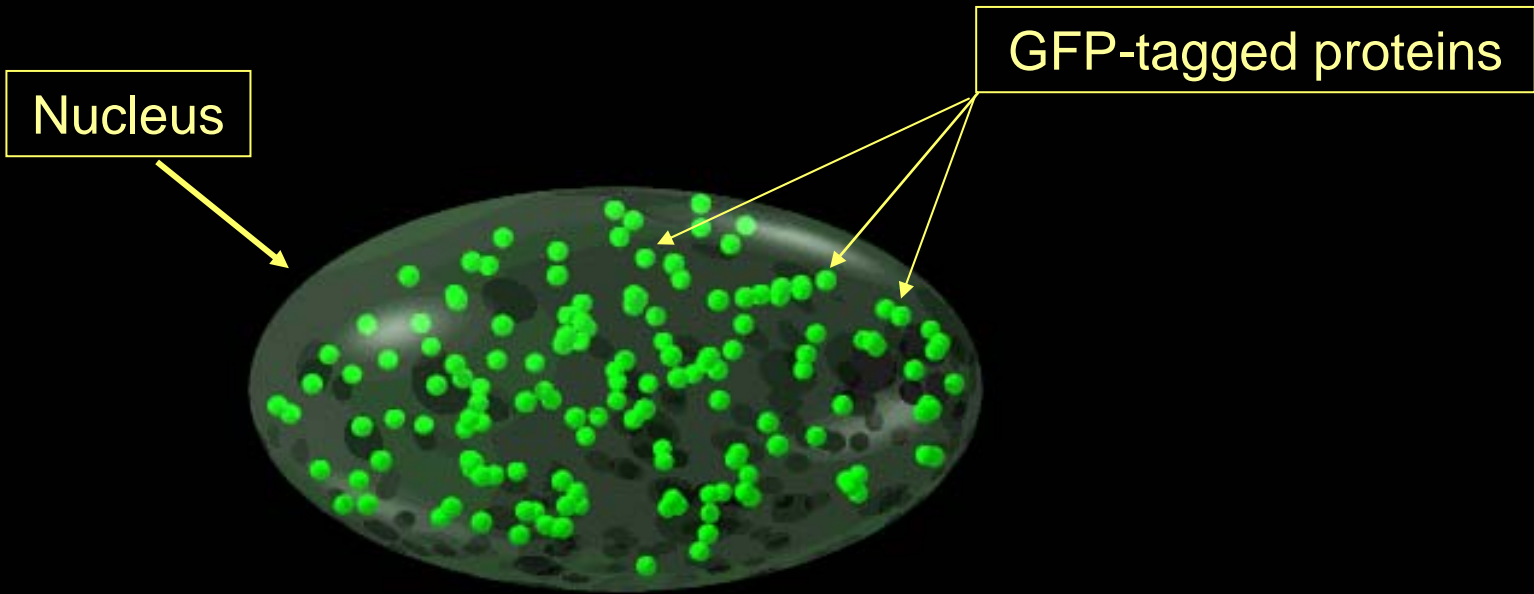
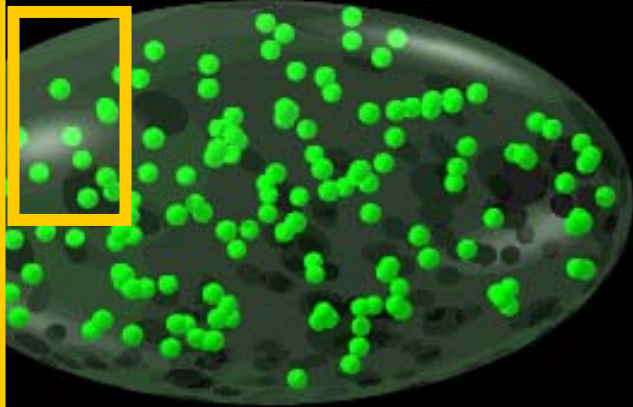
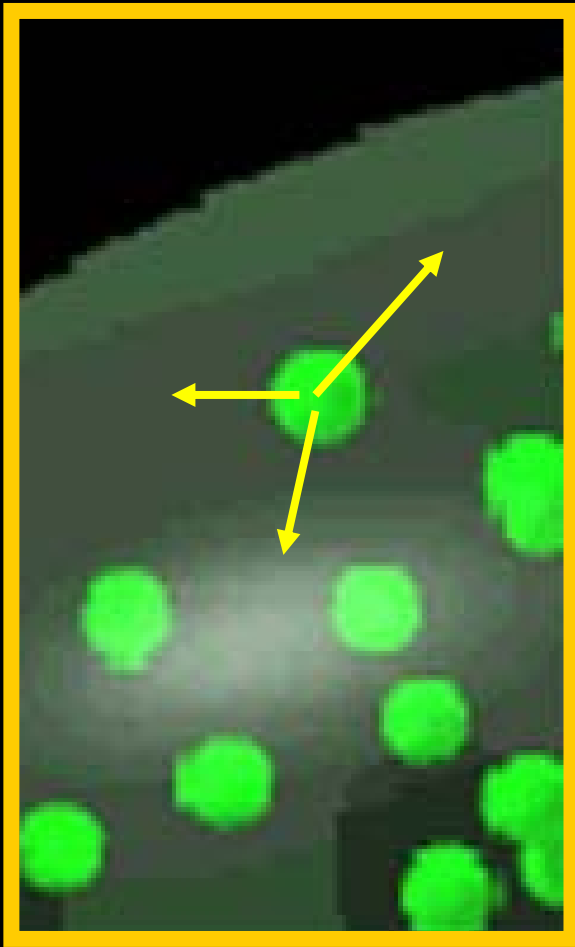
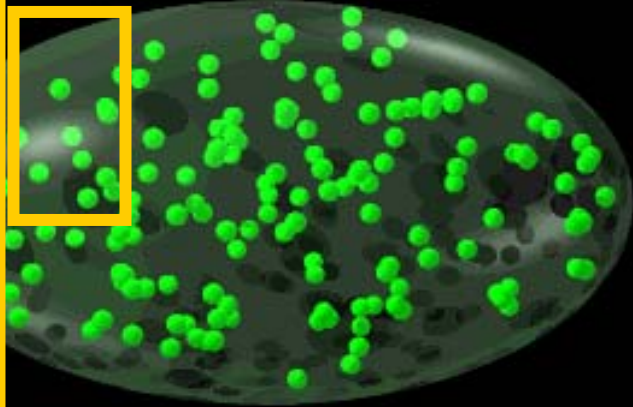
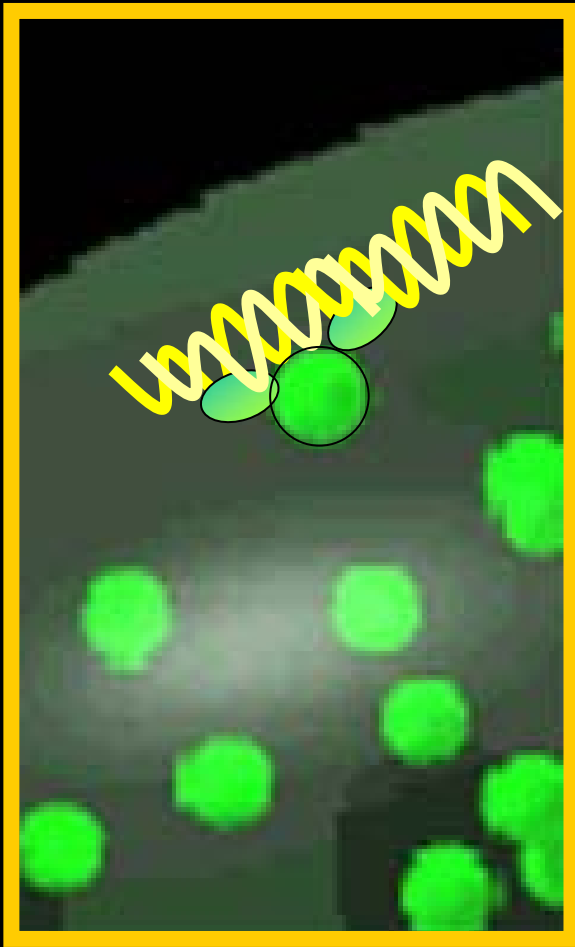


# Molecular mechanisms of DNA repair and transcription studied by FRAP

Adriaan Houtsmuller  
Josephine Nefkens Institute  
Erasmus Medical Centre  
Rotterdam  
The Netherlands

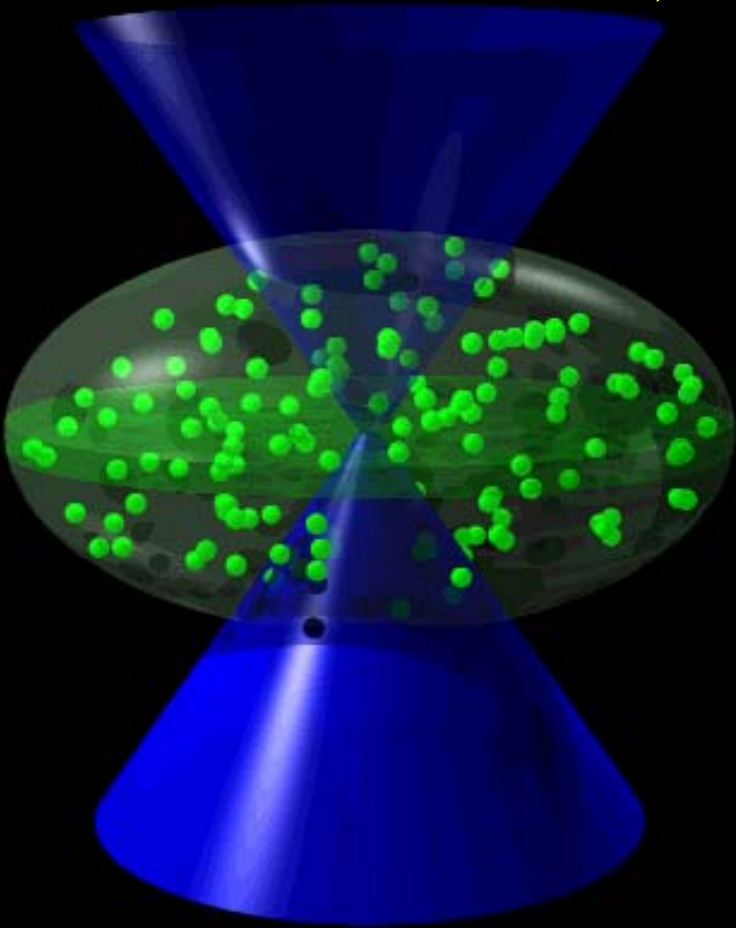




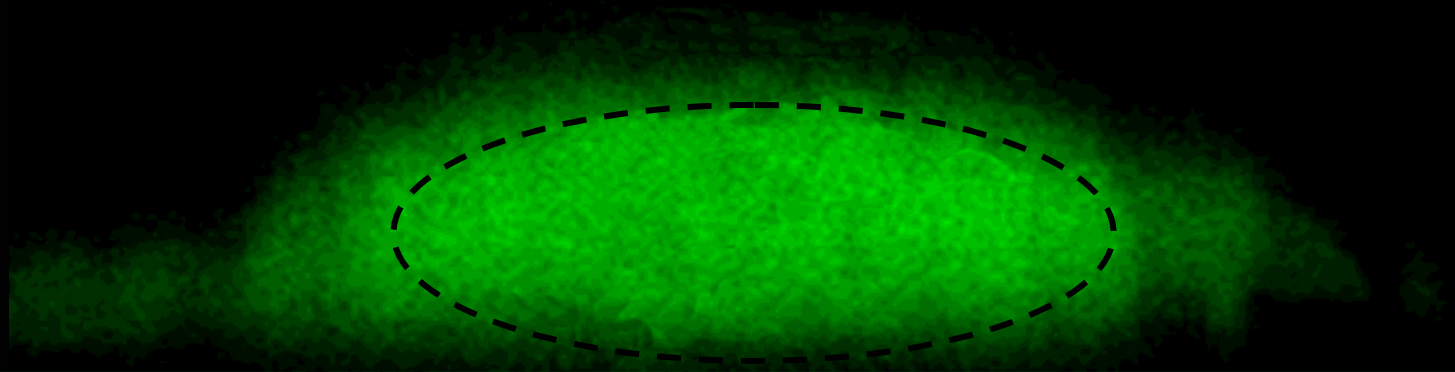


Focused laser beam

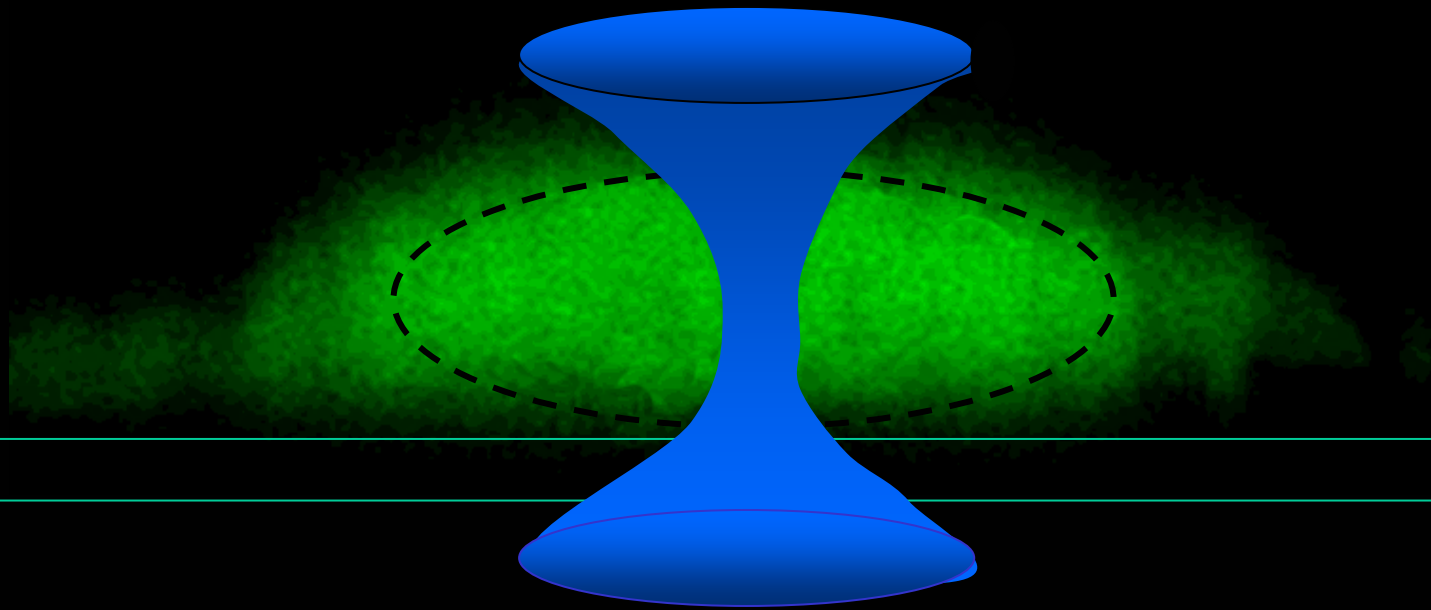
Confocal plane



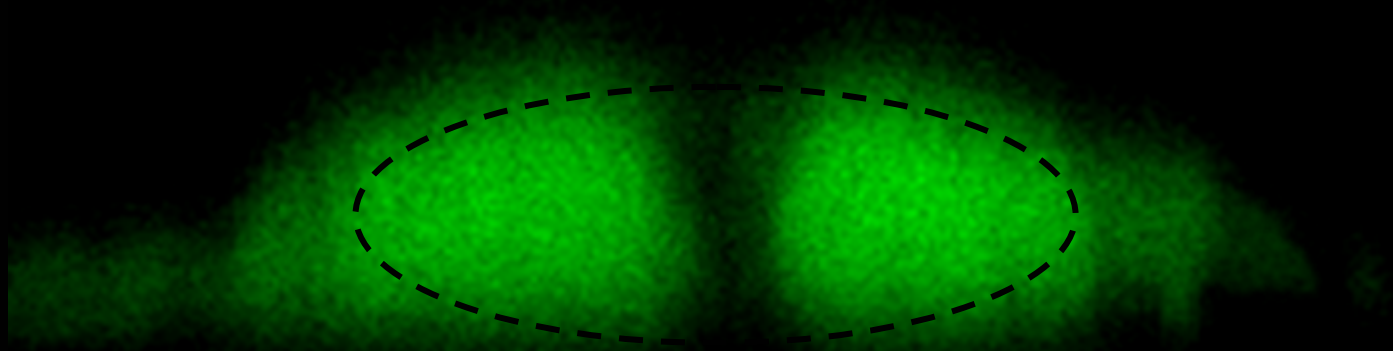
# Confocal side view of a fixed cell expressing GFP



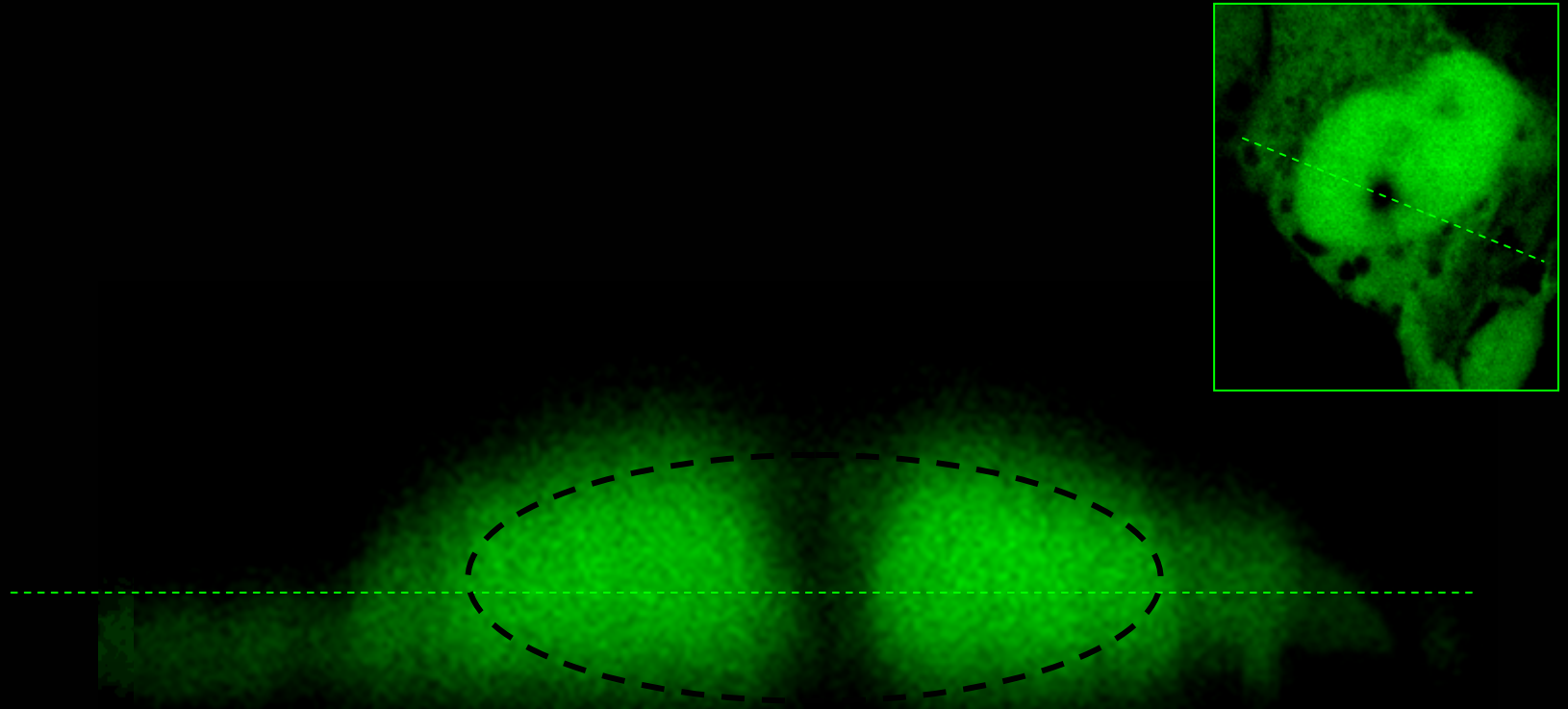
# Confocal side view of a fixed cell expressing GFP



