur Neuropsychopharmacol. 18: 682–691, IF: 3,661; MNiSW points: 40

Katarzyna Grymek, Magdalena Gąska, Sylwia Łukasiewicz, **Andrzej Górecki**, Marta Dziedzicka-Wasylewska (2008) "Fluorescencyjne metody badania oligomeryzacji receptorów związanych z białkami G" *Postępy Biochemii* **54** (4): 431-437, MNiSW points: 5

Marta Dziedzicka-Wasylewska, Agata Faron-Górecka, **Andrzej Górecki**, Maciej Kuśmider (2008) „Mechanism of action of clozapine in the context of dopamine D1-D2 receptor hetero-dimerization – a working hypothesis" *Pharmacological Reports* **60:** 581-587, IF: 2,167; MNiSW points: 25

These issues are also described in the chapters of the monograph:

Agata Faron-Górecka, Marta Szlachta, Magdalena Kolasa, Joanna Solich, **Andrzej Górecki**, Maciej Kuśmider, Dariusz Żurawek, Marta Dziedzicka-Wasylewska, Understanding GPCR dimerization, w: G Protein-Coupled Receptors, Part B, Methods in Cell Biology (red nauk. Arun K. Shukla), Volume 149, 2019, Elsevier

Анджей Гурецкий, Марта Дзедзицкая-Василевская, Физические методы молекулярного исследования механизмов функционирования мозга In red. nauk. Рышард Тадеусевич, Основы Нейрокибернетики, Moscow 2015, Горячая линия – Телеком

Andrzej Górecki, Piotr Bonarek, Badanie struktury białek, In red. nauk. Marta Dziedzicka-Wasylewska, Zaawansowane Metody Badania Mózgu, Kraków 2011, Instytut Farmakologii PAN w Krakowie

Andrzej Gorecki, Marta Dziedzicka-Wasylewska, Fizyczne metody badania mózgu, w: Neurocybernetyka teoretyczna (red. nauk. Ryszard Tadeusiewicz) Warszawa 2009, Wydawnictwo Uniwersytetu Warszawskiego

For similar reasons, I started a cooperation with dr. Arkadiusz Matwijczuk from the Department of Applied Physics of the University of Life Sciences in Lublin, where I applied time-resolved fluorescence spectroscopy to study the family of 1,3,4-thiadiazole compounds. The result of this cooperation is a series of nine publications:

Matwijczuk A, **Górecki A**, Kamiński D, Myśliwa-Kurdziel B, Fiedor L, Niewiadomy A, Karwasz GP, Gagoś M, (2015) „ Influence of Solvent Polarizability on the Keto-Enol Equilibrium in 4-[5-(naphthalen-1-ylmethyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol.", J Fluoresc, IF: 1,601; MNiSW points: 25

Arkadiusz Matwijczuk; Daniel Kamiński, **Andrzej Górecki**, Agnieszka Ludwiczuk; Andrzej Niewiadomy, Sebastian Maćkowski, Mariusz Gagos, (2015) "Spectroscopic Studies of Dual Fluorescence in 2-(4-fluorophenylamino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole", Journal of Physical Chemistry Part: Part A, J Phys Chem A. 119(44):10791-805, IF: 2,883; MNiSW points: 30

Matwijczuk A, Kluczyk D, **Górecki A**, Niewiadomy A, Gagoś M. (2016) "Solvent Effects on Molecular Aggregation in 4-(5-Heptyl-1,3,4-thiadiazol-2-yl)benzene-1,3-diol and 4-(5-Methyl-1,3,4-thiadiazol-2-yl)benzene-1,3-diol.", J Phys Chem B 18;120(32):7958-69, IF: 3,177; MNiSW points: 30

Kluczyk D, Matwijczuk AP, **Górecki A**, Karpińska MM, Szymanek M, Niewiadomy A, Gagos M. (2016) Molecular Organisation of Dipalmitoylphosphatidylcholine Bilayers Containing Bioactive Compounds 4- (5-heptyl-1,3,4-thiadiazol-2-yl) benzene-1,3-diol and 4-(5-methyl-1,3,4-thiadiazol-2-yl) benzene-1,3-diols, J Phys Chem B 1;120(47):12047-12063, IF: 3,177; MNiSW points: 30

Matwijczuk A, Kluczyk D, **Górecki A**, Niewiadomy A, Gagoś M. (2017) Spectroscopic Studies of Fluorescence Effects in Bioactive 4-(5-Heptyl-1,3,4-Thiadiazol-2-yl)Benzene-1,3-Diol and 4-(5-Methyl-1,3,4-Thiadiazol-2-yl)Benzene-1,3-Diol Molecules Induced by pH Changes in Aqueous Solutions. J Fluoresc. 27(4):1201-1212. IF: 1,665; MNiSW points: 25

