Poster Presentations
Studies on platinum(II) complexes of the forms: cis-PtL(NH\textsubscript{3})Cl\textsubscript{2} and cis-PtL\textsubscript{2}Cl\textsubscript{2} where L stands for a planaramine ligand

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Cisplatin is a highly used and successful drug in cancer chemotherapy. However, it suffers from two major drawbacks which are severe normal tissue toxicity and the frequent occurrence of initial and acquired resistance to treatment. The side-effects include neurotoxicity, nephrotoxicity, ototoxicity, nausea, vomiting and hair-loss. In an attempt to reduce toxicity and widen the spectrum of activity, thousands of cisplatin analogues have been prepared by varying the nature of the labile and non-labile ligands. However, it is found that all cisplatin analogues generally have similar spectrum of activity and develop similar resistance. Currently platinum compounds with markedly different structures are looked at with the idea that these may have distinctly different types of interaction with DNA and hence different spectrum of activity eg planaramine platinum complexes and compounds with multiple metal centres. It was suggested that the introduction of bulky planaramine ligands could activate trans-geometry by reducing the reactivity of the compounds. Indeed such compounds have been prepared. It has also been found that planaramineplatinum(II) complexes with cis-geometry can also be anticancer active (eg. AMD473. The aim of the present study is to prepare new cis-planaramineplatinum (II) complexes of the type cis-PtL\textsubscript{2}Cl\textsubscript{2} and cis-Pt(NH\textsubscript{3})LCl\textsubscript{2} [where L stands for 3-hydroxypyridine and 2,3-diaminepyridine], determine the activity of the designed complexes against a number of cancer cell-lines and quantify the nature of interaction with DNA. The complexes are synthesized using modified Dhara method. Interaction with DNA is studied using gel-electrophoresis, HPLC, AAS, UV-visible spectrophotometry and restriction enzyme digestion. Activity against a number of cisplatin-responsive and cisplatin-resistant cancer cell-lines is determined using MTT assay. All the three compounds are found to be less active than cisplatin. This poster will describe the results of the studies in terms of their structure and DNA binding profile.
Investigation of Indoleamine 2,3-Dioxygenase using Synchrotron Radiation Techniques.

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Indoleamine 2,3-dioxygenase (IDO) is a monomeric, glycoprotein (40-45 kDa) enzyme containing protoporphyrin IX.\textsuperscript{1-3} It is the enzyme involved in the initial and rate limiting step in the breakdown of L-tryptophan along the kynurenine pathway. Trp is the least abundant, essential amino acid required for protein synthesis. In its native ferric form IDO is inactive and requires reductive activation.\textsuperscript{4,5} Once in its active ferrous form, IDO has a high affinity for L-Trp. Both the Fe(III) and Fe(II) forms of IDO have been studied \textit{in vivo} and \textit{in vitro}. Spectroscopic evidence to date has shown that IDO is similar to typical monomeric hemeproteins such as Mb and Lb.\textsuperscript{6-8} As such, IDO is expected to exhibit properties like other hemeproteins with similar coordination environments.

While NO is a potentially toxic free radical, it is also an important signal and effector molecule in the body, and as such is involved in the immune, nervous and vascular systems of mammals. Recently, it has been found that there is a similarity, and possibly a relationship, between the L-Arg and L-Trp metabolic pathways, which are initiated by NOS and IDO, respectively.\textsuperscript{9} NO inhibits IDO \textit{in vitro} and it is postulated that it regulates IDO activity in vivo.

Using synchrotron radiation techniques the structure of various forms of IDO have been investigated. XAFS and XANES have been used to show a similarity of IDO binding sites to those in Lb and Mb in vitro. SRIXE and micro-XANES were then used to map and characterise the nature of IDO within endothelial cells exposed to the cytokine interferon-\textgreek{g}. 

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Hydrolysis of esters by zinc(II) complex of a tetradeptate ligand providing sulfur and nitrogen donors

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Zinc is the active metal in a lot of hydrolytic enzymes. Some of these enzymes, such as β-lactamase, contain sulfur atom as ligand.\(^1\) To understand the role of sulfur donor atom upon hydrolysis, some zinc complexes with the ligand providing sulfur donor atom have been synthesized as a model of the active site of zinc metalloenzymes.\(^2\) However, their reactivity toward hydrolysis is scarcely observed, therefore the role of sulfur donor atom upon hydrolysis remains to be elucidated.

In this report, we synthesized a monomeric zinc(II) complex with the sulfur-containing tetradeptate ligand, \([(\text{bpmta})\text{Zn(H}_2\text{O})]\text{(ClO}_4\text{)}\text{)}_2\) (bpmta = N-(3-methylthiopropyl)bis(2-pyridylmethyl)amine) (1). The hydrolytic reactivity of complex 1 toward p-nitrophenyl acetate (pNPA) and bis(4-nitrophenyl)phosphate (BNP) was investigated under aqueous acetonitrile media. Also, that was compared with that of the \([(\text{tpa})\text{Zn(H}_2\text{O})]\text{(ClO}_4\text{)}\text{)}_2\) complex (tpa = tris(2-pyridylmethyl)amine) (2).

The complex 1 was synthesized by treating the bpmta ligand with an equimolar amount of Zn(ClO\(_4\))\text{)}_2\text{•6H}_2\text{O} in ethanol. X-ray crystallographic analysis of 1 revealed a mononuclear nitrogen/sulfur-ligated zinc aqua complex in which the donor atoms of the bpmta ligand coordinated to the zinc center. The five-coordinate zinc ion in 1 exhibits a slightly distorted trigonal bipyramidal geometry. The complex 1 promoted the hydrolysis of pNPA and BNP. The pseudo-first-order rate constant of pNPA hydrolysis was estimated at pH 9.2 and 25°C under an excess of complex. Also, that of BNP hydrolysis was estimated at pH 10.2 and 30°C under an excess of BNP. The result revealed that complex 1 can hydrolyze those esters faster than complex 2 under the basic condition. The details of the hydrolysis reaction mechanism will be discussed in this presentation.

Increasing the cellular accumulation of platinum(IV) complexes

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Platinum(IV) drugs are thought to be promising due to their relatively low reactivity. This should enable the drug to arrive at the tumour site intact, and reduce the incidence of side reactions that may initiate some of the toxic side effects associated with chemotherapy. The addition of lipophilic axial functional groups to platinum(IV) moieties is expected to increase their cellular accumulation. However, we have found that a series of platinum(IV) complexes, encompassing a range of lipophilicities, displayed lower cellular accumulation than cisplatin.

A strategy by which to improve the cellular accumulation of platinum species is to tether the platinum moiety to an intercalator. Platinum-intercalator complexes have been shown to bind to DNA more rapidly than the analogous platinum complex due to the presence of the intercalator.[1] Hence it is anticipated that such compounds will display significant cellular accumulation.

Cellular distribution of a number of platinum complexes has been investigated using micro-SRIXE (synchrotron resonance induced X-ray emission).[2] This technique has been used for the platinum(II) – anthraquinone complexes shown below, revealing that their localisation is significantly greater than that of cisplatin. Cellular accumulation of the complexes will also be reported.

References
Dinuclear Ruthenium Complexes as Sequence- and Structure-Selective Binding Agents for DNA

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Mononuclear transition metal complexes have been extensively used to examine the principles of DNA recognition, in order to provide a basis for the rational design of molecules that can selectively target particular nucleic acid sequences and structures. An understanding of how molecules find a unique binding position on a segment of DNA ranks among the most fascinating and important current chemical or biochemical questions, and the rational design of such molecules clearly requires an understanding of the physical and chemical principles involved in DNA and RNA recognition. However, studies with mononuclear complexes have only provided limited information, due to their size and moderate DNA binding affinity. Dinuclear complexes were anticipated to have increased binding strengths and experiments by others and in our own laboratories support this. In addition to increased binding strength there are considerable advantages to be achieved from the larger range of stereoisomers in modular form that will allows us to make oligonuclear complexes which will bind with high affinity and selectivity.

We have developed systematic synthetic and stereochemical strategies of the three uniquely different systems of dinuclear complexes and have commenced studies of their interactions with oligonucleotides by NMR spectroscopy.

1. Bis-intercalating complexes, D,D-[(dpq)_2Ru(phen-n-SOS-n-phen)Ru(dpq)_2]^4+ (where n = 3, 4 or 5 and SOS = 2-mercaptoethyl ether; the ligand dpq is an intercalator).
2. Groove-binding rigid dinuclear complexes such as [{Ru(phen)}_2(µ-HAT)]^4+ or [{Ru(Me_2bpy)}_2(µ-bpm)]^4+.
3. Mixed intercalating/groove-binding complexes with an intercalating ligand on one metal centre and non-intercalating ligands on the other, with both flexible or rigid bridging ligands e.g. [{(bpy)(dpq)}Ru(µ-bpm)Ru(bpy)_2]^4+.

The development of a model laboratory experience for advanced undergraduates using a bioinorganic model complex of a dimeric $\mu$-oxo-bridged manganese complex

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Bioinorganic chemistry is an area that has grown recently due to the synthesis and characterization of new organometallic compounds, metal clusters and bioinorganic compounds. There is a need to provide beneficial laboratory experiences for undergraduates in bioinorganic chemistry that prepare them for careers in research and medicine. This work will describe a series of experiments that are suitable for advanced undergraduates and will introduce them to the preparation and characterization of a bioinorganic model complex. The experiments are relevant to photosynthetic water oxidation, a process catalyzed by photosystem II (PSII) at an active site that contains a tetrameric $\mu$-oxo-bridged manganese (Mn$_4$) cluster. Bioinorganic models of the Mn$_4$ cluster aid in understanding the mechanism of water oxidation by PSII and may also be useful in developing artificial water oxidation catalysts. Students explore the chemistry of a dimeric $\mu$-oxo-bridged manganese complex that is a functional model for the Mn$_4$ cluster. They synthesize the complex $\left(\text{terpy}\right)\left(\text{H}_2\text{O}\right)	ext{Mn}^{\text{III}}\left(\text{O}\right)\text{Mn}^{\text{IV}}\left(\text{OH}_2\right)\text{terpy}\right)^{3+}$ (terpy = 2,2':6,2"-terpyridine) (1), and characterize 1 and its oxidation to a one-electron oxidized form by UV-visible spectroscopy. The complex 1 catalytically oxidizes H$_2$O to O$_2$ when peroxymonosulfate (oxone, H SO$_5^-$) is used as the primary oxidant. Students also measure the rate of O$_2$ production and calculate the deuterium kinetic isotope effect when D$_2$O is used in place of H$_2$O as a substrate. Techniques and concepts emphasized include inorganic synthesis, UV-visible spectroscopy, titrations, catalytic mechanisms, oxidation/reduction reactions, and kinetics.

EPR of monoMn-, MnMn-, ZnMn-, and FeFe-Sites in $\gamma$-GCS plus Substrates or Inhibitors

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$\gamma$-Glutamylcysteine synthetase ($\gamma$-GCS) is a di-magnesium enzyme that catalyzes the rate limiting reaction of glutathione synthesis. Binding of L-BSO (buthionine sulfoximine), L-Glu, L-Cys, or $\alpha$-ABA ($\alpha$-aminobutyric acid) to $\gamma$-GCS from E. coli produces resolved hyperfine lines with 45 G splittings upfield and downfield from $g=2$, indicating spin-spin coupling of two Mn ions (1,2). Temperature dependence of the high field line for $\gamma$-GCS +/-substrates +/-inhibitors gives J values of ~2.3 to ~3.5 cm$^{-1}$ and analysis of the position of the high field line gives r(Mn-Mn) and D$_2$ values of 3.7 to 3.6 Angstrom and ~0.02 to ~0.04 cm$^{-1}$ respectively. A single equivalent of Mn binds to the tight binding site (n$_1$), however, in the presence of one equivalent of Zn, Mn binds to the n$_2$ site. Upon addition of ATP and $\alpha$-ABA to ZnMn-$\gamma$-GCS, five resolved transitions are used to estimate the ZFS parameters for n$_2$ Mn. A six line Mn signal from monomMn-$\gamma$-GCS plus BSO and the absence of a six line spectrum at g about 4 for ZnMn-$\gamma$-GCS plus BSO is used to assign the six line spectrum to the n$_1$ site. For FeFe-$\gamma$-GCS, a mixed valence EPR signal ($g$-values: 2.03,1.93,1.90) is observed. These EPR parameters are useful as probes of the metal sites, especially in the presence of substrates and inhibitors. Future work is directed towards comparing EPR parameters for mono and di-metallo-$\gamma$-GCS in E. coli to parameters from human $\gamma$-GCS to understand the difference in binding of substrates and inhibitors.

Reactivity of Platinum(II) Ammine Bonds Trans to Sulfur in Methionine and Thiolate Complexes

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Among the molecules in biological systems which are most reactive toward cisplatin and its metabolites are those containing sulfur donor atoms, such as methionine (Hmet), cysteine (H₂cys), and peptides and proteins containing these amino acid residues.¹² Diammineplatinum(II) complexes with these species bound are therefore important in the metabolism of cisplatin. One of the more interesting aspects of the chemistry of these complexes is the labilization of ammine trans to the sulfur donor atoms, which have high trans effect, as this effectively provides an additional coordination site which can react with biomolecules.¹ There has, however, been little systematic investigation of this type of reaction.

The ammine ligand trans to sulfur in the methionine chlate complex [Pt(NH₃)₂(Hmet-N,S)]²⁺ may be displaced by chloride. Kinetics of this reaction have been followed by ¹⁵N NMR, using starting materials with the ammine ligands highly enriched in ¹⁵N. The reaction is second order, depending on concentrations of the starting complex and of chloride ion. The second order rate constant, k₂, is pH-dependent, having the form

\[ k₂ = a + b[H^+] \]

Guanosine monophosphate (GMP) reacts faster than chloride, although the acid dependence is more complicated because of the variation in protonation state of GMP. The reaction also proceeds to [Pt(GMP)₂(Hmet-N,S)], in which both ammine ligands have been replaced.

Labilization of ammine by trans-thiolate sulfur is more effective in the complex with cysteine chelated, [Pt(NH₃)₂(Hcys-N,S)]⁺ than in complexes with cysteine bound monodentate through sulfur.

Rigid tetraazamacrocycle copper complexes: radiopharmaceutical applications

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Copper has a unique selection of radioisotopes ($^{60}$Cu, $^{61}$Cu, $^{62}$Cu, $^{64}$Cu and $^{67}$Cu) with half-lives ranging from 9.8min to 61.9h suitable for medical imaging and therapy. Macro cyclic chelators have been found to have enhanced in vivo stability over acyclic chelators such as ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA). The current chelators of choice for copper in peptide/antibody targeted radiotherapy and imaging are derivatives of 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA). TETA has been employed in clinical trials, however there is evidence of transchelation in vivo.1

A new series of structurally reinforced cross-bridged tetraazamacrocycles with pendant-arms has been synthesised. The formation of an ethylene bridge between nonadjacent nitrogen donors of cyclam forces a cross-bridged conformation with all four nitrogen lone pairs converging on a cavity suitable for copper complexation. Both Weisman2,3 and Busch4 have demonstrated that the cross-bridged cyclam eliminates trans-coordination and ensures a reduced strain conformation ideal for cis-folded coordination. The novel bifunctional copper chelating agents synthesised have been conjugated to biomolecules and may have subsequent use as radiopharmaceuticals in positron emission tomography diagnostic applications.

