

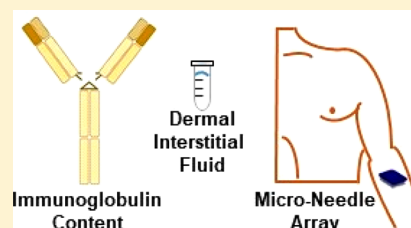
Proteomic Characterization of Immunoglobulin Content in Dermal Interstitial Fluid

Maria T. Arévalo,^{†,‡} Gabrielle M. Rizzo,[§] Ronen Polsky,^{||} Trevor Glaros,^{*,‡,§} and Phillip M. Mach^{*,‡,§}[†]Defense Threat Reduction Agency, Fort Belvoir, Virginia 22060, United States[‡]United States Army Combat Capabilities Development Command, Chemical Biological Center (CBC), Aberdeen Proving Ground, Maryland 21010, United States[§]Excet, Inc., 6225 Brandon Avenue, Suite 360, Springfield, Virginia 22150, United States^{||}Sandia National Laboratories, Albuquerque, New Mexico 87185, United States

S Supporting Information

ABSTRACT: Microneedles have been demonstrated to be a minimally invasive technique for sampling dermal interstitial fluid (ISF). Shotgun quantitative proteomics has already identified hundreds of proteins in ISF and quantitatively compared the proteome to matching serum and plasma. Interstitial fluid was determined to be a viable minimally invasive alternative to blood-derived fluids. In this communication, we re-examined the proteomic data from previous work to determine the diversity of immunoglobulins present compared with serum and plasma. Similar to our previous findings regarding the proteomic content across fluid types, ISF had a similar composition of IgG, IgA, IgD, and IgE antibodies as plasma or serum and lower quantities of IgM, which reflects the relative concentrations of dermal tissue T-cell and B-cell populations, indicating that the Ig's were likely locally derived. This work has significant implications for the utility of measuring Ig's in ISF for the clinical diagnosis of immunological diseases and skin infections. Data are available via ProteomeXchange with identifier PXD012658.

KEYWORDS: proteomics, interstitial fluid, wearable sensing, microneedles, immunoglobulin



INTRODUCTION

Blood and blood products are commonly used for the detection, diagnosis, and surveillance of disease. The detection and diagnosis of pathogens in the blood may involve the culture, isolation, or amplification of nucleic acids. However, the diagnosis of diseases, including those not caused by etiological agents, may also be accomplished by serological analyses. Serology, as used in disease diagnostics, involves the identification of antigen-specific immunoglobulins (Ig's) or antibodies. In a similar manner, serology can also be used to evaluate vaccine efficacy because the ability of the vaccine to elicit antibody responses is often used as a marker of immunogenicity.

Antibodies are composed of two light chains and two heavy chains that assemble into two antigen-binding regions and a single constant region¹ in a Y-shaped configuration.² There are two types of light chains (λ and κ) and five types of heavy chains (α , δ , ϵ , γ , and μ).³ The two heavy chains are held together via disulfide bonds, forming the trunk of the antibody, and one heavy chain is linked to one light chain by another disulfide bond, forming an arm of the antibody.³ Proteolytic cleavage of antibodies at the hinge regions yields two antigen-binding Fab fragments and the Fc constant fragment.¹ Whereas the Fab fragments are responsible for the specificity of antibodies to antigen, the Fc region mediates antibody effector functions via the interaction with Fc receptors (FcR) that are

expressed on the surface of leukocytes.^{4,5} On the basis of Fc regions, antibodies are divided into five main classes: IgA, IgD, IgE, IgG, and IgM,⁶ corresponding to heavy chains α , δ , ϵ , γ , and μ (Figure 1).³ IgG accounts for 75% of the Ig present in the serum, and in humans it is further subdivided into four subclasses, IgG1, IgG2, IgG3, and IgG4, with respective serum compositions of 67, 22, 7, and 4%.⁷ IgA accounts for 15% of serum antibodies and is also subdivided, but into two subclasses, IgA1 and IgA2.⁷ Finally, IgM is present as 10% of Ig's in the circulation, whereas IgD (<0.5%) and IgE (<0.01) are found at much lower levels.⁷

