BNFO 300: Summary of Experiment

Repelling class discrimination: ephrin-A5 binds to and activates EphB2 receptor signaling. Himanen, et al. (2004), Nature Neuroscience 7, 501 – 509

Link to article: http://www.nature.com/neuro/journal/v7/n5/full/nn1237.html

Nervous system function depends on the spatial arrangement of neurons and axons. If the patterns of connections between elements of the nervous system are inaccurate, various diseases may occur. Axon pathfinding is highly accurate, and the process behind it is poorly understood. The Eph receptors and ephrins are tyrosine kinases that transfer signals are one component of the machinery that determines neural cell migration and axon pathfinding. Eph and ephrins are each divided into two classes, A and B, based on which types of substrates they bind to: A ephrins bind to EphA receptors, and B ephrins bind to EphB receptors. These interactions are not completely understood. Himanen et al try to find an exception to the notion that A binds to A and B binds to B, by determining the extent to which ephrin-A5 binds to EphB2. Finding an exception to the rule would increase our understanding of the Eph/ephrin signaling pathway, and open up new possibilities for research in interactions that were previously thought not to exist.

To determine how much ephrin-A5 binds to the EphB2 receptor, Himanen et al used surface plasmon resonance (SPR). Metals have delocalized electrons on their surfaces, that is, electrons are able to move from one positive metal atom nucleus to another. These electrons move randomly about, and when a photon hits the surface of a metal, its electrons begin to oscillate at a certain frequency characteristic of the metal and whatever the metal is in contact with. A surface plasmon is a quantized oscillation of free electrons on the surface of metals in contact with something else. Surface plasmon resonance occurs when the frequency of a photon that hits the surface of a metal is the same as the frequency of the metal's surface plasmons. A useful property of surface plasmons is that they influence the interaction of metals with light.

In this experiment, a gold film coated a piece of glass. Gold has a high conductivity which is useful for SPR. On top of the gold was a dextran medium that contained many carboxyl groups which proteins could bind to. In two separate experiments, EphB2 and ephrin-A5 were added to the dextran medium. Then, a continuous solution of ephrin-A5 was added to the EphB2-coated medium, and a continuous solution of EphB2 was added to the ephrin-A5-coated medium. As particles of EphB2 and ephrin-A5 bound to each other in each experiment, the frequency of surface plasmon resonance on the gold surface changed, and this changed the index of refraction of the piece of glass. This change was proportional to the amount of EphB2 and ephrin-A5 binding that occurred. The change was measured continuously by firing a beam of light at a wide angle at the opposite side of the gold, dextran, and EphB2/ephrin-A5 medium. At one particular angle, the photon is absorbed by the piece of gold to create plasmon waves. The absorption angle is measured by a detector (Figure 1). This angle depends on the index of refraction which depends on the amount of binding, so SPR can be used to determine the amount EphB2 and ephrin-A5 bind to each other over time.

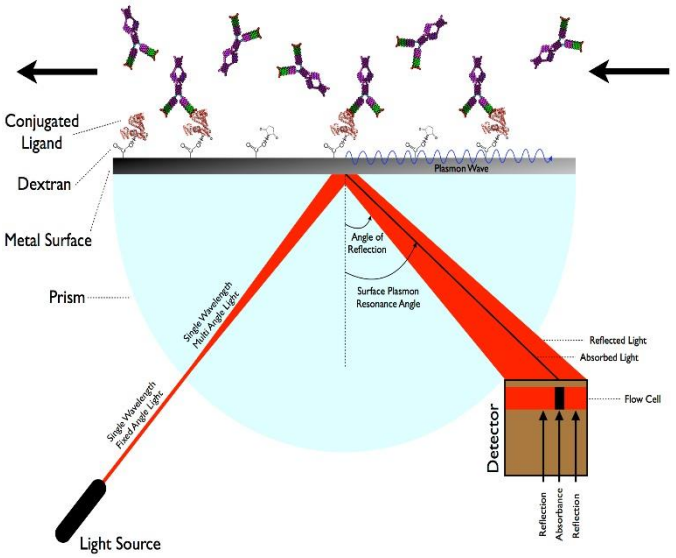

Figure 1: Surface plasmon resonance: the technique. Himanen et al used gold for the metal surface, and instead of a prism, they used a simple piece of glass and fired a wide-angle light beam. (Wikipedia article on Surface Plasmon Resonance)

