Review of: "Impact of crowded environments on binding between protein and single-stranded DNA"

Pinki Dey[1], Sangeeta Yadav[2]

1 University of New South Wales
2 Jawaharlal Nehru University

Potential competing interests: The author(s) declared that no potential competing interests exist.

This study investigates the impact of a crowded cellular environment on the binding of single stranded deoxyribonucleic acid (ssDNA) of variable chain lengths and the cold shock protein B (Csp-B). The Csp-B protein is selected as it has a well investigated three-dimensional structure and it preferentially binds to 6 - 7 nucleotide (nt) stretches of thymine (poly T sequence). To replicate the cellular crowding environment, this study makes use of macromolecular polyethylene glycol (PEG) and dextran along with their monomeric units, ethylene glycol (EG) and glucose respectively to incorporate the impact of crowder size and their chemical properties for the comparative study. As the presence of crowders is expected to increase the thermodynamic stability of proteins and hence can influence its interactions with the oligonucleotides, the authors here explore these dynamics of protein-DNA interactions in different crowded conditions by combining high resolution NMR and fluorescence spectroscopy. Moreover, the study involves the ssDNA length dependent investigations during their interaction with the protein.

The findings in the study show that the crowding environment has no significant impact on the protein structure, both in its ssDNA free and bound complex, however, some modest changes in ligand binding are observed on its interactions with the oligonucleotides when crowders are introduced. The study also suggests that the protein-ssDNA affinity decreases in the crowded environment constituted by EG, PEG1, or PEG8. However, crowding agents such as Dex20 and glucose have very little effect on the binding affinity. Also, the change in binding affinity is dependent on the ssDNA length as well as crowder concentration, which means it largely depends on the weight per volume fraction of the crowder molecule present, and not the size of the molecules used. To understand the above observation, the authors investigated the impact on protein-ssDNA association kinetics at different crowding concentration. They reported that the protein-ssDNA association rate constant is always lower compared to dilute media and it is independent of the type and size of the crowding agents, while the dissociation kinetics behaviour largely depends on the type of crowder molecule, each molecule showing different behaviour. This leads the authors to an implication that the protein-ssDNA binding affinity, association-dissociation kinetics has a dependency on the chemical properties and features of the crowder molecules such as difference in hydrogen bond formation capacity, polarity etc. for monomeric and macromolecular crowders, which can

interfere with the protein through soft interactions. As a result, the observed differential effect in protein-ligand dynamics cannot be solely explained by excluded volume theory which would have predicted the same effect for all macromolecules, but the observations are not in line with it except some excluded volume effect for PEG and Dex20.

To sum it up, the authors conclude that the sterically induced “excluded volume” is not the trivial factor that plays the role in the alteration in association-dissociation kinetics of protein and ssDNA molecules, but the chemical interactions of the crowding agents added in high concentration does so via modification of hydration shell around the protein. There is no evidence obtained for the direct interaction of crowder molecules with the protein and ligand (ssDNA) rather soft interactions such as vander Waals interactions, electrostatic interactions or hydrophobic interactions and protein solvation, etc. regulates the binding dynamics. These soft interactions depend on the chemical property of the crowding molecules and do not provide specific binding but participate in transient interactions.

The work done here is very elaborate and addresses the underlying mechanisms of protein-nucleic acid interactions in crowded environment. To increase the comprehensiveness of the study, some points could have been addressed such as the macromolecular crowders used in this study are mostly inert such as PEG and dextran, which are mainly found to exert excluded volume effects. This leads to a question that whether using macromolecular crowders that exert more enthalpic effect/ chemical interactions could have shown a larger impact on the protein-DNA association or dissociation kinetics. Also, some explanation on the use of dextran as an alternative to PEG for testing the effect of soft interactions on protein-ssDNA binding affinity would have been useful as both PEG and dextran are generally categorized under inert crowders. Lastly, the work has been conducted in a mostly homogenous medium, hence a future suggestion would be the use of a heterogeneous crowding environment to mimic the crowded cellular milieu more closely.