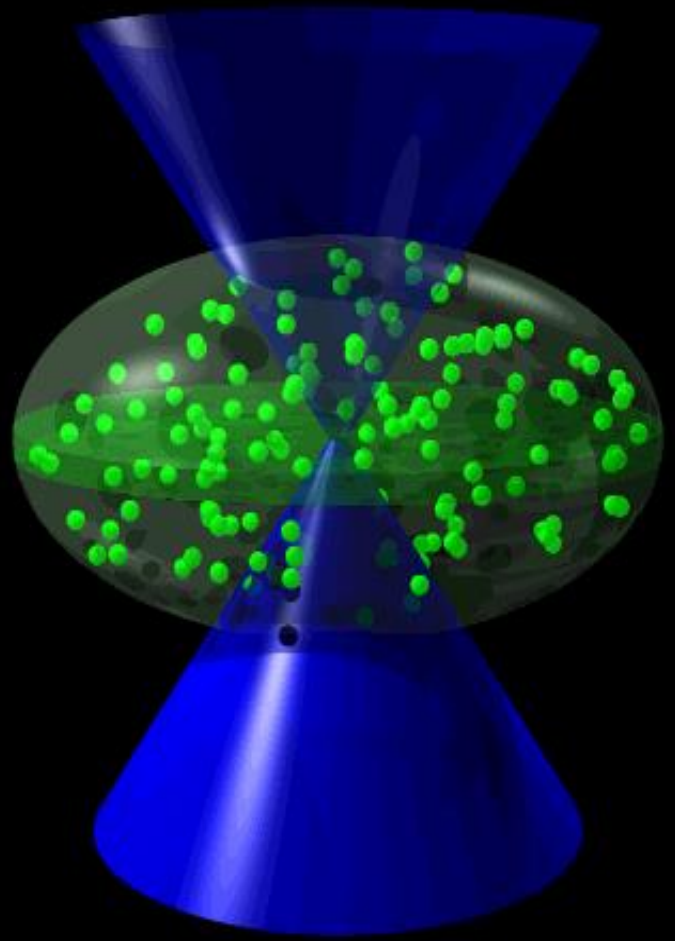
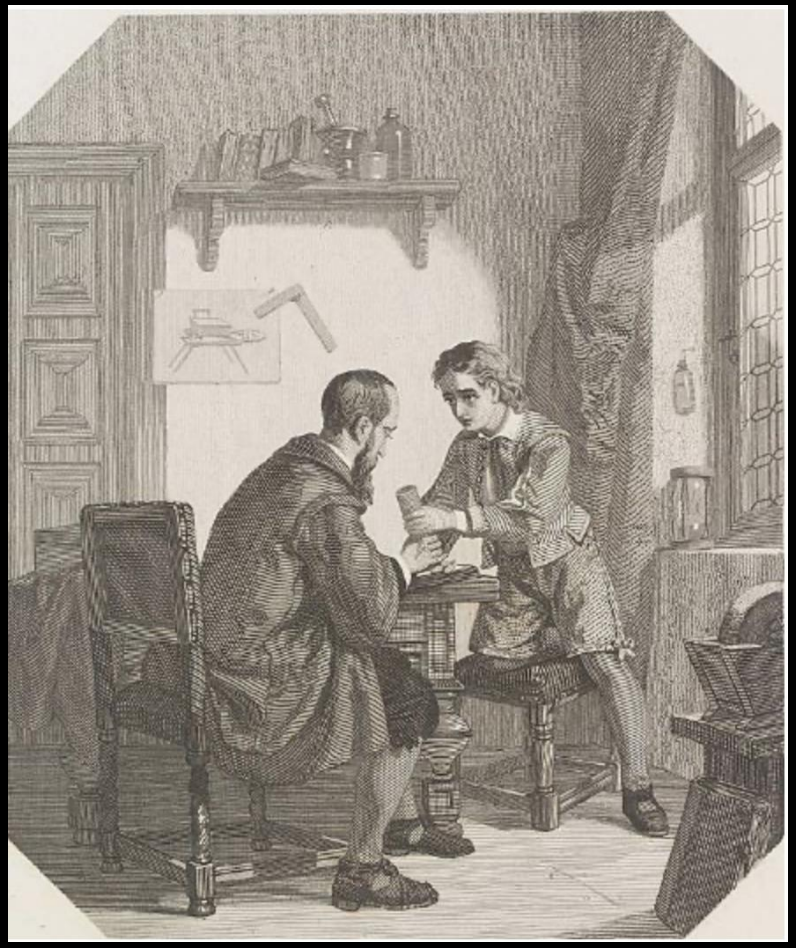
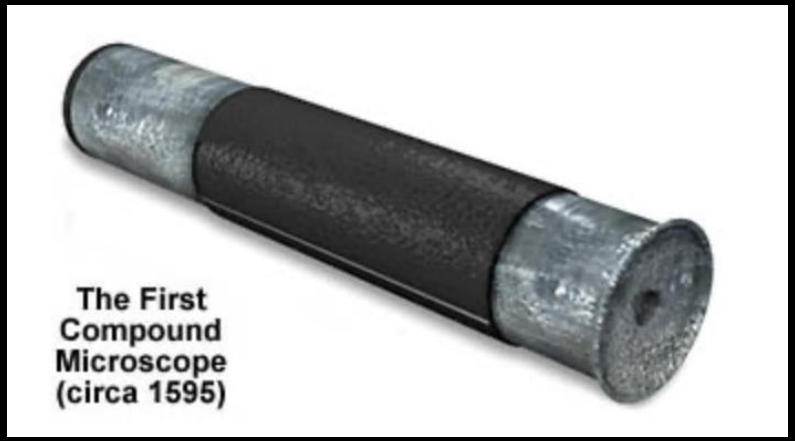
Fluorescence Recovery After Photo Bleaching

Adriaan Houtsmuller
Erasmus MC Rotterdam





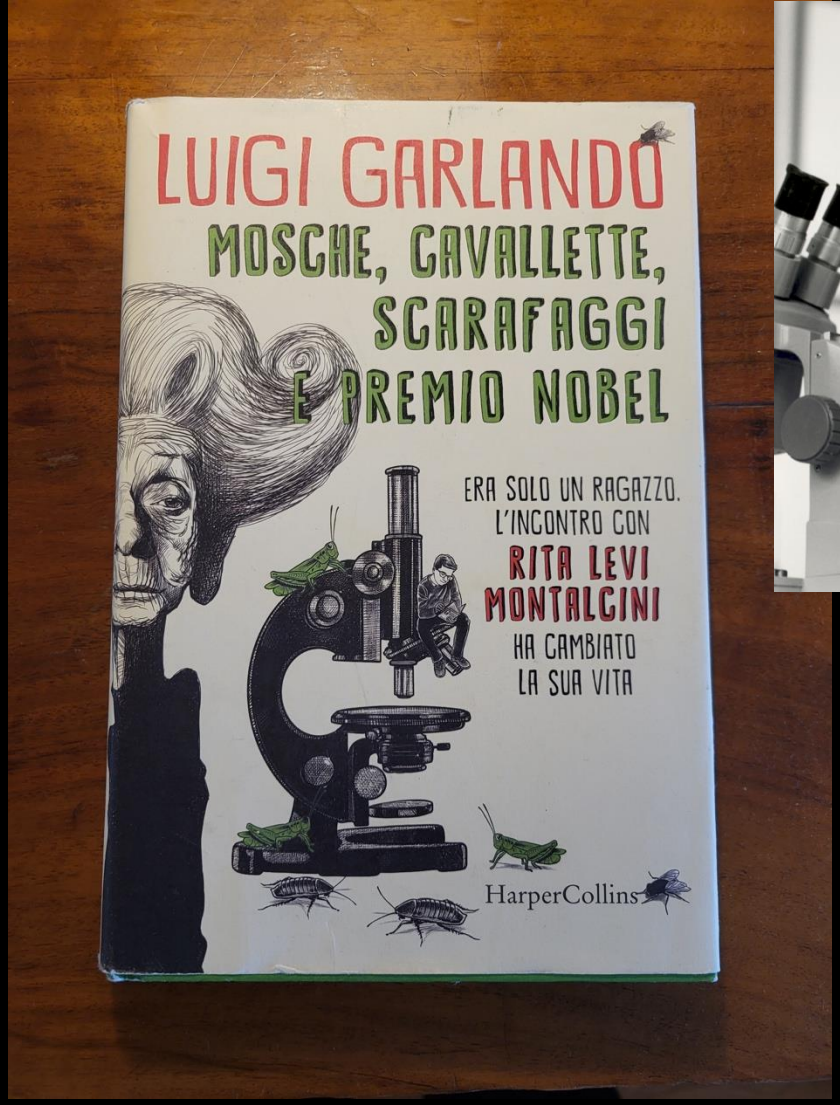


Zacharias Jansen (right, 1585-1632)





Antoni van Leeuwenhoek (1632-1723)



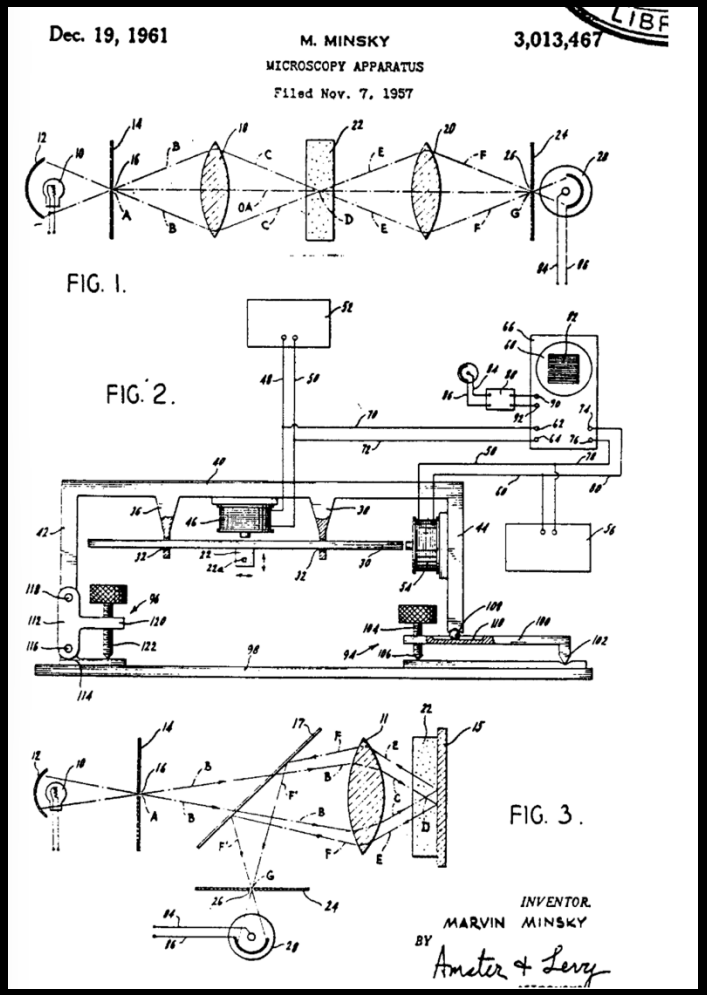




Rita Levi Montalcini (1909-2012)



Marvin Minsky (1927-2016)



Dec. 19, 1961 M. MINSKY 3,013,467

MICROSCOPY APPARATUS

Filed Nov. 7, 1957

FIG. 1.