# Confocal side view of a fixed cell expressing GFP



# Confocal side view of a fixed cell expressing GFP



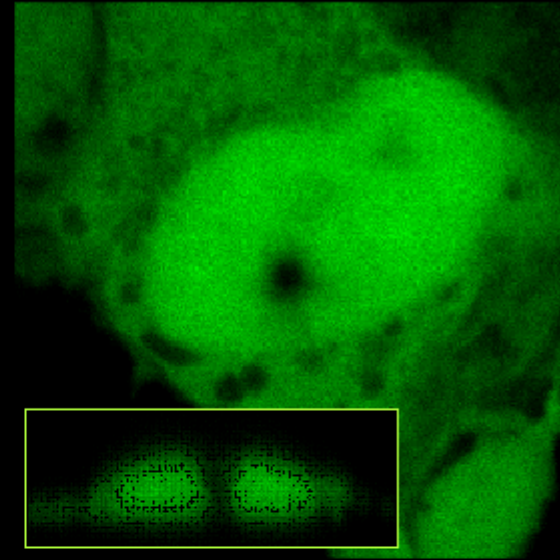
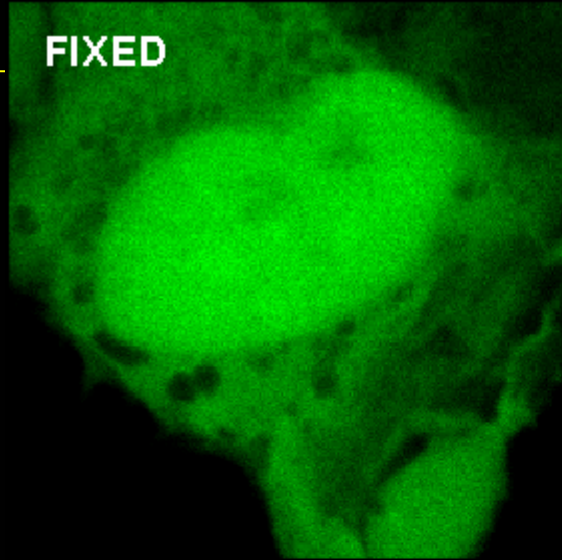
# Spot bleaching of GFP in nuclei

before bleach

after bleach

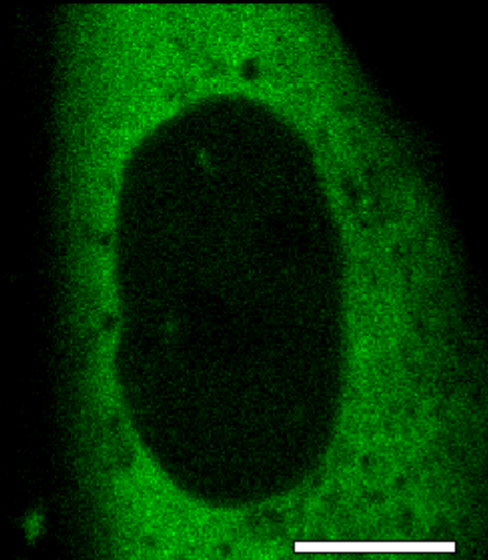
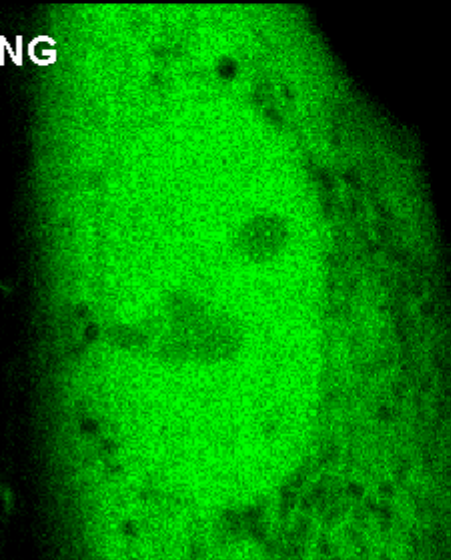
FIXED

immobile  
molecules



LIVING

mobile  
molecules



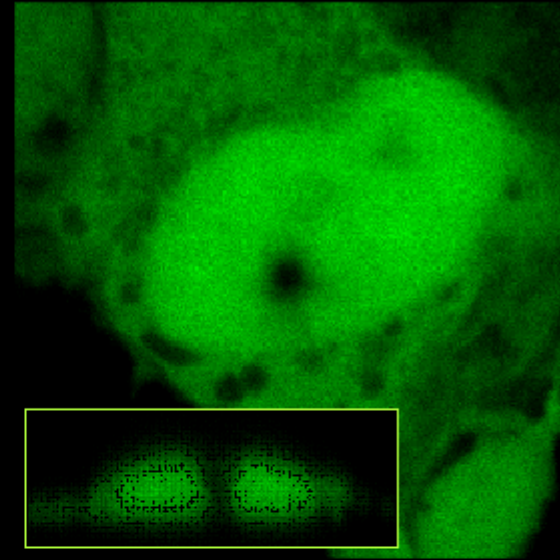
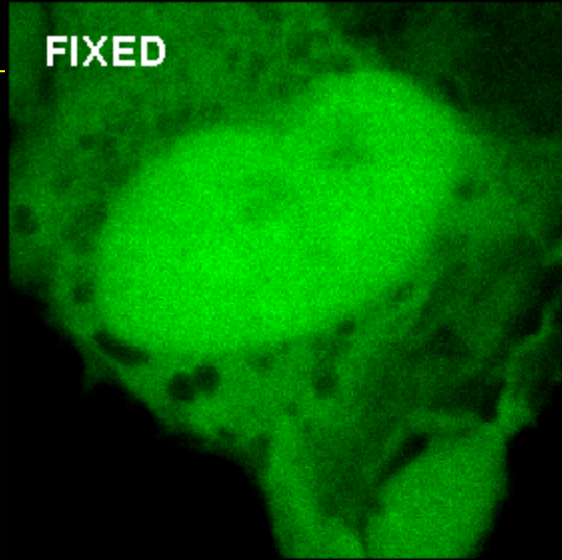
# Spot bleaching of GFP in nuclei

before bleach

after bleach

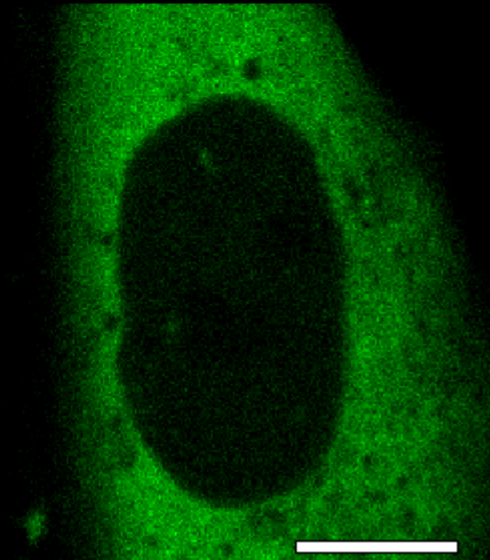
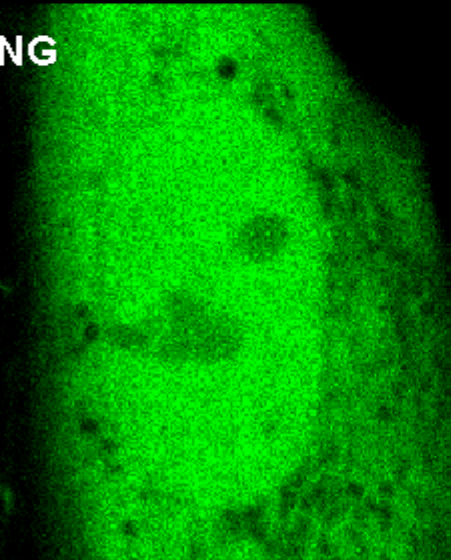
FIXED

immobile  
molecules



LIVING

mobile  
molecules



# Nucleotide excision repair

Single strand  
UV-damage

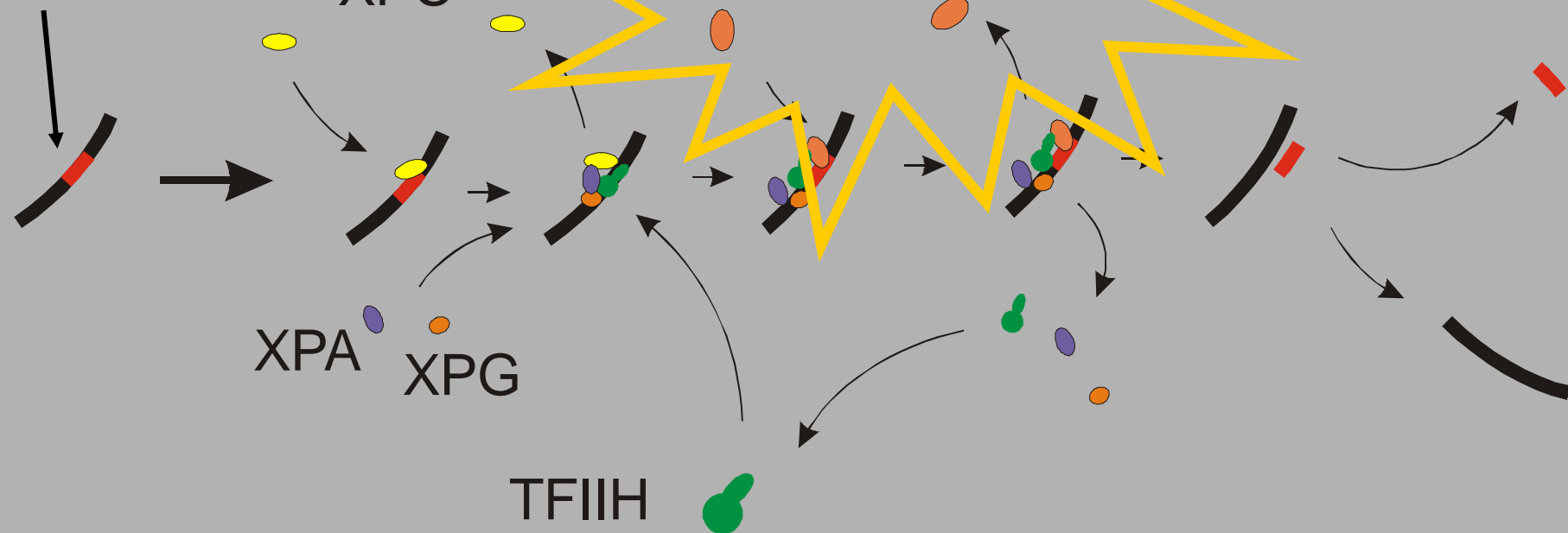
XPC

ERCC1/XPF

XPA

XPG

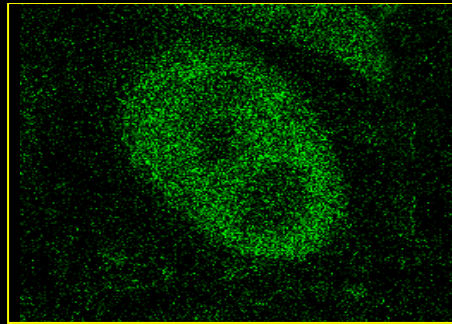
TFIIH



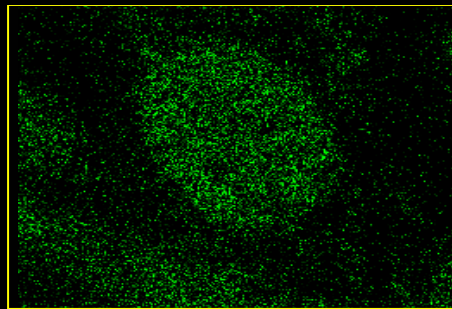
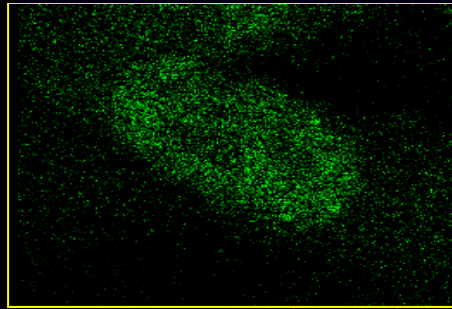
# Spotbleaching of cells expressing ERCC1-GFP

before bleach

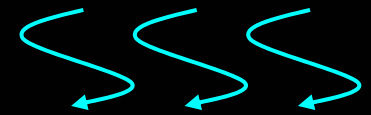
fixed



living



untreated

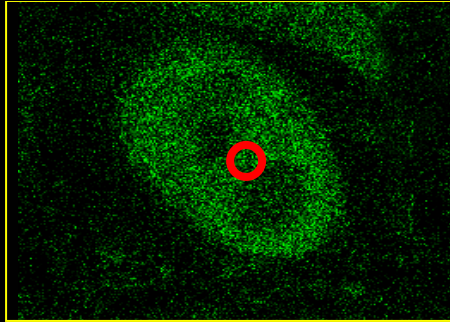


UV irradiated  
(8 J/m<sup>2</sup>)

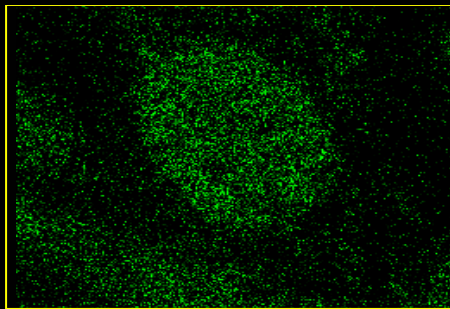
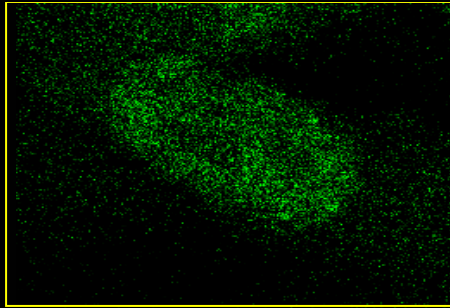
# Spotbleaching of cells expressing ERCC1-GFP

