Anthrax toxin pore inserted in lipid nanodiscs

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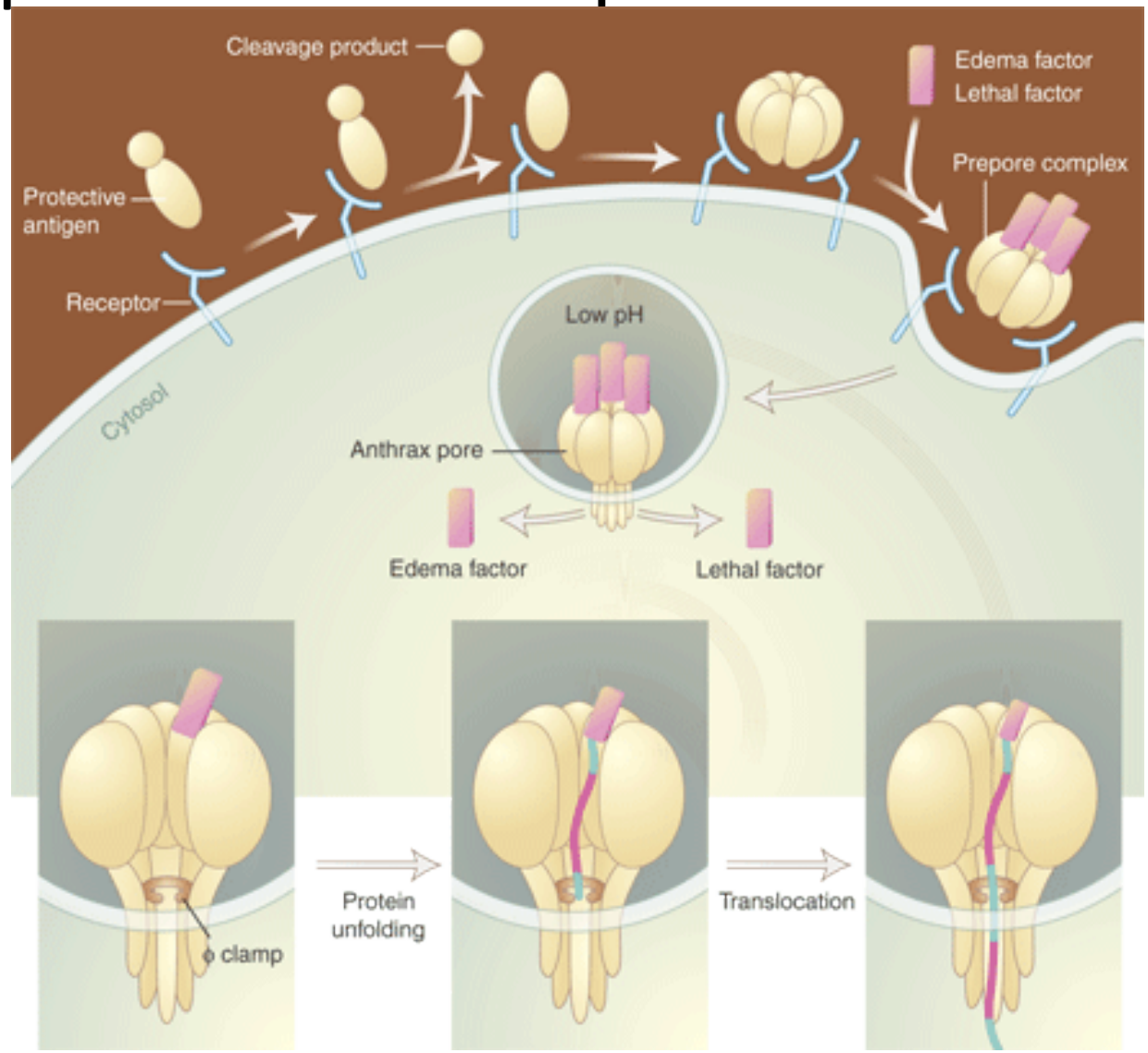
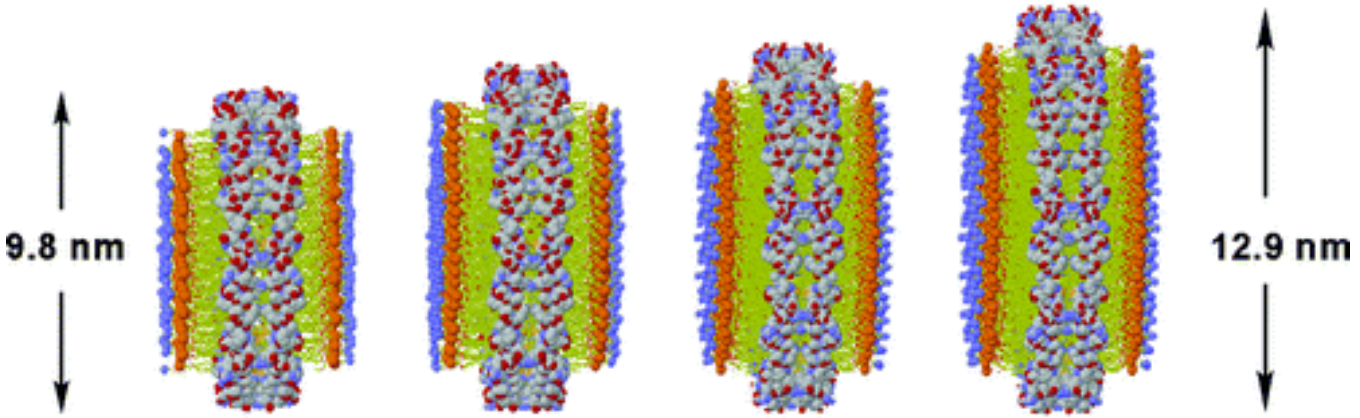
Protective antigen: pore forming component of the tripartite anthrax toxin



