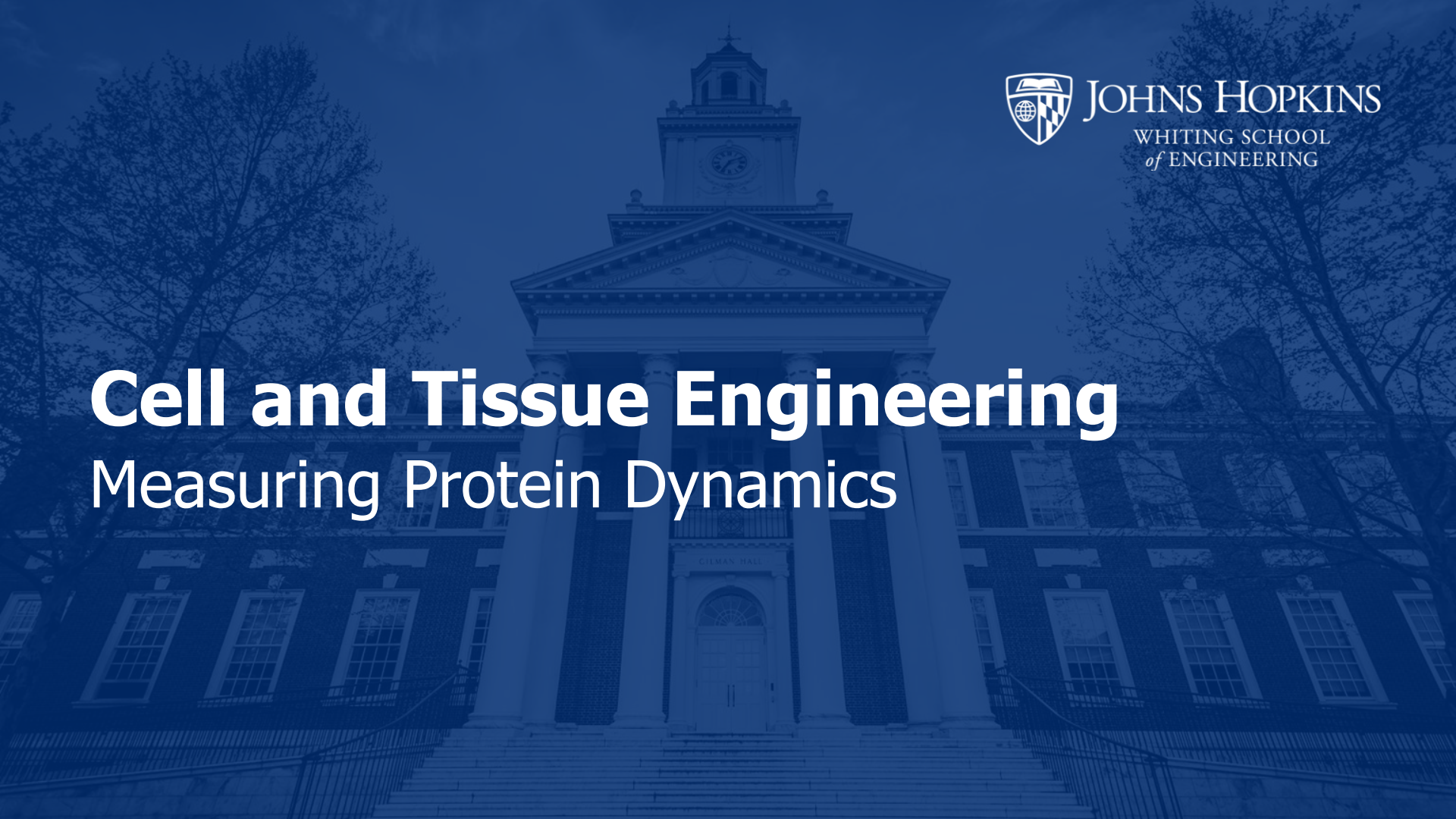
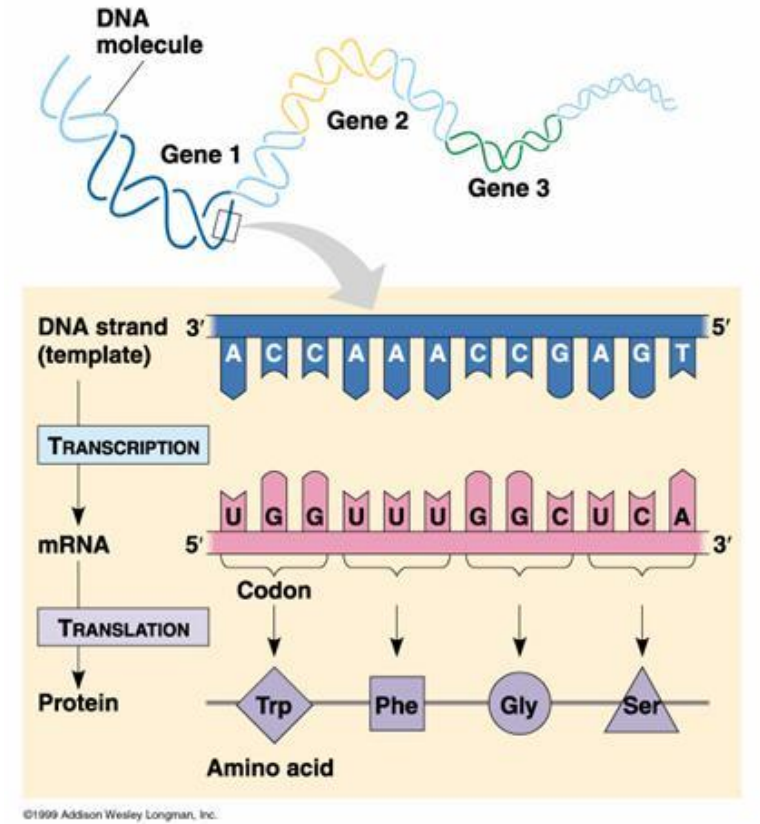


Cell and Tissue Engineering

Measuring Protein Dynamics

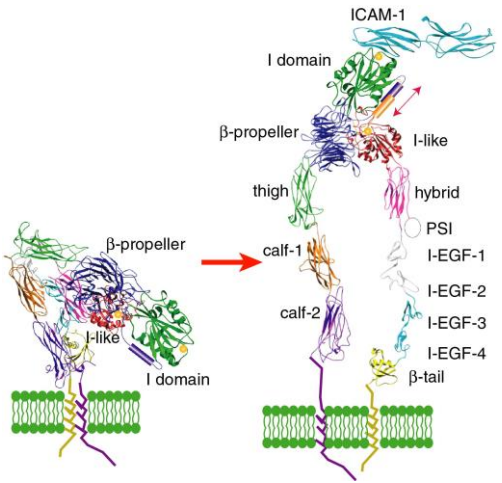
Protein dynamics → cellular dynamics

- What comes next?
- How do protein work?
- How can we study protein dynamics?

