



# PDE Specificity Illuminated: Monitoring cGMP and cAMP Levels in Living Cells

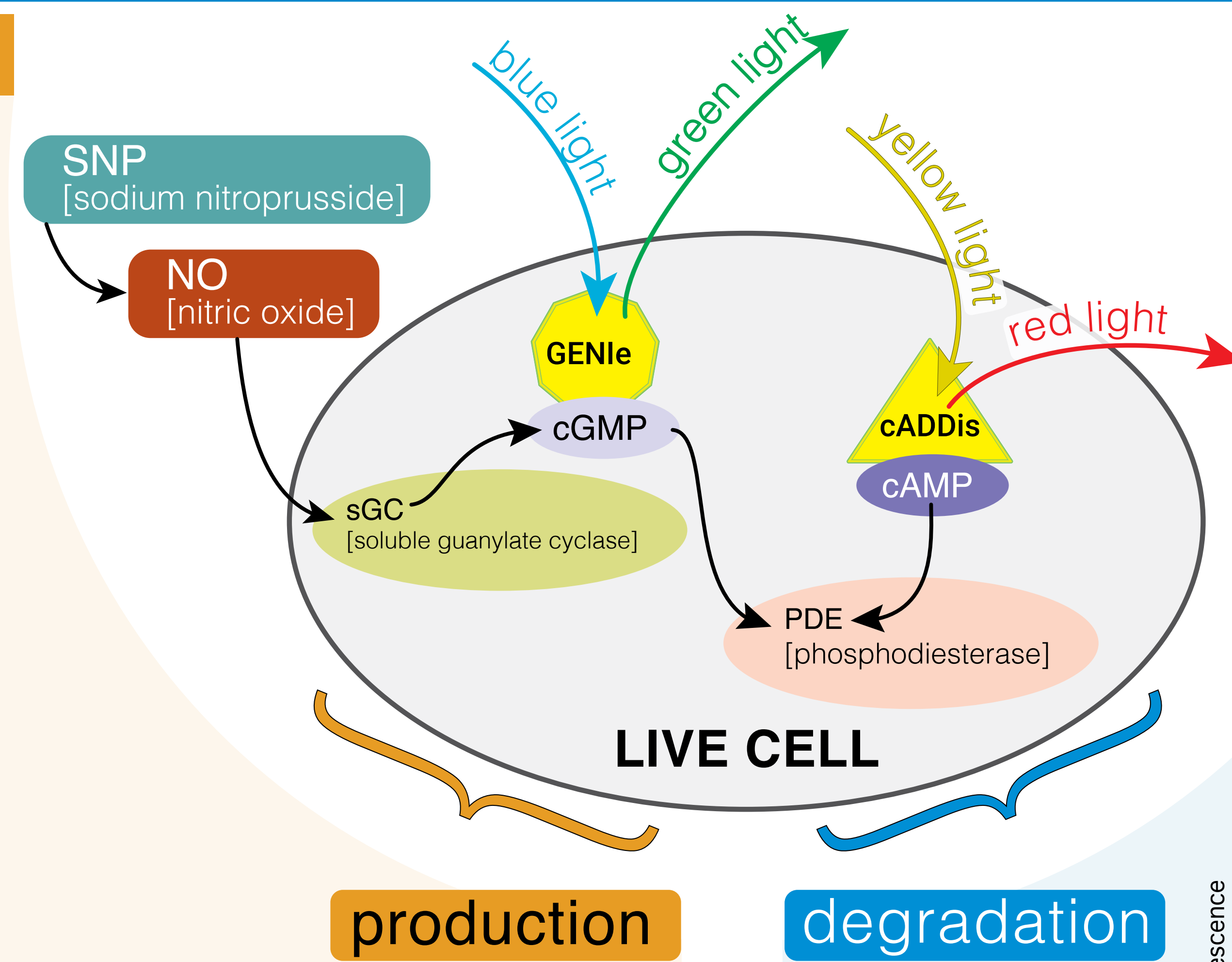
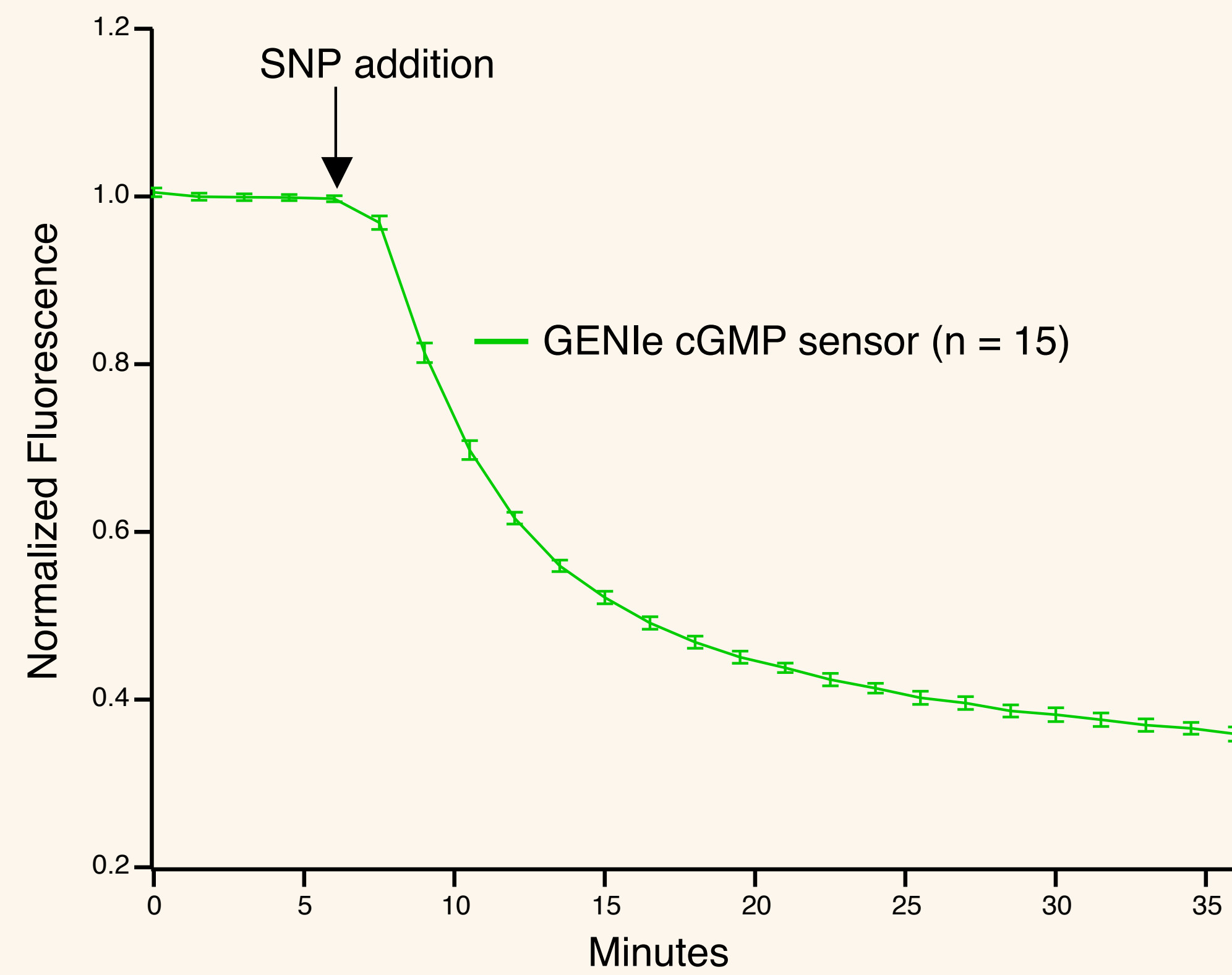
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