Coordination Chemistry Reviews 255 (2011) 459–472

Contents lists available at ScienceDirect

Coordination Chemistry Reviews

journal homepage: www.elsevier.com/locate/ccr

Review

Recent advances in biosensory and medicinal therapeutic applications of zinc(II) and copper(II) coordination complexes

Joel A. Drewry[a,b], Patrick T. Gunning[a,b,*]

[a] Department of Chemistry, University of Toronto Mississauga, 3359 Mississauga Road North, Mississauga, ON, L5L 1C6 Canada
[b] Department of Chemical and Physical Sciences, University of Toronto Mississauga, 3359 Mississauga Road North, Mississauga, ON, L5L 1C6 Canada

Contents

ARTICLE INFO

Article history:
Received 19 July 2010
Accepted 19 October 2010
Available online 28 October 2010

Keywords:
Copper(II) coordination complexes
Zinc(II) coordination complexes
Molecular recognition
Chemosensors
Biosensors
Protein–protein interactions
Molecular therapeutics
Protein denaturation
Phosphopeptide recognition

ABSTRACT

Historically, Lewis acidic metal coordination complexes have played a pivotal and defining role in the broad field of molecular recognition and more specifically in the sensing and sequestration of biologically relevant anionic species. More recently, with the expanding interest in chemical biology, there has been a resurgence in the use of coordination complexes, specifically, through their application as medicinal therapeutics and chemo/biosensors. From the disruption of oncogenic protein–protein interactions to the fluorescent sensing of PTP1B phosphatase enzyme activity, the powerful binding potency of coordination complexes has been harnessed to great effect. The ingenuity of the rationally designed coordinating ligands has facilitated the diversity of roles played by Lewis metal complexes. Herein, we will review the recent advances in the application of coordination complexes in medicinal and chemo/biosensory roles over the last decade. In particular, this review will focus on Cu(II) and Zn(II) coordination complexes.

1. Introduction

For thousands of years, metal complexes have played important and diverse roles in medicine [1]. From the antiseptic properties of copper complexes, to the long-standing application of gold complexes in Chinese and Arabic medicine [2], the unique and

* Corresponding author at: Department of Chemistry, University of Toronto Mississauga, 3359 Mississauga Road North, Mississauga, ON, L5L 1C6 Canada. Tel.: +1 905 828 5354; fax: +1 905 828 5425.
E-mail address: patrick.gunning@utoronto.ca (P.T. Gunning).

0010-8545/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ccr.2010.10.018

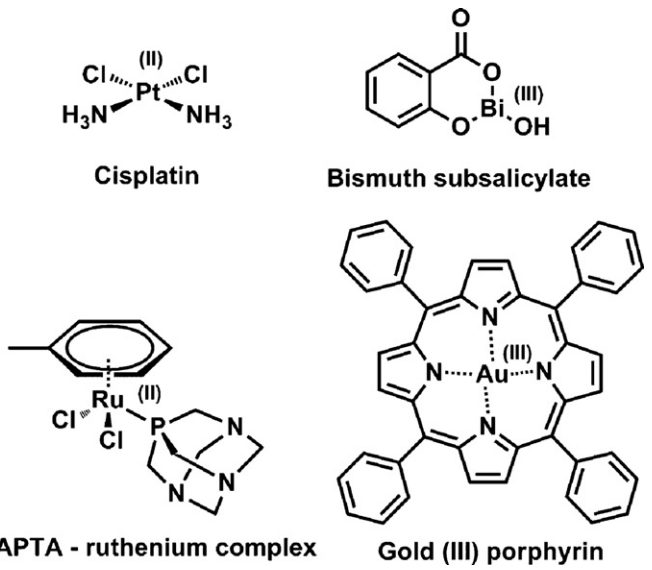


Fig. 1. Chemical structures of (A) cisplatin; (B) bismuth subsalicylate; (C) RAPTA ruthenium(II)–arene complex used to treat tumour metastasis; (D) Ag(III)–porphyrin, used to treat hepatocellular and nasopharyngeal carcinoma.

useful therapeutic benefits of metals have long been recognized and harnessed [3,4]. The therapeutic application of metal complexes in modern medicine was arguably initiated by the discovery of the anticancer properties of cisplatin (Fig. 1A, cis-diaminodichloroplatinum(II)) [5]. In addition to the continued clinical use of cisplatin against specific types of cancer, this discovery also inspired a new generation of effective, and in some cases selective [6], metal-based cancer therapeutics, thereby demonstrating the potential of metal complexes as alternatives to classical 'drug-like' small molecule inhibitors of human disease.

Currently, a variety of transition metal coordination complexes are employed in numerous medicinal roles. For example, bismuth complexes (Fig. 1B) have emerged as potent metalloenzyme inhibitors, anti-diarrheals (bismuth subsalicylate) [7] and anti-bacterials (Noviform) [8–12]. In addition, the promising therapeutic properties of gold complexes as antibiotic, antiarthritic and anti-cancer agents have been extensively investigated [4,13–16] (Fig. 1C). In a number of studies, ruthenium complexes have been shown to exhibit highly encouraging anti-cancer activity [4,13,17,18] (Fig. 1D). Furthermore, vanadium sulphate is being investigated in diabetes research as a potential alternative to insulin in type II diabetes [19–26]. More recently, metal coordination complexes have been employed in molecular recognition of biological substrates [27], specifically as protein biosensory probes and as inhibitors of protein complexation events and biological function. As a result, metal coordination complexes, with their uniquely powerful binding potential, have become important players in an emerging field of bioinorganic chemical biology.

The development of safe and effective treatments for human disease may be greatly assisted by the identification of probes which directly and accurately sense the cytological effect of agonist- or antagonist-mediated modulation of one or more specific biochemical pathways. To this end, the development of biosensors capable of selectively detecting important biological macromolecules and reporting on parameters such as concentration, conformation or activation state, has been an extremely active area of investigation. Often, the qualities or characteristics of a useful biosensor, including cell permeability, target affinity and target selectivity, are congruent to those of a potential molecular therapeutic. Accordingly, the potential of metal coordination complexes as viable, molecularly targeted drug molecules is increasingly being investigated, either through the repositioning of existing biosensors into a therapeutic role, or, by rationally designing metal complexes to inhibit specific biological targets.

The use of divalent Zn(II) and Cu(II) complexes as biosensors and molecular therapeutics has significantly increased over the past fifteen years. Both Cu(II)and Zn(II)metals are abundant in mammalian cells (*c.a.* 10–100 μM), and thus the risk of cytotoxic effects from exposure to these metals is lower than for other transition metal complexes. Furthermore, both Cu(II) and Zn(II) have been shown to display coordination chemistry ideal for targeting functional groups common to a cytosolic environment, such as imidazoles, sugars, phosphates and carboxylic acids [28–30]. Previous reviews have comprehensively detailed the application of coordination chemistry to the sequestration of important small biomolecules [25,27–29]. This review will focus on the use of Zn(II) and Cu(II) coordination complexes in the molecular recognition of biologically relevant peptides and proteins for biosensory and/or therapeutic medicinal purposes. While Zn(II) and Cu(II) complexes have been widely applied to nucleic acid recognition and phosphatase enzyme mimicry [30–40], such work is beyond the scope of this review.

2. Zn(II) and Cu(II) coordination complexes as protein and peptide biosensors

2.1. Fluorescent Zn(II)-coordination complexes in biosensory applications

Incorporating both organic and inorganic fluorophores into molecular recognition scaffolds has become a standard design feature of biosensory detection systems [41–46]. In the last decade, a plethora of fluorescently labelled Zn(II)coordination complexes have been synthesized and used for selective peptide and protein biosensing. A significant body of this work has been conducted by Hamachi and coworkers.

2.1.1. Fluorescent sensing of (*i*, *i*+*n*) bis-phosphorylated phosphopeptide sequences

Following the seminal pyrophosphate fluorescent-chemosensor studies of Vance and Czarnik [47], Hamachi and coworkers showed that anthracene-labelled Zn(II)–dipicolylamine (DPA) **1** and $Zn(II)_2$–bisdipicolylamine (BDPA) **2** chemosensors, were able to selectively bind polyanionic phosphopeptides relative to non-phosphorylated peptides under physiological conditions (Fig. 2A) [48]. Hamachi *et al.* showed that receptor binding to the phosphorylated peptide enhanced the fluorescence intensity of the anthracene fluorophore (Fig. 2B(A)). This was attributed to a decrease in partial cationic character of the flanking pyridines of the DPA ligand, and a corresponding reduction in photo-induced electron transfer (PET) quenching of the anthracene fluorophore's excited state [48]. Corresponding non-phosphorylated tyrosine containing sequences were shown to confer negligible changes in fluorescence. In a subsequent study, Hamachi and coworkers demonstrated that this approach could be successfully applied to the monitoring of protein tyrosine phosphatase 1B (PTP1B)-catalyzed dephosphorylation events by way of fluorescently sensing the phosphorylation state of consensus EGFR peptide sequences (DADE-pY-LIPNNG (988–998 fragment of EGFR)) known to act as substrates for phosphatases (Fig. 2B(B)) [49].

In addition, fluorescent coordination complexes have been employed to selectively recognize peptide sequences containing proximal phosphotyrosine residues (*i*, *i*+*n*) as compared to mono-phosphorylated sequences. Using a ditopic $Zn(II)_2$–BDPA system, where the linker between the two Zn(II)–DPA units can readily modulated, Hamachi and coworkers were able to

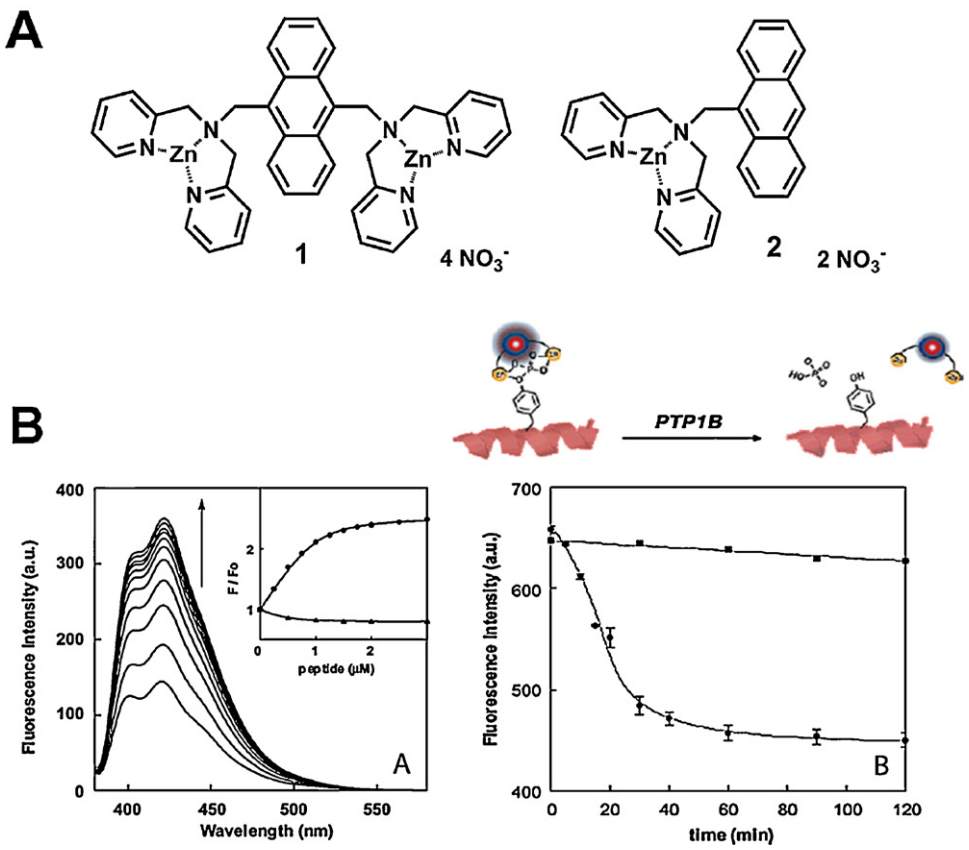


Fig. 2. (A) Chemical structures of anthracene-labelled Zn(II)–DPA (**1**) and $Zn(II)_2$–BDPA (**2**) chemosensors; (B) (A) fluorescence spectral change of **1** (1 μM) upon the incremental addition of phosphopeptide in 50 mM HEPES buffer, pH 7.2, 50 mM, NaCl at 20 °C; (B) time trace of the PTP1B-catalyzed dephosphorylation monitored by the emission at 420 nm of **1** (excited at 370 nm) with (circle) or without (square) PTP1B.
(Reprinted with permission from [48,49]. Copyright American Chemical Society).

