Use of an Nluc-BRET system to investigate ligand binding to the human β_1-adrenoceptor

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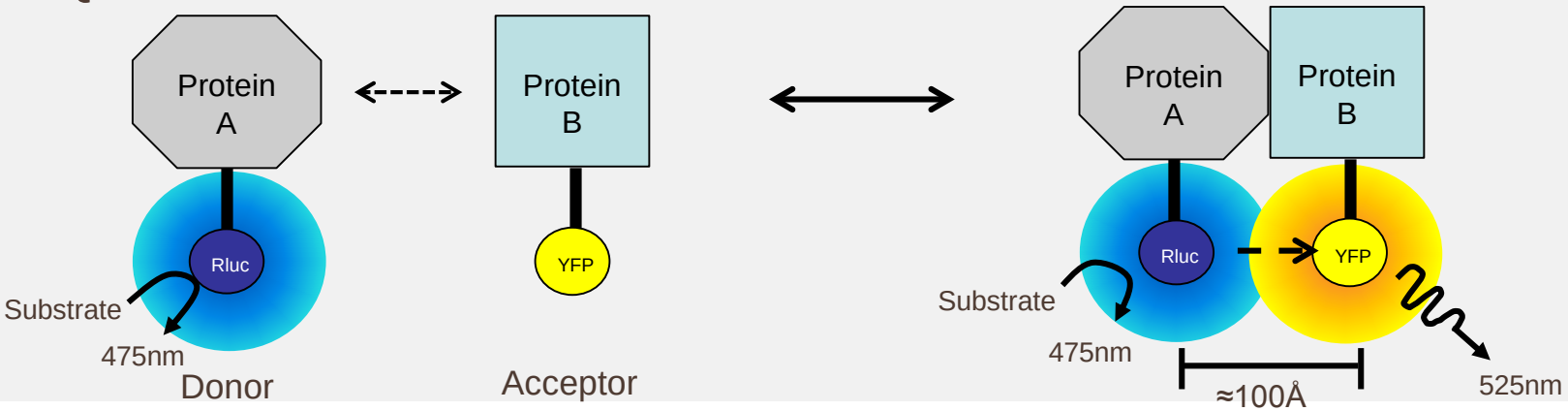
What is BRET?

- Bioluminescence Resonance Energy Transfer
- Non-radiative energy transfer from Donor luciferase to Acceptor fluorophore
- Quantified as ratio of fluorescence/luminescence



Why use BRET?

- Donor and acceptor must be in close proximity (up to 10nm)
  - Greatly reduces non-specific background signal

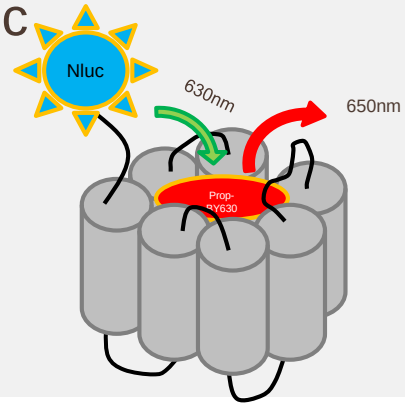
$$E = \frac{1}{1+\left(\frac{r}{R_0}\right)^6}$$

- No external excitation required:
  - Energy from the oxidation of luminescent substrate
  - No photobleaching of acceptor fluorophore
  - Unlike FRET, no simultaneous excitation of donor and acceptor fluorophores

