Expert Opinion

What has computer-aided molecular design ever done for drug discovery?

David E Clark
Argenta Discovery Ltd, 8/9 Spire Green Centre, Flex Meadow, Harlow, Essex, CM19 5TR, UK

This article assesses the contribution of computer-aided molecular design (CAMD) to the field of drug discovery. Several examples of ligand- and structure-based drug design are used to demonstrate the role of CAMD in the discovery of marketed drug compounds. Although CAMD is now an integral part of many drug discovery projects, there are significant challenges still facing its practitioners, particularly the prediction of binding affinity.

Keywords: computer-aided drug design, computer-aided molecular design, ligand-based drug design, structure-based drug design, virtual screening

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1. Introduction

The discipline of computer-aided molecular design (CAMD) has its roots in the 1960s with the development of quantitative structure–activity relationships (QSARs) by Hansch and Fujita [1]. With increasing computer power and graphical capabilities, the 1970s saw the gradual emergence of molecular modelling, but it was not until the late 1980s/early 1990s that advances in computer hardware and software, together with the increasing availability of protein structures of biochemical targets of pharmaceutical interest, brought CAMD into the mainstream of pharmaceutical research. Thus Snyder wrote in 1991: 'every major pharmaceutical firm conducting research in the 1980s introduced computational chemistry into the discovery matrix' [2]. Other articles from the same period testify to the growing impact of CAMD [3,4].

Given that CAMD has been an integral part of drug discovery for at least the last 15 years, the question 'what has CAMD ever done for drug discovery?' is a valid one and one that others have sought to answer in the past. As Boyd noted in 1999, 'because of the wide use of the term computer-aided drug design, computational chemists are often challenged about whether their computer-based approaches have actually designed a drug' [5]. This article aims to demonstrate that CAMD has indeed had a significant influence on the design and discovery of several marketed drugs and others that are currently in the clinic.

2. The influence of computer-aided molecular design on some marketed drugs

The role of CAMD in the discovery of six marketed drug compounds is briefly examined in this article. Three compounds are the result of ligand-based drug design (that is, no structural information on the biochemical target was available) and the other three are examples of structure-based drug design.

2.1 Ligand-based design

2.1.1 Norfloxacin

Norfloxacin (Figure 1) was the first fluoroquinolone antibiotic to reach the market, being launched in 1984. It is used (in particular) to treat infections of the urinary tract. Norfloxacin was discovered at Kyorin in Japan and subsequently licensed to

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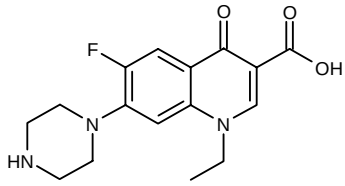

Figure 1. Two-dimensional structure of norfloxacin.

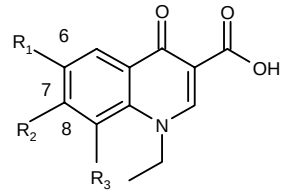

Figure 2. Generic structure showing numbering of substituents in norfloxacin-type compounds.

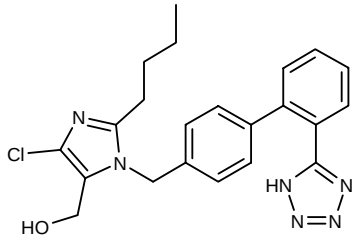

Figure 3. Two-dimensional structure of losartan.

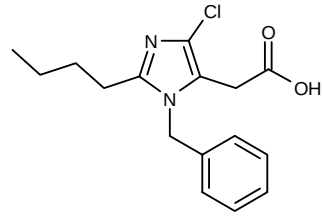

Figure 4. Lead compound, S-8307.

Merck. The story of its discovery and the role of CAMD therein have been described by Koga *et al.* [6].

The scientists at Kyorin had developed a QSAR model for 6-, 7- or 8-monosubstituted compounds relating antibacterial activity to steric parameters for R_1 (Taft's Es parameter) and R_3 (Verloop's B4 parameter; Figure 2). No relationship had been found for substituents at the R_2 position, but piperazine had been observed to be promising. The QSAR equation predicted that polysubstituted compounds might be more potent than the extant monosubstituted analogues. In particular, the 6-fluoro-7-(1-piperazinyl) derivative was predicted to be 10-fold more potent than its des-fluoro analogue. When this compound was synthesised and tested, the prediction was borne out: it was 16-times more potent. On the basis of further preclinical data, the compound was selected for clinical trials and subsequently became norfloxacin.

