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Journal Name

ARTICLE

Metal complexes as "*Protein Surface Mimetics*"

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A key challenge in chemical biology is to identify small molecule regulators for every single protein. However, protein surfaces are notoriously difficult to recognise with synthetic molecules, often having large flat surfaces that are poorly matched to traditional small molecules. In the surface mimetic approach, a supramolecular scaffold is used to project recognition groups in such a manner as to make multivalent non-covalent contacts over a large area of protein surface. Metal based supramolecular scaffolds offer unique advantages over conventional organic molecules for protein binding, inlcuding greater steroechemcial and geometrical diversity conferred through the metal centre and the potential for direct assessment of binding properties and even visualisation in cells without recourse to further functionalisation. This feature article will highlight the current state of the art in protein surface recognition using metal complexes as surface mimetics.

Introduction

The last decade has seen an increasing diversity of new methods to target protein function[1,2] including control of protein localisation,[3] and degradation.[4,5] The prevailing methods, however, still centre on development of ligands which prevent the protein of interest from engaging in interactions with substrates (e.g. small molecules or other proteins), through either an orthosteric or allosteric mode of action. Whilst methodologies to identify suitable chemical matter for established protein targets such as GPCRs and enzymes are well known,[6] the difficulty in achieving the goal of a "small molecule modulator for every protein",[7] has been most acutely demonstrated through efforts to identify inhibitors of protein-protein interactions (PPIs).[8–10] Supramolecular Chemical Biology[8] can offer solutions to this challenge: the surface mimetic approach involves the recognition of large areas of a protein surface, using a functionalized supramolecular scaffold capable of making multivalent non-covalent contacts to achieve strong and selective binding (Fig. 1).[11] Multivalency is widely exploited in nature, permitting an increased binding affinity by increasing the number of ligands and receptor sites, for example in signal transduction, cell membrane adherence, and immunological responses. A recent review by Ulrich *et al*. has outlined how multivalency can be used to inhibit enzymes.[12]

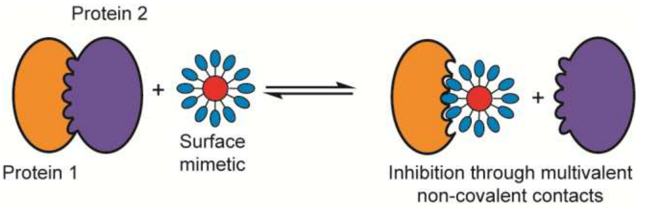

Figure 1 General schematic of the surface mimetic approach, a large multivalent, supramolecular molecule binds to a protein surface, potentially displacing a natural protein binding partner

This feature article will highlight the development of "*the surface mimetic approach*"[13,14] to protein surface recognition. The term "*surface mimetic*" is distinct from "*proteomimetic*"[15,16] which refers to small molecules mimicking a region of protein structure (usually a defined secondary structural motif such as an α-helix or β-sheet). The initial section of the article will focus on organic supramolecular scaffolds to illustrate the thinking in developing this approach, before moving on to metal co-ordination complex scaffolds as ligands for protein surfaces.

Metal complexes have had a huge impact on medicinal chemistry starting with the introduction of *cis*-platin. The discovery and development of cytotoxic organometallic small molecules and co-ordination complexes has been reviewed on numerous occasions previously.[17–20] Similarly, the use of co-ordination complexes as ligands for nucleic acids has seen extensive development and the reader is directed towards recent review articles.[21,22] In contrast metal complexes for protein-surface recognition are less well developed, however, metals offer distinct advantages for this challenging goal over conventional organic scaffolds (Fig 2.). Advantages of metal complexes include the ability to

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offer a variety of co-ordination numbers and geometries (Fig. 2), thus expanding the number of globular shapes available and allowing exploration of protein pockets and surfaces inaccessible to conventional small organic molecules.[23] Metal complexes also have the ability to exist in many more stereoisomers than organic molecules, for example an sp^3 carbon with 4 different substituents has only two possible stereoisomers whereas an octahedral metal centre with 6 different ligands can exist in up to 30 different stereoisomers (Fig. 2).[19] Critically, protein binding selectivity of small molecules has been shown to correlate with both shape and stereochemical complexity,[24] emphasizing how the diversity of metal complex ligands might be used for selective protein recognition. The use of metal complexes allows for combinatorial synthesis in order to generate a wide range of metal complexes using similar reactions,[19] thus permitting a variety of compounds to be screened readily. The metal centre itself can be used solely as a scaffold, for forming coordinative bonds with the biological macromolecule, and for its reactive capacity, thus expanding the scope of interactions possible to achieve binding.[19] In addition, the ligands on the metal play a role in the redox behaviour, bio-stability, absorption and delivery of the metal complex, and can be used to direct the synthesis towards particular stereoisomers (e.g. using the *trans* effect). Moreover, use of metal complexes provides direct entry to molecular sensors. Through judicious choice of metal scaffold, intrinsic luminescence can detect molecular recognition and cellular behaviour, e.g. ruthenium(II) and iridium(III) complexes are phosphorescent, allowing direct visualisation in both biological assays and cellular imaging.[25] In contrast, most traditional organic ligands need derivatization, often through lengthy syntheses, which result in changes to (molecular recognition and physicochemical) properties. Finally, although our own driver for this work has been to address deficiencies in the ability to identify inhibitors of protein-protein interactions, the exploitation of metal complexes for protein surface recognition has had influence more widely e.g. in developing kinase inhibitors with superior selectivity profiles.[19,26]

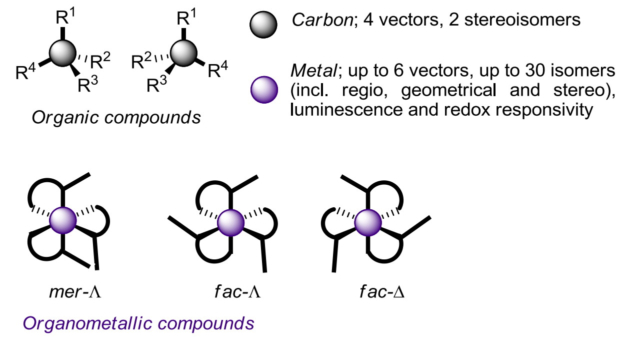

Figure 2 Comparison of organic and metal complex scaffolds illustrating advantages of metal complex based systems for protein surface recognition.