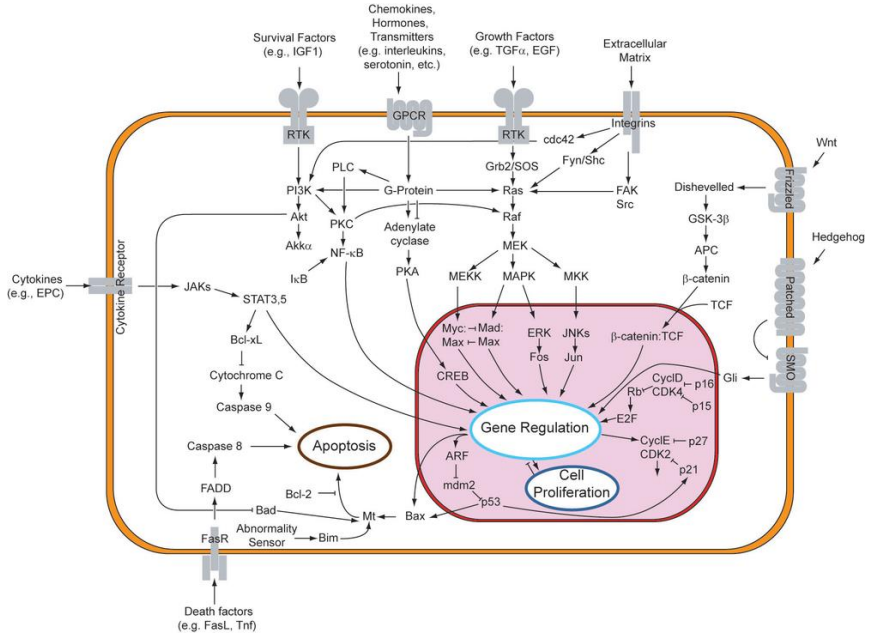


Proteins function dynamically

Conformational Changes



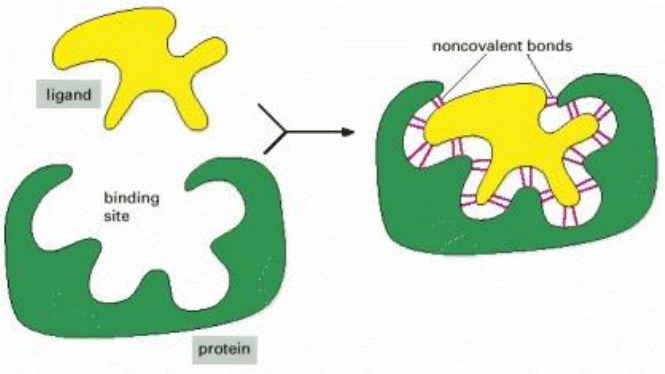
Movement and Interaction

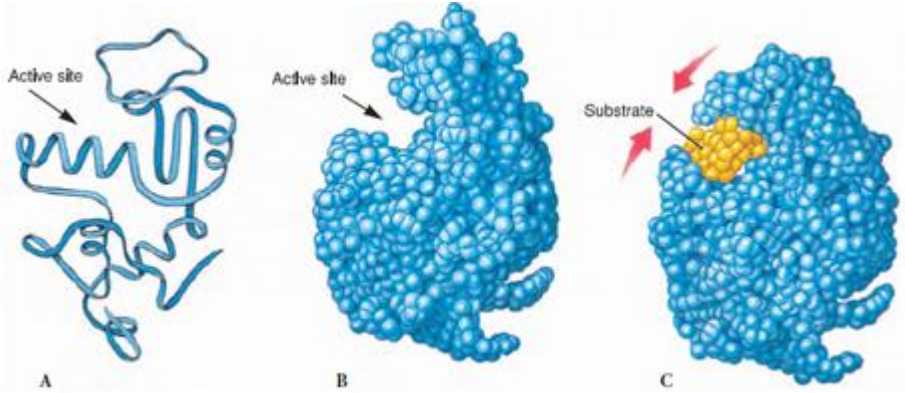


Proteins function dynamically (cont.)

http://publications.nigms.nih.gov/structlife/chapter1.html

The basics of protein function

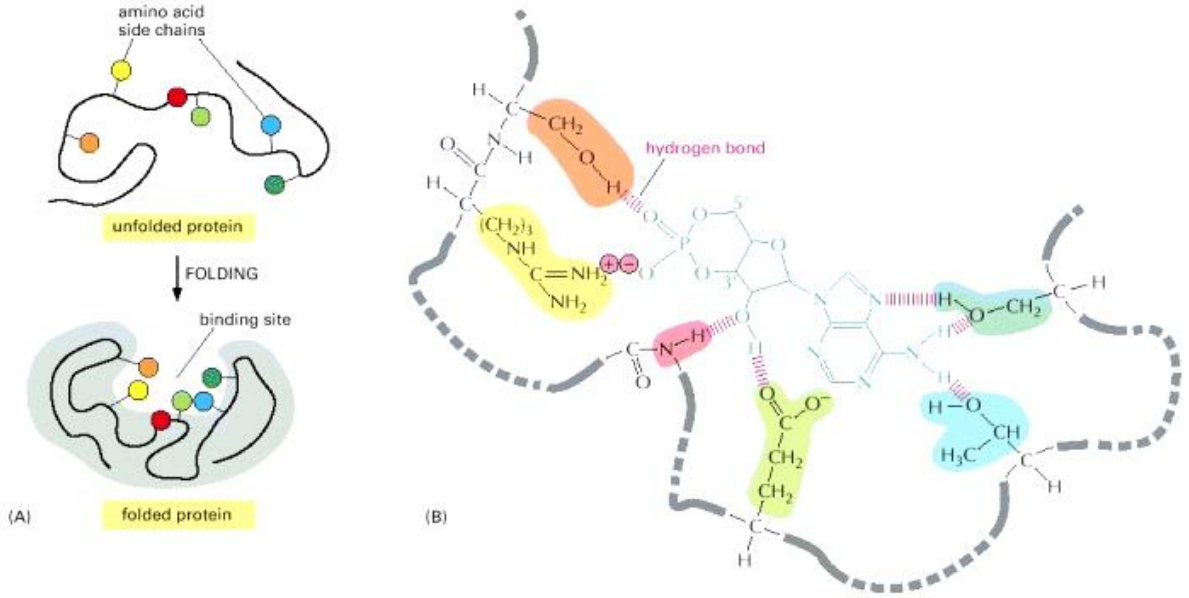




Molecular Biology of the Cell, 4th Edition, Action of Enzymes

The basics of protein function (cont.)

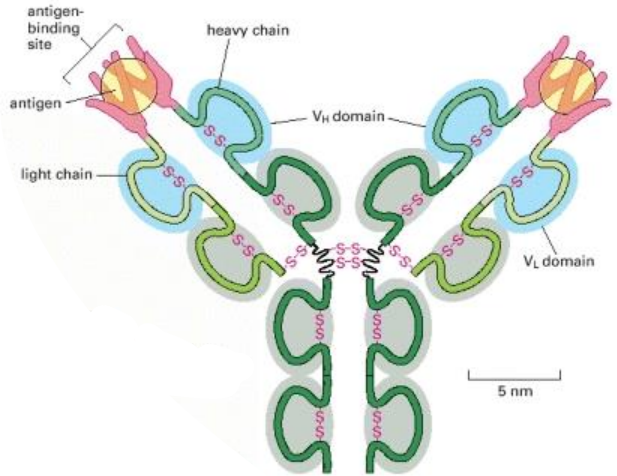
- Binding pockets in the tertiary structure of proteins



Molecular Biology of the Cell, 4th Edition

The basics of protein function (cont.)

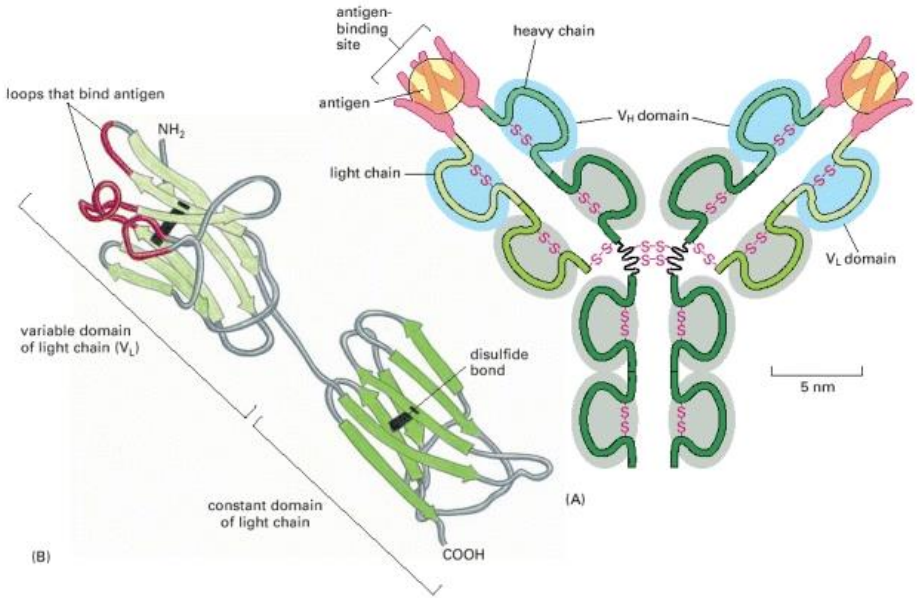
- Antibody structure allows for many noncovalent bonds to the antigen



Molecular Biology of the Cell, 4th Edition, www.pierce-antibodies.com

The basics of protein function (cont.)