Solid tumors are often hypoxic due to an insufficient blood supply. They draw their energy mainly from glycolysis, which results in production of large amounts of lactate. Therefore, the intracellular pH and the extracellular pH are decreasing with increasing tumor size. A high concentration of H⁺ outside the tumor cells is a problem for weak base drugs. On the other hand a major breakthrough in chemotherapy could be possible when taking advantage of the acidic pH in many tumors. An interesting class of compounds showing pH dependent behavior are diamineplatinum(II) complexes with N-hydroxyalkyl substituents. It was observed by NMR spectroscopy that cis-dichlorobis(2-hydroxyethylamine)platinum(II) and corresponding derivatives undergo intramolecular ligand exchange reactions in aqueous solution resulting in platinum species with one chelating ethanolatoamine ligand. Under more basic conditions, the reaction afforded selectively the double ring closed platinum(II) species.

NMR spectroscopic studies and investigations using CZE-ESI-MS have shown that the binding behavior of cis-dichlorobis(2-hydroxyethylamine)platinum(II) towards 5'-GMP is dramatically increased by decreasing the pH from 7.4 to 6.0. The half life of adduct formation with 5'-GMP was found to be 4.5 h at pH 6 compared to 28.5 h at pH 7.4. This fact is of great interest with regard to the before mentioned lower pH in solid tumors and is in accordance with a pH dependent ring closing/opening reaction of N-hydroxyethyl substituted cis-diaminedichloroplatinum(II) complexes. Fine tuning of important properties such as lipophilicity and reactivity can be driven by selection of the hydroxyalkylamine ligand.
In an attempt to overcome severe limitations of a platinum based chemotherapy, it is desirable to develop new anticancer drugs which show an improved clinical effectiveness and a reduced general toxicity. A class of platinum compounds which could fulfill these requirements are kinetically inert platinum(IV) complexes which display a reduced toxicological profile dependent on their axial ligands. Moreover, such compounds are also suitable for oral administration.

Whereas platinum(II) chemistry is based on ligand exchange reaction, platinum(IV) chemistry made considerable progress through the carboxylation of hydroxide coordinated to the platinum(IV) center. Reduction and release of axial ligands could turn out to be a problem when trying to couple carrier molecules via the coordinated hydroxo group or when important properties such as lipophilicity are depending on the nature of the axial ligands. Therefore, a new strategy was developed for the coupling of carrier molecules to kinetically inert tetrachloroplatinum(IV) complexes taking advantage of peripheral functional groups rather than hydroxo ligands. The typical class of carboxylation reagents widely applied in organic chemistry, the acyl chlorides, were introduced for analogous derivatization of 2-hydroxyethyl-substituted tetrachloro(ethane-1,2-diamine)platinum(IV) complexes. To set up a general reaction procedure and to optimize the reaction conditions, the before mentioned kinetically inert tetrachloroplatinum(IV) compounds and quite simple acyl chlorides have been chosen.
Synthesis, structure, spectroscopic and in vitro anti-tumor properties of triazolium salts of cis- and trans-tetrachlorobis(triazole)ruthenate(III)

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Ruthenium(III)-azole complexes of the type HL[trans-RuCl₄L₂] with L = imidazole or indazole show remarkable anti-tumor activity both in vitro and in vivo [1] The preclinical studies on the indazole derivative have reached an advanced stage and the complex is currently entering phase I of clinical trials as an anti-tumor drug. Searching for other potent antineoplastic agents with similar or even broader spectrum of indication, low toxic side effects and better water solubility, which would make the pharmacologic formulation easier than in the case of indazole species, we suggested to use 1H-1,2,4-triazole as heterocyclic azole ligand. This contains one ring nitrogen atom more when compared to imidazole or indazole. Increased hydrophilicity of triazole would result in better water solubility of the final complexes. In addition, the 1H-1,2,4-triazole exists in different tautomeric forms and adopts varied modes of coordination in metal complexes, which makes the coordination chemistry of this ligand towards ruthenium even more exciting. Herein we report on the synthesis of two isomeric compounds H₂trz[cis-RuCl₄(Htrz)₂]H₂O and H₂trz[trans-RuCl₄(Htrz)₂], which showed better aqueous solubility than the indazole derivative. The structure of compounds determined by X-ray diffraction methods and their spectroscopic properties are also discussed. The cytotoxic activity of both compounds has been tested in vitro on three human cancer cell lines HT-29 (colon carcinoma), SK-BR-3 (breast carcinoma) and SW480 (colon carcinoma) and these results will also be reported.


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Mechanisms of Redox-coupled Proton Transfer in Proteins: The Role of the Proximal Proline in Reactions of the [3Fe-4S] Cluster in Azotobacter vinelandii Ferredoxin I

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The 7Fe ferredoxin from Azotobacter vinelandii (AvFdI) contains a [3Fe-4S]^{+/-} cluster that binds a single proton in the one-electron reduced (0) level. AvFdI is amenable to a variety of discriminating investigative techniques, including protein film voltammetry, which can analyze, in considerable detail, the kinetics and energetics of coupled and gated electron-transfer reactions occurring at active sites. The [3Fe-4S] cluster is buried and inaccessible to solvent, yet proton transfer between the cluster and solvent is fast, mediated by the mobile carboxylate of an adjacent surface residue, aspartate-15, the pK of which is sensitive to the charge on the cluster (1). This paper describes structural and electrochemical experiments to examine the role of a nearby proline residue (proline-50) on the kinetics of proton transfer between the solvent and the cluster, and its coupling to electron transfer. In the P50A and P50G mutants a water molecule has entered the cluster binding region; it is hydrogen bonded to the to the backbone amide of Ala-50 or Gly-50, and to the carboxylate of Asp-15, and is 3.9 Å from the closest sulfur atom (S1) of the cluster. Despite the presence of the water molecule, which now links the cluster more directly to the solvent, proton transfer is slower than observed for the native protein. Detailed analysis reveals that the coupling between the pK values of the cluster and Asp-15 is significantly quenched, so that addition of an electron to the cluster no longer induces such a favorable shift in pK of the carboxylate.


Reactive Sulfur Species: Reactions of Hypothiocyanite

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The usual role of thiols in the cellular oxidant defense system can be compromised when reactive conjugates are formed. These so-called reactive sulfur species (RSS) can have beneficial or deleterious effects on the host.1 The biological reactive intermediate hypothiocyanite (OSC\text{N}^{-}), which can be generated by enzymatic oxidation of thiocyanate (SCN\text{−}) and by hydrolysis of chemical reagents such as thiocyanogen ((SCN)\text{2}), plays a role \textit{in vivo} as an antimicrobial agent and possibly a mechanism of shunting generation of more powerful and less selective oxidants such as hypochlorite (OCl\text{−}).2 The chemistry of OSC\text{N}^{-} has previously received little attention. Although many conjugate reactions of proteins have been attributed to OSC\text{N}^{-}, there is little precedence for such reactions in small-molecule chemistry. We have characterized alkylsulfenyl thiocyanates (RSSCN), sulfenic acids (RSH), and N-thiocarbamates (RSC=ONH\text{2}) among the reactive conjugates that are formed when solutions that are known to contain HOSCN/OSC\text{N}^{-} are reacted with small-molecule thiols such as penicillamine and cysteine-containing protein such as hemoglobin. Since different reactivities are observed when HOSCN/OSC\text{N}^{-} is generated by mammalian peroxidases (e.g., lactoperoxidase, LPO), plant peroxidases (e.g. horseradish peroxidase, HRP), hydrolysis of (SCN)\text{2}, and other reagents we have developed, we suggest that cocktails of RSS are in fact produced by such reactions. We report our findings regarding the product distributions that are observed.


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Spectroscopic Characterization of a Fe(IV) Intermediate with a Non-Porphyrin Ligand. Hydroxylation and Epoxidation Activity.

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Fe(III)-OOH et Fe(IV)=O species are proposed as key intermediates in the catalytic cycle of several heme, as well as non-heme, enzymes, despite the fact that they have not always been characterized. For example, Fe(IV)=O species have not been observed with non-heme natural systems. In synthetic models, aminopyridine ligands have been successfully used to obtain Fe(III)-OOH intermediates by adding H2O2 to a Fe(II) complex. By using of a new class of oxidant on these same complexes, we report the formation of a Fe(IV) intermediate. Its characterization by UV-visible and Mössbauer spectrosopies, together with its high specific reactivity, is consistent with a Fe(IV)=O structure. DFT calculations indicate a complete delocalisation of the two unpaired electrons over both the Fe and O centres. Therefore, the electronic description of this Fe(IV)=O species is analogous to the ground triplet state of dioxygen. This observation is fully consistent with the observed reactivity.

Resonance Raman studies of wild type and mutants of the oxygen sensor protein BjFixL. Relevance for oxygen binding and release.

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The FixL proteins are biological oxygen sensors that restrict the expression of specific genes to hypoxic conditions. FixL contains a heme sensor domain and a histidine kinase domain. Crystals structures have been obtained for the heme domains of BjFixL. Based on these structures, the mechanism of signal transduction has been suggested to involve the heme propionates and two arginine residues, one of which is hydrogen-bonded to heme-bound oxygen (Arg220). This arginine should be responsible for the very efficiency heme-O2 recombination in FixL compared to Mb. This implies that the heme pocket acts as a ligand-specific trap for FixL. In order to determine the exact role of arginine 220 in ligand fixation as well as signal transduction, we prepared three mutants: Q220R, I220R and E220R. In all cases, O2 fixation and heme pocket structural modifications were studied by Resonance Raman. The general implications for the functioning of heme-based ligand sensors will be discussed.
New developments on the coordination behaviour of 2-substitued benzimidazoles

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We have been interested in the coordination behaviour of biologically active ligands containing 2-substitued benzimidazoles. A series of heterocycles containing N, O and S, were substituted and investigated. There were obtained coordination compounds of Co(II), Ni(II) and Co(II) with 2-(2-benzothiazolylamino)-benzothiazole, 2-(2-aminobenzothiazolylamino)benzoxazole and 2-(2-benzothiazolyl- amino)benzimidazole (see fig.). The aromatic heterocycles were prepared from 2-aminobenzothiazole differing by the position of the heteroatom at position (S, O, N). The N-H group in all cases was deprotonated and coordination to the metal ion occurs with nitrogen atoms N(3) and N(13). The coordination compounds were characterized in the solid state by UV-Vis-NIR reflectance spectra, IR, X-ray diffraction and mass spectrometry. There were obtained compounds where the ligands are chelating the cobalt ions in a distorted tetrahedral arrangement. Nickel(II) compounds presented different structures, i.e. distorted pentacoordinated or in an octahedral arrangement.

Their coordination behaviour is compared with 2-substitued benzimidazoles with polyfunctional planar molecules with a delocalized p electronic system.

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Electrode Surface Structures for Studying Long-Range Electron Transfer in Biological Systems.

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Protein film voltammetry (PFV) is a dynamic electrochemical technique by which the active sites of adsorbed protein molecules may be directly observed and their reactions investigated. In this work, the scope of PFV is being extended in order to study large and complex membrane-bound redox proteins. Stable, conducting surface structures on gold electrodes are being developed that resemble biological membranes and are able to transfer electrons efficiently over distances greater than 30 Angstroms.

Following work published by Chidsey and coworkers [1], an ethyl terminated oligophenylenevinylene (OPV) thiol, 15 Angstroms in length, was synthesized and incorporated into self assembled monolayers (SAMs) on gold. The study of this system, by PFV, using the simple electron transfer protein, azurin, has demonstrated that the OPV SAM facilitates fast electron transfer between the protein and the electrode. We are now synthesizing OPV molecules having different terminal functionalities and much greater length (exceeding 30 Angstroms). As well as determining the abilities of these molecules to engage in very long-range electron transfer to different proteins, we are using scanning tunneling microscopy (STM) to elucidate the arrangement of the OPV molecules on the electrode surface.

Synthesis and biological activities of some new gold(I)-carbene complexes: potential antimitochondrial antitumour agents

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Mitochondria play a key role in the regulation of apoptosis and recent findings suggest that many apoptotic pathways converge at a single event – mitochondrial membrane permeabilization. As a result there has been considerable interest in targeting mitochondrial cell death pathways in the development of new chemotherapeutic agents. Significantly, carcinoma cells have an elevated mitochondrial membrane potential relative to normal cells. Tumour cells can be selectively targeted using lipophilic cationic molecules which preferentially accumulate in the aberrant mitochondria. In previous work we have investigated a series of Au(I) complexes of lipophilic bidentate phenyl- and pyridyl-phosphine ligands which possess significant antitumour properties. Importantly, it has been shown that the selectivity of these complexes for cancer cells over normal cells can be ‘tuned’ by adjusting their lipophilic/hydrophilic balance through ligand design.

In this work we are investigating a new class of lipophilic cationic Au(I) complexes based on carbene rather than phosphine ligands. The similar chemistry and structural features of the two classes of compounds allow us to assess whether the phosphine ligand is essential for the antitumour properties. The synthesis, structural analysis and antimitochondrial activities of these compounds will be described.


Ligand-Enhanced Lanthanide Ion Fluorescence as a Probe for Proteolytic Activity and Specificity

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Proteolytic enzymes play key roles in many biological pathways. One aim in the emerging field of proteomics is to delineate the roles of newly discovered proteases in biology. In order to achieve this aim, possible physiological and pathological substrates of the protease must be identified and validated. Once the function of a protease is known, rational drug design can be employed to create therapeutically relevant inhibitors. To this end, novel fluorogenic protease substrates have been developed utilizing ligand-enhanced lanthanide ion fluorescence as shown below. These substrates have proven useful for assaying the activity of a variety of proteases. In addition, libraries of peptide substrates have been synthesized, allowing a facile determination of the extended substrate specificity of proteolytic enzymes.

Mixed ligand complexes of the type \([\text{Co(en)}_2(L)\text{Br}_3\) (series I) and \([\text{Co(L)}_2\text{Cl}_2\text{Cl}\) (series II) where en = N, N' ethylenediamine and L = 1,10-phenanthroline (phen), 2,2'-bipyridine (bpy), 1,10-phenanthroline-5,6-dione (phendione), dipyrido[3,2-a:2',3'-c]phenazine (dppz) have been synthesized and characterized by elemental analyses, IR, UV-Vis, NMR spectroscopy and cyclic voltammetry. The X-ray structure of \([\text{Co(phendione)}_2\text{Cl}_2\text{Cl}\) has been solved and refined to \(R = 0.0646\) for 5115 reflections. The crystals are monoclinic of space group C2/c with cell constants \(a = 25.730(2)\) Å, \(b = 12.375(1)\) Å, \(c = 18.979(2)\) Å, \(b = 119.925(1)^\circ\), \(V = 5237.03(7)\) Å³ and \(Z = 8\). The cobalt(III) ion is in an octahedral environment with coordinated nitrogens from phendione and two cis-chlorides.

The DNA binding and photo cleavage characteristics of the complexes of series I have been investigated by absorption titration, competitive binding fluorescence measurements, viscosity measurements and gel electrophoresis respectively. Similar studies were carried out for the series II. The order of intercalative ability of the coordinated ligands is dppz > phendione > phen > bpy in both the series of complexes. All complexes, however, cleave plasmid pBR 322 DNA upon irradiation. The probable mechanism might be generation of a reactive oxygen species, viz. 1O2, formed in situ due perhaps to photolysis of water.
New N,O-Bidentate Pro-Ligands Capable of Stabilising a Phenoxyl Radical State as Free and when Bound to Cu(II) or Zn(II)

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A new family of N,O-bidentate, phenol-imidazole, pro-ligands (LH) has been designed and representative members synthesised and characterised. Each LH possesses no readily oxidisable position (other than the phenol) and involves o- and p-substituents on the phenol ring to prevent radical coupling reactions. Each LH undergoes a reversible one-electron oxidation to form the radical cation [LH]+. The unusual reversibility of the [LH]+/[LH] process has been attributed, in part, to a stabilisation of [LH]+ by intramolecular O-H...N hydrogen bonding between the phenol and the imidazole groups. This interaction is strikingly similar to that proposed between the Tyr160 radical and the adjacent His189 residue in PSII.

