Published in final edited form as:

J Am Soc Mass Spectrom. 2015 May ; 26(5): 808–817. doi:10.1007/s13361-015-1083-4.

Effective Application of Bicelles for Conformational Analysis of G Protein-Coupled Receptors by Hydrogen/deuterium Exchange Mass Spectrometry

Nguyen Minh Duc^{#1}, Yang Du^{#2}, Cheng Zhang², Su Youn Lee¹, Thor S. Thorsen², Brian K Kobilka^{*,2}, and Ka Young Chung^{*,1}

¹School of Pharmacy, Sungkyunkwan University, 2066 Seobu-ro, Jangan-gu, Suwon, South Korea

²Department of Molecular and Cellular Physiology, Stanford University, Medical School, 297 Campus Drive, Beckman Center, Stanford, California, USA

[#] These authors contributed equally to this work.

Introduction

G-protein coupled receptors (GPCRs) are the most important class of membrane receptors with over 800 GPCRs identified in the human, and many are involved in diseases such as oncologic, psychiatric, metabolic, neurodegenerative, cardiovascular and infectious diseases [1-2]. Approximately 40-50% of drugs circulated in the market targets GPCRs for treatment of various diseases [2]. Ligand binding induces conformational changes of GPCRs, which regulate the interaction with downstream signaling molecules such as heterotrimeric G-proteins or β -arrestins [3-4]. Understanding the conformational changes of GPCRs upon activation or inactivation would greatly benefit to understanding the mechanism of activation and inactivation induced by endogenous or synthetic ligands, which leads to design more effective and less toxic drugs.

Hence, enormous efforts have been put into the characterization of structures of GPCRs. X-ray crystallography and NMR spectroscopy are standard techniques for obtaining high-resolution structures of proteins. The recent breakthroughs in getting high-resolution X-ray crystal structures of GPCRs provide the most comprehensive insights into understanding the unique functional properties of the receptors in both of inactive and active states [5]. Although X-ray crystallography gives an important three-dimension overview of structure, there are limitations. X-ray crystal structure represents a single static conformational state, giving little information about the conformational changes or dynamics. Other major limitation is the crystallization process, which takes a lot of effort and time in GPCR engineering, co-ligand as well as detergent selection [5-6]. Additionally, the introduction of nonfunctional insertion, truncations or mutations to native GPCRs might affect the endogenous conformation. More importantly, the conditions under which proteins function

*Corresponding Authors. Address reprint requests to; Brian K. Kobilka, Postal address: 157 Beckman Center, 279, Campus Drive, Stanford, CA, USA, 94305, Tel: 650-723-7069, Fax: 650-498-5092, kobilka@stanford.edu, Ka Young Chung, Postal address: 2066 Seobu-ro, Jangan-gu, Suwon, South Korea, 440-746, Tel: 82-31-290-7787, Fax: 82-31-292-8800, kychung2@skku.edu.

are generally not compatible with conditions required for X-ray diffraction. For NMR method, there is still a great restriction in the protein size and in the sample preparation such as expression and isotope labeling of proteins [7]. Thus, the application of NMR to structural studies of GPCRs is currently very limited. Therefore, other techniques are needed to study the conformation of GPCRs.

HDX-MS measures the exchange rates of peptide amide hydrogen with deuterium in the solvent. In folded proteins, the exchange rate varies depending on the position of the amide hydrogen [8-9]. Exposed or highly dynamic regions will show rapid exchange rates while excluded and rigid regions will show slow exchange rates [8-9]. Thus, HDX-MS has been successfully used to study conformational changes [10-11], protein-protein interaction interface, protein-small molecule interaction sites, and protein folding [12-13]. Recently, it also has been proved to be a useful technique for conformational analysis of GPCR-interacting molecules (e.g. G proteins and β -arrestin) upon binding to GPCRs [14-16]. However, the conformational information of GPCRs was limited due to the technical challenges in studying membrane proteins by mass spectrometry [14, 16].

GPCRs are insoluble and unstable membrane proteins, and the use of detergents is obviously required for extraction and purification from cell systems. The detergents are used not only to solubilize and stabilize GPCRs but also to keep the purified GPCRs in functionally folded states without phospholipid membrane. Among many commercially available detergents, DDM is the most widely used detergent for GPCR studies (Fig 1b). In addition to the conventional detergents, various approaches have been made to solubilize and stabilize GPCRs [17]. Bicelles (bilayered micelles) are lipid-detergent assemblies with discrete bilayer fragments that are edge-stabilized by certain detergents (Fig 1a) [18]. They provide a lipid-rich medium mimicking natural phospholipid bilayer environment that is compatible with structural studies of membrane proteins [19]. Of note, bicelles express the attractive characteristics of both micellar and lipid bilayer systems in structural studies of membranous biomolecules [19-20]. To date, because of the advantage of bicelles, number of studies used them to maintain functional membrane proteins in EPR [21] and NMR [21-23] as well as X-ray crystallography [24]. Recently, there are several reports in which bicelles were utilized to study membrane proteins by mass spectrometry [25-26]. However, to our knowledge, there is no HDX-MS study using bicelles.