before bleach

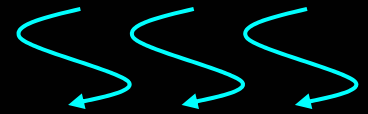
fixed



living

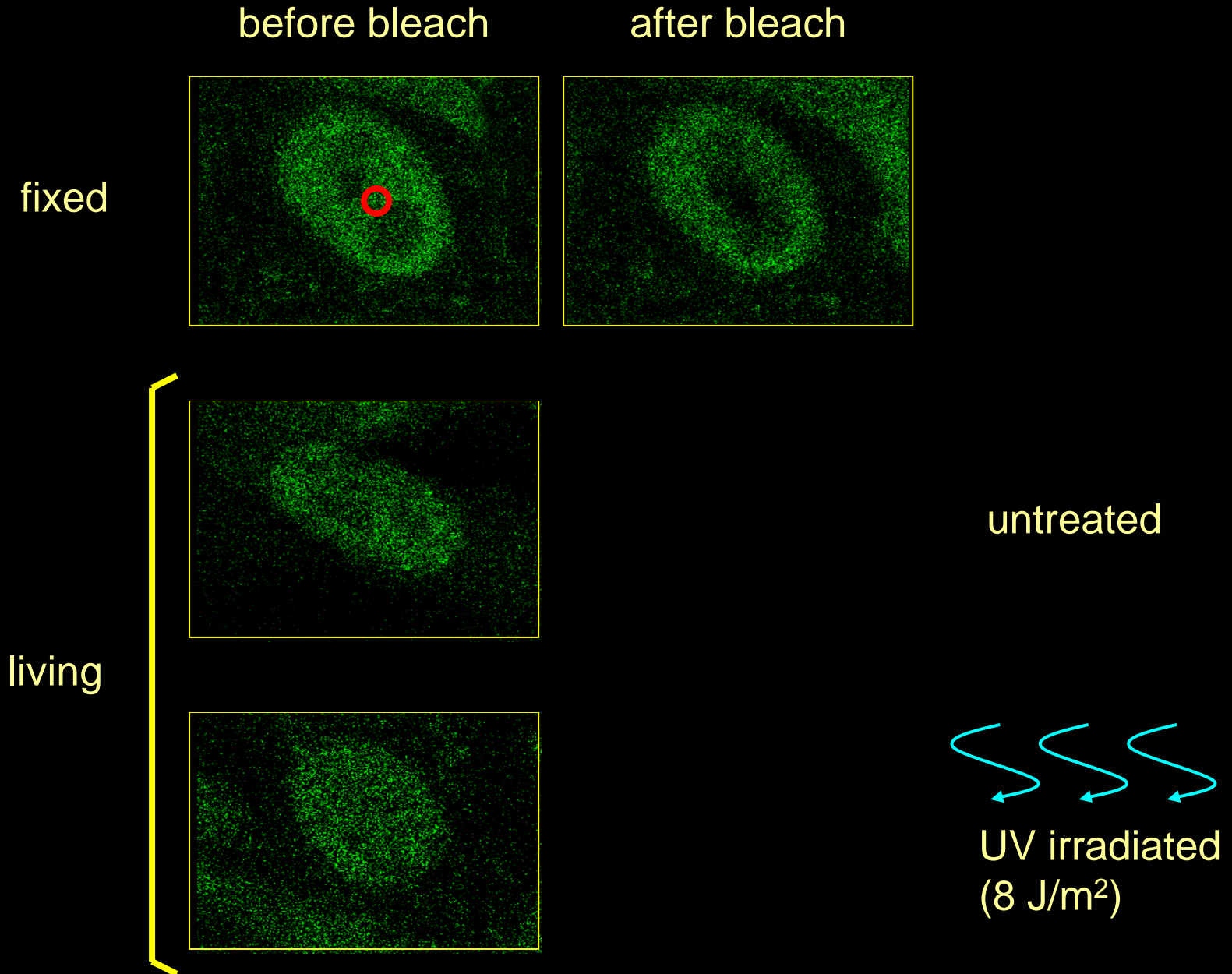


untreated

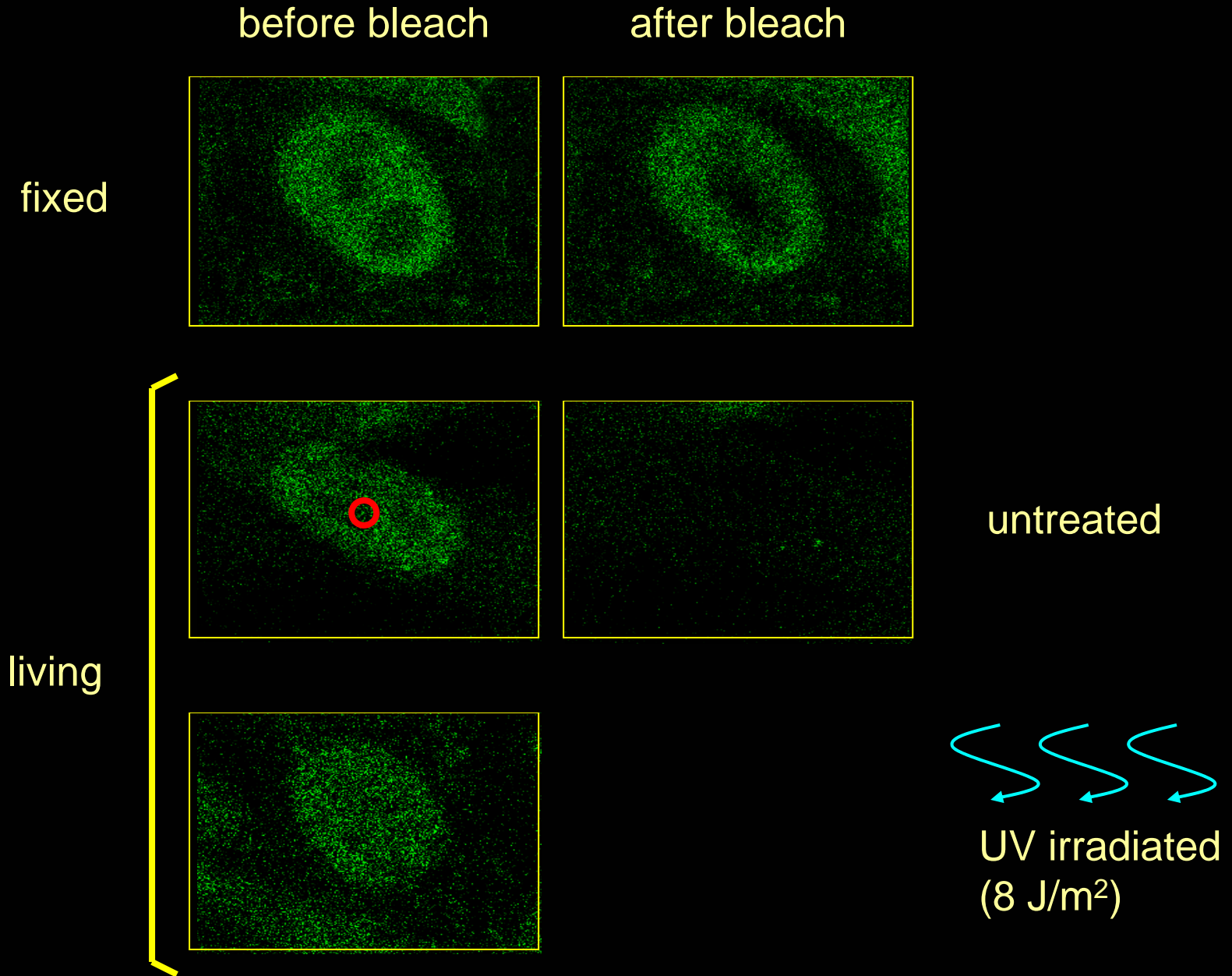


UV irradiated  
(8 J/m<sup>2</sup>)

# Spotbleaching of cells expressing ERCC1-GFP



# Spotbleaching of cells expressing ERCC1-GFP

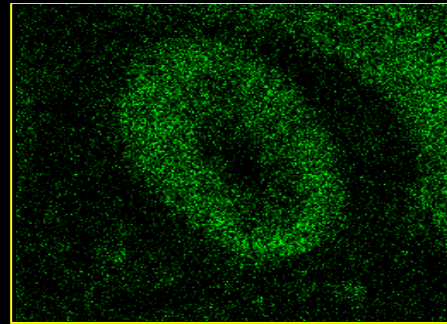
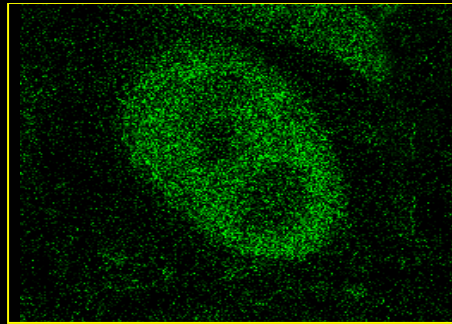


# Spotbleaching of cells expressing ERCC1-GFP

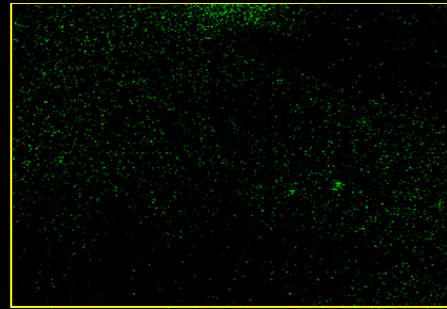
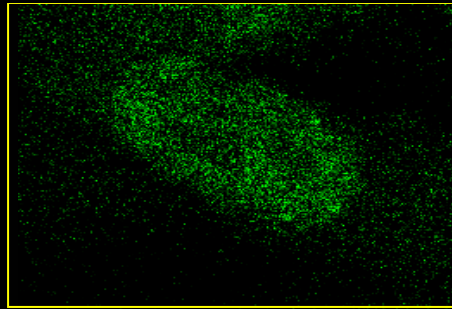
before bleach

after bleach

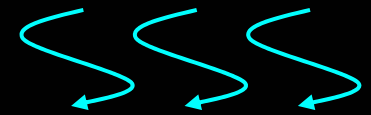
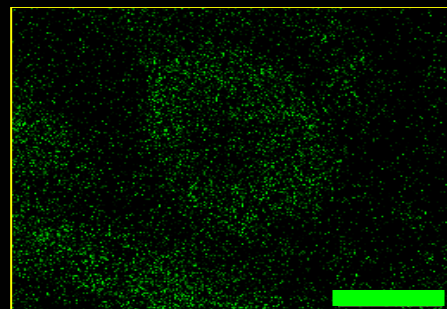
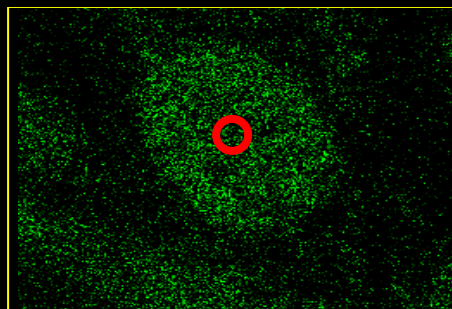
fixed



living

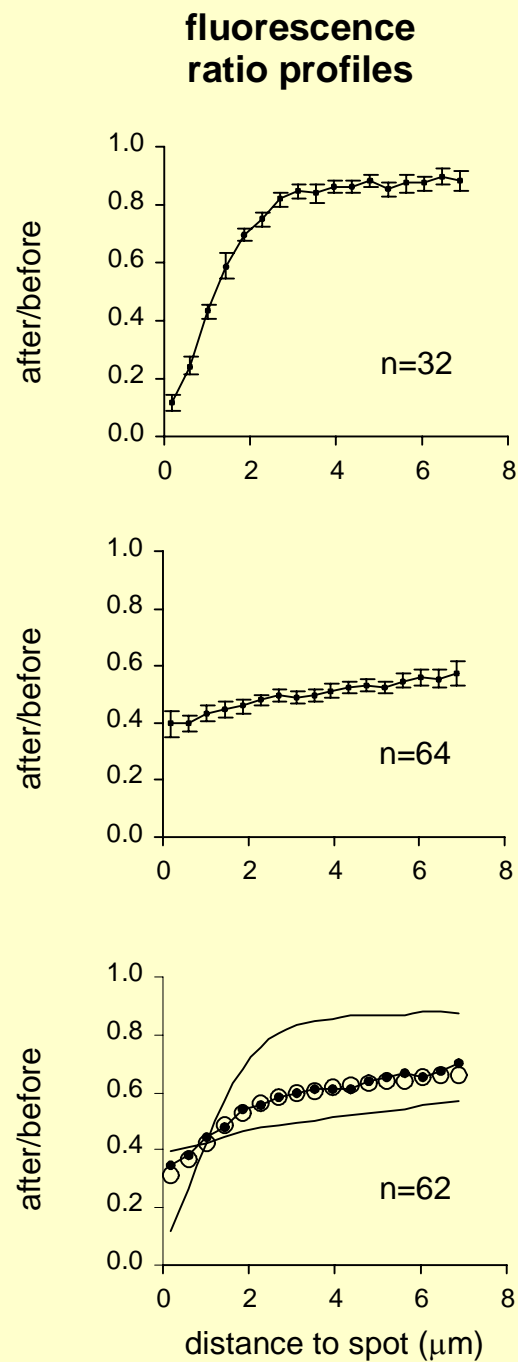
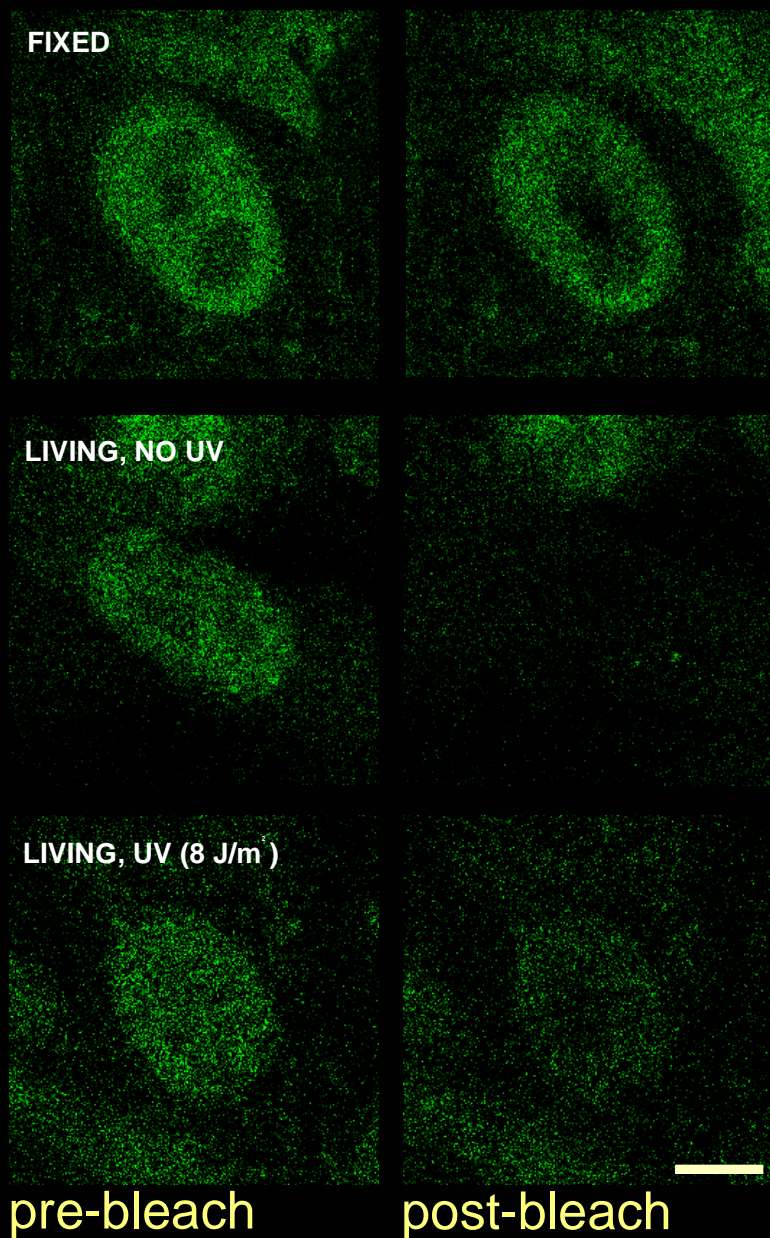


untreated

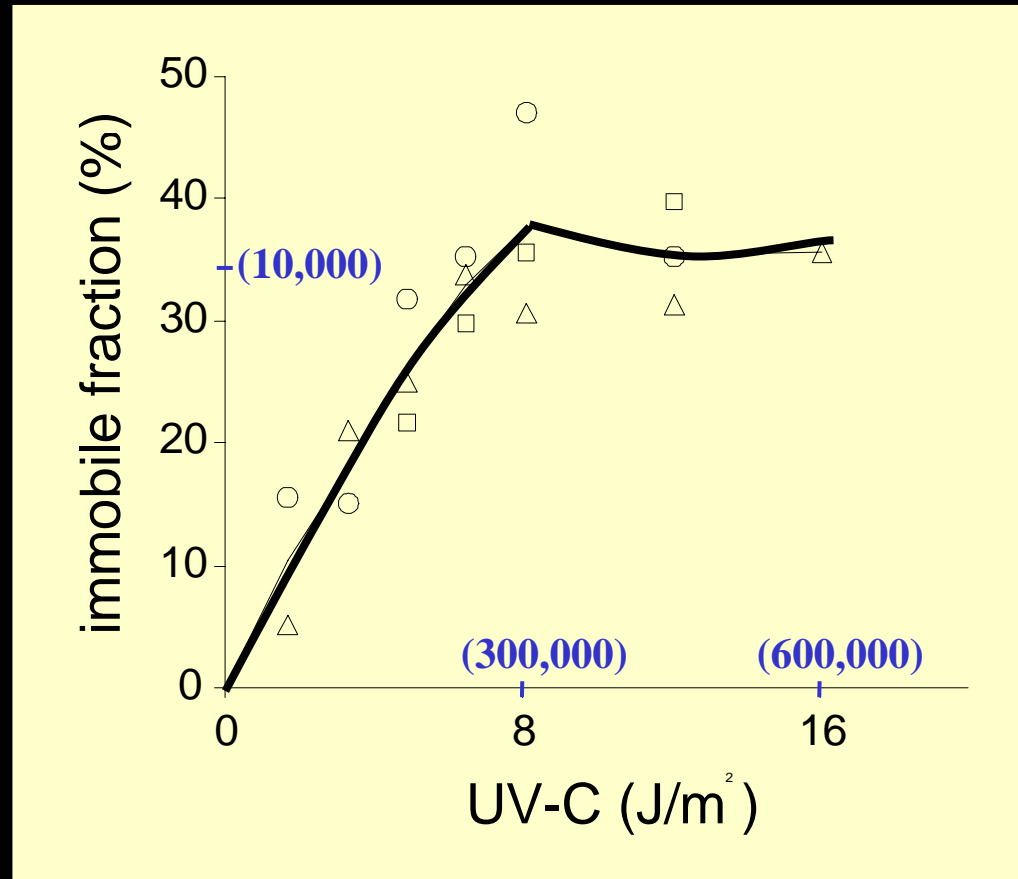


UV irradiated  
(8 J/m<sup>2</sup>)

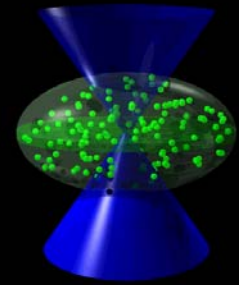
# Spot-FRAP on ERCC1-GFP expressing cells



# Immobilisation of ERCC1-GFP depends on UV-dose

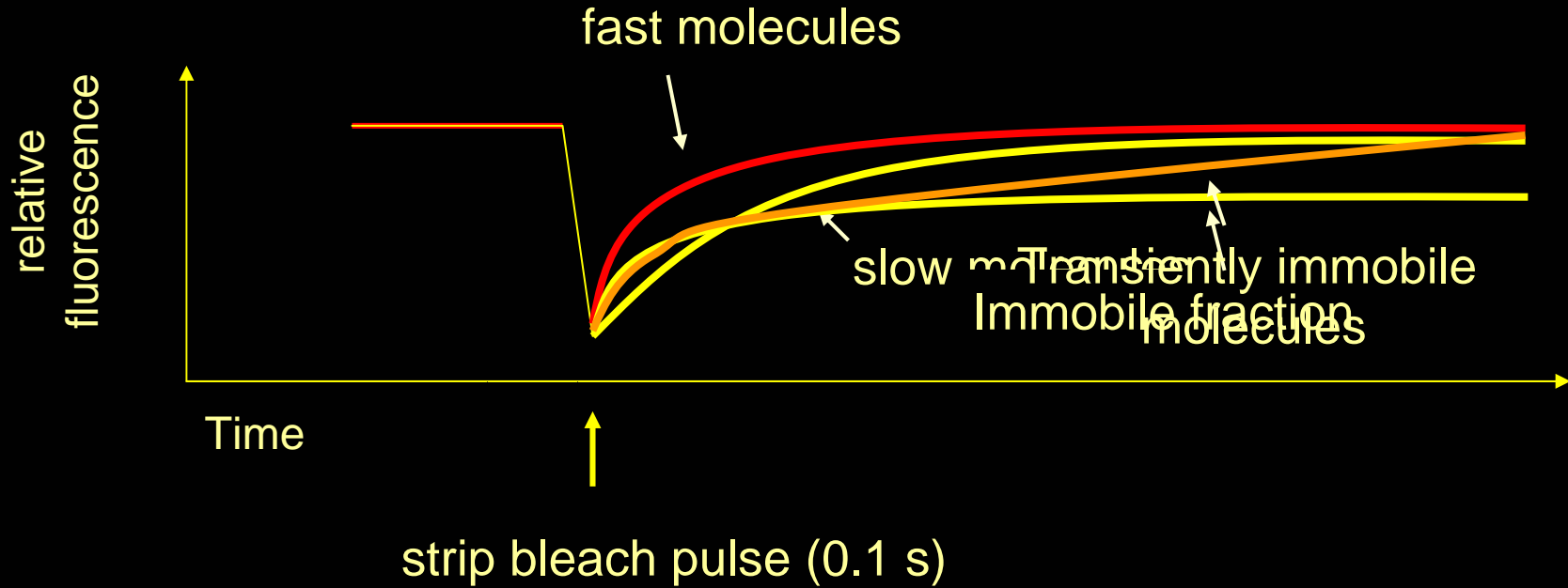
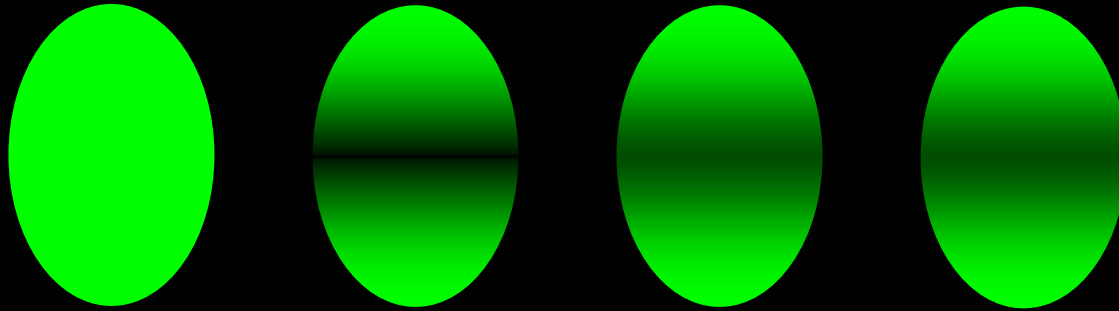


