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Direct Measurements of Gi signaling and its dynamic interplay with Gs using cADDis, a live-cell assay for cAMP with improved sensitivity.

Our goal was to develop a cAMP assay with the following characteristics: (1) it should detect both Gs and Gi signaling in living cells, in standard automated plate readers, (2) it should be capable of measuring the dynamic interplay between Gs and Gi signaling, (3) it should be as simple and biologically relevant as possible, requiring minimal liquid handling steps, co-factors, enzyme substrates, PDE inhibitors, or forskolin.

We have previously demonstrated that our cADDis cAMP biosensor can be used to detect Gs signaling in fluorescence plate readers with a Z' statistic of 0.8 or greater. The next step was to determine if cADDis could be used to detect Gi signaling. A variety of Gi-coupled receptors were expressed in HEK 293 cells, but known agonists produced no detectable change in cADDis fluorescence. Reasoning that this was due to low basal adenylyl cyclase activity, we increased Gs signaling by co-expressing a constitutively active Gs mutant. In this context, the cADDis sensor showed large changes in fluorescence, reported EC_{50} values consistent with literature, and yielded high Z' values in a standard automated fluorescence plate reader.

In living cells, cAMP levels are determined by the ever changing interplay of Gs and Gi signaling over time. To test whether the cADDis can detect this interplay, we first stimulated a Gs pathway and then rapidly followed with Gi activation. The cADDIS sensor could, in real time, demonstrate how Gi stimulation can occlude the typical Gs response. Raising cAMP levels with the Gs mutant made it possible to first stimulate the Gi pathway and then occlude this response with a secondary Gs stimulation. This offers a unique view into how cells balance adenylyl cyclase activity to tightly control cAMP levels.

Many Gi assays use forskolin to artificially raise cAMP levels. The cADDis sensor responded well in this context and revealed that, in some cases, false negatives can arise. For instance, known agonists for both the Adenosine1 and D2-dopaminergic receptors produced robust Gi responses in HEK293 cells when cAMP was elevated with isoproterenol, but failed to produce a response in the presence of forskolin.

The cADDis assay involves only three steps: 1) the addition of virus on day one, 2) washing the cells on day 2 and, 3) applying the compound. No PDE inhibitors or forskolin are necessary, no enzyme substrates are required. To measure inhibition of cAMP production through Gi, cAMP levels can be elevated prior to receptor activation with either constitutively active Gs proteins, stimulation of endogenous Gs pathways, or forskolin, if you must.