FRET

Fluorescence (Förster) Resonance Energy Transfer

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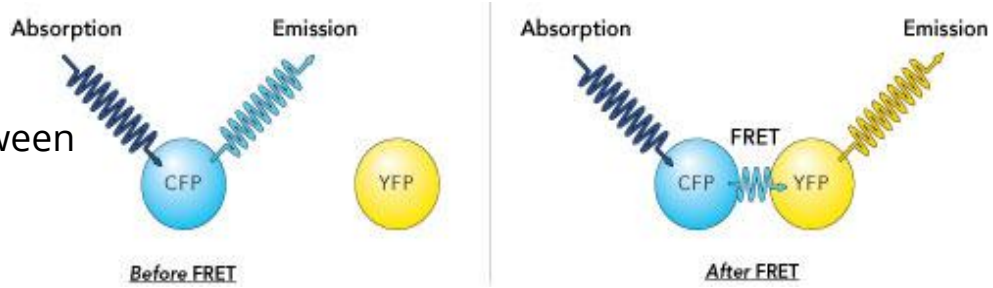
What is FRET?

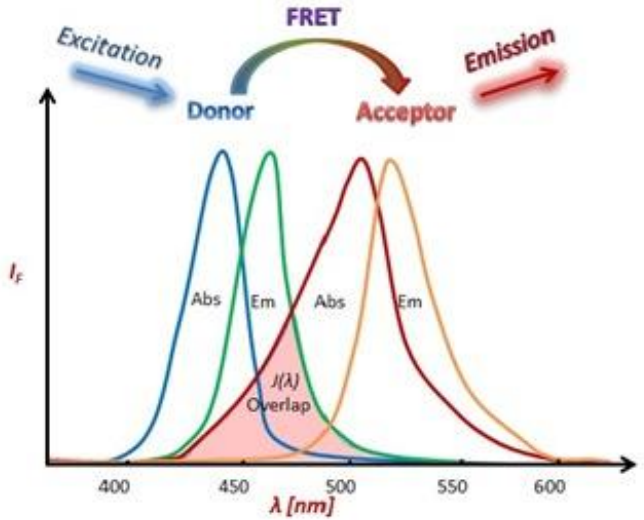
- Method to study physical interactions between proteins or DNA, as well as protein conformation.



How?

- Uses donor and acceptor fluorophores attached to biomolecules (proteins or DNA)

- Transfer efficiency is sensitive to distance (1 - 10 nm), so distance between fluorophores can be estimated by measuring FRET efficiency



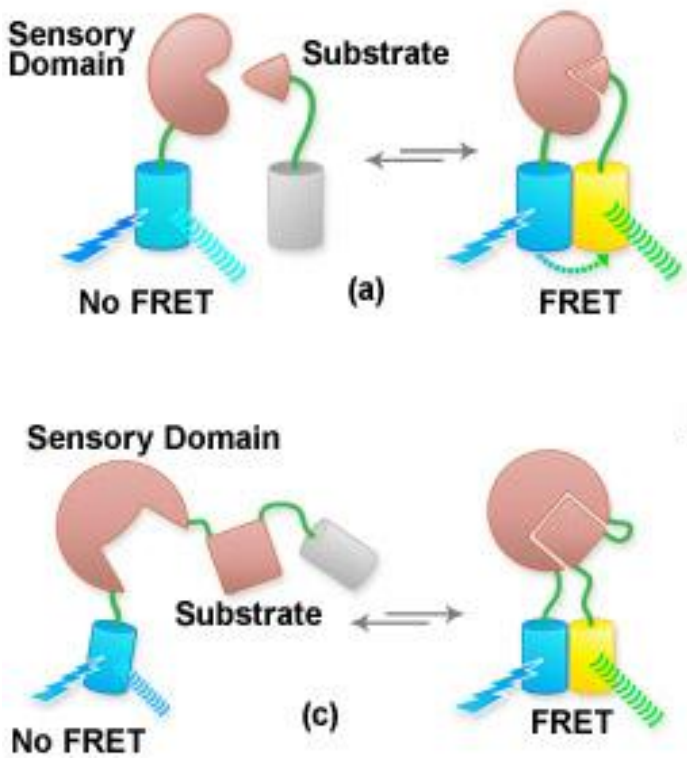
https://www.semrock.com/Data/Sites/1/semrockimages/drawings/fret_500px.jpg

What is FRET used for?

