**Structural and mechanistic studies of the CARD8 inflammasome**

**Background:** CARD8 is a small protein that cleaves itself in a novel function-to-find domain (FIIND). Following autoproteolysis, full-length CARD8 binds DPP9 to maintain an autoinhibited state. Upon activation, CARD8 forms an inflammasome, a signaling center that recruits innate immunity effector molecules, mediated by the C-terminal portion of the protein (C-terminus to the FIIND cleavage site).

**Biological Significance:** The biological pathway and regulation of CARD8 is poorly understood despite its relevance to innate immunity and disease. Structural information will aid in understanding its mechanism. Additionally, the FIIND is a novel domain of unknown structure, and thus high-resolution EM will be necessary to build a *de novo* model of this poorly characterized protein domain.

**Specimen Molecular Weight:** 60 kDa monomer (CARD8), 200 kDa dimer (DPP9)

**Specimen Dimensions:** ~20 nm bi-lobal dimer (DPP9) with—see PDB ID: 6EOQ. The binding stoichiometry CARD8 remains unknown, but the high expression of DPP9 and its high affinity for dimerization bias towards 2:1 DPP9:CARD8 complexes.

**Figure.** CARD8 regulation by DPP9. **A)** MST binding curves of alexa488-labeled DPP9 with either wild-type or autoproteolytic-deficient (S297A) CARD8 reveals that FIIND autoprocessing is dispensable for its interaction with DPP9. **B)** DPP9 inhibitors do not dissociate the CARD8-DPP9 complex on gel filtration. **C)** Structure-guided DPP9 mutants abolish or weaken its interaction with CARD8. **D)** Preliminary cryo-EM structure of the CARD8-DPP9 complex (arrow indicates the DPP9 active site) and the **E)** focused-refined map of CARD8 (FIIND and CARD visible).

