BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
Malmstadt, Noah	Professor of Chemical Engineering & Materials Science, Biomedical Engineering, and Chemistry
eRA COMMONS USER NAME MALMSTADT2	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
California Institute of Technology	B.S.	1997	Chemical Engineering
University of Washington	Ph.D.	2003	Bioengineering Mentors: Patrick S. Stayton and Allan S. Hoffman
University of California, Los Angeles	Postdoc	2004-2007	Bioengineering Mentor: Jacob J. Schmidt

A. Personal Statement

I have extensive research experience developing tools to probe the biophysical and biochemical properties of lipid bilayers in their context as the supermolecular structures that provide the architecture of cell membranes. In the past five years, my lab has published over 20 papers describing the development and application of new approaches to fabricate and analyze lipid bilayer structures. Our recent work has focused on the formation of giant unilamellar lipid vesicles (GUVs) by a hydrogel swelling method and the efficient incorporation of active membrane proteins into GUVs using this method (Hansen 2013). We have recently demonstrated the active incorporation of G protein-coupled receptors (GPCRs) in lipid bilayers using this method (Gutierrez 2014) and the use of this platform to measure the lipid-dependent activity of the 5-HT_{1A} serotonin receptor (Gutierrez 2016). We have performed mechanistic studies of the hydrogel swelling-driven lipid bilayer formation process, with the goal of producing controlled vesicles sizes, geometries, and patterns on the surface of hydrogel-coated substrates (Peruzzi 2016). In recent months, as described in this proposal, we have applied the hydrogel-swelling system to reconstitute adenosine 2a receptor ($A_{2a}R$) in GUVs of varying lipid composition and to measure its lipid-dependent activity. My research group's combined expertise in membrane protein biophysics and experimental tool development equips us ideally for the proposed research program, which seeks to develop an ambitious new high-throughput experimental platform and apply it to questions surrounding the dependence of GPCR function on lipid bilayer composition.

1) "Lipid directed intrinsic membrane protein segregation." Jesper S. Hansen, James R. Thompson, Claus Hélix-Nielsen, and Noah Malmstadt. *Journal of the American Chemical Society*. **135**(46):17294-17297. 2013.
2) "Human serotonin receptor 5-HT_{1A} preferentially segregates to the liquid disordered phase in synthetic lipid bilayers." M. Gertrude Gutierrez and Noah Malmstadt. *Journal of the American Chemical Society*. **136**(39):13530-13533. 2014.
3) "The functional activity of the human serotonin 5-HT_{1A} receptor is controlled by lipid bilayer composition." M. Gertrude Gutierrez, Kylee Mansfield, and Noah Malmstadt. *Biophysical Journal*. **110**(11):2486-2495. 2016.
4) "Dynamics of hydrogel-assisted giant unilamellar vesicle formation from unsaturated lipid systems." Justin Peruzzi, M. Gertrude Gutierrez, Kylee Mansfield, and Noah Malmstadt. *Langmuir*. **32**(40):12702-12709. 2016.

B. Positions and Honors.

Positions and Employment

2007-2014 Assistant Professor, Mork Family Department of Chemical Engineering and Materials Science University of Southern California

2009-present Joint (Courtesy) Appointment, Department of Biomedical Engineering, University of Southern California

2014-2018 Associate Professor, Mork Family Department of Chemical Engineering and Materials Science University of Southern California

2015-present Joint (Courtesy) Appointment, Department of Chemistry, University of Southern California

2018-present Professor, Mork Family Department of Chemical Engineering and Materials Science University of Southern California

2018-present Associate Chair for Graduate Programs, Mork Family Department of Chemical Engineering and Materials Science, University of Southern California

Other Experience and Professional Memberships

2006- Member, American Chemical Society

2007- Member, American Institute of Chemical Engineers

2007- Reviewer, *Journal of the American Chemical Society, Nature Chemistry, Scientific Reports, Lab on a Chip, Langmuir, Bioconjugate Chemistry, Analytical and Bioanalytical Chemistry, Journal of Membrane Science, ACS Applied Materials and Interfaces, Biomicrofluidics,* and *Journal of Physical Chemistry Letters*

