Bacterial Biosensors

Hormone dysfunction is a significant instigator of disorders ranging from cancer to infertility. Dr David Wood explains how biosensing techniques to identify compounds that mimic, or inhibit hormones, help in drug discovery

Could you begin by outlining the history of your research and your overall aims and objectives in the field of bacterial biosensors?

This work started several years ago with the development of a self-cleaving affinity tag method based on a self-splicing protein known as an 'intein'. We are able to engineer useful self-cleaving tags, but needed an additional way to control the cleaving reaction. Therefore, we started inserting small-molecule binding proteins into our intein to see if we could control cleaving by the addition of the small molecule. We were not able to generate the controllable intein cleaving that we were seeking, but instead found that we could create E. coli cells that were sensitive to the presence of various small molecules. In particular, when we made fusions between cleaving inteins and nuclear hormone receptor ligand-binding domains, we created E. coli cells that were growth sensitive to hormones and hormone-like compounds, including pharmaceuticals and endocrine-disrupting pollutants. At this point we see several potential applications for these sensors, ranging from drug discovery to early screening of potentially dangerous chemicals.

How have advances in affinity tag technologies enabled you to further your research?

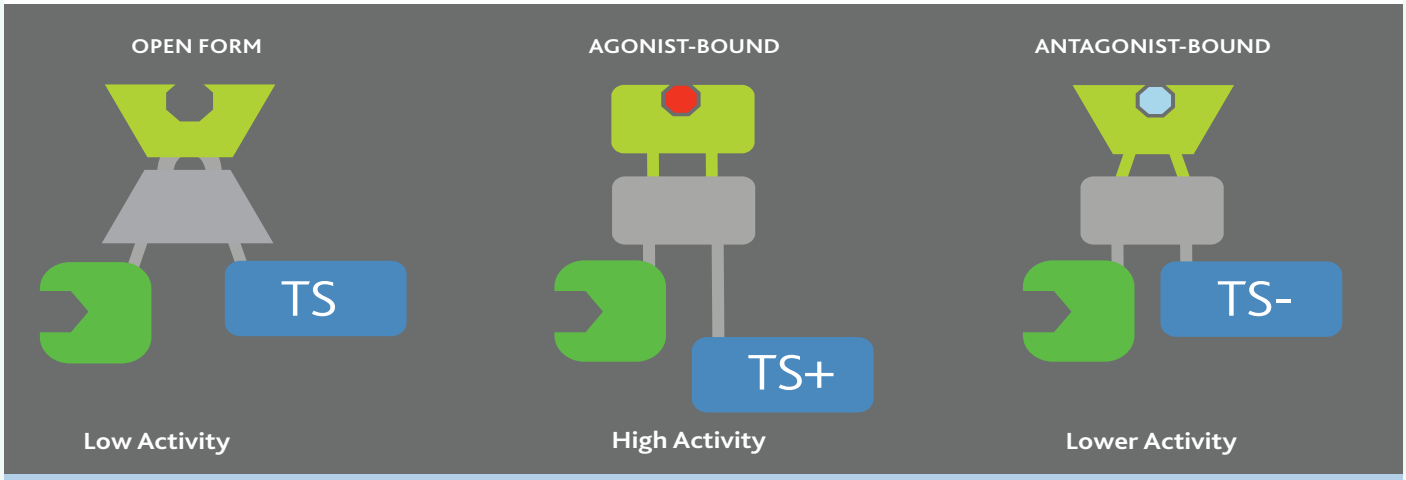
The core of my work in bioseparations has been to make affinity and other separations tags self-cleaving. Because this can be generalised, we have been greatly helped by the development of additional tags with additional capabilities. We have recently developed self-cleaving versions of a variety of tags, including non-chromatographic tags and strongly binding tags. Our goal is to eventually introduce self-cleaving purification tags to the pharmaceutical industry as a platform technology in biologics manufacturing. We have also adapted new rapid cloning technologies to our self-cleaving tags, which have the potential to greatly accelerate research on purified recombinant proteins.

Are you restricted in your work by the demands and pressures of the biotech industry?

Because of my engineering background, I generally like to focus on solving real problems for real products, and for this reason I have had a pretty good relationship with the biotech industry. For example, there has been quite a lot of excitement over some of my work in bioseparations, and I have been invited to

talk at many major conferences about how it might be applied to biopharmaceutical manufacturing. At these meetings I have come to realise that there are plenty of problems that this industry would like to solve, and although the pressure for innovation is relentless, these companies can be stymied by their own aversion to risk. One advantage of government funding is that it allows high-risk research to take place, even if the rewards may take some time. Indeed, the biosensor project itself grew unexpectedly out of my bioseparations work, and now it is a critical component of my research programme.

How important is it to have scientists from a number of different disciplines and laboratories assisting in your research?



