# 'Directing' evolution to identify potential drugs earlier in discovery

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Scientists have developed a technique that could significantly reduce the time of discovering potential new antibody-based drugs to treat disease.

Antibodies are produced by the body in response to the presence of a disease-causing agent. They can also be synthesised in the laboratory to mimic natural antibodies and are used to treat a number of diseases.

Antibody therapies can be highly effective, but challenges can arise when promising candidate antibodies are produced at a large scale. Stresses encountered during manufacturing can disrupt the structure of these fragile proteins leading to aggregation and loss of activity. This in turn prevents them from being made into a therapeutic.

New research from an eight-year collaboration between scientists at the University of Leeds and the biopharmaceutical company AstraZeneca has resulted in a technique that allows fragments of antibodies to be screened for susceptibility to aggregation caused by structure disruption much earlier in the drug discovery process.

The approach is described in the journal *Nature Communications*, published today.

Dr. David Brockwell, Associate Professor in the Astbury Centre for Structural Molecular Biology at the University of Leeds, led the research. He said: "Antibody therapeutics have revolutionised medicine. They can be designed to bind to almost any target and are highly specific.

"But a significant problem has been the failure rate of candidates upon manufacturing at industrial scale. This often only emerges at a very late stage in the development process—these drugs are failing at the last hurdle.

"But our research is turning that problem on its head."

When it comes to developing an antibody drug, scientists are not restricted to a single protein sequence. Fortunately, there is often an array of similar antibodies with the same ability of locking or binding tightly onto a disease-causing agent. That gives researchers a range of proteins to screen, to determine which are more likely to progress

through the development process.

Professor Sheena Radford, FRS, Director of the Astbury Centre for Structural Molecular Biology, said: "The collaboration that has existed between the team of scientists within the University of Leeds and AstraZeneca demonstrates the power of industry and academia working together to tackle what has been one of the major roadblocks preventing the efficient and rapid development of these powerful therapeutic molecules."

## How the target proteins are screened

The target proteins are cloned into the centre of an enzyme that breaks down antibiotics in the bacterium E.coli. This enables the scientists to directly link antibiotic resistance of the bacteria to how aggregation-prone the antibody fragment is. A simple readout—bacterial growth on an agar plate containing antibiotic—gives an indication of whether the target protein will survive the manufacturing process. If the antibody proteins are susceptible to stress, they will unfold or clump together, become inactive, and the antibiotic will kill the bacteria. But if the protein chain is more stable, the bacteria thrives and will display antimicrobial resistance and will grow in the presence of the antibiotic.

The scientists harvest the bacteria that have survived and identify the cloned protein sequence. That indicates which protein sequences to take forward in the development pipeline. The whole cycle takes about a month and increases the chances of success.

## Directed evolution

But the process can go a step further, using the idea of directed evolution.

Scientists use the idea of natural selection where mutations or changes take place in the proteins, sometimes making them more stable. Directed evolution could generate new better performing sequences that, at the current time, cannot even be imagined, let alone designed and manufactured. How does this method work? Like Darwin's natural selection, evolutionary pressure in this case is applied by the antibiotic and selects for the survival of bacteria that produce the protein variants that do not aggregate.

The protein sequences hosted in the bacterial cells that have shown resistance are harvested and their genes sequenced and scored, to select the best performing sequences. After a quick check to ensure that the new antibody sequences still retain their excellent binding capability for the original disease-causing target, they can be taken forward for further development.

Professor Radford said: "There is tremendous excitement about this approach. We are letting evolutionary selection change the sequence of the proteins for us and that might make some of them more useful as drug therapies. Importantly for industry, nature does the hard-work—obviating the need for so called rational engineering which is time- and resource-intensive.

"As we do this, we will be putting the sequence information we gather into a database. As the database gets bigger, it may well be possible with artificial intelligence and machine learning to be able to identify the patterns in protein sequences that tell us that a protein can be scaled up for pharmaceutical production without needing any experiments. That is our next challenge and one we are tackling right now."

Dr. David Lowe, who led the work at AstraZeneca, said: "The screening system that we have developed here is a great example of industry and academia working together to solve important challenges in the

development of potential new medicines.

"By combining AstraZeneca's antibody discovery and screening expertise, together with the Astbury Centre's world-leading knowledge of protein structure and aggregation, we have produced a high throughput method for rapidly evolving proteins with better biophysical properties that has the potential for wide scientific applicability."

**More information:** Jessica S. Ebo et al, An in vivo platform to select and evolve aggregation-resistant proteins, *Nature Communications* (2020). DOI: 10.1038/s41467-020-15667-1

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