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Unraveling proteoform complexity by native liquid chromatography-mass spectrometry

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Appendices

References

1. Ghilardi, N.; Pappu, R.; Aron, J. R.; Chan, A. C., 30 Years of Biotherapeutics Development-What Have We Learned? *Annu. Rev. Immunol.* **2020**, *38*, 249-287.
2. Quianzon, C. C.; Cheikh, I., History of insulin. *J. Community Hosp. Intern. Med. Perspect.* **2012**, *2* (2), 18701.
3. Jelkmann, W., Physiology and Pharmacology of Erythropoietin. *Transfus. Med. Hemother.* **2013**, *40* (5), 302-309.
4. Carter, P. J.; Lazar, G. A., Next generation antibody drugs: pursuit of the 'high-hanging fruit'. *Nat. Rev. Drug Discov.* **2018**, *17* (3), 197-223.
5. Singh, S.; Kumar, N. K.; Dwiwedi, P.; Charan, J.; Kaur, R.; Sidhu, P.; Chugh, V. K., Monoclonal Antibodies: A Review. *Curr. Clin. Pharmacol.* **2018**, *13* (2), 85-99.
6. van Beers, M. M. C.; Bardor, M., Minimizing immunogenicity of biopharmaceuticals by controlling critical quality attributes of proteins. *Biootechnol. J.* **2012**, *7*, 1433-1443.
7. Castelli, M. S.; McGonigle, P.; Hornby, P. J., The pharmacology and therapeutic applications of monoclonal antibodies. *Pharmacol. Res. Perspect* **2019**, *7* (6), e00535.
8. Köhler, G.; Milstein, C., Continuous cultures of fused cells secreting antibody of pre-defined specificity. *Nature* **1975**, *256*, 495-497
9. Pierpont, T. M.; Limper, C. B.; Richards, K. L., Past, Present, and Future of Rituximab-The World's First Oncology Monoclonal Antibody Therapy. *Front. Oncol.* **2018**, *8*, 163.
10. Grillo-Lopez, A. J., The first antibody therapy for cancer: a personal experience. *Expert Rev. Anticancer Ther.* **2013**, *13* (4), 399-406.
11. Mullard, A., FDA approves 100th monoclonal antibody product. *Nat. Rev. Drug Discov.* **2021**, *20* (7), 491-495.
12. Kaplon, H.; Chenoweth, A.; Crescioli, S.; Reichert, J. M., Antibodies to watch in 2022. *mAbs* **2022**, *14* (1), 2014296.
13. McGovern, P. E.; Zhang, J.; Tang, J.; Zhang, Z.; G.R., H.; Moreau, R. A.; Nunez, A.; Butrym, E. D.; Richards, M. P.; Wang, C.; Cheng, G.; Zhao, Z.; Wang, C., Fermented beverages of pre- and proto-historic China. *PNAS* **2004**, *101* (51), 17593-17598.
14. May, O., *Industrial Enzyme Applications*. Wiley-VCH: Weinheim, 2019.
15. Arnau, J.; Yaver, D.; Hjort, C. M., Strategies and Challenges for the Development of Industrial Enzymes Using Fungal Cell Factories. In *Grand Challenges in Fungal Biotechnology*, Nevalainen, H., Ed. Springer: Cham, 2020; pp 179-210.
16. Polaina, J.; MacCabe, A. P., *Industrial Enzymes Structure, Function and Applications*. Springer: Dordrecht, The Netherlands, 2007.
17. Leisola, M.; Jokela, J.; Pastinen, O.; Turunen, O.; Schoemaker, H. E., Industrial use of Enzymes. In *Physiology and maintainance*, Vol. 2.
18. Sharma, A.; Gupta, G.; Ahmad, T.; Mansoor, S.; Kaur, B., Enzyme Engineering: Current Trends and Future Perspectives. *Food Rev. Int.* **2019**, *37* (2), 121-154.
19. Patel, A. K.; Singhania, R. R.; Pandey, A., Novel enzymatic processes applied to the food industry. *Curr. Opin. Food Sci.* **2016**, *7*, 64-72.
20. Singh, R.; Kumar, M.; Mittal, A.; Mehta, P. K., Microbial enzymes: industrial progress in 21st century. *3 Biootech* **2016**, *6* (2), 174.
21. Melis, S.; Meza Morales, W. R.; Delcour, J. A., Lipases in wheat flour bread making: Importance of an appropriate balance between wheat endogenous lipids and their enzymatically released hydrolysis products. *Food Chem.* **2019**, *298*, 125002.
22. Butt, M. S.; Tahir-Nadeem, M.; Ahmad, Z.; Sultan, M. T., Xylanases and Their Applications in Baking Industry. *Food Technol. Biotechnol.* **2008**, *46* (1), 22-31.
23. Vemulapalli, V.; Miller, K. A.; Hoseney, R. C., Glucose Oxidase in Breadmaking Systems. *Cereal Chem.* **1998**, *75* (4), 439-442.
24. Hayward, S.; Cilliers, T.; Swart, P., Lipoxygenases: From Isolation to Application. *Compr. Rev. Food Sci. Food Saf.* **2017**, *16* (1), 199-211.
25. Lopez, M.; Edens, L., Effective Prevention of Chill-Haze in Beer Using an Acid Proline-Specific Endoprotease from *Aspergillus niger*. *J. Agric. Food Chem.* **2005**, *53*, 7944-7949.
26. Hemsworth, G. R.; Johnston, E. M.; Davies, G. J.; Walton, P. H., Lytic Polysaccharide Monooxygenases in Biomass Conversion. *Trends Biotechnol.* **2015**, *33* (12), 747-761.
27. Collins, F. S.; Lander, E. S.; Rogers, J.; R.H., W., Finishing the euchromatic sequence of the human genome. *Nature* **2004**, *431* (7011), 931-945.
28. Aebersold, R.; Agar, J. N.; Amster, I. J.; Baker, M. S.; Bertozzi, C. R.; Boja, E. S.; Costello,

- C. E.; Cravatt, B. F.; Fenselau, C.; Garcia, B. A.; Ge, Y.; Gunawardena, J.; Hendrickson, R. C.; Hergenrother, P. J.; Huber, C. G.; Ivanov, A. R.; Jensen, O. N.; Jewett, M. C.; Kelleher, N. L.; Kiessling, L. L.; Krogan, N. J.; Larsen, M. R.; Loo, J. A.; Ogorzalek Loo, R. R.; Lundberg, E.; MacCoss, M. J.; Mallick, P.; Mootha, V. K.; Mrksich, M.; Muir, T. W.; Patrie, S. M.; Pesavento, J. J.; Pitteri, S. J.; Rodriguez, H.; Saghatelian, A.; Sandoval, W.; Schluter, H.; Sechi, S.; Slavoff, S. A.; Smith, L. M.; Snyder, M. P.; Thomas, P. M.; Uhlen, M.; Van Eyk, J. E.; Vidal, M.; Walt, D. R.; White, F. M.; Williams, E. R.; Wohlschlager, T.; Wysocki, V. H.; Yates, N. A.; Young, N. L.; Zhang, B., How many human proteoforms are there? *Nat. Chem. Biol.* **2018**, *14* (3), 206-214.
29. Smith, L. M.; Kelleher, N. L.; Consortium for Top Down, P., Proteoform: a single term describing protein complexity. *Nat. Methods* **2013**, *10* (3), 186-187.
30. Toby, T. K.; Fornelli, L.; Kelleher, N. L., Progress in Top-Down Proteomics and the Analysis of Proteoforms. *Annu. Rev. Anal. Chem.* **2016**, *9* (1), 499-519.
31. Uversky, V. N., Posttranslational Modification. In *Brenner's Encyclopedia of Genetics (Second Edition)*, Maloy, S.; Hughes, K., Eds. Academic Press: San Diego, 2013; pp 425-430.
32. Helenius, A.; Aeby, M., Intracellular Functions of N-Linked Glycans. *Science* **2001**, *291*, 2364-2369.
33. Skropeta, D., The effect of individual N-glycans on enzyme activity. *Bioorg. Med. Chem.* **2009**, *17* (7), 2645-2653.
34. Geyer, H.; Geyer, R., Strategies for analysis of glycoprotein glycosylation. *Biochim. Biophys. Acta* **2006**, *1764* (12), 1853-1869.
35. Hanisch, F. G., O-Glycosylation of the Mucin Type. *Biol. Chem.* **2001**, *382*, 143-149.
36. Trill, J. J.; Shatzman, A. R.; Ganguly, S., Production of monoclonal antibodies in COS and CHO cells. *Curr. Opin. Biotechnol.* **1995**, *6*, 553-560.
37. Dordal, M. S.; Wang, F. F.; Goldwasser, E., The Role of Carbohydrate in Erythropoietin Action. *Endocrinology* **1985**, *116* (6), 2293-2299.
38. Szabo, Z.; Thayer, J. R.; Reusch, D.; Agroskin, Y.; Viner, R.; Rohrer, J.; Patil, S. P.; Krawitzky, M.; Huhmer, A.; Avdalovic, N.; Khan, S. H.; Liu, Y.; Pohl, C., High Performance Anion Exchange and Hydrophilic Interaction Liquid Chromatography Approaches for Comprehensive Mass Spectrometry-Based Characterization of the N-Glycome of a Recombinant Human Erythropoietin. *J. Proteome Res.* **2018**, *17* (4), 1559-1574.
39. Shields, R. L.; Lai, J.; Keck, R.; O'Connell, L. Y.; Hong, K.; Meng, Y. G.; Weikert, S. H.; Presta, L. G., Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fc γ RIII and antibody-dependent cellular toxicity. *J. Biol. Chem.* **2002**, *277* (30), 26733-26740.
40. Goetze, A. M.; Liu, Y. D.; Zhang, Z.; Shah, B.; Lee, E.; Bondarenko, P. V.; Flynn, G. C., High-mannose glycans on the Fc region of therapeutic IgG antibodies increase serum clearance in humans. *Glycobiology* **2011**, *21* (7), 949-959.
41. Sjogren, J.; Lood, R.; Nageli, A., On enzymatic remodeling of IgG glycosylation: unique tools with broad applications. *Glycobiology* **2020**, *30* (4), 254-267.
42. Demain, A. L.; Adrio, J. L., Contributions of microorganisms to industrial biology. *Mol. Biotechnol.* **2008**, *38* (1), 41-55.
43. Jafari-Aghdam, J.; Khajeh, K.; Ranjbar, B.; Nemat-Gorgani, M., Deglycosylation of glucoamylase from Aspergillus niger: effects on structure, activity and stability. *Biochim. Biophys. Acta* **2005**, *1750* (1), 61-68.
44. Chang, X.; Xu, B.; Bai, Y.; Luo, H.; Ma, R.; Shi, P.; Yao, B., Role of N-linked glycosylation in the enzymatic properties of a thermophilic GH 10 xylanase from Aspergillus fumigatus expressed in Pichia pastoris. *PLoS One* **2017**, *12* (2), e0171111.
45. Maillard, L. C., Action des acides amines sur le sucre: Formation des melanoidines par voie methodique. *C. R. Acad. Sci.* **1912**, *154*, 66-68.
46. Zhang, Q.; Ames, J. M.; Smith, R. D.; Baynes, J. W.; Metz, T. O., A perspective on the Maillard reaction and the analysis of protein glycation by mass spectrometry: probing the pathogenesis of chronic disease. *J. Proteome Res.* **2009**, *8* (2), 754-69.
47. Gstötter, C.; Reusch, D.; Haberger, M.; Dragan, I.; Van Veelen, P.; Kilgour, D. P. A.; Tsypbin, Y. O.; van der Burgt, Y. E. M.; Wuhrer, M.; Nicolardi, S., Monitoring glycation levels of a bispecific monoclonal antibody at subunit level by ultrahigh-resolution MALDI FT-ICR mass spectrometry. *mAbs* **2020**, *12* (1), 1682403.
48. Sun, F.; Suttipitugsakul, S.; Xiao, H.; Wu, R., Comprehensive Analysis of Protein Gly-

