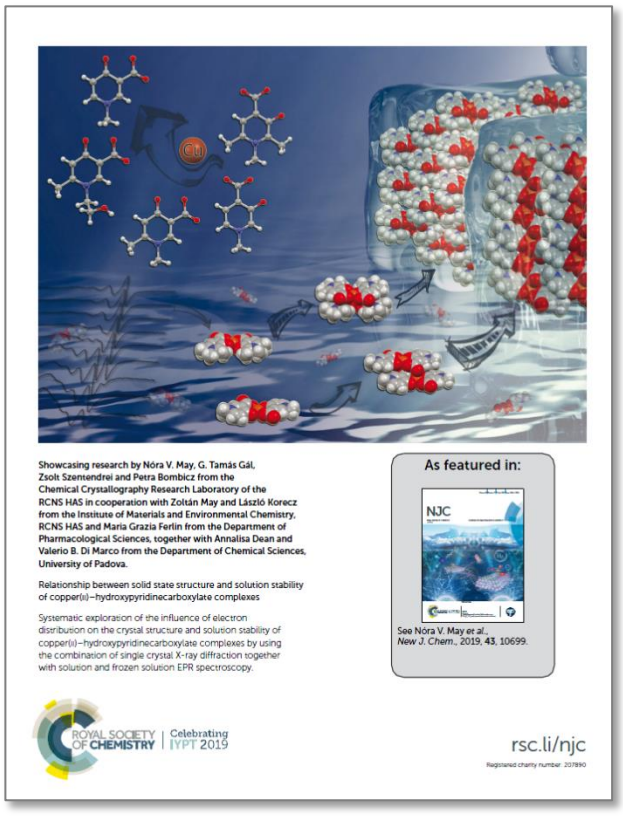
Final report

on the OTKA project 115762 entitled „Structure determination of bioligands and their functional metal complexes in solid and solution phases”

The objective of the project was to investigate the structural features of small molecules with potential biological relevance and their metal complexes in both solid and solution phases. Single crystal X-ray diffraction (SXRD) was used to study the coordination geometry of the complexes and conformations of bioligands and investigate the packing arrangements and main secondary interactions in the crystals. In case of paramagnetic metal complexes electronspin resonance spectroscopy (EPR) was the most powerful technique to reveal the speciation and the most plausible chemical forms in solutions. The synergetic combination of the SXRD and EPR methods provided reliable data for the structure and stoichiometry of the metal-containing compounds. Under this OTKA project 51 new crystal structures have been deposited in the Cambridge Structural Database (CSD) and around 20 copper(II) complexes have been investigated by EPR technique. The results have been published in 18 peer-reviewed articles with an overall impact factor of 53.62. The results have been presented in around 15 national and the same number of international conferences. During the four years of the project 5 MSc students joined the research and learned the theory and practice of SXRD and EPR techniques. The scientific outcome is the result of several collaborations with researchers from the MTA TTK, the Universities of Szeged, Debrecen, Padova, Vienna and Dublin.

1. Hydroxypyridinecarboxylic acids (HPC) as candidates in chelation therapy

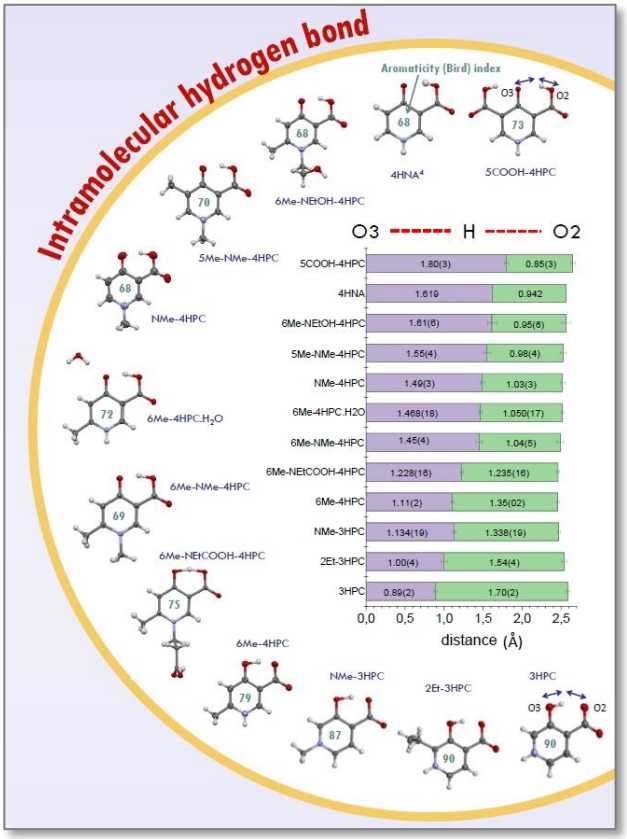
A series of hydroxypyridinecarboxylic acid derivatives, as potential candidates in chelation therapy of metal overloading conditions, was provided us by Prof. Valerio di Marco (University of Padova). The understanding of the structures and the supramolecular interactions (H-bomd, electrostatic and other secondary interactions) of this compounds and their copper(II) complexes will facilitate the fine-tuning of the structural properties in order to produce new substances with better coordination properties. The improved physico-chemical properties influence the formulation of the pharmaceuticals, their stability and also bioavailability.