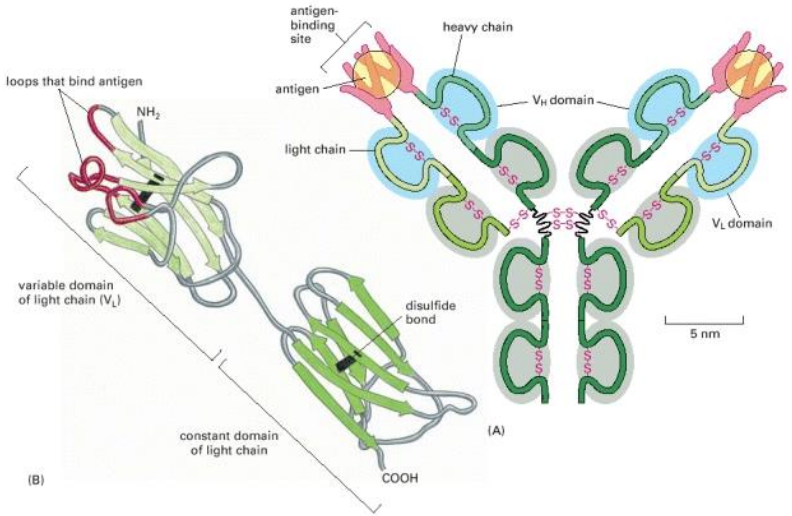
- Antibody structure allows for many noncovalent bonds to the antigen



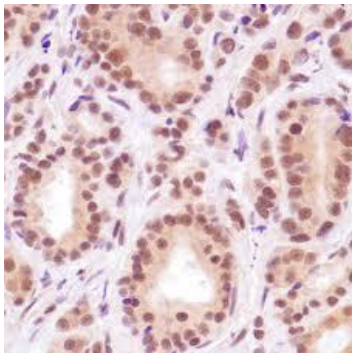
Molecular Biology of the Cell, 4th Edition, www.pierce-antibodies.com

The basics of protein function (cont.)

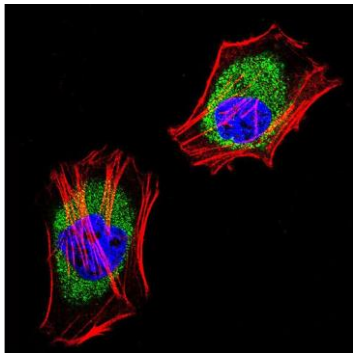
Antibody structure allows for many noncovalent bonds to the antigen



Immunohistochemistry

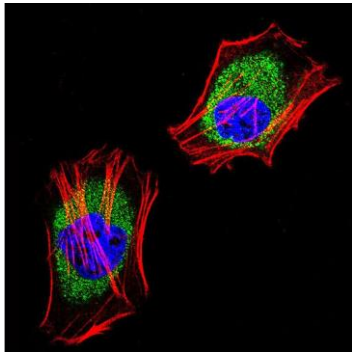


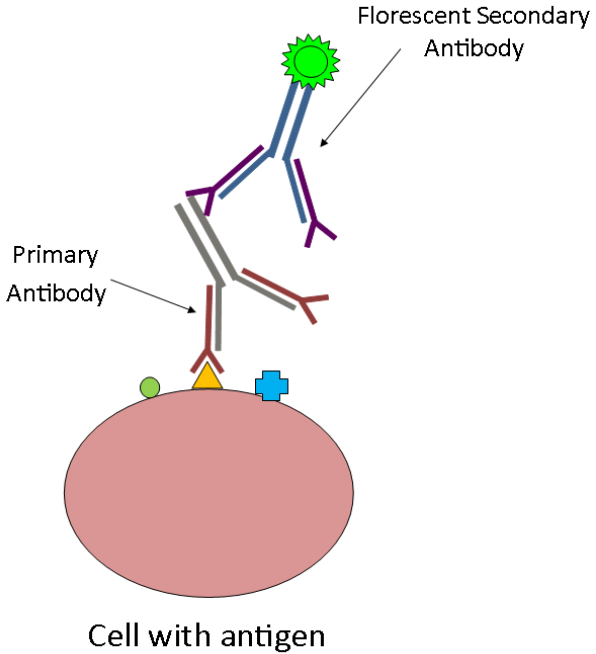
Immunofluorescence



Molecular Biology of the Cell, 4th Edition, www.pierce-antibodies.com

Immunofluorescence imaging





http://www.docstoc.com/docs/41551577/Immunofluorescence-and-Flow-Cytometry, www.pierce-antibodies.com

Immunofluorescence imaging (cont.)

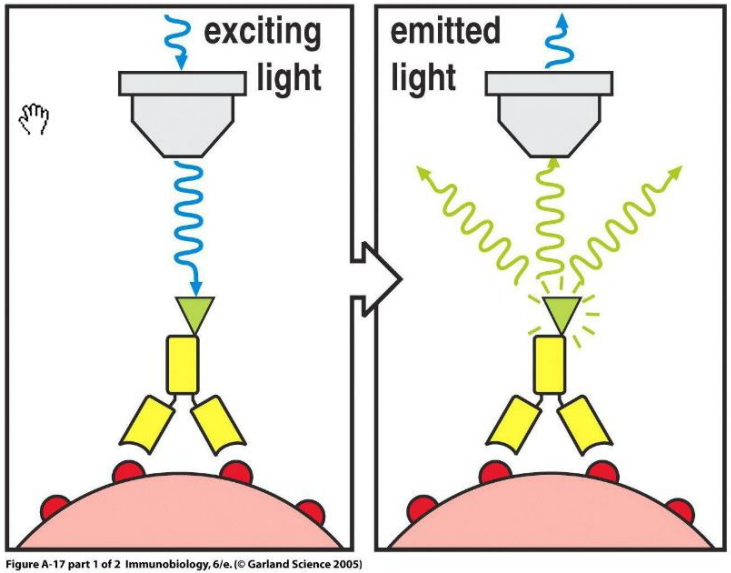
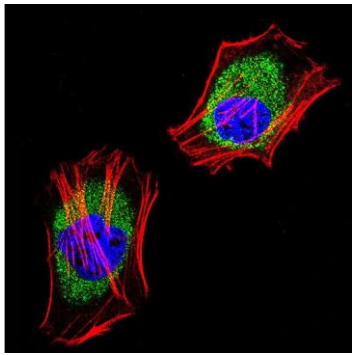
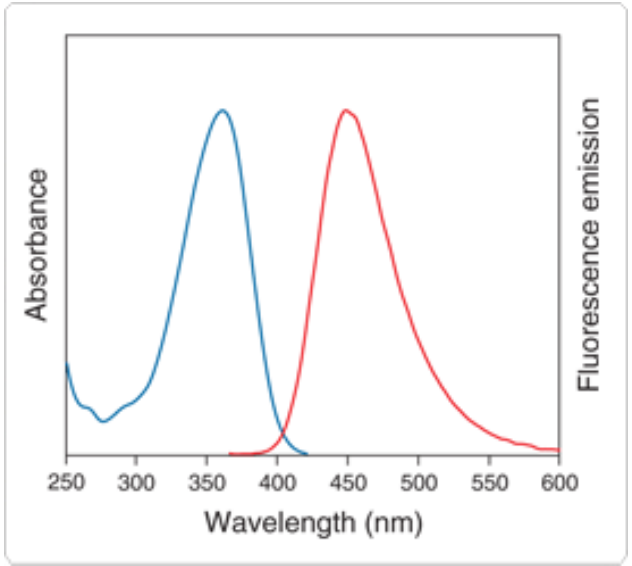


Figure A-17 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

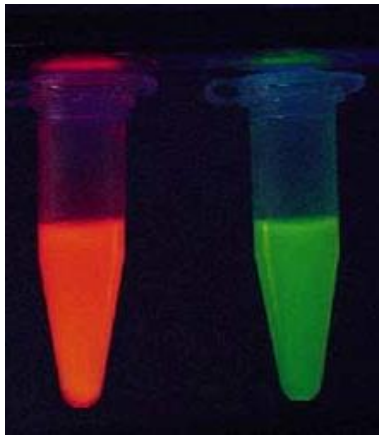


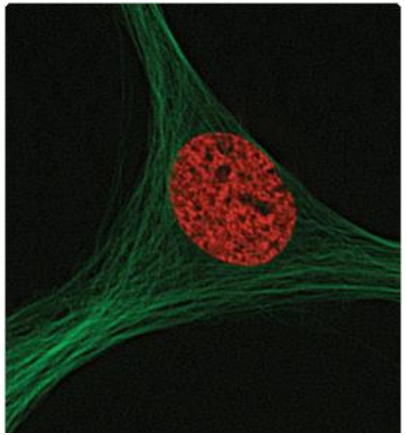
Excitation and
Emission

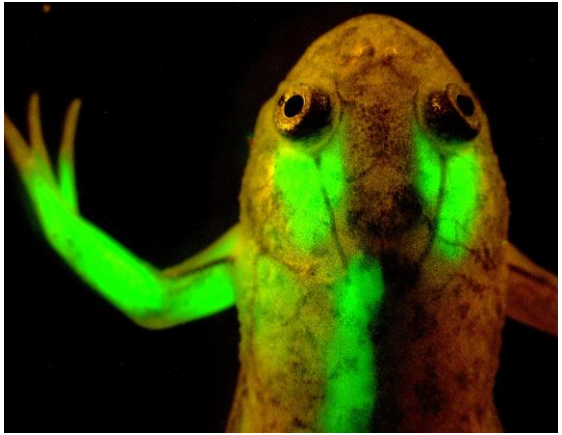


www.pierce-antibodies.com
http://www.docstoc.com/docs/41551577/Immunofluorescence-and-Flow-Cytometry
Thermo Fisher Scientific

