Final scientific report for project PD 115503

PI: Julianna Oláh

Executive Summary:

During the execution of the project we investigated the systems described in the proposal and set out new research directions as well. As this was a post-doctoral fellowship from which only the salary of the PI was paid, the fellowship also helped the PI in developing towards independence and setting up her own research group. The new research directions were initiated in this context and were part of international/national collaborations. Below. I will describe our results obtained in the course of the project and give a detailed description of the poster/oral presentation given during the course of the project. The publication list that is found in the EPR system shows all published, peer-reviewed publications.

Summary of the outcome of the research project:

Sci/Wos publications published during the course of the project	11
publications in Q1 of the journal ranking list	8
sum of impact factors (including expected values) of published papers	42.996
corresponding authorship on published papers	5
invited and oral presentations[§]	7
poster presentations[§]	20

[§]includes presentations by students as well

Research planned in the proposal:

1. **Role of estrogens in the initation of cancer (Year 1 and 2)**

   1.1 Bioactivation of estrogens by CYPs to DNA damaging agents

   (Chem. Res. Toxicol. 2017, 30, 583−594)

   Using a combination of structural analysis, docking, and quantum chemical calculations at the B3LYP/6-311+G* level we investigated the factors that influence the regioselectivity of estrogen metabolism in man. We studied the structure of human estrogen metabolizing enzymes (CYP1A1, CYP1A2, CYP1B1, and

CYP3A4) in complex with estrone using docking and investigated the susceptibility of estrone, equilin, and equilenin (which only differ in the unsaturation of ring B) to undergo 2- and 4-hydroxylation using several models of CYP enzymes (Compound I, methoxy, and phenoxy radical). We found that even the simplest models could account for the experimental difference between the 2- and 4- hydroxylation pathways and thus might be used for fast screening purposes. We also show that reactivity indices, specifically in this case the radical and nucleophilic condensed Fukui functions, also correctly predict the likeliness of estrogen derivatives to undergo 2- or 4-hydroxylation.

1.2 Redox cycling between cathecols and quinones derived from estrogens

We have started to write a publication from the results of sections 1.2 and 1.3

Using a set of experimental hydroquinone/cathecol-quinone datasets a BSc student (Kristóf Nagy) created a calibration diagram to predict 1 e and 2e/2H^+ reduction potentials from energy differences based on *J. Am. Chem. Soc.* 2016, 138, 49, 15903-15910. We can show that with increasing unsaturation of ring B the difference between the reduction potentials of 2,3 and 3,4 quinones significantly increases, and the 3,4-cathecol of equilinene is the most likely species to undergo oxidation. Using the Marcus equation we can show that this is the species that undergoes the fastest oxidation both to semiquinone and quinone. We are still working to get an estimate of the barrier for the reduction of quinones by NADPH. After that we will use our very recent experience in microkinetic modelling (see below) to model the redox cycles of estrogen cathecols and quinones and assess their ROS generating activity.

1.3. Reaction of estrogen o-quinones with the DNA.

Estrogen-o-quinones react with DNA and either stable complexes or depurinating adducts are formed. Peter Girnt, and MSc student has studied the mechanism of these reactions and prepared a thesis for the University Scientific Student Conference. We could show that depurination occurs very fast if the DNA base is doubly protonated and is faster in the case of adenine then of guanine. 2,3-estrogen quinones react differently from 3,4-quinones, but the aromaticity of ring B does not influence the reaction mechanism substiantially.

2. **CYP-mediated bioactivation of cyclophosphamide to DNA-alkylating** agents

This research was planned for Year 3 of the project (thus taking the maternity leave into account it would have meant the period of 2019April-2020March). Unfortunately, a significant publications dealing with the planned research appeared in 2017: Mono- and Di-Alkylation Processes of DNA Bases by Nitrogen Mustard Mechlorethamine ChemPhysChem 2017, 18, 3390 –3401. The major difference between the planned research and the one published is the substituent of the tertiary amine in the mustard, bus as this substitution does not influence the reaction mechanism, we decided to abandon our plans and focussed on other projects that we

considered to have more significant impact and lead to original research instead of repeating something that was already published.

