APS physics
HYBRID SATELLITE MEETING, ICTS, MARCH 14-18, 2022

Optogenetic modulation of real-time nanoscale dynamics of HCN channels using photoactivated adenylyl cyclases

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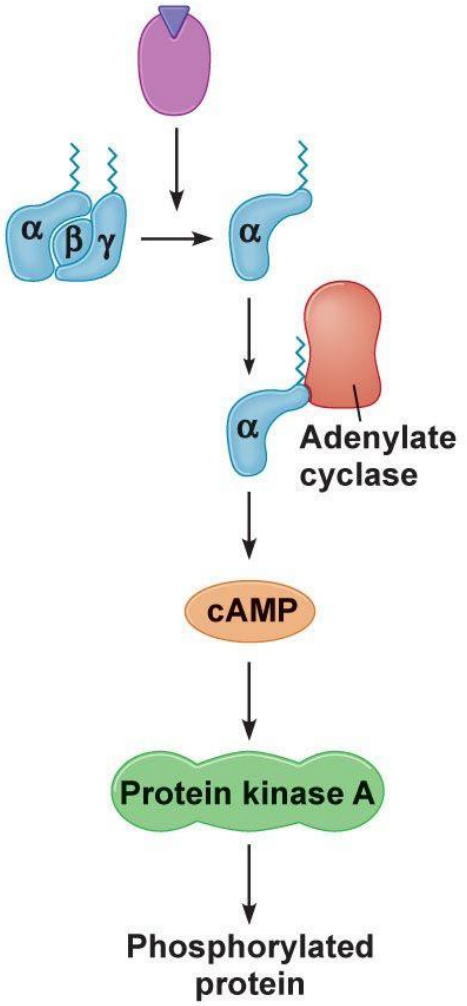
Tanwar et al, RSC Chemical Biology, 2021

2° MESSENGER PATHWAYS

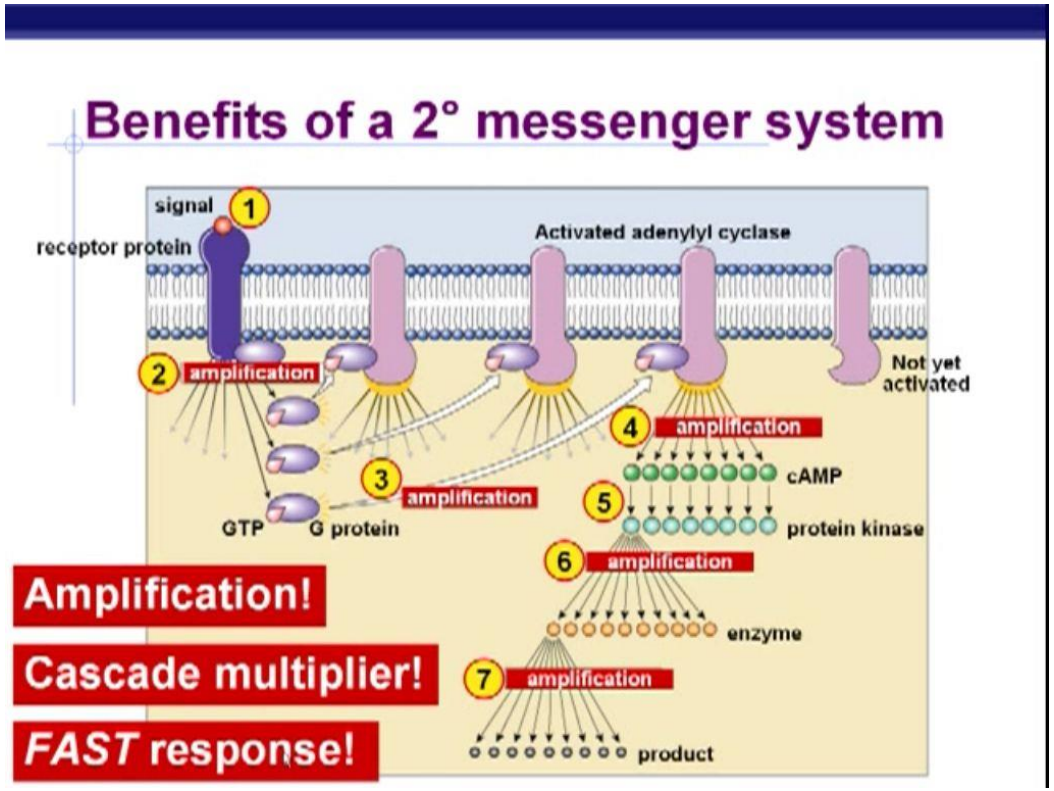


	Total number of product
One messenger binds to one receptor	1
Several G proteins are activated	10
Each G protein activates an adenylate cyclase	10
Each adenylate cyclase generates hundreds of cAMP molecules	5000
Each cAMP activates a protein kinase A	5000
Each protein kinase A phosphorylates hundreds of proteins	2,500,000

One messenger molecule

leads to…

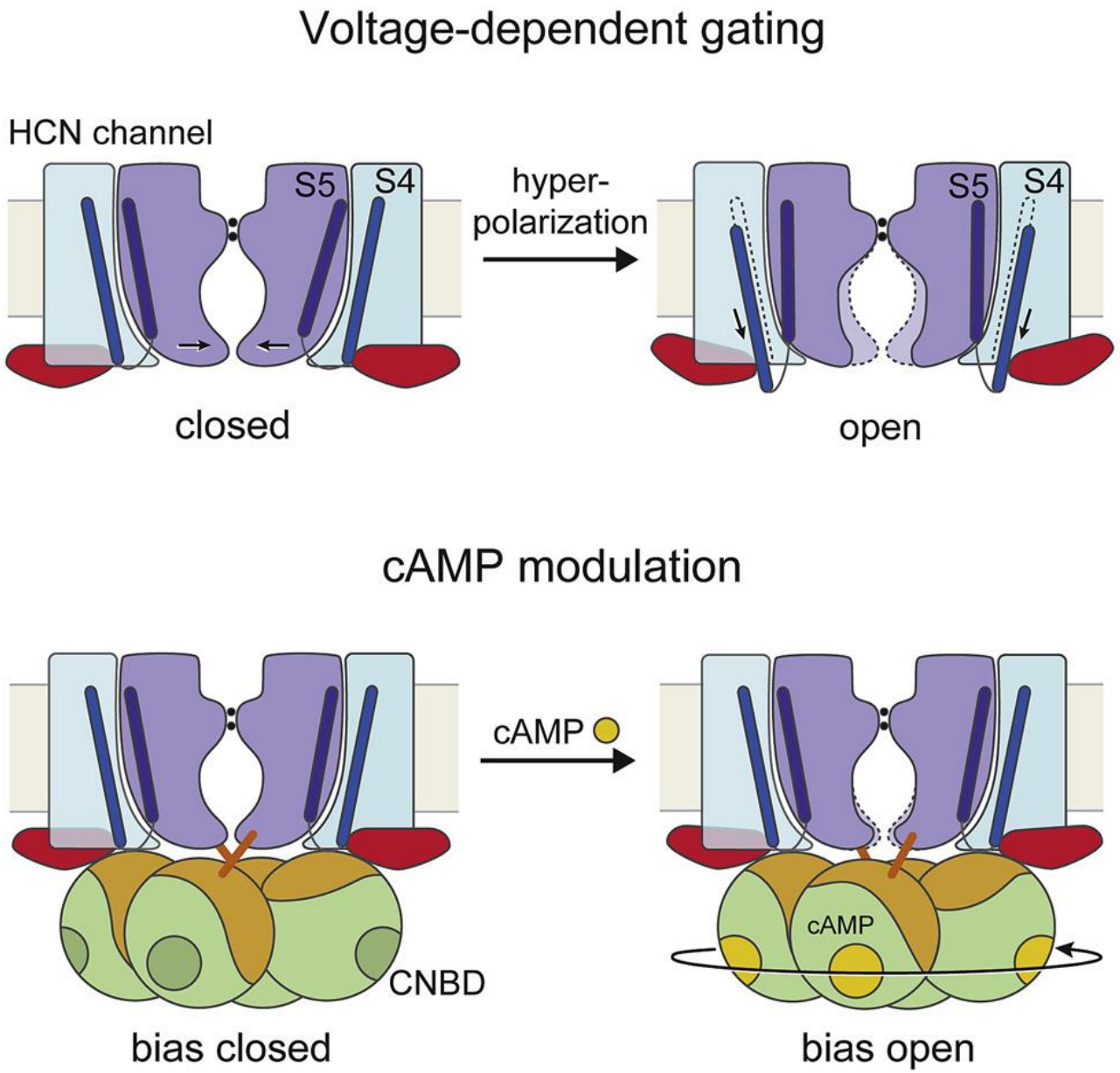
phosphorylation of millions of proteins



cAMP Effectors

Protein Kinase A (PKA), Exchange protein directly affected by cAMP (EPAC), Cyclic Nucleotide Gated ion channels (CNG), Hyperpolarization activated cyclic nucleotide gated ion channels (HCN)

