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: Qiao Lin

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

POSITION TITLE: Professor of Mechanical Engineering

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
Tsinghua University, Beijing, China	B.S.	1985	Engineering Mechanics
Tsinghua University, Beijing, China	M.S.	1988	Engineering Mechanics
California Institute of Technology, Pasadena, CA	Ph.D.	1998	Mechanical Engineering
California Institute of Technology, Pasadena, CA	Postdoctoral	2000	Electrical Engineering

A. Personal Statement

My research centers on micro/nanotechnologies as applied to biological sensing and manipulation, with an emphasis on controlling, sensing and characterizing biological systems by integrating microelectromechanical systems (MEMS) transducers with microfluidics. My group has developed micro/nanoscale biosensors and microfluidic manipulation devices for biomedical applications. Our biosensing effort has focused on affinity sensing of biomolecules in microstructures and on functional nanomaterials, being the first to report *in vivo*-validated affinity glucose microsensors for continuous glucose monitoring and calorimetric microdevices for quantitative thermodynamic measurements of biochemical reactions. My lab has also investigated microfluidic manipulation of live cells using both physically based and selective molecular recognition methods. Moreover, my group is among pioneers in microfluidic discovery and applications of nucleic acid aptamers, having devised methods for total integration of aptamer isolation processes, which can now be completed within a day, in contrast to the conventional month-long requirements.

In the proposed research, I will develop a technology for preparing specimens from submillisecond biomolecular reactions in time-resolved cryo-EM (TRCEM). TRCEM is highly desirable for studying short-lived biomolecular reaction events but is currently limited to reaction times larger than 20 ms, which is a highly limiting capability gap. The proposed technology will achieve tunable submillisecond reaction times at high resolution and low dispersion. I am passionate about developing this technology because of its potentially transformative utility for enabling TRCEM studies of biological systems such as many ion channels and receptors whose activation and gating times are on submillisecond scales. I have collaborated with Dr. Frank to demonstrate a microfluidic nozzle for cryo-EM specimen preparation as well as to conceive the proposed technology. I will continue to collaborate with him, as well as with Drs. Chris Boyce, Oliver Clarke and Andrew Marks, to develop and validate this technology.

Ongoing and recently completed projects that I would like to highlight include:

1R01EB032910-01A1
Stojanovic, Lin, Cremers and Mohan (Multiple PIs)
08/01/2022-07/31/2027
Towards Rapid Measurement of Antibiotics in Clinical Care Setting
Major goals of my effort in this project are to develop and integrate aptamer-functionalized graphene affinity nanosensors with microfluidic concentration for real-time measurements of antibiotics in critically ill patients.

NIH 1R21CA261775-01A1
Lin (PI)
05/01/2022-04/30/2025
A Practical Approach to Tumor-Specific Aptamers for B-Cell Hematologic Malignancies
Major goals are to demonstrate microfluidic selection of aptamers targeting variable regions (idiotypes) of cell surface immunoglobulins (sIgs) in patients with B-cell malignancies.

NIH 5R33CA196470-03
Lin (PI)
09/07/2016-07/31/2021
Validating Rapid Microfluidic Isolation of Personalized Aptamers for Monitoring Minimal Residual Disease in Multiple Myeloma
Major goals are to validate microfluidic selection of aptamers targeting tumor-specific monoclonal antibodies in individual patients of multiple myeloma for serum-based detection and monitoring of minimal residual disease.

Citations:

1. H. Sun, T. Olsen, J. Zhu, J. Tao, B. Ponnaiya, S. Amundson, D. Brenner, and Q Lin, "A Microfluidic Approach to Parallelized Transcriptional Profiling of Single Cells," *Microfluidics and Nanofluidics*, 19, 1429-1440, 2015. PMCID: PMC4868186.
2. H. Sun, T. Olsen, J. Zhu, J. Tao, B. Ponnaiya, S. Amundson, D. Brenner, and Q. Lin, "A Bead-Based Microfluidic Approach to Integrated Single-Cell Gene Expression Analysis By Quantitative RT-PCR," *RSC Advances*. 5: 4886-4893, 2015. PMCID: PMC4394375.
3. X. Feng, Z. Fu, Y. Jia, S. Kaledhonkar, B. Shah, A. Jin, Z. Liu, M. Sun, B. Chen, R. Grassucci, Y. Ren, H. Jian, J. Frank and Q. Lin, "A Fast and Effective Microfluidic Spraying-plunging Method for High-Resolution Single-Particle Cryo-EM," *Structure*, 25: 663-670, 2017. PMCID: PMC5382802.
4. X. Feng, Y. Jia, H. Jiang and Q. Lin, "Microfabrication-Based Isothermal Titration Calorimetry Using a Combined In-Mixing and Post-Mixing Titration Approach," *Analytical Methods*, 10: 4665- 4670, 2018.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2020 –	Associate Editor, Microfluidics and Nanofluidics, Springer
2018 –	Editorial Board, Journal of Micro-Bio Robotics, Springer
2018 –	Professor of Mechanical Engineering, Columbia University, New York, NY
2017 –	Associate Editor, Biomedical Microdevices, Springer
2009 – 2019	Associate Editor, Sensors and Actuators A: Physical, Elsevier
2009 – 2018	Associate Editor, Journal of Micro-Bio Robotics, Springer
2005 – 2018	Associate Professor of Mechanical Engineering, Columbia University, New York, NY
2003 – 2005	Assistant Professor of Mechanical Engineering and Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA
2000 – 2003	Assistant Professor of Mechanical Engineering, Carnegie Mellon University, Pittsburgh, PA
1998 – 2000	Postdoctoral Scholar, Electrical Engineering, California Institute of Technology
1998 – 1998	Postdoctoral Scholar, Mechanical Engineering, California Institute of Technology
1992 – 1998	Graduate Assistant, Mechanical Engineering, California Institute of Technology

Honors

2018 Cover Article, Analytical Methods. (X. Feng et al., "Microfabrication-Based Isothermal Titration Calorimetry Using a Combined In-Mixing and Post-Mixing Titration Approach," 10, 4665- 4670. Corresponding Author.)

2016 Cover Article, Analytical Methods. (J. Yang et al., "An Integrated Microfluidic Aptasensor for Mass Spectrometric Detection of Vasopressin in Human Plasma Ultrafiltrate," 8:5190-5196. Corresponding Author.)

2015 Huawei Best Sensor Paper, 9th IEEE International Conference on Nano/Molecular Medicine and Engineering (IEEE-NanoMed 2015), Honolulu, Hawaii, (Q. Lin et al. "MEMS-Based Differential Calorimetry for Biomolecular Characterization")

2014 Cover Article, Lab on a Chip. (X. Huang et al., "A Microfabricated Differential Dielectric Affinity Biosensor," 14: 294-301. Corresponding Author.)

2013 C.M. Ho Best Paper Award in Micro/Nano Fluidics, 8th IEEE International Conference on Nano/Micro Engineered and Molecular Systems (NEMS '13), Suzhou, China. (J. Zhu et al., "Physical Modulation Based Cell Manipulation in Microfluidic Devices," Corresponding Author.)
2011 Gold Prize, 11th Annual Diabetes Technology Meeting, Burlingame, CA. (X. Huang et al., "Miniaturized Differential Affinity Sensors for Continuous Glucose Monitoring," Corresponding Author.)
2010 Best Paper Finalist, 5th IEEE International Conference on Nano/Micro Engineered and Molecular Systems (NEMS '10), Xiamen, China. (B. Wang et al., "A Microfluidic Device for Pulsatile Transdermal Delivery for Neurobiological Drugs," Corresponding Author.)
2009 Best Student Paper, 4th IEEE International Conference on Nano/Micro Engineered and Molecular Systems (NEMS '09), Shenzhen, China. (X. Huang et al., "A Biocompatible Affinity MEMS Sensor for Continuous Monitoring of Glucose," Ph.D. Advisor.)
2006 Best Paper Finalist, 1st IEEE International Conference on Nano/Micro Engineered and Molecular Systems (NEMS '06), Zhuhai, China. (L. Wang and Q. Lin, "A MEMS Nanocalorimeter for Biomolecular Characterization," Corresponding Author.)
2003 First Prize, Best Posters, ASME Microfluidic/Biosensor Workshop, Philadelphia, PA. (R. Magargle et al., "Synthesis of Biofluidic Microsystems," Ph.D. Advisor.)

