Advances in Mass Spectrometry for Membrane Protein Pharmacology

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Abstract

Membrane proteins are important targets for pharmacological research, due to their involvement in various cellular processes, ranging from signal transduction to transport, and cellular communication. While they make up roughly 30% of the human proteome and are the target of more than half of all approved drug products, their hydrophobicity and low abundance pose significant challenges for drug discovery and development. Mass spectrometry has emerged as a powerful tool for studying membrane protein structure, dynamics, regulation, as well as their interaction with proteins, small molecules, and lipids. In this review, we summarize recent findings and methodological advances that facilitate the study of cell surface organization, the discovery of druggable membrane proteins, and the identification of binding sites.

1. Novel mass spectrometric methods for the identification of drug targets and binding sites

[1,2,3,4,5,6,7,8,9]

Accessibility approaches

[4,5,10,11,12,13,14]

Thermal proteome profiling

[3,15]

Proximity-based approaches and chemical cross-linking

[16,17,18,19,20,21,22,23]

Native and intact mass spectrometry of membrane proteins

[24,25,26,27,28,29,30,31,32,33]

2. Selective probing of membrane proteins with chemoproteomics

[34,35,36,37,38,39]

3. Mass spectrometry reveals the cell surface organization

[2,3,18,19,24,35,40,41]

4. Insights into G protein-coupled receptors

[3,7,24,27,29,30,32,33,42]

5. Remaining challenges and outlook

[8,33,43,44,45]

References

1. **Capture of the Mouse Organ Membrane Proteome Specificity in Peptidisc Libraries**
Frank Antony, Zora Brough, Zhiyu Zhao, Franck Duong van Hoa
Journal of Proteome Research (2024-01-17) https://doi.org/g5w3hs
DOI: 10.1021/acs.jproteome.3c00825 · PMID: 38232390

2. **A Peptidisc-Based Survey of the Plasma Membrane Proteome of a Mammalian Cell**
Zhiyu Zhao, Arshdeep Khurana, Frank Antony, John W Young, Keeley G Hewton, Zora Brough, Tianshuang Zhong, Seth J Parker, Franck Duong van Hoa
Molecular & Cellular Proteomics (2023-08) https://doi.org/g9ks7s
DOI: 10.1016/j.mcpro.2023.100588 · PMID: 37295717 · PMCID: PMC10416069

3. **Cell surface thermal proteome profiling tracks perturbations and drug targets on the plasma membrane**
Mathias Kalxdorf, Ina Günthner, Isabelle Becher, Nils Kurzawa, Sascha Knecht, Mikhail M Savitski, HChristian Eberl, Marcus Bantscheff
Nature Methods (2021-01) https://doi.org/ghswc8
DOI: 10.1038/s41592-020-01022-1 · PMID: 33398190

4. **Target Deconvolution by Limited Proteolysis Coupled to Mass Spectrometry**
Viviane Reber, Matthias Gstaiger
Methods in Molecular Biology (2023) https://doi.org/g9ks7q
DOI: 10.1007/978-1-0716-3397-7_13 · PMID: 37558949

5. **High-throughput peptide-centric local stability assay extends protein-ligand identification to membrane proteins, tissues, and bacteria**
Kejia Li, Clement M Potel, Isabelle Becher, Nico Hüttmann, Martin Garrido-Rodriguez, Jennifer Schwarz, Mikhail M Savitski
Cold Spring Harbor Laboratory (2025-04-29) https://doi.org/g9ks7v
DOI: 10.1101/2025.04.28.650974

6. **Effects of theophylline on ADCY5 activation—From cellular studies to improved therapeutic options for ADCY5-related dyskinesia patients**
Dirk Tänzler, Marc Kipping, Marcell Lederer, Wiebke F Günther, Christian Arlt, Stefan Hüttelmaier, Andreas Merkenschlager, Andrea Sinz
PLOS ONE (2023-03-03) https://doi.org/grv9ht
DOI: 10.1371/journal.pone.0282593 · PMID: 36867608 · PMCID: PMC9983822

7. **Phosphorylation Sites of the Gastric Inhibitory Polypeptide Receptor (GIPR) Revealed by Trapped-Ion-Mobility Spectrometry Coupled to Time-of-Flight Mass Spectrometry (TIMS-TOF MS)**
Kyle A Brown, Rylie K Morris, Samantha J Eckhardt, Ying Ge, Samuel H Gellman
Journal of the American Chemical Society (2023-12-13) https://doi.org/g9ks7t
DOI: 10.1021/jacs.3c09078 · PMID: 38091482 · PMCID: PMC10842860

