One discovery. Two discovery. Three discovery? More?

Professor Ed Bayer

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Professor Ed Bayer seems to constantly make new discoveries, starting during his PhD with avidin-biotin interactions (a technology used by thousands of scientists today) and continuing with the discovery of the tiny factories known as cellulosomes. Here he discusses the twists and turns of his career.

Could you describe your research background? What brought you into this field of study?

It is true I've had a varied path, and in retrospect, I probably did everything wrong. I started in Zoology, but was bored with anatomy (and other such topics) and wanted to know more about mechanisms. So I studied Biology. Same thing! I wanted to go deeper, so I went into Biochemistry and then Molecular Biology and Protein Chemistry. It probably would have been better to do it in the reverse, i.e., from the physical to the chemical to the biological. In any case, here I am! My PhD was on "The Biotin Transport System in Yeast", during which time I became interested in the avidin-biotin complex. Together with my mentor, Meir Wilchek, we invented, (as it were), avidin-biotin technology, which is still broadly used in biological and clinical research, and in industry. During my postdoctoral studies I worked on oil-degrading bacteria, developing various genetic and molecular technologies to address the interaction of microbes and insoluble substrates. Towards the end of my postdoc I connected with another young scientist, Raphael Lamed, who had been working on bacterial degradation of cellulose. We put the technologies I had developed in my postdoc to good use, and the rest has been documented in the literature.

What do you believe is the most significant outcome from your research career?

I would probably have to divide my research career into two major discoveries: The avidin-biotin system and the cellulosome. The first, the invention of the avidin-biotin system, has evolved to be a widely used tool in the biological sciences, and the second, the discovery of the cellulosome, embodies a major paradigm of plant cell wall degradation with a host of significant implications – environmental, biotechnological and for alternative energy.

You were one of the pioneers of cellulosome research – was there ever a 'breakthrough' moment?

There was a "Wow!" moment. When we started, we weren't even looking for a multi-enzyme complex at all! We started with a simple observation: the bacterium binds to the cellulose substrate before it starts to degrade it, and we were actually looking for what we then called a "cellulose-binding factor", or CBF. We had two things going for us: the unconventional approach that I had previously developed precluded predetermined opinions – we could simply let the research lead us; and we ignored dogma – when research led us in a unique direction we didn't just throw the results down the sink! (I suspect that others who tried to study this system before us might have done so…).

There were two surprising defining moments. The first occurred when we discovered that the CBF contained a multiplicity of proteins, (at the time we counted 14), and so realised that the CBF was a multi-protein complex. The second was when we discovered that most, but not all, of these proteins exhibited cellulose-degrading activity – thus we inferred that the CBF was a multi-enzyme complex. Fortunately we were astute enough at the time to propose a generic term, and so the "cellulosome" was born.

Have you thought about scaling-up the designer cellulosome system to develop into an industrial enzyme mix?

Despite that fact that over 30 years have elapsed since the discovery of the cellulosome, its use in industry currently is infeasible… As opposed to the free cellulase systems, produced by aerobic fungi and bacteria, cellulosomes are produced by anaerobic bacteria. Anaerobes just don't have enough energy to compete with aerobes, and the amount of protein produced is comparatively little. To circumvent these problems we developed 'designer cellulosome' technology, based on the principles of synthetic biology, to produce cellulosomal components in large quantities in aerobic systems and allow them to self-assemble into designer cellulosomes. The development of this technology has thus far taken 20 years and is ongoing. We have now produced designer cellulosomes that rival the activity levels of the native complex, nevertheless scaling-up of the system is still premature. We still need to address numerous other aspects of the technology before we can consider its broad application as a competitive strategy for cellulosic biomass conversion to biofuels.

Where would you like to take your research in future? Have you a 'dream goal', as it were?

I have a lot of dreams! It is true that a breakthrough in the development of renewable energy and viable solutions to the energy crisis would be admirable long-term objectives – it would be exceptionally gratifying to have contributed to these lofty goals for the betterment of human society. Nevertheless, as a scientist, my goals are much less lofty, more down-to-earth perhaps, and more science-oriented. My dream goals are more connected to the biochemistry and genetics of cellulosome production and assembly. I'd like to know more about how these multi-enzyme complexes are formed on the molecular level, and the secret to their highly efficient function. These are the topics that greet me when I wake up in the morning, occupy my daydreams, and penetrate my thoughts as I fall asleep at night…

The smallest factory in the world

The Dept. of Biological Chemistry at the Weizmann Institute of Science is home to over 24 research groups, each tied together by a common thread: a focus on the biochemistry of life. Here we look at Professor Bayer's work on natural, Nano-scale factories – cellulosomes.

Imagine a factory. Not as you usually would, there is no giant boxy shed full of machinery here. Instead you need to picture a long stretch of LEGO blocks, each manufacturing a certain product, clicking into place in a way that is both highly ordered and yet immensely flexible. Sounds like nothing you've ever heard of? That's because this particular factory is built at the molecular scale, comprised of enzymes which are simply invisible to our eyes. Although tiny, we can still build, modify and use them for our own purposes.

Paper, wood, cotton. Many industries rely on one vital, humble molecule: cellulose. New research may help us to do even more with this plentiful, renewable resource.

Where do these factories come from? And why do they look so strange? To understand this, we need to look at one of the most ubiquitous energy sources available – plants. Plants, trees, grasses, shrubberies, etc. All of these convert energy from sunlight into sugars (carbohydrates) which are then used both for energy storage and to provide the structure for the plant itself. The most common structural carbohydrate in plants is known as cellulose, and it plays an important role in the lives of organisms ranging from humans to bacteria.

