BIOGRAPHICAL SKETCH

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NAME: Shi, Yi

eRA COMMONS USER NAME (credential, e.g., agency login): YISHI2013

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Dalian University of Technology, China	B.S	07/2003	Civil Engineering
Baylor College of Medicine	Ph.D.	05/2011	Proteomics
The Rockefeller University	Postdoctoral Associate	12/2016	Mass Spectrometry

A. **Personal Statement**

Our lab is interested in the development of cutting-edge mass spectrometry-based proteomics technologies for the analysis of biomolecules and macromolecular assemblies. Recently, fascinated by the exciting biomedical potentials of camelid single-chain VHH antibodies (so-called nanobodies), we have begun to develop methods and informatics to revolutionize the discovery and characterization of nanobodies. In parallel, we are harnessing these tools and the potent biomolecules that we discovered to enable biomedical applications, disease diagnosis, and treatment. In collaboration with structural biologists and modelers, we are also interested in understanding the detailed mechanisms underlying antigen-nanobody interactions.

1. Xiang, Y., Nambulli, S., Xiao, Z., Liu, H., Sang, Z., Duprex, W.P., Schneidman-Duhovny, D., Zhang, C., and Shi, Y. (2020). Versatile and multivalent nanobodies efficiently neutralize SARS-CoV-2. Science. PMID: 33154108.
2. Xiang, Y., Sang, Z., Bitton, L., Xu, J., Liu, Y., Schneidman-Duhovny, D.*, and Shi, Y.* (2020b). Integrative proteomics reveals exceptional diversity and versatility of mammalian humoral immunity. bioRxiv, 2020.2008.2021.261917. (revision)
3. Shen Z, Xiang Y, Vegara S, Chen A, Xiao Z, Santiago U, Jin C, Sang Z, Luo J, Chen K, Schneidman-Duhovny D, Camacho C, Calero G, Hu B, Shi Y (2020) A robust and versatile nanobody platform for drug delivery. bioRxiv: 2020.08.19.257725. (revision)
4. Kim, S.J.*, Fernandez-Martinez, J.*, Nudelman, I.*, Shi, Y.*, Zhang, W.*, Raveh, B., Herricks, T., Slaughter, B.D., Hogan, J., Paula, U., Chemmama, I., Pallerin, R., Echeverria, I., Shivaraju, M., Chaudhury, A.S., Wang, J.J., Williams, R., Unruh, J.R., Greenberg, C.H., Jacobs, E.Y., Yu, Z., De la Cruz, M.J., Mironska, R., Strokes, D.L., Aitchison, J.D., Jarrold, M.F., Gerton, J.L., Ludtke, S.J., Akey, C.W., Chait, B.T., Sali, A., and Rout, M.P. Structure and Functional Anatomy of the Nuclear Pore Complex (2018). *Nature* 555, 475-482. PMID: 29539637

B. Positions and Honors

Positions and Employment

2011 - 2016 Postdoc Associate, The Rockefeller University, NYC

2017 - current Assistant Professor, Department of Cell Biology, University of Pittsburgh, Pittsburgh, PA

C. Contributions to Science

1. Ultrapotent Nanobody Cocktails for SARS-CoV-2 Neutralization and Potential Treatment of COVID-19. Using *in vivo* affinity immunization (camelid) and advanced proteomics we have recently identified a large repertoire of aniti-SARS-CoV-2 neutralizing nanobodies (Nbs). We discovered elite Nbs of pico-to-femtomolar affinities that inhibit viral infection at sub-ng/ml concentration, more potent than some of the best human neutralizing antibodies. We determined a crystal structure of such an elite neutralizing Nb in complex with RBD. Structural proteomics and integrative modeling revealed multiple distinct and non-overlapping epitopes and indicated an array of potential neutralization mechanisms. Structural characterization facilitated the bioengineering of novel multivalent Nb constructs into multi-epitope cocktails that achieved ultrahigh neutralization potency (IC50s as low as 0.058 ng/ml) - the *most potent agents* for SARS-CoV-2 to date, and may prevent mutational escape. These thermostable Nbs can be rapidly produced in bulk from microbes and resist lyophilization, and aerosolization. We are positively evaluating the preclinical efficacy of the ultrapotent Nbs for rapid translation into an efficient, cost-effective, and convenient therapy.

