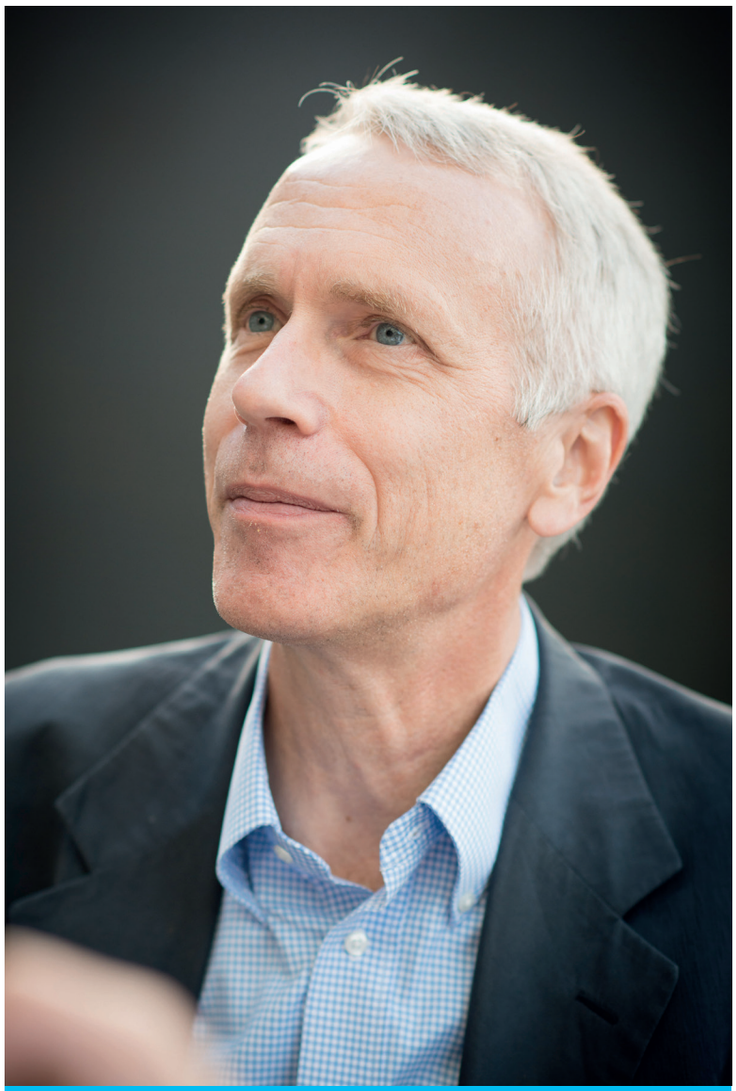
Interview with Brian Kobilka and Jan Steyaert

Brian Kobilka (Stanford University School of Medicine in California) shared the 2012 Nobel Prize in Chemistry with Robert Lefkowitz for their studies of G protein-coupled receptors. A couple of months ago Kobilka was in Brussels to give a talk about his work on invitation of Jan Steyaert (VIB Structural Biology Research Center, Vrije Universiteit brussel). The two scientists met 5 years ago, an encounter that would lead to a very rewarding collaboration. VIBnews had a talk with them.



Brian Kobilka

What are G protein-coupled receptors (GPCRs) and what makes them important?

Brian: GPCRs play a central role in how our cells respond to hormones and neurotransmitters. Localized on the surface of cells, they transmit signals to the inside of the cell, thus changing its behavior. This kind of cell communication allows us to function properly. GPCRs are involved in normal physiology but also in diseases, which makes them important drug targets. Actually about 30% of current drug targets are GPCRs. Understanding their structure may help the search for more selective and effective drugs.

I initially became interested in β-adrenergic receptors working as a physician in intensive care units where I used β-agonists to treat asthma and β-blockers to treat heart disease. This brought me to the lab of **Robert Lefkowitz**, the start of my career as a researcher.

How challenging was it to unravel the protein structure of GPCRs?

Brian: To determine the structure of proteins such as GPCRs it is necessary to crystallize the protein. When we set out in the early 1990s, we didn't know the first thing about crystallography or about the biochemical behavior of these proteins, for example whether they were dynamic or unstable. Using fluorescence spectroscopy we saw that the β-adrenergic receptor is flexible, especially when bound to an agonist. However, for proteins to crystallize they must all be in the same conformation, so this was a problem for obtaining the structure of the receptor in an active state.

