BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Nathaniel J Traaseth

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

POSITION TITLE: Professor of Chemistry

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
University of Minnesota-Duluth, Duluth, MN	B.S.	05/2003	Chemistry, Biochemistry, Political Science
University of Minnesota, Minneapolis, MN	Ph.D.	10/2007	Chemistry
University of Minnesota, Minneapolis, MN	Postdoc	04/2011	Biophysical Chemistry

A. Personal Statement

The goal of this proposal is to discover novel mechanisms of active transport in multidrug efflux pumps and to use this knowledge for inhibitor design. Over the last 11 years of my independent career, my group has accumulated expertise in molecular transporters involved in multidrug resistance, receptor tyrosine kinases that mediate cellular signaling, and the development and application of solution and solid-state NMR spectroscopy. The subject of this project began about eight years ago. Since this time, we have established collaborations with five groups at NYU who have expertise in cryo-EM (Da-Neng Wang), antibody engineering (Shohei Koide), *S. aureus* biology (Victor Torres), peptide synthesis and engineering (Paramjit Arora), and computational chemistry (Yingkai Zhang). The culmination of our efforts over the last few years recently manifested in the first structures of NorA bound to antibodies using cryo-EM. Furthermore, we have assigned functional roles to several hotspots within NorA's structure, which is essential for its function in *S. aureus*. The structures of NorA revealed an antibody CDR loop bound deeply inside the pocket, which suggested the possibility of protein-based efflux pump inhibitors. More recently, we designed and tested peptidomimetics of the antibody loops and showed inhibition against MRSA. Taken together with my group's expertise in NMR spectroscopy and multidrug efflux pumps, our collaborative team is ideally suited to accomplish the goals outlined in the proposal.

Ongoing funded projects I would like to highlight:

1. R01 AI165782 Traaseth (PI), Wang (MPI), Koide (MPI) 12/01/21 – 11/30/26
NIH-NIAID
Transport Mechanisms and Inhibition of Efflux Pumps in Pathogenic Organisms
The goal of this study is to establish the mechanisms of transport and inhibition of the *S. aureus* multidrug transporter NorA.

2. R01 AI108889 Traaseth (PI) 08/01/20 – 07/31/24
NIH-NIAID
Mechanisms of Allostery and Molecular Recognition in the Small Multidrug Resistance Family
The goal of this study is to elucidate the allosteric basis for multidrug resistance in bacteria conferred by the small multidrug resistant family of transporters.

3. NSF MCB1506420 Traaseth (PI) 08/01/19 – 07/31/23
Directorate for Biological Sciences - MCB
Tools for Probing Conformational Dynamics of Membrane Proteins
The goal of this award is to develop NMR tools to study membrane proteins dynamics.

Publications that highlight relevant work:

1. Brawley DN, Sauer DB, Li J, Zheng X, Koide A, Jedhe GS, Suwatthee T, Song J, Liu Z, Arora PS, Koide S, Torres VJ, Wang DN, **Traaseth NJ** (2022) Structural basis for inhibition of the drug efflux pump NorA from Staphylococcus aureus. *Nat Chem Biol*, 18, 706-712. *PMCID: PMC9246859*
2. Leninger M, Sae Her A, **Traaseth NJ** (2019) Inducing Conformational Preference of the Membrane Protein Transporter EmrE Through Conservative Mutations. *eLife*, 8:e48909. *PMCID: PMC6805155*
3. Gayen A, Leninger L, **Traaseth NJ** (2016) Protonation of a glutamate residue modulates the dynamics of the drug transporter EmrE. *Nat Chem Biol,* 12, 141-145. *PMCID: PMC4755857*
4. Li J, Sae Her A, **Traaseth NJ** (2020) Site-specific resolution of anionic residues in proteins using solid-state NMR spectroscopy. *J Biomolec NMR*, 74, 355-363. *PMCID: PMC7472563*

B. Positions and Honors

Positions and Employment

2001-2003	Undergraduate Researcher, University of Minnesota-Duluth (C. Giulivi)
2003-2007	Graduate Student, Department of Chemistry, University of Minnesota (G. Veglia)
2007-2011	Post-Doctoral Associate, Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota (G. Veglia)
2011-2017	Assistant Professor, Department of Chemistry, New York University
2017-2021	Associate Professor, Department of Chemistry, New York University
2018-2022	Director of Graduate Studies, Department of Chemistry, New York University
2021-present	Professor, Department of Chemistry, New York University

