Supporting Material

Third-party BRET indicates constitutive association of membrane proteins: application to class A GPCRs and G proteins

Sudhakiranmayi Kuravi, Tien-Hung Lan, Arnab Barik, and Nevin A. Lambert

Supporting Information

Third-party BRET indicates constitutive association of membrane proteins: application to class A GPCRs and G proteins

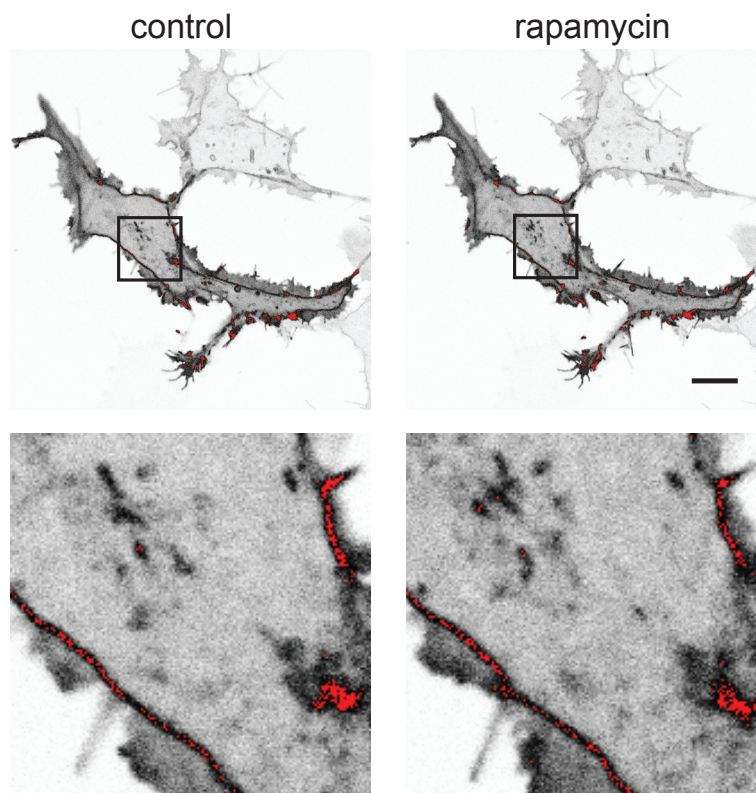Sudhakiranmayi Kuravi, Tien-Hung Lan, Arnab Barik and Nevin A. Lambert