In the present study, we tested the feasibility of bicelles for the structural analysis of GPCRs by HDX-MS. Three GPCRs including β_2 -adrenergic (β_2 AR), μ -opioid receptor (μ OR), and protease-activated receptor 1 (PAR-1) were used as GPCR models, and the HDX-MS profiles were compared between β_2 AR reconstituted in bicelles and in DDM.

Methods

Expression and purification of β_2 AR

Recombinant baculovirus was prepared using Bestbac expression system (Expression Systems Inc.) with pVL1392 as a vector, and β_2 AR is extracted and purified as previously described [27]. Briefly, cell pellets were lysed by stirring in lysis buffer (20 mM HEPES pH 7.5, 5 mM EDTA, 1 μ M alprenolol, 2.5 mg/ml leupeptin, 160 mg/ml benzamidine) for 20

min. The receptors were extracted from the cell membrane using douncing homogenization in solubilization buffer (20 mM HEPES, pH 7.5, 100 mM NaCl, 1% DDM, 1 μ M alprenolol, 2.5 mg/ml leupeptin, 160 mg/ml benzamidine) for 1hr at room temperature. After clarified by high-speed centrifugation at 18,000 $\times$ g for 30 mins, the receptors bearing the N-terminal FLAG epitope was then captured by M1 antibody affinity chromatography. The column was extensively washed with HMS-CHS buffer (20 mM HEPES pH 7.5, 350 mM NaCl, 0.1% DDM, 0.01% cholesterol hemisuccinate [CHS], 2mM CaCl₂), and then the receptors were eluted with HMS-CHS buffer supplemented with 5 mM EDTA and 200 μ g/ml free FLAG peptide. The eluted receptors were further purified by affinity chromatography using alprenolol-Sepharose as previously described [28] to select functional receptors. Size-exclusion chromatography (SEC) with Superdex-200 column (GE healthcare) equilibrated in HMS-CHS buffer was finally used to polish the receptor. The receptor was concentrated at 125 μ M and added with different ligands if needed. The purity of sample was higher than 95%, as assessed by SDS-PAGE.

Expression and purification of μ OR

The expression and purification of the construct was essentially the same as previous described by Manglik et al [29]. Briefly, the construct used was WT receptor from residue 6 fused to N-terminal signal FLAG-tag and a C-terminal 6 histidine His-tag. The construct was expressed in sf9 cells. Harvested cells were lysed in lysis buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA, 10 μ M naloxone). The cells pellet was then solubilized in solubilization buffer (20 mM HEPES pH 7.5, 0.5 M NaCl, 0.5% DDM, 0.3% 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulphonate, 0.03% CHS, 30% v/v glycerol, 10 μ M naloxone). Nickel-NTA agarose was added and stirred for 1h at 4 °C. The beads were recovered by centrifugation and washed in batch with nickel wash buffer, and then the receptors were eluted in wash buffer supplemented with 250 mM imidazole. The receptors were further purified by loading onto M1 antibody column. The column was then washed with wash buffer and eluted with elution buffer supplemented with 5mM EDTA and 0.2 mg/ml FLAG peptide. Finally, SEC with Superdex-200 column (GE healthcare) equilibrated in HMS-CHS buffer was used to polish the receptor.

Expression and purification of PAR-1

The human PAR-1 construct was prepared as a previous report with slight modification [30]. Briefly, the construct was expressed in Sf9 cells at 27°C for 48 hours before harvest. To purify the receptor, infected cells were lysed by osmotic shock in low-salt buffer containing 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 100 nM vorapaxar derivative and 100 μ M TCEP. Such vorapaxar derivative was generated by reducing the non-aromatic carbon-carbon double bond in vorapaxar. It showed much faster dissociation rate than vorapaxar in cell-based assays. The protein was further extracted from cell membranes with buffer 20 mM Hepes, pH 7.5, 500 mM NaCl, 1% DDM, 0.03% CHS, 0.2% sodium cholate, 15% glycerol, 100 nM vorapaxar derivative and 100 μ M TCEP. Cell debris was removed by high-speed centrifugation. From this point, 1 μ M vorapaxar derivative was added in all the following buffers used for purification except for the buffer used in size exclusion chromatography. Nickel-NTA agarose resin was added to the supernatant after homogenization and stirred for 1h at 4°C. The resin was then washed three times in batch with buffer comprised of 20 mM

HEPES, pH 7.5, 500 mM NaCl, 0.1% DDM, 0.02% CHS and 1 μ M vorapaxar derivative, and transferred to a glass column. The bound receptor was eluted with buffer containing 300 mM imidazole and loaded onto an anti-Flag M1 affinity column. After extensive washing with buffer comprised of 20 mM HEPES, pH 7.5, 500 mM NaCl, 0.1% DDM, 0.02% CHS, 1 μ M vorapaxar derivative and 2 mM Ca^{2+} , the receptor was eluted from M1 resin using the same buffer without Ca^{2+} but with 200 μ g/ml FLAG peptide and 5 mM EDTA. Size exclusion chromatography was used to obtain the final monodisperse receptor preparation. The running buffer contained 20 mM HEPES, pH 7.5, 100 mM NaCl, 0.1% DDM and 0.02% CHS. The flow rate was set at 0.2ml/min to give enough time to allow the vorapaxar derivative dissociate from the receptor.

