

Study into drug discovery and design

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Introduction

1. Background

Drug discovery and design is fuelled by the need for appropriate and effective treatment for disease. Initially discovery was achieved via empirical screening of vast libraries of molecules, which was incredibly effective. The majority of drugs currently in clinical use were discovered this way. However with increased technology, and a greater need for newer more effective medicines, structural biology has become a prominent tool.

The general principles behind drug discovery briefly discussed here include target identification and validation, and hit discovery or design to generate a lead which is then optimised.

1.1 Target Identification and Validation

A target is often a protein, however it can also be RNA, DNA or a carbohydrate. People who suffer degenerative, autoimmune and genetic diseases can be screened for genetic differences through genome wide association studies (Grupe et al., 2007) or systematic meta-analysis (Bertram et al., 2007). Infective organisms have genes that are very different to human genes that may be essential in the life or infective cycle of the organism and are thus useful targets that can be identified through bioinformatics analysis or loss of function mutant phenotype studies (Crellin et al., 2011). A structure-based technique includes structural genomics, which is the study of the structures of all proteins in a genome.

1. 2 Hit Identification and the Generation of a Lead Series

Once a target has been identified and validated, small molecules that bind and in some way alter function must be discovered or designed, again there are a number of ways in which this can be done. Empirical screening has identified a number of drugs, however structure-based techniques are more and more commonly being used. 3D structures from X-ray crystallography data and, to a lesser extent, nuclear magnetic resonance (NMR) have been used to generate the information required for computational methods involving docking and screening. This has been useful, for example in the in-silico screening of G-protein coupled receptor (GPCR) binding molecules (Richardson et al., 2007), however most structure-based drug designs have come from the design of compounds based on the 3D structure obtained from X-ray crystallography or NMR, or via biophysical screening techniques involving surface plasmon resonance (SPR) or NMR. Structure based screening methods often require fragment based libraries. These encompass a greater number of potential molecules, within smaller libraries of compounds, this is possible because there are no large functional groups that would inhibit binding, and so result in attractive starting points for hit discovery (Nordstrom et al., 2008).

To validate or measure the properties of the hits, crystal structures can be evaluated and additional information from secondary SPR screens, thermal information from isothermal titration calorimetry (ITC), and differential scanning fluorimetry (DSF) can be used to complement the data (Retra et al., 2010).

1.3 Lead Optimisation

Once validated, the structure is optimised. This can be based on ligand binding structures in NMR and X-ray crystal structures, or increasingly, in-silico modelling based on the pharmacophore hypothesis involving the evaluation of chemical and functional groups that may bind important sites of the target molecule (Voet et al., 2011).

The key structural techniques involved in structure-based drug design are X-ray crystallography and NMR, though mass spectrometry can also be used to observe proteins in multi-protein complex interactions. X-ray crystallography generates 3D structures of the protein of interest from crystals generated by altering conditions such as buffers, pH, temperature and the format; nanodrop, hanging drop as well as others (Giege & Sauter, 2010). These crystals are homogeneously packed and stored in cryo-protecting buffers so that they can be stored in liquid nitrogen which protects them from the X-rays used to generate the 3D structural information (Philippopoulos et al., 1999). Once obtained, if the ligand of interest is soluble and has relatively high affinity for the target protein, co-crystallisation studies can be used to look at interactions of the different ligands of interest with the target protein. This is not always possible; however there have been improvements since the advent of fragment based libraries. NMR based structures are more time consuming to construct, requiring the analysis of NMR peaks of different spectra to associate them with specific nuclei to generate restriction information to produce a structure. Though more time consuming, it is incredibly useful if other forms of structural information are not available (Zou, 2007). In structure-based drug design NMR has been more useful in

ligand protein interaction studies (Pellecchia, 2005), but has also been used in screening libraries for hit molecules (C. Murray et al., 2010). Mass spectrometry can be used in each stage of drug discovery (Deng & Sanyal, 2006), especially as technology advances, however it is much more limited (justification?) than those methods or techniques already mentioned and so will not be discussed in any great detail.

To complement these techniques there are a vast array of technologies available, a few of which are mentioned below. SPR measures interactions between the target protein and potential hits in biosensors, and can also be used in hit validation and optimisation in secondary screens (Retra et al., 2010). Since fragment based screening, SPR has become much more popular and will be discussed in greater detail later. Other complimentary techniques involves ITC which measures entropy and enthalpy to determine their contributions in ligand interaction, and therefore gives a clue as to what sorts of alterations would be required to optimise binding. DSF is also widely used, more often to measure where the hit compound is binding on the target molecule (Domigan et al., 2009).

