BIOGRAPHICAL SKETCH

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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	Postdoctoral	09/2017	Structural Biology

A. Personal Statement

As an Assistant Professor at Columbia University, I am well positioned to advance the proposed program of research. Broadly, my research as a postodoctoral scientist in the Hendrickson laboratory, and now in my capacity as an independent investigator has focused on the structural analysis of membrane proteins by single particle cryoelectron microscopy (cryoEM)[1-4], with a particular focus on ion channel gating and activation. The experience and skills I have gained in these works will be invaluable in ensuring successful completion of the work outlined in this proposal. Many of the issues that we anticipate may be encountered during processing of the data generated during the course of this proposal (variable symmetry; conformational and compositional heterogeneity; identification of ligand binding sites) are issues that I have already encountered and successfully addressed during my work on both the structure and gating mechanism of the RyR1 intracellular calcium release channel[1,3], the structure of the GABAB receptor[4], and the structure of the STRA6 receptor for retinol uptake[2].

1. 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.

2. Chen Y*, Clarke OB*, Kim J, Stowe S, Kim YK, Assur Z, Cavalier M, Godoy-Ruiz R, von Alpen DC, Manzini C, Blaner WS, Frank J, Quadro L, Weber DJ, Shapiro L, Hendrickson WA, Mancia F. Structure of the STRA6 receptor for retinol uptake. Science. 2016. 353(6302)

3. 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

4. Park et al**. Structure of human $GABA_B$ receptor in an inactive state. Nature. 2020.

*Authors contributed equally
** co-corresponding author

B. Positions and Honors

Positions and Employment

2012-2017 Postdoctoral Fellow, Columbia University, Dept. Biochem & Mol. Biophys.
2017- present Assistant Professor, Columbia University, Department of Anesthesiology (Secondary appointments in Physiology & Cellular Biophysics and the Irving Institute for Clinical and Translational Research)

Honors

2012 Overseas Biomedical Fellowship (NHMRC, Australia)
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 lead to an ongoing fascination with the structure and activation and gating mechanisms of ion channels, which I further explored in my main research project in the laboratory of Wayne Hendrickson, which entailed solving the structure of the ryanodine receptor, 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 receptor at 4.8 Å, and allowed me to gain experience in cryoEM data collection, processing, and the building and refinement of macromolecular models into EM maps. This work is ongoing; Having initially described the architecture of the closed state of the receptor, we have now obtained reconstructions at resolutions up to 3.7Å of multiple activated and ligand-bound states, helping elucidate the details of the conformational changes that occur during activation and gating.

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.

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.

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

2. *Structural analysis of an integral membrane receptor complex by cryoelectron microscopy.* A collaboration with the laboratory of Filippo Mancia provided me with further experience in the structural analysis of membrane protein complexes by cryoEM. The Mancia group had a longstanding interest in structural characterization of the receptor STRA6, which mediates the uptake of retinol from serum, where it is complexed with Retinol Binding Protein (RBP). I was the lead cryoelectron microscopist on this project, which resulted in a 3.9 Å reconstruction of the STRA6 receptor complex, into which I was able to build and refine a near complete atomic model. The structure revealed several surprises, the most immediate of which was that STRA6 functions as an obligate dimer in which the C-terminal tail of each protomer is intimately associated with a molecule of copurified calmodulin, an association which was entirely unexpected, but which Yunting Chen in the Mancia laboratory has since confirmed occurs in vivo. The STRA6/CaM association suggests the possibility of cross-talk between the Ca^{2+} and retinoid signaling pathways, an avenue of research which is being actively explored.

a. Chen Y*, Clarke OB*, Kim J, Stowe S, Kim YK, Assur Z, Cavalier M, Godoy-Ruiz R, von Alpen DC, Manzini C, Blaner WS, Frank J, Quadro L, Weber DJ, Shapiro L, Hendrickson WA, Mancia F. Structure of the STRA6 receptor for retinol uptake. Science. 2016. 353(6302)