2.1.2 *Losartan*

Losartan (Figure 3) was the first nonpeptide, oral angiotensin II receptor antagonist to reach the market; it was approved for the treatment of hypertension in 1995. The compound was discovered at DuPont, and then codeveloped and marketed by Merck. Losartan is a true 'blockbuster' drug, having forecast sales of ~ US$3 billion for 2006 [101]. The role of CAMD in the discovery of losartan has been described by Duncia *et al.* and also by Bhardwaj in a fascinating case study [7,102].

CAMD techniques were used to overlay an early, weakly potent lead (S-8307, IC_{50} = 150 µM; Figure 4) with a solution structure of the angiotensin II peptide (Figure 5). The alignment revealed that the *para* position of the benzyl group of S-8307 was a promising point from which to make substitutions that would increase the overlap of the molecule with angiotensin II. Ultimately, this led to the discovery of the now famous biphenyltetrazole motif, which is characteristic of the 'sartans'.

2.1.3 *Zolmitriptan*

Zolmitriptan (Figure 6) is a 5-$HT_{1B/1D}$ receptor agonist, indicated for the acute treatment of migraine. It is a leading second-generation 'triptan', having sales of US$352 million in 2005 [103]. Zolmitriptan was originally discovered at Wellcome, but was subsequently sold to AstraZeneca to comply with competition requirements following the Glaxo Wellcome merger in 1995. The discovery of zolmitriptan was heavily influenced by CAMD. A hint of this can be found in the fact that the first author of the paper describing its discovery is Robert ('Bobby') Glen [8], who was a molecular modeller at Wellcome at the time and is now Professor at the Unilever Centre for Molecular Sciences Informatics at Cambridge University, having spent some time in between with Tripos [104].

The 'active analogue approach' [9] was used to derive a pharmacophore model for 5-HT_{1D} binding (Figure 7). An additional 'selectivity volume' was also deduced by overlaying ligands that were selective and nonselective for the 5-HT_{2A} receptor, and identifying a region of space that was occupied by the selective (but not the nonselective) compounds. This model was used to help guide the design of potent and selective compounds. In addition to the

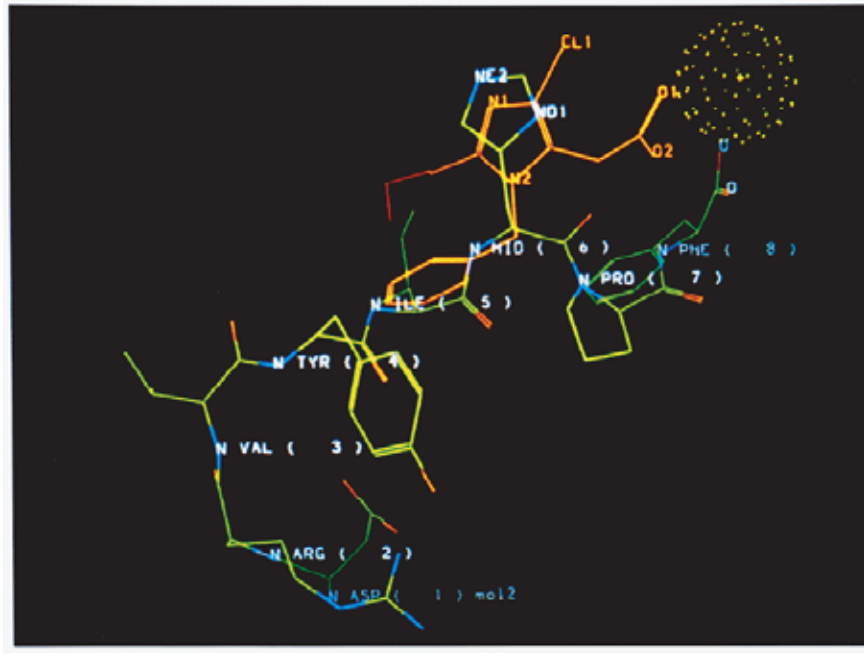

Figure 5. S-8307 (orange) overlaid with structure of angiotensin II (green). The discovery of potent nonpeptide angiotensin II receptor antagonists: a new class of potent antihypertensives.
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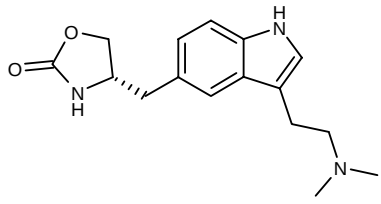

Figure 6. Two-dimensional structure of zolmitriptan.

optimisation of potency and selectivity, modelling was also applied to help optimise the pharmacokinetic properties of the compounds; for example it was noted that it was necessary to keep the calculated molar refractivity (a measure of molecular size) at < 9.5 to encourage absorption by paracellular transport across the intestinal membrane. In addition, logD (pH 7.4) calculations were used to guide the design of compounds that did not cross the blood–brain barrier and thus cause the unwanted activation of 5-HT_{1A} receptors.

