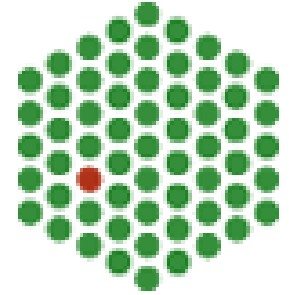




EAMNET practical course
,Imaging Molecular Dynamics'
EMBL Heidelberg, 19.-21. April 2004



Results: FRAP and FLIP analysis of Androgen Receptor (AR)

Reference:

Farla, P. et al. (2004): "The androgen receptor ligand-binding domain stabilizes DNA binding in living cells."
Journal of Structural Biology **147**(1): 50-61.

Biological System

In this part of the course cells stably transfected with AR-GFP wildtype or DBD mutant (Farla, P. et al. 2004) have been used to study the differences in mobility in presence of androgen. Only cells with low expression levels of GFP have been used for FRAP analysis to circumvent artifacts by overexpression.

These experiments were performed on a Carl Zeiss LSM510 META supervised by [Adriaan Houtsmuller](#).

Hardware settings:

- Objective lens: 40x1,3 oil
- A rectangular ROI (fig.1) was used for the FRAP experiment. Only the mean intensity of this rectangular ROI was recorded to gain sufficient speed (Mean ROI mode in the Zeiss time-lapse control)

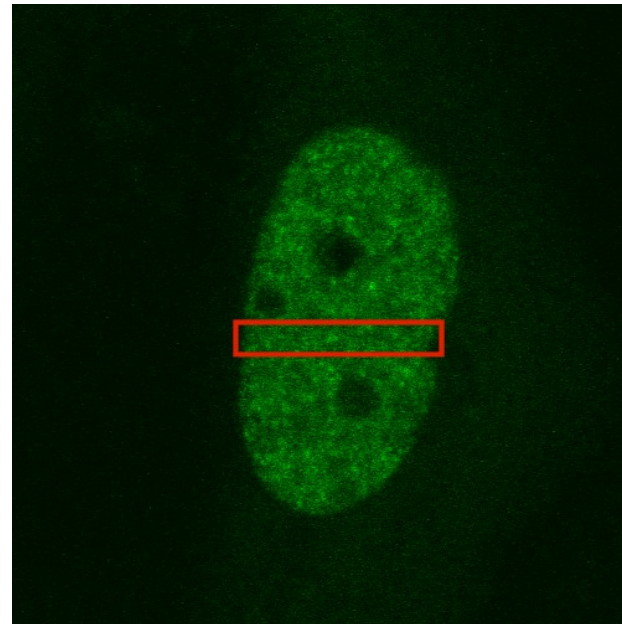
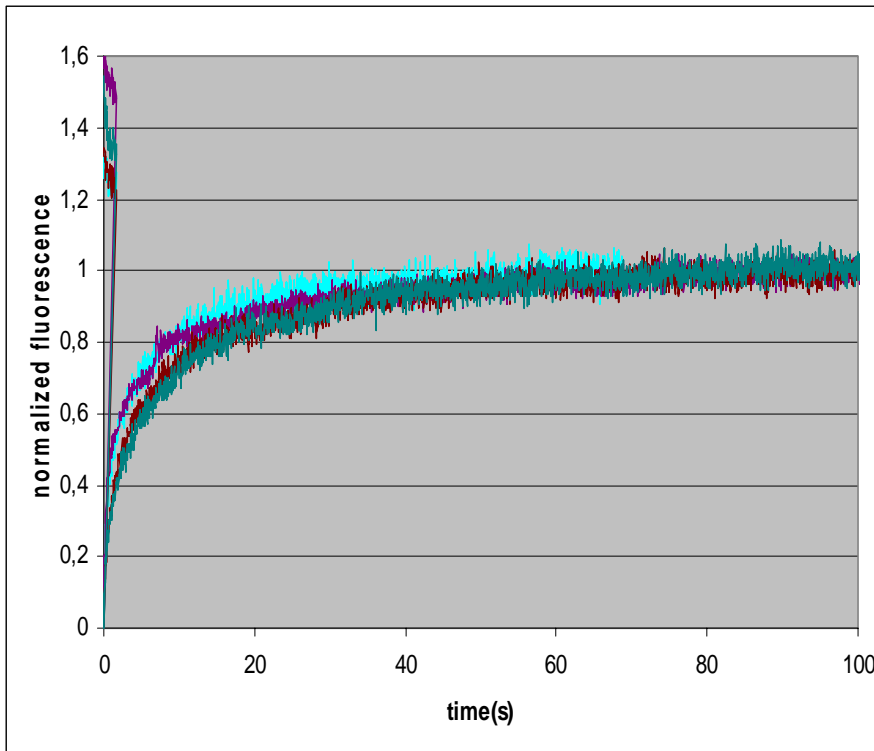