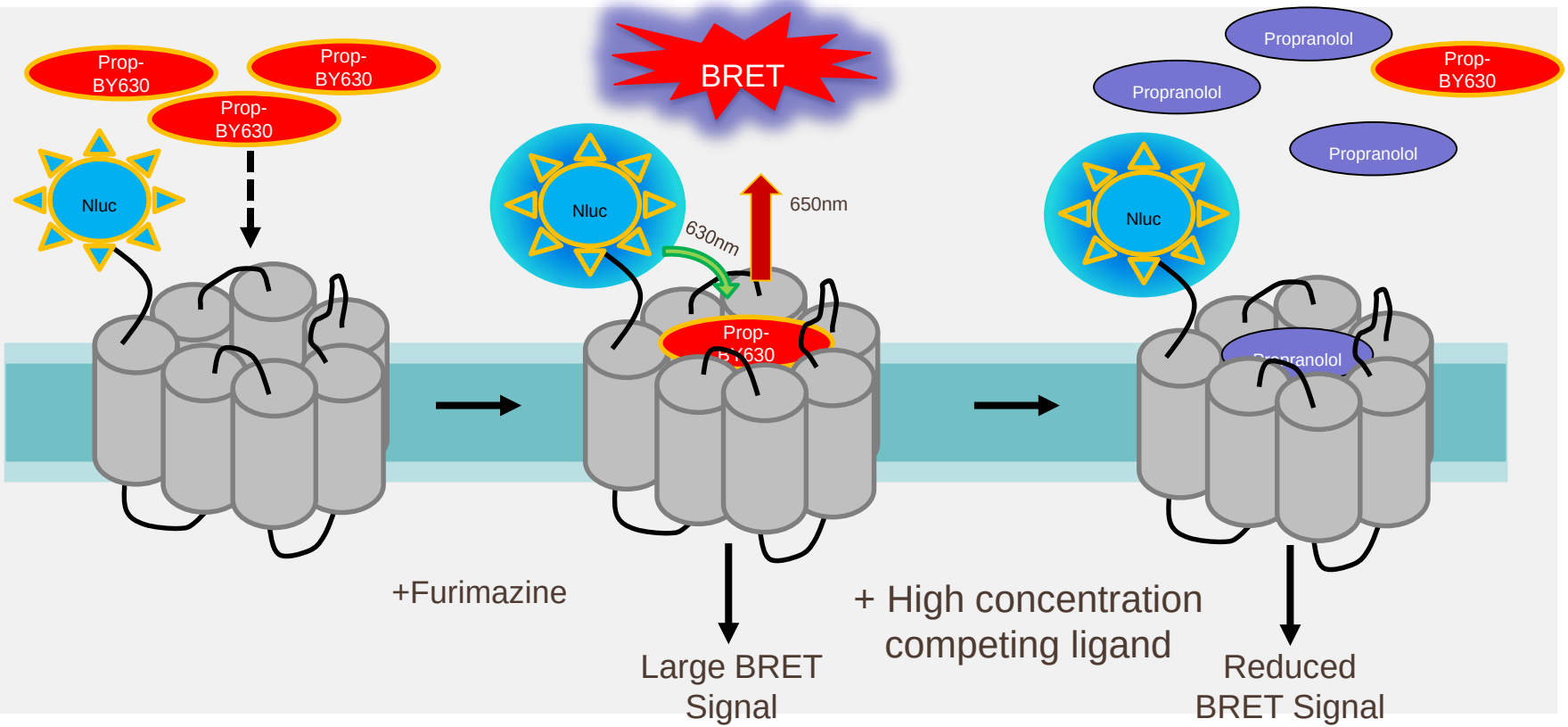
NlucBRET

- Nanoluc (Nluc) is a novel, very bright 19kDa luciferase developed by Promega from the deep sea shrimp *Oplophoros gracilirostris*

- Human β_1-adrenoceptor tagged at N-terminus with Nluc and expressed in HEK293 cells

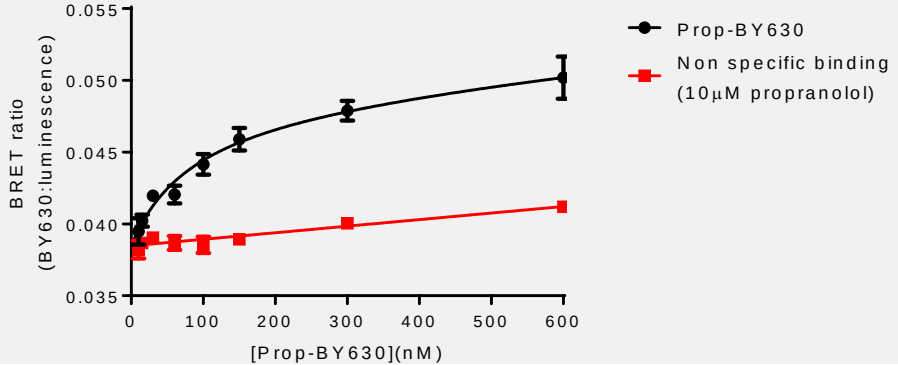


- Measured ligand binding with a propranolol-derived fluorescent ligand (Prop-BY630) on Pherastar plate reader (BMG Labtech)