2.2 Structure-based design

During the 1980s, the increasing availability of X-ray crystal structural information on targets of relevance to the pharmaceutical industry enabled structure-based approaches to be applied to the discovery of a number of drug compounds.

2.2.1 *Dorzolamide*

Dorzolamide (Figure 8) is a carbonic anhydrase (CA) II inhibitor that is used to treat conditions characterised by high intraocular pressure (IOP); for example, glaucoma. It was discovered at Merck and approved by the FDA in 1994. In 2005, it had sales of US$617 million [105]. The role of structural biology and CAMD in the discovery of dorzolamide was summarised in a perspective on structure-based drug design published in the mid-1990s [10].

A prototype compound, MK-927 (Figure 9), had been found to be water soluble and capable of lowering IOP in animal models. Resolution of the enantiomers of this compound showed that the *S*-enantiomer (K_i = 0.61 nM) was 100-fold more potent than the *R*-enantiomer (K_i = 71 nM). (K_i is a measure of potency of a compound against its biochemical target). Both enantiomers were cocrystallised with CA II and this revealed two conformational features that could account for the differing potencies of the enantiomers: a difference in the NSCS (nitrogen, sulfur, carbon, sulfur) torsional angles and a *trans* versus *gauche* geometry of the isobutylamine side chain. *Ab initio* calculations were used to demonstrate that the conformational features present in the *S*-enantiomer were more favourable than those in the *R*-enantiomer.

Attention was thus focused on the *S*-enantiomer. The X-ray cocrystal structure revealed that the isobutylamine side chain was in the less favoured pseudoaxial conformation. Further *ab initio* calculations suggested that the pseudoequatorial conformation would be preferred by

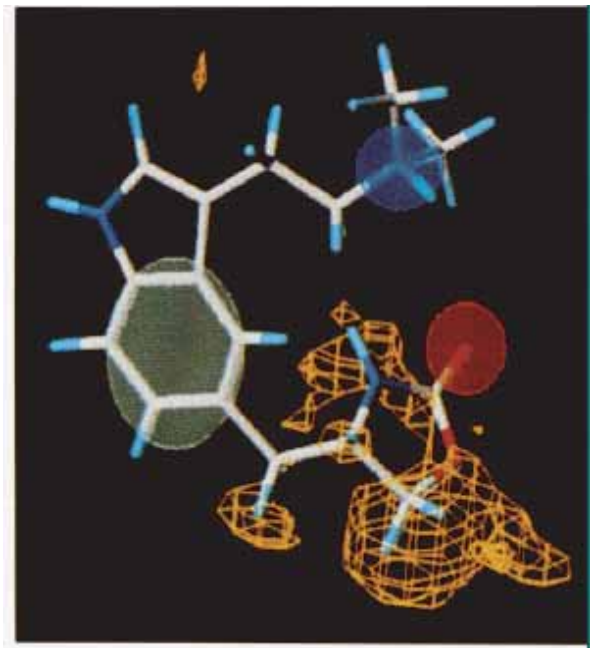

Figure 7. Zomig overlaid on (part of) pharmacophore model (blue: positive charge feature, red: hydrogen-bond acceptor feature and green: aromatic ring feature) and selectivity volume (orange). Computer-aided design and synthesis of 5-substituted tryptamines and their pharmacology at the 5-HT_{1D} receptor: discovery of compounds with potential anti-migraine properties.
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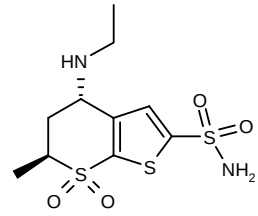

Figure 8. Two-dimensional structure of dorzolamide.

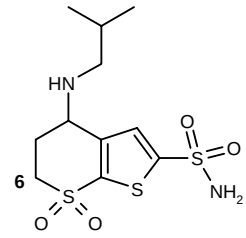

Figure 9. Structure of MK-927 showing 6-position.