The expression and secretion of the different antibody classes in response to an infection or antigen is temporal and dependent on the nature of the antigen. IgM and IgD are coexpressed on antigen-naïve B cells as monomers.⁷ As an initial response to a primary exposure to antigen, naïve B cells secrete pentameric IgM. The IgM opsonizes antigen and initiates the activation of the complement system, resulting in the phagocytosis or destruction of the antigen.^{7,8} Because IgM secretion is associated with primary antibody responses to antigen, it is often used for the detection or diagnosis of acute infections or acute exposure to antigen. Later on during the adaptive immune response, the B cells can undergo class-

Received: March 6, 2019

Published: May 15, 2019

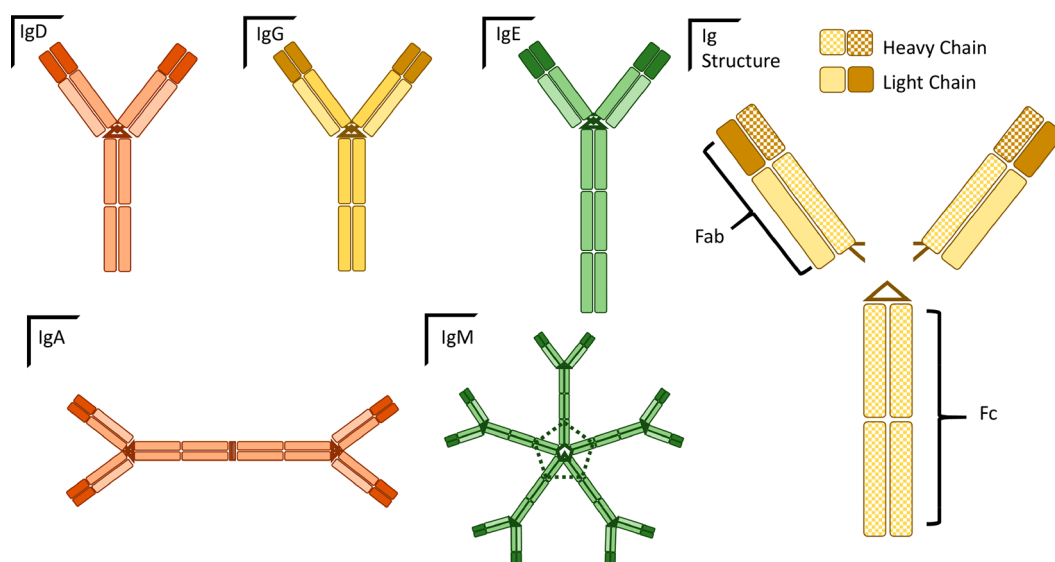


Figure 1. Five classes of immunoglobulins and generalized structure.

switching via T-dependent or T-independent cell mechanisms, resulting in the secretion of IgGs, IgA, and IgE.³ IgGs can recognize antigens and bind to Fc gamma receptors (FcγRs), which are differentially expressed by leukocytes. The IgG subclasses bind FcγRI-IV selectively and with varying affinities, and these interactions result in a broad range of effector functions that include antibody-dependent cytotoxicity, phagocytosis, and the modulation of immune factors such as proinflammatory cytokines.⁹ In addition, IgG can also bind C1q to initiate complement⁹ as well as neutralize viruses and toxins.⁷ IgG1 and IgG3 responses are triggered by soluble and membrane-bound proteins, whereas IgG2 is triggered by bacterial polysaccharide antigens and IgG4 is triggered by allergens.⁹ IgE also responds to allergens, binding to FcεRI on mast cells to trigger degranulation, as well as the synthesis of cytokines and chemokines.¹⁰ IgE is associated with allergic disorders, but it may also protect against parasitic infections.¹⁰ Whereas IgA is also present in the circulation, it is typically associated with mucosal immunity and is the predominant Ig found in milk, saliva, tears, and mucosal secretions.¹¹ Less is known about secreted IgD, but recent studies suggest a role in mucosal homeostasis.¹² In short, the different antibody classes and subclasses exist to fight exposure to a variety of antigens.