cooperatively bind (or 'cross link') two separate phosphorylated residues on a single sequence, and confer greater sequence specificity (conceptual strategy outlined in Fig. 3A) [50]. A bivalent $Zn(II)_2$–BDPA 'cross-linking' strategy has been successfully applied to selectively recognize and bind medicinally important bis-phosphorylated peptide sequences. For example, Hamachi *et al.* developed fluorescent 2,2′-bipyridine linked $Zn(II)_2$–BDPA chemosensors (Fig. 3B) for doubly phosphorylated peptides using three different α-helical model peptides containing two phosphorylated serine residues [50]. Relative to their selectivity for mono-phosphorylated peptides, $Zn(II)_2$–BDPA–5,5′-bipyridine linked receptors (representative example **3**, Fig. 3B) were highly selective for bis-phosphorylated peptide sequences with an inter-phosphate distance of ∼10 Å, approximating the distance between i and $i+7$ side chain residues in α-helices. In contrast, the selectivity amongst peptides phosphorylated in the (i, $i+7$) positions was moderate. Hamachi and coworkers successfully applied their cross linking strategy with lead $Zn(II)_2$–BDPA–5,5′-bipyridine complex **3**, against an insulin bisphosphorylated (i, $i+7$) receptor kinase (IRK) peptide [50]. In support of their model studies, the $Zn(II)_2$–BDPA–5,5′-bipyridine scaffold **3** showed a 1:1 binding ratio with the IRK sequence, as assessed by changes in fluorescence, and was reported to have an excellent binding affinity of $1.7 \times 10^6\ M^{-1}$.

In an analogous study, Hamachi and coworkers developed a sequence-selective fluorescent chemosensor for (i, $i+1$) bis-phosphorylated peptides [51]. In this case, the rigid stilbene-based scaffold **4** (Fig. 3B(ii)) orients two $Zn(II)_2$–DPA binding moieties in a conformation suitable for recognizing adjacent phosphorylated amino acid residues. Importantly, the authors demonstrated that this receptor was able to distinguish bis-phosphorylated peptides on the basis of the relative proximity of the two phosphate groups. Affinity constants, as measured by a dual-emission fluorescence signal change, were highest for the (i, $i+1$) bis-phosphorylated peptide Tau(400–409), containing a pYpS motif, while affinities for the (i, $i+n$) bis-phosphorylated peptide, where $n = 2$–6, decreased with increasing distance between phosphorylation sites. Encouragingly, mono-phosphorylated peptides did not induce a change in the fluorescent spectrum of the receptor [51].

In another example, a rationally designed $Zn(II)_2$–BDPA–based receptor **5** was reported to bind and non-covalently label a bis-phosphorylated peptide in the i and $i+4$ positions [52]. Boron-dipyrromethene(BODIPY)-tagged $Zn(II)_2$–DPA complexes were used to selectively label and detect neurofibrillary tangles (NFTs) of hyperphosphorylated tau proteins, commonly associated with Alzheimer's disease [53]. Compound **5** (shown in Fig. 3B(iii)), composed of two $Zn(II)_2$–DPA units tethered through a BODIPY fluorescent linker, enabled fluorescent visualization of NFTs (hyperphosphorylated) in hippocampus tissue taken from an Alzheimer's disease patient, differentiating between NFTs and prevalent β-amyloid plaques (non-phosphorylated). Specific for the i and $i+4$ bis-phosphorylation sites, this same complex was successfully used to monitor the activity of glycogen synthase kinase 3β(GSK 3β), a protein implicated in diabetes and neurodegeneration [54], for which tau is a natural substrate [52,55–57].

The hijacking of natural, endogenous proteins for biosensory purposes has emerged as an interesting strategy for developing sensors with a high degree of selectivity (conceptual model

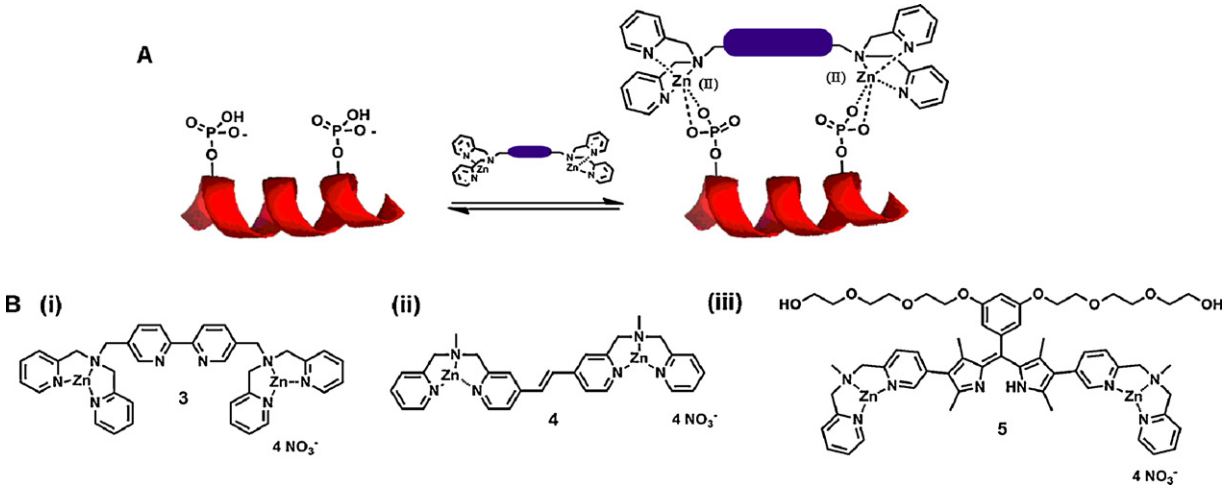


Fig. 3. (A) Schematic representation of bis-phosphopeptide biosensing using fluorescent ditopic receptors; (B) chemical structures of (i) $Zn(II)_2$–BDPA–5,5′-bipyridine (*i*, *i* + 7) receptor **3**; (ii) $Zn(II)_2$–BDPA–stilbene (*i*, *i* + 1) receptor **4**; (iii) boron-dipyrro-methene (BODIPY)-tagged $Zn(II)_2$–BDPA **5**.

illustrated in Fig. 4A). To this end, Hamachi et al. developed receptor **6**, composed of a Zn(II)–DPA complex coupled to a reactive maleimide group through a rigid stilbazole linker (Fig. 4B). In a proof-of-concept study, **6** was covalently incorporated (through the thiol group of a cysteine residue) into a Pin1 WW phosphoprotein binding domain, thereby furnishing a fluorescent hybrid biosensor capable of distinguishing between several biologically relevant phosphopeptides (Fig. 5) [58]. Pin1 is a critical regulator of transcriptional events in cancer cells [59]. The hybrid biosensor showed impressive binding ($K_a > 10^6\ M^{-1}$) to pS 6,9-CTD, a bis-phosphorylated peptide containing an internal sequence naturally recognized by the Pin1 WW domain. This sensor was used to discriminate effectively between similar bis-phosphorylated peptides while showing negligible binding to mono-phosphorylated peptides. In order to be recognized with high affinity by the hybrid sensor, the peptide had to (1) have a consensus sequence recognized by the Pin1 WW domain, including a phosphoserine, and (2) possess a second, nearby phosphorylated amino acid residue located at a specific distance/geometry relative to the domain-binding region. Thus, selectivity in this case was achieved through the cooperative action of both co-receptors [58].

2.1.2. Application of fluorescent Zn(II)-coordination complexes in novel peptide tag/probe pairs

The selective labelling of proteins in complicated biological systems generally requires both a peptide tag genetically incorporated into the protein, and a complementary molecular probe, usually fluorescent, which is introduced exogenously [60–64]. Although several peptide tag/probe pairs have been successfully developed, such as the Ni(II)/nitrilotriacetic acid (NTA) and FLAG/anti-FLAG systems [62], there is limited availability of pairs capable of real-time protein imaging *in vitro*. With an aim to develop a tag/probe pair orthogonal to existing methods for fluorescent protein imaging, Hamachi and coworkers designed an elegant system which uses multivalent coordination chemistry between a genetically encodable oligo-aspartate sequence (D4 tag), chosen for its numerous carboxylate metal binding groups, and a series of corresponding D4 binding, multinuclear $(Zn(II)_2$–$BDPA)_n$-based coordination complexes (Cy5-fluorescently labelled, example **7**, Fig. 6A) [65]. Synthetically, the authors prepared a series of ditopic $Zn(II)_2$–BDPA complexes linked via a series of amino acid and/or fluorescent linkers (cyanine 5 (Cy5)) and fluorescein isothiocyanate (FITC). Interestingly, the probes

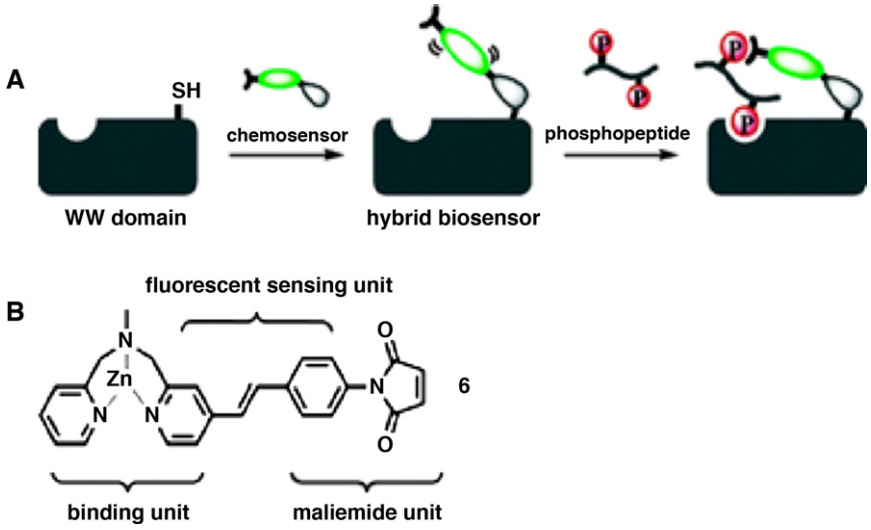


Fig. 4. (A) Schematic representation of WW domain hybrid biosensor assembly and application as a bis-phosphorylated peptide biosensor; (B) chemical structure of chemosensor **6**.
(Reprinted with permission from [58]. Copyright American Chemical Society).

