Optical Control of membrane receptors using µCELL Kinetic Image plate reader (Hamamatsu) & ARPEGE facility custom illumination system

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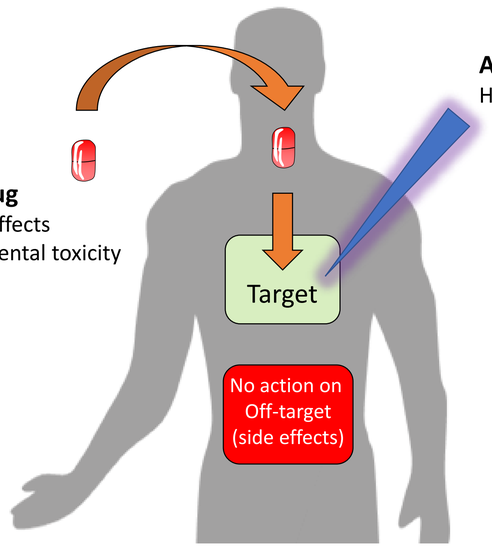
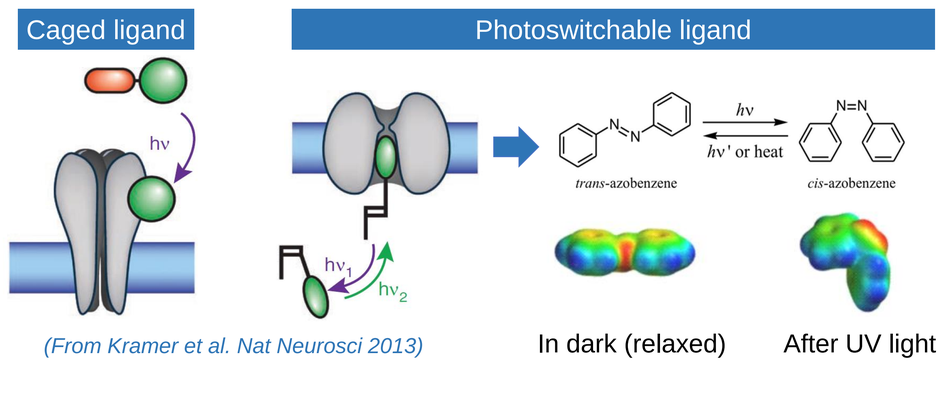
1 PHOTOPHARMACOLOGY

« Photopharmacology is a research tool to explore the physiological function of endogenous regulatory systems, with a high spatial and temporal resolution »