- cation Reveals Its Potential Impacts on Protein Degradation and Gene Expression in Human Cells. *J. Am. Soc. Mass Spectrom.* **2019**, *30* (12), 2480-2490.
49. Wei, B.; Berning, K.; Quan, C.; Zhang, Y. T., Glycation of antibodies: Modification, methods and potential effects on biological functions. *mAbs* **2017**, *9* (4), 586-594.
50. Misset, O.; van Dijk, A., Diagnosing the inactivating process of enzymes. In *Stability and Stabilization of Biocatalysts*, Ballesteros, A., Ed. Elsevier Science: 1998; Vol. 15.
51. Sutthirak, P.; Assavanig, A.; Dharmsthit, S.; Lertsiri, S., Changes in the Stability and Kinetic Parameters up on Glycation of Thermostable α -Amylase from *Bacillus Subtilis*. *J. Food Biochem.* **2010**, *34* (6), 1157-1171.
52. Mo, J.; Jin, R.; Yan, Q.; Sokolowska, I.; Lewis, M. J.; Hu, P., Quantitative analysis of glycation and its impact on antigen binding. *mAbs* **2018**, *10* (3), 406-415.
53. Sutthirak, P.; Dharmsthit, S.; Lertsiri, S., Effect of glycation on stability and kinetic parameters of thermostable glucoamylase from *Aspergillus niger*. *Process Biochem.* **2005**, *40* (8), 2821-2826.
54. Kuriakose, A.; Chirmule, N.; Nair, P., Immunogenicity of Biotherapeutics: Causes and Association with Posttranslational Modifications. *J Immunol Res* **2016**, *2016*, 1298473.
55. Hintersteiner, B.; Lingg, N.; Zhang, P.; Woen, S.; Hoi, K. M.; Stranner, S.; Wiederkum, S.; Mutschlechner, O.; Schuster, M.; Loibner, H.; Jungbauer, A., Charge heterogeneity: Basic antibody charge variants with increased binding to Fc receptors. *mAbs* **2016**, *8* (8), 1548-1560.
56. Wang, W.; Vlasak, J.; Li, Y.; Pristatsky, P.; Fang, Y.; Pittman, T.; Roman, J.; Wang, Y.; Prueksaritanont, T.; Ionescu, R., Impact of methionine oxidation in human IgG1 Fc on serum half-life of monoclonal antibodies. *Mol. Immunol.* **2011**, *48* (6-7), 860-866.
57. De Groot, A. S.; Scott, D. W., Immunogenicity of protein therapeutics. *Trends Immunol.* **2007**, *28* (11), 482-490.
58. Xu, H.; Wang, Y.; Lin, S.; Deng, W.; Peng, D.; Cui, Q.; Xue, Y., PTMD: A Database of Human Disease-associated Post-translational Modifications. *Genom. Proteom. Bioinform.* **2018**, *16* (4), 244-251.
59. Tenreiro, S.; Eckermann, K.; Outeiro, T. F., Protein phosphorylation in neurodegeneration: friend or foe? *Front. Mol. Neurosci.* **2014**, *7*, 42.
60. Jin, H.; Zangar, R. C., Protein Modifications as Potential Biomarkers in Breast Cancer. *Biomark. Insights* **2009**, *4*, 191-200.
61. Pinho, S. S.; Reis, C. A., Glycosylation in cancer: mechanisms and clinical implications. *Nat. Rev. Cancer* **2015**, *15* (9), 540-555.
62. Reilly, C.; Stewart, T. J.; Renfrow, M. B.; Novak, J., Glycosylation in health and disease. *Nat. Rev. Nephrol.* **2019**, *15* (6), 346-366.
63. Ciudad, S.; Puig, E.; Botzanowski, T.; Meigooni, M.; Arango, A. S.; Do, J.; Mayzel, M.; Bayoumi, M.; Chaignepain, S.; Maglia, G.; Cianferani, S.; Orekhov, V.; Tajkhorshid, E.; Bardiaux, B.; Carulla, N., $\text{A}\beta(1-42)$ tetramer and octamer structures reveal edge conductivity pores as a mechanism for membrane damage. *Nat. Commun.* **2020**, *11* (1), 3014.
64. Ebrahimi-Fakhari, D.; Wahlster, L.; McLean, P. J., Protein degradation pathways in Parkinson's disease: curse or blessing. *Acta Neuropathol.* **2012**, *124* (2), 153-172.
65. Walsh, G., Biopharmaceutical benchmarks 2018. *Nat. Biotechnol.* **2018**, *36* (12), 1136-1145.
66. Zhang, Y.; Fonslow, B. R.; Shan, B.; Baek, M. C.; Yates, J. R., 3rd, Protein analysis by shotgun/bottom-up proteomics. *Chem. Rev.* **2013**, *113* (4), 2343-2394.
67. Donnelly, D. P.; Rawlins, C. M.; DeHart, C. J.; Fornelli, L.; Schachner, L. F.; Lin, Z.; Lippeps, J. L.; Aluri, K. C.; Sarin, R.; Chen, B.; Lantz, C.; Jung, W.; Johnson, K. R.; Koller, A.; Wolff, J. J.; Campuzano, I. D. G.; Auclair, J. R.; Ivanov, A. R.; Whitelegge, J. P.; Pasa-Tolic, L.; Chamot-Rooke, J.; Danis, P. O.; Smith, L. M.; Tsbybin, Y. O.; Loo, J. A.; Ge, Y.; Kelleher, N. L.; Agar, J. N., Best practices and benchmarks for intact protein analysis for top-down mass spectrometry. *Nat. Methods* **2019**, *16* (7), 587-594.
68. Berkowitz, S. A.; Engen, J. R.; Mazzeo, J. R.; Jones, G. B., Analytical tools for characterizing biopharmaceuticals and the implications for biosimilars. *Nat. Rev. Drug Discov.* **2012**, *11* (7), 527-540.
69. Parr, M. K.; Montacir, O.; Montacir, H., Physicochemical characterization of biopharmaceuticals. *J. Pharm. Biomed. Anal.* **2016**, *130*, 366-389.
70. Skinner, O. S.; Haverland, N. A.; Fornelli, L.; Melani, R. D.; Do Vale, L. H. F.; Seckler, H.

- S.; Doubleday, P. F.; Schachner, L. F.; Srzentic, K.; Kelleher, N. L.; Compton, P. D., Top-down characterization of endogenous protein complexes with native proteomics. *Nat. Chem. Biol.* **2018**, 14 (1), 36-41.
71. Wohlschlager, T.; Scheffler, K.; Forstenlehner, I. C.; Skala, W.; Senn, S.; Damoc, E.; Holzmann, J.; Huber, C. G., Native mass spectrometry combined with enzymatic dissection unravels glycoform heterogeneity of biopharmaceuticals. *Nat. Commun.* **2018**, 9 (1), 1713.
72. Jooss, K.; McGee, J. P.; Melani, R. D.; Kelleher, N. L., Standard procedures for native CZE-MS of proteins and protein complexes up to 800 kDa. *Electrophoresis* **2021**, 42, 9-10.
73. Haberger, M.; Leiss, M.; Heidenreich, A. K.; Pester, O.; Hafenmair, G.; Hook, M.; Bonnington, L.; Wegele, H.; Haindl, M.; Reusch, D.; Bulau, P., Rapid characterization of biotherapeutic proteins by size-exclusion chromatography coupled to native mass spectrometry. *mAbs* **2015**, 8 (2), 331-339.
74. Bich, C.; Baer, S.; Jecklin, M. C.; Zenobi, R., Probing the hydrophobic effect of non-covalent complexes by mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2010**, 21 (2), 286-289.
75. Marie, A. L.; Dominguez-Vega, E.; Saller, F.; Plantier, J. L.; Urbain, R.; Borgel, D.; Tran, N. T.; Somsen, G. W.; Taverna, M., Characterization of conformers and dimers of antithrombin by capillary electrophoresis-quadrupole-time-of-flight mass spectrometry. *Anal. Chim. Acta* **2016**, 947, 58-65.
76. Konermann, L.; Ahadi, E.; Rodriguez, A. D.; Vahidi, S., Unraveling the mechanism of electrospray ionization. *Anal. Chem.* **2013**, 85 (1), 2-9.
77. Tong, W.; Wang, G., How can native mass spectrometry contribute to characterization of biomacromolecular higher-order structure and interactions? *Methods* **2018**, 144, 3-13.
78. Cech, N. B.; Enke, C. G., Practical implications of some recent studies in electrospray ionization fundamentals. *Mass Spectrom. Rev.* **2001**, 20 (6), 362-387.
79. Awad, H.; Khamis, M. M.; El-Aneed, A., Mass Spectrometry, Review of the Basics: Ionization. *Appl. Spectrosc.* **2014**, 50 (2), 158-175.
80. Belov, A. M.; Viner, R.; Santos, M. R.; Horn, D. M.; Bern, M.; Karger, B. L.; Ivanov, A. R., Analysis of Proteins, Protein Complexes, and Organellar Proteomes Using Sheathless Capillary Zone Electrophoresis - Native Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2017**, 28 (12), 2614-2634.
81. Konermann, L., Addressing a Common Misconception: Ammonium Acetate as Neutral pH "Buffer" for Native Electrospray Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2017**, 28 (9), 1827-1835.
82. Fekete, S.; Beck, A.; Veuthey, J. L.; Guillarme, D., Ion-exchange chromatography for the characterization of biopharmaceuticals. *J. Pharm. Biomed. Anal.* **2015**, 113, 43-55.
83. Queiroz, J. A.; Tomaz, C. T.; Cabral, J. M. S., Hydrophobic interaction chromatography of proteins. *J. Biotechnol.* **2001**, 87, 143-159.
84. Sterling, H. J.; Batchelor, J. D.; Wemmer, D. E.; Williams, E. R., Effects of buffer loading for electrospray ionization mass spectrometry of a noncovalent protein complex that requires high concentrations of essential salts. *J. Am. Soc. Mass Spectrom.* **2010**, 21 (6), 1045-1049.
85. Metwally, H.; McAllister, R. G.; Konermann, L., Exploring the mechanism of salt-induced signal suppression in protein electrospray mass spectrometry using experiments and molecular dynamics simulations. *Anal. Chem.* **2015**, 87 (4), 2434-2442.
86. Cassou, C. A.; Williams, E. R., Desalting protein ions in native mass spectrometry using supercharging reagents. *Analyst* **2014**, 139 (19), 4810-4819.
87. Susa, A. C.; Xia, Z.; Williams, E. R., Small Emitter Tips for Native Mass Spectrometry of Proteins and Protein Complexes from Nonvolatile Buffers That Mimic the Intracellular Environment. *Anal. Chem.* **2017**, 89 (5), 3116-3122.
88. Yan, Y.; Xing, T.; Wang, S.; Daly, T. J.; Li, N., Online coupling of analytical hydrophobic interaction chromatography with native mass spectrometry for the characterization of monoclonal antibodies and related products. *J. Pharm. Biomed. Anal.* **2020**, 186, 113313.
89. Yan, Y.; Xing, T.; Wang, S.; Li, N., Versatile, Sensitive, and Robust Native LC-MS Platform for Intact Mass Analysis of Protein Drugs. *J. Am. Soc. Mass Spectrom.* **2020**, 31 (10), 2171-2179.

