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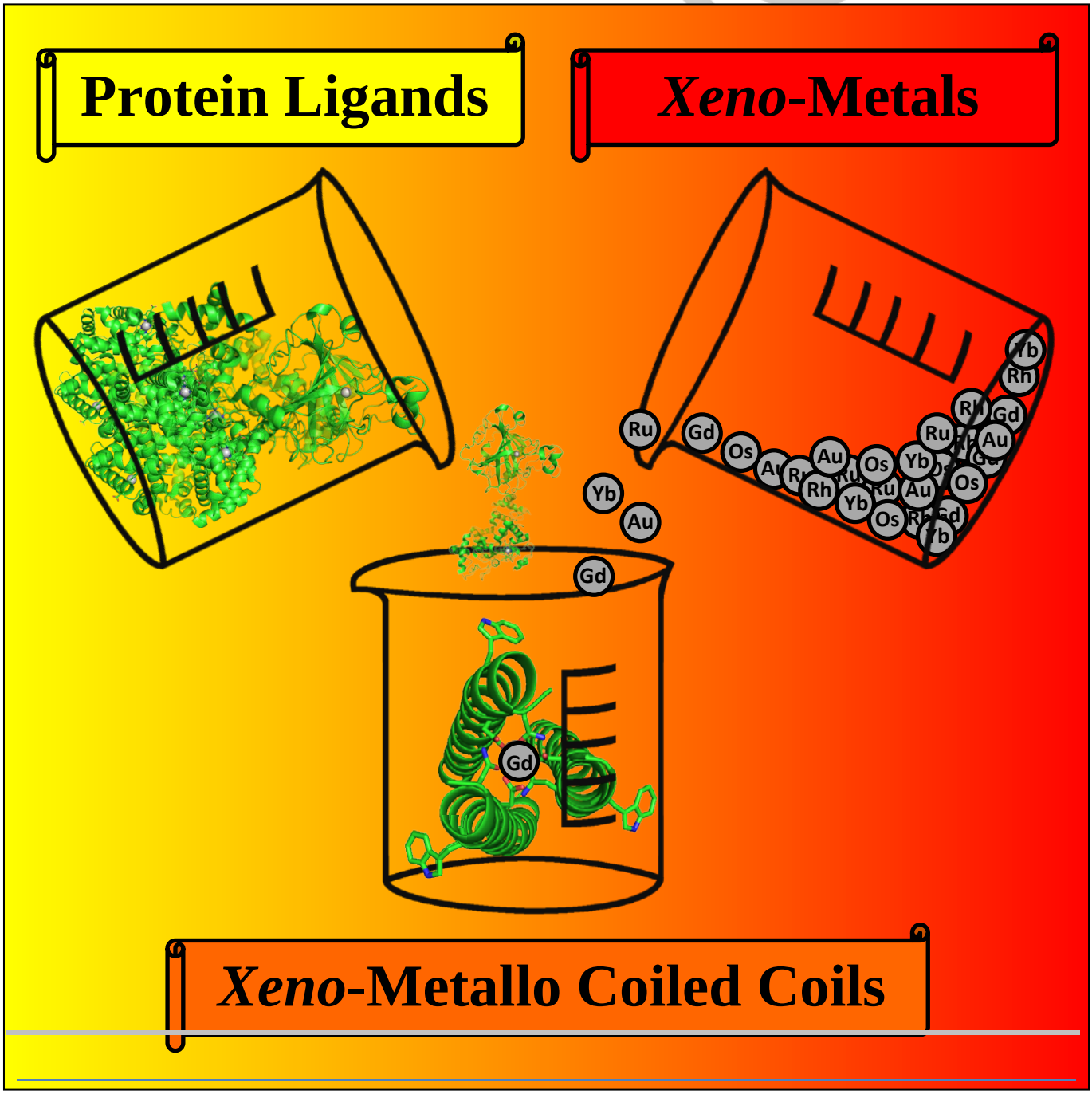
De Novo Design of *Xeno*-Metallo Coiled Coils

Louise N. Slope[a] and Anna F. A. Peacock*[a]



