Analysis of Binding Events and Diffusion in Living Cells

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This thesis is based on the following papers:

I. Elsner, M., Hashimoto, H., Simpson, J.C., Cassel, D., Nilsson, T., and Weiss, M. (2003).
Spatiotemporal dynamics of the COPI vesicle machinery.
EMBO Rep *4*, 1000-1004

II. Weiss, M.*, Elsner, M*., Kartberg, F., and Nilsson, T. (2004).
Anomalous subdiffusion is a measure for cytoplasmic crowding in living cells.
Biophys J *87*, 3518-3524.
**equal contribution*

III. Markus Elsner, Tommy Nilsson, Matthias Weiss
(2006)
Evidence for Golgi localization by oligomerization - kin recognition revisited
In manuscript

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It is well known that diffusion is the main mode of transport in living cells, but the consequences of diffusion in a complex cellular environment are not generally appreciated. In this thesis, we have investigated several aspects of how diffusion properties influence the observability of cellular binding kinetics and how they can be used to obtain information about the environment of proteins and other molecules.

First, the binding kinetics of the coat protein I (COPI) vesicles machinery were investigated. Three proteins are mainly responsible for the formation of COPI vesicles; coatomer, ARF1 and ARFGap1. From biochemical studies, it was expected that ARF1 and coatomer would show similar binding kinetics to the Golgi membranes. This was tested *in vivo* using GFP constructs in "fluorescence recovery after photobleaching" (FRAP) experiments. Surprisingly, the recovery constant of coatomer was twice that of ARF1. We could show that this did not reflect a difference in the actual binding kinetics, but difference due to a diffusion-limited exchange of coatomer between the cytosol and the membrane. For this we measured the diffusion coefficient of all three proteins with fluorescence correlation spectroscopy (FCS). We found that ARF1 and ARF-GAP1 are highly mobile in the cytosol, whereas coatomer diffuses 5–10 times more slowly than expected. Using computer simulations we could show that the slow diffusion of coatomer translates into a two times slower FRAP recovery then expected for the non-diffusion limited case.

Second, the unexpectedly slow diffusion of coatomer led to the idea of investigating the diffusion properties of inert tracers in the cytosol of livings cells. Fluorescently labelled dextrans showed normal diffusion in water, but strong anomalous subdiffusion when microinjected into cells. It could be ruled out that large scale structures like the cytoskeleton or the endoplasmic reticulum were responsible for the observed subdiffusion. Instead the emergence of subdiffusion could be attributed to macromolecular crowding using computer simulations and *in vitro* measurements in an artificially crowded solution. The fact that macromolecular crowding leads to anomalous diffusion can be used as a measure for the extent of crowding for a given solution.

In the third part of this thesis the focus is shifted from diffusion in the cytosol to diffusion in the membrane. Previously, it had been observed in FCS experiments that Golgi resident transmembrane proteins show anomalous subdiffusion. Since no consistent explanation for this phenomenon had been provided previously, we investigated whether the formation of dynamic oligomers can explain the observed subdiffusion. We constructed a computer model for two dimensional diffusion of particles that participate in oligomerisation reactions. It could indeed been shown that for the short time scales relevant for FCS experiments, anomalous diffusion can be observed. For long times the diffusion crossed over to normal diffusion. The extent of anomality and the crossover time depended on the equilibrium constant of the binding, the valence of the monomers and on the kinetics of the binding reaction.

Keywords: COPI, Golgi, Diffusion, Glycosyltransferases, sorting, molecular Crowding

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