# Cellular approaches for the detection and quantitation of protein-protein interactions : latest techniques and current limitations.

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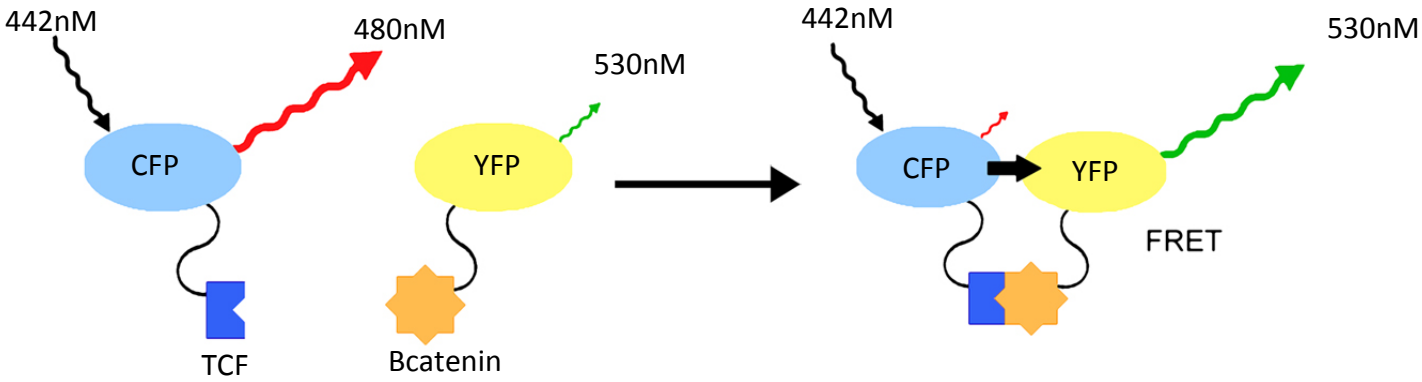
## Outline

1. Fluorescence resonance energy transfer (FRET)
2. Bioluminescence resonance energy transfer (BRET)
3. Protein fragment complement assays (PCA)
4. Others

# Fluorescence resonance energy transfer (FRET)

Mechanism:




Conditions:

1. Distance between two fluorophores must less than 10 nM
2. Donor emission spectra must overlap acceptor excitation spectra
3. The quantum yield of the donor must be high enough
4. Donor and acceptor transition dipole orientations must be approximately parallel

**CFP-YFP pairs**

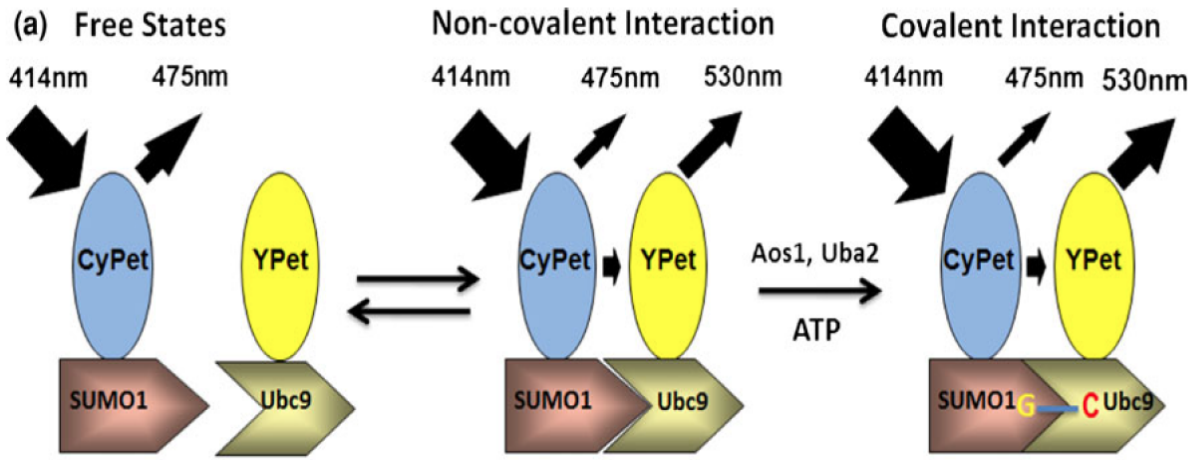
The most popular FRET pair for biological use is

cyan fluorescent protein (CFP) – yellow fluorescent protein (YFP) pair

Molecular Pharmacology, 2009, 75: 733-739.

# **Example:** Development of FRET Assay into Quantitative and High-through Screening Technology Platform for Protein-Protein Interactions

Mechanism:




CyPet and Ypet have much higher fluorescence quantum yield and FRET efficiency, 20 fold higher FRET signal than CFP-YFP.

