Synthetic approaches to rational drug design

a changing landscape

It is becoming clearer that newer synthetic approaches for rational drug design may hold the promise of taking us closer to industrialising drug discovery while, at the same time, overcoming the bottleneck of combinatorial discovery processes.

By Dr Shubh Sharma and Dr Carl Spana

The sequencing of the human genome was indeed an epoch-making endeavour that captivated the imagination of all humankind. The speed and swiftness with which it was accomplished also dazzled the scientific community. To many, it was a reflection of a high level of technological prowess who envisioned that the day would not be far off when all this would transcend to a robust medicine cabinet to cure the ills of infectious, autoimmune, as well as metabolic, disorders. After the dust settled, however, it quickly became apparent that the current tools of drug discovery lack the same power and fidelity to progress from a linear sequence of nucleotides into a dynamic disease-state-related, three-dimensional readout for developing a drug. Industrialisation of the drug discovery process, a phrase newly minted in the post-genomics era, can only be ushered in by a whole new set of very smart and creative target identification and drug design technologies. Such technologies must lift the weight of current non-linear human thought processes that often go into the drug design processes.

Combinatorial chemistry techniques perfected during the past decade for rapid identification of a lead molecule against a given target can be viewed as the first bold attempt towards industrialisation of drug discovery. Both chemically and biologically obtained libraries of highly diverse sets of an overwhelming number of compounds can now routinely be generated and screened in high throughput (HTS) assays. These libraries include peptides, peptidomimetics and small molecules. Peptide libraries are the most versatile in finding a lead against any target, irrespective of whether the natural ligand for the target is a peptide or non-peptide. Small molecular libraries often suffer from a lack of required diversity and quality which often is impacted by fidelity of the chemical processes for the synthesis of these libraries. Therefore, while these approaches can routinely generate weaker leads against most of the targets with a very low hit rate, it is now well recognised that these approaches most often lead to identification of antagonists. Also, it is now increasingly realised that a variety of newly discovered targets are recalcitrant to random approaches of combinatorial drug discovery processes. The combinatorial drug discovery process, therefore, has not fully lived up to its promise of industrialising drug discovery. However, combinatorial processes are extremely successful in lead optimisation rather than lead generation.