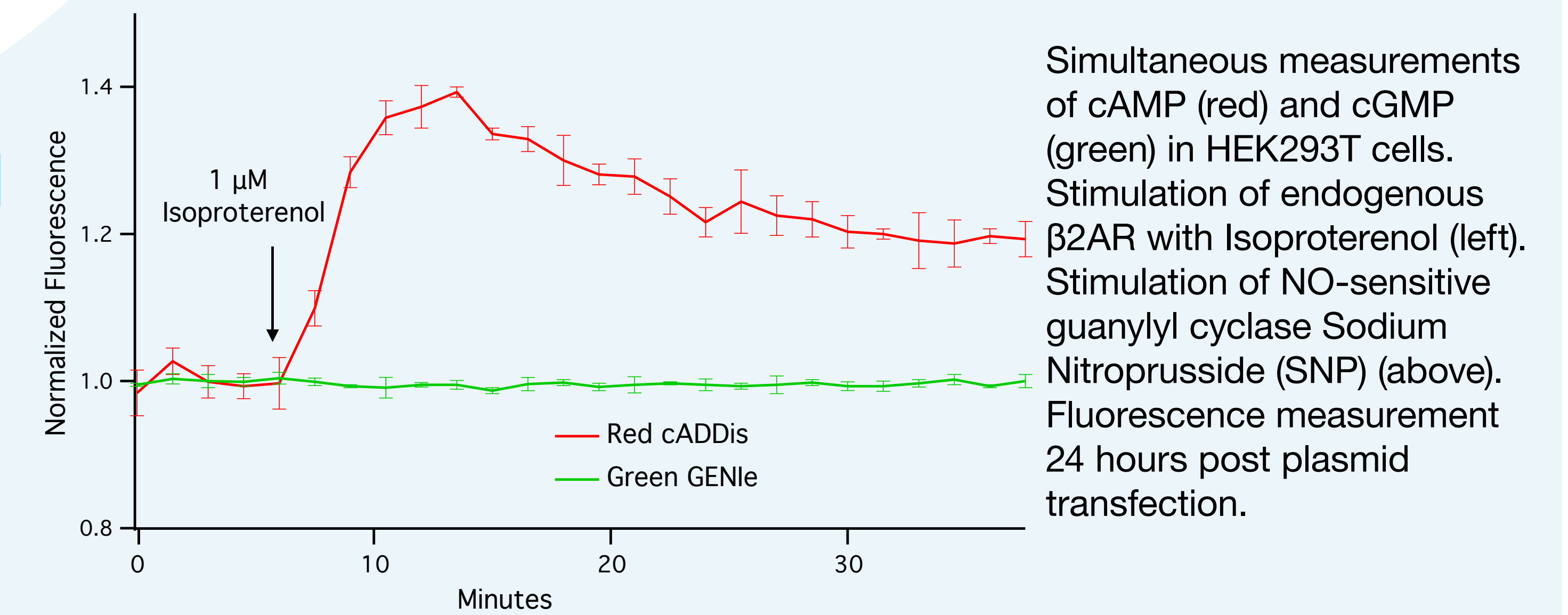
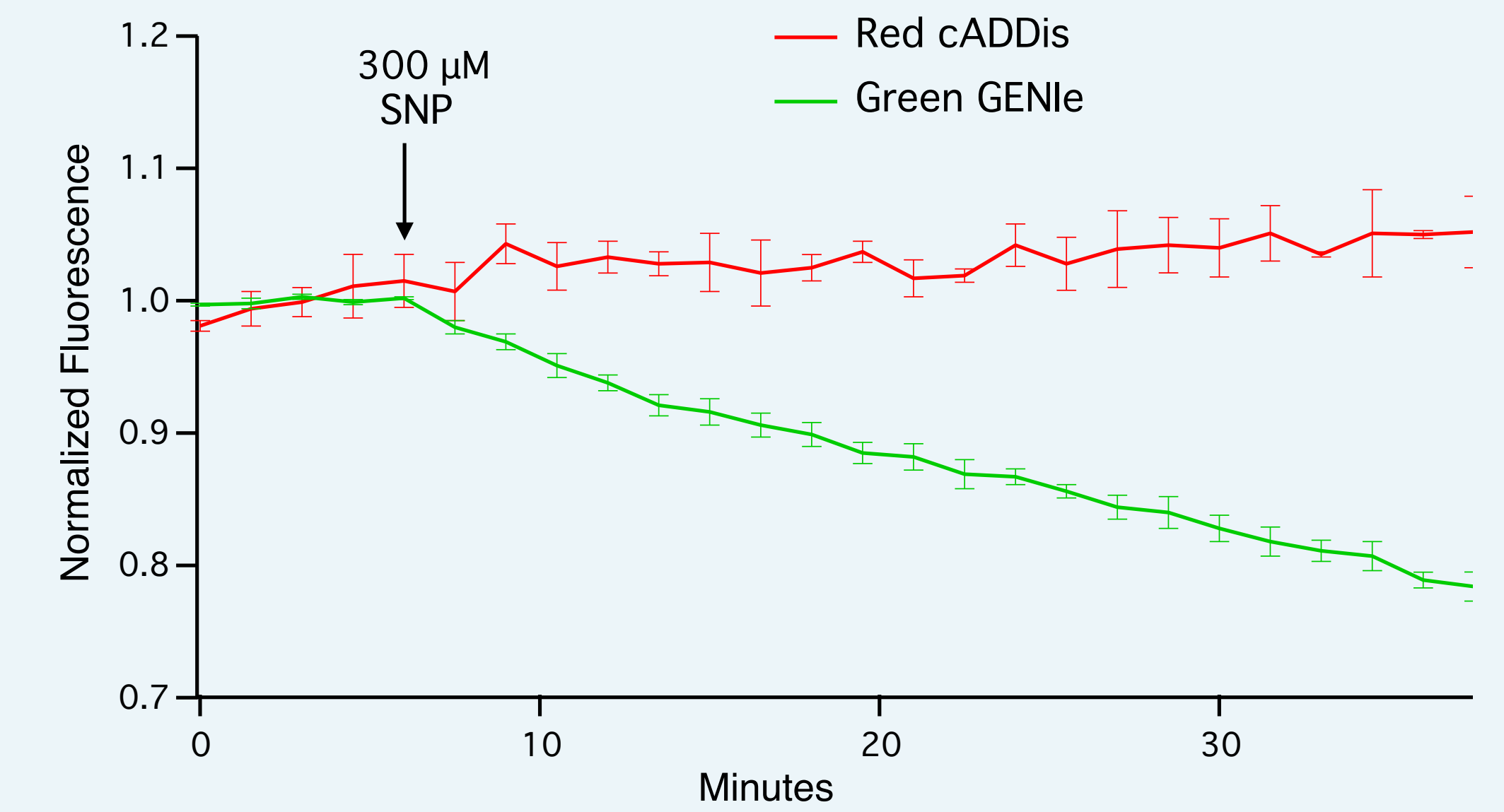
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## A bright green fluorescent sensor for cGMP: GENie

