New screening technique set to radically speed up hunt for cancer-fighting drugs

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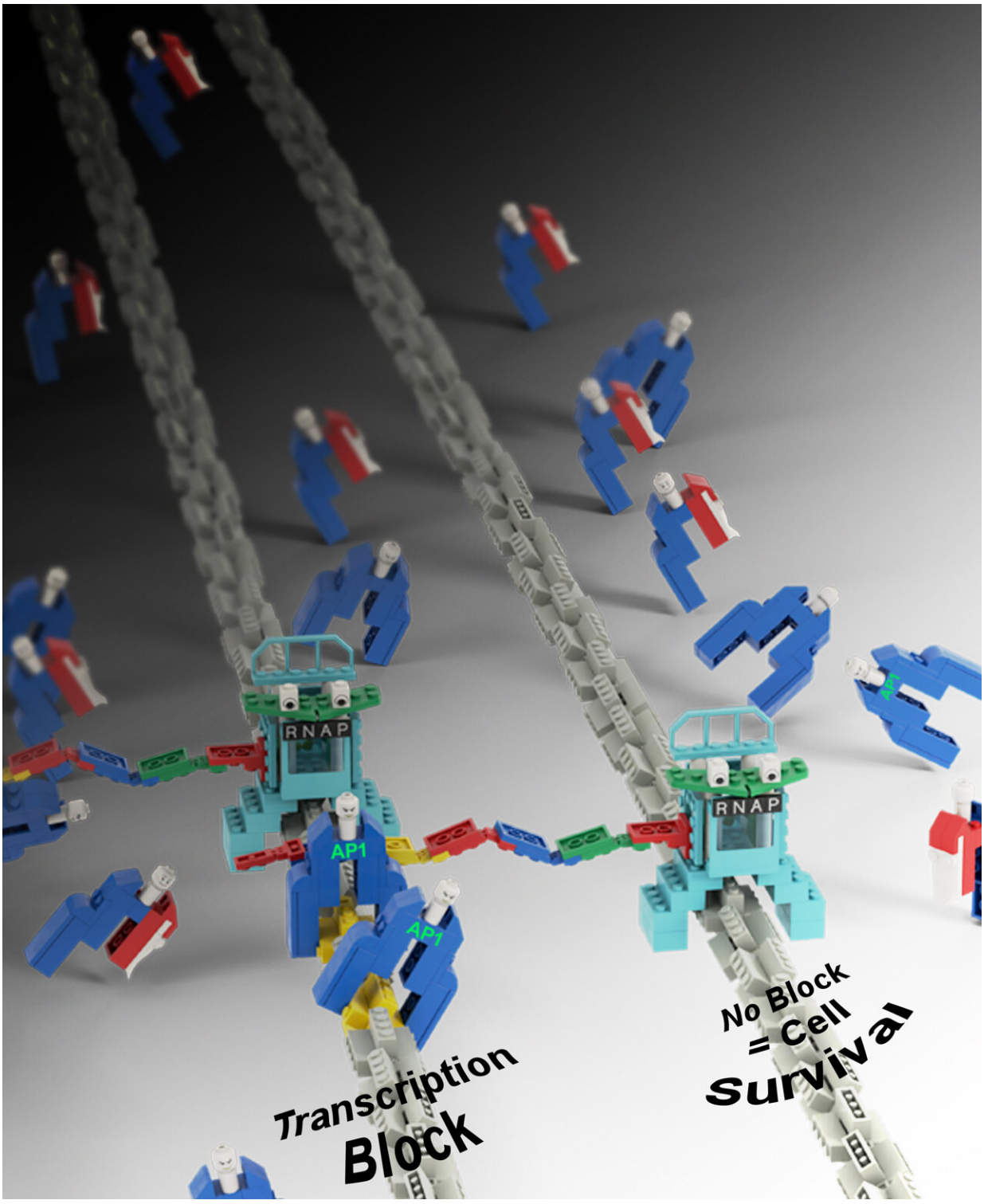

In a process known as transcription, DNA is transcribed into messenger RNA (mRNA) by the enzyme RNA polymerase (RNAP). During TBS, AP-1 molecules (blue) sit on key DNA sites (yellow), acting as roadblocks to this

process resulting in cell death. The roadblock is removed (right-hand image) when peptide molecules (red and white) bind to AP-1, both knocking it off the DNA strand and sequestering it. The cell then survives. Credit: Neil Kad, Professor of Molecular Biophysics, University of Kent

Scientists have developed a new technique to accelerate the discovery of cancer-fighting drugs. The technique identifies molecules that can shut down dangerous proteins before they wreak disease-triggering havoc, by blocking them from interacting with a cell's DNA.