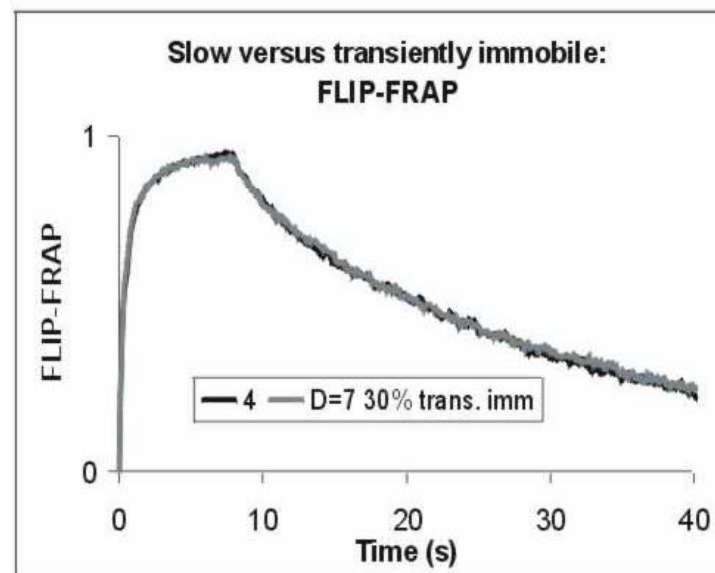
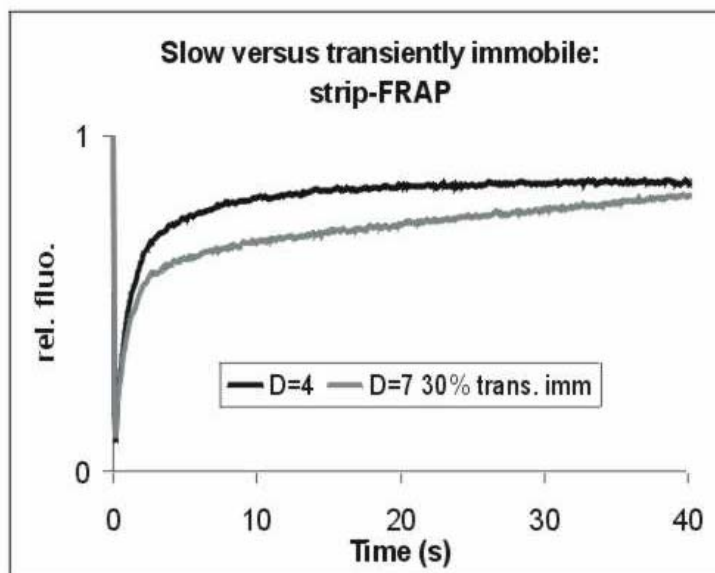
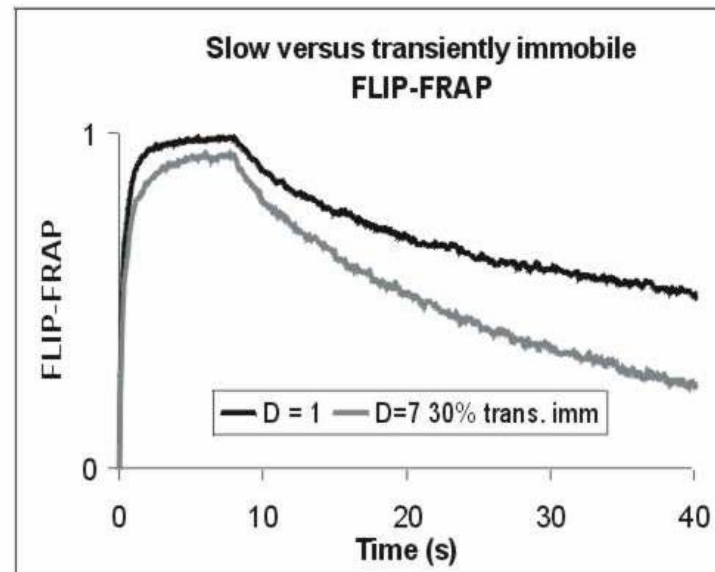
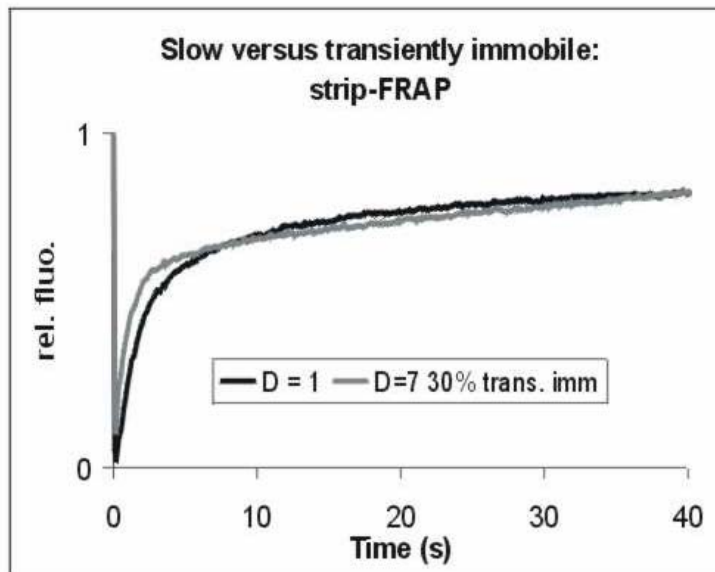
Houtsmuller et al., Science 1999



What if immobilisation  
is transient?

# Strip-FRAP

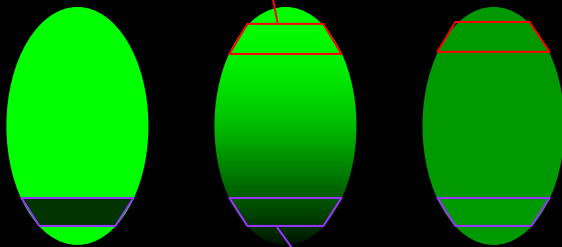




# Combined FRAP and FLIP

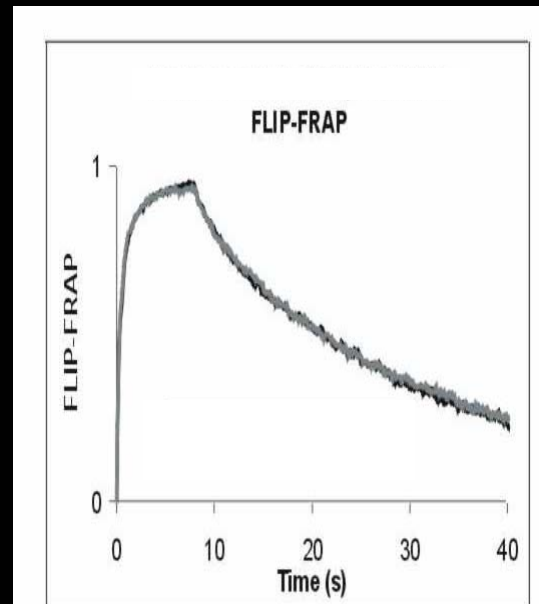
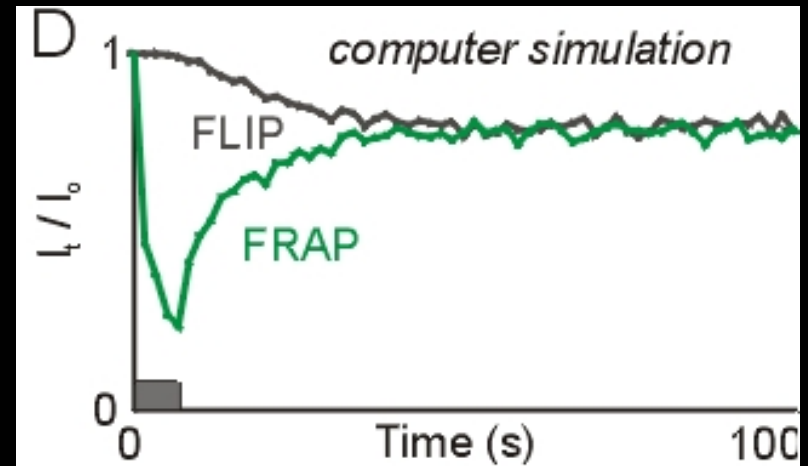
fluorescence loss in photobleaching

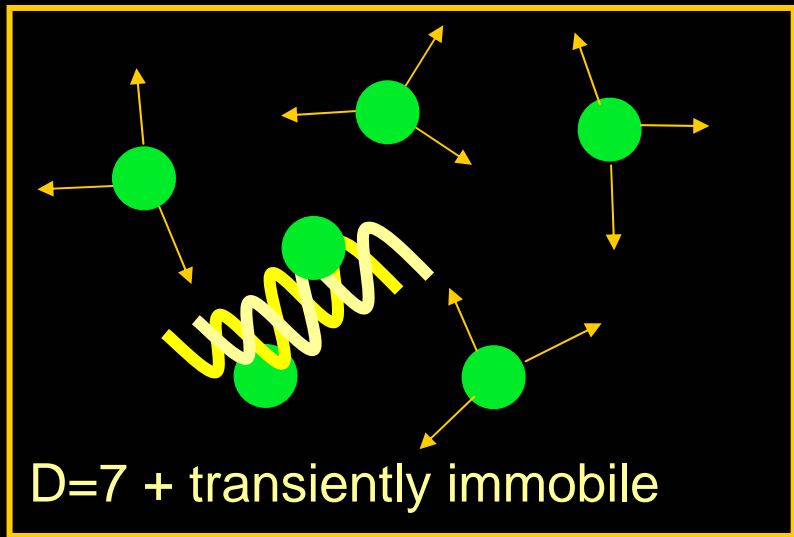
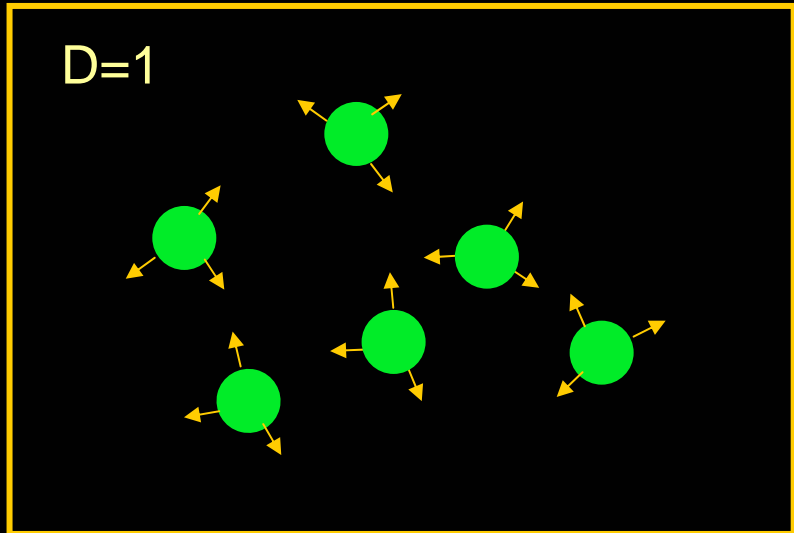
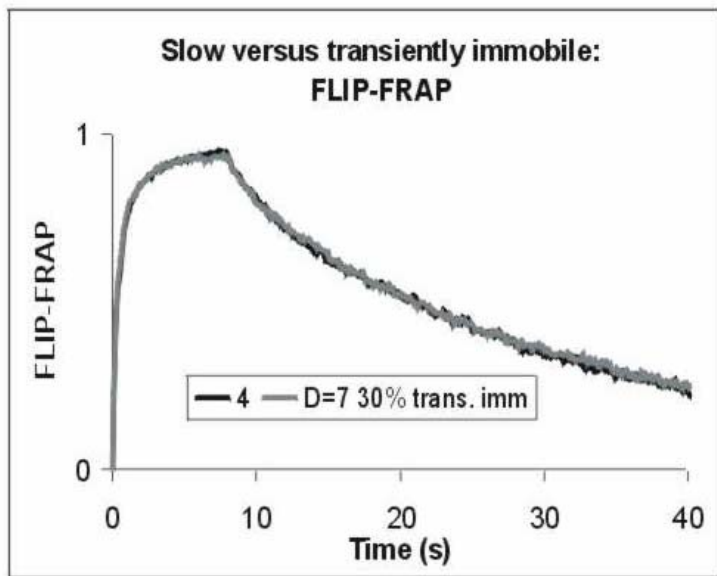
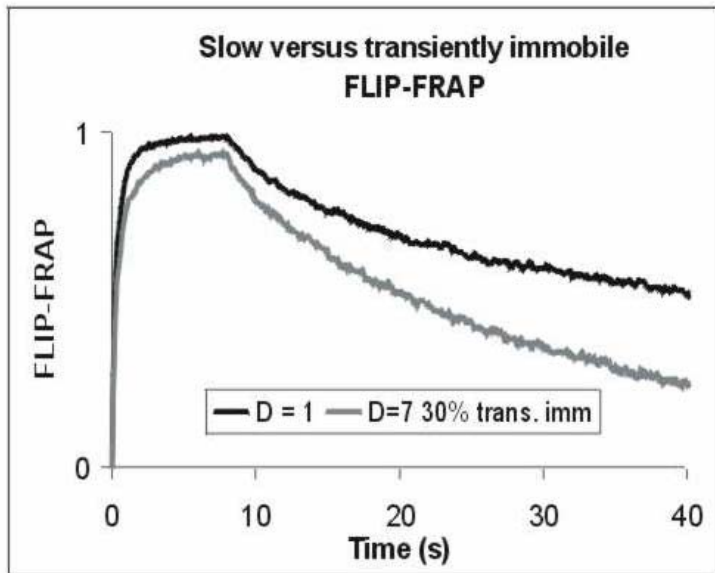
(FLIP)

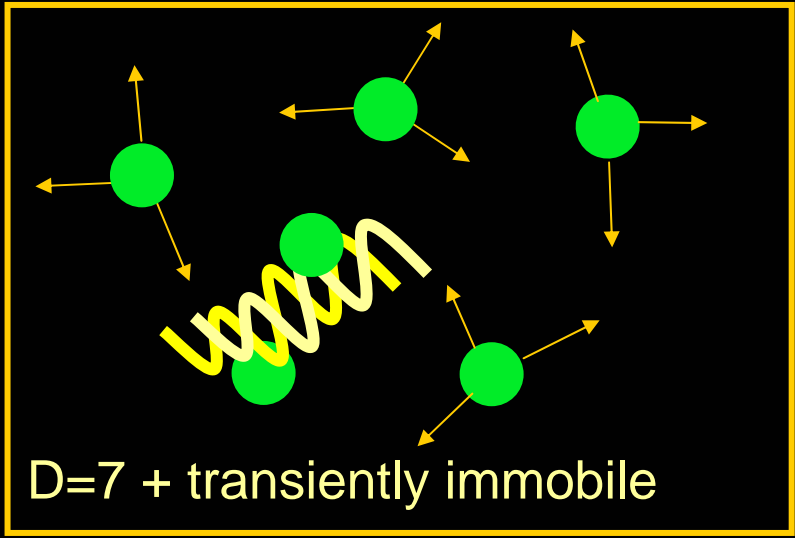
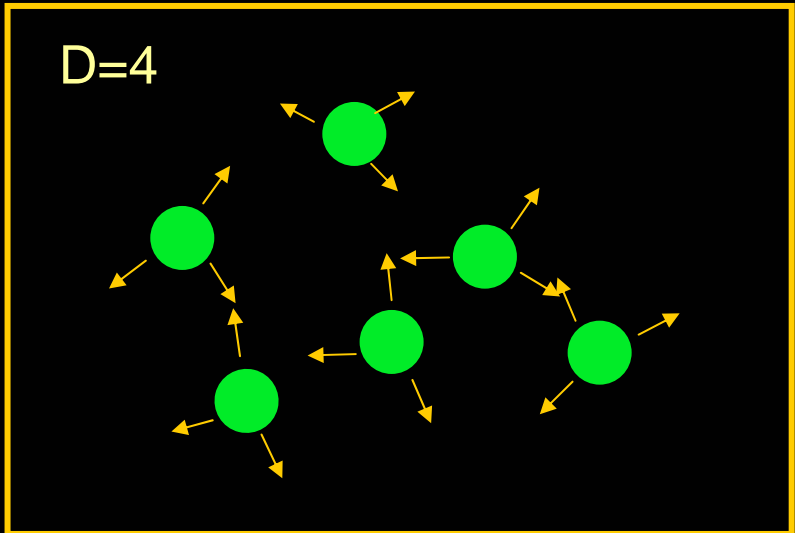
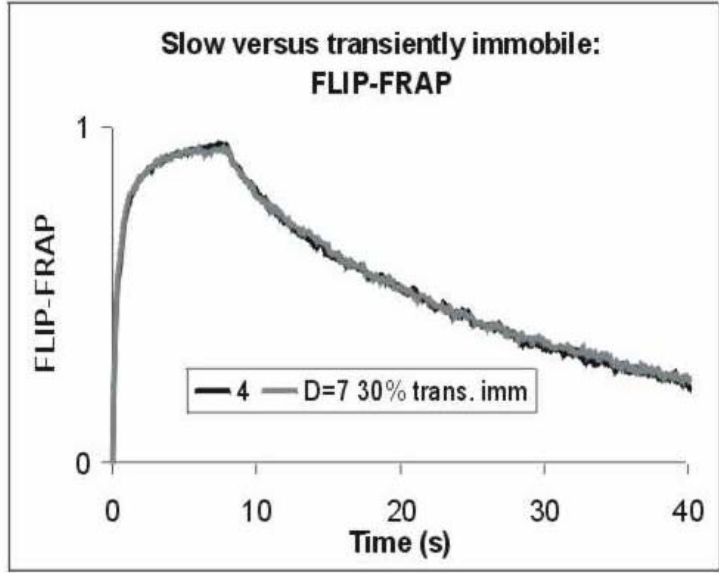
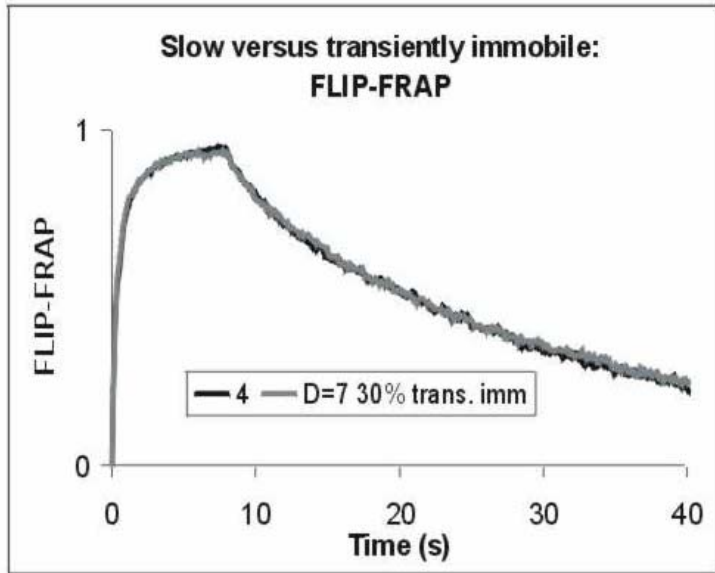