2008- Member, Biophysical Society

2009- NSF Proposal Review Panel member

Honors

1997 Graduated with Honors, California Institute of Technology

2000 Selected to NSF Summer Institute in Japan

2008 Powell Foundation Research Award

2012 Office of Naval Research Young Investigator

2015 USC Viterbi School of Engineering Junior Research Award

2016 USC PhD Student Mentoring Award

2016 Journal of Laboratory Automation JALA Ten Honoree

C. Contributions to Science

1) **Developed the hydrogel swelling technique to reconstitute integral membrane proteins in GUVs.** Of primary interest for the present proposal is my development of new techniques of membrane protein reconstitution in synthetic lipid bilayers to characterize the function of membrane proteins, particularly with regard to how lipid structure and composition affect protein function. The core technology here is a technique based on controllably swelling lipid bilayer-surrounded compartments from a lipid film cast on the surface of a hydrogel. This technique allows for unusually high-yield incorporation of integral membrane proteins in lipid bilayers of controlled composition. Here, we have published three major developments: a synthetic system for studying the functionality of the human glucose transfer GLUT1; the discovery that the human serotonin 5-HT_{1A} receptor co-segregates with liquid disordered lipids, in contradiction of earlier biochemical results; and a thorough analysis of the lipid dependence of the functional activity of the 5-HT_{1A} receptor using the same techniques we have proposed for the current application.

   a) "Lipid directed intrinsic membrane protein segregation." Jesper S. Hansen, James R. Thompson, Claus Hélix-Nielsen, and Noah Malmstadt. *Journal of the American Chemical Society*. **135**(46):17294-17297. 2013.
   b) "Human serotonin receptor 5-HT_{1A} preferentially segregates to the liquid disordered phase in synthetic lipid bilayers." M. Gertrude Gutierrez and Noah Malmstadt. *Journal of the American Chemical Society*. **136**(39):13530-13533. 2014.

c) "Glucose transport machinery reconstituted in cell models." Jesper S. Hansen, Karin Elbing, James R. Thompson, Noah Malmstadt, and Karin Lindkvist-Petersson. *Chemical Communications*. **51**(12):2316-2319. 2015.
d) "The functional activity of the human serotonin 5-HT_{1A} receptor is controlled by lipid bilayer composition." M. Gertrude Gutierrez, Kylee Mansfield, and Noah Malmstadt. *Biophysical Journal*. **110**(11):2486-2495. 2016.

2) **Developed new optical and microfluidic techniques to characterize lipid membrane biophysics.** Another focus of my work as an independent investigator has been the development of new techniques to understand the relationship between lipid bilayer composition and properties. This work has followed two primary routes: developing combined microfluidic and microscopy techniques to measure the permeability of lipid bilayer membranes and developing optical force techniques to measure the mechanical properties of lipid bilayer membranes. One application of both of these techniques has been to study the effects of lipid oxidation on the properties of the cell membrane. We have found two major results here: 1. Photooxidation alters lipid bilayer mechanics so as to facilitate the facile formation of large, long-lived pores. 2. Small levels of oxidation radically increase the passive permeability of cell membranes. Both of these results indicate that a major mechanism of oxidative damage to cell membranes may be to compromise the membranes' barrier properties. We have also recently published an optical force-based study that demonstrates that lipid vesicles behave as viscoelastic materials.

a) "The dynamics of giant unilamellar vesicle oxidation probed by morphological transitions." Shalene Sankhagowit, Shao-Hua Wu, Roshni Biswas, Carson T. Riche, Michelle L. Povinelli, and Noah Malmstadt. *Biochimica et Biophysica Acta – Biomembranes*. **1838**(10):2615-2624. 2014.
b) "Low levels of lipid oxidation radically increase the passive permeability of lipid bilayers." Kristina Runas and Noah Malmstadt. *Soft Matter*. **11**(3):499-505. 2015.
c) "Viscoelasetic deformation of lipid bilayer vesicles." Shao-Hua Wu, Shalene Sankhagowit, Roshni Biswas, Shuyang Wu, Michelle L. Povinelli, and Noah Malmstadt. *Soft Matter*. **11**:7385-7391. 2015.
d) "Oxidation of membrane curvature-regulating phophatidylethanolamine lipid results in formation of bilayer and cubic structures." Shalene Sankhagowit, Ernest Y. Lee, Gerard C.L. Wong, and Noah Malmstadt. *Langmuir*. **32**(10):2450-2457. 2016.