Annals of Biomedical Engineering, 2010, 39(4): 1224–1234

# Procedure

1. Protein expression

| Protein | Vector |
|---|---|
| CyPet-SUMO1 | **pET28a** |
| Ypet-Ubc9 | **pET28a** |
| CyPet-SUMO1 | **pCDNA3.1hygro** |
| Ypet-Ubc9 | **pCDNA3.1 V5His** |

2.FRET assay and Kd Measurement

a. Protein CyPet-SUMO1 concentration: 1 µM
Protein Ypet-Ubc9 concentration: 0 to 4 µM

b. Fluorescence plate reader
Excitation wavelengths: 414 nm to excite CyPet
Emission peak: 530 nm

c. Potential interaction interference of tag
CyPet and Ypet may have a weak dimerization activity.

Kd = 0.33± 0.04µM
Reported Kd = 0.25± 0.07µM

3. Establish stable cell line expressing CyPet-SUMO1 and Ypet-Ubc9

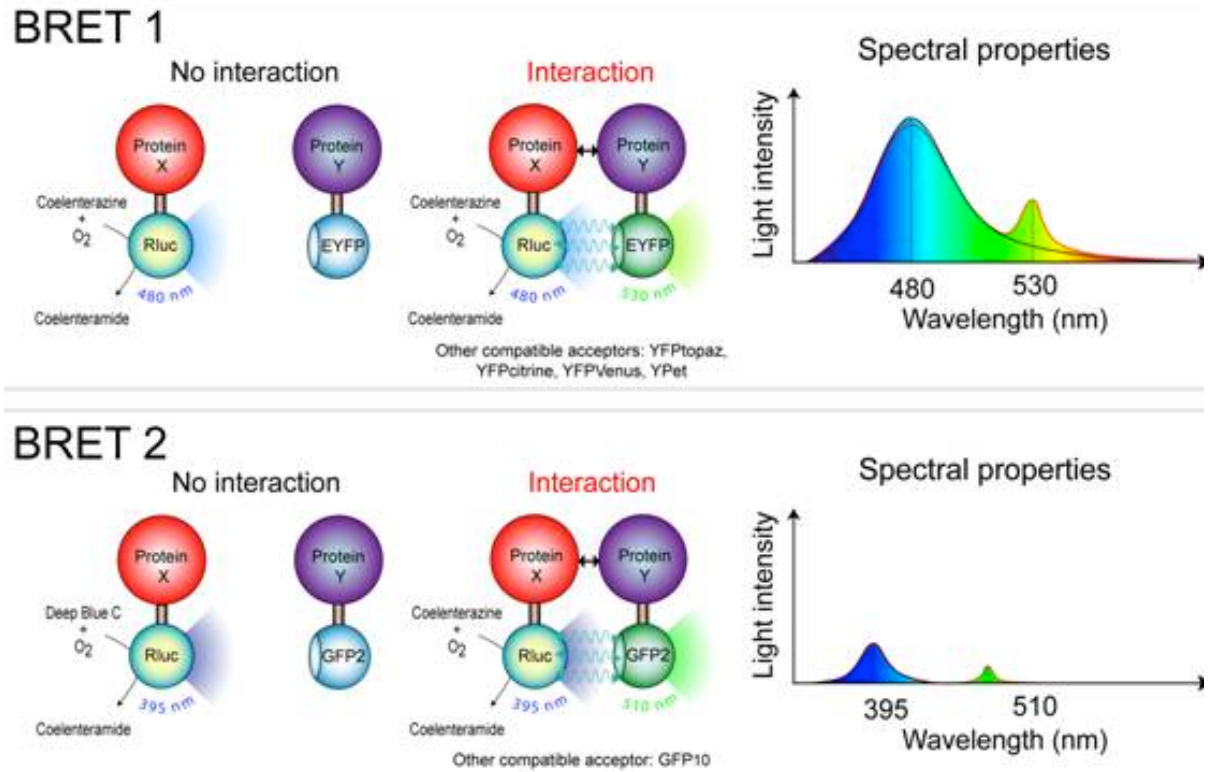
Emission ratio($Em_{530}/Em_{475}$) :
0.8-1.2 (1.14± 0.04)
Control cell ($_{CyPet-SUMO1}$): 0.4-0.6
(0.54± 0.04)

4. Compound Screening

2000 compounds
Hit standard: $Em_{530}/Em_{475}$)= 0.5± 0.45
136 hits ( filter out with self-fluorescence)
39 compounds

# Bioluminescence resonance energy transfer(BRET)

Mechanism:




Methods in Molecular biology,284: 271-286.

**Example**: Detecting protein-protein interactions in living cells: development of a bioluminescence resonance energy transfer assay to evaluate the PSD-95/NMDA receptor interaction

1. Proteins

C-terminal of NMDA receptor + GFP

PDZS of PSD-95 + Rluc

2. Cells

COS7 cells

3. BRET assay

Transient transfection: equal amount of donor and acceptor plasmids.

Emission fliters:

410nm(80nm bandwidth)

515nm(40 nm bandwidth).