FIG. 2.

FIG. 3.

INVENTOR.
MARVIN MINSKY
BY Ames & Levy

Ultimate machine (Marvin Minsky)



FRAP will be explained on the basis of application to two model systems

DNA Damage Repair

A large number of DNA repair proteins
exist that repair UV induced
DNA damage

XPC detects the damage

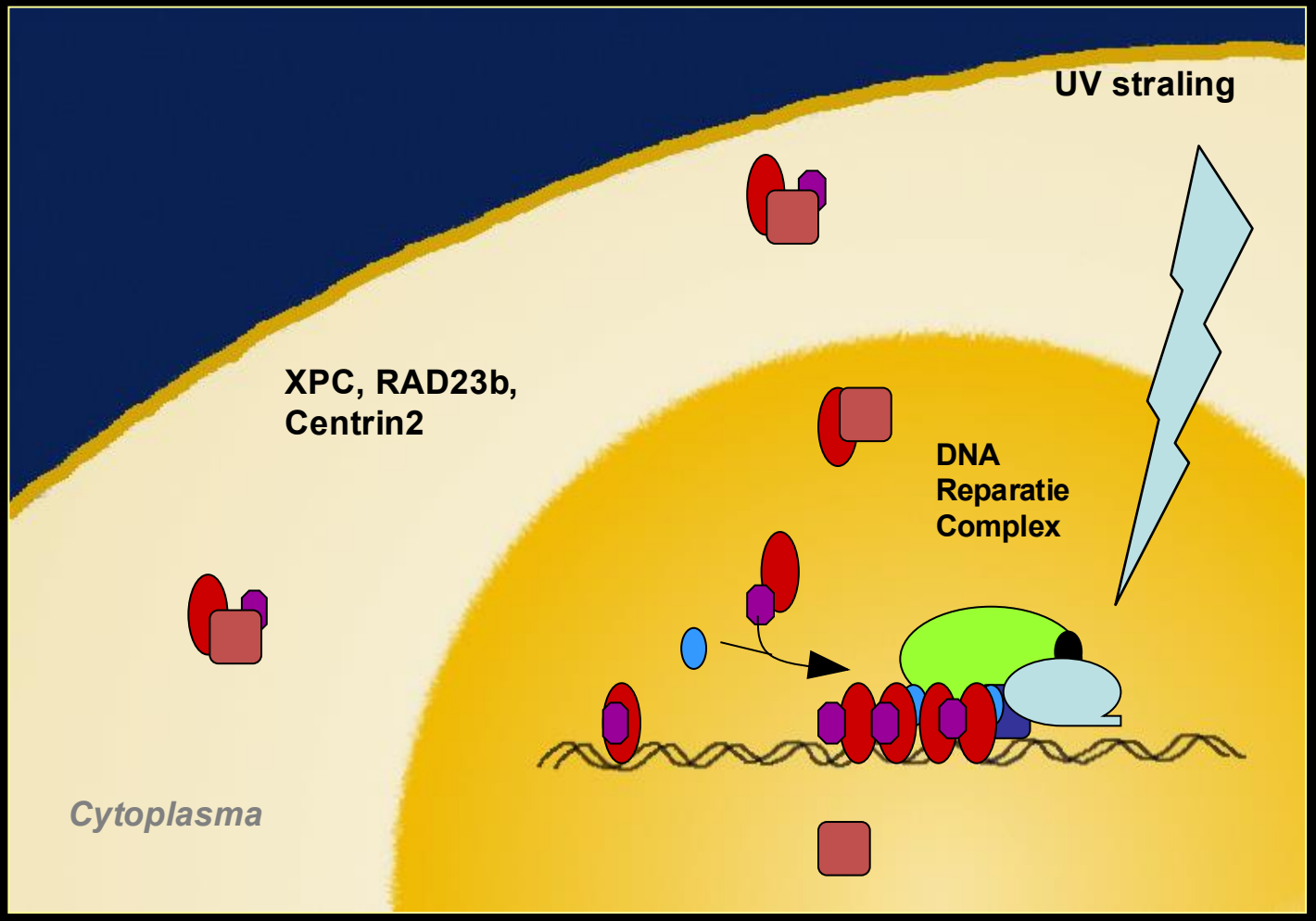
Transcription Regulation

genes involved in development and maintenance
of the male phenotype are
regulated by

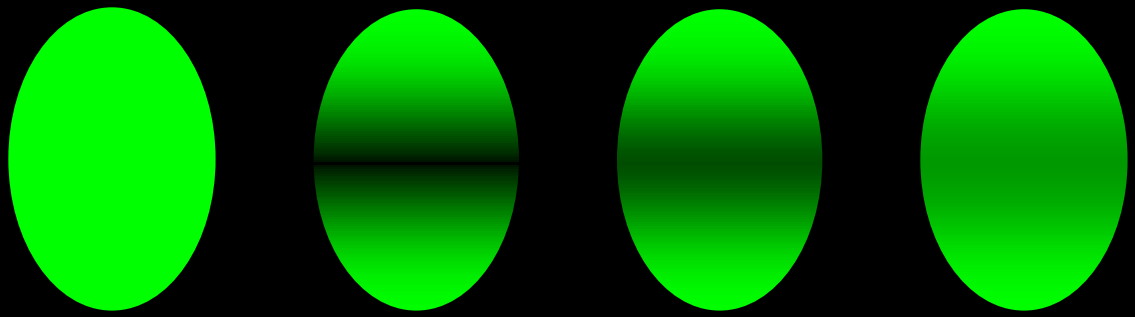
Androgen Receptor detects promoter sequences

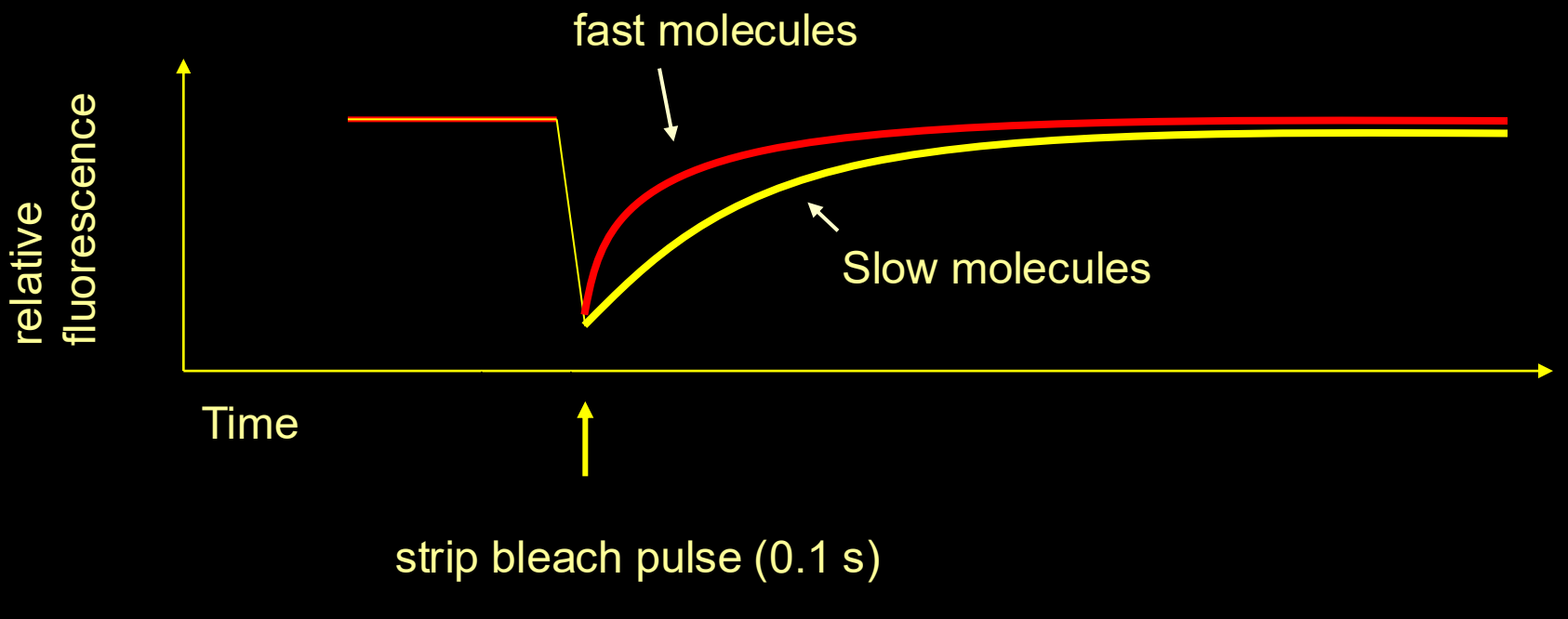
XPC and AR have in common that these are the ones to bind first to DNA to initiate protein complex formation

