ChemPlusChem

A Multidisciplinary Journal Centering on Chemistry

Chemistry Europe

European Chemical Societies Publishing

Accepted Article

Title: Anthracene-Bridged Detergents for Membrane Protein Studies

Authors: Li Song, Yili Dai, Xin Zhou, Yan Tan, Zijie Zhou, Xing Zheng, Fei Zhao, Meifang Yang, and Houchao Tao

To be cited as: *ChemPlusChem* **2025**, e202500320

Link to VoR: https://doi.org/10.1002/cplu.202500320

WILEY VCH

10.1002/cplu.202500320

WILEY VCH

RESEARCH ARTICLE

Anthracene-Bridged Detergents for Membrane Protein Studies

Li Song[a,b]†, Yili Dai[a,b]†, Xin Zhou[a], Yan Tan[a], Zijie Zhou[a], Xing Zheng[b], Fei Zhao[c], Meifang Yang[a*], and Houchao Tao[a*]

[a] L. Song, Y. Dai, X. Zhou, Y. Tan, Z. Zhou, M. Yang, and Prof. Dr. H. Tao
Shanghai Frontiers Science Center of TCM Chemical Biology, Innovation Research Institute of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China.
E-mail: yangmf@shutcm.edu.cn, houchao@shutcm.edu.cn

[b] L. Song, Y. Dai, Prof. Dr. X. Zheng
Institute of Pharmacy and Pharmacology, Hengyang Medicinal School, University of South China, Hengyang, Hunan 421001, China.

[c] Prof. Dr. F. Zhao
iHuman Institute, ShanghaiTech University, Shanghai 201210 P. R. China

Supporting information for this article is given via a link at the end of the document.

Abstract: Detergents are indispensable for membrane protein (MP) research, yet innovative designs are needed to meet the diverse requirements of MPs. Here, we present anthracene-bridged detergents (ABDs), featuring a curved anthracene-based scaffold with a hydrophobic core and hydrophilic exterior. The modular synthesis of ABDs facilitated a systematic exploration of their structure-property relationships. Evaluation across representative G protein-coupled receptors (GPCRs) demonstrated superior or comparable stabilization to conventional detergents. Notably, PP-ABD maintained complete homogeneity of A_{2A}AR after heat shock and exhibited strong potential for electron microscopy applications. This study establishes ABDs as versatile and effective tools for MP research, offering valuable insights into the rational design of next-generation detergents.

Introduction

Membrane proteins (MPs) constitute approximately one-third of the human proteome and are pivotal to fundamental and translational research efforts.[1] These proteins play critical roles as mediators of cell-cell communication and the exchange of information and materials between cells and their environment.[2] Notably, MPs serve as the targets for nearly half of all marketed pharmaceuticals.[3] Despite significant advances over recent decades in elucidating MP structures—such as those involved in signal transduction via G protein-coupled receptors (GPCRs)—a comprehensive understanding of their function and structure remains limited.[4] This limitation arises in part from the lack of suitable chemical tools, particularly lipid mimetics like detergents, which are used to replace the native lipid layer that supports MPs.[5] Identifying detergents capable of stabilizing MPs while maintaining their native activity has been a persistent challenge in the field. [6]

Continuous efforts in detergent development have led to the creation of a wide variety of novel detergents for membrane protein (MP) studies.[7] Topologically, these detergents can be categorized into several classes based on the arrangement of their hydrophilic heads and hydrophobic tails. Most commercially available detergents, such as dodecyl maltoside (DDM, Figure 1), follow the conventional one-head-one-tail model, characterized by structural simplicity. Recent advancements have focused on introducing innovative scaffolds and materials, leading to the development of multi-head, multi-tail detergents. Notable examples include neopentyl glycols (NGs),[8] trehalose detergents (DDTres),[9] norbornane-based maltosides (NBMs),[10] mannitol-based amphiphiles (MNAs),[11] and polymer surfactants.[12] Some of these detergents represent a unique class of facially arranged molecules, including tripod amphiphiles (TPAs),[13] cholane-derived facial amphiphiles (FAs),[14] scyllo inositol-cored trimaltosides (STM),[15] penta-saccharide amphiphiles (PSAs),[16] based on cyclodextrin,[17] calixarene,[18] and peptides[19] (Figure 1). These scaffolded detergents, along with others, like cyclic and fluorinated detergents[20], often feature relatively rigid structures, imparting unique physicochemical properties and superior stabilization capabilities for MPs. However, while many of these scaffolds are derived from natural sources, their synthesis

