

Cryo-EM reveals a *de novo* minibinder induces a conformational change in $\alpha 5\beta 1$ integrin



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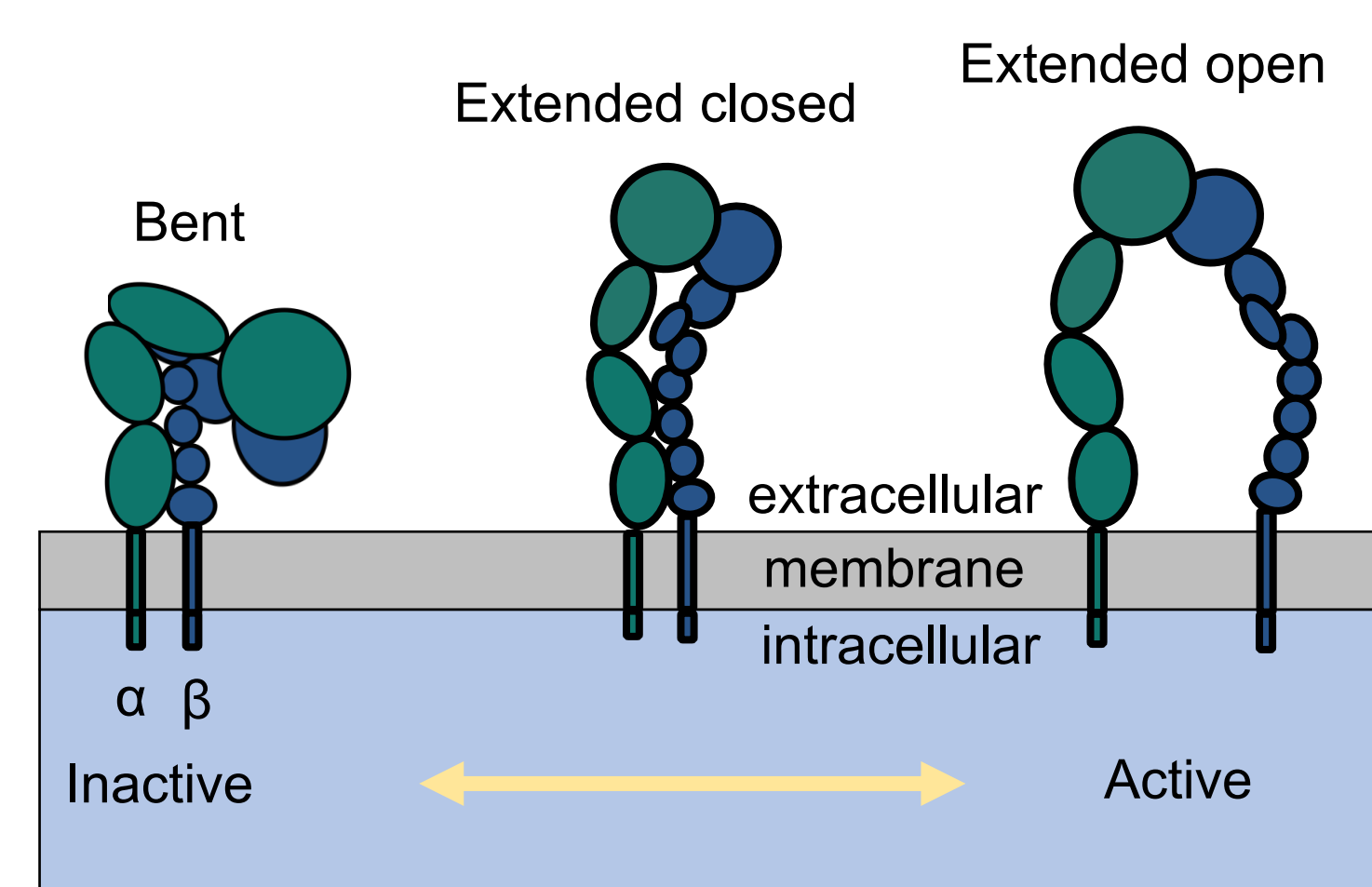
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BACKGROUND

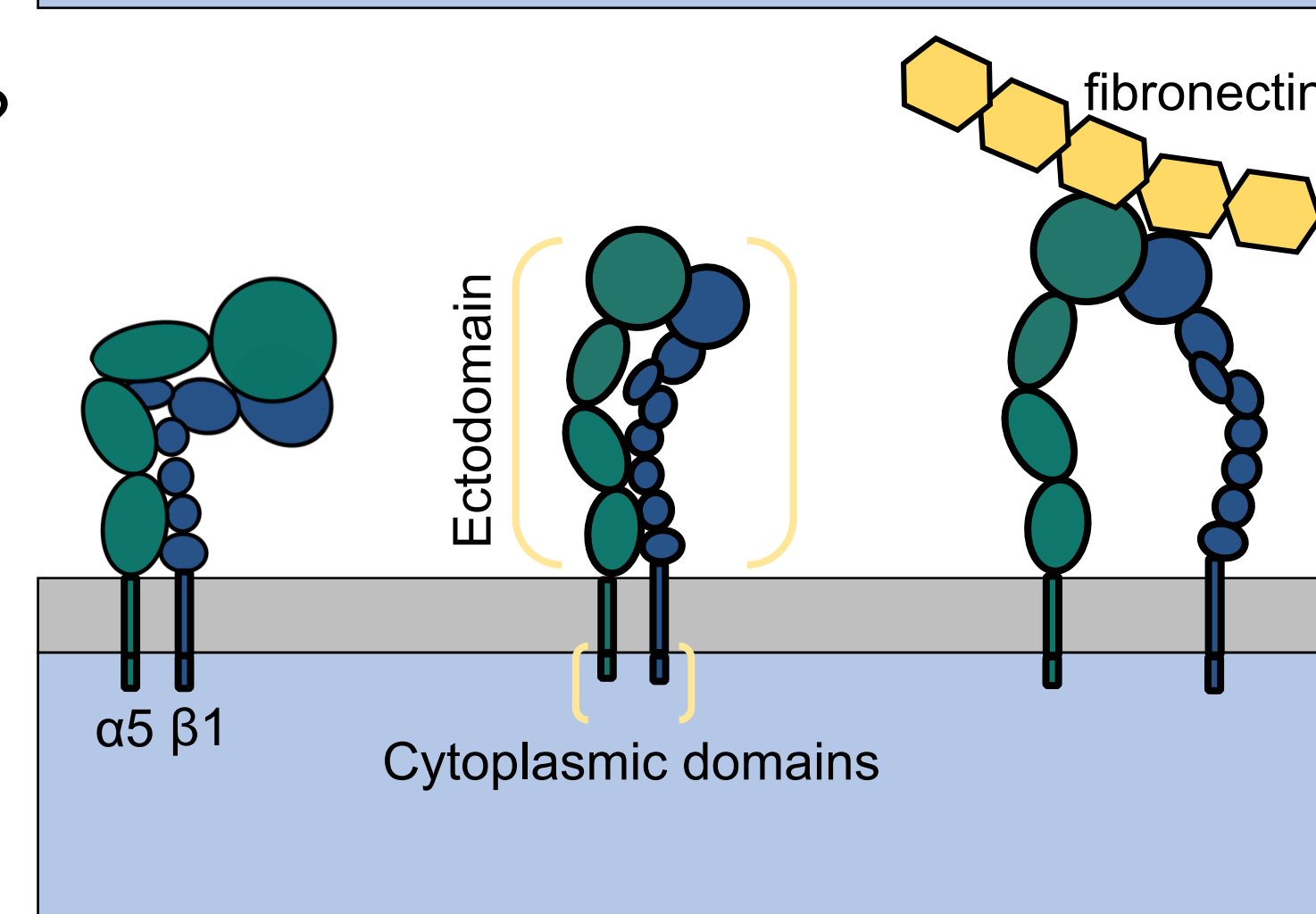
What is integrin?