Figure S1. Formation of mem-zip-Rluc8:mem-zip-FRB:mem-l-FKBP-V ternary complexes does not redistribute the third-party acceptor mem-l-FKBP-V. Confocal images of HEK 23 cells before (control) and after (rapamycin) addition of 5 μM rapamycin. Squares in the top panels correspond to the expanded regions shown in the bottom panels. Red pixels are intentionally saturated, and the comparable number of saturated pixels at the plasma membrane before and after addition rapamycin indicates that this third-party acceptor was not recruited to this compartment. Calibration bar = 10 μm.
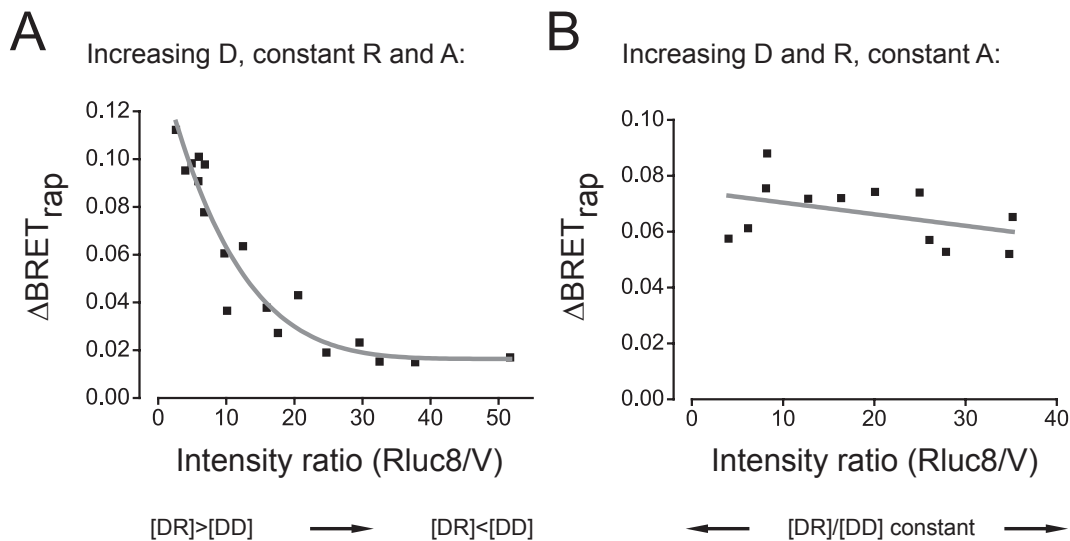
Figure S2. Third-party BRET is predictably sensitive to changes in the donor:recruiter ratio, but not to changes in total expression of proteins of interest. (*A*) Rapamycin-induced BRET ($\Delta BRET_{rap}$) is plotted against Rluc8/V intensity in cells expressing an increasing amount of the donor (D) mem-zip-Rluc8 together with a constant amount of the recruiter (R) mem-zip-FRB and acceptor (A) mem-link-FKBP-V. When donors are relatively scarce, most will associate with a recruiter and contribute to $\Delta BRET_{rap}$. When donors are present in excess, most will associate with each other, and will be unable to contribute to $\Delta BRET_{rap}$. The smooth line is a least-squares fit to a hyperbolic function. (*B*) $\Delta BRET_{rap}$ plotted across the same range of Rluc8/V intensity as in *A* in cells expressing an increasing amount of the donor *and* recruiter, together with a constant amount of the acceptor. $\Delta BRET_{rap}$ was relatively constant across an approximately 10-fold increase in donor expression, suggesting the fraction of donors associated with recruiters remained constant.
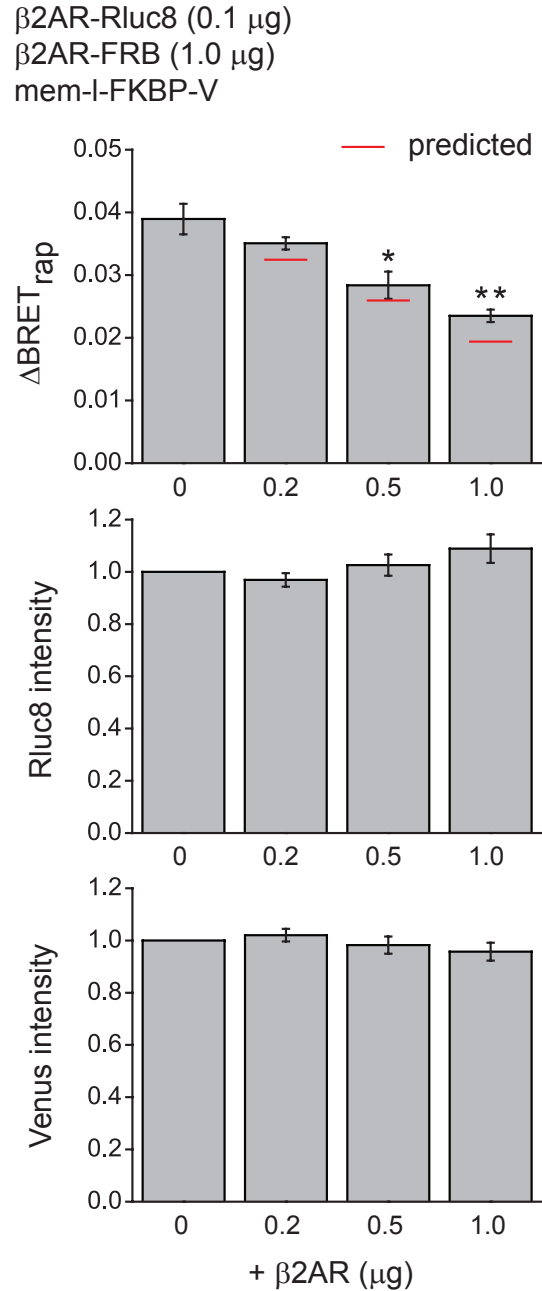
Figure S3. Third-party BRET involving β2AR-Rluc8 and β2AR-FRB is sensitive to competition from unlabeled β2AR. $\Delta BRET_{rap}$, β2AR-Rluc8 intensity, and venus intensity are plotted against the amount of cotransfected DNA expressing unlabeled β2AR (*n*=8). $\Delta BRET_{rap}$ decreases as β2AR expression increases, whereas Rluc8 and venus intensity are unchanged. Horizontal red lines indicate the predicted level of $\Delta BRET_{rap}$ assuming all β2AR constructs express at levels proportional to the amount of DNA transfected, and all β2AR proteins associate randomly; *, *P*<0.05; **, *P*<0.001.