This indicator decreases in fluorescence when cGMP rises.

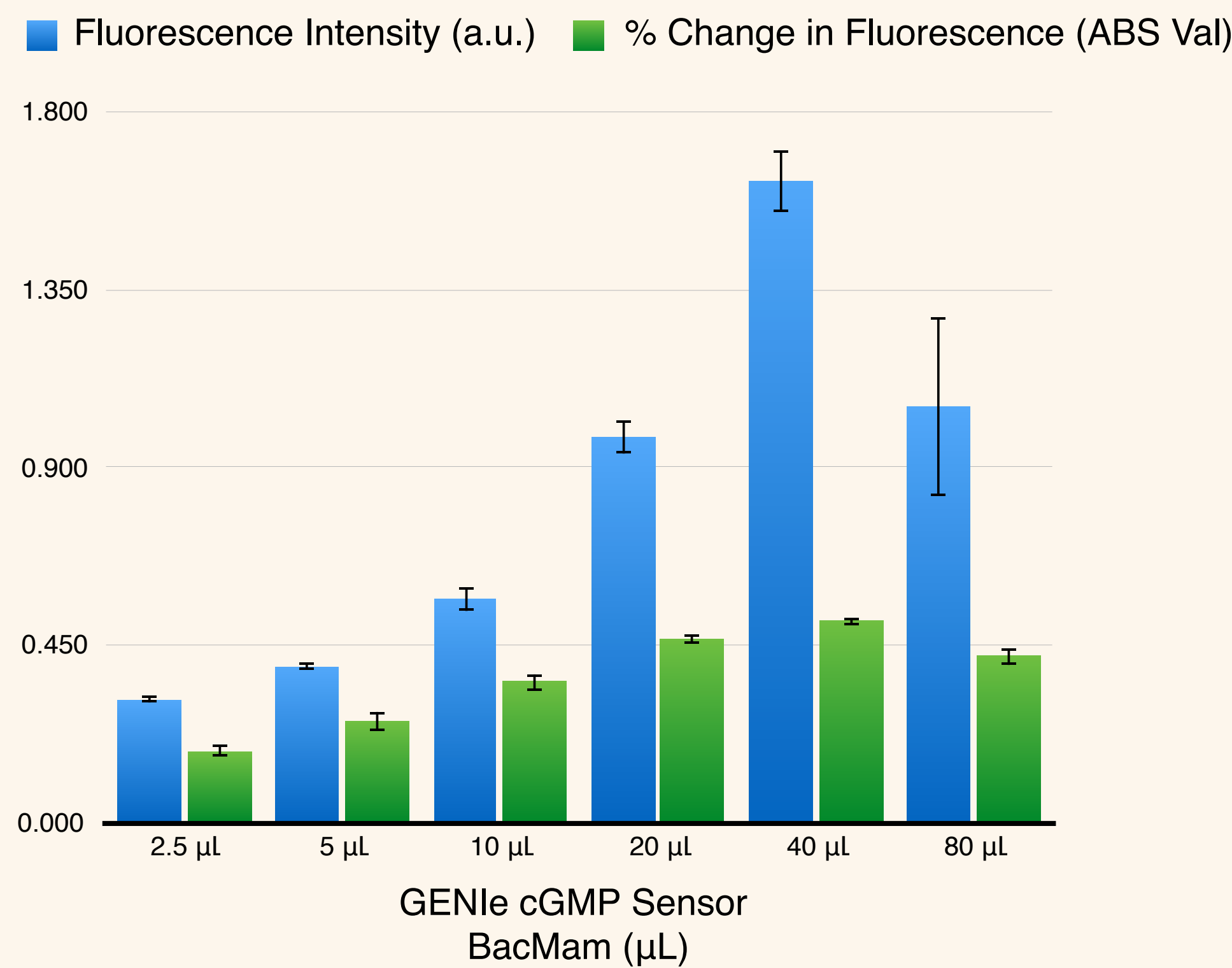


GENie can be paired with R-cADDis to compare cGMP and cAMP concentrations in living cells on a fluorescence plate reader.