1.1. Copper(II) complexes of HPCs studied by EPR and SXRD. Influence of electron distribution on the copper(II) complexation properties of a series of hydroxypyridinecarboxylate derivatives was the subject of this project. The crystal structure of the bis-ligand complexes of five different HPCs could be determined with $[O_{carb},O^-][O_{carb},O^-]$ equatorial coordination modes. The different substituents on the ligands however had an influence to the electron distribution on the donor oxygens changing the type of the axial coordination and the *cis* or *trans* arrangements of the donor atoms. While oligomerisation has been detected in solid state, EPR spectroscopy showed monomer complexes in

solutions at room temperature. The stability of the copper(II) complexes showed a positive correlation with the acidity of the hydroxyl group. In order to support the effect of the electron distribution on the detected stability order DFT quantum chemical calculations have also been performed. According to the detected stability order, the copper(II) binding strength of the studied compounds could be set up. This study illustrated well the potential of combined solid state/solution study of bioligand–copper(II) complexes by using single crystal X-ray diffraction together with EPR spectroscopy. The understanding of the solid state and solution structures of copper(II) complexes of small bioligands paves the way to design chelators with predicted coordination modes. These results have been published in the article*: **N. V. May*, G. T. Gál**, Zs. Szentendre, L. Korecz, Z. May, M. G. Ferlin, A. Dean, **P. Bombicz** and V. B. Di Marco: "Relationship between Solid State Structure and Solution Stability of Copper(II) – Hydroxypyridinecarboxylate Complexes", New Journal of Chemistry, 2019, 43, 10699-10710*, and we were offered the opportunity to highlight our results in the Back Cover page.

1.2 Crystal structure of HPC derivatives studied by SXRD. The crystal structures of 11 different HPC derivatives have been determined which led us to study the electrostatic and steric effect of H-donor and –acceptor substituents to the molecular self-assembly. Exploring the secondary interactions is important to the development of new pharmaceutical therapies by understanding the interactions at a drug binding site, in protein-protein interactions and also in drug encapsulation and targeted release mechanism. The position of the hydroxyl proton involved in an intramolecular hydrogen bond with the carboxylate oxygen inform us about the electron distribution on the oxygen donor atoms and about the aromaticity of the pyridine ring. These differences were also manifested in their complexation properties with copper(II), and it is very important as this proton is replaced by the metal ion during the action of chelation therapy. A continuous shift of the hydrogen position from the carboxylate oxygen (oxo form in 4HPC derivatives) to the hydroxyl oxygen (enol form in 3HPC derivatives) could be detected in the investigated series. 6Me-4HPC was the only 4HPC where enol form was detected, however in its hydrate crystal the oxo-form also appeared. In 6Me-NEtCOOH-4HPC the proton is positioned halfway of the two oxygen. To interpret the differences in the electron distributions ring aromaticity was calculated using Bird index. The Bird index is created in a way that for benzene this value is 100 and for the hypothetic cyclohexatrien it is 0. We have found that significantly lower aromaticity was found in 4HPC derivatives, where this value falls in the range 68-73; while it increased to 75 and 79 in 6Me-NEtCOOH-4HPC and 6Me-4HPC, respectively. Highest values were calculated for 3HPCs to be between 87 and 90. The main secondary interactions (π...π, C-O…π, C-H…O, N-H…O) have also been identified where synthon arrangements are preserved in the crystals of different substituents. These results could give a deeper understanding of intermolecular interactions and their effect on the arrangement of molecules in the solid phase influenced by addition of H-donor and

