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Formation of protein-complexes in crowded environments: from in vitro to in vivo[1]

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Rates of protein interactions are one to five orders of magnitude slower than the theoretically calculated collision rate of spheres of the same size. The rates can be increased by favorable electrostatic forces between the two proteins. Recent studies have established that the association reaction proceeds through transient complexes, which may be specific or diffusive in nature. To bring binding studies closer to the in vivo environment, we investigated the role of crowding on binding. For crowding we added various polymers to the solution, including Dextran and PEGs of different molecular weights. While crowding enhances oligomerization and polymerization of macromolecules, it has only a small effect on the binding rates and affinities of transient protein-protein interactions. We suggest that the limited effect of crowders, which is much bellow the expected from the increased viscosity of the solutions, is a result of the occluded volume effect in high crowder concentrations. Direct measurements of the stability of the encounter complex shows that crowders slow both k1 and k-1, resulting in an increased half-life of the encounter complex. High crowder concentrations also slow k2, suggesting an increased size of the encounter region. These results fit double-mutant cycle measurements on the activated complex, which suggest an increased size of the fruitful encounter region. These results are in line with the suggested occluded volume effect of crowders. We contrasted these with the effect of crowding on the weak binding pair CyPET-YPET. On this pair, aggregation, and not enhanced dimerization, was detected in PEG solutions. The results suggest that typical crowding agents have only a small effect on specific protein-protein dimerization reactions while promoting aggregation. To further validate these results, we performed real time binding assays in living cells, showing that even in the crowded cellular environment binding can be fast and specific.

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