a. Xiang, Y., Nambulli, S., Xiao, Z., Liu, H., Sang, Z., Duprex, W.P., Schneidman-Duhovny, D., Zhang, C., and Shi, Y. (2020). Versatile and multivalent nanobodies efficiently neutralize SARS-CoV-2. Science. PMID: 33154108.

2. Development of a robust proteomic platform for nanobody discovery and characterizations. We have recently overcome significant technical barriers to develop a transformative pipeline that integrates proteomics, informatics, and structural methods to enable global, quantitative and structural characterization of antigen-specific Nb proteomes. The robustness and sensitivity of our technologies have been rigorously evaluated with different antigens spanning three orders of magnitude in immune responses. Using this approach, thousands of divergent, high-quality Nbs could be confidently identified and classified based on their physicochemical properties. A significant fraction of our identifications had high-affinity and specificity comparable to therapeutic antibodies. A high-throughput structural approach based on computational docking and chemical cross-linking/mass spectrometry was developed for rapid epitope mapping through structural characterization of >100,000 antigen-Nb complexes. These technologies will open exciting possibilities of Nb-based biomedical applications.

a. Xiang, Y., Sang, Z., Bitton, L., Xu, J., Liu, Y., Schneidman-Duhovny, D.*, and Shi, Y.* (2020b). Integrative proteomics reveals exceptional diversity and versatility of mammalian humoral immunity. bioRxiv, 2020.2008.2021.261917.

3. Novel nanobody-based drug delivery: Therapeutic and diagnostic efficacies of numerous small biomolecules, nanobodies, and chemical compounds are hampered by the short half-lives. We have recently developed a repertoire of high-quality albumin-nanobodies (Nb_{HSA}) to facilitate drug delivery. We have systematically characterized the Nb_{HSA} for albumin binding, mapped the epitopes, and resolved the architecture of a tetrameric Nb-albumin complex. We employed quantitative proteomics for accurate, multiplex Nb pharmacokinetic analysis. Using a humanized albumin mouse model, we found that the Nb-HSA has outstanding pharmacokinetics; the most stable Nb-HSA has a 771-fold T1/2 improvement compared with control Nbs. Interestingly, the pharmacokinetics of Nb-HSA is related to their biophysical and structural properties. To demonstrate the utility of Nb-HSA, we developed a highly stable Nb-HSA-hIL-2 cytokine conjugate “Duraleukin” and confirmed its improved anticancer properties than hIL-2 alone. This high-quality Nb resource will advance research into novel biotherapeutics.

a. Shen Z, Xiang Y, Vegara S, Chen A, Xiao Z, Santiago U, Jin C, Sang Z, Luo J, Chen K, Schneidman-Duhovny D, Camacho C, Calero G, Hu B, **Shi Y** (2020) A robust and versatile nanobody platform for drug delivery. bioRxiv: 2020.08.19.257725.

4. **Structural determination of the eukaryotic nuclear pore complex (NPC):** The yeast nuclear pore complex (NPC) is an organelle-sized macromolecular assembly (> 550 proteins) that pays key roles in the nuclear-cytoplasmic transport of numerous biomolecules. The sheer size, complexity, and flexibility of the NPC are among the main challenges for a detailed structural characterization of the complex. In collaboration with structural biologists, we employed a highly interdisciplinary approach spanning cutting-edge structural proteomics, cryoEM, and modeling to resolve the complete structure of this organelle-sized protein assembly at unprecedented details. We have uncovered a cornucopia of new insights of, e.g., the NPC's construction principles; how it shapes the nuclear envelope and forms platforms for RNA processing and export; how the central gating machinery is organized and its remarkable evolutionary origin.