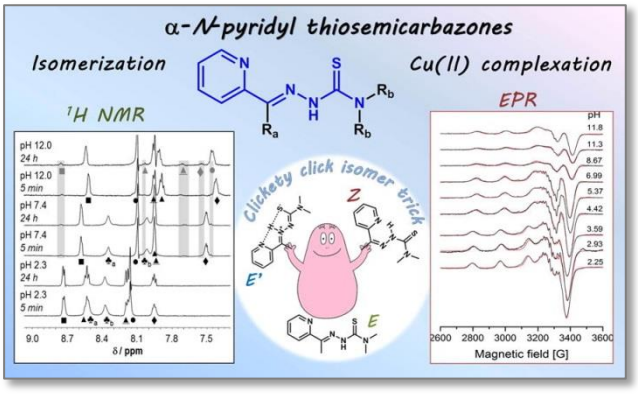


acceptor substituents. Increasing knowledge on the principles of supramolecular chemistry contributes to the crystal engineering and prediction ability and brings us closer to the manipulation of supramolecular packing architecture. Our results have been presented in the 32st European Crystallographic Meeting, 18-24 August 2019, Vienna, Austria, and a paper is under preparation: ***N. V. May*, G. T. Gál, T. Holczbauer****, V. B. Di Marco and* ***P. Bombicz****: „Gradual changes in the aromaticity vs. intramolecular H-bond interactions observed in a series of substituted β-hydroxypyridinecarboxylic acids."*

2. Complexes as potential anticancer agents

Cisplatin (cis-diamminedichloridoplatinum(II)) is one of the most powerful chemotherapeutic drugs used for the treatment of several kind of cancers (carcinoma of lung, testis, esophagus, ovarian, breast and prostate). Despite the great efficacy of cisplatin, it shows a high cytotoxicity also for normal tissues and causes several side-effects as neuro- and nephrotoxicity, moreover, the effectiveness of cisplatin as drug is limited by the development of drug resistance. To overcome these limits the study of thiosemicarbasones (TSCs) derived complexes with copper(II) have been the goal of this project which has expanded in the last years by other ligands as well as ruthenium(II) and rhodium(III) „piano-stool" complexes with (N,O) donor ligands.

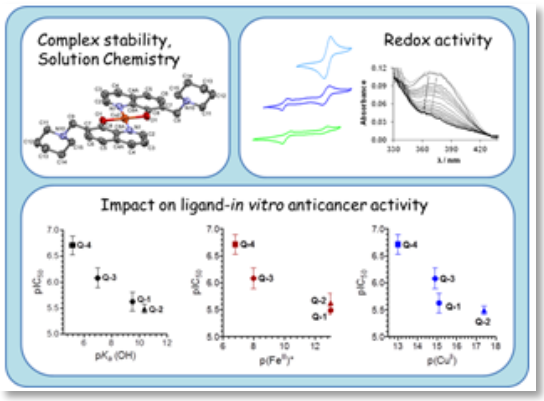
2.1 Copper(II) complexes studied by EPR and SXRD. Thiosemicarbazones (TSCs), and their metal complexes can be new candidates as antitumor agents owing to their topoisomerase IIα or ribonucleotide reductase inhibitor activity. One of the modern challenges is the design of novel active TSCs and their metal complexes with enhanced aqueous solubility and a priori oriented towards a cancer specific target. In this field we have been investigated 2-formylpyridine thiosemicarbazone (FTSC) and pyridine-2-carboxaldehyde N^4,N^4-dimethylthiosemicarbazone (PTSC) with copper(II). By the help of an in-situ pH-titration a series of EPR spectra have been recorded between pH 2-12 in order to follow the complexation properties of the ligands and detect the coexisting species of different stoichiometry. The stability and structural properties have been compared with previously studied TSC derivatives. FTSC acting as a tridentate ligand forming mono-ligand complexes such as $[CuLH]^{2+}$, $[CuL]^{+}$ and [CuL(OH)], and a bis complex $[CuL_2]$. Based on the results we could conclude $[CuL]^{+}$ is formed containing the (N_{pyr},N,S^{-}) donor set, $[CuL_2]$ is formed only at ligand excess and two isomers were identified in solution by the EPR spectroscopic measurements. In complexes $[CuL_2]$ two or three nitrogen donors are found at the equatorial positions in the major and in the minor isomer respectively, suggesting equatorial coordination of ($N_{pyridyl}$,N,S)(N) and ($N_{pyridyl}$,N,S)(S) donor sets (probably with additional axial coordination of this second ligand). The results are summarized in the article: *O. Dömötör,* ***N. V. May****, K. Pelivan, T. Kiss, B. K. Keppler, C. R. Kowold,* ***É. A. Enyedy****: "A comparative study of a-N-pyridyl thiosemicarbazones: spectroscopic properties, solution stability and copper(II) complexation" Inorganica Chimica Acta 472, 2018, 264-275.* This article received already four independent citations.