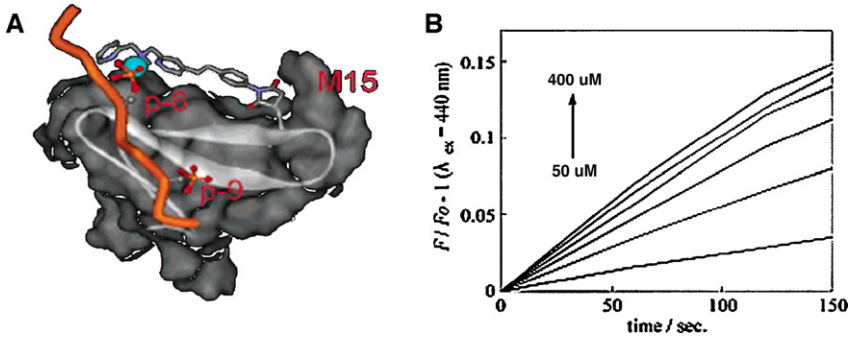


Fig. 5. (A) Computationally predicted structure of the hybrid biosensor bound to bis-phosphorylated p6,9-CTD peptide; (B) real-time fluorescence sensing of CDK9/T1 catalyzed phosphorylation of p9-CTD into p6,9-CTD.
(Reprinted with permission from [58]. Copyright American Chemical Society).

synthesized showed specificity of effect for D4 probes relative to corresponding alanine substituted D4 peptides (DADD and DAAD), indicating that effective recognition required the full D4 sequence. In addition, comparative ITC experiments against D2, D3, D4 and D5 tags with monotopic $Zn(II)_2$–BDPA–FITC showed highly selective binding for D4 ($K_{app} = 10^5\ M^{-1}$) over both D3 ($K_{app} = 10^4\ M^{-1}$) and D2 ($K_{app} = 10^3\ M^{-1}$) tags, highlighting the potential utility and specificity of this method. More impressively, Hamachi et al. showed that this approach is orthogonal to traditional His tag/Ni(II)-NTA protocols, with the tetra-nuclear $Zn(II)_4$–BDPA probe (Fig. 6A(i)) displaying relatively low affinity for the His tag (H-YHHHHHH-NH_2, $K_{app} < 10^3\ M^{-1}$) compared to the target D4 tag ($K_{app} > 10^7\ M^{-1}$). As a proof of concept, using confocal laser scanning microscopy, the authors demonstrated successful fluorescent labelling of triple-D4 ($(D4)_3$) tagged (exoplasmic N-terminus) muscarinic acetylcholine receptors ($(D4)_3$–mAChRs) in CHO cells with an FITC labelled $Zn(II)_4$–BDPA probe. To confirm the targeting of mAChR *in vitro*, Hamachi *et al.* carried out the same experiment using a EGFP-fluorescently labelled $(D4)_3$–mAChR receptor. Subsequent co-localization experiments using confocal laser scanning microscopy showed excellent overlap between the $(D4)_3$–EGFP–mAChR receptor and a Cy5-labelled $Zn(II)_4$–BDPA receptor (Fig. 6B and C) [65].

Later, in a separate study, Hamachi et al. constructed a dual-emission, excited dimer (or excimer)-based detection system for $(D4)_2$-tagged proteins by coupling a fluorescent pyrene tag to a dinuclear $Zn(II)_2$–BDPA complex. Proximal pyrene fluorophores exhibit strong excimer emission at 480 nm and was thus considered suitable for a monomer/excimer dual emission sensing probe. Since Hamachi et al. had previously discovered that $Zn(II)_2$–BDPA scaffolds bound the D4-tag with a 1:1 stoichiometry, the authors reasoned correctly that a $Zn(II)_2$–BDPA–pyrene tagged complex would bind a $(D4)_2$ tag (Ac-DDDDGDDDDGY-NH_2) with 2:1 binding ratio and elicit excimer emission. This unique approach was

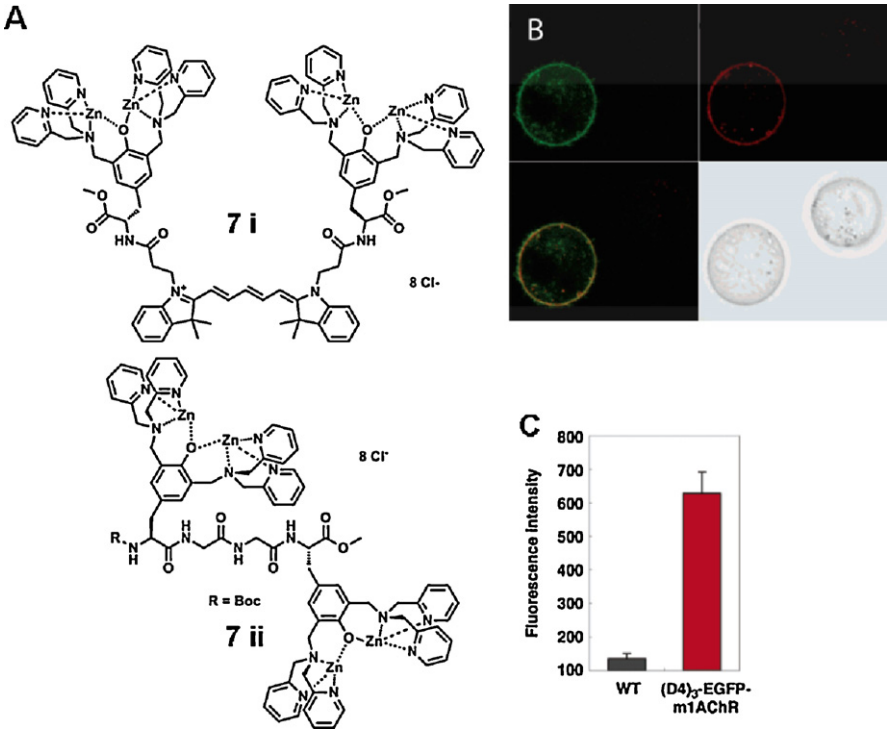


Fig. 6. (A) Chemical structures of (i) Cy5-labelled ditopic **7i** complex; (ii) $(Gly)_2$-linked $Zn(II)_4$–BDPA complex; (B) fluorescent labelling of $(D4)_3$–EGFP–m1AChR with **7**. Cells were labelled simultaneously for EGFP (top left) and Cy5 (top right). The overlay is shown in bottom left. Bottom right is a transmission micrograph; (C) relative fluorescence intensity (Cy5) for wild-type (WT) CHO cells, which do not express m1AChR, as compared to $(D4)_3$–EGFP–m1AChR transfected CHO cells.
(Reprinted with permission from [65]. Copyright American Chemical Society).

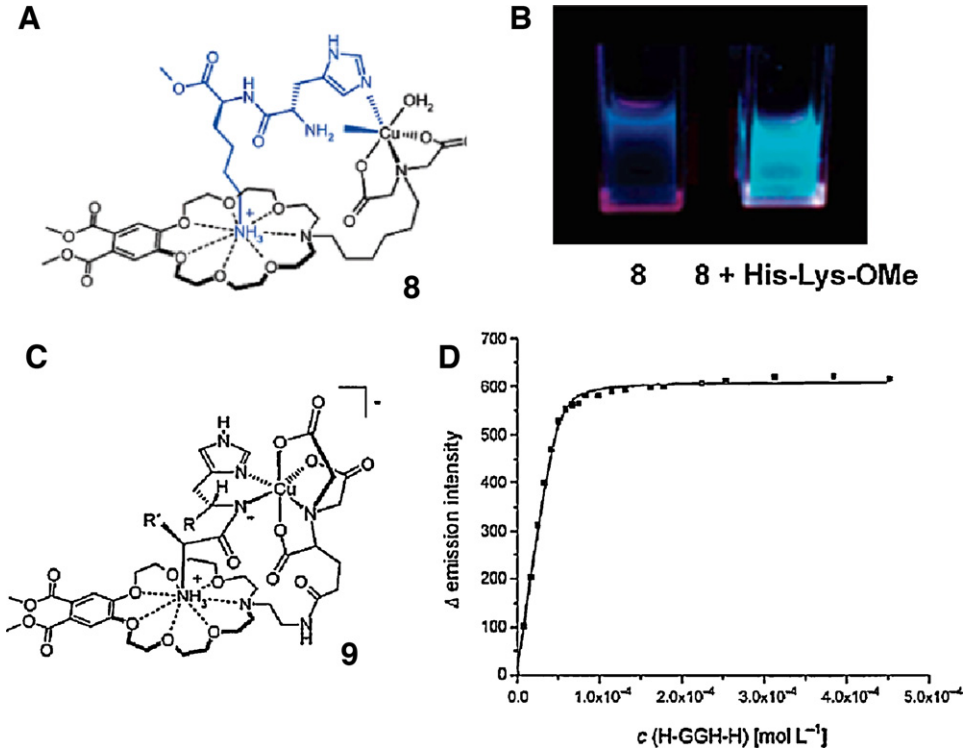


Fig. 7. (A) Proposed mode of binding of Cu(II)IDA-benzocrown ether (**8**) to His-Lys-OMe; (B) emission intensity changes in solutions of **8** in buffered water in the presence of His-Lys-OMe; (C) proposed structure of stable binding mode of peptides to Cu(II)–NTA/benzocrown ether (**9**); (D) change in emission intensity of **9** upon H–GGH–OH binding.
(Reprinted with permission from [68,69]. Copyright American Chemical Society and Wiley–VCH Verlag GmbH & Co. KGaA).

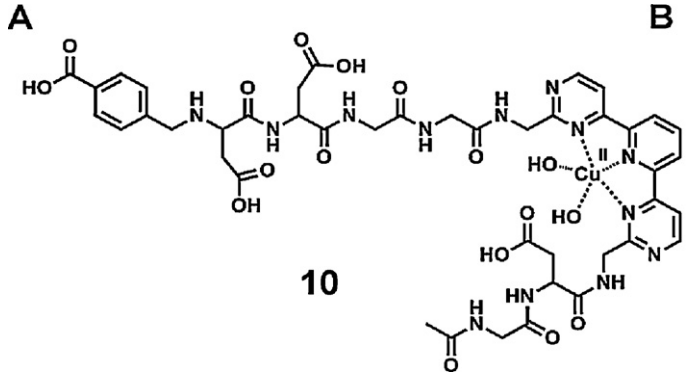
successfully applied in the selective detection of $(D4)_2$-tagged RNase [66]. Development of ratiometric detection systems based on binding-induced micro-pH changes is also a promising and ongoing area of research [67].

2.2. Cu(II) coordination complexes in biosensory applications

2.2.1. Peptide recognition via cooperative Cu(II) coordination and ion pairing

König and coworkers successfully developed a metal-based luminescent chemosensor of N-terminal histidine containing peptides in aqueous conditions. König *et al.* combined an N-terminal histidine-coordinating Cu(II)–iminodiacetic acid (IDA) metal complex with a fluorescent methyl isophthalate crown ether moiety (**8**, Fig. 7A) to selectively detect His-Lys-OMe and His-OMe in aqueous solution ($K_a = 1.65 \times 10^4\ M^{-1}$)[68]. To afford peptide/amino acid specificity, König's ditopic receptor **8** employs an ideal ammonium cation binding partner (crown ether) and a histidine binding Lewis acidic Cu(II) complex. By simultaneously binding the N-terminal of His and the corresponding side chain imidazole nitrogen (Fig. 7A), König and coworkers proposed that the divalent Cu(II) complex sequesters the peptide and facilitates subsequent intramolecular crown ether coordination to the lysine ammonium cation or, in the case of His-OMe, the backbone N-terminus [68]. Binding of the second ammonium side chain/N-terminal triggers the emission intensity of the fluorescent isophthalic ester [69], allowing this sensor to selectively detect species containing both an imidazole and an ammonium cation (Fig. 7B).

In a subsequent study, König and coworkers probed the peptide sequence specificity of a series of Cu(II)–IDA–benzocrown ether



B

analyte	binding constant
Ac-L-His	0
L-His-OMe	1.0×10^5
L-His	1.2×10^5
D/L-Cys	8.0×10^4
L-Met	3.0×10^3
L-Val	0
Gly	0
L-His-L-Lys-L-Lys	1.0×10^6
L-His-Gly-Gly	1.5×10^4
Gly-Gly-Gly	0
L-Cys-L-Lys-L-Lys	3.0×10^5
L-Cys-Gly-Gly	5.0×10^2
L-Met-L-Lys-L-Lys	1.0×10^5
L-Met-Gly-Gly	2.5×10^4
L-Lys-L-Lys-L-His	0

Fig. 8. (A) Chemical structure of receptor **10**; (B) binding constants (K_a, M^{-1}) for interactions between **10** and free amino acids, protected amino acids, and tripeptides (as measured at 25 °C).
(Reprinted with permission from [71]. Copyright American Chemical Society).