Early Approaches for Protein Surface Recognition

Several traditional supramolcular scaffolds have been developed for binding to protein surfaces. These include calixarenes, porphyrins, anthracenes, cyclodextrins, resorcinarenes and dendrimers.[13]

Calixarenes

Calixarenes are cone-like molecules with two distinct edges that can be functionalised with recognition elements for protein surface recognition.[27] Their biological use has been recently reviewed by Nimse and Kim.[27] The Hamilton group introduced the concept of protein-surface mimetics recognizing the potential of calix[4]arene derivatives for this purpose (Fig. 3a).[28] A series of derivatives were identified that bind to cytochrome (Cyt) *c*, α-chymotrypsin and platelet-derived growth factor (PDGF), acting as antibody mimics.[14,28–30] Most impressively, GFB-111, a PDGF binder with IC$_{50}$ ~ 250 nM was shown to inhibit tumour growth and angiogenesis *in vivo*.[30] More recently, Crowley and co-workers, have highlighted an active role for these scaffolds,[31] solving crystal structures of a *p*-sulfonatocalix[4]arene bound to Cyt *c* (Fig. 3b)[32] and lysozyme.[33] In the former, binding occurred at three different sites, with the calixarenes acting as mediators of the PPIs required for crystallisation.[31]

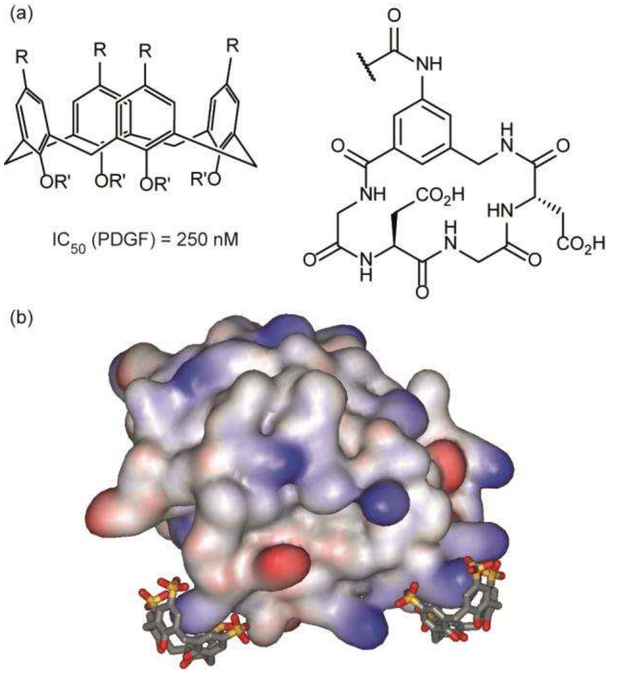

Figure 3 Use of Calixarenes for protein surface recognition (a) structure of GFB-111 a PDGF inhibitor (b) X-ray structure of *p*-sulfonatocalix[4]arene bound to cytochrome *c* (PDB ID: 3TYI)[32]

Calix[4]arene derivatives, have also been used to bind to and inhibit the acyl transfer enzyme transglutamase (with up to 62 % reduction in activity), by blocking the entrance of the substrate into the active site.[34] Finally, calixarenes along with readily-available dyes have been used to create indicator displacement assays/sensors for antibody free detection of histone modifications through lysine side chain recognition.[35,36]

Porphyrins

Since 1950, porphyrins have been used for protein-surface recognition, initially focussed on binding to human serum albumin,[37–39] but since then, many other targets including K_v potassium channels,[40–44] VEGF,[45] Cyt *c*[46,47] and lectins[48–50] have been studied. Hamilton and co-workers recognised the potential of functionalized porphyrin ligands as *bona fide* receptors for protein-surface recognition, developing potent ligands for Cyt *c*.[46,51] These studies are discussed in greater detail later in this article.

Trauner and coworkers rationally designed a tetraphenylporphyrin-based scaffold (Fig. 4a) which targets the K_v potassium channel with nanomolar affinity, and reduces the current through the channel.[41] The C_4 symmetry of the porphyrin was thought to be well-suited to the tetrameric nature of the potassium channel.[41] However it has since been shown, by solid state NMR, that the porphyrin lies perpendicular to the protein, projecting one of its cationic side chains into the channel.[40,52] The porphyrin blocks the ion conduction pathway and stabilises a closed K_v channel state upon interaction with the voltage sensor domain.[44] Further studies have been directed towards inhibiting specific K_v1 channels.[53]

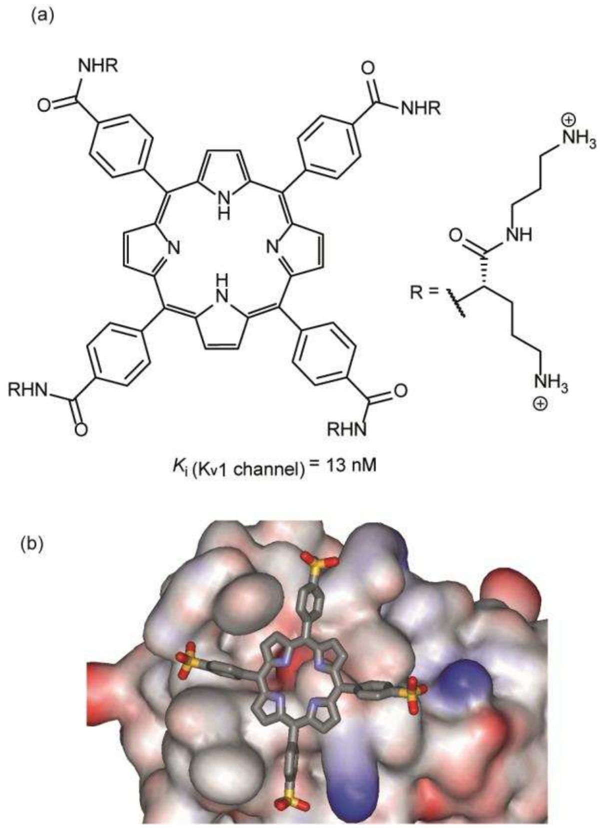

Figure 4 Porphyrins for protein surface recognition (a) structure of a porphyrin ligand for K_v potassium channels (X-ray structure of tetrasulfonatophenylporphyrin bound to Jacalin (PDB ID: 1PXD)

The Yayon group studied porphyrins that bind to fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF),[45] a protein important in tumour angiogenesis and metastasis,[54] with low micromolar affinity *in vitro*, *in cellulo*, and *in vivo* (mouse). They also showed these porphyrins were selective inhibitors of the VEGF/VEGFR PPI over the EGF/EGFR PPI.[45] Finally, the binding of porphyrins to lectins has been extensively studied[48–50,55–58] with crystal structures solved for H_2TPPS binding to Jacalin (Fig. 4b),[57] peanut lectin (PNA),[55] and concanavalin A.[59]

Resorcinarenes

Uchiyama and coworkers developed resorcinarene scaffolds for histone recognition.[60–63] They first developed compounds with 8 (monomeric) and 28 (tetrameric) (Fig. 5) peripheral carboxylates intended to match the basic surface of the histone.[60] This was followed by a more extended scaffold with 84 carboxylates.[61] These receptors were shown to be agglutinated by histone in a turbidity assay, and were shown to bind with K_a 4.2 × 10^5 M^{-1}, 1.3 × 10^7 M^{-1}, and 8.4 × 10^7 M^{-1} respectively by a kinetic analysis from a surface plasmon resonance (SPR) assay. Moreover the receptors were selective for histone over lysozyme and ovalbumin.[61] Subsequent studies adapted the system to (i) permit fluorescence based detection of binding,[62] (ii) establish binding to be electrostatically driven and (iii) exploit a mechanically interlocked rotaxane architecture for binding and FRET based histone detection.[63] In related studies, dipepetide substituted resorc[4]arenes have been exploited for binding to human serum albumin (HSA) and α-chymotrypsin.[64]

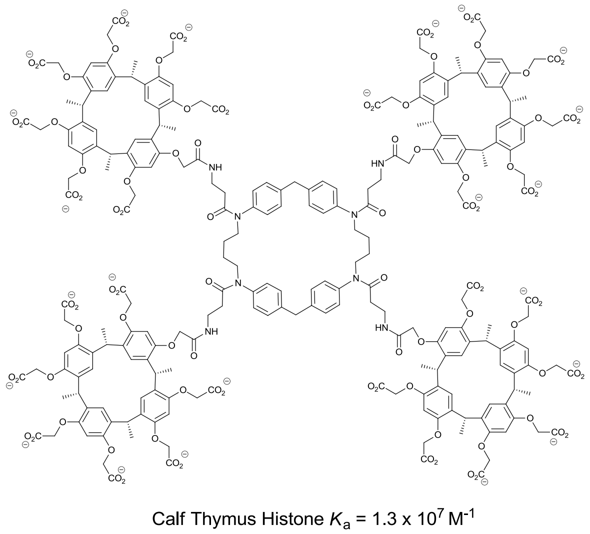

Figure 5 Structure of a resorcinarene based receptor for histones.

