How to do FRET?

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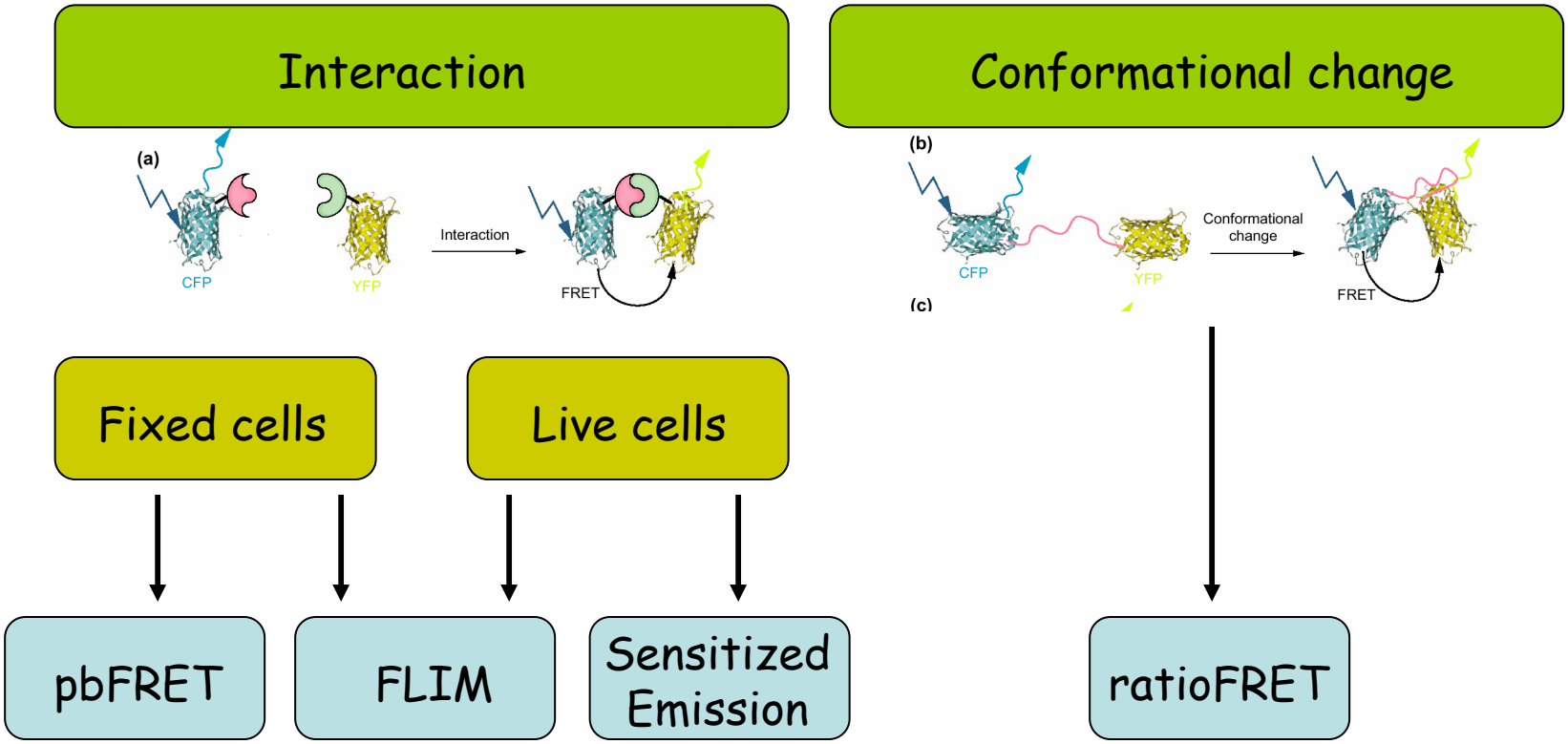
Which FRET pair should I use?



CFP YFP	++	− Double exponential decay of CFP +	++	++
GFP Cherry	n.d.	+++	− Weak acceptor fluorescence => difficult to detect changes	− Weak acceptor fluorescence => difficult to detect changes
YFP Cherry	n.d.	+++	−	−

How to prepare my constructs

FRET occurs in a distance range from 2 to ~8 nm. As smaller your proteins of interest are, as more likely it is having the fluorescent tags in a right distance range for FRET.