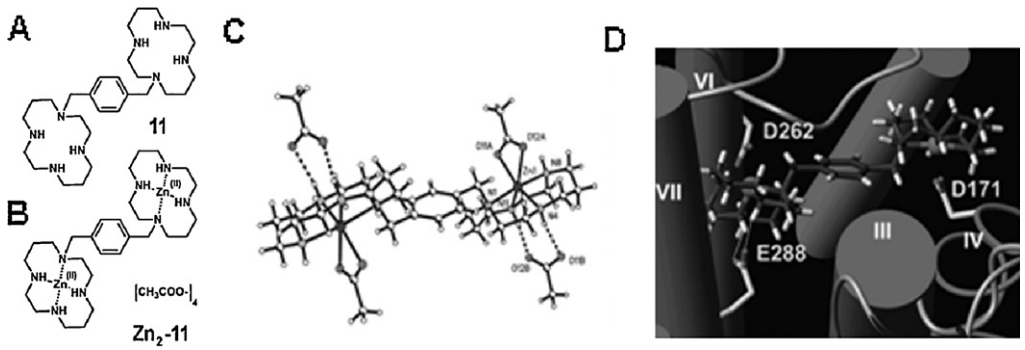


Fig. 9. (A) Chemical structure of **11**; (B) chemical structure of $Zn(II)_2$-**11**; (C) crystal structure of $Zn(II)_2$-**1**.diacetate, illustrating favourable *trans* geometry; (D) *in silico* docking of energy minimized $Zn(II)_2$-**11** with CXCR4. Image shows proposed binding to Asp_{171}/Asp_{262} residues and also indicated the participation of Glu288. (Reprinted with permission from [89]. Copyright American Chemical Society).

conjugates (representative example **9**, Fig. 7C) by screening against a small library of di-, tri- and tetrapeptides [70]. Unsurprisingly, as assessed by changes in emission intensity, negligible binding was reported for peptides lacking the Cu(II) chelating His residue, such as tetrapeptide QGGG. Negligible activity was reported for peptides in which the distance between the imidazole side chain and the peptide N-terminus becomes too distant (≥3 residues). The highest affinities were observed for the tripeptides Gly-Gly-His and Gly-His-Gly ($K_a > 10^5\ M^{-1}$) (Fig. 7D). N-terminal glycine modulation was well tolerated and did not compromise recognition potency. This observation was consistent with the authors' hypothesis that Cu(II)–IDA motifs anchor to the imidazole of a His residue and, if in close proximity, will facilitate intramolecular benzocrown ether binding to an N-terminal amino group, resulting in an increased emission intensity signal [70].

In addition, Wright and Anslyn developed a peptide recognition motif based on a rigid Cu(II)–polyazatricyclic scaffold, substituted with two variable oligopeptide chains for peptide sensing [71]. The authors postulated that individual tripeptides could be distinguished based on the charge complimentarity, as well as the presence or absence of a Cu(II)-coordinating terminal side chain residue (His or Cys). Using UV absorption binding experiments, Wright and Ansyln successfully showed that poly-anionic receptor **10** (Fig. 8A) was able to achieve tight and selective binding to L-His-L-Lys-L-Lys, with a $K_a > 10^6\ M^{-1}$ (Fig. 8B) [71].

These modular receptor systems were further investigated by Wrightet al. to produce a silicon microchip array to selectively detect different peptides on the basis of their primary sequence [72]. In this study, the authors generated a small library of resin-bound Cu(II)-polyazatricyclic receptors furnished with varying tripeptide appendages, and attached to a silicon microchip array (~30 receptors). An indicator-uptake assay was used for colorimetric quantification of the tripeptide/array binding interaction, and Principal Component Analysis (PCA), which is a useful analytical method for reducing the dimensionality of large, complex data sets, was used to achieve excellent discrimination and separation of tripeptides [72].

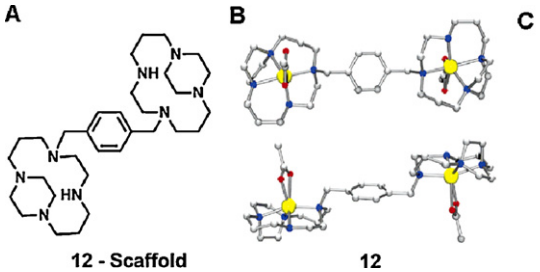
A similar pattern-based technique was developed by Ansyln and coworkers, wherein several phosphoserine containing peptides were successfully distinguished from one another [73]. In this case, a versatile C_3 symmetrical mono-nuclear amino-tris(pyridine) receptor [74,75], known to bind phosphomonoesters, was substituted with several different tripeptide sequences to create a diversified set of sensors, with each analog providing a unique microenvironment around the metal center. The authors reasoned that these microenvironments would allow for the selective binding of specific phosphorylated peptide sequences to each receptor, and thus facilitate pattern-based recognition. Potent and selective artificial peptide recognition systems could play an important role in the future of clinical diagnostics and provide early detection of peptidic human disease markers. Indicator displacement assays have been used extensively by Collins and Anslyn to develop standard and pattern-based detection systems [76] for individual amino acids on the basis of sequence structure [77,78] and stereochemistry [78,79]. It is also notable that cationic Cu(II)–porphyrin complexes have been successfully applied as sensitive reporters of the structural conformation of poly-L-glutamatic acid biopolymers [80].

3. Zn(II) and Cu(II) coordination complexes in therapeutic roles against peptide/protein targets

3.1. Zn(II) and Cu(II) complexes involved in competitive inhibition mechanisms

3.1.1. Zn(II) coordination complexes targeting CXCR4 receptor

Zn(II) complexes have been extensively investigated as inhibitors of the chemokine receptor CXCR4, which is known to be aberrantly involved in numerous human diseases, including breast cancer [81] and HIV [82]. While the unchelated xylyl–bicyclam



Anti-HIV Activities, Cytotoxicity and Selectivity Index in MT-4 Cells

Compound	HIV Strain	Av EC_{50} (μM)[a]	Av CC_{50} (μM)[b]	SI[c]
12 -scaffold	HIV-1 (III_B)	6.98	>225	>32
	HIV-2 (ROD)	23.2	>225	10
12	HIV-1 (III_B)	0.0025	60.56	24 225
	HIV-2 (ROD)	0.0040	60.56	15 140
AMD3100	HIV-1 (III_B)	0.011	>225	20 455
Zn_2AMD3100	HIV-1 (III_B)	0.008	>225	28 125

[a] Average effective concentration to reduce the HIV-induced cytopathic effect by 50% in MT-4 cells. [b] Concentration required to have a cytotoxic effect reducing MT-4 cell viability by 50%. [c] Selectivity index based on μM conversion.

Fig. 10. (A) Chemical structure of **12** scaffold; (B) X-ray crystal structure of conformationally restricted **12**, illustrating stable cis/trans geometry; (C) anti-HIV activity of **12** compared to free ligand, AMD3100 (**11**) and $Zn(II)_2$-**11**. (Reprinted with permission from [91]. Copyright American Chemical Society).

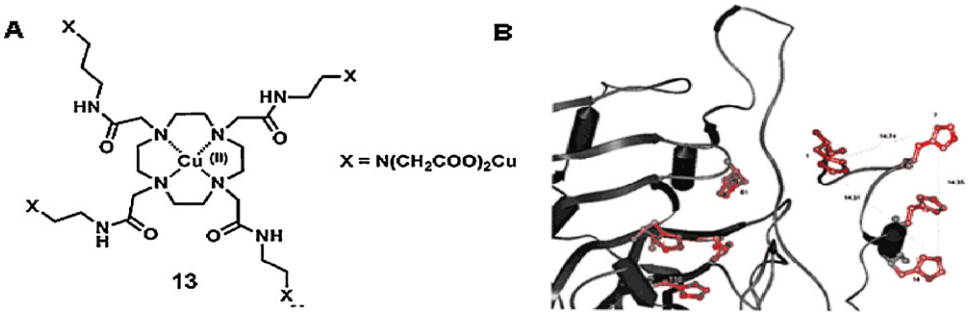


Fig. 11. (A) Chemical structure of polynuclear Cu(II)–IDA complex; (B) crystal structure of bovine carbonic anhydrase (PDB1V9E), with targeted, solvent-exposed histidine residues shown in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.) (Reprinted with permission from [93]. Copyright American Chemical Society).

ligand **11** (or AMD3100, Fig. 9A) is a known antagonist of the CXCR4 receptor [83–85], the corresponding Zn(II) complex of **11** has recently been identified as having a greater potency [86–88]. This observation lends credence to the emerging theory that the bioactive species of **11** might in fact be the dinuclear $Zn(II)_2$-**11** complex (Fig. 9B), given the concentrations of zinc in the cytoplasmic domain. Sadler and coworkers have comprehensively probed the molecular dynamics of $Zn(II)_2$-**11**'s interaction with CXCR4 *in vitro* using NMR, *in silico* molecular docking and computational modelling studies. Initial *in vitro* studies which sought to probe the conformational effects of ligand coordination to the two Zn(II)–cyclam metal centers found that the binding of acetate anions to $Zn(II)_2$-**11** induced a stabilized *trans* configuration, wherein the two Zn(II) metal centers are oriented in opposite directions (crystal structure shown in Fig. 9C). Sadler and coworkers demonstrated *in silico*, using computational docking studies, that adoption of the *trans* configuration facilitated binding to the CXCR4 residues Asp_{171}/Asp_{262} (Fig. 9D), which have been shown via mutational analysis to be essential for CXCR4 activity in HIV [89].

In a subsequent study, Sadler et al. confirmed that binding of the acetate anions to $Zn(II)_2$-**11** coordination complex induces a pronounced shift into the *trans* configuration in aqueous solution [90]. This observation offered insight into the nature of the $Zn(II)_2$–bicyclam/CXCR4 interaction, and suggested that a multistep pseudo-induced fit might take place between Asp_{171}/Asp_{262} and the metal complex upon binding. In a related study, Archibald and coworkers hypothesized that a conformationally restricted cyclam analog should have greater binding activity, and thus synthesized a small family of sterically constrained, alkyl bridging piperazinocyclam analogs [91]. The most promising compound, **12** (Fig. 10A), was shown to adopt a single conformation in aqueous solution, analogous to that of the proposed binding state adopted by $Zn(II)_2$-**11** in the CXCR4 binding site. Furthermore, in whole cell experiments **12** was highly effective in the prevention of HIV infection of highly sensitive human T-cells (MT-4) *in vitro* [91]. The large therapeutic window between the antiviral activity compared to the general cytotoxicity ($EC_{50} \sim 2.5$ nM *c.f.* $CC_{50} \sim 61$ μM), serves as evidence that targeting CXCR4 with $Zn(II)_2$–bicyclam derivatives is a valid therapeutic avenue in the treatment of HIV (Fig. 10C) [91].

Subsequently, Hamachi and coworkers have identified a number of potent, non-peptidic antagonists of CXCR4 based on the repositioning of previously synthesized $Zn(II)_2$–BDPA complexes [92]. Through individual screening, it was discovered that most of the reported complexes were shown to inhibit natural substrate binding in transfected CHO cells at nanomolar (nM) concentrations. Consistent with findings by Sadler et al., Hamachi et al. demonstrated that complexes able to adopt a stabilized *trans* geometry, where the respective coordination spheres of the two Zn(II) ions are oriented away from one another, were the most effective at binding CXCR4.

3.1.2. *Protein surface recognition with Cu(II) coordination complexes*

The selective recognition of proteins based on the distribution pattern of histidines on the protein surface has been pursued by Mallik and coworkers [93]. The authors designed polynuclear Cu(II)–IDA complexes (**13**, Fig. 11A) for the recognition of bovine carbonic anhydrase through the complementary interaction between Cu(II) sites and solvent-exposed histidine residues on the protein surface. High affinity binding constants ($K_a > 10^5\ M^{-1}$) were reported in aqueous solution (pH = 7.0, 25 °C, 25 mM HEPES). Through rigorous testing, Mallik et al. showed that spatial and geometric complementarities between the Cu(II) metal centers and the

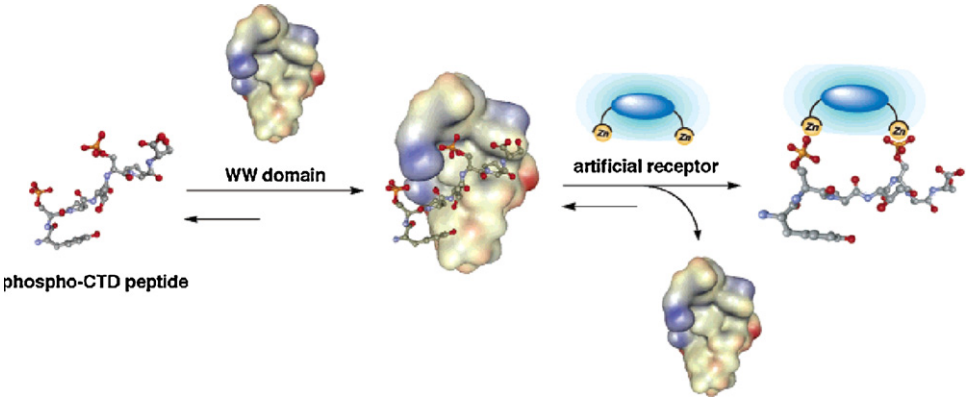


Fig. 12. Disruption and fluorescent sensing of bis-phosphopeptide–WW domain interactions using a $Zn(II)_2$–BDPA receptor. (Reprinted with permission from [100]. Copyright American Chemical Society).