Other scaffolds

The Hamilton group also investigated anthracene scaffolds as protein surface mimetics which bind to Cyt *c* and lysozyme.[65] Similarly, bivalent cyclodextrins have been synthesised by Breslow and co-workers, to inhibit aggregation of citrate synthase and L-lactate dehydrogenase, by binding to (and thus preventing the aggregation of) surface exposed hydrophobic patches.[66] In related studies, Kano and Ishida developed a polyanionic β-cyclodextrin capable of binding to Cyt *c*.[67] This concept was further developed by formation of a ternary complex with a porphyrin spanning two Cyt c binding cyclodextrins.[67]

Dendrimers

Dendrimers are supramolecular scaffolds with high valency (Fig. 6), possessing a central core that projects a branching network of repeating units culminating in terminal functionality which can be used for binding to proteins.[68] Protein recognition using dendrimers has recently been comprehensively reviewed by Marjorale *et al.*;[68] a few representative examples are highlighted here. Twyman and coworkers designed polyanionic poly(amidoamine) (PAMAM) dendrimers which bind to Cyt *c* and α-chymotrypsin.[69,70] PAMAM dendrimers have also been shown to bind to human serum albumin in an extensive study by the Giri group.[71] They studied binding constants, NMR (^{1}H, STD and DOSY) and molecular dynamic (MD) simulations of 19 PAMAM dendrimers in order to gain insight into the interactions, looking at differences in core, dendrimer generation and terminal group permitting detailed analyses of the key determinants of protein recognition.

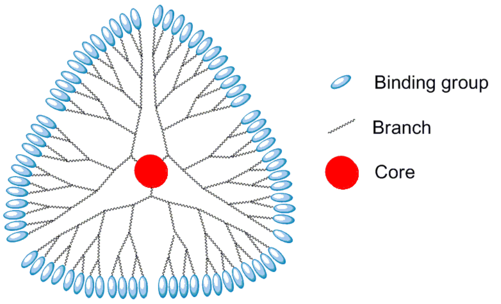

Figure 6 Schematic of a dendrimer.

Metal-based scaffolds

Metal coordination to peptides and proteins

Metal-ligand interactions are stronger (in water) than the conventionally used protein recognition interactions such as hydrogen-bonding, electrostatics and van der Waals contacts.[72] This makes metal-ligand interactions a potentially useful tool for recognition of proteins, as fewer interactions might be needed for selective and high affinity binding. The scope of this approach is limited to those amino acids and post-translational modifications, which are able to coordinate to a metal centre. Naturally, one such coordinative interaction is already widely exploited in the purification of proteins in the form of nickel or cobalt affinity chromatography which exploit the chelating ability of oligohistidine sequences.[73–75] Mallik and coworkers used this knowledge in an intelligent manner. They used molecules with copper(II)-iminodiacetate (IDA) arms (known histidine binding ligands)[76] to recognise patterns of surface-exposed histidine residues, resulting in recognition of bovine erythrocyte carbonic anhydrase (Fig. 7a).[77,78] A three Cu(II) system was used to bind three histidine side chains (Fig. 7a) on the *N*-terminus of the carbonic anhydrase, with the ligand alone showing no binding, highlighting the importance of the metal centre for recognition. The highest affinity compound (3 µM K_d by isothermal titration calorimetry (ITC)) was also found to be selective for carbonic anhydrase over chicken egg albumin which has the same number of surface histidine residues (six) but positioned in a spatially distinct manner on the protein surface.

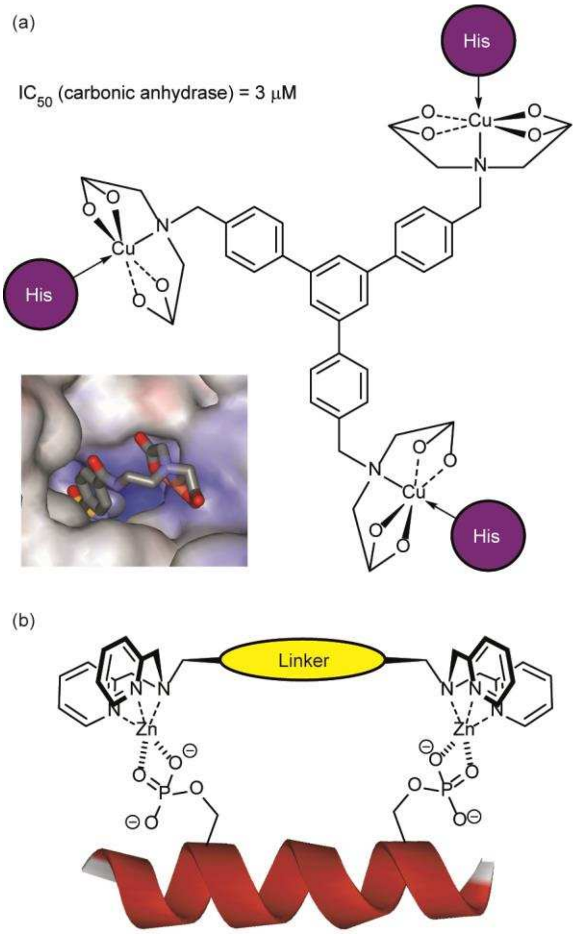

Figure 7 Scaffolds for metal coordination to peptides and proteins, (a) Receptor for carbonic anhydrase that functions through recognition of histidine residues on the protein surface (inset: example of a carbonic anhydrase ligand making use of a Cu(IDA) motif to achieve histidine co-ordination, (PDB ID: 2FOV)[76]; (b) Bis(Zn(II)dpa) receptor for recognition of phosphorylated peptides.

