How different are nanobody-stabilized GPCR structures from their G-protein-stabilized equivalents?

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The G-protein coupled receptor (GPCR) family constitutes the majority of drug targets.[1] Despite the diversity of their biological roles, GPCRs adapt the same structural architecture of seven transmembrane (TM) helices[2] and signal mainly through coupling to hetreotrimeric G-proteins.[3,4] However, G-proteins are extremely sensitive to detergents, pH and nucleotides, which are often needed to crystallize GPCRs for X-ray crystallography.[5] Nanobodies represent a successful alternative to G-proteins in stabilizing active-state GPCRs.[5,6] Their importance in GPCR structural biology is emphasized by their role in crystallization of all but one of the stabilized active-state, Class A rhodopsin, GPCRs.[7-9] Extensive molecular-dynamics simulations including metadynamics enhanced sampling were used to compare the effect of these nanobodies on the binding modes of the co-crystalized agonists and the organization of the binding pocket of the three nanobody-stabilized GPCR's crystals structures with the G-protein complexes. Our results show ligand-specific changes that can alter the agonist binding modes.

(1) Ma, P.; Zemmel, R. *Nature reviews. Drug discovery* **2002**, *1*, 571.

(2) Pierce, K. L.; Premont, R. T.; Lefkowitz, R. J. *Nature reviews. Molecular cell biology* **2002**, *3*, 639.

(3) Neves, S. R.; Ram, P. T.; Iyengar, R. *Science* **2002**, *296*, 1636.

(4) Oldham, W. M.; Hamm, H. E. *Nature reviews. Molecular cell biology* **2008**, *9*, 60.

(5) Steyaert, J.; Kobilka, B. K. *Current opinion in structural biology* **2011**, *21*, 567.

(6) Ghosh, E.; Kumari, P.; Jaiman, D.; Shukla, A. K. *Nature reviews. Molecular cell biology* **2015**, *16*, 69.

(7) Kruse, A. C.; Ring, A. M.; Manglik, A.; Hu, J.; Hu, K.; Eitel, K.; Hubner, H.; Pardon, E.; Valant, C.; Sexton, P. M.; Christopoulos, A.; Felder, C. C.; Gmeiner, P.; Steyaert, J.; Weis, W. I.; Garcia, K. C.; Wess, J.; Kobilka, B. K. *Nature* **2013**, *504*, 101.

(8) Huang, W.; Manglik, A.; Venkatakrishnan, A. J.; Laeremans, T.; Feinberg, E. N.; Sanborn, A. L.; Kato, H. E.; Livingston, K. E.; Thorsen, T. S.; Kling, R. C.; Granier, S.; Gmeiner, P.; Husbands, S. M.; Traynor, J. R.; Weis, W. I.; Steyaert, J.; Dror, R. O.; Kobilka, B. K. *Nature* **2015**.

(9) Rasmussen, S. G. F.; Choi, H. J.; Fung, J. J.; Pardon, E.; Casarosa, P.; Chae, P. S.; DeVree, B. T.; Rosenbaum, D. M.; Thian, F. S.; Kobilka, T. S.; Schnapp, A.; Konetzki, I.; Sunahara, R. K.; Gellman, S. H.; Pautsch, A.; Steyaert, J.; Weis, W. I.; Kobilka, B. K. *Nature* **2011**, *469*, 175.