8. **Integrative structural modeling of macromolecular complexes using Assembline**
Vasileios Rantos, Kai Karius, Jan Kosinski
Nature Protocols (2021-11-29) https://doi.org/gq586q
DOI: 10.1038/s41596-021-00640-z · PMID: 34845384

9. **Simple But Efficacious Enrichment of Integral Membrane Proteins and Their Interactions for In-Depth Membrane Proteomics**

Pornparn Kongpracha, Pattama Wiriyasermkul, Noriyoshi Isozumi, Satomi Moriyama, Yoshikatsu Kanai, Shushi Nagamori
Molecular & Cellular Proteomics (2022-05) https://doi.org/g9ks7r
DOI: 10.1016/j.mcpro.2022.100206 · PMID: 35085786 · PMCID: PMC9062332

10. **Structure of Mycobacterium tuberculosis Cya, an evolutionary ancestor of the mammalian membrane adenylyl cyclases**
Ved Mehta, Basavraj Khanppnavar, Dina Schuster, Ilayda Kantarci, Irene Vercellino, Angela Kosturanova, Tarun Iype, Sasa Stefanic, Paola Picotti, Volodymyr M Korkhov
eLife (2022-08-18) https://doi.org/gsx57q
DOI: 10.7554/elife.77032 · PMID: 35980026 · PMCID: PMC9433096

11. **Structural basis of calmodulin modulation of the rod cyclic nucleotide-gated channel**
Diane C A Barret, Dina Schuster, Matthew J Rodrigues, Alexander Leitner, Paola Picotti, Gebhard F X Schertler, U Benjamin Kaupp, Volodymyr M Korkhov, Jacopo Marino
Proceedings of the National Academy of Sciences (2023-04-03) https://doi.org/gs27gg
DOI: 10.1073/pnas.2300309120 · PMID: 37011209 · PMCID: PMC10104587

12. **Regulatory sites of CaM-sensitive adenylyl cyclase AC8 revealed by cryo-EM and structural proteomics**
Basavraj Khanppnavar, Dina Schuster, Pia Lavriha, Federico Uliana, Merve Özel, Ved Mehta, Alexander Leitner, Paola Picotti, Volodymyr M Korkhov
EMBO Reports (2024-02-13) https://doi.org/g9kwfz
DOI: 10.1038/s44319-024-00076-y · PMID: 38351373 · PMCID: PMC10933263

13. **A peptide-centric local stability assay enables proteome-scale identification of the protein targets and binding regions of diverse ligands**
Kejia Li, Shijie Chen, Keyun Wang, Yan Wang, Lianji Xue, Yuying Ye, Zheng Fang, Jiawen Lyu, Haiyang Zhu, Yanan Li, … Mingliang Ye
Nature Methods (2024-12-10) https://doi.org/g84jvq
DOI: 10.1038/s41592-024-02553-7 · PMID: 39658593

14. **In-Cell Fast Photochemical Oxidation Interrogates the Native Structure of Integral Membrane Proteins**
Jie Sun, Mierxiati Saimi, Don Rempel, Qing Cao, Mengqi Chai, Weikai Li, Michael L Gross
Angewandte Chemie International Edition (2025-03-09) https://doi.org/g9kwfm
DOI: 10.1002/anie.202424779 · PMID: 40033852 · PMCID: PMC12052488

15. **Thermal proteome profiling identifies the membrane-bound purinergic receptor P2X4 as a target of the autophagy inhibitor indophagolin**
Marjorie A Carnero Corrales, Sarah Zinken, Georgios Konstantinidis, Muhammad Rafehi, Aliaa Abdelrahman, Yao-Wen Wu, Petra Janning, Christa E Müller, Luca Laraia, Herbert Waldmann
Cell Chemical Biology (2021-12) https://doi.org/gmz72w
DOI: 10.1016/j.chembiol.2021.02.017 · PMID: 33725479

16. **Cross-Linking Mass Spectrometry to Capture Protein Network Dynamics of Cell Membranome**
Lucia Santorelli, Michele Costanzo, Sara Petrosino, Michele Santoro, Marianna Caterino, Margherita Ruoppolo, Paolo Grumati
Methods in Molecular Biology (2024-12-24) https://doi.org/g9kwfn
DOI: 10.1007/978-1-0716-4298-6_16 · PMID: 39716008

17. **Cross-link assisted spatial proteomics to map sub-organelle proteomes and membrane protein topologies**