Fluorescent markers used to study dynamic protein function



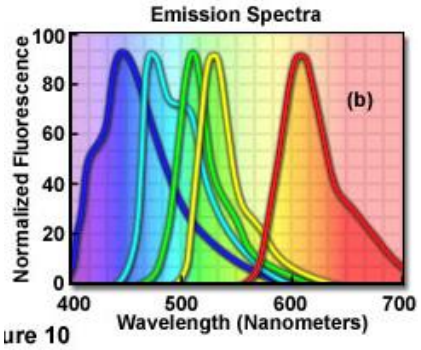


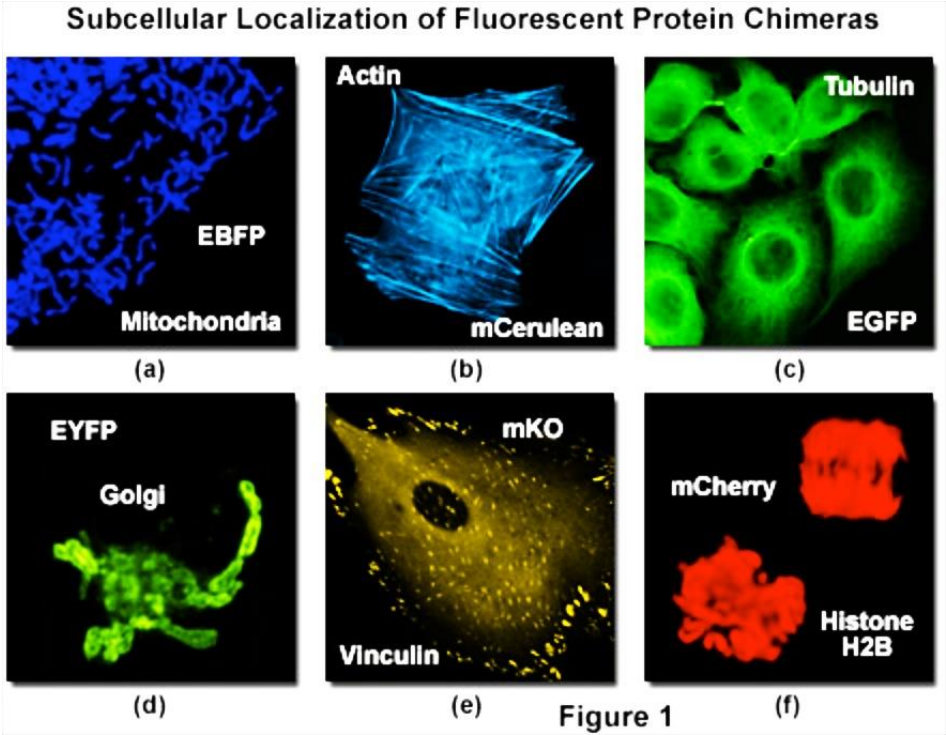


GFP – green fluorescent protein
RFP or DsRed – red fluorescent protein
Quantum dots

http://www.livescience.com/16752-gfp-protein-fluorescent-nih-nigms.htmlcc
Thermo Fischer, Millipore Sigma

Fluorescent markers used to study dynamic protein function (cont.)





Fluorescent markers used to study dynamic protein function (cont.)

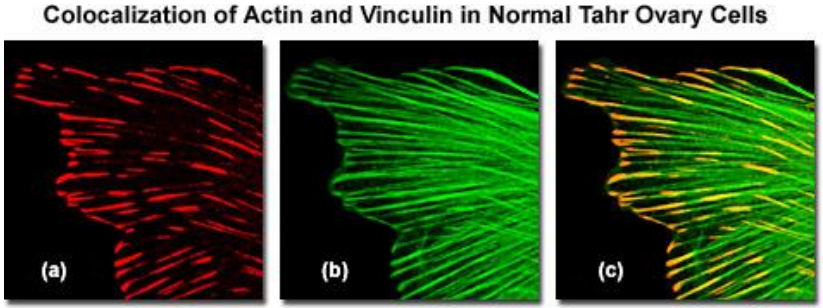
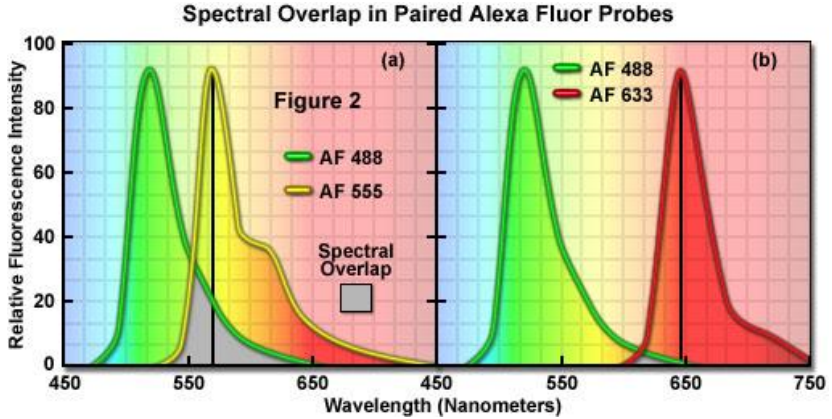


Figure 1



Fluorescent markers used to study dynamic protein function (cont.)

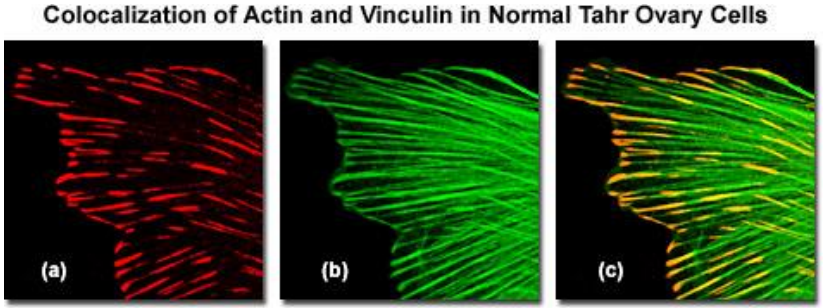
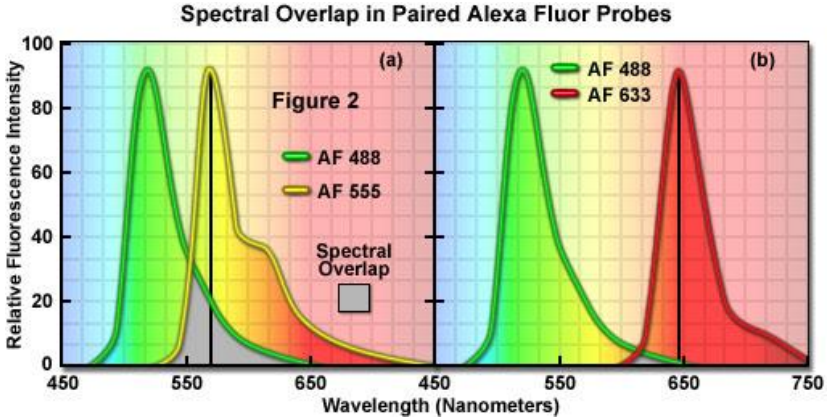
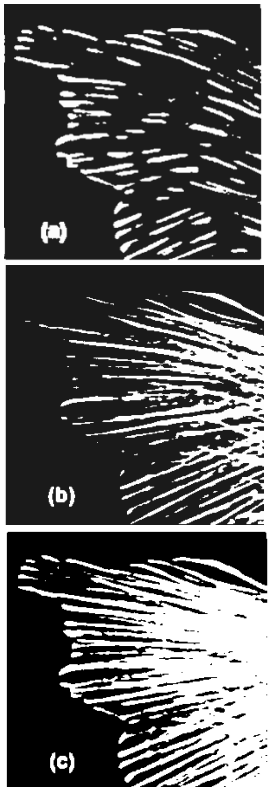
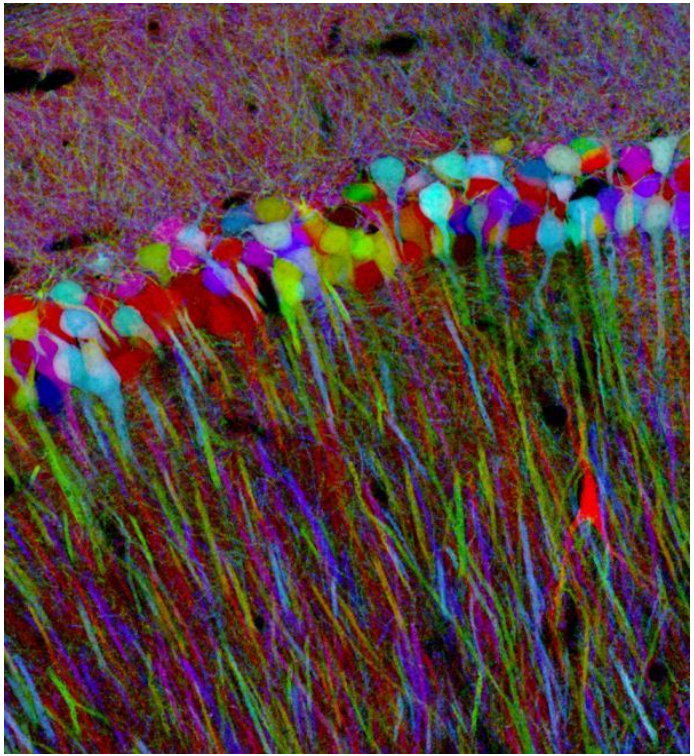