Lipid nanodiscs



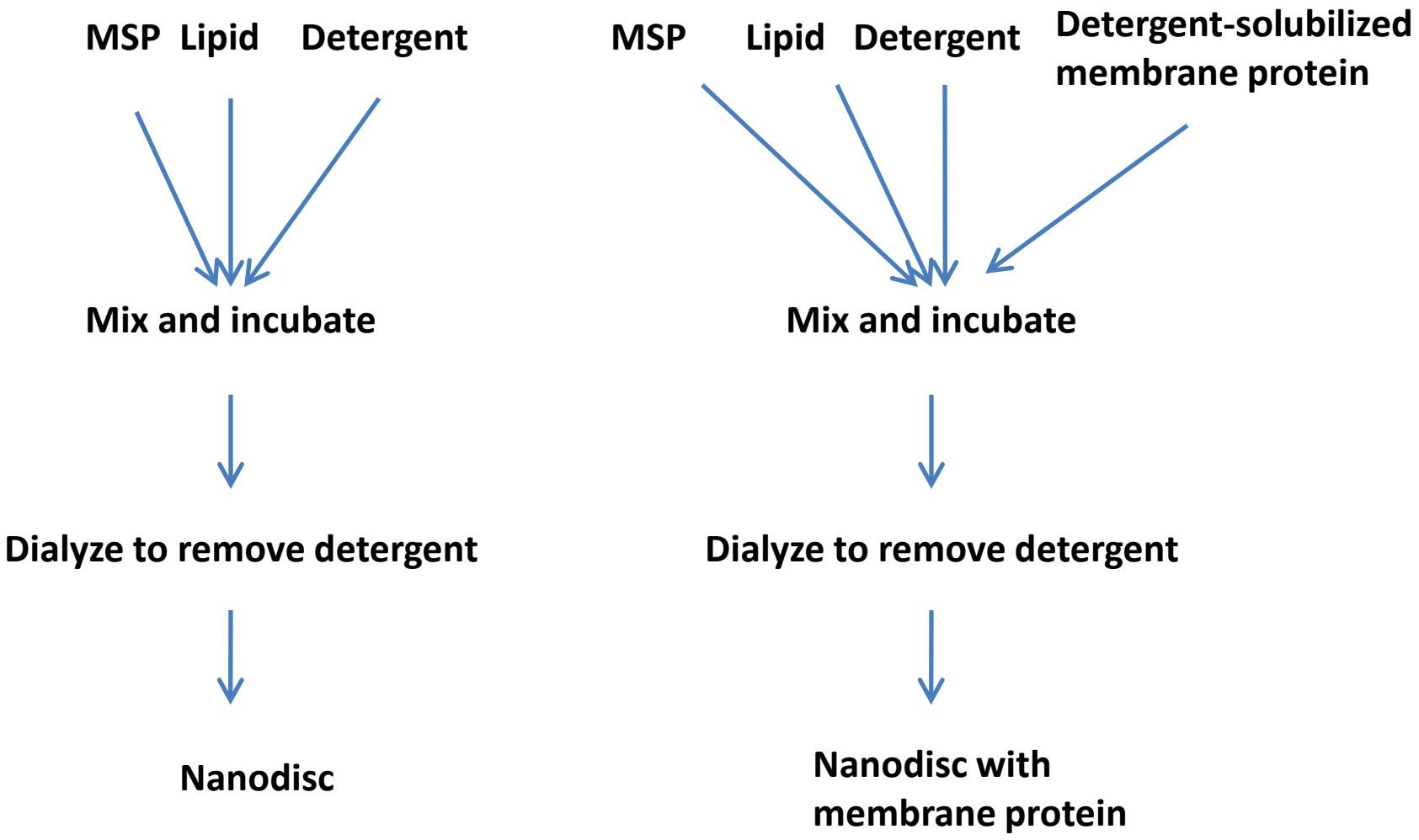
Apo A-I	GLOB-H1-H2-H3-H4-H4-H5-H6-H5-H6-H7-H8-H9-H10
MSP1T2 (MSP1D1)	HisTev-H0.5-H2-H3-H4-H5-H6-H7-H8-H9-H10