C. Contribution to Science

1. My research has addressed the creation of microfluidic devices that exploit aptamers, which are oligonucleotides that specifically bind to biological targets via affinity interactions, to manipulate biomolecules and cells. My lab has developed microfluidic systems that use surface-immobilized aptamers to enable the enrichment and detection of biomolecules and cells. These devices are highly selective to targets thanks to the specificity of aptamers. Moreover, these devices purposely exploit the stimulus-responsiveness, i.e., the strong environmental (e.g., temperature) dependence of affinity of aptamers to targets. For example, we have demonstrated the use of modest temperature changes to manipulate the reversible aptamer-analyte binding, thereby achieving thermally activated release and isocratic elution of biomolecules and cells after their specific enrichment. This significantly eliminates the need for solvent gradients typically required in traditional approaches. The eluted analytes are then detected via mass spectrometry and fluorescence microscopy, or by integrated nanobiosensing methods such as surface-enhanced Raman spectroscopy and graphene field-effect transistor measurements. (I served as the principal investigator for this effort and all other efforts described below.) A paper resulting from this effort was featured as a cover article in Analytical Methods (August 2016).
    a. C. Wang, J. Kim, J. Zhu, J. Yang, R. Pei, G. Liu, J. Hone, M. Stojanovic and Q. Lin, "An Aptameric Graphene Nanosensor for Label-Free Detection of Small-Molecule Biomarkers," *Biosensors and Bioelectronics*, 71, 222-229, 2015. PMCID: PMC4466219.
    b. J. Yang, J. Zhu, R. Pei, J. Oliver, D. Landry, M. Stojanovic and Q. Lin, "An Integrated Microfluidic Aptasensor for Mass Spectrometric Detection of Vasopressin in Human Plasma Ultrafiltrate," *Analytical Methods*, 8: 5190-5196, 2016. PMCID: PMC5228624.
    c. X. Wang, Y. Zhu, T. Olsen, N. Sun, W. Zhang, R. Pei and Q. Lin, "A Graphene Aptasensor for Biomarker Detection in Human Serum," *Electrochimica Acta*, 290, 356-363, 2018. PMCID: PMC7861490.
    d. Z. Wang, Z. Hao, S. Yu, C. De Moraes, L. Suh X. Zhao and Q. Lin, "An Ultra-Flexible and Stretchable Aptameric Graphene Nanosensor for Biomarker Detection and Monitoring," *Advanced Functional Materials*, doi: 10.1002/adfm.201905202, 2019. PMCID: PMC7861488.

2. Aptamers are highly attractive, but there is often a lack of aptamers for targets of interest. Aptamers are isolated from randomized oligonucleotide libraries through an *in vitro*, iterative process termed systematic evolution of ligands by exponential enrichment (SELEX). Conventional SELEX methods are labor-intensive and time-consuming, which is a major reason for the general lack of aptamers. We exploit microfluidic technology to integrate the SELEX process, with an aim to enable automated and rapid isolation of aptamers to be used as routinely available affinity reagents for both *in vitro* and *in vivo* applications. Our approach is based on a microbead-based protocol to affinity-select and amplify target-binding oligonucleotides. This protocol drastically simplifies the execution and coupling of the affinity selection and amplification procedures, thereby achieving the integration of the entire SELEX process on a single microchip. Using this approach, we have made early demonstration of microfluidic isolation of DNA aptamers against proteins, cells and small molecules, with the SELEX process completed within ~10

hours, a substantial reduction from ~1 month as required by conventional SELEX platforms. A paper resulting from this effort was recognized as a Finalist in the inaugural Best Paper competition of the IEEE International Conference on Micro Electro Mechanical Systems (January 2013).

