Speeding up directed evolution of molecules in the lab using a new robotic platform

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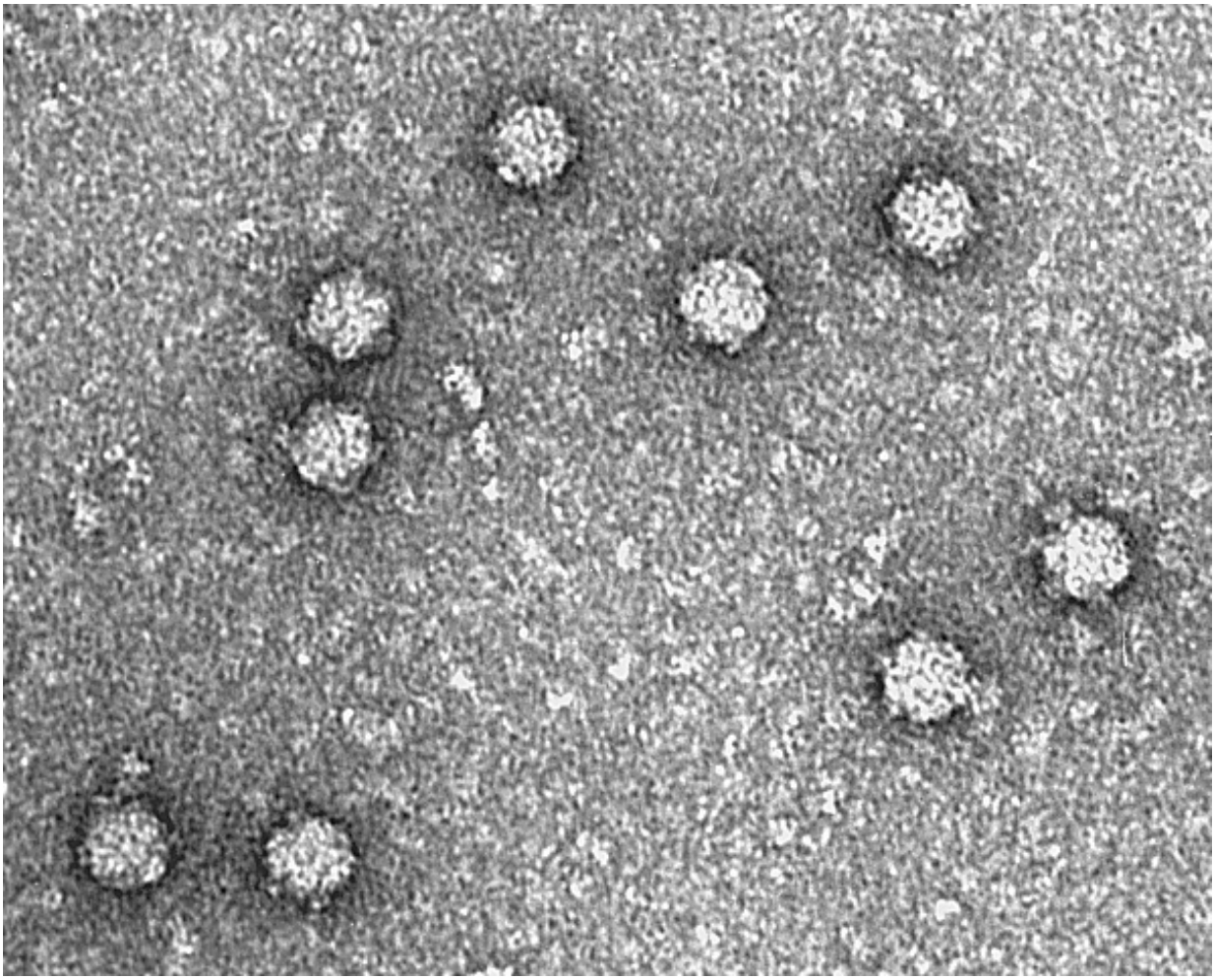

Bacteriophage Phi X 174 Electron micrograph. Credit: Wikipedia/CC BY-SA 4.0

Natural evolution is a slow process that relies on the gradual accumulation of genetic mutations. In recent years, scientists have found ways to speed up the process on a small scale, allowing them to rapidly create new proteins and other molecules in their lab.

This widely-used technique, known as directed evolution, has yielded new antibodies to treat cancer and other diseases, enzymes used in biofuel production, and imaging agents for magnetic resonance imaging (MRI).

