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

[<u>3</u>,<u>15</u>]

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 & Molecular 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: 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 & 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/gsx57g

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: 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: <u>10.1038/s41592-024-02553-7</u> · PMID: <u>39658593</u>

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: PMCI2052488

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: <u>10.1016/j.chembiol.2021.02.017</u> · PMID: <u>33725479</u>

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: <u>10.1007/978-1-0716-4298-6 16</u> · PMID: <u>39716008</u>

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/gg3hq5

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: <u>10.1038/s41589-024-01588-3</u> · PMID: <u>38528119</u> · PMCID: <u>PMC11365811</u>

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: <u>10.1021/acs.analchem.4c06994</u> · PMID: <u>40146057</u>

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: PMCID: PMC7995251

30. Evaluation of Drug Responses to Human β ₂AR 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: <u>10.1101/2024.08.20.608660</u> · PMID: <u>39229057</u> · PMCID: <u>PMC11370482</u>

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: <u>10.1038/s41589-021-00907-2</u> · PMID: <u>34799735</u> · PMCID: <u>PMC8607797</u>

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: <u>10.1021/acs.analchem.4c04134</u> · PMID: <u>39805041</u> · PMCID: <u>PMC11780575</u>

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: <u>10.1021/jacs.4c03922</u> · PMID: <u>39001877</u>

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: <u>10.1126/sciadv.adl4628</u> · PMID: <u>38354247</u> · PMCID: <u>PMC10866560</u>

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: <u>10.1021/acs.analchem.3c03228</u> · PMID: <u>37732836</u> · PMCID: <u>PMC10568529</u>

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: <u>10.1039/d4cc01583e</u> · PMID: <u>39081146</u>