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Reprint

New Trends in Computer-Aided Drug Design
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New Trends in Computer-Aided Drug Design

On-line Integration of Super-Computers

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Drug molecule in a binding pocket of a protein (for an interactive inspection via internet see http://www.molcad.de/customerscenes/)

Realisation Jens Gimmler,
MOLCAD GmbH
Computer-Aided Drug design (CADD) is not new. The Journal of Computer-Aided Molecular Design (Springer) was founded in 1987, when computers in the worldwide top 500 were slower than today’s smart phones. This makes the field a quarter of a century old. Generally, scientific disciplines of this age have become mature, the major developments have been made and procedures have become routine. Superficially, this is also the case for CADD. However, the environment for all computer-based scientific disciplines has changed rapidly and continuously in the past quarter century. Our phones and automobiles have more CPU power that supercomputers 25 years ago and importantly, can also speak to each other more easily. This situation is exciting. We can do things that we couldn’t dream of in 1987. This should mean that CADD is also a rapidly expanding field in which new compute-intensive techniques are being introduced continuously to improve performance and applicability. Sadly, this is not the case. CADD has not used the possibilities open to it. However, there are signs that things are beginning to change.

In the traditional CADD scenario the number crunching was decoupled from the interactive data processing and knowledge generation process. Visualization was the domain of specialized graphics computers that were able to handle the extreme demands of visualizing proteins and their properties. That was before the games industry. Top quality molecular graphics are now at the bottom end of the demands placed on graphics hardware. At the risk of being boring, even smart phones can produce interactive 3D-molecular graphics that would have been beyond the first generation of raster-graphics machines. New web-based technology, such as Molcad’s web3d molecule visualizer can make high-quality interactive 3D molecular graphics available anywhere at any time. This is only the beginning; interactive 3D graphics that can be manipulated in real time can be shared by anyone with a suitable web browser. Moreover, the new Web based technologies open completely new direction in the simulation scenario: Number crunching can be directly integrated in the real time treatment of CADD-activities, even when these processes are performed at locations thousands of miles apart.

The in many ways unimaginable increase in the speed of computations opens possibilities of using theoretical techniques that were unthinkable in the early days of CADD. Sadly, very little use has been made of these possibilities. The vast majority of CADD-techniques today rely on atomistic classical mechanical models that were designed in the 1970’s for the hard- and software of the time. One of the most important interactions between molecules (electrostatics) is treated in this model by assigning a single charge at the center of each atom. This model is completely unsuitable for elements like...
chlorine, bromine and iodine, which appear negative from some directions and positive from others. These elements are generally assigned a negative charge, meaning that they repel other negative atoms such as the oxygens in the backbone chains of proteins in the model. This is the reverse of the true situation. In the correct orientation, backbone oxygen atoms attract atoms such as chlorine, bromine and iodine.

We are thus confronted with the situation that the models that we have used for CADD for a quarter century give the wrong sign for an important interaction (very many current drugs contain chlorine). How can this happen? The answer is probably that we have confused our models with reality. “Everybody knows” that chlorines in drug molecules “are negative”. That is true if you approach them perpendicular to the carbon-chlorine bond, but chlorine atoms “are positive” if you approach them from opposite the carbon to which they are bound. Currently, many attempts are being published to correct this situation by adding an extra positive charge to chlorine in the model.

This will fix the problem — but what about the next one? One of Thomas Kuhn’s signs of a failing paradigm is that it needs increasing numbers of ad hoc fixes. So why stick to classical mechanical models? We have a variety of quantum mechanical methods that all reproduce the chlorine-oxygen interaction correctly. Why not use them? The answer, sadly, is that they used far too much computer time in the 1970’s and 1980’s. Today’s hard- and software are easily capable of using standard quantum mechanical calculations to treat pharmaceutical databases of hundreds of thousands of molecule or even to calculate an entire protein target. Our challenge is therefore to combine modern theoretical techniques from other branches of chemistry with high-performance hard- and software to improve the performance and reliability of CADD.