Honors

2003	American Institute of Chemist's Award
2003	Graduated with Honors from the Departments of Chemistry and Political Science
2003	Graduated *Magna Cum Laude*
2003-2004	Izaak Koltoff Fellowship
2004-2005	NIH Neuro-Physical-Computational Sciences Grant
2007	Overend Award in recognition of outstanding graduate student researcher physical chemistry
2005-2007	American Heart Association Pre-Doctoral Fellowship
2008	Paul Boyer Award for significant research contributions in biochemistry as a postdoc.
2010	NIH NRSA Postdoctoral Fellowship (*declined*)
2011-2013	NIH (NIAID) National Research Scholar Development K22 Award

C. Contribution to Science

1. Transport mechanisms of multidrug efflux pumps. A long-term goal of my lab is to reveal novel mechanisms used by multidrug transporters and to harness this knowledge to predict and control function, including in design of conformationally specific efflux pump inhibitors. Our approach is to study transporters within lipid bilayers, which provides the best mimic of the native environment and offers conditions in which dynamics can be quantitatively measured. Our studies have primarily focused on EmrE, which serves as a model transporter from the small multidrug resistance family to understand the ability of multidrug pumps to promiscuously bind substrates and to use anionic residues to couple with the pH gradient. We discovered that the drug-free form of the transporter exhibits significant conformational plasticity that we hypothesize enables it to bind and efflux a wide variety of substrates varying in size, shape, and charge. Our latest findings were used to derive a correlation between the acid dissociation constant of a conserved glutamic acid residue and the phenotype conferred to *E. coli*. We found that an elevated acid dissociation constant was needed to transport

and provide resistance to toxins. We also discovered that the protonation state plays a role in modulating allostery and in the global conformational exchange required for transfer of drugs across the membrane. These findings were used to derive a model whereby we hypothesize that the inward-open conformation of the transporter is favored in the presence of a pH gradient. Namely, when the pH values on the cytoplasm and periplasm are centered around those of the acid dissociation constant, EmrE is positioned toward the cytoplasm and poised to bind to toxic compounds. This leads to a preferred resting conformation of the transporter, which is a novel finding in the transport field.

(a) Brawley DN, Sauer DB, Li J,Zheng X, Koide A, Jedhe GS, Suwatthee T, Song J, Liu Z, Arora PS, Koide S, Torres VJ, Wang DN, **Traaseth NJ**. Structural basis for inhibition of the drug efflux pump NorA from *Staphylococcus aureus*. *Nat Chem Biol*, in-press. *PMID: 35361990*

(b) Li J^+, Sae Her A^+, **Traaseth NJ** (2021). Asymmetric protonation of glutamate residues drives a preferred transport pathway in EmrE. *Proc Natl Acad Sci*, 118: e2110790118. *PMCID: PMC8521673*

(c) Leninger M, Sae Her A, **Traaseth NJ** (2019) Inducing Conformational Preference of the Membrane Protein Transporter EmrE Through Conservative Mutations. *eLife*, 8:e48909. *PMCID: PMC6805155*

(d) Gayen A, Leninger L, **Traaseth NJ** (2016) Protonation of a Glutamate Residue Modulates the Dynamics of the Drug Transporter EmrE. *Nat Chem Biol,* 12, 141-145. *PMCID: PMC4755857*

2. NMR methods for probing structure and dynamics of biomolecules. My group has actively developed solution and solid-state NMR methods to be used to acquire high-resolution structural information for complex biomolecules, including membrane proteins. While the solid-state NMR approach is a preferred way to study membrane protein structure and dynamics due to the ability to characterize the proteins in lipid bilayers, the two primary problems associated with widespread usage are reduced spectral resolution and sensitivity. We have developed new approaches to address both limitations. The first is the *Afterglow* method that makes use of residual magnetization from the cross-polarization sequence that is widely used in solid-state NMR. This magnetization is stored and used for acquisition of a second dataset, thus improving the sensitivity by a significant amount (i.e., *two for the price of one*). The second method we developed is a set of isotopic labeling schemes to be used in conjunction with the *Afterglow* technology. In this approach, we create a defined set of pairwise connectivity through the careful choice of label incorporation and apply spectroscopic filters to reduce the spectral complexity. These developments have been instrumental in increasing the speed of obtaining structural information for large, polytopic membrane transport proteins. In addition, we have developed a novel technique that can reduce the time needed to acquire chemical exchange saturation transfer (CEST) experiments that are used to probe motion on the msec to sec timescale. These approaches are time consuming because they require a series of 2D datasets (i.e., pseudo 3D acquisition). Recently, we introduced a multifrequency approach for CEST that can reduce the data acquisition by an integer factor of 3-4-fold, which will enable solution NMR users a way to *speed-up* data acquisition.