90. Konermann, L.; Metwally, H.; Duez, Q.; Peters, I., Charging and supercharging of proteins for mass spectrometry: recent insights into the mechanisms of electrospray ionization. *Analyst* **2019**, 144 (21), 6157-6171.
91. Felitsyn, N.; Peschke, M.; Kebarle, P., Origin and number of charges observed on multiply-protonated native proteins produced by ESI. *Int. J. Mass Spectrom.* **2002**, 219, 39-62.
92. Ventouri, I. K.; Malheiro, D. B. A.; Voeten, R. L. C.; Kok, S.; Honing, M.; Somsen, G. W.; Haselberg, R., Probing Protein Denaturation during Size-Exclusion Chromatography Using Native Mass Spectrometry. *Anal. Chem.* **2020**, 92 (6), 4292-4300.
93. Hedges, J. B.; Vahidi, S.; Yue, X.; Konermann, L., Effects of ammonium bicarbonate on the electrospray mass spectra of proteins: evidence for bubble-induced unfolding. *Anal. Chem.* **2013**, 85 (13), 6469-6476.
94. Benesch, J. L. P.; Ruotolo, D. T.; Simmons, D. A.; Robinson, C. V., Protein Complexes in the Gas Phase: Technology for Structural Genomics and Proteomics. *Chem. Rev.* **2007**, 107, 3544-3567.
95. Karas, M.; Bahr, U.; Dülcks, T., Nano-electrospray ionization mass spectrometry: addressing analytical problems beyond routine. *Fresenius J. Anal. Chem.* **2000**, 366, 669-676.
96. Muneeruddin, K.; Nazzaro, M.; Kaltashov, I. A., Characterization of Intact Protein Conjugates and Biopharmaceuticals Using Ion-Exchange Chromatography with Online Detection by Native Electrospray Ionization Mass Spectrometry and Top-Down Tandem Mass Spectrometry. *Anal. Chem.* **2015**, 87 (19), 10138-10145.
97. Ehkirch, A.; Hernandez-Alba, O.; Colas, O.; Beck, A.; Guillarme, D.; Cianferani, S., Hyphenation of size exclusion chromatography to native ion mobility mass spectrometry for the analytical characterization of therapeutic antibodies and related products. *J. Chromatogr. B* **2018**, 1086, 176-183.
98. Chen, B.; Lin, Z.; Alpert, A. J.; Fu, C.; Zhang, Q.; Pritts, W. A.; Ge, Y., Online Hydrophobic Interaction Chromatography-Mass Spectrometry for the Analysis of Intact Monoclonal Antibodies. *Anal. Chem.* **2018**, 90 (12), 7135-7138.
99. Chen, B.; Peng, Y.; Valeja, S. G.; Xiu, L.; Alpert, A. J.; Ge, Y., Online Hydrophobic Interaction Chromatography-Mass Spectrometry for Top-Down Proteomics. *Anal. Chem.* **2016**, 88 (3), 1885-1891.
100. Ehkirch, A.; D'Atri, V.; Rouviere, F.; Hernandez-Alba, O.; Goyon, A.; Colas, O.; Sarrut, M.; Beck, A.; Guillarme, D.; Heinisch, S.; Cianferani, S., An Online Four-Dimensional HICxSEC-IMxMS Methodology for Proof-of-Concept Characterization of Antibody Drug Conjugates. *Anal. Chem.* **2018**, 90 (3), 1578-1586.
101. van Schaick, G.; Gstöttner, C.; Büttner, A.; Reusch, D.; Wuhrer, M.; Domínguez-Vega, E., Anion Exchange Chromatography – Mass Spectrometry for Monitoring Multiple Quality Attributes of Erythropoietin Biopharmaceuticals. *Anal. Chim. Acta* **2021**, 1143, 166-172.
102. Yan, Y.; Liu, A. P.; Wang, S.; Daly, T. J.; Li, N., Ultrasensitive Characterization of Charge Heterogeneity of Therapeutic Monoclonal Antibodies Using Strong Cation Exchange Chromatography Coupled to Native Mass Spectrometry. *Anal. Chem.* **2018**, 90 (21), 13013-13020.
103. Nguyen, G. T. H.; Tran, T. N.; Podgorski, M. N.; Bell, S. G.; Supuran, C. T.; Donald, W. A., Nanoscale Ion Emitters in Native Mass Spectrometry for Measuring Ligand-Protein Binding Affinities. *ACS Cent. Sci.* **2019**, 5 (2), 308-318.
104. Bertoletti, L.; Schappeler, J.; Colombo, R.; Rudaz, S.; Haselberg, R.; Domínguez-Vega, E.; Raimondi, S.; Somsen, G. W.; De Lorenzi, E., Evaluation of capillary electrophoresis-mass spectrometry for the analysis of the conformational heterogeneity of intact proteins using beta2-microglobulin as model compound. *Anal. Chim. Acta* **2016**, 945, 102-109.
105. Gahoual, R.; Busnel, J. M.; Wolff, P.; Francois, Y. N.; Leize-Wagner, E., Novel sheathless CE-MS interface as an original and powerful infusion platform for nanoESI study: from intact proteins to high molecular mass noncovalent complexes. *Anal. Bioanal. Chem.* **2014**, 406 (4), 1029-1038.
106. Nguyen, A.; Moini, M., Analysis of major protein-protein and protein-metal complexes of erythrocytes directly from cell lysate utilizing capillary electrophoresis mass spectrometry. *Anal. Chem.* **2008**, 80, 7169-7173.
107. Shen, X.; Liang, Z.; Xu, T.; Yang, Z.; Wang, Q.; Chen, D.; Pham, L.; Du, W.; Sun, L.,

- Investigating native capillary zone electrophoresis-mass spectrometry on a high-end quadrupole-time-of-flight mass spectrometer for the characterization of monoclonal antibodies. *Int. J. Mass Spectrom.* **2021**, *462*, 116541.
108. Farsang, E.; Murisier, A.; Horvath, K.; Beck, A.; Kormany, R.; Guillarme, D.; Fekete, S., Tuning selectivity in cation-exchange chromatography applied for monoclonal antibody separations, part 1: Alternative mobile phases and fine tuning of the separation. *J. Pharm. Biomed. Anal.* **2019**, *168*, 138-147.
109. Hopper, J. T.; Sokratous, K.; Oldham, N. J., Charge state and adduct reduction in electrospray ionization-mass spectrometry using solvent vapor exposure. *Anal. Biochem.* **2012**, *421* (2), 788-790.
110. MehaFFEY, M. R.; Xia, Q.; Brodbelt, J. S., Uniting Native Capillary Electrophoresis and Multistage Ultraviolet Photodissociation Mass Spectrometry for Online Separation and Characterization of *Escherichia coli* Ribosomal Proteins and Protein Complexes. *Anal. Chem.* **2020**, *92* (22), 15202-15211.
111. Ehkirch, A.; Goyon, A.; Hernandez-Alba, O.; Rouviere, F.; D'Atri, V.; Dreyfus, C.; Haeuw, J. F.; Diemer, H.; Beck, A.; Heinisch, S.; Guillarme, D.; Cianferani, S., A Novel Online Four-Dimensional SECxSEC-IMxMS Methodology for Characterization of Monoclonal Antibody Size Variants. *Anal. Chem.* **2018**, *90* (23), 13929-13937.
112. Gilroy, J. J.; Eakin, C. M., Characterization of drug load variants in a thiol linked antibody-drug conjugate using multidimensional chromatography. *J. Chromatogr. B* **2017**, *1060*, 182-189.
113. Zhou, Z.; Zhang, J.; Xing, J.; Bai, Y.; Liao, Y.; Liu, H., Membrane-based continuous remover of trifluoroacetic acid in mobile phase for LC-ESI-MS analysis of small molecules and proteins. *J. Am. Soc. Mass Spectrom.* **2012**, *23* (7), 1289-1292.
114. Wouters, S.; Eeltink, S.; Haselberg, R.; Somsen, G. W.; Gargano, A. F. G., Microfluidic ion stripper for removal of trifluoroacetic acid from mobile phases used in HILIC-MS of intact proteins. *Anal. Bioanal. Chem.* **2021**, *413* (17), 4379-4386.
115. Füssl, F.; Cook, K.; Scheffler, K.; Farrell, A.; Mittermayr, S.; Bones, J., Charge Variant Analysis of Monoclonal Antibodies Using Direct Coupled pH Gradient Cation Exchange Chromatography to High-Resolution Native Mass Spectrometry. *Anal. Chem.* **2018**, *90* (7), 4669-4676.
116. Zhang, L.; Vasicek, L. A.; Hsieh, S.; P., B. K.; J., H., Top-down LC-MS quantitation of intact denatured and native monoclonal antibodies in biological samples. *Bioanalysis* **2018**, *10* (13), 1039-1054.
117. Kaddis, C. S.; Loo, J. A., Native protein MS an ion mobility: Large flying proteins with ESI. *Anal. Chem.* **2007**, *79* (5), 1778-1784.
118. Lossl, P.; Snijder, J.; Heck, A. J., Boundaries of mass resolution in native mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2014**, *25* (6), 906-917.
119. Tamara, S.; den Boer, M. A.; Heck, A. J. R., High-Resolution Native Mass Spectrometry. *Chem. Rev.* **2021**, *122* (8), 7269-7326.
120. Fort, K. L.; van de Waterbeemd, M.; Boll, D.; Reinhardt-Szyba, M.; Belov, M. E.; Sasaki, E.; Zschoche, R.; Hilvert, D.; Makarov, A. A.; Heck, A. J. R., Expanding the structural analysis capabilities on an Orbitrap-based mass spectrometer for large macromolecular complexes. *Analyst* **2017**, *143* (1), 100-105.
121. Campuzano, I. D. G.; Nshanian, M.; Spahr, C.; Lantz, C.; Netirojanakul, C.; Li, H.; Wongkongkathep, P.; Wolff, J. J.; Loo, J. A., High Mass Analysis with a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer: From Inorganic Salt Clusters to Antibody Conjugates and Beyond. *J. Am. Soc. Mass Spectrom.* **2020**, *31* (5), 1155-1162.
122. Mallis, C. S.; Zheng, X.; Qiu, X.; McCabe, J. W.; Shirzadeh, M.; Lyu, J.; Laganowsky, A.; Russell, D. H., Development of Native MS Capabilities on an Extended Mass Range Q-TOF MS. *Int. J. Mass Spectrom.* **2020**, *458*, 116451.
123. Terral, G.; Beck, A.; Cianferani, S., Insights from native mass spectrometry and ion mobility-mass spectrometry for antibody and antibody-based product characterization. *J. Chromatogr. B* **2016**, *1032*, 79-90.
124. Leney, A. C.; Heck, A. J., Native Mass Spectrometry: What is in the Name? *J. Am. Soc. Mass Spectrom.* **2017**, *28* (1), 5-13.
125. Rose, R. J.; Damoc, E.; Denisov, E.; Makarov, A.; Heck, A. J. R., High-sensitivity Orbitrap mass analysis of intact macromolecular assemblies. *Nat. Methods* **2012**, *9* (11), 1084-1086.

126. Barth, M.; Schmidt, C., Native mass spectrometry-A valuable tool in structural biology. *J. Mass Spectrom.* **2020**, 55 (10), e4578.
127. Zhou, M.; Lantz, C.; Brown, K. A.; Ge, Y.; Pasa-Tolic, L.; Loo, J. A.; Lermyte, F., Higher-order structural characterisation of native proteins and complexes by top-down mass spectrometry. *Chem. Sci.* **2020**, 11 (48), 12918-12936.
128. Belov, M. E.; Damoc, E.; Denisov, E.; Compton, P. D.; Horning, S.; Makarov, A. A.; Kelleher, N. L., From protein complexes to subunit backbone fragments: a multi-stage approach to native mass spectrometry. *Anal. Chem.* **2013**, 85 (23), 11163-11173.
129. Konijnenberg, A.; Bannwarth, L.; Yilmaz, D.; Kocer, A.; Venien-Bryan, C.; Sobott, F., Top-down mass spectrometry of intact membrane protein complexes reveals oligomeric state and sequence information in a single experiment. *Protein Sci.* **2015**, 24 (8), 1292-1300.
130. Macias, L. A.; Santos, I. C.; Brodbelt, J. S., Ion Activation Methods for Peptides and Proteins. *Anal. Chem.* **2020**, 92 (1), 227-251.
131. Brodbelt, J. S.; Morrison, L. J.; Santos, I., Ultraviolet Photodissociation Mass Spectrometry for Analysis of Biological Molecules. *Chem. Rev.* **2020**, 120 (7), 3328-3380.
132. Ruotolo, B. T.; Giles, K.; Campuzano, I.; Sandercock, A. M.; Bateman, R. H.; Robinson, C. V., Evidence for Macromolecular Protein Rings in the Absence of Bulk Water. *Science* **2005**, 310, 1658-1661.
133. Deslignière, E.; Ley, M.; Bourguet, M.; Ehkirch, A.; Botzanowski, T.; Erb, S.; Hernandez-Alba, O.; Cianferani, S., Pushing the limits of native MS: Online SEC-native MS for structural biology applications. *Int. J. Mass Spectrom.* **2021**, 461, 116502.
134. Deslignière, E.; Ehkirch, A.; Botzanowski, T.; Beck, A.; Hernandez-Alba, O.; Cianferani, S., Toward Automation of Collision-Induced Unfolding Experiments through Online Size Exclusion Chromatography Coupled to Native Mass Spectrometry. *Anal. Chem.* **2020**, 92 (19), 12900-12908.
135. Deslignière, E.; Ehkirch, A.; Duivelshof, B. L.; Toftevall, H.; Sjogren, J.; Guillarme, D.; D'Atri, V.; Beck, A.; Hernandez-Alba, O.; Cianferani, S., State-of-the-Art Native Mass Spectrometry and Ion Mobility Methods to Monitor Homogeneous Site-Specific Antibody-Drug Conjugates Synthesis. *Pharmaceutics* **2021**, 14 (6), 498.
136. D'Atri, V.; Causon, T.; Hernandez-Alba, O.; Mutabazi, A.; Veuthey, J. L.; Cianferani, S.; Guillarme, D., Adding a new separation dimension to MS and LC-MS: What is the utility of ion mobility spectrometry? *J. Sep. Sci.* **2018**, 41 (1), 20-67.
137. Bobaly, B.; Fleury-Souverain, S.; Beck, A.; Veuthey, J. L.; Guillarme, D.; Fekete, S., Current possibilities of liquid chromatography for the characterization of antibody-drug conjugates. *J. Pharm. Biomed. Anal.* **2018**, 147, 493-505.
138. Munneeruddin, K.; Thomas, J. J.; Salinas, P. A.; Kaltashov, I. A., Characterization of small protein aggregates and oligomers using size exclusion chromatography with online detection by native electrospray ionization mass spectrometry. *Anal. Chem.* **2014**, 86 (21), 10692-10699.
139. Astefanei, A.; Dapic, I.; Camenzuli, M., Different Stationary Phase Selectivities and Morphologies for Intact Protein Separations. *Chromatographia* **2017**, 80 (5), 665-687.
140. Goyon, A.; Beck, A.; Colas, O.; Sandra, K.; Guillarme, D.; Fekete, S., Evaluation of size exclusion chromatography columns packed with sub-3μm particles for the analysis of biopharmaceutical proteins. *J. Chromatogr. A* **2017**, 1498, 80-89.
141. Goyon, A.; Fekete, S.; Beck, A.; Veuthey, J. L.; Guillarme, D., Unraveling the mysteries of modern size exclusion chromatography - the way to achieve confident characterization of therapeutic proteins. *J. Chromatogr. B* **2018**, 1092, 368-378.
142. Goyon, A.; D'Atri, V.; Colas, O.; Fekete, S.; Beck, A.; Guillarme, D., Characterization of 30 therapeutic antibodies and related products by size exclusion chromatography: Feasibility assessment for future mass spectrometry hyphenation. *J. Chromatogr. B* **2017**, 1065-1066, 35-43.
143. Fekete, S.; Beck, A.; Veuthey, J. L.; Guillarme, D., Theory and practice of size exclusion chromatography for the analysis of protein aggregates. *J. Pharm. Biomed. Anal.* **2014**, 101, 161-173.
144. Lakayan, D.; Haselberg, R.; Gahoual, R.; Somsen, G. W.; Kool, J., Affinity profiling of monoclonal antibody and antibody-drug-conjugate preparations by coupled liquid chromatography-surface plasmon resonance biosensing. *Anal. Bioanal. Chem.* **2018**, 410 (30), 7837-7848.