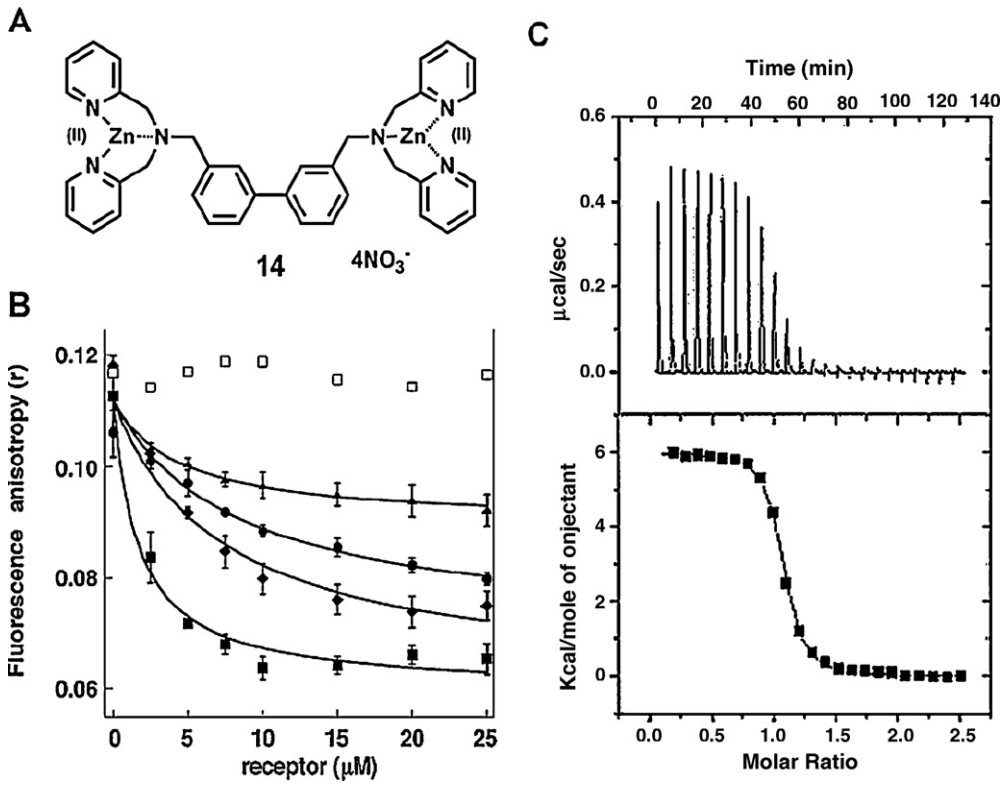


Fig. 13. (A) Chemical structure of **14**; (B) FP data showing potent displacement of the Pin1 WW domain (25 μM) by **14** (black square) from a fluorescently labelled pS 2,6 bis-phosphorylated peptide; (C) ITC trace for **14** interacting with the pS 6,9 bis-phosphopeptide.
(Reprinted with permission from [100]. Copyright American Chemical Society).

exposed surface histidine residues were essential for high affinity binding (Fig. 11B) [93]. Similar research has been done by Mallik et al. involving the application of histidine–Cu(II) complex matching to peptide recognition [94,95].

3.1.3. *Disrupting phosphopeptide–protein and protein–protein interactions with Zn(II) and Cu(II) coordination complexes*

Inhibition of cancer-promoting, constitutive protein–protein interactions via disruption of binding interfaces offers significant value as a molecular-targeted therapy [96,97]. Critically, protein complexation events are often initiated and maintained through phosphorylation of critical tyrosine or serine amino acid residues. Proteins containing key phosphorylated tyrosine residues are often recognized and bound by specific phosphopeptide binding modules, *e.g.* the Src Homology 2 (SH2) domain [98] or the WW domain [99], of the complementary protein-binding partner. In a seminal paper [100], Hamachi and coworkers introduced the concept of using metal coordination complexes to disrupt phosphotyrosine mediated protein complexation events via targeting of the phosphopeptide and not, as has traditionally been the case, through targeting of the phosphopeptide binding domain by phosphopeptide mimetics (new conceptual strategy outlined in Fig. 12). Building on previous work in bis-phosphopeptide recognition [101], Hamachi and coworkers successfully used a ditopic Zn(II)–DPA receptor linked through a biphenyl unit (**14**, Fig. 13A) to disrupt a phosphopeptide–protein interaction between a Pin1 WW domain, a small, well characterized protein module which recognizes proline-rich phosphoserine-containing peptides [102], and a bis-phosphorylated peptide known to function as a Pin1 WW binding partner (Fig. 12) [103,104]. As observed in previous cross-linking investigations involving bis-phosphopeptide recognition, the strongest binding constants were achieved with receptors where the distance between the linked Zn(II)–DPA units matched the distance between the two phosphoserines on the target peptide. Hamachi et al. used a fluorescence polarization (FP) assay (Fig. 13B), ITC (Fig. 13C) and circular dichroism to characterize the strong receptor–phosphopeptide binding event ($K_a > 10^6\ M^{-1}$ as assessed by ITC) and successfully demonstrated the disruption of a phosphopeptide–protein complex ($K_i = 1.84 \times 10^6\ M^{-1}$) (Fig. 13). This strategy is now emerging as a valid therapeutic avenue for the targeting of cancer promoting protein–protein interactions mediated by phosphotyrosine recognition.

Similarly, Gunning and coworkers recently reported the use of asymmetrically functionalized $Cu(II)_2$–DPA scaffolds [105] to disrupt oncogenic Signal Transducer and Activator of Transcription 3 (Stat3) protein–protein interactions [106]. Stat3 is a well recognized master regulator of the cancer phenotype and is aberrantly activated in numerous human cancers. Stat3 promotes the expression of anti-apoptotic target genes such as Bcl-xL, Myc and survivin, preventing cancer cell death and thus increasing resistance to current chemotherapeutics [96]. Stat3 is phosphorylated on a key tyrosine residue (Tyr705), which leads to the formation of transcriptionally active Stat3–Stat3 homo-dimers which are mediated through reciprocal SH2 domain–pTyr-705 interactions. After translocation to the nucleus, dimeric Stat3 complexes bind to specific DNA response elements and promote target gene transcription. Inhibition of these cancer-promoting, constitutive Stat3–Stat3 protein complexes via disruption of binding interfaces offers significant value as a molecular-targeted therapy for the treatment of human cancers. Gunning et al. postulated that the reciprocal phosphotyrosine–SH2 domain interaction could be disrupted by binding the key pY705 with a metal coordination complex and effectively displacing the cognate Stat3 binding partner (Fig. 14A) [106]. Nuclear extracts from cells containing constitutively acti-

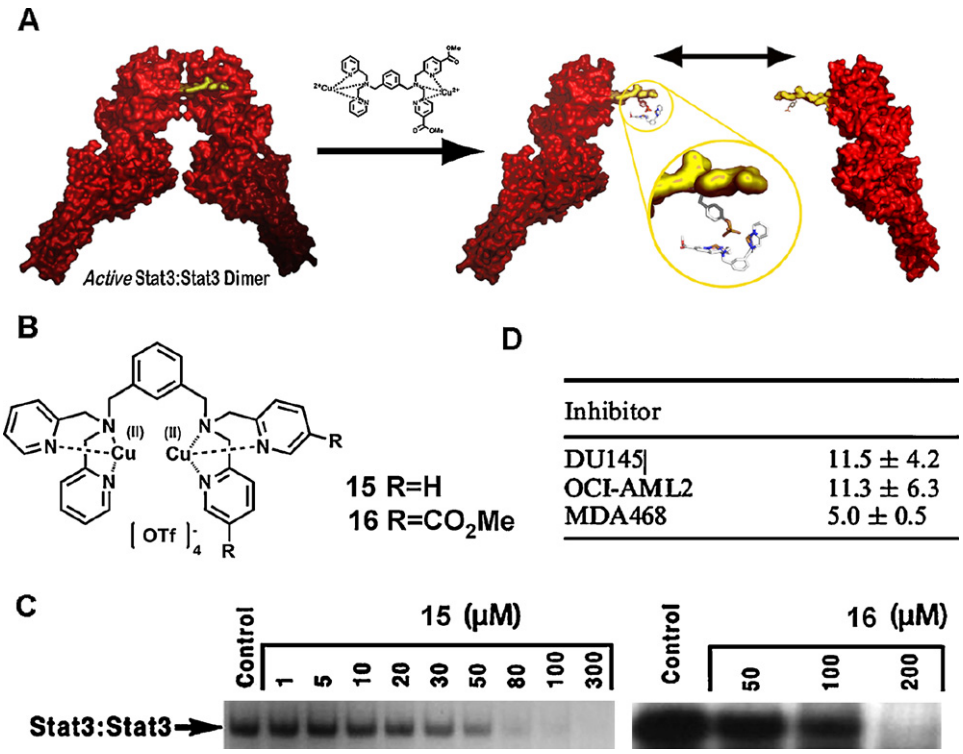


Fig. 14. (A) Schematic representation of the disruption of Stat3–Stat3 dimer using a functionalized $Cu(II)_2$–BDPA complex; (B) chemical structures of inhibitors **15** and **16**; (C) EMSA analysis of vSrc/NIH-3T3 cells treated with increasing concentrations of **15** and **16**; (D) table of IC_{50} values for **16** against prostate cancer (DU145), acute myeloid leukemia (OCI-AML) and solid breast cancer tumour (MDA 468) (as assessed by MTS assay).
(Reprinted with permission from [106]. Copyright Royal Society of Chemistry).

vated Stat3 (NIH3T3/v-Src (v-Src-transformed mouse fibroblasts)) [107] were treated with functionalized $Cu(II)_2$–BDPA complexes (Fig. 14B), and then analyzed for Stat3–Stat3:DNA binding *in vitro* using a STAT specific radiolabelled hSIE probe and electrophoretic mobility shift assay (EMSA). The authors showed significant levels of $Cu(II)_2$–BDPA-induced Stat3 dimer disruption (Fig. 14C). Interestingly, asymmetrically functionalized inhibitor **16** showed Stat3 specific suppression of Stat3–Stat3:DNA binding activity with an IC_{50} value of 61 ± 4.4 μM, inhibiting Stat3 complexes and thus preventing DNA binding. Moreover, Gunning et al. found that inhibitor **16** displayed isoform selectivity for Stat3 over Stat1 protein complexes; mimetic **16** showed a three-fold preference for Stat3 (Stat3 $IC_{50} = 61 \pm 4$ *cf.* Stat1 $IC_{50} = 176 \pm 24$ μM). Further FP [108] and ITC binding experiments confirmed the binding potency of the $Cu(II)_2$–BDPA complexes for the cognate phosphopeptide sequence ($K_a \sim 10^4$ to $10^5\ M^{-1}$). To determine the therapeutic potential of **16**, screening against a range of cancer cell lines known to contain activated Stat3 protein was conducted, including breast cancer (MDA468), prostate cancer (DU145) and acute myeloid leukemia (OCI-AML) (Fig. 14D). Screening identified **16** as a promising anticancer agent, exhibiting low single digit μM activity against all three cell lines. Most promisingly, inhibitor **16** was calculated to have an $IC_{50} = 5$ μM against MDA468 breast cancer cells [106]. Biochemical and biological investigations are currently ongoing to identify the mechanisms of observed inhibition.

In similar work, König and coworkers have developed artificial ditopic phosphopeptide receptors, composed of two Zn(II)–cyclen units bound to a triazine core substituted with oligopeptide sequences. Each sequence was equipped with either a terminal guanidinium (**17**) or Zn-NTA (**18**) group (Fig. 15), for selective recognition of phosphoserine-peptides containing a glutamic acid residue in the (pS + 3) position [109]. Most encouragingly, the authors reported one of the highest binding affinity constants ever published between a phosphopeptide and an artificial receptor. In subsequent work, the same authors used a fluorescence anisotropy assay to demonstrate that suitably functionalized analogous architectures were able to effectively disrupt a tight phosphopeptide–protein interaction between an interferon-γ-derived phosphopeptide, and a Stat1 transcription factor at high micromolar concentrations [110].