a. J. Hilton, T. Olsen, J. Kim, J. Zhu, T. Nguyen, M. Barbu, R. Pei, M. Stojanovic and Q. Lin, "Isolation of Thermally Sensitive Protein-Binding Oligonucleotides on a Microchip," *Microfluidics and Nanofluidics*, 19, 795-804, 2015. PMCID: PMC6586242
b. J. Kim, J.P. Hilton, K. Yang, R. Pei, M. Stojanovic and Q. Lin, "Integrated Microfluidic Isolation of Aptamers Using Electrophoretic Oligonucleotide Manipulation," *Scientific Reports*, 6, 26139, 2016. PMCID: PMC4877600.
c. T. Olsen, J. Zhu, J. Kim, R. Pei, M. Stojanovic, and Q. Lin, "An Integrated Microfluidic SELEX Approach Using Combined Electrokinetic and Hydrodynamic Manipulation," *SLAS Technology*, 22: 63-72, 2017. PMCID: PMC5417355.
d. T. Olsen, Z. Zhang, R. Pei, M. Stojanovic and Q. Lin, "Integrated Microfluidic SELEX Using Free Solution Electrokinetics," *J. of the Electrochemical Society*, 164: B3122-B3129, 2017. PMCID: PMC5697788.

3. My research has also produced microfluidic devices for biofluid and cellular manipulation. These devices leverage microflow control devices (e.g., valves and pumps) fabricated of polymeric materials such as polydimethylsiloxane (PDMS) to enable effective handling of fluids, thereby achieving integrated manipulation and interrogation of biomolecules and cells on a single microchip. For example, we have developed microfluidic devices for pulsatile delivery of minute volumes of drug solution in brief time periods into mouse brain and for controlled isolation and immobilization of single or prespecified small numbers of live cells. We have also demonstrated microfluidic devices for integrated genotyping of single nucleotide polymorphisms (SNPs) using microbead- or solution-based procedures. Moreover, we have developed microfluidic approaches that use microbead-based protocols to achieve fully integrated gene profile analysis by reverse transcription–quantitative polymerase chain reaction (RT-qPCR) of low abundance mRNA from a single cell or an array of single cells. More recently, we have developed microfluidic devices to enable spraying-plunging-based preparation of cryoelectron microscopy (cryo-EM) grids with vitreous ice of controllable, highly consistent thickness for time-resolved cryo-EM studies. A paper resulting from this effort was honored as the C.M. Ho Best Paper in Micro/Nano Fluidics at the IEEE International Conference on Nano/Micro Engineered and Molecular Systems (April 2013).
    a. B. Wang, J. Ni, Y. Litvin, D. Pfaff and Q. Lin, "A Microfluidic Approach to Pulsatile Transcranial Delivery of Drugs for Neurobiological Studies," *J. Microelectromechanical Systems*, 21: 53-61, 2012. PMCID: PMC3840590.
    b. J. Zhu, J. Shang, T. Olsen, K. Liu, D. Brenner and Q. Lin. "A Mechanically Tunable Cell-Trapping Device," *Sensors and Actuators A: Physical*, 215, 197–203, 2014. PMCID: PMC4371545.
    c. H. Sun, T. Olsen, J. Zhu, J. Tao, B. Ponnaiya, S. Amundson, D. Brenner and Q. Lin, "A Microfluidic Approach to Parallelized Transcriptional Profiling of Single Cells," *Microfluidics and Nanofluidics*, 19, 1429-1440, 2015. PMCID: PMC4868186.
    d. X. Feng, Z. Fu, Y. Jia, S. Kaledhonkar, B. Shah, A. Jin, Z. Liu, M. Sun, B. Chen, R. Grassucci, Y. Ren, H. Jian, J. Frank and Q. Lin, "A Fast and Effective Microfluidic Spraying-plunging Method for High-Resolution Single-Particle Cryo-EM," *Structure*, 25: 663-670, 2017. PMCID: PMC5382802.