145. Yan, Y.; Xing, T.; Wang, S.; Daly, T. J.; Li, N., Coupling Mixed-Mode Size Exclusion Chromatography with Native Mass Spectrometry for Sensitive Detection and Quantitation of Homodimer Impurities in Bispecific IgG. *Anal. Chem.* **2019**, 91 (17), 11417-11424.
146. Valliere-Douglass, J. F.; McFee, W. A.; Salas-Solano, O., Native intact mass determination of antibodies conjugated with monomethyl Auristatin E and F at interchain cysteine residues. *Anal. Chem.* **2012**, 84 (6), 2843-2849.
147. VanAernum, Z. L.; Busch, F.; Jones, B. J.; Jia, M.; Chen, Z.; Boyken, S. E.; Sahasrabuddhe, A.; Baker, D.; Wysocki, V. H., Rapid online buffer exchange for screening of proteins, protein complexes and cell lysates by native mass spectrometry. *Nat. Protoc.* **2020**, 15 (3), 1132-1157.
148. Cavanagh, J.; Benson, L. M.; Thompson, R.; Naylor, S., In-Line Desalting Mass Spectrometry for the Study of Noncovalent Biological Complexes. *Anal. Chem.* **2003**, 75, 3281-3286.
149. Fekete, S.; Ganzler, K.; Guillarme, D., Critical evaluation of fast size exclusion chromatographic separations of protein aggregates, applying sub-2 μm particles. *J. Pharm. Biomed. Anal.* **2013**, 78-79, 141-149.
150. Botzanowski, T.; Erb, S.; Hernandez-Alba, O.; Ehkirch, A.; Colas, O.; Wagner-Rousset, E.; Rabuka, D.; Beck, A.; Drake, P. M.; Cianferani, S., Insights from native mass spectrometry approaches for top- and middle- level characterization of site-specific antibody-drug conjugates. *mAbs* **2017**, 9 (5), 801-811.
151. van der Rest, G.; Halgand, F., Size Exclusion Chromatography-Ion Mobility-Mass Spectrometry Coupling: a Step Toward Structural Biology. *J. Am. Soc. Mass Spectrom.* **2017**, 28 (11), 2519-2522.
152. Haberger, M.; Bomans, K.; Diepold, K.; Hook, M.; Gassner, J.; Schlothauer, T.; Zwick, A.; Spick, C.; Kepert, J. F.; Hienz, B.; Wiedmann, M.; Beck, H.; Metzger, P.; Molhoj, M.; Knoblich, C.; Grauschoopf, U.; Reusch, D.; Bulau, P., Assessment of chemical modifications of sites in the CDRs of recombinant antibodies: Susceptibility vs. functionality of critical quality attributes. *mAbs* **2014**, 6 (2), 327-339.
153. Leblanc, Y.; Ramon, C.; Bihoreau, N.; Chevreux, G., Charge variants characterization of a monoclonal antibody by ion exchange chromatography coupled on-line to native mass spectrometry: Case study after a long-term storage at +5 degrees C. *J. Chromatogr. B* **2017**, 1048, 130-139.
154. Bertoletti, L.; Regazzoni, L.; Aldini, G.; Colombo, R.; Abballe, F.; Caccialanza, G.; De Lorenzi, E., Separation and characterisation of beta2-microglobulin folding conformers by ion-exchange liquid chromatography and ion-exchange liquid chromatography-mass spectrometry. *Anal. Chim. Acta* **2013**, 771, 108-114.
155. Leblanc, Y.; Bihoreau, N.; Chevreux, G., Characterization of Human Serum Albumin isoforms by ion exchange chromatography coupled on-line to native mass spectrometry. *J. Chromatogr. B* **2018**, 1095, 87-93.
156. Munneeruddin, K.; Bobst, C. E.; Frenkel, R.; Houde, D.; Turyan, I.; Sosic, Z.; Kaltashov, I. A., Characterization of a PEGylated protein therapeutic by ion exchange chromatography with on-line detection by native ESI MS and MS/MS. *Analyst* **2017**, 142 (2), 336-344.
157. Füssl, F.; Trappe, A.; Cook, K.; Scheffler, K.; Fitzgerald, O.; Bones, J., Comprehensive characterisation of the heterogeneity of adalimumab via charge variant analysis hyphenated on-line to native high resolution Orbitrap mass spectrometry. *mAbs* **2019**, 11 (1), 116-128.
158. Sankaran, P. K.; Kabadi, P. G.; Honnappa, C. G.; Subbarao, M.; Pai, H. V.; Adhikary, L.; Palanivelu, D. V., Identification and quantification of product-related quality attributes in bio-therapeutic monoclonal antibody via a simple, and robust cation-exchange HPLC method compatible with direct online detection of UV and native ESI-QTOF-MS analysis. *J. Chromatogr. B* **2018**, 1102-1103, 83-95.
159. Bailey, A. O.; Han, G.; Phung, W.; Gazis, P.; Sutton, J.; Josephs, J. L.; Sandoval, W., Charge variant native mass spectrometry benefits mass precision and dynamic range of monoclonal antibody intact mass analysis. *mAbs* **2018**, 10 (8), 1214-1225.
160. Füssl, F.; Trappe, A.; Carillo, S.; Jakes, C.; Bones, J., Comparative Elucidation of Cetuximab Heterogeneity on the Intact Protein Level by Cation Exchange Chromatography and Capillary Electrophoresis Coupled to Mass Spectrometry. *Anal. Chem.* **2020**, 92 (7), 5431-5438.

161. Haberger, M.; Heidenreich, A. K.; Hook, M.; Fichtl, J.; Lang, R.; Cymer, F.; Adibzadeh, M.; Kuhne, F.; Wegele, H.; Reusch, D.; Bonnington, L.; Bulau, P., Multiattribute Monitoring of Antibody Charge Variants by Cation-Exchange Chromatography Coupled to Native Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2021**, 32 (8), 2062-2071.
162. Füssl, F.; Criscuolo, A.; Cook, K.; Scheffler, K.; Bones, J., Cracking Proteoform Complexity of Ovalbumin with Anion-Exchange Chromatography-High-Resolution Mass Spectrometry under Native Conditions. *J. Proteome Res.* **2019**, 18 (10), 3689-3702.
163. Leblanc, Y.; Faid, V.; Lauber, M. A.; Wang, Q.; Bioreau, N.; Chevreux, G., A generic method for intact and subunit level characterization of mAb charge variants by native mass spectrometry. *J. Chromatogr. B* **2019**, 1133, 121814.
164. Sharma, P.; Panchal, A.; Yadav, N.; Narang, J., Analytical techniques for the detection of glycated haemoglobin underlining the sensors. *Int. J. Biol. Macromol.* **2020**, 155, 685-696.
165. Yan, J.; Springsteen, G.; Deeter, S.; Wang, B., The relationship among pKa, pH, and binding constants in the interactions between boronic acids and diols—it is not as simple as it appears. *Tetrahedron* **2004**, 60 (49), 11205-11209.
166. Haverick, M.; Mengisen, S.; Shameem, M.; Ambrogelly, A., Separation of mAbs molecular variants by analytical hydrophobic interaction chromatography HPLC: overview and applications. *mAbs* **2014**, 6 (4), 852-858.
167. Lamanna, W. C.; Mayer, R. E.; Rupprechter, A.; Fuchs, M.; Higel, F.; Fritsch, C.; Vogelsang, C.; Seidl, A.; Toll, H.; Schiestl, M.; Holzmann, J., The structure-function relationship of disulfide bonds in etanercept. *Sci. Rep.* **2017**, 7 (1), 3951.
168. Fekete, S.; Veuthey, J. L.; Beck, A.; Guillarme, D., Hydrophobic interaction chromatography for the characterization of monoclonal antibodies and related products. *J. Pharm. Biomed. Anal.* **2016**, 130, 3-18.
169. Boyd, D.; Kaschak, T.; Yan, B., HIC resolution of an IgG1 with an oxidized Trp in a complementarity determining region. *J. Chromatogr. B* **2011**, 879 (13-14), 955-960.
170. Xiu, L.; Valeja, S. G.; Alpert, A. J.; Jin, S.; Ge, Y., Effective protein separation by coupling hydrophobic interaction and reverse phase chromatography for top-down proteomics. *Anal. Chem.* **2014**, 86 (15), 7899-7906.
171. Wei, B.; Han, G.; Tang, J.; Sandoval, W.; Zhang, Y. T., Native Hydrophobic Interaction Chromatography Hyphenated to Mass Spectrometry for Characterization of Monoclonal Antibody Minor Variants. *Anal. Chem.* **2019**, 91 (24), 15360-15364.
172. Haselberg, R.; de Jong, G. J.; Somsen, G. W., Capillary electrophoresis-mass spectrometry for the analysis of intact proteins. *J. Chromatogr. A* **2007**, 1159 (1-2), 81-109.
173. Le-Minh, V.; Tran, N. T.; Makky, A.; Rosilio, V.; Taverna, M.; Smadja, C., Capillary zone electrophoresis-native mass spectrometry for the quality control of intact therapeutic monoclonal antibodies. *J. Chromatogr. A* **2019**, 1601, 375-384.
174. Shen, X.; Yang, Z.; McCool, E. N.; Lubeckyj, R. A.; Chen, D.; Sun, L., Capillary zone electrophoresis-mass spectrometry for top-down proteomics. *Trends Analyt. Chem.* **2019**, 120, 115644.
175. Bonvin, G.; Schappeler, J.; Rudaz, S., Capillary electrophoresis-electrospray ionization-mass spectrometry interfaces: fundamental concepts and technical developments. *J. Chromatogr. A* **2012**, 1267, 17-31.
176. Shen, X.; Kou, Q.; Guo, R.; Yang, Z.; Chen, D.; Liu, X.; Hong, H.; Sun, L., Native Proteomics in Discovery Mode Using Size-Exclusion Chromatography-Capillary Zone Electrophoresis-Tandem Mass Spectrometry. *Anal. Chem.* **2018**, 90 (17), 10095-10099.
177. Stepanova, S.; Kasicka, V., Recent applications of capillary electromigration methods to separation and analysis of proteins. *Anal. Chim. Acta* **2016**, 933, 23-42.
178. Marie, A. L.; Przybylski, C.; Gonnet, F.; Daniel, R.; Urbain, R.; Chevreux, G.; Jorieux, S.; Taverna, M., Capillary zone electrophoresis and capillary electrophoresis-mass spectrometry for analyzing qualitative and quantitative variations in therapeutic albumin. *Anal. Chim. Acta* **2013**, 800, 103-110.
179. Shen, Y.; Zhao, X.; Wang, G.; Chen, D. D. Y., Differential Hydrogen/Deuterium Exchange during Proteoform Separation Enables Characterization of Conformational Differences between Coexisting Protein States. *Anal. Chem.* **2019**, 91 (6), 3805-3809.
180. Sun, L.; Zhu, G.; Zhao, Y.; Yan, X.; Mou, S.; Dovichi, N. J., Ultrasensitive and fast bottom-up analysis of femtogram amounts of complex proteome digests. *Angew. Chem. Int. Ed. Engl.* **2013**, 52 (51), 13661-13664.