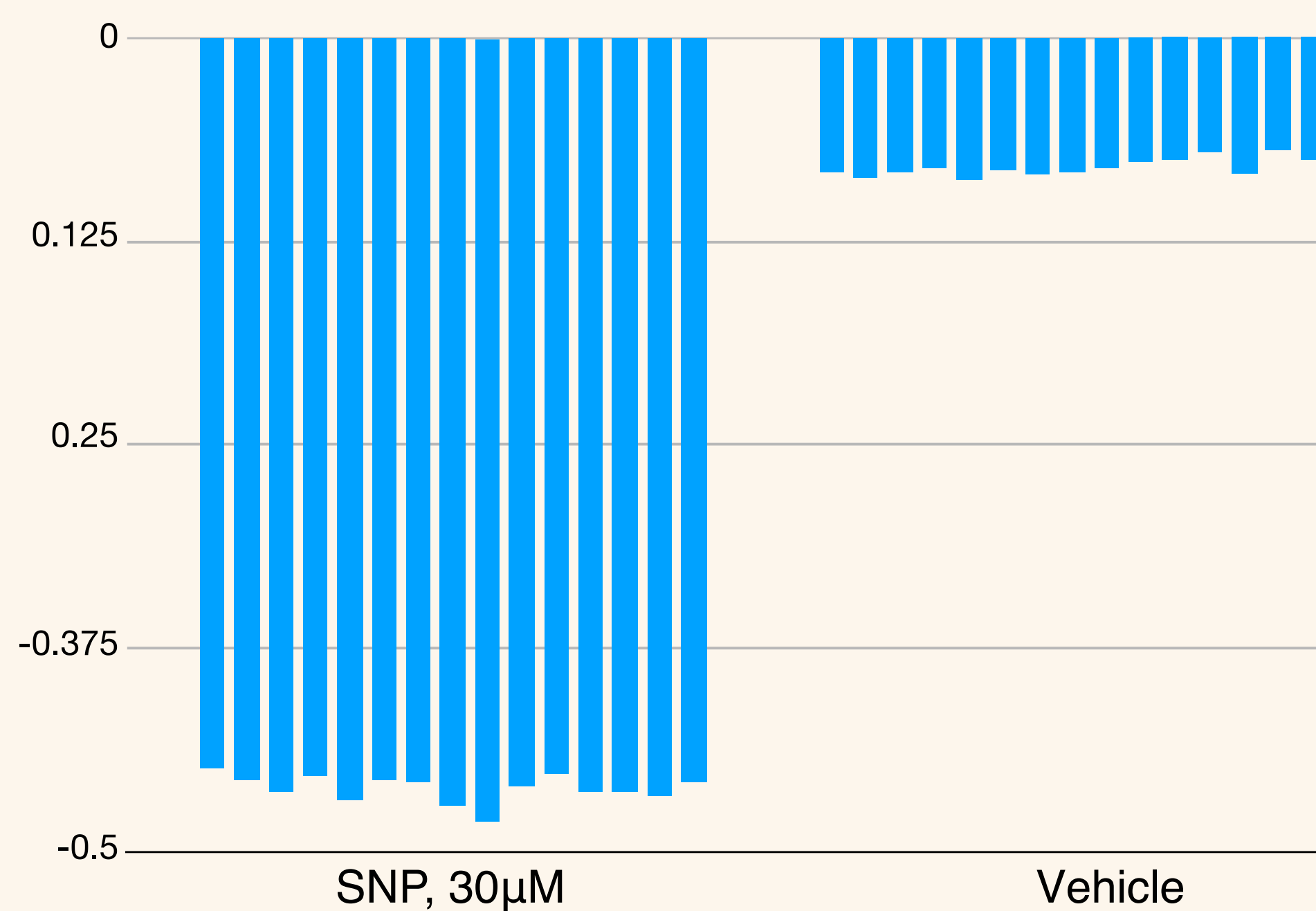


Simultaneous measurements of cAMP (red) and cGMP (green) in HEK293T cells. Stimulation of endogenous  $\beta$ 2AR with Isoproterenol (left). Stimulation of NO-sensitive guanylyl cyclase Sodium Nitroprusside (SNP) (above). Fluorescence measurement 24 hours post plasmid transfection.

GENie is a bright sensor compatible with standard fluorescent plate readers that produces a robust assay.