Why do we need to improve such a well established field? After all, CADD is used every day in dozens of pharmaceutical companies, apparently with good results. Well, another consequence of a paradigm at the end of its days is that it is defended strongly in the face of hard evidence that it does not work. A published test of docking and scoring algorithms used to estimate the binding affinity of drugs to their protein targets concluded with a table in which ten different docking-and-scoring techniques were used on datasets for ten different targets. The table reported the correlation coefficients between the experimental and calculated binding affinities. Of one hundred entries in the table, 64 were negative (i.e. they predicted the reverse trend to that found experimentally). Of the remaining 36, the highest was approximately 0.2. This is hardly acceptable performance but nonetheless, pharmaceutical companies rely on docking and scoring and dozens of papers using the technique without experimental confirmation are published every day. The whole situation is reminiscent of “the emperor’s new clothes”.

So what needs to be done? We need to rethink the way that we do CADD. Over the last decade, the emphasis has been placed on using existing techniques for increasing numbers of compounds. Given the results discussed above, this amounts to collecting even larger numbers of wrong predictions. Of course, the likelihood of a few predictions being correct increases with the number of predictions made. What we have been doing is buying more and more lottery tickets.

Is there a “system” that allows CADD to “win” and become predictive? We don’t know. What we do know is that there are theories of intermolecular interactions (which are what really interest us) that are far more accurate and above all general than the ones we currently use in CADD. A variety of quantum mechanical methods range from semiempirical MO-theory (which can calculate hundreds of thousands of molecules or complete proteins) to high-level ab initio theory (which for small molecules is usually more accurate than experiment). Density-functional theory (DFT) has become the workhorse of computational quantum chemistry and can easily calculate drug-sized molecules within a few minutes on a modern multicore node. It has been parameterized extensively in the last decade so that the newest functionals are very accurate for everyday molecules like drugs.
Tim Clark was born in 1949 in England and obtained his Ph.D. from the Queen’s University Belfast in 1973. He is Director of the Computer-Chemie-Centrum in Erlangen and the Centre for Molecular Design at the University of Portsmouth, UK. He develops and applies modelling and simulation techniques in chemistry, materials science and biology. He is the author of 300 research articles and two books and is the founding editor of the Journal of Molecular Modeling. In 2009, he was awarded the Klaus-Wilhelm von der Lieth Medal.

Jürgen Brickmann, born 1939 in Schwerin, studied physics 1959-1965 (Uni Munich, Uni Innsbruck, Tu Munich) and obtained his Ph.D. from the TU Munich in 1967. In 1973 he became a Private Docent for physical chemistry at the Uni Freiburg, from 1974 to 1978 he was Professor for chemical dynamics at the Uni Konstanz. From 1979 to 2004 hold a chair for physical chemistry at Darmstadt University of Technology. He was guest scientist at different Universities (FU Berlin, Tel Aviv University, Hebrew University, Jerusalem). Presently he is principal owner and CEO of MOLCAD GmbH as well scientific director of SUCCIDIA AG, a publication- and communication company in Darmstadt (Germany).

Treating intermolecular interactions properly is only half the battle. Biological systems are flexible and dynamic at physiological temperatures. This means that we must consider all the conformations that are present in the living system. This is the so-called conformational sampling problem, which means that we must use molecular-dynamics simulations to allow the molecules to move and to adopt all possible conformations open to them. This is a major computational task, for which special dedicated hardware has been built. Considering conformational sampling adequately involves a true paradigm shift in CADD from thinking about static single structures to considering what the real moving molecules are doing.

Finally, to make things even worse, biology happens in aqueous solution that is full of ions, small molecules, proteins, nucleic acids etc. Simulating the effect of the solvent water is very expensive computationally. It can be done by simulating a system that includes the water solvent explicitly but a liter of water contains more than $3 \times 10^{25}$ molecules. We don’t need to simulate a liter but there are still going to be a lot of water molecules. One solution would be to represent the solvent as a continuous medium or continuum. This would be fine and very efficient if it were accurate. The problem is that water is a very complicated molecule and doesn’t look much like a simple continuum. We therefore also need new calculational models in which the water solvent is treated implicitly and accurately.