Table S1. Basal and rapamycin-induced BRET between engineered membrane-associated proteins (± SEM).

third-party acceptor/donor	recruiter	donor/acceptor	BRET$_{basal}$	ΔBRET$_{rap}$	P	n
mem-l-FKBP-V	mem-zip-FRB	mem-zip-Rluc8	0.14±0.02	0.07±0.01	<0.00003	7
mem-l-FKBP-V	mem-zip-FRB	mem-l-Rluc8	0.06±0.01	0.00±0.00	0.552	7
mem-l-FKBP-V	mem-l-FRB	mem-zip-Rluc8	0.13±0.01	0.01±0.00	0.072	8
mem-l-FKBP-V	mem-l-FRB	mem-l-Rluc8	0.05±0.00	0.00±0.00	0.718	4
mem-l-FRB-V	mem-zip-FKBP	mem-zip-Rluc8	0.13±0.02	0.05±0.00	<0.00001	7
mem-l-FKBP-Rluc8	mem-zip-FRB	mem-zip-V	0.09±0.01	0.08±0.01	<0.00002	7

Table S2. Basal and rapamycin-induced BRET between mem-l-FKBP-Rluc8/mem-zip-FRB and various membrane protein acceptors (± SEM).

third-party donor	recruiter	acceptor	BRET$_{basal}$	ΔBRET$_{rap}$	P	n
mem-l-FKBP-Rluc8	mem-zip-FRB	mem-zip-V	0.11±0.01	0.12±0.01	<0.0004	5
		D2R-V	0.07±0.01	-0.01±0.00	0.009	5
		GIRK1/2-V	0.02±0.00	-0.00±0.00	0.129	5
		C-TM-V	0.05±0.00	-0.01±0.00	0.015	5
		masGRKct-V	0.14±0.01	-0.03±0.01	0.002	5
		caveolin1-V	0.02±0.00	-0.01±0.00	0.011	5
		G$\beta_1\gamma_2$-V	0.02±0.00	-0.01±0.00	0.025	5
		Gα_{oA}-V	0.04±0.00	-0.00±0.00	0.042	5
		V-kras	0.18±0.01	-0.01±0.01	0.099	5

Table S3. Basal and rapamycin-induced BRET between mem-l-FKBP-V/β2AR-FRB and various membrane protein acceptors (± SEM).

third-party acceptor	recruiter	donor	BRET$_{basal}$	ΔBRET$_{rap}$	P	n
mem-l-FKBP-V	β2AR-FRB	β2AR-Rluc8	0.22±0.02	0.05±0.01	0.001	5
		mem-l-Rluc8	0.13±0.02	0.00±0.00	0.103	5
		Rluc8-kras	0.12±0.01	0.00±0.00	0.683	4
		Rluc8-PH	0.16±0.02	-0.00±0.00	0.603	5
		masGRKct-Rluc8	0.20±0.02	-0.01±0.00	0.236	5

Table S4. Basal and rapamycin-induced BRET between mem-l-FKBP-V/β2AR-FRB and various class A GPCRs (± SEM).

third-party acceptor	recruiter	donor	BRET$_{basal}$	ΔBRET$_{rap}$	P	n
mem-l-FKBP-V	β2AR-FRB	β2AR-Rluc8	0.18±0.01	0.04±0.00	<0.0001	10
		D1R-Rluc8	0.19±0.00	0.01±0.00	<0.0001	5
		D2R-Rluc8	0.14±0.01	-0.00±0.00	0.650	5
		α2AR-Rluc8	0.14±0.01	0.01±0.00	<0.0001	5
		M3R-Rluc8	0.27±0.01	-0.03±0.00	0.0002	5
		M4R-Rluc8	0.14±0.00	-0.02±0.00	<0.0001	5
		MOR-Rluc8	0.01±0.00	0.02±0.00	<0.0001	5
		CB1R-Rluc8	0.08±0.00	0.02±0.00	0.0003	5
		mem-l-Rluc8	0.07±0.00	0.00±0.00	0.330	5