- Nanodiscs are lipid bilayer discs surrounded by membrane scaffold proteins (MSP)
- MSP is derived from human apolipoprotein Apo A-I
- MSP consists of a series of amphipathic helices.

Advantages of nanodisc

- Advantages over detergents
  - Near native lipid bilayer
- Advantages over lipid vesicles
  - Uniform size
  - His-tagged
  - Smaller (more particles in a field)
  - Can be formed around membrane proteins

How to make nanodiscs



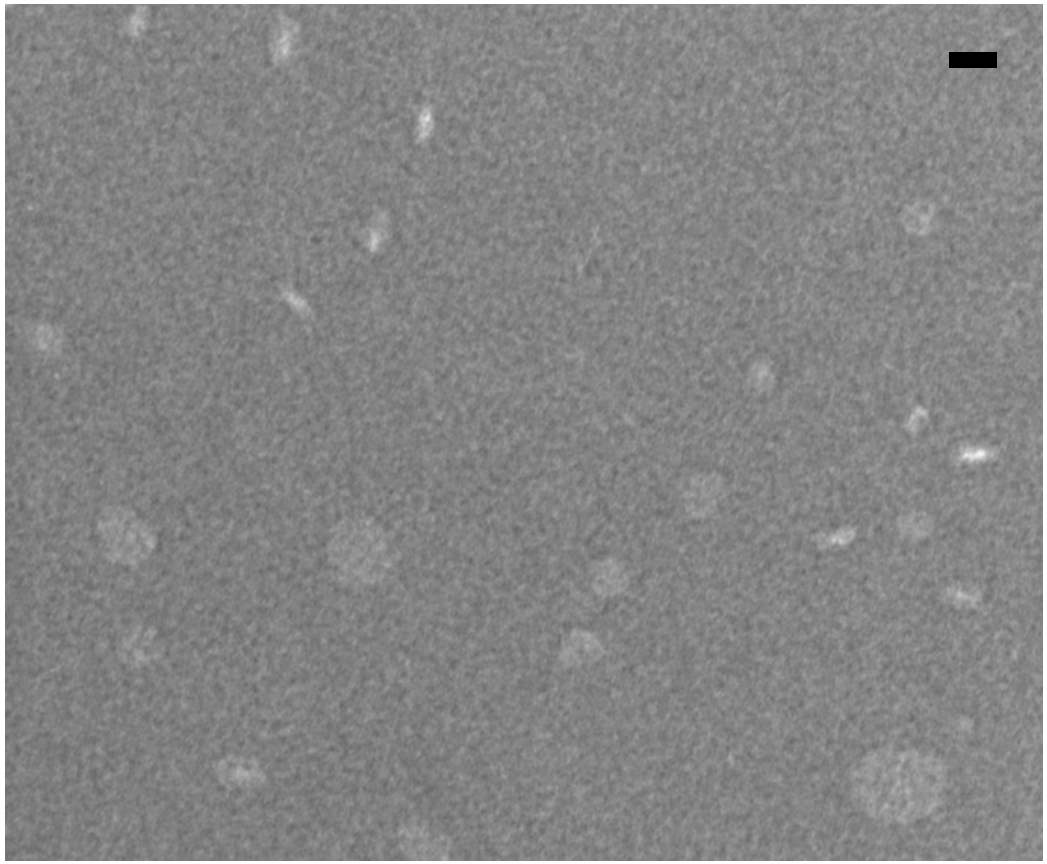
Two options to make PA/nanodisc complex

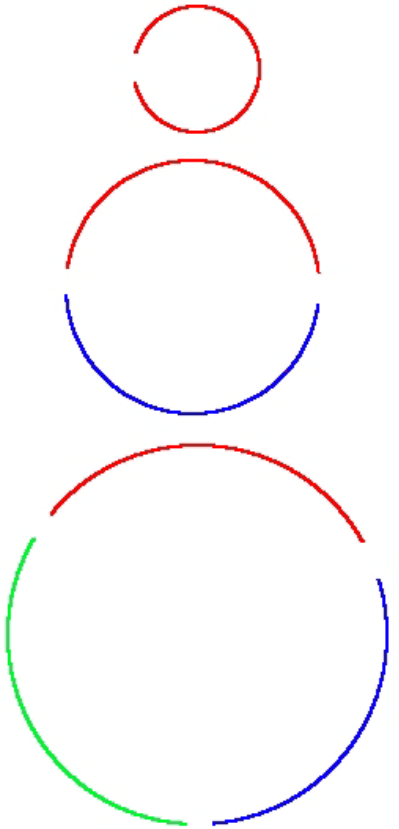
1. Form nanodiscs first, then insert PA pore
   - PA is a pore forming toxin that inserts itself into membrane
   - Insertion is not very efficient without receptors
   - Majority of the molecules ended up in aggregation when tested with lipid vesicles

2. Form nanodiscs in the presence of PA pore
   - There is no known detergent that can solubilize PA pore without disrupting the heptamer.