(a) Li J, Sae Her A, **Traaseth NJ** (2020) Site-specific resolution of anionic residues in proteins using solid-state NMR spectroscopy. *J Biomolec NMR*, 74, 355-363. *PMCID: PMC7472563*

(b) Leninger M, Marsiglia WM, Jerschow A, **Traaseth NJ** (2018) Multiple frequency saturation pulses reduce CEST acquisition time for quantifying conformational exchange in biomolecules. *J Biomolec NMR*, 71, 19-30. *PMCID: PMC5989009*

(c) Banigan JR, Gayen A, **Traaseth NJ** (2013). Combination of ^{15}N Reverse Labeling and Afterglow Spectroscopy for Assigning Membrane Protein Spectra by Magic-Angle-Spinning Solid-State NMR: Application to the Multidrug Resistance Protein EmrE. *J Biomolec NMR*, 55, 391-9. *PMCID: PMC3747971*

(d) Banigan JR, **Traaseth NJ** (2012). Using Afterglow Magnetization from Cross-Polarization Magic-Angle-Spinning Solid-State NMR Spectroscopy to Obtain Simultaneous Heteronuclear Multidimensional Spectra. J Phys Chem B, 116, 7138-44. *PMCID: PMC3418334*

3. Role of intrinsic kinase dynamics in biological activity. We elucidated allosteric control mechanisms underpinning receptor tyrosine kinase (RTK) regulation by using the fibroblast growth factor receptor (FGFR) kinase subfamily as a model system in collaboration with Moosa Mohammadi. In our initial work at NYU, we discovered that pathogenic mutations in the kinase activation loop (A-loop) altered the conformational dynamics, which shifted the equilibrium toward the active form of the enzyme by introducing intramolecular contacts that stabilize the active state (*Cell Reports* 2013). Building on this work, we proposed a detailed allosteric network involving four key hotspots that are involved in regulating the transition between

autoinhibited and activated states (*eLife* 2017). Using a combination of NMR, bioinformatics, and MD simulations in collaboration with Yingkai Zhang (NYU), we showed how pathogenic mutations at the kinase hinge bypass the autoinhibitory network at the molecular brake to lead to activation (*Structure* 2019). In our most recent work, we showed how A-loop tyrosine transphosphorylation of a pathogenic mutation of the FGF receptor kinase proceeds via an asymmetric complex that is thermodynamically disadvantaged because of an electrostatic repulsion between enzyme and substrate kinases (*Nature Chemical Biology* 2020). We believe this mode of A-loop tyrosine transphosphorylation is shared in wild-type FGFR as well as other RTKs.

(a) Chen L, Marsiglia WM, Chen H, Katigbak J, Erdjument-Bromage H, Kemble DJ, Fu L, Ma J, Sun G, Zhang Y, Liang G, Neubert TA, Li X, **Traaseth NJ*,** Mohammadi M* (2020) Molecular basis for receptor tyrosine kinase A-loop tyrosine transphosphorylation. *Nat Chem Biol*, 16(3), 267-277. *PMCID: PMC7040854*

(b) Marsiglia WM, Katigbak J, Zheng S, Mohammadi M, Zhang Y, **Traaseth NJ** (2019). A Conserved Allosteric Pathway in Tyrosine Kinase Regulation. *Structure*, 27(8), 1308-1315. *PMCID: PMC6687525*

(c) Chen H, Marsiglia WM, Cho M-K, Huang Z, Deng J, Blais SP, Gai W, Bhattacharya S, Neubert TA, **Traaseth NJ***, Mohammadi M* (2017) Elucidation of a four-site allosteric network in fibroblast growth factor receptor tyrosine kinases. eLife, 6, pii: e21137. doi: 10.7554/eLife.21137. *PMCID: PMC5293489*

(d) Chen H, Huang Z, Dutta K, Blais S, Neubert TA, Li X, Cowburn D, **Traaseth NJ**, Mohammadi M (2013) Cracking the Molecular Origin of Intrinsic Tyrosine Kinase Activity through Pathogenic Gain-of-Function Mutations. *Cell Reports*, 4(2), 376-84. *PMCID: PMC3752781*