3. *The structure and mechanism of CDP-alcohol phosphotransferases and other integral membrane enzymes.* The CDP-alcohol phosphotransferase (CDP-AP) family of integral membrane enzymes catalyses the transfer of a substituted phosphate group from a CDP-linked donor to an alcohol acceptor. This is an essential reaction for phospholipid biosynthesis, and it is catalysed solely by CDP-APs. As part of a collaboration with the laboratory of Filippo Mancia, I helped determine the first structure of a CDP, of the archaeal orphan enzyme AF2299, which processes two soluble substrates of unknown identity, but the structure of which nevertheless gave insight into the mode of CDP-binding and the role of the signature sequence shared by all members of the family. AF2299 is an unusual CDP-AP, in that it possesses a soluble domain that many other CDP-APs lack. CDP-APs that process lipidic substrates were initially recalcitrant to crystallization, and I hypothesized that the soluble domain might be the key factor in the successful crystallization of AF2299. After fusing the soluble domain of AF2299 to the N-terminus of a bona fide lipid-processing CDP-AP, phosphatidylinositolphosphate synthase from *Renibacterium salmoninarum*, crystals were readily obtained from lipidic cubic phase trials, leading to a structure of the enzyme alone at 2.5Å, and of the enzyme in complex with the lipid-linked donor CDP-diacylglycerol at 3.6Å, revealing the location of the acceptor substrate site, and the molecular determinants of substrate specificity and catalysis. In addition to my work on the CDP-alcohol phosphotransferase family of integral membrane enzymes, I have also contributed to the structural analysis of other families of lipid-modifying enzymes studied within the Mancia lab, including the glycosyltransferases GtrB and ArnT.

a. Sciara G*, Clarke OB*, Tomasek D*, Kloss B, Tabuso S, Byfield R, Cohn R, Banerjee S, Rajashankar KR, Slavkovic V, Graziano JH, Shapiro L, Mancia F. Structural basis for catalysis in a CDP-alcohol phosphotransferase. Nat Comms. 2014; 5:4068.

b. Clarke OB, Tomasek D, Jorge CD, Dufrisne MB, Kim M, Banerjee S, Rajashankar KR, Shapiro L, Hendrickson WA, Santos H, Mancia F. Structural basis for phosphatidylinositol-phosphate biosynthesis. Nat Comms. 2015; 6:8505.

c. Ardiccioni C*, Clarke OB*, Tomasek D, Issa HA, von Alpen DC, Pond HL, Banerjee S, Rajashankar KR, Liu Q, Guan Z, Li C, Kloss B, Bruni R, Kloppmann E, Rost B, Manzini MC, Shapiro L, Mancia F. Structure of the polyisoprenyl-phosphate glycosyltransferase GtrB and insights into the mechanism of catalysis. Nature communications. 2016; 7:10175.

d. Petrou VI, Herrera CM, Schultz KM, Clarke OB, Vendome J, Tomasek D, Banerjee S, Rajashankar KR, Belcher Dufrisne M, Kloss B, Kloppmann E, Rost B, Klug CS, Trent MS, Shapiro L, Mancia F. Structures of aminoarabinose transferase ArnT suggest a molecular basis for lipid A glycosylation. Science (New York, N.Y.). 2016; 351(6273):608-12.

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

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

Ongoing Research Support:

(New Award)
R01AR077720 (Clarke) 04/01/21-03/31/22

Structural basis for allosteric regulation of RyR1
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.

R01 NS109366-01A1 08/15/19-06/30/24
Structural studies of HCN channels in health and disease
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 HL145473 08/23/19-04/30/23
Structure-function analysis for elucidating pathogenicity of cardiac ryanodine receptor genetic variants
This project is aimed at understanding using structure/function approaches how pathogenic mutations in RyR2 lead to channel activation.
Role: CoI

American Thyroid Association Research Grant
Structure determination of mammalian thyroglobulin by cryoEM 06/01/19-06/01/21
The goal of this project is to obtain a structure of mammalian thyroglobulin in multiple states, in order to understand the structural basis of thyroid hormone biogenesis. Towards this end, we have obtained a 2.5 Å structure of bovine thyroglobulin by cryoEM, and are finalizing a manuscript describing these findings, including a structural description of one of the primary sites of hormone biogenesis.
Role: PI