Researchers at MIT have now developed a robotic platform that can perform 100 times as many directed-evolution experiments in parallel, giving many more populations the chance to come up with a solution, while monitoring their progress in real-time. In addition to helping researchers develop new molecules more rapidly, the technique could also be used to simulate natural evolution and answer fundamental questions about how it works.

"Traditionally, directed evolution has been much more of an art than a science, let alone an engineering discipline. And that remains true until you can systematically explore different permutations and observe the results," says Kevin Esvelt, an assistant professor in MIT's Media Lab and the senior author of the new study.

MIT graduate student Erika DeBenedictis and postdoc Emma Chory are the lead authors of the paper, which appears today in *Nature Methods*.

Rapid evolution

Directed evolution works by speeding up the accumulation and selection of novel mutations. For example, if scientists wanted to create an antibody that binds to a cancerous protein, they would start with a test tube of hundreds of millions of yeast cells or other microbes that have

been engineered to express mammalian antibodies on their surfaces. These cells would be exposed to the cancer protein that the researchers want the antibody to bind to, and researchers would pick out those that bind the best.

Scientists would then introduce random mutations into the antibody sequence and screen these new proteins again. The process can be repeated many times until the best candidate emerges.

About 10 years ago, as a graduate student at Harvard University, Esvelt developed a way to speed up directed evolution. This approach harnesses bacteriophages (viruses that infect bacteria) to help proteins evolve faster toward a desired function. The gene that the researchers hope to optimize is linked to a gene needed for bacteriophage survival, and the viruses compete against each other to optimize the protein. The selection process is run continuously, shortening each mutation round to the lifespan of the bacteriophage (which is about 20 minutes), and can be repeated many times, with no human intervention needed.

Using this method, known as phage-assisted continuous evolution (PACE), directed evolution can be performed 1 billion times faster than traditional directed evolution experiments. However, evolution often fails to come up with a solution, requiring the researchers to guess which new set of conditions will do better.

The technique described in the new *Nature Methods* paper, which the researchers have named phage and robotics-assisted near-continuous evolution (PRANCE), can evolve 100 times as many populations in parallel, using different conditions.

In the new PRANCE system, bacteriophage populations (which can only infect a specific strain of bacteria) are grown in wells of a 96-well plate, instead of a single bioreactor. This allows for many more evolutionary

trajectories to occur simultaneously. Each viral population is monitored by a robot as it goes through the evolution process. When the virus succeeds in generating the desired protein, it produces a fluorescent protein that the robot can detect.

"The robot can babysit this population of viruses by measuring this readout, which allows it to see whether the viruses are performing well, or whether they're really struggling and something needs to be done to help them," DeBenedictis says.

If the viruses are struggling to survive, meaning that the target protein is not evolving in the desired way, the robot can help save them from extinction by replacing the bacteria they're infecting with a different strain that makes it easier for the viruses to replicate. This prevents the population from dying out, which is a cause of failure for many directed evolution experiments.

"We can tune these evolutions in real-time, in direct response to how well these evolutions are occurring," Chory says. "We can tell when an experiment is succeeding and we can change the environment, which gives us many more shots on goal, which is great from both a bioengineering perspective and a basic science perspective."

Novel molecules

In this study, the researchers used their new platform to engineer a molecule that allows viruses to encode their genes in a new way. The genetic code of all living organisms stipulates that three DNA base pairs specify one amino acid. However, the MIT team was able to evolve several viral transfer RNA (tRNA) molecules that read four DNA base pairs instead of three.

In another experiment, they evolved a molecule that allows viruses to

incorporate a synthetic amino acid into the proteins they make. All viruses and living cells use the same 20 naturally occurring amino acids to build their proteins, but the MIT team was able to generate an enzyme that can incorporate an additional amino acid called Boc-lysine.

The researchers are now using PRANCE to try to make novel small-molecule drugs. Other possible applications for this kind of large-scale directed evolution include trying to evolve enzymes that degrade plastic more efficiently, or molecules that can edit the epigenome, similarly to how CRISPR can edit the genome, the researchers say.

With this system, scientists can also gain a better understanding of the step-by-step process that leads to a particular evolutionary outcome. Because they can study so many populations in parallel, they can tweak factors such as the mutation rate, size of original population, and environmental conditions, and then analyze how those variations affect the outcome. This type of large-scale, controlled experiment could allow them to potentially answer fundamental questions about how evolution naturally occurs.

"Our system allows us to actually perform these evolutions with substantially more understanding of what's happening in the system," Chory says. "We can learn about the history of the evolution, not just the end point."

More information: Erika A. DeBenedictis et al, Systematic molecular evolution enables robust biomolecule discovery, *Nature Methods* (2021). DOI: 10.1038/s41592-021-01348-4

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