| Multiple ways to the 'Final Destination', starring benzene and mutant T4 lysozyme |

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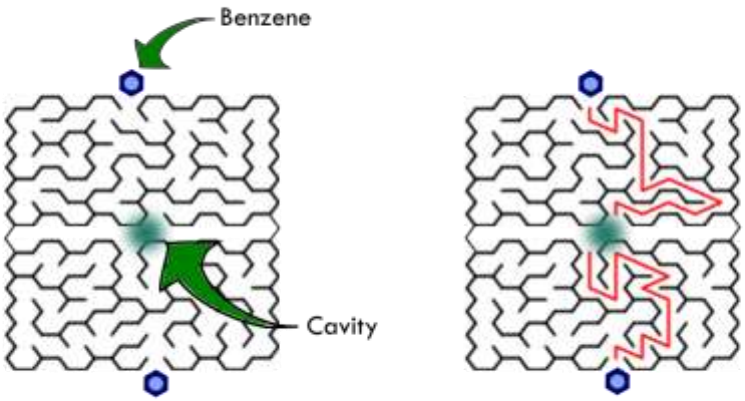
"You are the Yin to my Yang", said every receptor to its ligand; and they lived triggering cellular responses happily ever after.

Binding of ligands to their respective receptors is of crucial importance in preventing the human body from snowballing into a state of disorder. There are multiple kinds of receptors, located either inside a cell or on its surface. When a ligand binds to its respective receptor, it triggers a cascade of cellular responses. For example, the number of olfactory receptors in the human nose is around four hundred and each contributes to your sense of smell. Though one could go on and on about the different kinds of receptors and their effect on all biological processes inside your body, this article is centred around the fundamental question of how a ligand reaches and binds to a seemingly inaccessible cavity inside its complementary receptor.

Dr. Jagannath Mondal from TIFR Hyderabad teamed up with his colleague, Dr. Pramodh Vallurupalli, to investigate this problem. They took the classical example of a small benzene molecule which finds its way to a deeply buried cavity of a mutant form of T4 lysozyme. The 99^{th} amino acid residue in this lysozyme is mutated from Leucine to Alanine. This mutation causes a slight change in the conformation of T4 lysozyme, resulting in the formation of a small cavity (~150 Å). This small cavity provides enough room for a ligand such as benzene, thus, stabilizing the ligand-receptor complex. This reaction provides an excellent model for studying the kinetics of ligand-receptor recognition. Previous studies from multiple groups have unearthed detailed observations regarding the chemical kinetics of this reaction. However, the process by which the ligand found its way into the cavity was still unclear. This was primarily due to difficulties in extracting data, in high resolution, from rapidly changing protein conformations.

This study adopted a computational approach to address the question. The crystal structures of both bound and unbound states of T4 lysozyme were available. With the help of this information, Dr. Jagannath Mondal's group designed molecular dynamics (MD) simulations, spanning in the order of microseconds (2-8 μs). The simulations beautifully show how the hydrophobic benzene molecule successfully finds its way into the solvent-inaccessible cavity, despite beginning its journey from different points. One can draw comparisons to a maze with multiple entry points and paths to the target. The different entry points denote the initial placements of benzene. The seeker may take any of the several paths to the deeply buried, not-so-easily reachable target.



Mazes generated and adapted from www.mazegenerator.net

MD simulations (in the order of μs) require considerable computing power. Conventional high-performance computing facilities use multiple central processing units (CPUs), thus obtaining parallel processing. Given the requirements of this study, Dr. Mondal came up with a relatively low-cost computing solution to run the simulations. He used a single workstation i.e. a single CPU with eight GeForce GTX 980 graphics card for more efficient computing power. For those who are avid lovers of GTA4, Shadow of Mordor and Counter Strike, these graphics cards are familiar. Therefore, with the help of gaming cards and GROMACS (an open source programme), a part of the simulations were run in the CPU while the more time-consuming non-bonding interactions' calculations were done in the graphics processing units (GPU). Dr. Mondal says that this methodology has shaped his perspective towards approaching molecular dynamics simulations.

The simulations in this study show three different paths taken by the benzene molecule to reach the cavity in the T4 lysozyme. It is important to note that each interaction did not require radical conformational changes in the receptor protein. Subtle fluctuations resulting in helix-gate opening of the receptor protein was enough for the binding to occur. This piece of work sets the tone for future characterizations of ligand-receptor interactions at the atomic level. Given the increasing exigency of such studies for the discovery of novel drugs, Dr. Mondal and Dr. Vallurupalli's research efforts provide significant impetus to the said field.

Reference:

Jagannath Mondal , Navjeet Ahalawat, Subhendu Pandit, Lewis E. Kay, Pramodh Vallurupalli*, Atomic resolution mechanism of ligand binding to a solvent inaccessible cavity in T4 lysozyme*, PLoS Comput. Biol. (2018) https://doi.org/10.1371/journal.pcbi.1006180