3) **Developed novel techniques for fabricating GUVs.** Another major topic of focus has been the development of new systems for forming giant unilamellar lipid vesicles (GUVs). GUVs are essential tools for studying the biophysics of the cell membrane, but techniques for fabricating them have been severely lacking in terms of reproducibility, universality, and controllability. We have several new systems for forming GUVs with better control. One is microfluidic: a microfluidic GUV fabrication system that allows for the formation of GUVs with compositionally asymmetric bilayers (mimicking the lipid compositional asymmetry of the eukaryotic plasma membrane). We have also pioneered the application of synthetic polymer hydrogels as substrates for swelling lipid films into GUVs. This latter approach has been extended to the functional reconstitution of membrane proteins in vesicles fabricated from synthetic block copolymers rather than lipids.

a) "Microfluidic fabrication of asymmetric giant lipid vesicles." Peichi Hu, Su Li, and Noah Malmstadt. *ACS Applied Materials and Interfaces.* **3**(5):1434-1440. 2011.
b) "G protein-coupled receptors incorporated into rehydrated diblock copolymer vesicles retain functionality." M. Gertrude Gutierrez, Farzah Jalali-Yazdi, Justin Peruzzi, Carson T. Riche, Richard W. Roberts, and Noah Malmstadt. *Small*. **12**(38):5256-5260. 2016.
c) "Evaluation of dextran(ethylene glycol) hydrogen films for giant unilamellar vesicle production and their application for the encapsulation of polymersomes." Nestor Lopez Mora, Yue Gao, M. Gertrude Gutierrez, Justin Peruzzi, Ivan Bakker, Ruud J.R.W. Peters, Bianka Siewert, Sylvestre Bonnet, Roxanne E. Kieltykia, Jan C.M. van Hest, Noah Malmstadt, and Alexander Kros. *Soft Matter.* **13**:5580-5588. 2017.
d) "Photolithographic patterned surface forms size-controlled lipid vesicles." M. Gertrude Gutierrez, Shotaro Yoshida, Noah Malmstadt, and Shoji Takeuchi. *APL Bioengineering.* **2**:016104. 2018.

4) **Developed microfluidic technologies for automated bioanalysis.** I have put considerable effort into developing new microfluidic systems with predictable and well controlled flow behavior. In 2014, I led the development of a modular microfluidic system that provides a novel approach to microfluidic system design. This technology allows for the facile assembly of complex microfluidic systems that route fluids in three dimensions. Since it is based on 3D-printed parts, it eliminates the need for expensive and time-consuming cleanroom fabrication steps. Since the initial demonstration of the system, my lab has published extensively regarding design rules for modular microfluidics, control of surface properties in this system, and approaches to integrating off-the-shelf electronic components to add complex functionality to modular microfluidics. Our experience in developing automated microfluidic analytical systems is directly applicable to the system automation and data processing pipeline development that will be necessary to accomplish the proposed work.

   a) "Discrete elements for 3D microfluidics." Krisna C. Bhargava, Bryant Thompson, and Noah Malmstadt. *Proceedings of the National Academy of Sciences.* 111(42):15013-15018. 2014.
   b) "Predicting the behavior of microfluidic circuits made from discrete elements." Krisna C. Bhargava, Bryant Thompson, Danish Iqbal, and Noah Malmstadt. *Scientific Reports.* **5**:15609. 2015.
   c) "Temperature sensing in modular microfluidic architectures." Krisna C. Bhargava, Bryant Thompson, Anoop Tembhekar, and Noah Malmstadt. *Micromachines*. **7**(1):11. 2016.
   d) "Engineered hydrophobicity of discrete microfluidic elements for double emulsion generation." Bryant Thompson, Carson T. Riche, Nareh Mouvesesian, Krisna C. Bhargava, Malancha Gupta, and Noah Malmstadt. *Microfluidics and Nanofluidics*. **20**:78. 2016.

