Biochemical reactions in crowded environment: separating the influence of depletion interactions and ion complexation on the example of DNA hybridization

Every one of us has hundreds of trillions of cells in the body that maintain life through biochemical processes. The mechanisms that keep us alive are not yet fully known, especially when it comes to interactions between specific molecules. These interactions take place in a highly crowded environment, where biomolecules occupy up to 50-weight % of a cell's mass and react in various manners. That is why understanding complex cellular behavior is difficult and needs deeper investigation.

To study any specific reaction outside of a cell, researchers mimic its highly packed environment by applying inert molecules like non-ionic polymers. However, these inert compounds are less inert than expected. Recently, we shed light on their behavior in aqueous solutions. It appeared that these inert molecules, called crowders, indeed "steal" ions from the solution, which may create severe problems for scientists and generate errors. Even a minor change in ions concentration may vitally impact biochemical processes. For example, the decrease in the concentration of cations destabilizes the double-stranded form of negatively charged DNA. In this way, for crowder concentrations at 50-weight %, no double-stranded form appears in some crowder solutions.

On the other hand, the presence of crowders itself causes the opposite effect. Every crowder molecule in the solution "wants" to have as much room as possible. If there are many of them, they compete for space and, ultimately, squeeze other biomolecules like DNA together. In this situation, the double-stranded form of DNA is stabilized. These interactions are called depletion interactions and dominate for low crowder concentrations (15 wt.%).

To date, no model exists that could predict the contribution of depletion interactions and the "ion stealing" effect by crowders to biochemical reactions. In the number of experiments, we will study the influence of crowder (polyethylene glycol – PEG) size and concentration on DNA hybridization. These results will give us information about the joint effect of both phenomena. Next, we will measure the interactions of sodium and potassium ions with PEGs and the impact of these ions on DNA hybridization. By that means, we will calculate only the "ion stealing" effect. Finally, by comparing results from the first and second series of measurements, we will derive the contribution of pure depletion interactions and model the forces governing the crowded environment.

The model will be especially crucial in 1) designing artificial cells, 2) developing new diagnostic therapies that aim for specific biomolecules or gene loci, such as CRISPR-Cas, and 3) tissue engineering, as macromolecular crowding enhances and accelerates tissue-specific extracellular matrix formation. Moreover, the outcome of the studies will allow a better understanding of a cell-like environment and the cellular processes, such as protein folding, enzyme-substrate interactions, and transport of biomolecules.

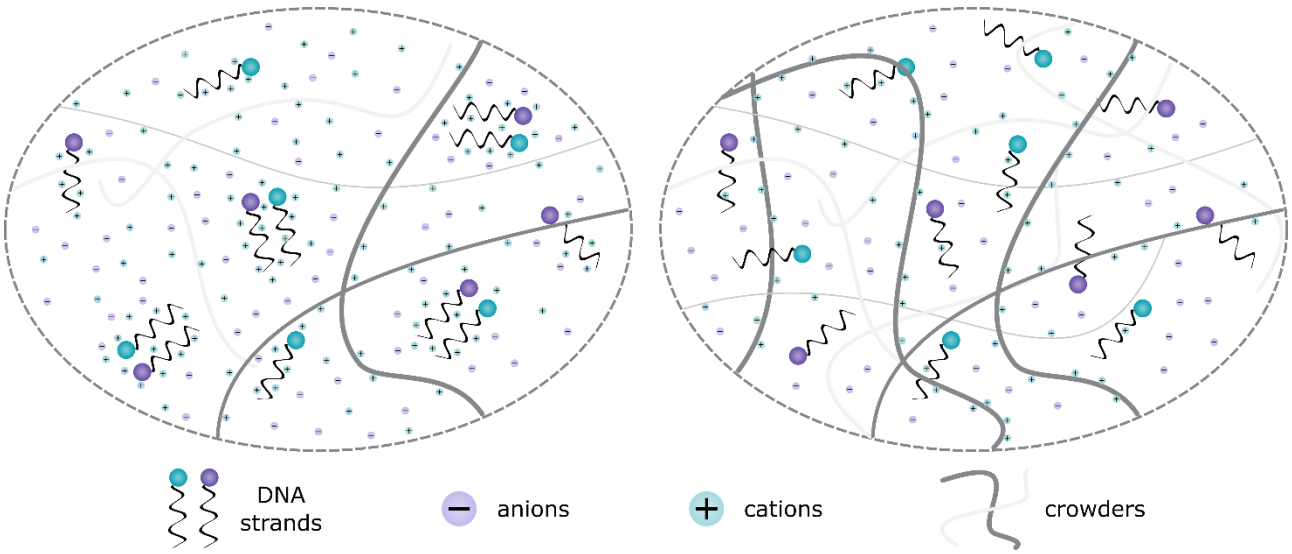

Figure 1: Two different situations caused by opposing phenomena in a similar system. In low crowder concentrations (left panel), dominates depletion interactions squeezing biomolecules together. In high crowder concentrations (right panel), dominates the "ion stealing" effect caused by crowders. In the results, like-charged DNA strands repel each other.