New research directions opened during the course of the project

1. **Study of CYP-related heme containing systems and other iron-containing systems**

1.1. Redox potential-basicity relationship of Compound II of heme enzymes

Results have been published in the form of an oral talk (Seventh scientific workshop (ECOSTBio COST Action CM1305), 2018) and a manuscript is in preparation.

The axial ligand of iron greatly influences the properties of the heme centre: cysteinate-ligated CYP enzymes directly oxidize strong C–H bonds via hydrogen atom transfer mechanisms, while histidine-ligated peroxidases primarily act upon phenol-like substrates following PCET reaction mechanisms. We investigated their basicity and redox potential relationships by performing MD simulations and QM/MM calculations on thiolate-ligated (CYP119, CYP158A2, and an aromatic peroxygenase) and histidine-ligated (horseradish and lignin peroxidases) enzymes. We calculated protonation energies and electron affinities and also determined the pKa of Fe=O functionality and the reduction potential of the active site. We found that a high cpd-II basicity is always coupled to a suppressed redox potential and we offer intuitive support for the notion that CYP enzymes avoid non-productive PCET reactions by shifting their active site properties to a low-redox-potential – high pKa regime. By plotting density difference distributions for vertical electron attachment processes we revealed two conceptually distinct redox scenarios; namely, the reduction is sulphur-based in cysteine ligated enzymes while porphyrin-based in the case of histidine-ligated proteins.

1.2. First principles calculation of the reaction rates for ligand binding to myoglobin: the cases of NO and CO

(Chem. Eur. J. 2018, 24, 5350-5358)

Gas-sensing of O_2, CO and NO by heme proteins is a fundamental process in living organisms. As the overall process involves steps occurring on different time scales, MD simulations were performed to address the diffusion of the ligand through the enzyme, and DFT calculations in combination with statistical rate calculation to investigate the spin-forbidden reaction of the formation of bond between iron and the ligand. The calculations yielded rate constants in qualitative agreement with experiments and revealed that the bottleneck of NO and CO binding is different; for NO, diffusion was found to be rate-limiting, whereas for CO, the spin-forbidden step is the slowest.

1.3. **Study on artificial nitrogenases**

(Inorg. Chem. 2018, 57, 8499-8508, Inorg. Chem. 2019, 58, 7969-7977, and a third manuscript in preparation)

Biomimetic nitrogen fixation provides an attractive alternative for the century-old Haber−Bosch process; however, the performance of the currently available molecular biomimetic catalysts is very limited. We decided to investigate the catalytic cycle of singlesite iron complexes with EPPP tetradentate ligands (E = B, Si) that were synthesized in the group of Jonas Peters (Caltech, USA) and showed to be able to convert N_2 to NH_3 in a homogenous mixture using reductants and proton sources. Unfortunately, the selectivity of the currently known biomimetic catalysts is poor, as they also catalyze the unproductive hydrogen evolution reaction (HER).

First, we calculated the Gibbs free energy of all elementary reaction steps of homogeneous dinitrogen reduction to NH_3 (N_2RR nitrogen reduction reactions) and examined all possible mechanisms. We found that the catalytic mechanism depends on the applied axial ligand and that the distal pathway observed with E = B is the most favorable route regarding the catalytic performance. The lack of thermodynamic driving force in the last steps of the catalytic cycle may be responsible for the low catalytic activity of the studied biomimetic catalysts.

In our second contribution we examined the hydrogen evolution reaction (HER) activity of early intermediates of N_2RR intermediates in EPPP (E = B, Si) ligated single-site biomimetic iron complexes by calculating and comparing the activation Gibbs free energies of HER and N_2RR elementary steps. We showed that early N_2RR intermediates are not likely sources of HER under turnover conditions, as the barriers of the competing N_2RR steps are significantly lower.

