

Pioneering engineering

Professor José Avalos is engineering the powerhouses of the cell – mitochondria. Here, he discusses his inadvertent entry into the field of mitochondrial engineering, and his plans to open a new interdisciplinary lab in 2015



To begin, could you provide an overview of your research?

Eukaryotic cells are characterised by subcellular compartments with essential and unique functions. Each compartment has different environments, enzymes, metabolites and cofactors. Most previous efforts in yeast metabolic engineering have focused on targeting specific pathways in the cytoplasm, while relatively little attention has been paid to the potential of subcellular compartments, where the pathways of interest may in fact work more effectively.

However, when engineering cells, it is important that subcellular compartmentalisation is not ignored. One must pay attention not only to what is occurring in the cell, but also where it is happening.

Can you describe your professional background and what led you to this area of study?

I obtained an undergraduate degree in Chemical Engineering, during which time I became passionate about two topics: environmental remediation and biotechnology. After graduating, I wanted to satisfy my curiosity in

biology, so I completed an MSc in Biochemical Research at Imperial College, London, UK, after which I decided to pursue a PhD in Biochemistry and Biophysics at Johns Hopkins University, USA, with Professors Cynthia Wolberger and Jef Boeke. After my PhD, I joined Rod MacKinnon's lab at Rockefeller University, USA, looking to be immersed in the world of ion channels.

I loved my experiences at Johns Hopkins and Rockefeller, because they allowed me to blend my passions for chemistry, biology and physics. However, my original passion for environmental issues was missing. I decided to join Professors Greg Stephanopoulos and Gerry Fink at MIT, drawn by their work on biofuels, to reconnect with my original desire to be involved in environment-related research. My landing on mitochondrial engineering was, in a sense, accidental. I realised that to engineer yeast to make the biofuels I was pursuing, I could not ignore the natural involvement of mitochondria. I decided instead to embrace mitochondria, and engineer it for the benefit of my pathway.

How do your studies differ to those conducted by other research groups?

Others have targeted mitochondria for different purposes, for example, to tap into a metabolite or enzymatic activity, or to avoid side-product reactions. However, these efforts have recruited the mitochondria into pathways that are fragmented throughout different compartments in the cell. We showed that an entire pathway compartmentalised in the mitochondria can be functional, and outperform the identical pathway in which enzymes are targeted to their native compartments.

Do you foresee any specific difficulties in attempting to engineer mitochondria?

There are many things we know about mitochondrial physiology and metabolism, which offer us a lot of potential leads. However, one of the biggest challenges I foresee is our incomplete understanding of yeast mitochondria. This means when we try

to engineer some aspect of this organelle, we might be surprised about the outcomes of our manipulations. While this is a challenge for our engineering efforts, it is also a goldmine of opportunities for new discoveries.

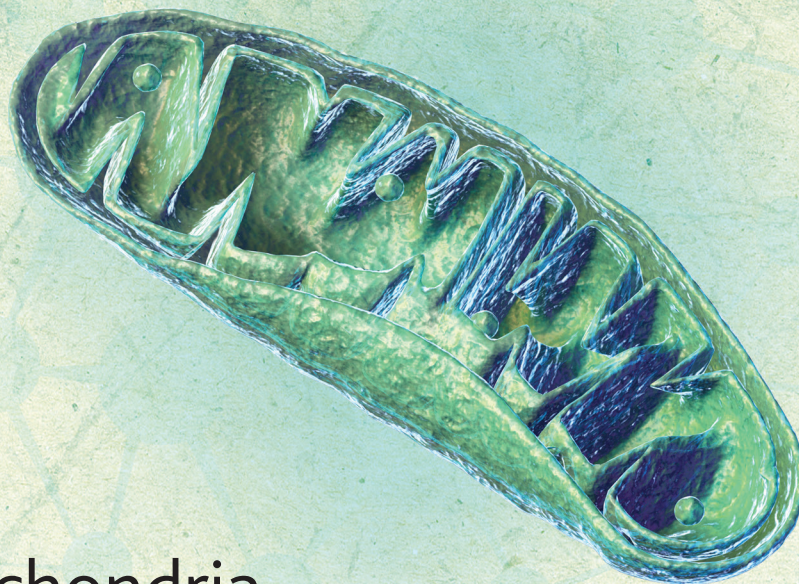
Another challenge is that of metabolite transport. Raw materials have to enter the mitochondria, and the products must exit. It might be necessary to engineer transporters targeted to the mitochondrial membrane in order to catalyse these processes.

By what means do you hope to integrate multiple disciplines to establish a laboratory of synthetic biology and metabolic engineering?