A WORLD OF OPPORTUNITY IN A SINGLE PLANT

As an exceptionally common molecule, cellulose is a valuable potential energy source for many different organisms. However, due to the particularly intractable nature of cellulose, it is often too difficult to degrade into the component sugars and thus provides very limited nutritional value. It instead passes through the intestines effectively untouched, providing us with the dietary fibre which our body needs. For those species which can degrade cellulose, however, a whole new world of carbohydrate energy opens up. This is a particular advantage for fungi which colonise dead and decaying wood, allowing them to feed off the energy stored within the structure itself. Many bacteria have also evolved the ability to degrade cellulose, allowing them to colonise otherwise unavailable niches (cow stomachs, for example, where they are vital for energy extraction from plant matter).

Enzymes which degrade cellulose are known, generally, as cellulases. The action and format of these enzymes vary, as does the amount of enzyme which is produced by any one bacteria. Aerobic bacteria live in oxygen-filled environments, such as on the wooden log outside your door, and tend to produce vast amounts of cellulases. Anaerobic bacteria survive in the absence of oxygen, such as within a cow's rumen, and due to the generally low-energy environment tend to be more efficient in how they produce these enzymes. In particular, they usually produce lower numbers of enzymes but organise these enzymes together into clusters known a cellulosomes. This was first noted in an anaerobic bacterium known as *Clostridium thermocellum,* which produces an entire enzyme complex which is capable of, given time, degrading even tough cotton fibre into soluble sugar.

How does this actually work in practice? The first stage, the LEGO baseplate as it were, is made from a protein known as scaffoldin, which attaches itself to the cellulose fibre at one end via the aptly named carbohydrate-binding module, or CBM. Scattered along the protein are cohesin modules, which act as attachment points for other parts of the growing complex. Cellulase enzymes then bind to these cohesin locations via their own dockerin modules, each of which allows strong and specific binding at one or more locations. The earliest observed cellulosomes were very simple

constructs, with a single base and 5-9 cellulase 'blocks'. However, the complexes can become immensely complicated as further scaffoldin proteins attach themselves to the initial scaffold, each of which can then branch further and attach even more enzymes to the complex. Bacteria such as *Clostridium clariflavum* can have up to 160 enzymes in a single complex, all working together to form a giant cellulose-degrading machine.

The major advantage of this rather intricate approach is that it brings together a number of diverse enzymes together into one common location.

How do we actually know this? Predominantly due to the pioneering work of Professor Edward Bayer, who discovered the cellulosome system with Professor Lamed in the early 1980's. Following on from this discovery he began to focus on characterising the multitude of parts involved in the system, thus beginning a long and successful career in cellulosome research which continues to this day. One of the areas in which his lab is focused is in the development of 'designer cellulosomes' – whereby target-binding domains are modified to create a specific complex for each possible need. As each cohesin/dockerin pair has a set binding specificity, it is possible to construct a human-defined molecular factory. Professor Bayer's group has already designed novel cellulosomes and shown that they can be produced at small scales – improved yields could help with the growing problem of cellulose waste.

"HOMO CELLULOSIS"

The strength and stability of cellulose are vital to a number of human endeavours. As Professor Bayer comments "Cellulose has been closely woven into the fabric of our society through the development of the wood, paper and textile industries". The oldest of these is construction, with wood providing one of the earliest building materials and one which is still in constant use today. It is also vital for the textile industry (cotton being almost pure cellulose, for example) and paper industry (paper being, essentially, cellulose fibres stuck together). While its stability is the driving force behind the utility of cellulose, it is precisely this stability which leads to problems in disposal. While paper and cloth recycling systems currently exist, they are not applicable to a number of industrial processes which involve the production of large amounts of plant waste (as for example in fruit juice production, which already involves the heavy use of less-effective cellulase enzymes).

Efficient degradation of cellulose to produce free sugars also allows it to be used as feedstock for further biotech processes, such as enzyme production or bioethanol manufacture. Current technology involves the use of free-floating cellulases, a process which is not yet truly cost effective. Professor Bayer sees potential in the incorporation of designer cellulosome systems: "the two systems – free versus cellulosomal – act in very different, but complementary ways" he comments. "Perhaps the secret to high efficiency lies in a combination of the two". Now that the initial steps have been taken, the next step is to scale the system up, to see how well it can compare to current technology used by industrial producers such as DuPont and Novozymes. While promising, Professor Bayer is cautious, "the development of this technology has thus far taken 20 years and is ongoing… We still need to address numerous aspects of designer cellulosome technology before we can consider its broad application".

Despite thousands of years' experience in working with cellulose-containing substances, we remain puzzled by efficient degradation – the sheer toughness that we prize often returns to haunt us. Plant matter, full of cellulose, remains a plentiful, renewable resource. Recent research into the cellulosome by groups such as Ed Bayer's may allow us to gain the maximum amount of value from each tree.

Researcher Profile

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Professor Edward Bayer received his PhD in Biophysics from the Weizmann Institute of Science. His career has encompassed two major discoveries; the existence of the avidin-biotin interaction system and the discovery of the cellulosome. He has authored almost 400 publications and serves as an editor-in-chief, editor, and/or on the editorial board of several journals in the fields of biotechnology and microbiology. For his work in pushing the boundary of knowledge he has been awarded both the Sarstedt Award and the Ulitzky Prize, as well as being elected to the Fellowship of the American Academy of Microbiology and the European Academy of Microbiology.

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