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FlincGs: novel, non-FRET cGMP biosensors with nanomolar sensitivity for NO-induced signaling

Lydia WM Nausch* and Wolfgang R Dostmann

Address: University of Vermont, College of Medicine, Department of Pharmacology, Burlington, VT, USA

Email: Lydia WM Nausch* - lgruber@uvm.edu

* Corresponding author

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Previously, our lab has developed FRET-based cGMP-indicators (cygnets) to study the spatial and temporal dynamics of intracellular cGMP [1]. Cygnets have been proven to advance our understanding of NO/cGMP signaling in vascular smooth muscle cells [2]. However, FRET based indicators suffer from intrinsic technical difficulties: they require a dual emission detection system, show overall low cyan/yellow emission ratio changes, and are generally insensitive to physiological (low nanomolar) stimuli of NO. These restrictions prompted us to develop several novel, non-FRET based cGMP biosensors (FlincG:fluorescence indicator of cyclic GMP), which are composed of a single circular permutated EGFP (cpEGFP) fused to regulatory fragments of cGMP-dependent protein kinase (PKG). Based on the different PKG type I isoforms, we designed α-FlincG and β-FlincG. A third construct (δ-FlincG), had the entire N-terminal region truncated. All three indicators were expressed and purified from *E. coli* and showed cGMP selective changes in total 510 nm emission intensities of 50–200% with apparent $K_{D,cGMP}$ values ranging from 40 nM to 1.2 μM. Furthermore, we observed a >1000 fold selectivity for cGMP over cAMP. Interestingly, in adenovirus transfected vascular smooth muscle cells (P0), FlincG indicators detected cGMP responses to sub-nanomolar NO. The apparent EC_{50} values were determined as 0.3 nM, 12 nM and 7 nM for α-FlincG, β-FlincG and δ-FlincG, respectively. Due to their superior kinetic characteristics, FlincG biosensors may serve as ideal tools to elucidate cGMP signaling in cultured smooth muscle cells and in intact arteries using conventional epi-fluorescence microscopy as well as confocal fluorescence microscopy.

References

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