Progress toward *in vivo* identification of enhancer occupancy in the developing retina

Authors: Angelina Grebe[1,2], Tarsisius Tiyani[2,3], Kevin H. Gardner, PhD[2,3,4,5,6] and Mark M. Emerson, PhD[1,2,6]

Author affiliations: [1]Department of Biology, The City College of New York; [2]Biochemistry PhD Program, The Graduate Center, CUNY; [3]Structural Biology Initiative, CUNY ASRC; [4]Department of Chemistry and Biochemistry, The City College of New York; [5]Chemistry PhD Program, The Graduate Center, CUNY; [6]Biology PhD Program, The Graduate Center, CUNY

In order to develop treatments for retinal diseases, it is important to understand the cellular events that drive differentiation from a pool of retinal progenitor cells. Binding of transcription factors to specific DNA elements, known as *cis*-regulator elements (CREs), contributes to controlling cell type-specific gene expression; a way to experimentally determine CRE occupancy would help elucidate these mechanisms. Given that protein-DNA interactions happen on the nanoscale level, the approach to this problem must come with sufficient resolution. My research seeks to address these issues by covalently attaching a proximity labeling enzyme, APEX2, to a plasmid containing a CRE of interest. This plasmid-protein complex will then be introduced into cells, with the enzyme preferentially labeling transcription factors and other proteins that associate with the DNA sequence of interest. APEX2 will be attached to the plasmid by utilizing a HaloTag-APEX2 fusion protein; HaloTag is an engineered protein that forms covalent bonds with haloalkanes.[1] A haloalkane will be attached to the plasmid by reaction of a choloralkane-succinimidyl ester molecule to a primary amine-modified nucleotide. This nucleotide will be incorporated into the plasmid proximal to the DNA sequence of interest. I have conducted initial work to optimize these reactions within my system and have also begun exploring the optimal method by which to attach a protein to plasmid DNA.

Once developed, this novel methodology will first be tested using a CRE previously identified to be active in retinal progenitors that give rise to cones and horizontal cells, named ThrbCRM1. Previous rigorous experimentation has indicated that two transcription factors (TFs), Otx2 and Onecut1, bind this element to drive gene expression.[2] A plasmid with the Otx2 and Onecut1 known binding sites mutated has been developed and will be used as a negative control. After successful development of this novel methodology, I will use it to probe the occupancy of other CREs that are involved in retinal development. This will allow for identification of the TFs that play a role in many of the gene-regulatory networks important for retinal development. Furthermore, this method will help expedite full characterization of any subsequently identified CREs.

(1) Lam, S. S.; Martell, J. D.; Kamer, K. J.; Deerinck, T. J.; Ellisman, M. H.; Mootha, V. K.; Ting, A. Y. Directed Evolution of APEX2 for Electron Microscopy and Proximity Labeling. *Nat. Methods* **2015**, *12* (1), 51–54. https://doi.org/10.1038/nmeth.3179.

(2) Souferi, B.; Emerson, M. M. Quantitative Analysis of the ThrbCRM1-Centered Gene Regulatory Network. *Biol. Open* **2019**, *8* (4), bio039115. https://doi.org/10.1242/bio.039115.