I have a diverse background, spanning chemical engineering, biochemistry, biophysics, molecular biology, genetics and metabolic engineering. I plan to establish a lab in which our technological developments are guided by basic research on the phenomenon or item we are engineering (a protein, metabolic pathway, organelle, etc.). We plan to complement our efforts in metabolic and mitochondrial engineering with the development of biosensors and genetic switches, which will help us to monitor and control the functions we are engineering. Furthermore, I will tap into my background in biochemistry and biophysics to study *in vitro* the proteins that are relevant to the synthetic functions we are working on. I feel incredibly fortunate to have the opportunity to establish a new lab at Princeton University, USA, where I will blend all my experiences and passions.

Finally, are there any discoveries of which you are particularly proud?

I have been fortunate to participate in various research projects of which I am proud, including elucidating the structure-based molecular mechanisms of sirtuins at Johns Hopkins; helping to solve the first full-length crystal structure of a eukaryotic inward-rectifying potassium channel; and, most recently, contributing to the development of mitochondrial engineering.



Synthetic mitochondria

Researchers from **Princeton University** in the US are creating novel subcellular engineering methods to synthesise a range of molecules, from drugs to fuel. If successful, their work could lead to the first ever synthetic mitochondria and further broaden the applications of subcellular engineering in general

THE FIELD OF cellular engineering, which applies the principles and techniques of engineering to living cells, has grown rapidly in recent years. As biological engineering has increasingly focused on subcellular compartments, it has developed into an important scientific discipline, enabling scientists to develop cells with traits for medical, industrial and environment benefit. Cells can be 'hijacked' to manufacture a range of biochemicals, and have long been engineered to synthesise therapeutics and fuels.

Professor José Avalos, based at Princeton University, USA, plans to change the cellular engineering paradigm. Delving even deeper, he is engineering at the subcellular level, within compartments known as organelles. Focusing on mitochondria, the 'power plants' of the cell, he is able to synthesise high-demand products with increased efficiency.

USING EUKARYOTES

Yeast (which are eukaryotes) are commonly used for this cellular form of engineering. They offer numerous advantages over bacterial hosts (prokaryotes) for industry; they are more tolerant to stress and better able to express eukaryotic genes, for example. However, they also present a number of challenges.

The most obvious of these challenges relates to cellular architecture. Bacteria have a simple cellular set up, comprising mostly of a single cytoplasmic area that contains the majority of enzymes, metabolites and nucleic acids needed by the cell. In contrast, eukaryotic cells have a much more complex architecture. Their cells have several different compartments, or organelles, separating the biomolecules. Not entirely separate entities, these organelles are connected to each other through elaborate signalling and transport networks. This means the location of a molecule or function of interest must be considered – a complication not found in the single compartments of bacteria: "There are also other challenges not found in groups like bacteria, such as the need to express genes individually," Avalos adds. Researchers have been studying these issues for many years, but it is only recently that science has begun to harness compartmentalisation for engineering benefit.

THE CHALLENGES OF COMPARTMENTALISATION

Indeed, because of the challenges associated with the complexities of eukaryotic cells, most metabolic engineering efforts have left subcellular compartments virtually untouched, instead focusing on engineering pathways

within the cytoplasm. This represents huge untapped potential for Avalos. "Different compartments have different environments, which may be better for the engineered functions than the cytoplasm. Targeting organelles enables us to take advantage of the enzymes, cofactors or metabolites present there," he explains. For example, by targeting the synthesis pathway in a specific organelle it is possible to use transport bottlenecks associated with metabolites entering or exiting the organelle to our advantage, and limit the loss of metabolites to competing pathways. "One can also see organelles as isolated vessels, where a metabolic pathway can be placed to keep its intermediates from reacting with cytoplasmic components, confer pathway specificity, or avoid the toxicity of intermediates to other parts of the cell," Avalos comments. It is clear that such an approach has many added benefits. By concentrating enzymes into the smaller volume of an organelle, their local concentrations are increased, thereby increasing the productivity of the pathway.

TARGETED SYNTHESIS

Avalos is focusing on one organelle in particular: the mitochondrion. These highly dynamic organelles offer vast engineering potential.

Mitochondria are the result of what is called an endosymbiotic event, whereby a free-living organism capable of respiration (the combustion of food to energy) was engulfed by another cell around 2 billion years ago. This conferred the ability to carry out respiration to the eukaryote. Today, mitochondria still contain many of the original cellular components from the prokaryote, including their own DNA, cellular membrane, enzymes and the machinery necessary to replicate DNA and synthesise proteins. Despite this high level of autonomy, the majority of genes underlying these processes are found in the nucleus of the cell.