- ❖ Membrane proteins expressed on the surface of all nucleated cells¹
- ❖ Go through drastic conformational changes associated with function¹
- ❖ Different cations, like Mn^{2+} can activate/induce the extended open state¹



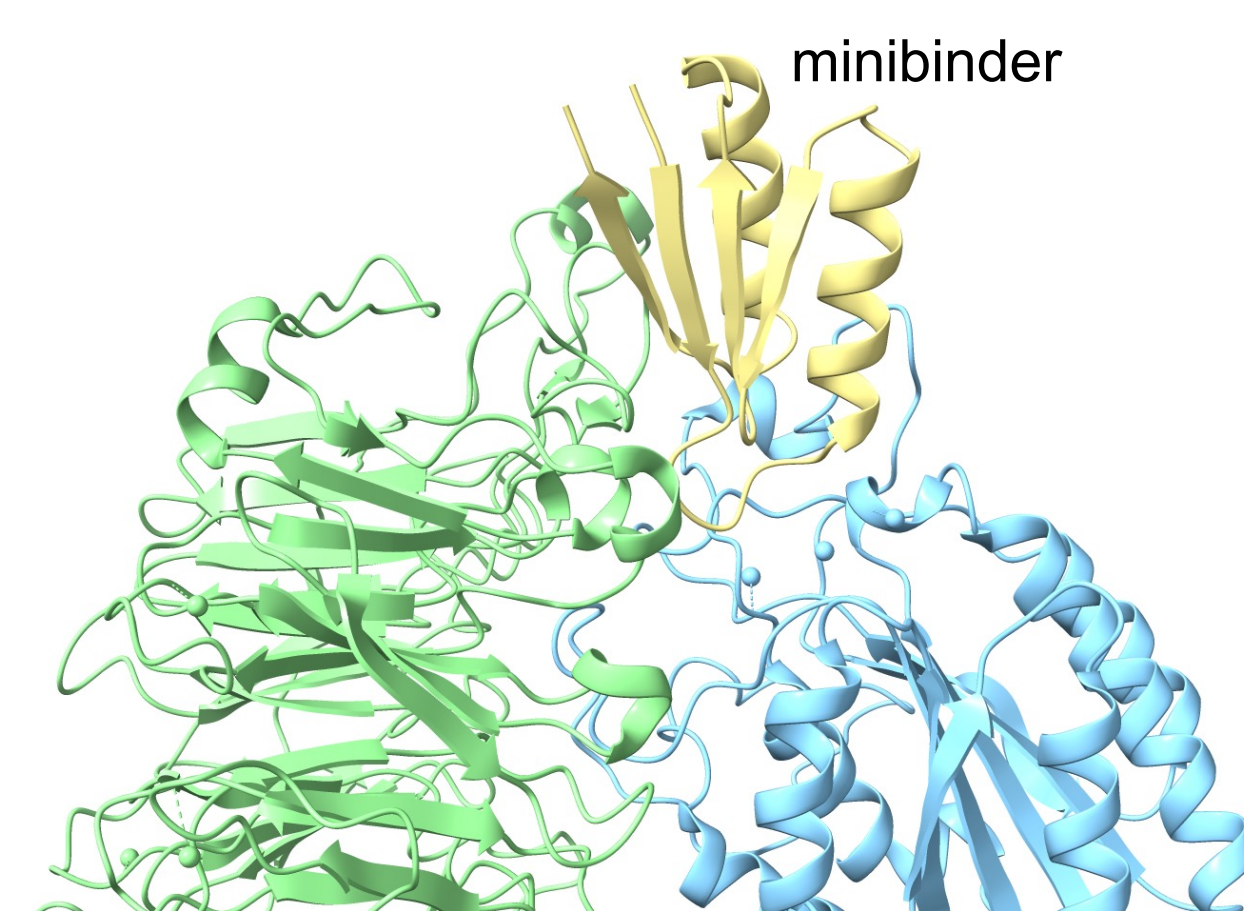
What is $\alpha 5\beta 1$ integrin?

