Montana Molecular
Fluorescent Biosensors for Live Cell Discovery

Pharmechanics
We help you analyze your pharmacology data

Biosensor Data Analysis Overview

Sam Hoare

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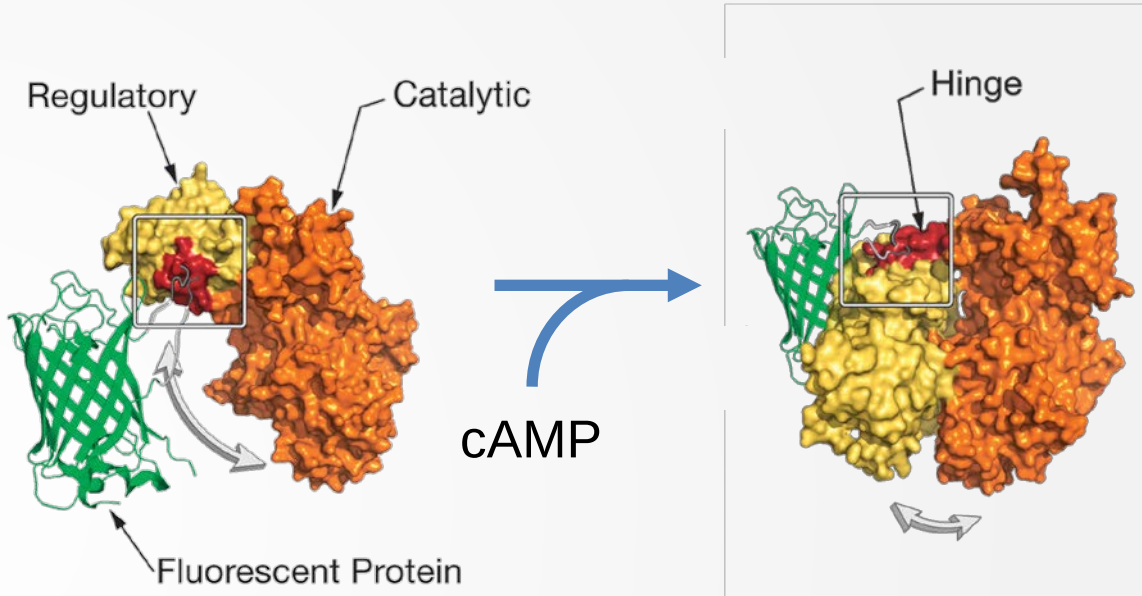
sam.hoare@pharmechanics.com

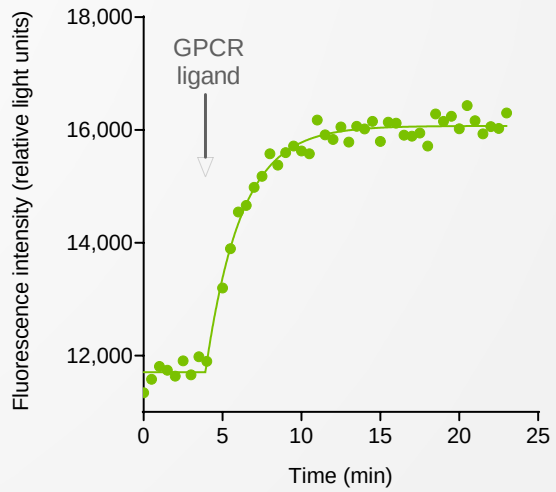
Background

Fluorescent biosensors report biological events in live cells. The event, e.g. generation of cAMP by a GPCR, results in a change in fluorescence that can be recorded in a plate reader or microscope.

This can be done repeatedly from the same well of cells, enabling convenient recording of the time course (waveform) of the activity.

Here the data handling and analysis steps are described for Montana Molecular's fluorescent biosensors of GPCR signaling.



