

*Certain small glycols as cryptic pocket finders in proteins*Harsh Bansia<sup>1</sup>, Pranjal Mahanta<sup>1</sup>, Suryanarayanarao Ramakumar<sup>1</sup><sup>1</sup>Department Of Physics, Indian Institute Of Science, Bangalore, India

E-mail: harshu@physics.iisc.ernet.in

Cryptic pockets are sites on proteins which are not apparent in the unliganded form of proteins [1]. They become visible only when a small molecule/inhibitor/drug binds to its target site on protein and are considered important from the perspective of drug design. Recently a cryptic pocket was found in K-RAS, the most common oncogene in human cancers, and was successfully targeted [2].

In our laboratory we have a long standing program of investigating the factors which contribute to the thermal stability of proteins and as a part of which we have determined crystal structures of a thermostable recombinant xylanase (RBSX) and its mutants [3]. While investigating the importance of aromatic residues in the thermal stability of RBSX, we observed the presence of a surface pocket in the crystal structure of RBSX Trp6 to Ala (RBSX-W6A) mutant containing a GlycolA molecule (RBSX-W6A-GlycolA). GlycolA was used as a cryoprotectant in the diffraction experiment. Notably, this pocket was found to be absent when the crystal structure of the same protein (RBSX-W6A) was determined without the cryoprotectant GlycolA (RBSX-W6A-sans-GlycolA). In RBSX-W6A-sans-GlycolA structure, neighboring Phe4 side-chain moves and covers the cryptic pocket so created by substituting a bulky Trp6 residue with Ala. This cryptic pocket reappeared containing GlycolB when the crystal structure of RBSX-W6A was determined in the presence of cryoprotectant GlycolB (RBSX-W6A-GlycolB). The rationale for choosing GlycolB is its structural similarity with GlycolA.

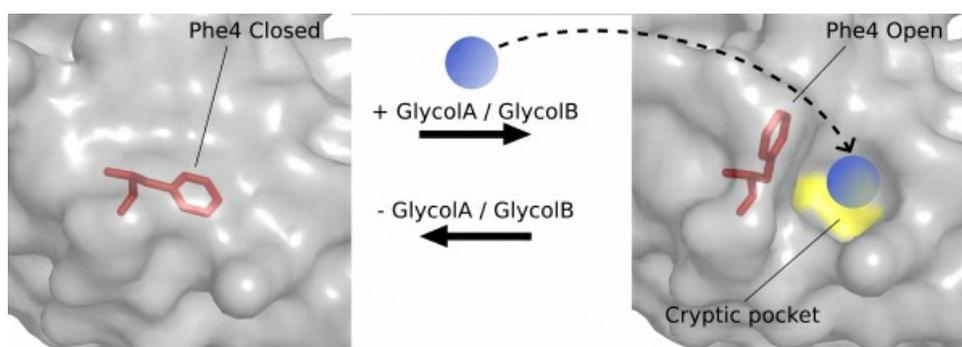
We define state of Phe4 side-chain as open with respect to the cryptic pocket in RBSX-W6A-GlycolA/GlycolB structure while closed in RBSX-W6A-sans-GlycolA/GlycolB structure. To determine whether water molecules can uncover this cryptic pocket, we performed 120 ns explicit solvent MD simulations with closed state of Phe4 side-chain as starting structure. Our simulations showed that Phe4 side-chain remained in the closed state throughout the length of MD simulations. Interestingly, when MD simulations were performed with phe4 side-chain in open state, it changed from open to closed state after 30 ns and remained as such for further 90 ns.

Based on crystal structure analyses and MD simulations we conclude that in RBSX-W6A mutant, Phe4 side-chain covers the cryptic pocket created by substituting Trp6 with Ala and when GlycolA/GlycolB is introduced in diffraction experiment, it is able to displace Phe4 side-chain and uncover the cryptic pocket while the same is not true for water molecules. Thus, we show that these glycols help in identifying cryptic pockets in proteins and therefore could benefit the drug design approaches.

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[2] Ostrem, J. M. et al. (2013). Nature, 503, 548– 551.

[3] Mahanta, P. et al. (2015). FEBS J, 282, 3543–3555.



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