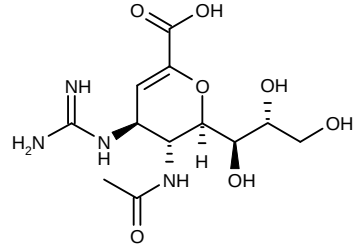

Figure 10. Two-dimensional structure of zanamivir.

1 kcal/M. Similar calculations were used to propose that the introduction of a methyl substituent at the 6-position would eliminate the preference for the pseudoequatorial conformation and thus decrease the energy penalty experienced by the compound on binding to the enzyme. Therefore, the methyl group was introduced and the isobutylamine truncated to an ethylamine to compensate for the increased lipophilicity. Of the four possible diastereomers of the resulting compound, the *trans*-(*S*,*S*) form (dorzolamide) was preferred, having a K_i value of 0.37 nM.

2.2.2 *Zanamivir*

Zanamivir (Figure 10) was the first neuraminidase (sialidase) inhibitor to be marketed for the treatment of influenza. The compound was developed through research funded by GlaxoSmithKline (GSK) in collaboration with a group of Australian institutions including Biota Holdings, the CSIRO and the Victorian College of Pharmacy. Zanamivir was approved by the FDA in 1999, but sales have been disappointing (£19 million for 2004). However, it is conceivable that the current fears of an avian flu pandemic could lead to an upturn in sales, at least in the short term. The discovery of zanamivir is a classic example of the application of CAMD to drug discovery and has been described by von Itzstein *et al.* [11].

Colman's group at the CSIRO Division of Biomolecular Engineering had solved the X-ray structure of influenza virus neuraminidase in complex with an unsaturated sialic acid analogue (known as DANA). The GRID program was then used to analyse the active site and predict energetically favourable substitutions that could be made to DANA (Figure 11). One of the predicted substitutions was to replace the hydroxyl at the 4-position of the ring by an amine, with the aim of improving interactions with two neighbouring glutamic acid residues. When the 4-guanidino compound was synthesised, it was found to be a highly potent inhibitor of the enzyme (K_i = 0.2 nM) and was subsequently developed as zanamivir.

The main drawback of the compound is that it is not orally bioavailable due to the presence of the strongly basic guanidine group. Thus zanamivir is administered by

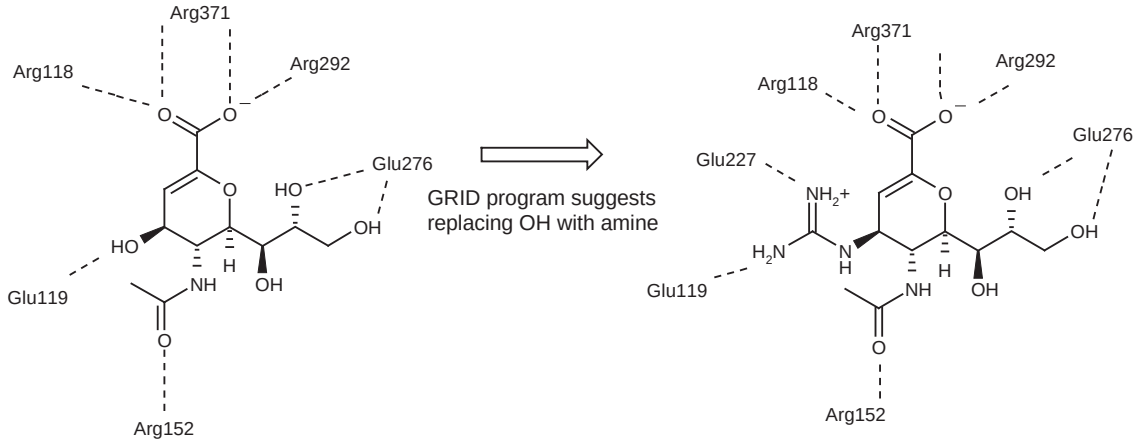

Figure 11. Role of the GRID program in the design of zanamivir.

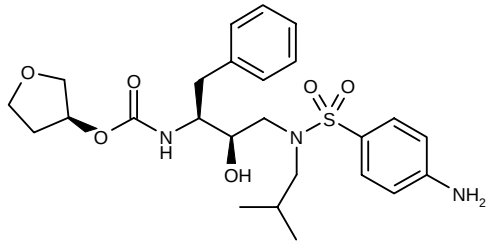

Figure 12. Two-dimensional structure of amprenavir.

inhalation. The success of Roche's neuraminidase inhibitor, oseltamivir, probably derives largely from its oral bioavailability and is at least partly responsible for the disappointing sales of zanamivir.