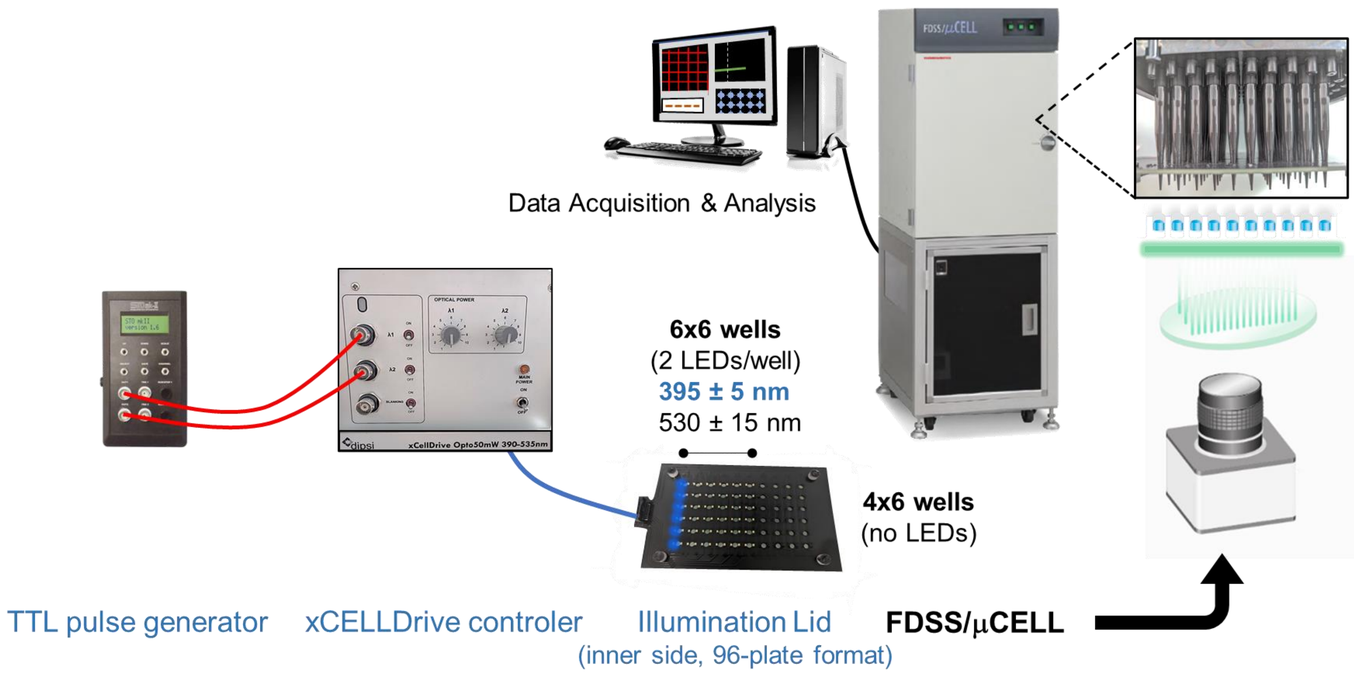


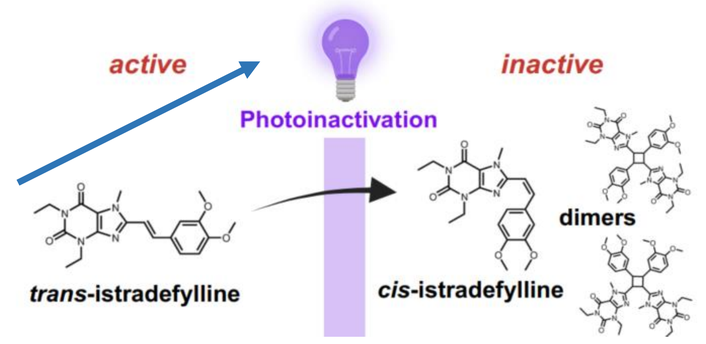
(From Kramer et al. Nat Neurosci 2013)

- **Small, diffusible, drug-like photo-controlled ligands**
  - ➢ No need of transgenic expression of exogenous protein
  - ➢ Endogenous targets in their native environment
- **Versatility**
  - ➢ Receptors
  - ➢ Ions channels
  - ➢ Lipids
  - ➢ Enzymes
  - ➢ Microtubules
- **Accurate spatiotemporal photo-control of drug/target activity**
- **Fundamental research**
  - ➢ Exploring/validating target function in preclinical models

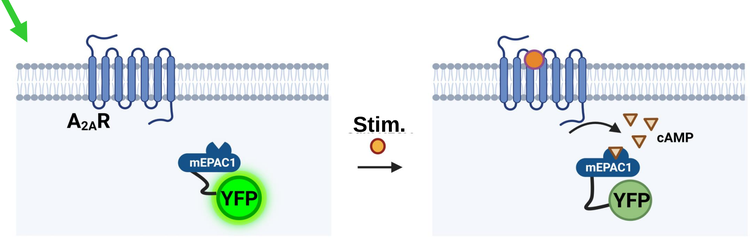
Lack of selectivity is caused by the inability to control drug activity in time and space. By incorporating photosensitive groups (caged, switchable) into the molecular structure of bioactive compounds it becomes possible to control with high spatiotemporal precision drug activity by using an external light source.

2 CUSTOMIZED EXTERNAL ILLUMINATION SYSTEM COMBINED WITH FDSS/µCELL PLATE READER TO SWITCH PHOTO-SENSITIVE MOLECULES





Istradefylline is a styryl-xanthine analogue related to caffeine and a selective/potent competitive Adenosine 2A receptor (A2AR) antagonist.

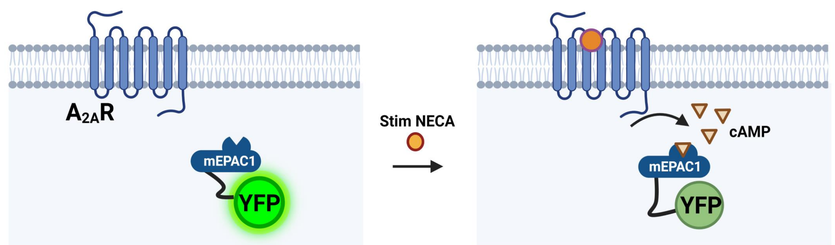


Our customized external light source is equipped with a TTL pulse generator to control the frequency of the light pulses, an xCELLDrive controller to adjust the light energy delivered by the LEDs, an illumination lid containing 36 wells equipped with 2 LEDs (395 nm & 530 nm) each able to be switched ON and OFF independently, and 24 wells with no LEDs (negative control).

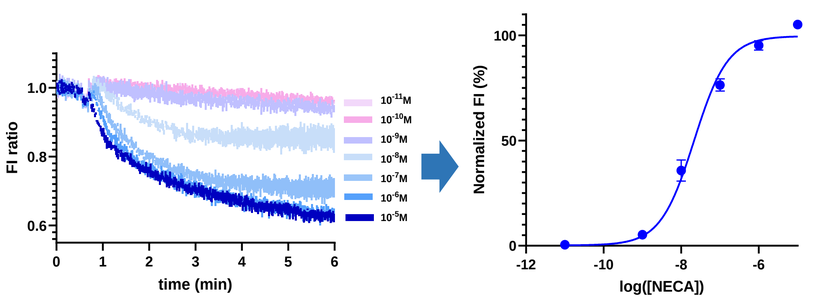
A double stable cell line containing both the A2AR and the cAMP fluorescent indicator Flamindo2 was developed for this study. Flamindo2 is a yellow fluorescent protein-based cAMP indicator that has an absorption peak at 480 nm and emission maximum at 510 nm, which are fully compatible with istradefylline photochemistry. Flamindo2 fluorescence is inversely correlated with the cAMP concentration, decreasing as the cAMP concentration increases