As a follow-up to the above-mentioned two projects we wanted to explore the origin of HER we studied further reaction routes and microkinetic modelling of the overall catalytic cycle and side reactions. We propose a side-reaction cycle (amplified hydrogen evolution reaction (aHER)) that can lead to H_2 production by EPPP. This manuscript is still in preparation, but will be submitted soon.

1.4. Spin crossover behavior in a homologous series of Iron (II) complexes based on functionalized Bipyridyl ligands

(Inorg. Chem. 2018, 57, 9880-9891)

A series of bulky substituted bipyridine-related iron(II) complexes $[Fe(H_2Bpz_2)_2(L)]$ (pz = pyrazolyl) were prepared and their X-Ray structures determined in the group of Prof. Garcia (UCLouvain, Belgium). They also studied the spin crossover properties of the studied molecules and showed that some of the complexes display incomplete spin crossover (SCO) behavior because of a freezing-in effect, some undergo gradual and incomplete SCO behaviors, while two of them were shown to exhibit steep SCO. Such different SCO behaviors were attributed to an electronic substituent effect in the bipyridyl ligand conformation and a crystal packing effect. Importantly, the electronic substituent effect of the isopropyl acetate group and C–H···O supramolecular interactions in **4** contribute to a highly cooperative behavior, which leads to an abrupt thermally induced spin transition. My task in the project was the computational investigation of the electronic structure of the synthesized compounds using DFT calculations.

2. **Modelling of the hydrogen-bond network in mixtures and around insulin**

2.1 Hydration sphere structure of proteins: A theoretical study

(J. Mol. Liquids 2017, 238, 462-469)

We studied the water network around insulin (as a model protein) in aqueous NaCl solutions using molecular dynamics simulations and statistical analysis of the topological properties (hydrogen bond neighbor number and the interaction energy between hydrogen-bonded water molecules) of the water network. We proposed a simple method to define the hydration layers around proteins. Water molecules in the first and second layers form significantly less, but stronger hydrogen bonds with each other than in the bulk phase. Furthermore, water molecules over the hydrophilic and hydrophobic surface of the protein possess slightly different H-bonding properties, supporting the hypothesis of structural and dynamical heterogeneity of the water molecules over protein surface. The protein molecule perturbs the solvent structure at least up to the fourth-fifth hydration layer. Our data suggest the peculiar role of the second hydration shell.

2.2 **Water-formamide mixtures: Topology of the hydrogen-bonded network**

(J. Mol. Liquids 2017, 228, 25-31)

Using neutron diffraction measurements and molecular dynamics simulations we studied the hydrogen bonded structure of water-formamide mixtures. The calculated and measured total neutron diffraction radial distribution function agreed very well. After evaluation of this function, the hydrogen-bonded structure of mixtures with various compositions has been studied, and the clustering properties and the topology of the hydrogen-bonded network were investigated. The mixtures exhibit an extended range structure in solution. In water-formamide mixtures the average number of hydrogen bonded neighbors (water, formamide) and the distribution of the number of H-bonded neighbors do not change significantly as a function of the formamide mole fraction. Molecules are shown to form percolated networks at each concentration. The composition of cyclic entities in these systems is very close to being ideal; that is, these systems are microscopically homogeneous.

2.3 **How can we detect hydrogen bond local cooperativity in liquid water: A simulation study**

(J. Mol. Liquids 2017, 245, 140-146)

We investigated the static correlation of the hydrogen bond network in liquid water at various temperatures and densities in a series of molecular dynamics simulations using descriptors derived from network science. We found that the structure of the subsystems of water molecules with 3 and 4 hydrogen-bonds is distinctly different at low temperature, 3-hydrogen-bonded water molecules form branched chain structures at all temperature. Deconvolution of the descriptors of the mixing pattern of water molecules according to their donor and acceptor numbers showed that species with complementary hydrogen bonding properties are likely to correlate and form H-bonds with each other, while species with similar H-bond pattern tend to

avoid each other. Pearson's coefficient of the studied networks suggests that at normal density the H-bonded network in liquid water is an uncorrelated.