- ❖ Fibronectin receptor²
- ❖ Does not follow typical integrin conformational changes²
- ❖ Expressed on the surface of epithelial cells²
- ❖ Main functions are in cell adhesion and angiogenesis²



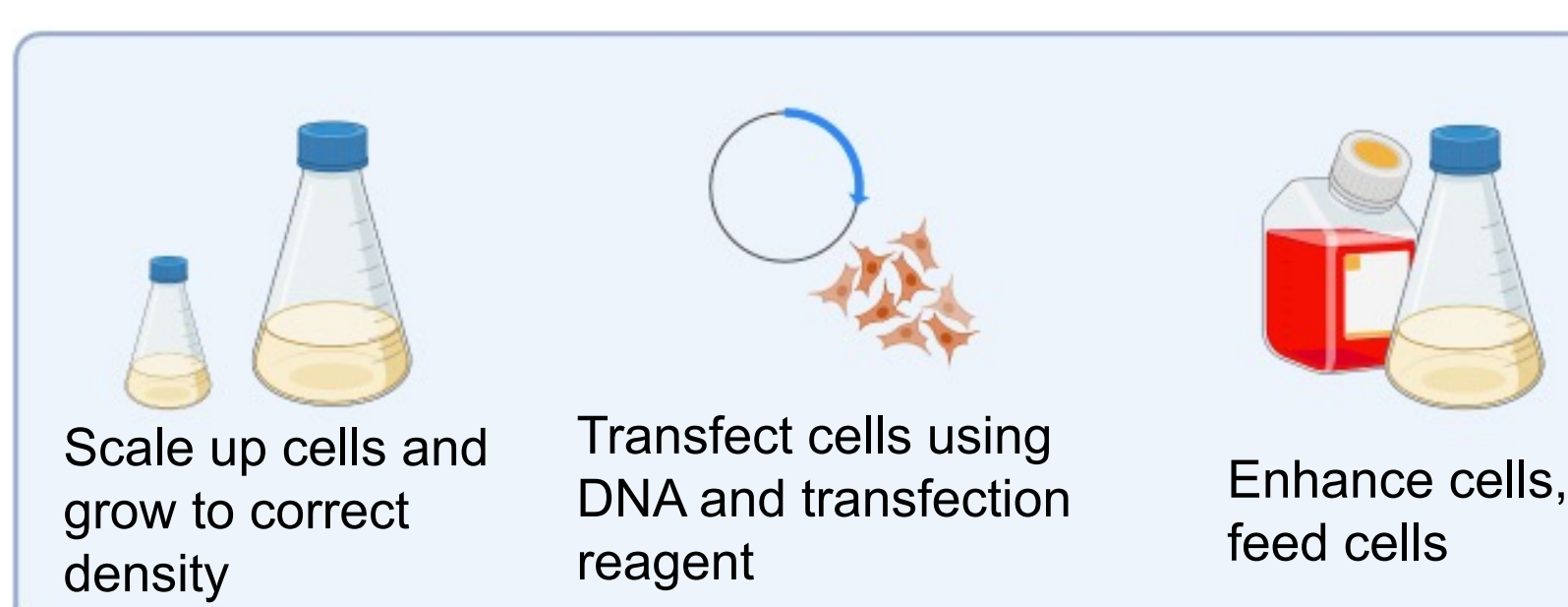
What is a minibinder?

- ❖ *De novo*, computationally designed proteins
- ❖ Small (~7 kDa) and stable protein
- ❖ For therapeutic application, it can be used as an alternative to antibody therapy
- ❖ Preliminary experiments have shown that V5 minibinder activates integrin

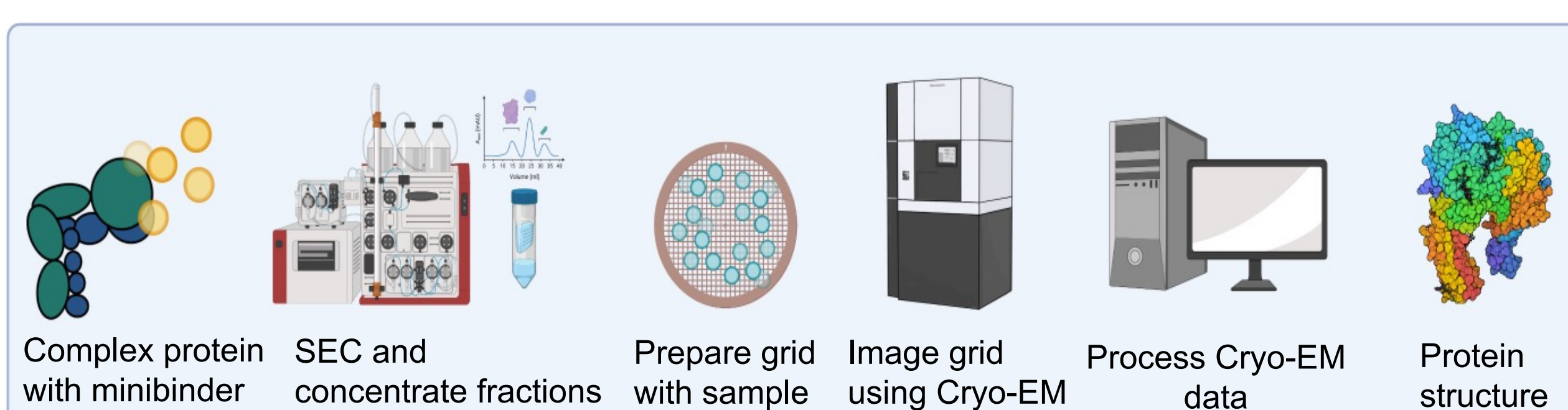
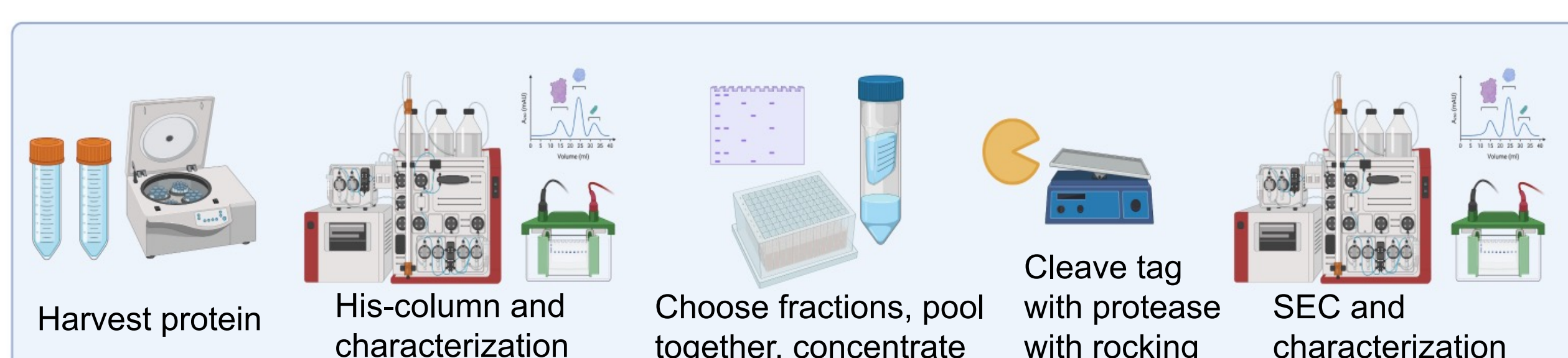