cAMP effector: HCN ion channels



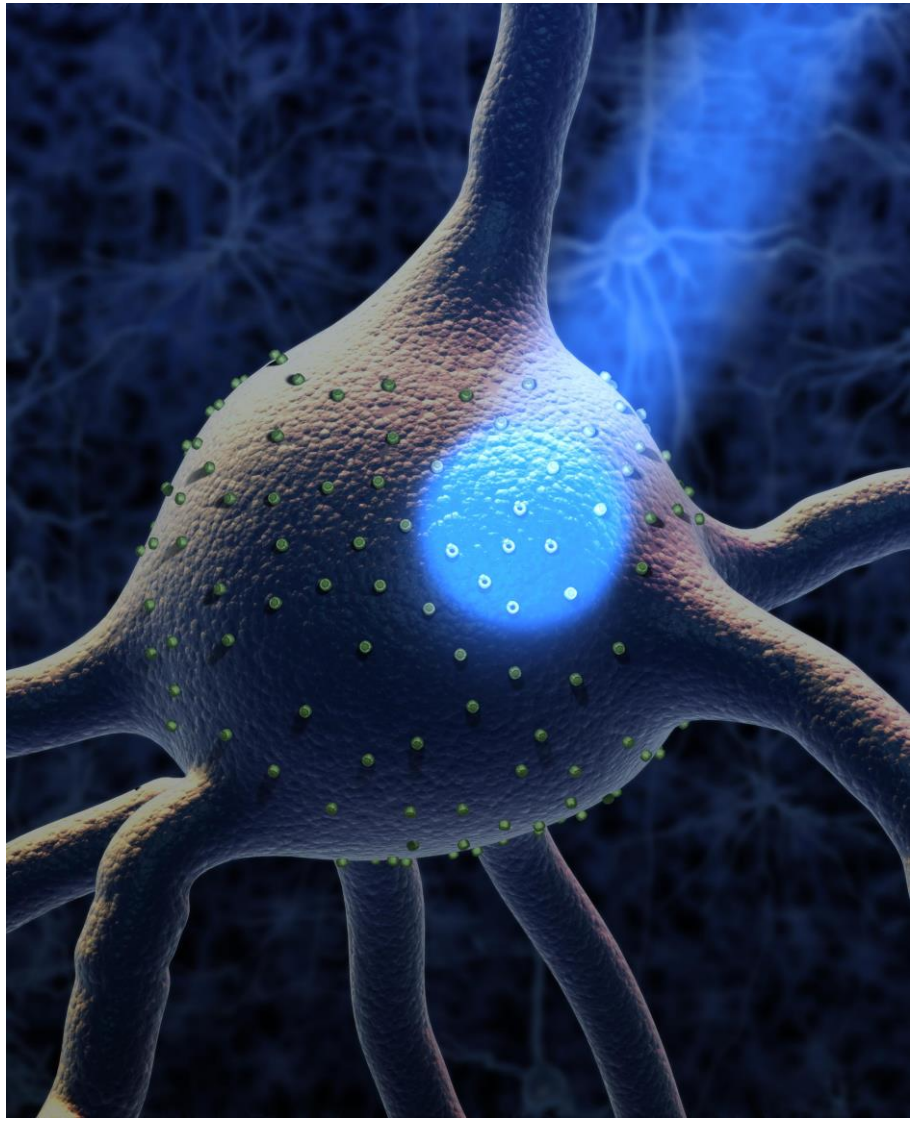
Trans-membrane molecules
4 subunits (identical/non-identical):
6 trans-membrane domains each
Votage sensor, pore region (Na+, K+),
Cyclic nucleotide binding domain (CNBD)

Various combinations/conformations
of 4 subunits

HCN isoforms: HCN1, HCN2, HCN3, HCN4

HCN2 has highest affinity to cAMP

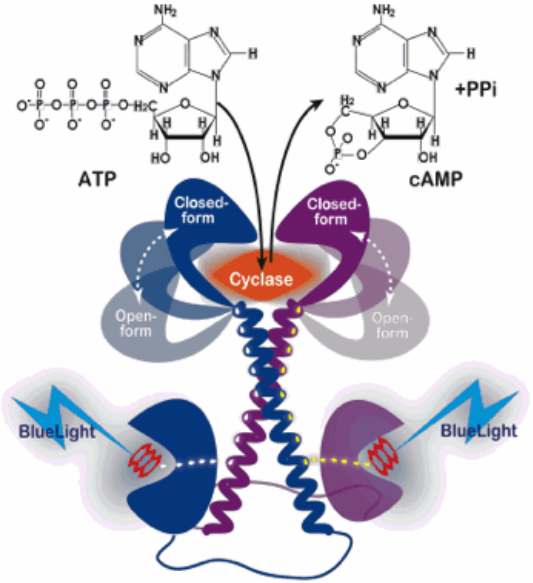
OPTOGENETICS



Structure of Photoactivated adenylate cyclase (PAC)

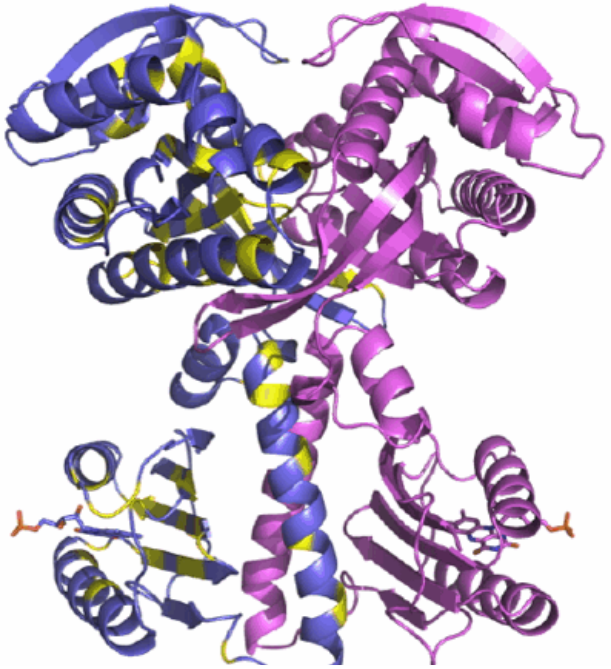
OaPAC protein controls the fundamental second messenger cyclic-AMP (cAMP) activities in response to blue light

Molecular characteristics of OaPAC



Adenylyl cylase homology domain

AC-domain



Blue light using FAD(BLUF)

