Traffic in crowded environments and osmosensing mechanisms of membrane transport proteins

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Cells regulate their internal pH, ionic strength, molecular crowding, water activity and other physicochemical parameters within narrow ranges. Changes in the osmolality of the environment have an immediate impact on the volume of a cell and thus the internal concentrations of solutes and macromolecules. Upon osmotic upshift, traffic inside the cell slows down and metabolic activity decreases, which is detrimental if the cell is unable to counteract the consequence of the stress. Osmoregulatory transporters are still active under these conditions and allow cells to recover by accumulating compatible solutes (co-solvents) that rehydrate the cell and stabilize macromolecules. I will present our recent work on molecule diffusion inside live cells and the mechanism of sensing of osmotic stress by ATP-binding cassette (ABC) transporters. The ABC transport systems have been reconstituted in synthetic lipid vesicles and bilayer nanodiscs, and conditions equivalent to those in living cells have been mimicked in these membrane model systems. The importance of anionic lipids, ionic strength and macromolecular crowding in sensing of osmotic stress will be presented. Also, the (dis)advantages of different *in vitro* reconstitution systems for assaying translocation reactions will be discussed.

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