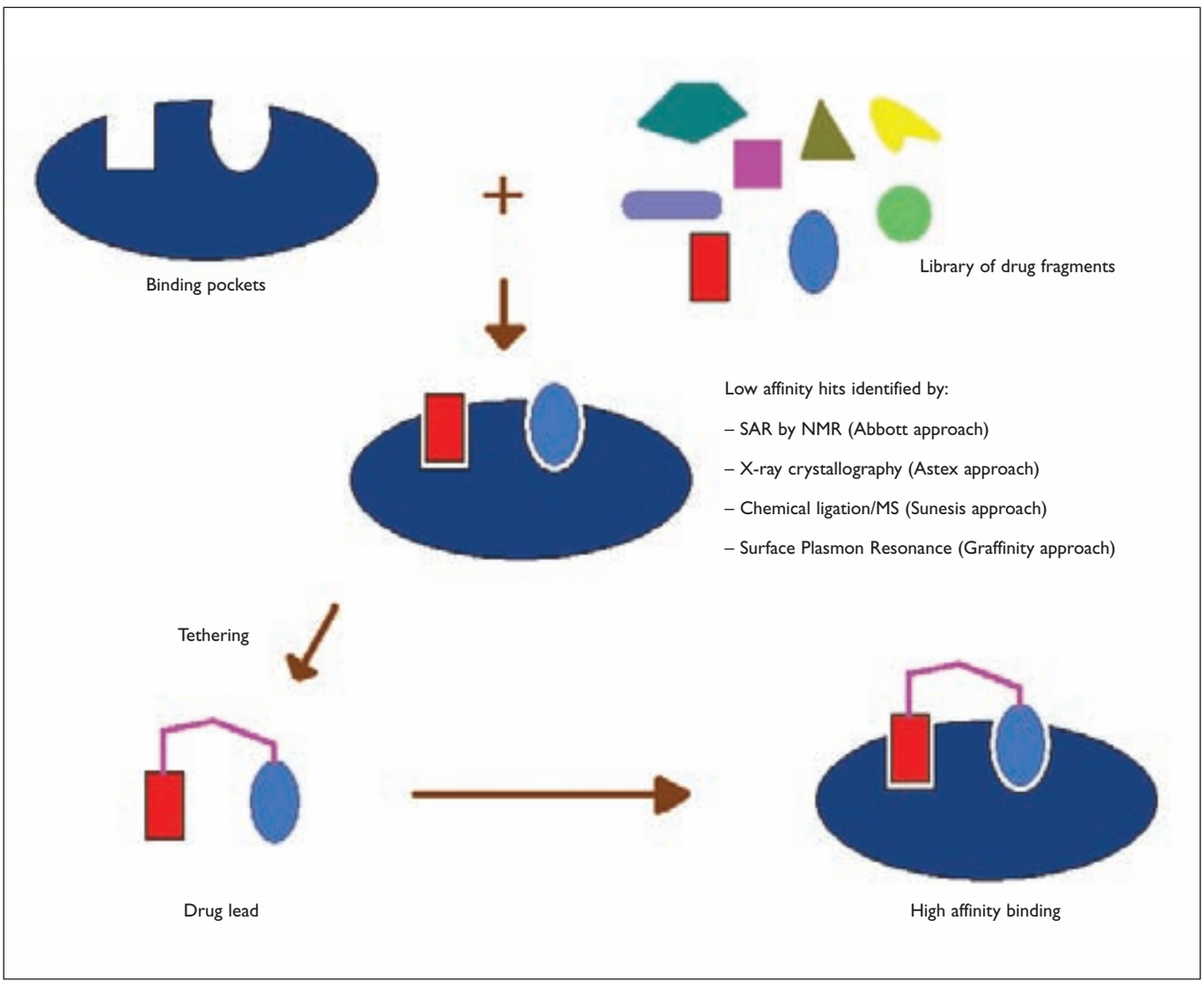

Figure 1
Conceptual outline of various tethering-based technologies. Each technology employs a unique approach to identify the weak chemotype hits

In this post-combinatorial chemistry era, the industry has placed an emphasis on rational design of drugs. A number of innovative, rational drug design approaches have been invented with the potential of much wider applicability to allow routine discovery of target-based leads. The central theme of all these approaches is to discern a three-dimensional chemical space preferred by the drug molecule. The starting point, the approach and the choice of a technique to conceptually map the so-called pharmacophore vary from one technology to another. The most notable of these approaches are reviewed here. These include a novel metallopeptide-based drug design (MIDAS™) approach using a peptide as a starting ligand and a number of technologies for *de novo* identification of bits and pieces of a pharmacophore (individual drug molecule fragments or chemotypes) that are then tethered together into a complete drug molecule (**Figure 1**).

SAR by NMR as a tool for drug design

Stephen Fesik and his colleagues at Abbott Laboratories (Abbott Park, Illinois, USA) pioneered a fascinating technology for *de novo* design of small molecule drugs using NMR as a structure-based pharmacophore mapping tool[1]. The approach termed 'Structure Activity Relationship by NMR' is based on mapping a set of individual small chemotypes to their individual small binding pockets at the active site of a protein. Using NMR, weak interactions established by each chemotype with the binding pocket can be picked up which usually cannot be measured by standard ligand-binding assays. Using this approach, first a map of key hot spots

on the target protein is established based on their interactions with a known natural or similar other ligand in an NMR study. These NMR studies performed using an all N-15 labelled soluble protein allow an unambiguous mapping of these interaction sites. Appropriate key NMR signals altered upon protein-ligand interaction are picked to design a SAR study using a set of small molecule chemotypes. The small molecules used here are selected randomly as potential mimics of putative fragments of a soon-to-be-designed drug molecule. Two or more chemotypes species that interact with different binding pockets in the protein with low affinities are identified and then tethered together using appropriate chemical linkers and spacers. The combined molecule after optimisation of the chemical linkers usually turns out to be a potent low nanomolar ligand. The SAR by NMR is applicable to well-characterised protein targets that can be cloned and produced as N-15 labelled proteins in large quantities to allow several NMR studies with different chemotypes. However, the prerequisite here is a structural model of the target protein for developing *in silico* screening parameters.

Virtual SAR by NMR

Refinements to SAR by NMR have led to the development of *in silico* screening and scoring algorithms where chemotypes are individually docked in the binding pockets either alone or in tandem. For example, Peter Rose and colleagues (Agouron Pharmaceuticals Inc, La Jolla, California, USA) have validated this approach experimentally using FK-506 binding protein and stromelysin[2]. The best fit set of chemotypes, in this case are also tethered together to form a potent lead molecule. Virtual screening of chemotypes alleviates some of the protein-related handicaps of experimental SAR by NMR technique.