2.2.3 Amprenavir

Amprenavir (Figure 12) is a HIV-1 protease inhibitor for the treatment of HIV/AIDS. It was discovered by Vertex Pharmaceuticals (one of the pioneers of structure-based drug design) and later codeveloped and marketed with GSK and Kissei (Japan). It was first made available to patients via an early access programme in 1998 and approved in 1999. According to GSK's 2005 annual report [106], amprenavir had sales of £112 million in 2005. In a paper written by the drug discovery team at Vertex, it was stated that amprenavir was the result of a 'focused programme of structure-based drug design' [12].

This publication makes the role of CAMD in the discovery of amprenavir very clear; for example, the authors note the 'ready incorporation and testing of modelling and structural insights'. Such as molecular dynamics calculations were used to rationalise the experimental observation that the P1′ amide NH of substrate sequences was not obligatory for binding and productive catalysis. The simulations revealed that this formed the weakest hydrogen bond to the enzyme of all those made by an early inhibitor. One of the key decisions in the project (supported by modelling) was the choice of the *N*,*N*-dialkyl sulfonamide moiety, which was intended to bind to the 'flap' water molecule and to act as a scaffold for the P1′ and P2′ groups. Searches of the Cambridge Structural Database were used to provide information on the likely conformations of the *N*,*N*-dialkyl sulfonamide and show that these were consistent with those needed to bind to the enzyme in a low energy conformation.

Gratifyingly, when representative compounds were cocrystallised with the enzyme, 'the bound conformation of the inhibitor backbones were substantially similar to those suggested by computational analysis'. Furthermore, 'good hydrogen bond distances between [the flap water] and the sulfonamide oxygens were observed in all cases, supporting our modelling prediction' [12].

3. Virtual screening successes

Since the turn of the century, virtual screening has become a very popular technique for lead discovery, particularly for smaller companies that do not possess the necessary compound collections or infrastructure required for high-throughput screening. The first compounds whose discovery can be attributed to virtual screening are now in clinical trials. Two such compounds are described in this section.

3.1 SC-12267

SC-12267 is a novel inhibitor of the enzyme dihydroorotate dehydrogenase (DHODH) for the treatment of rheumatoid arthritis that was discovered by 4SC AG. The company started the DHODH project at the end of 2001 using its proprietary 4SCan®/Propose [13,14] virtual high-throughput screening technology. A library of commercially available

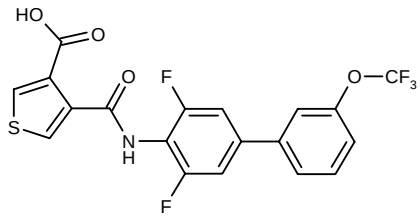

Figure 13. 4SC DHODH inhibitor.

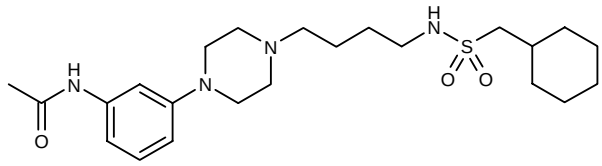

Figure 14. Structure of PRX-00023.

compounds was docked into a publicly available X-ray structure of DHODH and this led to the identification of a series of novel cyclic aliphatic carboxylic acids [15], which has been subsequently optimised [16,17]. Approximately 28 months after the inception of the project, SC-12267 was brought to clinical trials. The Phase I trial was completed in March 2005 and a Phase IIa trial is scheduled to commence in 2006. This project was the subject of a deal between Serono and 4SC, that was announced in May 2004. However, all rights were returned to 4SC in March 2005. The structure of SC-12267 as not yet been disclosed, but it may be related to the compound shown in Figure 13, which has an IC_{50} of 10 nM against DHODH, and has shown potent antiproliferative activity on peripheral blood mononuclear cells [16].