Nanodisc formed without PA

-Nanodiscs in different sizes

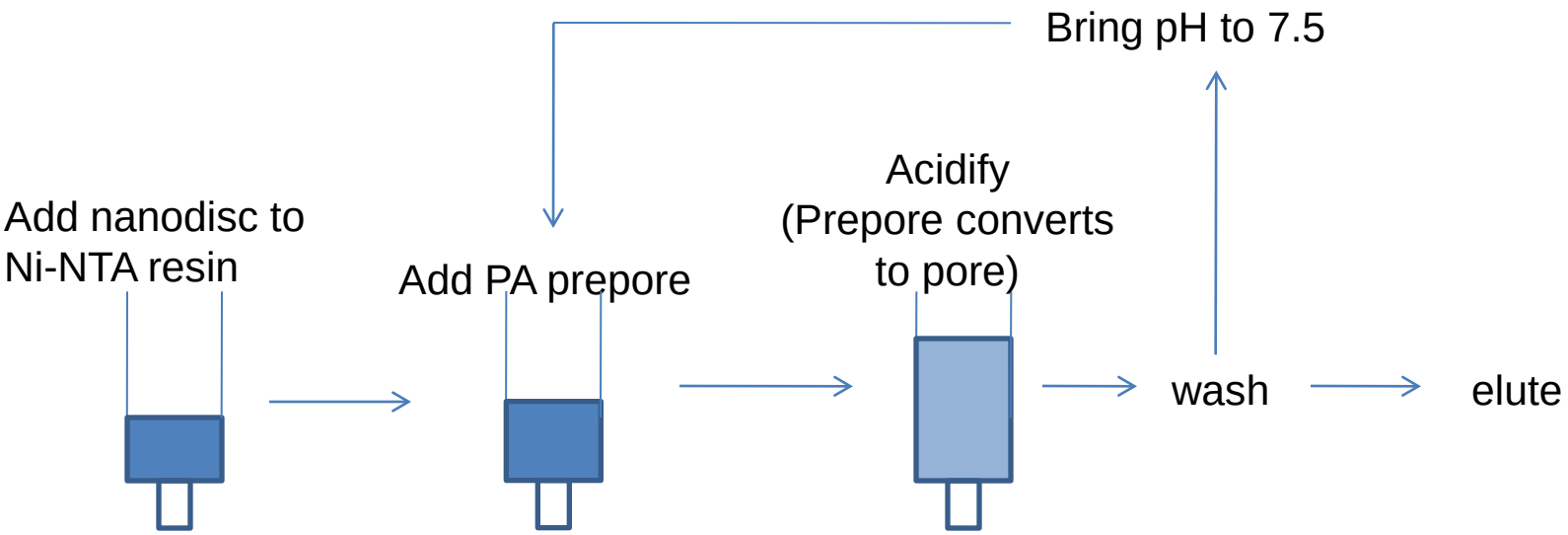




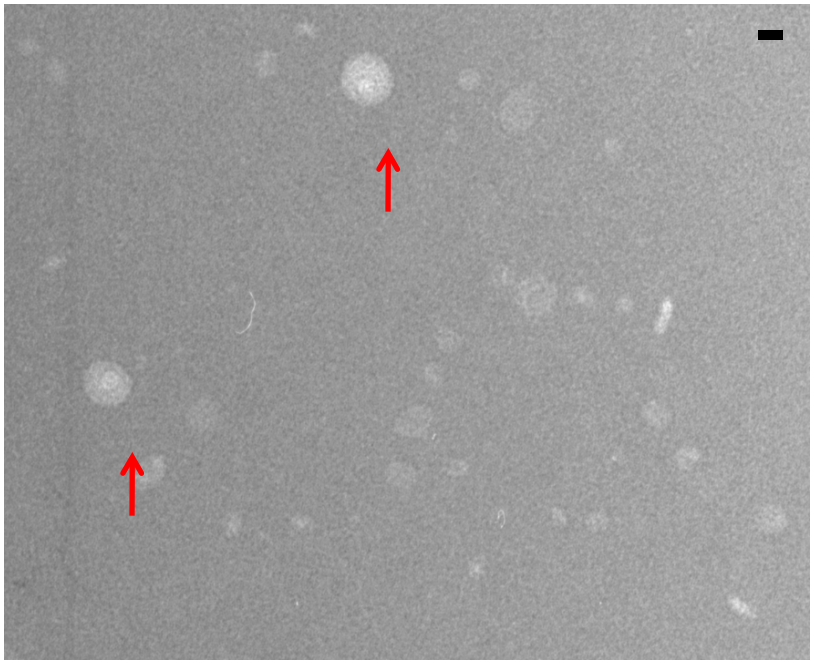