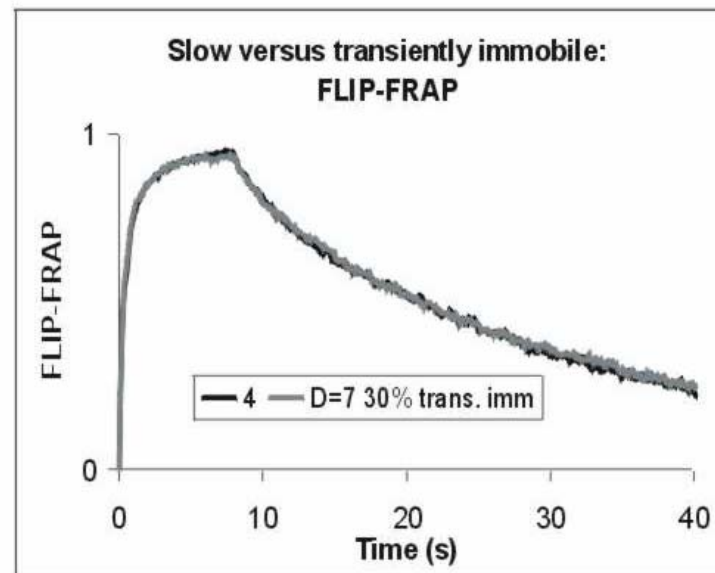
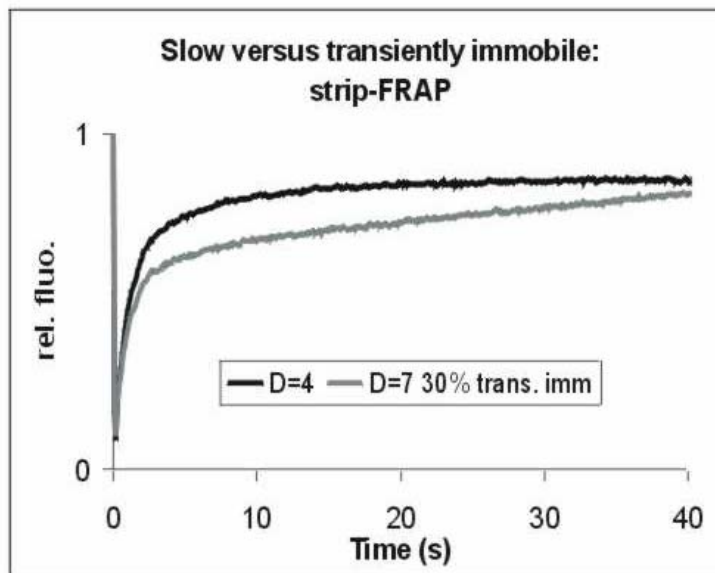
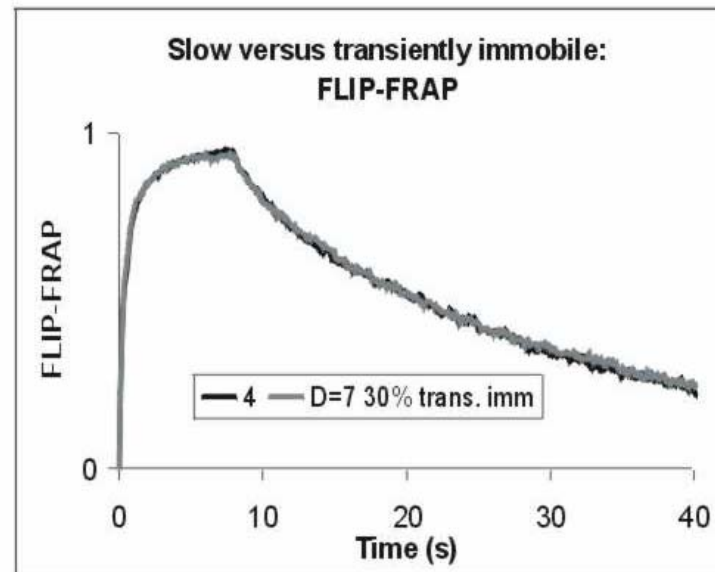
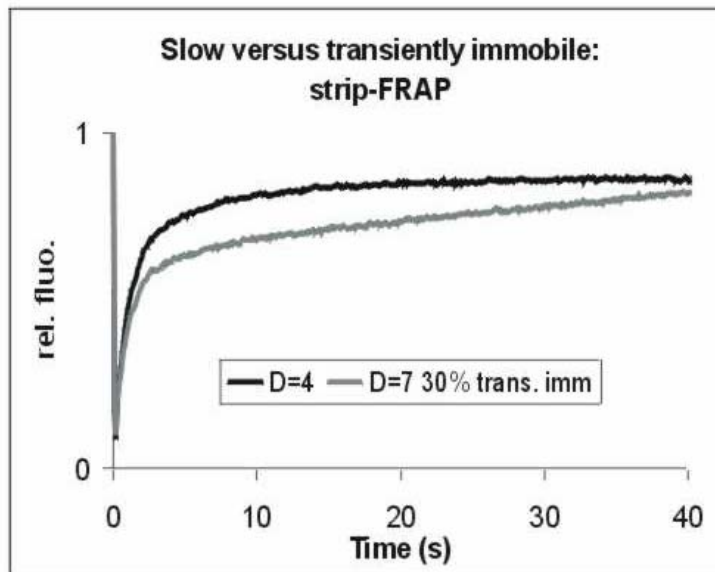
(FRAP)

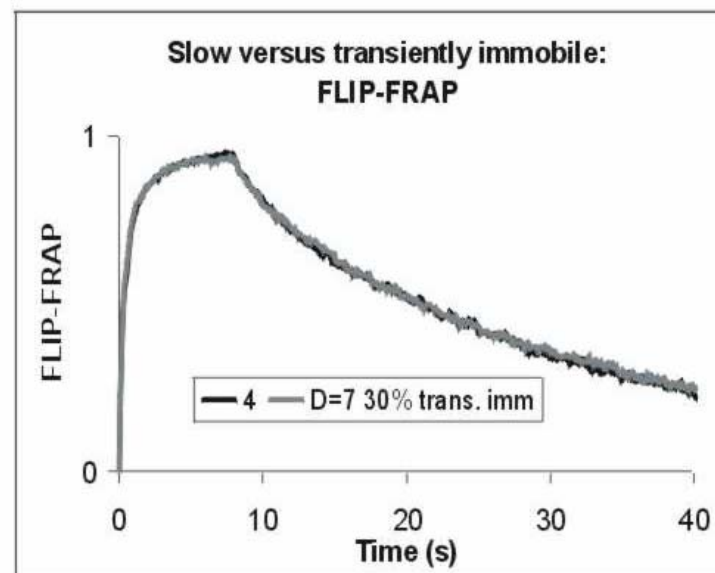
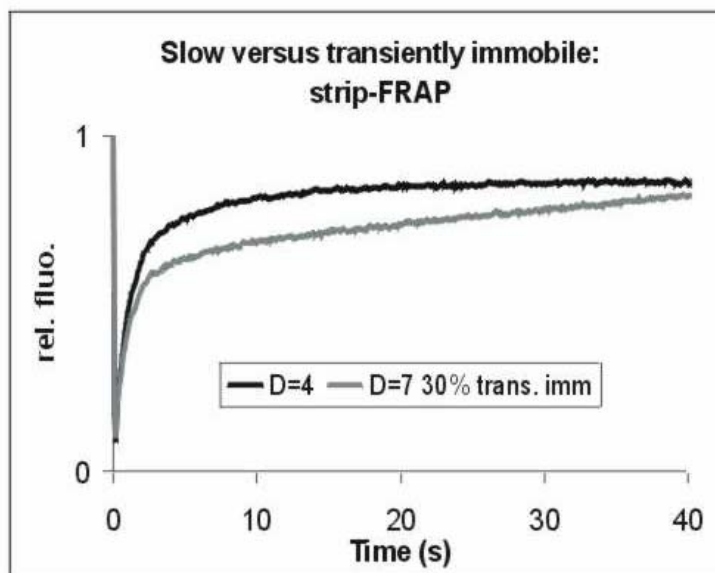
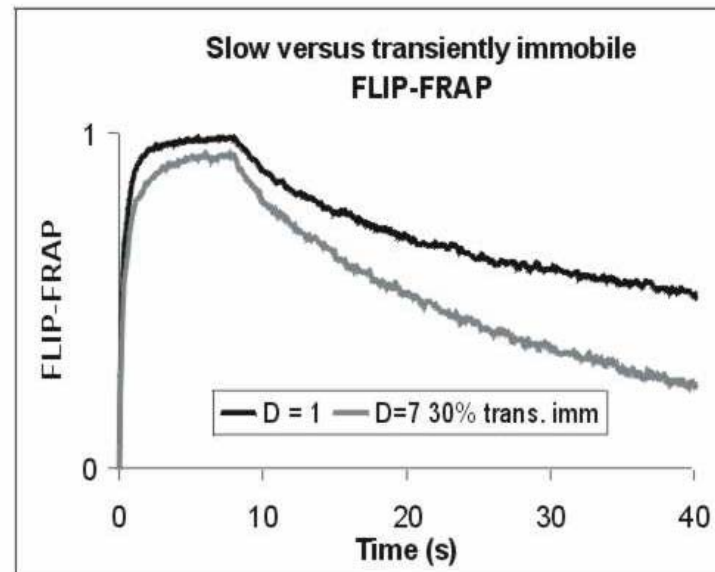
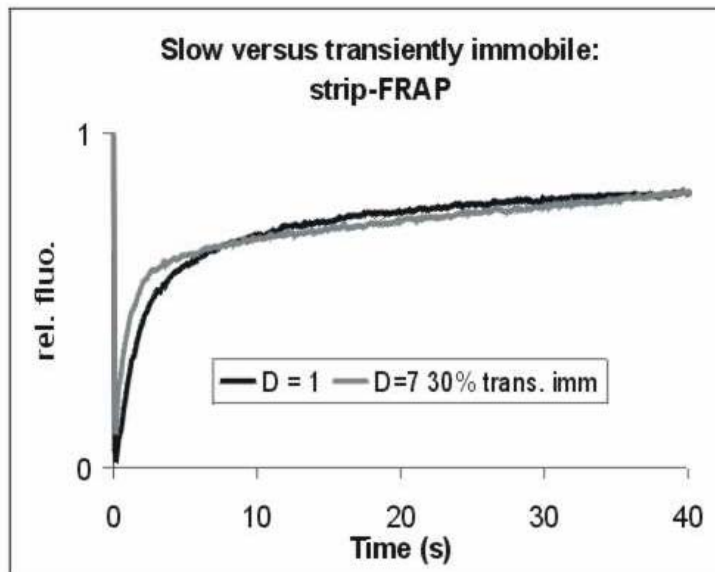
fluorescence redistribution  
after photobleaching





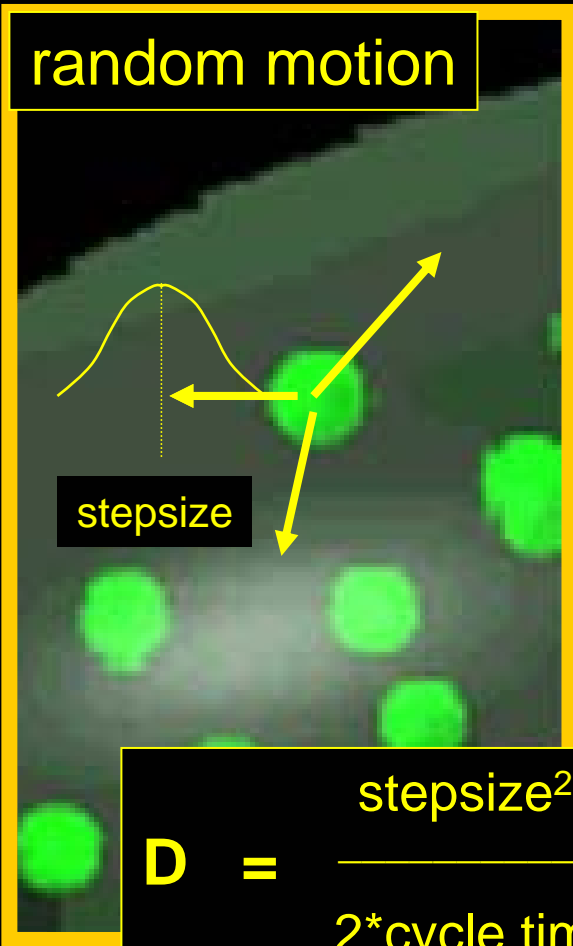




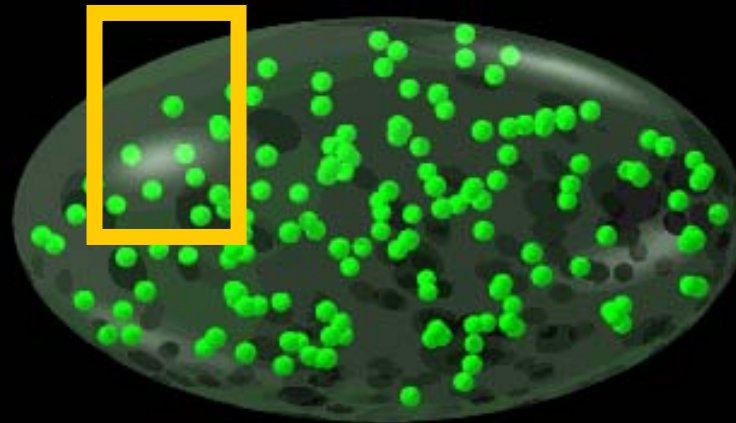


# COMPUTER SIMULATION OF FRAP ON FLUORESCENT NUCLEAR PROTEINS

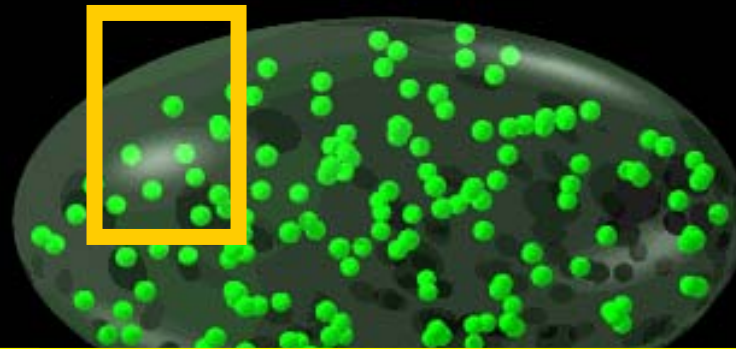
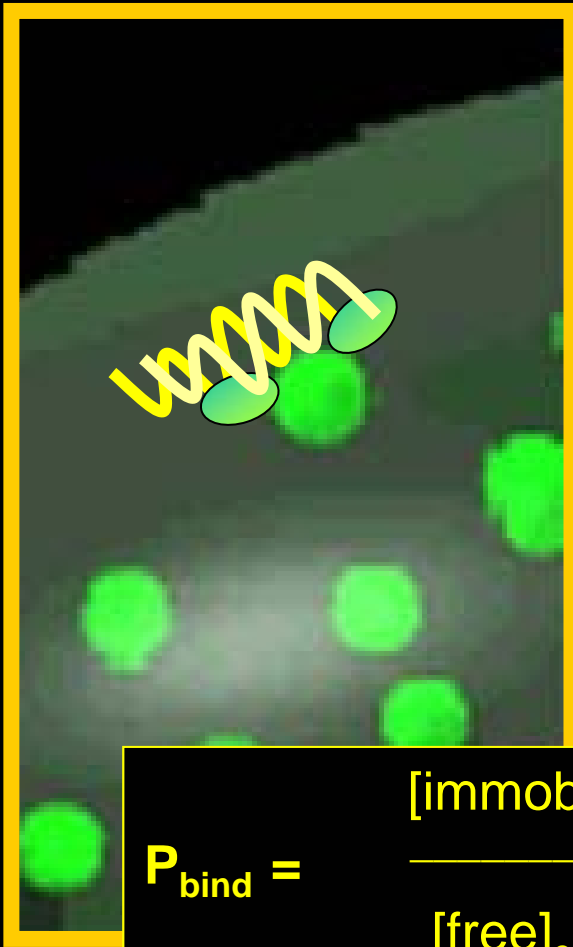
random motion



$$D = \frac{\text{stepsize}^2}{2 * \text{cycle time}}$$



# COMPUTER SIMULATION OF FRAP ON FLUORESCENT NUCLEAR PROTEINS

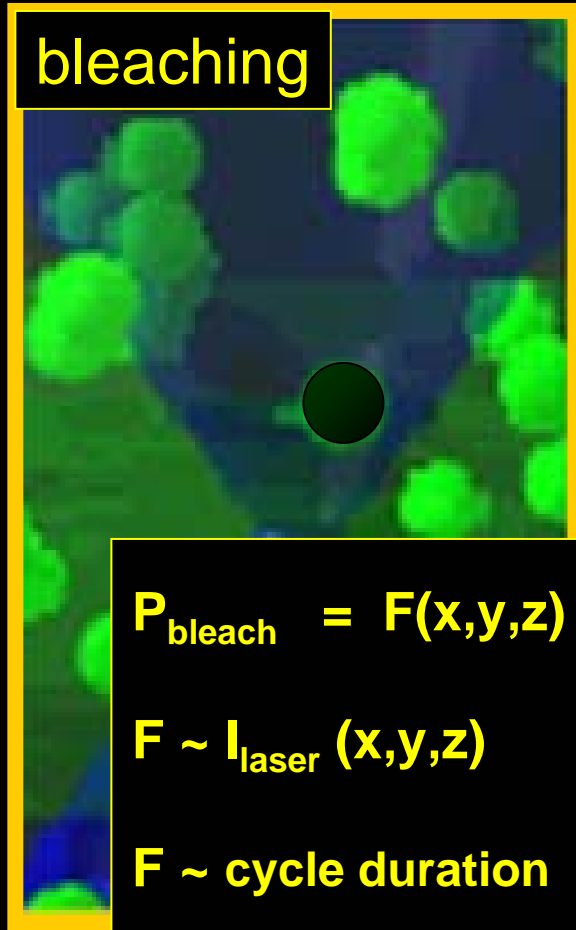


$$P_{\text{bind}} = \frac{[\text{immobile}]_{\text{eq}}}{[\text{free}]_{\text{eq}}} * \text{average immobilisation time}^{-1}$$