When bound, does V5 minibinder induce a conformational change in $\alpha 5\beta 1$ integrin?

METHODS

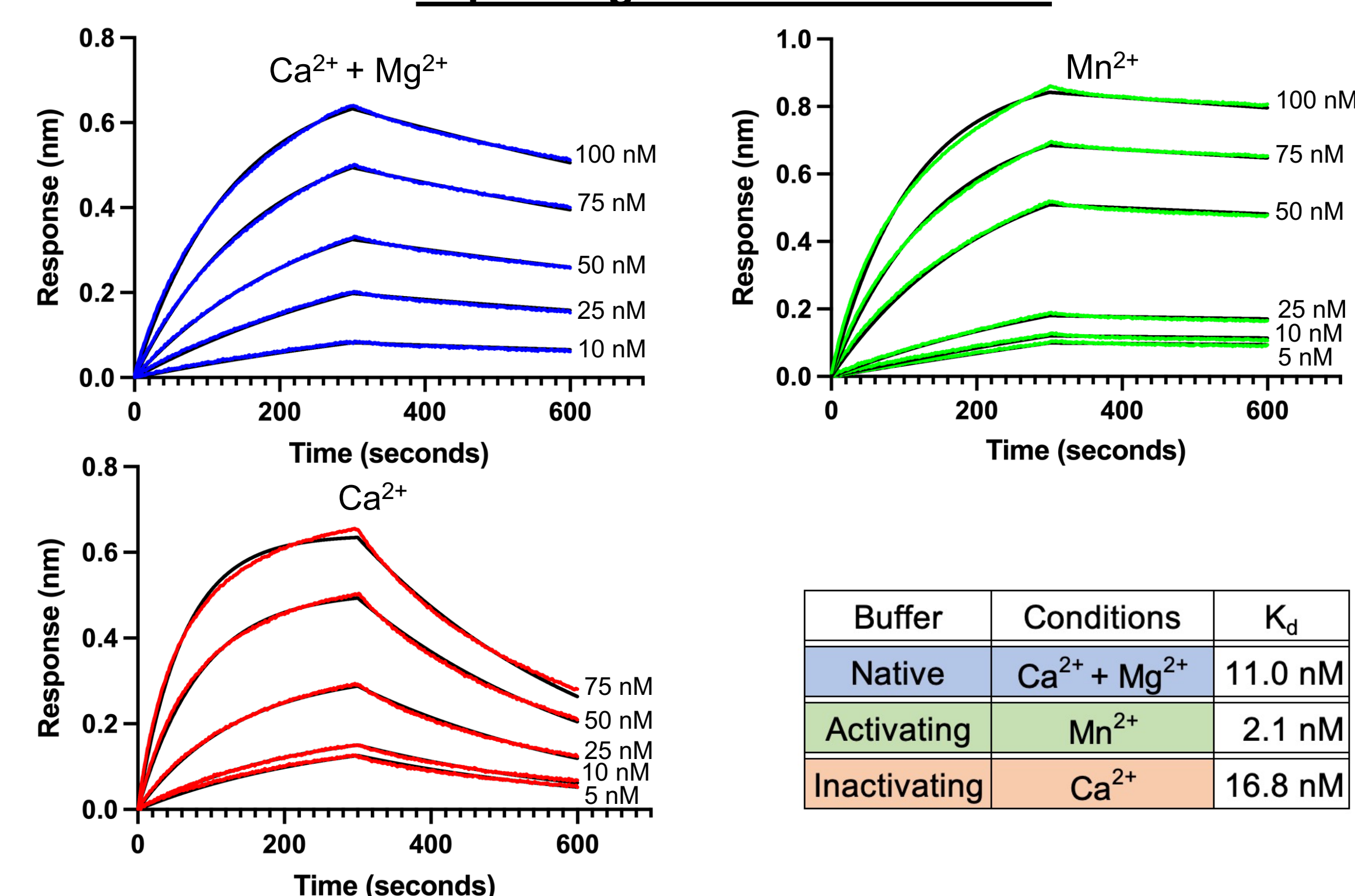


Experimental workflow for Cryogenic Electron Microscopy (Cryo-EM)