In comparing the tridentate ligands with a bidentate TSC derivative, the pyrazolyl thiosemicarbazone (Ph-pyrTSC) have been investigated. The crystal of $[Cu(Ph\text{-}pyrTSCH_{-1})_2]$ complex could be successfully crystallized and the structure was determined by SXRD. The structure confirm the bidentate coordination of

the ligands via (N,S$^-$) donor set with deprotonated hydrazinic nitrogens. Because of low solubility in water, the solution structure of this complex was investigated in DMSO by EPR spectroscopy. The solution study revealed that dissolution of the bis-complex preserves the coordination mode. In vitro cytotoxicity studies showed that complexation with the metal ions has increased the cytotoxic activity in human colonic adenocarcinoma and human embryonal lung fibroblast in comparing with the free ligands. These results have been recently accepted for publication in *Journal of Inorganic Biochemistry*: *O. Dömötör, M. A. Kiss,* ***G. T. Gál, N. V. May****, G. Spengler, M. Nóvé, A. Č. Gašparović, É. Frank,* ***É. A. Enyedy****:" Solution equilibrium, structural and cytotoxicity studies on Ru(η^6-p-cymene) and copper complexes of pyrazolyl thiosemicarbazones".*

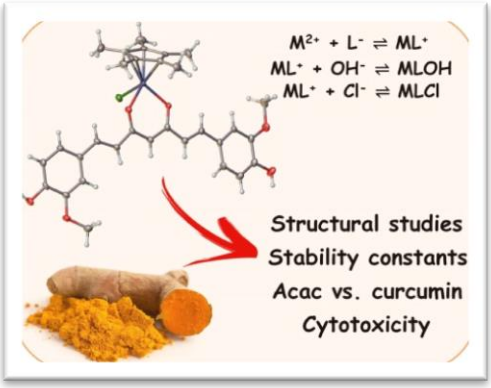
Selected 8-hydroxyquinolines as bidentate (N,O) ligands were also studied. The anticancer activity of 8-hydroxyquinolines relies on complex formation with redox active copper and iron ions. For this, copper(II) complexes of 8-hydroxyquinoline derivatives have been studied by the comparative structural investigation techniques of SXRD and EPR. EPR spectroscopy was used to compare complex formation processes of the reference compound 8-hydroxyquinoline (Q-1) and three related Mannich bases with copper(II) to reveal possible correlations with biological activity. The studied derivatives contains a CH_2–N moiety at position 7 linked to morpholine (Q-2), piperidine (Q-3), and chlorine and fluorobenzylamino (Q-4) substituents. Solid phase structures of Q-3, Q-4·HCl·H_2O, [(Cu(HQ-2)$_2$)$_2$]·2(CH_3OH)·4Cl·2(H_2O), [Cu(Q-3)$_2$]·2Cl and [Cu(HQ-4)$_2$(CH_3OH)]·$ZnCl_4$·CH_3OH were characterized by single-crystal X-ray diffraction analysis. Correlation analysis of the anticancer activity and the metal binding properties of the compound series indicates that, at physiological pH, weaker copper(II) and iron(III) binding results in elevated cytotoxicity. A linear relationship between the pK_a (OH) and IC_{50} values of the studied 8-hydroxyquinolines was found. In summary, we identifed Q-4 as a potent and selective anticancer candidate with significant toxicity in drug resistant cells. This study was performed together with the Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences and University of Szeged and have been published in the paper: *V. F. S. Pape,* ***N. V. May, G. T. Gál****, I. Szatmári, F. Szeri, F. Fülöp, G. Szakács and* ***É. A. Enyedy****: "Impact of copper and iron binding properties on the anticancer activity of 8-hydroxyquinoline derived Mannich bases", Dalton Trans., 2018, 47, 17032–17045.*



