Fluorescent molecular rotors may revolutionize the search for new anticancer drugs

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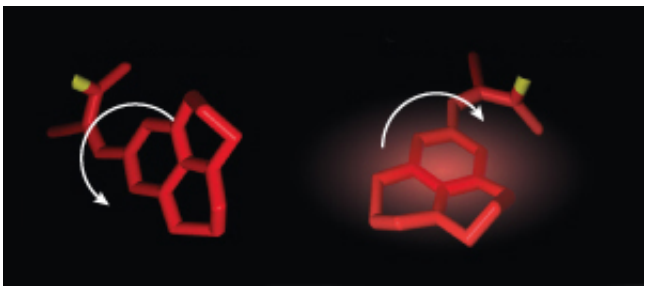


Fluorescent molecular rotors are molecules with a twisted structure. In their twisted state, their intrinsic fluorescence is quenched (left), but when their rotation is hindered, such as in highly viscous solutions, the molecule becomes untwisted and fluoresces (right). Credit: American Chemical Society

When Nobel Laureate Sydney Brenner established the A*STAR Molecular Engineering Laboratory five years ago, his aim was to bring together a small team of young, high-caliber researchers from disparate disciplines to catalyze game-changing advances in science and technology. His venture has now borne fruit with the laboratory's development of fluorescent molecular rotors—used for almost 20 years to measure physicochemical properties such as viscosity—to probe the molecular interactions between proteins involved in cancer1.

This new tool for cancer researchers makes it possible to directly

observe the disruption of target protein–protein reactions, taking much of the labor and guesswork out of early drug development. "Interdisciplinary research is the key to making truly revolutionary advances in science," notes Brenner. "This molecular rotor strategy provides such an example, with great potential for many applications, particularly for drug discovery."

Joining the dots

Fluorescent molecular rotors belong to a class of naturally twisted compounds that emit fluorescent light when untwisted. For decades, researchers have known that this unwinding can occur when the rotors are placed in highly viscous micro-environments, making them useful for probing properties such as viscosity and the dynamics of polymerization. Yet despite knowledge of their unique properties, the use of fluorescent molecular rotors for biological research has been limited.

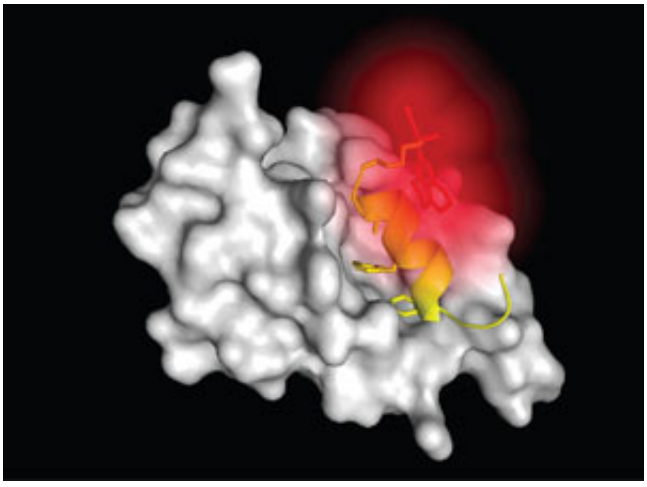
Brenner's idea was to see if rotors could be used to assay biological interactions, explains Yin Nah Teo, who led the research team on this project.

"His initial thought was that perhaps we could use molecular rotors for detecting interactions between biomolecules. So we established a research team to investigate whether environmental effects through interaction with proteins could also result in the same change in fluorescence properties."

The idea led Brenner and Teo to consider the types of protein interactions that could benefit most from such a molecular probe. A natural target, given A*STAR's world-leading expertise in the area, was the protein known as p53, a 'tumor-suppressor' that plays a critical role in cancer development.

"Protein–protein interactions are the key mechanisms of cell signaling pathways in living systems," explains Teo. "We formed a multidisciplinary project, in close collaboration with Farid Ghadessy and the A*STAR p53 Laboratory, looking at potential uses of the rotors for specific interactions involving this important protein."