Knowing where, when, and why certain antibodies are secreted is useful in the detection and diagnosis of pathogens and disease. As mentioned before, blood is commonly used in a variety of methods to detect or diagnose infections and diseases. However, blood draws are invasive procedures that require trained personnel, may be painful and uncomfortable to the patient, and have little utility for real-time monitoring applications. Moreover, blood requires additional laboratory processing to separate into useful components like serum, plasma, or cells for analyses. An alternative to blood may be found in the interstitial fluid (ISF) that can be sampled using a minimally invasive and painless microneedle approach.^{13,14} Skin also possesses twice the concentration of T cells than are present in blood circulation at any one point¹⁵ as well as fixed memory T cells to control immune responses and tissue homeostasis.¹⁶ Efforts have used skin applied arrays to detect *P. falciparum* rPfHRP2 and total IgG.¹⁷ Furthermore, a wearable immunoassay patch allowed for selective IgG

sampling.¹⁸ Additional studies have been begun to evaluate pharmacokinetics and biomarkers in ISF.¹⁹ Previously, we used a shotgun quantitative proteomic approach to characterize the dermal ISF and found that it was similar in composition to plasma and serum.²⁰ In this study, we further analyzed ISF samples for immunoglobulin content and compared it to matching plasma and serum.

■ EXPERIMENTAL SECTION

The previously collected spectra²⁰ were reanalyzed against a FASTA database containing 35 unique sequences. This FASTA file (Supporting Information) was generated by downloading all sequences from UniProt that contained the keywords “immunoglobulin heavy constant” and “*Homo sapiens*”. The database used to search the data against was simplified to streamline data analysis. All data were processed using the SEQUEST HT algorithm²¹ using Proteome Discoverer 2.1 SP1 software (Thermo Fisher Scientific, Waltham, MA). Search parameters of the analysis included a precursor mass tolerance of 10 ppm, a fragment mass tolerance of 0.6 Da, a maximum of two missed cleavage sites, and a minimum peptide length of 6. Modifications included dynamic modifications of oxidized methionine [+15.99 Da] and acetylated N-protein terminus [+42.011 Da] and static modifications of carbamidomethylation of cysteine [+57.02 Da], N-terminal TMT 6plex [+229.163 Da], and TMT labeling of lysine [+229.16 Da]. Peptide spectrum matches (PSMs) were based on *q* value (FDR = 0.01) using the Percolator algorithm.²² Peptide identifications were also manually confirmed. Additional filters were set to obtain only identifications with high-confidence PSMs, high protein confidence (≤1% FDR), and a minimum of two peptides and one protein-unique peptide identified in a protein, with only unique/razor peptides considered for quantitation. MS2 spectra above a coisolation interference threshold of 50 and MS3 average reporter signal-to-noise (S/N) threshold of 10 were selected for reporter quantification. Reporter ion quantification was based on MS3 spectra at an integration tolerance of 20 ppm, with the peak integration method based on the most confident centroid.

Table 1. Identified Immunoglobulins from Interstitial Fluid and Their Quantitative Comparison to Plasma and Serum

immunoglobulin	description	accession	% coverage	no. PSMs	no. unique peptides	serum/ISF	plasma/ISF
IgA1	heavy constant alpha 1	P01876	63	369	8	1.3	1.3
IgA2	alpha-2 heavy chain	P0DOX2	57	230	8	1.0	1.0
IgA2	heavy constant alpha 2 (fragment)	A0A0G2JMB2	70	256	1	1.1	1.2
IgD	delta heavy chain	P0DOX3	40	32	3	1.0	0.9
IgE	epsilon heavy chain	P0DOX4	4	2	2	1.0	1.3
IgG1	gamma-1 heavy chain	P0DOX5	63	1026	15	0.8	0.9
IgG2	heavy constant gamma 2	P01859	60	828	7	0.8	0.8
IgG3	heavy constant gamma 3	P01860	66	696	10	0.9	0.9
IgG4	heavy constant gamma 4 (fragment)	A0A286YFJ8	43	593	5	1.0	1.0
IgM	heavy constant mu	P01871	72	357	9	1.5	1.5
IgM	mu heavy chain	P0DOX6	44	251	2	1.6	1.5

