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Allostery in Drug Discovery

NanoBRET assay

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Abstract: The NanoBRET assay is a competition-based ligand-binding assay that can be used on various receptors.

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The NanoBRET (Bioluminescence Resonance Energy Transfer) assay is a competition-based ligand binding assay which utilizes the properties of the NanoLuc sequence and TAMRA (5-Carboxytetramethylrhodamine). Indeed, a bioluminescent signal is produced by their proximity to each other, meaning that, theoretically, when TAMRA-labeled ligand binds to a protein N-terminally fused with a NanoLuc sequence this signal will be emitted. Then the subsequent addition of a series of concentrations of the ligand to be tested will result in the signal being lost if the ligand in question binds to the same ligand pocket, as well as show how high its affinity is for the given protein.

At the Scheerer lab, this assay was found to be highly successful for the melanocortin-4 and -5 receptors (MC4R and MC5R), however it is recommended to first test if the TAMRA-labeled ligand and the NanoLuc-fused protein are showing the expected bioluminescent signal at the right concentration before attempting to perform the NanoBRET assay.

Protocol:

Around 24h before the start of the experiment the HEK293T cells should be transfected with the NanoLuc fused target protein then placed in a 96-well plate. Keep in the incubator at 37°C.

The dilution plate should be prepared in a dark room under red light only as TAMRA is very light sensitive:

- 90 µl measurement buffer is added to all wells of a new 96-well plate (the dilution plate). This measurement buffer contains the TAMRA-labelled ligand at an appropriate concentration in OptiMEM (around 10ml per plate). For MC4R and MC5R, 50 nM of TAMRA-NDP-α-MSH is typically used.
- Add the 10µl of the ligands to be tested in row A at an appropriate concentration (typically 1mM) for the experiment (4 ligands can be tested in triplicate per plate).
- Perform serial dilution steps by taking 10 µl of row A to row B with a multichannel pipette, pipetting up and down then switching tips and repeating the process from B to C, C to D etc… until G to H.
- Take the plate with the transfected cells (the measurement plate) out of the incubator and aspirate all cell medium from the plate (with a multichannel pipette).
- In the dark room, transfer 75 µl from each well from the dilution plate to the measurement plate using a multichannel pipette.
- Cover the measurement plate with aluminum foil so it is not exposed to light and place it back into the incubator at 37°C for 2h.
- In the meantime, prepare the Fluorofurimazine from the Nano-Glo Promega kit by diluting it 1:100 in OptiMEM.

- At the plate reader add 25µl per well under red light using a multichannel pipette.
- Measure at a wavelength of 530-40 nm and at a wavelength of 620-10 nm. The BRET ratio calculated are the results of the 620 nm wavelength over the 530 nm wavelength for each individual well.

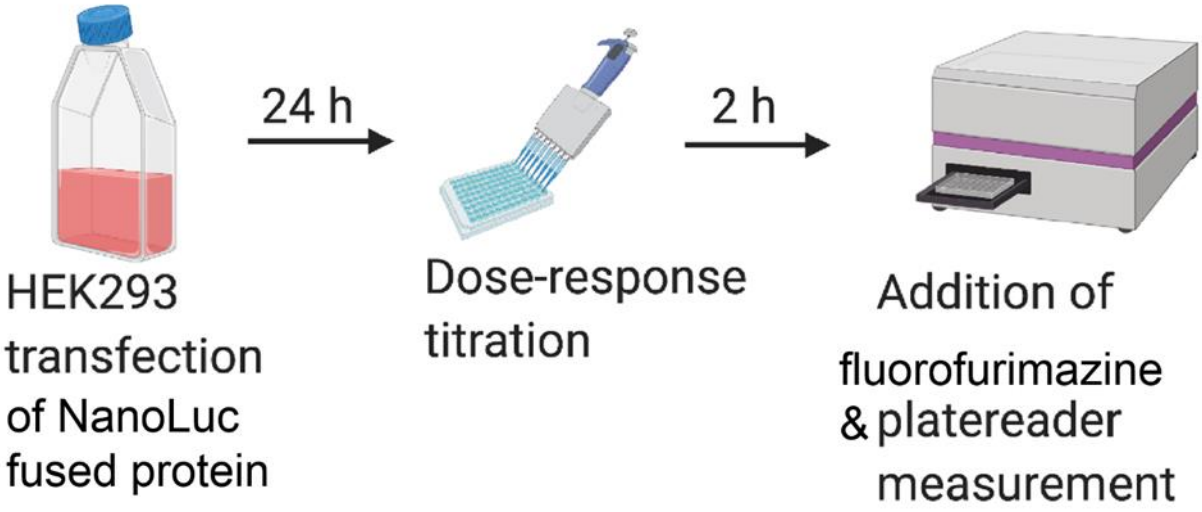

Figure 1: NanoBRET assay workflow.