BIOGRAPHICAL SKETCH

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NAME: Ewan Kenneth Strome McRae

eRA COMMONS USER NAME (credential, e.g., agency login): EMCRAE2023

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 Manitoba	B.Sc. Hons	01/2015	Biochemistry
University of Manitoba	Ph.D.	05/2019	Biochemistry
Aarhus University	Postdoctoral	05/2022	Structural Biology
LMB-MRC	Postdoctoral	01/2023	Structural Biology

A. Personal Statement

I am an early career researcher in the Center for RNA Therapeutics at the Houston Methodist Research Institute (HMRI). I have recently started my lab (in March 2023) with the support of a “Recruitment of first-time tenure-track faculty members” award from the Cancer Prevention Research Institute of Texas (CPRIT).

As a Ph.D student I learned a broad range of wet-lab biochemical and biophysical assays to study RNA-protein interactions, which allowed me to characterize novel RNA-protein interactions and identify new enzymatic activities of the helicase enzyme DDX21. As part of this work, I used a diverse set of mammalian cell culture and molecular biology techniques to investigate mechanisms of post-transcriptional gene expression regulation and identify mRNA that DDX21 regulates. This experience makes me well prepared to manage the currently proposed project where we will measure RNA-protein interactions using BLI and culture HUVEC cells for Calcium mobilization assays.

As a post-doctoral fellow, I focused on RNA structure design and determination using the RNA origami method and cryogenic electron microscopy, respectively. As part of this work, I established a structural biology pipeline for cryo-EM that allowed for the routine characterization of RNA samples at the electron microscope. During this time, I learned principles for RNA structure design as well as how to effectively use the RNA Origami Automated Design (ROAD) software from its inventors Cody Geary and Ebbe Andersen. The RNA origami method serves as the basis for the modular platform we are developing in this proposal. Among the projects I worked on in the Andersen lab was the design and structural characterization of a structure switching RNA origami that utilized toe-hold mediated strand displacement to turn on and off fluorescence as triggered by arbitrary RNA sequences. This work, now published in Science Advances, is directly relevant to the currently proposed project where we will use the ROAD software to design structure switching aptamers. While the Andersen Lab continues to use this system for synthetic biology related research, it is my aim to utilize these design methods for applications in medicine.

I would like to highlight one grant and these manuscripts which are directly relevant to the work proposed here:

1) RR230015 - Recruitment of First-Time, Tenure-Track Faculty Members, **McRae, E.K.S.** (PI) - The Methodist, Hospital Research Institute, Structure based design of RNA therapeutics, (03/2023-02/2028)
2) **McRae, E. K. S.** *et al.* Structure, folding and flexibility of co-transcriptional RNA origami. Nat. Nanotechnol. 1–10 (2023) doi:10.1038/s41565-023-01321-6 PMID: 36849548.
3) Sampedro Vallina, N., **McRae, E. K. S.**, Geary, C. & Andersen, E. S. An RNA Paranemic Crossover Triangle as A 3D Module for Cotranscriptional Nanoassembly. Small 19, 2204651 (2023) PMID: 36526605**.**
4) Vallina, N. S., **McRae, E. K. S.**, Geary, C. & Andersen, E. S. An RNA origami robot that traps and releases a fluorescent aptamer. Sci. Adv. 10, eadk1250 (2024) PMID: 38507482.

B. Positions, Scientific Appointments, and Honors

Positions & Scientific Appointments:

2023/3 - present Assistant professor, Houston Methodist Research Institute, Houston, TX, USA
2022/9 - 2023/1 Research Scientist, MRC-LMB, Cambridge, UK
2019/5 - 2022/6 Post Doctoral Fellow at iNano, Aarhus University, Denmark
2015/1 - 2019/5 Ph.D. Student at Department of Chemistry, University of Manitoba
2017/9 - 2018/3 Teaching assistant - Advanced Biochem Lab, The University of Manitoba
2014/9 - 2015/4 Teaching Assistant - Biochem 1 & 2 Labs, The University of Manitoba
2014/9 - 2015/1 Undergraduate Researcher Chemistry, The University of Manitoba

Awards and Honors:

2023/2 - **CPRIT recruitment of first-time tenure track faculty award $2,000,000 over 5 years**
2022/4 - FNANO conference - Best Oral presentation Award
2020/5 - Distinguished Dissertation award – University of Manitoba
2019/9 - **Post Doctoral Fellowship - Natural Sciences & Engineering Research Council of Canada - $140,000 over 2 years**
2018/4 - Best Oral Presentation Award - RiboWest / RiboClub
2017/9 - **Alexander Graham Bell Ph.D. Scholarship - Natural Sciences & Engineering Research Council of Canada - $140,000 over 4 years**
2016/9 - PhD Studentship - Research Manitoba
2015/9 - University of Manitoba Graduate Fellowship
2015/2 - Faculty of Science award, University of Manitoba

C. Contributions to Science

1. Advancements in Cryo-EM for RNA Structure Determination

My research in the Andersen lab has focused on cryogenic electron microscopy, this method allowed me to investigate the structure of our designed RNA in a native hydrated state where previously they had only been observed attached to a surface (i.e. by AFM or NS-TEM). The 3D RNA structures revealed that the designs were twisted compared to our predicted models. A high-resolution structure of one of our designs allowed us to ascertain that one of the core structural motifs that we use in our RNA origami structures was folded differently than had been reported in the crystal structure, on which we based our designs. By modifying our designs to account for this deviation I produced a twist-corrected RNA tile that has improved fidelity to our

design [a]. This work is some of the first cryo-electron microscopy to be performed on RNA only samples and has also led to better understanding of the dynamics of RNA structure and the limits of cryo-EM for structure determination of RNA.