RESULTS AND DISCUSSION

Our analysis revealed that IgM, IgD, IgG1–4, IgA1–2, and IgE were all present in the ISF based on the identification of peptides that corresponded to their respective heavy-chain regions (Table 1). Both kappa and lambda light chains were also identified from our database search but are not associated with a particular Ig; as such, they were excluded from this analysis. Additionally, we identified two other sequences during our analysis, accession numbers A0A0A0MS09 and P01764. However, because these sequences were not associated with a particular Ig or were not quantitated across all TMT channels, they were removed from the data presented in Table 1. Furthermore, the relative quantification of IgG1–4 in the serum or plasma to the ISF yielded serum/ISF and plasma/ISF ratios of 0.8 to 1.0, suggesting that IgG content in the ISF is similar to IgG content in the blood. IgAs, IgE, and IgD were also present in similar levels within ISF with ratios ranging from 0.9 to 1.3. Interestingly, IgM was the only Ig that was found to vary from blood, with levels in ISF found to be 50–60% lower compared with plasma and serum. This is explained by the naturally lower levels of skin-derived B cells found in healthy subjects over that in normal systemic circulation.²³ However, circulating B cells will migrate from circulation to peripheral tissues upon infection as a secondary immune response that dramatically increases cytokine production as well as the levels of IgMs and T-cell-communicative Ig's. The size of the Ig's indicates that they are too large to normally diffuse across capillary membranes and are therefore likely locally derived from skin T-cell and B-cell populations, which makes the skin particularly appealing as a unique source for the clinical diagnosis of Ig's.

CONCLUSIONS

ISF may be a viable alternative to blood for use in the detection and diagnosis of pathogens and disease by leveraging the immunoglobulins present. Using proteomic analysis, we discovered that ISF had a similar composition of IgG, IgA, IgD, and IgE antibodies as plasma or sera. In contrast, IgM was less abundant in the ISF compared with plasma or sera, likely due to the lower concentration of B cells in healthy skin. The relatively large size of these molecules makes it highly improbable that they could passively diffuse from the vasculature into the ISF, as is the case with glucose;²⁴ therefore, they are likely locally derived from T-cell and B-cell populations found in the skin. However, increased vascular pressure from the device used²⁰ could cause endothelial walls to expand and allow macromolecules to diffuse that would otherwise be confined due to their size.²⁵ The size of secreted

Ig's ranges from ~150 kDa for monomeric antibodies such as those of the IgG class to ~1000 kDa for pentameric IgM.²⁶ It is possible that the Ig's are moving into the ISF by utilizing a transport receptor that has been described for transcytosis of polymeric Ig's into mucosal secretions. Dimeric IgA or pentameric IgM is bound by a polymeric Ig receptor that is expressed on the basolateral surface of mucosal epithelial cells; the complex is internalized and transported through vesicles.^{11,27} IgGs can also be transported by neonatal Fc receptor (FcRn), which is expressed on vascular endothelial cells and intestinal epithelial cells and coordinates bidirectional transfer between the circulation and tissues.²⁸ FcRn is also present in placental syncytiotrophoblasts, making it possible for the transport of maternal IgG to the fetus.²⁸

The current study analyzed three sets of ISF, serum, and plasma samples from healthy individuals. However, it would be more valuable for future analyses to study how the overall protein composition (including Ig content) changes in the ISF in response to infection, vaccination, or other exposure to antigen. The characterization of changes in the Ig composition based on the type of antigen (e.g., bacterial, viral, allergen, self) and route of exposure (e.g., airborne vs vector-borne) would help in the assessment of ISF as an alternative to blood for the detection of diseases.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jproteome.9b00155.

FASTA file utilized (PDF)

Related Articles

This Communication serves as a follow-up to Tran, B. Q.; Miller, P. R.; Taylor, R. M.; Boyd, G.; Mach, P. M.; Rosenzweig, C. N.; Baca, J. T.; Polsky, R.; Glaros, T. Proteomic Characterization of Dermal Interstitial Fluid Extracted Using a Novel Microneedle-Assisted Technique. *J. Proteome Res.* **2018**, *17*, 479–485. DOI: 10.1021/acs.jproteome.7b00642.²⁰

AUTHOR INFORMATION

Corresponding Authors

*T.G.: Trevor.G.Glaros.civ@mail.mil.

*P.M.M.: Phillip.M.Mach.civ@mail.mil.