4. Molecular mechanism of cardiac contractility regulation by phospholamban. Prior to my independent career at NYU, my research focused on elucidating the regulatory mechanism on the Ca^{2+}-ATPase (SERCA) from phospholamban (PLN). PLN is a single-pass membrane protein that inhibits SERCA, an ATP-driven pump that translocates calcium ions into the lumen of the sarcoplasmic reticulum, initiating muscle relaxation. PLN binds SERCA through intramembrane interactions, impeding calcium translocation. While phosphorylation of PLN at Ser-16 and/or Thr-17 reestablishes calcium flux, the molecular details of inhibition and relief of inhibition remained elusive prior to our studies. While SERCA had been crystallized in several different states along the enzymatic reaction coordinates, the lack of high-resolution crystals in the presence of PLN and SLN limited the understanding of the regulatory mechanism. Our use of NMR and EPR experiments showed that PLN regulates SERCA through a series of conformational rearrangements involving membrane interactions and the folding and unfolding of domains within the juxtamembrane and cytoplasmic regions of PLN. These results have improved our understanding of the calcium translocation process and are the basis for designing novel therapeutic approaches to ameliorate muscle malfunctions.

(a) **Traaseth NJ**, Verardi R, Torgersen KD, Karim CB, Thomas DD, Veglia G (2007). Spectroscopic Validation of the Pentameric Structure of Phospholamban. *Proc Natl Acad Sci,* 104, 14676-81. *PMCID: PMC1976191*

(b) Gustavsson M, Verardi R, Mullen DG, Mote KR, **Traaseth NJ**, Gopinath T, Veglia G. (2013) Allosteric Regulation of SERCA by Phosphorylation-Mediated Conformational Shift of Phospholamban. *Proc Natl Acad Sci*, 110, 17338-43. *PMCID: PMC3808617*

(c) Verardi R, Shi L, **Traaseth NJ,** Walsh N, Veglia G (2011) Structural Topology of Phospholamban Pentamer in Lipid Bilayers by a Hybrid Solution and Solid-State NMR Method. *Proc Natl Acad Sci,* 108, 9101-6. *PMCID: PMC3107283*

(d) **Traaseth NJ**, Thomas DD, Veglia G (2006) Effects of Ser16 phosphorylation on the allosteric transitions of phospholamban/Ca(2+)-ATPase complex. *J Mol Biol*, 358, 1041-50. *PMID: 16564056*

5. Structure Determination Protocol for Membrane Proteins. To fully describe the fold space and ultimately the biological function of membrane proteins, it is necessary to determine the specific interactions of the protein with the membrane. As a post-doctoral associate, I spearheaded work to develop a structure determination protocol that simultaneously defined the structure, orientation, and depth of insertion of membrane proteins in the lipid bilayer. This hybrid objective function was incorporated into XPLOR-NIH and was the first to combine solution and solid-state NMR restraints in a quantitative fashion to calculate membrane protein structures with respect to the lipid bilayer. This approach was originally demonstrated with the cardiac membrane protein phospholamban and has since been applied to other systems.

(a) **Traaseth NJ**, Shi L, Verardi R, Mullen D, Barany G, Veglia G (2009) Determination of Membrane Protein Structure and Topology Using a Hybrid Solution and Solid-State NMR approach. *Proc Natl Acad Sci* 106, 10165-70. *PMCID: PMC2700893*

(b) Shi L, **Traaseth NJ**, Verardi R, Gustavsson M, Gao J, Veglia G (2011) Paramagnetic-based NMR restraints lift residual dipolar coupling degeneracy in multidomain detergent-solubilized membrane proteins. *J Am Chem Soc,* 133, 2232-41. *PMCID: PMC3328396*

(c) Shi L, **Traaseth NJ**, Verardi R, Cembran A, Gao J, Veglia G (2009) A refinement protocol to determine structure, topology, and depth of insertion of membrane proteins using hybrid solution and solid-state NMR restraints. *J Biomol NMR,* 44, 195-205. *PMCID: PMC2824793*

(d) **Traaseth NJ**, Verardi R, Veglia G (2008) Asymmetric methyl group labeling as a probe of membrane protein homo-oligomers by NMR spectroscopy. *J Am Chem Soc,* 130, 2400-1. *PMCID: PMC2699765*

Complete List of Published Work in My Bibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/nathaniel.traaseth.1/bibliography/47380351/public/?sort=date&direction=descending