Tech Note 07 / March 15, 2016

Binding Kinetics of a GPCR

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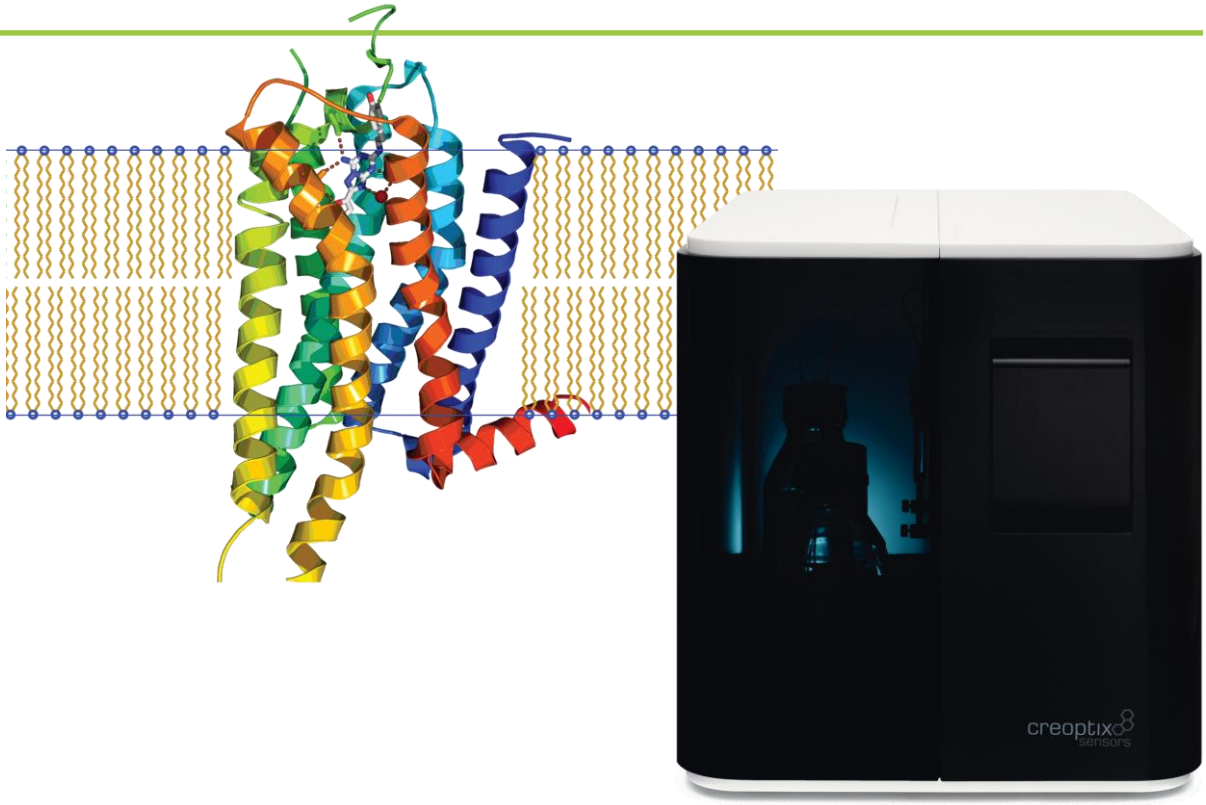

G-protein coupled receptors (GPCRs) comprise a large class of integral membrane proteins, crucial for the transduction of extracellular stimuli across the plasma membrane to elicit molecular and cellular responses. They regulate a variety of physiological processes in eukaryotes and represent the largest group of therapeutic targets, with more than 30% of available pharmaceuticals targeting GPCRs.

Membrane proteins are notoriously difficult to study due to the requirement for a membrane-mimicking environment and their instability once extracted from a cellular membrane. Here we show the capability of the WAVE to measure the interaction of a peptide ligand agonist (NTA11) with a thermo-stabilized variant of the neurotensin receptor 1 (NTSR1)[1] at highest resolution. This GPCR mediates the multiple functions of neurotensin, such as hypotension, hyperglycemia, hypothermia, antinoci-ception, and regulation of intestinal motility and secretion[2].