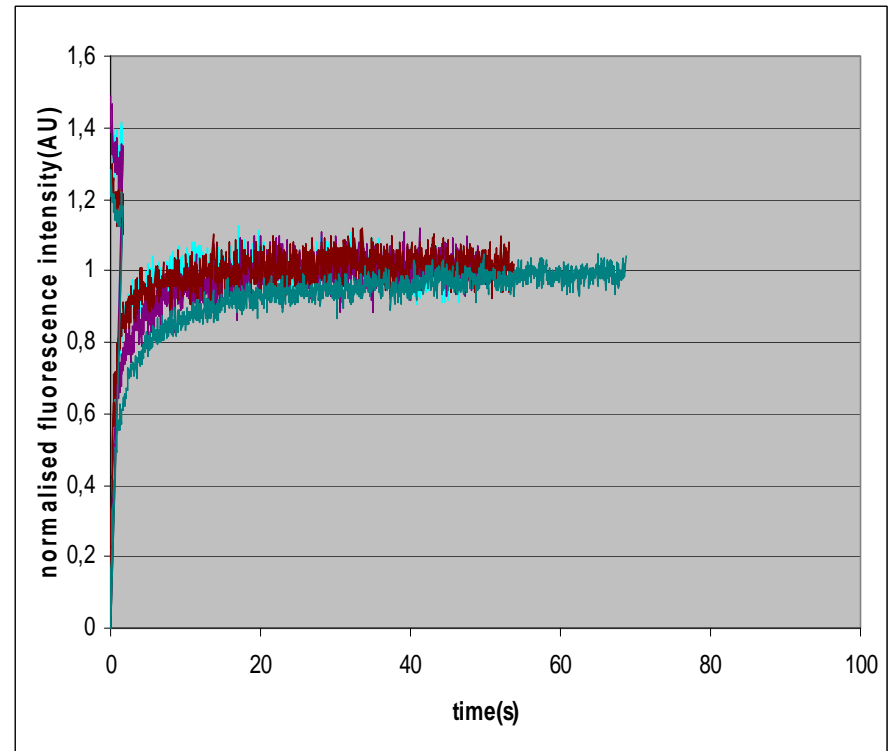
Fig 1: AR-GFP cell with used rectangle ROI For FRAP experiment.

Strip FRAP of AR-GFP

Comparison of AR wildtype and a mutant lacking the DNA-binding domain capacity (DBD mutant):