Ying Zhu, Kerem Can Akkaya, Julia Ruta, Nanako Yokoyama, Cong Wang, Max Ruwolt, Diogo Borges Lima, Martin Lehmann, Fan Liu
Nature Communications (2024-04-17) https://doi.org/g9kwfv
DOI: 10.1038/s41467-024-47569-x · PMID: 38632225 · PMCID: PMC11024108

18. **Glycan–protein cross-linking mass spectrometry reveals sialic acid-mediated protein networks on cell surfaces**
Yixuan Xie, Siyu Chen, Qiongyu Li, Ying Sheng, Michael Russelle Alvarez, Joeriggo Reyes, Gege Xu, Kemal Solakyildirim, Carlito B Lebrilla
Chemical Science (2021) https://doi.org/g9kwf3
DOI: 10.1039/d1sc00814e · PMID: 34257876 · PMCID: PMC8246274

19. **In situ cell-type-specific cell-surface proteomic profiling in mice**
SAndrew Shuster, Jiefu Li, URee Chon, Miley C Sinantha-Hu, David J Luginbuhl, Namrata D Udeshi, Dominique Kiki Carey, Yukari H Takeo, Qijing Xie, Chuanyun Xu, … Liqun Luo
Neuron (2022-12) https://doi.org/gq3hq5
DOI: 10.1016/j.neuron.2022.09.025 · PMID: 36220098 · PMCID: PMC9742329

20. **Profiling the proximal proteome of the activated μ-opioid receptor**
Benjamin J Polacco, Braden T Lobingier, Emily E Blythe, Nohely Abreu, Prachi Khare, Matthew K Howard, Alberto J Gonzalez-Hernandez, Jiewei Xu, Qiongyu Li, Brandon Novy, … Ruth Hüttenhain
Nature Chemical Biology (2024-03-25) https://doi.org/gtqbbp
DOI: 10.1038/s41589-024-01588-3 · PMID: 38528119 · PMCID: PMC11365811

21. **Chromatographic Phospholipid Trapping for Automated H/D Exchange Mass Spectrometry of Membrane Protein–Lipid Assemblies**
Dietmar Hammerschmid, Valeria Calvaresi, Chloe Bailey, Benjamin Russell Lewis, Argyris Politis, Michael Morris, Laetitia Denbigh, Malcolm Anderson, Eamonn Reading
Analytical Chemistry (2023-01-27) https://doi.org/g9kwms
DOI: 10.1021/acs.analchem.2c04876 · PMID: 36706021 · PMCID: PMC9909672

22. **Molecular characterization of a complex of apoptosis-inducing factor 1 with cytochrome c oxidase of the mitochondrial respiratory chain**
Johannes F Hevler, Riccardo Zenezeni Chiozzi, Alfredo Cabrera-Orefice, Ulrich Brandt, Susanne Arnold, Albert JR Heck
Proceedings of the National Academy of Sciences (2021-09-21) https://doi.org/g9kwf5
DOI: 10.1073/pnas.2106950118 · PMID: 34548399 · PMCID: PMC8488679

23. **A proximity proteomics pipeline with improved reproducibility and throughput**
Xiaofang Zhong, Qiongyu Li, Benjamin J Polacco, Trupti Patil, Aaron Marley, Helene Foussard, Prachi Khare, Rasika Vartak, Jiewei Xu, Jeffrey F DiBerto, … Ruth Hüttenhain
Molecular Systems Biology (2024-07-01) https://doi.org/g9kwf2
DOI: 10.1038/s44320-024-00049-2 · PMID: 38951684 · PMCID: PMC11297269

24. **Capturing a rhodopsin receptor signalling cascade across a native membrane**
Siyun Chen, Tamar Getter, David Salom, Di Wu, Daniel Quetschlich, Dror S Chorev, Krzysztof Palczewski, Carol V Robinson
Nature (2022-04-06) https://doi.org/g9kwfw
DOI: 10.1038/s41586-022-04547-x · PMID: 35388214 · PMCID: PMC9007743

25. **Native Mass Spectrometry of Membrane Protein–Lipid Interactions in Different Detergent Environments**
Smriti Kumar, Lauren Stover, Lie Wang, Hanieh Bahramimoghaddam, Ming Zhou, David H Russell, Arthur Laganowsky

Analytical Chemistry (2024-10-12) https://doi.org/g9kwfr
DOI: 10.1021/acs.analchem.4c03312 · PMID: 39394983 · PMCID: PMC11503522