Abstract: Bioinorganic chemists aspire to achieve the same exquisite and highly controlled inorganic chemistry, featured in Biology. An exciting mimetic approach involves the use of miniature artificial protein scaffolds designed *de novo* (often based on the coiled coil (CC) scaffold), for reproducing native metal ion sites and their function. Recently, there is increased interest, instead, in the design of *xeno*-metal sites within CC assemblies. This involves incorporating either non-biological metal ions, cofactors or non-proteinogenic amino acid ligands, for metal ion coordination, whilst retaining a minimal CC protein scaffold. Using this approach, one should be able to create functional designs with unique and unusual properties, which combine the advantages of both biology and 'traditional' non-biological inorganic chemistry. It is the recent progress with respect to the design of *xeno*-metallo CCs which will be discussed in this focus review.

1. Introduction

It has been estimated that roughly a third of all native proteins contain metal ions which are essential for their function,[1] making the study of metalloproteins an attractive and worthwhile goal. The ability to artificially replicate challenging metal ion sites, both structurally and functionally, as well as recreating the exquisite bioselectivity and control displayed in biology, is one of the principle challenges of bioinorganic chemistry.

This challenge has often been addressed through the synthesis of small molecule complexes, which resemble the biological metal ion site of interest. However, in some cases, small molecule complexes are unable to reproduce the chemistry of these sites, highlighting the importance of the protein scaffold. *De novo* peptide design can sometimes be adopted to mimic biology, and resulting miniature protein folds can be used to elucidate important structure-function relationships.[2] This approach has been successfully expanded to the *de novo* design of mimetic metallopeptides, yielding some state-of-the-art mimics,[3] and represents an exciting opportunity to bridge the gap between small synthetic inorganic complexes and complex native metalloproteins.

In view of the above, it is thus also attractive to explore the coordination of *xeno*-metals, metals with no known biological role, as well as the introduction of other non-biological features, such as cofactors or non-proteinogenic amino acids, in to the design.[4] These hybrids could couple the diverse array of chemistry afforded by non-biological metal ion complexes, with the enormous benefits associated with the use of proteins as ligands for metal ions. As well as providing a sophisticated scaffold that can be used to delicately tune the properties of the metal ion, proteins can act as multidentate chelating ligands, providing spatial fixation and rigidity, and serve as a medium with well-defined dielectric properties. Ultimately, it is our belief that this approach will realize the true potential of bottom-up synthetic biology with respect to inorganic chemistry, yielding new metalloprotein hybrids (here we will focus on the CC motif) with novel properties. This focus review, which is far from exhaustive, will feature some key examples to illustrate this topic. The interested reader is directed to more comprehensive reviews on metallopeptide design.[5]

[a] Dr. A. F. A. Peacock and Miss L. N. Slope
School of Chemistry
University of Birmingham
Edgbaston, B15 2TT (UK)
E-mail: a.f.a.peacock@bham.ac.uk

Louise N. Slope obtained her MSci degree at The University of Birmingham in 2013. She is currently a PhD student in the group of Dr Anna F. A. Peacock at the same university. Her research spans the *de novo* design of metallopeptides, centred around Ln(III) selectivity within CCs.

Anna F. A. Peacock received her MChem degree from the University of York (2003) and her PhD in chemistry (2007) from the University of Edinburgh (advisor: Prof. Peter J. Sadler FRS). After a two-year postdoctoral fellowship at the University of Michigan, in the group of Prof. Vincent L. Pecoraro, she was appointed as a Lecturer in Chemistry at the University of Birmingham (2009). Her research interests are centred on bioinorganic chemistry, and in particular metallopeptide/protein design and engineering.

2. *Xeno*-metals

Although metal ions are essential for many biological processes, Nature selects from a rather limited range. Contrast this with the inorganic chemists' toolbox, which has the breadth of elements across the Periodic Table to choose from, and consequently the potential to develop systems with a much more diverse range of properties and potential applications. This section focuses on some key examples where *xeno* binding sites, inspired by non-biological Inorganic Chemistry, have been engineered into a CC scaffold.

