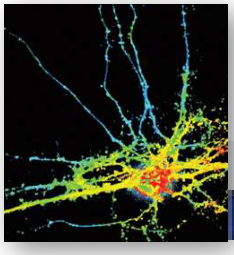
Bioimaging core facility
Faculté de Médecine
Université de Genève



How to visualize (the dynamics of) molecular interactions?

Förster or Fluorescence Resonance Energy Transfer (FRET)

Intramolecular FRET (biosensors)

METHOD

Ratiometric imaging approach
(Donor/Acceptor)

MICROSCOPES

- confocal: Nipkow/ spinning disk, Nikon A1r spectral/CLSM
- widefield: Bioflux

SOFTWARES

NIS-Elements, Metafluor, Metamorph, ImageJ

CONDITIONS

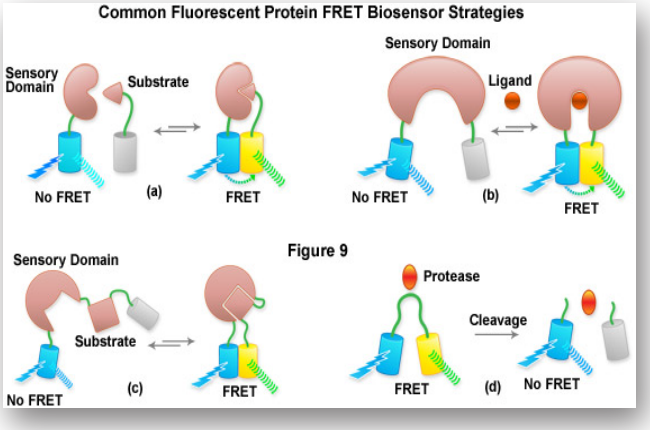
Key factors are:
- Distance A-D <100 Å (Ro)
- Overlap λem(Donor) - λex(Acceptor)
- Fluorophore orientation
- Biological context
- Transfection level

Controls are required to define the FRET efficiency (lowest and highest values, FRET range):
- positive control (high FRET efficiency, biosensor constitutively active)
- negative control (low FRET efficiency, biosensor constitutively inactive)

PROTOCOLE

Kardash et al. (2011). Imaging protein activity in live embryo using fluorescence resonance energy transfer biosensors. Nat. Protoc. Nov 3;6(12):1835-46

http://zeiss-campus.magnet.fsu.edu/articles/spectralimaging/spectralfret.html



Intermolecular FRET

METHODS

- **FRET by intensity measurement**
- FRET by Acceptor photobleaching
- FRET by Donor photobleaching
- FRET by lifetime measurement
  (TCSPC, Time Correlated Single Photon Counting)
- FRET by fluorescence anisotropy

MICROSCOPES

- **Confocal: Nikon A1r spectral/CLSM**
- FLIM (Fluorescence Lifetime Imaging Microscopy) by single-photon counting
- FCS/ FCCS
  (Fluorescence (Cross-) Correlation Spectroscopy)

SOFTWARES

NIS-Elements, ImageJ

CONDITIONS

Key factors are:
- Distance A-D <100 Å (Ro)
- Overlap λem(Donor) - λex(Acceptor)
- Stoechiometry D-A 1:1
- Fluorophore orientation
- Biological context
- Transfection level

Controls are required to define the FRET efficiency (lowest and highest values):
- D alone, A alone, D+A (constructs)

PROTOCOLE

https://www.unige.ch/medecine/bioimaging/en/information/tutorials/
and go to: "F-techniques> * How to perform FRET experiments?"

Broussard et al. (2013). Fluorescence resonance energy transfer microscopy as demonstarted by measuring the activation of the serine/threononce kinase Akt. Nat. Protoc. 8, Jan 10, 265-281.