Bioengineered Yeast Could Be Key to New and Better Medicines

A research team at Sylvester Comprehensive Cancer Center at the University of Miami Miller School of Medicine has shown that bioengineered *Saccharomyces cerevisiae* (baker’s yeast) can help identify signaling molecules (ligands) for a class of important drug targets called G protein-coupled receptors (GPCRs).
In a paper published in *PNAS*, the group described how this yeast-based system, called dynamic cyan induction by functional integrated receptors (DCyFIR), can rapidly find potentially therapeutic molecules. In addition, the team also identified ligands that regulate so-called pharmacologically dark receptors, which have been considered undruggable.

“There are 800 GPCRs, half of which are in the nose and eyes and tongue. The other half are distributed throughout the body and essentially every tissue,” said Daniel Isom, Ph.D., assistant professor of molecular and cellular pharmacology and...
senior author of the paper. “As a result, they are the most therapeutically-targeted class of receptors on the cell surface for cardiovascular drugs, psychiatric drugs, diabetes drugs – you name it.”

GPCRs live on outer membranes and are key components in an elaborate cellular communication network. When a signaling molecule binds to a GPCR, the receptor sends a message through a chain of intermediary proteins, the first of which is called $G_\alpha$. This signaling modulates cell behavior, making them promising targets for therapeutic interventions.

While researchers and drug companies have spent decades searching for signaling molecules that activate GPCRs, these efforts have been slow and often ineffective. Of the 400 most interesting GPCRs, about a third are considered pharmacologically dark. Around 30% of approved drugs target GPCRs.

To help solve this problem, Dr. Isom’s team turned to *Saccharomyces cerevisiae* because it has only one GPCR pathway. In the *PNAS* paper, the scientists described using CRISPR gene editing to introduce human GPCRs into the yeast. They also added a reporter gene to make the receptor glow when activated. Molecules that activate a GPCR could potentially be refined into an effective medicine.

“All of the receptors are put into one test tube and exposed to a drug, and then any that are activated make the cells glow cyan,” Dr. Isom said. “We can identify which receptors were in the active pool. This scales up, so we can do it for any number of ligands. Every time we do an experiment, we’re doing something no one else has ever done before: Testing a drug or
a metabolite against many receptors all at once.”

But the researchers took this one step further. Along with each GPCR, they engineered ten Ga protein variations into the yeast model. As a result, while the study looked at 30 receptors, it investigated 300 different combinations. In addition to identifying which receptors responded to a specific molecule, DCyFIR also showed which Ga proteins reacted to the coupling.

The lab used DCyFIR to test 320 human metabolites and found multiple hits, including on some receptors that were considered undruggable. In other cases, they found molecules that activated a receptor better than a known ligand.

This approach has tremendous implications for drug discovery, potentially identifying an enormous pool of molecules that can modulate GPCRs.

However, this proof of concept paper was only a start. In another study, published in the *Journal of Biological Chemistry*, Dr. Isom’s team synthetically engineered four gene editing land pads in the *Saccharomyces cerevisiae* genome.

These CRISPR-addressable landing pads provide a “safe harbor” for gene edits because they are not highly regulated by the yeast’s epigenome. In other words, there is less risk the yeast will turn off those genes after scientists have gone to the trouble of adding them in. This work essentially provides a gene-editing manual for yeast researchers.

“Since we have four of those sites, people can just automatically hit them with high precision every time because
they're CRISPR addressable,” Dr. Isom said. “So, we've solved that problem, and now we think we're going to have that standardized.”

While these results can be used to optimize the DCyFIR system, they have far wider applications, giving researchers new tools to interrogate a variety of systems. With so many options, researchers can edit in a gene for a receptor, as well as a gene for the peptide that activates it, providing incredible research opportunities.

“This really allows us to explore small metabolic pathways,” Dr. Isom said. “Imagine putting three or four enzymes that make vitamin A, for example, into the pads. Then you'd be doing synthetic biology easily just using that strain.”

In the near future, Dr. Isom and his group hope to use these tools to study how different drugs function at specific pH levels. This could lead to therapies that better target inflammation or tumors, both of which tend to thrive in more acidic environments, while leaving healthy tissues alone.

“The importance of this is that tumor microenvironments are more acidic,” Dr. Isom said. “So, we can develop drugs that target these tumor microenvironments and touch the receptors there, but don't touch a receptor in a normal microenvironment.”

In addition to Dr. Isom, the research was performed by graduate students Jacob Rowe, who was first author on the JBC paper, Nicholas Kapolka and Geoffrey Taghon, who shared co-first authorship with Jacob Rowe on the PNAS study; research associates William Morgan and Jay Enten; and Nevin Lambert,
Ph.D., professor of pharmacology and toxicology at Augusta University.