$$P_{\text{release}} \sim t_{\text{after binding}} \pm \sigma$$

# COMPUTER SIMULATION OF FRAP ON FLUORESCENT NUCLEAR PROTEINS

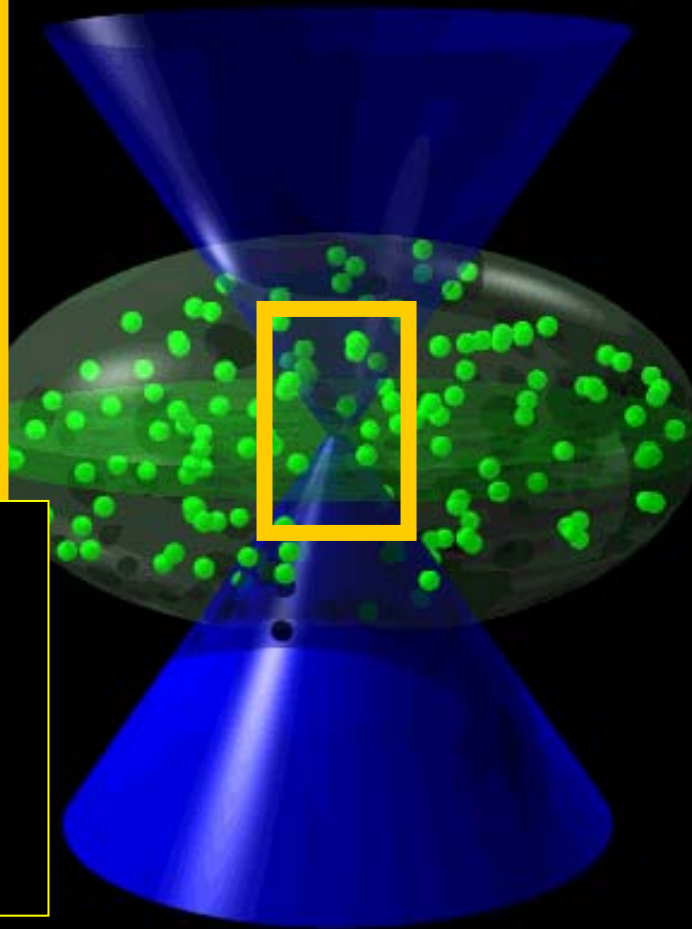
bleaching



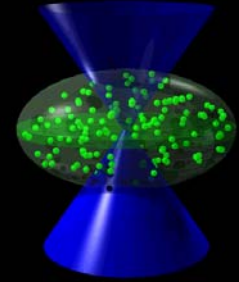
$$P_{\text{bleach}} = F(x,y,z)$$

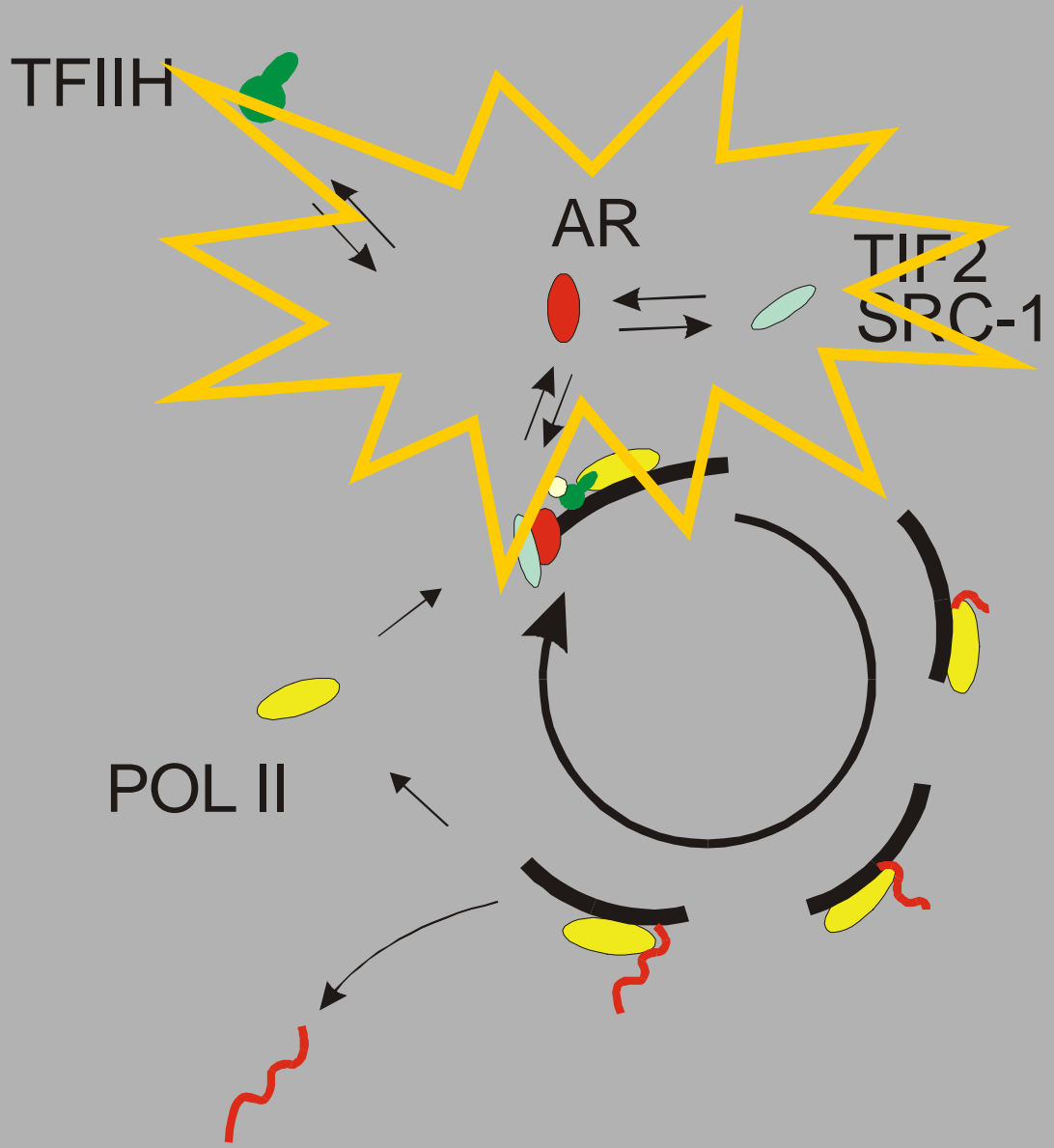
$$F \sim I_{\text{laser}}(x,y,z)$$

$$F \sim \text{cycle duration}$$



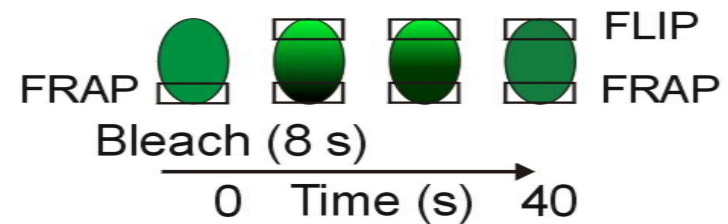
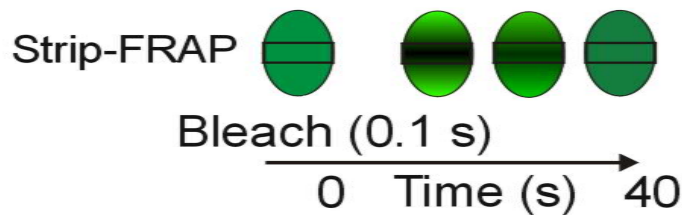
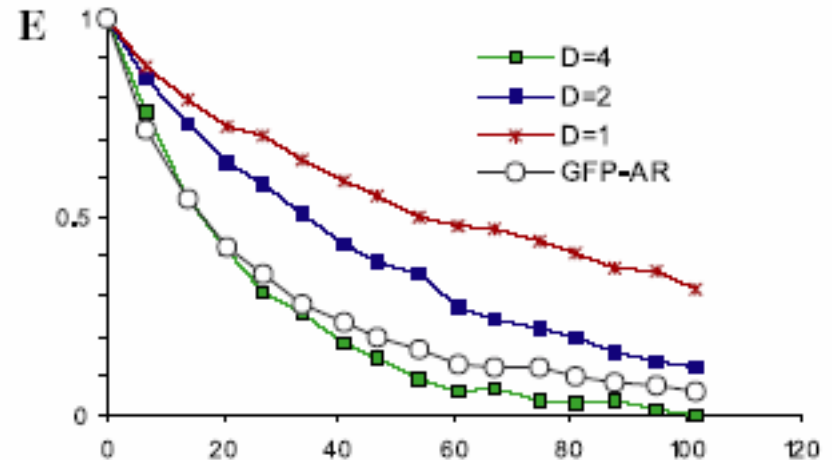
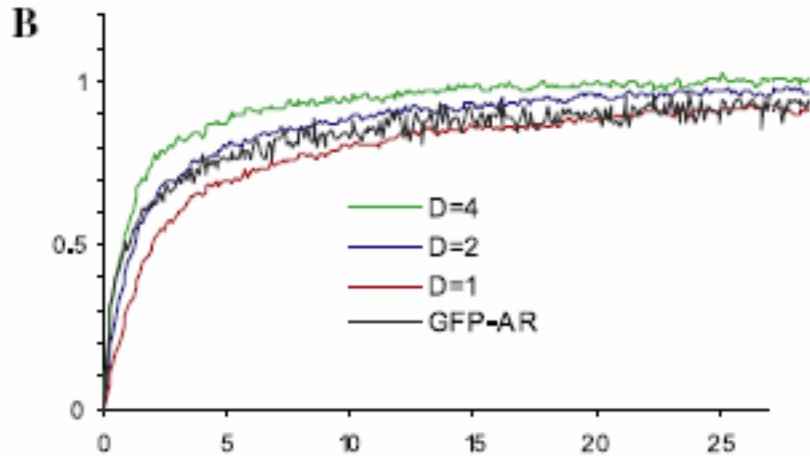
# Computer simulation DEMO



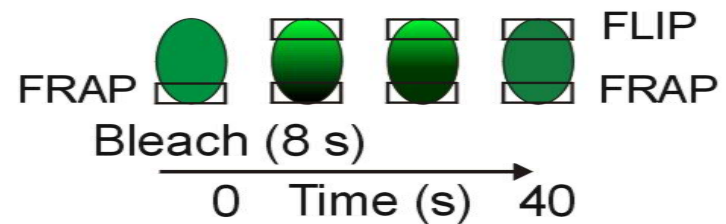
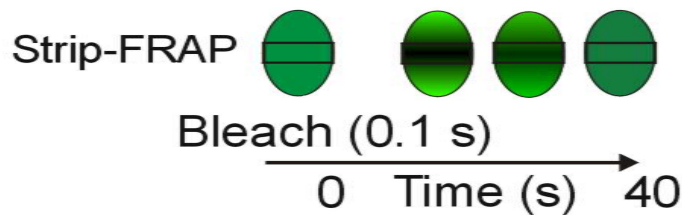
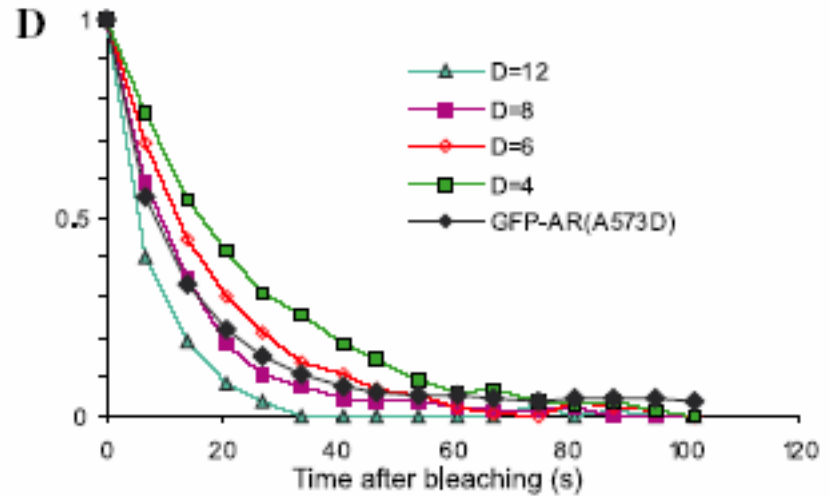
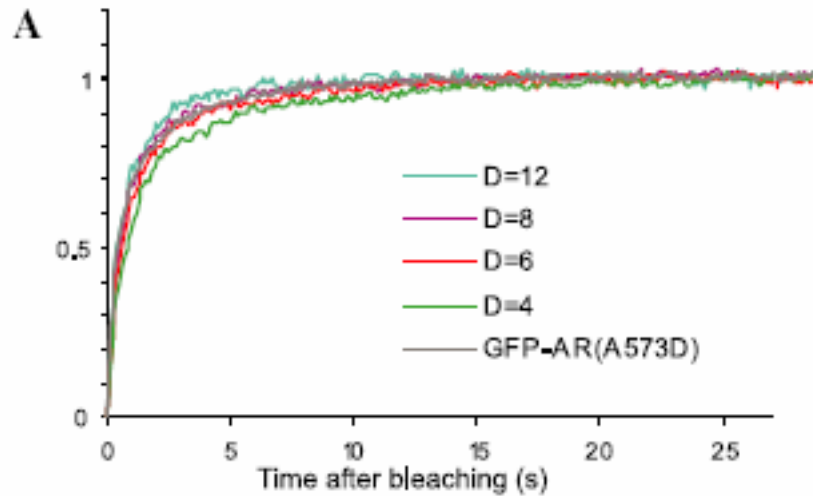


**RNA POL II transcription**

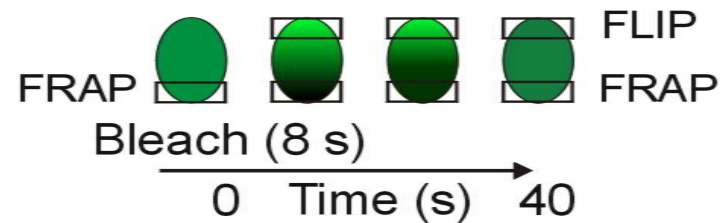
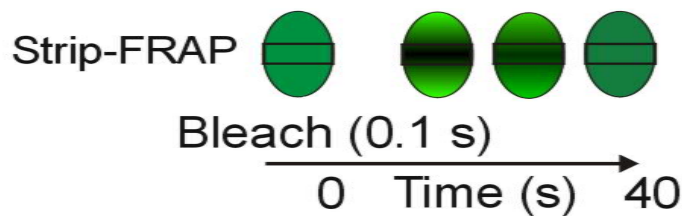
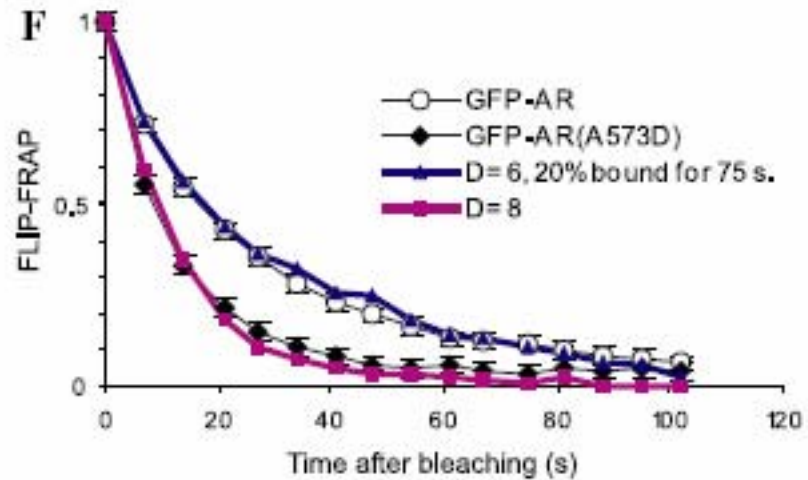
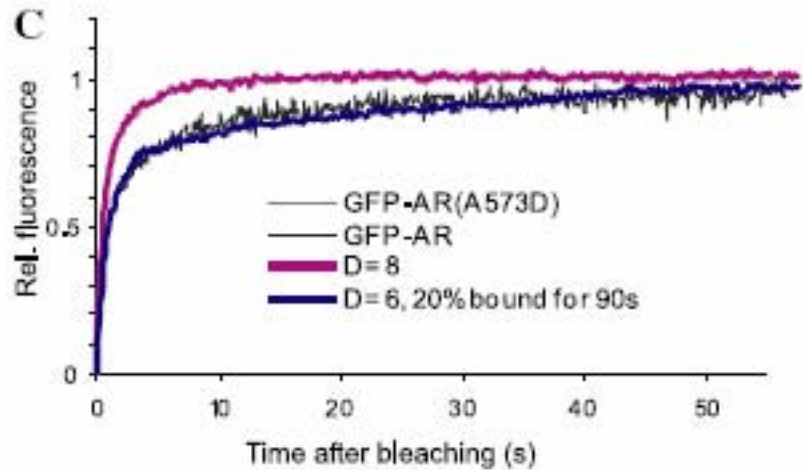
# Mobility of the Androgen Receptor (AR) studied by strip-FRAP and FLIP-FRAP

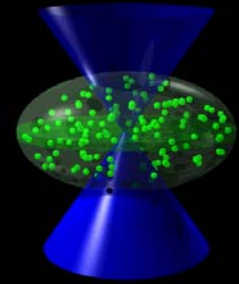


# Mobility of an Androgen Receptor mutant that cannot bind DNA

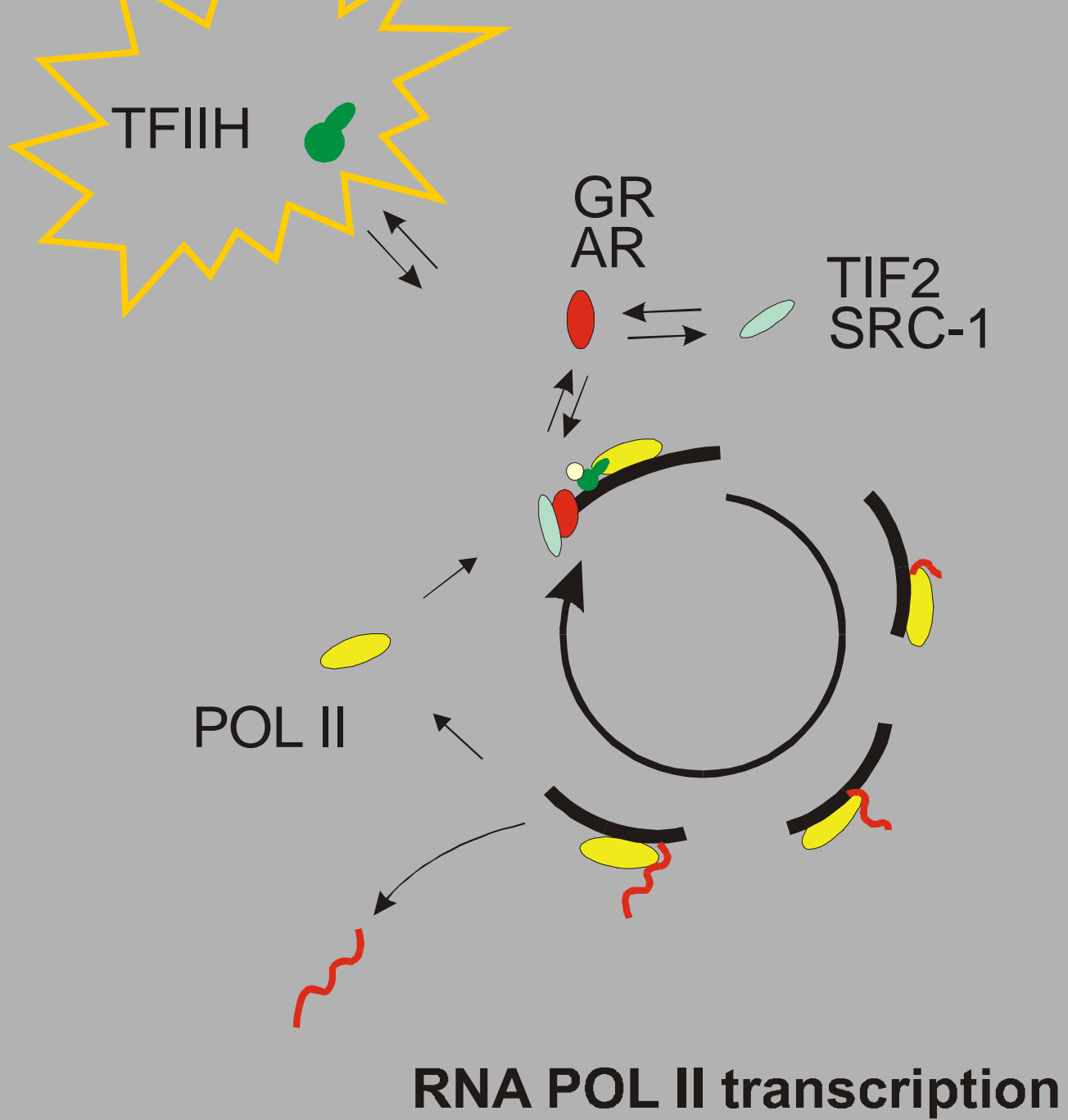


# Mobility of the Androgen Receptor (AR) and a non-DNA-binding mutant





What if immobilisation  
is too short to be distinguished  
by FRAP?