2.1. Lanthanides

One class of *xeno*-metals (with only a single example of a biological role reported[6]) are the lanthanide ions. Having a similar size and bonding preference, Ln(III) ions are often capable of substituting Ca(II) sites to yield Ln(III) metallopeptides, in which the attractive photophysical and magnetic properties of the Ln(III) can be exploited.[7] Of relevance to this focus review, is the early work by Hodges and

co-workers[8] who reported the *de novo* design of a two stranded CC, bridged by a disulphide bond (owing to Cys residues in position two), for Ln(III) coordination. In the apo form the inter-helical ionic repulsions across the dimer interface, between negatively charged carboxylic groups in the *i* and *i'+5* positions (*e* and *g* sites), prevent peptide folding. However, Ln(III) binding to these glutamic acid (Glu, E) residues, neutralizes the repulsive charges and instead bridges the two Glu, triggering CC formation. Only a modest increase in folding was observed, due to the Ln(III) affinity for the site being relatively weak.[8b] A subsequent study using the non-proteogenic amino acid, γ-carboxyglutamic acid (Gla, see section 3.1), in place of Glu, led to both a greater Ln(III) affinity and associated increase in folding.[7e]

The first report of a Ln(III) site generated within a CC interior, involved the use of the non-proteinogenic Gla,[7e] and again focused on how Ln(III) coordination induced CC formation. Whilst Ln(III) binding was achieved, no detailed analysis of coordination chemistry was reported. More recently, we reported the design of a Ln(III) binding site within a CC interior using natural asparagine (Asn, N) and aspartic acid (Asp, D) residues (Figure 1). All Ln(III) ions tested (including Tb(III), Gd(III), Ce(III), Nd(III), Eu(III), Dy(III), Er(III) and Yb(III)) were found to bind.[9] Furthermore, we interrogated the coordination chemistry, and despite finding no evidence of inner sphere water, often assumed to be a prerequisite for Gd(III) MRI contrast agents, the Gd(III) CC, the first ever to be reported, displayed superior MRI relaxivity than the clinically employed Dotarem.[9] This example illustrates how a *xeno*-metal can be introduced, so that the resulting metallo-CC can be explored for a non-biological application, in this case medical imaging.

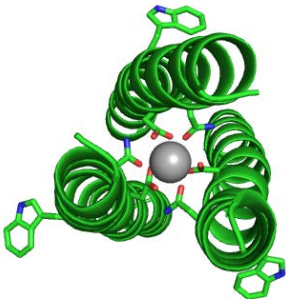


Figure 1. Pymol model of a designed Ln(III) binding site within the hydrophobic core of a three-stranded CC. Shown are the main chain atoms represented as helical ribbons (green), the Asn, Asp and Trp side chains in stick form (oxygen in red and nitrogen in blue), and the Ln(III) ion as a grey sphere.[9]

2.2. Uranyl

To the best of our knowledge, the only actinide so far to be coordinated to a CC is uranium. Uranyl (UO_2^{2+}), the predominant aerobic form of uranium, is present at a relatively high concentration in seawater. Whilst the vast array of metals with a similar size and charge to uranyl make it difficult to sequester effectively, a helical bundle has been engineered to selectively bind the uranyl with femtomolar affinity, and unprecedented selectivity (Figure 2).[10] A pentagonal bipyramidal binding site was computationally designed into the interior of a three-helical bundle, and has been verified experimentally from the crystal structures of both the apo and uranyl-bound forms (see Figure 2).[10] The fact that a protein can be designed to display such impressive selectivity for a *xeno*-metal, is an exciting achievement for the community.