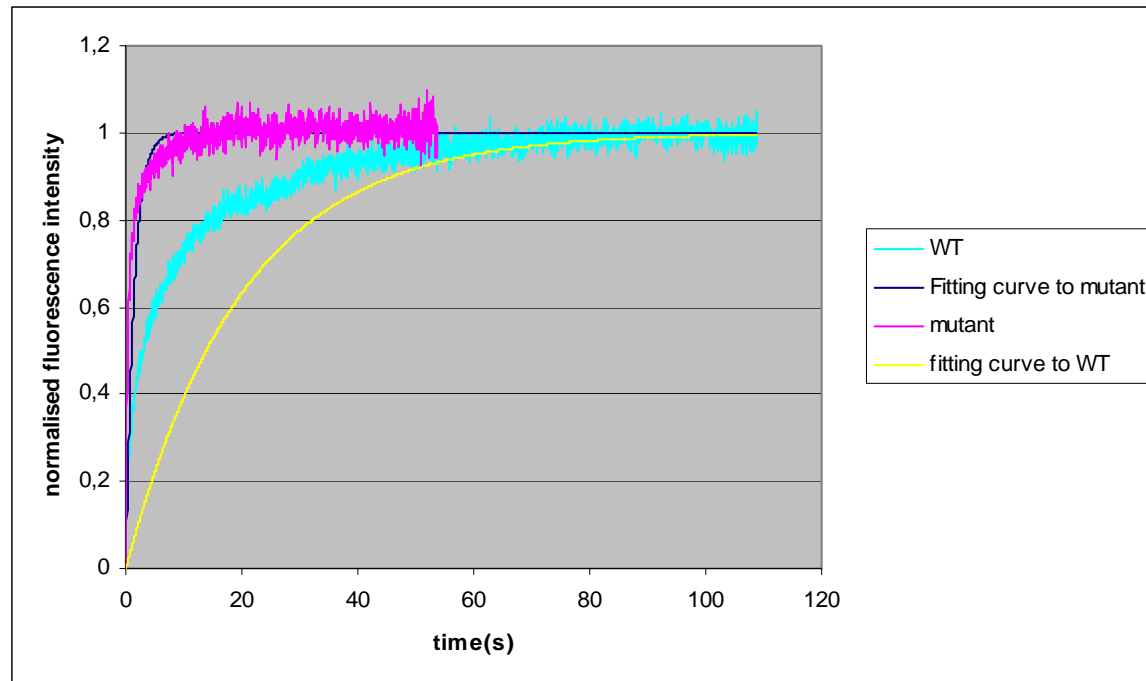
AR wildtype



AR DBD mutant

Even though the wildtype and the DBD mutant have the same diffusion constant (Farla et al. 2004), the mutant shows much faster recovery rates due to less transient Immobilization by DNA binding.