Completed Research Support
Columbia University CTSA Pilot Award 06/01/18 - 05/31/19
Novel approach to modeling pathogenicity of CPVT mutations for improving clinical management
The goal of the project was to use structural and bioinformatic data on the ryanodine receptor to improve predictions of the pathogenicity of variants of unknown significance (VUSs).
Role: PI

BIOGRAPHICAL SKETCH

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NAME: Vallese, Francesca

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

POSITION TITLE: Associate Research Scientist

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 Padova	BS	03/2009	Biotechnology
University of Padova	Ph.D.	03/2013	Biosciences and Biotechnology
University of Padova	Post Doc	07/2019	Structural Biology
Columbia University	Ass. Reserch	09/2021	Cryo-EM

A. Personal Statement

As an Associate Research Scientist at Columbia University, I have the possibility access all the instruments and facilities that can help in this research. My past research as a postdoctoral scientist has focused on the biochemical characterization and structural analysis of membrane proteins, complexes and soluble proteins. The experience and skills I have gained in expression and purification of proteins in different expression systems and the use of different technique for the biochemical characterization of different proteins and protein complexes will be invaluable in ensuring successful my contribution in this proposal. In the past two years I learned how to prepare samples for cryo-EM and how to use microscopes for screening and collection of images.

B. Positions and Honors

2020 Finalist at the Finalist in Anesthesiology Academic Evening
2020 Finalist at the ISSNAF YI Awards
2019-present Associate Research Scientist, Columbia University, Department of Anesthesiology
2015-2019 Postdoctoral Fellow, University of Padova, Dept. Biomedical Science.
2014-2015 Postdoctoral Fellow, Telethon grant, project N°GGP14187A, University of Padova, Dept. Biomedical Science.

C. Contributions to Science

1. Biochemical characterization of [FeFe]-hydrogenase maturation pathway. During my graduate work, I was involved in the characterization of the [FeFe]-hydrogenase maturation pathway, an important factor for hydrogen production. In particular, the aim of my project was to investigate the interactions between proteins involved in [FeFe]-hydrogenase maturation and to solve the three-dimensional structure of HydF, the key protein of the system of maturation, and the study of its domain involved in the binding of an FeS

cluster. In that period, I learned molecular biology techniques such as cloning and mutagenesis, expression in *E.coli*, size exclusion chromatography, DLS, and circular dichroism spectroscopy. this experience has made me independent in the expression, purification and characterization of proteins and protein complexes.

a)Albertini M, Galazzo L, Maso L, Vallese F, Berto P, De Rosa E, Di Valentin M, Costantini P, Carbonera D. "Characterization of the [FeFe]-Hydrogenase Maturation Protein HydF by EPR Techniques: Insights into the Catalytic Mechanism". Topic in catalysis. 2015. 58(12-13).

b) Vallese F*, Berto P, Ruzzene M, Cendron L, Sarno S, De Rosa E, Giacometti GM, Costantini P. Biochemical analysis of the interactions between the proteins involved in the [FeFe]-hydrogenase maturation process.*J Biol Chem*. 2012 Oct 19;287(43):36544-55.

c) Berto P, Di Valentin M, Cendron L, Vallese F, Albertini M, Salvadori E, Giacometti GM, Carbonera D, Costantini P. "The [4Fe-4S]-cluster coordination of [FeFe]-hydrogenase maturation protein HydF as revealed by EPR and HYSCORE spectroscopies". *Biochim Biophys Acta.* 2012 Dec;1817(12):2149-57.

d) Cendron L, Berto P, D'Adamo S, Vallese F, Govoni C, Posewitz MC, Giacometti GM, Costantini P, Zanotti G. "Crystal structure of HydF scaffold protein provides insights into [FeFe]-hydrogenase maturation". *J Biol Chem.* 2011 Dec 23;286(51):43944-50.