Reconstitution of receptors into bicelles

Ten % bicelle stock was prepared with 3:1 molar ratio between DMPC and CHAPSO. One aliquot of the bicelles was thawed at room temperature until the phase changes to a clear gel, and then was transferred on ice to liquefy. The aliquot was vortexed briefly to reestablish a homogenous phase and placed back on ice. One μ l of receptor was added to 9 μ l 10% bicelle stock with final 1% working concentration of bicelles. The protein-bicell smixture was gently pipetted up and down until the solution becomes clear and homogenous. The mixture was incubated on ice for about 30 min to allow for complete reconstitution of receptor into bicelles and kept on ice for the following sample preparation for HDX-MS studies.

Hydrogen/Deuterium Exchange and Mass Spectrometry

The purified proteins were prepared in the concentration of 125 pmole/ μ L. Hydrogen/deuterium exchange was initiated by mixing 2 μ L of proteins with 28 μ L of the D_2O buffer (20 mM HEPES, pD 7.4, 100 mM NaCl, and 0.1% DDM or bicelles in D_2O), and the mixtures were incubated at various time intervals including 10s, 100s, 1000s and 10000s on ice. At the indicated time points, the mixtures were quenched by adding 30 μ L of ice-cold quench buffer (100 mM KH_2PO_4 , 20mM TCEP, pH 2.01) and immediately placed in dry ice for frozen. For non-deuterated samples, 2 μ L of purified protein was mixed with 28 μ L of H_2O buffer (20 mM HEPES, pH 7.4, 100 mM NaCl, and 0.1% DDM or bicelles in H_2O), and 30 μ L of ice-cold quench buffer was added. The quenched samples were digested online by passing through an immobilized pepsin column (2.1 $\times$ 30 mm) (Life Technologies, Carlsbad, CA) at a flow rate of 100 μ l/ min with 0.05% formic acid in H_2O at 10 $^\circ\text{C}$. Peptide fragments were subsequently collected on a C18 VanGuard trap column (1.7 μ m $\times$ 30 mm) (Waters, Milford, MA) for desalting with 0.05% formic acid in H_2O and then separated by ultra-pressure liquid chromatography using Acquity UPLC C18 column (1.7 μ m, 1.0 $\times$ 100 mm) (Waters, Milford, MA) at a flow rate of 40 μ l/ min with an acetonitril gradient by using two pumps, which started with 8% B and increased to 85% B over the next 8.5 minutes. The mobile phase A was 0.1% formic acid in H_2O , and solvent B was acetonitril containing 0.1 % formic acid. To minimize the back-exchange of deuterium to hydrogen, the sample, solvents, trap and UPLC column were all maintained at pH 2.5 and 0.5 $^\circ\text{C}$ during analysis. Mass spectral analyses were performed with a Xevo G2 Qtof equipped with a standard ESI source (Waters, Milford, MA). The mass spectra were acquired in the range of m/z 100-2000 for 10 minutes in the positive ion mode.

Peptide identification and HDX-MS data processing

Peptic peptides in non-deuterated samples were identified with ProteinLynx Global Server 2.4 (Waters, Milford, MA). Searches were run with variable methionine oxidation modification. To process HDX-MS data, the amount of deuterium in each peptide was determined by measuring the centroid of the isotopic distribution using software, DynamX (Waters, Milford, MA). The EX1 kinetics was determined by visually inspecting the presence of two isotopic distributions. All experiments were conducted in more than triplicate.

Results

Successful HDX-MS analysis is often determined by the sequence coverage that is derived from the identified peptic peptides of non-deuterated samples. It is, therefore, necessary to optimize buffers, digestion condition, and LC-MS condition to get the highest sequence coverage. Here, we tested quench buffer (100 mM KH_2PO_4 , pH 2.01) that was supplemental with various concentrations of denaturant (guanidine-HCl, 0.0-4.0 M) and reductant (TCEP, 0.01 - 0.2 M). We found that 20 mM TCEP is optimal, and addition of guanidine-HCl has deleterious effect (data not shown). Based on this result, we chose the quench buffer composed of 100 mM KH_2PO_4 , pH 2.01, and 20 mM TCEP.