The Sunesis chemical capture and tethering technology

Sunesis Pharmaceuticals (South San Francisco, California, USA) has pioneered a similar tethering approach. Instead of NMR, this approach relies on a clever chemical ligation of a chemotype fragment molecule to a cysteine residue specifically placed in the target protein sequence[3]. This approach identifies few low molecular weight chemotype compounds (approximate MW250) that bind to different sites in a target protein. These individual species representing pharmacophore fragments of a ligand are then tethered together into one biologically potent lead molecule. The technology involves a small molecular library (8-15 members) of thiol-reactive chemotype compounds that

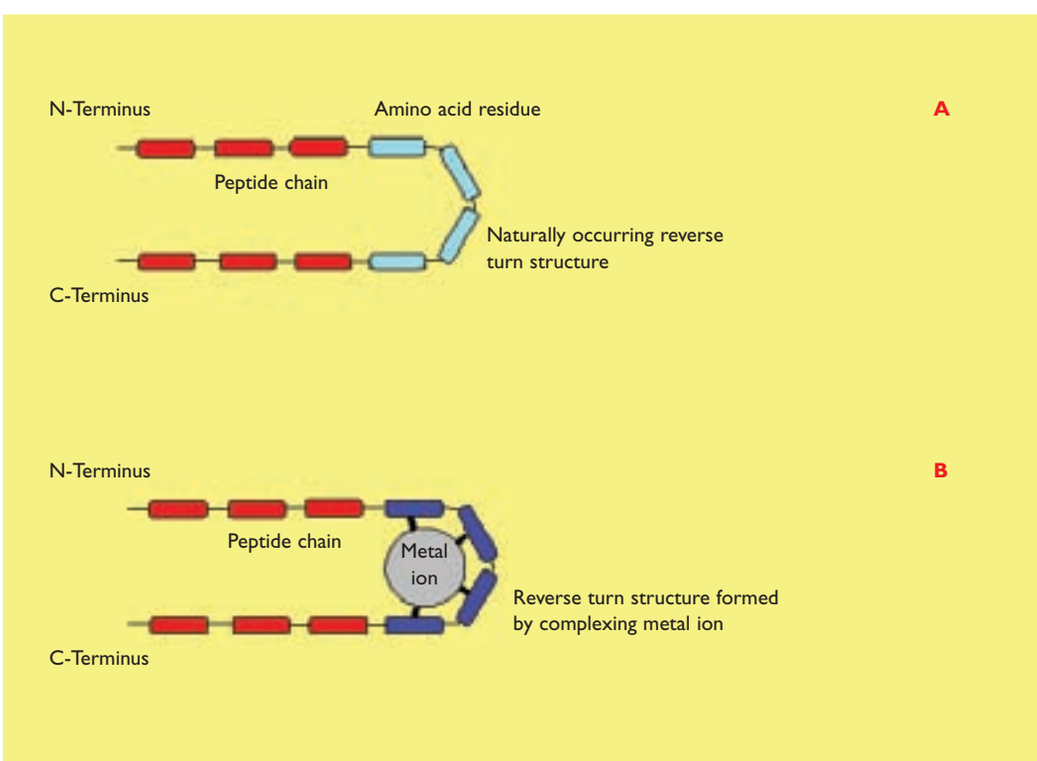

Figure 2
MIDAS mimics and deciphers the natural paradigms of biologically active peptides

are exposed to the cysteine-engineered target protein to select and chemically ligate a best fit chemotype molecule. The identification of the ligated molecule is made by subsequent mass spectrometric analysis of the targeted protein-chemotype conjugate. This approach has been validated on several protein targets, such as thymidylate synthase, capsase, IL-2, etc. As with SAR by NMR approach, this technology is applicable to well-characterised single chain target proteins that can be produced with a cysteine mutation at a suitable position in its sequence.

Surface Plasmon Resonance (SPR)-assisted screening of chemotype microarrays

Graffinity Technologies (Germany) has also pioneered another tethering technology in which individual drug fragment chemotypes are identified using a change in surface plasmon resonance upon binding of a chemotype anchored on a gold-coated glass chip to a target protein[4]. One such chip contains as many as 9,216 microarrays of various fragment chemotypes that are carefully organised in pre-determined spatial locations. The interaction of a soluble target protein with one or more chemotype species as discerned by SPR, leads to identification of its structure. The fragments are then tethered together to form the drug lead. As with all the other techniques described above, this approach is only applicable to soluble proteins.