-Studying colocalization/interactions of two proteins

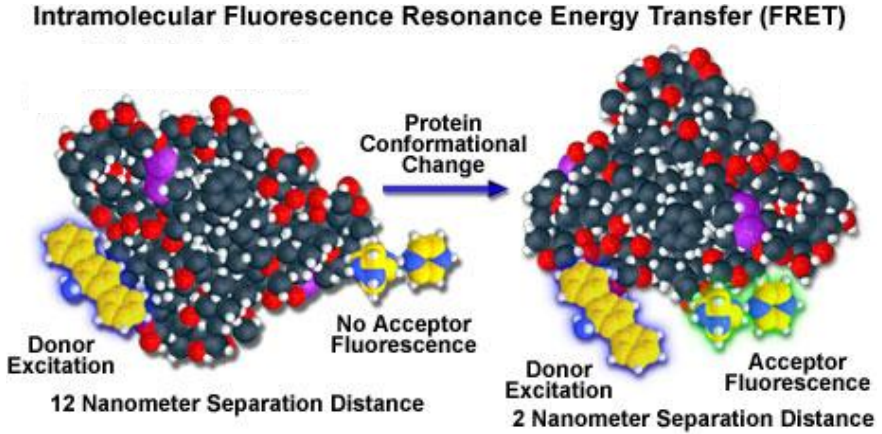
-Studying colocalization/interactions of a protein and a particular DNA sequence

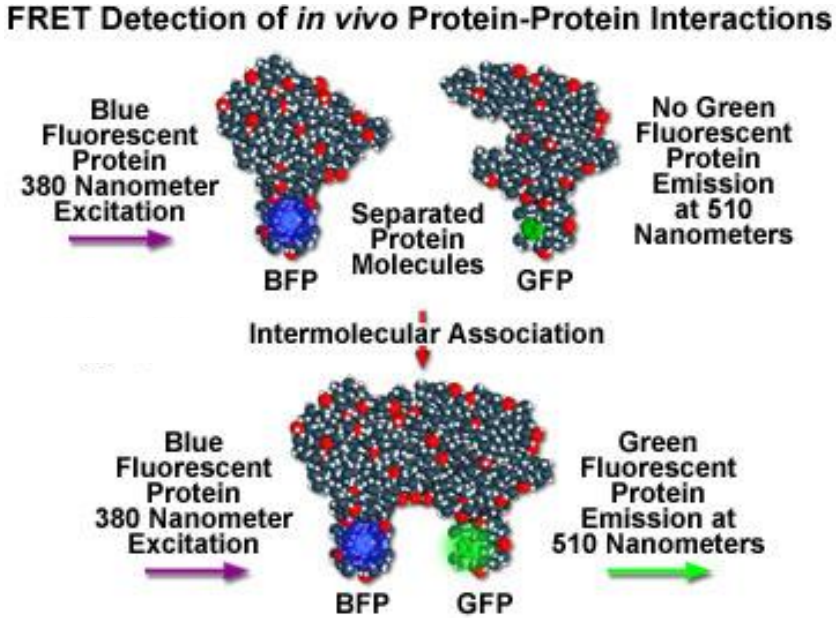
-Measuring intramolecular distance within one protein (structure and conformation)



http://zeiss-campus.magnet.fsu.edu/tutorials/spectralimaging/fretbiosensors/fretbiosensorsfigure1.jpg

Using FRET



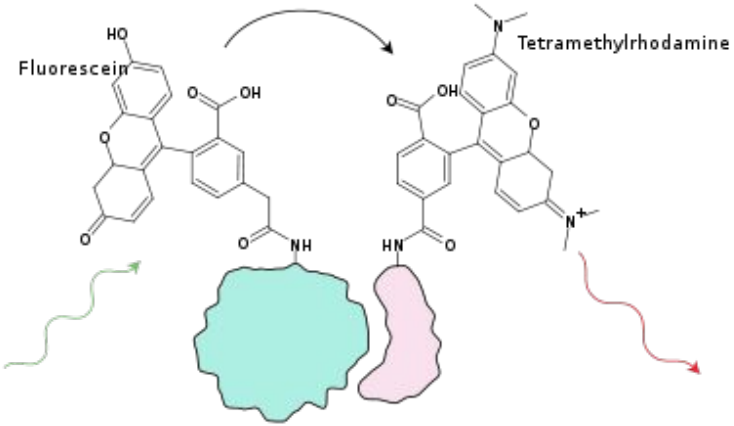


http://www.olympusmicro.com/primer/techniques/fluorescence/fret/images/fretintrofigure1.jpg

http://www.olympusmicro.com/primer/techniques/fluorescence/fret/images/fretintrofigure2.jpg

Fluorescent tagging: Post-Translational

- Donor chromophore and acceptor chromophore (donor-acceptor pair)
- Attach fluorescent markers to proteins post-translationally



