Optimization of cryoEM grids for full-length integrin: Using an established integrin cryo-grid optimization pipeline, we optimized grid conditions for purified full-length integrin (Figure 1) and collected preliminary cryoEM data (~1,000 images) on our 200 kV Glacios and (~3,000 images) on 300 KV Krios microscope. We have a good full-length sample and well-optimized freezing conditions to support massive data collection for potential atomic resolution structures.

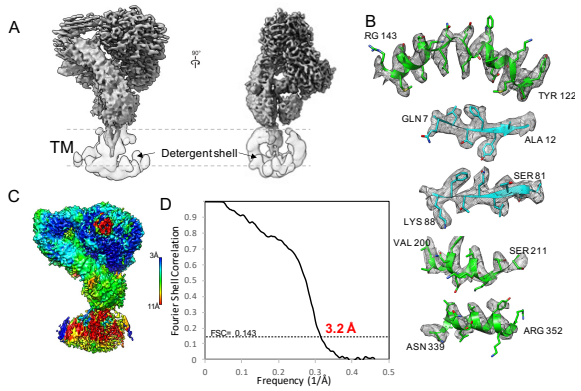

Figure 1. (A) CryoEM structure of the detergent solubilized, purified full-length human integrin heterodimer. The density map shown for two different thresholds represents the relatively low-resolution TM region in the density map. (B) Representative regions revealing atomic-level details of the cryoEM density map and its corresponding docking model from X-ray crystal structure. (C) Resolution variation within the EM density (representing a lower threshold) estimated by ResMap. (D) The overall resolutions of the density maps is 3.2 Å, determined using Fourier shell correlations (FSC) with a 0.143 cutoff.

A near-atomic resolution structure of full-length human integrin by cryoEM: In preliminary data processing, we have boxed out over 300,000 particles and performed a 3D refinement from scratch, resolving a structure at high resolution of ~3 Å (Figure 1). We show the first overall architecture of a full-length integrin at a near-atomic resolution. The resolution variation map for the first time reveal an overall structural dynamics in the full-length integrin which suggest the headpiece adopts a more rigid and single conformation compare to other domains in the basal condition. Each functional domain is clearly identified with sufficient detail and clarity to identify the secondary structures.

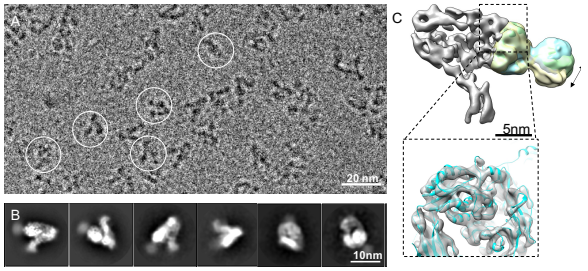

Figure 2. (A) CryoEM image of αI-integrin ectodomain, (B) 2D class average, and (C) 3D reconstruction of ectodomain by single-particle cryoEM represented in a bent closed state. Green and yellow densities present different conformations in the ligand-binding domain resolved from different classification maps.

Extended cryoEM study of αI-integrins: We have used cryoEM with the purified leuko-integrin ectodomain, and optimized grid conditions to solve a near-atomic structure at ~5 Å, presenting a bent closed state (**Figure 2).** This clearly reveals domain-specific conformational dynamics (e.g., ligand-binding domain and EGF-1-4 domains), and further suggests that all the crystal structures might have been stabilized in the lowest energy state by crystal lattice contacts. These images demonstrate our ability to map the dynamic landscape of integrin conformations using cryoEM.