Table S5. Basal and rapamycin-induced BRET between mem-l-FKBP-Rluc8/FRB-G$\beta_1\gamma_2$ and various acceptors (± SEM).

third-party donor	recruiter	acceptor	BRET$_{basal}$	ΔBRET$_{rap}$	P	n
mem-l-FKBP-Rluc8	FRB-Gγ_2+Gβ_1	Gα_{oA}-V	0.02±0.00	0.05±0.00	0.001	4
	FRB-Gγ_2+Gβ_1	masGRKct-V	0.05±0.00	0.03±0.00	<0.0006	4
	FRB-Gγ_2+Gβ_1	V-GIRK1+GIRK2	0.00±0.00	0.01±0.00	<0.0001	3
	FRB-Gγ_2+Gβ_1+Gα_{i1}	α2AR-V	0.04±0.01	-0.01±0.00	0.014	4
	FRB-Gγ_2+Gβ_1+Gα_{i1}	mem-zip-V	0.07±0.01	-0.01±0.00	0.003	4
	FRB-Gγ_2+Gβ_1+Gα_{i1}	V-kras	0.11±0.02	-0.02±0.00	0.003	4

Table S6. Basal Em535/480 and rapamycin-induced BRET between engineered membrane-associated proteins calculated with propagated errors (± 95% confidence).

third-party acceptor/donor	recruiter	donor/acceptor	Em535/480$_{basal}$	Em535/480$_{rap}$	ΔBRET$_{rap}$	*n*
mem-l-FKBP-V	mem-zip-FRB	mem-zip-Rluc8	0.54±0.01	0.61±0.01	0.07±0.01	7
mem-l-FKBP-V	mem-zip-FRB	mem-l-Rluc8	0.46±0.00	0.46±0.00	0.00±0.01	7
mem-l-FKBP-V	mem-l-FRB	mem-zip-Rluc8	0.55±0.01	0.56±0.01	0.01±0.01	8
mem-l-FKBP-V	mem-l-FRB	mem-l-Rluc8	0.45±0.00	0.45±0.00	0.00±0.01	4
mem-l-FRB-V	mem-zip-FKBP	mem-zip-Rluc8	0.56±0.01	0.61±0.01	0.05±0.01	7
mem-l-FKBP-Rluc8	mem-zip-FRB	mem-zip-V	0.50±0.01	0.59±0.01	0.10±0.01	7

Table S7. Basal Em535/480 and rapamycin-induced BRET between mem-l-FKBP-Rluc8/mem-zip-FRB and membrane protein acceptors calculated with propagated errors (± 95% confidence).

third-party donor	recruiter	acceptor	Em535/480$_{basal}$	Em535/480$_{rap}$	ΔBRET$_{rap}$	*n*
mem-l-FKBP-Rluc8	mem-zip-FRB	mem-zip-V	0.51±0.00	0.63±0.00	0.12±0.01	5
		D2R-V	0.47±0.01	0.46±0.01	-0.01±0.02	5
		GIRK1/2-V	0.43±0.01	0.43±0.01	-0.00±0.01	5
		C-TM-V	0.46±0.01	0.46±0.01	-0.01±0.01	5
		masGRKct-V	0.55±0.01	0.52±0.01	-0.03±0.01	5
		caveolin1-V	0.43±0.01	0.42±0.01	-0.01±0.01	5
		G$\beta_1\gamma_2$-V	0.43±0.01	0.42±0.01	-0.00±0.01	5
		Gα_{oA}-V	0.44±0.00	0.44±0.00	-0.00±0.01	5
		V-kras	0.60±0.02	0.57±0.01	-0.03±0.02	5

Table S8. Basal Em535/480 and rapamycin-induced BRET between mem-l-FKBP-V/β2AR-FRB and membrane protein acceptors calculated with propagated errors (± 95% confidence).