ML2 compounds (M = CuII, ZnII; L = BzL, PhL, PhOMeL) have been synthesised, and structurally and spectroscopically characterised. Each ML2 undergoes two reversible, one-electron, ligand-based oxidation, to form [M(L)(L′)]+ and [M(2L′)]2+, respectively. [M(L)(L′)]+ cations have been generated by electrochemical and chemical oxidation, and their [ML2][BF4] salts isolated as air-stable solids. The UV/vis, EPR, and magnetic characteristics of these compounds, together with the structural characterisation of [M(pL)(pOMeL)][BF4], are consistent with the cation of each involving an MII (M = Cu or Zn) centre ligated by a phenoxide (L−) and a phenoxyl radical (L′) ligand. Thus, we have isolated and characterised complexes that contain a CuII bound to a phenoxyl radical, a chemical combination that is crucial for the catalytic activity of the enzyme galactose oxidase (GAO).

The spectroscopic properties and the relative stability of the radical cations [LH]+ and [M(L)(L′)]+ will be discussed and particular attention will be drawn to their relevance to the tyrosyl radical contained in PSII and the active form of GAO, respectively.

References


Acknowledgements
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Direct Electrochemistry of Bacterial Molybdoenzymes

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Mononuclear molybdenum enzymes are found in all forms of life. In all known cases the Mo ion at the active site is bound by either one or two pterin-dithiolene ligands. The mononuclear Mo-enzymes fall into three distinct groups comprising the xanthine oxidase, sulfite oxidase and DMSO reductase families (below). The ligand ‘X’ in the DMSO reductase family is provided by a serine, cysteine or selenocysteine residue.

Mo-enzymes perform a diverse range of oxygen transfer reactions on small organic or inorganic substrates. Despite the intense interest in the catalytic properties of Mo-enzymes, direct electrochemical responses from the active sites of these enzymes remained elusive for many years. Recently we have been successful in obtaining voltammetric responses from a number of bacterial Mo-enzymes under both non-turnover and catalytic conditions [1,2]. Here we shall present some of our work in this area, where we have applied protein film voltammetry to provide insight into electron and atom transfer reactions at the Mo active site.

References

An in vitro study of DNA damage due to a mixture of Fe$^{2+}$ and ascorbate

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Iron is a transition element that is essential to all forms of life. It performs many essential functions including the reversible binding with molecular oxygen, the activation of oxygen and dismutation of superoxide. Life in an oxygen-rich environment is not without hazard as oxygen-derived free radicals are formed. These free radicals can attack biomolecules including DNA. Antioxidants such as vitamin C normally protect the DNA from oxidative damage. However, there is some evidence suggesting that in presence of Fe$^{2+}$ that can undergo redox cycling, ascorbate can act as a prooxidant. The aim of this study is to investigate the damage to salmon sperm DNA and pBR322 plasmid DNA due to their interaction with mixtures of iron (II) ammonium sulfate and ascorbate around pH 6.5.

It is found that at [Fe$^{2+}$] ranging from 5 to 10 mM, as the concentration of ascorbate is increased, an increasing damage to the DNA takes place. No DNA band is observed when [ascorbate] equals 1 mM or greater. The results suggest that in presence of Fe$^{2+}$, under the conditions of the experiment, ascorbate is acting only as a prooxidant. It is also seen that in presence of Fe$^{3+}$ alone, DNA is changed from a mixture of Forms I and II to a mixture of Forms II and III.
Structural control of copper and zinc exchange dynamics in bacterial transport and storage proteins

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The cyanobacterial protein SmtA from Synechococcus PCC 7942 contains a metallothionein-like cluster, in which four Zn\textsuperscript{2+} ions are bound to nine cysteine sulfurs and two histidine nitrogens.\textsuperscript{1} One of the cluster Zn\textsuperscript{2+} ions simultaneously is part of a GATA-like zinc finger, and displays unexpected metal exchange dynamics.

SmtA is the prototype of a novel class of bacterial zinc (cluster)-finger proteins. In a structural genetics approach, several putative members of this new class of proteins have been characterised. NMR spectroscopy confirmed the similarity of their folds to that of SmtA, and electrospray mass spectrometry has provided insight into metal binding stoichiometry and exchange dynamics. The new class can be divided into three groups with different metal-binding properties. Members of group A are found in cyanobacteria, and contain all 11 protein Zn\textsuperscript{2+} ligands. Group B proteins are found, e.g., in pseudomonads, and contain either 10 or 11 ligands. Group C proteins are found in E. coli and salmonellae, and retain the zinc finger only. The figure shows characteristic features of the zinc finger site in SmtA.

The cyanobacterium Synechocystis PCC 6803 expresses a protein with 20-28\% sequence homology to eukaryotic and prokaryotic copper chaperones. The cyanobacterial ATX1 is ca. 8 residues shorter than these and contains a unique histidine (His-61) residue close to the C-terminus. We have combined NMR spectroscopy and homology modelling to explore the involvement of His-61 in copper binding and transfer.

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Preliminary study of some chemical reactions catalysed by DNA

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DNA-templated ligation reactions of chemically modified oligonucleotides are highly sequence-selective and have recently been applied to gene diagnosis and chemical replication systems. Applicability is limited by the lack of efficient amplification due to product inhibition. This limitation may be overcome by replacing the covalent ligation by a cleavage or group exchange reaction.

Here we present the first examples of DNA-templated disulfide cleavage reaction by thiol or phosphine modified oligonucleotide analogs (peptide nucleic acids, PNAs) and DNA-templated ester cleavage by metal complex-PNA conjugates.

Nucleic acid templated cleavage reactions are applicable both in vitro and (potentially) in living cells and may inspire the development of novel chemical detection systems for nucleic acids or – if a cytotoxic agent is catalytically released – of gene-selective prodrugs.
Structure of the binuclear copper centre in hemocyanins and in related models by multiple scattering XAS calculations

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The XAS approach allows deriving fundamental structural information in the case of hemocyanins (Hcs) and their related model compounds. Due to difficult crystallization and high molecular weight, both XRD and NMR are not suited for the structural investigation of these proteins, which are also EPR silent in native and derivative forms.

Our study [1] on the met- and met-azido-Hcs forms from Octopus vulgaris (mollusc) and Carcinus aestuarii (arthropod) at pH 7.5 and on some related binuclear models without XRD [2] has considered and resolved some fundamental aspects (i.e. correct values of the Cu-Cu distances, apical distortion when present, at the copper site, presence and type of bridging groups). This study has shown that only by applying calculation methods with XAS programming codes using multiple scattering (MS) it is possible to improve the investigation of the high energy regions of the absorption spectra (EXAFS modulation). This study has also shown the importance of a parallel characterization, even if in a qualitative mode, of the low energy region of the absorption spectra (XANES edge region). The quantitative analysis of this region of the XAS spectrum is usually a complex problem, which becomes very difficult for a binuclear site with local structural contributions due to the two different metal atom centres.

In this contribution, we will show how, only by advanced MS calculations it has been possible to refine the EXAFS modulation of the absorption spectra and overcome some severe problems that mainly derive from the presence of two absorbing atoms and from the fact that the metal-metal contribution in the absorption spectra overlaps with the Cu-His signals. We will also present some results indicating the role of the MS calculations in the XANES edge region, which show how it is possible to extract quantitative structural information from the absorption data also in a binuclear core in order to refine the fine structure of the site.


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Pyruvate formate-lyase activating enzyme (PFL-AE), which generates the catalytically essential glycyl radical on PFL, is a representative member of an emerging group of enzymes that utilize iron-sulfur clusters and S-adenosylmethionine (AdoMet) as required cofactors in radical generation. Though diverse in function, these enzymes have been proposed to have in common key mechanistic features including the generation of an intermediate 5'-deoxyadenosyl radical that initiates catalysis by hydrogen atom abstraction. As an initial step towards understanding the role of the iron-sulfur cluster in deoxyadenosyl radical generation, we have utilized Mössbauer and electron nuclear double resonance (ENDOR) spectroscopies to probe the interaction of AdoMet with the [4Fe-4S] cluster of PFL-AE. The [4Fe-4S] cluster of PFL-AE is a site-differentiated cluster, and a dramatic perturbation of the Mössbauer parameters of the unique site is observed in the presence of AdoMet, suggesting coordination of AdoMet to the unique site. 2H, 13C, 17O, and 15N-ENDOR of site-specifically labelled AdoMet in complex with PFL-AE have been used to develop a picture of the interaction of AdoMet with the [4Fe-4S] cluster of PFL-AE. A mechanism is proposed in which the unique site of the cluster serves to anchor AdoMet in position such that inner-sphere electron transfer can occur via an interaction between the sulfonium of AdoMet and μ-3 bridging sulfide of the cluster.
Synthesis and DNA binding of bisintercalating complexes of the type [(dpq)$_2$Ru(phen-n-SOS-n-phen)Ru(dpq)$_2$]$^{4+}$

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Over the past five decades, interest in ruthenium(II) polypyridyl complexes as DNA probes has resulted in the synthesis of many mononuclear complexes. The effectiveness these complexes as chemotherapeutic agents is restricted due to their relatively small size, low cytotoxicity and modest DNA binding affinity limits. Dinuclear metallointercalators are expected to overcome the limitations of mononuclear complexes and have a much greater DNA binding affinity in addition to slower DNA-dissociation rates. Work so far indicates that dinuclear complexes are capable of binding to DNA at a strength 100 times greater than the equivalent mononuclear complex, in part due to the species charge. It is expected that the increase in DNA binding affinity will afford an appreciable increase in the effectiveness of these complexes as potential chemotherapeutic agents.

We report the synthesis and characterisation of each of the dimeric complexes [(dpq)$_2$Ru(phen-n-SOS-n-phen)Ru(dpq)$_2$]$^{4+}$ (where n = 3, 4 or 5, phen = 1,10-phenanthroline; dpq = dipyrido[6,7-d:2',3'-f]quinoxaline and SOS = 2-mercaptoethyl ether) (see Figure). The examination of the comparative DNA affinity of these compounds requires an array of techniques. Some of these include the determination of DNA binding constants and thermal melting studies.

Use Of Induced Circular Dichroism Studies To Predict The Biological Activity Of Platinum Intercalators.

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Circular Dichroism (CD) spectroscopy is a valuable tool in the investigation of nucleic acid structure due to its sensitivity. The usefulness of CD in the secondary structure estimation of nucleic acids is well recognised and is frequently used as a macroscopic technique to probe changes in the conformation. When an achiral molecule interacts with DNA, the resulting induced circular dichroism (ICD) is due solely to changes in the macromolecule structure. The simplest use of ICD is to identify structural changes in DNA and therefore conclude that the molecule interacts with DNA.

Here we report the study of DNA interactions with two series of platinum metallointercalators, one chiral and one achiral. We have determined equilibrium binding constants from titrations of DNA with the platinum complexes. In addition, we have conducted viscosity experiments and carried out a cell growth inhibition assay against Murine Leukaemia (L1210) cells in order to determine IC50 values for each compound. From careful observation of changes in the ICD spectra generated from titration, a correlation can be drawn between the biological activity (IC50) and the induced changes in the CD spectra of the molecule.

Conformational flexibility and reactivity towards dioxygen of macrocyclic dinuclear complexes

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Dinuclear copper and iron proteins are important for the biological transport and activation of dioxygen. Some of these proteins undergo dramatic conformational changes on substrate binding (induced fit), e.g. the Cu – Cu distance in transport protein hemocyanine reduces from 460 to 360 pm on binding of dioxygen. In others such as ascorbat oxidase (a tricopper enzyme) changes of intermetal distance on reaction with dioxygen are less pronounced. To assess the influence of conformational flexibility on reactivity in a simple low molecular weight model, we have designed a flexible, dinucleating macrocyclic ligand L. The accessible conformational space of complexes LM2 is controlled by programmed secondary interactions of substituents R in some distance to the reaction center. Copper and iron complexes of L and their reactivity towards dioxygen will be presented.

Figure: LM2 and crystal structure of [Fe(III)(L)(µ-O)]^{3+}(R=Methylbenzylamine)
Investigation of Rotamers About the Pt-N7 Bonds in Bulky [Pt(d(GpG))(diamine)] Complexes

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Cisplatin binds to DNA primarily as a GpG bifunctional adduct and this is believed to be responsible for its pharmacotherapeutic action. A range of complexes of the form [Pt(d(GpG))(diamine)] have been investigated as models for these adducts with a view to rational drug design. Marzilli and co-workers examined the rotation about the Pt-Guanine bond and found there to be four rotamers [1]. Here, we have used a combination of modelling and HPLC to gain an increased understanding of the behaviour of these adducts.

Compounds such as 5,5′-dimethyl-2,2′-bipyridineplatinum(II) were designed to have a bulky nature such that there are steric interactions which slow down the rotation allowing these to be resolved via HPLC.

2D contour maps of the strain energy versus torsion angle revealed the thermodynamically stable conformers allowing identification of the high and low energy pathways between conformations. The HPLC and GF-AAS profiles of the dinucleotides indicate that up to four rotamers are present. Further [Pt(d(GpG))(diamine)] complexes are currently being investigated. These include complexes of the substituted bipyridine ligand and complexes of substituted versions of the phenanthroline ligand. The HPLC and GF-AAS are proving to be useful tools in identifying the number of the [Pt(d(GpG))(diamine)] adduct conformations. Presently, the stable conformations identified are being purified for NMR studies, in which a solution structure of the conformations will be elucidated.

Mechanism of Action of Platinum Complex Binding to DNA

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Cisplatin (cis-[PtCl2(NH3)2]) is one of the most potent antitumour drugs currently in clinical use [1]. The precise mechanism of action of cisplatin is unknown, although much research has been done on the aquation and preassociation of the multistage process of binding to DNA [2].

In this work we have used theoretical and experimental techniques to uncover more information about these processes. The QM/MM hybrid method of ONIOM was adapted to investigate the sequence selectivity of cisplatin. The key step in this selectivity is believed to be the monofunctional adduct formation, proposed to be partially controlled by long-range electrostatic interactions. The correlation between experimentally determined rate constants and the electrostatic potential was investigated, specifically with respect to the preference for guanine over adenine in the monofunctional adduct formation.

In the experimental study we have compared specifically the adducts formed by cisplatin and the diaquated form by reacting them each in stoichiometric amounts with three self-complementary 52-base-pair oligonucleotide duplexes. The oligonucleotides were designed to have only three d(GpG), d(ApG) or d(GpA) sites per strand separated from each other by non-target bases to allow only intrastrand binding to these sites. By varying the reaction conditions we were able to compare the binding preferences of cisplatin and its diaquated form for d(ApG), d(GpA) and d(GpG). HPLC and GF-AAS results were used to determine the adduct profile.


Bis(pyrazol-1-yl)acetato ligands: models for the 2-his-1-carboxylate facial triad

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Bis(pyrazol-1-yl)acetates such as bis(3,5-dimethylpyrazol-1-yl)acetate (bdmpza) represent a new class of tripodal ‘scorpionate’ ligands in coordination chemistry.[1,2] Here we report on the syntheses of achiral, chiral and even enantiopure bis(pyrazol-1-yl)acetato ligands (Fig. 1)[1-4], and on complexes of bis(pyrazol-1-yl)acetato ligands coordinated to bio-relevant metals (Fe, Zn). According to their X-ray structures some complexes such as [(bdtpza)ZnCl] and [(bdtpza)FeCl]2 (bdtpza: bis(3,5-di-tert-butylpyrazol-1-yl)acetate) can be regarded as structural model complexes.[1,5] This discussion will focus on their similarity to the 2-Histidin-1-Carboxylate motif often found in metallo-enzymes such as thermolysin (Zn), isopenicillin N synthase IPNS (Fe) (Fig. 2)[6] or 2-oxoglutarate dependent iron enzymes e.g. taurine dioxygenase TauD[7]. We will further report on iron(III) complexes[5] accessible from [NEt4][Cl3FeOFeCl3] and ruthenium model complexes for the 2-oxoglutarate dependent enzymes and IPNS easily prepared from [(bdmpza)RuCl(PPh3)2].[8,9]

Fig. 1: A chiral zine-methyl complex [3]  Fig. 2: Active site of IPNS [6]  Fig. 3: [(bdmpza)O2CC(O)PPh3] [9]

Modifying the specificity of the HMA motif.