Arkadiusz Paweł Matwijczuk, **Andrzej Górecki**, Marcin Makowski, Katarzyna Pustuła, Joanna Waś, Andrzej Niewiadomy, Mariusz Gagoś (2018) Spectroscopic And Theoretical Studies of Fluorescence Effects in 2-methylamino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole Induced by Molecular Aggregation, J Fluoresc 28(1):65-77, IF: 1,665; MNiSW points: 25

Arkadiusz Matwijczuk, Dariusz Karcz, Katarzyna Pustuła, Marcin Makowski, **Andrzej Górecki**, Dariusz Kluczyk, Monika M. Karpińska, Andrzej Niewiadomy, Mariusz Gagoś (2018) "Spectroscopic and Theoretical Studies of Fluorescence Effects in Bio-Active: 4-(5-(methyl-1,3,4-thiadiazol-2-yl))benzene-1,3-diol and 4-(5-(methylamino-1,3,4-thiadiazol-2-yl))benzene-1,3-diol Compounds: Effect of Molecular Aggregation and Amino Group Position" Journal of Luminescence 201:44-56, IF: 2,732; MNiSW points: 35

Grzegorz Czernel, Arkadiusz Matwijczuk, Dariusz Karcz, **Andrzej Górecki**, Agnieszka Niemczynowicz, Aleksandra Szcześ, Grzegorz Gładyszewski, Alicja Matwijczuk, Bożena Gładyszewska, Andrzej Niewiadomy (2018), „Spectroscopic Studies of Dual Fluorescence in 2-(4-fluorophenylamino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole: Effect of Molecular Aggregation in a Micellar System", Molecules, 2;23(11), IF: 3,098; MNiSW points: 30

Agnieszka Niemczynowicz, Grzegorz Czernel, Arkadiusz Matwijczuk, Marcin Makowski, Katarzyna Pustuła, Dariusz Karcz, Alicja Matwijczuk, **Andrzej Górecki**, Agnieszka I. Piotrowicz-Cieślak (2019) "Spectroscopic and theoretical studies of dual fluorescence in 2-hydroxy-n-(2-henylethyl)benzamide induced by ESIPT process – Solvent effects", Journal of Luminescence 208:125-134, IF: 2,732; MNiSW points: 35

My interests in the field of circular dichroism spectroscopy used to study protein structure and my experience in the use of this technique have become the basis for cooperation with prof. Andrzej Ożyhar from the Department of Biochemistry, Faculty of Chemistry at the Wrocław University of Technology. The interests of prof. Ożyhar focused on the molecular basis of the relationship between the structure and function of proteins and so are particularly close to my interests. Our cooperation resulted in two publications:

Dziedzic-Letka A, Rymarczyk G, Kapłon TM, **Górecki A**, Szamborska-Gbur A, Wojtas M, Dobryszycki P, Ożyhar A. (2011) Intrinsic disorder of Drosophila melanogaster hormone receptor 38 N-terminal domain. Proteins. 79(2):376-92, IF: 3,392; MNiSW points: 30

Szamborska-Gbur A, Rymarczyk G, Orłowski M, Kuzynowski T, Jakób M, Dziedzic-Letka A, Górecki A, Dobryszycki P, Ożyhar A (2014) The molecular basis of conformational instability of the ecdysone receptor DNA binding domain studied by in silico and in vitro experiments. PlosONE 2014 Jan 23;9(1):e86052. doi: 10.137/journal.pone.0086052. eCollection 2014 Jan 23, IF: 3,234; MNiSW points: 40

The interest in fluorescence spectroscopy techniques applied to the broadly understood protein research allowed me also to cooperate with other research groups within the Jagiellonian University. The result of these collaborations are following publications:

Loch J, Polit A, **Górecki A**, Bonarek P, Kurpiewska K, Dziedzicka-Wasylewska M, Lewiński K. (2011) Two modes of fatty acid binding to bovine Beth-lactoglobulin-crystallographic and spectroscopic studies. J Mol Recognit. 24(2):341-9, IF: 3,31; MNiSW points: 25

Wybieralska E, Szpak K, **Górecki A**, Bonarek P, Miękus K, Drukała J, Majka M, Reiss K, Madeja Z, Czyż J. (2011) Fenofibrate attenuates contact-stimulated cell motility and gap junctional coupling in DU-145 human prostate cancer cell populations. Oncol Rep. 26(2):447-53, IF: 1,835; MNiSW points: 20