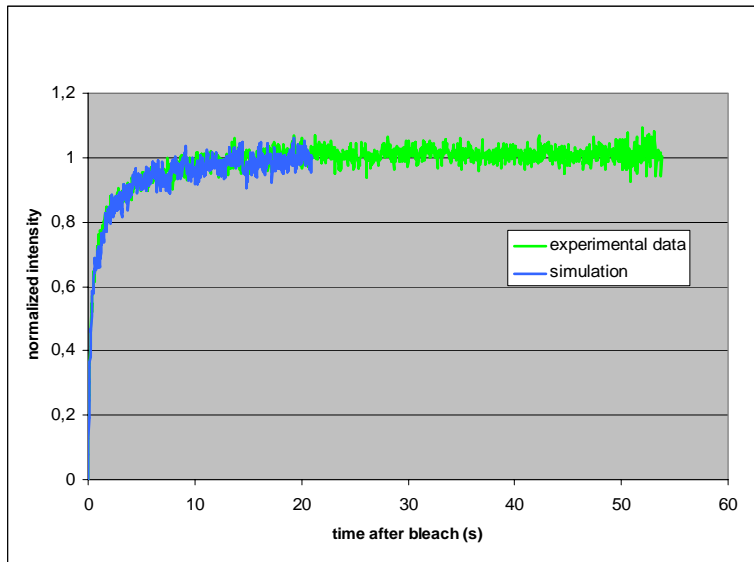
WT and mutant plus fitting curves



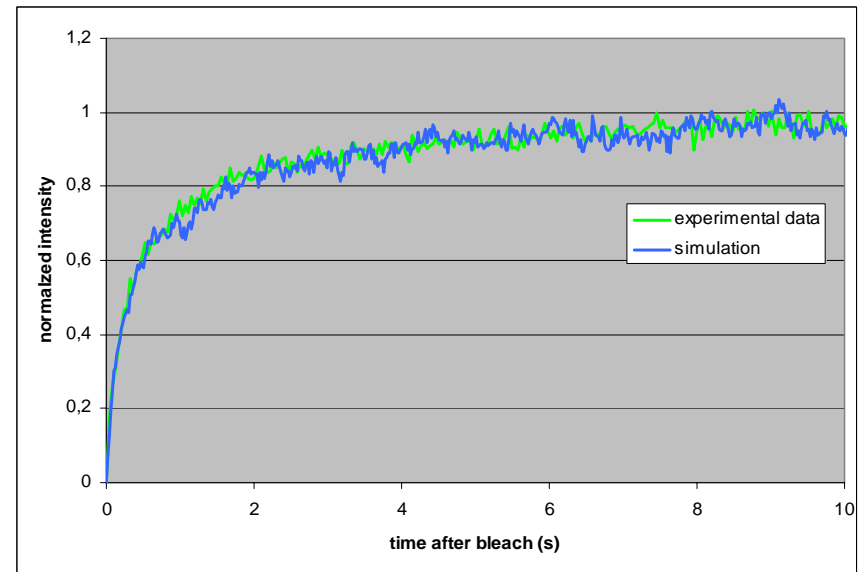
The FRAP curves were fitted with a single exponential function to test how well the data is fitted. The curve of the DBD mutant is fitted by this simple (and not optimal!) function much better than the data of the Wildtype. This is due to the binding of the wildtype to DNA which immobilizes the molecules for several seconds.

Fitting curve of the mutant generated by simulation

To further analyze the FRAP data, simulations of FRAP kinetics were done with a monte-carlo simulation (Farla et al. 2004).

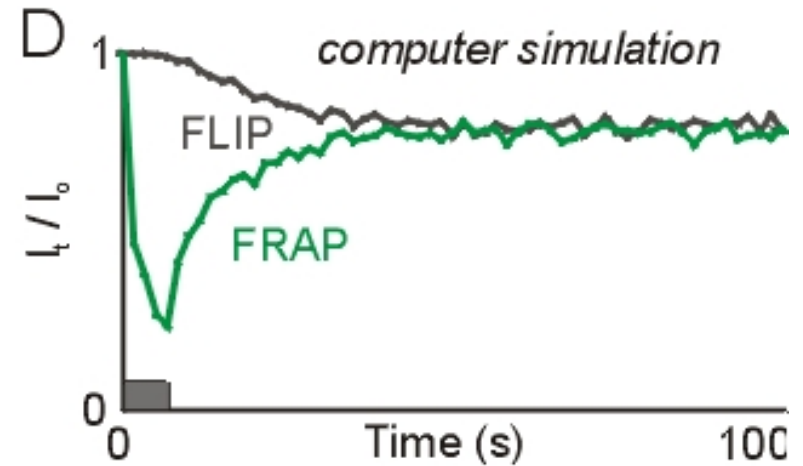
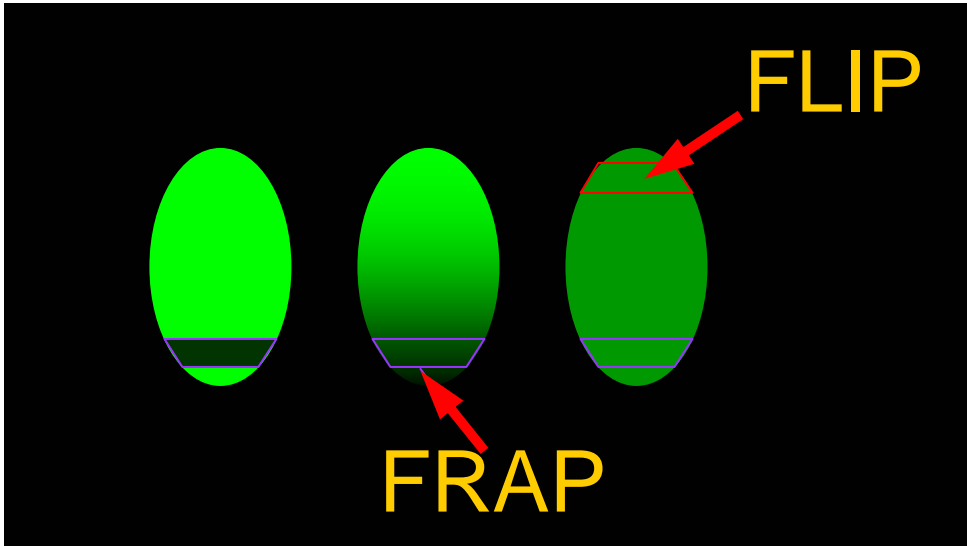