SINGLE MOLECULE BASED SUPER RESOLUTION MICROSCOPY

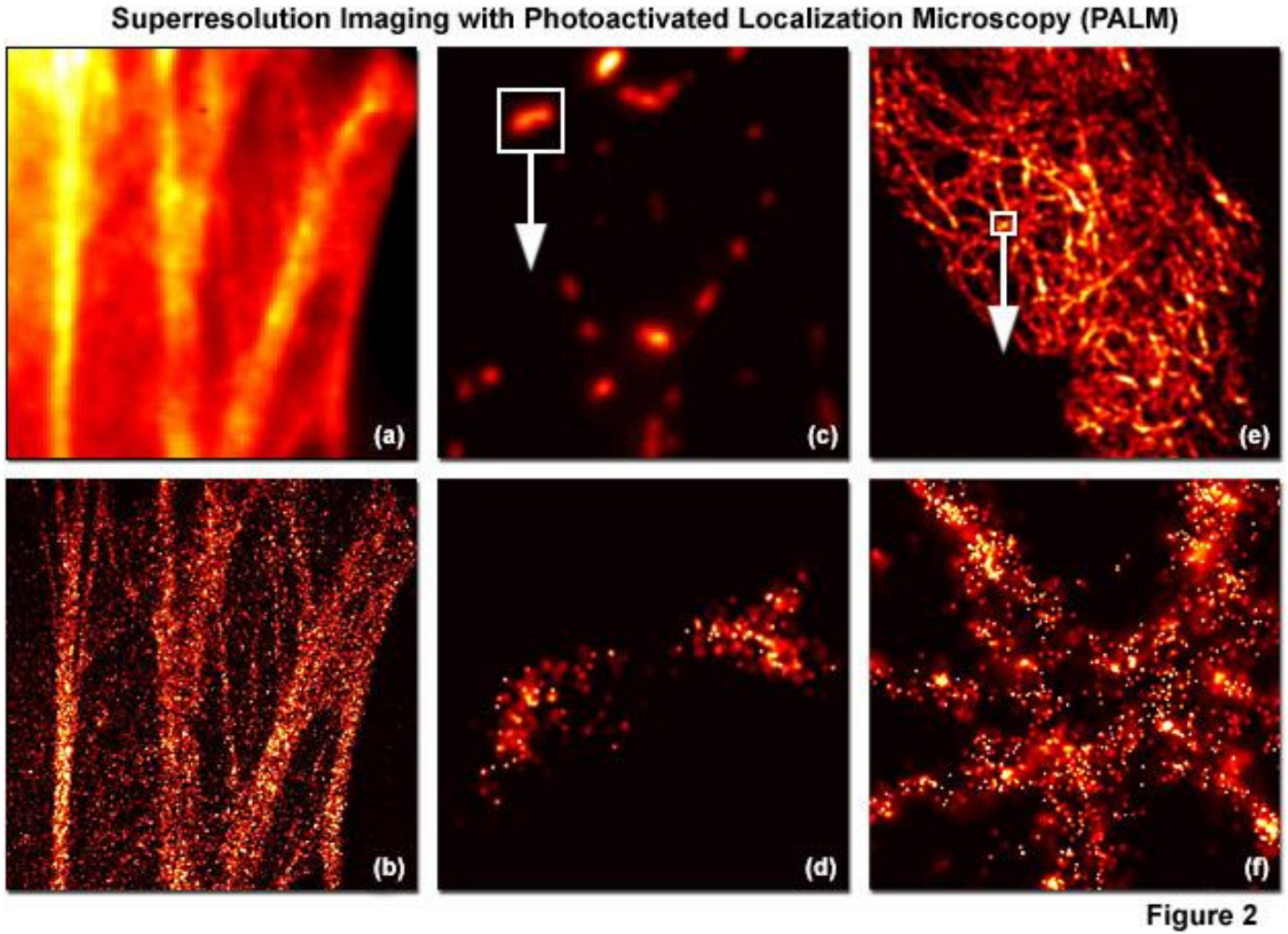


Figure 2

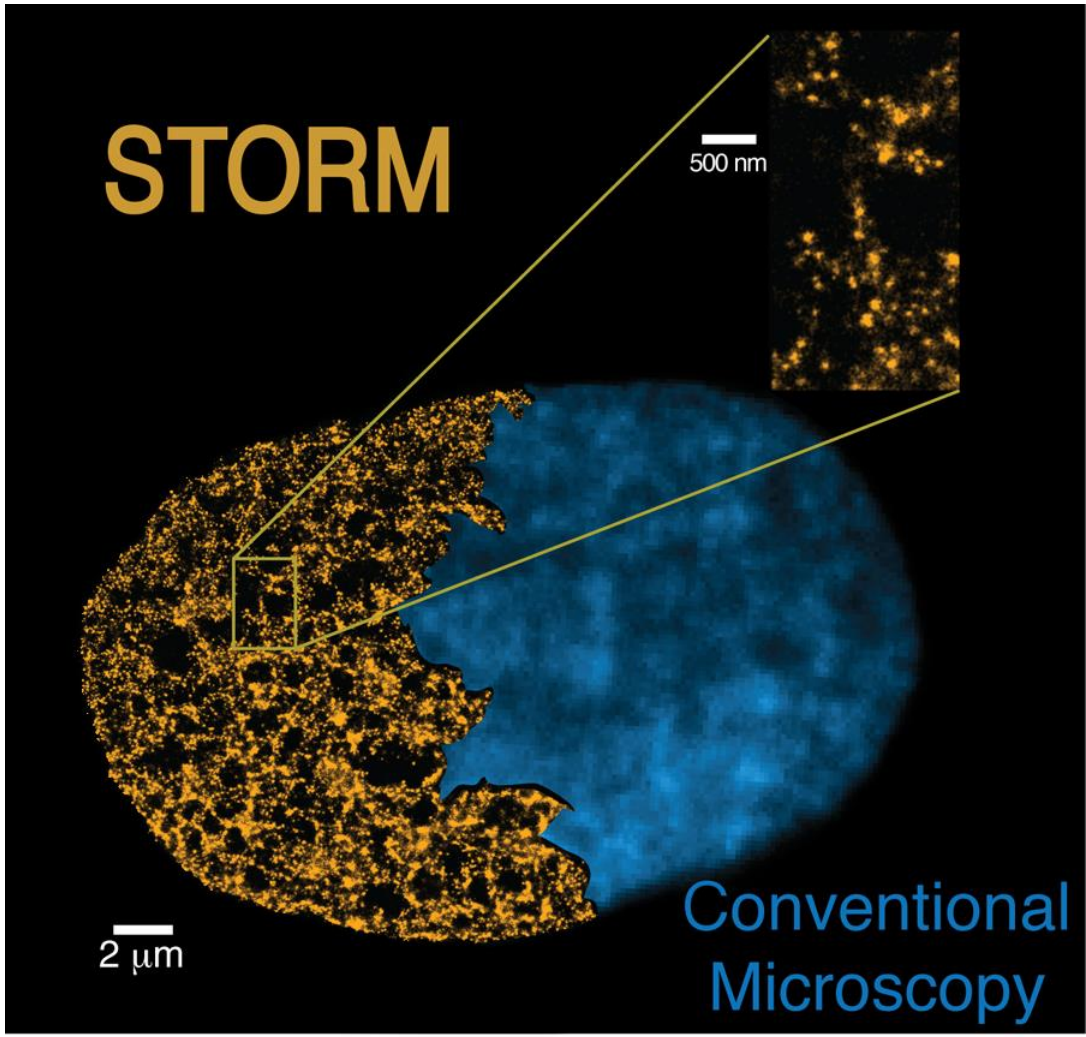
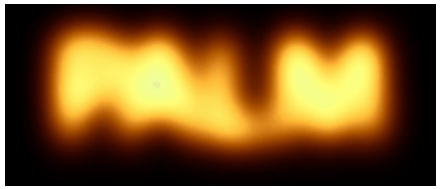


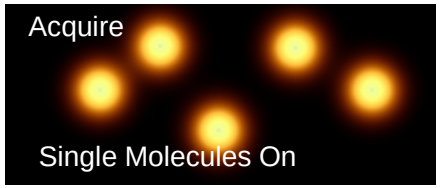
Photo-Activation Localization Microscopy

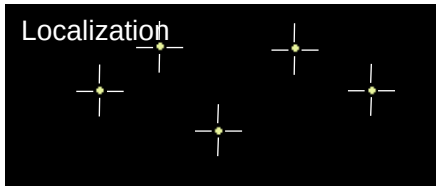
(Betzig et al., Science, 2006)