181. Peuchen, E. H.; Zhu, G.; Sun, L.; Dovichi, N. J., Evaluation of a commercial electro-kinetically pumped sheath-flow nanospray interface coupled to an automated capillary zone electrophoresis system. *Anal. Bioanal. Chem.* **2017**, *409* (7), 1789-1795.
182. Haselberg, R.; Ratnayake, C. K.; de Jong, G. J.; Somsen, G. W., Performance of a sheathless porous tip sprayer for capillary electrophoresis-electrospray ionization-mass spectrometry of intact proteins. *J. Chromatogr. A* **2010**, *1217*, 7605-7611.
183. Jooss, K.; Schachner, L. F.; Watson, R.; Gillespie, Z. B.; Howard, S. A.; Cheek, M. A.; Meiners, M. J.; Sobh, A.; Licht, J. D.; Keogh, M. C.; Kelleher, N. L., Separation and Characterization of Endogenous Nucleosomes by Native Capillary Zone Electrophoresis-Top-Down Mass Spectrometry. *Anal. Chem.* **2021**, *93* (12), 5151-5160.
184. He, M.; Luo, P.; Hong, J.; Wang, X.; Wu, H.; Zhang, R.; Qu, F.; Xiang, Y.; Xu, W., Structural Analysis of Biomolecules through a Combination of Mobility Capillary Electrophoresis and Mass Spectrometry. *ACS Omega* **2019**, *4* (1), 2377-2386.
185. Lucy, C. A.; MacDonald, A. M.; Gulcev, M. D., Non-covalent capillary coatings for protein separations in capillary electrophoresis. *J. Chromatogr. A* **2008**, *1184*, 81-105.
186. Hajba, L.; Guttman, A., Recent advances in column coatings for capillary electrophoresis of proteins. *Trends Analyt. Chem.* **2017**, *90*, 38-44.
187. Haselberg, R.; Brinks, V.; Hawe, A.; de Jong, G. J.; Somsen, G. W., Capillary electrophoresis-mass spectrometry using noncovalently coated capillaries for the analysis of biopharmaceuticals. *Anal. Bioanal. Chem.* **2011**, *400* (1), 295-303.
188. Haselberg, R.; de Jong, G. J.; Somsen, G. W., Low-Flow Sheathless Capillary Electrophoresis-Mass Spectrometry for Sensitive Glycoform Profiling of Intact Pharmaceutical Proteins. *Anal. Chem.* **2013**, *85* (4), 2289-2296.
189. Grodzki, A. C.; Berenstein, E., Antibody Purification: Affinity Chromatography – Protein A and Protein G Sepharose. In *Immunocytochemical Methods and Protocols*, Oliver, C.; Jamur, M. C., Eds. Humana Press: Totowa, NJ, 2010; pp 33-41.
190. Dashivets, T.; Thomann, M.; Rueger, P.; Knaupp, A.; Buchner, J.; Schlothauer, T., Multi-Angle Effector Function Analysis of Human Monoclonal IgG Glycovariants. *PLoS One* **2015**, *10* (12), e0143520.
191. Schlothauer, T.; Rueger, P.; Stracke, J. O.; Hertenberger, H.; Fingas, F.; Kling, L.; Emrich, T.; Drabner, G.; Seeber, S.; Auer, J.; Koch, S.; Papadimitriou, A., Analytical FcRn affinity chromatography for functional characterization of monoclonal antibodies. *mAbs* **2014**, *5* (4), 576-586.
192. Lippold, S.; Nicolardi, S.; Dominguez-Vega, E.; Heidenreich, A. K.; Vidarsson, G.; Reusch, D.; Haberger, M.; Wuhrer, M.; Falck, D., Glycoform-resolved FcRIIIa affinity chromatography-mass spectrometry. *mAbs* **2019**, *11* (7), 1191-1196.
193. Thomann, M.; Schlothauer, T.; Dashivets, T.; Malik, S.; Avenal, C.; Bulau, P.; Ruger, P.; Reusch, D., In vitro glycoengineering of IgG1 and its effect on Fc receptor binding and ADCC activity. *PLoS One* **2015**, *10* (8), e0134949.
194. Gahoual, R.; Heidenreich, A. K.; Somsen, G. W.; Bulau, P.; Reusch, D.; Wuhrer, M.; Haberger, M., Detailed Characterization of Monoclonal Antibody Receptor Interaction Using Affinity Liquid Chromatography Hyphenated to Native Mass Spectrometry. *Anal. Chem.* **2017**, *89* (10), 5404-5412.
195. Roopenian, D. C.; Akilesh, S., FcRn: the neonatal Fc receptor comes of age. *Nat. Rev. Immunol.* **2007**, *7* (9), 715-725.
196. Stracke, J.; Emrich, T.; Rueger, P.; Schlothauer, T.; Kling, L.; Knaupp, A.; Hertenberger, H.; Wolfert, A.; Spick, C.; Lau, W.; Drabner, G.; Reiff, U.; Koll, H.; Papadimitriou, A., A novel approach to investigate the effect of methionine oxidation on pharmacokinetic properties of therapeutic antibodies. *mAbs* **2014**, *6* (5), 1229-1242.
197. Bao, J.; Krylova, S. M.; Cherney, L. T.; Le Blanc, J. C.; Pribil, P.; Johnson, P. E.; Wilson, D. J.; Krylov, S. N., Pre-equilibration kinetic size-exclusion chromatography with mass spectrometry detection (peKSEC-MS) for label-free solution-based kinetic analysis of protein-small molecule interactions. *Analyst* **2015**, *140* (4), 990-994.
198. Bao, J.; Krylova, S. M.; Cherney, L. T.; LeBlanc, J. C.; Pribil, P.; Johnson, P. E.; Wilson, D. J.; Krylov, S. N., Kinetic size-exclusion chromatography with mass spectrometry detection: an approach for solution-based label-free kinetic analysis of protein-small molecule interactions. *Anal. Chem.* **2014**, *86* (20), 10016-10020.
199. Ren, C.; Bailey, A. O.; VanderPorten, E.; Oh, A.; Phung, W.; Mulvihill, M. M.; Harris, S. F.; Liu, Y.; Han, G.; Sandoval, W., Quantitative Determination of Protein-Ligand Affinity

- by Size Exclusion Chromatography Directly Coupled to High-Resolution Native Mass Spectrometry. *Anal. Chem.* **2019**, 91 (1), 903-911.
200. Wang, B.; Lv, W.; Chang, M.; Zhao, C.; Shi, X.; Xu, G., Untargeted Defining Protein-Metabolites Interaction Based on Label-Free Kinetic Size Exclusion Chromatography-Mass Spectrometry. *Anal. Chem.* **2020**, 92 (11), 7657-7665.
201. Dubsky, P.; Dvorak, M.; Ansorge, M., Affinity capillary electrophoresis: the theory of electromigration. *Anal. Bioanal. Chem.* **2016**, 408 (30), 8623-8641.
202. Chen, Z.; Weber, S. G., Determination of binding constants by affinity capillary electrophoresis, electrospray ionization mass spectrometry and phase-distribution methods. *Trends Analyt. Chem.* **2008**, 27 (9), 738-748.
203. Vuignier, K.; Schappeler, J.; Veuthey, J. L.; Carrupt, P.; Martel, S., Improvement of a capillary electrophoresis/frontal analysis (CE/FA) method for determining binding constants: Discussion on relevant parameters. *J. Pharm. Biomed. Anal.* **2010**, 53, 1288-1297.
204. Dominguez-Vega, E.; Haselberg, R.; Somsen, G. W.; de Jong, G. J., Simultaneous assessment of protein heterogeneity and affinity by capillary electrophoresis-mass spectrometry. *Anal. Chem.* **2015**, 87 (17), 8781-8788.
205. Vuignier, K.; Veuthey, J. L.; Carrupt, P. A.; Schappeler, J., Characterization of drug-protein interactions by capillary electrophoresis hyphenated to mass spectrometry. *Electrophoresis* **2012**, 33 (22), 3306-3315.
206. Sladkov, V., Effect of non-thermostated capillary inlet in affinity capillary electrophoresis: uranyl-selenate system at variable temperatures. *J. Chromatogr. A* **2012**, 1263, 189-193.
207. Wang, X.; Dou, Z.; Yuan, Y.; Man, S.; Wolfs, K.; Adams, E.; Van Schepdael, A., On-line screening of matrix metalloproteinase inhibitors by capillary electrophoresis coupled to ESI mass spectrometry. *J. Chromatogr. B* **2013**, 930, 48-53.
208. Mironov, G. G.; Clouthier, C. M.; Akbar, A.; Keillor, J. W.; Berezovski, M. V., Simultaneous analysis of enzyme structure and activity by kinetic capillary electrophoresis-MS. *Nat. Chem. Biol.* **2016**, 12 (11), 918-922.
209. Fermas, S.; Gonnet, F.; Sutton, A.; Charnaux, N.; Mulloy, B.; Du, Y.; Baleux, F.; Daniel, R., Sulfated oligosaccharides (heparin and fucoidan) binding and dimerization of stromal cell-derived factor-1 (SDF-1/CXCL 12) are coupled as evidenced by affinity CE-MS analysis. *Glycobiology* **2008**, 18 (12), 1054-1064.
210. Langmajerová, M.; Řemínek, R.; Pelcová, M.; Foret, F.; Glatz, Z., Combination of on-line CE assay with MS detection for the study of drug metabolism by cytochromes P450. *Electrophoresis* **2015**, 36, 1365-1373.
211. Jiang, C.; Armstrong, D. W., Use of CE for the determination of binding constants. *Electrophoresis* **2010**, 31 (1), 17-27.
212. Wang, Y.; Adeoye, D. I.; Ogunkunle, E. O.; Wei, I. A.; Filla, R. T.; Roper, M. G., Affinity Capillary Electrophoresis: A Critical Review of the Literature from 2018 to 2020. *Anal. Chem.* **2020**, 93 (1), 295-310.
213. Fayad, S.; Nehmé, R.; Langmajerová, M.; Ayela, B.; Colas, C.; Maunit, B.; Jacquinet, J.; Vibert, A.; Lopin-Bon, C.; Zdeněk, G.; Morin, P., Hyaluronidase reaction kinetics evaluated by capillary electrophoresis with UV and high-resolution mass spectrometry (HRMS) detection. *Anal. Chim. Acta* **2017**, 951, 140-150.
214. Jones, J.; Pack, L.; Hunter, J. H.; Valliere-Douglass, J. F., Native size-exclusion chromatography-mass spectrometry: suitability for antibody-drug conjugate drug-to-antibody ratio quantitation across a range of chemotypes and drug-loading levels. *mAbs* **2020**, 12 (1), 1682895.
215. van Schaick, G.; Domínguez-Vega, E.; Gstöttner, C.; van den Berg-Verleg, J. H.; Schouten, O.; Akeroyd, M.; Olsthoorn, M. M. A.; Wuhrer, M.; Heck, A. J. R.; Abello, N.; Franc, V., Native structural and functional proteoform characterization of the prolyl-alanyl-specific endoprotease EndoPro from *Aspergillus niger*. *J. Proteome Res.* **2021**, 20 (10), 4875-4885.
216. Gomes, F. P.; Yates III, J. R., Recent trends of capillary electrophoresis-mass spectrometry in proteomics research. *Mass Spectrom. Rev.* **2019**, 38 (6), 445-460.
217. van Schaick, G.; Haselberg, R.; Somsen, G. W.; Wuhrer, M.; Domínguez-Vega, E., Studying protein structure and function by native separation-mass spectrometry. *Nat. Rev. Chem.* **2022**, 6, 215-231.
218. Madunic, K.; Wagt, S.; Zhang, T.; Wuhrer, M.; Lageveen-Kammeijer, G. S. M., Dop-