Comparison of experimental data of AR DBD mutant (green) and simulated data (blue)

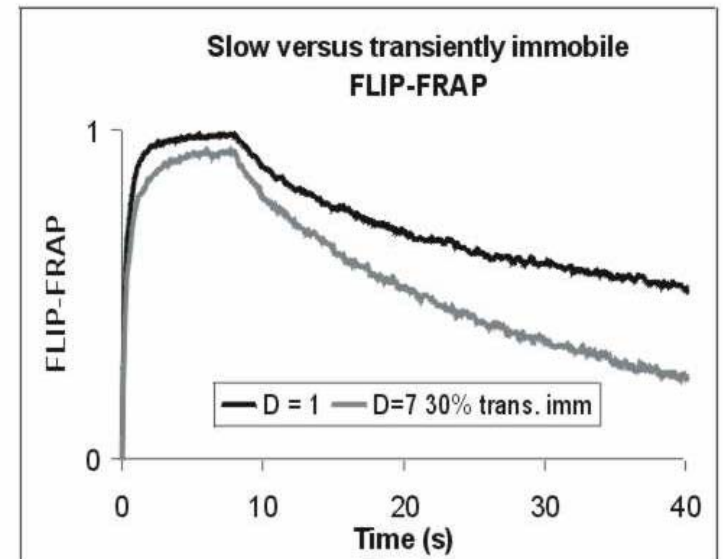


Zoom in on the first 10 seconds of the same Data to show the overlap.

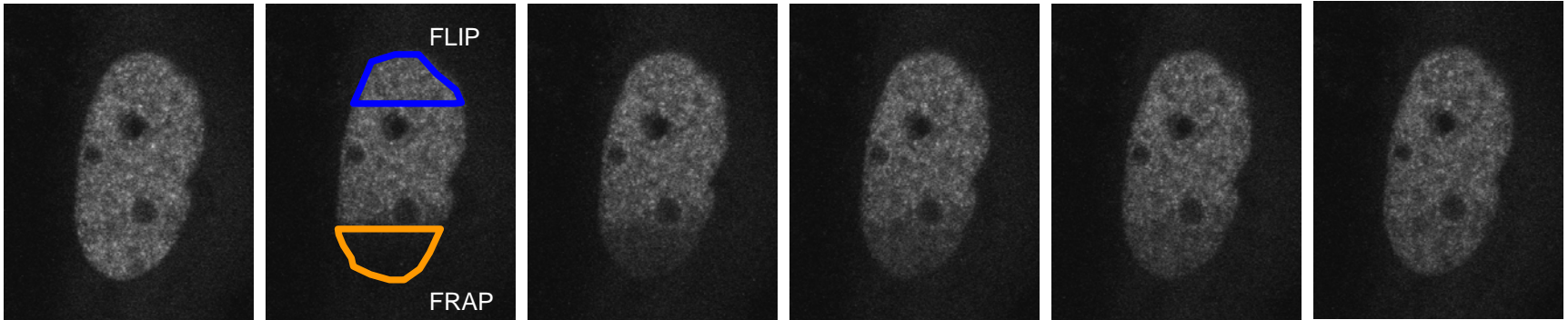
FLIP-FRAP



A combination of Fluorescence Recovery after Photobleaching (FRAP) and Fluorescence Loss in Photobleaching (FLIP) enables distinction between slow non-immobilized and fast transiently immobilized molecules (figures from [presentation by A.Houtsmuller](#) during the course).



FLIP-FRAP of AR-GFP



Example images and curve of a FLIP-FRAP experiment during the course.

[example movie](#)

For further questions please mail to eamnet@embl.de