UV-induced DNA Damage

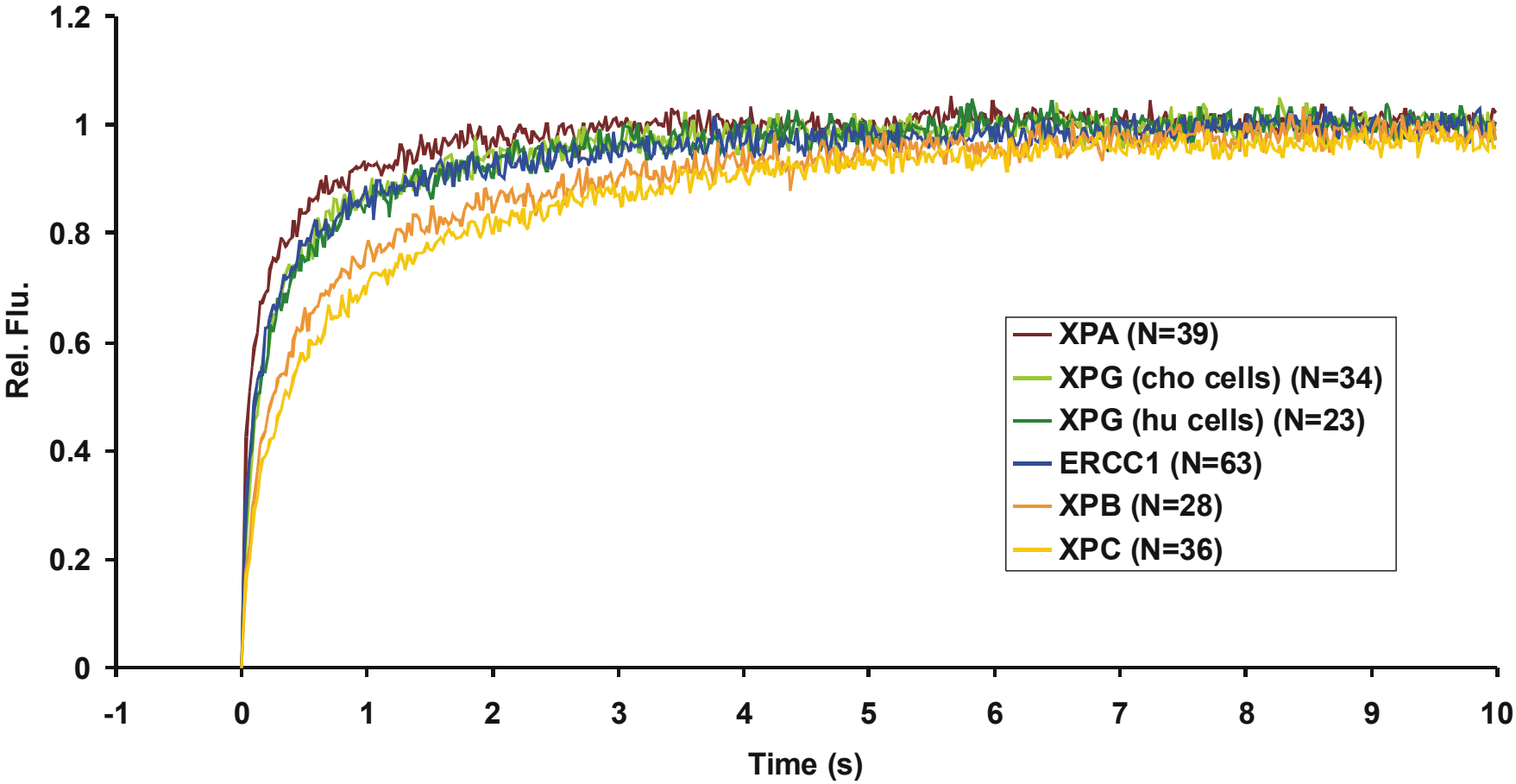


Fluorescence Recovery After Photobleaching FRAP



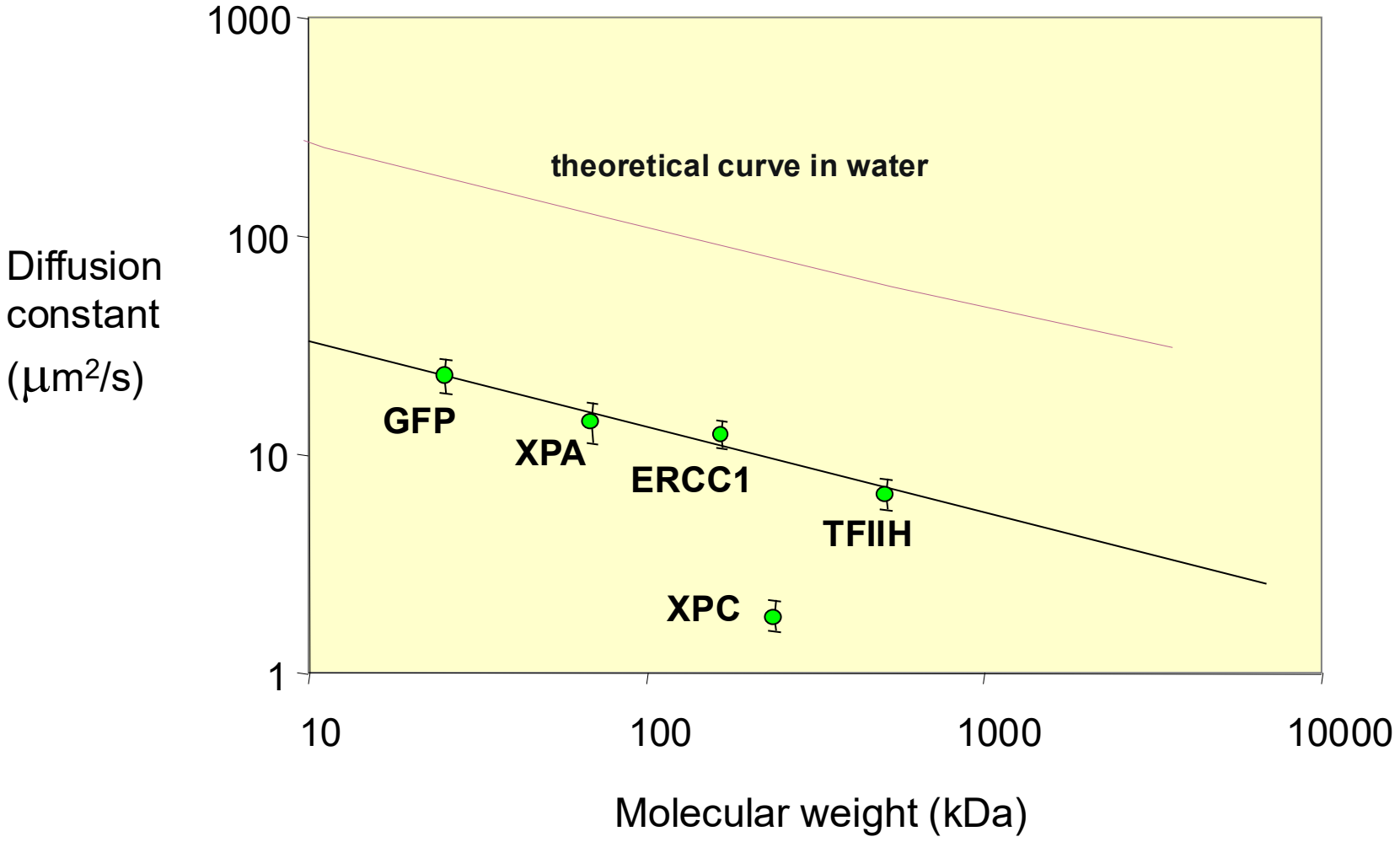


FRAP applied to inactive DNA-repair proteins

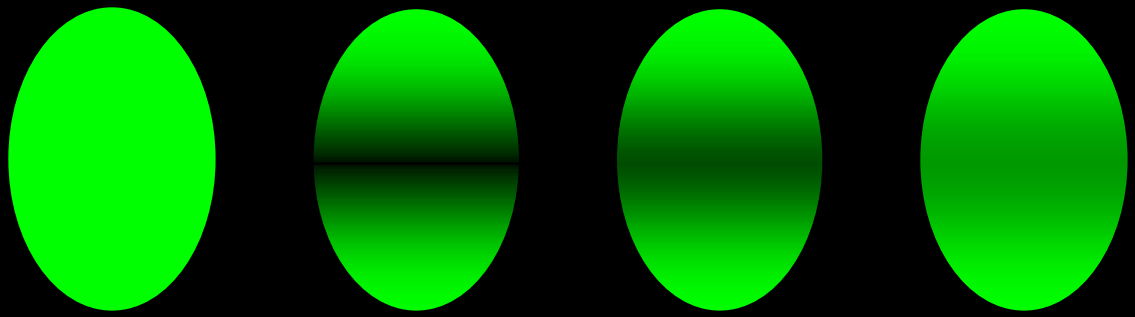


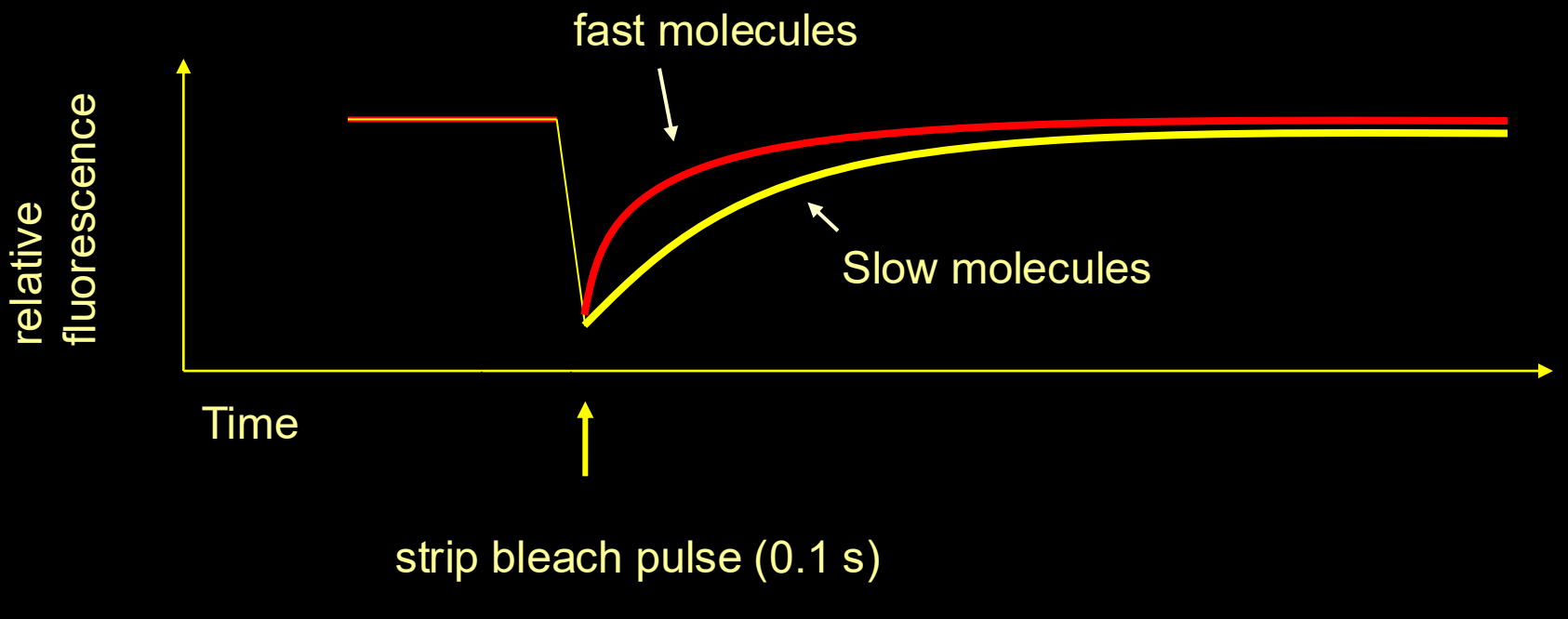
Houtsmuller et al., Science, 1999
Hoogstraten et al., Mol Cell 2002
van den Boom et al., J Cell Biol 2004
Zotter et al., J Cell Sci., 2007
Van Royen et al., Chromosome Res, 2010
Bergink et al., J Cell Bio2012
Van Cuijk et al., Nat Communications, 2015
Steurer et al., PNAS, 2018