3.2. Zn(II) and Cu(II) coordination complexes in non-traditional inhibition mechanisms

Zn(II) and Cu(II) metal complexes have also been used to inhibit biological targets in a number of non-classical mechanisms. With the long term goal of achieving therapeutically beneficial results, coordination complexes have been demonstrated to denature protein structure, stabilize misfolded 'non-functional' protein states, and replicate proteolytic enzymes for the breakdown of medicinally important protein and peptide targets.

3.2.1. Cu(II) coordination complex mediated protein denaturation

Expanding on previous studies involving substituted porphyrins in protein recognition [111–114], Hamilton and coworkers reported polyanionic Cu(II)-centered porphyrins able to selectively denature cytochrome *c*, a protein involved in cellular respiration and the regulation of apoptosis [115], at near-physiological conditions (pH = 7.4, 5 mM P_i, 50 mM NaCl, rt). Hamilton and coworkers showed that Cu(II)–porphyrins, which are believed to exist as dimers in aqueous solution, lowered the temperature at which cytochrome *c* undergoes denaturation (T_M) by ~50 °C, as determined by UV absorbance and CD [116]. In subsequent work, Hamilton et al. characterized polyanionic Cu(II)–porphyrin dimer mediated catalysis of the denaturation-mediated proteolysis of cytochrome *c* under physiological conditions by natural proteases

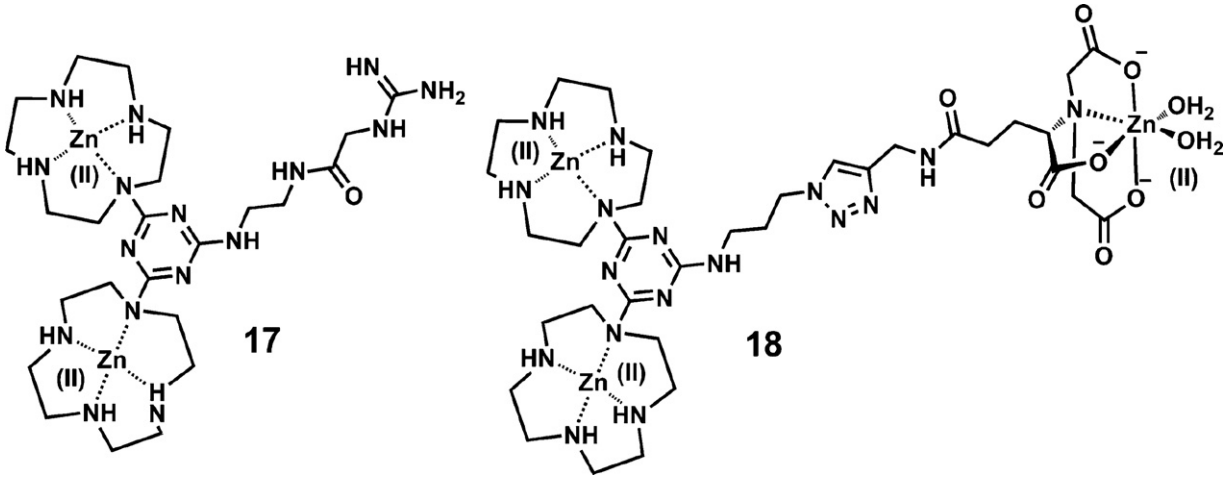


Fig. 15. Triazine-based ditopic receptors developed for phosphopeptide recognition.

(**19**, Fig. 16B). Binding-induced denaturation by **19** resulted in a favorable 2.4 kcal/mol decrease in the $\Delta G_{conform}$ for proteolysis. Moreover, SDS-PAGE and CD were used to show that, under milder than physiological conditions, a general ablation of the secondary and tertiary structures occurred upon Cu(II)–porphyrin dimer binding, resulting in non-specific proteolysis of the peptide backbone by trypsin [117].

Agents which are able to selectively denature the tertiary structure of target proteins could be of tremendous benefit in the treatment of human prion diseases, which are characterized by the propagation of native proteins misfolding into highly stable, inactive proteins that are resistant to cellular degradation processes [118].

3.2.2. Zn(II) complexes involved in stabilizing protein conformational states

Stabilization of active-state conformers, recently reported by König and coworkers, is also an interesting and unprecedented application of metal complexes in the modulation of biochemical pathways. König *et al.* used ^{31}P NMR to monitor the effect of Zn(II)–cyclen on the conformational state of RAS protein bound to a non-hydrolysable guanosine triphosphate (GTP) analog [119]. RAS is an oncogenic protein that is aberrantly activated in approximately 30% of all human tumours. RAS normally exists in two conformations in the GTP-bound state, one which can bind effector proteins (State II, or the 'on' state), and another which cannot (State I, or the 'off' state) [120–125]. Excitingly, König and

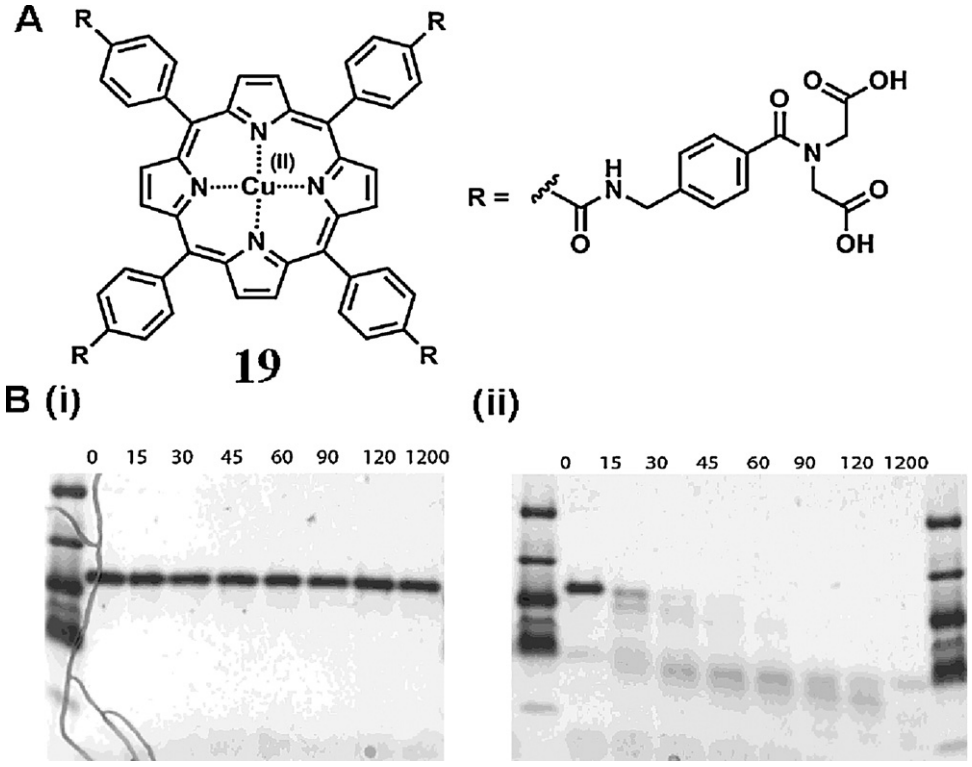


Fig. 16. (A) Chemical structure of copper porphyrin **19**; (B) trypsin catalyzed hydrolysis of cytochrome *c* over 20 h; (i) trypsin alone; (ii) trypsin in the presence of four equivalents of **19**. Incubation time shown in minutes.
(Reprinted with permission from [117]. Copyright American Chemistry Society).

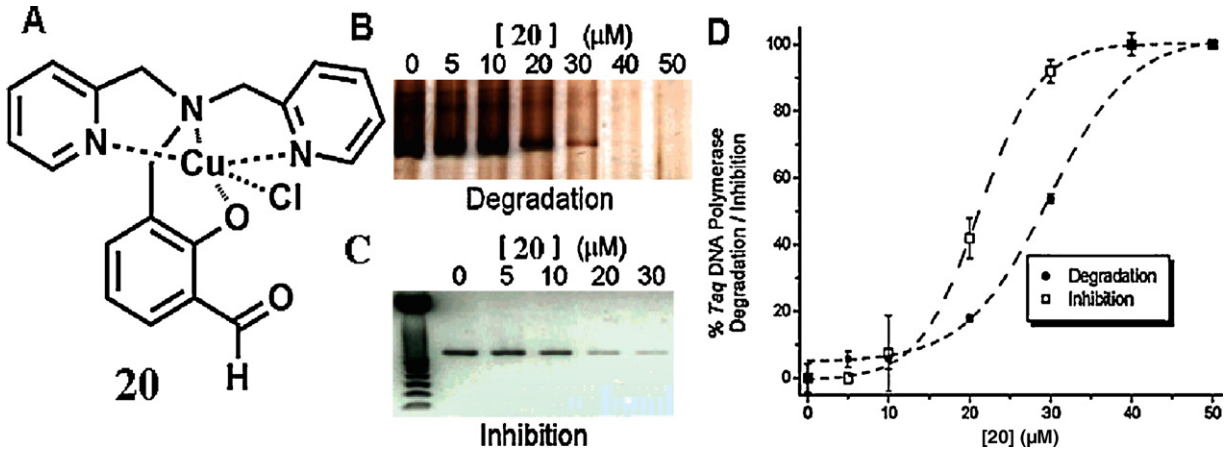


Fig. 17. (A) Structure of proteolytic catalyst **20**; (B) concentration-dependent proteolytic degradation of TAQ polymerase; (C) inhibition of PCR(TAQ)-mediated DNA replication by tripodal hydrolysis catalyst **20**; (D) correlation between TAQ proteolysis and inhibition of DNA replication by **20**.
(Reprinted with permission from [127]. Copyright American Chemical Society).

coworkers reported that Zn(II)–cyclen stabilizes the weak binding state (State I, 'off' state), shifting the equilibrium between the two states to favor State I exclusively at millimolar concentrations [119]. This work may lead to the discovery of novel RAS targeting therapeutics. Although the binding location and mechanism have yet to be determined, this work is the first example of a novel inhibitory approach wherein a single non-competent conformer of a protein's excited state was stabilized, leading to an equilibrium shift away from the competent excited state.

3.2.3. *Mimicking enzyme-mediated proteolytic function with Cu(II) coordination complexes*

Another emerging area of investigation involves the development of artificial proteases capable of degrading specific folded proteins *in vitro*. Providing that reasonable target specificity could be attained, such molecules could represent a new and promising class of molecular therapeutics [126]. Soares and coworkers synthesized and structurally characterized a tripodal Cu(II) complex of 2-[(bis(pyridylmethyl)amino)methyl]-4-methyl-6-formylphenol (HL), **20** (Fig. 17A), that functions as an efficient artificial protease under mild conditions (50 °C, pH 7.0) *in vitro* [127]. In proof-of-principal work, a Cu(II)–HL complex was demonstrated to hydrolyse thermostable TAQ polymerase (Fig. 17B and D), and consequently impede PCR-mediated gene amplification (Fig. 17C and D). The complex was also shown to hydrolyse bovine serum albumin (BSA) in specific structural positions, likely corresponding to disordered, solvent-exposed regions on the protein's surface.

In similar work, Suh and coworkers [128] have made significant progress in the development of both selective and non-selective proteases based on Cu(II) complexes [126]. Notably, this group developed the first artificial protease specific to a target protein, in this particular case myoglobin. Suh et al. optimized a peptide nucleic acid (PNA) ligand with a high degree of specificity for myoglobin, and covalently attached a Cu(II)–cyclen moiety (Fig. 18). The resultant complex, **21**, was able to efficiently and selectively hydrolyse myoglobin at low micromolar concentrations [129]. Later work involving the variation of the coordinating ligand revealed that the proteolytic activity of the complex was specific to Cu(II)–cyclen [130]. This work demonstrated that, through a cooperative interaction between a protein binding moiety and a hydrolysis catalyst (such as Cu(II)–cyclen), selective proteolytic activity for specific proteins can be achieved.