But you managed to freeze the beta2-adrenergic receptor in one position?

Brian: For many years, we tested different antibody formats to freeze the conformation of the receptor. After trying conventional antibodies, synthetic single

chain Fvs and even chicken antibodies, I got to know **Jan Steyaert** at a Gordon conference in Italy. When I met him, he was pioneering the application of Nanobodies® as facilitators of protein crystallogenesis.

Shortly after the conference I sent Jan purified, agonist-bound b2AR reconstituted into phospholipid vesicles for immunizing llamas. By November 2010 we had our first Nanobodies® and my postdoctoral fellow **Søren Rasmussen** identified one that exhibits G-protein-like properties: Nb80. The beta2-adrenergic receptor–Nb80 complex gave us the first picture of the active-state conformation of the b2AR.

Our ultimate goal however was to solve the structure of the b2AR–Gs transmembrane signaling complex. Together with the Steyaert lab we developed another Nanobody (Nb35) that binds to the interface between the α and the β subunits of the heterotrimeric Gs protein.

The first crystals of the elusive complex were obtained in April 2011. It is the crystal structure of the β2 adrenergic receptor in complex with its G protein which I believe was instrumental in my being awarded the 2012 Nobel Prize in Chemistry.

Jan: This remarkable success is for a large part thanks to the quality of the proteins Brian gave us to immunize the llamas. When generating Nanobodies the quality of starting material is crucial.

> “I often compare Brian with a spider in the middle of a web, in the good sense.”

The tough part in preparing proteins for research is the biochemistry. Often underestimated, but we definitely need more good biochemists.

What were other crucial factors for this success?

Brian: Difficult to say. There are so many different pieces that were essential. To come to the final crystal structure is the result of a big team effort. I collaborated with many people, all of them with key contributions to this work. I'm happy I managed to meet the right people. All together I think that we are about 20 people who had an active role in this story. It was a tough decision to make when I could only choose 14 to accompany me in Sweden to receive the Nobel Prize (note of the editor: Jan Steyaert was one of them).

So Networking and collaborations are essential to top science?

Jan: Definitely. I often compare Brian with a spider in the middle of a web, in the good sense. He knows how to attract and interact with the right people. Brian always communicates very clearly and transparently. This is very important in collaborations, but not always evident. I also admire him for doing the things he does with such a small group. Brian's group prooves that it is not size but the quality of the people that matters. I'm trying to follow his example by going for a smaller team of top people, with better funding per headcount.

These two breakthrough papers in which **Jan Steyaert** and **Brian Kobilka** are collaborating are highly cited. Published less than 4 years ago, both papers collected together over 1000 citations to date (dec 2014) indicating the breakthrough value of using Nanobodies® in determining protein structures.

- Crystal structure of the beta2 adrenergic receptor-Gs protein complex, Rasmussen *et al. Nature,* 2011
  - 599 citations
  - Featured in Natures '365 days: 2011 in review' as a fundamental breakthrough
- Structure of a nanobody-stabilized active state of the β2 adrenoceptor, Rasmussen *et al. Nature,* 2011
  - 492 citations

Brian: What I consider very important within my group is that people communicate and work together. Nowadays you see too much competition, even within labs. This isn't productive. I try to teach my team members that sharing and working together is much better. Especially in current times where funding is more limited and problems need to be tackled in a multidisciplinary way, it is important to collaborate in an honest way.

This is also the kind of collaboration between Jan and me. Jan is always confident and straightforward about what is possible and what not. When a deal is made, he always delivers what he promised. And that is rare. You feel he can rely on his people and has a lot of confidence in them. It is a no nonsense relation that I appreciate a lot.

How does it feel to be a Nobel laureate?

Brian: It never occurred to me that I might win it until 2012 when I did. I'm honored for receiving this recognition, but it also has its disadvantages. It has been very disruptive, in part because I accepted too many invitations to speak at conferences. The volume of e-mail increased dramatically and as a result I wasn't spending enough time focusing on my research.
Jan: There is also a huge difference in the perception of a Nobel Prize between the US and here.
In the US there are more Nobel Prizes, which makes them less exceptional. Here every university dies to have a Nobel Prize.

Jan Steyaert and *Brian Kobilka*