26. **Native mass spectrometry of proteoliposomes containing integral and peripheral membrane proteins**
Yun Zhu, Sangho D Yun, Tianqi Zhang, Jing-Yuan Chang, Lauren Stover, Arthur Laganowsky
Chemical Science (2023) https://doi.org/g9kwf4
DOI: 10.1039/d3sc04938h · PMID: 38098719 · PMCID: PMC10718073

27. **Capillary Zone Electrophoresis-Mass Spectrometry of Intact G Protein-Coupled Receptors Enables Proteoform Profiling**
Ashley N Ives, Kevin Jooß, Rafael D Melani, Ryan T Fellers, John Janetzko, Neil L Kelleher
Analytical Chemistry (2025-03-27) https://doi.org/g9kwfs
DOI: 10.1021/acs.analchem.4c06994 · PMID: 40146057

28. **Capillary Zone Electrophoresis-Tandem Mass Spectrometry for Top-Down Proteomics of Mouse Brain Integral Membrane Proteins**
Qianjie Wang, Tian Xu, Fei Fang, Qianyi Wang, Peter Lundquist, Liangliang Sun
Analytical Chemistry (2023-08-18) https://doi.org/g9kwfq
DOI: 10.1021/acs.analchem.3c02346 · PMID: 37595263 · PMCID: PMC10540247

29. **The Effects of Sodium Ions on Ligand Binding and Conformational States of G Protein-Coupled Receptors—Insights from Mass Spectrometry**
Mark T Agasid, Lars Sørensen, Leonhard H Urner, Jun Yan, Carol V Robinson
Journal of the American Chemical Society (2021-03-12) https://doi.org/gjf7x5
DOI: 10.1021/jacs.0c11837 · PMID: 33711230 · PMCID: PMC7995251

30. **Evaluation of Drug Responses to Human β₂AR Using Native Mass Spectrometry**
Michiko Tajiri, Shunsuke Imai, Tsuyoshi Konuma, Keiko Shimamoto, Ichio Shimada, Satoko Akashi
ACS Omega (2023-06-28) https://doi.org/g9kwft
DOI: 10.1021/acsomega.3c02737 · PMID: 37457453 · PMCID: PMC10339329

31. **Native mass spectrometry and structural studies reveal modulation of MsbA–nucleotide interactions by lipids**
Tianqi Zhang, Jixing Lyu, Bowei Yang, Sangho D Yun, Elena Scott, Minglei Zhao, Arthur Laganowsky
Nature Communications (2024-07-15) https://doi.org/gt4mfb
DOI: 10.1038/s41467-024-50350-9 · PMID: 39009687 · PMCID: PMC11251056

32. **Mass spectrometry captures biased signalling and allosteric modulation of a G-protein-coupled receptor**
Hsin-Yung Yen, Idlir Liko, Wanling Song, Parth Kapoor, Fernando Almeida, Joanna Toporowska, Karolina Gherbi, Jonathan TS Hopper, Steven J Charlton, Argyris Politis, … Carol V Robinson
Nature Chemistry (2022-11-10) https://doi.org/jmm7
DOI: 10.1038/s41557-022-01041-9 · PMID: 36357787 · PMCID: PMC9758051

33. **Native mass spectrometry prescreening of G protein-coupled receptor complexes for cryo-EM structure determination**
Donggyun Kim, Weijing Liu, Rosa Viner, Vadim Cherezov
Structure (2024-12) https://doi.org/g9kwfp
DOI: 10.1016/j.str.2024.10.004 · PMID: 39471802 · PMCID: PMC11625002

34. **Lipid- and protein-directed photosensitizer proximity labeling captures the cholesterol interactome**
Andrew P Becker, Elijah Biletch, John Paul Kennelly, Ashley R Julio, Miranda Villaneuva, Rohith T Nagari, Daniel W Turner, Nikolas R Burton, Tomoyuki Fukuta, Liujuan Cui, … Keriann M Backus
Cold Spring Harbor Laboratory (2024-08-20) https://doi.org/g9kwhm
DOI: 10.1101/2024.08.20.608660 · PMID: 39229057 · PMCID: PMC11370482

35. **A chemical proteomics approach for global mapping of functional lysines on cell surface of living cell**
Ting Wang, Shiyun Ma, Guanghui Ji, Guoli Wang, Yang Liu, Lei Zhang, Ying Zhang, Haojie Lu
Nature Communications (2024-04-08) https://doi.org/g9kwhj
DOI: 10.1038/s41467-024-47033-w · PMID: 38589397 · PMCID: PMC11001985

