Strength in weakness

Professor Sten Ohlson describes his new approach to drug discovery, in which he investigates overlooked drug candidates that bind to their targets in a more transient manner than traditional drugs

Can you discuss the overarching goals of your research?

The basic theme of my research efforts is to explore the role of weak or transient interactions in biological systems. As transient interactions are hard to see and evaluate in an ocean of other interactions, a lot of my efforts have centred around developing and applying technology to measure weak interactions. Focus here has been on weak affinity chromatography, surface plasmon resonance and fluorescence spectroscopy.

Another key goal for me is to apply the knowledge gained on transient biological interactions to mainly clinical diagnostics and drug discovery. In clinical diagnostics the objective is to develop continuous biosensors such as for monitoring glucose in diabetes patients and in drug discovery to develop transient drugs.

The overall goal of my work generally is to convince the individual researcher in life sciences that weak or transient interactions are extremely relevant and they should not be regarded as non-specific and of little value.

What are the main bottlenecks currently faced in the development of new drugs generally?

There are many reasons for the failure of most drug candidates. First

A natural affinity

A new approach to drug discovery is exploring previously overlooked weak transient biological interactions which could provide relief to a multitude of diseases, including schizophrenia

THE INTERACTION OF biological molecules is essential for life. Weak (transient) biological interactions occur throughout the human body and are only now starting to be appreciated by researchers who have noticed their significance in a range of complex biological networks.

Almost all molecules, including nucleic acids, peptides/proteins, carbohydrates and small organic molecules, are involved in these complex networks. Ongoing research suggests that the interactome (the whole range of biological interactions) is probably much larger than previously thought. Of these interactions, transient interactions could make up the majority.

Multisite interactions are very frequent and play an important role in parallel specific recognition in biological systems. Professor Sten Ohlson, an expert in biotechnology from Linnaeus University, Sweden, explains this in more detail: "Polyvalent interactions are the simultaneous binding of multiple sites of a ligand to multiple sites on a specific target such as a protein or a cell surface. The individual binding sites are typically weak or transient in their nature and by collective interactions they become progressively stronger".

One example of the importance of transient interactions is the binding of cells to other cells. When white blood cells are recruited to a site of inflammation, they are directed by polyvalent interactions with other cells. In the blood stream the white blood cells roll along endothelial cells that form the internal wall of the blood vessels by numerous polyvalent weak interactions of special proteins with carbohydrate units of other proteins.

However, whilst transient interactions have been shown to be extremely important, their significance has not yet been acknowledged by drug companies and many individuals within the scientific community, who continue along more traditional lines.

TRADITIONAL DRUG DISCOVERY

Most researchers working in drug discovery focus on the tightest drug binder (highest affinity) which shows the best potency (efficacy) and selectivity in relation to some particular disease.

and foremost, drug discovery is a complex process that requires huge investment both in terms of time and money (we are talking at the scale of billions of euros). In light of this, the pharmaceutical industry has had a tendency to turn away from long-term drug discovery in its purest sense, aiming instead at the more accessible development of short-term 'me-too' or 'little better' medicines. Furthermore, 'small diseases' have been neglected by the industry, as the prospects for sufficient economical return are slim.

In addition, traditional thinking in drug discovery is also a barrier, where the reductionistic paradigm of the 'magic bullet' approach to drug discovery is still prevailing. We need to dare to think more outside the box; for example, by considering the prospects of multiple (magic shot gun) and transient drugs (mild molecules) to treat complex diseases.

Can you elaborate on the new tool you are developing to enable efficient fragment screening?

Fragment-based screening has become an established tool in the pharmaceutical industry as is evidenced by the number of clinical trials performed on drug candidates originating from it. Now, I would like to add a new kid on the block for fragment screening by introducing zonal weak affinity chromatography combined with mass spectroscopy. Historically, affinity chromatography has been used as a powerful purification procedure, but it could also be favourably used for analytical purposes such as fragment screening.

Have you encountered any particular issues in the project to date?

It is a constant and healthy debate to discuss the pros and cons of a transient drug and the context in which it can be used. Certain aspects of the project, eg. how to screen difficult targets such as a membrane protein, are challenging, and efforts are currently underway in our laboratories to determine different ways of solving these problems.

How might transient binding be of great value when screening for drug side effects?

It can be argued that certain drug side effects can be caused by cumulative transient binding to various targets. However, our understanding of this is virtually unknown at this point. Up- or down-regulation of proteins, such as cytochrome P450 enzymes, may be affected by excessive weak interactions over long periods of time.

A lot of research is still needed to discover the role of weak persistent interactions with a drug especially with regard to its possible adverse reactions. Now that techniques are available to identify weak interactions in biological systems, I think we will see major progress in this area.

How do you see your research moving forward?

My initial research projects on transient drugs will be focused on central nervous system (CNS) diseases and novel methods for fragment screening of weak binders. Hopefully this will be followed by other projects in collaboration with academia and the pharmaceutical industry. I will place all my efforts on this area of transient drug discovery and the research will only be limited by the resources gained to realise these goals.

By doing so, the theory is that the new drug should minimise any undesirable side effects. The goal has therefore been to find candidates that bind in the submicromolar (<μM) range to assure tight binding to the target.

Although tremendous efforts in the drug industry have been invested in rational drug design and high throughput screening methods, the rate that new original drugs have been reaching the market has fallen over the last decade.

It might not come as a surprise, then, that new rationales for drug discovery have started to come to the fore. Instead of the 'magic bullet' approach which has become increasingly unsuccessful, a more balanced overall view of the biological system has been sought by many researchers.