Relationship between diffusion and molecular weight

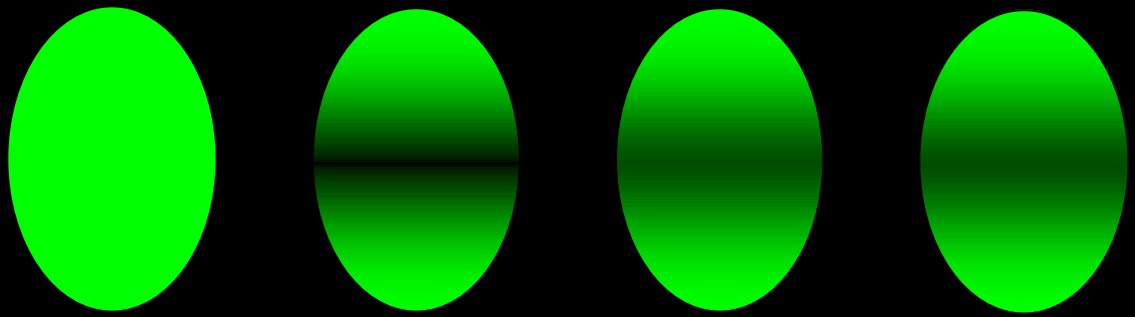


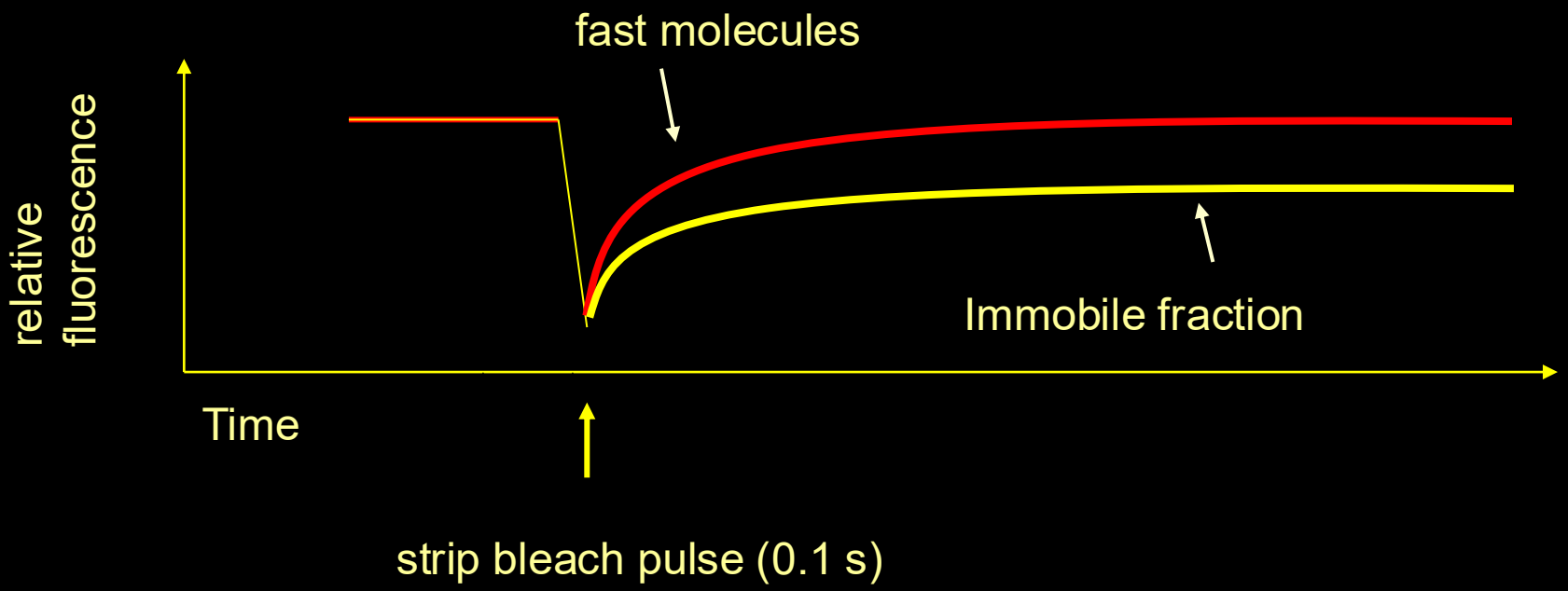
Fluorescence Recovery After Photobleaching FRAP



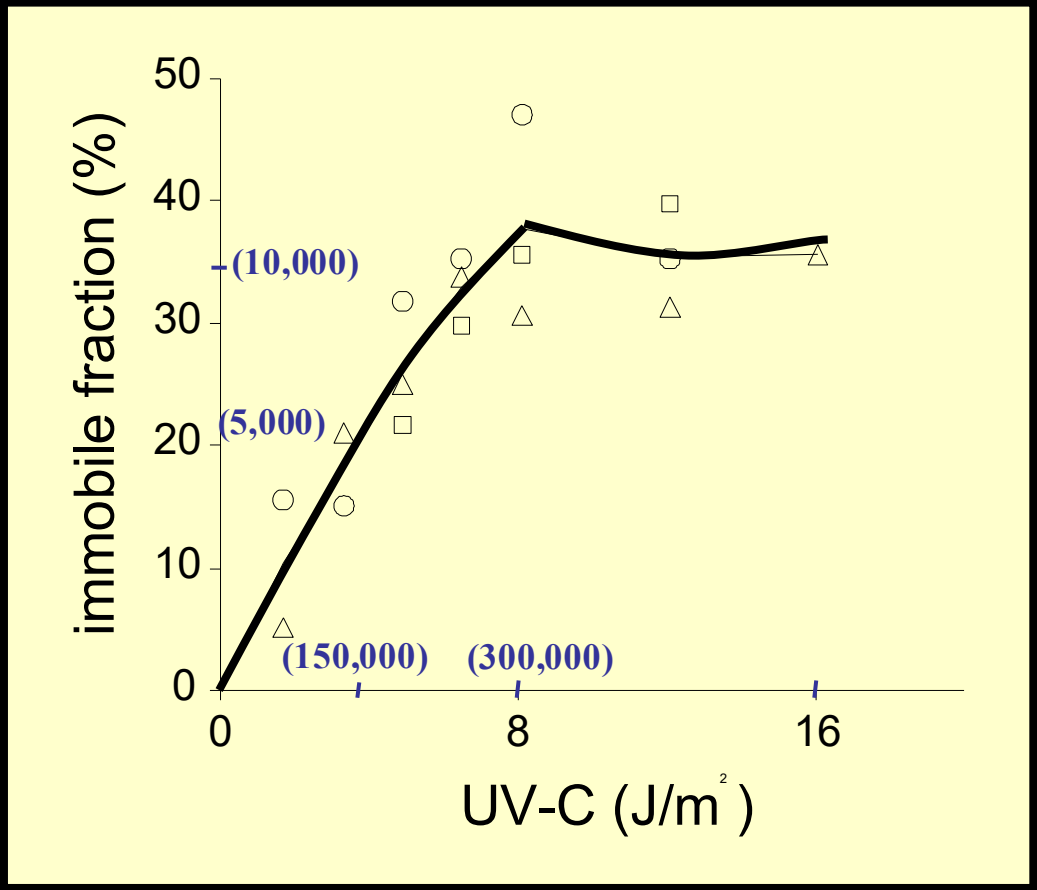


FRAP



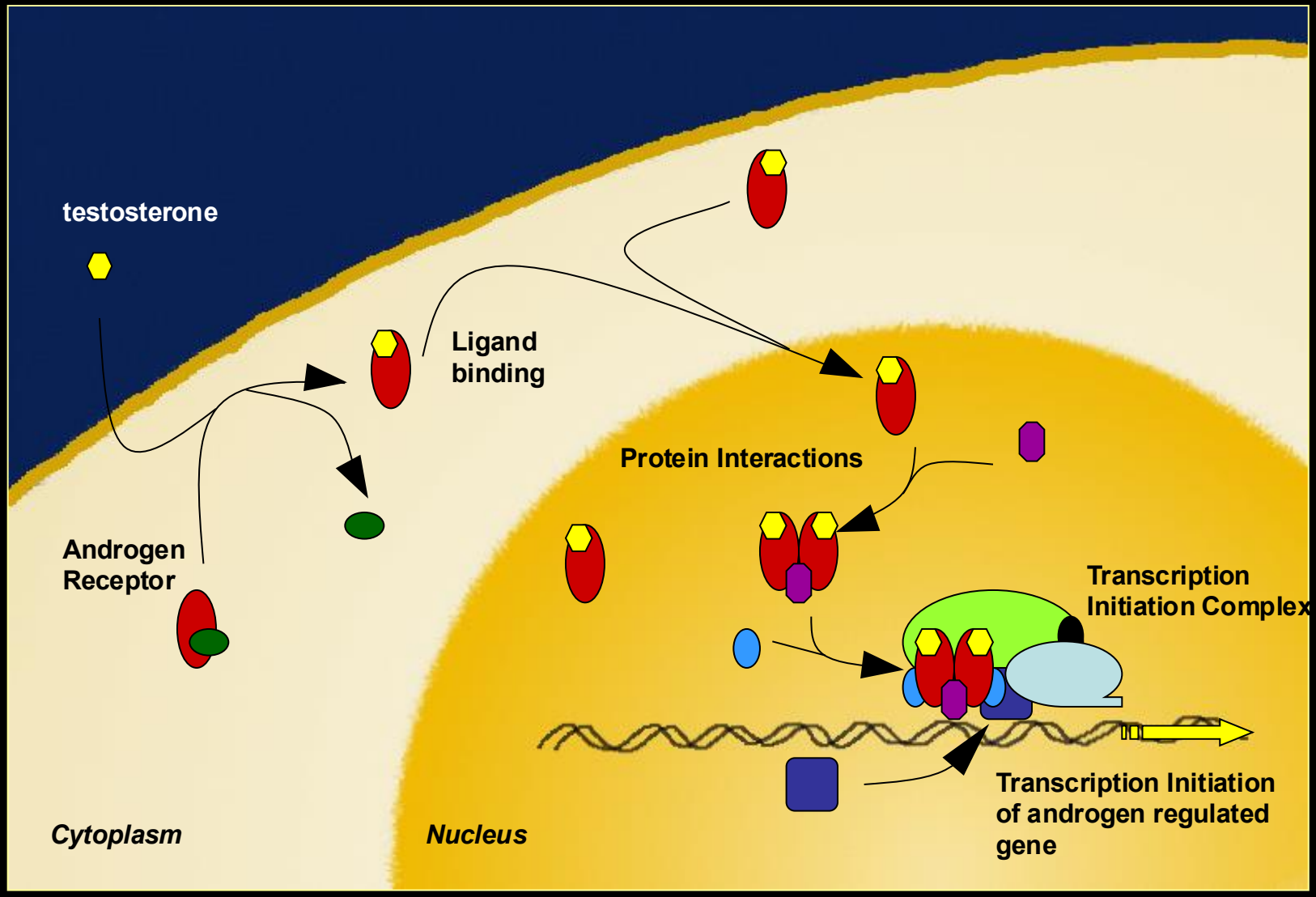

fast molecules
relative fluorescence
Immobile fraction
Time
strip bleach pulse (0.1 s)