*Methylamine tungstate stain. Scale bar = 10nm

Multiple MSP for one disc results in larger nanodiscs

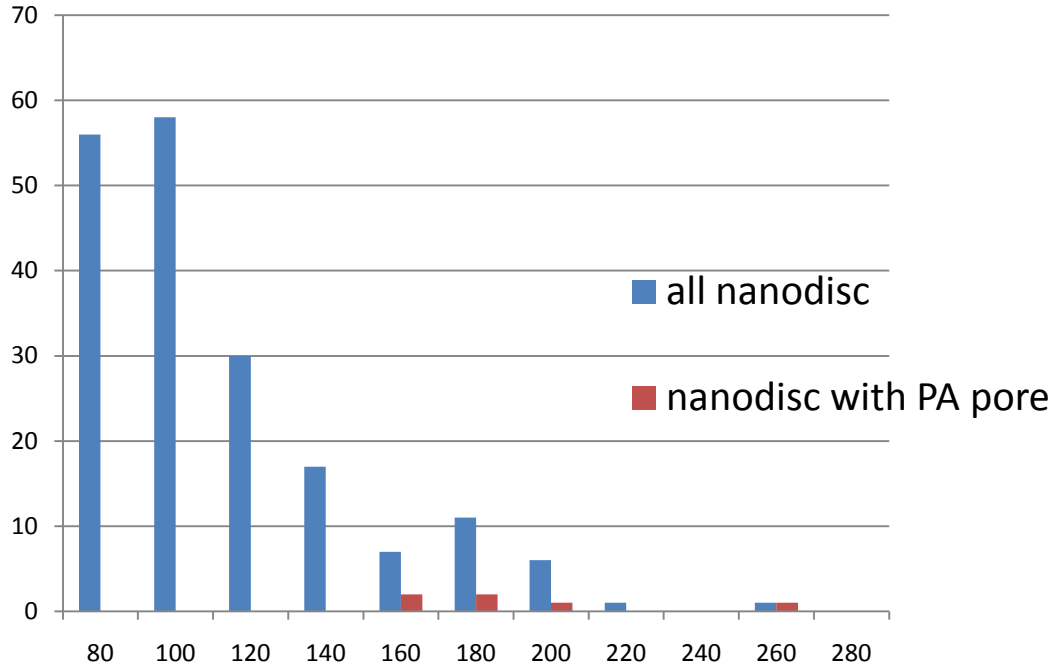
Multiple additions to increase insertion