USING TRANSIENT BIOLOGICAL INTERACTIONS

The use of transient interactions as a tool for drug discovery (and other areas) in biological sciences has long been neglected for a number of reasons. For example, the binding can be very subtle and the amount of binding is low. This can generate detection problems when studying weak interactions. Interference from non-specific binding effects can also pose problems.

However, some researchers are now actively studying and characterising transient interactions, which they ultimately want to use in drug discovery and other areas of research. Ohlson leads a study of transient biological interactions based on this new way of thinking about drug discovery. "I have postulated that instead of developing a traditional drug with strong or tight binding to a target, an alternative is to develop a drug (typically a low molecular weight molecule) which is characterised by weaker and dynamic binding (typically μM binding strength)," he reveals.

PSYCHIATRIC DISEASE

Ohlson's project looks specifically at mono-/multi-target transient (weak) affinity drugs to treat psychiatric diseases, which will lead to new knowledge of how to screen for hard-to-see biological transient interactions and,

TRANSIENT MONO/MULTI-BINDING DRUGS: A NEW WAY FOR PHARMACEUTICAL SCIENCE

OBJECTIVES

To understand the role and impact of weak interactions in biological systems and to apply this knowledge to the development of transient drugs, personalised medicine and clinical diagnostics.

PARTNERS

Uppsala University; Linkoping University; Lund University; Swedish University of Agricultural Sciences; Astra & Zeneca; Transientic Interactions, Sweden • **Nanyang Technological University**, Singapore • **Utah State University**, USA • **Agilent Technologies**, Germany • **Mipsalus**, Denmark

KEY COLLABORATORS

Professor Katarina Edwards, Uppsala University • **Professor Roland Isaksson**, Linnaeus University • **Professor Peter Påhlsson**, Linkoping University • **Professor Carl-Fredrik Mandenius**, Linköping University • **Professor Mats Ohlin**, Lund University • **Dr Tomas Fex**, Astra & Zeneca • **Dr Gerard Rozing**, Agilent Technologies

FUNDING

Linnaeus University • Swedish Research Council • The Knowledge Foundation • The Crafoord Foundation • Vinnova • Life-science industry

CONTACT

Professor Sten Ohlson
Research leader

Division of Biochemistry/Biotechnology
School of Natural Sciences
Linnaeus University
SE-391 82 Kalmar, Sweden

T +46 480 446 242
F +46 480 446 262
E sten.ohlson@lnu.se

STEN OHLSON graduated from Lund Institute of Technology, Lund University, Sweden in 1981 with a PhD in Chemical Engineering. He has spent 13 years in the life science industry (Gambro, Perstorp and HyClone laboratories) as vice president/director of R&D. In 1993 he joined the University of Kalmar in Sweden as associate professor in applied biochemistry, becoming professor in 1999. He is now professor and head of the Biochemistry/Biotechnology division at Linnaeus University, Sweden.

based on that, a novel concept for drug design. In particular, Ohlson hopes to find a new range of drug candidates for the treatment of schizophrenia.

These drug candidate molecules need to show binding to a number of selected brain receptor molecules alone or simultaneously. Ohlson and colleagues will use high-throughput screening in an affinity-based, miniaturised and parallel separation platform to select molecules with potential. The molecules will then be evaluated for their biological effect in functional assays to identify activation or inhibition of the selected brain receptor molecules individually and a further few will be evaluated via behavioural animal models.

SUCCESSFUL SCREENING

One of the reasons for the recent rapid development in our understanding of transient interactions has been the introduction of methods including x-ray, nuclear magnetic resonance (NMR), surface plasmon resonance (SPR), thermal shift and isothermal titration calorimetry (ITC). Alongside these, Ohlson has invented another screening method: zonal weak affinity chromatography (WAC) combined with mass spectroscopy (MS). The technique has significant advantages over these other screening methods, notes Ohlson: "When performing WAC, a fragment at an individual and low concentration (<mM) can be screened and immediate output is received in terms of affinity and kinetics to its target. The whole range of weak and medium affinities can be covered from 10 mM to µM". In addition, WAC-MS possesses several advantages when compared with other methods for fragment screening. These include robustness, simplicity, high-quality data, high throughput and affordability.

WAC-MS technology is now operational, having fully demonstrated its potential in a number of applications, such as in enzymes (proteases and kinases, two important drug targets), and further improvements are on the cards: "There is still considerable development work ahead to show that the technology works on membrane proteins (eg. embedded in the membranes of cells) such as of G protein-coupled receptors (GPCRs) – another important drug target," Ohlson explains.

WAC-MS technology is now under patent-pending and has been licensed to Ohlson's offshoot company, Transientic Interactions AB (TIAB), which plans in the immediate future to use this tool for fragment screening in the pharmaceutical industry.

CONTINUOUS BIOSENSORS

Transient interactions could also be beneficial to diagnostics. One avenue that Ohlson and colleagues are exploring is creating continuous biosensors: "When binding is weak between target and analyte the amount of bound analyte to the target is instantly related to the surrounding analyte concentration which means that the concentration of the analyte can be continuously measured second-by-second," he observes. In addition, in some cases a weaker binder is more specific than a strong binder, especially when the cross reactivity of a target is high towards the analyte and to non-relevant analytes.

SCHIZOPHRENIA AND BEYOND

The research so far has shown particular promise in treating schizophrenia, for which current medication is not entirely effective. Ohlson and his collaborators are using a cocktail of transiently interacting molecules specific to a variety of receptors involved in psychotic diseases. Whilst this is a serious challenge, it could prove to be a very successful way to treat psychotic diseases. By varying the concentration of a selection of weakly interacting molecules it could be possible to mildly stimulate or inhibit individual receptors involved in a disease.

> I have postulated that instead of developing a traditional drug with strong or tight binding to a target, an alternative is to develop a drug which is characterised by a weaker and dynamic binding