Similarly, the Hamachi and Kasagi groups used bis(Zn(II)-dipicolylamine) (Dpa) derivatives to bind histidine residues on the surface of α-helical peptides, thus stabilising the α-helical conformation.[79,80] This lead to the use of the bis(Zn(II)Dpa) complexes in the binding of both mono- and multi-phosphorylated peptides *via* bidentate binding between the Zn(II) and the phosphate groups (Fig. 7b), resulting in conformational stabilisation.[81,82]

Recognition of phosphate groups on protein surfaces is significant given the role of protein phosphorylation in regulating signaling pathways. The bis(Zn(II)Dpa) receptors have been used as chemosensors by varying the bridging group between the two Zn(II) centres resulting in fluorescence changes on binding.[81–84] With doubly phosphorylated model α-helical peptides it was shown, by circular dichroism (CD), that with appropriately spaced Zn(II) centres, the α-helical content of the peptide increases, and that that there is 10-fold selectivity for doubly phosphorylated peptides over mono-phosphorylated peptides.[82,85] This approach has subsequently been used to develop an inhibitor (IC$_{50}$ = 5.6 μM) of the phosphoprotein-protein interaction between the phosphorylated CTD peptide and the Pin1 WW domain.[86] A more rigid diazastilbene linker was subsequently used in a receptor for doubly phosphorylated peptides.[87] More recently complexes based on these scaffolds have been linked to a bis[(4,6-diflurophenyl)pyridanto-*N*,C$^{2'}$] iridium(III) picolinate motif to generate a phosphorescent sensor for phosphorylated peptides with markedly improved selectivity over ATP.[88]

Building on Hamachi's work, Gunning and coworkers used bis(Cu(II)Dpa) and bis(Zn(II)Dpa) complexes to bind to phosphotyrosine on signal transduction and activator of transcription 3 (STAT3), thus inhibiting STAT3/STAT3 dimerisation.[89,90] ITC and fluorescence polarisation (FP) data demonstrated the copper(II) complexes bound to a phosphopeptide (with micromolar K$_d$), thus inhibiting the phosphopeptide-protein complex, with micromolar K$_i$.[89] The copper(II) complexes were further shown to inhibit STAT3/STAT3:DNA binding in an electrophoretic mobility shift assay (EMSA) with IC$_{50}$ = 8.2 μM. They also exhibited low micromolar IC$_{50}$s in 3 different cancer cell lines but much lower inhibition, and low cytotoxicity, in healthy NIH3T3 cells, thus highlighting their potential therapeutic utility.[89] Later, the same group illustrated the use of bis(Zn(II)Dpa) complexes as mimics of *src* homology domain 2 (SH2) domains; fluorescence quenching experiments demonstrated binding of these complexes to phosphotyrosine containing peptides, with K$_d$ ~10^{-7} M and some sequence identity discrimination.[90] Several of these compounds were also shown to be cytotoxic in three types of cancer cell.[90]

Co-ordination complexes as ligands for protein surfaces

Several surface mimetics use metals as a core structural unit, while the ligands surrounding the metal are used for protein binding. Using metals in a purely structural capacity, especially in thermodynamically or kinetically inert compounds, allows for their use *in cellulo*, as the metal is unable to non-specifically co-ordinate to biomacromolecules and exert a toxic effect.

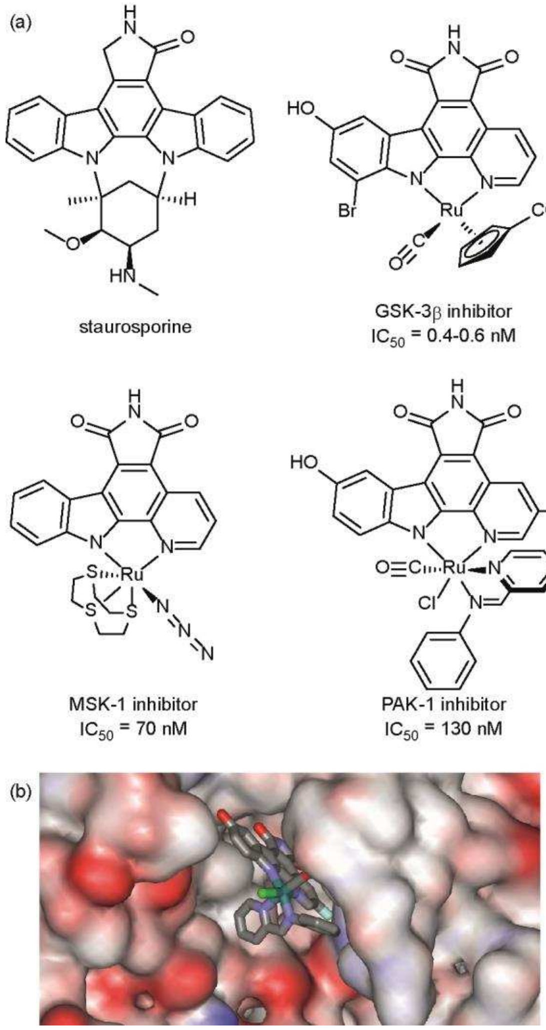

Figure 8 Co-ordination complexes for kinase recognition (a) structures of different inhibitors highlighting similarity to natural product staurosporine – a pan kinase inhibitor (b) X-ray crystal structure of a ruthenium complex bound to PAK1 kinase domain (PDB ID: 3FXZ)[103]

Metal complexes for kinase surface recognition

The use of metal co-ordination complexes as scaffolds has been pioneered by the Meggers group; they have focussed primarily on ruthenium(II) complexes, but more recently on rhodium(III),[91,92] iridium(III),[93,94] osmium(II)[95] and platinum(II)[96] coordination complexes. These have been used for inhibition of multiple protein kinases (Fig 8a) including Pim1,[97,98] glycogen synthase kinase 3β (GSK3β),[99] MSK1,[97] BRAF kinase,[100] and PAK1.[101] X-ray crystal structures have been solved for several of these compounds bound to their target protein kinase, demonstrating the metals act solely in a structural capacity (Fig. 8b).[98,102,103] The majority of these co-ordination complexes function mechanistically as ATP mimics, being based on staurosporine, a widely studied ATP mimic that acts as a pan kinase inhibitor,[104] but non-ATP mimics have been studied more recently,[105] as have inhibitors of other nucleotide binding proteins including the human repair enzyme 7,8-dihydro-8-oxoguanosine triphosphatase,[106] and the lipid kinase PI3K.[107] This approach has been informative in highlighting the utility of metal complexes for projecting recognition groups along vectors to gain additional non-covalent contacts with target proteins in a manner that is not possible using organic molecules.

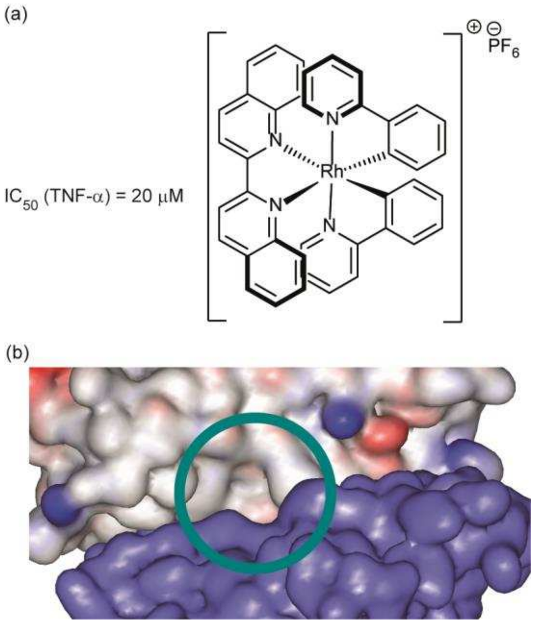

Figure 9 Co-ordination complexes as TNF-α binders (a) structure of Rh(III) complex TNF-α trimerisation inhibitor (a) Dimer structure of TNF-α (PDB ID: 2AZ5) highlighting hypothesized site of small molecule binding.