Immobilisation of UV damage repair factor ERCC1-GFP depends on UV-dose



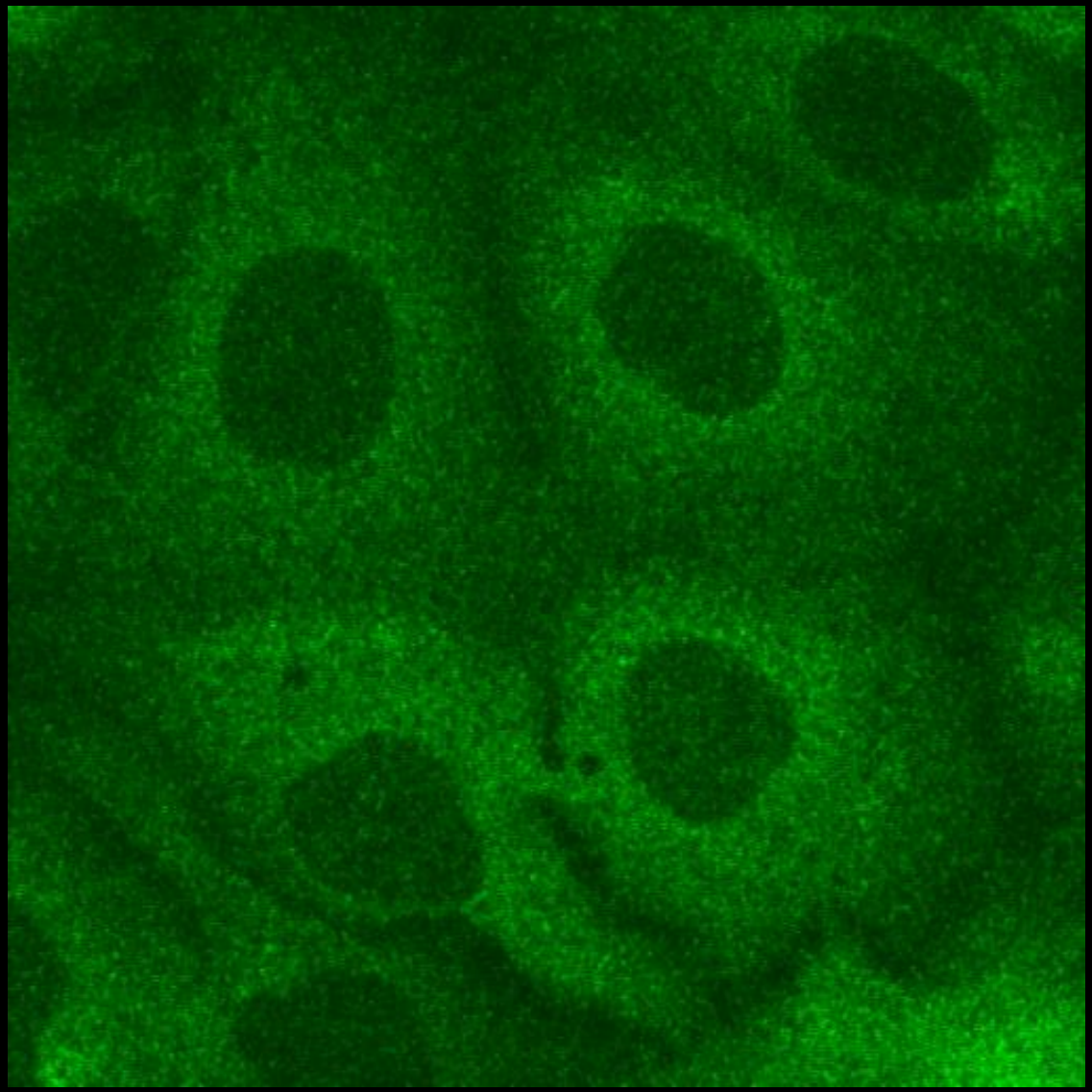
Transcription regulation

the *Androgen Receptor* is a protein that activates genes involved in development and maintenance of the male phenotype