Only large nanodiscs had PA pore inserted



*Methylamine tungstate stain. Scale bar = 10nm

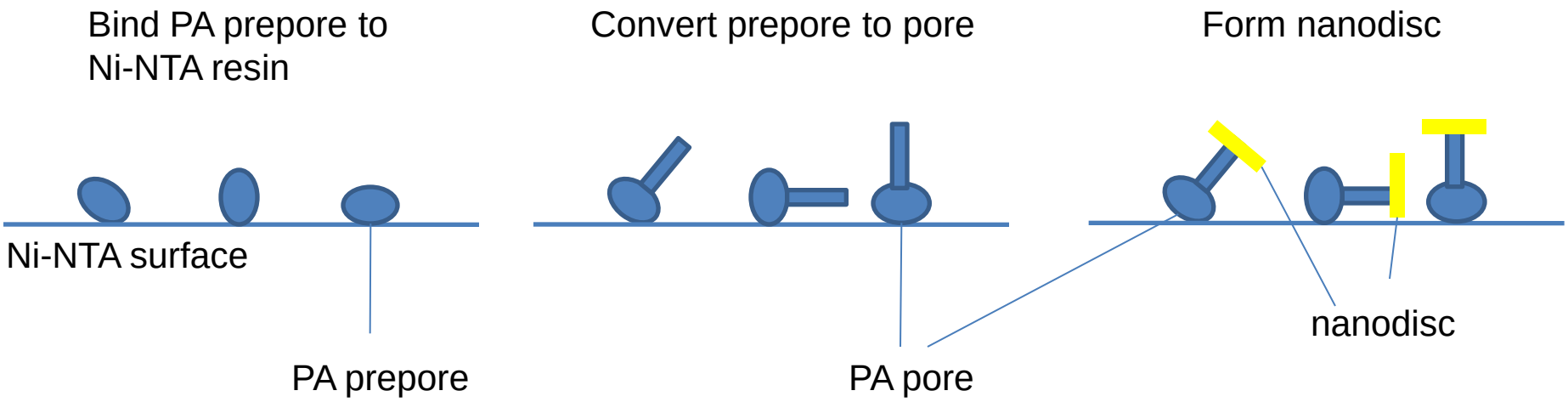


•PA stem may occupy so much area when inserted to nanodisc while the circumference of the disc is define by MSP and cannot stretch.

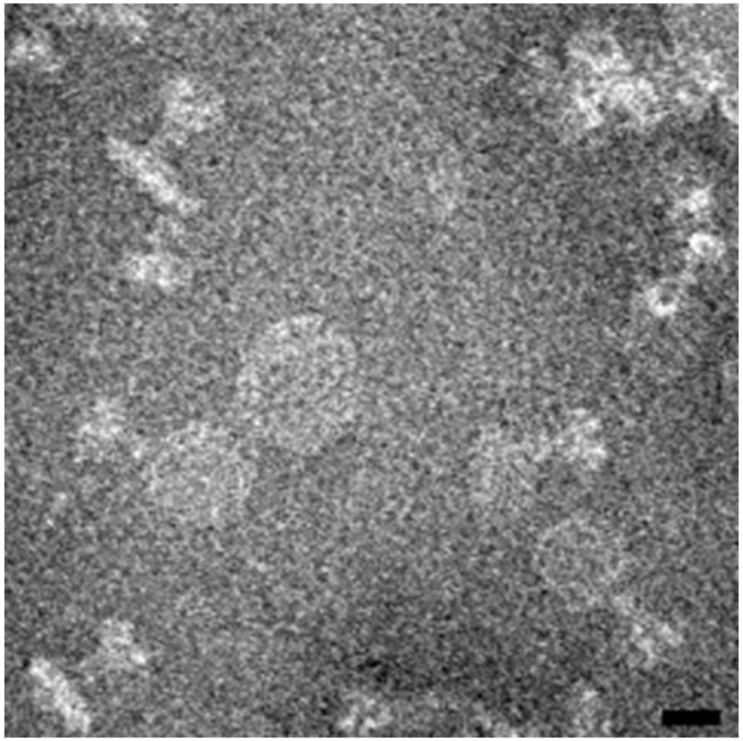
•Larger nanodiscs may help, and we are obtaining longer MSP.

