New 'protein nano-switch' method promises rapid and reliable development of diagnostic tests

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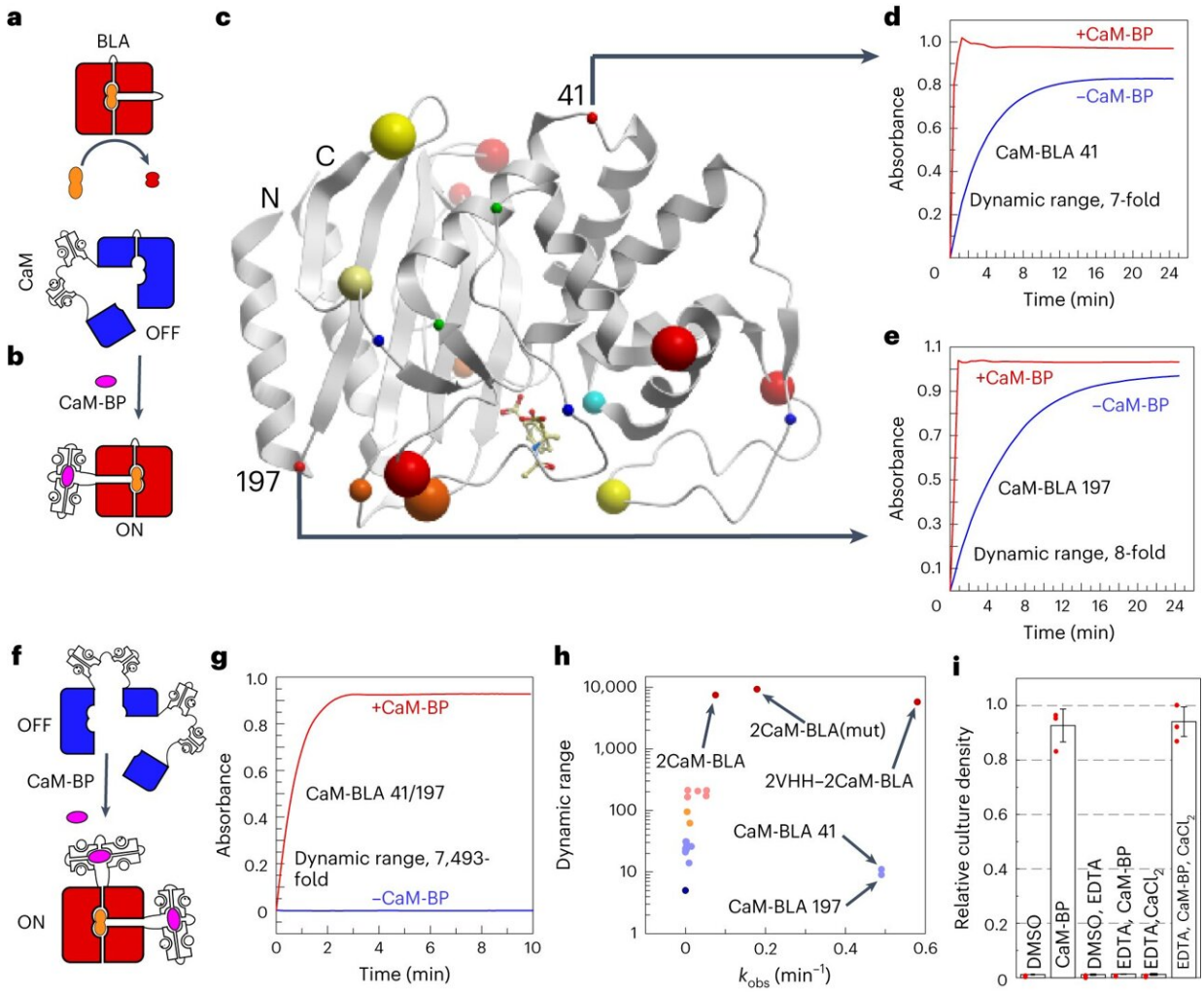

YES gate allosteric switch based on TEM-1 BLA. **a**, Schematic representation of BLA mediating β-lactam cleavage. **b**, Schematic of CaM-BLA chimera in the