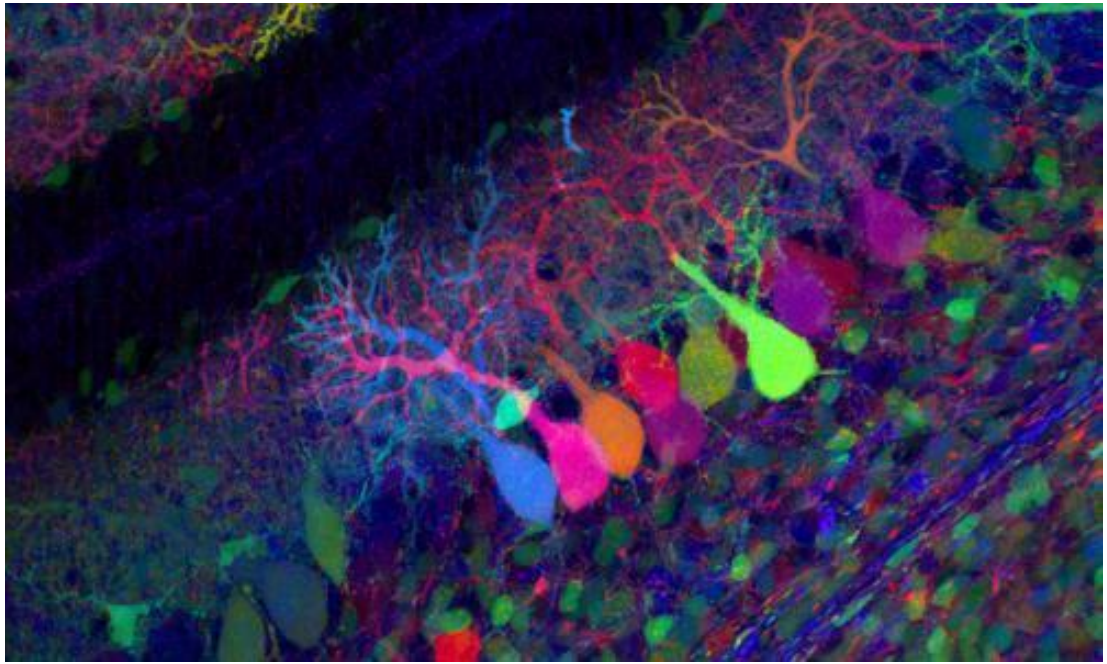
Figure 1





“Brainbow”





FRAP, FRET and FCS

Fluorescent imaging methods assist the study of protein dynamics

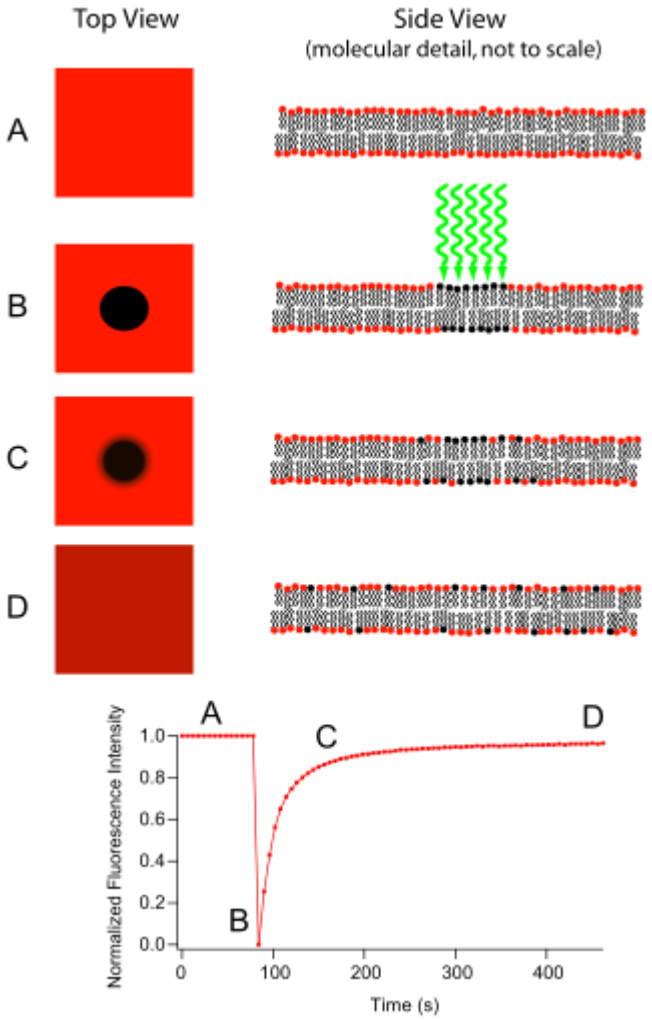
FRAP: fluorescence recovery after photobleaching

FRET: fluorescence (or Förster) resonance energy transfer

FCS: fluorescence correlation spectroscopy

	FRAP	FRET	FCS
Diffusion Rates	✓ Yes	No	✓ Yes
Multicomponent Diffusion	No	No	✓ Yes
Mobile Fraction	✓ Yes	No	No
Concentration	No	No	✓ Yes
Complexing	No	✓ Yes	✓ Yes
Complex Stoichiometry	No	No	✓ Yes
Binding Kinetics	✓ Yes	✓ Yes	✓ Yes

Show movement (passive or active) and protein complexing



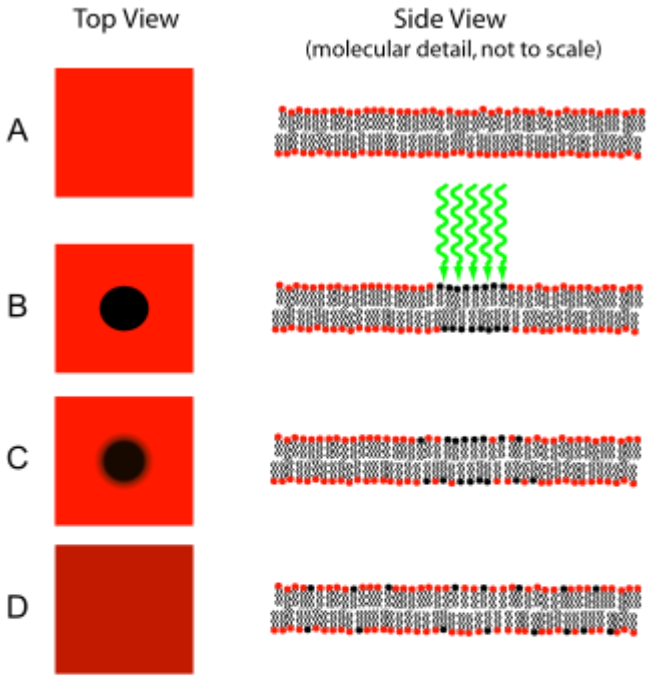
FRAP 1

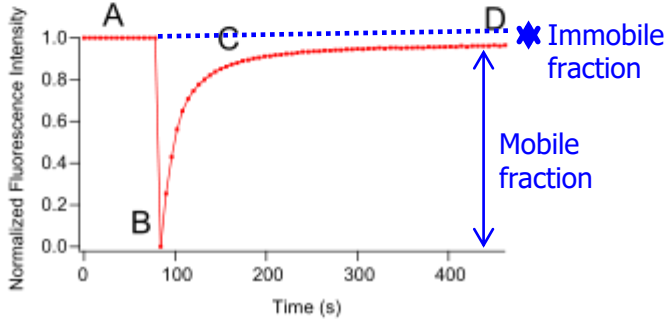
Assesses kinetic properties of proteins

	FRAP
Diffusion Rates	✔ Yes
Multicomponent Diffusion	No
Mobile Fraction	✔ Yes
Concentration	No
Complexing	No
Complex Stoichiometry	No
Binding Kinetics	✔ Yes