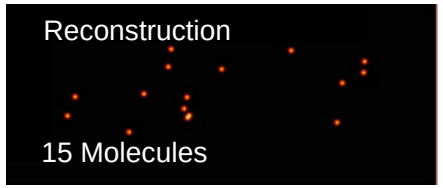


Bulk fluorescence image

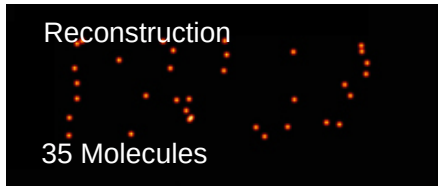
Activate



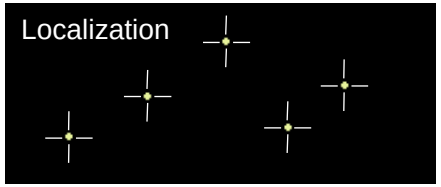


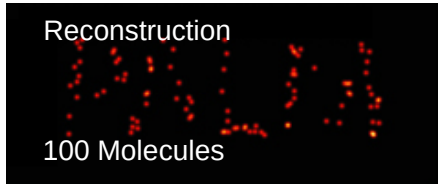


Bleach

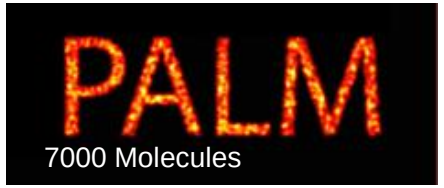


Activate



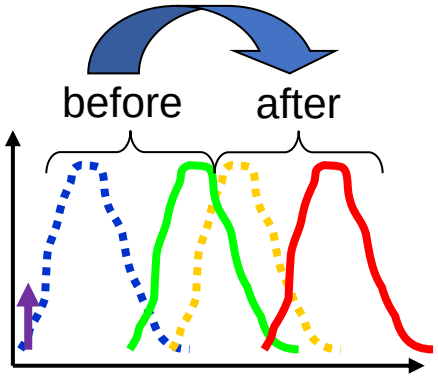


Bleach

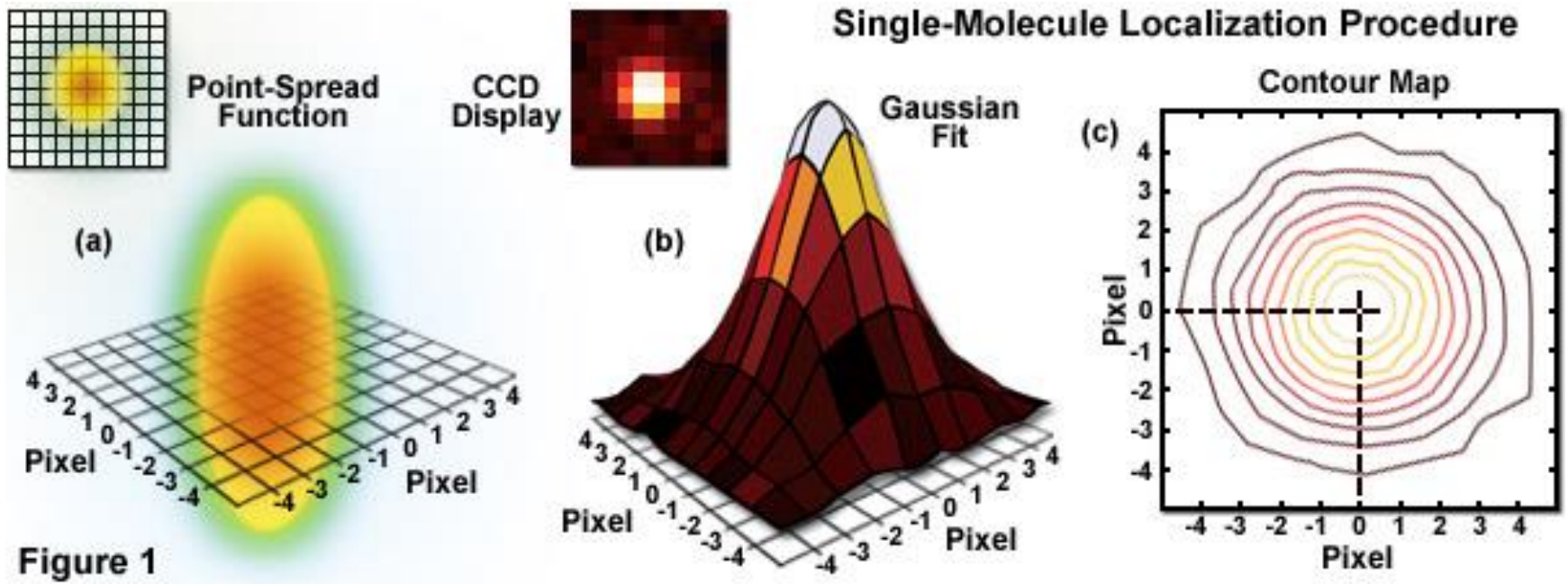


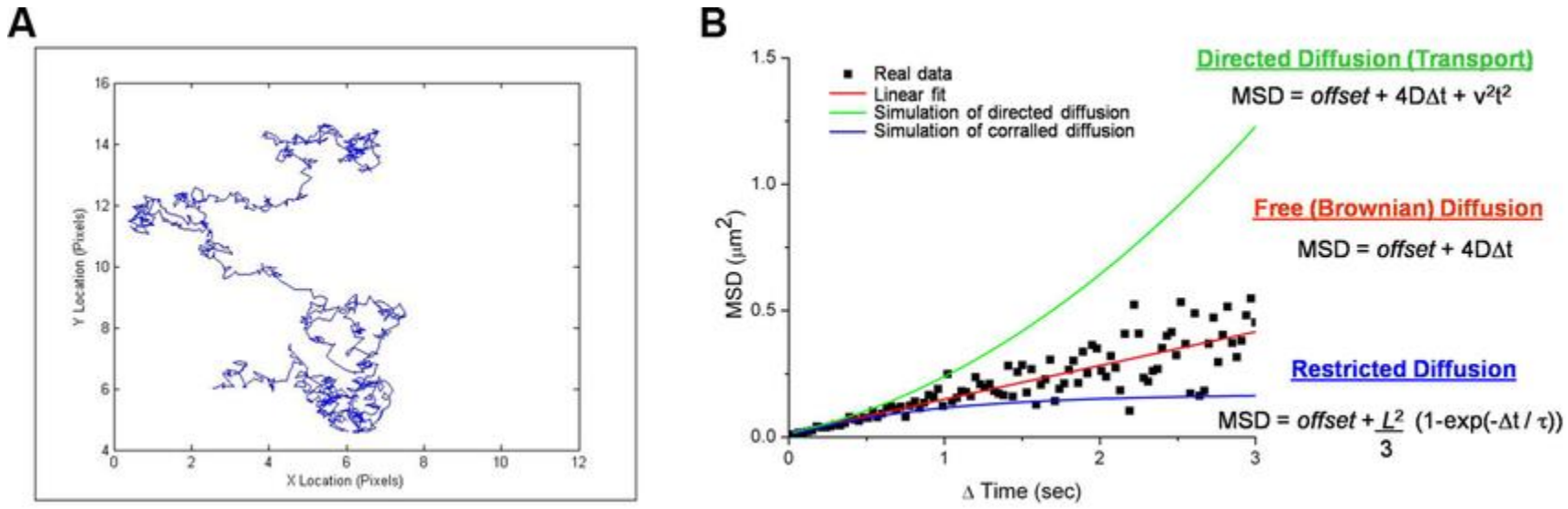
Super-resolution image

Photoswitching



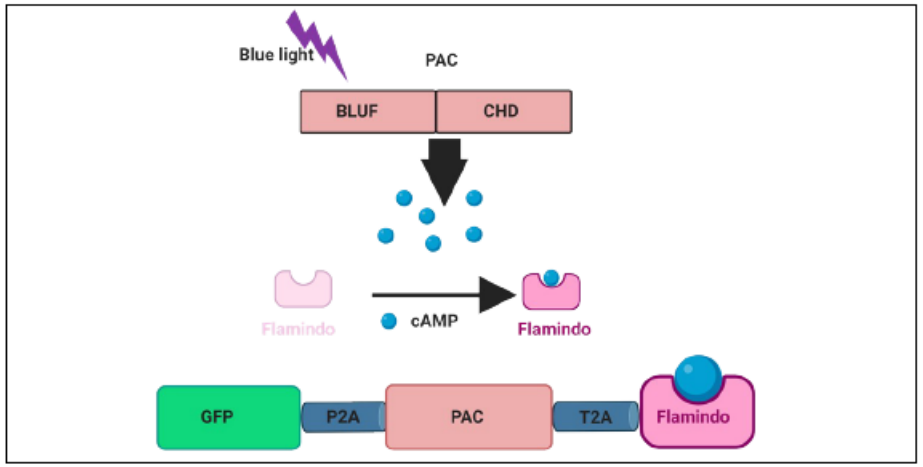
SINGLE MOLECULE LOCALIZATION AND KINETICS



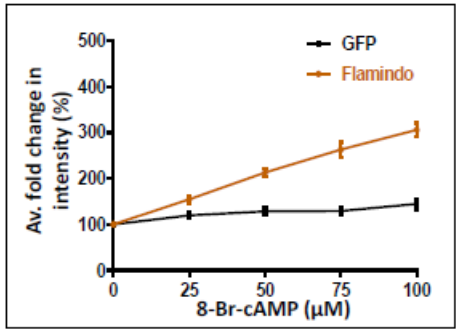


PHOTOPHYSICAL CHARACTERIZATION OF THE PAC VARIANTS

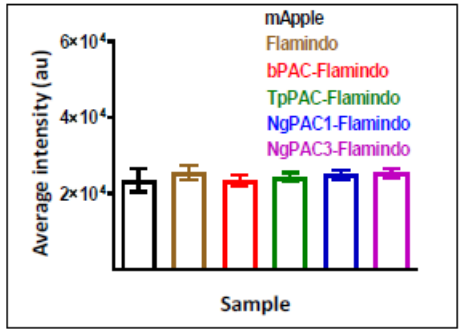
A)



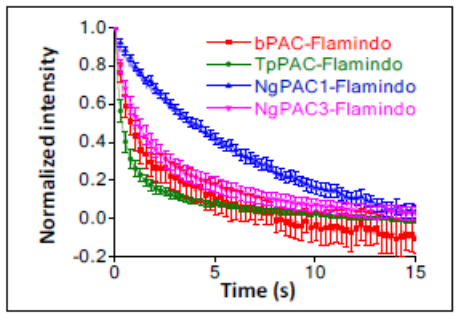
B)