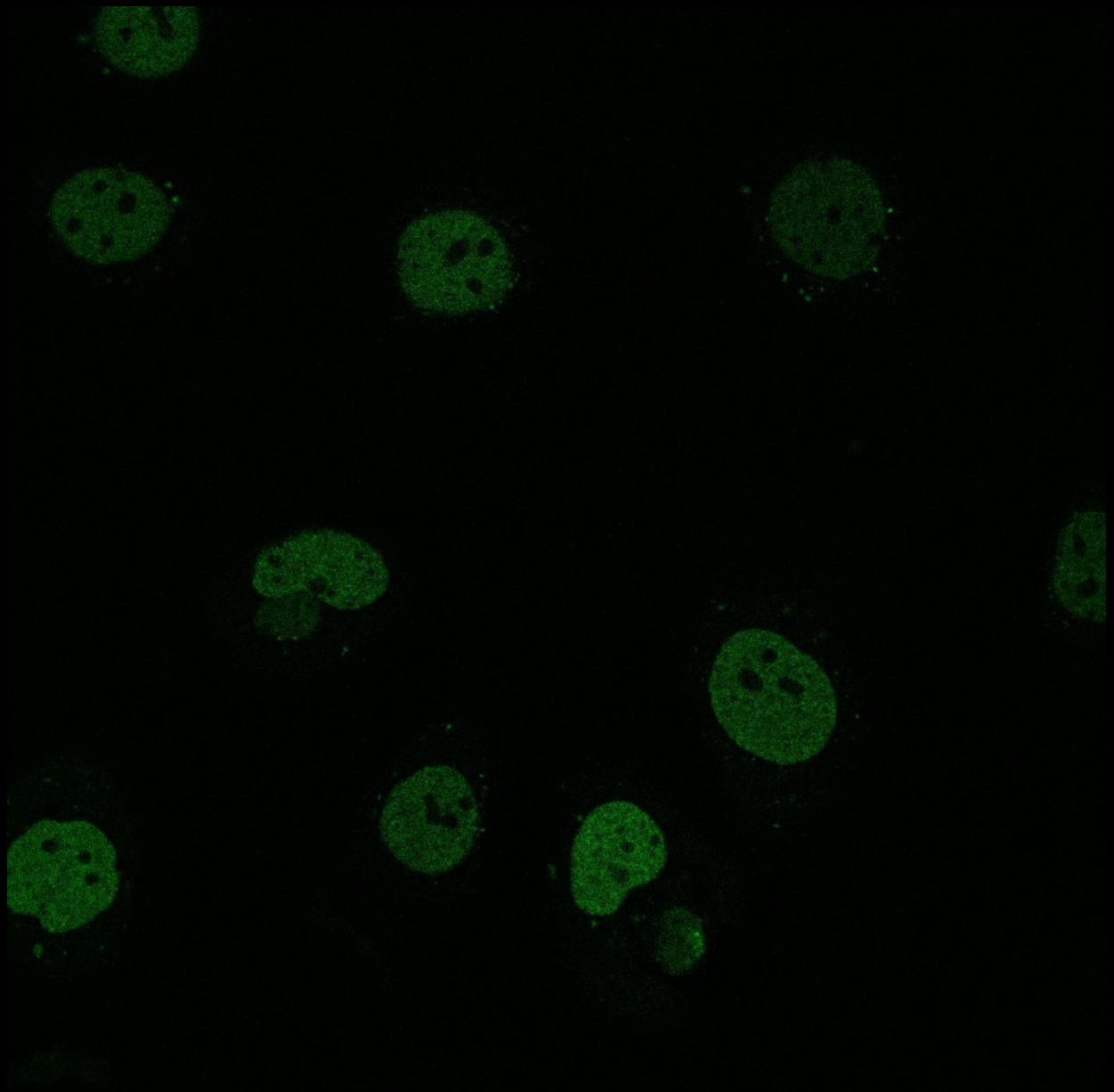


Activated AR-GFP translocates to the nucleus

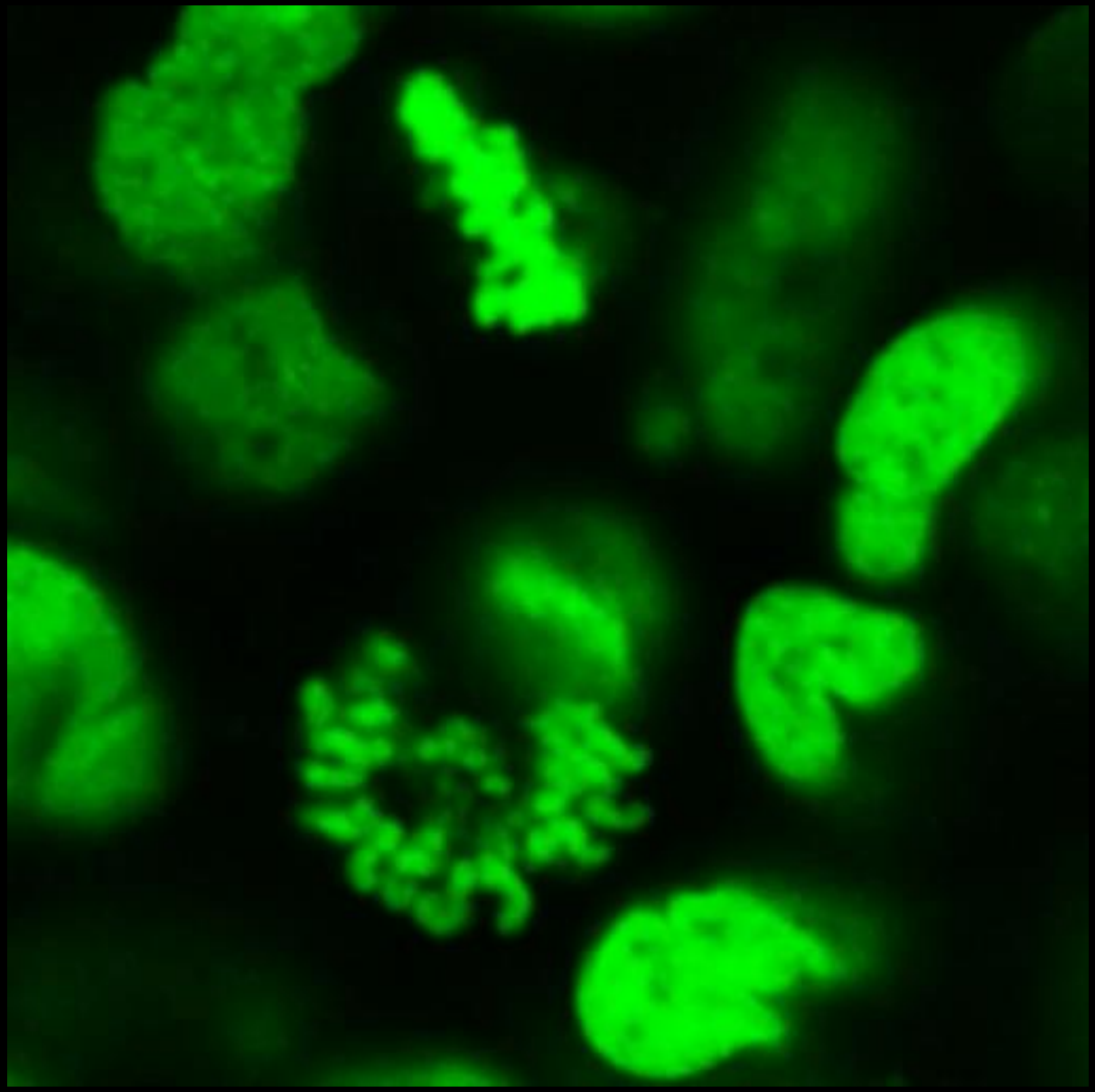
Activated AR-GFP translocates to the nucleus



GFP-AR expressing cells divide

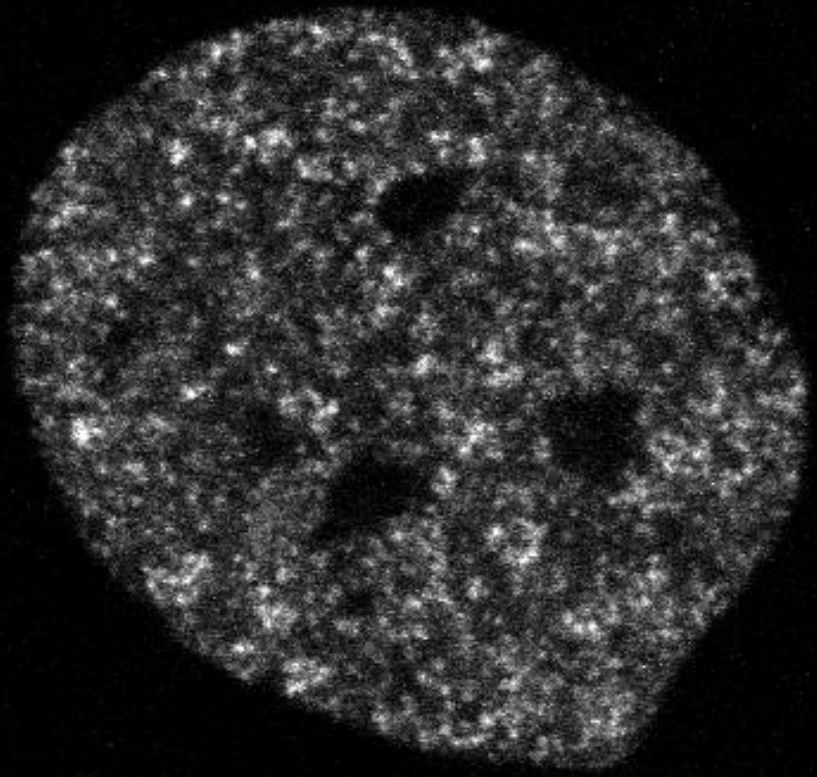


GFP-H2B expressing cells divide



GFP-AR accumulates in speckles

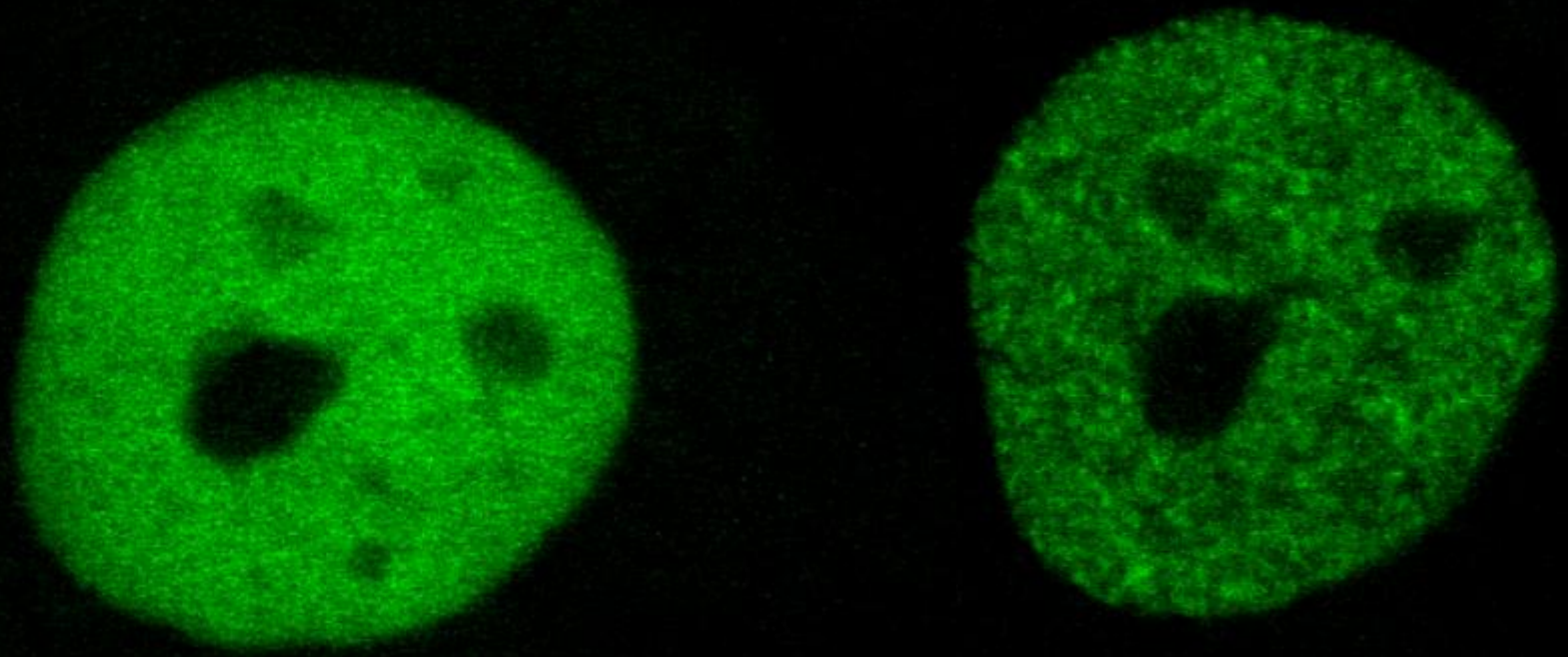
GFP-AR accumulates in speckles



Confocal images of living Hep3-B cell nuclei expressing AR-GFP

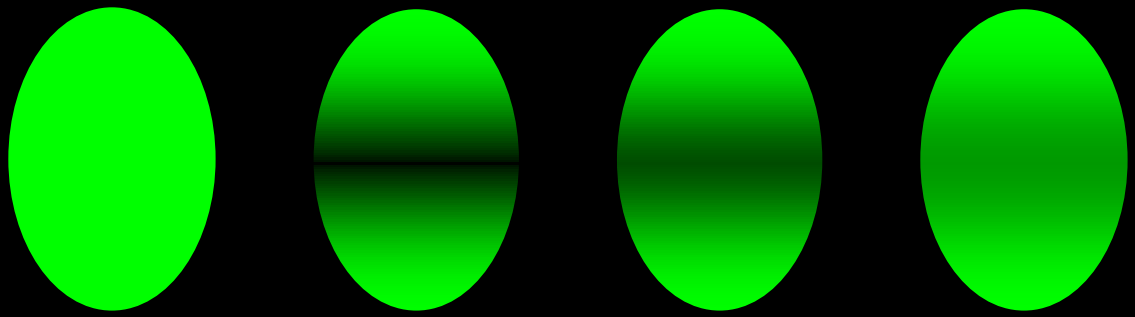
Before activation by testosterone
in presence of anti-androgen