Group 9 metal complexes as PPI inhibitors

The Leung group have studied a series of iridium(III) and rhodium(III) co-ordination complexes with a view to identification of inhibitors of protein-protein interactions.[108] In an important proof of concept, cyclometalated iridium(III) complexes were shown to be capable of binding to tumour necrosis factor-α (TNF-α) (Fig. 9).[109] The authors postulated that the complex utilises the aromatic bidentate ligands 2-phenylpyridinato (ppy) and 2,2'-biquinoline (biq), in order to target a hydrophobic binding site of the TNF-α dimer (Fig. 9b), preventing active trimer formation. Both enantiomers of the complex were found, by ELISA, to have an IC_{50} in the region of 20 μM, comparable to that of SPD304,[110] one of the strongest inhibitors of TNF-α. Structure activity relationships have since been performed, using 22 iridium(III) complexes with ligands of different shapes and sizes in order to generate low micromolar inhibitors (seen in an *in cellulo* inhibition of TNF-α induced NF-κB luciferase assay in HEP G2 cells).[111] They also looked at the effect of stereochemistry, comparing the Δ and Λ isomers, showing that the Λ isomers had increased cellular activity (3.4 μM versus 9.9 μM IC_{50} in the cellular assay) and binding affinity (30 versus 57 μM IC_{50} in an *in vitro* assay).[111]

In a subsequent study the group synthesised iridium(III) and rhodium(III) compounds capable of binding to, and preventing dimerization and phosphorylation of the STAT3 (Fig. 10a).[112] The most potent Rh(III) compound was found to have anti-tumour activity in a mouse xenograft tumour model and was found to bind to the SH2 domain of STAT3 with an IC_{50} of 4.8 μM. STAT3 pull-down assays demonstrated inhibition of STAT3 dimerisation whilst Western blotting confirmed inhibition of STAT3 phosphorylation. The group have also screened a series of iridium complexes as inhibitors of the p53/*h*DM2 interaction (Fig. 10b).[113] One compound was shown to be a 16 μM inhibitor in a fluorescence anisotropy competition assay. Subsequent cellular analysis confirmed the induction of p21 (a downstream target of p53) and apoptosis.

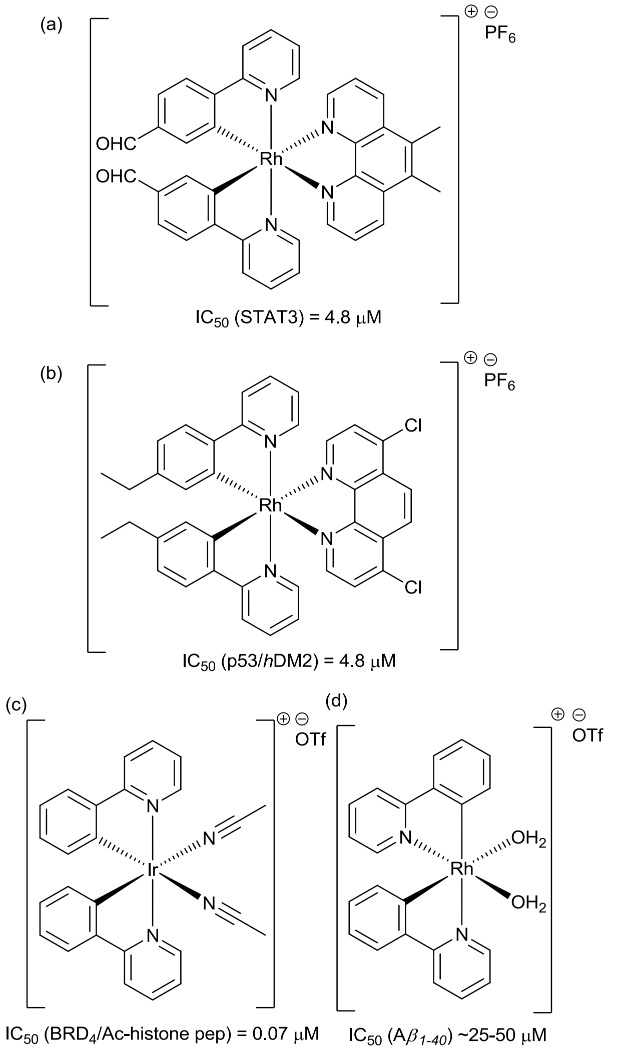

Figure 10 Leung's Ir(III) and Rh(III) protein binders for (a) STAT3, (b) p53/*h*DM2, (c) BRD4 (d) $A\beta_{1\text{-}40}$

The group have extended this strategy which is based only on molecular recognition between protein and metal complex to develop irreversible Ir(III) and Rh(III) inhibitors which also exploit co-ordinative interaction between the two. An Ir(III) based irreversible inhibitor of the interaction between bromodomain-containing protein 4 (BRD4) and acetylated histone peptide (Fig. 10c) has been developed.[114] The group initially screened 27 compounds and found a compound capable of modulating the interaction between BRD4 and chromatin *in vitro* and *in vivo.* The compound was found to bind to histidine residues, with the loss of acetonitrile ligands, and was found to be selective over the other histidine containing proteins STAT3 and caspase-6. The group have also developed Ir(III) and Rh(III) complexes that inhibit the aggregation of $A\beta_{1-40}$,[115] a peptide implicated in neurodegeneration in Alzheimer's disease (Fig. 10d). The authors proposed the compounds bind to histidine residues on the peptide, displacing the water ligands, and allowing further interactions of the hydrophobic ligands with hydrophobic residues at the *N*-terminus of the peptide. The compounds can also serve as luminescent probes for $A\beta_{1-40}$.

The use of metals to modify the properties of surface mimetics

A number of conventional supramolecular scaffolds can be easily modified through the addition of a metal. Such compounds offer the advantage of fluorescence or phosphorescence, which may be exploited to detect binding without the need for peripheral functionalization as required for conventional small molecules. The metal may also modify the binding behaviour e.g. by providing an additional coordination site where one or more ligands on the surface mimetic are labile or by modulating the electrostatic surface proximal to the site of coordination. The following section outlines where this has been explored for porphyrin-derived protein surface mimetics.