BRET signal = $(E_{515}$- background $_{515})/(E_{410}$- background$_{410})$

= 134 ± 49 to 375± 23

4. Confirmation of specific interaction

Mutant NMDA(no binding motif)+ GFP

Or PDZS of PSD-95(no binding motif) + Rluc

BRET signal = 21±15 to 101±36

5. Effect of a peptide

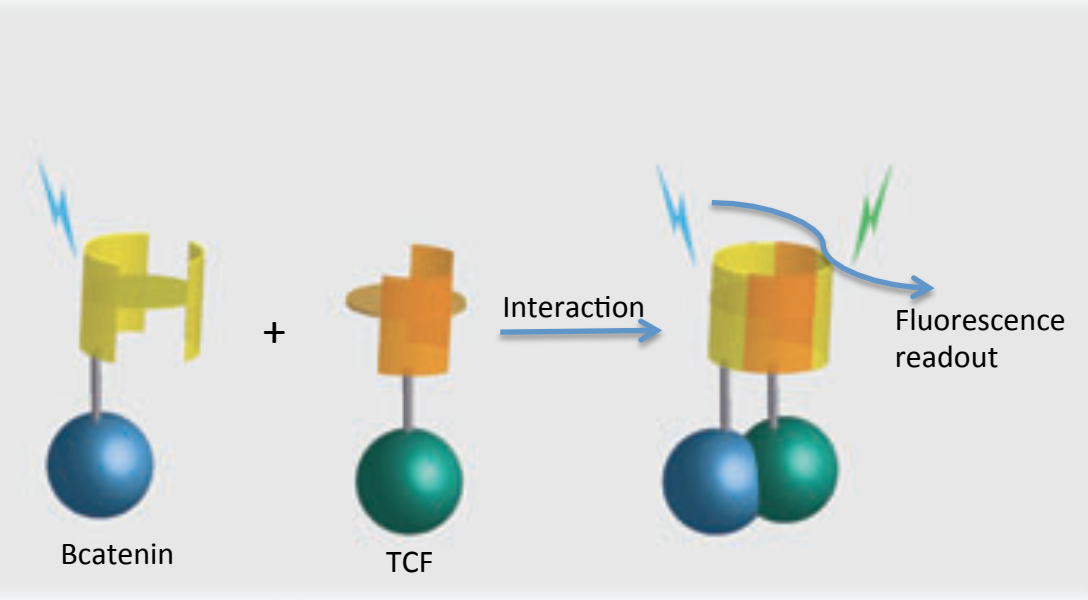
Peptide: 100 µM

BRET signal decrease 53±4%

Neurochem Res (2009) 34:1729–1737

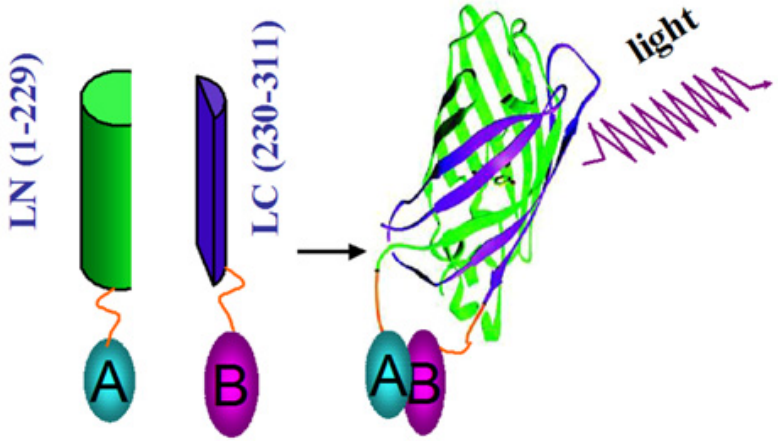
# Protein fragment complement assays (PCA)

Mechanism:




Nature Reviews, 2007, 6: 569-582.

# **Example**: Application of a split luciferase complementation assay for the detection of viral protein-protein interactions




1. Protein: Renilla luciferase(RL)
   RL N-terminal 1-229 + influenza B PA
   RL C-terminal 230-311 + influenza B PB

2. Cell: COS1 cell

3. Transfection: transient transfection
   euqal amount of plasmids (0.5ug)

4. Result:
   Signal: around 40 fold increase of luciferase activity against the background

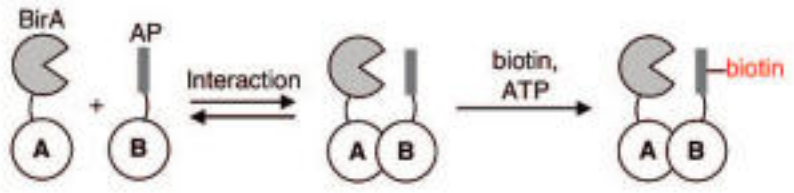
5. Confirmation

   Mutant PA or PB(site-directed mutagenesis)
   Signal decrease 65% to 73%

Journal of Virological Methods 2011, 176:108-111

# Others

- Proximity Biotinylation




**Enzyme / substrate pair**
Enzyme: the E.Coli enzyme biotin ligase (BirA)
Substrate: BirA's acceptor peptide (AP) substrate

JACS, 2008,130: 9251-9253.