Through collaboration with Berkeley researcher Gang Ren we have produced a series of 120 individual particle electron tomography reconstructions of an RNA origami as it folds. These reconstructions use data from individual molecules, with no averaging, and show unprecedented resolution of secondary structural elements that allow us to observe rare folding intermediates that would never be observed by conventional single particle averaging methods. [b]

Additionally, I used the structural biology pipeline that I established in Aarhus to characterize RNA structures from 4 different collaborative projects that are currently at various stages of manuscript preparation and peer review. Previously, RNA has been considered as too flexible for structural analysis, or too small to be observed by electron microscopy. My work on these projects, as well as work from other independent research groups, has shown that the previous concepts regarding the size limitations of cryo-EM do not hold true for nucleic acids. The phosphodiester backbone of RNA has much greater electron density than proteins, which increases the contrast in electron micrographs of RNA. Furthermore, my work demonstrates that the inherent dynamics of RNA are not a major problem for cryo-EM structural analysis and, in fact, one of the benefits of cryo-EM as a structural biology tool is its ability to characterize many different states within the same sample.

a) **McRae E.K.S.,** Helena Rasmussen, Jianfang Liu, Andreas Boggild, Michael Nguyen, Nestor Vallina, Thomas Boesen, Jan Skov Pedersen, Gang Gary Ren, and Ebbe Andersen., Structure, folding and flexibility of co-transcriptional RNA origami, (2023) Nature NanoTechnology, 1-10
b) Jianfang Liu, **Ewan K.S. McRae**, Meng Zhang, Cody Geary, Ebbe Sloth Andersen, Gang Ren, Non-averaged single-molecule tertiary structures reveal RNA self-folding through individual-particle cryo-electron tomography. (2024) Nature Communications – in Press

2. RNA Structural Design and Folding Optimization

During my time with the Andersen lab I had the opportunity to help troubleshoot and optimize the latest generation of RNA design software. I helped validate the structure of our designer RNA using negative stain transmission electron microscopy (NS-TEM). By using size exclusion chromatography to purify the RNA structures, I was also able to assess the relative yield of different designs and test the veracity of our design principles. To avoid topological barriers and to correct RNA folding, one must optimize the 3D location of the 5' and 3' and ends of the RNA within the structure. Although this insight was critical, it was not immediately obvious or intuitive and reviewers questioned whether our optimized start sites and strand paths were necessary. In response I designed a series of RNAs that should form the same structure but have different folding pathways. I characterized the yield of each design and observed folding defects by NS-TEM, verifying that our algorithm was indeed detecting and aiding to avoid topological traps in the folding pathway.

a) Geary, C., Grossi, G., **McRae, E.K.S.**, Rothemund, P.W.K., Andersen E.S. "RNA origami design tools enable cotranscriptional folding of kilobase-sized nanoscaffolds." Nature Chemistry – 13(6):549-558

3. Elucidating the Structural Basis of RNA Polymerase Ribozyme Activity

I have also initiated a collaboration with the Holliger lab in Cambridge, UK. The Holliger lab investigates the origin of life through *in vitro* selection experiments whereby they have evolved an RNA enzyme capable of self-templated RNA synthesis. This activity is a key prediction of the RNA World hypothesis and is widely believed to have been a central pillar of the emergence of life's first genetic system and possibly even life itself. Sequencing experiments revealed that the in vitro evolution experiments had selected for a system where two RNA molecules work symbiotically to improve the self-synthesis capabilities of the system. One RNA molecule is catalytically active on its own, the other is not. However, the catalytically inactive species dramatically enhances both the rate and fidelity of RNA templated RNA synthesis when partnered with the active species. The nature of this activity enhancement has been a quandary for many years. Despite previous challenges in structural determination for this RNA, I was able to determine the structure of the heterodimeric complex to 5 Angstrom resolution using cryo-EM. This proved to be sufficient to trace the entire backbone of both RNA strands and, remarkably, demonstrate the mechanism by which the inactive subunit acts as a scaffold to

position the template binding region of the active subunit near the active site and enhance fidelity and enzymatic activity of the hetero-dimeric species. To follow up on this collaboration I had a short-term position at the MRC-LMB to continue work on this project before starting my own lab at the HMRI.

a) **McRae EKS**, Wan CJK, Kristoffersen EL, Hansen K, Gianni E, Gallego I, Curran JF, Attwater J, Holliger P, Andersen ES. Cryo-EM structure and functional landscape of an RNA polymerase ribozyme. *Proc Natl Acad Sci* 2024;**121**:e2313332121.

D. Bibliography

https://www.ncbi.nlm.nih.gov/myncbi/ewan.mcrae.2/bibliography/public/