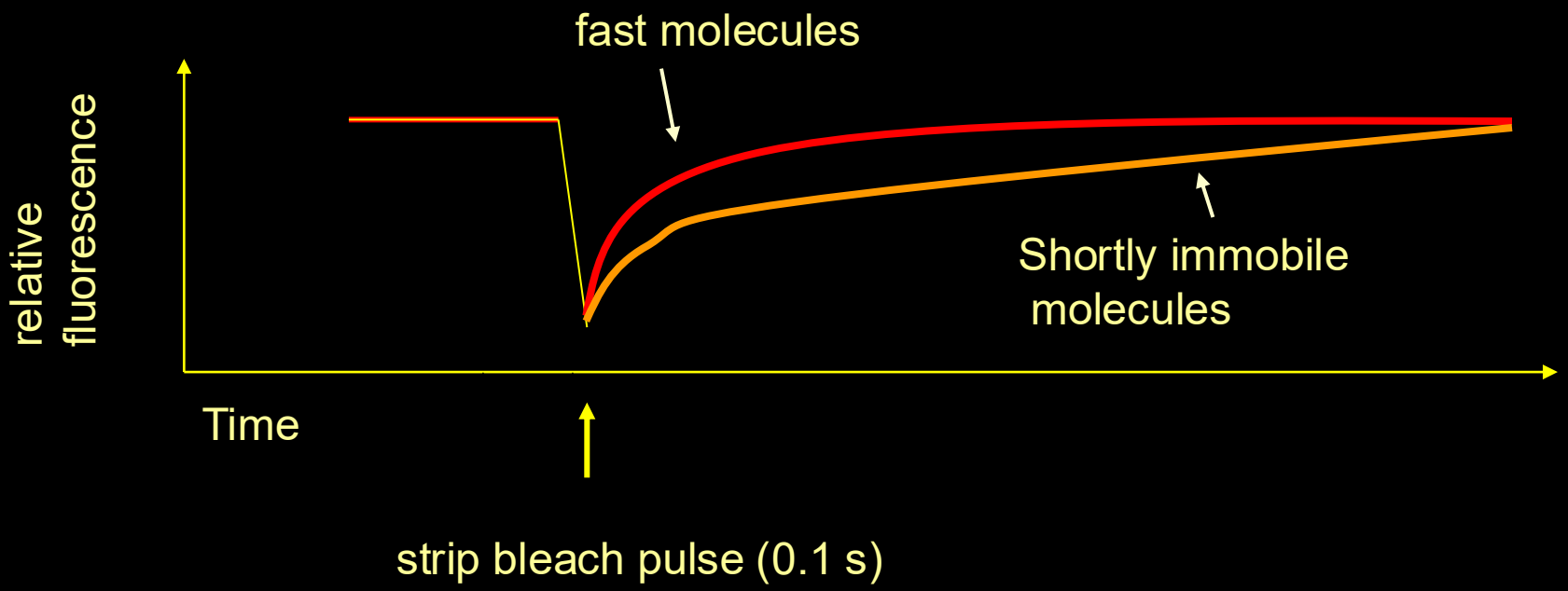
After activation by testosterone

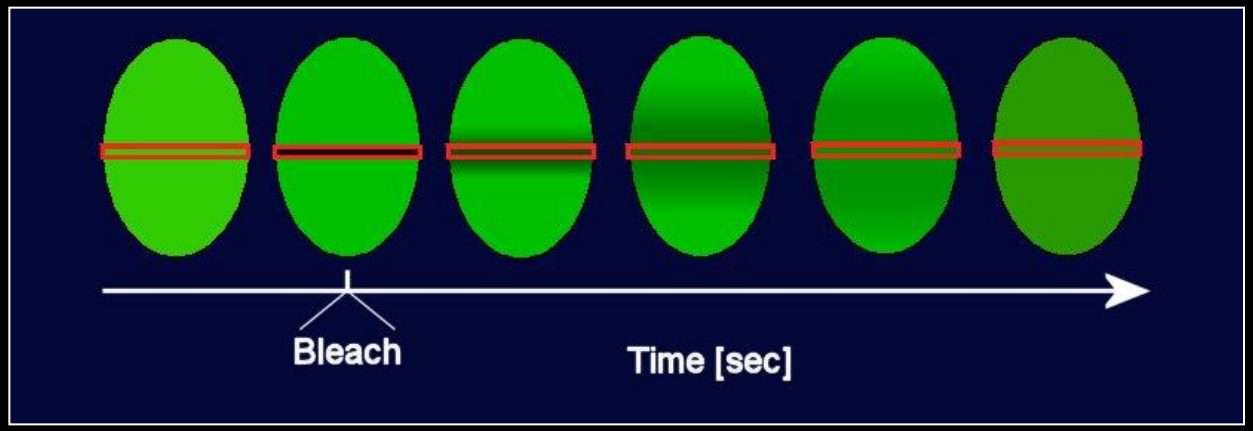


10 μm

FRAP

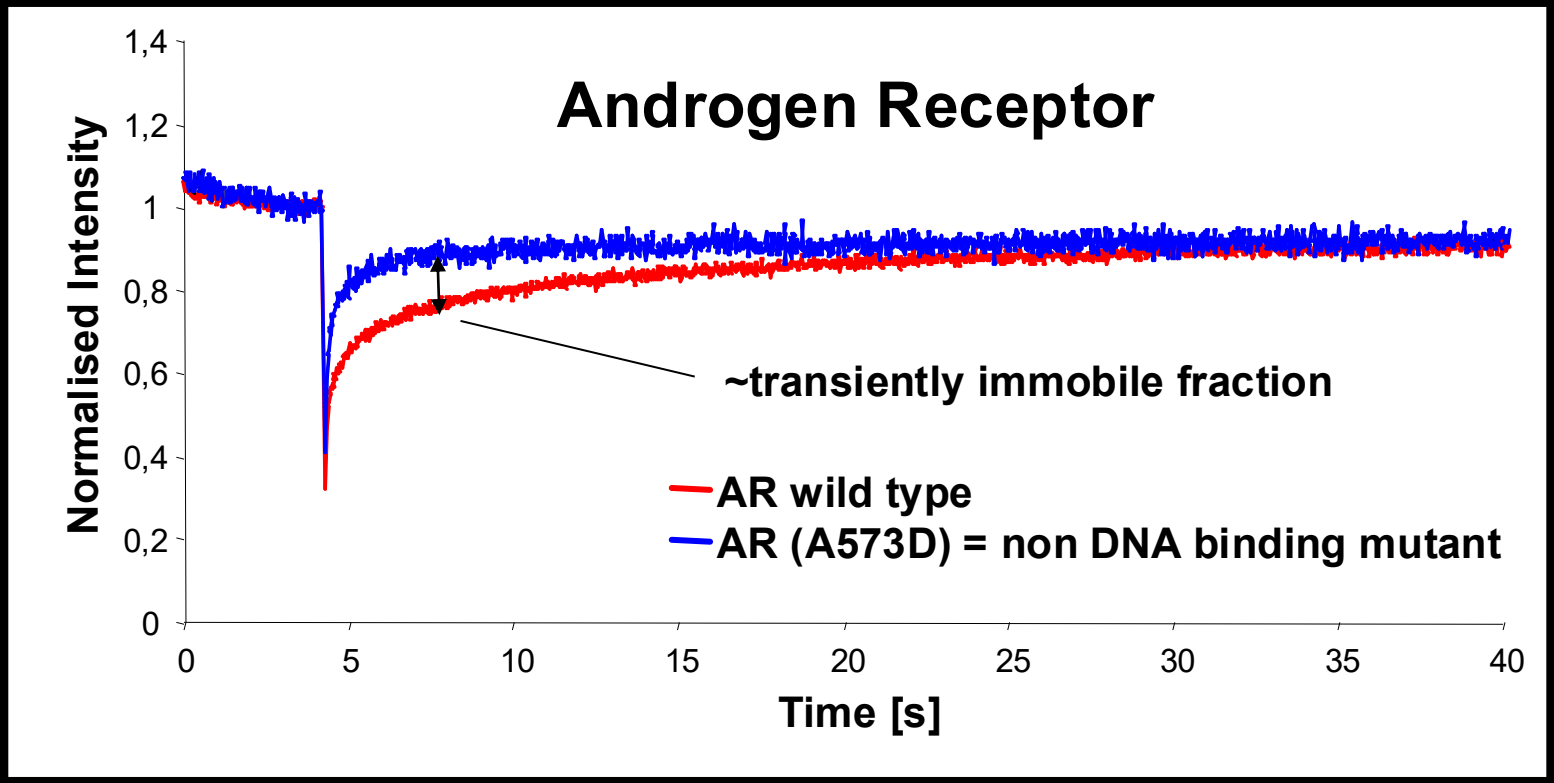



fast molecules
relative fluorescence
Shortly immobile molecules
Time
strip bleach pulse (0.1 s)



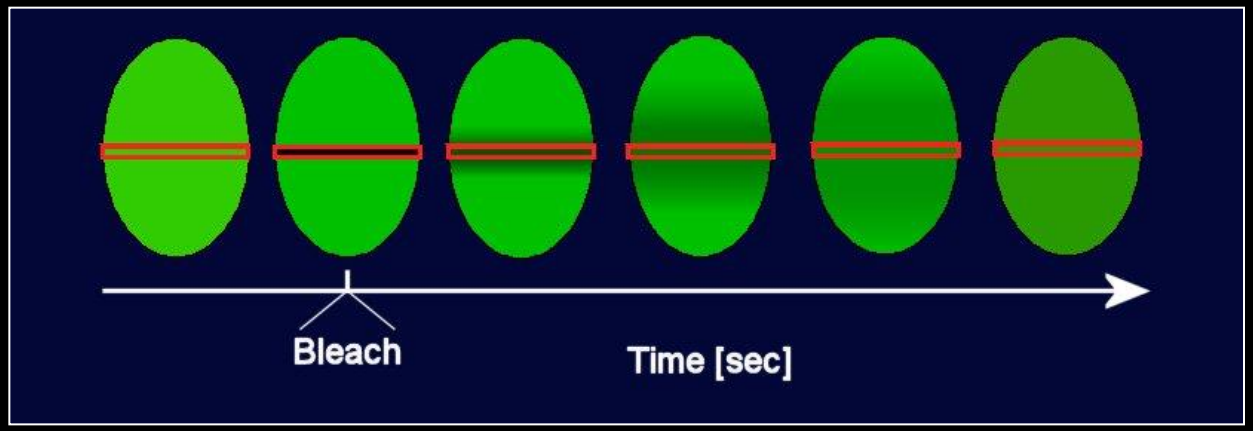
FRAP

(Fluorescence
Recovery
After
Photobleaching)