1. 4 Summary and Aims

To summarise, the stages of drug design include target identification and validation; hit identification and generation of the lead molecule; and then the optimisation of the lead into a drug for testing and then clinical trials. Target identification may utilise such structural techniques as structural genomics. Hit identification makes better use of structural information from X-ray crystallography or NMR and the design of drugs and computational in-silico drug design, or screening methods including high throughput screening

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(HTS), SPR library screens, and in silico computational screening methods. Optimisation generally uses structural information taken from X-ray crystallography as well as computational methods and in some cases NMR aided by SPR, DSF and ITC to increase binding affinity and then the pharmacokinetic properties.

To assess their usefulness in structure-based drug discovery and design, case studies will be analysed to look at how these techniques have been used to further the production of clinically used drugs, or at least increase our understanding so that we may be able to use it in future drug design attempts.

2. Case Studies

2.1 Nuclear Magnetic Resonance in Fragment Based Library Screening and X-ray Crystallography in the Design and Optimisation of Hsp90 Inhibitors

The heat shock protein 90 (Hsp90) is a human chaperone which is involved in stress responses, but is also required in the essential process of client protein maturation. Many of its client proteins are involved in cell signalling, proliferation and growth (Biamonte et al., 2010), which have been associated with a number of different cancers. The overexpression or inappropriate activation of Hsp90 is also therefore associated with cancer, therefore a number of drugs have been produced which aim to inhibit the essential ATPase activity of Hsp90. Hsp90's combinatorial in so many different client proteins makes it a good target for drug development, therefore many drugs are already available that target Hsp90. However there are a number of problems concerning bioavailability, toxicity and increased resistance and so newer more effective drugs are required (Van Montfort & Workman, 2009).

As can be seen in figure 1 Hsp90 is active as a dimer, and in the N terminal of each subunit is a functionally essential ATPase site (Prodromou et al., 1997), the middle domain regulates the interaction of Hsp90 with its client proteins (Meyer et al., 2003) and the C terminal region is responsible for dimerization (Minami et al., 1994).

Initially drugs for Hsp90 were discovered using the binding and cell based assays, however more recently there have been drugs that have entered clinical trials that were generated using structure-based techniques. These have targeted the ATP binding site essential for function, and so required a good understanding of this site. As can be seen in figure 2 there are critical hydrogen bonds between the adenine of the ATP bound, and the side chain of the amino acid residues Thr184 and Asp93 . These would therefore be ideal targets in the design of an inhibitor molecule (Obermann, 1998).

There are examples where inhibitors have been identified using NMR and X-ray crystallography screening methods of fragment libraries, and as has been described, fragment based libraries generate useful starting hits (Hartshorn et al., 2005). In an NMR fragment based library scan, the displacement of low concentrations of ADP (the product compound of the ATPase domain) was measured using NMR waterLOGSY (Water-Ligand Observed Via Gradient Spectroscopy) (Dalvit et al., 2001), which indicated when a fragment had bound which could be chosen for further study (Murray et al., 2010).

Murray et al. discovered a number of binding fragments, 2 of which became lead compounds. The first was compound 1 (fig3) which made extensive

hydrogen bonding interactions with key residue Asp93 (as seen in figure 2) and a number of water molecules found deep within the binding pocket as can be seen in figure 4a. However, as can be seen in figure 4b the compound 1 doesn't efficiently fill the lipophilic pocket defined by the residues Met98, Leu107, Val150, Phe138 and Val186, additionally it was found that compound 1 wasn't particularly stable, as it was twisted about the bond between the pyridine and pyrimidine.

Virtual screening for analogues was used initially to produce more stable forms of compound 1, and though this yielded higher affinity binding molecules, their torsion profiles indicated steric clashes between the methoxy group at position R2 (fig5a) would result in unfavourable binding. Instead using SAR (specific absorption rate) analysis, it was predicted that exchanging the methoxy for chloro improved it significantly, resulting in compound 9, the basis of further optimisation outline as a chemical structure in figure 5b, with the positions for optimisation labelled as R4 and R5. This was done using computer based modelling techniques, and illustrates how useful it can be when enough information regarding the target protein and the current ligands is available. The methoxy and chloro groups added to positions 4 and 5 of the upper phenyl ring increased binding affinity to the lipophilic binding pocket to 12nM.