C)



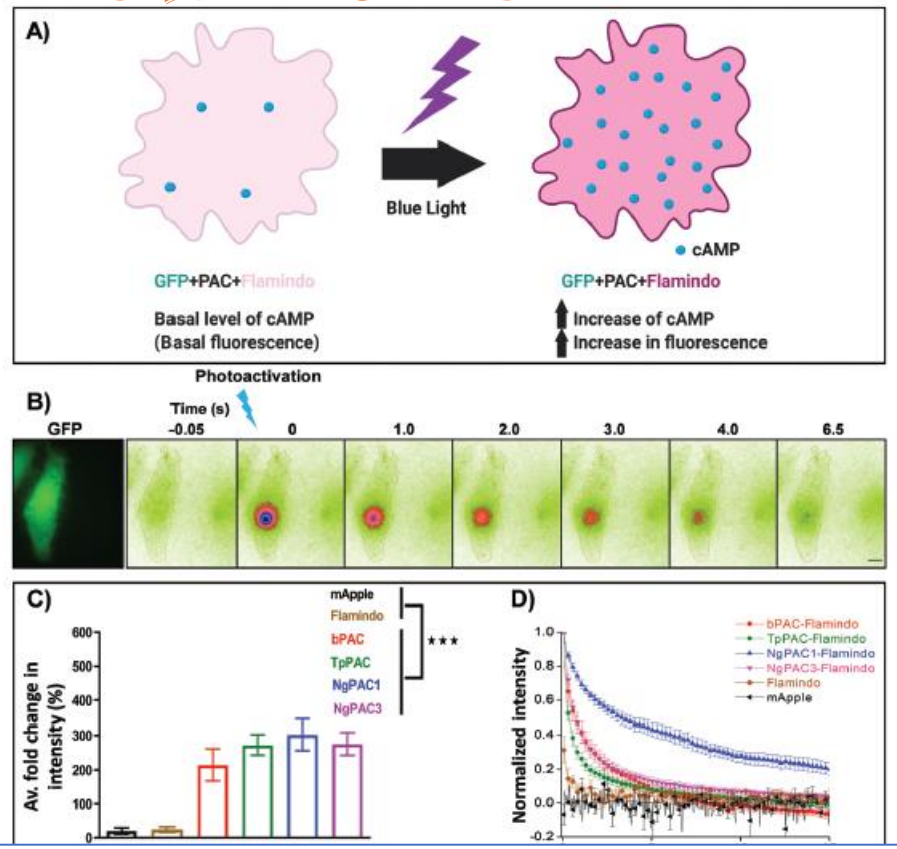
D)



E)

Construct	A1	τ_1 (s)	A2	τ_2 (s)	τ_{av} (s)
bPAC-Flamindo	1.71	0.50	1.23	3.39	1.71
TpPAC-Flamindo	1.08	0.33	0.33	5.05	1.43
NgPAC1-Flamindo	0.57	3.78	0.91	19.32	13.32
NgPAC3-Flamindo	1.68	0.65	1.36	4.20	2.24

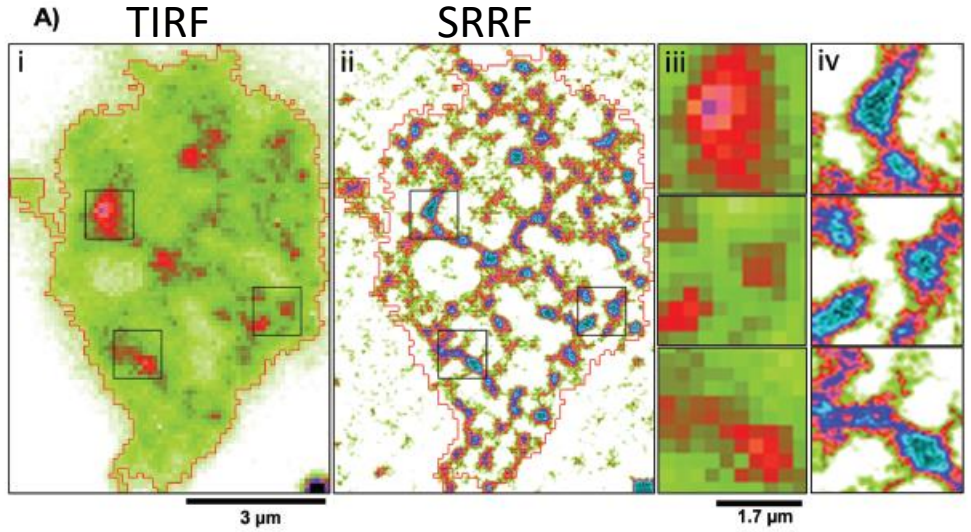
OPTOGENETIC ACTIVATION AND SENSING OF TRANSIENT CHANGES IN CAMP LEVELS IN LIVE NEURO-2A CELLS



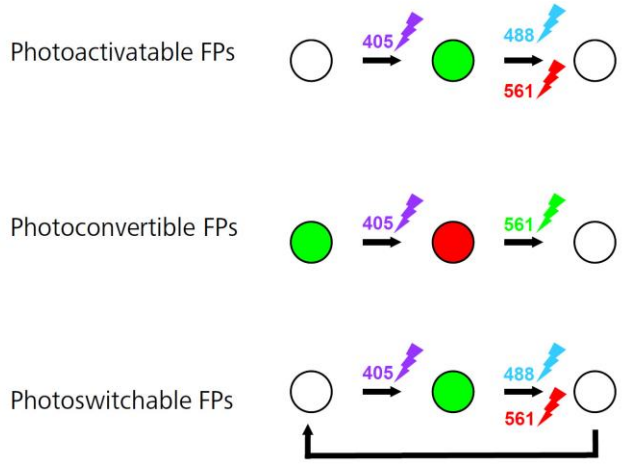
All PACs showed distinct characteristics in terms of fold change, duration of recovery (recovery kinetics), and fractional components of life-times (A1 and A2 corresponding to t1 and t2,respectively).

cAMP levels increased from bPAC to NgPAC1 activation.

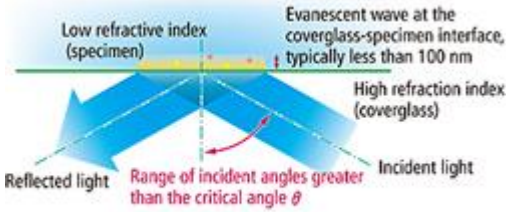
SPATIALLY CONFINED ACTIVATION OF PACS RESULTS IN DIFFERENTIAL MOBILITY OF HCN CHANNELS (HCN2)



HCN2 ::photoconvertible fluorescent protein mEos2



Imaging in TIRF mode



HCN2 diffusion was the fastest for bPAC, which reduced for TpPAC and significantly reduced further for NgPAC1, consistent with our previous observations where cAMP levels increased from bPAC to NgPAC1.

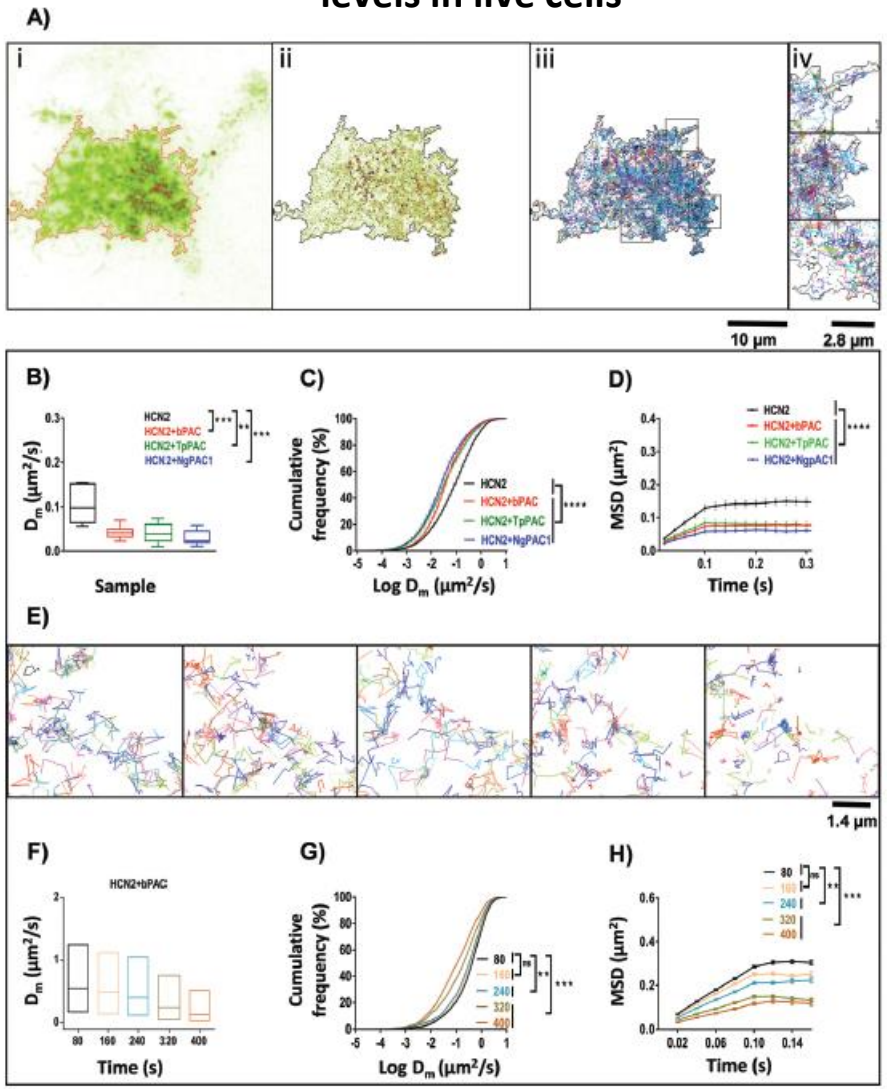
The PAC variants modulated the mobility of HCN2, correlated with their photodynamics and amplitude of the light-gated cAMP level altered by the respective PAC.