GFP

en.wikipedia.org
Confocal Microscopy





	FRAP
Diffusion Rates	✔ Yes
Multicomponent Diffusion	No
Mobile Fraction	✔ Yes
Concentration	No
Complexing	No
Complex Stoichiometry	No
Binding Kinetics	✔ Yes

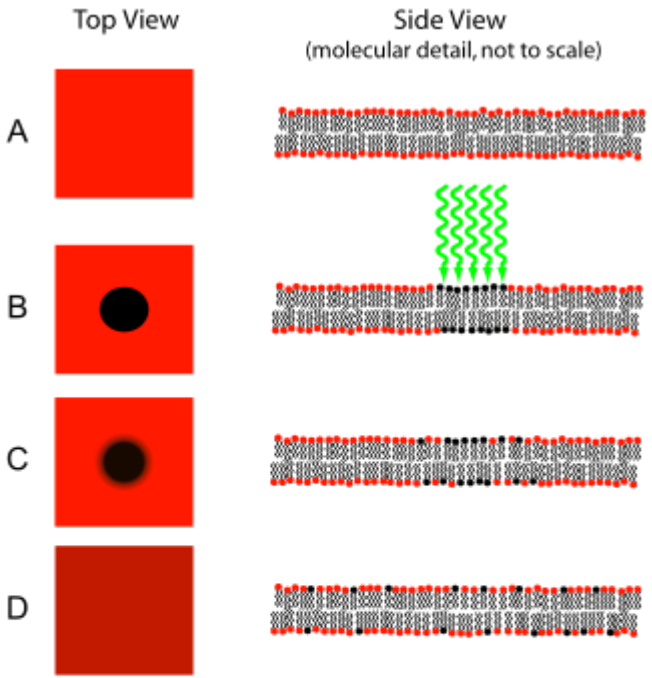
FRAP 2

M_f mobile fraction, % of recovery

D diffusion constant

en.wikipedia.org
Confocal Microscopy

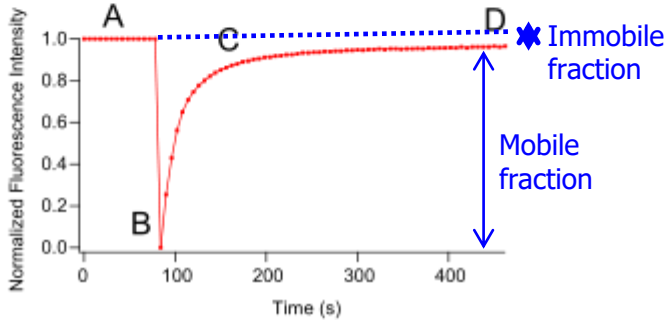
FRAP 3



	FRAP
Diffusion Rates	✓ Yes
Multicomponent Diffusion	No
Mobile Fraction	✓ Yes
Concentration	No
Complexing	No
Complex Stoichiometry	No
Binding Kinetics	✓ Yes

M_f mobile fraction, % of recovery
D diffusion constant

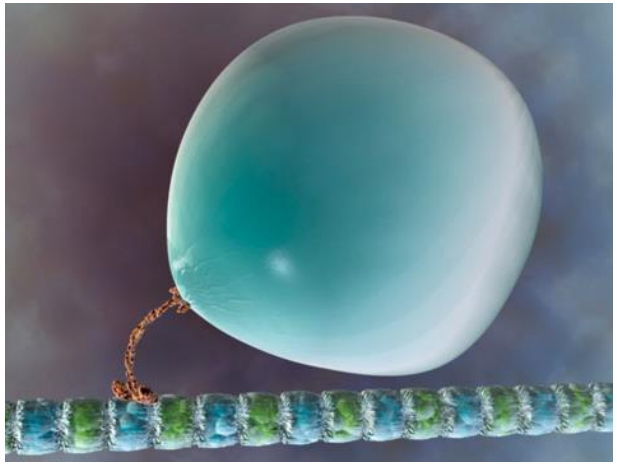
$$D = \frac{kT}{6\pi\eta R} = \frac{w^2}{2\eta\tau_D}$$



T absolute temperature
η viscosity of solution
η k Boltzman's constant
R hydrodynamic radius of the molecule
w radius of the bleached area
τ_D diffusion time

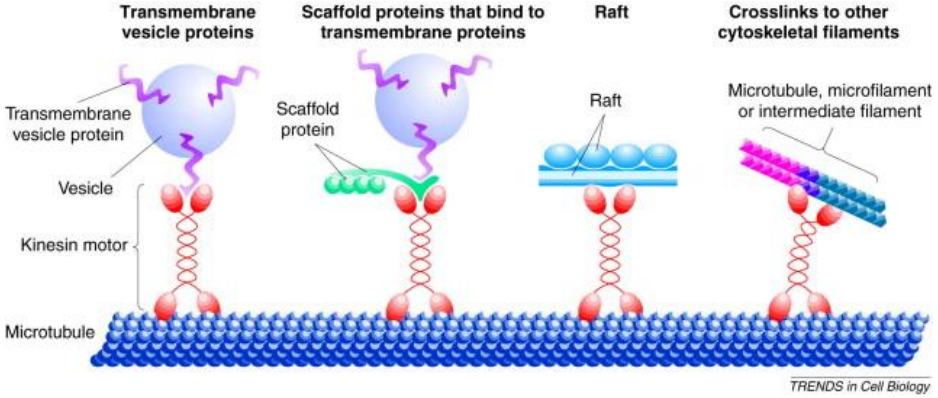
en.wikipedia.org
Confocal Microscopy

FRAP 4

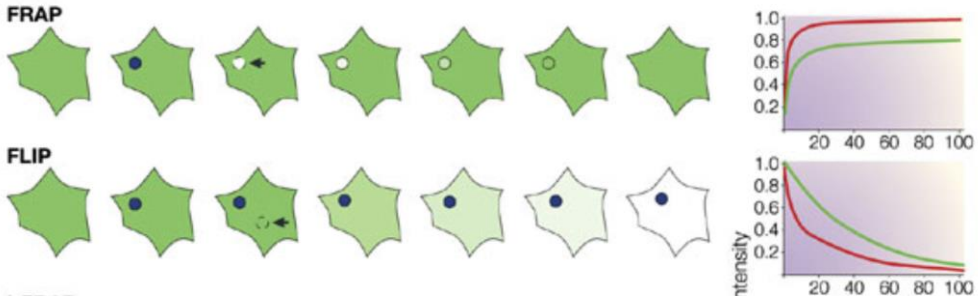


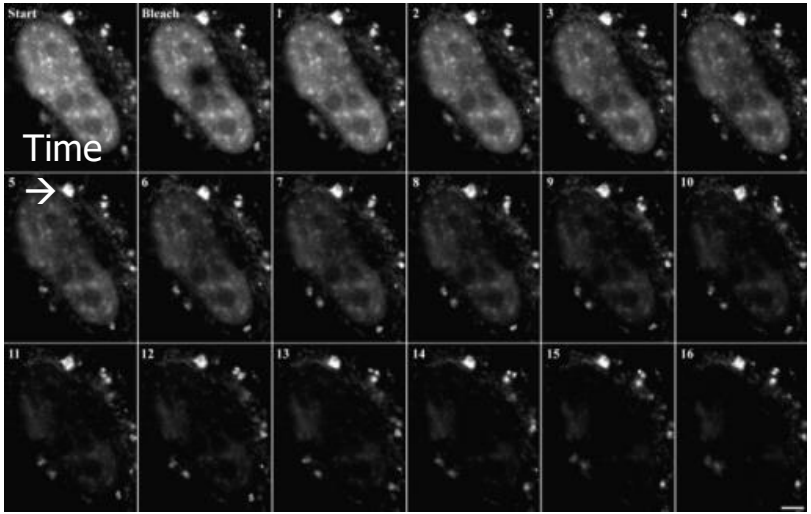
	FRAP
Diffusion Rates	✔ Yes
Multicomponent Diffusion	No
Mobile Fraction	✔ Yes
Concentration	No
Complexing	No
Complex Stoichiometry	No
Binding Kinetics	✔ Yes