Once the affinity was increased, cellular activity had to be improved and this was achieved by adding a morpholine group to position 5 outlined in figure 5b, a decision based on the crystal structure, and this resulted in compound 14 which is currently going through clinical trials for the treatment of

different cancers. As can be seen in figure 6, compound 14 in blue binds in much the same way as compound 1 in orange, but makes more extensive interactions with the lipophilic pocket via an extended phenyl ring.

The second line of lead compounds Murray et al. followed initiated from compound 3 (fig7), which using their initial NMRwaterLOGSY screening method appeared to bind rather inefficiently. However, upon observation of the X-ray crystal structure of Hsp90 bound to compound 3 (fig8) it was decided that it provided a quick and attractive optimization route. It's binding with water molecules and one of the key residues Thr184, though on its own provided a relatively weak interaction, if optimised could also make direct interactions with the alternative key residue Asp93, and also with additional endogenous water molecules.

Using trial and error, the writers found that a tetra-butyl group filled the lipophilic pocket appropriately with fewer steric clashes, and this resulted in compound 18, the lead compound that was further optimized to make more effective interactions within the lipophilic pocket. Using modelling studies, interactions with the side chain of residue Lys58 was approved. Compound 24, an isoindoline filled the pocket with a phenyl ring which interacts with residues Ala55, Lys58 and Ile96 completely displacing Lys58 side chain as can be seen in figure 9a.

In other inhibitors a position 2 OH (hydroxyl group) resulted in the greater affinities, however compound 18 had a position 4 OH, and a replacement with an OH at position 2 resulted in a lower affinity compound. Addition of an OH at position 2 to compound 24 as well as the OH at position 4 resulted in

compound 31 which enabled interactions directly with Asp93, retaining interactions with Thr184 as well as increasing hydrogen bonding with water molecules as can be seen in figure 9b. It also , illustrates compound 31 in blue binding in much the same way as compound 3, but it fills the lipophilic pocket more efficiently, and makes more extensive interactions. This greatly increased binding affinity and compound 31 is now going through clinical trials.

This helps illustrate the importance of structure-based approaches such as NMR and X-ray crystallography in the identification and optimization of lead compounds, as well as the input computer based methods can have. X-ray structures were particularly helpful in the case of compound 3, as without this structure compound 3 would have been dismissed as an inefficient binding compound. Additionally, all kinetic data which helped support the optimization and validation steps, was obtained using ITC.

Further work on improvements to the pharmacokinetic properties as well as drug-tissue distribution should be concentrated on.

2. 2 Crystal Structures from X-ray Crystallography and Nuclear Magnetic Resonance in In-Silico Drug Design, and 3D Drug Development – Human Immunodeficiency Virus

HIV (the human immunodeficiency Virus) is the causative agent of the acquired immune deficiency syndrome (AIDS) and statistics show that by 2005, approximately 38 million people worldwide were living with HIV (Beyrer, 2007). HAART (highly active anti-retroviral therapy) established in the 1990's makes living with HIV bearable by keeping viraemia low, and CD4+ (cluster of differentiation 4) cells at a high enough level to protect

from opportunistic pathogens. However, with increasing resistance and the negative side effects of current drugs, constant improvement and newer drugs are required. The protease inhibitors were revolutionary in HIV treatment, starting with the rationally designed Saquinavir approved for use in 1995 (Roberts et al., 1990). HIV protease is a good target, essential in the life cycle of the virus, and though Saquinavir was very successful, resistance quickly arose, and so a greater understanding of the protease structure and biochemistry was required. This was necessary not only to try and target residues that would be less likely to result in resistance, but also to improve the pharmacokinetic properties, producing non-peptidic as opposed to peptidic drugs to reduce toxicity and improve half-life (Arung Ghosh et al., 2008).

There have been multiple inhibitors designed with the use of X-ray crystallography, to enter clinical trials and be approved by the FDA (Food and Drug Administration) for use in HAART. It was determined that by targeting the protease backbone residues, it would be possible to generate drugs that would be less likely to result in resistance because mutations are rare, and those that occur do not often distort the overall conformation. Such a site is therefore more conserved and a better drug target (Ghosh et al., 2011).