## 1. Diffusion at different temperatures:

Diffusion slows down linearly with temperature:

$$D \sim T$$

from 310 K (37 °C) to 300 K (27 °C) D will only go down ~3%

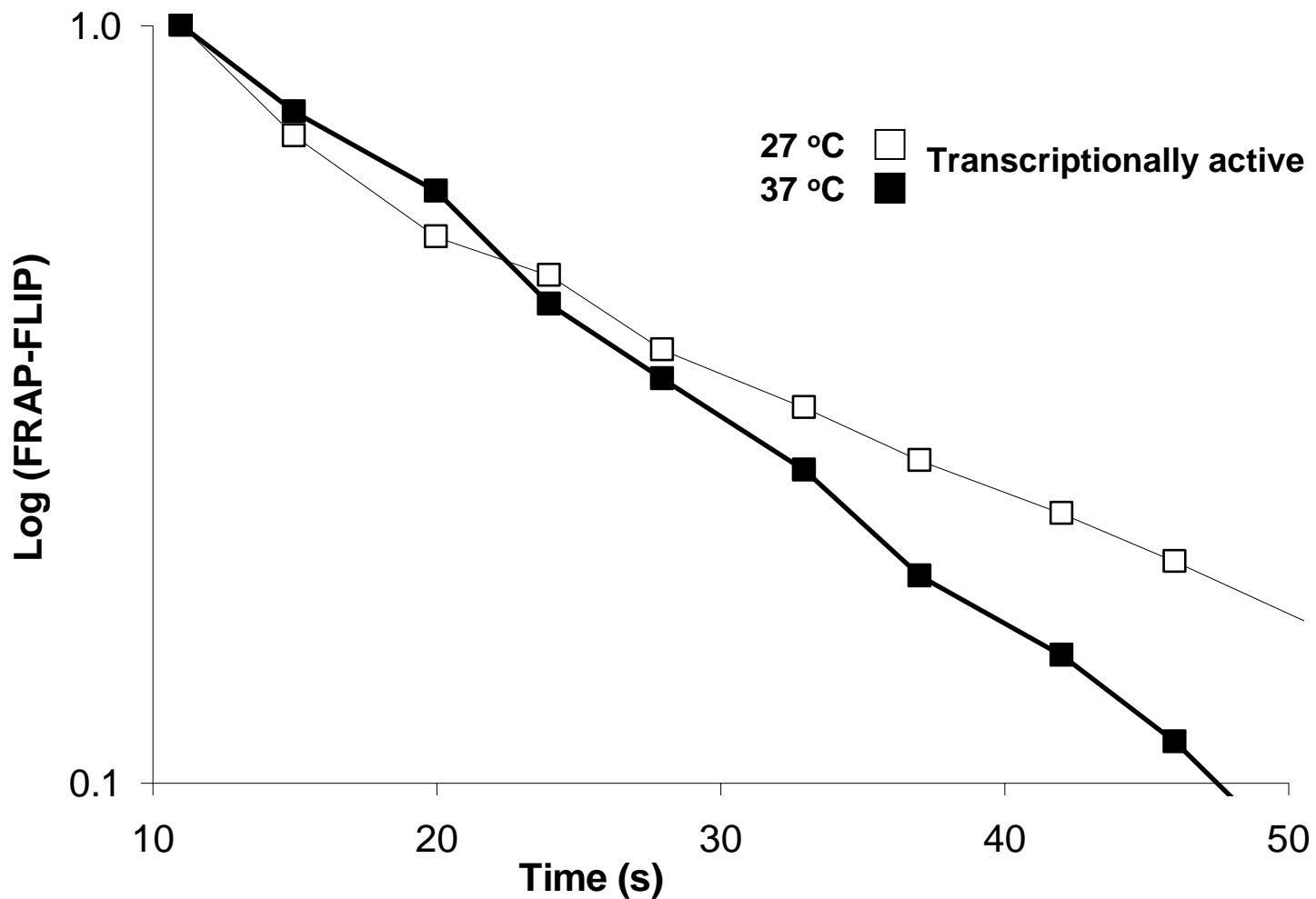
(Phair and Misteli, 2000; Politz et al, 1998)

## 2. Immobilisation time at different temperatures:

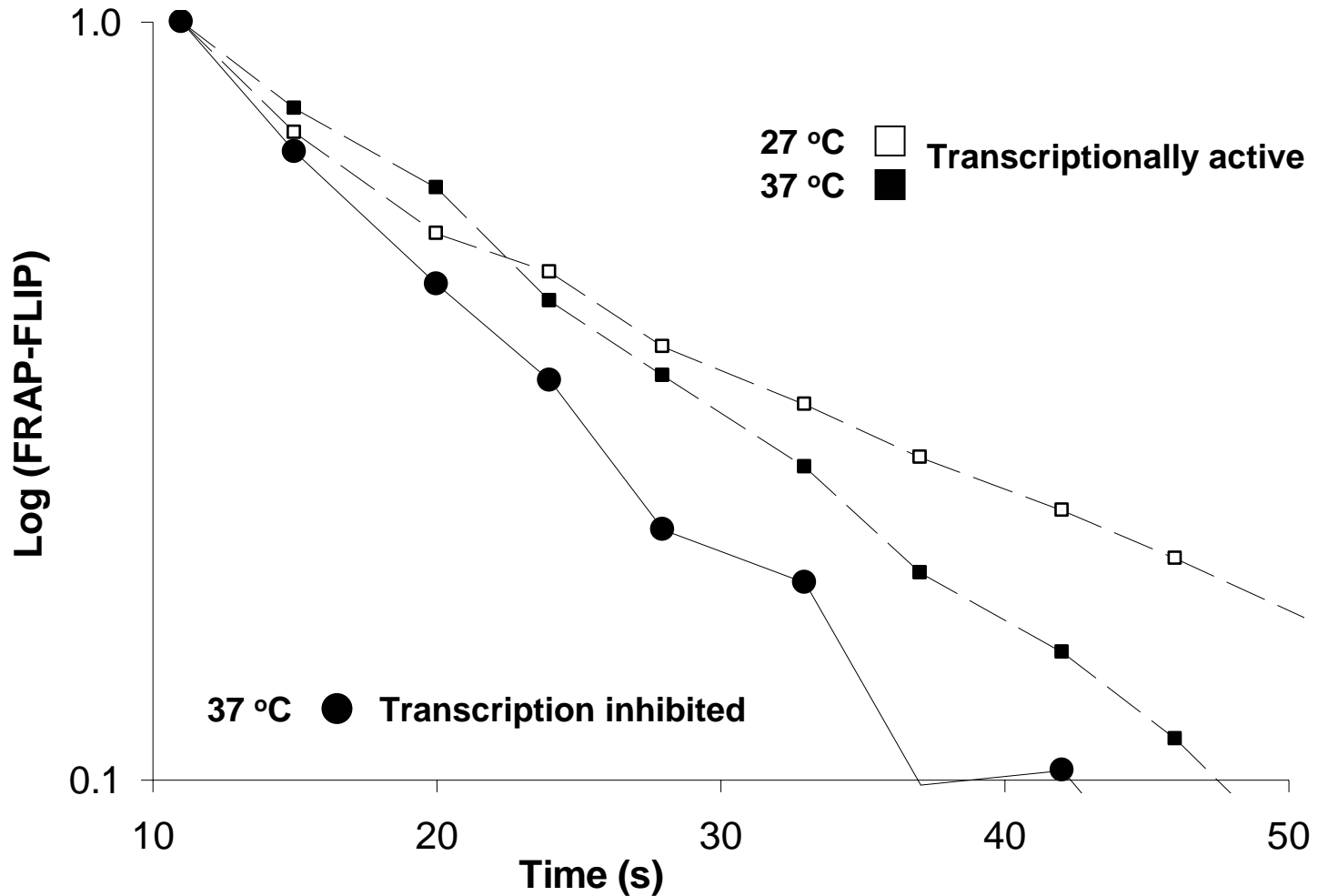
Proteins involved in a temperature dependent process will be immobilised much longer when temperature is dropped 10 degrees

-> Overall mobility of proteins will only significantly decrease with temperature when they are transiently involved in a temperature dependent process in which they are immobilised (e.g. transcription)

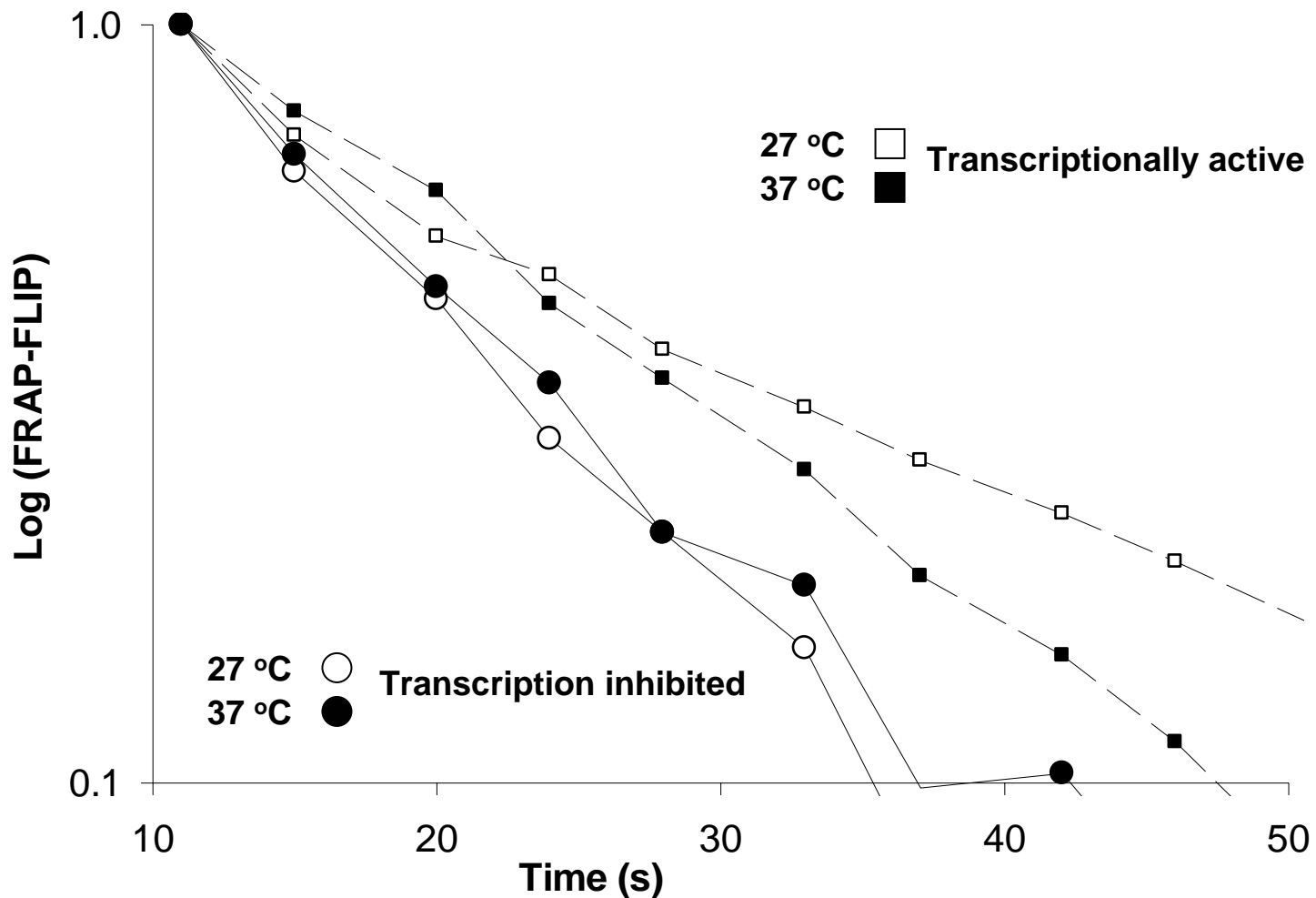
# Mobility of transcription factor TFIIH at different temperatures

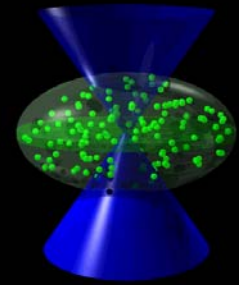


# Mobility of transcription factor TFIIH at different temperatures



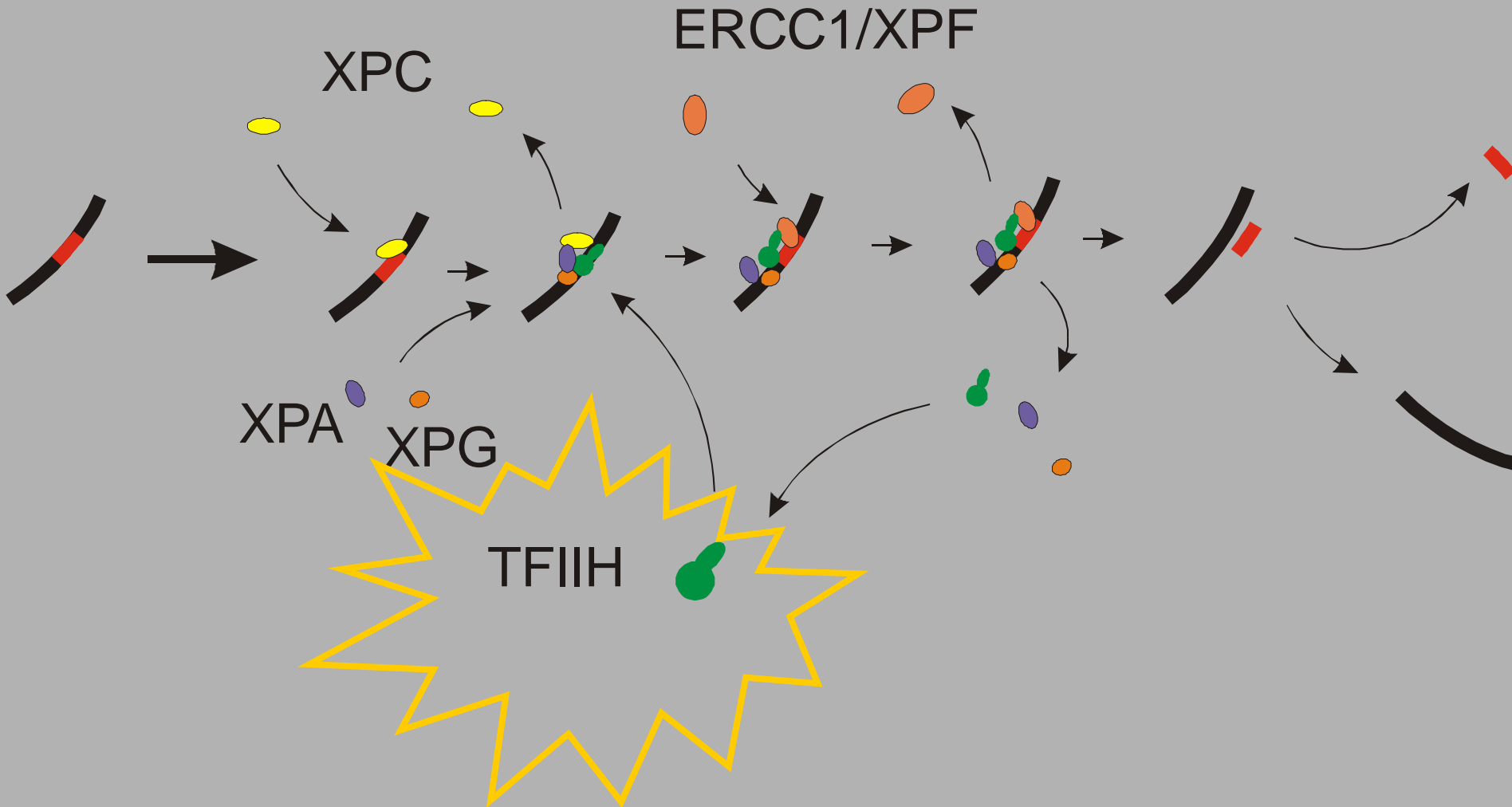
# Mobility of transcription factor TFIIH at different temperatures



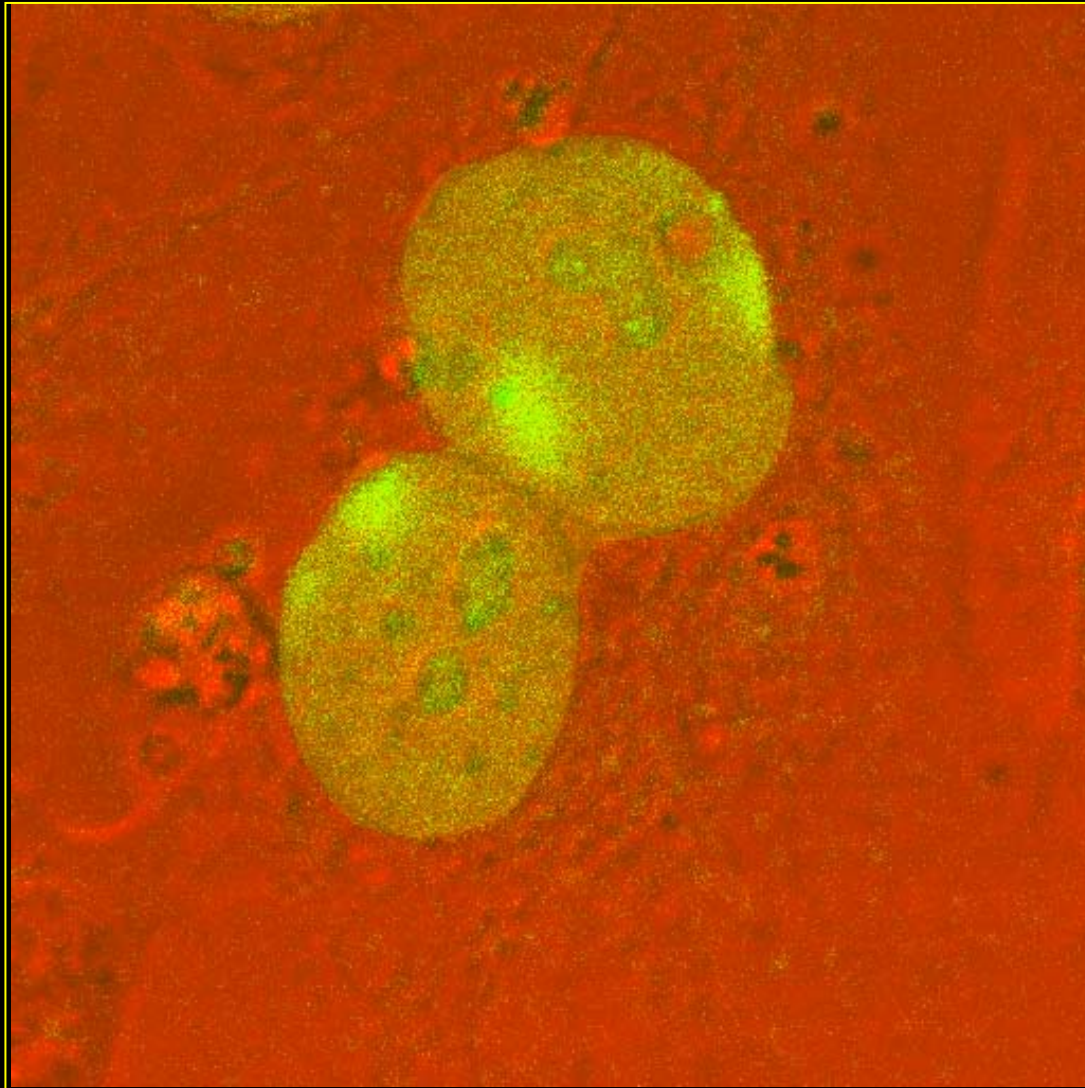


What if immobilisation  
is too long for FRAP?