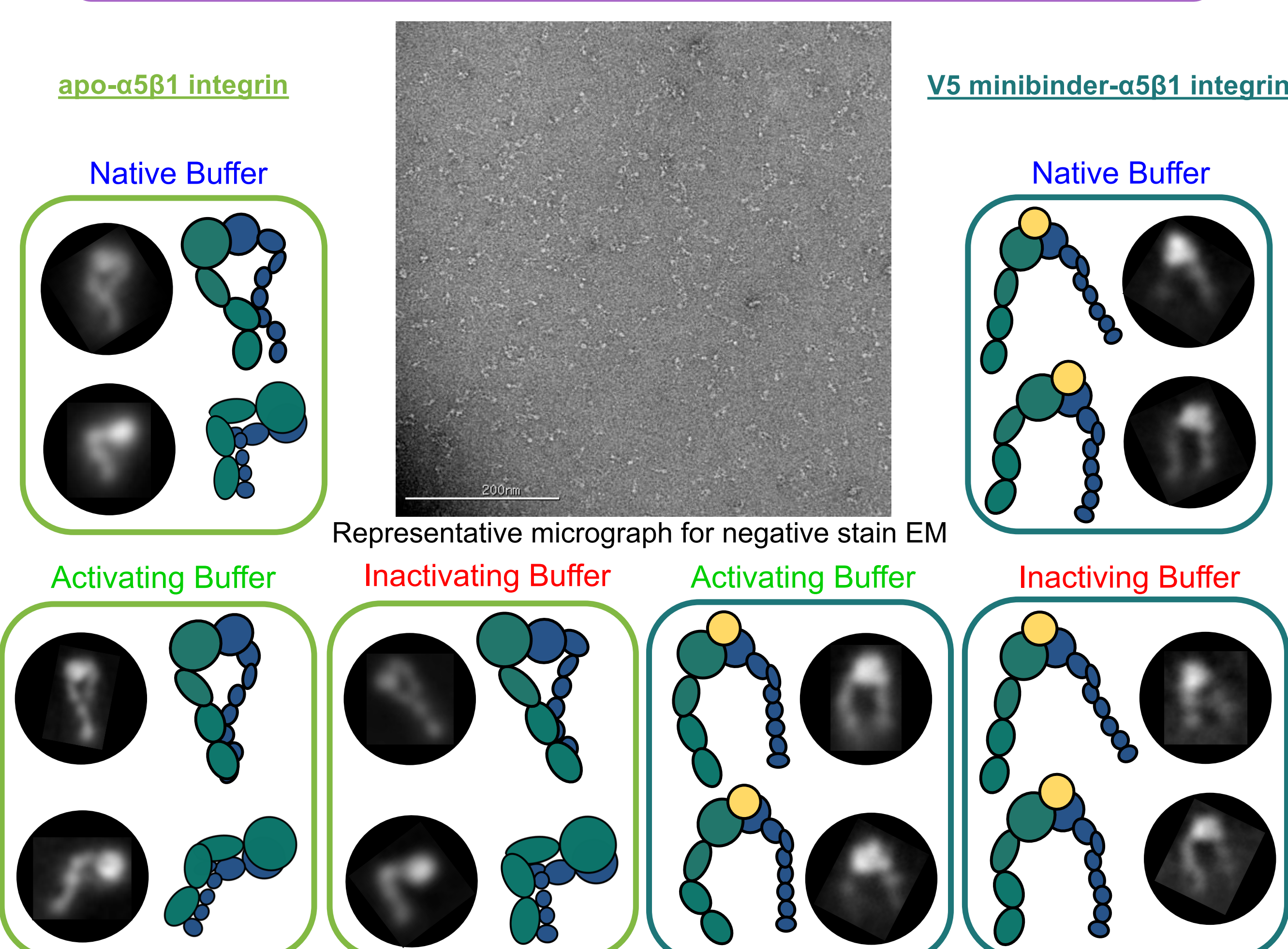
MINIBINDER HAS HIGH BINDING AFFINITY

$\alpha 5\beta 1$ integrin + V5 minibinder



Using bioluminescence resonance energy transfer, the V5 minibinder is shown to have high binding affinity in all cation conditions and varying $\alpha 5\beta 1$ integrin concentrations.

MINIBINDER INDUCES OPEN-EXTENDED CONFORMATION



Using negative stain, conformational analysis was done on ligand-free (apo- $\alpha 5\beta 1$ integrin) and ligand-bound (V5 minibinder- $\alpha 5\beta 1$ integrin) $\alpha 5\beta 1$ integrin under different cation conditions. Under all conditions the minibinder induced a conformational change in $\alpha 5\beta 1$ integrin to the extended open state.

REFERENCES & ACKNOWLEDGEMENTS

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2. Schumacher, S. *et al.* Structural insights into integrin $\alpha 5\beta 1$ opening by fibronectin ligand. *Sci. Adv.* **7**, (2021).
3. Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nat. Methods* **14**, 290–296 (2017).

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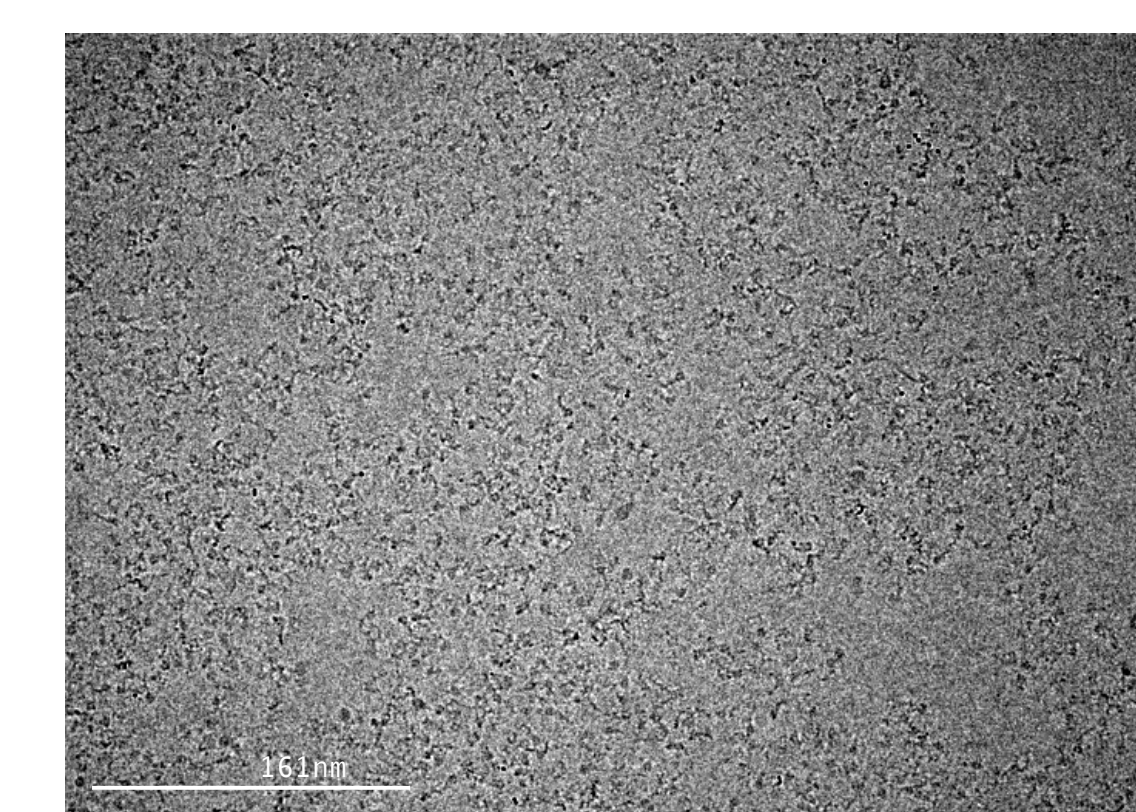
Thank you to Caleigh Azumaya and rest of Cryo-EM core for helping collect Cryo-EM data and training me!