Metalloporphyrins

Considerable effort in the 1980s and 90s was devoted to the study of electron transfer between both metallo and non-metallo anionic porphyrins and Cyt *c*.[116–123] Jameson *et al.* compared two types of these porphyrins: uroporphyrins (URO) and tetracarboxyporphyrin (4CP).[121] 4CP was shown to have higher quenching rates, possibly due to a difference in Cyt c binding orientation for the two porphyrins, as evidenced by differences in the induced CD of the porphyrins on binding to Cyt c. The Rodgers group also used cationic metalloporphyrins as extrinsic probes to study peptide aggregation by analysing photoinduced electron transfer from tyrosine or tryptophan residues in the protein to the metalloporphyrin.[124,125]

Following Fisher's initial observation that tetra carboxyphenylporphyrin bound to Cyt *c*, selectively over acetylated Cyt *c*, with K_d in the region 0.05 μM – 5 μM using a flavodoxin competition assay,[116] the Hamilton group developed higher affinity Cyt *c* ligands (Fig 11).[46,47] Tetraphenyl porphyrin scaffolds provide a large, flat, semi-rigid molecular surface of ~300 – 400 Å^2 which with anionic substituents on the periphery bind to Cyt *c* in a 1:1 stoichiometry.[46,47] The compounds were found to be selective for Cyt *c* over the related proteins Cyt c_{551} (a protein with a similar function, shape and secondary structure to Cyt *c* but lacking surface lysines) and ferredoxin.[47] Crowley and coworkers later analysed sulfonato-porphyrins binding to Cyt *c* by ^{1}H, ^{15}N HSQC NMR, with the results backed up by docking studies.[126] Theses analyses pointed to a dynamic ensemble of energetically similar interactions with the porphyrin occupying several different patches on the surface.[126]

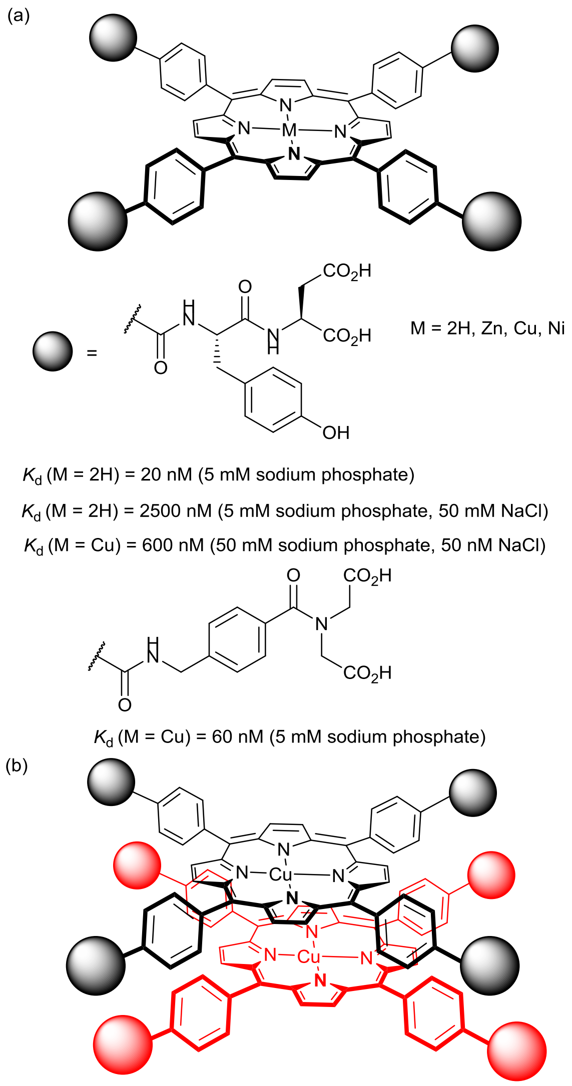

Figure 11 Hamilton's copper(II) porphyrins. a) The two best Cyt c binders and denaturants, b) Schematic of how the porphyrins dimerise

One observation made during these studies was that suitably functionalized proteins lowered the melting temperature of Cyt *c*,[127] by up to 50 °C.[47] The porphyrins did not cause lowered melting temperature for acetylated Cyt *c* or Cyt c_{551}, showing charge complementarity to be key to the "denaturing" effect. It was hypothesized that the effect arose due to the porphyrin binding preferentially to the unfolded state of the protein. Critically, metal ions were subsequently shown to dramatically control the binding behaviour of the porphyrin towards Cyt *c*, in particular copper(II) porphyrins (Fig. 11b).[128,129] Metalloporphyrins tend to dimerise/ aggregate more readily in water when compared to their free base analogues (Fig. 11b) due to enhanced π-π stacking.[130,131] The exception are the zinc(II) variants which prefer to adopt a five co-ordinate geometry with an axial water molecule, thus retarding dimer formation. Consequently, the copper(II) derivative of the originally identified Cyt *c* receptor was shown to have higher affinity for Cyt *c* with accurate K_d values not being able to be obtained without increasing the ionic strength. The copper(II) derivatives bind in a 2:1 stoichiometry porphyrin:protein. The copper(II) porphyrins were shown to denature Cyt *c* at room temperature and do so selectively over α-lactalbumin, Bcl-x_L, Cyt c_{551}, myoglobin and RNAse A. This ability to bind preferentially to the unfolded state of the protein resulted in an acceleration of the rate of tryptic proteolysis. This was first shown to occur in the presence of stoichiometric quantities of porphyrin and then catalytically (0.1 equivalents). In contrast, the free base and zinc(II) pophyrins did not do this presumably arising due to the dimeric nature of the copper(II) variant and hence higher charge density. Subsequently, copper(II) porphyrins were shown to denature both myoglobin and haemoglobin, seen by a decrease in melting temperature, increased trypsin digestion and decrease in the α-helical content by CD.[132]

The Hamilton group subsequently employed families of functionalized porphyrins in a protein sensing array for protein detection,[133,134] whilst zinc(II) and iron(III) metalloporphyrins have also been shown to multimerise Cyt c7 from *Geobacter sulfureducens*, lysozyme and Cyt *c* at high (millimolar) porphyrin and protein concentrations,[135] as observed by SAXS and rationalised by molecular dynamics (MD) simulations.

Metallodendrimers

Zinc(II) porphyrin-based dendrimers have also been developed, with the fluorescent metalloporphyrin-core being utilised for detection/ sensing.[136] These large multivalent nanoscale structures have been used to bind to Cyt *c*, with the Cyt *c*/dendrimer complex being more stable than the native Cyt *c*/Cyt b_5 PPI as demonstrated by 20 % fluorescence recovery (of the dendrimer) on addition of 14 equivalents of Cyt b_5 to the Cyt *c*/dendrimer complex. One of these original Zn(II)-porphyrin dendrimers, and subsequent generations, were subsequently shown to improve cell viability when cells were subjected to an apoptotic stimulus.[137] It has been hypothesised that the dendrimers trap Cyt *c*, preventing it from interacting with Apaf1 to form the apoptosome, thus inhibiting apoptosis.