X-ray crystallography approach for discerning drug fragment chemotypes

Astex Technologies (Cambridge, UK) is utilising x-ray crystallography to examine the interaction of target protein-drug fragment chemotype complex[5,6]. HTS methods of x-ray crystallography are utilised to study an array of protein-chemotype complexes that bind specific binding pockets. Appropriate chemotypes binding adjacent binding pockets are then tethered together to design an optimised drug molecule.

Metallopeptide approach of rational drug design

Palatin Technologies Inc (Cranbury, NJ, USA) has pioneered an entirely new concept in peptide-based

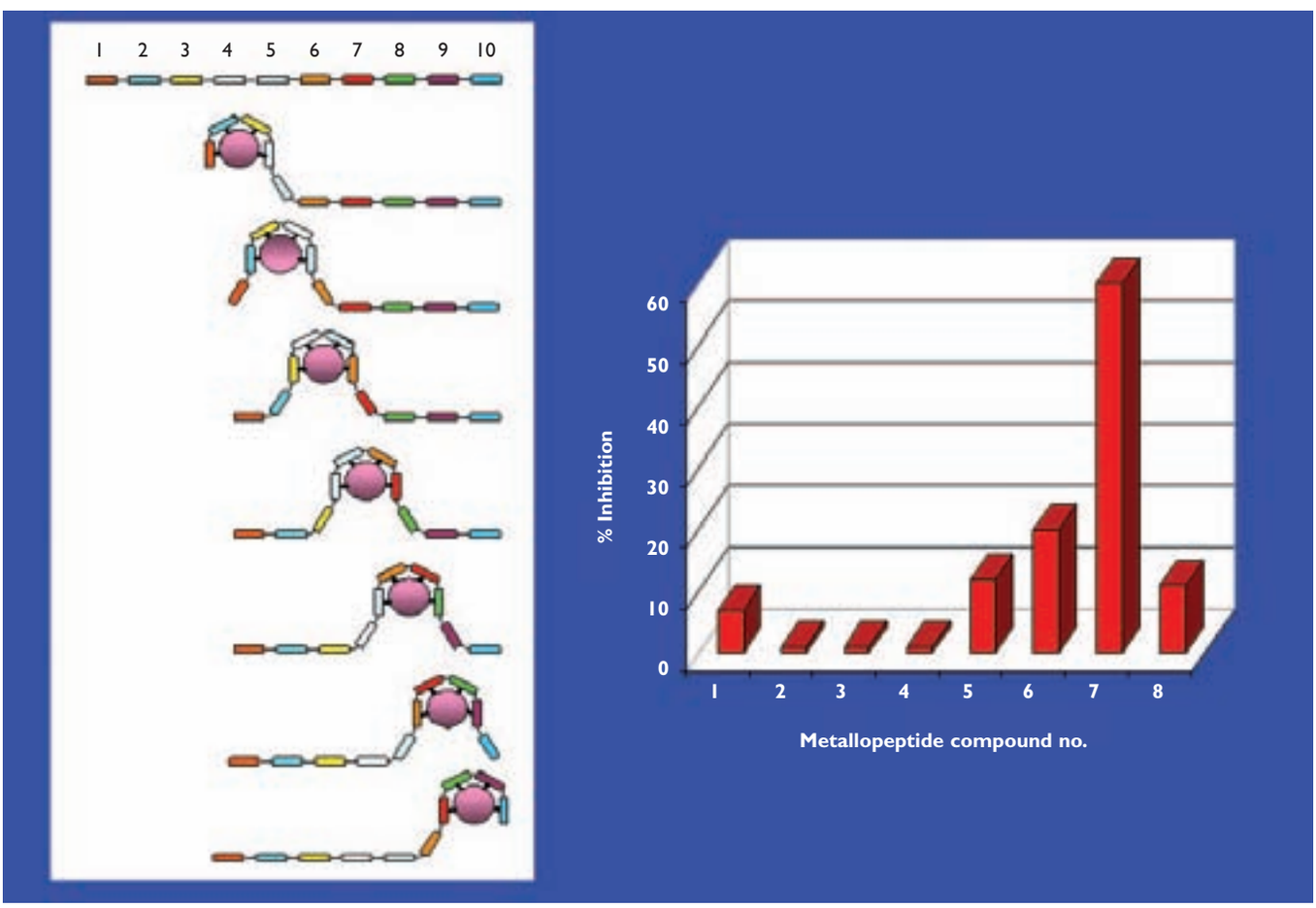

Figure 3
Conformational walk allows for pre-organising unique structural motifs at each amino acid position (left). Note the unique presentation of a colour-coded amino acid in each compound to the biological target. The graph (right) showing a typical readout from a conformational walk series in screening assays. Usually one molecule with its unique structural organisation is biologically active