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The MerP protein from transposon Tn501 is a small periplasmic protein involved in uptake of mercuric ions. It contains a single Heavy Metal Associated (HMA) motif, GMXCXXC, which is also found in the N-terminal domain of several metal transporters. There is some controversy over whether the metal specificity of the metal transporters resides solely in the transmembrane region or whether the HMA motif also contributes [1].

Seventeen amino acids including the HMA of MerP were replaced by the equivalent regions of Atx1, a copper chaperone [2], or CadA, a cadmium efflux protein [3]. Data on metal binding were generated by Immobilised Metal Affinity Chromatography (IMAC) and Electrospray Mass Spectrometry (ESI-MS).

MerP(Atx1) binds Cu(II) and Ag(I) more strongly than does MerP, and can also bind Cd(II) and Pb(II), but to a lesser extent. Mercury binding is reduced but not abolished. Metal interactions were also evaluated in vivo, and cells showed enhanced tolerance to copper ions but there was no change to the minimal inhibitory concentration of the metal.

E. coli W3110 cells expressing the MerP(CadA) protein were more sensitive to CdCl2 than those expressing MerP. Purified MerP(CadA) showed reduced affinity for Hg(II) and significantly increased affinities for Cd(II) and Cu(II) compared to MerP. Moreover, changes in the position of the two cysteines of MerPCadA, from CANC to CACN, enhanced its affinity for Cd(II).

A similar approach has been applied to generate chimeric proteins containing the HMA motifs of PbrA, ZntA and Hah1, and their metal affinities were evaluated by ESI-MS. We conclude that the HMA motif does have a direct effect on the metal-binding preferences of the protein.


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Crystal structure of AphA class B acid phosphatase/phosphotransferase from E. coli

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The crystal structure of the AphA aspecific acid phosphatase from the periplasm of E. coli has been determined at 2.2 Å resolution by MAD measurements on a NaBr derivative. The resolution of the structure has been extended to 1.4 Å on a AuCl3 derivative. The quaternary structure of the active enzyme consists of a homotetramer built by using the first 23 N-terminal residues which intertwine the monomers in a stable holoenzyme using only a relatively minimal interaction surface. The homotetramer is the stable form present in solution. The N-terminal forms a hook which represents a new structural motif used to build a stable, although flexible multimeric enzyme. The active site of the native enzyme as prepared hosts a magnesium ion which can be replaced by other metal ions. The structure explains the aspecific behaviour of AphA towards substrates.
The Particulate Methane Monooxygenase from Methylococcus capsulatus (Bath)

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The membrane-bound or particulate methane monooxygenase (pMMO) from Methylococcus capsulatus (Bath) mediates the controlled oxidation of methane to methanol under ambient conditions of temperature and pressure. Experiments on cryptically chiral ethanes and deuterated butanes have shown that this hydroxylation chemistry proceeds with total retention of configuration at the carbon center attacked. The H/D and $^{13}$C/$^{12}$C kinetic isotope effects on the hydroxylation have been measured to be ~ 5.5 and 1.000, respectively. These results are interpreted in terms of a concerted oxo-transfer mechanism based on side-on singlet ‘oxene’ insertion across the ‘C-H’ bond similar to that previously noted for singlet carbene insertion (JACS 114, 7590 (1992)).

The pMMO hydroxylase consists of three subunits of molecular mass 45, 27 and 25 kDa. It is a copper protein, with ~15 copper ions arranged into 5 trinuclear copper clusters. These copper ions are fully reduced in the functional form of the enzyme. The 5 clusters could be divided into 2 catalytic clusters or C-clusters and 3 E-clusters or electron-transfer clusters. The C-clusters are involved in dioxygen activation and alkane hydroxylation; the reduced copper ions of the E-clusters apparently provide a reservoir of reducing equivalents to reduce the catalytic clusters following dioxygen reduction and alkane hydroxylation.

Finally, we have undertaken a detailed characterization of the pMMO using a diverse range of physical methods, including EPR, X-ray absorption spectroscopy, and metal analyses. We have also followed the turnover of the C-clusters and E-clusters under a variety of conditions, including alkane hydroxylation, dioxygen activation, and redox titrations using various oxidants and reductants. These results will be described.

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Synthetic Models for the Metal binding site of Iron Superoxide Dismutase

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The superoxide dismutases (SODs) are a class of metalloenzymes which offer protection to all biological systems from oxidative damage. Although many studies aimed at understanding the structure and superoxide dismutase mechanism for the Cu-Zn containing SODs have been carried out, relatively less work has been carried out with the Mn and Fe dependent SODs. For Mn and Fe, the metal binding sites are similar and for the active form of the enzyme the coordination geometry has been found to be trigonal bipyramidal with two Histidine nitrogen and one carboxylate oxygen occupying the equatorial positions and one Histidine nitrogen and one hydroxide ligand located at the axial positions. It is believed that this conserved geometry and donor set are necessary for the enzymes to carry out its function. In this report we will present the synthesis and structures of iron complexes supported by ligands which possess the correct donor set and which enforce trigonal bipyramidal geometry. The use of steric interactions is employed to maintain mononuclearity and coordinative unsaturation. In addition, attachment of ligand substituents with amino acid-like functionalities will also be explored to mimic the second coordination sphere of the enzyme active site. The properties and reactivities of these complexes will be presented. Insights provided by these studies relative to iron superoxide dismutase will also be discussed.
Preparation and Characterization of a (Cu, Zn)-pMMO from Methylococcus capsulatus (Bath)

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We report the preparation of a (Zn, Cu)-pMMO in which the bulk of the copper ions of the E-clusters has been replaced by divalent Zn ions. The Cu and Zn contents in the (Zn, Cu)-pMMO were determined by both ICP-MS and x-ray absorption K-edge spectroscopy. Further characterization of the (Zn, Cu)-pMMO was provided by low temperature electron paramagnetic spectroscopy during reductive titration and reoxidation experiments. These studies indicate that the (Zn, Cu)-pMMO is still capable of supporting the activation of dioxygen, but that the replacement of the E-cluster copper ions has compromised the ability of the protein to mediate the transfer of reducing equivalents to the C-clusters. These observations provide strong support for the electron transfer and catalytic roles that we have previously proposed for the E-cluster and C-cluster copper ions, respectively.

Chiral Dinuclear Bis Ru(η6-arene) Anticancer Complexes: Diastereoselectivity in Guanine N7 Recognition

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Organometallic ruthenium(II) complexes of the type of [Ru(arene)(en)Cl]+ (en = ethylenediamine) are effective inhibitors of the growth of cisplatin-resistant cell lines [1]. They specially target guanine (G) of oligomers and form monofunctional adducts [1]. This high site-selectivity appears to be strongly influenced by the stereospecific H-bonding of en NH with G O6 and by arene-nucleobase stacking [2]. We have initiated here the design of polyfunctional dinuclear Ru(arene) anticancer agents. They exhibit a distinct profile of antitumour activity compared with their corresponding mononuclear complexes [3], which is likely to be associated with inter- and intra-strand cross-linking of DNA. We have studied the structures, kinetics and mechanism of binding of the chiral dinuclear complex [{(η6-biphenyl)RuCl(en)}2(CH2)6]2+ (Ru-Ru) and the chiral mononuclear analogue [{(η6-biphenyl)RuCl(en-ethyl)}+ to 9-ethylguanine(9EtG), using crystallography and NMR methods. Stepwise coordination of 9EtG to ruthenium was observed for the dinuclear complex with formation of the intermediate Ru-Ru(9EtG) and conversion to the final product (9EtG)N7-Ru-Ru(9EtG). A novel epimerization occurs at the ruthenium centre to accommodate the G base. This epimerization appears to be irreversible after G-N7 coordination with the formation of stereospecific H-bonding of the tetraamine NH with G O6, leading to high diastereoselectivity in guanine-N7 binding to the chiral bis Ru(arene) complex.

We thank the CVCP (ORS award for HC), EPSRC, ERI, and EC COST D8/D20 for their support for this work, and Dr Duncan Jodrell and colleagues (University of Edinburgh Cancer Research UK centre) and Professor Viktor Brabec and colleagues (Institute of Biophysics of the Czech Republic, Brno) for collaborative biological studies.

Puriﬁcation of the Particulate Methane Monooxygenase from Methylococcus capsulatus (Bath)

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The pMMO isolated from Methylococcus capsulatus (Bath) consists of a three-subunit hydroxylase (45, 27 and 23 kDa). The hydroxylase is a copper protein, with 15 copper ions arranged in five trinuclear copper clusters. The functional form of the enzyme is the fully or partially reduced copper hydroxylase. The pMMO exhibits unusual substrate speciﬁcity: only small normal alkanes are hydroxylated and similar alkenes epoxidated. In addition, the chemistry is highly regiospeciﬁc as well as stereoselective.

By cultivating Methylococcus capsulatus (Bath) under methane stress conditions and high copper levels in the growth medium, membranes highly enriched in the pMMO can be isolated from these cells. Puriﬁed pMMO can be subsequently obtained from these membrane preparations using protocols in which an excess of reductants and anaerobic conditions were maintained during membrane solubilization by dodecyl b-D-maltoside and puriﬁcation by size-exclusion chromatography only. The pMMO was found to be the major constituent in these membranes, constituting 80-90% of total membrane proteins. The dominant species of the pMMO was found to consist of three subunits\(^{a,b}\), with an apparent molecular mass of 45, 27, and 23 kDa, respectively.

From elemental analysis and X-ray near edge absorption spectroscopy, the puriﬁed pMMO is a copper-containing protein only and shows a requirement for Cu(I) ions. Approximately 12-15 Cu ions were determined per 94kDa protein. X-ray absorption near edge spectra indicate that puriﬁed pMMO as isolated contains a mixture of copper ions in Cu(I) and Cu(II) oxidation states. Finally, Extended X-ray Absorption Fine Structure (EXAFS) data are best ﬁt with oxygen/nitrogen ligands (coordination number is 2.5) at an average distance of 2.35Å and a 2.95Å Cu-Cu interaction (coordination number is 1.5), providing direct evidence for the existence of copper-clusters in pMMO.

Characterization of Isomerism between The \(\mu-\eta^2: \eta^2\)-Peroxo and Bis(\(\mu\)-oxo)Dicopper Cores by Density Functional Theory

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Bonding interactions between dicopper and dioxygen of both \(\mu-\eta^2: \eta^2\)-peroxo and bis(\(\mu\)-oxo) dicopper cores in model compounds [(NH\(_3\))\(_2\)Cu\(_2\)O\(_2\)]\(^{2+}\) and [(NH\(_3\))\(_3\)Cu\(_2\)O\(_2\)]\(^{2+}\) are analyzed in the framework of approximate density functional theory with the use of the quantitative energy decomposition scheme \(\Delta E (\text{total energy}) = \Delta E_{\text{prep}} + \Delta E_{\text{int}} \), \(\Delta E_{\text{int}} = \Delta E_{\text{elstat}} + \Delta E_{\text{pauli}} + \Delta E_{\text{orb}}\). The energy decomposition analysis of the potential energy surface along the interconversion coordinate of [(NH\(_3\))\(_2\)Cu\(_2\)O\(_2\)]\(^{2+}\) has been examined. An opposite behavior is obtained for \(\Delta E_{\text{int}}\) and \(\Delta E_{\text{prep}}\), indicating that it is the competition between \(\Delta E_{\text{int}}\) and \(\Delta E_{\text{prep}}\) that governs the formation of the \(\mu-\eta^2: \eta^2\)-peroxo vs the bis(\(\mu\)-oxo) dicopper complex. The term \(\Delta E_{\text{prep}}\) is destabilizing with decreasing distance between two copper fragments. \(\Delta E_{\text{int}}\) is dominated by \(\Delta E_{\text{orb}}\), the orbital interaction energy between the copper and oxygen fragments. This orbital interaction energy could be divided into two terms, \(\Delta E(\sigma^*)\) and \(\Delta E(\pi^*\sigma)\), by the symmetry of bonding. Both these two interactions \(\Delta E(\sigma^*)\) and \(\Delta E(\pi^*\sigma)\) between dicopper and dioxygen are bonding, and are nearly the same for 4- and 5-coordinate \(\mu-\eta^2: \eta^2\)-peroxo or bis(\(\mu\)-oxo) dicopper complexes. These results indicate that the details of the di-oxygen bonding in the model compounds do not correlate directly with the coordination numbers of the supporting ligands, but rather depend mostly on the differences in \(\Delta E(\sigma^*)\) and \(\Delta E(\pi^*\sigma)\) between \(\mu-\eta^2: \eta^2\)-peroxo and bis(\(\mu\)-oxo) dicopper complexation.

References:
DNA hydrolysis by Tris-triazacyclononanes Metal Complexes

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The use of multi-nuclear metal complexes is getting interests in mimicking enzyme functions. In DNA hydrolysis, metal ions are known to act as a Lewis acid to stabilize the transition state and to form a metal-hydroxide as a nucleophile to attack the phosphodiester bonds. The synthetic bimetallic complexes containing both factors were found to enhance the cleavage of double-stranded DNA at 37°C and pH 8. Recently, trinuclear copper complex shows the specific recognition with a DNA junction between single- and double-stranded regions. The use of double Lewis acids and the third metal ion as a nucleophile may improve the reactivity and selectivity of multi-nuclear metal complexes in DNA hydrolysis. Therefore, a novel coordinate ligand was designed and synthesized to contain three 1,4,7-triazacyclononanes (TACN) in the pre-organized position. The formation of metal complexes with different metal ions such as Zn\(^{2+}\), Co\(^{2+}\), Cu\(^{2+}\), La\(^{3+}\), Tb\(^{3+}\), and Ce\(^{3+/4+}\) was characterized by electronic absorption and ESI-mass spectroscopy. The hydrolysis product analyzed by HPLC will be discussed. The ability of DNA hydrolysis and the sequence selectivity with different metal complexes were studied by agarose gel and polyacrylamide gel electrophoresis in different buffers, pH, and temperatures.

Studies on new homonuclear and heteronuclear metal complexes with multiple centres

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Currently platinum compounds with structures different from that of cisplatin are being considered with the idea that they would have a different spectrum of activity and might not develop cross-resistance to cisplatin. One such compound is BBR3464 which is characterized by activity in human tumour (e.g. ovarian) resistant to cisplatin and alkylating agents, a high activity in a broad spectrum of tumours commonly insensitive to chemotherapeutic intervention. It is currently in the second stage of clinical trial. BBR3464 has three trans-platinum units connected together by alkyl diamine chains. Whereas the two terminal platinum units bind covalently with nucleobases in DNA, the central platinum unit is believed to undergo only non-covalent interactions including electrostatic interaction and H-bonding.

We hypothesized that while the replacement of the central platinum unit with other suitable metal units or platinum bonded to one or more planaramines instead of ammonia ligands would not markedly alter the covalent interactions of the terminal units with DNA, it would have a subtle effect on the non-covalent interactions of the central unit and this may be enough to result in anticancer active compounds with a different spectrum of activity. The aim of this study is to design new homonuclear and heteronuclear metal complexes with multiple metal centres and to quantify their activity and nature of interaction with DNA.