ORCID

Trevor Glaros: 0000-0001-9922-6330

Phillip M. Mach: 0000-0003-0842-3844

Notes

The authors declare no competing financial interest. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the data set identifier PXD012658.^{29,30}

ACKNOWLEDGMENTS

Conclusions and opinions presented here are those of the authors and are not the official policy of the U.S. Army, CCDC – Chemical Biological Center, or the U.S. Government. Information in this report is cleared for public release and distribution is unlimited. We thank Dr. Justin T. Baca and Dr. Robert M. Taylor of the Department of Emergency Medicine, The University of New Mexico for their assistance in the collection of samples. This work was supported by a grants (CB10434 and 189920) from the Joint Science and Technology Office (JSTO) and the CBA Division of the Defense Threat Reduction Agency (DTRA). DTRA is a Combat Support Agency and a Defense Agency with a three-pronged mission: (1) to counter the threats posed by the full spectrum of weapons of mass destruction (WMD), including chemical, biological, radiological, nuclear, and high-yield explosives; (2) to counter the threats posed by the growing, evolving categories of improvised threats, including improvised explosive devices, car bombs, and weaponized consumer drones, as well as the tactics, technologies, and networks that put them on the battlefield; and (3) to ensure the U.S. military maintains a safe, secure, effective, and credible nuclear weapons deterrent.