Prop-BY630
Prop-BY630
Prop-BY630
Nluc
+Furimazine
BRET
Nluc
630nm
650nm
Prop-BY630
Large BRET Signal
Propranolol
Propranolol
Propranolol
Prop-BY630
Nluc
+ High concentration competing ligand
Reduced BRET Signal

Saturation binding

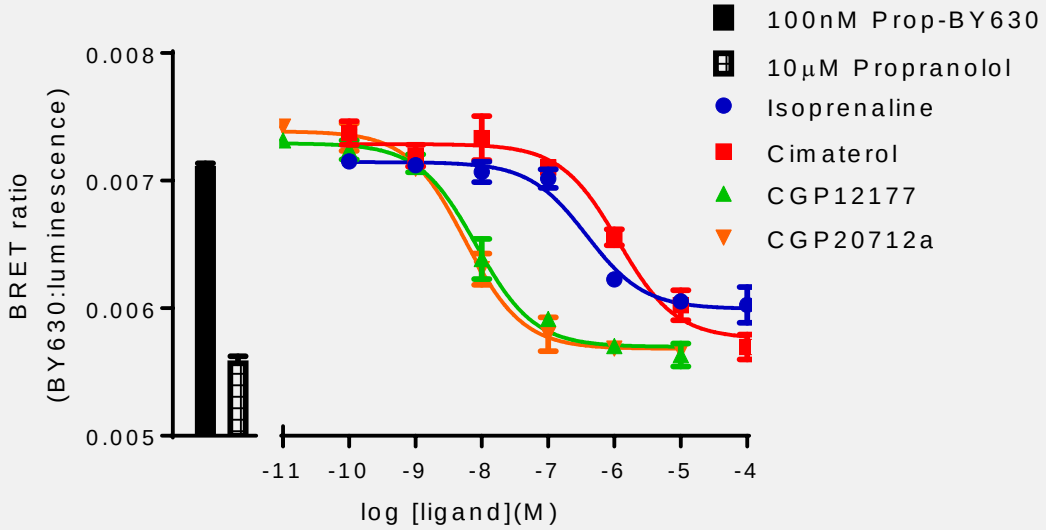
- Simultaneous addition of a range of Prop-BY630 concentrations with and without a fixed concentration of propranolol
- Plate incubated for 60 minutes at 37ºC in dark
- BRET ratio defined as fluorescence/luminescence



pK_D 7.06±0.08
n= 8

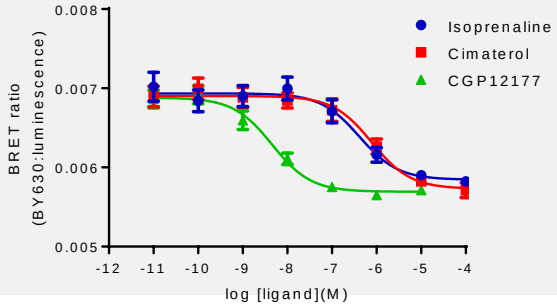
Competition binding

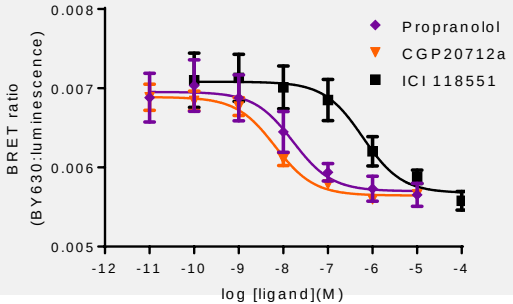
- Simultaneous addition of 100nM Prop-BY630 and competing β-adrenoceptor ligands in a range of concentrations