The feasibility of bicelles for mass spectrometry was tested and compared with DDM. The sequence coverages were analyzed for the selected GPCRs (*i.e.* $\beta_2\text{AR}$, μOR , and PAR-1) prepared in either bicelles or DDM. GPCRs prepared in bicelles showed improved sequence coverages with higher redundancy than GPCRs prepared in DDM (Table 1). Interestingly, an even worse sequence coverage was obtained when $\beta_2\text{AR}$ was solubilized in maltose neopentyl glycol-3 (MNG-3) (data not shown), a recently introduced detergent for GPCR structural studies [31]. Higher redundancy is an important parameter because we can use more peptides for the analysis of one region, which makes us more confident with evaluating deuterium uptake level of this region. The sequences coverage maps of $\beta_2\text{AR}$ as a representative are shown in Fig 1c and 1d. Hydrophobic peptides are generally difficult to be analyzed by mass spectrometry than hydrophilic peptides [32]. As expected, more peptides were identified in intracellular or extracellular regions of $\beta_2\text{AR}$ than transmembrane (TM) regions for both bicelles and DDM samples (Fig 1c and 1d). The very small number of peptides in TM regions for $\beta_2\text{AR}$ prepared in DDM attributes to the low sequence coverage (Fig 1d). In contrast, $\beta_2\text{AR}$ prepared in bicelles has decent number of peptides covering TM regions and a lot more peptides covering intracellular or extracellular regions (Fig 1c).

To test the compatibility of bicelles and DDM for HDX-MS, we analyzed the HDX profile of $\beta_2\text{AR}$ prepared in bicelles and in DDM (Fig 2). As shown in Fig 2, TM regions showed lower deuterium uptake than intracellular or extracellular region for both bicelles- and DDM-prepared $\beta_2\text{AR}$. This is expected because TM regions are relatively protected by bicelles or DDM and hydrogen bonds in the α -helices also prevent fast hydrogen/deuterium exchange (HDX). These results suggest that both bicelles and DDM are compatible with HDX-MS. Since bicelles gives better sequence coverage than DDM, we could get more HDX information in more regions (*i.e.* TM1, TM3, and TM7) from $\beta_2\text{AR}$ prepared in

bicelles than DDM (Fig 2). These results (Table 1, Fig 1, and Fig 2) suggest that bicelles are better solubilization method than DDM for HDX-MS analysis of GPCRs.

As bicelles are proved to be a good system for HDX-MS analysis of GPCRs, we compared the conformation of β_2 AR prepared in bicelles with that in DDM. HDX profile is dependent on the peptic peptides used for the analysis. Therefore, we chose the peptides commonly found in both bicelles and DDM samples for side by side comparison. New heat maps are generated using these common peptides (Fig 3a), and deuterium uptake level differences between bicelles and DDM samples at 1000 time point are illustrated in the snake map (Fig 3b). Most regions showed similar deuterium uptake level except one region at ICL3. ICL3 showed slightly lower deuterium uptake level in bicelles than in DDM (Fig 3a and 3b red boxes, and 3c). These results indicate that the conformation of β_2 AR is similar between bicelle-prepared and DDM prepared samples in most regions except ICL3. ICL3 is more dynamic in β_2 AR prepared in DDM than bicelles.

The kinetics (*i.e.* EX1 and EX2 kinetics) of HDX profile provides information on the folding status of a protein [33]. EX2 kinetics occurred when the rate of HDX is much slower than the rate of protein unfolding, which might represent local conformational fluctuations closed to the surface of proteins [34]. In contrast, the EX1 kinetics occurs when the rate of protein unfolding reaction is slower than the rate of HDX, which might represent the global unfolding/refolding of proteins [34-35]. In physiological conditions, the EX1 kinetics is rare but very interesting because it helps us to understand the dynamics of a protein [35]. We found a peptide from C-terminus of TM6/ECL3 (IVNIVHVIQDNL) showing intermediate EX1 and EX2 kinetics from β_2 AR prepared both in bicelles (Fig 4, left) and DDM (Fig 4, right), where both bimodal isotopic distribution (EX1) and time-dependent mass shifts (EX2) exist.

Discussion

In this study, we could get more than 90% sequence coverage of GPCRs in HDX-MS experimental setup by solubilizing GPCRs with bicelles. Importantly, the data was observed by testing 3 unrelated GPCRs including PAR-1, μ OR and β_2 AR with 97%, 96%, and 94% sequence coverage respectively. Of note, not only the sequence coverage but also the redundancy covering an amino acid improved significantly. Redundancy is an important parameter because more peptides covering a same region gives higher quality and more trustworthy HDX-MS profile. Good sequence coverage and increased redundancy lead to successful analysis of β_2 AR by HDX-MS suggesting that bicelles are good GPCR-solubilization method for HDX-MS studies.