The SPR results are shown in Figure 2. The first part of the curve between around 120 and 300 seconds represents analyte being added to the ligands: as more is added, more binding occurs. The second part of the curve after 300 seconds represents dissociation of the analyte after the addition is completed. The binding constant was calculated by comparing the association rate (the first curve) to the dissociation rate (the second curve). Affinity and specificity were also measured using these curves. These results show that EphB2 binds to ephrinB2 and ephrinB1 more than ephrinA5 (Figure 2c). Figure 2d shows that EphB2 binds to ephrinA5 with high affinity. The curves are also used to calculate the binding constant between EphB2 and ephrinA5. Note that the binding constant in Figure 2c is approximately 1 divided by the binding constant in Figure 2d.

Himanen et al's results show that a class A ephrin binds to a class B Eph receptor. Though EphB2 prefers binding by ephrin-B1 and ephrin-B2, ephrin-A5 also binds to it. The results show that interclass interaction between ephrin and Eph is more common than postulated and should be studied further, but the authors caution that cross-class binding is still probably rare. From other experiments in this study, the ephrin-A5 and EphB2 interaction may also regulate movement of neurons in the visual processing area of the brain.

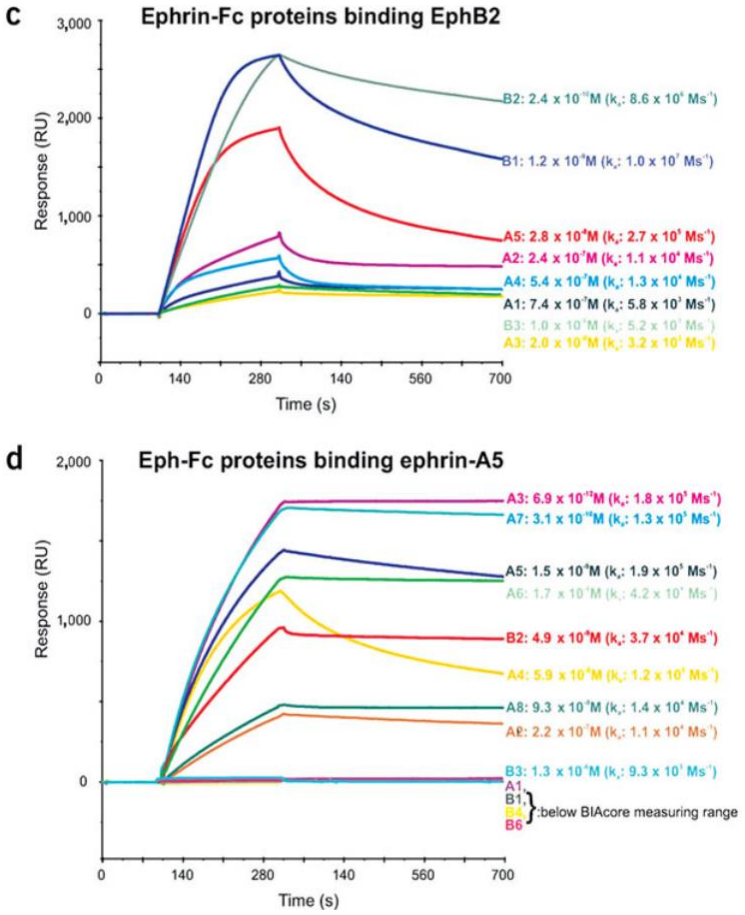

Figure 2. SPR results. One RU (vertical axis) is equal to 1 pg/mm^2 of binding. In this summary, I only talk about the SPR measurements of EphB2 and ephrin-A5, which are red in both sub-figures.
(From article)