2. *Structural analysis of H. pylori* proteins. During my postdoc I studied proteins from *H. pylori*, an organism that chronically infects the gastric mucosa of the majority of the human population, and it is implicated in the development of severe gastroduodenal diseases, including active chronic gastritis, peptic ulcers, gastric adenocarcinoma and mucosa-associated lymphoma. This bacterium is interesting not only because it is a poorly characterized human pathogen, but also for other reasons: it lives only in a very special niche, the acidic environment of the human stomach, and during evolution it has adapted to this peculiar and harsh environment. In that period in Professor Zanotti's Lab. I solved the structure of five *H. pylori* proteins involved in pathogenicity, colonization and persistence.

a) Capitani N*, Codolo G*, Vallese F, Minervini G, Grassi A, Cianchi F, Troilo A, Fischer W, Zanotti G, Baldari CT, de Bernard M, D'Elios MM. "The lipoprotein HP1454 of Helicobacter pylori regulates T-cell response by shaping T-cell receptor signaling". Cell Microbiol. 2019 Jan 15.

b) Cieri D*, Vicario M*, Vallese F, Berto P, Grinzato A, Brini M, Cali' T. "Tau localizes within mitochondrial sub-compartments and its caspase cleavage affects ER-mitochondria interactions and cellular Ca2+ handling" Biochim Biophys Acta Mol Basis Dis. 2018 Jul 11.

c) Vallese F*, Mishra NM*, Pagliari M, Berto P, Codolo G, de Bernard M, Zanotti G. "Helicobacter pylori antigenic Lpp20 is a structural homologue of Tipα and promotes epithelial-mesenchymal transition". Biochim Biophys Acta. 2017 Sep 22.

d) D'Elios MM, Vallese F, Capitani N, Benagiano M, Bernardini ML, Rossi M, Rossi GP, Ferrari M, Baldari CT, Zanotti G, de Bernard M, Codolo G. "The Helicobacter cinaedi antigen CAIP participates in atherosclerotic inflammation by promoting the differentiation of macrophages in foam cells". Sci Rep. 2017 Jan 11.

e) Compostella ME, Berto P, Vallese F, Zanotti G. "Structure of a-carbonic anhydrase from the human pathogen Helicobacter pylori" Acta Cryst F. 2015.

f) Vallese F*, Percudani R, Fischer W, Zanotti G. "The crystal structure of Helicobacter pylori HP1029 highlights the functional diversity of the sialic acid-related DUF386 family" FEBS J. 2015

3. Purification of MICUs protein involved in MCU regulation. A collaboration with the laboratory of Professor Rosario Rizzuto provided me further experience in the biochemical analysis of membrane protein complexes. The Rizzuto group had a longstanding interest in functional characterization of the mitochondrial calcium uniporter (MCU) complex, a milestone on the route towards a deeper comprehension of the complexity of global Ca^{2+} signaling. Thanks to this collaboration I learned to use protein expression systems such as mammalian cells and *Lactococcus lactis*. I was involved in biochemical, biophysical and structural studies of the proteins that are part of the MCU complex. The Identification of drugs that regulate the channel was part of this project which resulted in the identification of two negative regulators of the calcium channel. I also

characterized a Micu1 splice variant expressed in some tissue which was found to be of fundamental importance in the homeostasis of calcium in skeletal muscle.

a) Di Marco G, Vallese F, Jourde B, Bergsdorf C, Sturlese M, De Mario A, Techer-Etienne V, Haasen D, Oberhauser B, Schleeger S, Minetti G, Moro S, Rizzuto R, De Stefani D, Fornaro M, Mammucari C. “A High-Throughput Screening Identifies MICU1 Targeting Compounds”. Cell Rep. 2020 Feb 18;30(7):2321-2331.e6. doi: 10.1016/j.celrep.2020.01.081.PMID: 32075766 Free PMC article.
b) Vecelio Reane D, Vallese F, Checchetto V, Acquasaliente L, De Filippis V, Zanotti G, Rizzuto R, Raffaello A. “A MICU1 splice variant confers high sensitivity to the Ca2+ uptake machinery of skeletal muscle”. Molecular Cell. 2016 Nov 3.

Complete list of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/12uaYs8W5ofQos/bibliography/public/

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