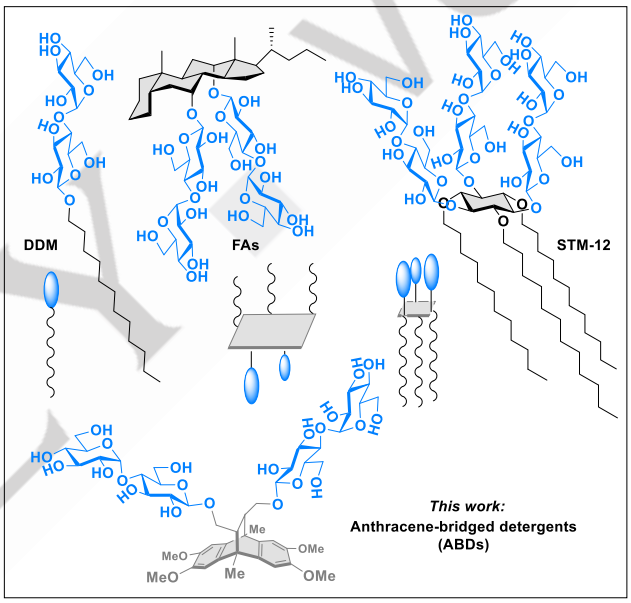


Figure 1. Detergent evolutions from conventional one-head-one-tail detergent to facial detergents.

typically requires laborious protective group transformations. Additionally, adjusting their hydrophobicity to optimize performance can be challenging.[21]

In this report, we introduce a novel design based on an anthracene-bridged scaffold. This rigid framework might minimize the dynamic flexibility and therefore benefit the stabilization of MPs. Meanwhile, it also allows for the stepwise incorporation of flexible chains with varying lengths, facilitating systematic investigations of structure-property relationships. The anthracene-based scaffold features a defined curved profile and a clean facial surface for hydrophilic modifications.[22] Through thermal stabilization assays,[23] the optimal detergent, PP-ABD, was identified and successfully applied in electron microscopy (EM) studies[24] of $A_{2A}AR$, demonstrating its potential as a promising tool for MP research.

Results and Discussion

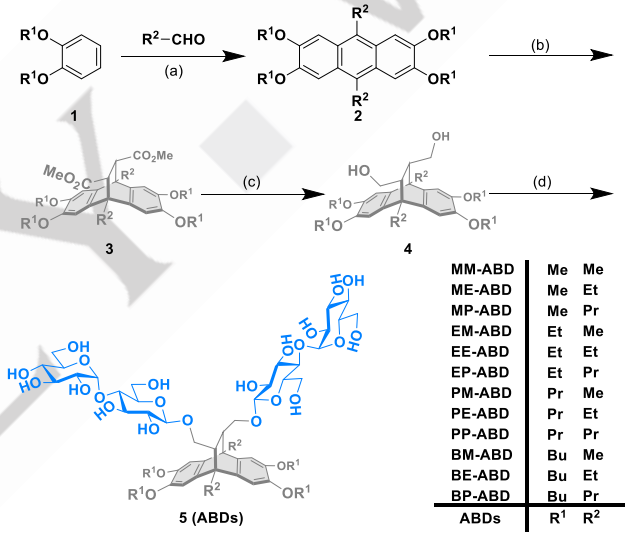
Synthesis of anthracene-bridged detergents (ABDs).

The synthesis of anthracene-bridged detergents (ABDs) began with the condensation[25] of dialkylated pyrocatechols (**1a**–**1d**) with various aldehydes under acidic conditions (Scheme 1), yielding a series of anthracene cores (**2a**–**2i**). By varying the alkyl chains on pyrocatechols and aldehydes, we generated a diverse set of anthracene intermediates. These intermediates underwent a Diels-Alder reaction[26] with dimethyl fumarate to construct the anthracene-bridged scaffolds (**3a**–**3i**). Reduction of these scaffolds with lithium aluminum hydride[27] yielded dialcohol derivatives (**4a**–**4i**) in high purity. To functionalize the dialcohols, maltosyl groups were introduced via glycosylation, as maltosides are well-known for their superior performance in membrane protein (MP) studies.[28] A perbenzoylated maltosyl bromide proved to be the optimal donor for bi-glycosylation, outperforming other glycosyl donors such as trichloroacetimidate, *N*-phenyltrifluoroacetimidate and thioglycoside[14c]. The final step involved global deprotection to produce the target ABDs (**5**).