Active transport via membrane-bound vesicles that track along cytoskeletal fibers

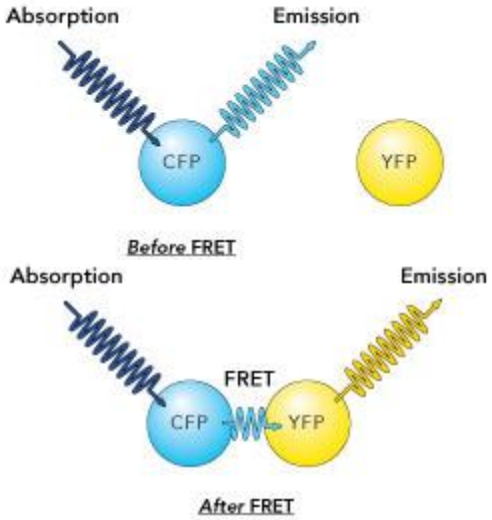


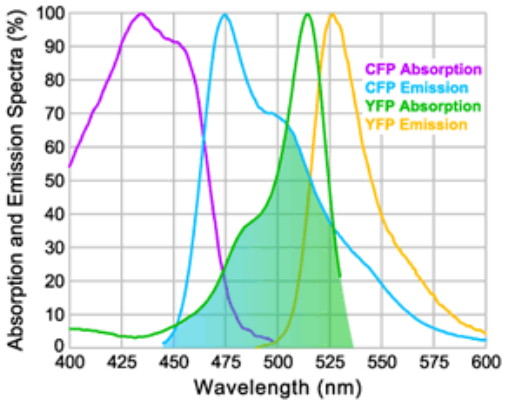
FLIP: Fluorescence Loss In Photobleaching





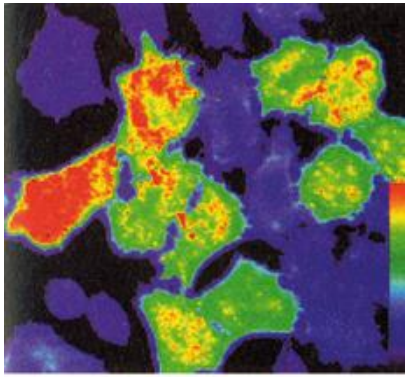
Nature
http://openi.nlm.nih.gov/detailedresult.php?img=2185567_JCB9911078.f8&req=4

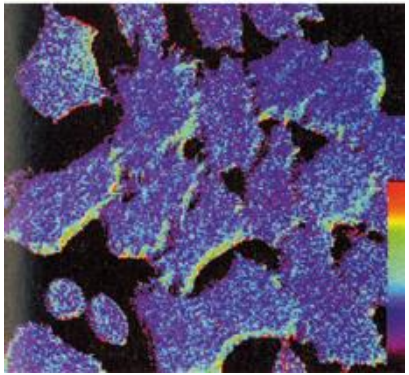




FRET 1

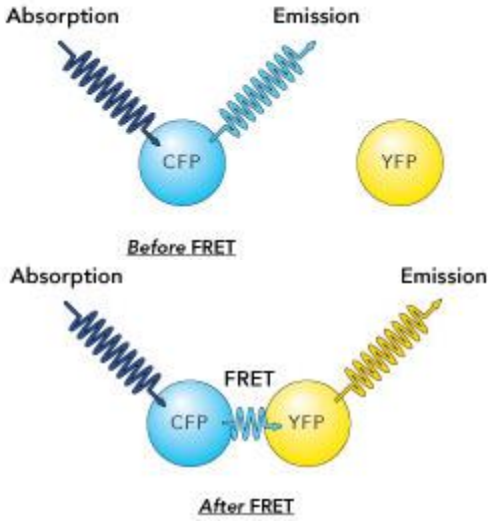
Measures protein-protein interactions

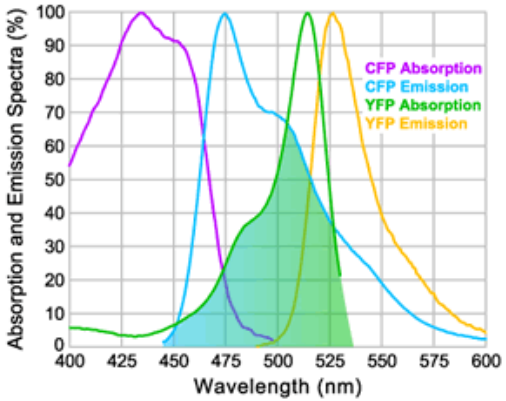




	FRET
Diffusion Rates	No
Multicomponent Diffusion	No
Mobile Fraction	No
Concentration	No
Complexing	✔ Yes
Complex Stoichiometry	No
Binding Kinetics	✔ Yes

http://www.semrock.com/fret.aspx,
http://www.nature.com/milestones/milelight/full/milelight11.html





http://www.semrock.com/fret.asp

FRET 2

Measures protein-protein interactions

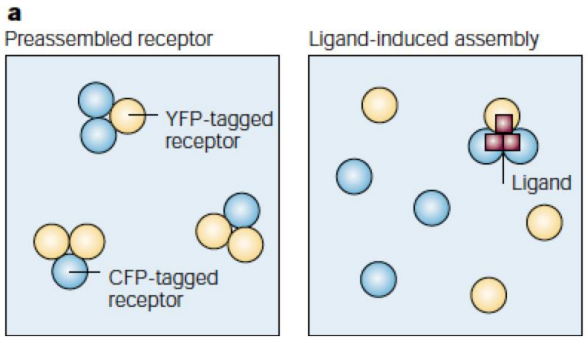
	FRET
Diffusion Rates	No
Multicomponent Diffusion	No
Mobile Fraction	No
Concentration	No
Complexing	✔ Yes
Complex Stoichiometry	No
Binding Kinetics	✔ Yes

$$k_F \sim \left(\frac{r_0}{r} \right)^6 . k_D$$

k_F rate of energy transfer from donor to acceptor
k_D radiative decay rate of donor fluorophore
r distance between the two molecules
r_0 Förester distance (50% efficiency point of energy transfer)

FRET 3

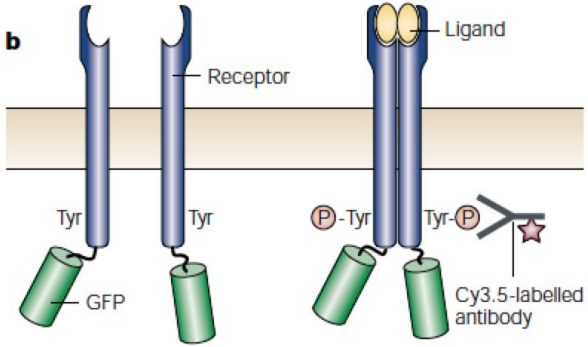
	FRET
Diffusion Rates	No
Multicomponent Diffusion	No
Mobile Fraction	No
Concentration	No
Complexing	✔ Yes
Complex Stoichiometry	No
Binding Kinetics	✔ Yes



http://www.semrock.com/fret.asp

FRET 4

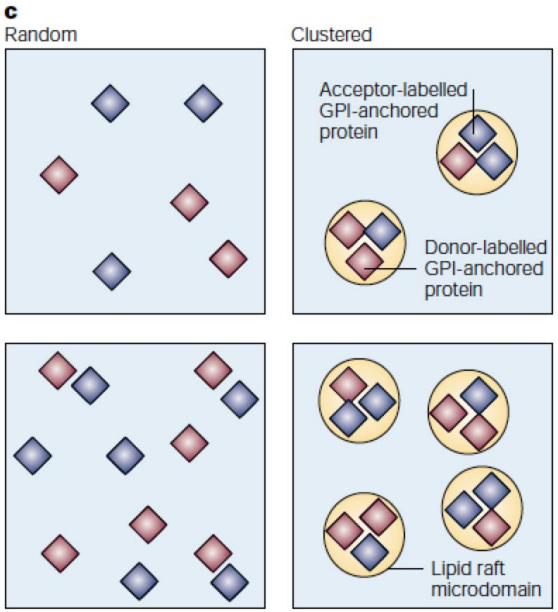
	FRET
Diffusion Rates	No
Multicomponent Diffusion	No
Mobile Fraction	No
Concentration	No
Complexing	✔ Yes
Complex Stoichiometry	No
Binding Kinetics	✔ Yes