Saquinavir, though a peptidic drug with poor pharmacokinetic properties did bind the backbone residues (though relatively weakly) it also bound outside of the binding envelope, the region which locates the gag-pol polyprotein for cleavage. Mutations are far more common and tolerated outside of the envelope region. Mutations would therefore not reduce virion viability but

would prevent inhibitor binding (King et al., 2004). The development of Aprenavir, with a single-ringed tetrahydrofuran (THF) group was designed using Saquinavir as a scaffold, to generate a related, but non-peptidic cyclic compound that would bind and inhibit the active site of the protease, much in the same way as Saquinavir but with increased half-life, better pharmacokinetic properties, increased backbone binding and a more specific binding to the active site envelope. The chemical structure of Aprenavir as seen in figure 9, binds the S1S2 S1'S2' binding envelope of the protease, closely interacting with the backbone residues Asp29 and Asp30, as well as many other residues (Kim, 1995). The interactions with Asp29/30 were relatively weak, and the THF group, believed to be involved in increasing favourable enthalpy interactions, if increased in size was thought to be able to improve backbone and hydrophobic interactions with the residues that make up the lipophilic flap.

Using Aprenavir as a scaffold, Darunavir was developed, a bis-THF compound with a double ring, as can be seen in figure 10. This single ringed to double ringed evolution resulted in more extensive interactions with the key backbone residues (Tie et al., 2004) as can be seen in figure 10, as there are far more hydrogen bonds present between the bis-THF complex in pink with the backbone residues than there are between the single ringed THF complex in green.

To measure the ability of Darunavir to withstand mutations in HIV protease, Tie et al. co-crystallised Darunavir with a wild type protease and a mutant version. As can be seen in figure 11, the wild type hydrogen bonds at 4.1 Å

indicated by the purple dashed lines is retained in the mutant distance of 3.8 Å in blue. This suggests that Darunavir is robust, and will continue to be active against resistant strains of HIV.

The inherently high mutation rate of the HIV genome due to the accident prone polymerase means that there will be strains that will become resistant to Darunavir in the future, and it is always necessary to stay one step ahead. Darunavir has thus been used in modelling studies to design optimised structures which are incredibly potent, more so than Darunavir retaining the favourable pharmacokinetic and cellular properties (Ghosh et al., 2011).

Figure 12 details the position of compound 1b in green– a Darunavir like compound in the hydrophobic pocket of the HIV-1 protease, and as can be seen, it makes a number of Van der Waals interactions with residues Ile47, Val32, Ile84, Leu76 and Ile50' which make up the hydrophobic flap as well as hydrogen bonds with Asp30 (3.5 Å long) and Asp29 (2.9 Å long). To improve the interaction distance with Asp30's NH group, Ghosh et al. modelled an increase in phenyl ring size of the P2 ligand in an attempt to also increase flexibility of the structure. This was achieved with the addition of an amide group which also increased the hydrophobic interactions with the lipophilic pocket residues. The pink structure of compound 35a as seen in figure 12 binds in much the same way as compound 1b, but makes more extensive interactions with the key residues and fills out the lipophilic pocket more effectively. This compound was then generated and its K_i and IC_{50} values calculated to measure it against 1b, it was a far more efficient inhibitor, and thus a potential clinical candidate.

There are many examples of proteins that cannot be crystallised, and to obtain structural information so rather than using X-ray crystallography, NMR can be used. As an example, the HIV protease structure was constructed using NMR (fig 13). An X-ray crystal structures is a static representation of a dynamic system in a relatively unnatural environment, whereas NMR is in solution and is believed to be more biologically relevant, and can in some circumstances be used to observe dynamic protein systems (Zou, 2007). NMR is far more time consuming however, and the inherent flexibility of proteins results in areas of low resolution in structures, more so than with X-ray crystallography.

NMR has been used more successfully in hit identification, as has been discussed in the example of Hsp90 inhibitors.

2.3 The use of Surface Plasmon Resonance, Isothermal Titration Calorimetry and In-Silico Drug Design to Complement Structural Techniques Such as X-Ray Crystallography and Nuclear Magnetic Resonance

As technology improves newer methods have evolved that complement the existing, this includes such techniques as SPR which detects the interactions between the target protein and ligand, used in primary fragment based library screens to identify hits, or secondary screens to identify or validate hits (Retra et al., 2010). As previously discussed, fragment based screening methods can result in attractive starting points for lead optimisation (Erlanson, 2006). SPR can be used in a number of ways, in chemical micro arrays, SPR imaging, secondary screens of hits found through high throughput screens and also in primary biosensor screens.