M(bpy)$_3$ scaffolds for multipoint surface recognition

In the 1950s and 60s Dwyer and coworkers showed that simple bipyridine (bpy) and phenanthroline (phen) ruthenium(II) complexes ellicit bacteriostatic and bacteriocidal activities and also inhibit tumour growth, thus highlighting the potential use of these complexes.[138,139] In an early designed approach Sasaki *et al.* described a saccharide substituted Fe(II)(bpy)$_3$ complex capable of binding to lectins,[140] thus introducing the idea of using metal *tris*-bipyridines to project recognition domains over a protein surface to make multivalent non-covalent contacts and achieve binding.[140] Fe(II)(bpy)$_3$ complexes are relatively dynamic in aqueous solution, this allows for the use of dynamic combinatorial chemistry around the Fe(II) core. This has been used by the Sasaki and de Mendoza groups in order to generate lectin binding complexes.[141,142] Sasaki and coworkers generated an Fe(II) complex with a mono GalNAc substituted bipyridine, which altered its stereochemical configuration in solution resulting in the enrichment of higher affinity compounds for various different lectins.[141] De Mendoza and co-workers used bipyridines functionalised with 3 different sugars complexed them to Fe(II) then incubated them with the mannose binding lectin, Concanavalin A (ConA), this resulted in enrichment of the mannose functionalised complex (detected by LCMS), as predicted.[142]

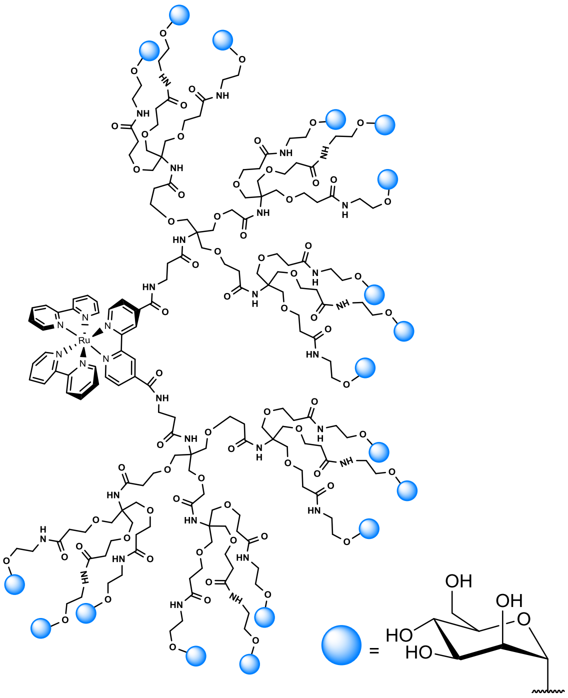

Figure 12 Seeberger's mannose functionalised Ru(bpy)$_3$s for ConA/GNA binding

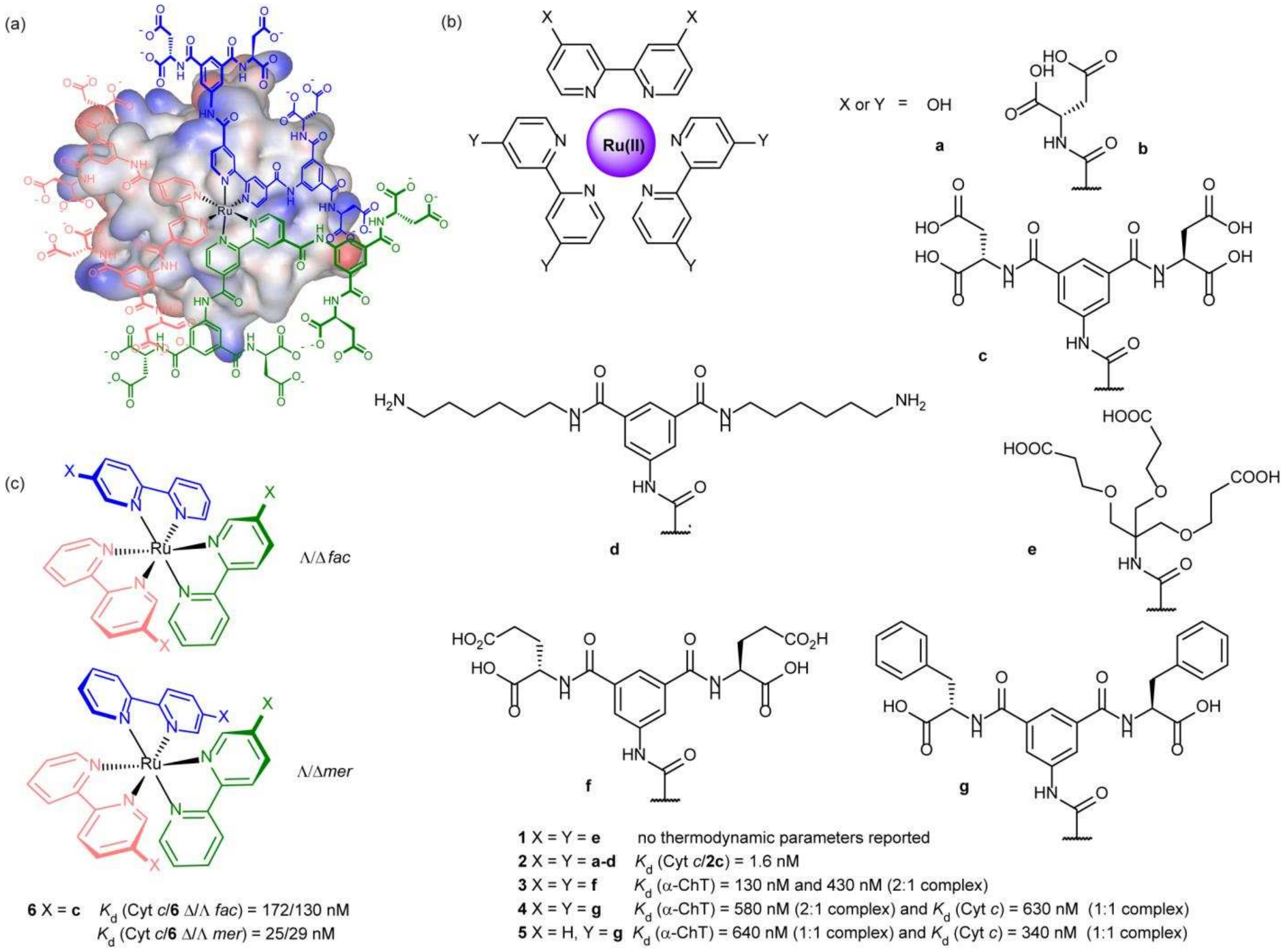

Figure 13 Ru(bpy)$_3$ surface mimetics for protein recognition (a) schematic depicting proposed mode of recognition between Ru(bpy)$_3$ surface mimetics e.g. **3c** and Cyt *c* (b) 4,4' bipyridine ruthenium complexes used by the Hamachi, Ohkanda and Wilson groups, (c) The mono 5' substituted bipyridine complexes analysed by the Wilson group.

While the labile nature of the Fe(II) core can be useful for the generation of high affinity protein receptors, the inert nature of the ruthenium(II) core is attractive as decomplexation will not occur in biological media in dilute solution.[143] Moreover, the ruthenium(II) core permits detection of binding events through the metal to ligand charge transfer (MLCT) luminescence.

