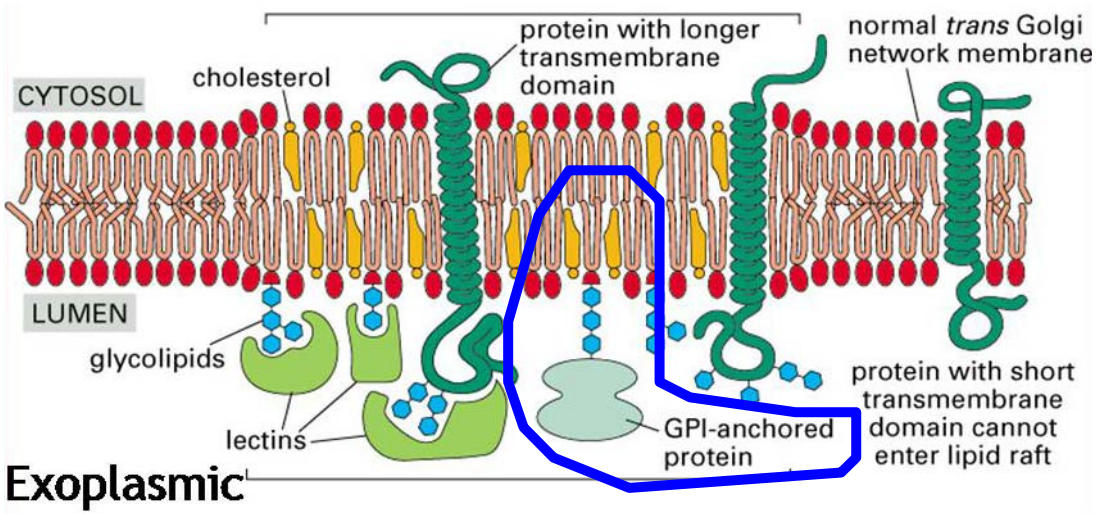
EAMNET practical course
‚**Imaging Molecular Dynamics**'
EMBL Heidelberg, 19.-21. April 2004

Results:
FRAP on gpi anchored GFP

Bleaching of Membrane Proteins

In this part of the course glycosylphosphatidylinositol (GPI)-anchored GFP was used for Photobleaching studies. This molecule is membrane bound on the exoplasmic side of the plasma membrane and its recovery is restricted to the two dimensional flow of the membrane.



gpi-GFP (Leica SIRIUS)

Photobleaching experiments were performed on a Leica SP2 AOBS SIRIUS. This system is equipped with stronger lasers (488 nm and 532 nm) to enable a significantly shorter bleach time. A circular ROI was bleached with one bleaching iteration (fig 1, green ROI).

Raw data of one example experiment:

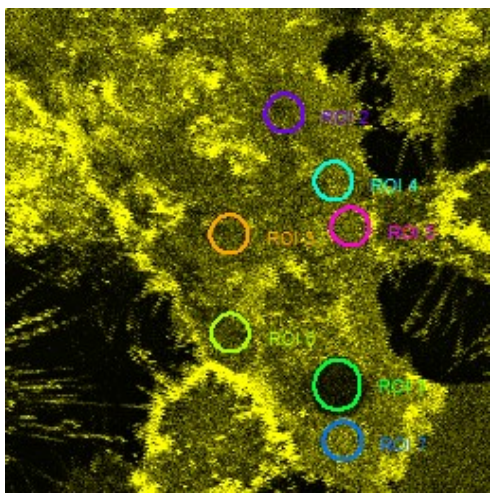


Fig.1: first afterbleach image,
Green ROI: bleached area
Other ROIs: reference areas

Leica FRAP wizard Values
Time Constant 28.62 s
Halftime 19.84 s
Recovery Rate 0.03 Hz

Fig 2.: Raw data of the bleach and reference ROIs plotted by the Leica FRAP wizard

Curve Fitting with IgorPro Macro

The raw data should be corrected by subtracting the average background intensity from the measured average ROI value. Correction for fluorescence loss by the bleach pulse and unwanted bleaching during postbleach acquisition can be corrected by dividing the background corrected values of the bleach region by the total cell fluorescence.

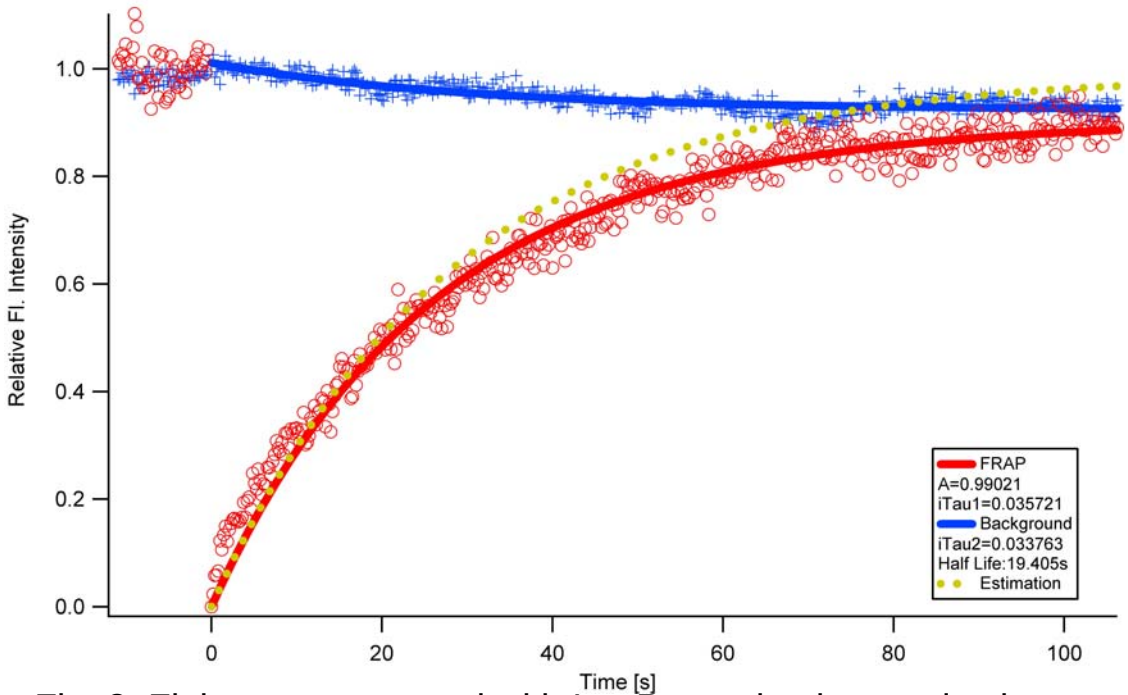


Fig. 3: Fitting curve created with IgorPro on background subtracted data (red). The yellow dotted curve is corrected for fluorescence loss due to acquisition photobleaching.

IgorPro macro result
Mobile fraction: **99 %**
Halftime 19.4 s
D_{eff}: ~1.2 µm^2 s^{-1} comparable to Kenworthy et al. 2004

Literature

- Kenworthy, A. K. et al. (2004): J. Cell Biol. **165**(5): 735-746.
- Stadler, J., T. W. et al. (1989):*EMBO J.* 8:371-377
- Simons, K. and E. Ikonen (1997): Nature **387**: 569-572