4. Continuous glucose monitoring (CGM) involves monitoring blood glucose concentrations, on a continuous basis, in patients with diabetes. My lab aims to address the urgent need of CGM for improved reliability and accuracy by combining specific recognition of glucose, via its reversible affinity binding to a synthetic polymer, with integrated microscale transducers created by microelectromechanical systems (MEMS) technology. This effort has resulted in subcutaneously implantable affinity MEMS biosensors. These miniaturized devices determine glucose concentrations in interstitial fluid within subcutaneous tissue by using MEMS transducers to measure changes in physical properties of a synthetic polymer, such as poly(acrylamide-ran-3-acrylamidophenylboronic acid) (PAA-ran-PAAPBA), as it binds reversibly to glucose via affinity interactions. For example, we have reported MEMS affinity sensors that perform vibration-based measurements of changes in the viscosity of a polymer solution due to glucose binding, and that make capacitive measurements of glucose binding-induced changes in the dielectric coefficient of the polymer

solution. Testing in laboratory mice has demonstrated that these devices hold the promise to enable rapid, accurate and reliable continuous monitoring of glucose concentrations. Results from this research were awarded the Gold Prize at the Diabetes Technology Meeting (Burlingame, CA, October 2011), and featured as a cover article in *Lab on a Chip* (February 2014).

a. X. Huang, J. Oxsher, C. LeDuc, Y. Ravussin, Q. Wang, D. Li, D. Accili, R. Leibel and Q. Lin, "A MEMS Differential Viscometric Sensor for Affinity Glucose Detection in Continuous Glucose Monitoring," *J. of Micromechanics and Microengineering*. 23 (5): 055020, 2013. PMCID: PMC3743269.
b. X. Huang, S. Li, E. Davis, D. Li, Q. Wang and Q. Lin, "A MEMS Dielectric Affinity Glucose Biosensor," *J. of Microelectromechanical Systems*. 23: 14-20, 2014. PMCID: PMC3915936.
c. X. Huang, J. Oxsher, C. LeDuc, Y. Ravussin, Q. Wang, D. Accili, R. Leibel and Q. Lin, "A Microfabricated Differential Dielectric Affinity Biosensor," *Lab on a Chip*. 14: 294-301, 2014. PMCID: PMC3893139.
d. Z. Zhang, J. Shang, J. Yan, Q. Wang and Q. Lin, "A Dielectric Affinity Glucose Microsensor Using Hydrogel-Functionalized Coplanar Electrodes," *Microfluidics and Nanofluidics*, 21: 93-100, 2017. PMCID: PMC6586246.

5. Biomolecular interactions and conformational transitions are in general temperature-dependent; understanding such behavior can offer valuable insight for both basic studies and practical applications. My lab has pursued MEMS-based characterization of temperature-dependent biomolecular behavior, such as measurements of thermally active biochemical reactions and studies of temperature-dependent kinetics of biomolecules. These in particular include differential biocalorimetry, which measures heat evolved in biological processes in solution in a label-free manner. Conventional calorimetry methods are expensive, use complicated design and construction, and require long analysis times. By integrating highly sensitive MEMS thermal transduction with microfluidic liquid handling, my lab has demonstrated miniaturized differential calorimeters. We have demonstrated MEMS devices for differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC). These devices feature thermally isolated calorimetric structures integrated with highly sensitive thermoelectric sensors, allowing DSC and ITC measurements of biomolecular interactions in small, well-defined volumes for quantitative determination of thermodynamic properties of biomolecular binding. Results from this research received the Huawei Best Sensor Paper award at the IEEE International Conference on Nano/Molecular Medicine and Engineering (November 2015), and were featured as a cover article in Analytical Methods (October 2018).

a. B. Wang, J. Fei, R. Gonzalez and Q. Lin, "A Microfluidic Approach for Investigating the Temperature Dependence of Biomolecular Activity with Single-Molecule Resolution," *Lab on a Chip*, 11: 274-281, 2011. PMCID: PMC3766768.
b. B. Wang and Q. Lin, "A MEMS Differential Scanning Calorimeter for Thermodynamic Characterization of Biomolecules," *J. Microelectromechanical Systems*, 21: 1165-1171, 2012.
c. Y. Jia, Z. Zhang and Q. Lin, "Isothermal Titration Calorimetry in a Polymeric Microdevice," *Microfluidics and Nanofluidics*, 21:90, 2017.
d. X. Feng, Y. Jia, H. Jiang and Q. Lin, "Microfabrication-Based Isothermal Titration Calorimetry Using a Combined In-mixing and Post-mixing Titration Approach," *Analytical Methods*, 10, 4665-4670, 2018.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/qiao.lin.2/bibliography/public/