Recently, thanks to the development of instrument and technology, HDX-MS has gradually become a potent technique for studying dynamic structures of various proteins. Analysis of membrane proteins such as GPCRs are, however, still challenging mainly due to the poor compatibility of membrane proteins with LC/MS [32]. Recent collaborative studies by the Woods lab successfully analyzed the conformational changes of Gs protein or β -arrestin-1 upon binding to β_2 AR [14, 16]. However, the conformational information of β_2 AR was limited due to poor sequence coverage of β_2 AR in contrast to the fairly good sequence

coverages of Gs protein or β -arrestin-1. In these studies, the proteins are prepared in newly introduced detergents, MNG-3 or LMNG [14, 16]. Extensive condition-optimization by the Griffin lab could achieve approximately 89% sequence coverage of β_2 AR solubilized in DDM [36]. However, this result was achieved by extending the LC gradient up to 120 min. Since the back-exchange of deuterium to hydrogen occurs continually in the quenched sample even at low pH and temperature, the analysis process should be performed as quickly as possible after quench to preserve as much deuterium as possible [37], and 120 min LC gradient is too long to prevent back-exchange. Consequently, the Griffin lab tested more suitable condition for HDX-MS method (9.5 min LC gradient), and the sequence coverage of β_2 AR reduced to 71% [38].

Previous reports showed that careful selection of the detergent and the proper concentration as well as optimization of variable conditions can enhance the mass spectral analysis of proteins and peptides that are difficult to be solubilized, such as membrane proteins [25, 36]. In the present study, we tested bicelles for HDX-MS compatibility, and we could get more than 90% sequence coverage for GPCRs solubilized in bicelles. It is important that these results were obtained by using quick on-line digestion (1 min) and rapid peptide separation system (8.5 min LC gradient), which are suitable for minimizing the back-exchange. Interestingly, both DDM and MNG-3 gave worse sequence coverage than bicelles, and this might lead to the generalization that bicelles are more compatible with mass spectrometric analysis of GPCRs than conventional detergents.

This study, for the first time, adopted bicelles as a solubilizing method for HDX-MS-based conformational analysis of GPCRs. It is well-known that the GPCR-G protein coupling is slower in detergent-solubilized GPCRs than in phospholipid bilayer membrane [39]. Hence, more physiological GPCR conformations will be obtained in phospholipid bilayer-mimicking systems than in detergents. Bicelles are the most multipurpose model mimicking phospholipid membrane system presently available [23]. They mimic physiological phospholipid bilayer environment and enable the natural folding of membrane proteins better than detergent micelles [40]. Nowadays, the structural properties and formation of bicelles have been understood in detail so that they are routinely utilized in a number of structural studies of membrane proteins by EPR [21] and NMR [21-23], X-ray crystallography [24], and drug formulations[41]. rHDL particles are another well-characterized GPCR-solubilization method that mimics phospholipid bilayer very well [42]. However, the reconstitution process of rHDL particle is more complex than that of bicelles, and furthermore it uses a dimer of ApoA-I as a scaffolding protein to surround the lipid bilayer [42]. This extra protein might give more complexity to the HDX-MS data analysis, which makes rHDL particle less attractive than bicelles. The advantages of bicelles, combined with HDX-MS, are therefore well suited to investigate the conformation of GPCRs.

The HDX-MS profile of β_2 AR showed higher HDX in intracellular of extracellular regions and lower HDX in TM regions, which is well correlated with the overall seven-transmembrane structure of β_2 AR and other GPCR HDX-MS studies [36]. Interestingly, we observed EX1 kinetics at C-terminus of TM6/ECL3 region, which suggests that C-terminus of TM6/ECL3 region slowly exchange the folded and unfolded conformation. To our

knowledge, this is the first EX1 kinetics reported from GPCRs. TM6 undergoes outward movement upon GPCR activation leading to G protein coupling and has an important role in GPCR oligomerization [27, 43-46]. The importance of this EX1 kinetics on the GPCR function will be an interesting field to be explored.

Conclusion

In conclusion, the present study suggests bicelles as a better GPCR-solubilization tool for HDX-MS studies than conventional detergents such as DDM or MNG-3. It will be interesting to investigate the mechanism why bicelles are more compatible with HDX-MS than detergents such as DDM or MNG-3. Understanding the mechanism would lead to development of HDX-MS-compatible detergents for GPCRs and other membrane proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2012R1A1A1039220) and under the framework of an international cooperation program managed by the National Research Foundation of Korea (2012K2A1A2031900).