This efficient, stepwise synthesis facilitated the preparation of well-defined anthracene-based scaffolds while allowing convenient tailoring of hydrophobicity through the introduction of alkyl chains of varying lengths. Each ABD was named based on the substituents attached to the anthracene core (R^1-R^2-ABD). For example, when R^1 and R^2 are methyl and ethyl groups, respectively, the detergent is referred to as ME-ABD. This systematic approach underscores the tunable nature of ABDs, making them versatile candidates for MP stabilization and structural studies.

Solubility studies revealed that ABDs with shorter alkyl chains, such as MM-, ME-, MP-, EE-, and EP-ABDs, exhibited good aqueous solubility (>2% w/v, Table S1). In contrast, ABDs with longer chains showed reduced solubility and were used as saturated solutions for subsequent experiments. The concentrations of these less soluble ABDs were quantified using UV-visible spectroscopy (Figure S1), and their concentrations as follows: PE-ABD: 0.08 mM; PP-ABD: 0.04 mM; BM-ABD: 0.05 mM; BE-ABD: 0.03 mM; BP-ABD: 0.17 mM (Table S3). This reduced solubility likely results from the combined effects like strong π-π interactions, the rigid ABD scaffold, and the hydrophobic interactions from flexible alkyl chains. Interestingly, BP-ABD, despite having the longest alkyl chains, exhibited higher solubility, likely due to a self-assembly behavior more similar to traditional detergents with flexible chains.

We attempted to determine the critical aggregation ~~micelle~~ concentration (CAC) for all ABD detergents. Interestingly, only EE-ABD and PM-ABD exhibited a measurable CAC of 0.02 mM and 0.15 mM, respectively, while ABDs with either fewer or more carbons showed minimal changes in measurement signals, preventing accurate CAC determination (Table S1). Despite these challenges, dynamic light scattering (DLS) measurements indicated the formation of aggregates in the more soluble ABDs, albeit in large sizes (Table S1). Small-angle X-ray scattering (SAXS) experiments were conducted to characterize the morphology of these aggregates, but no definitive structural information could be obtained due to weak scattering signals. In subsequent EM studies of MP-detergent complex, some irregularly shaped particles were observed (Figure 4B).



Scheme 1. Synthesis of anthracene-bridged detergents.

Reagents and conditions: (a) Aldehydes, 84% H_2SO_4, CH_3CN, 21%~54%; (b) Dimethylfumarate, $AlCl_3$, CH_2Cl_2, 0 °C, 16%~97%; (c) $LiAlH_4$, THF, 45%~95%; (d) i) Perbenzoylated maltosylbromide, AgOTf, CH_2Cl_2, 2,4,6-collidine, -45 °C→r.t.; ii) NaOMe, MeOH, 37%~95% (two steps).

Thermal stabilization evaluations on G protein-coupled receptors (GPCRs).

GPCRs are the largest and most diverse family of MPs in the human genome, involved in a wide range of physiological processes.[29] Their ability to transduce extracellular signals into intracellular responses makes them essential mediators of cell communication.[30] GPCRs are particularly significant as therapeutic targets,[31] with approximately 35% of all FDA-approved drugs targeting these receptors. Notable examples include the adenosine A_{2A} receptor ($A_{2A}AR$),[32] a class A GPCR involved in cardiovascular and neurological disorders; the smoothened receptor (SMO),[33] a class F GPCR and a key signal transducer in the Hedgehog pathway implicated in cancer; and the glucagon-like peptide-1 receptor (GLP-1R),[34] a class B GPCR and a critical target for managing type 2 diabetes and

10.1002/cplu.202500320

obesity. Their structural diversity and functional importance have made GPCRs a cornerstone of drug discovery and development, underscoring the need for advanced tools and strategies to study and manipulate their functions effectively.