Forming nanodisc in the presence of PA pore

- PA pore rapidly aggregates in solution. Aggregation must be prevented while nanodisc is forming.
- PA weakly interact with Ni-NTA resin (no his-tag). The binding is strong enough for changing solution and washing, and it can be released with imidazol.
- Immobilizing PA to Ni-NTA prevent it from aggregating while nanodiscs are forming.



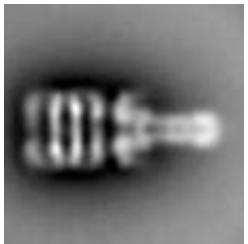
More PA pore was found in nanodiscs



Methylamine tungstate stain.
Scale bar = 10nm

Aggregations and lipid vesicles are also present.
Aggregations come from PA prepore that did not convert to pore on the resin, and pore that did not get capped with nanodiscs.

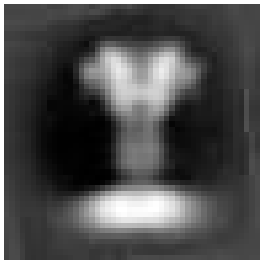
To enrich PA/nanodisc population (ongoing)
- Purification
- Orientation specific binding – GroEL immobilized beads

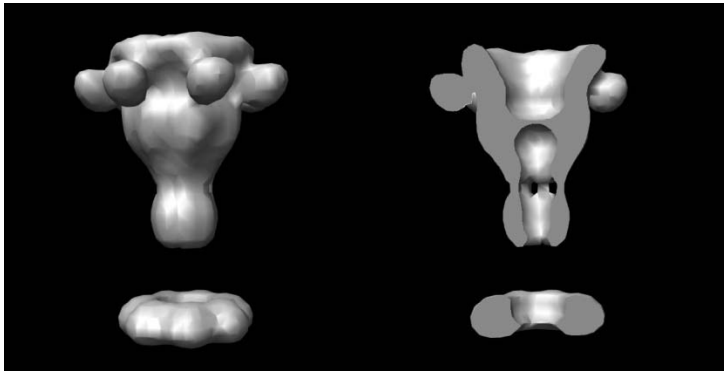


Preliminary image analysis

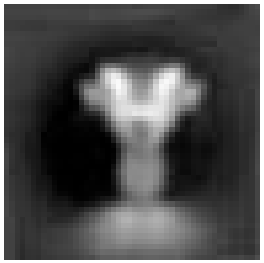
Structures at 22Å resolution

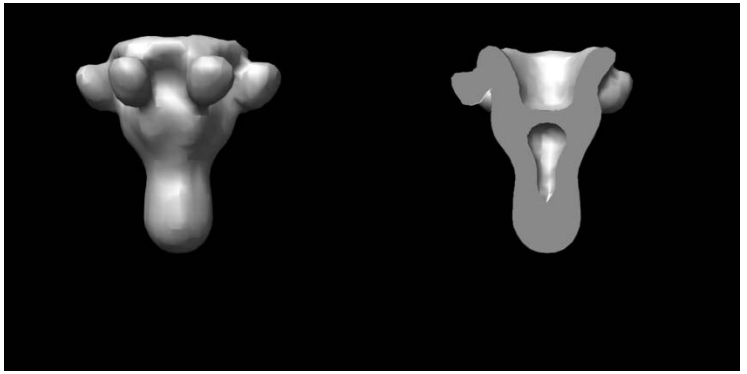
PA pore in nanodisc





PA pore in vesicle





*2D average and 3D reconstructions from **negatively stained images**

In surface representation, the entire vesicle did not appear, and there is a gap between nanodisc and the stem of PA pore. The gap may be due to high stain accumulation on the surface of the nanodisc.

Summary and implications

- Nanodisc can incorporate PA pore and nanodisc/PA complex is observable with EM
- PA pore only inserts to larger nanodiscs when nanodiscs are pre-formed.
- Immobilizing PA pore decreased aggregation and enabled nanodisc to form around the pore stem
- Immobilization method can bypass detergent solubilization.
- Similar approaches may be applicable to other membrane proteins with different immobilization methods such as affinity tags.

Acknowledgement

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