Donor and Acceptor Fluorophores

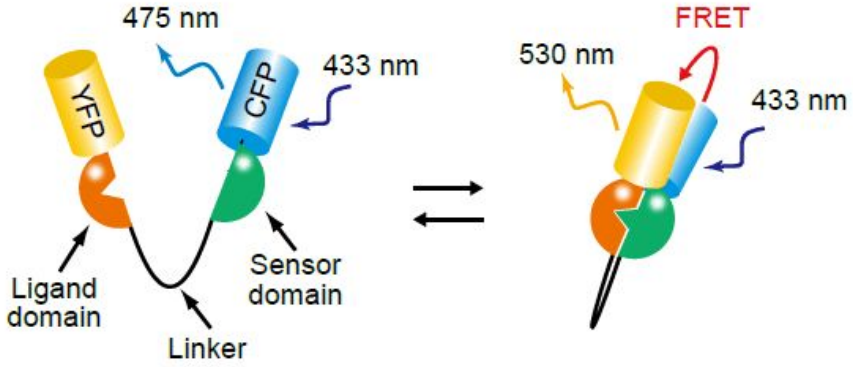
Donor and Acceptor Fluorescent Nucleotides

https://en.wikipedia.org/wiki/Fluorescent_glucose_biosensor#/media/File:Squid%27s_fret.svg

Fluorescent tagging: GFP Constructs

-GFP: Protein recombinant is constructed by genetic modification

-The recombinant proteins of interest are inherently fluorescent (do not need post-transcriptional attachment of fluorophores)

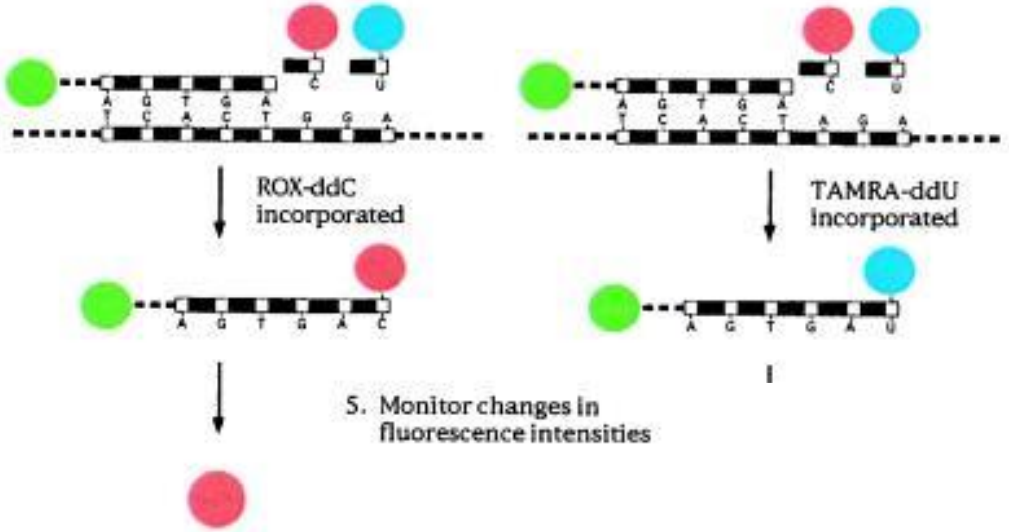


Donor and Acceptor Fluorescent Proteins

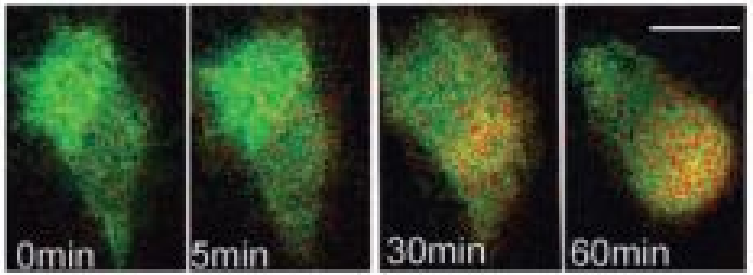
http://www.fret.lif.kyoto-u.ac.jp/e-phogemon/images/fig4103.gif

Examples of FRET in research

- Used to study the colocalization of glucocorticoid and mineralocorticoid receptors[3]
- Used to determine if a mutation is present on a gene for cystic fibrosis[1]



Chen, X. *et al.* (1997)



Nishi, M. *et al.* (2004)

Further Resources/References

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3. Nishi, M., Tanaka, M., Matsuda, K., Sunaguchi, M., Kawata, M. (2004). “Visualization of Glucocorticoid Receptor and Mineralocorticoid Receptor Interactions in Living Cells with GFP-Based Fluorescence Resonance Energy Transfer.” *The Journal of Neuroscience. 24(21):* 4918-4927. doi: 10.1523/JNEUROSCI.5495-03.2004
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5. Seegar, T., Barton, W. (2010). “Imaging Protein-protein Interactions *in vivo*.” *J. Vis. Exp. (44), e2149,* doi:10.3791/2149 (2010).
6. Sekar, R. B., Periasamy, A. (2003). “Fluorescence resonance energy transfer (FRET) microscopy imaging of live cell protein localizations.” *J. Cell. Biol. 160(5):* 629-633. DOI: 10.1083/jcb.200210140.
7. Sprenger, J. U., Perera, R. K., Götz, K. R., Nikolaev, V. O. (2012). “FRET Microscopy for Real-time Monitoring of Signaling Events in Live Cells Using Unimolecular Biosensors.” *J. Vis. Exp.* 66, *e4081* doi:10.3791/4081.