REFERENCES

- (1) Rajpal, A.; Strop, P.; Yeung, Y. A.; Chaparro-Riggers, J.; Pons, J. Introduction: Antibody structure and function. *Therapeutic Fc-Fusion Proteins* **2013**, 1–44.
- (2) Kaur, H.; Salunke, D. M. Antibody promiscuity: Understanding the paradigm shift in antigen recognition. *IUBMB Life* **2015**, 67 (7), 498–505.
- (3) Janeway, C. A.; Travers, P.; Walport, M.; Shlomchik, M. J. *Immunobiology: The Immune System in Health and Disease*, 5th ed.; Garland Publishing: New York, 2001.
- (4) Bournazos, S.; Ravetch, J. V. Diversification of IgG effector functions. *Int. Immunol.* **2017**, 29 (7), 303–310.
- (5) Bournazos, S.; Ravetch, J. V. Fcγ receptor function and the design of vaccination strategies. *Immunity* **2017**, 47 (2), 224–233.
- (6) Tiller, K. E.; Tessier, P. M. Advances in antibody design. *Annu. Rev. Biomed. Eng.* **2015**, 17, 191–216.
- (7) Schroeder, H. W., Jr.; Cavacini, L. Structure and function of immunoglobulins. *J. Allergy Clin. Immunol.* **2010**, 125 (2), S41–S52.
- (8) Díaz-Zaragoza, M.; Hernández-Ávila, R.; Viedma-Rodríguez, R.; Arenas-Aranda, D.; Ostoa-Saloma, P. Natural and adaptive IgM antibodies in the recognition of tumor-associated antigens of breast cancer. *Oncol. Rep.* **2015**, 34 (3), 1106–1114.
- (9) Vidarsson, G.; Dekkers, G.; Rispen, T. IgG subclasses and allotypes: from structure to effector functions. *Front. Immunol.* **2014**, 5, 520.
- (10) Mukai, K.; Tsai, M.; Starkl, P.; Marichal, T.; Galli, S. J. IgE and mast cells in host defense against parasites and venoms. *Semin. Immunopathol.* **2016**, 38 (5), 581–603.
- (11) Woof, J.; Russell, M. Structure and function relationships in IgA. *Mucosal Immunol.* **2011**, 4 (6), 590.
- (12) Gutzeit, C.; Chen, K.; Cerutti, A. The enigmatic function of IgD: some answers at last. *Eur. J. Immunol.* **2018**, 48, 1101.
- (13) Miller, P. R.; Taylor, R. M.; Tran, B. Q.; Boyd, G.; Glaros, T.; Chavez, V. H.; Krishnakumar, R.; Sinha, A.; Poorey, K.; Williams, K. P.; et al. Extraction and biomolecular analysis of dermal interstitial fluid collected with hollow microneedles. *Commun. Biol.* **2018**, 1 (1), 173.
- (14) Miller, P. R.; Narayan, R. J.; Polsky, R. Microneedle-based sensors for medical diagnosis. *J. Mater. Chem. B* **2016**, 4 (8), 1379–1383.
- (15) Clark, R. A. Skin-Resident T Cells: The Ups and Downs of On Site Immunity. *J. Invest. Dermatol.* **2010**, 130 (2), 362–370.
- (16) Sanchez Rodriguez, R.; Pauli, M. L.; Neuhaus, I. M.; Yu, S. S.; Arron, S. T.; Harris, H. W.; Yang, S. H.-Y.; Anthony, B. A.; Sverdrup, F. M.; Krow-Lucal, E.; et al. Memory regulatory T cells reside in human skin. *J. Clin. Invest.* **2014**, 124 (3), 1027–1036.
- (17) Lee, K. T.; Muller, D. A.; Coffey, J. W.; Robinson, K. J.; McCarthy, J. S.; Kendall, M. A. F.; Corrie, S. R. Capture of the Circulating Plasmodium falciparum Biomarker HRP2 in a Multiplexed Format, via a Wearable Skin Patch. *Anal. Chem.* **2014**, 86 (20), 10474–10483.
- (18) Coffey, J. W.; Corrie, S. R.; Kendall, M. A. F. Rapid and selective sampling of IgG from skin in less than 1 min using a high surface area wearable immunoassay patch. *Biomaterials* **2018**, 170, 49–57.
- (19) Kolluru, C.; Williams, M.; Yeh, J. S.; Noel, R. K.; Knaack, J.; Prausnitz, M. R. Monitoring drug pharmacokinetics and immunologic biomarkers in dermal interstitial fluid using a microneedle patch. *Biomed. Microdevices* **2019**, 21 (1), 14.
- (20) Tran, B. Q.; Miller, P. R.; Taylor, R. M.; Boyd, G.; Mach, P. M.; Rosenzweig, C. N.; Baca, J. T.; Polsky, R.; Glaros, T. Proteomic Characterization of Dermal Interstitial Fluid Extracted Using a Novel Microneedle-Assisted Technique. *J. Proteome Res.* **2018**, 17 (1), 479–485.
- (21) Eng, J. K.; Fischer, B.; Grossmann, J.; MacCoss, M. J. A fast SEQUEST cross correlation algorithm. *J. Proteome Res.* **2008**, 7 (10), 4598–4602.
- (22) Käll, L.; Canterbury, J. D.; Weston, J.; Noble, W. S.; MacCoss, M. J. Semi-supervised learning for peptide identification from shotgun proteomics datasets. *Nat. Methods* **2007**, 4 (11), 923.
- (23) Egbuniwe, I. U.; Karagiannis, S. N.; Nestle, F. O.; Lacy, K. E. Revisiting the role of B cells in skin immune surveillance. *Trends Immunol.* **2015**, 36 (2), 102–111.
- (24) Cengiz, E.; Tamborlane, W. V. A tale of two compartments: interstitial versus blood glucose monitoring. *Diabetes Technol. Ther.* **2009**, 11 (S1), S-11–S-16.
- (25) Levick, J. R.; Michel, C. C. Microvascular fluid exchange and the revised Starling principle. *Cardiovasc. Res.* **2010**, 87 (2), 198–210.
- (26) Roberts-Thomson, P. J.; Shepherd, K. Molecular size heterogeneity of immunoglobulins in health and disease. *Clin. Exp. Immunol.* **1990**, 79 (3), 328–334.
- (27) Stadtmueller, B. M.; Huey-Tubman, K. E.; López, C. J.; Yang, Z.; Hubbell, W. L.; Bjorkman, P. J. The structure and dynamics of secretory component and its interactions with polymeric immunoglobulins. *eLife* **2016**, 5, No. e10640.
- (28) Rosales, C. Fcγ receptor heterogeneity in leukocyte functional responses. *Front. Immunol.* **2017**, 8, 280.
- (29) Vizcaino, J. A.; Csordas, A.; Del-Toro, N.; Dienes, J. A.; Griss, J.; Lavidas, I.; Mayer, G.; Perez-Riverol, Y.; Reisinger, F.; Ternent, T.; et al. 2016 update of the PRIDE database and its related tools. *Nucleic Acids Res.* **2016**, 44 (D1), D447–D456.
- (30) Vizcaino, J. A.; Deutsch, E. W.; Wang, R.; Csordas, A.; Reisinger, F.; Rios, D.; Dienes, J. A.; Sun, Z.; Farrah, T.; Bandeira, N.; et al. ProteomeXchange provides globally coordinated proteomics data submission and dissemination. *Nat. Biotechnol.* **2014**, 32 (3), 223.