Kaboyashi and coworkers,[143,144] generated a series of glycofunctionalised Fe(bpy)$_3$ and Ru(bpy)$_3$ compounds, showing that the ruthenium glycoclusters had high lectin affinity and increased luminescence on lectin binding. Similarly, the Seeberger group developed sugar functionalised Ru(bpy)$_3$ complexes (Fig. 12) that bind to the mannose-binding lectins ConA and *galanthus nivilis agglutinin* (GNA).[145] A follow-on study used digital logic analysis to determine the best lectin binders for further study: this was achieved by assessing the increase in luminescence output of the Ru(II)glycodendrimers in the presence of different lectins.[146] The complexes with surface bound lectins have also been used as luminescent sensors for measuring monosaccharide and oligosaccharide concentrations, by using the displacement of the Ru(II)glycodendrimers from a lectin surface by the sugar.[147] In a different approach the same group used related scaffolds functionalised with adamantane units, to recruit mannose functionalised β-cyclodextrin in a "supramolecular click" strategy to achieve high affinity binding of ConA K_d = 0.14 μM as determined by SPR.[148] Finally, the Okada group also used galactose functionalised Ru(bpy)$_3$ complexes to bind to *peanut agglutinin* (PNA) and glucose functionalised Ru(bpy)$_3$ complexes to bind to ConA (K_d = 18 μM), using fluorescence emission and fluorescence polarisation.[149]

Electron transfer experiments between Cyt *c* and Ru(bpy)$_3$ complexes (as well as Ru(phen)$_3$, Os(bpy)$_3$ and Os(phen)$_3$ complexes) were initially reported by Cho in the 1980s.[150] Subsequently Hamachi developed carboxylate functionalised Ru(bpy)$_3$ derivatives **1** (Fig. 13b) that could bind to and photoreduce Cyt c, selectively over a series of less basic proteins (myoglobin, horseradish peroxidase and Cyt *b$_{562}$*).[151] The compounds were observed to bind to Cyt *c* using an ultrafiltration binding assay with the compound with the highest number of carboxylic acids (18 COOH) being shown to bind an order of magnitude more tightly than an unfunctionalised Ru(bpy)$_3$ complex. The Ru(bpy)$_3$

complexes were capable of photoreducing Cyt *c* with the most effective being a heteroleptic complex.[151]

Subsequent to Hamachi's initial observations, both the Ohkanda and Wilson groups further established selective binding of Ru(bpy)$_3$ complexes to Cyt *c* and α-chymotrypsin (α-ChT) (Fig. 13b). The Wilson group developed both mono- (5′) **6** (Fig. 13b) and di- (4,4′) **2** (Fig. 13a) substituted Ru(bpy)$_3$ complexes, which were shown to bind Cyt *c*.[152,153] Using a fluorescence quenching assay, the highest affinity complex **2c** was shown to bind to Cyt *c* with K_d = 1.6 nM (5 mM sodium phosphate, pH 7.4).[152] As with Hamilton's porphyrins,[46] negatively charged substituents (based on aspartic acid moieties) were shown to promote high affinity binding in fluorescence quenching assays.[152] Notably, negative cooperativity was observed with increasing numbers of carboxylates[152] (i.e. as the overall affinity increases, the affinity per carboxylate decreases) presumably reflecting the fact that the roughly spherical shape of the ruthenium complex would prevent all carboxylates from simultaneously engaging the protein surface.

In subsequent studies focused on the role of geometrical and stereochemcial isomers, the *mer* isomers of the 5′-monosubstituted complexes **6** showed ~10 fold better binding affinity compared to the *fac* isomers e.g. 25 (Δ–*mer) versus* 172 (Δ-*fac*) nM for Cyt *c* (5 mM sodium phosphate, pH 7.4). In contrast, the Δ and Λ isomers bound Cyt c with little difference in their affinities (25 vs 29 nM for the *mer* isomers).[153] Further analysis using a functional ascorbate reduction assay demonstrated that both the (4,4′) disubstituted and 5′ monosubstituted bipyridine complexes slow the rate of reduction of Cyt *c*, probably as a consequence of blocking the approach of the reducing agent to the solvent exposed haem group on the surface of Cyt *c*, which is surrounded by basic amino acid residues.[152] The absence of binding to 60 % *acetylated* Cyt *c* confirmed this charge complementarity to be key for binding.[152,153] Further analyses of the complex **2c**, in a manner similar to Hamilton's porphyrins,[128] revealed it lowered the melting temperature of Cyt *c* by 25 °C and show an increased rate of proteolytic degradation at room temperature in both stoichiometric and substoichiometric quantities of the complex.[154] A change in the binding with a change from a 1:1 binding to a 2:1 (protein:complex) stoichiometry was observed on increasing the temperature from 25 to 70 °C. This result in particular adds to the original conceptual observation from the Hamilton group,[128] in that it implies negative co-operative binding to the unfolded form of Cyt *c* is favoured.

In cellulo studies have also been performed with these complexes (Fig. 14). Meaningful analyses on the 5′ monosubstituted derivatives **6** was limited by their lower quantum yield, however the 4,4′ complexes **2** exhibited 95% efficiency of transfection into HEK-293T cells at 10 μM concentration.[155] The complexes appeared to be taken into cells by endocytosis and were shown to localise to the lysosome. In the case of the anionic derivatives, they were also shown to be non-cytotoxic.[155]

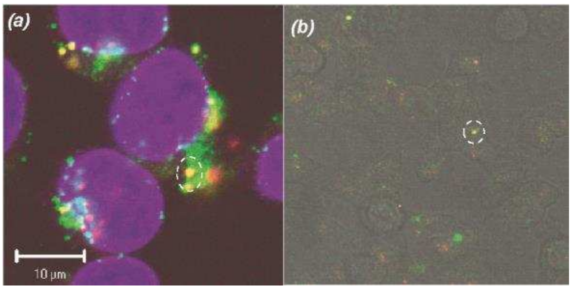

Figure 14 Cell localisation behaviour of compound **2c**; (a) **2c** (emits pink/red), antibody for LAMP1 (emits green) and propidium iodide (denotes nucleus in blue/purple) in fixed cells (b) **2c** and lysotracker in living cells (antibody emits green and denotes lysosomes). Co-localisation is denoted by a dashed white circle

Simultaneously Ohkanda and co-workers developed dendritic Ru(bpy)$_3$ complexes **3-5** (Fig. 13a) that bind to α-chymotrypsin in a mixed 1:1 and 1:2 (complex:α-chymotrypsin) stoichiometry (e.g. **3** K_d = 130 and 430 nM (5 mM phosphate, pH 7.4) for the first and second equilibrium step respectively.[156] These surface mimetics inhibited the enzyme by non-competitive inhibition.[156] They later synthesised homo and heteroleptic complexes **4** and **5** for binding to both α-chymotrypsin and Cyt *c,* with submicromolar afinity.[157] Molecular modelling indicated that three isophthalic arms interact with α-chymotrypsin, and four interact with Cyt *c*.[157] *In cellulo* studies also highlighted a capacity for these compounds to enter cells.[157]

Conclusions

The development of protein surface mimetics has emerged as a novel approach for the inhibition of protein-protein interactions in chemical biology. Within this group of supramolecular receptors, organometallic and coordination complexes offer unique advantages. These unique properties have been demonstrated through the development of protein-surface mimetics that achieve binding through direct coordinative interactions with surface exposed ligands, by exploiting the additional vectoral presentation of functional groups in metal complexes to achieve binding, and, by using a metal complex to project binding groups across a large surface area resulting in multivalent contacts. Despite these successes, many challenges remain, in particular, to refine, using computational modelling as appropriate, the structural diversity and asymmetry of these types of complexes so that their recognition of protein-targets is highly specific and of higher affinity. Beyond this it will be necessary to apply these approaches to the development of selective ligands for a far greater range of protein targets, and finally, to demonstrate more extensively a biological effect *in cellulo* and *in vivo*.

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