# Nucleotide excision repair

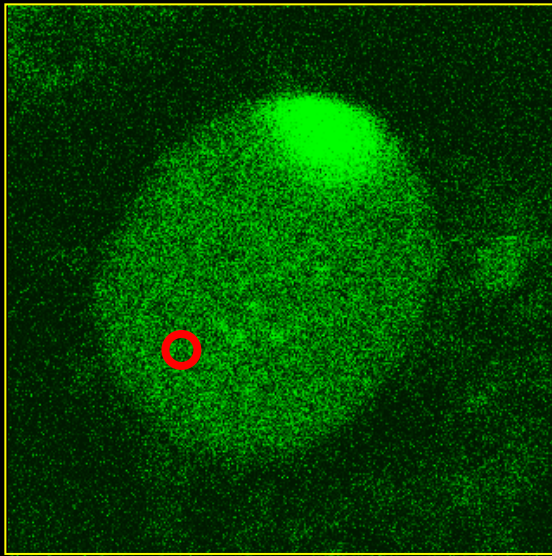


# Accumulation of TFIIH (XPB-GFP) in UV irradiated spots and in nucleoli

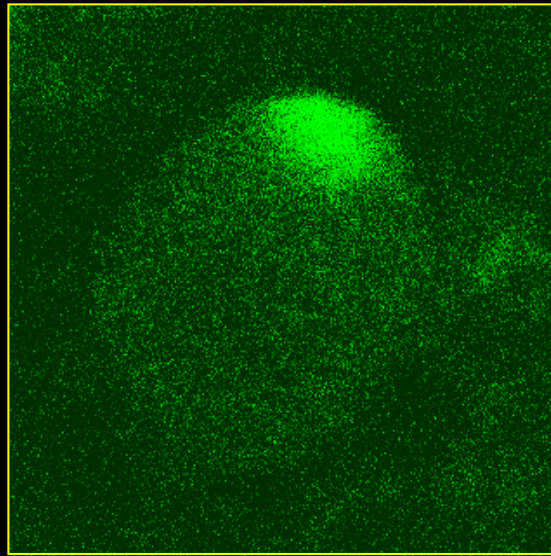


# Fluorescence loss in photobleaching (FLIP) of TFIID in a partly UV-irradiated cell

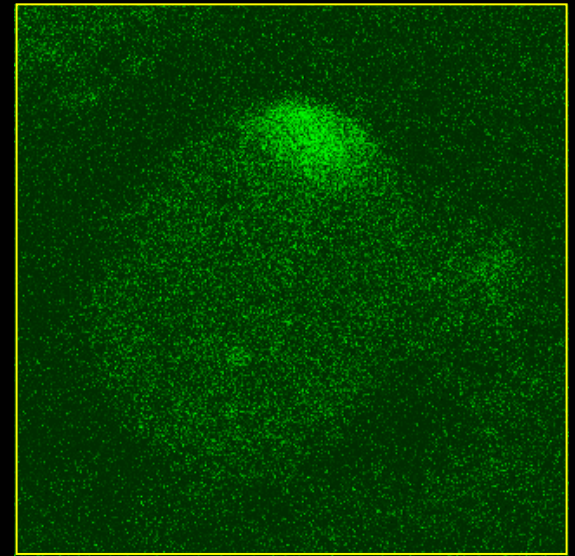
before bleach



after bleach

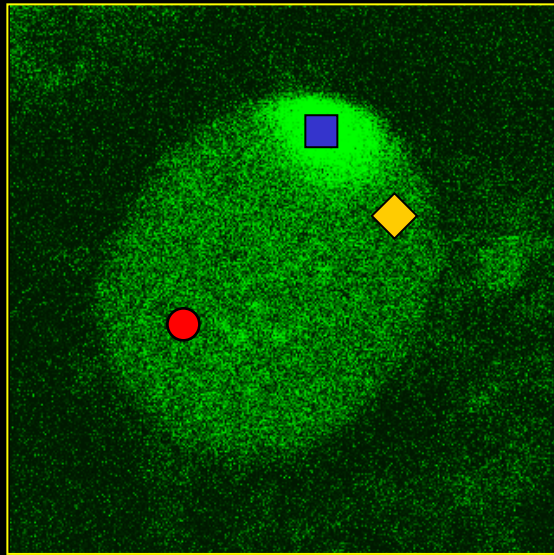


equilibrium

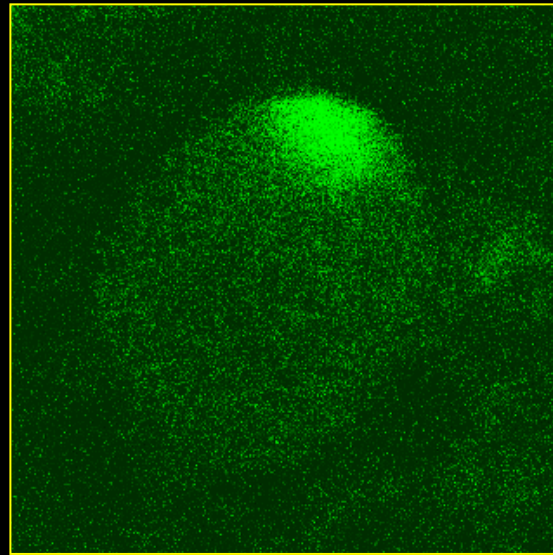


# FLIP of a cell expressing TFIIH-GFP

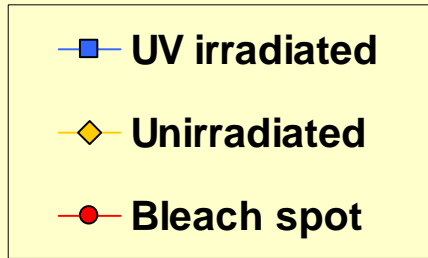
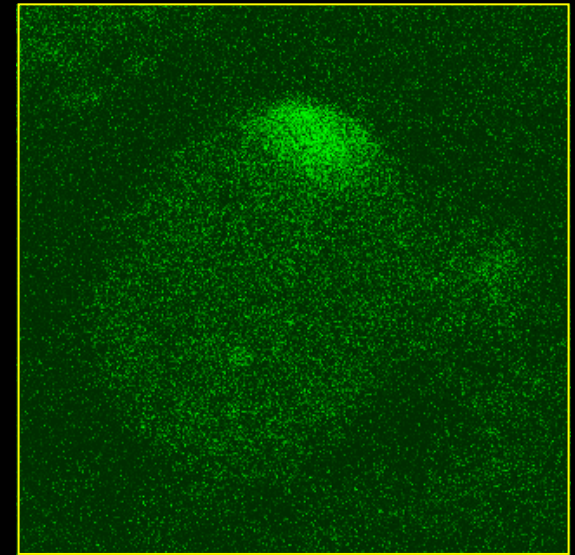
before bleach



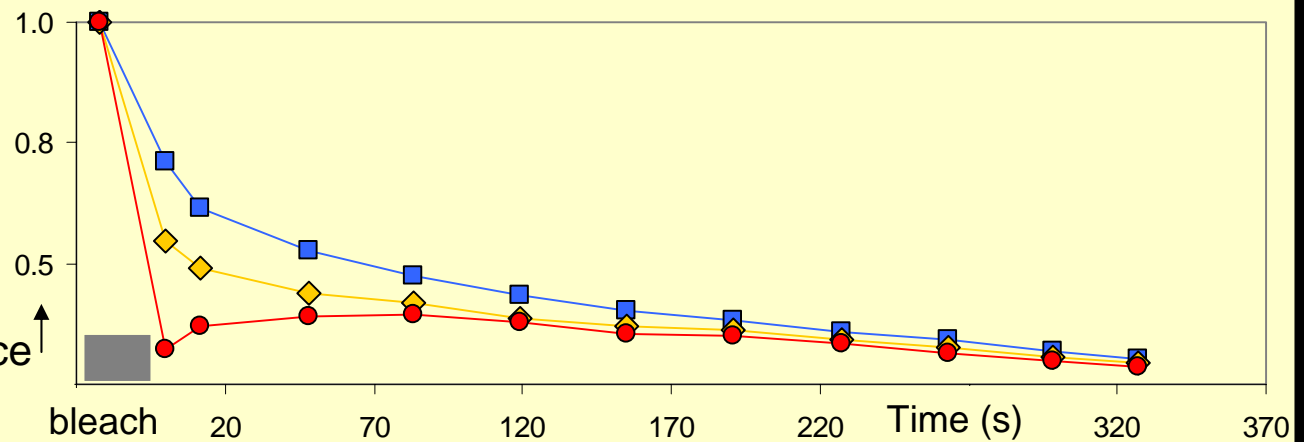
after bleach



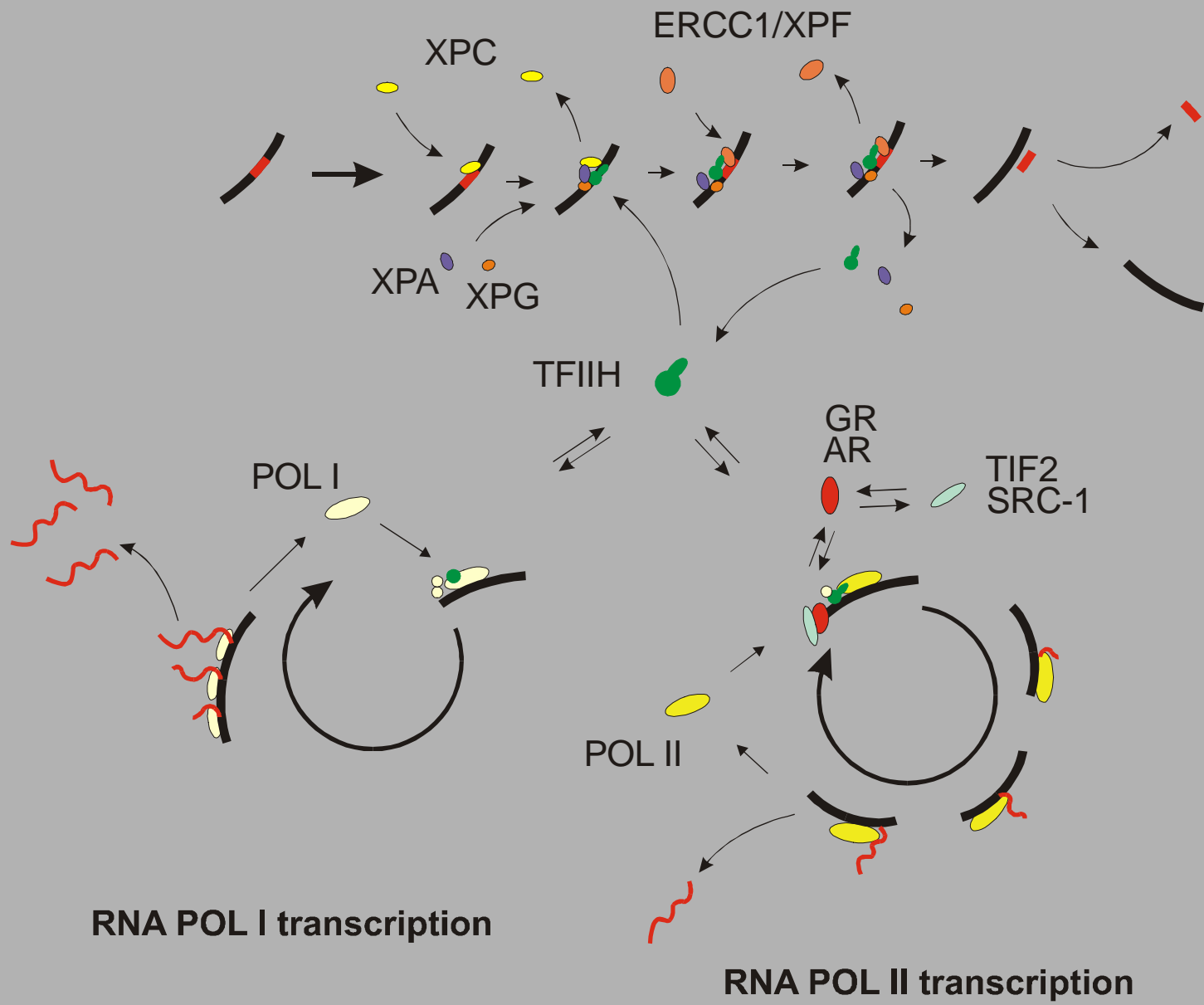
equilibrium (300 s)



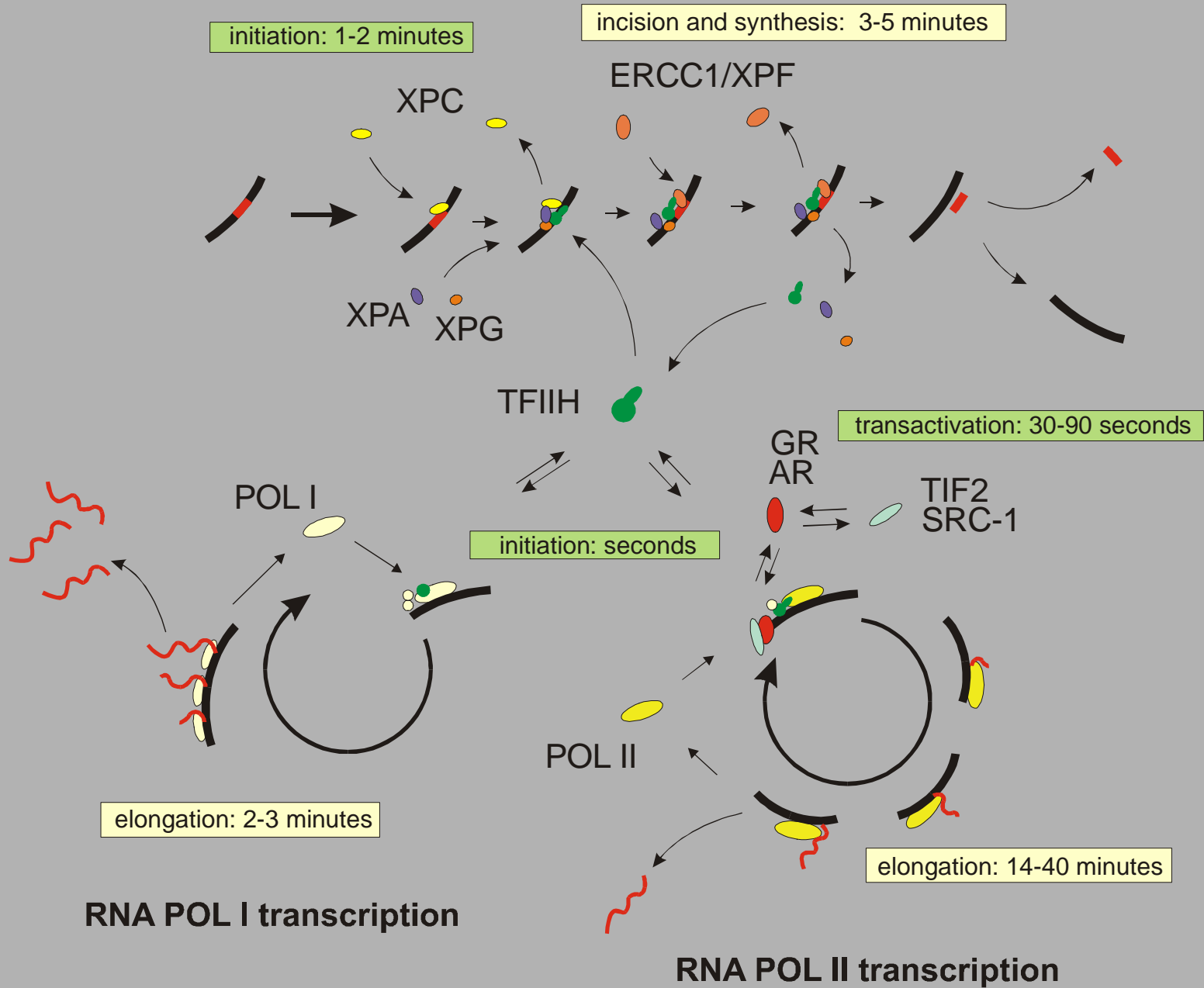
relative fluorescence  
( $I_t/I_0$ )



# Nucleotide excision repair



# Nucleotide excision repair



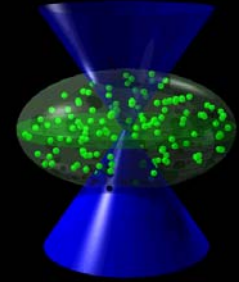
- Repair and transcription factors move freely through the nucleus and have access to most regions of the nucleus
- Interaction of repair and transcription factors with DNA is dynamic allowing a rapid response to changing conditions; e.g. TFIIH can readily switch between transcription and repair
- Repair complexes are more stable than transcription initiation complexes

- **TRANSCRIPTION**

- transactivation by steroid receptors: 30-90 seconds (~20%)
- initiation by TFIIH: 2-10 seconds (~30-60%)
- elongation: 10-60 minutes

- **DNA REPAIR**

- initiation: 2-3 minutes (~40%)
- excision and synthesis: 3-5 minutes (~30%)



## • FRAP and FLIP

- are well suited to determine 'mobility parameters':

- diffusion coefficient
- immobile fraction
- average duration of immobilisation

- Application is especially powerful in inducible systems like DNA repair and steroid receptor transcription initiation

## • Computer simulation

- -understand and develop FRAP and FLIP assays
- estimation of 'mobility parameters'
- simulate cellular processes and develop new assays to verify/falsify simulated models