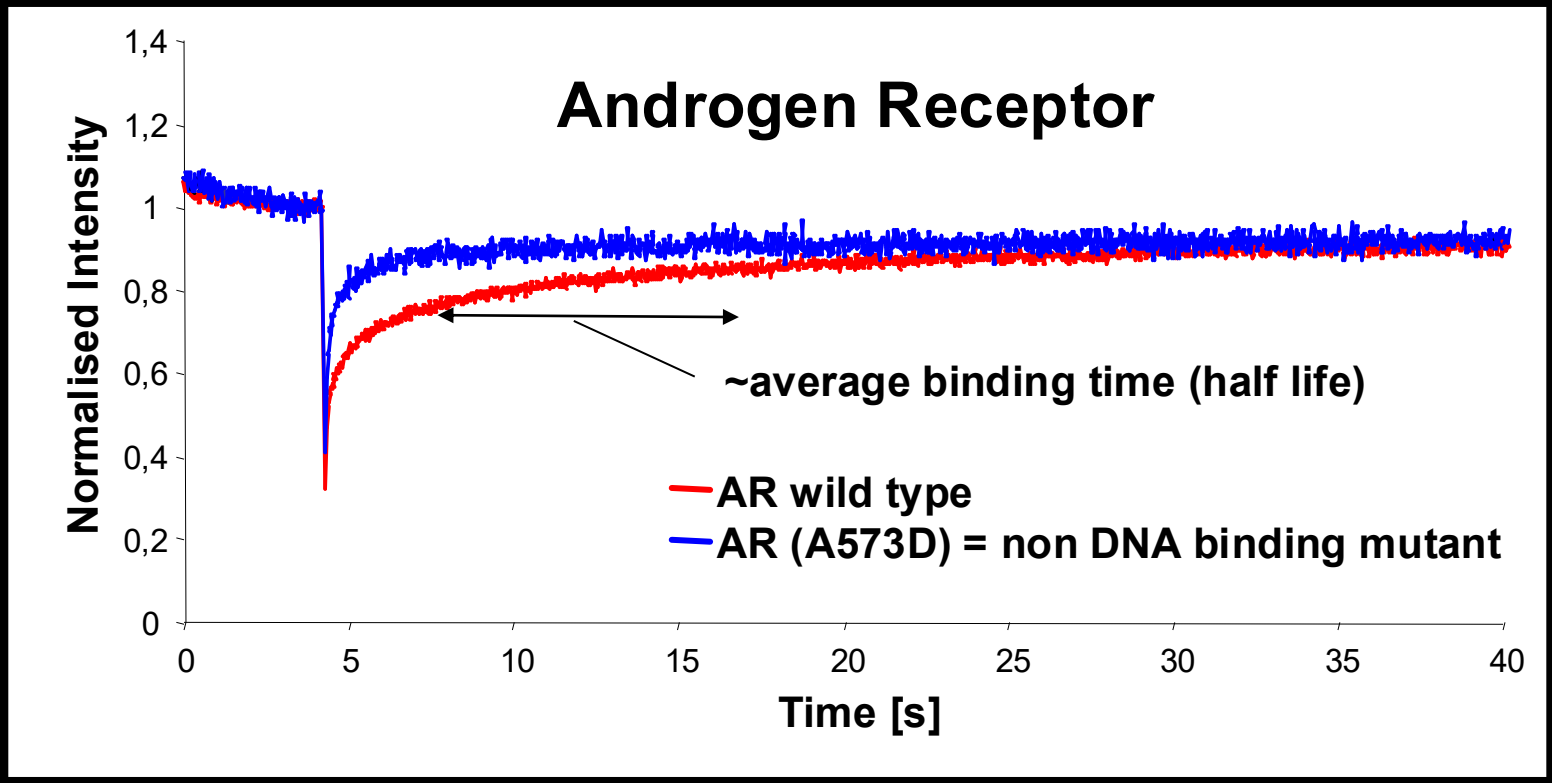
Farla et al. 2004,
J Struct. Biol

Farla et al. 2005,
J Cell Sci



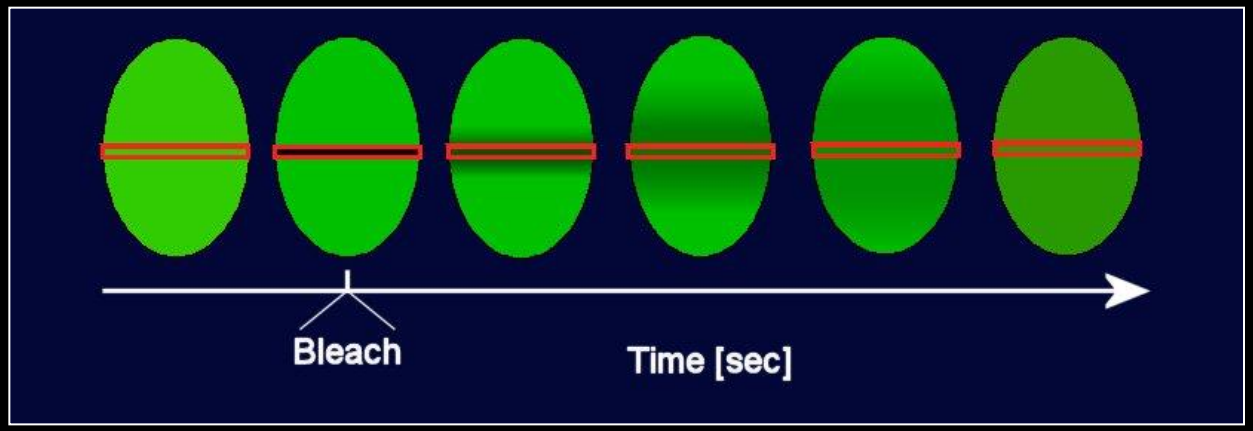
FRAP

(Fluorescence
Recovery
After
Photobleaching)



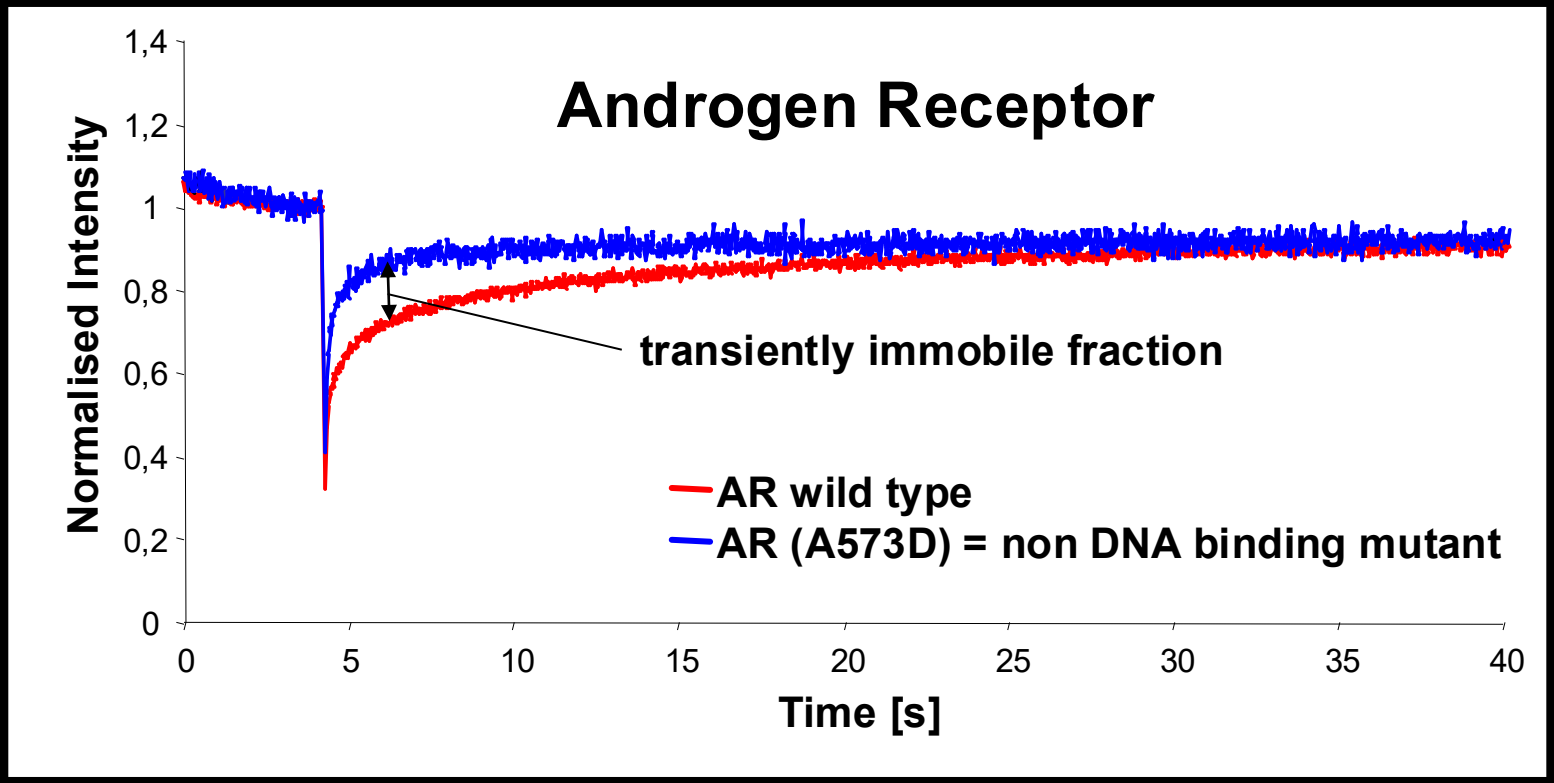
Farla et al. 2004,
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J Cell Sci



FRAP

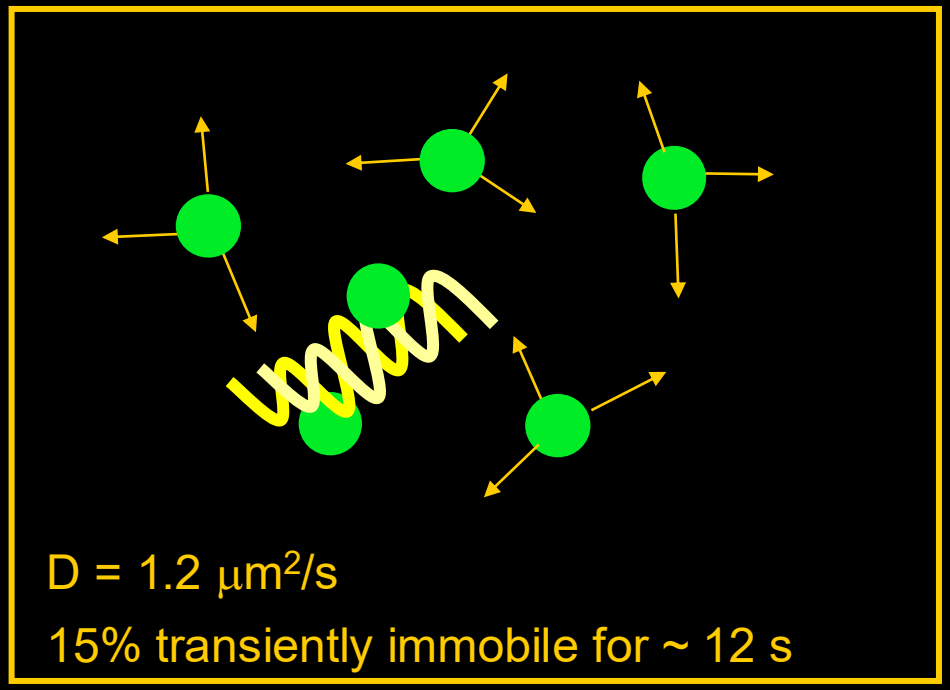
(Fluorescence
Recovery
After
Photobleaching)



Farla et al. 2004,
J Struct. Biol

Farla et al. 2005,
J Cell Sci

Wild-type AR



non-DNA-binding AR mutant