3 ADENOSINE 2A RECEPTOR / ISTRADEFYLLINE INTERACTION PHOTOCONTROL

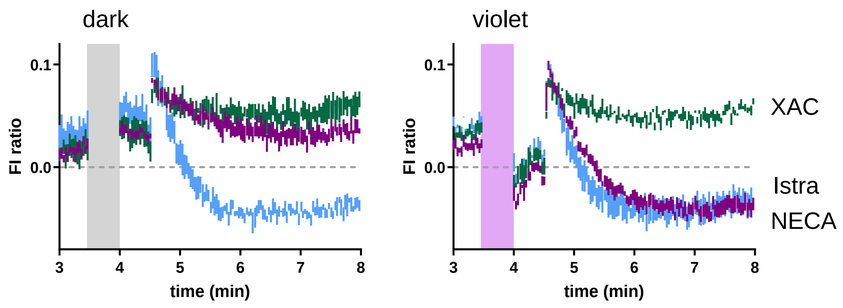
(doi.org/10.1021/acschemneuro.3c00721)

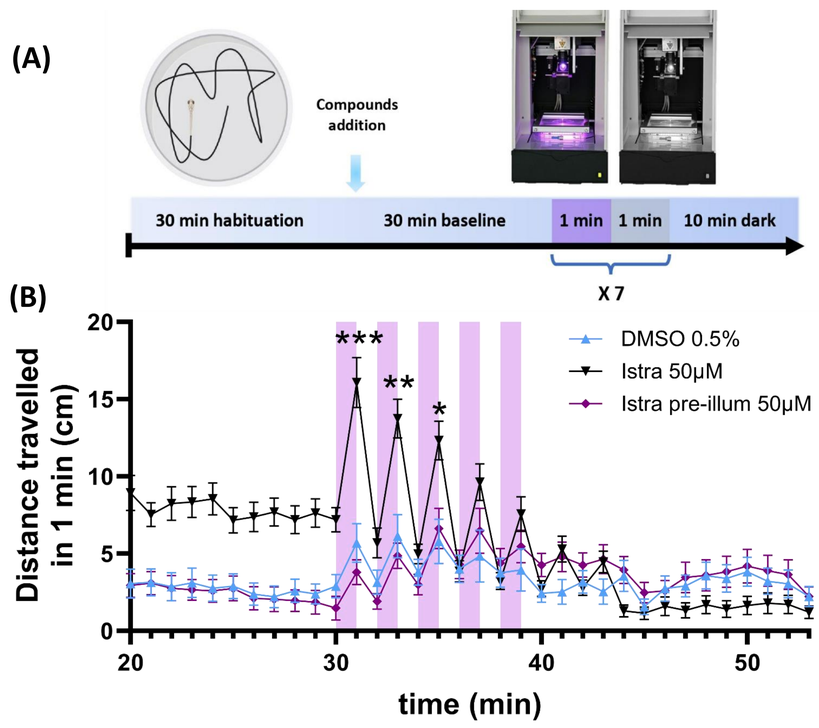


Time-course of fluorescence following A2AR stimulation with different concentrations of the agonist NECA in the CHO-A2AR-Flamindo2 cell line.



Time-course of fluorescence induced by 1 µM NECA in CHO-A2AR-Flamindo2 cells, alone (blue trace) or in the presence of 1 µM of istradefylline (violet trace) or 1 µM of the nonphotosensitive A2A antagonist XAC (green trace), with 30 s dark or violet light (395 nm, 14 mW) treatment (left and right panels, respectively).





(A) Experimental timeline: one 5 day old zebrafish larva was placed in a well of a 96-well plate. Following 30 min of habituation, larvae were exposed for 30 min to vehicle (DMSO 0.5%), 50 µM istradefylline (kept in the dark), or 50 µM UV-pretreated istradefylline (Istra preillum), directly added to the well. After 30 min, zebrafish received seven UV/dark illumination cycles (1 min UV (365 nm, 8 mW) and 1 min dark). (B) Integration of the total free-swimming distance for every minute. Each data point corresponds to the mean ± SEM of the total distance swum during 1 min by 15-16 zebrafish larvae.

The FDSS/µCELL kinetic image plate reader is a high-performance instrument enabling the simultaneous acquisition of 96 cell samples at a time, with no measurement delay between samples. Our customized external stimulation system enables photosensitive molecules to be stimulated at 395 & 530 nm, while measuring intracellular responses. As a proof of concept, we have shown that the blockade of A2AR function by istradefylline antagonist can be rapidly abolished by near-UV light (395 nm) in living CHO cells and Zebrafish in a spatiotemporal-controlled manner.