Are we doing anything about improving CADD and using the capabilities of modern computers? The answer is a cautious “yes”. The hpCADD project (www.hpcadd.com) involves partners from academia (the Universities of Erlangen-Nürnberg and Dortmund) and industry (Sanofi, Frankfurt) and is funded by the German Ministry of Education and Research. The € 1.5 million, three year project involves computational and theoretical chemists, computer scientists who specialize in high-performance computing and a pharmaceutical company to test and validate new methods in real life. The aim of the project is exactly that outlined above – to drag CADD into the 21st century by allowing it to make use of modern high performance highly parallel computers and real time manipulation of the simulation scenarios via internet and modern graphical user interfaces.

We like to thank Jens Gimmler (Molcad GmbH) and Matthias Henneimann (Copos InSilico) for computational assistance.

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CADDLEs are a revolutionary new way to model drug molecules, their activity and ADME-properties. CADDLEs are completely web-based apps that require only a suitable browser on the client machine. They require absolutely no software installation apart from on the central server but nevertheless provide molecular modeling and visualization on the highest level. They can be used on any hardware with a suitable browser (desktop, tablet, smart phone).

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Dr. Joanne Laukart demonstrates the new weighing method. Developers and employees at Mettler-Toledo, the company specialising in weighing technology, are proud of the innovative gravimetric system that can now dose liquids as well as powder.
The Right Weight*

The new world of automated dosing

Every scientist studying at university was confronted in chemical practicals with the problem of dissolving a given powdered substance in a given solution and producing a solution with the prescribed concentration. The first step was to weigh. Armed with a spatula and a small sheet of weighing paper, a fixed amount of the substance was weighed on usually outdated laboratory scales and then transferred (as completely as possible) from the paper to a graduated flask, which was then filled up with solvent up to the calibration mark and stirred well. The solution was then ready. Depending on skill and experience, that would take a few minutes or longer. If many such solutions need to be made up, a tedious and time-consuming business. One may well have cursed and yearned for a device that would do this work automatically. The technology has now been around for some years to automate these activities.

The scientific director of SUCCIDA AG, Prof. Dr. Jürgen Brickmann, spoke in Greifensee near Zurich to Dr. Joanne Laukart of Mettler-Toledo AG, a company that has brought just such a system on the market, about various aspects of automated dosing.

* in contrast to Joseph Roth, The Wrong Weight, 1957
Prof. Jürgen Brickmann: How did it all start with the idea of automated powder dosing?
Dr. Joanne Laukart: It goes back a long time. Analytical balances have been around for a very long time. We conducted a survey in different industries to identify everywhere that analytical balances are used, as well as for which purposes. In the pharmaceutical industry, practically 90% of balances are used for powder dosing. We measured the time involved and estimated the reliability of the dosing. People are spending 10–20% of their working time sitting in intense concentration at their balances and dosing powder. That is tedious and not particularly sexy.

When was that?
Six to seven years ago.

Who provided the impetus for this development?
We established that we could make the use of our analytical balances significantly easier and more effective for our customers.

But there must have been a point when someone made the step from spatula to dosing head?
One of the engineers, who had been working at Mettler for 35 years and was considered a bit of a guru in his department, had the idea that sparked it all off: the transport of powder in flour mills was the all-important inspiration and the eureka moment. This moment of inspiration, a good measure of dogged persistence and the expertise on hand in the construction of balances finally resulted in the Quantos automated dosing system.

Up to what size particle can you still dose?
Particles in the order of 900 micrometers (1 micrometer = 10^-6 m) can still be easily processed.

What are more typical particle sizes?
30–40 micrometers. Sometimes even 2–3 micrometers. In terms of accuracy, the smaller, the better.

Where do customers for these instruments come from?
Until now, practically 80% come from the pharmaceutical industry.