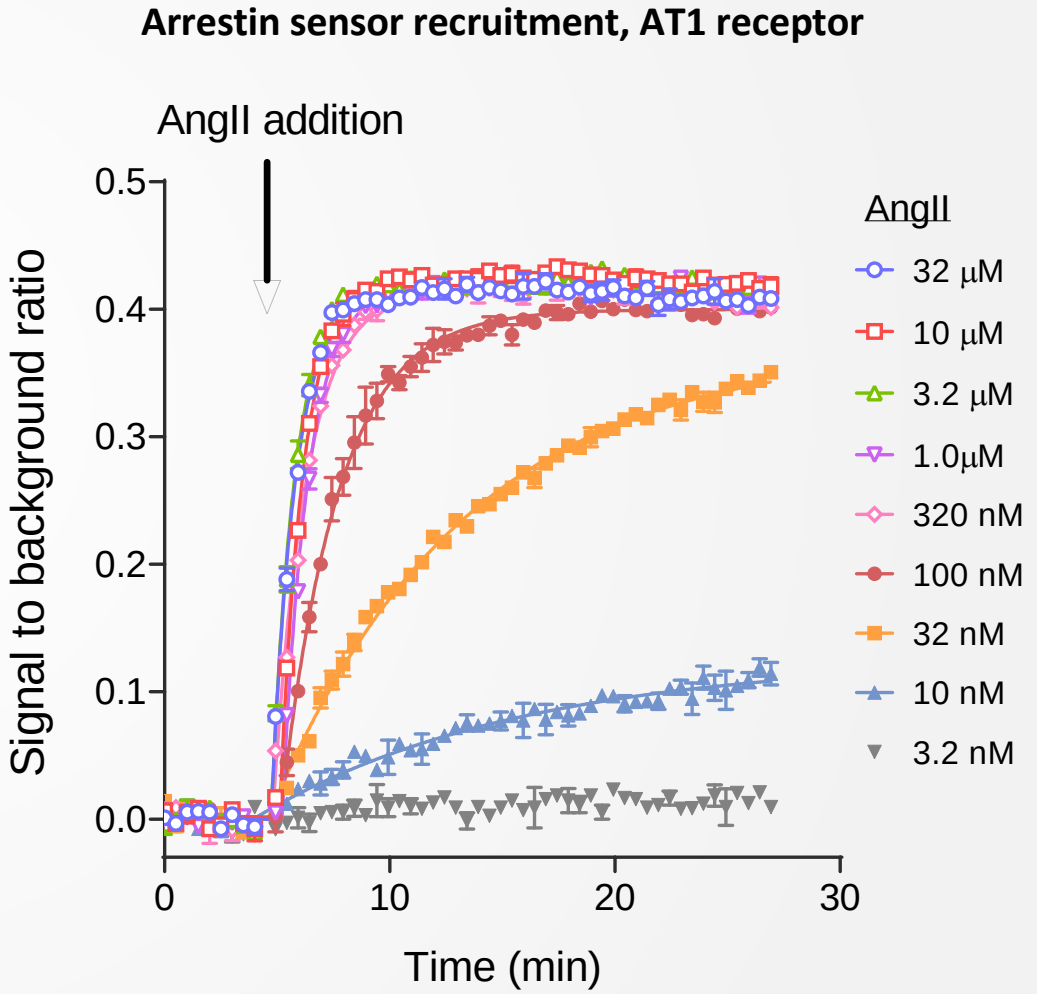


Data handling – signal to background considerations

In the experiment, the baseline fluorescence is recorded in the plate reader before the addition of the GPCR agonist. This provides an internal control for the amount of sensor / cells in the well and greatly improves the variability between technical replicates, i.e. replicate wells.

This enables much smaller signal-to-background ratios to be used, compared with other assay modalities, e.g. antibody-based detection methods.

S:B ratios as low as 0.1 provide tight enough data for robust drug activity measurements. S:B ratios of 0.5 to 3 are typical for biosensor assays.