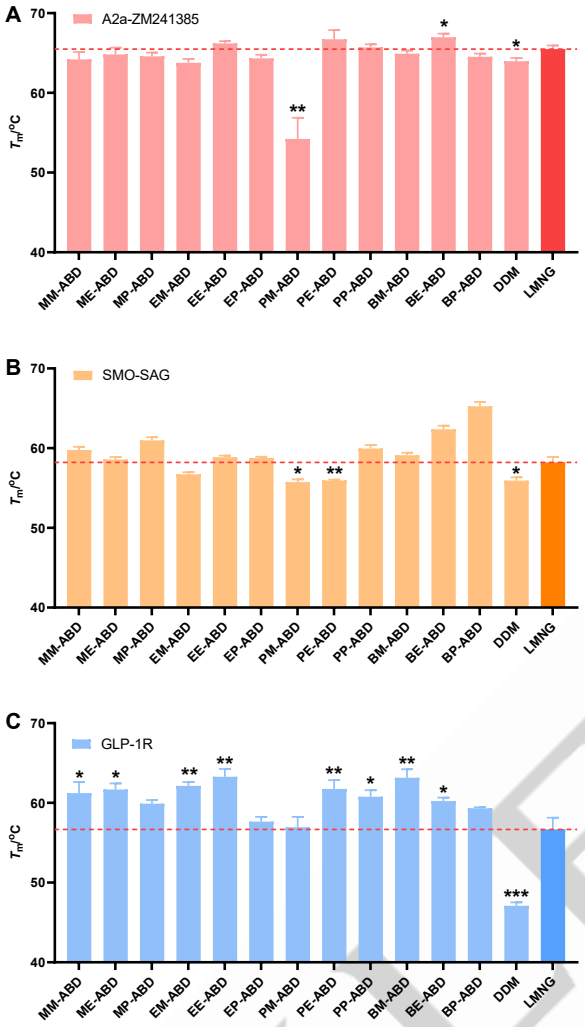


Figure 2. Thermal stabilization evaluations of anthracene-bridged detergents on selected GPCRs. The concentrations used in this assay were set at 0.5 mM for soluble ABD detergents, as well as for the conventional detergents DDM and LMNG. For ABD detergents with low solubility (PE-, PP-, BM-, BE-, and BP-ABD), saturated solutions were used instead at ranges from 0.03 mM to 0.17 mM (Table S3). Thermal stability revealed by the T_m values in CPM assay for A_{2A}AR-ZM241385 (A), SMO-SAG (B) and GLP-1R (C). Error bars represent SEM, n = 6. *P<0.05, **P <0.01 and ***P <0.001.

The thermal stability of selected GPCRs was evaluated using the CPM assay, which employs the thiol-specific fluorogenic dye *N*-[4-(7-diethylamino-4-methyl-3-coumarinyl)phenyl]maleimide (CPM).[35] During thermal denaturation, as the temperature increases, exposed cysteine residues react with the CPM dye, resulting in fluorescence activation. The thermal stability of proteins is quantified by the midpoint temperature (T_m), where 50% of the maximum fluorescence is reached, a well-established metric for assessing protein stability. For ABDs with extended hydrophobic chains with poor solubility, CACs were not determined (Table S1). These detergents were therefore tested as saturated solutions. The other detergents were set at 0.5 mM.

For A_{2A}AR, initially solubilized in DDM and stabilized with the antagonist ZM241385, samples were diluted into buffers containing different ABDs. As shown (Figure 2A), except PM-ABD, other ABDs demonstrated comparable stabilization to LMNG and DDM, with EE-, PE-, and BE-ABDs showing slightly superior performance, suggesting that ethyl was the optimal R^2 group, regardless of R^1. This observation highlights that hydrophobicity is not the dominant factor for stabilization in this detergent class. Intriguingly, similar behavior was observed for A_{2A}AR thermal stabilization in the presence of agonist NECA (Figure S2A). For SMO which was initially solubilized in LMNG with the presence of agonist SAG, the dimeric ABDs consistently outperformed monomeric DDM and exhibited significant stabilization improvements over LMNG, except for EM-, PM- and PE-ABDs (Figure 2B). In the R^1=butyl group series, stability notably improved with increasing R^2 chain length, as indicated by higher CPM values. Conversely, when R^2=propyl, the results emphasized its importance for SMO stabilization, differing from A_{2A}AR results. However, for SMO with the presence of antagonist LY294680, similar preference of MP-, BE- and BP-ABD was observed when compared to SMO with agonist SAG (Figure S2B). For GLP-1R, all dimeric detergents, including LMNG, showed greater stabilization superiority over DDM (Figure 2C). Remarkably, all ABDs outperformed or comparable (for EP- and PM-ABDs) to LMNG, highlighting their enhanced ability to stabilize this receptor. These results collectively demonstrate that ABDs, particularly those with optimized R^1 and R^2 groups, exhibit superior thermal stabilization for a range of GPCRs, surpassing traditional detergents like DDM and LMNG in most cases. These findings underscore the varying detergent preferences across different MPs while also proving the versatility and efficacy of ABDs in addressing diverse stabilization requirements for future applications.

Homogeneity evaluations of A_{2A}AR within ABDs.