What are the limitations?
When the substance tends to clump together, for example, or absorbs water very quickly. We tested approximately 4,000 samples. In 90% of all powders the dosing system worked perfectly. In about 10% of the samples we were unable to give doses within the tolerance limit we had set ourselves of 0.5 mg. Customers are often happy with a tolerance of 2 mg but we set ourselves this narrower limit to be able to offer customers clear improvements in processing.

Why aren’t these solutions produced on a large scale and then dosed in liquid form? Wouldn’t that perhaps be more efficient?
In theory, yes, but not in practice!

You will have to explain that to me.
It often happens in practice that users know little about the stability of the substances they are using. As a rule,
substances in powder form are more stable than in liquid form. If I produce a solution today and then store it for a week, I perhaps don’t know if the substance has changed or not.

**So, producing the solutions as and when they are required is still the better option.**

Yes, there’s no getting away from it.

**The system now also doses liquids. How does the dosing of powders compare with the dosing of liquids when it comes to safety?**

In general, the dosing of liquids manually is safer than the dosing of powders. You see when something is split and can wipe it away. With powders, it is not so easy. You can assume that about 10% does not end up where it should be. It may get into the air and then become a significant health risk if harmful substances, for example, are inhaled. With the dosing system, you dose directly into the container in which you later also produce the solution. That means that there is no weighing paper onto which an initial dose is placed. That is much safer as there is then little opportunity for the powder to scatter.

**What are the most important advantages of automated dosing?**

The time-factor: the dosing is about six times quicker than one performed manually. Then, there is the accuracy and, finally, the fact that significantly smaller amounts of solution can be produced. All the different phases of the process are documented automatically. What is particularly exciting is that there is no need for expensive volumetric flasks in liquid dosing as the solvent is weighed directly in the container. Studies have shown, moreover, that volumetric flasks are a source of errors which occur as a result of either inaccurate reading of the calibrated mark or of mixing-up one flask with another or of cross-contamination of a flask that has not been properly cleaned.

**How accurately do the balances in your systems weigh?**

Quantos has an accuracy of 5 µg in a target zone of 220 g. That means we can produce a solution with a weight of 200 g with an accuracy of 0.000005 g. Our balances have also now achieved a level of performance which means that only very small amounts of a substance are necessary to be able to perform an extremely accurate measurement. It is practically only just such an automated process that allows dosing in such small amounts. That is particularly important in the use of very expensive or highly toxic substances.

**Joanne Laukart**

studied Biotechnology at Leicester Polytechnic (UK) und graduated in Bioelectrochemistry from the Cranfield Institute of Technology (UK) in 1988. She has been working at Mettler-Toledo since 2006 and was initially responsible for Business Development at Quantos. Her main area of responsibility lies in determining and integrating customer needs/application processes and developing the Quantos product portfolio accordingly.

That is really impressive.

We are also very proud of this.

I would like to raise one more issue. If you look at the dosing head, mechanically it does not seem to be all that complicated. That means that it could be copied relatively easily and could certainly tempt product counterfeiters onto the scene. Are you already aware of any such activities?

So far, no. There is a chip on each dosing head. The system wouldn’t work without it. Moreover, the dosing system is controlled by motors that are in turn guided by computer. Such complexity is not so easily copied.

**The chip in the dosing head counts the number of doses. What is the upper limit?**

After 999 doses the lifespan of the dosing head is at an end. As a rule, significantly fewer doses are undertaken with one head.

**You couldn’t therefore dose diamond dust?**

Some customers do, but then only up to 40 doses. After 39 doses, the system then signals that only one more dose can be undertaken. The hardness of the powder is the problem here, the limit is set to ensure no detrimental effect to the powder. This is possible thanks to the intelligence built into the dosing head.

Thank you for talking so openly.
The revolutionary Quantos dosing technology reduces sample material and solvent quantities required by at least 60%. This means less waste and a higher yield from your precious sample. More than 60% of leading pharmaceutical companies are already using Quantos. When are you planning to?

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