3.2 PRX-00023

PRX-00023 (Figure 14) is a novel, highly selective, nonazapirone 5-HT_{1A} agonist discovered by Predix Pharmaceuticals. The compound was identified using PREDICT™, the company's proprietary GPCR modelling, screening and lead optimisation technology [18]. PREDICT was used to build a three-dimensional *de novo* model of the 5-HT_{1A} receptor and a library of 40,000 screening compounds was docked into this model using DOCK. A total of 78 compounds were selected for screening and 16 of these were confirmed as hits in an *in vitro* assay with K_i values of < 5 µM (a 21% hit rate). In addition, these 16 hits represented 5 distinct chemical series, which is always advantageous when starting a hit-to-lead programme. The best hit was a novel 1 nM compound (PRX-93009), although optimisation was needed to address some of the selectivity issues. This led ultimately to the discovery of PRX-00023, which entered a pivotal Phase III clinical trial in patients with generalised anxiety disorder in August 2005. Results of this trial are expected in the second half of 2006.

4. Some current challenges in computer-aided molecular design

The examples presented in this article demonstrate that CAMD can contribute significantly, and sometimes decisively, to a drug discovery project. However, there are many occasions when modelling fails to make such an impact and there is certainly no room for complacency or arrogance among CAMD practitioners [19]. Of all the challenges facing the modelling community, the prediction of binding mode and binding affinity stand out as being particularly significant in an era when structure-based drug design and virtual screening are being applied routinely to discovery projects.

4.1 Binding mode prediction

The current generation of docking programs can successfully reproduce the binding mode of ligands, as observed in cocrystal structures in 80% of cases [20]. Thus ~ 20% of cases are docked 'wrongly' (although sometimes additional binding modes that are not yet detected by crystallography may be possible). A pressing challenge is how to treat protein flexibility. Protein structures are treated as rigid bodies by most docking programs, whereas in reality they are dynamic, flexible entities that may often mould themselves to fit the binding ligand (the phenomenon known as 'induced fit'). Some programs are beginning to account for a certain degree of protein motion [21], but at a considerable computational cost, which makes them impractical for high-throughput virtual screening purposes. Water molecules observed in the protein binding site can also complicate matters. There is no general rule on how to treat these, although they are ignored or deleted frequently. The best approach is to examine each water molecule in the site carefully, preferably with reference to the crystallographic B factors, local environment and number of hydrogen bonds formed by the water molecule [22].

4.2 Binding affinity prediction

If binding mode prediction presents challenges, the prediction of binding free energy is even more fraught with difficulties. Although free energy perturbation approaches were once thought to hold promise [23], these have not progressed beyond the hands of a few specialists, a situation that seems unlikely to change in the short term. Recently, linear interaction energy methods have been applied with success to certain systems [24], but have yet to prove themselves when applied more generally. There has been a great deal of research into scoring functions suitable for structure-based virtual screening over the last decade [25]; however, it has to be admitted that these are only capable of yielding 'enrichment' and rarely give a good correlation with observed binding affinities.

Both of these challenges point to the underlying complexity of molecular recognition and to the incomplete ability to model its constituent phenomena. Therefore, more basic research to increase the understanding of these is still required.

5. Expert opinion

CAMD can make valuable contributions to drug discovery programmes and certainly works best when closely integrated (and preferably colocated) with allied disciplines such as medicinal chemistry and structural biology [26]. Although automated *de novo* design programs are not yet at a stage where they can be routinely applied (although progress is being made [27]), modelling still often acts as an 'ideas generator' leading to the design of compounds that otherwise might not have been considered. Being accstomed to viewing compounds in three dimensions (whereas the *lingua franca* of the medicinal chemist remains the two-dimensional structure diagram), modellers can bring a unique perspective to drug discovery teams. However, although the perspective may be unique, it should always be remembered that it is not infallible. After all, the discipline is termed 'molecular modelling' for valid reasons. Given the challenges that remain in CAMD, it is wise to maintain a degree of suspicion when presented with modelling results, especially those that seem to be counter-intuitive. The power of appealing computer graphics to bewitch should not be underestimated.

Nonetheless, it is clear that even from its earliest days, CAMD (and perhaps just as importantly, its practitioners) have been making key contributions to the discovery of marketed drugs and continue to do so. This is particularly evident in the current 'structure-based era'. Indeed, as a recent article states: 'with little fanfare, structure-based drug design is filling development pipelines' [28].

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Affiliation

David E Clark
Argenta Discovery Ltd, 8/9 Spire Green Centre, Flex Meadow, Harlow, Essex, CM19 5TR, UK
Tel: +44 (0)1279 645 645;
Fax: +44 (0)1279 645 646;
E-mail: david.clark@argentadiscovery.com