absence and presence of CaM-BP, which induces a conformational change and restores the chimera's catalytic activity. **c**, Ribbon representation of TEM-1 BLA where CaM insertion sites are denoted as space-filling balls. The size of the balls correlates with the dynamic range of the resulting chimera (small balls represent low dynamic range) while the color coding reflects maximal catalytic activity (blue is the lowest and red the highest). The active site is marked by a boronic acid inhibitor (from PDB no. 1ERQ). **d**, Activity analysis of 50 nM CaM-BLA 41 chimera in the presence (red line) or the absence (blue line) of a saturating concentration (1 μM) of CaM-BP. **e**, As in **d**, but using the CaM-BLA 197 chimera. **f**, Schematic of a double CaM-BLA chimera in the absence and presence of CaM-BP. **g**, Activity analysis of CaM-BLA 41 or 197 chimera, assayed as in **d** or **e**. **h**, A plot of catalytic activities and dynamic ranges of CaM-BLA chimeras performed using 25 nM purified chimera and 1 μM CaM-BP. The '2CaM-BLA(mut)' represents a thermostabilized variant (Supplementary Fig. 3f–k). The variants are colored blue to red to denote an increase in the dynamic range. **i**, Densities of *E. coli* expressing a 2CaM-BLA switch grown overnight in Luria-Bertani broth medium suspension culture containing 100 μg ml^{-1} of ampicillin and combinations of the following compounds: 0.1% (v/v) dimethylsulfoxide (DMSO), 1 μM CaM-BP, 12.5 mM $CaCl_2$ and 10 mM ethylenediaminetetraacetic acid (EDTA). The bars represent values of an average of three independent experiments performed as a part of the same experimental set. The individual data points are shown as red dots. The error bars denote positive and negative boundaries of the standard error of the mean. Credit: *Nature Nanotechnology* (2023). DOI: 10.1038/s41565-023-01450-y

QUT researchers have developed a new approach for designing molecular ON-OFF switches based on proteins which can be used in a multitude of biotechnological, biomedical and bioengineering applications.

The research team demonstrated that this novel approach allows them to design and build faster and more accurate diagnostic tests for detecting diseases, monitoring water quality and detecting environmental

pollutants.

Professor Kirill Alexandrov, of the QUT School of Biology and Environmental Science, lead scientist on the CSIRO-QUT Synthetic Biology Alliance and a researcher with the ARC Centre of Excellence in Synthetic Biology, said that the new technique published in *Nature Nanotechnology* demonstrated that protein switches could be engineered in a predictable way.

Professor Alexandrov said currently available 'point of care' diagnostic tests which provided immediate results, such as blood glucose, pregnancy, and COVID test kits, used protein-sensing systems to detect the presence of sugar, pregnancy hormones, and COVID proteins.

"These, however, represent only a tiny fraction of what is needed in patient-focused health care model," Professor Alexandrov said.

"However, developing new sensing systems is a challenging and time-consuming trial-and-error process."

"The new 'protein nano-switch' method can massively accelerate development of similar diagnostics by decreasing the time and increasing the success rate. It uses proteins modified to behave like ON/OFF switches in response to specific targets."

"The advantage of our approach is that the system is modular, similar to building with Lego bricks, so you can replace parts easily to target something else—another drug or a medical biomarker, for example."

Professor Alexandrov said the method offered the possibility of building many different diagnostic and analytic tests, with a wide range of possible applications including diagnostics in human and animal health, testing kits for water contamination, and detecting rare earth metals in

samples to direct mining efforts.

The multidisciplinary research team included scientists from QUT and the ARC Centre of Excellence in Synthetic Biology, consisting of lead researcher Professor Kirill Alexandrov, Dr. Zhong Guo, Cagla Ergun Ayva, Patricia Walden and Adjunct Professor Claudia Vickers.

The QUT team collaborated with leading electrochemists Evgeny Katz and Oleh Smutok from Clarkson University, in New York, and chemical pathologist Dr. Jacobus Ungerer from Queensland Health.

To demonstrate the technology, the team focused on a cancer chemotherapy drug that is toxic and requires constant measurement to ensure patient welfare.

"Too little of the drug won't kill the cancer, but too much could kill the patient," Professor Alexandrov said.

The sensor the team designed for the drug uses a color change to identify and quantify the drug.

Professor Alexandrov said the next step was the for the sensor to be tested in Queensland Health laboratories for approval for use in clinical setting.

"It's really exciting, because it's the first time an artificially designed protein biosensor may be actually suitable for a real-life diagnostic application" Professor Alexandrov said.

Dr. Ungerer said the protein-engineering technology developed by the research team provided a novel means to create laboratory tests.

"This has the potential to improve and expand laboratory testing, which

will result in substantial health and economic benefits," Dr. Ungerer said.

Dr. Guo said these advancements were made possible by an international and interdisciplinary team and excellent teamwork.

Professor Alexandrov said that the next step was to take this approach and standardize and scale it, to then start building more sophisticated sub-systems. He said there are two future directions for the work.

"One is to develop computer models that allow us to design and build the switches even more rapidly and precisely," he said.

"The other is to demonstrate the scale and potential of the technology by building many switches for different diagnostic applications."

Professor Alexandrov said the team were currently modifying existing proteins, but in the future, they could use the same principles to develop components that did not exist and would be designed from scratch.

"The new technique provides scientists unprecedented control over construction of protein-based sensing systems," he said.

The article 'Development of epistatic YES and protein logic gates and their assembly into signalling cascades' is published in *Nature Nanotechnology*.

More information: Guo, Z. et al. Development of epistatic YES and AND protein logic gates and their assembly into signalling cascades, *Nature Nanotechnology* (2023). DOI: 10.1038/s41565-023-01450-y. www.nature.com/articles/s41565-023-01450-y