- ant-Enriched Nitrogen Gas for Enhanced Electrospray Ionization of Released Glycans in Negative Ion Mode. *Anal. Chem.* **2021**, 93 (18), 6919-6923.
219. Marie, A. L.; Ray, S.; Lu, S.; Jones, J.; Ghiran, I.; Ivanov, A. R., High-Sensitivity Glycan Profiling of Blood-Derived Immunoglobulin G, Plasma, and Extracellular Vesicle Isolates with Capillary Zone Electrophoresis-Mass Spectrometry. *Anal. Chem.* **2021**, 93 (4), 1991-2002.
220. Kammeijer, G. S.; Kohler, I.; Jansen, B. C.; Hensbergen, P. J.; Mayboroda, O. A.; Falk, D.; Wührer, M., Dopant Enriched Nitrogen Gas Combined with Sheathless Capillary Electrophoresis-Electrospray Ionization-Mass Spectrometry for Improved Sensitivity and Repeatability in Glycopeptide Analysis. *Anal. Chem.* **2016**, 88 (11), 5849-5856.
221. Alagesan, K.; Kolarich, D., To enrich or not to enrich: Enhancing (glyco)peptide ionization using the CaptiveSpray nanoBooster. *bioRxiv* **2019**.
222. Wang, S.; Xing, T.; Liu, A. P.; He, Z.; Yan, Y.; Daly, T. J.; Li, N., Simple Approach for Improved LC-MS Analysis of Protein Biopharmaceuticals via Modification of Desolvation Gas. *Anal. Chem.* **2019**, 91 (4), 3156-3162.
223. Chen, J.; Liu, Z.; Wang, F.; Mao, J.; Zhou, Y.; Liu, J.; Zou, H.; Zhang, Y., Enhancing the performance of LC-MS for intact protein analysis by counteracting the signal suppression effects of trifluoroacetic acid during electrospray. *Chem. Commun.* **2015**, 51 (79), 14758-14760.
224. Gargano, A. F. G.; Roca, L. S.; Fellers, R. T.; Bocxe, M.; Dominguez-Vega, E.; Somsen, G. W., Capillary HILIC-MS: A New Tool for Sensitive Top-Down Proteomics. *Anal. Chem.* **2018**, 90 (11), 6601-6609.
225. Lin, Y. H.; Franc, V.; Heck, A. J. R., Similar Albeit Not the Same: In-Depth Analysis of Proteoforms of Human Serum, Bovine Serum, and Recombinant Human Fetuin. *J. Proteome Res.* **2018**, 17 (8), 2861-2869.
226. Gstöttner, C.; Vergoossen, D. L. E.; Wührer, M.; Huijbers, M. G. M.; Dominguez-Vega, E., Sheathless CE-MS as a tool for monitoring exchange efficiency and stability of bispecific antibodies. *Electrophoresis* **2021**, 42 (1-2), 171-176.
227. Yang, Y.; Barendregt, A.; Kamerling, J. P.; Heck, A. J., Analyzing protein micro-heterogeneity in chicken ovalbumin by high-resolution native mass spectrometry exposes qualitatively and semi-quantitatively 59 proteoforms. *Anal. Chem.* **2013**, 85 (24), 12037-12045.
228. Prien, J. M.; Ashline, D. J.; Lapadula, A. J.; Zhang, H.; Reinhold, V. N., The high mannose glycans from bovine ribonuclease B isomer characterization by ion trap MS. *J. Am. Soc. Mass Spectrom.* **2009**, 20 (4), 539-556.
229. Gargano, A. F. G.; Schouten, O.; van Schaick, G.; Roca, L. S.; van den Berg-Verleg, J. H.; Haselberg, R.; Akeroyd, M.; Abello, N.; Somsen, G. W., Profiling of a high mannose-type N-glycosylated lipase using hydrophilic interaction chromatography-mass spectrometry. *Anal. Chim. Acta* **2020**, 1109, 69-77.
230. Wilm, M., Principles of electrospray ionization. *Mol. Cell Proteomics* **2011**, 10 (7), M111 009407.
231. Tatkare, D. *Erythropoietin Drugs Market by Product Types (Epoetin-alfa, Epoetin-beta, Darbepoetin-alfa) and Applications (Anemia, Kidney Disorders, Neural Disease and Wound healing) - Global Opportunity Analysis and Industry Forecast, 2013 - 2020; 2015.*
232. Lai, P.; Everett, R.; Wang, F.; Arakawa, T.; Goldwasser, E., Structural Characterization of Human Erythropoietin. *J. Biol. Chem.* **1986**, 261 (7), 3116-3121.
233. Buettner, A.; Maier, M.; Bonnington, L.; Bulau, P.; Reusch, D., Multi-Attribute Monitoring of Complex Erythropoietin Beta Glycosylation by GluC Liquid Chromatography-Mass Spectrometry Peptide Mapping. *Anal. Chem.* **2020**, 92 (11), 7574-7580.
234. Delorme, E.; Lorenzini, T.; Giffin, J.; Martin, F.; Jacobsen, F.; Boone, T.; Elliott, S., Role of glycosylation on the secretion and biological activity of erythropoietin. *Biochemistry* **1992**, 31 (41), 9871-9876.
235. Banks, D. D., The effect of glycosylation on the folding kinetics of erythropoietin. *J. Mol. Biol.* **2011**, 412 (3), 536-550.
236. Zhou, Q.; Qiu, H., The Mechanistic Impact of N-Glycosylation on Stability, Pharmacokinetics, and Immunogenicity of Therapeutic Proteins. *J. Pharm. Sciences* **2019**, 108 (4), 1366-1377.
237. Yamaguchi, K.; Akai, K.; Kawanishi, G.; Ueda, M.; Masuda, S.; Sasaki, R., Effects of Site-directed Removal of N-Glycosylation Sites in Human Erythropoietin on Its Product

- tion and Biological Properties. *J. Biol. Chem.* **1991**, *266* (30), 20434-20439.
238. Elliott, S.; Lorenzini, T.; Chang, D.; Barzilay, J.; Delorme, E., Mapping of the active site of recombinant human erythropoietin. *Blood* **1997**, *89* (2), 493-502.
239. Song, K. E.; Byeon, J.; Moon, D. B.; Kim, H. H.; Choi, Y. J.; Suh, J. K., Structural identification of modified amino acids on the interface between EPO and its receptor from EPO BRF, human recombinant erythropoietin by LC/MS analysis. *Molecules and cells* **2014**, *37* (11), 819-826.
240. European Pharmacopoeia 1316; 2002; pp 1123-1128.
241. Zimmermann, H.; Gerhard, D.; Hothorn, L. A.; Dingermann, T., An Alternative to Animal Testing in the Quality Control of Erythropoietin. *Pharmer. Bio. Sci. Notes* **2011**, *1*, 66-80.
242. Yang, Y.; Liu, F.; Franc, V.; Halim, L. A.; Schellekens, H.; Heck, A. J. R., Hybrid mass spectrometry approaches in glycoprotein analysis and their usage in scoring biosimilarity. *Nat. Commun.* **2016**, *7*, 13397.
243. Alley, W.; Tao, L.; Shion, H.; Yu, Y. Q.; Rao, C.; Chen, W., UPLC-MS assessment on the structural similarity of recombinant human erythropoietin (rhEPO) analogues from manufacturers in China for attribute monitoring. *Talanta* **2020**, *220*, 121335.
244. Byeon, J.; Lim, Y. R.; Kim, H. H.; Suh, J. K., Structural Identification of a Non-Glycosylated Variant at Ser126 for O-Glycosylation Site from EPO BRF, Human Recombinant Erythropoietin by LC/MS Analysis. *Molecules and cells* **2015**, *38* (6), 496-505.
245. Harazono, A.; Hashii, N.; Kuribayashi, R.; Nakazawa, S.; Kawasaki, N., Mass spectrometric glycoform profiling of the innovator and biosimilar erythropoietin and darbepoetin by LC/ESI-MS. *J. Pharm. Biomed. Anal.* **2013**, *83*, 65-74.
246. Fekete, S.; Beck, A.; Fekete, J.; Guillarme, D., Method development for the separation of monoclonal antibody charge variants in cation exchange chromatography, Part II: pH gradient approach. *J. Pharm. Biomed. Anal.* **2015**, *102*, 282-289.
247. Hernandez-Alba, O.; Wagner-Rousset, E.; Beck, A.; Cianferani, S., Native Mass Spectrometry, Ion Mobility, and Collision-Induced Unfolding for Conformational Characterization of IgG4 Monoclonal Antibodies. *Anal. Chem.* **2018**, *90* (15), 8865-8872.
248. Beyer, B.; Schuster, M.; Jungbauer, A.; Lingg, N., Microheterogeneity of Recombinant Antibodies: Analytics and Functional Impact. *Biotechnol. J.* **2018**, *13* (1), 1700476.
249. Liu, H.; Ponniah, G.; Zhang, H. M.; Nowak, C.; Neill, A.; Gonzalez-Lopez, N.; Patel, R.; Cheng, G.; Kita, A. Z.; Andrien, B., In vitro and in vivo modifications of recombinant and human IgG antibodies. *mAbs* **2014**, *6* (5), 1145-1154.
250. Murisier, A.; Duivelshof, B. L.; Fekete, S.; Bourquin, J.; Schmidlach, A.; Lauber, M. A.; Nguyen, J. M.; Beck, A.; Guillarme, D.; D'Atri, V., Towards a simple on-line coupling of ion exchange chromatography and native mass spectrometry for the detailed characterization of monoclonal antibodies. *J. Chromatogr. A* **2021**, *1655*, 462499.
251. Zhang, L.; Patapoff, T.; Farnan, D.; Zhang, B., Improving pH gradient cation-exchange chromatography of monoclonal antibodies by controlling ionic strength. *J. Chromatogr. A* **2013**, *1272*, 56-64.
252. Di Marco, F.; Berger, T.; Esser-Skala, W.; Rapp, E.; Regl, C.; Huber, C. G., Simultaneous Monitoring of Monoclonal Antibody Variants by Strong Cation-Exchange Chromatography Hyphenated to Mass Spectrometry to Assess Quality Attributes of Rituximab-Based Biotherapeutics. *Int. J. Mol. Sci.* **2021**, *22* (16), 9072.
253. Dixit, S. M.; Polasky, D. A.; Ruotolo, B. T., Collision induced unfolding of isolated proteins in the gas phase: past, present, and future. *Curr. Opin. Chem. Biol.* **2018**, *42*, 93-100.
254. Pringle, S. D.; Giles, K.; Wildgoose, J. L.; Williams, J. P.; Slade, S. E.; Thalassinos, K.; Bateman, R. H.; Bowers, M. T.; Scrivens, J. H., An investigation of the mobility separation of some peptide and protein ions using a new hybrid quadrupole/travelling wave IMS/oa-ToF instrument. *Int. J. Mass Spectrom.* **2007**, *261* (1), 1-12.
255. Botzanowski, T.; Hernandez-Alba, O.; Malissard, M.; Wagner-Rousset, E.; Desligniere, E.; Colas, O.; Haeuw, J. F.; Beck, A.; Cianferani, S., Middle Level IM-MS and CIU Experiments for Improved Therapeutic Immunoglobulin Subclass Fingerprinting. *Anal. Chem.* **2020**, *92* (13), 8827-8835.
256. Tian, Y.; Han, L.; Buckner, A. C.; Ruotolo, B. T., Collision Induced Unfolding of Intact Antibodies: Rapid Characterization of Disulfide Bonding Patterns, Glycosylation, and Structures. *Anal. Chem.* **2015**, *87* (22), 11509-11515.
257. Ferguson, C. N.; Gucinski-Ruth, A. C., Evaluation of Ion Mobility-Mass Spectrometry for Comparative Analysis of Monoclonal Antibodies. *J. Am. Soc. Mass Spectrom.* **2016**, *27*