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: Clarke, Oliver B

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

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
University of Melbourne	BSc(Hons)	11/2007	Chemistry & Biochemistry
University of Melbourne (Walter & Eliza Hall Institute of Medical Research)	PhD	07/2011	Structural Biology
Columbia University	Posdoctoral training	09/2017	Structural Biology

A. Personal Statement

My research has focused on the structural analysis of membrane proteins by single particle cryoelectron microscopy (cryoEM), with a particular interest in ion channel gating and activation. I am currently PI on an NIH R01 grant aimed at understanding the structural basis of regulation of the skeletal muscle ryanodine receptor by ligands and protein binding partners, and also serve as CoI on a number of other NIH-funded grants. My laboratory has also been engaged in work to understand the molecular architecture of the red blood cell membrane, for the continuance of which we recently obtained an R01 grant from NHLBI, with a particular focus on the ankyrin complex, which clusters key membrane proteins involved in membrane transport and links the membrane to the cytoskeleton. I have trained two graduate students (Kookjoo Kim and Emmanuel Afriyie), as well as two postdoctoral researchers (Dr. Francesca Vallese and Dr. Huan Li) in cryo-electron microscopy and structural analysis since starting my lab in November 2017. In addition, I have mentored numerous undergraduate students in summer placements, including five students from underrepresented groups mentored as part of the SPURS program at Columbia.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2017- present Assistant Professor, Columbia University, Department of Anesthesiology (Secondary appointments in Physiology & Cellular Biophysics and the Irving Institute for Clinical and Translational Research)

2012-2017 Postdoctoral Fellow, Columbia University, Dept. Biochem & Mol. Biophys.

Honors

2023 Irma T. Hirschl Research Award

2014 Charles H. Revson Senior Fellowship

C. Contributions to Science

1. *Ion channel architecture and gating*. During my graduate work, I described the structure of multiple conformational states of a bacterial inwardly rectifying postassium channel, KirBac 3.1, determined by X-ray crystallography. This work led to an ongoing fascination with the structure and activation and gating mechanisms of ion channels, which I further explored during my postdoctoral work in the laboratory of Wayne Hendrickson, which entailed solving the structure of the ryanodine receptor RyR1, an intracellular calcium release channel of exceptional size and complexity. This project, initially a collaboration between the Hendrickson lab and that of Andrew Marks, was initially focused on the use of X-ray crystallography to solve the structure of the channel, but obtaining well-diffracting crystals proved difficult. As a result, I switched techniques, initiating a collaboration with the laboratory of Joachim Frank, which resulted in a reconstruction of the closed, ligand-free state of the receptor at 4.8 Å, and later the structures of multiple ligand-bound conformations including open states at resolutions up to 3.7 Å. This work allowed me to gain experience in cryoEM data collection, processing, and the building and refinement of macromolecular models into EM maps. I have continued structural studies of the skeletal muscle ryanodine receptor in my own laboratory, with a focus on understanding how ligands and binding partners binding in peripheral regions of the receptor allosterically regulate channel gating. In recent, unpublished work, we have solved cryoEM structures of the receptor at resolutions up to 2.1Å in complex with the malignant hyperthermia therapeutic dantrolene, unexpectedly showing that dantrolene binds to the first tandem repeat (RY12) domain of RyR1 in concert with adenine nucleotides. In follow up work we determined that this site is a novel binding site for ATP and ADP, even in the absence of dantrolene, and we are currently investigating the functional and physiological significance of this site.