A series of copper(II) complexes of coumarin derived Schiff base ligands was studied for their pro- and antioxidant behaviour in the MCF-7 human breast cancer cell line in the Centre of Applied Science and Health (TU Dublin, Ireland). At the request of Bernadette S. Creaven two Schiff base ligands have been investigated by EPR spectroscopic methods. The solution studies indicated that monomeric species are present in the Cu(II) – L1 system at neutral pH, whereas dinuclear species were observed in the case of the Cu(II) – L7 system. As in the later case the EPR spectra disappeared, we proposed that the OH group on the R1 position is able to bind to another copper complex and vice versa forming an EPR-silent dimer species. This difference in speciation was reflected in their relative cytotoxicities with the copper(II) complex of L1, showing significant cytotoxicity against MCF-7 cells whilst the complex of L7 was inactive. The results were published in the article: *L. MacLean, D. Karcz, H. Jenkins, S. McClean, M. Devereux, O. Howe, M. D. Pereira,* ***N. V. May****,* ***É. A. Enyedy****, B. S. Creaven:" Copper(II) complexes of coumarin-derived Schiff base ligands: Pro- or Antioxidant activity in mammalian cells?" Journal of Inorganic Biochemistry 197 (2019) 110702.*

2.2 Ruthenium and Rhodium half-sandwich complexes studied by SXRD. With the goal to improve the selectivity and open new way of actions Ru(II)/(III) complexes have been identified as promising alternatives to anticancer Pt compounds (some ruthenium compounds are currently undergoing clinical trials as potential anticancer drugs). Due to these results "piano-stool" organometallic complexes has become a mainstream research area, and numerous complexes were found to have antitumor activity. These compounds often cause less side effects compared to platinum drugs and are selective for cancer cells. During this project complex formation of Rh(η^5-C_5Me_5) and Ru(η^6-*p*-cymene) organometallic cations with different 8-hydroxyquinoline (HQ) ligands were studied. The solid phase structure of the [Rh(η^5-C_5Me_5)(8-quinolinolato)(Cl)] complex was characterized by single-crystal X-ray diffraction analysis. As it is expected, the rhodium(III) centre exhibits a pseudooctahedral ("piano-stool") geometry, and the C_5Me_5 moiety occupies facially three coordination sites, while the deprotonated ligand is bidentate via its (N,O) donor atoms and the coordination sphere is completed with a chlorido ligand. The binding of the different donor groups resulted in a pseudochiral centre around Rh, nevertheless the complex crystallized in a racemic form. Similar cytotoxicity of the ligands and their Ru(η^6-*p*-cymene) and Rh(η^5-C_5Me_5) complexes was obtained against human uterine sarcoma cell line and its multidrug resistant counterpart (MES-SA/Dx5). These results can be found in the article: *O. Dömötör, V. F. S. Pape,* ***N. V. May****, G. Szakács and* ***É. A. Enyedy****,*:" Comparative solution equilibrium studies of antitumor ruthenium(η^6-p-cymene) and rhodium((η^5-C_5Me_5) complexes of 8-hydroxyquinolines" Dalton Trans., 2017,46, 4382-4396.* The great interest is shown by the fact that this article received 8 independent citations so far.

Curcumin and its metal complexes are extensively investigated bioactive compounds. We have studied the speciation and structure of its half-sandwich organometallic complexes with Rh(η^5-C_5Me_5). Acetylacetone (Hacac), as the simplest β-diketone ligand bearing (O,O) donor set, was involved in the comparison. Formation constant of [Rh(η^5-C_5Me_5)(H_2curcumin)(H_2O)]$^+$ reveals similar solution stability to that of the acac complex. We have determined single crystal structures of two complexes by X-ray diffraction. The results can be found in the paper: *J. P. Mészáros, J. M. Poljarević,* ***G. T. Gál, N. V. May****, G. Spengler and* ***É. A. Enyedy*****:" Comparative solution and structural studies of half-sandwich rhodium and ruthenium complexes bearing curcumin and acetylacetone" Journal of Inorganic Biochemistry 195 (2019) 91–100.*