References

1. Lundstrom K. An overview on GPCRs and drug discovery: structure-based drug design and structural biology on GPCRs. *Methods Mol Biol.* 2009; 552:51–66. [PubMed: 19513641]
2. Lagerström, Malin C.; Schiöth, HB. Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nature Reviews Drug Discovery.* 2008; 7(4):339–357. [PubMed: 18382464]
3. Rajagopal S, Rajagopal K, Lefkowitz RJ. Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nature reviews. Drug discovery.* 2010; 9(5):373–386. [PubMed: 20431569]
4. Kahsai AW, Xiao K, Rajagopal S, Ahn S, Shukla AK, Sun J, Oas TG, Lefkowitz RJ. Multiple ligand-specific conformations of the β 2-adrenergic receptor. *Nature Chemical Biology.* 2011; 7(10):692–700. [PubMed: 21857662]
5. Venkatakrishnan AJ, Deupi Xavier, Lebon Guillaume, Tate Christopher G, Schertler GF, Babu MM. Molecular signatures of G-protein-coupled receptors. *Nature.* 2013; 494:185–194. [PubMed: 23407534]
6. Kobilka B. The Structural Basis of G-Protein-Coupled Receptor Signaling (Nobel Lecture). *Angewandte Chemie International Edition.* 2013; 52(25):6380–6388.
7. Granier, Sébastien; Kobilka, B. A new era of GPCR structural and chemical biology. *Nature Chemical Biology.* 2012; 8(8):670–673. [PubMed: 22810761]
8. Percy AJ, Rey M, Burns KM, Schriemer DC. Probing protein interactions with hydrogen/deuterium exchange and mass spectrometry-a review. *Anal Chim Acta.* 2012; 721:7–21. [PubMed: 22405295]
9. Marcisisin SR, Engen JR. Hydrogen exchange mass spectrometry: what is it and what can it tell us? *Anal Bioanal Chem.* 2010; 397(3):967–972. [PubMed: 20195578]
10. Resing KA, Ahn NG. Deuterium Exchange Mass Spectrometry as a Probe of Protein Kinase Activation. Analysis of Wild-Type and Constitutively Active Mutants of MAP Kinase Kinase-1. *Biochemistry.* 1998; 37(2):463–475. [PubMed: 9425067]

11. Engen, John R.; Smithgall, Thomas E.; Gmeiner, William H.; Smith, DL. Comparison of SH3 and SH2 domain dynamics when expressed alone or in an SH(3 + 2) construct: the role of protein dynamics in functional regulation. *Journal of Molecular Biology*. 1999; 287(3):645–656. [PubMed: 10092465]
12. Heidary DK, Gross LA, Roy M, Jennings PA. Evidence for an obligatory intermediate in the folding of interleukin-1 beta. *Nat Struct Biol*. 1997; 4(9):725–731. [PubMed: 9303000]
13. Yang, Houjun; Smith, DL. Kinetics of Cytochrome c Folding Examined by Hydrogen Exchange and Mass Spectrometry. *Biochemistry*. 1997; 36(48):14992–14999. [PubMed: 9398224]
14. Chung KY, Rasmussen SGF, Liu T, Li S, DeVree BT, Chae PS, Calinski D, Kobilka BK, Woods VL, Sunahara RK. Conformational changes in the G protein Gs induced by the β_2 adrenergic receptor. *Nature*. 2011; 477(7366):611–615. [PubMed: 21956331]
15. Orban T, Jastrzebska B, Gupta S, Wang B, Miyagi M, Chance MR, Palczewski K. Conformational dynamics of activation for the pentameric complex of dimeric G protein-coupled receptor and heterotrimeric G protein. *Structure*. 2012; 20(5):826–840. [PubMed: 22579250]
16. Shukla AK, Westfield GH, Xiao K, Reis RI, Huang LY, Tripathi-Shukla P, Qian J, Li S, Blanc A, Oleskie AN, Dosey AM, Su M, Liang CR, Gu LL, Shan JM, Chen X, Hanna R, Choi M, Yao XJ, Klink BU, Kahsai AW, Sidhu SS, Koide S, Penczek PA, Kossiakoff AA, Woods VL Jr, Kobilka BK, Skiniotis G, Lefkowitz RJ. Visualization of arrestin recruitment by a G-protein-coupled receptor. *Nature*. 2014
17. Zhao Q, Wu BL. Ice breaking in GPCR structural biology. *Acta Pharmacol Sin*. 2012; 33(3):324–334. [PubMed: 22286917]
18. Sanders CR, Prosser RS. Bicelles: a model membrane system for all seasons? *Structure*. 1998; 6(10):1227–1234. [PubMed: 9782059]
19. Sanders, Charles R.; Prosser, RS. Bicelles: a model membrane system for all seasons? *Structure*. 1998; 6:1227–1234. [PubMed: 9782059]
20. Czerski, Lech; Sanders, CR. Functionality of a Membrane Protein in Bicelles. *Analytical Biochemistry*. 2000; 284:327–333. [PubMed: 10964416]
21. Dürr, Ulrich H.N.; Soong, Ronald; Ramamoorthy, A. When detergent meets bilayer: Birth and coming of age of lipid bicelles. *Progress in Nuclear Magnetic Resonance Spectroscopy*. 2013; 69:1–22. [PubMed: 23465641]
22. Park SH, Prytulla S, De Angelis AA, Brown JM, Kiefer H. High-resolution NMR spectroscopy of a GPCR in aligned bicelles. *Journal of the American chemical society*. 2006; 128(23):7402–7403. SJ, O. [PubMed: 16756269]
23. Dürr, Ulrich H. N.; Gildenberg, Melissa; Ramamoorthy, A. The Magic of Bicelles Lights Up Membrane Protein Structure. *Chemical reviews*. 2012; 112:6054–6074. [PubMed: 22920148]
24. Ujwal R, Bowie JU. Crystallizing membrane proteins using lipidic bicelles. *Methods*. 2011; 55:337–341. [PubMed: 21982781]
25. Hopper, Jonathan T S.; Yu, Yvonne Ting-Chun; Li, Dianfan; Raymond, Alison; Bostock, Mark; Liko, Idlir; Mikhailov, Victor; Laganowsky, Arthur; Benesch, Justin L P.; Caffrey, Martin; Nietlispach, Daniel; Carol, V. Robinson Detergent-free mass spectrometry of membrane protein complexes. *Nature methods*. 2013; 10(12):1206–1208. [PubMed: 24122040]
26. Pan, Yan; Brown, Leonid; Konermann, L. Kinetic Folding Mechanism of an Integral Membrane Protein Examined by Pulsed Oxidative Labeling and Mass Spectrometry. *Journal of Molecular Biology*. 2011; 410(1):146–158. [PubMed: 21570983]
27. Rasmussen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, Thian FS, Chae PS, Pardon E, Calinski D, Mathiesen JM, Shah ST, Lyons JA, Caffrey M, Gellman SH, Steyaert J, Skiniotis G, Weis WI, Sunahara RK, BK K. Crystal structure of the β_2 adrenergic receptor-Gs protein complex. *Nature*. 2011; 477(7366):549–555. [PubMed: 21772288]
28. Rosenbaum DM1, Cherezov V, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Yao XJ, Weis WI, Stevens RC, BK K. GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. *Science*. 2007; 318(5854):1266–1273. [PubMed: 17962519]
29. Manglik, Aashish; Kruse, Andrew C.; Kobilka, Tong Sun; Thian, Foon Sun; Mathiesen, Jesper M.; Sunahara, Roger K.; Pardo, Leonardo; Weis, William I.; Kobilka, BK.; Granier, S. Crystal