To assess the ability of ABDs to preserve A_{2A}AR homogeneity after heat shock, the receptor was subjected to a brief heat shock at 50 °C for three minutes, followed by analysis using size exclusion chromatography (SEC) (Figure 3). Most ABD detergents outperformed DDM, which showed partial aggregation of A_{2A}AR. Specifically, A_{2A}AR solubilized in PP-ABD retained complete homogeneity, with its SEC profile identical to that before heating. Other ABDs, such as MP-, EE-, BE- and BP-ABDs, displayed slight peak shifts but maintained homogeneous profiles. In contrast, PE-, PM-, PE- and BM-ABD caused significant aggregation, indicating reduced efficiency in preserving protein integrity. Interestingly, these findings show some discrepancies with the CPM assay results, where PP-ABD demonstrated favorable stabilization performance. This inconsistency highlights the complementary nature of these methods, with the CPM assay measuring thermal unfolding and SEC focusing on aggregation and homogeneity after heat shock. Together, the results underline the remarkable ability of ABDs, particularly PP-ABD, to stabilize A_{2A}AR under thermal stress, while reinforcing the necessity of using diverse approaches to fully evaluate detergent performance.

10.1002/cplu.202500320

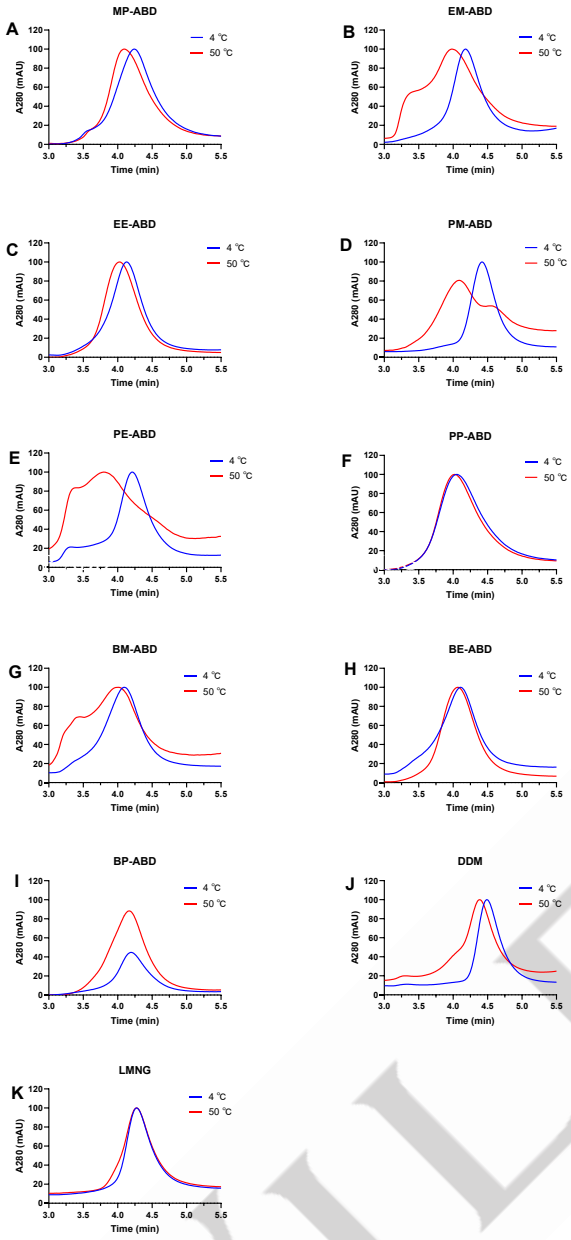


Figure 3. Homogeneity evaluation of $A_{2A}AR$ within selected ABDs upon heat shock. (A) MP-ABD, (B) EM-ABD, (C) EE-ABD, (D) PM-ABD, (E) PE-ABD, (F) PP-ABD, (G) BM-ABD, (H) BE-ABD, (I) BP-ABD, (J) DDM, (K) LMNG.

EM studies of $A_{2A}AR$ using PP-ABDs.

Considering the superior performance of ABD detergents in stabilizing and preserving $A_{2A}AR$, we carried out negative stain electron microscopy (EM) studies. $A_{2A}AR$ samples prepared in selected ABDs and stained with uranyl salts were visualized by EM, yielding high-quality EM images that revealed uniform, monodisperse particles (Figure 4). Consistent with our previous work using β-sheet peptide detergents[19a, 19c, 19e], low solubility did not hinder effective membrane protein stabilization or EM visualization. Unlike LMNG-stabilized samples, which predominantly form spherical particles, ABD-stabilized samples adopted more irregular shapes. From the EM images of PP-ABD prepared samples (Figure 4B), two-dimensional classification of approximately 20,721 automated anatomical particles were picked, enabling clear identification of different conformations of $A_{2A}AR$-G complex. This result is promising, demonstrating low protein clustering and enhanced clarity, which are comparable to samples prepared using LMNG under similar conditions (Figure 4A). In addition, as preliminarily indicated in our mass photometry analysis, PP-ABD-prepared samples primarily contained $A_{2A}AR$ monomers (Figure S3). However, the broad peak observed in the mass photometry data may have masked the presence of $A_{2A}AR$ dimers that could not be clearly resolved. Whereas LMNG-prepared samples evidently exhibited both monomers and dimers, together with higher percentage of empty aggregates (Figure S3).