ACUTE AND CHRONIC PHOTOACTIVATION OF PACS ALTERS LATERAL DIFFUSION OF HCN2 CHANNELS

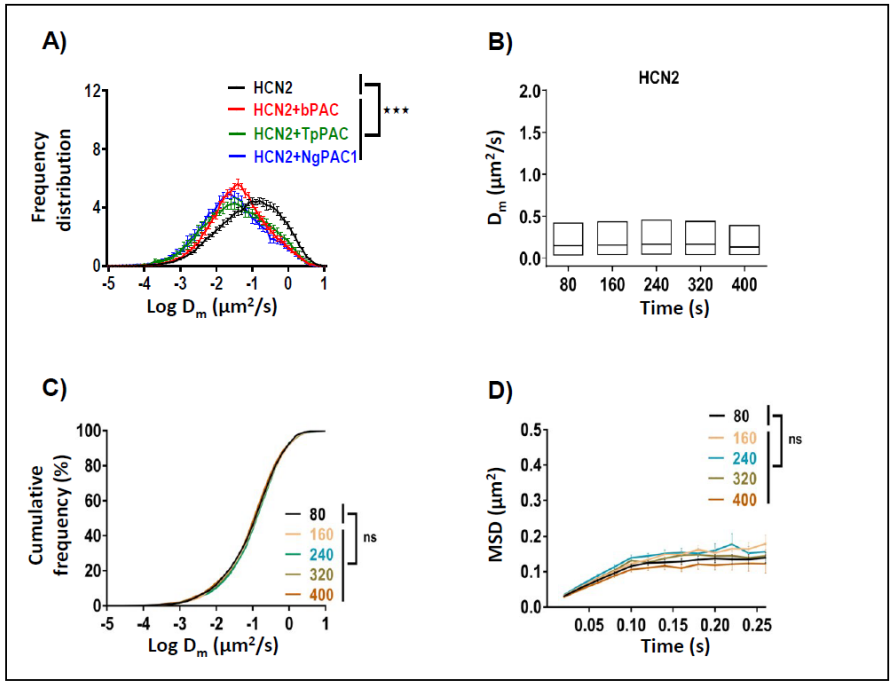
Acute activation
High power, short duration

Chronic activation
Low power, continuous

Global spatio-temporal control of HCN2 mobility by modulating cAMP levels in live cells



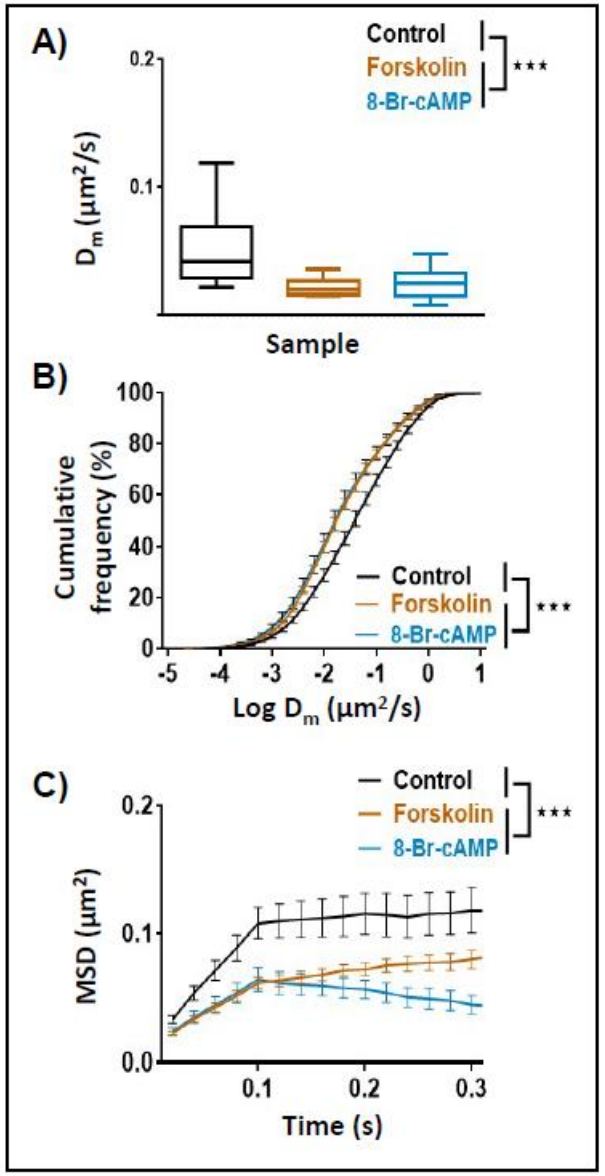
Time dependent mobility characteristics of mEos::HCN2 in live cells



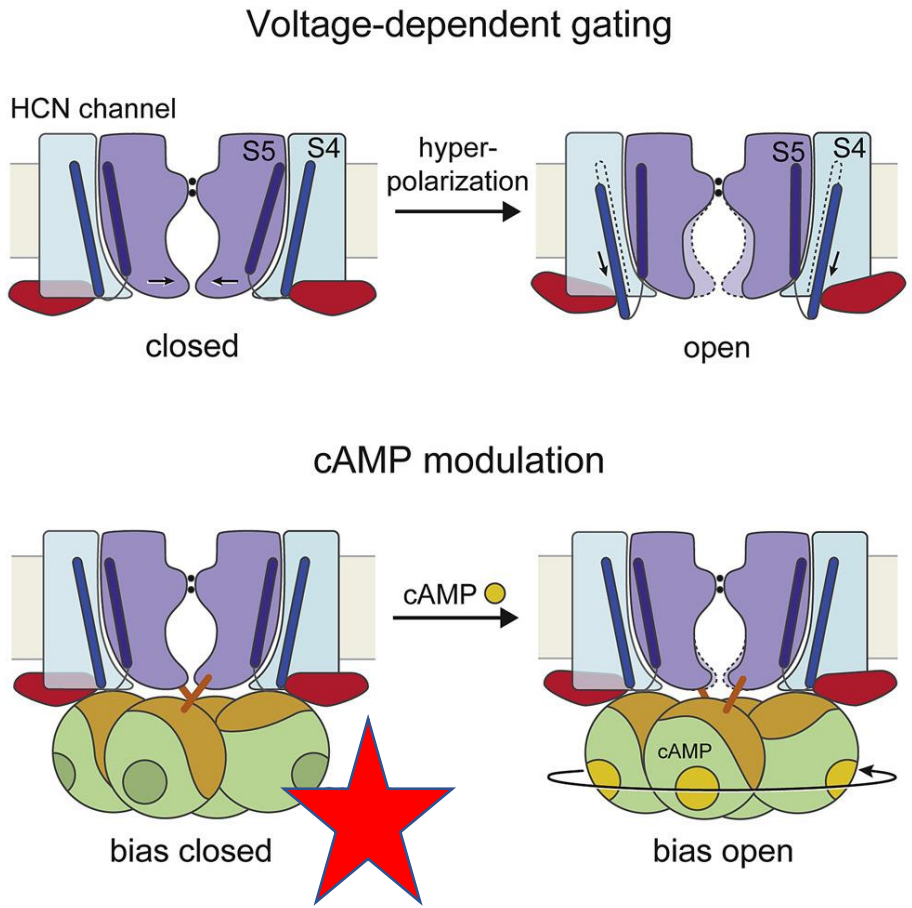
A targeted stimulation resulted in slower mobility of HCN2 in the presence of PACs, especially NgPAC1, indicating confinement of HCN2 resulting from immobilization or trapping.