However, a number of studies have shown that the proteins encoded in the nucleus can be targeted to the mitochondria. This includes enzymes, which can be targeted to the mitochondria to produce important compounds including hydrocortisone, commonly used to treat skin conditions dermatitis and eczema; and terpenoids, traditional herbal remedies with potential antibacterial activity.

Work to date though has involved pathways fragmented across a number of subcellular compartments. Challenging this model, Avalos' research, published in *Nature Biotechnology* last year, focused on one. His team compared the performance of synthetic pathways for advanced biofuels targeted to the mitochondria alone, and to their original compartments (fragmented between the mitochondria and the cytoplasm), allowing them to directly measure the effect of mitochondrial targeting on the performance of engineered pathways. Strikingly, they found mitochondrial targeting led to a significant improvement in the production of

three advanced biofuels, reaching a 500 per cent increase in some cases.

MEDICAL PROMISE

Avalos' work in mitochondrial engineering, and subcellular engineering more broadly, will advance the field of metabolic engineering. The real-world applications are numerous, including the production of advanced fuels, chemicals and therapeutic compounds. Importantly, this work comes at a particularly opportune time, one of a 'mitochondrial renaissance'.

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Due to its vital and varied roles in human physiology, and links with ageing, there has been significant renewed interest in mitochondria for medical research. As a result of these improved efforts, there have been a flurry of fundamental discoveries in mitochondrial physiology, metabolism and interactions. Consequently, we now understand much more about its role in a number of human disorders, from Parkinson's disease to diabetes. It is therefore possible that Avalos' work on yeast mitochondrial engineering, used today for industrial purposes, could one day be used to develop novel treatments for human disease.

THE ROAD AHEAD

Through their intensive research efforts, Avalos' group has shown not only that exogenous enzymes can function inside yeast mitochondria, but also that entire pathways targeted to mitochondria can remain functional, and furthermore, can outperform identical pathways targeted to their natural compartments.

Although significant progress has been made, many obstacles must be overcome, as the field of mitochondrial engineering remains in its nascent stages. Specific challenges lie in determining to what extent the advantages of mitochondrial engineering can be exploited, and how widely the technology can be applied. The team has successfully engineered mitochondrial metabolism, but they are yet to engineer other aspects of the organelle, such as its physiology, signalling networks and dynamics. In order to reach the full potential of mitochondrial engineering, Avalos hopes to ultimately develop fully engineered, or synthetic, organelles: "Synthetic organelles have engineered metabolisms, physiologies and behaviours that work to generate a beneficial function. I plan to expand my research to make this a reality," he concludes.

INTELLIGENCE

MITOCHONDRIAL ENGINEERING

OBJECTIVES

To engineer organisms with new desirable traits of medical, industrial and environmental interest.

The laboratory will be purpose-built to:

- Engineer cells forging established methods in metabolic engineering and synthetic biology, with new technologies developed in the lab, including mitochondrial engineering
- Address fundamental questions of protein structure and function, cellular physiology and metabolism that either currently limit efforts in cellular engineering or offer opportunities for new technologies

FUNDING

Previous funders include the US National Institutes of Health and Shell Global Solutions

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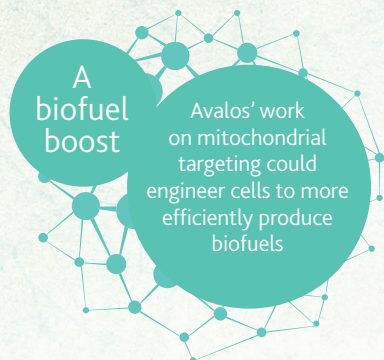
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JOSÉ AVALOS is an assistant professor in the Department of Chemical and Biological Engineering at Princeton University in the USA. He has formal training in chemical engineering, biochemistry, biophysics, and metabolic engineering. He received his PhD from Johns Hopkins University, and carried out postdoctoral studies at Rockefeller University in New York, and MIT/Whitehead Institute in Cambridge, Massachusetts. He has received various awards, including the Weintraub Graduate Research Award, the Damon Runyon Cancer Research Fellowship and the Ruth Kirschstein National Research Service Award.



Biofuels, such as isobutanol, are promising and renewable alternatives to gasoline. Targeting the biofuel biosynthetic pathways to mitochondria could enhance their production, providing a route to producing large quantities, reliably and from renewable sources.

Yeast traditionally makes isobutanol in small amounts, but Avalos' team found a way to boost production, by engineering yeast so that isobutanol synthesis only takes place within mitochondria.

This principle could be applied to other molecules and other organelles, a strategy that will advance progress in metabolic engineering.