a. **Clarke OB**, Caputo AT, Hill AP, Vandenberg JI, Smith BJ, Gulbis JM. Domain reorientation and rotation of an intracellular assembly regulate conduction in Kir potassium channels. Cell. 2010; 141(6):1018-29. PMID: 20564790

b. Zalk R[*], **Clarke OB**[*], des Georges A[*], Grassucci RA, Reiken S, Mancia F, Hendrickson WA, Frank J, Marks AR. Structure of a mammalian ryanodine receptor. Nature. 2015; 517(7532):44-9. PMCID: PMC4300236

c. des Georges A[*], **Clarke OB**[*], Zalk R[*], Yuan Q, Condon KJ, Grassucci RA, Hendrickson WA, Marks AR, Frank J. Structural Basis for Gating and Activation of RyR1. Cell. 2016; 167(1):145-157.e17. PMCID: PMC5142848

d. Melville Z, Kim K, **Clarke OB**, Marks AR. High-resolution structure of the membrane-embedded skeletal muscle ryanodine receptor. Structure. 2022 Jan 6;30(1):172-180.e3. PMCID: PMC8741649

2. *Structure and conformational dynamics of class C GPCRs*. In collaborative effort lead by Qing Fan and her team, we have been involved in structural studies of two class C GPCRs, the $GABA_B$ receptor and the Calcium sensing receptor (CaSR). Unlike GPCRs from better characterized groups such as the class A receptors, which have ligand binding sites within the classical 7TM bundle, class C GPCRs sense signals via ligand binding to a large extracellular domain. Until recently, there has been little structural information available for intact class C receptors, as the flexible hinge between the transmembrane region and the ligand binding domains makes them recalcitrant to crystallization, and difficult targets even for single particle cryoEM. In our initial work, we have solved the structure of the heterodimeric human $GABA_B$ receptor by cryoEM, in an inactive state. The structure reveals the binding site of multiple ligands of the receptor, including the unexpected presence of native lipids in a transmembrane cleft. Recently, we solved the structure of a second class C GPCR, the homodimeric CaSR, in both active and inactive states, giving insights into the mechanism of receptor activation and modulation by ligands. Much of the cryoEM data processing was performed in my laboratory, and I am co-corresponding author on both of the published works.

a. Park J, Fu Z, Frangaj A, Liu J, Mosyak L, Shen T, Slavkovich VN, Ray KM, Taura J, Cao B, Geng Y, Zuo H, Kou Y, Grassucci R, Chen S, Liu Z, Lin X, Williams JP, Rice WJ, Eng ET, Huang RK, Soni RK, Kloss

B, Yu Z, Javitch JA, Hendrickson WA, Slesinger PA, Quick M, Graziano J, Yu H, Fiehn O, **Clarke OB**[*], Frank J[*], Fan QR[*]. Structure of human $GABA_B$ receptor in an inactive state. Nature. 2020 Aug;584(7820):304-309. PMCID: PMC7725281

b. Park J, Zuo H, Frangaj A, Fu Z, Yen LY, Zhang Z, Mosyak L, Slavkovich VN, Liu J, Ray KM, Cao B, Vallese F, Geng Y, Chen S, Grassucci R, Dandey VP, Tan YZ, Eng E, Lee Y, Kloss B, Liu Z, Hendrickson WA[*], Potter CS, Carragher B, Graziano J, Conigrave AD[*], Frank J[*], **Clarke OB**[*], Fan QR[*]. Symmetric activation and modulation of the human calcium-sensing receptor. Proc Natl Acad Sci U S A. 2021 Dec 21;118(51) PMID: 34916296

3. *Molecular architecture of the red blood cell membrane*. In a new direction for my laboratory, we are investigating the molecular architecture of the red blood cell membrane, as it represents both a tractable model system for understanding the organization and architecture of eukaryotic plasma membranes in general, and is also of substantial physiological and biomedical significance in its own right. We are taking a top-down approach to this problem, involving differential solubilization of the membrane using mild detergents, followed by density gradient centrifugation and size exclusion chromatography to separate out large membrane protein complexes. In initial results, we have solved the structure of the 1.2MDa erythrocyte ankyrin complex, which acts to mechanically stabilize the membrane by linking the spectrin-actin cytoskeleton to the membrane, as well as to cluster key membrane proteins involved in ion transport, pH regulation and regulation of cell shape and volume.

a. Vallese F, Kim K, Yen LY, Johnston JD, Noble AJ, Calì T, **Clarke OB**. Architecture of the Human Erythrocyte Ankyrin-1 Complex. Nature Structural & Molecular Biology 29, no. 7 (July 1, 2022): 706–18.

