

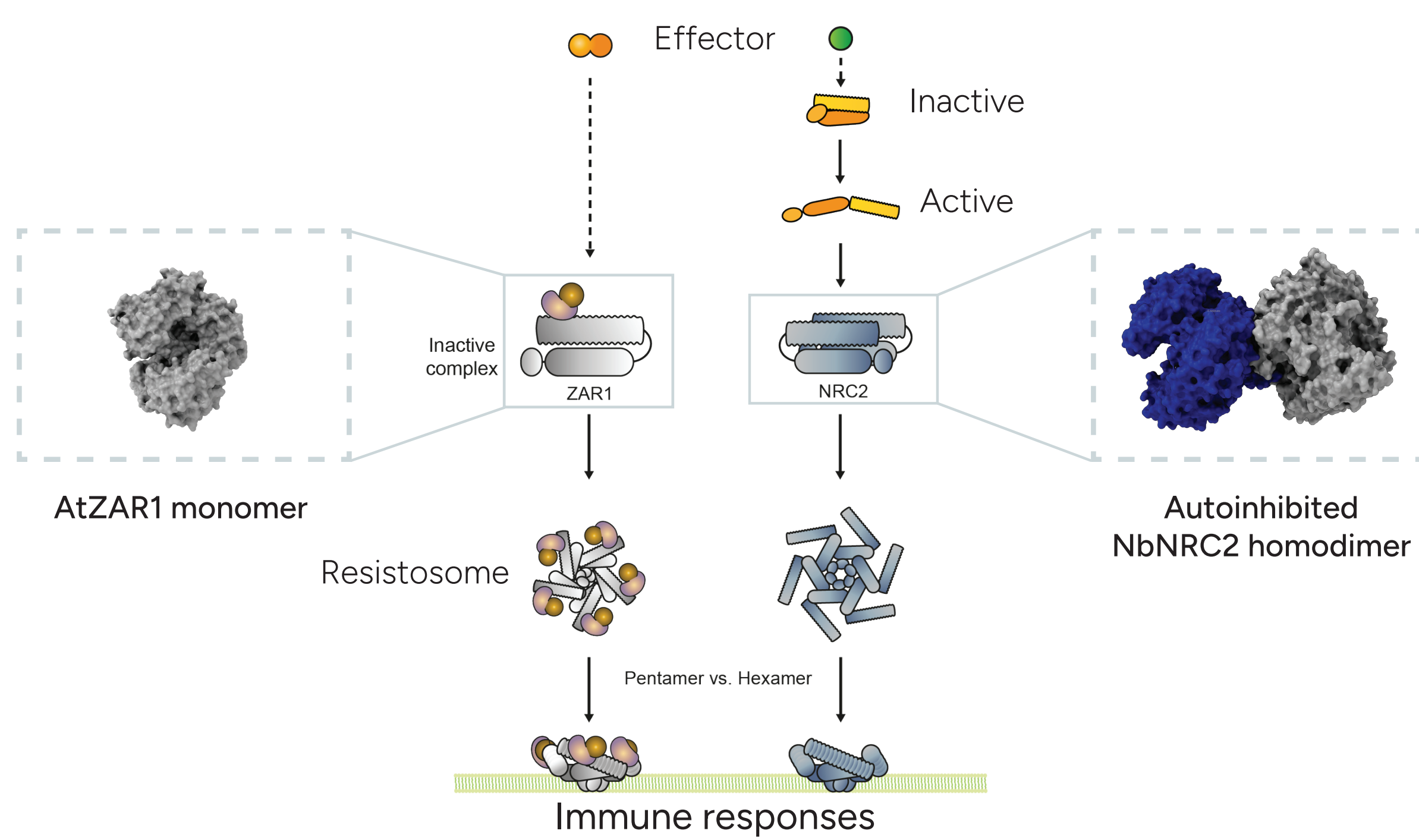


No Rest for the Resting State: Unresolved Structures of NRC immune proteins

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1