Thank you Xinru Wang and the Baker lab for collaborating and providing the minibinders!

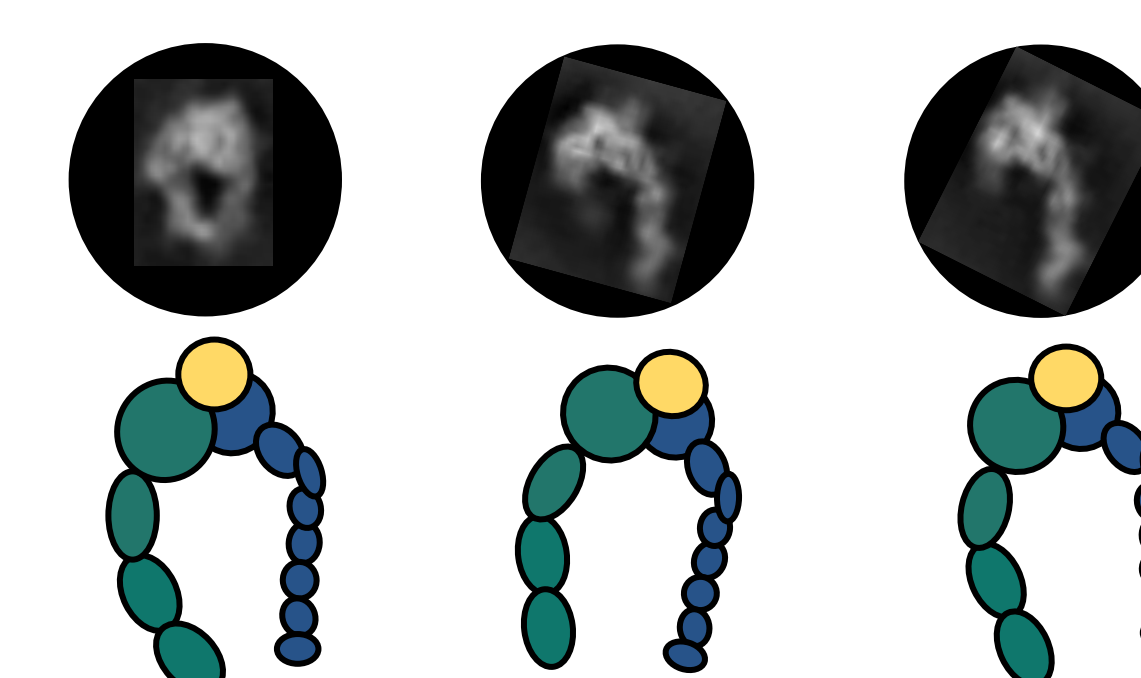
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STRUCTURAL VALIDATION USING CRYO-EM