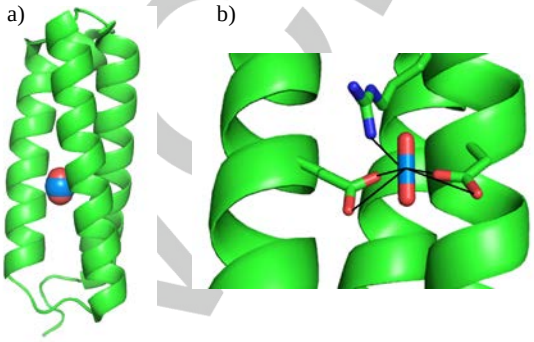


Figure 2. Pymol image of a) the designed uranyl bound helical bundle; b) the uranyl ion binding pocket showing the pentagonal bipyramidal geometry. The main chain is shown as a helical ribbon (green), with the uranyl ion represented as a) spheres or b) in stick form (uranium in light blue and oxygen in red), the binding residues Asp68, Arg71 and Glu17 are also shown in stick form (nitrogen atoms in blue). Based on PDB code 4FZP.[10]

2.3. Rhodium

Ball and co-workers have extensively investigated dirhodium metallopeptides for catalysis,[11] in an effort to couple the catalytic activity of Rh(II) complexes, to the selectivity commonly encountered in enzymes, so as to achieve reactivity inaccessible to traditional transition metal catalysts.[11] Rh(II) carboxylates, bound through peptide Glu and Asp side chains, were found to be stable and catalytically active in water.[11b] Furthermore, by careful design of the peptide component, specifically the use of CC domains, designed dirhodium peptides were able to couple molecular recognition with catalytic activity, so as to allow for the formation of site-specific chemical modifications. Changes to the metallopeptide sequence, which altered recognition patterns, were found to have a strong effect on catalytic selectivity, and could therefore be used to control the chemistry of the coordinated *xeno*-metal complex.[11c] One potential limitation of these designs is the use of Glu and Asp residues for Rh(II) coordination, which are abundant in proteins and might therefore limit the selective introduction of the dirhodium catalyst into more complicated peptide assemblies.

2.4. Ruthenium

Ruthenium is widely employed in inorganic chemistry, with applications including, but not limited to, anticancer drug design, catalysis and photochemistry. Two main routes to the synthesis of Ru(II) coordinated CCs, involve the introduction of either 1) an intact Ru(II) complex, or 2) a non-standard amino acid for subsequent Ru(II) coordination (see section 3.2).

Our first example features the introduction of an intact ruthenium complex, for the photochemistry it affords. A photolabile biselectrophile Ru(II) complex, Figure 3, that can be used for homo- and hetero-dimerization of Cys containing peptides, was reported by Mascareñas and co-workers.[12] The bromine-functionalized Ru(II) complex was used to assemble homo- and hetero- two stranded CCs, based on DNA binding bZIP derivatives, reversibly using light.[12]

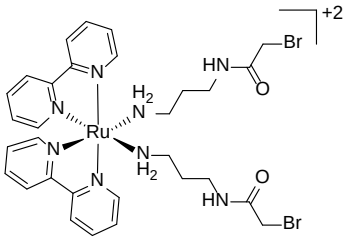


Figure 3. Structure of the electrophilic Ru(II) linker developed by Mascareñas and co-workers.[12]

Ogawa and co-workers similarly introduced a [Ru(bpy)(phen-ClA)] complex (where bpy and phen-ClA correspond to 2,2′-bipyridine and 5-chloroacetamido-1,10-phenanthroline, respectively), covalently into a CC by alkylation of a Cys side chain.[13] Intriguingly, when the Cys residue was located at the helical interface (but not when located on the CC exterior), the resulting metallopeptide diastereomers (altered chirality of the Ru(II) complex) could be separated by reversed phase-HPLC, proposed to be due to the restricted conformational environment.[13a] The authors also introduced a pentamine ruthenium complex through direct coordination to a His. Using these two different approaches the two different ruthenium redox active complexes could be selectively introduced at opposite ends of the CC. These systems were subsequently used to demonstrate that the rate of electron transfer was independent of helix dipole direction.

Further examples of ruthenium complexes introduced by complexation with non-proteinogenic amino acids, will be discussed in section 3.2.