Starting materials, transplatin and its analogue, have been prepared by modified Dhara’s method. Attempts have been made to synthesis a number of compounds of the form: \([\text{trans-Cl(NH}_3\text{)}_2\text{-Pt-H}_2\text{N-R-NH}_2\text{-Cl(NH}_3\text{)}_2\text{Pt}]X_4\), where M stands for Pt\(^{2+}\), Cu\(^{2+}\) and Co\(^{3+}\), L stands for ligands such as hydroxypridines or imidazole, R stands for a carbon chain and X stands for a singly charged negative ion. The complexes are synthesized using step-up method of synthesis and purified by repeated dissolution and crystallization. Interaction with DNA is studied using gel-electrophoresis, HPLC, AAS, UV-visible spectrophotometry and restriction enzyme digestion. Activity against a number of cisplatin-sensitive and cisplatin-resistant cancer cell-lines is determined using MTT assay.
Correlation between Calculated Fermi Contact Spin Densities and Experimental Contact Shifts of Paramagnetic Ironporphyrins. A Novel Approach to the Electronic Structure of Intermediate-Spin Iron(II) Porphyrin

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Four-coordinate iron(II) porphyrin complexes with an intermediate-spin state have been studied extensively by paramagnetic NMR spectroscopy. Analyses of the contact shift confirmed the nature of P to Fe pi spin transfer mechanism consistent with both \(^1\)A2g and \(^3\)Eg ground state electronic structures. Calculation of the Fermi contact spin densities revealed that a- and b-C are of opposite sign for these two states, and assignment of these two peaks in the corresponding 13C NMR spectra should be critical to the identification of the ground state.

1H and 13C NMR spectra have been fully assigned for a series of four-coordinate iron(II) porphyrin complexes FeIP (P = TPP, TMP, OEP, OETPP) with different macrocycle deformation. Separation of the contact shift from both metal- and ligand-centered dipolar shifts was approached through theoretical calculations. Calculated Fermi contact spin densities for \(^3\)A2g inevitably show better correlation with contact shifts, and suggest \(^3\)A2g to be the leading contributor to the ground state of FeP.

Symmetry controlled bonding interactions between iron(II) and porphyrin will also be analyzed in the framework of DFT with the use of the quantitative energy decomposition scheme in combination with removing the vacant pi* orbitals of the porphyrin from the valence space. Elucidation of the bonding characteristics provides a solid foundation for ligand-field based rationalization and will facilitate the assignment of the electronic structure.

Model complexes for catechol dioxygenases supported by a tridentate hydrotris(pyrazolyl)borate ligand

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Catechol dioxygenases are non-heme iron enzymes which catalyze the oxidative cleavage of catechols.\(^1\) Although a number of biomimetic model complexes derived from various supporting ligands have been reported as structural and functional mimics for the enzymes, model complexes supported by tridentate ligands have received relatively less attention.\(^2,3\) Herein we report the synthesis, structure, and reactivity of a series of iron(III)-catecholate complexes containing the tridentate scorpionate ligand hydrotris(3,5-dimethylpyrazolyl)borate (TpMe2-).

The Fe(III) complex (1) was readily prepared by reacting FeCl\(_3\)•6H\(_2\)O, 3,5-dimethylpyrazole (pzMe2H), and NaTpMe2 in MeOH. Subsequent treatment of (1) with the appropriate catecholate ligands in toluene afforded [Fe(TpMe2)(pzMe2H)(Cat)] [Cat = C\(_6\)H\(_4\)O\(_2\) (2a), 3,5-But\(_2\)C\(_6\)H\(_4\)O\(_2\) (2b), C\(_6\)Cl\(_4\)O\(_2\) (2c)] as purple crystals. The molecular structures of (1) and (2b) have been elucidated by single-crystal X-ray crystallography. The reactivities of complexes (2) towards dioxygen have also been investigated.

References

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First Isolation and Crystal Structure of a Peroxo-Bridged Heme-Copper Complex

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1. Reaction of dioxygen with transition metal complexes is important from the standpoint of $O_2$ binding and activation in biological systems. Cytochrome c oxidase (CcO), which catalyzes four-electron reduction of $O_2$ to water, has a unique heme/non-heme copper dinuclear core in its active site. A number of heme-based iron-copper dinuclear complexes have been synthesized in conjunction with $O_2$ reduction in CcO. We have reported the reaction of $O_2$ with tetraphenylporphyrin (TPP)Fe$^{II}$-linked tris(2-pyridylmethyl)amine (TPA)Cu$I$ dinuclear complexes and the formation of peroxo-bridged dinuclear species Fe-($O_2$)-Cu. However, the isolation and structure of such a peroxo-bridged hetero dinuclear complex has never been reported. Here, we report the isolation and structure determination of a peroxo-bridged heme-copper dinuclear complex. 2. We prepared a dinuclear complex [(TMP)Fe$^{II}$-(5MeTPA)Cu$I$]BPh$_4$ (1). 1 was reacted with $O_2$ in CH$_3$CN at -30 °C and the resulting solution was kept at -30 °C for several days, to give dark-purple crystals [(TMP)Fe$^{III}$-(O$_2$)-(5MeTPA)Cu$I$]BPh$_4$ (2). The successful isolation of 2 was confirmed by the elemental analysis and various spectroscopic methods. The complex 2 was assigned to a paramagnetic species with a $S = 2$ spin state from the magnetic studies, since the bridging peroxo ligand mediated the strong antiferromagnetic coupling between the high-spin iron(III) and the copper(II) ions. 3. The crystal structure of 2 was determined by X-ray analysis. The Fe-O$_2$-Cu moiety has a characteristic $\mu$-$\eta^2:\eta^1$ coordination mode; both the two oxygen atoms of the peroxo ligand bind to the iron ion while only one oxygen atom binds to the copper ion.

Studies on planaramineplatinum(II) complexes of the form: PtCL$_3$ and trans-PtCL$_2$L$_2$ where L stands for 3-hydroxypyridine

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Transplatin [transdiamminochloroplatinum(II)], the trans-isomer of the commonly used anticancer drug cisplatin is not therapeutically active rather it is found to be toxic. Thus the classic structure antitumor activity relationship namely cis-geometry, the presence of two labile ligands such as Cl- and two non-labile ligands such as NH$_3$ was established. The reason why transplatin is inactive is believed to be due to its higher reactivity as compared to cisplatin. Thus it was suggested by Farrell that the trans-geometry could be activated due to the introduction of bulky ligands so as to reduce the reactivity. Indeed many trans-complexes, studied in vitro against a number of cancer cell-lines exhibited activity comparable to cisplatin. As a general feature it is expected that the trans-platinum compounds with bulky planar ligands would not show cross-resistant to cisplatin, as they would form different types of adducts with DNA. The major DNA adducts for cisplatin are the intrastrand bifunctional adducts such as Pt(GG), while the trans-analogues would form mainly interstrand adducts. This study describes the syntheses, DNA binding profile and activity of two platinum(II) compounds of the form: PtCIL$_3$ and trans-PtCl$_2$L$_2$ where L stands for 3-hydroxypyridine.
Orally Active Chelators for the Treatment of Iron Overload Diseases

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Iron, although crucial for life, is toxic if present in excessive amounts in the human body. Patients suffering from diseases, including hemochromatosis and thalassemia-major, accumulate, either directly or through repeated blood transfusion, high levels of iron in their vital organs, which rapidly leads to death through cardiac failure if not treated. Currently, desferrioxamine (DFO), a hexadentate hydroxamic acid, is still the only clinically approved iron chelator. This drug is very expensive and orally ineffective so patients must endure long periods (12-24 h/day, 5-6 days/week) of subcutaneous infusion of DFO to excrete these excessive levels of iron. These inherent limitations of DFO have prompted research in the search for alternatives.1

We have recently developed a new aroylhydrazone class of iron chelators based on the parent ligand 2-pyridinecarbaldehyde isonicotinoyl hydrazone (HPCIH). Studies on ligands from this family have revealed much greater iron-chelating efficacy than DFO in vitro and are promising as orally effective iron chelators.2 However, we also observed the unexpected and unprecedented oxidation of Fe(PCIH)₂ to give the ligand H₂IPH complexed to Fe(III), namely [Fe(HIPH)(IPH)] (crystal structure shown in the Figure).3 This has spawned a series of N,N-diaryldiarylhydrazines. Our preliminary studies have shown that some of these N,N-diarylhydrazine chelators exhibit similar iron-chelating efficacy to their hydrazone analogs and are much more effective than DFO.3 We are currently studying the coordination chemistry of these ligands and their metal complexes to achieve better understanding of their biological activity.

References
Molybdenum and tungsten are the only second- and third-row transition elements, respectively, in the Periodic Table known to be essential to biological systems. These metals feature at the active site of a class of enzymes, called metallo-oxotransferases, which catalyse metabolically critical carbon-, nitrogen- and sulfur-based redox reactions in all organisms: archaea, bacteria, plants and humans (1). Although the majority of metallo-oxotransferases characterised from mesophilic (‘middle-loving’; optimal metabolism occurring at temperatures ~25-40 °C) organisms contain Mo at the active site, several enzymes from hyperthermophilic (‘high-temperature loving’; optimal metabolism occurring at temperatures >85 °C) organisms, instead contain W.

It has recently been shown that W-substituted forms of mesophilic metallo-oxotransferases retain catalytic competency (2); also, a native W-containing metallo-oxotransferase has been characterised from a mesophilic organism (3). These reports challenge the folklore that exists in this class of enzyme linking W or Mo to hyperthermophilicity or mesophilicity, respectively. We are seeking more insight into metallo-isoenzyme forms of metallo-oxotransferases using chemical and biochemical approaches. Our electronic absorption and electron paramagnetic resonance (EPR) spectroscopic studies of the distribution of oxoMo(V) and oxoW(V) dithiolene species under temperatures that model those occurring at hydrothermal vents will provide further insight into Nature’s selectivity with respect to these ions. Here, we will present a selection of preliminary results from these studies and/or from density functional calculations and bioinformatics.

Substrate-tethered Probes for Investigating the Active Site of Myeloperoxidase

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Myeloperoxidase, a mammalian heme enzyme found in human neutrophils, functions as a bactericide via catalysis of the two-electron oxidation of Cl⁻ by H₂O₂ to give HOCl. This process, which requires potentials of ~+1.1 V (vs. NHE), proceeds via the intermediacy of a high potential MPO-I intermediate. The current status of our efforts towards the design, synthesis, characterization, and use of a series of MPO specific probes based on salicylate substrates will be described including (1) ‘dark to light’ optical probes incorporating Fluorescein, Rhodamine, and Dansyl salicylates, (2) redox probes incorporating Re(CO)₅(py), and (3) substrate tethered graphite electrodes.

Ligand Topology Tuning of Non-Heme Iron-Catalyzed Hydrocarbon Oxidations

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Nature has evolved a number of nonheme iron oxygenases capable of the stereoselective oxidation of C-H and C=C bonds. Still far from being understood are the factors that control the ability of iron centers to catalyze a range of reactions including alkane hydroxylation, olefin epoxidation and olefin cis-dihydroxylation. In our effort to develop bio-inspired nonheme iron catalysts, we have discovered a family of iron complexes with tetradentate pyridine/amine ligands that, in combination with H₂O₂, are capable of carrying out the above transformations with high stereoselectivity. Enantioselectivity has also been obtained with the chiral ligand N,N’-bis(2-pyridylmethyl)-N,N’-dimethyl-trans-1,2-diaminocyclohexane (bpmcn). We have undertaken a systematic effort to uncover the factors that control the catalytic reactivity of the nonheme iron center in this family of complexes. Herein we report the unexpectedly distinct oxidation behavior of two [Fe(bpmcn)(OTf)] complexes in both cis-α and cis-β ligand topologies. These results demonstrate the exquisite role ligands can play in fine tuning of the reactivity of an iron catalyst. Such fine tuning may also serve as a precedent to understand the diversity on the reactions of hydrocarbon oxidation catalyzed by non-heme iron enzymes.
Studies on heteronuclear metal complexes as anticancer drugs

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Currently platinum complexes with structures different from that of cisplatin are being considered with idea that they would have a different spectrum of activity and would not develop cross-resistance to cisplatin. A novel trinuclear platinum complex code named BBR3464, which is now under clinical trial, shows outstanding cytotoxicity against different cell lines both sensitive and resistant to cisplatin. It has three trans-platinum units connected together by alkyl diamine chains. The two terminal platinum units bind covalently with nucleobases in DNA, whereas the central platinum unit is believed to undergo only non-covalent interactions. We hypothesized that while the replacement of central platinum unit with the corresponding palladium unit would not markedly alter the covalent interactions of terminal units with DNA, it could have a subtle effect on non-covalent interactions of the central unit and this might be enough to result into anticancer active compounds with a different spectrum of activity. This poster describes the syntheses, activity and cell-uptake of a number of trinuclear Pt-Pd-Pt complexes and one Pt-Pd dinuclear complex, where the metal centres are linked together by alkyldiamino chains. The complexes have been characterized by elemental microanalysis, Raman and Infrared spectral studies, and have been evaluated for anti-tumour activity as compared to that for cisplatin against ovary cell lines (A2780) sensitive and resistant to cisplatin, A2780 resistant to ZD0473, lung (NCI-H640) and melanoma (ME-10538). Cell uptake, DNA binding was studied on two cell lines (A2780) sensitive and resistant to cisplatin. Interaction with DNA has been studied using gel electrophoresis, HPLC, AAS, UV-visible spectrophotometer and restriction enzyme digestion.

Two of the complexes are found to be significantly more active than cisplatin and all of the compounds have resistance factors better than that of cisplatin. It is also found the both activity and the solubility in water change with the change in the length of alkyl diamine chain. One of the designed compounds is found to be readily soluble in water.

Interstrand and 1,3-intrastrand crosslinking of cisplatin to guanosine bases: mechanistic and conformational insights

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The anticancer properties of cisplatin are believed to arise from its binding to intracellular DNA, inducing a conformational change, that renders the DNA immune to repair enzymes and results in apoptosis. Platination is concentrated at intrastrand GpG (65%) and ApG (25%) sites, and is also seen at lower levels at intrastrand GpNpG and interstrand G-G positions. As platination is irreversible under normal conditions, the product distribution is under kinetic control; the adducts at which binding occurs most rapidly are those that are most abundant.

Our previous work has determined the rate constants for each consecutive step in the binding of cisplatin to defined oligonucleotides in which a 1,2- intrastrand GpG, ApG or GpA grouping is provided as the preferred binding site. This work has shown a definite kinetic preference towards the 3'- guanine of a purine-purine intrastrand grouping, as well as slow bifunctional crosslink formation from the 5' base irrespective of the base to which binding was occurring.

This poster details the rate constants for the consecutive hydrolysis, covalent binding and bifunctional crosslinking steps of cisplatin coordinating to 14- base pair oligonucleotides in which the preferred binding site is a 1,2- interstrand crosslink (in both 5' – 3' and 3' – 5' directions) or a 1,3- GTG intrastrand crosslink. Both reactions are characterised by the presence of conformational isomers of the monofunctional adducts, indicating restricted rotation about the Pt-G(N7) bond; a phenomenon not observed with the adducts at purine-purine sequences. The nature of the final adducts is also described.

A loose inverse correlation is found between the half-life of the cisplatin aquation reaction and the percentage of adducts found in vitro. The aquation rate constant is sequence dependent, consistent with precovalent association between cisplatin and the oligonucleotide, which affects the entry of water to the Pt coordination sphere and hence the commensurate aquation reaction step.
Salt Effects and DNA-binding Specificity of NZF-1, a CCHC Zinc Binding Protein.

