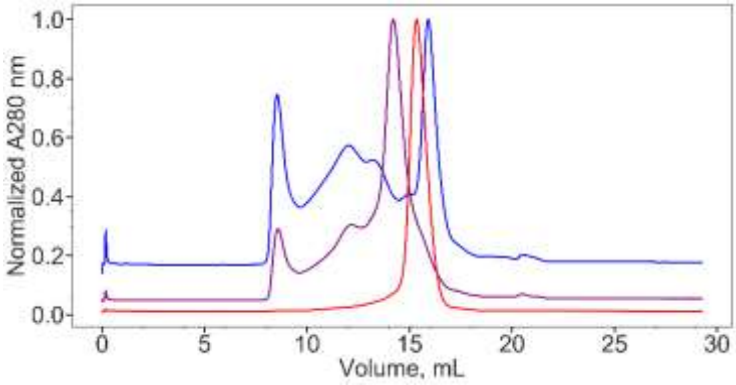
**Structural and mechanistic studies of the CARD8 inflammasome**

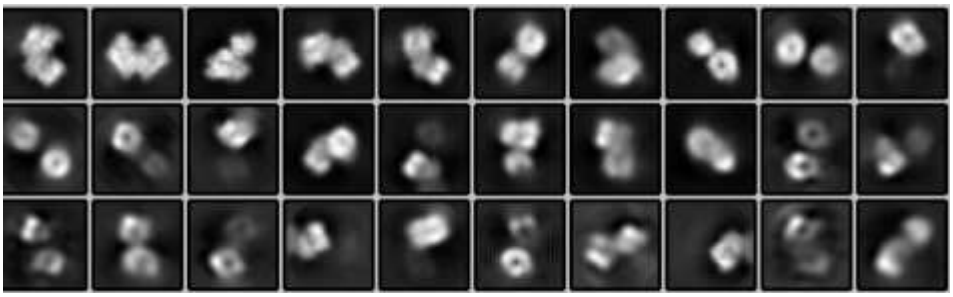
**Background:** CARD8 is a small protein that cleaves itself in a novel function-to-find domain (FIIND). Following autoproteolysis, full-length CARD8 binds either DPP9 or DPP8 to maintain autoinhibition. Upon activation, CARD8 forms an inflammasome, a signaling center that recruits innate immunity effector 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 (DPP8/9)
**Specimen Dimensions:** ~20 nm bi-lobal dimer (DPP8/9) with—see PDB ID: 6EOQ. The binding stoichiometry CARD8 remains unknown, but initial data processing suggests a well-ordered binding domain on each copy of the DPP9 dimer (low-resolution 3D reconstruction not shown). Likely C2-symmetric.




Size exclusion chromatograms (superose 6, normalized to 1) of DPP9 (red), the DPP9-CARD8 complex (purple) and CARD8 alone (blue) showing well-behaved protein at elution volumes consistent with each respective molecular weight.



Preliminary 2D classes of the CARD8-DPP9 complex from data processing in Relion3. 3500 micrographs were collected on a 200 kV Talos Artica microscope.