SCHEMATIC REPRESENTATION OF AN ALLOSTERIC BIOSENSOR FUSION PROTEIN. BINDING OF AN AGONIST OF ANTAGONIST SMALL MOLECULE BY THE SENSOR ALLOSTERICALLY AFFECTS THE ACTIVITY OF THE THYMIDYLATE SYNTHASE (TS) REPORTER ENZYME. THIS CHANGE CAN BE CHARACTERISED BY OBSERVING THE GROWTH PHENOTYPES OF THE EXPRESSING CELLS

Collaborations are critical to my work. Although I have expertise in protein engineering and microbial genetics, I have very little background in relevant disease targets and many other potential applications of my work. Even short conversations with endocrinologists and clinicians have opened up entirely new avenues. Over the course of this work I have had several collaborations with scientists and medicinal chemists at several institutes in the U.S. and Europe, and these efforts have led to several co-authored papers and patent applications on previously unreported oestrogenic compounds. Indeed, one of the main advantages of these biosensors is that they are so simple that theorists with minimal laboratory resources can use them to evaluate their model predictions.

Could you offer an insight into your work with nuclear hormone receptors (NHRs) and how this can be translated into treatments for breast cancer and fertility problems?

Many important disorders are related to hormone dysfunctions in humans and animals. These include several forms of cancer, as well as osteoporosis and infertility. One way to treat these illnesses is to provide small molecules that can mimic, or in other cases inhibit, the action of specific hormones. For example, the anticancer drug tamoxifen selectively inhibits one subtype of the oestrogen receptor in breast tissue. This allows it to serve as a treatment for breast cancer with reduced side effects in other tissues. Importantly, this type of subtype-selective behaviour can be detected by our biosensors. The discovery of these sorts of compounds is a major focus of pharmaceutical development worldwide, and the availability of simple and general methods to identify subtype-selective ligands for a given hormone target will accelerate these efforts. We are particularly excited that the modular design of this sensor may facilitate the examination of receptors that have been difficult of impossible to study in conventional systems, which may allow novel treatments for many diseases to be developed.

Have you achieved any results into your research in the field of autism spectrum disorder (ASD)?

We have constructed a few biosensors using protein targets that may be associated with ASD. Currently we are working to characterise these sensors and improve their reliability. This project ties in with my previous oestrogen and thyroid biosensor work in that several potential ASD related proteins are nuclear hormone receptors, and several additional potential

Understanding Autism Spectrum Disorder

It is estimated that 1.5 million Americans are affected by autism. Dr David Wood explains how a better understanding of the causes of autism at a genetic level can offer new treatments and improve the lives of people affected by the condition

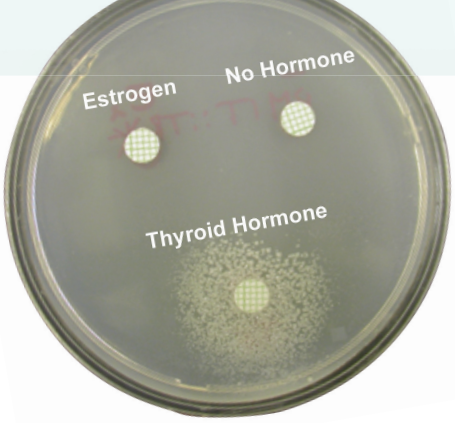
AUTISM SPECTRUM DISORDER (ASD) is now considered to be primarily genetic in origin, and several associated chromosomal regions have recently been identified. However; despite these findings, environmental factors have not been ruled out in contributing to ASD risk in susceptible individuals, and several compounds have been scrutinised over the past few decades. The emerging picture is that ASD susceptibility, resulting from complex combinations of potentially dozens of genes, can combine with specific environmental exposures to elicit the onset of ASD. Close to a dozen chromosomal regions have been associated with ASD. Within these regions lie approximately 5300 genes. Among these are encoded several known ASD-associated proteins, including transporters, receptors, transcription factors, secreted signal proteins and kinases, as well as several other types of products. Some of these proteins have the potential to bind various ligands, while others act as ligands for other protein targets, and many are involved in neurodevelopment at various levels. In addition to these proteins, there are over 100 'environmentally responsive' genes in these regions, most of which have known non-silent single-nucleotide polymorphisms, and have not been studied relative to ASD. The complexity of these interactions in the context of human development, however, has made their characterisation close to impossible, and suggests a need for simple and systematic approaches to identify specific important interactions at the molecular level.

THE APPROACH

To address these needs we have developed a novel assay, which relies on an engineered multi-domain allosteric protein expressed in a bacterial background. The engineered protein includes the ligand-binding domain of the suspected target as part of a modular design. In our previous work we have used this strategy to develop a series of E. coli strains that can detect and identify a variety of human and insect hormones, as well as natural and synthetic compounds with hormone-like effects. A critical capability of our system is that the design of the allosteric fusion protein stabilises the structure of the target protein, allowing human protein targets to fold and retain binding activity in the bacterial context. We have used these strains to detect ligands for the oestrogen and thyroid receptors, as well as the insect ecdysone receptor – all in a simple E. coli growth phenotype context. The modular design of the fusion also greatly simplifies the development of new sensors, which are generated by simple domain-swapping and minor optimisation. Finally, our sensor proteins are particularly good at detecting interactions with weakly binding compounds, which are generally missed by most conventional assays. We have used these sensors to identify several environmental oestrogen disruptors, as well as unreported drug-like estrogen and thyroid compounds, and even detect significant oestrogenicity in several popular perfumes. In our most recent work, we have identified several potential ASD-associated target proteins and have begun to incorporate them into our biosensor. These include additional thyroid hormone receptors, as well as PPARG, PPARD and neurexin, and we are currently optimising their performance. A primary goal of the present work is to understand the mechanism of this biosensor in order to systematise the incorporation of new classes of protein targets.