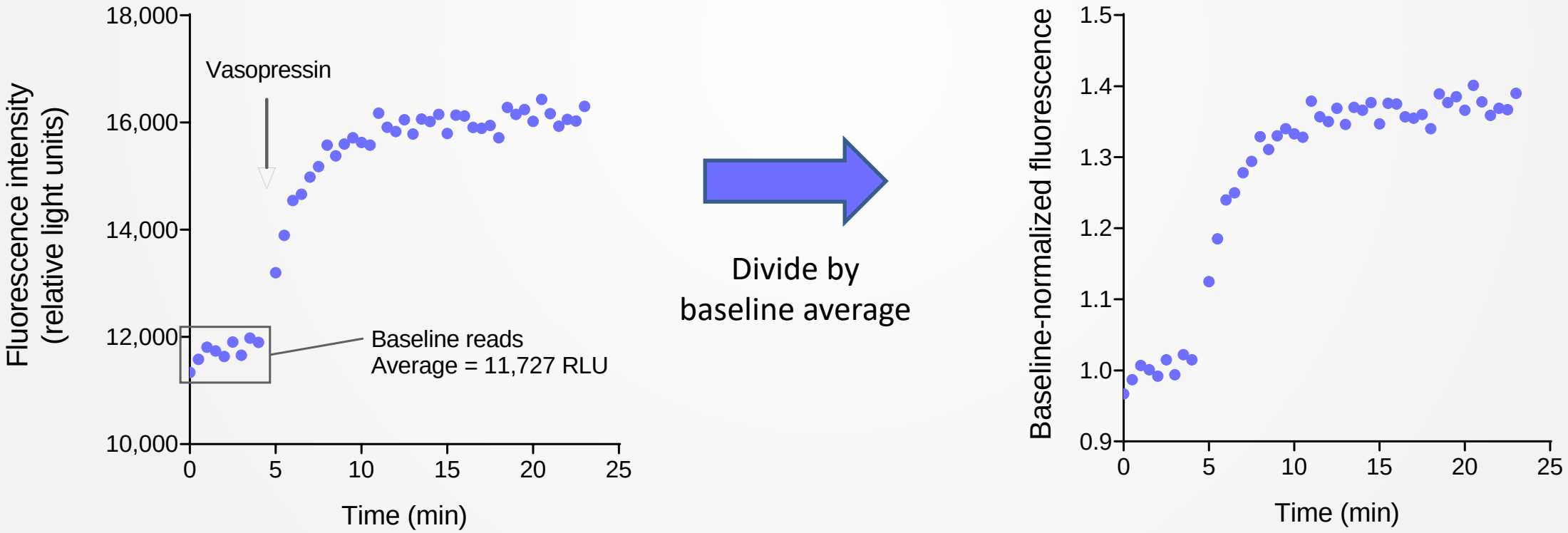


Normalizing to baseline

The signal after addition of GPCR agonist is normalized to the baseline signal before addition of agonist.

Baseline fluorescence is measured over several timepoints then the average of these timepoints taken to obtain the baseline fluorescence value.

Then data for each time point divided by this average baseline value to obtain the baseline-normalized fluorescence. This normalizes the baseline to a value of 1.



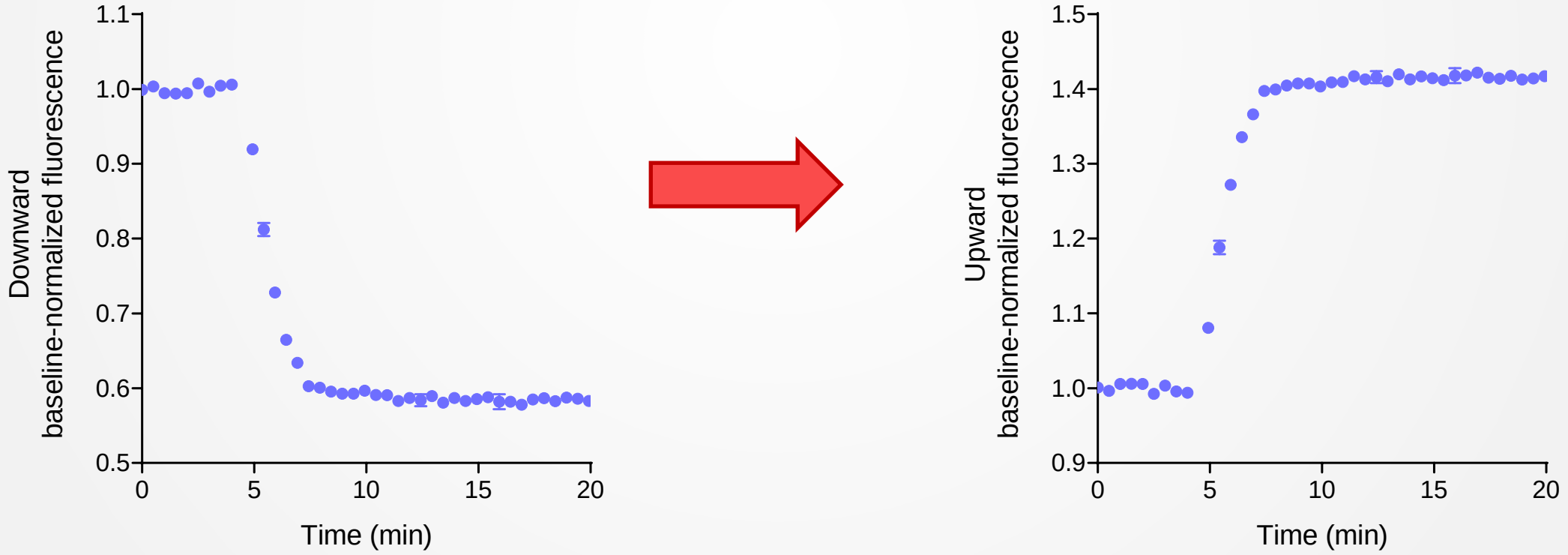
cAMP red upward cADDis, V2 vasopressin receptor

Downward sensors – additional step

The fluorescence of some sensors decreases when interacting with the signaling analyte, e.g. downward cADDis for cAMP, and the arrestin sensors.