The new drug-discovery platform—named Transcription Block Survival (TBS)—has been developed by scientists at the Universities of Bath and Kent in the UK and has the potential to dramatically accelerate the hunt for cures of deadly cancers. This breakthrough has wider implications too, as the proteins involved in cancer also play a central role in many other diseases, including osteoporosis and inflammatory diseases like rheumatoid arthritis and psoriasis.

"Identifying processes that can make it faster for scientists to find new drug candidates for severe diseases, as we are able to do using TBS, is a huge benefit to research," says principal investigator Professor Jody Mason, who is based in the university's Department of Biology & Biochemistry.

Drug discovery is slow, costly and complex. Often, researchers are on a quest to find pharmaceutical molecules that can bind to sites on disease-causing proteins. But binding to a target site is not enough—a therapeutic molecule must also have the ability to shut down the dangerous protein. Most importantly, it must succeed in doing so in a live cell, without too many side effects.

"A big challenge is finding ways to ensure functional loss of detrimental protein activity within the demanding environment of a cell," says Professor Mason.

He adds that "using the TBS approach, at the very first pass we're able to eliminate molecules that stick to the cell target but that ultimately fail to knock out the function of the disease-causing protein. By removing molecules from the screening process that ultimately have little or no therapeutic value, we'll save a lot of time and money."

Although TBS can be applied to the discovery of drug candidates for any number of diseases, Professor Mason's new research centers around finding molecules called peptides (short chains of amino acids—the building blocks of proteins) that permanently suppress the activity of a protein called Activator Protein-1 (AP-1). AP-1 is present naturally in the body and is important in 'switching on' genes involved in a number of cellular processes, but when out of control, it becomes a major player in cancer.

It's all in the binding

Genes work by making copies of themselves in a process known as transcription. These copies, which take the form of messenger RNA (mRNA), then turn the genetic information into proteins—the building blocks of life that carry out the instructions encoded within the genes. AP-1 promotes the growth of cancerous cells first by binding to gene promotors in specific sections of a cell's DNA, and then hijacking the expression of key genes by permanently switching them on. In other words, AP-1 forces a gene to make mRNA and corresponding proteins at the wrong times and amounts.

It is these proteins, when over-expressed, that are implicated in the proliferation of cancer. Conversely, through the activity of a cancer-

fighting peptide, AP-1 can be stopped from binding to a cell's DNA, preventing it from switching on genes.

Study first author Dr. Andrew Brennan, also from Bath's Department of Biology & Biochemistry, says that "using the TBS screening platform, researchers can find peptides that bind AP-1 in such a way as to guarantee that it cannot overstimulate cancer-related genes. These peptides can both block AP-1 from binding to DNA or kick AP-1 off genes it has already paired with, allowing them to turn off the cancerous signal in vulnerable cells."

Drug molecules that work doubly hard

Established drug-screening techniques already allow scientists to identify cancer-beating peptides by their ability to bind AP-1. A major strength and distinguishing feature of the new drug-screening technique, however, is that it allows scientists to identify peptides that have a dual function: they can recognize/bind to AP-1 both before it has bound to DNA and when it is in a DNA-bound state, ultimately freeing AP-1 from DNA and shutting down its function altogether.

"This ability to distinguish between AP-1 binders and those that are capable of shutting down AP-1 function is unique to this technique and addresses a problem that until now has hampered the search for 'functionally active' inhibitors," says Professor Mason.

Another distinguishing feature of TBS is that the screening technique happens within live cells and without modifying either the protein target or the peptide library with tags (molecular labels that are typically added to help with the identification process) that may alter function, a common issue with other techniques. Most established screening methods involve testing peptides in vitro (i.e. outside of live cells), meaning target binding is the only factor under consideration. This can

result in false positives.

"What you often find when screening is done using traditional methods is that a peptide appears to work on an isolated protein but doesn't have the same effect when it's used within a cellular context, and it certainly does not guarantee the functional loss of the protein" says Professor Mason.

In this work, recently published in *JACS Au*, Professor Mason and his team screened over 130,000 different peptides to identify one that is functionally active (red and white in the image) in potently blocking AP-1 (blue) from binding to specific DNA sequences (yellow). This action effectively blocks AP-1's ability to promote gene transcription.

The patented TBS approach can be applied to find therapeutic molecules that can target a wide range of DNA-binding proteins implicated in disease.

More information: Andrew Brennan et al, An Approach to Derive Functional Peptide Inhibitors of Transcription Factor Activity, *JACS Au* (2022). DOI: 10.1021/jacsau.2c00105

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