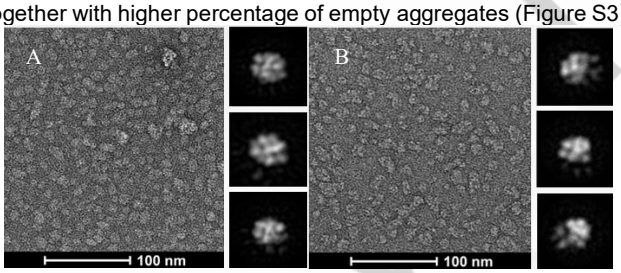


Figure 4. EM studies of $A_{2A}AR$. Single particle EM of negative-stained $A_{2A}AR$ solubilized in LMNG (A) or in PP-ABD (B).

Conclusion

In this study, we successfully designed and synthesized a series of anthracene-bridged detergents (ABDs) with tunable hydrophobic and hydrophilic properties. By leveraging a stepwise synthetic approach, we constructed well-defined scaffolds that allowed for systematic exploration of structure-property relationships. Our results demonstrate that ABD detergents, despite their solubility challenges, were effective in stabilizing GPCRs such as $A_{2A}AR$, SMO, and GLP-1R. Compared to traditional detergents like DDM and LMNG, several ABD variants exhibited comparable or superior stabilization effects. While some ABDs with extended alkyl substituents exhibited lower solubility, we addressed this limitation by using saturated solutions, which remained effective in MP assays. However, due to their limited solubility, these detergents could not reach the concentrations typically required for membrane protein extraction and purification—representing a key limitation of the current design. This highlights the need for further optimization. Looking ahead, introducing carboxylic acid, or amide, or more sugar as head groups may enhance solubility, providing a potential direction for future optimization. Notably, PP-ABD maintained $A_{2A}AR$ homogeneity after thermal stress and proved suitable for EM studies. Moreover, mass photometry analysis suggested that $A_{2A}AR$ solubilized in PP-ABD retained relatively homogeneous population, mitigating initial concerns on its solubility. These results highlight the versatility and effectiveness of ABDs across different MPs, underscoring their potential as useful tools in structural and functional studies of challenging MPs. Beyond introducing a promising class of detergents, this work provides valuable insights into the rational design of novel amphiphiles, paving the way for next-generation detergents tailored to address the complexities of MP stabilization and structural biology.

Acknowledgements

The authors gratefully acknowledge NSF of China (21672147) and Shanghai University of Traditional Chinese Medicine for financial support.

Keywords: anthracene-bridged detergents • membrane proteins • facial amphiphiles

[1] (a) N. Bloemeke, K. Meighen-Berger, M. Hitzenberger, N. C. Bach, M. Parr, J. P. Coelho, D. Frishman, M. Zacharias, S. A. Sieber, M. J. Feige, *EMBO J.* **2022**, *41*, e110959; (b) L. E. Hedin, K. Illergård, A. Elofsson, *J. Proteome Res.* **2011**, *10*, 3324-3331.

[2] E. Wallin, G. von Heijne, *Protein Sci.* **1998**, *7*, 1029-1038.

[3] J. P. Overington, B. Al-Lazikani, A. L. Hopkins, *Nat. Rev. Drug Discovery* **2006**, *5*, 993-996.

[4] K. Lundstrom, *Trends Biotechnol.* **2005**, *23*, 103-108.

[5] R. M. Garavito, S. Ferguson-Miller, *J. Biol. Chem.* **2001**, *276*, 32403-32406.

[6] A. M. Seddon, P. Curnow, P. J. Booth, *Biochim Biophys Acta.* **2004**, *1666*, 105-117.

[7] H. J. Lee, H. S. Lee, T. Youn, B. Byrne, P. S. Chae, *Chem* **2022**, *8*, 980-1013.

[8] P. S. Chae, R. R. Rana, K. Gotfryd, S. G. F. Rasmussen, A. C. Kruse, K. H. Cho, S. Capaldi, E. Carlsson, B. Kobilka, C. J. Loland, U. Gether, S. Banerjee, B. Byrne, J. K. Lee, S. H. Gellman, *Chem. Commun.* **2013**, *49*, 2287-2289.