- (5), 822-833.
258. Polasky, D. A.; Dixit, S. M.; Fantin, S. M.; Ruotolo, B. T., CIUSuite 2: Next-Generation Software for the Analysis of Gas-Phase Protein Unfolding Data. *Anal. Chem.* **2019**, 91 (4), 3147-3155.
259. Migas, L. G.; France, A. P.; Bellina, B.; Barran, P. E., ORIGAMI: A software suite for activated ion mobility mass spectrometry (alIM-MS) applied to multimeric protein assemblies. *Int. J. Mass Spectrom.* **2018**, 427, 20-28.
260. Bush, M. F.; Hall, Z.; Giles, K.; Hoyes, J.; Robinson, C. V.; Ruotolo, B. T., Collision Cross Sections of Proteins and Their Complexes: A Calibration Framework and Database for Gas-Phase Structural Biology. *Anal. Chem.* **2010**, 82, 9557-9565.
261. Harris, R. J.; Kabakoff, B.; Macchi, F. D.; Shen, F. J.; Kwong, M.; Andya, J. D.; Shire, S. J.; Bjork, N.; Totpal, K.; Chen, A. B., Identification of multiple sources of charge heterogeneity in a recombinant antibody. *J. Chromatogr. B* **2001**, 752, 233-245.
262. von Pawel-Rammingen, U.; Johansson, B. P.; Bjork, L., IdeS, a novel streptococcal cysteine proteinase with unique specificity for immunoglobulin G. *EMBO J.* **2002**, 21 (7), 1607-1615.
263. Wagner-Rousset, E.; Fekete, S.; Morel-Chevillet, L.; Colas, O.; Corvaia, N.; Cianferani, S.; Guillarme, D.; Beck, A., Development of a fast workflow to screen the charge variants of therapeutic antibodies. *J. Chromatogr. A* **2017**, 1498, 147-154.
264. Kim, J.; Kim, Y. J.; Cao, M.; De Mel, N.; Albarghouthi, M.; Miller, K.; Bee, J. S.; Wang, J.; Wang, X., Analytical characterization of coformulated antibodies as combination therapy. *mAbs* **2020**, 12 (1), 1738691.
265. Goyon, A.; Excoffier, M.; Janin-Bussat, M. C.; Bobaly, B.; Fekete, S.; Guillarme, D.; Beck, A., Determination of isoelectric points and relative charge variants of 23 therapeutic monoclonal antibodies. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2017**, 1065-1066, 119-128.
266. Vallejo, D. D.; Polasky, D. A.; Kurulugama, R. T.; Eschweiler, J. D.; Fjeldsted, J. C.; Ruotolo, B. T., A Modified Drift Tube Ion Mobility-Mass Spectrometer for Charge-Multiplexed Collision-Induced Unfolding. *Anal. Chem.* **2019**, 91 (13), 8137-8146.
267. Tian, Y.; Ruotolo, B. T., Collision induced unfolding detects subtle differences in intact antibody glycoforms and associated fragments. *Int. J. Mass Spectrom.* **2018**, 425, 1-9.
268. Zheng, K.; Bantog, C.; Bayer, R., The impact of glycosylation on monoclonal antibody conformation and stability. *mAbs* **2011**, 3 (6), 568-576.
269. Upton, R.; Migas, L. G.; Pacholatz, K. J.; Beniston, R. G.; Estdale, S.; Firth, D.; Barran, P. E., Hybrid mass spectrometry methods reveal lot-to-lot differences and delineate the effects of glycosylation on the tertiary structure of Herceptin(R). *Chem. Sci.* **2019**, 10 (9), 2811-2820.
270. Zheng, K.; Yarmarkovich, M.; Bantog, C.; Bayer, R.; Patapoff, T. W., Influence of glycosylation pattern on the molecular properties of monoclonal antibodies. *mAbs* **2014**, 6 (3), 649-658.
271. Houde, D.; Peng, Y.; Berkowitz, S. A.; Engen, J. R., Post-translational modifications differentially affect IgG1 conformation and receptor binding. *Mol. Cell Proteomics* **2010**, 9 (8), 1716-1728.
272. Wada, R.; Matsui, M.; Kawasaki, N., Influence of N-glycosylation on effector functions and thermal stability of glycoengineered IgG1 monoclonal antibody with homogeneous glycoforms. *mAbs* **2019**, 11 (2), 350-372.
273. Raveendran, S.; Parameswaran, B.; Ummalyma, S. B.; Abraham, A.; Mathew, A. K.; Madhavan, A.; Rebello, S.; Pandey, A., Applications of Microbial Enzymes in Food Industry. *Food Technol. Biotechnol.* **2018**, 56 (1), 16-30.
274. Demain, A. L.; Vaishnav, Production of recombinant proteins by microbes and higher organisms. *Biotechnol. Adv.* **2009**, 27, 297-306.
275. Yang, M.; Yu, X. W.; Zheng, H.; Sha, C.; Zhao, C.; Qian, M.; Xu, Y., Role of N-linked glycosylation in the secretion and enzymatic properties of Rhizopus chinensis lipase expressed in *Pichia pastoris*. *Microb. Cell Fact.* **2015**, 14, 40.
276. Čaval, T.; Heck, A. J. R.; Reiding, K. R., Meta-heterogeneity: evaluating and describing the diversity in glycosylation between sites on the same glycoprotein. *Mol. Cell. Proteomics* **2021**, 20, 100010.
277. Samodova, D.; Hostfield, C. M.; Cramer, C. N.; Giuli, M. V.; Cappellini, E.; Franciosa, G.; Rosenblatt, M. M.; Kelstrup, C. D.; Olsen, J. V., ProAlanase is an Effective Alternative to

- Trypsin for Proteomics Applications and Disulfide Bond Mapping. *Mol. Cell Proteomics* **2020**, *19* (12), 2139-2157.
278. Tsatsiani, L.; Akeroyd, M.; Olsthoorn, M.; Heck, A. J. R., Aspergillus nigerProlyl Endoprotease for Hydrogen–Deuterium Exchange Mass Spectrometry and Protein Structural Studies. *Anal. Chem.* **2017**, *89* (15), 7966-7973.
279. Šebela, M.; Řehulka, P.; Kábrt, J.; Řehulková, H.; Oždian, T.; Raus, M.; Franc, V.; Chmelík, J., Identification of N-glycosylation in prolyl endoprotease from Aspergillus niger and evaluation of the enzyme for its possible application in proteomics. *J. Mass Spectrom.* **2009**, *44* (11), 1587-1595.
280. van der Laarse, S. A. M.; van Gelder, C. A. G. H.; Bern, M.; Akeroyd, M.; Olsthoorn, M. M. A.; Heck, A. J. R., Targeting Proline in (Phospho)Proteomics. *FEBS J.* **2020**, *287* (14), 2979-2997.
281. Fekete, S.; Beck, A.; Fekete, J.; Guillarme, D., Method development for the separation of monoclonal antibody charge variants in cation exchange chromatography, Part I: salt gradient approach. *J. Pharm. Biomed. Anal.* **2015**, *102*, 33-44.
282. Loke, I.; Packer, N. H.; Thayesen-Andersen, M., Complementary LC-MS/MS-Based N-Glycan, N-Glycopeptide, and Intact N-Glycoprotein Profiling Reveals Unconventional Asn71-Glycosylation of Human Neutrophil Cathepsin G. *Biomolecules* **2015**, *5* (3), 1832-1854.
283. Yang, Y.; Franc, V.; Heck, A. J. R., Glycoproteomics: A Balance between High-Throughput and In-Depth Analysis. *Trends Biotechnol.* **2017**, *35* (7), 598-609.
284. Tjondro, H. C.; Ugonotti, J.; Kawahara, R.; Chatterjee, S.; Loke, I.; Chen, S.; Soltermann, F.; Hinneburg, H.; Parker, B. L.; Venkatakrishnan, V.; Dieckmann, R.; Grant, O. C.; Bylund, J.; Rodger, A.; Woods, R. J.; Karlsson-Bengtsson, A.; Struwe, W. B.; Thayesen-Andersen, M., Hyper-truncated Asn355- and Asn391-glycans modulate the activity of neutrophil granule myeloperoxidase. *J. Biol. Chem.* **2021**, *296*, 100144.
285. Selman, M. H.; Hemayatkar, M.; Deelder, A. M.; Wuhrer, M., Cotton HILIC SPE microtips for microscale purification and enrichment of glycans and glycopeptides. *Anal. Chem.* **2011**, *83* (7), 2492-2499.
286. Reiding, K. R.; Blank, D.; Kuijper, D. M.; Deelder, A. M.; Wuhrer, M., High-throughput profiling of protein N-glycosylation by MALDI-TOF-MS employing linkage-specific sialic acid esterification. *Anal. Chem.* **2014**, *86* (12), 5784-5793.
287. Schneider, C. A.; Rasband, W. S.; Eliceiri, K. W., NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012**, *9* (7), 671-675.
288. MacLean, B.; Tomazela, D. M.; Shulman, N.; Chambers, M.; Finney, G. L.; Frewen, B.; Kern, R.; Tabb, D. L.; Liebler, D. C.; MacCoss, M. J., Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics* **2010**, *26* (7), 966-968.
289. Lin, Y. H.; Zhu, J.; Meijer, S.; Franc, V.; Heck, A. J. R., Glycoproteogenomics: A Frequent Gene Polymorphism Affects the Glycosylation Pattern of the Human Serum Fetuin/alpha-2-HS-Glycoprotein. *Mol. Cell Proteomics* **2019**, *18* (8), 1479-1490.
290. Doering, T. L.; Cummings, R. D.; Aebl, M., Fungi. In *Essentials of Glycobiology*, 3rd ed.; Varki A, C. R., Esko JD, et al., Ed. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 2017.
291. Edens, L.; Dekker, P.; Van Der Hoeven, R.; Deen, F.; De Roos, A.; Floris, R., Extracellular Prolyl Endoprotease from Aspergillus niger and Its Use in the Debittering of Protein Hydrolysates. *J. Agric. Food Chem.* **2005**, *53*, 7950-7957.
292. Reiding, K. R.; Bondt, A.; Franc, V.; Heck, A. J. R., The benefits of hybrid fragmentation methods for glycoproteomics. *Trends Analyt. Chem.* **2018**, *108*, 260-268.
293. Pazur, J. H., The determination of the anomeric configuration of glycosyl-phosphoryl linkages of immunogenic phosphoglycans. *Anal. Biochem.* **1985**, *145*, 385-392.
294. Stals, I.; Sandra, K.; Geysens, S.; Contreras, R.; Van Beeumen, J.; Claeysseens, M., Factors influencing glycosylation of Trichoderma reesei cellulases. I: Postsecretorial changes of the O- and N-glycosylation pattern of Cel7A. *Glycobiology* **2004**, *14* (8), 713-724.
295. Thieme, T. R.; Bailou, C. E., Nature of the Phosphodiester Linkage of the Phosphomannan from the Yeast Kloeckera brevis. *Biochemistry* **1971**, *10* (22), 4121-4129.
296. Maras, M.; De Bruyn, A.; Schraml, J.; Herdewijn, P.; Claeysseens, M.; Fiers, W.; Contreras, R., Structural characterization of N-linked oligosaccharides from cellobiohydrolase I secreted by filamentous fungus Trichoderma reesei RUTC 30. *Eur. J. Biochem.* **1997**, *245*,