In primary screens, a biosensor is set up with the target molecules immobilised on chips and this has been successfully used in the identification of hits without the requirement of other forms of structural information (Nordstrom et al., 2008). The hit molecules can then be integrated into lead series and optimised using other structural techniques such X-ray crystallography and NMR to obtain clinical candidates (Huber, 2005).

The matrix metalloproteinases (MMPs) are a group of proteins found in many different species; in humans there are approximately 12 that are involved in tissue remodelling, and degrading extra cellular matrix molecules such as elastin, collagen and laminin (Demedts et al., 2006). MMP-12, involved in various human diseases such as emphysema and chronic obstructive pulmonary disease (COPD) is the target of a number of therapeutic drugs, all of which have harmful side effects and so new drugs are required (Nordstrom et al., 2008). Using SPR and ITC in conjunction with NMR or X-ray crystal structures Nordstrom et al. produced an in silico drug design based on the binding sites identified by the crystal structures, using pharmacophore properties to model a binding molecule. Mutant proteins were designed in silico and then generated, immobilised on chips along with wild type proteins as depicted in figure 14. Molecules designed in silico could then be screened against the different proteins on the chip.

For screening purposes SPR is limited, the number of molecules screened against the biosensor is relatively small as the proteins become degraded; only a couple of hundred molecules can be screened, compared to thousands in HTS. The library must therefore be carefully designed, using in

silico modelling, docking and screening, or with a vast knowledge and understanding of the target.

Alternatively, SPR can be used in hit validation for lead series initiation, assessing the enthalpy and kinetics of binding, as was the case for campstatin analogues to increase binding affinity for C3b in the treatment of multiple human disorders involving the over-activation of complement (Qu et al., 2010). C3b is an appropriate target because it is involved in so many disorders such as neurodegenerative, sepsis and has also been linked to stroke. Campstatin is a good peptidic protein inhibitor, binding and inhibiting C3b regardless of the initiation pathway. However, due to its peptidic nature, Campstatin is not very stable with a short half-life in vivo, and due to the low concentrations of C3b found in plasma, a higher affinity compound with better pharmacokinetic properties would be ideal.

N-methylations were analysed at different positions on the Campstatin scaffold and changes in binding affinity measured using SPR, and confirmed using ITC to conclude that by generating a compound that retains a rigid structure both in solution and in a bound state, it would bind with increased enthalpy, without decreasing the entropy as had other previous designs (Qu et al., 2010).

This demonstrates the powerful applications SPR and ITC can have in drug discovery or design, and how in conjunction with in-silico computer based techniques, they can complement X-ray crystallography and NMR techniques.

2. 4 The Difficulties Associated with Membrane Protein – The B2 Adrenergic Receptor: an Example of A G-Protein Coupled Receptor

Crystallisation seems to be at the heart of structural biology and even with the option of NMR there are still severe limitations that mean many proteins, particularly membrane bound proteins, cannot be crystallised and thus cannot be visualised as a 3D structure. This is particularly problematic for structure-based drug design, as some 50% of drugs target G-protein coupled receptors (GPCRs) alone, not including the many other families of membrane bound proteins. GPCRs are a superfamily of proteins which all have 7 transmembrane helices found in eukaryotes important in many crucial signalling processes (Lundstrom, 2005).

The problem with studying membrane proteins in general is the difficulties in solubilising them and getting enough protein to work with. To obtain this large amount of protein, recombinant protein is required and for human protein this is a particularly difficult task (Mancia et al., 2007). The lack of structural information limits our understanding of ligand binding, as well as allosteric control and active site location (Summers, 2010). There have been major advances in obtaining the structures of GPCRs recently, with structural information on rhodopsin, A2A adenosine receptor, B1 adrenoreceptor and the B2 adrenergic receptor. The problems to overcome were obtaining enough usable protein, thus an appropriate expression system, the intrinsic flexibility and therefore excessive instability, and obtaining the exact solubilising formula for each protein. Once achieved, the crystallisation process for membrane proteins is no different than for globular proteins (Velipekka et al, 2010).

To stabilise the different GPCRs, mutagenesis was used in rounds for B1 adrenoreceptor (Warne et al., 2008), or in the case of the B2 adrenergic receptor and A2A adenosine receptor, the flexible intracellular loops were stabilised by replacing them with the easily crystallised and inherently stable T4 lysozyme (Rosenbaum et al., 2007).