A complete list of my publications can be found on MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/noah.malmstadt.1/bibliography/40867884/public/?sort=date&direction=ascending.

D. Research Support

Ongoing Research Support

1R01GM120351 Lyman (PI) 9/1/17 – 8/31/22
National Institutes of Health
"Effects of lipidomic diversity on GPCR activity."
The goal of this project is to understand how differences in the lipid composition of the cell plasma membrane alter the behavior of G protein-coupled receptors, with an emphasis on the adenosine 2A receptor.
Role: co-Investigator

FP00209340 Boron (PI) 6/1/16 – 5/31/21
Office of Naval Research
"MURI: Molecular mechanisms and pathways for gas transport across biological membranes and implications for physiology and performance."
The goal of this project is to identify and characterize modes of transport of dissolved gases across lipid bilayers, including transport routes through channel proteins.
Role: co-Investigator

1R21CA204708 Malmstadt (PI) 5/1/17 – 4/30/20
National Institutes of Health
"A Target-Directed Reagent Pipeline via Microfluidic mRNA Display."
The goal of this project is to adapt the versatile molecular selection system called mRNA Display to a fully automated microfluidic discovery pipeline.
Role: PI

CMMI-1728649 Malmstadt (PI) 7/15/17 – 7/14/20
NSF
"Highly Parallel 3D Microfluidic Architectures for Manufacturing Catalytic Nanoparticles."

The goal of this project is to develop novel devices architectures and control strategies that will allow for chemical reactions that produce nanoparticles to be scaled via the massive parallelization of microfluidic devices.
Role: PI

N00014-16-1-2382 Malmstadt (PI) 7/15/16 – 7/14/20
Office of Naval Research
"Connecting lipid oxidation to cellular dysfunction in hyperbaric oxygen toxicity."
The goal of this project is to identify how oxidative processes alter the lipidome of cells in culture and to connect these changes to alterations in the biophysical properties of the lipid bilayer that forms the structure of the plasma membrane.
Role: PI

Past Research Support

11028663 Malmstadt (PI) 4/1/12 – 12/31/15
Office of Naval Research (Young Investigator Award)
"Probing the molecular origins of cell membrane damage in hyperbaric oxygen toxicity."
The goal of this project is to understand how high partial pressures of oxygen can damage cell membranes, leading to altered phase behavior and compromised barrier properties.
Role: PI

1R01GM093279 Malmstadt (PI) 9/15/10 – 9/15/16
NIH
"Connecting plasma membrane function to lipid structure and organization with asymmetric lipid vesicles."
The goal of this project is to observe the behavior of compositionally asymmetric lipid bilayer membranes in terms of small-molecule transport, intrinsic curvature, lipid-peptide interactions, and macromolecular crowding.
Role: PI

CMMI-1068212 Malmstadt (PI) 4/15/11 – 4/14/15
NSF
"Cholesterol flip-flop dynamics and nanomechanical response of deformed biomembranes: Experiments and petascale simulation."
The goal of this project is to understand how changes in the rate of cholesterol transport across the lipid bilayer can alter the mechanics of lipid bilayer membranes.
Role: PI

CBET-1067021 Malmstadt (PI) 5/1/11 – 4/30/15
NSF
"Uncovering fundamental relationships between molecular structure and passive cell membrane transport."
The goal of this project is to understand how the variety of lipid molecular structures present in cell plasma membranes can affect the passive transport properties of these membranes.
Role: PI

1R21AG033890 Malmstadt (PI) 5/1/09 – 4/30/12
NIH
"Biomimetic systems for studying nanoscale structure formation in cell membranes."
The goal of this project is to develop novel synthetic lipid model systems and microscopy tools for observing nanoscale lipid-lipid interactions.
Role: PI