- 617-625.
297. Gemmill, T. R.; Trimble, R. B., Overview of N- and O-linked oligosaccharide structures found in various yeast species. *Biochim. Biophys. Acta* **1999**, *1426*, 227-237.
298. Deshpande, N.; Wilkins, M. R.; Packer, N.; Nevalainen, H., Protein glycosylation pathways in filamentous fungi. *Glycobiology* **2008**, *18* (8), 626-637.
299. Kirk, O.; Borchert, T. V.; Fuglsang, C. C., Industrial enzyme applications. *Curr. Opin. Biotechnol.* **2002**, *13* (4), 345-351.
300. Viikari, L.; Kantelinen, A.; Sundquist, J.; Linko, M., Xylanases in bleaching: From an idea to the industry. *FEMS Microbiol. Rev.* **1994**, *13*, 335-350.
301. Motta, F. L.; Andrade, C. C. P.; Santana, M. H. A., A Review of Xylanase Production by the Fermentation of Xylan: Classification, Characterization and Applications. In *Sustainable Degradation of Lignocellulosic Biomass—Techniques, Applications and Commercialization*, Chandel, A.; Silva, S. S. D., Eds. IntechOpen: London, UK, 2013.
302. Juturu, V.; Wu, J. C., Microbial xylanases: engineering, production and industrial applications. *Biotechnol. Adv.* **2012**, *30* (6), 1219-1227.
303. Collins, T.; Gerday, C.; Feller, G., Xylanases, xylanase families and extremophilic xylanases. *FEMS Microbiol. Rev.* **2005**, *29* (1), 3-23.
304. Bhardwaj, N.; Kumar, B.; Verma, P., A detailed overview of xylanases: an emerging biomolecule for current and future prospective. *Bioresources and Bioprocessing* **2019**, *6* (1), 40.
305. Zhang, Q.; Ames, J. M.; Smith, R. D.; Baynes, J. W.; Metz, T. O., A perspective on the Maillard reaction and the analysis of protein glycation by mass spectrometry: probing the pathogenesis of chronic disease. *J. Proteome Res.* **2009**, *8* (2), 754-769.
306. Priego-Capote, F.; Ramirez-Boo, M.; Finamore, F.; Gluck, F.; Sanchez, J. C., Quantitative analysis of glycated proteins. *J. Proteome Res.* **2014**, *13* (2), 336-347.
307. Siegel, D., Applications of reversible covalent chemistry in analytical sample preparation. *Analyst* **2012**, *137* (23), 5457-5482.
308. Viski, K.; Gengeliczki, Z.; Lenkey, K.; Baranyane Ganzler, K., Parallel development of chromatographic and mass-spectrometric methods for quantitative analysis of glycation on an IgG1 monoclonal antibody. *J. Chromatogr. B* **2016**, *1032*, 198-204.
309. Lhota, G.; Sissolak, B.; Striedner, G.; Sommeregger, W.; Vorauer-Uhl, K., Quantification of glycated IgG in CHO supernatants: A practical approach. *Biotechnol. Prog.* **2021**, *37*, e3124.
310. Zhang, L.; Zhang, Q., Glycated Plasma Proteins as More Sensitive Markers for Glycemic Control in Type 1 Diabetes. *Proteomics Clin. Appl.* **2020**, *14* (2), e1900104.
311. Milkovska-Stamenova, S.; Hoffmann, R., Hexose-derived glycation sites in processed bovine milk. *J. Proteomics* **2016**, *134*, 102-111.
312. Nicolardi, S.; Kilgour, D. P. A.; van der Burgt, Y. E. M.; Wuhrer, M., Improved N- and C-Terminal Sequencing of Proteins by Combining Positive and Negative Ion MALDI In-Source Decay Mass Spectrometry. *Anal. Chem.* **2020**, *92* (18), 12429-12436.
313. van der Burgt, Y. E. M.; Kilgour, D. P. A.; Tsybin, Y. O.; Srzentic, K.; Fornelli, L.; Beck, A.; Wuhrer, M.; Nicolardi, S., Structural Analysis of Monoclonal Antibodies by Ultrahigh Resolution MALDI In-Source Decay FT-ICR Mass Spectrometry. *Anal. Chem.* **2019**, *91* (3), 2079-2085.
314. Teodorowicz, M.; Hendriks, W. H.; Wicher, H. J.; Savelkoul, H. F. J., Immunomodulation by Processed Animal Feed: The Role of Maillard Reaction Products and Advanced Glycation End-Products (AGEs). *Front. Immunol.* **2018**, *9*, 2088.
315. Bilova, T.; Lukasheva, E.; Brauch, D.; Greifenhagen, U.; Paudel, G.; Tarakhovskaya, E.; Frolova, N.; Mittasch, J.; Balcke, G. U.; Tissier, A.; Osmolovskaya, N.; Vogt, T.; Wessjohann, L. A.; Birkemeyer, C.; Milkowski, C.; Frolov, A., A Snapshot of the Plant Glycated Proteome: structural, functional, and mechanistic aspects. *J. Biol. Chem.* **2016**, *291* (14), 7621-7636.
316. Li, D.; Chen, Y.; Liu, Z., Boronate affinity materials for separation and molecular recognition: structure, properties and applications. *Chem. Soc. Rev.* **2015**, *44* (22), 8097-8123.
317. Frolov, A.; Bluher, M.; Hoffmann, R., Glycation sites of human plasma proteins are affected to different extents by hyperglycemic conditions in type 2 diabetes mellitus. *Anal. Bioanal. Chem.* **2014**, *406* (24), 5755-5763.
318. Zhang, L.; Liu, C. W.; Zhang, Q., Online 2D-LC-MS/MS Platform for Analysis of Glycated Proteome. *Anal. Chem.* **2018**, *90* (2), 1081-1086.

319. Quan, C.; Alcala, E.; Petkovska, I.; Matthews, D.; Canova-Davis, E.; Taticek, R.; Ma, S., A study in glycation of a therapeutic recombinant humanized monoclonal antibody: where it is, how it got there, and how it affects charge-based behavior. *Anal. Biochem.* **2008**, 373 (2), 179-191.
320. Duivelshof, B. L.; Fekete, S.; Guillarme, D.; D'Atri, V., A generic workflow for the characterization of therapeutic monoclonal antibodies-application to daratumumab. *Anal. Bioanal. Chem.* **2019**, 411 (19), 4615-4627.
321. Rouby, G.; Tran, N. T.; Leblanc, Y.; Taverna, M.; Bihoreau, N., Investigation of monoclonal antibody dimers in a final formulated drug by separation techniques coupled to native mass spectrometry. *mAbs* **2020**, 12 (1), e1781743.
322. Duivelshof, B. L.; Beck, A.; Guillarme, D.; D'Atri, V., Bispecific antibody characterization by a combination of intact and site-specific/chain-specific LC/MS techniques. *Talanta* **2022**, 236, 122836.
323. van Schaick, G.; el Hajjouti, N.; Nicolardi, S.; den Hartog, J.; Jansen, R.; van der Hoeven, R.; Bijleveld, W.; Abello, N.; Wuhrer, M.; Olsthoorn, M. M. A.; Domínguez-Vega, E., Native liquid chromatography and mass spectrometry to structurally and functionally characterize endo-xylanase proteoforms. *Int. J. Mol. Sci.* **2022**, 23, 1307.
324. Priego Capote, F.; Sanchez, J. C., Strategies for proteomic analysis of non-enzymatically glycated proteins. *Mass Spectrom. Rev.* **2009**, 28 (1), 135-146.
325. Cardoso, H. B.; Wierenga, P. A.; Gruppen, H.; Schols, H. A., Maillard induced glycation behaviour of individual milk proteins. *Food Chem.* **2018**, 252, 311-317.
326. Liu, J.; Ru, Q.; Ding, Y., Glycation a promising method for food protein modification: Physicochemical properties and structure, a review. *Food Res. Int.* **2002**, 49, 170-183.
327. Koval, D.; Kasicka, V.; Cottet, H., Analysis of glycated hemoglobin A1c by capillary electrophoresis and capillary isoelectric focusing. *Anal. Biochem.* **2011**, 413 (1), 8-15.
328. Hall, D. G., Structure, Properties, and Preparation of Boronic Acid Derivatives. In *Boronic Acids: Preparation and Applications in Organic Synthesis, Medicine and Materials*, Wiley-VCH Verlag GmbH & Co: 2011.
329. Li, Y.; Larsson, E. L.; Jungvid , H.; Galaev, I. Y.; Mattiasson, B., Shielding of protein-boronate interactions during boronate chromatography of neoglycoproteins. *J. Chromatogr. A* **2001**, 909, 137-145.
330. Espina-Benitez, M. B.; Randon, J.; Demesmay, C.; Dugas, V., Back to BAC: Insights into Boronate Affinity Chromatography Interaction Mechanisms. *Sep. Purif. Rev.* **2017**, 47 (3), 214-228.
331. Togashi, D. M.; Ryder, A. G.; O'Shaughnessy, D., Monitoring local unfolding of bovine serum albumin during denaturation using steady-state and time-resolved fluorescence spectroscopy. *J. Fluoresc.* **2010**, 20 (2), 441-452.
332. Tessier, F. J., The Maillard reaction in the human body. The main discoveries and factors that affect glycation. *Pathol. Biol.* **2010**, 58 (3), 214-219.
333. Chen, J.-H.; Lin, X.; Bu, C.; Zhang, X., Role of advanced glycation end products in mobility and considerations in possible dietary and nutritional intervention strategies. *Nutr. Metab.* **2018**, 15 (1), 72.
334. Bosch, L.; Alegria, A.; Farre, R.; Clemente, G., Fluorescence and color as markers for the Maillard reaction in milk-cereal based infant foods during storage. *Food Chem.* **2007**, 105 (3), 1135-1143.
335. Matiacevich, S. B.; Santagapita, P. R.; Buera, M. P., Fluorescence from the maillard reaction and its potential applications in food science. *Crit. Rev. Food Sci. Nutr.* **2005**, 45 (6), 483-495.
336. Murzin, A. G., Metamorphic Proteins. *Science* **2008**, 320 (5884), 1725-1726.
337. Skolnick, J.; Gao, M.; Zhou, H., How special is the biochemical function of native proteins? *F1000Res* **2016**, 5.
338. Alberts, B.; Johnson, A.; Lewis, J., Protein Function. In *Molecular Biology of the Cell*, 4th ed.; Garland Science: New York, 2002.
339. Berggard, T.; Linse, S.; James, P., Methods for the detection and analysis of protein-protein interactions. *Proteomics* **2007**, 7 (16), 2833-2842.
340. Tassi, M.; De Vos, J.; Chatterjee, S.; Sobott, F.; Bones, J.; Eeltink, S., Advances in native high-performance liquid chromatography and intact mass. *J. Sep. Sci.* **2018**, 41, 125-144.
341. Susa, A. C.; Xia, Z.; Williams, E. R., Native Mass Spectrometry from Common Buffers with

- Salts That Mimic the Extracellular Environment. *Angew. Chem. Int. Ed. Engl.* **2017**, *56* (27), 7912-7915.
342. Rolland, A. D.; Prell, J. S., Approaches to Heterogeneity in Native Mass Spectrometry. *Chem. Rev.* **2022**, *122* (8), 7909-7951.
343. Ben-Nissan, G.; Sharon, M., The application of ion-mobility mass spectrometry for structure/function investigation of protein complexes. *Curr. Opin. Chem. Biol.* **2018**, *42*, 25-33.
344. Frolov, A.; Hoffmann, R., Analysis of amadori peptides enriched by boronic acid affinity chromatography. *Ann. N. Y. Acad. Sci.* **2008**, *1126*, 253-256.
345. Brady, L. J.; Martinez, T.; Balland, A., Characterization of Nonenzymatic Glycation on a Monoclonal Antibody. *Anal. Chem.* **2007**, *79*, 9403-9413.
346. Fischer, S.; Hoernschemeyer, J.; Mahler, H. C., Glycation during storage and administration of monoclonal antibody formulations. *Eur. J. Pharm. Biopharm.* **2008**, *70* (1), 42-50.
347. Wouters, B.; Curran, S. A.; Abdulhussain, N.; Hankemeier, T.; Schoenmakers, P. J., Immobilized-enzyme reactors integrated into analytical platforms: Recent advances and challenges. *Trends Analyt. Chem.* **2021**, *144*.
348. Gstöttner, C.; Klemm, D.; Haberger, M.; Bathke, A.; Wegele, H.; Bell, C.; Kopf, R., Fast and Automated Characterization of Antibody Variants with 4D HPLC/MS. *Anal. Chem.* **2018**, *90* (3), 2119-2125.
349. Camperi, J.; Goyon, A.; Guillarme, D.; Zhang, K.; Stella, C., Multi-dimensional LC-MS: the next generation characterization of antibody-based therapeutics by unified online bottom-up, middle-up and intact approaches. *Analyst* **2021**, *146* (3), 747-769.
350. Liu, Z.; He, H., Synthesis and Applications of Boronate Affinity Materials: From Class Selectivity to Biomimetic Specificity, *Acc. Chem. Res.* **2017**, *50* (9), 2185-2193.
351. Chen, Y.; Huang, A.; Zhang, Y.; Bie, Z., Recent advances of boronate affinity materials in sample preparation. *Anal. Chim. Acta* **2019**, *1076*, 1-17.
352. Li, H.; He, H.; Liu, Z., Recent progress and application of boronate affinity materials in bioanalysis. *Trends Anal. Chem.* **2021**, *140*, 116271.
353. Brooks, W. L.; Sumerlin, B. S., Synthesis and Applications of Boronic Acid-Containing Polymers: From Materials to Medicine. *Chem. Rev.* **2016**, *116* (3), 1375-1397.
354. Tomsho, J. W.; Pal, A.; Hall, D. G.; Benkovic, S. J., Ring Structure and Aromatic Substituent Effects on the pK a of the Benzoxaborole Pharmacophore. *ACS Med Chem Lett* **2012**, *3* (1), 48-52.
355. Wang, D.; Qu, Y.; Wang, F.; Li, Q.; Cao, Q., One-pot synthesis of highly selective phenylboronic acid-functionalized organic polymers for the enrichment of cis-diol containing molecules. *J. Mater. Sci.* **2022**, *57* (11), 6182-6195.
356. Ren, L.; Liu, Z.; Liu, Y.; Dou, P.; Chen, H. Y., Ring-opening polymerization with synergistic co-monomers: access to a boronate-functionalized polymeric monolith for the specific capture of cis-diol-containing biomolecules under neutral conditions. *Angew. Chem. Int. Ed. Engl.* **2009**, *48* (36), 6704-6707.
357. Wu, C.; Liang, Y.; Zhao, Q.; Qu, Y.; Zhang, S.; Wu, Q.; Liang, Z.; Zhang, L.; Zhang, Y., Boronate affinity monolith with a gold nanoparticle-modified hydrophilic polymer as a matrix for the highly specific capture of glycoproteins. *Chemistry* **2014**, *20* (28), 8737-8743.
358. Kyte, J.; Doolittle, R.F., A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **1982**, *157*, 105-132.