Therapeutics aimed at A2A adenosine receptor could help in the treatment of seizure, asthma, Parkinson's, pain and many other neurological problems (Jaakola et al., 2009). The crystal structure of the A2A adenosine receptor with the antagonist ZM241385 enabled the determination of important residues in ligand binding, and thus generated the information required to use computational modelling studies to suggest residues that would be important in inhibitor binding. Figure 15 depicts the binding of the antagonist, hydrogen bonded to Asn253, aromatically stacked against Phe168 as well as hydrophobically interacting with Ile274. An understanding of these interactions greatly helps in the elucidation of therapeutically important binding molecules (Jaakola et al., 2009).

B2- adrenergic receptors, a class of GPCR are important in smooth muscle related diseases such as asthma (Cherezov et al., 2007). Cherezov et al. made a B2-adrenergic receptor T4 lysozyme fusion protein to enable crystallisation with Carazolol at 2.4 Å. Carazolol has high affinity for the receptor, lying adjacent to, and making significant interaction with the residues Phe289, Phe290 and Trp286 as seen in figure 16b and reduces basal level of activity of the receptor via its interactions with Phe289/290 which result in the inactive Trp286 state as seen in figure 16.

This understanding of agonist binding and an in depth knowledge of the residues involved, if expanded upon could increase the possibilities for structure-based drug design and modelling.

3. Conclusion

3.1 Summary of Main Points and Advantages of Structure-Based Techniques

The power of structural biology is apparent; it provides a clear physical picture of the target protein. It enables the identification of hit compounds via X-ray crystallography and more commonly NMR, supported by the complimentary techniques – computational analysis, SPR, ITC and DSF. Such techniques can validate those hit compounds to enter them into lead series and they can then be used to optimise leads to generate clinically usable compounds.

The importance of structural biology is therefore easy to see as it has been successful in generating clinically used drugs, Darunavir for the treatment of HIV as a protease inhibitor being just one of many examples.

3.2 The Limitations of Structure-Based Techniques

Of course they are not without their limitations. X-ray crystal structures are static freeze frame shots of a dynamic system, so we cannot be certain that what we see is biologically relevant or simply artefacts. Both X-ray crystallography and NMR suffer with the inherent instability and flexibility of proteins. There are methods to improve the 3D structures, as seen in the crystallisation of membrane protein – the B2 adrenergic receptor (Rosenbaum et al., 2007), suggesting that these limitations are not permanent, and can be overcome. Many proteins cannot be crystallised, and

though there has been recent breakthroughs as with the case of the GPCRs, the vast majority have not been visualised and yet 50% of drugs are aimed at them.

Complementary techniques such as SPR, ITC and DSF have successfully been used to identify hit molecules (Nordstrom et al., 2008) and to validate or optimise leads (Huber, 2005). Unfortunately these too are not without their faults, requiring smaller screening libraries, and the proteins involved to be constantly replaced during screens.

To overcome this there have been computer based in-silico screening and design processes, which under certain circumstances has been used efficiently as was the case with the optimization of Darunavir (Ghosh et al., 2011), however there have been huge limitations. The first human GPCR crystallised, rhodopsin was a model for all GPCRs and in-silico modelling studies utilised it to generate binding molecules, but with the visualisation of the A2A adenosine receptor via X-ray crystal images, it became apparent that this was a far too over-simplified view (Jaakola et al., 2009).

3.3 Concluding Thoughts and Future Advances

To conclude, there are clear limitations concerning the structure-based design of therapeutic drugs, requiring further advances in technology and understanding to be made before we can easily utilise every form of technology efficiently and in an integrated fashion. Structure-based techniques do not speed up the process of drug discovery, however, there are also clear advances that have been made through the use of such structural biology techniques. They should therefore continue to be used in

conjunction with current technologies to ever improve the therapeutics in use.

Future advances should include improved recombinant protein technologies and purification procedures to obtain the large quantities of protein required, improved detergent mixtures for membrane proteins as well as better crystallisation procedures in general to increase resolution. As well as finding hits for lead series of molecules, structural techniques should also focus on increasing the number of targets, so that whole new sets of drugs can be made to add to combinatorial drug therapies such as HAART in the treatment of HIV, in an attempt to overcome the problems of resistance.