The A*STAR p53 Laboratory, headed by chief scientist Sir David Lane, is a world-leading center for p53-based cancer research. In about half of all cases of cancer in humans, the p53 gene is mutated, resulting in the production of a faulty p53 protein. And in cancer patients with an apparently normal p53 gene, the p53 pathway is believed to be faulty. One of p53's normal functions is to catalyze DNA repair in damaged or mutated cells, thereby suppressing tumor formation. Faulty p53, or a fault in the protein's functional pathway, undermines this protective process.



A molecular rotor (red) that binds to a specific peptide on the p53 protein (white) and fluoresces only when p53 complexes with the Mdm2 protein (yellow). Credit: American Chemical Society

With insights gained from Ghadessy's team at the A*STAR p53 Laboratory, the interaction between p53 and one of its regulators, the protein Mdm2, was selected as the target for the molecular probe. "Farid Ghadessy is an expert in p53 and Mdm2, and had a lot of ideas about how we could use this probe, such as in drug screening."

Mdm2 inhibits the overproduction of p53 in healthy individuals. High levels of Mdm2, however, can result in a loss of p53 activity. Studying the interactions between p53 and Mdm2 is one of the most active areas of research at the A*STAR p53 Laboratory, providing a clear target for Teo's molecular rotor project.

"The team we pulled together was very multidisciplinary, involving chemists and biologists from the A*STAR Molecular Engineering Laboratory and the A*STAR p53 Laboratory, as well as bioinformaticians from the A*STAR Bioinformatics Institute," says Teo.

Finding the right parts

Adapting the fluorescent molecular rotors for the detection of specific protein–protein interactions meant finding ways to bind or 'conjugate' the rotors to peptide sequences on the target proteins so that rotor rotation would be restricted only when the proteins underwent specific interactions with each other—something that had never been attempted before.

"It took several months to synthesize different versions of the rotors," explains Teo. "We tested multiple molecular rotor designs, methods of conjugation and peptide sequences in search of a probe design that would give the best change in fluorescence signal. Min Yen Lee in our research team was instrumental in realizing these changes."

Eventually, the research team was able to design and synthesize a

molecular rotor that binds specifically to a p53 peptide and fluoresces only when it interacts with Mdm2. "Our results show that biomolecular interactions indeed restrict intramolecular rotation, resulting in fluorescence," says Teo. "The rotor provides a direct readout through fluorescence intensity for studying the interactions of proteins with small molecules, peptides or other proteins."

By applying the newly designed rotor in a rapid, high-throughput assay, Walter Goh at the A*STAR p53 Laboratory was able to quickly screen a large library of protein fragments for molecules that inhibit the interactions between p53 and Mdm2. In doing so, the researchers discovered 15 potential inhibitors. Seven of these could not be detected by standard techniques, highlighting the specific sensitivity of the rotor-based assay.

A world of potential

Brenner is understandably proud of the team's achievement with the fluorescent molecular rotor strategy and the success of such a strong multidisciplinary and inter-laboratory project. "Through our skills in chemistry and by enlisting the support of groups outside the A*STAR Molecular Engineering Laboratory, Yin Nah Teo has been able to develop a very useful assay," says Brenner.

"Unlike lower-throughput methods, our simple turn-on fluorescent probes provide rapid and sensitive readout," notes Teo. "High-throughput screening for small molecules that bind to therapeutically important proteins is therefore one of the most promising applications of our work."

The fluorescent molecular rotor strategy represents an invaluable new research tool that could significantly accelerate cancer research by allowing specific protein interactions to be explored in unprecedented

depth. The strategy is not limited, however, to specific protein–protein interactions or for sole use within the A*STAR p53 Laboratory—it is also broadly applicable to many possible molecular interactions, making it a very exciting development indeed. "This work exemplifies how the translational convergence of chemistry and biology is giving rise to exciting new systems with wide-ranging applications," says Ghadessy.

More information: Goh, W. L., Lee, M. Y., Joseph, T. L., Quah, S. T., Brown, C. J., Verma, C., Brenner, S., Ghadessy, F. J. & Teo, Y. N. "Molecular rotors as conditionally fluorescent labels for rapid detection of biomolecular interactions." *Journal of the American Chemical Society* 136, 6159–6162 (2014). dx.doi.org/10.1021/ja413031h

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