Competition binding

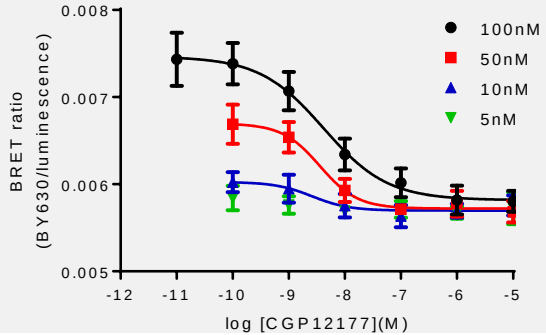
Compound	Nluc pK_i (mean ± SEM)	n	Radioligand pK_i (mean ± SEM)
ICI 118551	6.55 ± 0.19	4	6.12 ± 0.13
Cimaterol	6.44 ± 0.11	7	6.13 ± 0.05
Isoprenaline	6.72 ± 0.12	7	6.29 ± 0.07
Propranolol	8.11 ± 0.12	4	8.04 ± 0.09
CGP 20712a	8.52 ± 0.09	7	8.04 ± 0.12
CGP 12177	8.67 ± 0.05	7	8.87 ± 0.07





Competition binding

- Simultaneous addition of increasing concentrations of Prop-BY630 and CGP12177 in a range of concentrations
- Increasing [Prop-BY630] resulted in an increase in BRET ratio with no change in affinity, representing increased proportion of receptor occupancy



[Prop-BY630]	pK_i (mean ± SEM)	BRET ratio (mean ± SEM)
100nM	8.69 ± 0.12	0.0075 ± 0.0002
50nM	8.62 ± 0.18	0.0069 ± 0.0001
10nM	8.83 ± 0.29	0.0060 ± 0.0001
5nM	-	-

Conclusions

- Developed a BRET assay to monitor fluorescent ligand binding to human β_1-adrenoceptor
- Specific binding was able to be displaced by 10μM propranolol
- Nluc fluorescent ligand binding closely match radioligand binding data in the same cellular environment

Acknowledgements

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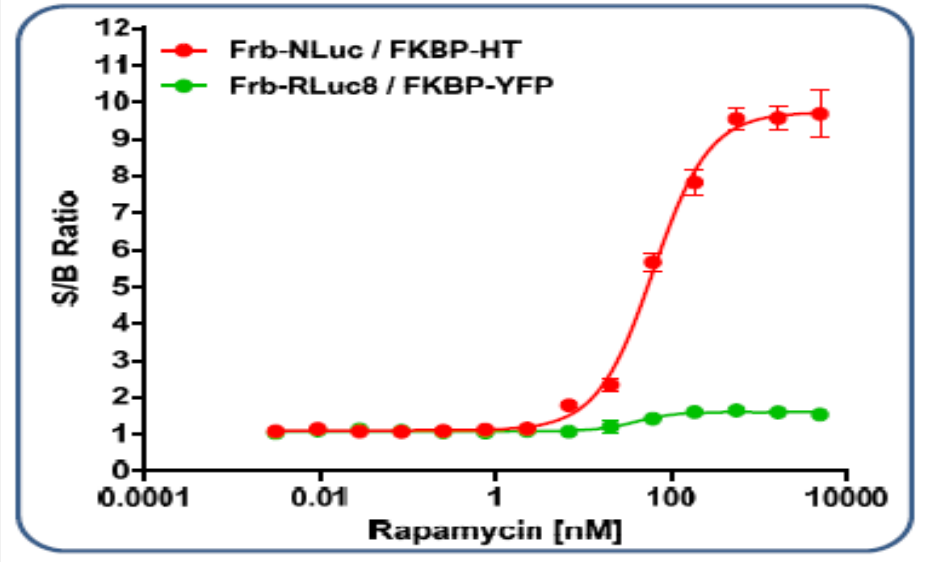
E is the BRET efficiency