   a. Kim, S.J.*, Fernandez-Martinez, J.*, Nudelman, I.*, Shi, Y.*, Zhang, W.*, Raveh, B., Herricks, T., Slaughter, B.D., Hogan, J., Paula, U., Chemmama, I., Pallerin, R., Echeverria, I., Shivaraju, M., Chaudhury, A.S., Wang, J.J., Williams, R., Unruh, J.R., Greenberg, C.H., Jacobs, E.Y., Yu, Z., De la Cruz, M.J., Mironska, R., Strokes, D.L., Aitchison, J.D., Jarrold, M.F., Gerton, J.L., Ludtke, S.J., Akey, C.W., Chait, B.T., Sali, A., and Rout, M.P. Structure and Functional Anatomy of the Nuclear Pore Complex (2018). *Nature* 555, 475-482. PMID: 29539637

   b. Fernandez-Martinez, J.*, Kim, S.J.*, Shi, Y.*, Upla, P.*, Pellarin*, R., Gagnon, M., Chemmama, I.E., Wang, J., Nudelman, I., Zhang, W., et al. (2016). Structure and Function of the Nuclear Pore Complex Cytoplasmic mRNA Export Platform. *Cell* 167, 1215-1228. PMID: 27839866

   c. Shi, Y.*, Fernandez-Martinez, J.*, Tjioe, E.*, Pellarin, R.*, Kim, S.J.*, Williams, R., Schneidman-Duhovny, D., Sali, A., Rout, M.P., and Chait, B.T. (2014). Structural Characterization by Cross-linking Reveals the Detailed Architecture of a Coatomer-related Heptameric Module from the Nuclear Pore Complex. *Molecular & Cellular Proteomics 13*, 2927-2943. PMID: 25161197

5. **Development of integrative structural proteomics:** I have developed robust proteomic tools for hybrid structural dissection of endogenous complexes. The proteomic technologies will help illuminate the structure-functions of many large, low-abundance, flexible and dynamic macromolecular assemblies in the cell.

   a. Shi, Y., Pellarin, R., Fridy, P.C., Fernandez-Martinez, J., Thompson, M.K., Li, Y., Wang, Q.J., Sali, A., Rout, M.P., and Chait, B.T. (2015). A strategy for dissecting the architectures of native macromolecular assemblies. *Nature Methods 12*, 1135-1138. PMID: 26436480

   b. Chait, B.T., Cadene, M., Olinares, P.D., Rout, M.P. & Shi, Y. (2016). Revealing Higher Order Protein Structure Using Mass Spectrometry. *J Am Soc Mass Spectrom* 27, 952-965. PMID: 27080007

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/1xSmyjv4ibo5E/bibliography/public/

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R35GM137905 Shi (PI) 2020/07/01-2025/06/30
Development of the next generation antibody technologies and their applications
The goal of this study is to develop proteomic technologies and informatics for nanobody identification, characterization and cutting-edge biomedical applications.
Role: PI

MJFF and Alzheimer's Association (co-funded) Shi (PI) 2019/06/01-2020/12/31
Development of novel blood-brain-barrier nanobodies

The goal of this study is to develop nanobodies to efficiently penetrate the blood-brain-barrier for advanced PET-based brain imaging.
Role: PI

University of Pittsburgh (CTSI) Shi (PI) 2020/05/01-2021/04/30
Therapeutic Nanobodies for SARS-CoV-2 Viral Neutralization
The goal of this study is to develop ultrapotent, cost-effective agents to neutralize SARS-CoV-2 for the potential treatment of COVID-19
Role: PI

Other Support

I have established collaborations with scientists listed below in foreign countries. My collaborators provide my group with necessary analytic support for the ongoing projects. I do not receive any foreign financial support for myself and for the ongoing projects in my lab.

Dr. Dina Schneidman-Dohovny, assistant professor at Hebrew University of Jerusalem, Israel. My lab has extensive collaborations with Dohovny on the large-scale structural modeling of antigen-antibody complexes.