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Design and characterization of FRET-based cGMP indicators

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The intracellular signalling molecule cGMP is involved in a variety of physiological processes such as smooth muscle relaxation and inhibition of platelet aggregation. The combination of different cGMP-generating and cGMP-degrading enzymes, guanylyl cyclases and phosphodiesterases, respectively, determines amplitude and shape of cGMP signals in a given tissue. Fast and transient cGMP signals that reach up to hundredfold elevated peak concentrations within seconds and rapidly return to almost baseline levels have been observed in neuroblastoma cells, platelets and smooth muscle cells. Thus, indicators allowing continuous measurement of cGMP signals in living cells are clearly demanded.

Here, we report on an approach to create FRET-based cGMP indicators using the two known types of cGMP-binding domains, cNMP-BD and GAF, occurring in the cGMP-dependent protein kinases and phosphodiesterase 5, respectively. More than 50 constructs containing different regions of either isolated or tandem cGMP-binding domains were created and tested in vitro. Interestingly, only tandem cGMP-binding domains arranged as in the cGMP effector proteins were cGMP-responsive. The GAF-derived indicators showed extremely slow FRET changes upon cGMP binding and dissociation making them unsuitable for monitoring fast intracellular cGMP signals. From the tandem cNMP-BDs of cGMP-dependent protein kinase a range of functional constructs was obtained. Three of these indicators with affinities between 500 nM and 6 μM were chosen to cover a range of intracellular cGMP concentrations. These indicators feature twice the dynamic range of existing cGMP sensors, exhibit fast kinetics, and a high selectivity for cGMP. The in vivo performance is demonstrated in a HEK293 line stably expressing NO-sensitive guanylyl cyclase and phosphodiesterase 5 which displays cGMP signals comparable to the ones of smooth muscle cells. The signals obtained with the three cGMP indicators are validated by comparison with cGMP dynamics measured by radioimmunoassays.