Benzene-based probes highlight two hidden binding sites on an anticancer drug target

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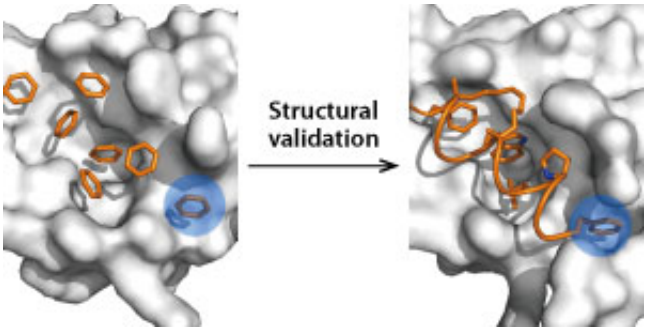

Binding pocket detection using benzene molecules as probes (left) and structural validation using stapled peptides (right). Credit: Y. S. Tan et al.

In the quest for new cancer therapies, A*STAR researchers have devised a computational strategy that unearths any previously unknown binding sites or 'pockets' on drug targets.

More effective cancer treatments are likely to emerge from the drug development pipeline. Cancer drug discovery hinges on identifying and characterizing binding pockets in target proteins. Typically, this evaluation uses computational techniques that rely on static protein structures. However, proteins have an inherent flexibility that causes a tendency to change shape upon contact with the drugs. Certain binding pockets remain undetectable unless they interact with an appropriate substance and, therefore, are missed by conventional simulations. These hidden pockets, however, are usually water-repelling or hydrophobic

sites that only open when there are low polarity substances.

To tackle this, Yaw Sing Tan and Chandra Verma from the Bioinformatics Institute have developed a probe-based method called ligand-mapping molecular dynamics (LMMD). They used this technique to seek hidden binding pockets in the anticancer target protein MDM2. The resulting predictions were experimentally validated by long-standing collaborators from A*STAR's p53 Laboratory and Institute of Chemical and Engineering Sciences as well as structural biologists from Newcastle University, UK.

Tan explains that initially he had designed this probe-based method for another target protein and successfully used it to find a hidden binding pocket that stayed closed in conventional simulations. "We then decided to apply this approach to MDM2 to see if we could discover any previously unknown binding sites that could enhance the potency of existing MDM2 inhibitors," he adds.

Using benzene molecules as hydrophobic pocket detection probes, the researchers computationally identified two new binding sites on MDM2. "We were excited to see that these sites lie very close to the binding pocket of the tumor suppressor protein p53," says Tan.

Furthermore, the researchers expect the newly found sites to lead to more potent stapled peptides—these are amino acid helices chemically stabilized by a hydrocarbon chain that have recently emerged as powerful p53 activators. Consequently, they created stapled peptides from analogs known to tightly bind MDM2 and reactivate p53, and determined the affinity of these peptides to MDM2. Their simulations showed that the peptides bound MDM2 more strongly than p53 in the pockets and matched biophysical and X-ray crystallography experiments.

"This method could be used to interrogate other anticancer protein

targets to uncover novel binding sites that could be targeted for inhibition," says Tan. The team is now working to expand the reach of LMMD probes to other ligand types.

More information: Yaw Sing Tan et al. Benzene Probes in Molecular Dynamics Simulations Reveal Novel Binding Sites for Ligand Design, *The Journal of Physical Chemistry Letters* (2016). DOI: 10.1021/acs.jpclett.6b01525

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