3. **Miscellaneous**

3.1 **Quantum chemical calculations support pseudouridine synthase reaction through a glycal intermediate and provide details of the mechanism**
(Theor. Chem. Acc. 2018, 137, 162)

We studied the mechanism of pseudouridylation, the most important RNA modification, using quantum mechanical calculations. Results suggest that the Michael addition scheme is unlikely, as well as the nucleophilic substitution scheme. Our results are in favor of the glycal scheme and provide details for the mechanism that is likely to start with the glycosidic bond cleavage between the ribose and uracil, followed by or coupled to the deprotonation of the C2′-atom of the sugar by the conserved catalytic aspartate. A possible role of the latter step is suggested to be the regulation of the intermediate reactivity: C2′ deprotonation leads to a low-energy intermediate with sufficient lifetime to allow base repositioning before reattachment to ribose by C–C bond formation.

3.2 **DFT study of formation and properties of dinuclear zirconocene cations: Effects of ligand structure, solvent, and metal on the dimerization process**
(J. Organomet. Chem. 2020, 905, 121024)

We studied zirconocene catalysts with Profs. Meelua and Jitonnom from Thailand. Using DFT methods we studied the unfavorable dimerization proccesses of the catalysts as a function of their ligand structures, solvent polarity and metal type. .. The results show that in general the dimer structure in *trans* form is more stable than in the *cis* form. Steric hindrance of the ligand destabilizes as well as increasing polarity of the solvent destabilizes the dimerization process. Vibrational frequencies were calculated for the dimers and the changes in frequencies of the Zr-(μ-Me) stretching vibration were found to correlate well with the dimerization energies ($R^2 = 0.88$).

3.3 **Synthesis, experimental and theoretical studies on the factors influencing the pKa values of new crown ethers containing a diarylphosphinic acid unit**
(Tetrahedron 2016, 72, 8593-8602)

Open chain and crown ethers containing a diarylphosphinic acid unit were synthesized and their pKa values measured in the group of Prof. Peter Huszthy. I was asked to explain explain the discrepancies observed in the pKa values of nitro-substituted crown ethers compared to the analogous open chain ether derivatives. I used MD simulations and DFT calculations to address the question of the measured high pKa of the crown-ether with 4 nitro-groups. The two fundamentally methodologied showed in agreement that the acidic form of this species exists in one major, stable conformation (in contrast to all other observed species) and it causes the experimentally observed high pKa value of the compound in contrast to basic organic chemistry expectations.

International Conference Participation

The name of the presenter is marked with *, while the name of the PI is shown in bold.

Invited presentations:

1. Section talk at 13th European Biological Inorganic Chemistry (EuroBIC 13) Conference, August 28- 1 Sept, 2016, Budapest, Hungary
   title: BIOACTIVATION OF ESTROGENS BY CYTOCHROME P450 ENZYMES
   author: **J. Oláh**, * A. Lábas
2. Plenary talk at SFB 813 Women in Science@Spin Centers, July. 11– 13, 2016, Ihringen, Germany
   title: Multi-scale modelling of cytochrome P450 enzymes and related systems
   authors: **J. Oláh***
3. Plenary talk at 16th International Conference on Theoretical Aspects of Catalysis, Zakopane, Poland, June. 19-23, 2016
   title: Multiscale modelling of enzymatic reactivity
   author: **J. Oláh**,* A. Lábas

Oral Presentations:

1. Seventh scientific workshop (ECOSTBio COST Action CM1305) Dublin (Irország), December 14-15, 2017
   title: REDOX POTENTIAL-BASICITY RELATIONSHIP OF COMPOUND II OF HEME ENZYMES: A QM/MM STUDY
   author: A. Lábas, B. Pintér, **J. Oláh***
2. Girona Seminar 2018, Young Researcher's Symposium, Girona (Spain), April 3, 2018
   title: IDENTIFYING THE CRITICAL STEPS OF N2 FIXATION ON IRON COMPLEXES
   WITH TRI(PHOSPHINO)BORATE AND TRI(PHOSPHINO)SILYL LIGANDS
   author: Z. Benedek*, M. Papp, **J. Oláh** and T. Szilvás
3. XV. International Conference Students for Students, Kolozsvár, (Romania). April 18-22, 2018.
   title: THEORETICAL STUDY ON THE CATALYTIC CYCLE OF ARTIFICIAL NITROGENASES BEARING TRI(PHOSPHINO)BORANE AND TRI(PHOSPHINO)SILYL LIGANDS
   author: M. Papp*, Z. Benedek, **J. Oláh** and T. Szilvási
4. Chemistry towards Biology (CTB9), Budapest, Hungary 24-27 Sept. 2018
   title: MULTISCALE MODELLING OF PROTEIN-LIGAND INTERACTIONS IN HEME ENZYMES
   author: A. Labas, D.K. Menyhárd, J. N. Harvey, **J. Olah**,*

Poster presentations:

1. Girona Seminar: Predictive Catalysis: Transition-Metal Reactivity by Design, Girona, Spain, April 17-20, 2016
   title: BIOACTIVATION OF ESTROGENS BY CYPS TO TOXIC AGENTS
   author: A. Lábas,* B. Krámos, **J. Oláh**
2. 16th International Conference on Theoretical Aspects of Catalysis, Zakopane, Poland, Jún. 19, 2016 – Jún. 23, 2016
   title: Computational study of the reaction mechanism of the box H/ACA pseudouridine synthase catalysed uridine isomerisation
   author: D. J. Kiss,* J. Oláh, G. Tóth, Cs. Magyar, D. Karancsiné Menyhárd, G. G. Ferenczy
3. 16th International Conference on Theoretical Aspects of Catalysis, Zakopane, Poland, Jún. 19, 2016 – Jún. 23, 2016
   title: Why are eukaryotic dUTPases thermally less stable than prokaryotic dUTPases? – a molecular dynamics study
   author: Z. Benedek,* A. Lábas, G. N. Nagy, B. G. Vértessy, **J. Oláh**
4. 13th European Biological Inorganic Chemistry (EuroBIC 13) Conference, august 28-1 Szept., Budapest, Hungary
   title: The role of Mg2+ in the quaternary structure of human dUTPase – an MD study
   authors: Z. Benedek,* A. Lábas, G. N. Nagy, B. G. Vértessy, **J. Oláh**
5. 13th European Biological Inorganic Chemistry (EuroBIC 13) Conference, august 28-1 Szept., 2016, Budapest, Hungary
   title: SUBSTITUENT-EFFECTS IN THE BIOACTIVATION OF CONJUGATED ESTROGENS BY CYTOCHROME P450 ENZYMES
   author: Attila L. Oláh,* **J. Oláh**
6. Seventh scientific workshop (ECOSTBio COST Action CM1305), Dublin (Ireland), December 14-15, 2017
   title: RATIONAL DESIGN OF TETRADENTATE TRANSITION METAL LIGANDS FOR ATMOSPHERIC PRESSURE AMMONIA SYNTHESIS
   author: Z. Benedek*, M. Papp, T. Szilvási and **J. Oláh**
7. 2nd International Conference on Phosphorus Chemistry (ICPC) , Budapest (Hungary). Júl. 8–13, 2018
   title: DETERMINATION OF THE DEACTIVATION MECHANISM OF HOMOGENEOUS
   AMMONIA SYNTHESIS CATALYSTS WITH TRI(PHOSPHINO)BORATE AND TRI(PHOSPHINO)SILYL LIGANDS
   author: M. Papp*, Z. Benedek, T. Szilvási and **J. Oláh**
8. 2nd International Conference on Phosphorus Chemistry (ICPC), Budapest (Hungary). Júl. 8–13, 2018
   title: IDENTIFYING THE CRITICAL STEPS OF N 2 FIXATION ON IRON COMPLEXES
   WITH TRI(PHOSPHINO)BORATE AND TRI(PHOSPHINO)SILYL LIGANDS
   author: Z. Benedek*, M. Papp, T. Szilvási and **J. Oláh**