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The first type of zinc-binding domain described in transcription factors contains a Cys_His, motif. Recently, new families of transcription factors characterized by a CCHC zinc binding motif have been identified. Here we describe further characterization of a two-domain fragment, NZF1-2D, from the transcription factor, Neural Zinc Finger Factor 1 (NZF-1). NZF-1 contains six conserved CCHHC zinc-binding motifs and is involved in the development of the nervous system in eukaryotes by binding preferentially to the beta-retinoic acid receptor (β-RARE) DNA sequence. Gel-shift studies identified a seven-oligonucleotide tandem repeat within the β-RARE DNA sequence that appears to be essential for NZF-1 binding, with an estimated Kd of 10^-6 M. We present here the quantitative determination of this binding interaction using fluorescence anisotropy. In addition, we have investigated the effect of sodium chloride concentration on the specificity of β-RARE – NZF1-2D binding and have determined that the binding interaction is significantly compromised at salt concentrations less than and greater than 0.1 M. These data will be useful in continued structural studies of the β-RARE – NZF1-2D complex.

Characterization of the diiron(III) site in stearoyl-acp desaturase through EPR and ENDOR of the protein radiolytically reduced at 77k: effect of substrate.

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The diiron center in stearoyl-ACP desaturase (DS) from castor plant catalyzes the dioxygen and NADPH-dependent introduction of a cis-double bond between C9 and C10 of stearoyl-ACP. Radiolytic reduction of diferric DS at 77K produces an EPR detectable mixed-valence center trapped in the conformation of the diferric precursor that is a sensitive EPR/ENDOR probe for structural study of the diamagnetic diiron(III) state. Cryoreduced DS shows two distinct EPR signals suggesting the presence of two conformers with the μ-oxo(major) and μ-hydroxo(minor) bridged diiron centers. ENDOR studies show that in dominant conformer each Fe(III) coordinates a histidine and a water along with other ligands. Addition of stoichiometric amounts of stearoyl-ACP results in pronounced changes in the EPR and ENDOR spectra of cryoreduced DS. EPR spectra of cryoreduced DS-substrate complex disclose two distinct conformational states for DS. ENDOR studies show that in the major conformer each iron of the diferric cluster coordinates a μ-oxo ligand, water and histidine. In the minor conformer, one of the iron of the active site loses the terminal water ligand. The μ-oxo bridge in the major cryoreduced DS species is protonated on annealing to 230K, while no protonation of the μ-oxo bridge is observed upon annealing cryoreduced DS-substrate complex. The mixed-valence states of DS disappear at T>240K likely via disproportionation. The alterations in the diferric site of DS induced by the substrate are suggested to be mediated by conformational changes in the polypeptide chain produced by substrate binding. These structural alterations may provide DS with an additional mechanism for tuning the redox potential of the active site.
EPR and ENDOR Probing of Oxyheme Centers in Human Hemoglobin Reduced at 77K: Conformational Substates of the Oxyheme Sites in the α and β Subunits

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Exposure of frozen solutions of oxyhemoglobin to γ-irradiation at 77K yields EPR active one-electron reduced oxyheme centers [R. Kappl et al. BBA 827, 327 (1985)] which retain the conformation of the diamagnetic precursor. We have used this technique for EPR and ENDOR probing of oxyheme sites in human HbO2A, isolated αO2 and βO2 chains as well as αO2β(Zn), α(Zn)βO2 and αO2β(Fe3+) hybrids. EPR spectra of cryoreduced HbO2 reveal two spectroscopically distinct cryoreduced [FeO2] centers A and B. The relative populations of the centers in cryoreduced HbO2A is significantly changed on addition of glycols and independent of fractional production of cryoreduced oxyheme centers within the HbO2 tetramer. Influence of the glycol additives on the populations of these centers is substantially modulated by the R-T quaternary state transition induced by the allosteric effector IHP. EPR spectra of cryoreduced isolated αO2 and βO2 chains as well as αO2β(Zn), α(Zn)βO2 and αO2β(Fe3+) hybrids reveal that the αO2 and βO2 subunits both exhibit the A and B FeO2 centers. The data show that both the αO2 and βO2 subunits exhibit two major A and B conformational substates (αO2A (minor species) and αO2B (major species); βO2A (major species) and βO2B (minor species)) and the relative populations of the substates depend on the quaternary state of the HbO2 tetramer and affected by polyhydric alcohol additives. The H ENDOR spectra from the distal histidine proton hydrogen-bonded to the peroxo ligand show very different isotropic coupling for the A and B cryoreduced [FeO2] centers suggesting that these centers represent different orientations of the oxyheme O2 ligand relative to the distal histidine. It is likely that the A and B conformational substates in αO2 and βO2 subunits differ in their tertiary structures and their affinities to O2.

Spectroscopic Investigation of Stellacyanin Mutants: Axial Ligand Interactions at the Blue Copper Site

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Detailed electronic and geometric structural descriptions of wild-type (WT) stellacyanin and its Q99M and Q99L axial mutants have been obtained using a combination of XAS, resonance Raman, MCD, EPR, and DFT calculations. The results show that the origin of the short Cu-S(Cys) bond in blue copper proteins is the weakened axial interaction, which leads to a shorter Cu-S bond (from EXAFS results) that is more covalent (based on S K-edge XAS). EPR and XAS pre-edge energies show that the effective nuclear charge on the copper increases going from O(Gln) to S(Met) to no axial (Leu) ligand, indicating that the weakened axial ligand is not fully compensated for by the increased donation from the thiolate. MCD data show that the decreased axial interaction leads to an increase in the equatorial ligand field indicating that the site becomes a more trigonally distorted tetrahedral site. These geometric and electronic structural changes, which result from weakening the axial ligand, allow the site to maintain efficient electron transfer, while modulating the redox potential of the site to a biologically relevant range.
Mononuclear non-heme iron active sites catalyze a wide variety of essential biological functions requiring the binding and activation of dioxygen. In order to gain insight into the catalytic mechanism on a molecular level, the geometric and electronic structure of the oxygen intermediates and their reactivity need to be understood. The first trapped and well-characterized oxygen intermediate in a non-heme iron environment is that for the anti-cancer drug bleomycin (BLM). FeII-BLM reacts with dioxygen and an exogenous electron to form activated bleomycin (ABLM), a low-spin, end-on FeIII-hydroperoxo complex. Activated BLM is the last intermediate detected prior to DNA strand scission by H-atom abstraction from the C4' position of the DNA ribose sugar.

However, little is known about the mechanistic steps or catalytically active species of this H-atom abstraction reaction. Our previous work has focused on the homolytic cleavage of the O-O bond in ABLM and other non-heme FeIII-peroxo complexes.1,2 We now expand these studies to other possible pathways and investigate the thermodynamic and kinetic aspects of the reactivity of ABLM using spectroscopic and quantum chemical methods. In addition, the electronic structure and the reactivity of the bleomycin ligand is compared to non-heme and heme ligand sets.

This comprehensive study will provide a deeper level of understanding of the reactivity of oxygen intermediates in non-heme iron systems.

References:

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Copper-mediated Cyclization and Oxygenation of Heterocyclic Aldimines

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Multicopper proteins play a major role in biological dioxygen activation. Among other things they are abounded in oxidases and oxygenases\(^1\), noteworthy members of which include tyrosinase, dopamin \& beta-hydroxylase, laccase, and ascorbate oxidase. Multinuclear copper enzymes are involved also in the physiologically relevant oxidative heterocyclizations, such as phenoxazinone synthase or sulochrin oxidase. Recently, we were able to show that pyridine substituted aldimines and hydrazones undergo copper-mediated oxidative cyclization forming heterobicycles\(^2\). This reaction \(\text{(A)}\) has been carried out also with catalytic amounts of copper(II) by atmospheric oxygen\(^3\). Mechanistic studies on this reaction are presented showing that intermediate dinuclear or multinuclear copper complexes should be involved in the oxidation. Furthermore, we show that these multinuclear copper complexes not only activate the oxygen but also influence the selectivity of the reaction depending on the substitution pattern of the substrates. Using tridentate heterocyclic aldimines as educts concurrent reactions took place. Starting from the methyl derivative and in addition to reaction pathway \(\text{A}\), we found under an excess of copper(II) the formation of trisubstituted pyridine derivatives \(\text{(B)}\). In the presence of oxygen and catalytical amounts of copper the phenyl derivative underwent also an oxygenation forming the corresponding amide \(\text{(C)}\). On the basis of isolated intermediates, the selectivity and the mechanism of the concurrent reaction pathways will be discussed.

Models of the Molybdenum Hydroxylase Enzyme Family

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The Xanthine Oxidase (XO) family of enzymes are ubiquitous in biology. Crystallographic and spectroscopic data indicate that the active site of these enzymes contains a mononuclear cis-[MoVOS]^{2+} centre which cycles through a MoV intermediate during substrate turnover. [1] A comprehensive model of this enzyme which includes the reduced counterpart of the active site has proved elusive. [2]

Previously we communicated the synthesis and spectroscopic characterisation of an XO model system TpPrMoOS(X) (TpPr = hydrotris(3-isopropylpyrazol-1-yl)borate; X = OPh) (1), which features interconvertible cis-[MoVOS]^{2+} and cis-[Mo^VOS]^{+} moieties. [3] However, X-ray Absorption and IR spectroscopy, and X-ray crystallographic studies indicate that in the solid state (1) dimerises to form a biomimetically irrelevant µ-di-sulfido MoV complex.

Recently we have fine-tuned the properties of the coligand X to achieve mononuclear cis-[Mo^VOS]^{2+} centres in both solution and solid states. Substitution of the phenol with large alkyl groups in the 2 position (eg. -OC_6H_4^tBu-2 and -OC_6H_4^sBu-2) concomitantly tunes the reduction potential of the metal and provides a steric barrier for dimerisation. This paper describes the structural and spectroscopic characterisation of Tp^νMo^VOS(OC_6H_4^tBu-2) (Figure 1).
Of oxidases and peroxidases: new studies using protein film voltammetry

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David Arciero, University of Minnesota, United States
William Montfort, University of Arizona, United States
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With a few notable exceptions, the electrochemical characterization of oxidases and peroxidases has been difficult at best. This poster presents our attempts to explore oxidase and peroxidase chemistry at an electrode, as demonstrated by the two enzymes CueO (from E. coli) and NeCCP (from N. europaea), respectively. Both enzymes adsorb to electrodes of pyrolytic graphite edge, and in the case of CueO, alkane-thiol modified gold. In the case of the multi-copper oxidase CueO, this work reveals the reduction potentials of the CueO cofactors, as observed in the absence of substrate, and with inhibitors (azide, cyanide) bound. The context for these findings regarding other multi-copper oxidases will be presented. Regarding NeCCP, protein films of this protein demonstrate highly active catalytic reduction of hydrogen peroxide. The analysis of the waveform reveals an n=1 process governs catalysis, and at a reduction potential of approximately 540 mV (vs. NHE). Michaelis-Menton treatment of the substrate dependence gives values of $K_m$ of $55 \times M$, and $k_{cat}$ of $\sim 10000 \, s^{-1}$. The pH-dependence of this feature, along with its relationship to the current understanding of the mechanism of NeCCP will be presented.

Calculation of the Redox Potential of Iron-Sulfur Proteins

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There are many redox proteins having the same redox-active cofactor bound, but differing in redox potential. How the protein modulates the redox potential of the cofactor is not well understood.

We developed a procedure to compute redox potentials in proteins accurately by first principle methods. Our approach is to combine quantum mechanical calculations on the DFT level with continuum electrostatic methods. Our results on the two iron-sulfur proteins, Rubredoxin and Ferredoxin, show excellent agreement with experiments.

For Ferredoxin, it was shown by x-ray crystallography, that a peptide bond rotates comparing oxidized and reduced crystal structure. Our calculations show, that this structural change controls the redox potential of Ferredoxin.

Paramagnetic NMR studies determined the iron of the 2Fe2S center which changes its redox state. Our calculations are in agreement with the experiment, but the two reduced states only differ in energy when a sufficiently large part of the protein is treated quantum-mechanically (see Figure).

The redox potential can be decomposed to gain insight into which contributions modulate the redox potential and what their relative importance is. Hydrogen bond formation close to the 2Fe2S center are found to be of major importance for the redox potential. Charge polarization effects also play an important role.

In Rubredoxin, there was evidence for a leucine modulating the redox potential by permitting solvent access to the center only in the reduced state. Our calculations indicate roughly a cancellation of enthalpic and entropic contributions. Therefore water penetration does not have a strong effect on the redox potential. In agreement, very recent NMRD data indicates the possibility of water penetration also in the oxidized state.

Taking into account the experimental difficulties in measuring an ‘absolute’ redox potential accurately and the theoretical challenge to compute it, the agreement obtained is striking.

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For many years proof that the hypoxic nature of malignant tumours can be used to selectively target anticancer drugs has been sought. Several classes of potential redox activated anticancer drugs have been developed to take advantage of the reducing environment resulting from the hypoxia. Drug complexes with redox active metal centres as carriers have been investigated, but have largely been employed with cytotoxic drugs that require release of the drug intracellularly, complicating the design of such complexes. MMP inhibitors, a new class of anticancer drug, conversely act in the extracellular environment and we have investigated inhibitor complexes with several redox active transition metals.

Marimastat is an MMP inhibitor with potent in-vitro anti-metastatic activity and was recently in Phase III clinical trials for a variety of cancer types. We have synthesised a Co(III) complex of marimastat incorporating the tetradentate ligand tpa (tris(2-methylpyridyl)amine) as a carrier ligand. The complex was structurally characterised in the solid state by single crystal X-ray diffraction, the first example of a crystal structure containing marimastat. 2D COSY and NOESY NMR spectra showed that the complex exists in two isomeric forms in solution, corresponding to the cis and trans isomers yet only crystallises in one of these forms. Biological testing of the complex in mice with 4T1.2 tumours showed interesting and unexpected outcomes. Initial results of the tumour growth inhibition study showed that a significant inhibition of growth was exhibited by the complex over the free inhibitor and the control. However, the metastatic potential of both free marimastat and the complex were higher than the control indicating likely problems with the experimental protocol. Further experiments are needed to determine the potential of such complexes as hypoxia activated prodrugs but there appears at least to be some promise.
Studies on trinuclear palladium complexes

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BBR3464 is a trinuclear platinum(II) compound that has shown a very high activity against a number of both cisplatin-responsive and cisplatin-resistant cancer cell-lines; in some cases the activity is 100 times as high as that of cisplatin. It is composed of three trans-platinum units linked together by alkyl diamine chains. Although palladium complexes are generally found to be toxic rather than anticancer active because of their higher reactivity, it is thought the analogy may or may not be true for polynuclear complexes. In any case, it is thought that useful information may be obtained from quantifying the activity (or the lack of it) and the nature of interaction with DNA of polynuclear palladium complexes.

Two tri-nuclear palladium analogues of BBR3464, code named MH1 and MH2 have been synthesized and characterized by elemental analyses, IR and Raman spectral studies. The activity of the designed complexes has been determined against both cisplatin-responsive and cisplatin-resistant ovarian cancer cell-lines (A2780). Both the compounds are found to be less active than cisplatin, MH1 however being more activity than MH2.

The nature of interaction of both the compounds with salmon sperm and pBR322 plasmid DNAs have also been studied using gel electrophoresis.

This poster presentation will describe the results of the studies.
Amaranth intercalation in DNA – a study using terbium probe fluorescence.

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Many azo compounds are genotoxic in short-term tests and carcinogenic in laboratory animals[1]. The alkaline (pH>13) comet assay introduced by Singh et al.[2] is a rapid and sensitive procedure for quantitating DNA lesions in mammalian cells. We present our results of an investigation of intercalation compound formation between calf thymus DNA and amaranth, a frequently used food colorant in Brazil. Compound formation was accompanied by UV/Vis, electrophoreses in agarose gel and Tb$^{3+}$ fluorescence as chemical probe for DNA.