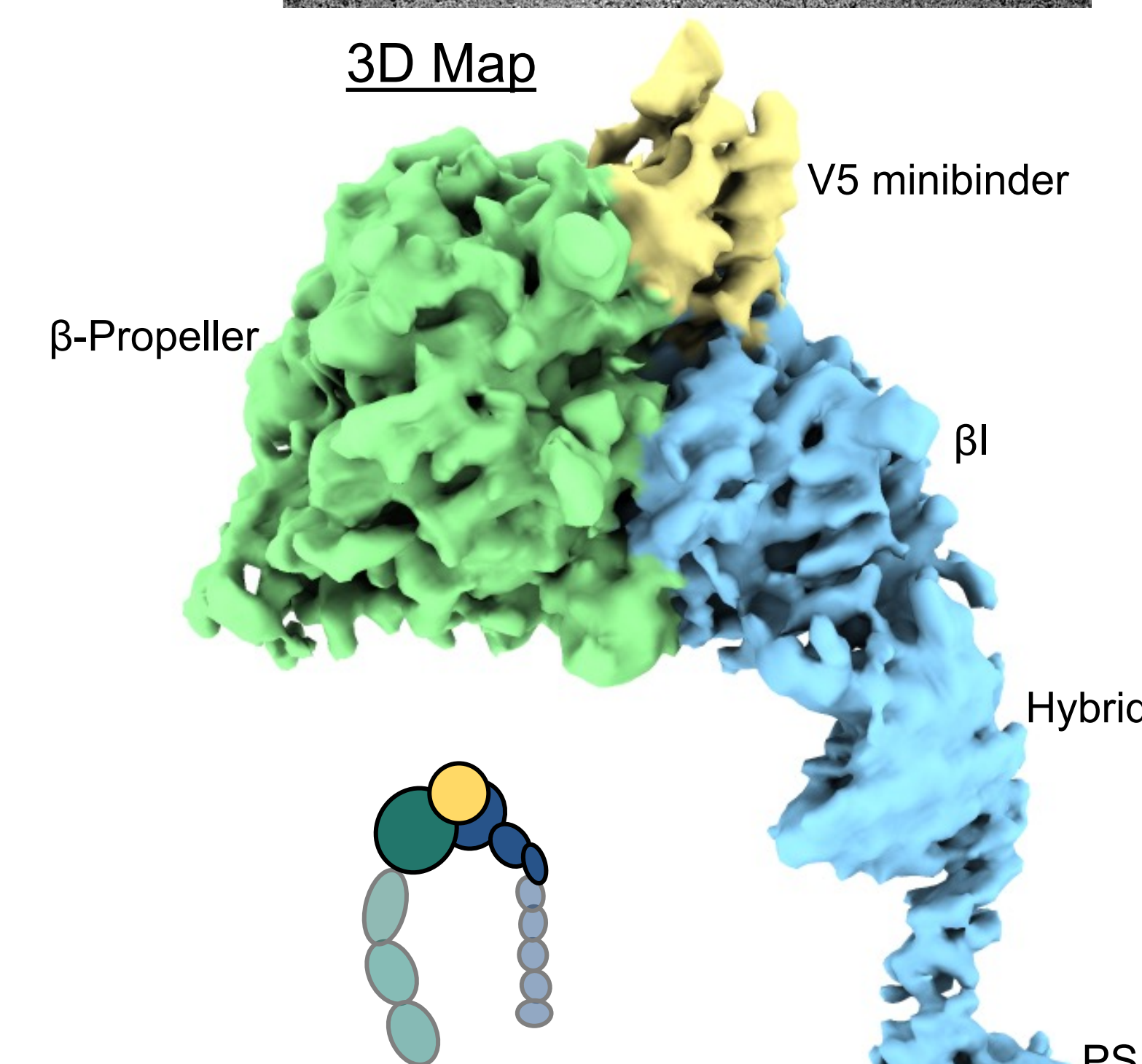
Representative Cryo-EM micrograph



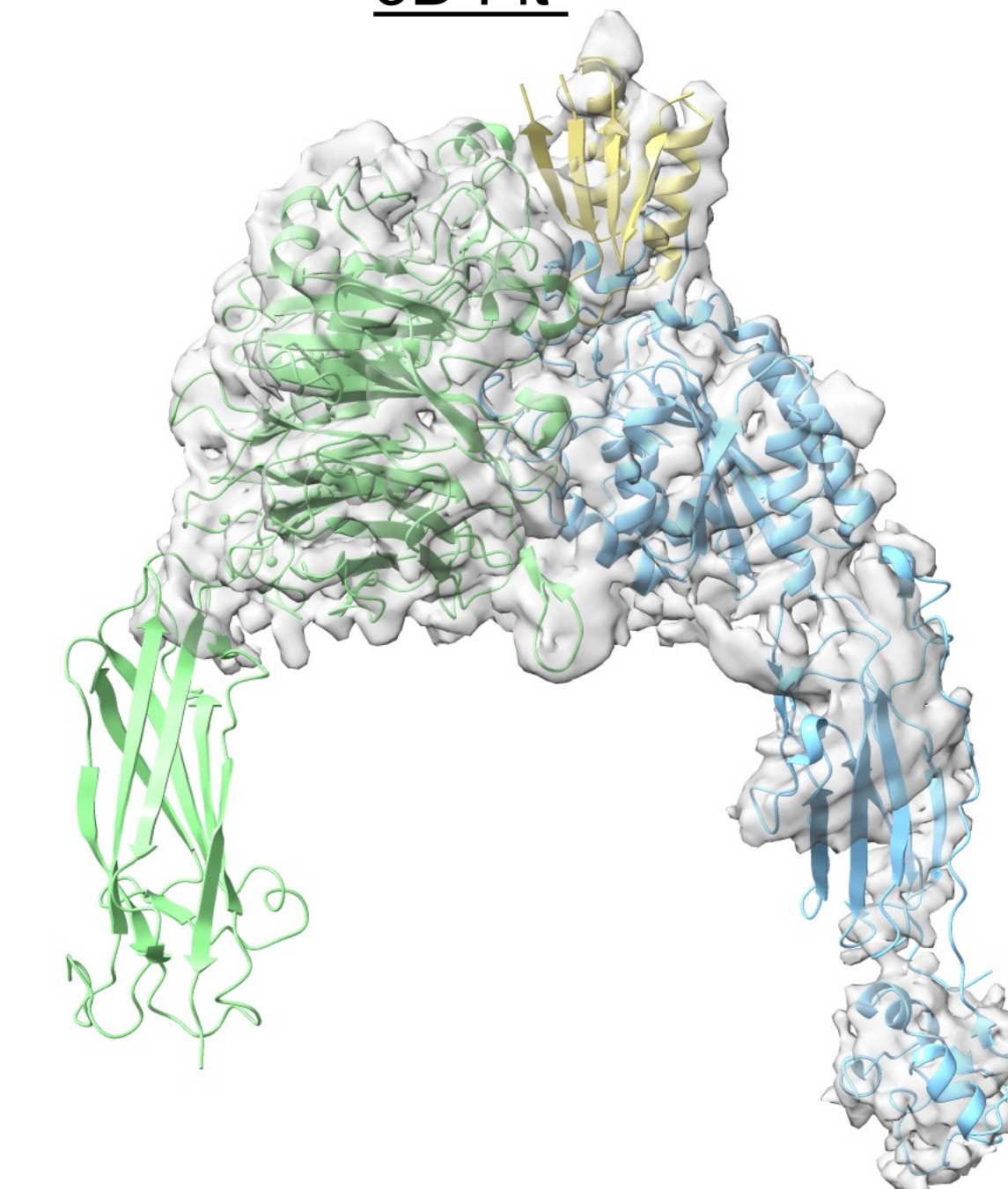
2D Classes



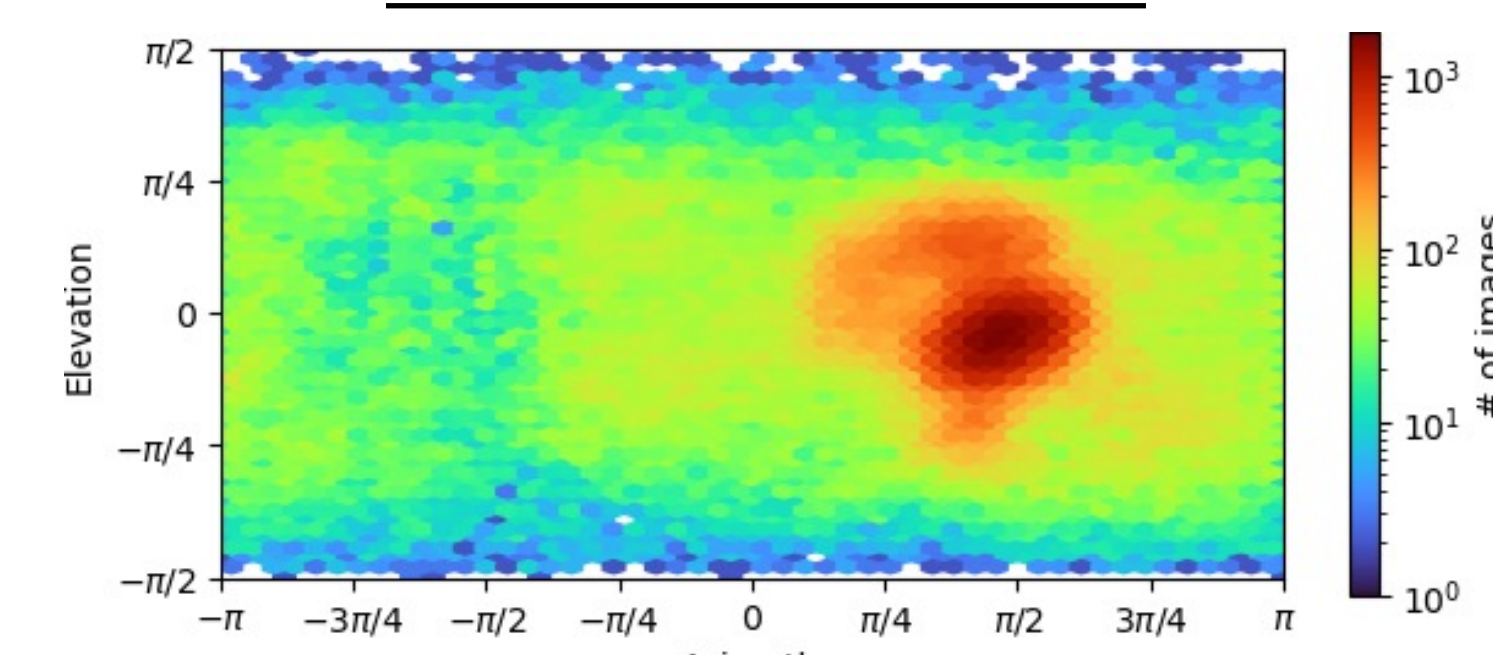
3D Map



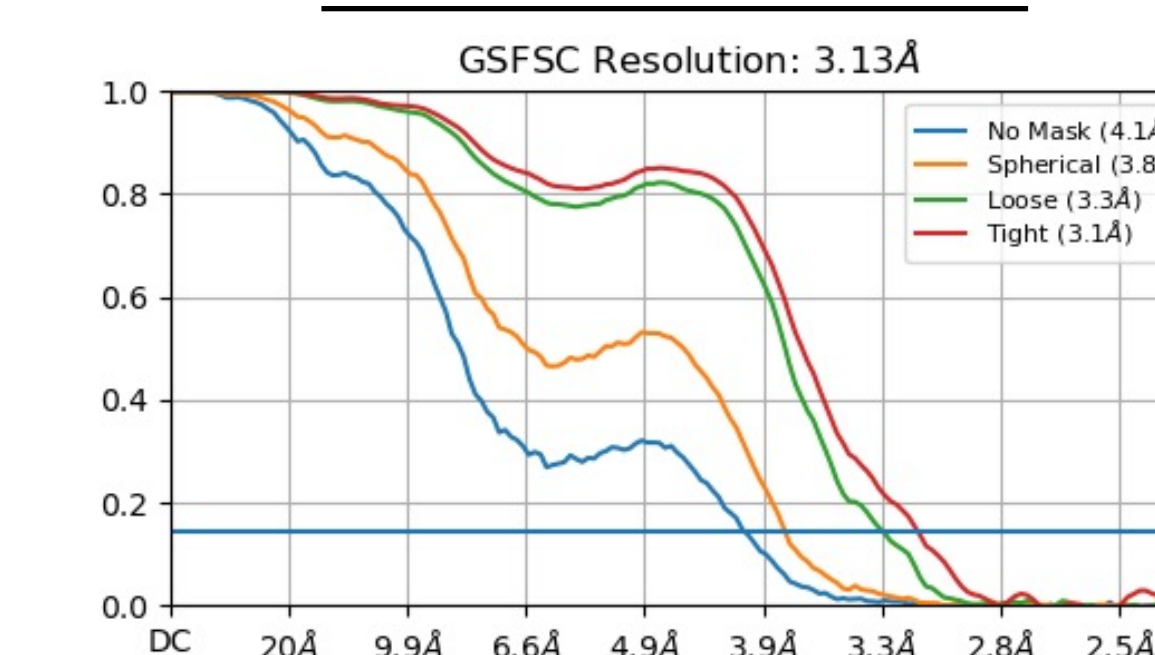
3D Fit



Orientation Distribution



Estimated Resolution



Using a Glacios electron microscope and K3 detector (camera), we verified that V5 minibinder is bound to $\alpha 5\beta 1$ integrin and does induce the extended open state in inactivating buffer (Ca^{2+}) with 3.1 Å resolution 3D map, but certain orientations are favored more (red). $\alpha 5\beta 1$ integrin model from PDB ID: 7NWL.

FUTURE WORK

- ❖ Experiment with different sample preparation and collection methods to improve the distributions of orientations for Cryo-EM (see image on right).
- ❖ With an improved Cryo-EM density, model the protein into the density
- ❖ Perform Cryo-EM on next-generation minibinders with improved characteristics such as higher binding affinity or specificity