These downward data are converted to upward data. The downward baseline-normalized fluorescence values, calculated as described on the previous slide, are entered into the following formula:

Upward baseline-normalized fluorescence = 2 – downward baseline-normalized fluorescence



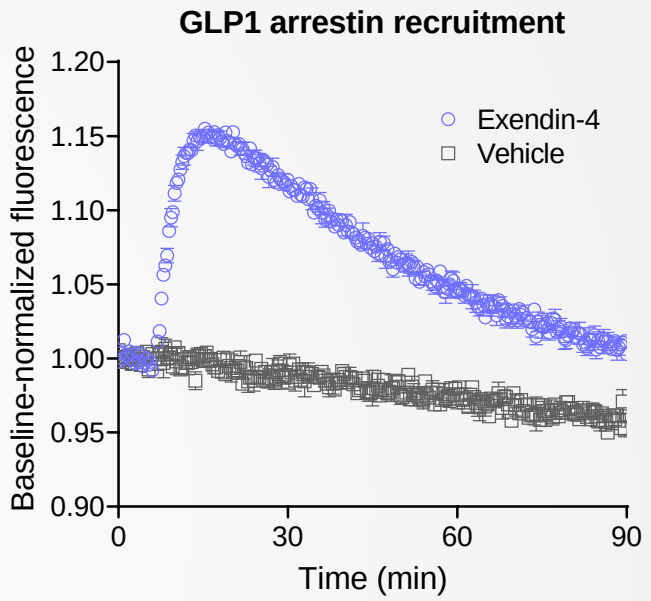
Handling baseline drift

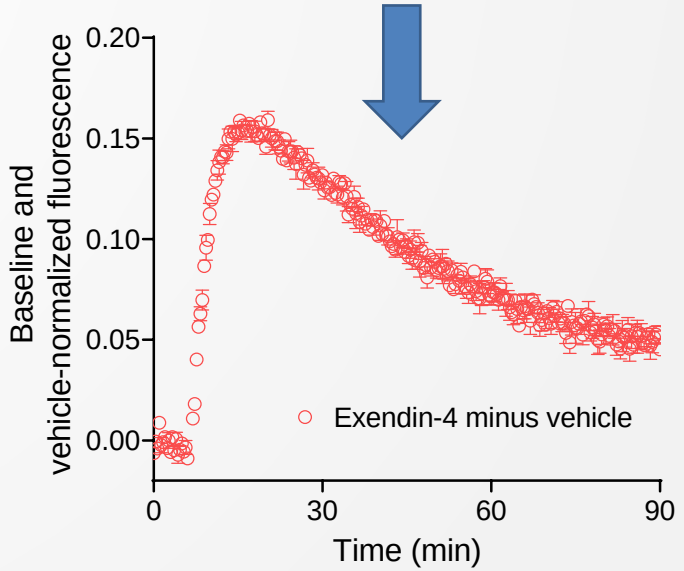
An issue with biosensors is a change of the baseline fluorescence over time. This can result in distortions of the waveform that are artifacts of the sensor (e.g. photobleaching) rather than reflecting biological phenomena (e.g. desensitization).

Montana Molecular's biosenors have been designed to minimize baseline-drift (being very bright and so minimizing photobleaching) but for advanced waveform analysis we have observed in some cases that slight drift of the sensors can impact the analysis.

Baseline drift can be handled two ways:

1. Subtract the drifting baseline from the data before the curve fitting analysis. This requires a vehicle control to be run. The baseline-normalized fluorescence of the vehicle is subtracted from the baseline-normalized fluorescence of the drug treatment. Prism contains a convenient means to do this. See here.
2. Incorporate a drift factor into curve fitting analysis. Pharmechanics has developed equations for this purpose. See next slide.





Curve fitting the time course waveform data

It can be valuable to analyze the time course waveform by curve fitting.

See here for a presentation on curve fitting time course data using Prism ("Custom time course equations background info") and here for a video workshop.

See here for a paper on the theoretical basis of the analysis.