Absorbance values are shown in Table 1. Compared to pure DNA, samples in the presence of the colorant show a 29% increase in absorbance. Simultaneously, amaranth absorbance decreased ~14%. These results suggest that amaranth is incorporated into the DNA nucleotide. Electrophoreses (Fig. 1) attested DNA integrity after its interaction with amaranth. Fluorescence spectra are shown in Fig. 2. The results suggest the sites occupied by amaranth are the same as those occupied by Tb$^{3+}$. Since the terbium probe fluorescence is primarily due to the guanine binding[3], we can conclude that amaranth intercalate in DNA mainly occupying the guanine sites.


Table 1. UV/Vis absorbance of DNA, Amaranth and DNA-Amaranth aqueous solutions.

<table>
<thead>
<tr>
<th>Aqueous solutions</th>
<th>Concentration</th>
<th>$\lambda_{\text{máx}}$</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>6 $\mu$g/L</td>
<td>262</td>
<td>0.135</td>
</tr>
<tr>
<td>Amaranth</td>
<td>1,0 $\times$10$^7$ / (mol/L')</td>
<td>522</td>
<td>0.103</td>
</tr>
<tr>
<td>DNA + Amaranth</td>
<td>same</td>
<td>262</td>
<td>0.174</td>
</tr>
<tr>
<td></td>
<td>same</td>
<td>522</td>
<td>0.089</td>
</tr>
</tbody>
</table>
Allosteric control of internal electron transfer in Cytochrome cd1 nitrite reductase

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Cytochrome cd1 nitrite reductase is a bifunctional multiheme enzyme catalyzing the one-electron reduction of nitrite to nitric oxide and the four-electron reduction of dioxygen to water. Kinetics and thermodynamics of the internal electron transfer process in the Pseudomonas stutzeri enzyme have been studied and found to be dominated by pronounced interactions between the c- and the d1- hemes. The interactions are expressed in both dramatic changes in the internal electron transfer rates between these sites and in marked cooperativity in their electron affinity. The results constitute a prime example of intra-protein control of the electron transfer rates by allosteric interactions.

Reference:
Ole Farver, Peter M.H. Kroneck, Walter G. Zumft & Israel Pecht, Intramolecular electron transfer in cytochrome cd1 nitrite reductase from Pseudomonas stutzeri; kinetics and thermodynamics, Biophys. Chem. 98 (2002) 27-34

Biological effects of low-level lead exposition on a human body.

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Lead is a heavy metal, which injures many organs and systems of the human body: gastrointestinal and nervous systems, kidneys, skeleton and muscles and so on. This information is dedicated to the influence of low levels of lead on human body and effectiveness of preventive measures against lead intoxication.

It has been established that small levels of lead lead to the several stages of consecutive fluctuation of parameters in a human body. Risk factors for poisoning reinforce lipid peroxidization and decrease antioxidant system activity, inclination to trombocytopenia and also contact with lead for more than during 5 years.

The dynamical observation on workers’ health at the modern factory producing small electric accumulators and at the soldering sections of two radioelectronic enterprises has shown the effectiveness of proper and adequate health education. Activation of lipid peroxidization and depression of antioxidant system, inclination for trombocytopenia and contact with lead for more than 5 years are indices of toxic risk.

Key words: antioxidant system, biochemical methods, heavy metals, intoxication, lipid peroxidization, low levels, preventive measures, risk factors.
Ruthenium(II) arene anticancer complexes: select recognition of nucleobases and control of hydrolysis rates

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We are investigating relationships between the chemical and biological activity of Ru(II) arene complexes of the type shown in Figure 1, including variations of the substituted $\eta^6$-arene, the chelating ligand (X-Y) and leaving group (Z).

When the chelating ligand (X-Y) is 1,2-ethylenediamine and the leaving group (Z) is Cl, the complexes exhibit anticancer activity [1] and bind to DNA [2]. In aqueous solution, $[\text{Ru}(\text{arene})(\text{en})\text{Cl}]^{+}$ binds specifically to guanine (G) when in competition with adenine (A), cytidine (C) and thymine (T) monophosphates [3,4]. The reaction proceeds via initial aquation of the chloro complex. We show that the rate of aquation can be controlled over a range of several orders of magnitude by variation in the arene and the leaving group. Aquation rates comparable with that of cisplatin and its analogues can be achieved.

Guanine adducts of $\{\text{Ru}(\text{arene})(\text{en})\}^{2+}$ are stabilized by binding to N7 of G and by C6O···HN(en) H-bonding, as well as $\pi$-π arene-purine stacking if the arene is large enough, and destabilized by en NH$_2$ – base NH$_2$ interactions [3,4]. By changing the chelating ligand X-Y to an acetylacetonate derivative, we show that binding can now occur to A as well as G with similar affinities. In general the hydrolysis rates of the acetylacetonate complexes appear to be faster than those of the ethylenediamine complexes.

We thank the EU (Marie Curie Fellowship for RF and COST D20), Edinburgh Technology Fund, and EPSRC for support, Dr Simon Parsons and colleagues for X-ray crystal structures, Dr Duncan Jodrell and colleagues (University of Edinburgh Cancer Research UK Centre) and Professor Viktor Brabec and colleagues (Institute of Biophysics of the Czech Republic, Brno) for collaborative biological studies on these compounds.

Platinum(II) Intercalators as Potential Anti-Cancer Agents

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A series of platinum(II) compounds have been designed to interact with DNA via a different binding mode than that of current anti-cancer compounds (e.g. cisplatin). Compounds like cisplatin tend to bind covalently to DNA, whereas, these new compounds intercalate between the base pairs of the DNA molecule. Binding studies with DNA have been conducted using viscometry, electrophoresis, circular and linear dichroism, and UV-vis spectroscopy to confirm the intercalating properties of these complexes.

Square-planar metallointercalators are composed of two parts (see figure): an intercalating moiety (I); and a non-intercalating (or ancillary) group (A). These components are typically neutral, nitrogen-based ligands. The planar intercalating segment is comprised of at least three aromatic rings fused together, whereas, there are no structural requirements for the ancillary portion. Both chiral and achiral ancillary ligands have been utilised.

The associated effects of placing substituents on the ancillary and intercalating ligands have also been investigated. Subtle changes in the structure of the complex induce differing levels of biological activity. The biological activity of these complexes has been determined through in vitro testing on human cell lines. Results have shown most compounds to be biologically active, but to varying degrees.

Some of the compounds synthesised and characterised to date have displayed a higher level of activity and greater solubility than cisplatin. These features suggest that such compounds may demonstrate higher clinical effectiveness and lower toxicity than current platinum-based compounds in clinical use.

Development of Lanthanide-Binding Peptides as Natively Expressed Protein Probes

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Biophysical probes that integrate into the covalent architecture of proteins are key tools for analysis of protein expression levels, investigation of protein function, and elucidation of protein structure. We have developed Lanthanide Binding Tags (LBTs) as natively expressed, minimally invasive and multitasking protein probes. A combination of peptide design and combinatorial screening enabled the identification of novel peptide sequences that feature greater than two orders of magnitude tighter affinity for Tb(III) and over 6-fold brighter luminescence intensity compared with existing peptides. Luminescence lifetime measurements, X-ray crystallography and NMR spectroscopy are being used to characterize these lanthanide-peptide complexes. Incorporating the LBT sequences into fusion proteins subsequently allows for powerful new techniques for visualizing proteins in both SDS-PAGE gels and in complex mixtures in solution. Future applications of the LBT strategy include their use to probe cell surfaces and as sensors of kinase activity.
New Crystallographic Studies of Copper Amine Oxidases

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Copper amine oxidases (CuAOs) catalyse the oxidative deamination of primary amines:

\[
\text{RCH}_2\text{NH}_2 + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{RCHO} + \text{NH}_3 + \text{H}_2\text{O}_2
\]

The active site of CuAOs includes both a Cu(II) atom and an organic cofactor. In the best-characterised sub-class of the enzymes, the cofactor is 2,4,5-trihydroxyphenyl-alanine quinone (TPQ). Crystal structure have been reported for CuAOs from the bacteria Escherichia coli (Leeds) and Arthrobacter globiformis (Osaka/Sydney), the yeasts Hansenula polymorpha (St. Louis) and Pichia pastoris (Sydney), and a higher plant Pisum sativum (pea seedlings, Sydney). A number of reaction intermediates (Leeds) and inhibitor complexes (Osaka) have also been characterized structurally.

Here we report new results of current crystal structure analyses of CuAOs, including:

1. A complex of A. globiformis AO with a potent inhibitor, 4-(2-naphthyloxy)-2-butyn-1-amine (NOBA), in which the inhibitor has interacted with the TPQ cofactor in an unexpected fashion.
2. Pichia pastoris AO, a CuAO with the unique ability to catalyse the oxidation of polypeptide lysyl side chains.
3. A Xe derivative of Pichia pastoris AO, in which a number of hydrophobic pockets are identified.

One of the main (but still conflicting) theories for the CuAO enzyme mechanism requires O2 to move to the active site via one or more hydrophobic pockets.

The crystal structure of Nitrobenzene 1,2-dioxygenase

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Nitroaromatic compounds are used extensively as industrial Feedstocks for many manufacturing processes, including the production of pesticides, dyes, and explosives. Due to improper storage, use, and disposal, nitroaromatic compounds have been released in the environment, where they are considered environmental pollutants.

Nitrobenzene dioxygenase (NBDO) from Comamonas sp. strain JS765 catalyzes the first step in the degradation of nitrobenzene yielding the oxidized reaction product catechol.

NBDO belongs to the naphthalene family of Rieske nonheme iron oxygenases.

Like naphthalene dioxygenase from Pseudomonas sp. (NDO) is NBDO characterized by the extensive range of substrates it can oxidize.

NBDO shares an 82% sequence identity with NDO which crystal structure is known. NDO has an \( \alpha_3 \beta_3 \) composition, and each \( \alpha \) subunit contains a Rieske [2Fe-2S] center and mononuclear iron at the active site.

Recent work has focused on identifying amino acids in the catalytic domain of the \( \alpha \) subunit that controls the regioselectivity and enantioselectivity of NDO. The structure of NDO shows that 17 amino acids line the active site. Several of these amino acids are present in NBDO. However, the residues at positions 206, 253 and 295 (NDO numbering) are unique to NBDO when compared to related dioxygenases.

The crystal structure of NBDO reveals how these structural differences leads to different substrate specificities.
Time resolved spectroscopic determination of a sub-microsecond, 1:1 copper dioxygen interaction in which the rate exceeds that for hemes.

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Donald V Scaltrito, Johns Hopkins University, United States
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The photolability of CO from the complex [Cu(tmpa)(CO)]\(^+\) (tmpa = tris[(2-pyridyl)methyl]amine) at low temperatures (-83°C to -58°C) in the presence of CO/O\(_2\) mixtures has resulted in the ability to observe the formation of [Cu(tmpa)(O\(_2\)-)]\(^+\) (\(\lambda_{\text{max}} = 425\) nm). In addition, kinetic analyses provided rate constants for the binding of O\(_2\) to copper (k\(_{O_2}\)) and the dissociation of O\(_2\) from copper (k\(_{-O_2}\)) as well as the corresponding binding constant (K\(_{O_2}\)). Activation parameters for k\(_{O_2}\) (∆H\(_a\) = 14.6 kJ mol\(^{-1}\) and ∆S\(_a\) = -10.7 J mol\(^{-1}\) K\(^{-1}\)) aid in the extrapolation of a room temperature rate constant, k\(_{O_2}\) = 4.9 x 10\(^9\) M\(^{-1}\) s\(^{-1}\), a value that exceeds the number of any heme or copper system previously reported. Thermodynamic parameters (∆H° = -44 kJ mol\(^{-1}\) and ∆S° = -116 J mol\(^{-1}\) K\(^{-1}\)) match well with those previously determined via stopped-flow spectroscopic measurements proving the validity of the experiments.

A Sterically Hindered Salen Iron Complex as a Model for Active Sites of Mononuclear Non-Heme Iron Enzymes

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Salen iron complexes 1-3 are prepared as a model for a mononuclear non-heme iron enzyme involving in dioxygen activation. In these complexes, a bulky mesityl substituent is attached to the salen ligand in order to isolate the mononuclear iron center. Indeed, the ferric aqua complex 3 is found to be monomeric from the X-ray crystallographic and EPR study. Intriguingly, the ferric iron site of 3 is considerably distorted to exhibit a trigonal-bipyramidal structure. The trigonal bipyramidal ferric center of 3 is very close to the active site structure of protocatechurate 3,4-dioxygenase. Mechanistic details of the distortion at the ferric center are investigated. Comparison of the crystal structure of 1-3 indicates that the magnitude of the distortion depends on the external ligand. The iron(III) site of 1 is square-pyramidal, which is common for five-coordinate salen iron complexes. On the other hand, the distortion of the square-pyramidal structure is observed for the ferric ethoxide complex 2, and the ferric site of 3 is distorted to the largest extent. The order of Cl cp: corresponds to the spectrochemical series, suggesting that the distortion caused by the external ligand comes from an electronic factor. The DFT calculation supports this idea. We propose that the s-donation from the external ligand affect the d orbital of the central iron, leading to the distortion to the trigonal-bipyramidal structure. The isolated iron center of the sterically hindered salen iron complex is also utilized to study active species generated from the iron center and oxidants. We successfully obtained the transient blue-green intermediate from 3 at a low temperature. The detailed structure is now being investigated and will be discussed.
Pentacoordinated iron complexes with carboxamido nitrogens and thiolate sulfurs: As models for Fe-containing nitrile hydratase

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Iron-containing nitrile hydratases (NHases) are bacterial metalloenzymes that catalyze the hydrolysis of nitriles to their corresponding amides. Their active site has an unusual coordination environment that consists of two amide nitrogen atoms provided by the main chain of peptide and three sulfur atoms from cysteine residues. In addition, two of the metal-bound sulfur atoms are post-translationally modified to sulfinate and sulfenate groups. The sixth coordination site is occupied by NO in the inactive form and is vacant or occupied by solvent in the activated form. Towards characterization of the iron(III) site in such a unique environment, we have synthesized pentacoordinated iron complexes with ligands having two amide moieties and N3S2 and N2S3 donor sets as structural models for NHases. We have prepared the iron complexes with both protonated and deprotonated carboxamido nitrogens and have investigated the complexes by spectroscopic (UV-Vis, EPR, FT-IR, and Mossbauer) and electrochemical methods. The results of this research and comparison with those of NHases will be discussed.

Oxidation Activity of Hydroperoxo-copper(II) Complex as a DβH

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Hydroperoxo-copper(II) complexes are very important as active intermediates in some oxygenases such as dopamine β-hydroxylase (DβH) and peptidylglycine α-hydroxylating monooxygenase (PHM). Previously we reported first preparation, crystal structure, and spectroscopic characterization of stable hydroperoxo-copper(II) complex with a tripodal tetradentate ligand, bis(6-pivalamido-2-pyridylmethyl)(2-pyridylmethyl)amine (BPPA), which was stabilized by hydrogen bonding interaction between two NH hydrogens of pivalamido amine and coordinating hydroperoxide oxygen. However this complex did not show any reactivity for organic substrates. In order to develop catalytic activity in oxidations, we employed tridentate ligand, bis(2-pyridylmethyl)tert-butylamine (BPBA). Preparation of Cu-BPBA-OOH species was confirmed on the basis of the spectroscopic characterization of the Cu(II) complexes with BPBA ligand (Fig 1). The reaction of some organic substrates, sulfides, olefins, or alkyl benzenes, with the Cu-BPBA complex in the presence of H2O2 gave the corresponding oxidation products. In the case of cyclohexene, cyclohexenol as an allylic oxidation product was mainly obtained. In the oxidation of alkyl benzene derivatives, especially, selective oxidation of benzylic compounds was observed, which resembles the reactivity of DβH.