Chronic stimulation showed a temporal increase in the fraction of HCN2 molecules being immobilized at low illumination intensities of optogenetic activation, which tended to saturate with longer duration of activation.

Diffusion dynamics of MEOS::HCN2 after pharmacological modulation of cAMP in live cells



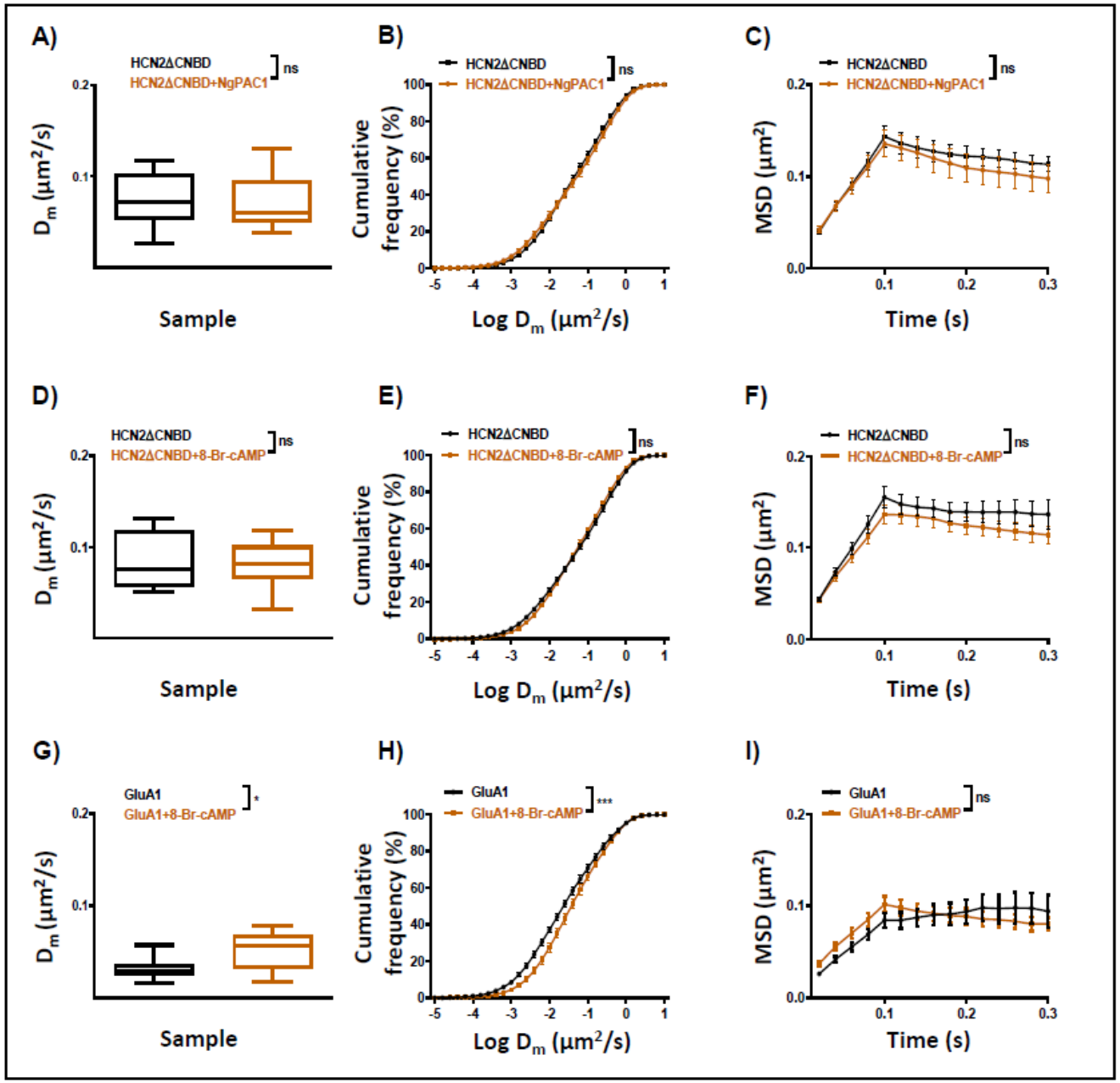
Generation of HCN mutants with altered cAMP sensitivity



Deletion mutant
Point mutants

Pharmacological treatment confirmed diffusional modifications of HCN2 were indeed due to alterations in cellular cAMP levels

DIFFUSION DYNAMICS OF MEOS::HCN2ΔCNBD AND GLUA1::MEOS UPON CAMP MODULATION IN LIVE CELLS

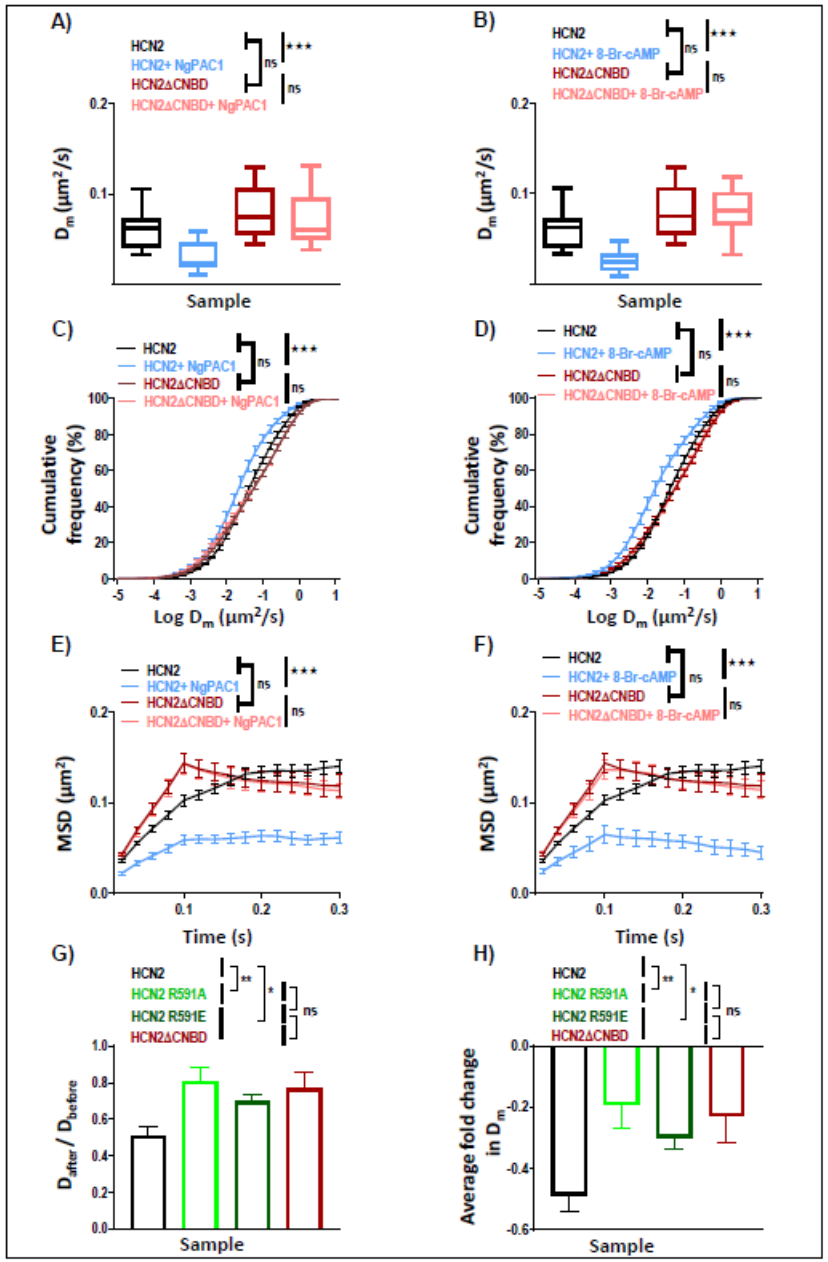


Results confirmed the cAMP dependent alterations in HCN2 lateral diffusion to be mediated by its cyclic nucleotide binding domain.

A slight cAMP dependent regulation of GluA1 dynamics and increase in mobility was observed, contrary to HCN2.