36. **Probing Monotopic Phosphoglycosyl Transferases from Complex Cellular Milieu**
Alyssa J Anderson, Leah M Seebald, Christine A Arbour, Barbara Imperiali
ACS Chemical Biology (2022-11-08) https://doi.org/g6g3mb
DOI: 10.1021/acschembio.2c00648 · PMID: 36346917 · PMCID: PMC9703085

37. **Dual-Probe Activity-Based Protein Profiling Reveals Site-Specific Differences in Protein Binding of EGFR-Directed Drugs**
Wouter van Bergen, Kristina Žuna, Jan Fiala, Elena E Pohl, Albert JR Heck, Marc P Baggelaar
ACS Chemical Biology (2024-07-25) https://doi.org/g9kwhh
DOI: 10.1021/acschembio.3c00637 · PMID: 39052621 · PMCID: PMC11334109

38. **Defining the Cell Surface Cysteinome Using Two-Step Enrichment Proteomics**
Tianyang Yan, Lisa M Boatner, Liujuan Cui, Peter J Tontonoz, Keriann M Backus
JACS Au (2023-12-13) https://doi.org/g5c55k
DOI: 10.1021/jacsau.3c00707 · PMID: 38155636 · PMCID: PMC10751780

39. **A proteome-wide map of 20(S)-hydroxycholesterol interactors in cell membranes**
Yu-Shiuan Cheng, Tianyi Zhang, Xiang Ma, Sarida Pratuangtham, Grace C Zhang, Alexander A Ondrus, Amirhossein Mafi, Brett Lomenick, Jeffrey J Jones, Alison E Ondrus
Nature Chemical Biology (2021-11-19) https://doi.org/g9kwhk
DOI: 10.1038/s41589-021-00907-2 · PMID: 34799735 · PMCID: PMC8607797

40. **Cell Surface Engineering Enables Surfaceome Profiling**
Zak Vilen, Abigail E Reeves, Timothy R O'Leary, Eugene Joeh, Naomi Kamasawa, Mia L Huang
ACS Chemical Biology (2022-04-20) https://doi.org/g9kwmw
DOI: 10.1021/acschembio.1c00865 · PMID: 35443134 · PMCID: PMC9901301

41. **Quantitative Glycan-Protein Cross-Linking Mass Spectrometry Using Enrichable Linkers Reveals Extensive Glycan-Mediated Protein Interaction Networks**
Siyu Chen, Yixuan Xie, Michael Russelle Alvarez, Ying Sheng, Yasmine Bouchibti, Vincent Chang, Carlito B Lebrilla
Analytical Chemistry (2025-01-13) https://doi.org/g9kwmv
DOI: 10.1021/acs.analchem.4c04134 · PMID: 39805041 · PMCID: PMC11780575

42. **Structural Mass Spectrometry Captures Residue-Resolved Comprehensive Conformational Rearrangements of a G Protein-Coupled Receptor**
Hongyue Liu, Pengfei Yan, Zhaoyu Zhang, Hongbo Han, Qingtong Zhou, Jie Zheng, Jian Zhang, Fei Xu, Wenqing Shui
Journal of the American Chemical Society (2024-07-13) https://doi.org/g9kwmx
DOI: 10.1021/jacs.4c03922 · PMID: 39001877

43. **Cryo-EM of soft-landed β-galactosidase: Gas-phase and native structures are remarkably similar**
Tim K Esser, Jan Böhning, Alpcan Önür, Dinesh K Chinthapalli, Lukas Eriksson, Marko Grabarics, Paul Fremdling, Albert Konijnenberg, Alexander Makarov, Aurelien Botman, … Stephan Rauschenbach
Science Advances (2024-02-16) https://doi.org/gth2cp
DOI: 10.1126/sciadv.adl4628 · PMID: 38354247 · PMCID: PMC10866560

44. **Cryogenic Soft Landing Improves Structural Preservation of Protein Complexes**
Michael S Westphall, Kenneth W Lee, Colin Hemme, Austin Z Salome, Keaton Mertz, Timothy Grant, Joshua J Coon
Analytical Chemistry (2023-09-21) https://doi.org/g9kwmt
DOI: 10.1021/acs.analchem.3c03228 · PMID: 37732836 · PMCID: PMC10568529

45. **Generating cysteine-trypsin cleavage sites with 2-chloroacetamidine capping**
Samuel Ofori, Heta S Desai, Flowreen Shikwana, Lisa M Boatner, Emil R Dominguez III, José O Castellón, Keriann M Backus
Chemical Communications (2024) https://doi.org/g9kwmz
DOI: 10.1039/d4cc01583e · PMID: 39081146