On the Mechanisms of Oxygenations of Catechols by Oxygenases and Their Model Complexes

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Mechanism of oxygenation by catecholdioxygenases and their model systems are one of the most important targets in the field of bioinorganic chemistry. We have first reported the importance of formation of the chelate-bound catecholatoiron(III) species [1] and their activation to semiquinonateiron(II) species [2] for the reaction with oxygen, that is generally accepted in the mechanism of oxygenation. Discussions at present are on the procedure of activation of oxygen and insertion into the C-C bond. The direct attack of molecular oxygen to the catecholate ligand carbon in the intradiol oxygenations has been favored, but we have proposed the alternative process, that involves the first attack of oxygen to the iron center. This mechanism is believed to be very probable in the extradiol oxygenations by iron(II) enzymes, but is also proposed in the extradiol oxygenation by model iron(III) complexes [3]. We wish to report supports for the oxygen activation by the metal center both in the intra- and extradiol oxygenation. We have observed the reversible binding of oxygen to the Mn(II) center in the Mn(II)-semiquinonate complex, that is oxygenatively cleaved to give mainly intradiol products with some extent of the extradiol product. The same phenomenon was observed even in the iron complex at low temperature. The oxygenation mechanism will be discussed on these new results.


Introduction of Tetrahedral Distortion into the Copper-dioxygen Complexes: The Remarkable Effects on Their Formation and Spectroscopic Properties

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The copper-dioxygen complexes had been found in model studies of dinuclear copper enzymes. These dioxygen adduct complexes are generally formed by oxygenation of the corresponding copper(I) complexes. The further progressed studies had established the Cu^{III}_{2}(µ-O)_{2} species, which formed by way of the subsequent O-O bond cleavage of Cu^{II}_{2}(µ-η^{2}:η^{2}-O_{2}) core[1]. The X-ray crystal structure analyses of Cu^{II}_{2}(µ-O)_{2} complexes had shown that their copper centers have square-based 4- or 5-coordinated geometries. Such the planar core structures have been affecting the motif of high-valent O₂-activating intermediate in dimetal systems. In this study, pursuing a new structural motif of M^{III}_{2}(µ-O)_{2} core, we have introduced a tetrahedral distortion into the Cu^{III}_{2}(µ-O)_{2} core by using (-)-sparteine (Sp) and its stereoisomer as bidentate ligands[2]. X-ray studies of these copper complexes with Sp revealed that these copper centers have tetrahedrally distorted structures regardless of their oxidation states, and monomer and bridged dimer forms, due to the steric requirements of the ligand. The Cu^{III}_{2}(µ-O)_{2} complexes with Sp was rapidly formed in solution at -80 °C, exhibiting unique spectroscopic properties. Carried out theoretical studies about these systems, the relationships between the physical properties and distorted structures have been discussed.

Introduction of Tetrahedral Distortion into the Copper-Dioxygen Complexes: The Remarkable Effects on Their Formation and Spectroscopic Properties

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The copper-dioxygen complexes had been found in model studies of dinuclear copper enzymes. The further progressed studies had established the Cu$^{	ext{III}}$(µ-O)$_2$ species, which formed by way of the subsequent O-O bond cleavage of Cu$^{	ext{II}}$(µ-η$^2$-O$_2$) core$^1$. The X-ray crystal structure analyses of Cu$^{	ext{III}}$(µ-O)$_2$ complexes had shown that their copper centers have square-based 4- or 5-coordinated geometries. Such the planar core structures have been affecting the motif of high-valent O$_2^-$ activating intermediate in other dimetal systems. In this study, pursuing a new structural motif of M$_2$(µ-O)$_2$ core, we have introduced a tetrahedral distortion into the Cu$^{	ext{III}}$(µ-O)$_2$ core by using (-)-sparteine (Sp) and its stereoisomer as bidentate ligands$^2$. X-ray studies of these copper complexes with Sp revealed that these copper centers have tetrahedrally distorted structures in Cu$^{	ext{I}}$ and Cu$^{	ext{II}}$ states, and in monomer and bridged dimer forms, due to the steric requirements. The Cu$^{	ext{III}}$(µ-O)$_2$ complexes with Sp was rapidly formed in solution at -80 °C, exhibiting unique spectroscopic properties. Carried out theoretical studies about these systems, the relationships between the physical properties and distorted structures have been discussed.


Studies of the Interaction of Potassium(I), Calcium(II), Magnesium(II) and Copper(II) with Cyclosporin A

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The metal binding properties of the immunosuppressant drug cyclosporin A (CsA) have been investigated. Complexation studies in acetonitrile solution using H NMR and CD spectroscopy yielded 1:1 metal-peptide binding constants(log$_\text{K}$) for potassium(I) (cp: in which it is proposed that the oxygen donor atoms are bound to the metal ion. The CD titrations support previous findings that Ca(II) forms a well defined 1:1 complex with CsA while Mg(II) does not.

The interaction of copper(II) salts with cyclosporin A in methanol was investigated with UV/VIS and EPR spectroscopy. The reaction of CsA with copper(II) requires the presence of base for complexation to occur and only monomeric copper(II) complexes are formed. The results are attributed to the number and type of donor atoms available, and the conformation of CsA which prevents the formation of dinuclear copper(II) complexes.
Silencing of Gadolinium(III)-Phosphonate MRI Contrast by Hydroxyapatite Binding

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Gd(DOTP)₅⁻ is a highly charged, bone-seeking paramagnetic complex that could potentially detect bone lesions by acting as a MRI contrast agent. To date, its pharmacokinetics, effects on organ relaxivity, and interaction with hydroxyapatite (HA) have not been described. Liver, kidney and bone MRI images were obtained on male white rabbits after administration of GdDOTP₅⁻, or a gold standard MRI contrast agent GdDTPA₂⁻. Signal intensity vs. time curves were generated and enhancement patterns compared. Parallel in vitro experiments quantified the effect of hydroxyapatite (HA) binding on GdDOTP₅⁻-induced changes in relaxivity. Dynamic gamma scintigraphic imaging and biodistribution studies were carried out on Wistar rats injected with ⁴⁰⁰⁰Sm-DOTP₅⁻ and ¹¹¹In-DOTP₅⁻ radioactive tracers. Binding studies of these tracers on HA surfaces were also undertaken.

It was found that GdDOTP₅⁻ undergoes fast elimination by glomerular filtration, similar to GdDTPA₂⁻ and other extracellular non-specific contrast agents. However, a second, slow washout compartment corresponding to approximately 20% of the total injected dose was also evident from the clearance data. Visceral signal enhancement by the two compounds was similar. However, no enhancement of bone was evident with GdDOTP₅⁻ despite confirmation of bone and HA binding of the radioactive ⁴⁰⁰⁰Sm-DOTP₅⁻ and ¹¹¹In-DOTP₅⁻ derivatives (Fig.1). In vitro experiments demonstrated that GdDOTP₅⁻ induced changes in relaxivity were silenced upon HA binding, but could be recovered by acid elution of the complex. Thus, GdDOTP₅⁻ has characteristics similar to other low molecular weight, extracellular MRI contrast agents but fails in its potential to provide diagnostic information about pathological bone processes. HA binding assays revealed that the complex is essentially MR silent when bound to bone, likely due to exclusion of all outer sphere water molecules from the surface of the complex. These data suggest a novel strategy for creating ‘stealth’ MRI contrast agents.

Fig.1. Biodistribution of ⁴⁰⁰⁰Sm-DOTP and ¹¹¹In-DOTP a) 30 minutes and b) 48 h after injection in Wistar rats.
**Superoxide Reactivity of KatG from M. Tuberculosis: Implications for Isoniazid Resistance**

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Isoniazid (INH) is a frontline antibiotic used in the treatment of tuberculosis (TB). As a prodrug, INH requires activation (via oxidation) to an as-of-yet undetermined form by the enzyme KatG, a hemoprotein possessing catalase-peroxidase, Mn²⁺-peroxidase, peroxynitritase and P450-oxygenase activities. Downstream targets of activated isoniazid include InhA and KasA, two enzymes which are involved in the synthesis of mycolic acids that are constituents of the mycobacterium cell wall.

Recent work has established a role for superoxide which correlates well with INH susceptibility. When superoxide (generated by SOTS-1 or xanthine/xanthine oxidase) is employed as the oxidant, the rate of INH oxidation for KatG(S315T), whose serine to threonine point mutation confers isoniazid resistance to M. tuberculosis, is 5-7 fold diminished as compared to WT KatG. By contrast, the rates of INH oxidation are nearly identical for WT vs. KatG(S315T) when organic hydroperoxides are employed, indicating a role for oxyferrous KatG over peroxidative compound I/II in resistance-dependent INH activation.

We have employed pulse radiolysis as the means to explore superoxide binding to KatG on the microsecond time-scale. Initial analysis of the data indicates that ferric KatG binds superoxide within 10 µs yielding an oxyferrous intermediate, with rates nearly identical for WT vs. KatG(S315T), indicating that step I in the proposed mechanism of INH activation (see figure) is not the origin of INH resistance. Furthermore, as mentioned above, previous work indicates that the peroxidative pathway yields rates of INH oxidation nearly identical for WT and mutant KatG, indicating step III of the proposed mechanism is also not the origin of INH resistance. Thus, as our data suggests, step II, which involves a proton-coupled electron-transfer to initiate O-O bond cleavage of the oxyferrous intermediate, is the origin of INH resistance in M. tuberculosis strains containing the KatG(S315T) mutation. Details of these and other studies which support the proposed mechanism will be presented.

**Nonnatural Amino Acid Ligands in Heme Protein Design**

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We are expanding heme protein design beyond the limits of genomic biology by increasing the repertoire of ligands available by using nonnatural amino acids. Here, we employ 4-β-(pyridyl)-L-alanine, β-(tetrazoyl)-L-alanine and 3-methyl-histidine as a heme ligands in a de novo designed four helix bundle to evaluate the thermodynamic consequences on Fe(III) and Fe(II) heme binding and electrochemistry. The ferric heme affinity for the bis-pyridylalanine peptide is significantly weaker, the first dissociation constant, Kd1, measured by UV-vis is 60µM as compared with the 135 pM value for the analogous bis-histidine site. The Fe(II)protoporphyrin IX affinity for the pyridylalanine peptide is significantly stronger, Kd1 of < 1nM, compared to 42 nM for the bis-histidine bundle. The weaker ferric binding constant and stronger ferrous binding constant elevates the heme equilibrium midpoint reduction potential at pH 7, Em7, to +58 mV, a value > 250 mV more positive than the bis-histidine protein.
Is Ag(I) an adequate probe for Cu(I) in structural copper-metallothionein studies? The binding features of Ag(I) to mammalian metallothionein 1

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The binding abilities of silver(I) to mammalian MT 1 have been studied and compared with those of copper(I), recently reported, with the aim of analyzing the suitability of Ag(I) as a Cu(I) probe in Cu-MT studies. The Zn/Ag replacement in recombinant mouse Zn₇-MT 1 and corresponding Zn₇-αMT 1 and Zn₇-βMT 1 fragments, as well as the stepwise incorporation of Ag(I) to the corresponding apo-MTs, have been followed in parallel by various spectroscopic techniques including electronic absorption (UV-vis), circular dichroism (CD) and electrospray mass spectrometry coupled to capillary zone electrophoresis (CZE-ESI-MS). A comparative analysis of the sets of data obtained in the titrations of the three proteins with AgClO₄ at pH 7.5 and 2.5 has led to the corresponding reaction pathways and has provided evidence of unprecedented stoichiometries and structural features for the species formed during the above processes. Thus, the Zn/Ag replacement in Zn₇-MT 1 at pH 7.5 has revealed the subsequent formation of the following MT species: Ag₄Zn₅-MT, Ag₇Zn₃-MT, Ag₈Zn₂-MT, Ag₁₀Zn₁-MT, Agₓ-MT, x = 14-19, whose structure consists of two additive domains only if Zn(II) remains coordinated to the protein. A second structural role for Zn(II) has been deduced from the different folding found for the Agₓ-MT species of the same stoichiometry formed at pH 7.5 or 2.5. Comparison of the binding features of Cu(I) and Ag(I) to the entire MT at pH 7.5 shows that, among all the MₓZn₇-MT (0 ≤ x ≤ 7) metal replacement, show comparable 3D structures; thus, they are the only species where Ag(I) ions can be predicted to be an adequate probe for Cu(I).
Bioinorganic chemistry of corrole metal complexes: interactions with proteins and cells

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With only one exception, corroles and their metal complexes were never used in biological systems because of severe limitations in their syntheses and the lack of appropriately substituted derivatives.[1] Following our invention about the solvent-free synthesis of corroles from pyrrole and aldehydes,[2] we have recently discovered the facile synthesis of corroles with polar head groups at specific positions on only one half of the macrocycle.[3] This provides a unique access to amphiphilic corroles and their metal complexes, allowing for utilization of the novel photophysical and catalytic properties that we found for lipophylic corroles in biological systems.[4, 5]

In particular, we will present the strong and metal-dependent association of the corroles shown in the Chart with proteins and the effectiveness of such conjugates for detection and/or selective damage to cancer cells.

References:

**Chart:** The structure of an amphiphilic corrole (M = H₄) and its metal complexes (M = Ga, Co, Fe, and Cr) and examples demonstrating their interaction with proteins (HSA = human serum albumin) and selective damage to cancer cell lines.
Does Platinum(IV) Survive in Tumour Cells?

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The rates and mechanism of reduction of platinum(IV) complexes by endogenous biomolecules in vitro have been extensively investigated.[1] However, no reliable spectroscopic method for observing the reduction of platinum(IV) complexes in biological systems has been reported. Current techniques do not allow for facile in situ determination of the average or component oxidation states of a system. Here we describe the use of X-ray absorption near edge spectroscopy (XANES) to provide information about the relative proportions of platinum(II) and platinum(IV) complexes in biological systems. Using XANES, the intracellular reduction of platinum(IV) complexes in cancer cells has been observed directly, and the proportion of reduction after 2 h was found to correlate with their reduction potentials. The localisation of a number of platinum(IV) complexes has been investigated using micro-SRIXE imaging of ovarian cancer cells, revealing that cellular distribution of the complexes is similar to that of cisplatin. The cytotoxicity and lipophilicity of platinum(IV) complexes with a range of reduction potentials have been determined to elucidate the importance of these parameters on their cytotoxicity.


Design and synthesis of a novel magnetic resonance imaging (MRI) contrast agent for selective sensing of zinc ion

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Light-based microscope imaging techniques using fluorescence sensor molecules sometimes suffer from photobleaching and light scattering, whereas magnetic resonance imaging (MRI) can provide a three-dimensional imaging without these problems. Zinc is an essential component of many enzymes, transcription factors and synaptic vesicles in excitatory nerve terminals. So imaging of chelatable zinc is of interest. Recently, gadolinium complexes have been used as MRI contrast agents. On the basis of these gadolinium complexes, we designed and synthesized the first Zn(II)-sensitive MRI contrast agent, which shows a dose-dependent decrease of the R1 relaxivity in the presence of Zn(II). The R1 relaxivity decreased ~30% when Zn(II) was added. This contrast agent also had high selectivity for Zn(II) against Ca(II) and Mg(II). Moreover, we examined the mechanism of the decrease of the R1 relaxivity of solutions of this contrast agent upon adding Zn(II). The presumed complex stoichiometry of Zn(II) was supported by the results of UV-visible spectra and coldspray ionization (CSI) mass spectrometry in the solution phase. This novel Zn(II)-sensitive MRI contrast agent seems to be an excellent candidate for incorporation into sensors designed for selective detection of Zn(II) in biological applications.