[9] (a) M. Das, Y. Du, J. S. Mortensen, M. Ramos, L. Ghani, H. J. Lee, H. E. Bae, B. Byrne, L. Guan, C. J. Loland, B. K. Kobilka, P. S. Chae, *Org. Biomol. Chem.* **2019**, *17*, 3249-3257; (b) H. Tao, Y. Fu, A. Thompson, S. C. Lee, N. Mahoney, R. C. Stevens, Q. Zhang, *Langmuir* **2012**, *28*, 11173-11181.

[10] M. Das, Y. Du, O. Ribeiro, P. Hariharan, J. S. Mortensen, D. Patra, G. Skiniotis, C. J. Loland, L. Guan, B. K. Kobilka, B. Byrne, P. S. Chae, *J. Am. Chem. Soc.* **2017**, *139*, 3072-3081.

[11] H. Hussain, T. Helton, Y. Du, J. S. Mortensen, P. Hariharan, M. Ehsan, B. Byrne, C. J. Loland, B. K. Kobilka, L. Guan, P. S. Chae, *Analyst* **2018**, *143*, 5702-5710.

[12] J. L. Popot, E. A. Berry, D. Charvolin, C. Creuzenet, C. Ebel, D. M. Engelman, M. Flötenmeyer, F. Giusti, Y. Gohon, P. Hervé, Q. Hong, J. H. Lakey, K. Leonard, H. A. Shuman, P. Timmins, D. E. Warschawski, F. Zito, M. Zoonens, B. Pucci, C. Tribet, *Cell. Mol. Life Sci.* **2003**, *60*, 1559-1574.

[13] P. S. Chae, A. C. Kruse, K. Gotfryd, R. R. Rana, K. H. Cho, S. G. F. Rasmussen, H. E. Bae, R. Chandra, U. Gether, L. Guan, B. K. Kobilka, C. J. Loland, B. Byrne, S. H. Gellman, *Chem. - Eur. J.* **2013**, *19*, 15645-15651.

[14] (a) Q. Zhang, X. Ma, A. Ward, W.-X. Hong, V.-P. Jaakola, R. C. Stevens, M. G. Finn, G. Chang, *Angew. Chem., Int. Ed.* **2007**, *46*, 7023-7025; (b) M. Ikematsu, Y. Sugiyama, M. Iseki, E. Muneyuki, A. Mizukami, *Biophys. Chem.* **1995**, *54*, 155-164; (c) S. C. Lee, B. C. Bennett, W.-X. Hong, Y. Fu, K. A. Baker, J. Marcoux, C. V. Robinson, A. B. Ward, J. R. Halpert, R. C. Stevens, C. D. Stout, M. J. Yeager, Q. Zhang, *Proc. Natl. Acad. Sci* **2013**, *110*, E1203-E1211.

[15] A. Sadaf, M. Ramos, J. S. Mortensen, Y. Du, H. E. Bae, C. F. Munk, P. Hariharan, B. Byrne, B. K. Kobilka, C. J. Loland, L. Guan, P. S. Chae, *ACS Chem. Biol.* **2019**, *14*, 1717-1726.

[16] M. Ehsan, L. Ghani, Y. Du, P. Hariharan, J. S. Mortensen, O. Ribeiro, H. L. Hu, G. Skiniotis, C. J. Loland, L. Guan, B. K. Kobilka, B. Byrne, P. S. Chae, *Analyst* **2017**, *142*, 3889-3898.

[17] M. Textor, C. Vargas, S. Keller, *Methods* **2015**, *76*, 183-193.

[18] J. Dauvergne, E. M. Desuzinges, C. Faugier, S. Igonet, M. Soulié, E. Grousson, D. Cornut, F. Bonneté, G. Durand, E. Dejean, A. Jawhari, *Chemistryselect* **2019**, *4*, 5535-5539.

[19] (a) H. Tao, S. C. Lee, A. Moeller, R. S. Roy, F. Y. Siu, J. Zimmermann, R. C. Stevens, C. S. Potter, B. Carragher, Q. Zhang, *Nat. Methods* **2013**, *10*, 759-761; (b) C. L. McGregor, L. Chen, N. C. Pomroy, P. Hwang, S. Go, A. Chakrabartty, G. G. Privé, *Nat. Biotechnol.* **2003**, *21*, 171-176; (c) M. Yang, Y. Dai, F. Zhou, X. Zhou, Y. Qiu, Y. Tan, S. Zhao, D. Xue, F. Zhao, H. Tao, *Chem. Eur. J.* **2025**, *31*, e202404520; (d) F. Zhao, Z. Zhu, L. Xie, F. Luo, H. Wang, Y. Qiu, W. Luo, F. Zhou, D. Xue, Z. Zhang, T. Hua, D. Wu, Z.-J. Liu, Z. Le, H. Tao, *Chem. Eur. J.* **2022**, *28*, e202201388; (e) M. Yang, W. Luo, W. Zhang, H. Wang, D. Xue, Y. Wu, S. Zhao, F. Zhao, X. Zheng, H. Tao, *Chem. Asian. J.* **2022**, *17*, e202200372.