Rajendiran and coworkers also reported a mixed-ligand Cu(II)(tdp)(tmp) complex **22** (Fig. 19A), where tdp is 2-[(2-(2-hydroxyethylamino)ethylimino)methyl]-phenol (Fig. 19B) and tmp is 3,4,7,8-tetramethyl-1,10-phenanthroline (Fig. 19C), that exhibits remarkable proteolytic activity. Rajendiran et al. demonstrated the reactivity of **22** through the proteolytic cleavage of bovine serum albumin (BSA) protein and lysozyme by 5- and 4-kDa fragments respectively. Moreover, the reported complex **22** was able to achieve site specific proteolysis in a matter of only minutes under physiological conditions (Fig. 19D). In a subsequent investigation to probe the nature of the observed catalysis, Rajendiran et al. disproved a radical-mediated hydrolytic cleavage mechanism; instead, it was postulated that the tmp ligand served to recognize specific, hydrophobic residues near the cleavage site [131,132].

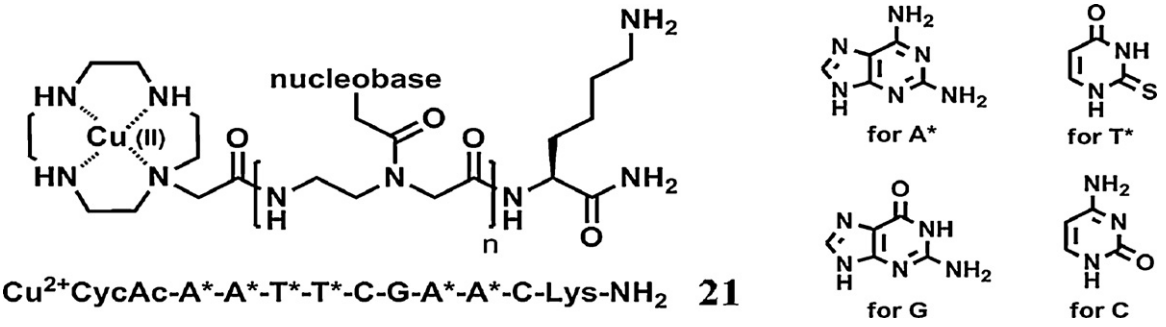


Fig. 18. Chemical structure of ditopic receptor system **21**, consisting of a PNA-based myoglobin recognition motif, and a proteolytic Cu(II) cyclen unit.

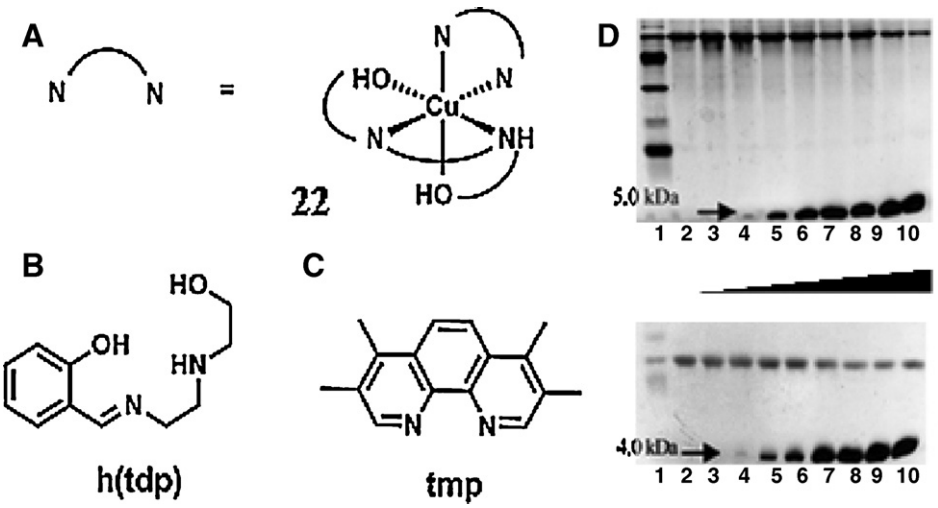


Fig. 19. (A) Structure of compound **22**; (B and C) structure of h(tdp) and tmp ligands, respectively; (D) SDS-PAGE analysis of concentration-dependent protein degradation (top – BSA, bottom – lysozyme) by **22** in a 10% DMF/10 mM HEPES buffer (pH 7.3) at 37 °C for 4 h: lane 1, molecular weight standard for the mass values given on the left; lane 2, control proteins; lanes 3–10, proteins (15 μM) incubated with **22** (0.05, 0.1, 0.2, 0.3, 0.5, 0.7, 1.0, and 1.2 mM).
(Reprinted with permission from [131]. Copyright American Chemical Society).

4. Conclusion

The past decade has seen an explosion of growth in the application of Zn(II) and Cu(II) complexes in the field of chemical biology, specifically in their application as powerful sensory tools as well as emerging medicinal therapeutics for human cancers and HIV. In this review we have attempted to demonstrate the impressive diversity of applications in which metal complexes play an important role. Numerous research groups have developed metal complexes for a wide variety of fluorescent and luminescent biosensory applications, including protein labelling and sequence-specific peptide recognition. Moreover, rationally designed Zn(II) and Cu(II) metal complexes have been successfully employed to achieve directed biochemical effects through proteolytic peptide bond cleavage, protein denaturation and stabilization of inactivated protein states. Rationally designed coordination complexes are thus gaining traction as alternative classes of innovative molecular therapeutics. In particular, their potential to disrupt notoriously difficult protein–protein interactions is highly appealing, especially given the limited success achieved by small molecule approaches thus far. While the biosensory and medicinal chemistry roles of Zn(II) and Cu(II) complexes are still evolving, the innovative and ground breaking work reviewed herein suggests that coordination complexes will have a major role to play in both clinical medicinal chemistry and biosensory molecular recognition.

Acknowledgments

We gratefully acknowledge the Leukemia and Lymphoma Society of Canada, NSERC, University of Toronto and the Connaught Foundation for financial support of our work in this field.

References

[1] C. Orvig, M.J. Abrams, Chem. Rev. 99 (1999) 2201.
[2] P.J. Sadler, R.E. Sue, Met. Based Drugs 1 (1994) 107.
[3] P.J. Sadler, Met. Based Drugs 4 (1997) 119.
[4] P.C. Bruijnincx, P.J. Sadler, Curr. Opin. Chem. Biol. 12 (2008) 197.
[5] S.M. Cohen, S.J. Lippard, Prog. Nucleic Acid Res. Mol. Biol. 67 (2001) 93.
[6] L. Kelland, Nat. Rev. Cancer 7 (2007) 573.
[7] D.W. Bierer, Rev. Infect. Dis. 12 (Suppl. 1) (1990) S3.
[8] P.J. Sadler, H.Y. Li, H.Z. Sun, Coord. Chem. Rev. 186 (1999) 689.
[9] L. Zhang, K.Y. Szeto, W.B. Wong, T.T. Loh, P.J. Sadler, H. Sun, Biochemistry 40 (2001) 13281.
[10] M.C. Steinhoff, R.G. Douglas Jr., H.B. Greenberg, D.R. Callahan, Gastroenterology 78 (1980) 1495.
[11] H.Z. Sun, H.Y. Li, P.J. Sadler, Chem. Berichte Recueil 130 (1997) 669.
[12] P.J. Sadler, Z.J. Guo, Pure Appl. Chem. 70 (1998) 863.
[13] C.-M. Che, F.-M. Siu, Curr. Opin. Chem. Biol. 14 (2010) 255.
[14] N.R. Panyala, E.M. Pena-Mendez, J. Havel, J. Appl. Biomed. 7 (2009) 75.
[15] V. Milacic, D. Fregona, Q.P. Dou, Histol. Histopathol. 23 (2008) 101.
[16] E.R.T. Tiekink, Crit. Rev. Oncol. Hematol. 42 (2002) 225.
[17] Y.K. Yan, M. Melchart, A. Habtemariam, P.J. Sadler, Chem. Commun. (Camb.) (2005) 4764.
[18] N.J. Farrer, L. Salassa, P.J. Sadler, Dalton Trans. (2009) 10690.
[19] C. Orvig, K.H. Thompson, M. Battell, J.H. McNeill, Met. Ions Biol. Syst. 31 (1995) 575.
[20] K.H. Thompson, J. Lichter, C. LeBel, M.C. Scaife, J.H. McNeill, C. Orvig, J. Inorg. Biochem. 103 (2009) 554.
[21] K.H. Thompson, J. Chiles, V.G. Yuen, J. Tse, J.H. McNeill, C. Orvig, J. Inorg. Biochem. 98 (2004) 683.
[22] V.G. Yuen, S. Bhanot, M.L. Battell, C. Orvig, J.H. McNeill, Can. J. Physiol. Pharmacol. 81 (2003) 1049.
[23] V.G. Yuen, P. Caravan, L. Gelmini, N. Glover, J.H. McNeill, I.A. Setyawati, Y. Zhou, C. Orvig, J. Inorg. Biochem. 68 (1997) 109.
[24] K.H. Thompson, J.H. McNeill, C. Orvig, Chem. Rev. 99 (1999) 2561.
[25] K.H. Thompson, C. Orvig, Met. Ions Biol. Syst. 41 (2004) 221.
[26] K.H. Thompson, C. Orvig, J. Inorg. Biochem. 100 (2006) 1925.
[27] S. Fletcher, A.D. Hamilton, Curr. Opin. Chem. Biol. 9 (2005) 632.
[28] M. Kruppa, B. Konig, Chem. Rev. 106 (2006) 3520.
[29] E.J. O'Neil, H. Jiang, B.D. Smith, Abst. Papers Am. Chem. Soc. 231 (2006).
[30] S. Aoki, E. Kimura, J. Biotechnol. 90 (2002) 129.
[31] E. Kikuta, S. Aoki, E. Kimura, J. Am. Chem. Soc. 123 (2001) 7911.
[32] H. Chen, J.A. Parkinson, R.E. Morris, P.J. Sadler, J. Am. Chem. Soc. 125 (2003) 173.
[33] S. Aoki, E. Kimura, Chem. Rev. 104 (2004) 769.
[34] E. Kimura, S. Aoki, Biometals 14 (2001) 191.
[35] E. Kimura, E. Kikuta, J. Biol. Inorg. Chem. 5 (2000) 139.
[36] K.J. Humphreys, A.E. Johnson, K.D. Karlin, S.E. Rokita, J. Biol. Inorg. Chem. 7 (2002) 835.
[37] X.M. Li, H.Q. Ju, C.F. Ding, S.S. Zhang, Anal. Chim. Acta 582 (2007) 158.
[38] Y. Li, Y. Wu, J. Zhao, P. Yang, J. Inorg. Biochem. 101 (2007) 283.
[39] K. Matsumura, S. Kina, J. Sumaoka, S. Tobey, E.V. Anslyn, M. Komiyama, Nucleic Acids Res. Suppl. (2001) 85.
[40] L. Li, N.N. Murthy, J. Telser, L.N. Zakharov, G.P. Yap, A.L. Rheingold, K.D. Karlin, S.E. Rokita, Inorg. Chem. 45 (2006) 7144.
[41] I.L. Medintz, H. Mattoussi, A.R. Clapp, Int. J. Nanomed. 3 (2008) 151.
[42] L. Choulier, K. Enander, Sensors 10 (2010) 3126.
[43] K. Tainaka, R. Sakaguchi, H. Hayashi, S. Nakano, F.F. Liew, T. Morii, Sensors 10 (2010) 1355.
[44] M.C. Morris, Cell Biochem. Biophys. 56 (2010) 19.
[45] I.T. Li, E. Pham, K. Truong, Biotechnol. Lett. 28 (2006) 1971.
[46] J.C. Pickup, F. Hussain, N.D. Evans, O.J. Rolinski, D.J.S. Birch, Biosens. Bioelectron. 20 (2005) 2555.
[47] D.H. Vance, A.W. Czarnik, J. Am. Chem. Soc. 116 (1994) 9397.
[48] A. Ojida, Y. Mito-Oka, M.A. Inoue, I. Hamachi, J. Am. Chem. Soc. 124 (2002) 6256.
[49] A. Ojida, Y. Mito-oka, K. Sada, I. Hamachi, J. Am. Chem. Soc. 126 (2004) 2454.
[50] A. Ojida, M.A. Inoue, Y. Mito-Oka, I. Hamachi, J. Am. Chem. Soc. 125 (2003) 10184.