The results signified the importance of cAMP dependent differential regulation of diverse ion channels, indicating a universal mechanism for controlling membrane excitability.

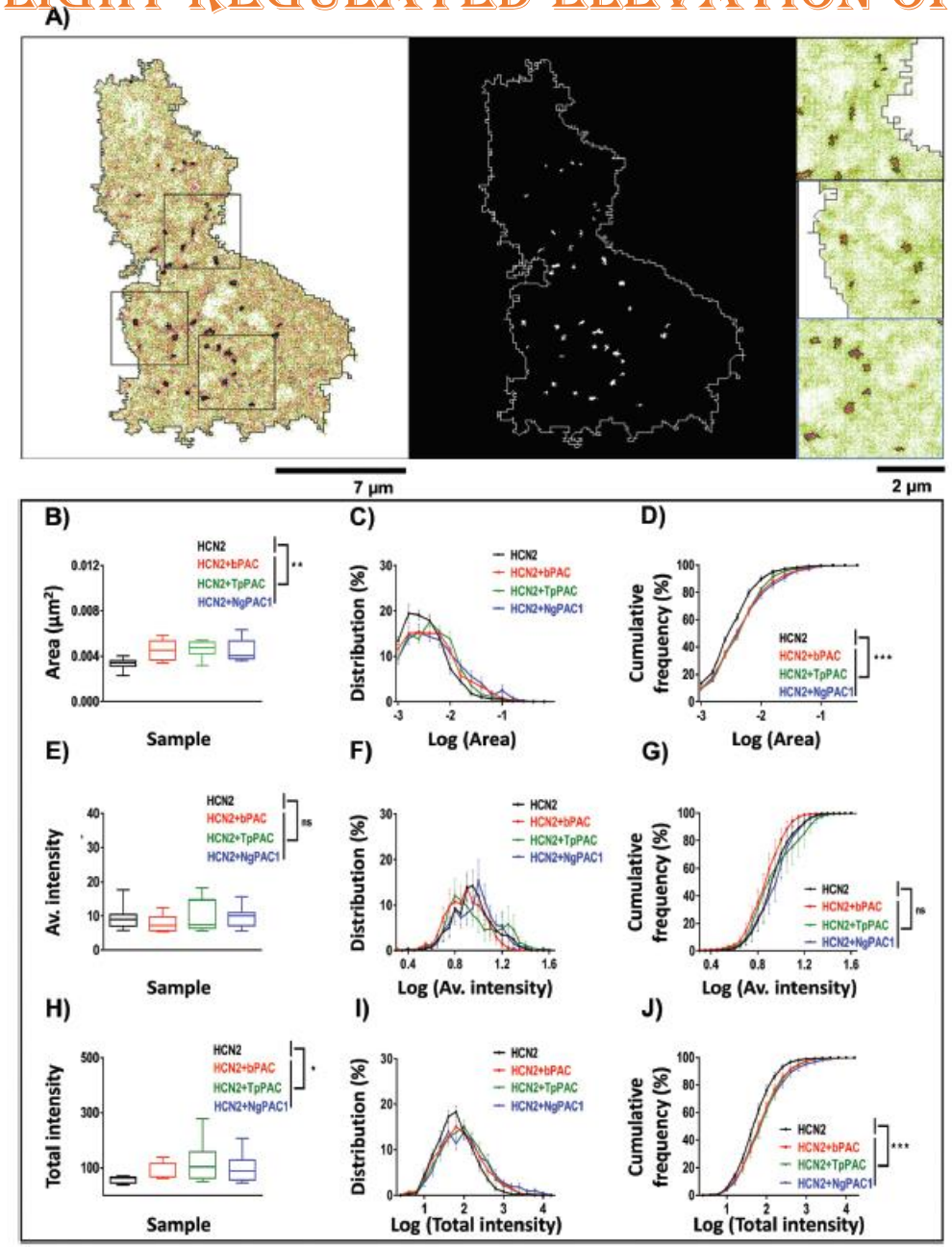
COMPARATIVE ANALYSIS OF MOBILITY KINETICS OF HCN2, ITS DELETION CONSTRUCT LACKING CNBD AND HCN2 POINT MUTANTS UPON STIMULATION



In contrast to wild type HCN2 whose mobility was significantly reduced to half by elevated cAMP levels, the point mutants (R591A and R591E) displayed slight alteration in their mobility upon stimulation, similar to the deletion construct (HCN2DCNBD).

Our results confirmed that the deviations in HCN2 lateral diffusion was indeed specific and predominantly mediated by direct binding of cAMP.

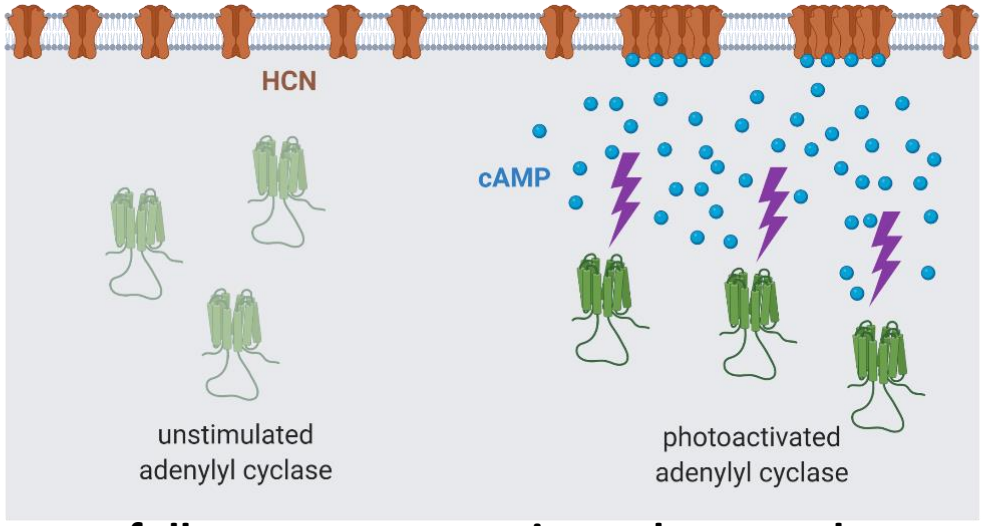
NANOSCALE AGGREGATION OF HCN2 CLUSTERS UPON LIGHT REGULATED ELEVATION OF CAMP



An increase in the area and intensity of HCN2 clusters was observed upon both optical and pharmacological stimulation of ectopically expressed as well as endogenous protein, overruling any over-expression artefacts and confirming that HCN2 channels responded to altering cAMP levels by modulating their localization and kinetics.

All these results emphasize a novel behavior of HCN2 i.e. its tendency to get immobilized with increasing levels of cAMP and self-organize into sub 100 nm sized nanoclusters.

MODULATION OF REAL-TIME REVERSIBLE NANOSCALE DISTRIBUTION OF HCN CHANNELS USING OPTOGENETIC ACTIVATION OF CAMP IN LIVE CELLS



We demonstrate a paradigm to successfully use optogenetic probes to observe different physiochemical properties of a molecule of interest at nanometer resolution in living cells.

We demonstrate that both acute and chronic stimulation of PACs can have differential effects on the HCN2 channel kinetics and clustering.

Here, with the help of optogenetic approaches, we were able to control Brownian diffusion of molecules and modulate both the nanoscale localization and real-time mobility of HCN2 channels.

Though these mechanisms are vague at present, our studies open up the possibility to use a genetically encodable optical approach to study real-time molecular changes in nano-organization which could be extended to different molecular complexes, giving a higher control on manipulating their localization and trafficking dynamics to understand the directionality of molecular signalling.

XXX

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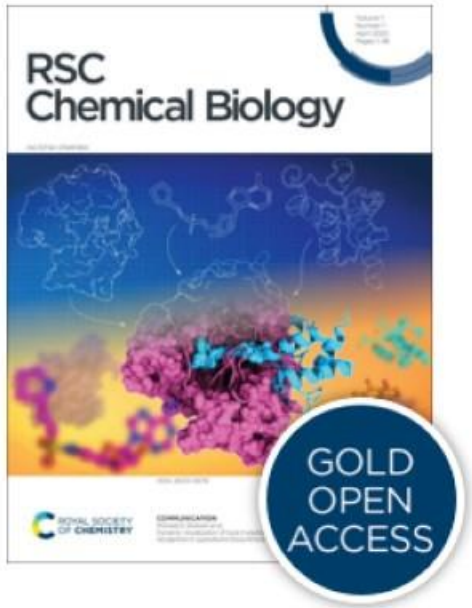
RSC Chem. Biol., 2021, xxx
DOI: 10.1039/xxx



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सत्यमेव जयते

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Thank you