9. Hungarian Molecular Life Sciences Conference 2019, Eger (Hungary). March 29-31, 2019
   title ATOMISTIC MODELLING OF GAS MOLECULE DIFFUSION IN H-NOX PROTEINS
   author: A. Rozza,* D. K. Menyhárd, **J. Oláh**
10. Hungarian Molecular Life Sciences Conference 2019, Eger (Hungary). March 29-31, 2019
    title: MULTISCALE MODELLING OF PROTEIN-LIGAND INTERACTIONS IN HEME ENZYMES
    author: A. Lábas, D. K. Menyhárd, J. N. Harvey, **J. Oláh***
11. Catalysis Towards Greener Chemistry: Mulheim, (Germany). May 20-23, 2019.
    title: Determination of the deactivation mechanism of biomimetic single-site Fe-nitrogenases
    author: M. Papp*, Z. Benedek T. Szilvási and **J. Oláh**
12. 17th Central European Symposium on Theoretical Chemistry CESTC 2019 Stadtschlaining (Austria). September 9–12, 2019
    title: Estrogen-quinone-induced DNA modifications: A computational study
    author: P. Girnt* és **J. Oláh**
13. 17th Central European Symposium on Theoretical Chemistry CESTC 2019 Stadtschlaining (Austria). September 9–12, 2019
    title: Exploring Hydrogen Amplification Reaction and Deactivation Mechanism of Biomimetic Fe-nitrogenases
    author: M. Papp,* Z. Benedek, T. Szilvási és **J. Oláh**
14. 17th Central European Symposium on Theoretical Chemistry CESTC 2019 Stadtschlaining (Austria). September 9–12, 2019
    title: MAPPING PATHWAYS OF DIATOMIC LIGANDS MIGRATION INTO H-NOX DOMAINS FOR AS A MODEL OF ACTIVATION OF SGC ACTIVATION
    author: A. Rozza,* D. K. Menyhárd, **J. Oláh**
15. Hungarian Molecular Life Sciences Conference 2019, Eger (Hungary). March 29-31, 2019
    title ATOMISTIC MODELLING OF GAS MOLECULE DIFFUSION IN H-NOX PROTEINS
    author: A. Rozza,* D. K. Menyhárd, **J. Oláh**
16. Hungarian Molecular Life Sciences Conference 2019, Eger (Hungary). March 29-31, 2019
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    author: A. Lábas, D. K. Menyhárd, J. N. Harvey, **J. Oláh***
17. Catalysis Towards Greener Chemistry: Mulheim, (Germany). May 20-23, 2019.
    title: Determination of the deactivation mechanism of biomimetic single-site Fe-nitrogenases
    author: M. Papp*, Z. Benedek T. Szilvási and **J. Oláh**
18. 17th Central European Symposium on Theoretical Chemistry CESTC 2019 Stadtschlaining (Austria). September 9–12, 2019

title: Estrogen-quinone-induced DNA modifications: A computational study
author: P. Girnt* és **J. Oláh**

19. 17th Central European Symposium on Theoretical Chemistry CESTC 2019 Stadtschlaining (Austria). September 9–12, 2019
title: Exploring Hydrogen Amplification Reaction and Deactivation Mechanism of Biomimetic Fe-nitrogenases
author: M. Papp,* Z. Benedek, T. Szilvási és **J. Oláh**
20. 17th Central European Symposium on Theoretical Chemistry CESTC 2019 Stadtschlaining (Austria). September 9–12, 2019
title: MAPPING PATHWAYS OF DIATOMIC LIGANDS MIGRATION INTO H-NOX DOMAINS FOR AS A MODEL OF ACTIVATION OF SGC ACTIVATION
author: P. Girnt* és **J. Oláh**