drug design. Its MIDAS™ (Metal ion Induced Distinctive Array of Structure) technology is a synthetically-driven approach of rationally transforming peptides into stable peptidomimetics as well as small molecular drugs[7,8].

Unlike contemporary methods of rational drug design, MIDAS does not require any of the NMR, x-ray, *in silico* computer modelling approaches or related physiochemical tools. Instead, MIDAS approach is based on creating privileged templates of pre-determined structures that are essentially derived from amino acid side chains and peptide backbones and inducting them into a peptide of interest. Site-specific complexation of a metal ion to the peptide backbone is used to create one such template. This easily creates a very rigid template where the amino acid side chains remain flexible to establish contact with the binding site of the target. The structure of such a template is pre-destined based on well-established geometry of the metal ion co-ordination sphere. It has been shown that these discrete sets of metallo-peptide templates as assembled in this manner resemble in certain aspects certain secondary structural characteristics found in peptides and proteins. In particular, metallopeptide templates can mimic the topology around a hairpin turn in peptides (**Figure 2**). This analysis has qualified these templates as viable scaffolds for drug design.

Furthermore these metallopeptide templates have been used in a unique manner to map the pharmacophore determinants in a biologically active peptide. The approach, termed a conformational walk, calls for synthesising all the possible metallopeptides for a given starting peptide by placing a metal ion at each of the amino acid positions in the peptide and screening these against the given receptor. This generates a very small discrete set of molecules, each presenting a unique metal-ion-induced secondary structure to the receptor site (**Figure 3**). Each individual amino acid side chain in this series of metallopeptide has the opportunity of presenting itself to the receptor site in a specific rigid configuration. Within such a series of metallopeptides, usually one of the molecules with its discreet secondary structure is biologically active thereby defining a biologically relevant pharmacophore (**Figure 3**). The structure and topology of this molecule is pre-determined based on the co-ordinates of the metal ion. Because of rigidity of the metallopeptide scaffold, the theoretically discerned structural parameters are precise enough to enable transformation of these into small molecules (**Figure 4**). The conformational walk approach is a robust method of determining the site of structural organisation in a peptide as well as identification of the key amino acid side chains that are crucial for biological activity. Typically a very small number of molecules (10-100) need to be synthesised to develop a potent lead molecule.

One of the most interesting aspects of the conformational walk approach of MIDAS is that this technology is a turn key approach that has wider applicability. All that one needs as a starting point is the linear amino acid sequence of a peptide and the rational synthetic approach of MIDAS discerns its three-dimensional pharmacophore. Starting peptide used here could be a natural peptide, a potent analog thereof, or a peptide lead previously identified through other well-established technologies such as phage display peptide libraries. Further, it is noteworthy that as compared to the other technologies described above, MIDAS is applicable to all sorts of biological targets such as soluble, membrane-bound single chain or multiple chain protein targets. Also, unlike most tethering technologies described above, application of MIDAS does not require isolation or purification of the target proteins.

In order to drive this synthetically-driven platform technology efficiently, robust methods for the synthesis of MIDAS metallopeptides have been developed. Both solid and solution phase synthetic methods have been optimised that allow for high throughput synthesis of metallopeptides using automated peptide synthesisers. MIDAS molecules can be developed simultaneously in radioactive as well as non-radioactive versions. This is possible by the use of a radioactive isotope of the metal ion for complexation to the peptide. The metallopeptides, like most peptides and organic compounds, are a chemically stable class of compounds. Unlike most peptides, however, these compounds are highly resistant to proteolysis. These compounds, therefore, can be used *in vivo* for efficacy and target validation processes. In certain therapeutic areas, these characteristics of MIDAS molecules may even allow their use as drug candidates.