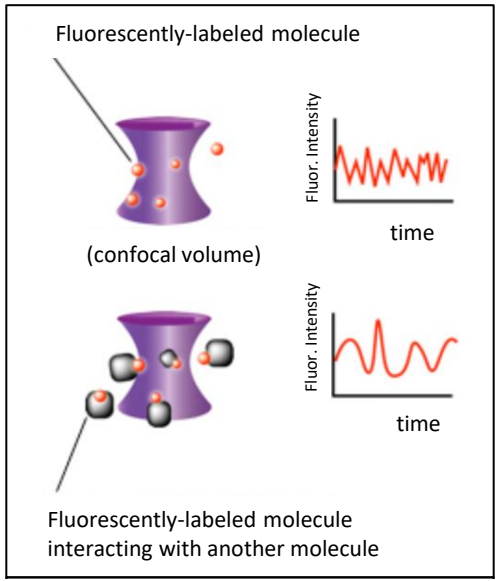
http://www.semrock.com/fret.aspx

FRET 5

	FRET
Diffusion Rates	No
Multicomponent Diffusion	No
Mobile Fraction	No
Concentration	No
Complexing	✔ Yes
Complex Stoichiometry	No
Binding Kinetics	✔ Yes



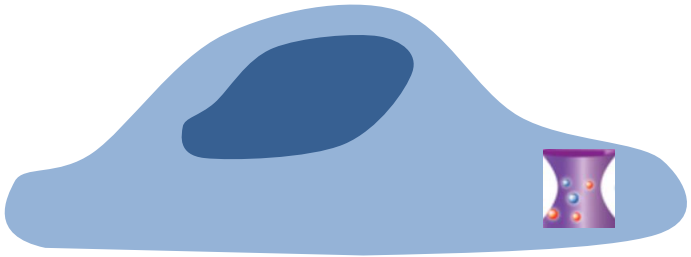
http://www.semrock.com/fret.aspx



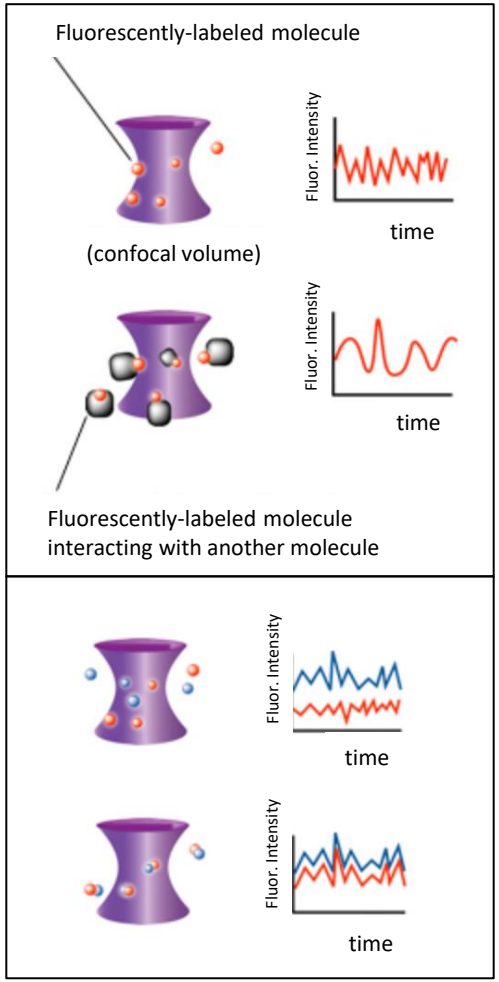
FCS and FCCS 1

Fluorescence correlation spectroscopy
Fluorescence cross-correlation spectroscopy
(dual-color FCS)

	FCS
Diffusion Rates	✓ Yes
Multicomponent Diffusion	✓ Yes
Mobile Fraction	No
Concentration	✓ Yes
Complexing	✓ Yes
Complex Stoichiometry	✓ Yes
Binding Kinetics	✓ Yes



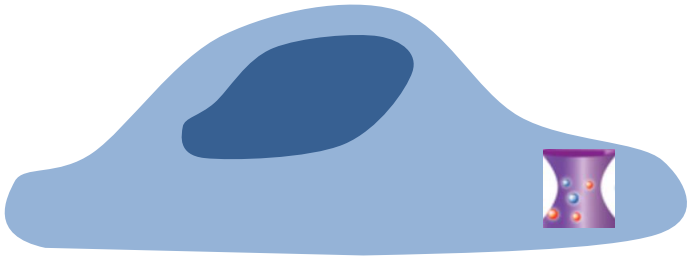
http://www.amalgaam.co.jp/support/application/fluc_deux_trypsinized_protein.html



FCS and FCCS 2

Fluorescence correlation spectroscopy
Fluorescence cross-correlation spectroscopy
(dual-color FCS)

	FCS
Diffusion Rates	✓ Yes
Multicomponent Diffusion	✓ Yes
Mobile Fraction	No
Concentration	✓ Yes
Complexing	✓ Yes
Complex Stoichiometry	✓ Yes
Binding Kinetics	✓ Yes



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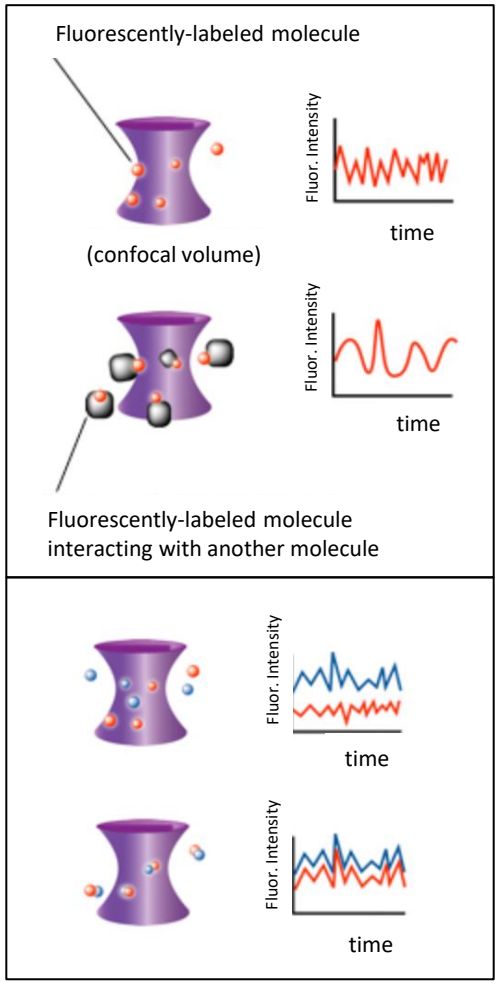
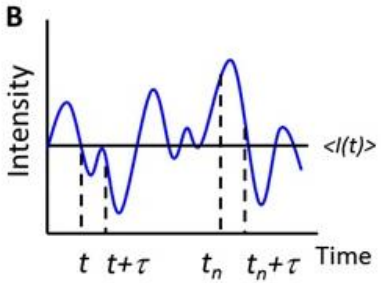
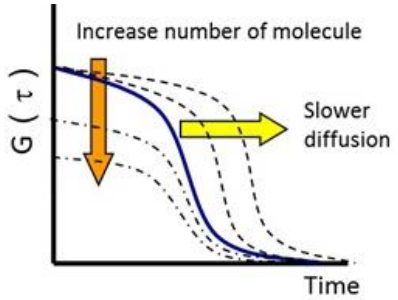


FCS and FCCS 3

Fluorescence correlation spectroscopy
Fluorescence cross-correlation spectroscopy
(dual-color FCS)

	FCS
Diffusion Rates	✓ Yes
Multicomponent Diffusion	✓ Yes
Mobile Fraction	No
Concentration	✓ Yes
Complexing	✓ Yes
Complex Stoichiometry	✓ Yes
Binding Kinetics	✓ Yes





G(τ) autocorrelation function

http://www.amalgaam.co.jp/support/application/fluc_deux_trypsinized_protein.html

What We Know Now