- structure of the μ -opioid receptor bound to a morphinan antagonist. *Nature*. 2012; 485(7398):321–326. [PubMed: 22437502]
30. Zhang, Cheng; Srinivasan, Yoga; Arlow, Daniel H.; Fung, Juan Jose; Palmer, Daniel; Zheng, Yaowu; Green, Hillary F.; Pandey, Anjali; Dror, Ron O.; Shaw, David E.; Weis, William I.; Coughlin, Shaun R.; Kobilka, BK. High-resolution crystal structure of human protease-activated receptor 1. *Nature*. 2012; 492(7429):387–392. [PubMed: 23222541]
31. Chae PS, Rasmussen SG, Rana RR, Gotfryd K, Chandra R, Goren MA, Kruse AC, Nurva S, Loland CJ, Pierre Y, Drew D, Popot JL, Picot D, Fox BG, Guan L, Gether U, Byrne B, Kobilka B, Gellman SH. Maltose-neopentyl glycol (MNG) amphiphiles for solubilization, stabilization and crystallization of membrane proteins. *Nat Methods*. 2010; 7(12):1003–1008. [PubMed: 21037590]
32. Rabilloud T. Membrane proteins and proteomics: love is possible, but so difficult. *Electrophoresis*. 2009; 30(Suppl 1):S174–180. [PubMed: 19517508]
33. Konermann, Lars; Tong, Xin; Pan, Y. Protein structure and dynamics studied by mass spectrometry: H/D exchange, hydroxyl radical labeling, and related approaches. *Journal of Mass Spectrometry*. 2008; 43(8):1021–1036. [PubMed: 18523973]
34. Konermann L, Tong X, Pan Y. Protein structure and dynamics studied by mass spectrometry: H/D exchange, hydroxyl radical labeling, and related approaches. *J Mass Spectrom*. 2008; 43(8):1021–1036. [PubMed: 18523973]
35. Weis DD, Wales TE, Engen JR, Hotchko M, LF TE. Identification and characterization of EX1 kinetics in H/D exchange mass spectrometry by peak width analysis. *Journal of the American Society for Mass Spectrometry*. 2006; 17(11):1498–1509. [PubMed: 16875839]
36. Zhang X, Chien EYT, Chalmers MJ, Pascal BD, Gatchalian J, Stevens RC, Griffin PR. Dynamics of the β_2 -adrenergic G-protein coupled receptor revealed by hydrogen-deuterium exchange. *analytical chemistry*. 2010; 82(3):1100–1108. [PubMed: 20058880]
37. Wu, Yan; Kaveti, Suma; Engen, JR. Extensive deuterium back-exchange in certain immobilized pepsin columns used for H/D exchange mass spectrometry. *analytical chemistry*. 2006; 78(5):1719–1723. [PubMed: 16503628]
38. West GM, Chien EYT, Katritch V, Gatchalian J, Chalmers MJ, Stevens RC, Griffin PR. Ligand-Dependent Perturbation of the Conformational Ensemble for the GPCR β_2 Adrenergic Receptor Revealed by HDX. *Structure*. 2011; 19(10):1424–1432. [PubMed: 21889352]
39. Jastrzebska B, Debinski A, Filipek S, Palczewski K. Role of membrane integrity on G protein-coupled receptors: Rhodopsin stability and function. *Prog Lipid Res*. 2011; 50(3):267–277. [PubMed: 21435354]
40. Durr UH, Soong R, Ramamoorthy A. When detergent meets bilayer: birth and coming of age of lipid bicelles. *Prog Nucl Magn Reson Spectrosc*. 2013; 69:1–22. [PubMed: 23465641]
41. Rubio L, Alonso C, Rodríguez G, Barbosa-Barros L, Coderch L, De la Maza A, Parra JL. Bicellar systems for in vitro percutaneous absorption of diclofenac. *International Journal of Pharmaceutics*. 2010; 386(1-2):108–113. O, L. [PubMed: 19922782]
42. Whorton MR, Bokoch MP, Rasmussen SG, Huang B, Zare RN, Kobilka B, Sunahara RK. A monomeric G protein-coupled receptor isolated in a high-density lipoprotein particle efficiently activates its G protein. *Proc Natl Acad Sci U S A*. 2007; 104(18):7682–7687. [PubMed: 17452637]
43. R. SG, C. HJ, Fung JJ, Pardon E, Casarosa P, Chae PS, Devree BT, Rosenbaum DM, Thian FS, Kobilka TS, Schnapp A, Konetzki I, Sunahara RK, Gellman SH, Pautsch A, Steyaert J, Weis WI, BK K. Structure of a nanobody-stabilized active state of the β_2 -adrenoceptor. *Nature*. 2011; 469(7329):175–180. [PubMed: 21228869]
44. Hebert TE, Moffett S, Morello JP, Loisel TP, Bichet DG, Barret C, Bouvier M. A peptide derived from a β_2 -adrenergic receptor transmembrane domain inhibits both receptor dimerization and activation. *J Biol Chem*. 1996; 271(27):16384–16392. [PubMed: 8663163]
45. Fung JJ, Deupi X, Pardo L, Yao XJ, Velez-Ruiz GA, Devree BT, Sunahara RK, Kobilka BK. Ligand-regulated oligomerization of β_2 -adrenoceptors in a model lipid bilayer. *EMBO J*. 2009; 28(21):3315–3328. [PubMed: 19763081]