Monika Janczak, Michal Bukowski, **Andrzej Górecki**, Grzegorz Dubin, Adam Dubin, Benedykt Wladyka (2015) "A systematic investigation of the stability of green fluorescent protein fusion proteins" , Acta Biochimica Polonica – Acta Biochim Pol. 62(3):413-21, IF: 1,187; MNiSW points: 15

Doe to my experience in the production and purification of recombinant proteins, I was invited to cooperate with ADAMED pharmaceutical group. Our cooperation included the development of processes of the fermentation, purification and formulation of artificial recombinant proteins, as well as determination of their structural parameters. Although this work was mainly confidential, it was possible to identify the part that formed the basis of the doctoral dissertation prepared by Małgorzata Figiel, entitled "Analysis of the structure and stability of TRAIL protein variants with increased cytotoxic activity", defended in 2015, where I served the role of the co-supervisor.

Part of the research was also published in the paper:

Figiel M, Bonarek P, **Górecki A**, Pawlak SD, Zerek B, Checinska B, Pieczykolan J, Dziedzicka-Wasylewska M., (2018) "Improved cytotoxicity of novel TRAIL-Bid fusion proteins", PEDS 31(2):37-46, IF: 1,881; MNiSW points: 30

However, protein regulation on the cellular level is much more close to my scientific area of interest. Therefore I willingly undertook cooperation with the group of prof. Jolanta Jura from the Department of General Biochemistry, Faculty of BBiB UJ, who deals with the importance of Regnaza 1 (MCPIP1) in the process of mRNA destabilization. The cooperation resulted in the doctoral dissertation of Mateusz Wilamowski (planned defense on 1/03/2019) entitled: "Characteristics of the specificity of transcription degradation by MCPIP1", where I act as an co-supervisor, and two original scientific publications:

Lipert B, Wilamowski M, **Gorecki A**, Jura J. (2017) MCPIP1, alias Regnase-1 binds and cleaves mRNA of C/EBPβ. PLoS One. 2017 Mar 22;12(3):e0174381, IF: 2,766; MNiSW points: 40

Mateusz Wilamowski, **Andrzej Górecki**, Marta Dziedzicka-Wasylewska, Jolanta Jura (2018) "Substrate specificity of human MCPIP1 endoribonuclease", Sci Rep. 8(1):7381l, IF: 4,122; MNiSW points: 40

Summary

My current scientific interests focus on proteins and their fermentation, purification and modification of their fluorescent properties. I treat these issues as tools enabling the study of the dependence of their structure and function.

I also readily use spectroscopic techniques such as circular dichroism and various stationary and time-resolved fluorescence techniques, as tools in my protein research.

In terms of biochemical processes, my interests are currently focused mainly on the protein regulation on the cellular level , in particular on the process of transcription regulation. My research also involves the meaning and significance of these processes in the carcinogenesis.

Out of all protein groups, I am mostly interested in transcription factors, also due to the possibility to treat them as model proteins for the study of allostery.

Regarding the structural groups of the proteins, I am most focused on intrinsically disordered proteins (IDP), for which conformational changes, which are often forgotten in the case of globular proteins, are the key to understanding their function.

4. Didactic and organizational activity

Didactics is a very important element of my professional work and gives me a lot of satisfaction. I particularly value the possibility of using my physical education in the teaching process. I start virtually every didactic contact with students from pure physical issues, after outlining the significance of the discussed topic, in order to build their knowledge on solid foundations. In my opinion, this approach gives the students the opportunity to deeply understand the processes described by physical biochemistry, which is my major subject. I put a special emphasis on a comprehensive understanding of the subject, with a little less attention given to the details, unless they are important to understand the given issue. Although this approach may seem not very affordable to the contemporary students, in fact it rather suits them, as evidenced by the Rector's Award of the Jagiellonian University, which I received for high quality didactic work in the academic year 2016/2017 (based on student questionnaires assessing the quality of the educational process), and also a large number of promoted masters and bachelor students.

In the early stages of my academic career (since 2001) I mainly conducted basic laboratory classes in the field of physical biochemistry and protein engineering. As part of the physical biochemistry course, my classes most often concern the study of protein structure using circular dichroism, fast kinetics measurements by the stopped flow method, and fluorescence techniques to study the structure and stability of proteins. As part of the protein engineering course, my classes included the fermentation process as well as the structure of recombinant proteins.