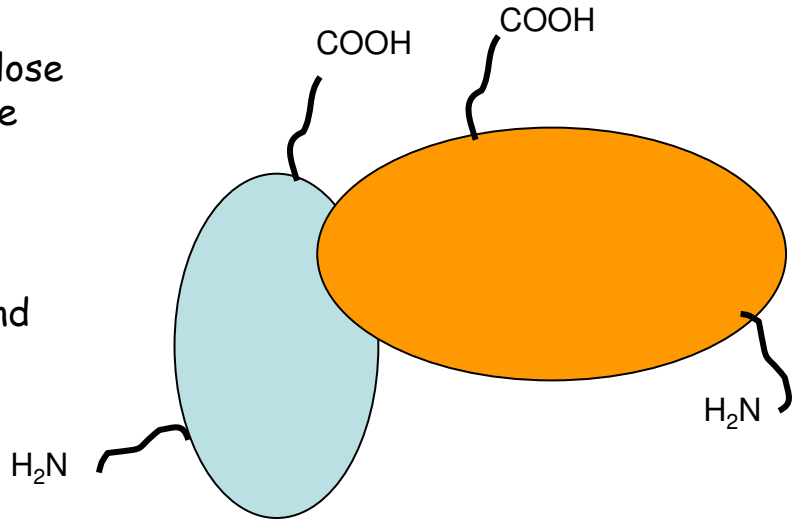
A Globular protein with a mass of ~ 50 kDa has a diameter of ~5 nm. However, also larger proteins con work. Just try it J .

But how should I start?

In most cases you don't know the structure of the proteins. But sometimes the interacting domains and their positions (close to C- or N-terminus) are known.

This is a good starting point and you should fuse the FP close to those domains. However, the tag can interfere with the function of your protein. Furthermore, maybe the other terminus of the protein is in a more favorable distance and/or orientation for FRET.

At the beginning always tag your proteins at both sites and check all 4 possible combination for a FRET signal.



Which protein should be tagged with the FRET donor?

If both proteins are expressed at similar levels then it doesn't matter. Otherwise, you should always tag the less abundant protein with the FRET donor.

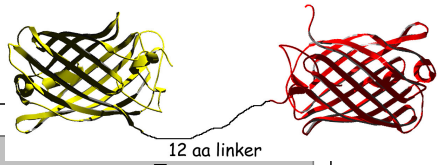
How does the mounting medium affect my measurements?

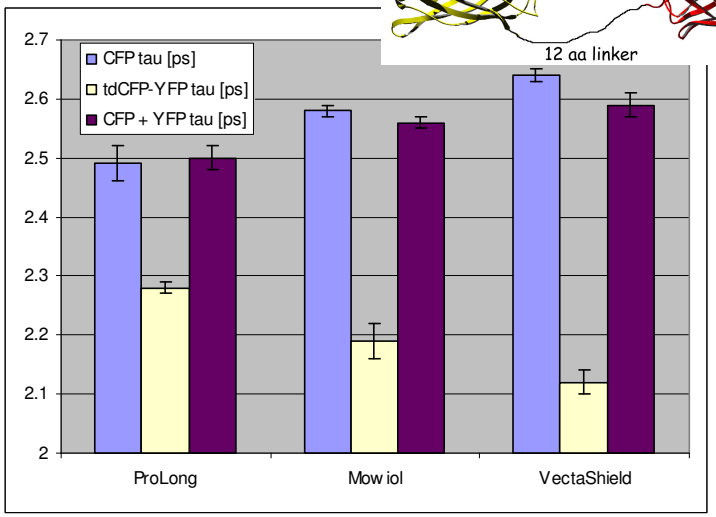
Some mounting media are glycerol-based (n ~1.47) and some are more aqueous (n ~1.33). Since the fluorescence lifetime is strongly sensitive to the direct environment, changes in the absolute numbers are expected.
Also the FRET efficiency is related to the refraction index n.

$$R_0 = 9790 \cdot \left(\kappa^2 \; Q_D \; J(\lambda) \; n^{-4}\right)^{\frac{1}{6}} \text{Å}$$

ProLong Gold Antifade	–
Mowiol	+
VectaShield	+

FLIM-FRET





pbFRET