third-party acceptor	recruiter	donor	Em535/480$_{basal}$	Em535/480$_{rap}$	ΔBRET$_{rap}$	*n*
mem-l-FKBP-V	β2AR-FRB	β2AR-Rluc8	0.60±0.01	0.65±0.01	0.05±0.01	5
		mem-l-Rluc8	0.52±0.00	0.52±0.00	0.00±0.00	5
		Rluc8-kras	0.65±0.00	0.65±0.01	0.00±0.01	4
		Rluc8-PH	0.56±0.00	0.55±0.00	-0.00±0.00	5
		masGRKct-Rluc8	0.58±0.00	0.58±0.00	-0.01±0.01	5

Table S9. Basal Em535/480 and rapamycin-induced BRET between mem-l-FKBP-V/β2AR-FRB and various class A GPCRs calculated with propagated errors (± 95% confidence).

third-party acceptor	recruiter	donor	Em535/480$_{basal}$	Em535/480$_{rap}$	ΔBRET$_{rap}$	*n*
mem-l-FKBP-V	β2AR-FRB	β2AR-Rluc8	0.58±0.01	0.62±0.01	0.04±0.01	10
		D1R-Rluc8	0.59±0.00	0.60±0.00	0.01±0.01	5
		D2R-Rluc8	0.55±0.01	0.55±0.01	0.00±0.01	5
		α2AR-Rluc8	0.54±0.01	0.56±0.01	0.01±0.01	5
		M3R-Rluc8	0.67±0.01	0.64±0.01	-0.03±0.02	5
		M4R-Rluc8	0.54±0.01	0.52±0.01	-0.02±0.01	5
		MOR-Rluc8	0.41±0.01	0.43±0.01	0.02±0.01	5
		CB1R-Rluc8	0.49±0.02	0.50±0.01	0.02±0.02	5
		mem-l-Rluc8	0.47±0.00	0.47±0.00	0.00±0.01	5

Table S10. Basal Em535/480 and rapamycin-induced BRET between mem-l-FKBP-Rluc8/FRB-G$\beta_1\gamma_2$ and acceptors calculated with propagated errors (± 95% confidence).

third-party donor	recruiter	acceptor	Em535/480$_{basal}$	Em535/480$_{rap}$	ΔBRET$_{rap}$	*n*
mem-l-FKBP-Rluc8	FRB-Gγ_2+Gβ_1	Gα_{oA}-V	0.42±0.00	0.47±0.00	0.05±0.01	4
	FRB-Gγ_2+Gβ_1	masGRKct-V	0.45±0.01	0.48±0.01	0.03±0.01	4
	FRB-Gγ_2+Gβ_1	V-GIRK1+GIRK2	0.40±0.01	0.41±0.01	0.01±0.01	3
	FRB-Gγ_2+Gβ_1+Gα_{i1}	α2AR-V	0.44±0.01	0.43±0.01	-0.01±0.01	4
	FRB-Gγ_2+Gβ_1+Gα_{i1}	mem-zip-V	0.47±0.00	0.46±0.00	-0.01±0.01	4
	FRB-Gγ_2+Gβ_1+Gα_{i1}	V-kras	0.51±0.01	0.49±0.01	-0.02±0.01	4

Table S11. Rapamycin-induced BRET between GPCRs and G proteins calculated with propagated errors (± 95% confidence).

GPCR	control	+agonist	GXP-depleted	depleted+agonist	+GTPγS	*n*
α2AR-V	-0.01±0.01	0.00±0.01	-0.01±0.01	0.05±0.01	-0.01±0.01	5
α2AR-Rluc8	-0.01±0.01	0.01±0.01	-0.01±0.01	0.04±0.01	0.01±0.01	4
α2AR-FRB	-0.00±0.01	0.01±0.01	-0.01±0.01	0.02±0.01	0.01±0.01	4
D2R-Rluc8	-0.02±0.01	-0.00±0.01	-0.02±0.01	0.01±0.01	-0.01±0.02	3
M4R-Rluc8	-0.03±0.01	0.01±0.01	-0.04±0.01	0.02±0.01	-0.03±0.02	3
CB1R-Rluc8	0.00±0.03	0.03±0.03	0.02±0.02	0.06±0.03	0.02±0.04	3

Table S12. Agonist-induced BRET between GPCRs and G proteins calculated with propagated errors (± 95% confidence).

GPCRs	UK 14,304	carbachol	UK 14,304+carbachol	*n*
α2AR-Rluc8 + M4R	0.01±0.01	-0.00±0.01	0.00±0.01	8
α2AR + M4R-Rluc8	-0.00±0.01	0.02±0.01	0.00±0.01	8