GENie is packaged in a BacMam vector that can be titrated to optimize the assay for a particular cell type. Here, HEK293T cells are transduced with increasing amounts of GENie. 2  $\mu$ L of sGC BacMam and 30  $\mu$ M SNP stimulates cGMP production in each condition. n = 10 wells per condition.



Z' = 0.89

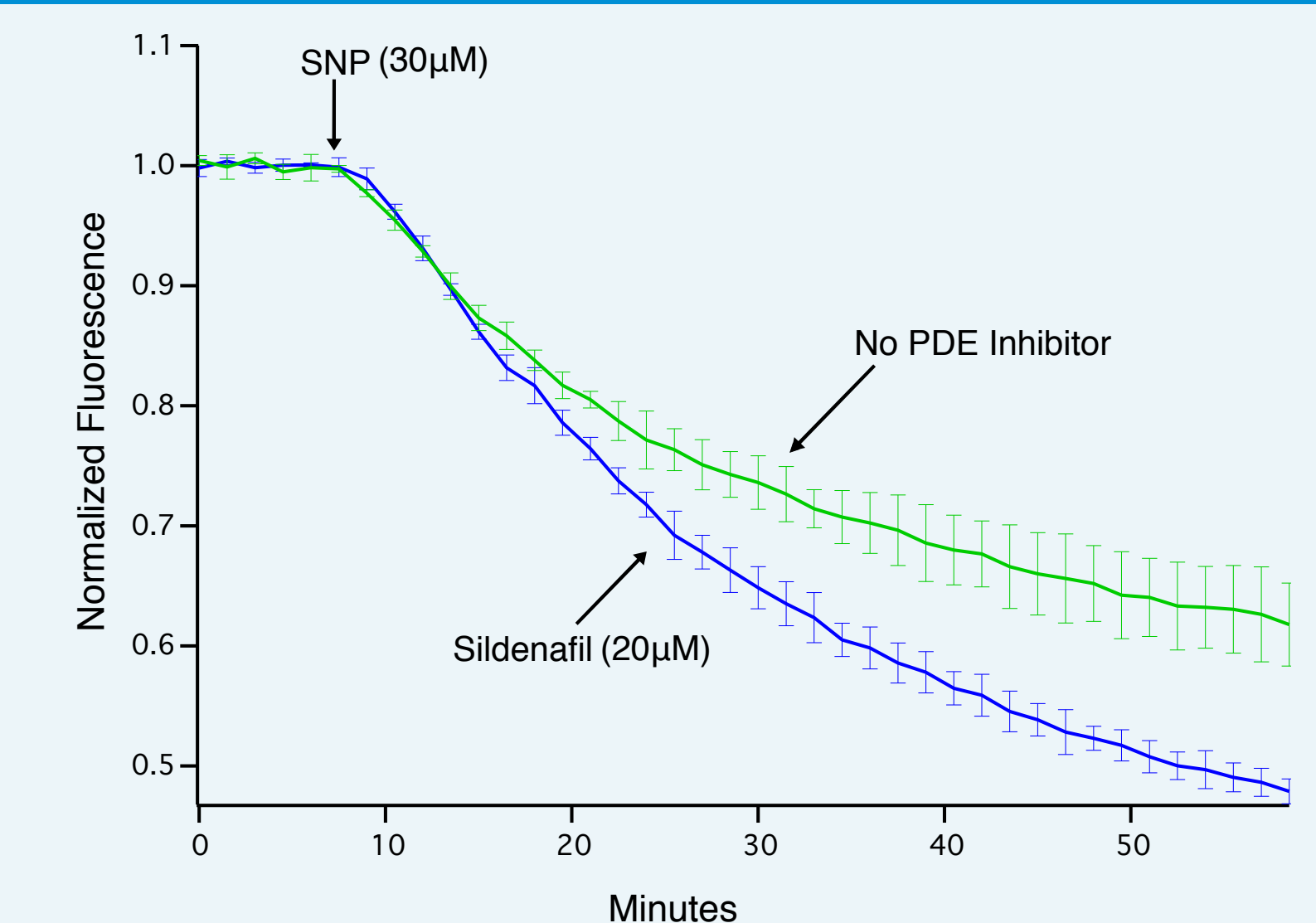
cGMP production stimulated by SNP. HEK293T cells transduced with BacMam vectors (2  $\mu$ L sGC and 25  $\mu$ L of GENie). Fluorescence measurements in 96 well format on a Biotek Synergy MX plate reader.