THE FUTURE

The ultimate goal of this research is to use our general protein engineering approach to create a series of molecular biosensors to detect active compounds against ASD-associated targets. Based on previous work, it is clear that these sensor proteins can be used to rapidly screen large numbers of compounds and mixtures against various human targets, and thus will have utility in identifying ASD-relevant environmental interactions. The simple genetic background of the sensor, and its flexibility in incorporating new target proteins, provide strong advantages in examining genetic and environmental interactions in the etiology of this complex disorder.



THE CHANGES IN GROWTH PHENOTYPE ARE SHOWN IN A PETRI DISH, WHERE CELLS WITH A THYROID BIOSENSOR ARE GROWING IN RESPONSE TO THE PRESENCE OF THYROID HORMONE

ASD related proteins are known to bind small-molecule ligands. The development of sensors to identify these specific interactions may be very helpful in determining if any environmental factors are involved with ASD, and what they may be. It is one of many approaches being used to study this complex condition, and hopefully will provide some help.

What are the potential clinical applications of your work?

We have had some interest in using these sensors to monitor hormone and drug levels in complex mixtures. Although the selectivity of our bacteria makes them well suited to this application, we would like to increase the sensitivity of the assays. We are also hoping to see these sensors used as a first-line screen for potential endocrine disrupting compounds in industry and in consumer product testing. Because it does not require animals, and can provide some important preliminary insight into the behaviour of a particular chemical, it may be a very good compromise between full animal studies and simply not testing these compounds at all. Additionally this method is inexpensive and simple, making it attractive to those who generally would be averse to experimental examination of uncharacterised compounds.

What are the main criteria by which you evaluate the success of your research? How important is the dissemination of your findings?

At the most basic level, the success of a specific biosensor design can be easily evaluated by its ability to detect the correct control compounds. In drug discovery, success is measured by the ability of our sensors to predict the behaviour of test compounds in more complex and expensive secondary assays. At a higher level, I would consider this work to be successful if we were able to generate biosensors that provide information that would have been impossible to obtain otherwise. These might include the determination of new ligands for orphan receptors, or possibly for binding module subdomains of complex proteins involved in important diseases. These sensors may also someday be used to identify the action of a highly relevant industrial chemical on an obscure but important target protein in the body. These are the types of findings that have impact in the non-scientific world, and can help society in general to make better decisions. Proper dissemination is critical to this goal, as well as for its continuation through increased funding and potential for collaborations.

How close are you to achieving your current goal of understanding the structure, energy and kinetics of your designed proteins?

Unfortunately, we are not very close. We have several hypotheses regarding the mechanism of the biosensor behaviour, generally based on crystal structures of the individual components of our biosensor proteins. These are not supported by any molecular dynamics models or direct structural information on the biosensors themselves. This is an active area of research in my laboratory, and we are approaching it from several angles.

Has your research been affected by the current economic climate?

I feel I have been pretty lucky with funding lately, although my research group is still quite small. I think that all scientists can think of projects that they would pursue with unlimited funding, and I can certainly think of a few as well, but the actual proposals that I have written in the last few years have done quite well. It is something that concerns all academic and government scientists though, and I have definitely lost sleep over it.

What is your vision for the future of your research and development in bacterial biosensors?

My primary goal is to identify design principles for generating biosensors for virtually any type of small-molecule target protein. The ability to generate bacterial versions of human membrane receptors would be particularly exciting. We would also like to understand the design and behaviour of these sensors to a point where we can confidently design functional sensors based purely on rational design principles. This would allow us to generate optimised sensors for targets that have no known ligands, and therefore cannot be validated independently. This would generate many new opportunities in drug discovery as well as in basic research.

INTELLIGENCE

BACTERIAL BIOSENSORS FOR ENDOCRINE DISRUPTING COMPOUNDS

OBJECTIVES

To develop and validate a highly flexible bacterial biosensor for the detection and characterisation of hormone-like endocrine-disrupting compounds in the environment.

FUNDING

National Institute of Environmental Health Sciences (NIEHS)

The Nancy Lurie Marks Family Foundation (NLMFF)

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DAVID WOOD recently joined the faculty at Ohio State University as an associate professor in the department of Chemical and Biomolecular Engineering. His work focuses on creating new and useful technologies at the biomolecular level by recombining functional domains of existing proteins. The proteins generated in the Wood lab have now been requested by over 100 laboratories worldwide, and he has active collaborations with over a dozen biotech companies.

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