[20] (a) M. B. Shah, H. H. Jang, P. R. Wilderman, D. Lee, S. Li, Q. H. Zhang, C. D. Stout, J. R. Halpert, *Biophys. Chem.* **2016**, *216*, 1-8; (b) E. Frotscher, B. Danielczak, C. Vargas, A. Meister, G. Durand, S. Keller, *Angew. Chem. Int. Ed.* **2015**, *54*, 5069-5073; (c) J. W. Missel, N. Salustros, E. R. Becares, J. H. Steffen, A. G. Laursen, A. S. Garcia, M. M. Garcia-Alai, C. Kolar, P. Gourdon, K. Gotfryd, *Curr. Res. Struct. Biol.* **2021**, *3*, 85-94.

[21] J. Breibeck, A. Rompel, *Biochim. Biophys. Acta, Gen. Subj.* **2019**, *1863*, 437-455.

[22] (a) M. Endo, S. Aoyama, Y. Tsuchido, L. Catti, M. Yoshizawa, *Angew. Chem., Int. Ed.* **2024**, *63*, e202404088; (b) A. Suzuki, K. Kondo, M. Akita, M. Yoshizawa, *Angew. Chem. Int. Ed.* **2013**, *52*, 8120-8123.

[23] S. P. D. Harborne, M. S. King, E. R. S. Kunji, in *Methods Mol. Biol.* (Eds.: V. L. G. Postis, A. Goldman), Springer US, New York, NY, **2020**, pp. 105-121.

[24] N. Thonghin, V. Kargas, J. Clews, R. C. Ford, *Methods* **2018**, *147*, 176-186.

[25] R. Ostaszewski, *Tetrahedron* **1998**, *54*, 6897-6902.

[26] N. Jain, A. V. Bedekar, *Tetrahedron: Asymmetry* **2011**, *22*, 1176-1179.

[27] C. H. Low, J. D. Nobbs, M. van Meurs, L. P. Stubbs, E. Drent, S. Aitipamula, M. H. L. Pung, *Organometallics* **2015**, *34*, 4281-4292.

[28] P. S. Chae, S. G. F. Rasmussen, R. R. Rana, K. Gotfryd, R. Chandra, M. A. Goren, A. C. Kruse, S. Nurva, C. J. Loland, Y. Pierre, D. Drew, J.-L. Popot, D. Picot, B. G. Fox, L. Guan, U. Gether, B. Byrne, B. Kobilka, S. H. Gellman, *Nat. Methods* **2010**, *7*, 1003-1008.

[29] D. M. Thal, A. Glukhova, P. M. Sexton, A. Christopoulos, *Nature* **2018**, *559*, 45-53.

[30] B. K. Kobilka, *Biochim. Biophys. Acta* **2007**, *1768*, 794-807.

[31] D. H. Yang, Q. T. Zhou, V. Labroska, S. S. Qin, S. Darbalaei, Y. R. Wu, E. Yuliantie, L. S. Xie, H. C. Tao, J. J. Cheng, Q. Liu, S. W. Zhao, W. Q. Shui, Y. Jiang, M. W. Wang, *Signal Transduct. Target. Ther.* **2021**, *6*.

[32] B. Carpenter, R. Nehmé, T. Warne, A. G. W. Leslie, C. G. Tate, *Nature* **2016**, *536*, 104-107.

[33] J. Jiang, *Semin. Cancer Biol.* **2022**, *85*, 107-122.

[34] S. Sisley, R. Gutierrez-Aguilar, M. Scott, D. A. D'Alessio, D. A. Sandova, R. J. Seeley, *J. Clin. Invest.* **2014**, *124*, 2456-2463.

[35] W. D. Picking, W. Kudlicki, G. Kramer, B. Hardesty, J. R. Vandenheede, W. Merlevede, I. K. Park, A. DePaoli-Roach, *Biochem.* **1991**, *30*, 10280-10287.

10.1002/cplu.202500320

WILEY VCH

RESEARCH ARTICLE

Entry for the Table of Contents