[51] Y. Ishida, M.A. Inoue, T. Inoue, A. Ojida, I. Hamachi, Chem. Commun. (Camb.) (2009) 2848.
[52] A. Ojida, T. Sakamoto, M.A. Inoue, S.H. Fujishima, G. Lippens, I. Hamachi, J. Am. Chem. Soc. 131 (2009) 6543.
[53] M. Goedert, M.G. Spillantini, Biochem. Soc. Symp. (2001) 59.
[54] H. Eldar-Finkelman, A. Licht-Murava, S. Pietrokovski, M. Eisenstein, Biochim. Biophys. Acta (1804) 598.
[55] T. Sakamoto, M.A. Inoue, A. Ojida, I. Hamachi, Bioorg. Med. Chem. Lett. 19 (2009) 4175.
[56] P. Cohen, M. Goedert, Nat. Rev. Drug Discov. 3 (2004) 479.
[57] M.P. Mazanetz, P.M. Fischer, Nat. Rev. Drug Discov. 6 (2007) 464.
[58] T. Anai, E. Nakata, Y. Koshi, A. Ojida, I. Hamachi, J. Am. Chem. Soc. 129 (2007) 6232.
[59] G.M. Wulf, A. Ryo, G.G. Wulf, S.W. Lee, T. Niu, V. Petkova, K.P. Lu, EMBO J. 20 (2001) 3459.
[60] R.Y. Tsien, FEBS Lett. 579 (2005) 927.
[61] A. Miyawaki, A. Sawano, T. Kogure, Nat. Cell Biol. Suppl. (2003) S1.
[62] K. Terpe, Appl. Microbiol. Biotechnol. 60 (2003) 523.
[63] I. Chen, M. Howarth, W. Lin, A.Y. Ting, Nat. Methods 2 (2005) 99.
[64] I. Chen, A.Y. Ting, Curr. Opin. Biotechnol. 16 (2005) 35.
[65] A. Ojida, K. Honda, D. Shinmi, S. Kiyonaka, Y. Mori, I. Hamachi, J. Am. Chem. Soc. 128 (2006) 10452.
[66] K. Honda, S.H. Fujishima, A. Ojida, I. Hamachi, Chembiochem 8 (2007) 1370.
[67] K. Honda, E. Nakata, A. Ojida, I. Hamachi, Chem. Commun. (Camb.) (2006) 4024.
[68] M. Kruppa, C. Mandl, S. Miltschitzky, B. Konig, J. Am. Chem. Soc. 127 (2005) 3362.
[69] C.P. Mandl, B. Konig, J. Org. Chem. 70 (2005) 670.
[70] S. Stadlbauer, A. Riechers, A. Spath, B. Konig, Chemistry 14 (2008) 2536.
[71] A.T. Wright, E.V. Anslyn, Org. Lett. 6 (2004) 1341.
[72] A.T. Wright, E.V. Anslyn, J.T. McDevitt, J. Am. Chem. Soc. 127 (2005) 17405.
[73] T. Zhang, N.Y. Edwards, M. Bonizzoni, E.V. Anslyn, J. Am. Chem. Soc. 131 (2009) 11976.
[74] G. Hennrich, V.M. Lynch, E.V. Anslyn, Chemistry 8 (2002) 2274.
[75] S.L. Tobey, B.D. Jones, E.V. Anslyn, J. Am. Chem. Soc. 125 (2003) 4026.
[76] B.E. Collins, E.V. Anslyn, Chemistry 13 (2007) 4700.
[77] J.F. Folmer-Andersen, V.M. Lynch, E.V. Anslyn, Chemistry 11 (2005) 5319.
[78] J.F. Folmer-Andersen, M. Kitamura, E.V. Anslyn, J. Am. Chem. Soc. 128 (2006) 5652.
[79] J.F. Folmer-Andersen, V.M. Lynch, E.V. Anslyn, J. Am. Chem. Soc. 127 (2005) 7986.
[80] G. De Luca, A. Romeo, L.M. Scolaro, R.F. Pasternack, Chem. Commun. (Camb.) 46 (2010) 389.
[81] A. Zlotnik, J. Pathol. 215 (2008) 211.
[82] H. Tamamura, A. Otaka, N. Fujii, Curr. HIV Res. 3 (2005) 289.
[83] E. De Clercq, Biochem. Pharmacol. 77 (2009) 1655.
[84] E. De Clercq, Mini Rev. Med. Chem. 5 (2005) 805.
[85] E. De Clercq, Nat. Rev. Drug Discov. 2 (2003) 581.
[86] L.O. Gerlach, J.S. Jakobsen, K.P. Jensen, M.R. Rosenkilde, R.T. Skerlj, U. Ryde, G.J. Bridger, T.W. Schwartz, Biochemistry 42 (2003) 710.
[87] E. De Clercq, Med. Res. Rev. 22 (2002) 531.
[88] E. De Clercq, Curr. Med. Chem. 8 (2001) 1543.
[89] X. Liang, J.A. Parkinson, M. Weishaupl, R.O. Gould, S.J. Paisey, H.S. Park, T.M. Hunter, C.A. Blindauer, S. Parsons, P.J. Sadler, J. Am. Chem. Soc. 124 (2002) 9105.
[90] X. Liang, M. Weishaupl, J.A. Parkinson, S. Parsons, P.A. McGregor, P.J. Sadler, Chemistry 9 (2003) 4709.
[91] G.C. Valks, G. McRobbie, E.A. Lewis, T.J. Hubin, T.M. Hunter, P.J. Sadler, C. Pannecouque, E. De Clercq, S.J. Archibald, J. Med. Chem. 49 (2006) 6162.
[92] H. Tamamura, A. Ojida, T. Ogawa, H. Tsutsumi, H. Masuno, H. Nakashima, N. Yamamoto, I. Hamachi, N. Fujii, J. Med. Chem. 49 (2006) 3412.
[93] M.A. Fazal, B.C. Roy, S. Sun, S. Mallik, K.R. Rodgers, J. Am. Chem. Soc. 123 (2001) 6283.
[94] S. Sun, M. Abul Fazal, B.C. Roy, S. Mallik, Org. Lett. 2 (2000) 911.
[95] S. Sun, M.A. Fazal, B.C. Roy, B. Chandra, S. Mallik, Inorg. Chem. 41 (2002) 1584.
[96] S. Fletcher, J.A. Drewry, V.M. Shahani, B.D. Page, P.T. Gunning, Biochem. Cell Biol. 87 (2009) 825.
[97] S. Fletcher, J. Turkson, P.T. Gunning, ChemMedChem 3 (2008) 1159.
[98] Z. Songyang, S.E. Shoelson, M. Chaudhuri, G. Gish, T. Pawson, W.G. Haser, F. King, T. Roberts, S. Ratnofsky, R.J. Lechleider, et al., Cell 72 (1993) 767.
[99] M. Sudol, H.I. Chen, C. Bougeret, A. Einbond, P. Bork, FEBS Lett. 369 (1995) 67.
[100] A. Ojida, M.A. Inoue, Y. Mito-oka, H. Tsutsumi, K. Sada, I. Hamachi, J. Am. Chem. Soc. 128 (2006) 2052.
[101] A. Ojida, Y. Miyahara, T. Kohira, I. Hamachi, Biopolymers 76 (2004) 177.
[102] P.-J. Lu, X.Z. Zhou, M. Shen, K.P. Lu, Science 283 (1999) 1325.
[103] G. Cesareni, S. Panni, G. Nardelli, L. Castagnoli, FEBS Lett. 513 (2002) 38.
[104] J.L. Ilsley, M. Sudol, S.J. Winder, Cell. Signal. 14 (2002) 183.
[105] J.A. Drewry, S. Fletcher, H. Hassan, P.T. Gunning, Org. Biomol. Chem. 7 (2009) 5074.
[106] J.A. Drewry, S. Fletcher, P. Yue, D. Marushchak, W. Zhao, S. Sharmeen, X. Zhang, A.D. Schimmer, C. Gradinaru, J. Turkson, P.T. Gunning, Chem. Commun. (Camb.) 46 (2010) 892.
[107] Y. Zhang, J. Turkson, C. Carter-Su, T. Smithgall, A. Levitzki, A. Kraker, J.J. Krolewski, P. Medveczky, R. Jove, J. Biol. Chem. 275 (2000) 24935.
[108] J. Schust, T. Berg, Anal. Biochem. 330 (2004) 114.
[109] A. Grauer, A. Riechers, S. Ritter, B. Konig, Chemistry 14 (2008) 8922.
[110] A. Riechers, A. Grauer, S. Ritter, B. Sperl, T. Berg, B. Konig, J. Mol. Recognit. 23 (2010) 329.
[111] R.K. Jain, A.D. Hamilton, Org. Lett. 2 (2000) 1721.
[112] M.A. Blaskovich, Q. Lin, F.L. Delarue, J. Sun, H.S. Park, D. Coppola, A.D. Hamilton, S.M. Sebti, Nat. Biotechnol. 18 (2000) 1065.
[113] R.K. Jain, A.D. Hamilton, Angew. Chem. Int. Ed. 41 (2002), 641-+.
[114] H.S. Park, Q. Lin, A.D. Hamilton, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 5105.
[115] Y.-L.P. Ow, D.R. Green, Z. Hao, T.W. Mak, Nat. Rev. Mol. Cell Biol. 9 (2008) 532.
[116] A.J. Wilson, K. Groves, R.K. Jain, H.S. Park, A.D. Hamilton, J. Am. Chem. Soc. 125 (2003) 4420.
[117] K. Groves, A.J. Wilson, A.D. Hamilton, J. Am. Chem. Soc. 126 (2004) 12833.
[118] A.L. Horwich, J.S. Weissman, Cell 89 (1997) 499.
[119] M. Spoerner, T. Graf, B. Konig, H.R. Kalbitzer, Biochem. Biophys. Res. Commun. 334 (2005) 709.
[120] A. Iuga, M. Spoerner, C. Ader, E. Brunner, H.R. Kalbitzer, Biochem. Biophys. Res. Commun. 346 (2006) 301.
[121] M. Spoerner, A. Nuehs, P. Ganser, C. Herrmann, A. Wittinghofer, H.R. Kalbitzer, Biochemistry 44 (2005) 2225.
[122] M. Spoerner, A. Nuehs, C. Herrmann, G. Steiner, H.R. Kalbitzer, FEBS J. 274 (2007) 1419.
[123] M. Spoerner, A. Wittinghofer, H.R. Kalbitzer, FEBS Lett. 578 (2004) 305.
[124] A. Iuga, M. Spoerner, H.R. Kalbitzer, E. Brunner, J. Mol. Biol. 342 (2004) 1033.
[125] M. Spoerner, C. Herrmann, I.R. Vetter, H.R. Kalbitzer, A. Wittinghofer, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 4944.
[126] J. Suh, W.S. Chei, Curr. Opin. Chem. Biol. 12 (2008) 207.
[127] M.C.B. de Oliveira, M. Scarpellini, A. Neves, H. Terenzi, A.J. Bortoluzzi, B. Szpoganics, A. Greatti, A.S. Mangrich, E.M. de Souza, P.M. Fernandez, M.R. Soares, Inorg. Chem. 44 (2005) 921.
[128] S.H. Yoo, B.J. Lee, H. Kim, J. Suh, J. Am. Chem. Soc. 127 (2005) 9593.
[129] J.W. Jeon, S.J. Son, C.E. Yoo, I.S. Hong, J.B. Song, J. Suh, Org. Lett. 4 (2002) 4155.
[130] J.W. Jeon, S.J. Son, C.E. Yoo, I.S. Hong, J. Suh, Bioorg. Med. Chem. 11 (2003) 2901.
[131] V. Rajendiran, M. Palaniandavar, P. Swaminathan, L. Uma, Inorg. Chem. 46 (2007) 10446.
[132] V. Rajendiran, R. Karthik, M. Palaniandavar, H. Stoeckli-Evans, V.S. Periasamy, M.A. Akbarsha, B.S. Srinag, H. Krishnamurthy, Inorg. Chem. 46 (2007) 8208.