2.5. Gold

Auranofin, [AuCl(PEt$_3$)], a drug currently used in the clinic to treat rheumatoid arthritis, has shown promising anticancer and HIV-activity.[14] The [Au(PEt$_3$)] fragment has been coordinated to peptides including a CC, through reaction of [AuCl(PEt$_3$)] with a Cys side chain under basic conditions. Coordination was found to be reversible with pH, and could represent a potential trigger or activation mechanism for the delivery of the potentially active [Au(PEt$_3$)]$^+$ species.[15]

Rather than the introduction of a single metal ion or complex, it has also been possible to generate different Au(0) nanoclusters within the interior of peptide trimers, tetramers and hexamers.[16] These were found to be able to accommodate six, eight and twelve Au(I) ions respectively, and on addition of a reducing agent, a series of highly stable, photoluminescent Au(0) nanoclusters were prepared.[16]

Finally, CC formation can also be used to assemble Au-nanoparticles. Two halves of a two-stranded CC, one basic and one acidic, were attached to Au-nanoparticles via a terminal Cys. CC formation and associated Au-nanoparticle assembly, could be controlled under mild conditions. Furthermore, peptide design could be used to generate structures with differing stabilities. CC assembly could offer a means by which new materials and nano-technological devices could be developed.[17]

2.6. Metallo-porphyrins

DeGrado and co-workers computationally designed nanostructure metalloporphyrin arrays of varying length based on the α-helical CC motif.[18] Antiparallel tetramer CCs with two or four non-biological DPP-Fe(II/III) (DPP = 5,15-di[(4-carboxymethyleneoxy)phenyl]porphinato) cofactors, were designed with specifically placed metalloporphyrins.[18a] The variation in length and positioning, allows for the tuning of the electrical and optical properties of the porphyrin arrays, and these designs were found to display functional redox properties previously unobtainable with natural cofactors.[18b]

The same group also incorporated two *xeno* iron diphenylporphyrins into a transmembrane CC, thereby creating a multicentered pathway for transmembrane electron transfer, important for photosynthesis and ATP production.[19] The designed transmembrane CC scaffold was again used to position the redox-active cofactors sufficiently close for electron transfer.[19]

Whilst most metallo-porphyrins contain Fe(II/III), there are examples with alternative metals. Four stranded CCs were also found to bind Zn(II)-porphyrins[20] in the interior of the scaffold, and one example of a Co(II)-porphyrin[21] sees it binding to the exterior of the assembly. An advancement in the incorporation of porphyrins into peptide scaffolds, saw Fairman and co-workers create a system that allows for different metallated porphyrins to be incorporated, by taking advantage of non-covalent binding between the porphyrin pendant groups and amino acid functional groups.[22] An anionic porphyrin, *meso*-tetrakis(4-sulfonatophenyl)porphine (TPPS$_4$), was introduced in this way, as the conducting material along the CC, through favorable electrostatic interactions between Lys side chains and three of the four sulfonate groups on the porphyrin.[22]

The above examples demonstrate how CCs are sophisticated scaffolds with which the supramolecular assembly of different metalloporphyrins can be achieved in a highly controlled fashion. The resulting x*eno*-porphyrin CCs are promising for the design of new photoelectronically active biomaterials.

3. *Non-proteinogenic amino acids*

An important advantage of *de novo* designed peptides, is that they tend to be amenable to solid-phase peptide synthesis

(SPPS), which allows for the introduction of non-standard, amino acid building blocks. This represents an important opportunity for protein engineering, and may provide a mechanism by which synthetic biology can surpass natural evolution. Though the use of non-proteinogenic amino acids is extensively reviewed elsewhere,[5c] of relevance to this focus review are the use of non-proteinogenic amino acids in CCs, which either feature ligands for metal ions, impact on its coordination chemistry, or through which intact complexes can be introduced.