The MIDAS technology has been successfully applied to a variety of drug targets that include enzymes and GPCRs. A variety of targets recalcitrant to combinatorial drug discovery, have been successfully approached by MIDAS. Target-based anti-inflammatory, anti-obesity and anti-cancer drug leads developed using this technology is currently in pre-clinical development. These include

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development of potent agonists and antagonists for the melanocortin family of receptors (MCRs). This family of receptors includes MC-1R, MC-3R, MC-4R and MC-5R that are considered valid targets for treatment of inflammatory diseases as well as feeding disorders. It remains to be seen if the metallopeptide approach is applicable to large size peptides with multiple structural motifs.

It is becoming clearer that the newer techniques of rational drug discovery may hold the promise of taking us a step closer towards industrialising drug discovery. Several of the approaches described above overcome the bottleneck of combinatorial discovery processes. There are several hurdles still to overcome. Approaches need to be developed for addressing multiple subunits and membrane bound targets. The MIDAS approach holds a tremendous promise towards streamlining peptide-based drug design due to its rational concept, wider applicability, simplicity and turnkey approach. DDW

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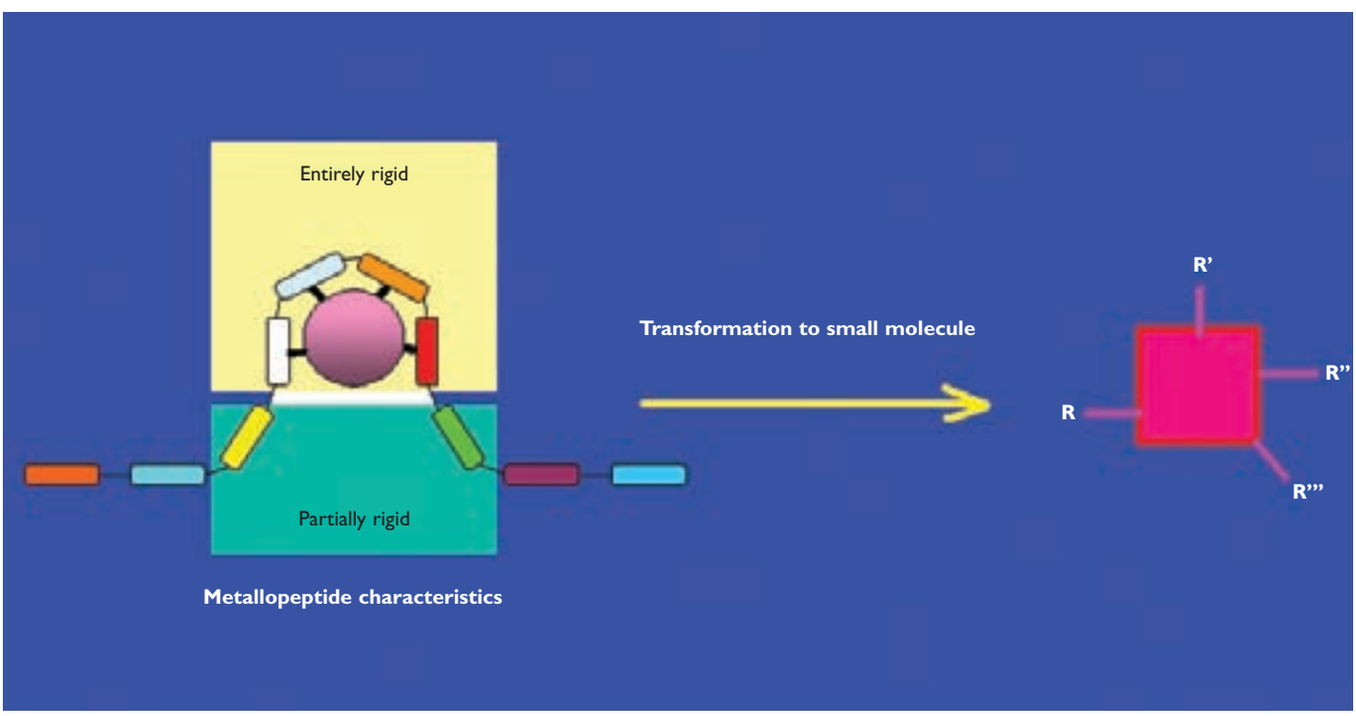

Figure 4
Rigid structure of metallopeptide provides fairly precise atomic co-ordinates of the biologically active structure of a peptide that are used to develop small molecule drugs