Clarification of PI effort on R00 project:

Per Dr. Pinello's job contract with the University of Wyoming, during the 9-month academic calendar, her effort toward this project will be 75%. This is equivalent to 6.75 person months. Because Dr. Pinello will have no teaching responsibilities during the summer term, her effort towards this project during the summer will be 100% . This is equivalent to 3 person months. Therefore, during the 12-month calendar year, Dr. Pinello's effort towards this project will be 9.75 person months.

Details of Foreign Collaboration:

a. Collaborator full names, email, and phone #:
   Félix A. Rey
   felix.rey@pasteur.fr
   336 4211 0051

b. Foreign institution name, city, and address.
   Institut Pasteur
   25-28 Rue du Dr Roux
   75015 Paris, France

c. Country name.
   France

d. Human/Animal involvement.
   Not applicable

e. Dollars at country level, if applicable.
   $0

f. Rationale of the foreign collaboration – a short paragraph describing the role and contribution of foreign collaborators and how that is related to the aims/scope of this project.

Dr. Félix Rey is an expert in structural virology, specializing in the same structural class of membrane fusion proteins (Class II) that the gamete fusion protein HAP2 belongs too. Dr. Rey solved the first structure of a Class II membrane fusion protein from viruses and was first to discover that eukaryotic cells also possess Class II fusion proteins used for developmental cell-cell fusion events (EFF-1). Furthermore, his group obtained and published the first structure of HAP2, showing that it too had a Class II fusion protein fold – evidence which helped solidify HAP2 as the first and only known eukaryotic gamete membrane fusion protein. With this background, he and his research group can provide my lab and this project with unparalleled and essential structural insight, information, and reagents that will support the success of this project. Specifically, they will provide my lab with purified recombinant protein and antibody reagents for Aim 3 of this project to test whether MAR1-FUS1 binding is sufficient to elicit the activation of HAP2 to form homotrimers required for fusion and I will send them *Chlamydomonas* HAP2 protein purified from native gamete membranes for our efforts in Aim 2 of this project to obtain a structure of HAP2 in its pre-fusion conformation.

Dr. Félix Rey and his colleagues at the Institut Pasteur have been long-term collaborators of mine and my K99 sponsor, Dr. William Snell on past projects, for example, our work finding that HAP2 must form homotrimers essential for gamete fusion during *Chlamydomonas* fertilization (PMID: 34282138). Currently, we are editing a structure-function manuscript for submission which describes a key binding site of a gamete membrane adhesion protein pair essential for fertilization (MAR1-FUS1 - specific to Aim 1 of this project). We have in vivo functional support (via bioassays with a *Chlamydomonas* MAR1 mutant strain and recombinant protein competition studies) for a heterotetrametric MAR1-FUS1 co-crystal structure that Dr. Rey's laboratory solved. In sum, I feel Dr. Rey and I have a good working relationship and a strong mutual interest in pushing these studies and our understanding HAP2 function during fertilization forward.