4. *Structure of mammalian thyroglobulin.* Thyroglobulin is a large, flexible, homodimeric protein that acts as both scaffold and substrate for the synthesis of the thyroid hormones, thyroxine and triiodothyronine, as well as serving as a major site of iodine storage. Until recently, no structural information was available concerning the architecture of the protein or the mechanism of hormonogenesis. In order to address this gap in knowledge, we sought to use single particle cryo-EM to solve the structure of bovine thyroglobulin, in collaboration with a leading laboratory in the field, the group of Peter Arvan at the University of Michigan. We solved the structure of bovine thyroglobulin in a natively iodinated state at 2.5Å, identifying two key acceptor-donor tyrosine pairs, and visualizing the bound hormone in the context of the thyroglobulin structure.

a. Kim K, Kopylov M, Bobe D, Kelley K, Eng ET, Arvan P, **Clarke OB**. The structure of natively iodinated bovine thyroglobulin. Acta Crystallogr D Struct Biol. 2021 Nov 1;77(Pt 11):1451-1459.

5. *Structure of cytochrome bo_3.* After accidentally purified E. coli cytochrome bo_3 ubiquinol oxidase and reconstituting it into lipid nanodiscs, while attempting to express another membrane protein of interest, we solved the structure of this important respiratory enzyme to 2.2 Å by cryo-EM, revealing the arrangement of metal redox centers and the binding mode of the ubqiquinone-8 substrate. This work which was conducted in collaboration with the Gennis, Zhang, Zhu and Tajkhorshid laboratories was published earlier this year in *PNAS*. I am co-corresponding author on this publication.

a. Li J, Han L, Vallese F, Ding Z, Choi SK, Hong S, Luo Y, Liu B, Chan CK, Tajkhorshid E, Zhu J[*], **Clarke O**[*], Zhang K[*], Gennis R[*]. Cryo-EM structures of *Escherichia coli* cytochrome bo_3 reveal bound phospholipids and ubiquinone-8 in a dynamic substrate binding site. Proc Natl Acad Sci U S A. 2021 Aug 24;118(34). doi: 10.1073/pnas.2106750118.

Complete list of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1Nur6sw6gnmkw/bibliography/public/

D. Grant support

Active:

R01AR077720

04/01/21-03/31/26

Structural basis for allosteric regulation of RyR1

Major goals: The goal of this proposal is to understand how small molecules and protein binding partners regulate RyR1 activity by binding to peripheral sites, using a combination of cryoEM and functional approaches.

Role: PI

R01HL168178

08/20/23-08/20/27

Architecture, dynamics and regulation of erythrocyte ankyrin-1 complexes

Major goals: The goal of this proposal is to use both structural and functional approaches to interrogate the role of ankyrin complexes in both modulating membrane curvature and the activity of the band 3 anion exchanger, a key element of the erythrocyte ankyrin-1 complex.

Role: PI

R01 NS109366

08/15/19-06/30/24

Structural studies of HCN channels in health and disease

Major goals: This project is aimed at understanding the structural basis of gating in the HCN4 channel, and regulation of gating by accessory proteins.

Role: CoI

R01 GM144620

9/22/2023 – 8/31/2024

Microfluidic Preparation of Specimens to Enable Submillisecond Time-Resolved Cryo-EM

Major goals: To develop a microfluidic tool for preparing specimens with reaction times down to submilliseconds for time-resolved cryo-EM studies of short-lived intermediates of biomolecular systems.

Role: CoI

Recently completed:

R01 HL145473

08/23/19-04/30/23

Structure-function analysis for elucidating pathogenicity of cardiac ryanodine receptor genetic variants

This project was aimed at understanding using structure/function approaches how pathogenic mutations in RyR2 lead to channel activation.

Role: CoI