46. Ghosh A, Sonavane U, Joshi R. Multiscale modelling to understand the self-assembly mechanism of human beta2-adrenergic receptor in lipid bilayer. *Comput Biol Chem.* 2014; 48:29–39. [PubMed: 24291490]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig 1. Sequence coverage of β_2 AR

Illustration of β_2 AR in bicelle (a) and DDM (b). The sequence coverage of β_2 AR prepared in bicelles (c) and DDM (d). Blue bars represent peptic peptides identified. The figures are representative of four independent experiments.

Fig 2. HDX profile of β_2 AR

The heat map of β_2 AR prepared in bicelles (a) and DDM (b). Incorporation of deuterium at 10 sec, 100 sec, 1000 sec, and 10000 sec are indicated by color coded blocks underlining the amino acid sequence. The color legend shows the HDX levels. Snake maps showing the deuterium uptake of β_2 AR at 1000 sec prepared in bicelles (c) and DDM (d). The figures are representative of four independent experiments.

Fig 3. Comparison of HDX profile of β_2 AR prepared in bicelles and DDM

The heat map of β_2 AR prepared in bicelles and DDM analyzed from common peptides (a). Incorporation of deuterium at 10 sec, 100 sec, 1000 sec, and 10000 sec are indicated by color coded blocks underlining the amino acid sequence. The color legend shows the HDX levels. Snake maps showing the deuterium uptake difference between β_2 AR prepared in bicelles and DDM (b). The color legend shows the difference of HDX levels. Color legend toward blue means more deuterium uptake, and color legend toward red means less deuterium uptake in bicelles than DDM samples. Regions showing differences between bicelles and DDM samples are highlighted with red boxes. The deuterium uptake plot (c). Error bars are standard error of four independent experiments. * p < 0.05.

Fig 4. EX1 kinetics observed from a peptide for C-terminus of TM6/ECL3 region
EX1 kinetics observed from a peptide from β_2 AR prepared in bicelles (right pannel) and DDM (left pannel). The figures are representative of four independent experiments.

Table 1

Sequence coverage comparison between GPCRs prepared in Bicelles and DDM

	β2AR		μOR		PAR-1	
	Bicelles	DDM	Bicelles	DDM	Bicelles	DDM
Sequence coverage (%)	94.4	75.2	97.5	72.5	96.6	91.1
Number of peptic peptides	122	60	187	71	99	86
Redundancy*	4.41	2.47	9.11	3.42	3.59	3.12

* Number of peptides covering same amino acids.