Material and Methods

The receptor was in vivo biotinylated via an avi-tag and captured on a streptavidin pre-coated sensor (WAVEchip DXH-S). For the measured peptide agonist, a mutated and truncated form of neurotensin (Mw 725 Da) comprising residues 8-13 with a Y11A substitution was used. Dose-response curves were recorded for 7 different analyte concentrations of a 3-fold dilution series with 300 nM being the highest concentration. The flow rate was set to 30 µL/min. The running buffer was 50 mM Tris pH 7.5, 150 mM NaCl, and 0.1% (w/v) of the detergent lauryl maltose-neopentyl glycol (L-MNG)[3]. All measurements were carried out at 25°C.

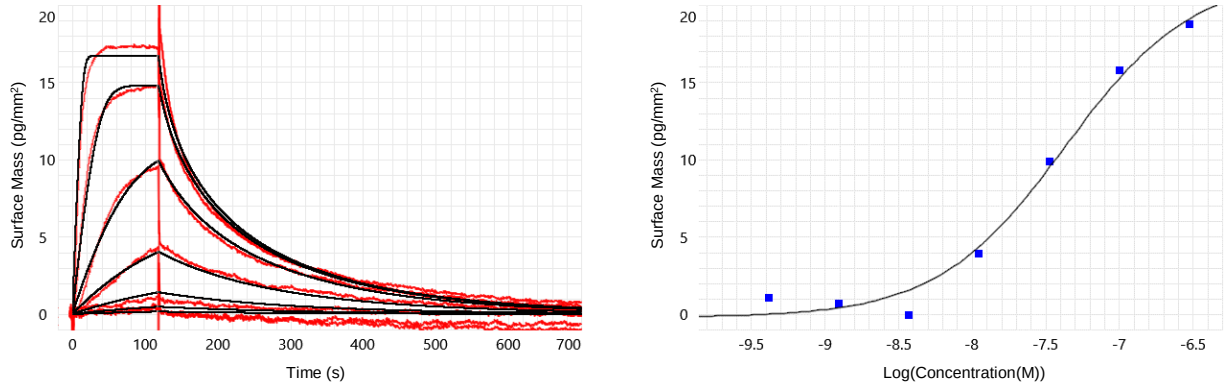

Figure 1: Sensorgrams of the interaction between a modified neurotensin peptide (Mw 725 Da) as analyte and a thermostabilized neurotensin receptor (NTSR1, 70 kDa protein/detergent complex) as ligand. The data were fitted with a model for a 1:1 interaction including a term for mass transport limitation in the WAVEcontrol software.

Kinetic determination				Equilibrium determination	
R_{max} (pg/mm²)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_d (nM)	R_{max} (pg/mm²)	K_d (nM)
17.982	1.681E6	3.497E-2	20.8	22.083	42.6

Table 1: Kinetic rates and dissociation constant for the NTA11 agonist obtained with a 1:1 interaction model including a term for mass transport limitation.

Results

Purified and biotinylated neurotensin receptor was captured on a streptavidin pre-coated sensor (WAVEchip DXH-S) at a density of 1350 pg/mm2. Dose-response curves for binding of peptide agonist were recorded (Figure 1), yielding binding data that could be well fitted with a model for a 1:1 interaction including a term for mass transport. The obtained kinetic rates and equilibrium constant are summarized in Table 1.

References

(1) Egloff, P., Hillenbrand, M., Klenk, C., Batyuk, A., Heine, P., Balada, S., Schlinkmann, K. M., Scott, D. J., Schütz, M., and Plückthun, A. (2014) Structure of signaling-competent neurotensin receptor 1 obtained by directed evolution in Escherichia coli. Proc. Natl. Acad. Sci. U. S. A. 111, E655–62.
(2) Krumm, B. E., and Grisshammer, R. (2015) Peptide ligand recognition by G protein-coupled receptors. Front. Pharmacol.
(3) Chae, P. S., Rasmussen, S. G. F., Rana, R. R., Gotfryd, K., Chandra, R., Goren, M. a, Kruse, A. C., Nurva, S., Loland, C. J., Pierre, Y., Drew, D., Popot, J., Picot, D., Fox, B. G., Guan, L., Gether, U., Byrne, B., Kobilka, B., and Gellman, S. H. (2010) Maltose-neopentyl glycol (MNG) amphiphiles for solubilization, stabilization and crystallization of membrane proteins. Nat. Methods 7, 1003–1008.