biochemical and biophysical approaches support this notion. These include co-immunoprecipitation, various types of FRET, atomic force microscopy, covalent cross-linking, gel filtration, neutron scattering experiments, functional complementation, cell biology studies demonstrating cross-internalization and co-processing of GPCRs as well as binding studies showing positive and negative cooperativity. These approaches, their relative strengths and caveats, including methodological considerations and potential functional outcomes, have recently been reviewed[16,17]. It is therefore premature to dismiss the GPCR oligomer hypothesis based on the interpretations of a single BRET study.

In conclusion, we believe that the results reported in the article by James *et al.* can be interpreted in different ways and that more controls would have been necessary to challenge the multidisciplinary work conducted on this topic by many groups over the past ten years. Clearly, BRET is gaining popularity in assessment of protein-protein interactions in living cells, and additional quantitative approaches will certainly be forthcoming. Maybe more importantly, additional studies performed in native tissues are needed to establish the generality of GPCR dimerization in physiologically relevant systems.

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James and Davis reply: Bouvier *et al.* claim that several theoretical and/or technical limitations undermine our study[1] of class-A G protein-coupled receptor (GPCR) oligomerization using bioluminescence resonance energy transfer (BRET). First, they propose that in order to characterize the dependence of BRET efficiency ($BRET_{eff}$) on acceptor/donor ratio it is not strictly necessary to increase the acceptor/donor ratio by holding the combined amount of acceptor and donor constant and decreasing the amount of donor. They claim that it is equally valid to hold donor levels constant and increase the amount of acceptor. The reason why the acceptor/donor ratio should not be varied in this way is that $BRET_{eff}$ will increase for both randomly interacting monomers and oligomers owing to the concomitant increase in overall protein density, preventing discrimination between the two types of interactions. Second, it is true that in the new method donor and acceptor densities each vary. The important point is that the acceptor density is effectively constant above a certain acceptor/donor ratio threshold, so that, in the case of randomly interacting donors and acceptors, each donor 'experiences' the same acceptor environment as the acceptor/donor ratio increases beyond this threshold. Third, they argue that we gave insufficient consideration to receptor expression level, but we actually show that GPCRs give similarly low acceptor/donor ratio–independent $BRET_{eff}$ irrespective of overall expression from subphysiological amounts to levels 10–100-fold higher than that observed *in vivo*. Fourth, they claim that we were over-reliant on absolute $BRET_{eff}$ levels when in fact we list four criteria that should be used to establish stoichiometry, including absolute $BRET_{eff}$ levels. Fifth, they propose that in 'type-2' BRET experiments (our nomenclature) it is the slope of the line that is important, whereas our control experiments and theory indicate that the defining factor is whether or not the intercept is zero.

Bouvier *et al.* then raise the question of what is to be made of all the biochemical and biophysical data ostensibly supporting class-A GPCR dimerization. We have no direct experience of working with multipass membrane proteins ourselves, but we suspect that these extremely hydrophobic molecules would be prone to artefactual behavior and would exhibit, for example, a tendency to aggregate once extracted from their native membrane environments. The focus of our paper was on the proper implementation of BRET, which, in principle, allows the organizational properties of cell-surface receptors to be examined *in situ* in living cells, free from potential artefacts of this nature. We also note that the controversy surrounding GPCR oligomerization is hardly new[2,3].

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