Support:

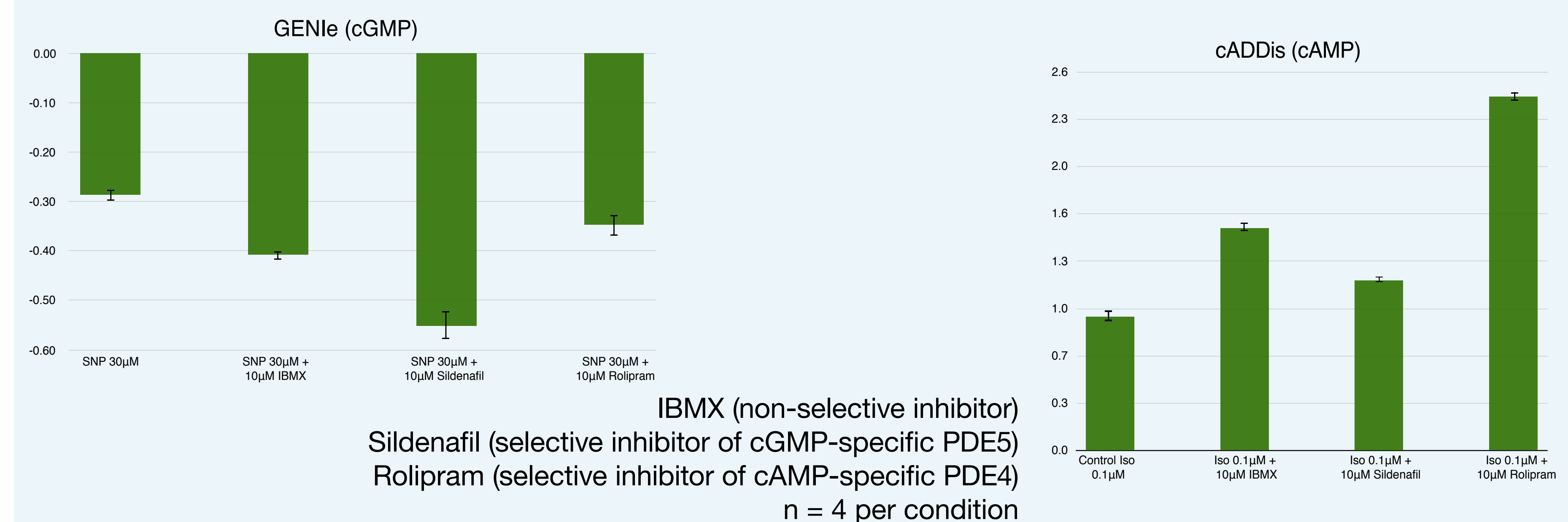
Funding from NSF SBIR Phase IIB 1430878

## GENie can detect the action of PDE inhibitors

The PDE inhibitor Sildenafil increases GENie fluorescence indicating that cGMP concentrations are rising faster when the degradation of cGMP is inhibited. HEK293T cells transduced with 25  $\mu$ L of GENie. Endogenous sGC activated with SNP. n = 5 wells per condition.



## A comparison of the GENie and cADDis sensors reveals the specificity of PDE inhibitors.



IBMX (non-selective inhibitor)  
Sildenafil (selective inhibitor of cGMP-specific PDE5)  
Rolipram (selective inhibitor of cAMP-specific PDE4)  
n = 4 per condition