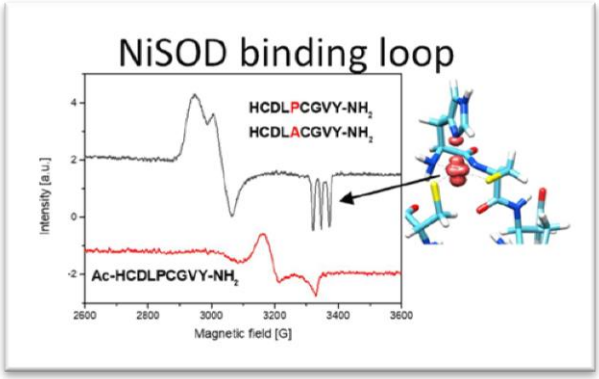


The Ru(η^6-toluene)(Cl) complexes of picolinate, substituted with different electron attracting and withdrawing groups, have been investigated and a positive correlation was found between the Ru-Cl bond distances and the electron attracting nature of the substituents. This suggests that more electron attracting groups can increase the Cl – H_2O exchange rate in solution which was proved to have significant impact on the biological activity. These results have been published in the paper*: J. M. Poljarević,* ***T. G. Gál, N. V. May****, G. Spengler, O. Dömötör, A. R. Savić, S. Grgurić-Šipka,* ***É. A. Enyedy*****:"Comparative solution equilibrium and structural studies of half-sandwich ruthenium(II)(η^6-toluene) complexes of picolinate derivatives", Journal of Inorganic Biochemistry 181 (2018) 74–85.*

3. Investigating coordination compounds as enzyme models

A large number of proteins contain metal ions to execute their functions. Metalloproteins have many different functions in cells for example storage and transport of proteins, or they catalyze reactions that are difficult to achieve in organic chemistry. Their common structural feature is that a metal ion is bound to the protein in a pocket whose shape fits the substrate. This pocket is usually investigated by low molecular weight enzyme mimics to understand the structure of the pocket and the mechanism of their function.

3.1 SOD-mimicking oligopeptides studied by EPR. We have been investigated the SOD-mimicking oligopeptides of Cu(II) and Ni(II)/(III) in cooperation with Norbert Lihi (University of Debrecen). In situ oxidation of the Ni(II) complexes yielded Ni(III) transient species in the case of nonapeptides. The square-pyramidal coordination environment with axial imidazole ligation provides the active structure of the oxidized form of NiSOD in the case of N-terminally free peptides which was proved by EPR spectroscopy. Consequently, these ligands are promising candidates for modeling NiSOD. The results have been published in the paper: *N. Lihi, G. Csire, B. Szakács,* ***N. V. May****, K. Várnagy, I. Sóvágó and I. Fábián, "Stabilization of the Nickel Binding Loop in NiSOD and Related Model Complexes: Thermodynamic and Structural Features", Inorg. Chem. 2019, 58, 1414−1424.*



3.2 Copper(II) and Zinc(II) complexes of Polydentate ligands studied by SXRD and EPR. New polydentate ligands have been investigated as enzyme mimics for Cu(II) and Zn(II) containing metalloproteins in collaboration with Tamás Gajda (University of Szeged). In the copper(II)-ligand equilibrium systems EPR spectroscopy was used to follow the stepwise deprotonation of the multidentate ligands forming complexes with different stoichiometry in a highly overlapped equilibrium system. In these systems crystallization is usually limited to the predominant species or is impossible owing to the long and flexible non-coordinating part of the ligand. Copper(II) complexes of the tripodal ligand trenpyz (tris[2-(5-pyrazolylmethyl)aminoethyl]amine) were characterized in both solution and solid states. A combined evaluation of potentiometric, UV-Vis and EPR data provided both thermodynamic and structural information on the complexes formed in aqueous solution. Copper(II) complexes of $[Cu(trenpyz)](ClO_4)_2$ could be crystallized which were measured by SXRD. Tripodal peptides containing non-protected N-terminal (tren3his) and C-terminal (nta3his) histidines have also been synthesized in order to combine the structuring effect of tripodal scaffolds and the strong metal binding properties of histidine moieties. EPR spectroscopy was used to reveal the mono and dinuclear species in solutions of copper(II) and the ligands at different metal-to-ligand concentration ratios. These results have been published in the articles: *F. Matyuska,* ***N. V. May****, A. Bényei and T. Gajda, "Control of structure, stability and catechol oxidase activity of copper(II) complexes by the denticity of tripodal platforms" New J. Chem 2017, 41, 11647-11660* and *Á. Dancs,* ***N. V. May****, K. Selmeczi, Z. Darula, A. Szorcsik, F. Matyuska, T. Páli and T. Gajda: "Tuning the coordination properties of multi-histidine peptides by using a tripodal scaffold: solution chemical study and catechol oxidase mimicking" New J. Chem., 2017,41, 808-823*