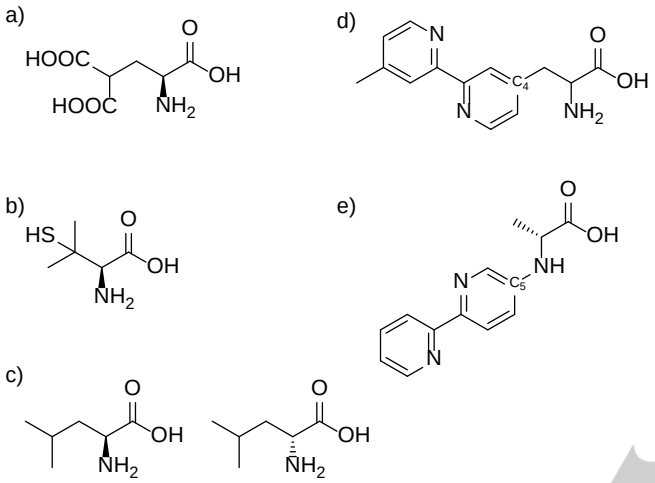


Figure 4. Some examples of non-proteinogenic amino acids used in metallo CCs; a) γ-carboxy glutamic acid (Gla), b) L-penicillamine (L-Pen), c) residues with altered chirality (L- and D-Leu), d) 2-amino-3-(4'-methyl-2,2'-bipyridin-4-yl) propanoic acid, and e) (2,2'-bipyridin-5-yl)alanine.

3.1. Ligands for Ln(III) ions

As described in section 2.1, a number of designed Ln(III) binding sites have employed the non-proteinogenic amino acid, Gla (Figure 4a).[7e,8b] Gla can be viewed as a derivative of Glu, which contains an additional carboxylate entity, and importantly is compatible with CC design, having been successfully introduced at both the CC helical interface, and buried within the hydrophobic core.[7e,8b] It is due to both its higher charge and denticity, that Gla (compared to Glu) binds Ln(III) tightly and selectively over Ca(II). The latter being important in view of the biological toxicity associated with the majority of free Ln(III) ions.

With protein structure and folding at the focus of peptide design, Samiappan *et al.*[23] explored the allosteric effects associated with CC folding and Ln(III) binding, by introducing Ln(III) chelating groups (hydroxyl-pheny oxazoline), either through the N-terminus or ε-amine of a lysine (Lys, K) side chain. They observed the formation of partially folded trimeric CCs, the formation of which induced Ln(III) coordination, which in turn yielded a stabilized 3D structure.[23]

3.2. Heterocyclic derivatives

Considering the prevalence of 2,2'-bipyridine (bpy) and other heterocyclic ligands in 'traditional' inorganic chemistry, it is not surprising that non-proteinogenic amino acids containing bpy side chains, are routinely used for the incorporation of *xeno*-metal binding sites within peptide scaffolds.[24] Though biology features a heterocycle, His, within its tool-box, the chelating bpy can be used to form more stable complexes, and allows one to reproduce interesting 'traditional' inorganic chemistry complexes. Whilst bpy attachment at position 6 leads to steric hindrance and poor metal binding,[25] when bound in positions 4 or 5, metal binding is readily achieved (Figures 4d and 4e).[26]

Bpy is a common ligand in ruthenium coordination chemistry, and Ru(II) coordination has been used to trigger CC assembly. Similar to the Ln(III) induced folding work described in section 2.1 and 3.1, Ghadiri and co-workers were the first to report how a 15 residue peptide with a bpy located at its N-terminus, could spontaneously self-assemble in the presence of Ru(II), to yield a stable three-stranded CC on formation of a $Ru(bpy)_3$ unit.[27] The latter being robust due to the slow ligand exchange kinetics associated with Ru(II).

3.3. Penicillamine and D-amino acids – modification of steric bulk

Pecoraro and co-workers have extensively studied the coordination of Cd(II) to thiolate sites engineered into CCs.[28] They have also studied the use of non-proteinogenic amino acids as a means by which one can control steric bulk, and thereby the coordination chemistry and physical properties of these sites. For example, replacing L-Cys with L-penicillamine (Figure 4b), a bulky analogue with methyl groups in place of the β-methylene protons, enhances the bulk around the metal centre, to yield a coordinatively unsaturated trigonal planar CdS_3 site (excluding water molecules from coordinating).[29] The same result could be achieved by altering the chirality of second sphere amino acids (L-Leu vs D-Leu, see Figures 4c and 5), reorientating the non-coordinating Leu towards the metal binding site and again excluding the coordination of water molecules to generate the CdS_3 site.[30] These examples demonstrate how non-standard amino acids can be used to modify steric bulk, when introduced in both a coordinating and non-coordinating site within a CC, so as to control metal ion coordination chemistry and related properties.