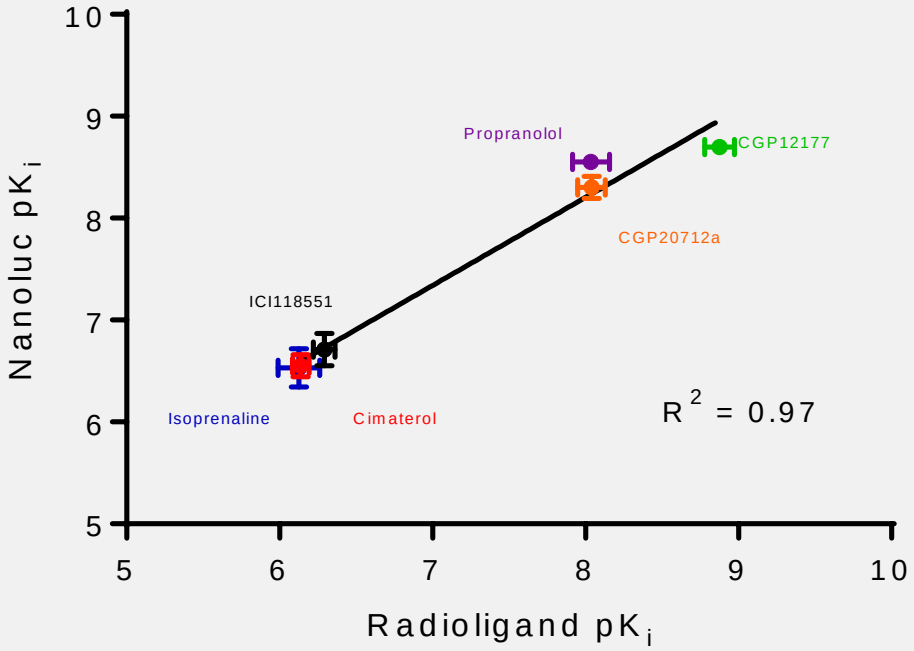
$$E = \frac{1}{1+\left(\frac{r}{R_0}\right)^6}$$

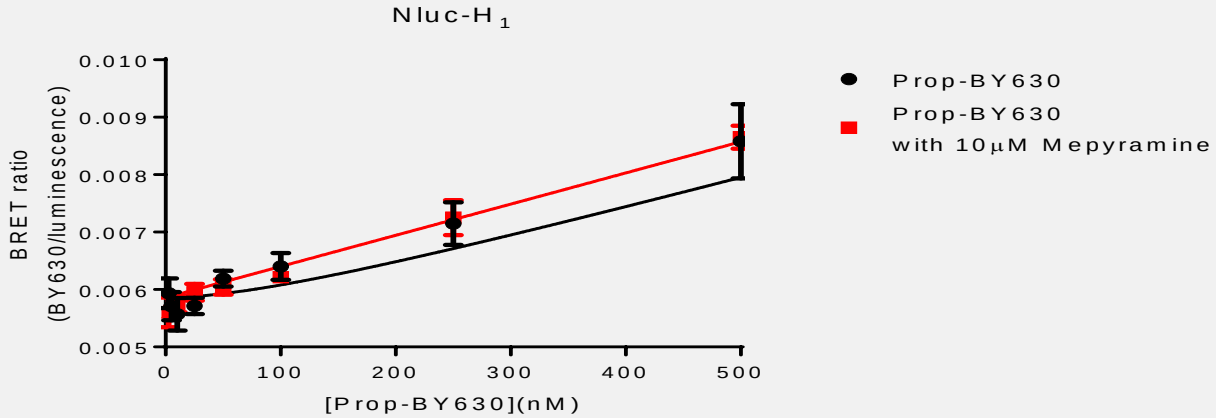
r is the donor-acceptor separation distance

R_0 is the Föster distance of the donor-acceptor pair. It depends on:

- Quantum yield of donor
- Dipole orientation
- Overlap between donor emission spectrum and acceptor excitation spectrum


Frb-NLuc / FKBP-HT
Frb-RLuc8 / FKBP-YFP
S/B Ratio
0
1
2
3
4
5
6
7
8
9
10
11
12
0.0001
0.01
1
100
10000
Rapamycin [nM]


10
9
8
7
6
5
5
6
7
8
9
10
Nanoluc pK$_i$
Radioligand pK$_i$
Propranolol
CGP12177
CGP20712a
ICI118551
Isoprenaline
Cimaterol
R^2 = 0.97


Nluc-H1
BRET ratio
(BY630/luminescence)
0.010
0.009
0.008
0.007
0.006
0.005
0
100
200
300
400
500
[Prop-BY630](nM)
Prop-BY630
Prop-BY630
with 10μM Mepyramine