3.3 Investigation of Copper(II)-histidyl-glycine coordination isomers by EPR. In metalloenzimes histidine side chains are usually coordinates to the metal centre but in oligopeptides the coordination of the backbone amide is more pronounced. The two type of coordination modes can co-exist as coordination isomers in small oligopeptides. The detection of these isomers are usually challenging with spectroscopic

techniques, since the difference between the individual spectra are usually small and the deconvolution of the superimposed spectra is not always possible. The isomerisation process of histidyl-glycine $[CuL]^+$ complex has been investigated by temperature dependent EPR and CV measurements in collaboration with Béla Gyurcsik (University of Szeged). In this complex the ligand can coordinate in a histamine- or in a peptide-type way depending on the protonation of the amide or the imidazole nitrogen. Only the histamine type of coordination was proved by the SXRD structure of the $[CuL_2]$ complex so far. Our results give the first spectroscopic evidence for the coexistence of these coordination isomers and show that the amount of the peptide-type complex is increasing with the temperature. See the article: *E. Tóth, **N. V. May**, A. Rockenbauer, G. Peintler and B. Gyurcsik:" Exploring the boundaries of direct detection and characterization of labile isomers – a case study of copper(II)–dipeptide systems" Dalton Trans., 2017, 46, 8157-8166.*

4. Organic and inorganic catalysts studied by SXRD

SXRD is a powerful method to determine the structure of both organic and inorganic catalysts which are crucial for the synthesis of new drug candidates. Structural analysis of some catalysts, as well as the compounds received by the application of these catalysts have been performed.

Chiral phase transfer catalysts have been studied in cooperation with the group of Tibor Soós (Institute of Organic Chemistry RCNS HAS). The rigid structure of these molecules resulted in highly porous hydrogen bond assisted ionic organic frameworks (iHOF). We described the polymorphism and solvatomorphism of porous cationic molecular crystals constructed by the assistance of C–H···Br− and $Br^-\cdots\pi$ interactions. The results have been published in CrystEngComm and they were highlighted in the Back Cover page of the journal: *D. V. Horváth, **T. Holczbauer, * L. Bereczki**, R. Palkó, **N. V. May**, T. Soós and **P. Bombicz** "Polymorphism of a porous hydrogen bond-assisted ionic organic framework" CrystEngComm, 2018, 20, 1779-1782.*

The dimeric structure of a Pd-complex synthesised by Zoltán Novák (MTA-ELTE "Lendület" Catalysis and Organic Synthesis Research Group) was verified by SXRD which was used in a palladium catalyzed C-H activation processes: *S. Kovács, B. L. Tóth, G. Borsik, T. Bihari, **N. V. May**, A. Stirling,* Z. Novák: "Direct ortho-Trifluoroethylation of Aromatic Ureas by Palladium Catalyzed C-H activation: A Missing Piece of Aromatic Substitutions", Adv. Synth. Catal. 2017, 359, 527-532.*

A compound with four $[Ag(py)_2]^+$ and one $[Ag(py)_4]^+$ cations and having four coordinated and one non-coordinated permanganate anion has been synthesized by László Kótai and coworkers (at the Institute of Materials and Environmental Chemistry, RCNS HAS), as potential oxydants in organic chemistry. The SXRD structure of this compound was determined. This compound has proved to be a mild and efficient oxidant toward benzyl alcohols*. G. B. Kovács, **N. V. May, P. Bombicz**, S. Klébert, P. Németh, A. Menyhárd, G. Novodárszki, V. Petrusevski, F. P. Franguelli, J. Magyari, K. Béres, I. M. Szilágyi and L. Kótai: „An unknown component of a selective and mild oxidant: structure and oxidative ability of a double salt-type complex having κ^1O-coordinated permanganate anions and three- and four-fold coordinated silver cations", RSC Advances, 9, 28387 - 28398.*

Several small organic compounds, by-products of the works detailed above, were investigated and their SXRD structures have been published in Acta Crystallographica Section E during the years in the frame of this research project.