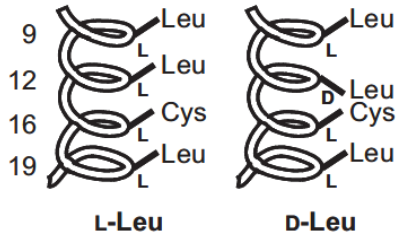


Figure 5. Chemdraw schematic showing the increase in steric bulk around the Cys16 residue on mutation of L-Leu12 to the D-Leu12 derivative. © 2008 by The National Academy of Sciences, USA.[30]

3.4 'Click' chemistry

'Click' chemistry is becoming routinely employed, including in the peptide design community, for the covalent linking of two units by an easy and efficient reaction, such as the azide alkyne Huisgen cycloaddition catalyzed by copper. Not surprisingly non-proteinogenic amino acids with side chains which feature either the alkyne or azide, have been developed for use in SPPS. For example, Waters and co-workers introduced Ru(II) and Os(II) complexes, covalently linked to the CC via azide-alkyne 'click' chemistry, at well-defined distances from one another.[31] The shape and rigidity of the CC scaffold allowed them to control the spatial alignment between the two centers, positioning the chromophores for efficient energy transfer, a theme which has previously been explored in this focus review[31]

4. Conclusions and perspectives

Using a *de novo* or "first-principles" approach to designing metallopeptide constructs, is a rapidly developing field, with metallo-CCs an attractive intermediary between 'traditional' small molecule complexes and complex native metallo-proteins. Though much effort has been directed towards using this approach to develop mimetics of native metallo-protein binding sites, here we show the increased interest in the study of *xeno*-, or non-biological, metal binding sites. The resulting *xeno* metallo-CCs can feature examples of non-biological metal ions, such as lanthanides, gold and rhodium, in an effort to introduce the chemical properties afforded by these metals, or may instead harness the control afforded by CC assembly. Alternatively, non-proteinogenic amino acid building blocks have been introduced, which either feature; non-biological ligands for metal ion coordination, so as to be able to generate mimics of 'traditional' small molecule inorganic complexes; chemical functionality with which to introduce non-biological metal complexes; or altered sterics to control metal ion coordination chemistry. As a result of these efforts, *xeno* metallo-CCs, which are beginning to explore the breadth of inorganic chemistry, have been reported with a wide range of potential applications, ranging from medical imaging, non-biological catalysis and photoenergy conversion. Indeed new, currently unforeseen applications are likely to come from adopting this approach, and this therefore represents an exciting area of metallopeptide design.

Acknowledgements

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Keywords: Bioinorganic Chemistry • *De Novo* Coiled Coils • Metalloproteins • *Non-Proteinogenic* Amino Acids • *Xeno*-Metals

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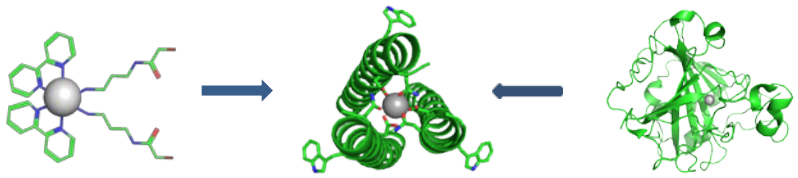

*Louise N. Slope and Anna F. A. Peacock**

Page No. – Page No.

De Novo Design of _Xeno_-Metallo Coiled Coils

Expanding the bioinorganic chemists toolbox. The use of *xeno*-metals and non-proteinogenic amino acids in *de novo* peptide design, will allow for the assembly of new bioinorganic complexes which should combine the advantages of both inorganic chemistry and biology into a single hybrid.