In 2003, I started conducting advanced classes in physical biochemistry, where I teach advanced kinetics using the stopped flow and temperature jump method, as well as the structure of proteins using time-corelated fluorescence techniques to study changes in protein structure. I also occasionally conducted micro-calorimetry classes, teaching ITC and DSC techniques.

In the following year I was included to the group of teachers for postgraduate students for whom I conduct "protein structure" lab exercises.

In 2006, I started conducting lectures on two courses: "Physical Biochemistry" and "Protein Engineering". In the case of the first one, I give lectures on the issues focused on protein-ligand interaction and techniques that allow to determine the

structure of macromolecules with atomic resolution, and occasionally also the basics of fluorescence techniques. As part of the course on protein engineering, I give a lecture on the issues related to the modification of fluorescent properties of recombinant proteins, protein stability and specificity of the ligand binding.

Since 2006, I also teach and coordinate the course of physical biochemistry dedicated to biophysicists. In this course, more emphasis is placed on the physical basis of biochemistry.

Since 2013, I have been conducting a specialist seminar for biochemists regarding intrinsically disordered proteins (IDPs).

Since 2015, I also co-conduct the course "Introduction to physical biochemistry", dedicated to master students who have not met with physical biochemistry as part of their previous didactic history.

In 2015, I also started classes in structural biology. I am the co-author and coordinator of this course. Within its framework, I focus mainly on the importance of NMR techniques and spectroscopic techniques in the study of macromolecules.

An important part of my didactic activity is also the course sponsored by the Federation of European Biochemical Societies (FEBS) entitled "Ligand-Binding Theory and Practice. Advanced Course". This course is held every two years since 2014 in the Czech Republic in Nové Hrady. I participate in the course from the first edition, lecturing on absorption and fluorescence spectroscopy. However, the core of the course is laboratory classes and consultations, during which I assist the participants in designing and conducting experiments, data analysis and interpretation of the obtained results. This course is dedicated mainly to PhD students and graduate students. The attendants come from most European countries, as well as from Asia, both Americas, Africa and Australia. In my opinion, it is also equally important that the course has also become an excellent platform for direct meetings with other teachers, most of whom are leading researchers in their specialties.

Since 2010 I am also a supervisor of BA and MA theses. I supervise students of biochemistry, biophysics and biotechnology, as well as chemistry and molecular biotechnology lectured in English. I have promoted a total of 12 undergraduate and 20 graduate students.

My organizational activities at the Faculty of BBB result from a natural desire to influence to the quality and efficiency of our work. I am happy to join in projects that I am able to meet at the current stage of my development.

In 2006 - 2013, I was a member of the FBBB teaching committee.

In the years 2006 - 2013 I was the head of part-time studies in biotechnology at the FBBB.

In 2008, I co-organized the XXXV Winter School of the Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University

In the years 2008 - 2012 I was one of the coordinators implementing the project : "Ordering education in technical, mathematical and natural sciences - piloting" - Priority IV PO KL.

In the years 2008 - 2014, I coordinated the cooperation between the Department of Biochemical Physics of WBBiB UJ and ADAMED Group as part of the project "Searching for a biotechnology, a targeted anti-cancer drug".

In the years 2013-2014, I was a member of the committee coordinating the implementation of the project "Million for Biochemistry".

From 2012 I am the supervisor of the "N.zyme" research circle for students of Biochemistry.

Since 2013, I am a member of the Laboratory Equipment Repair Commission at the FBBB.

In 2018 I was the organizer of the thematic session at the XLV Winter School of the Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University entitled: "Signaling in transcription regulation"

From 2019 I'm the Dean's Representative for student internships at the Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University

5. Summary of the whole acquis

I am a co-author of 30 original research papers, 4 review papers, 4 chapters in monographs and 54 conference presentations. Among all the papers in a collaboration, three times I was the first author and eight times the corresponding author.

I have promoted 20 masters and 12 licensees. I also took part in doctoral dissertations, three times acting as an supplementary supervisor and once as a scientific supervisor.

The summary of bibliographic data is presented in the table below.

Table 2. List of bibliographic data.

	before PhD			After PhD			together		
	liczba	IF	MNiSW	liczba	IF	MNiSW	liczba	IF	MNiSW
Oryginal	3	11,041	60	27	69,34	755	30	80,381	815
Reviews	-	-	-	4	4,453	50	4	4,453	50
Chapters in monography	-	-	-	4	-	-	4	-	-
together	3	11,041	60	31	73,793	800	34	84,834	865
Hirsch index									12
The number of citations on 27.02.2019r.							total		351
							without self citations		305

Gniadi