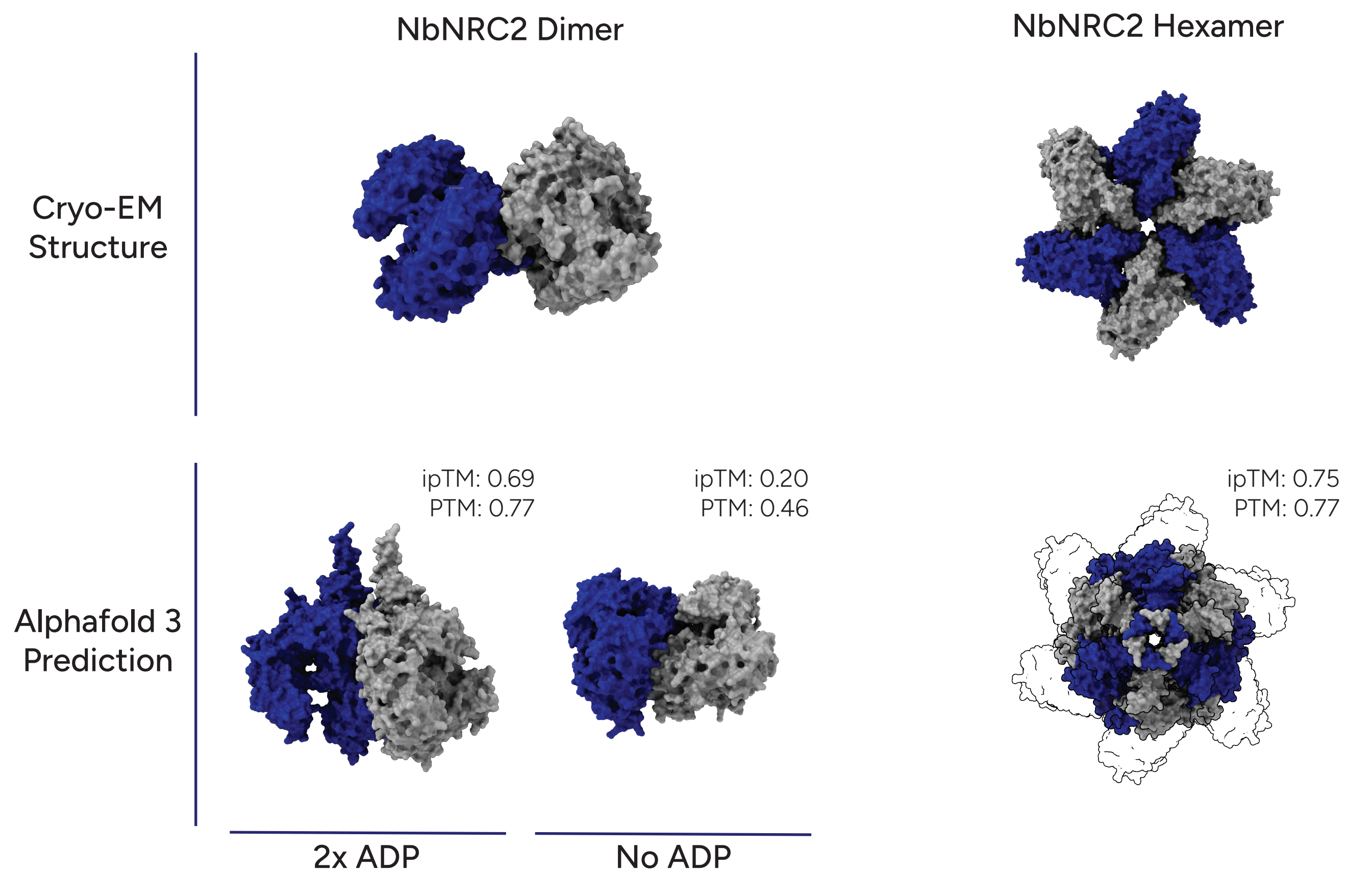
Plant NLRs exist in resting states and, upon activation oligomerize into higher-order resistosomes



So far monomeric and homodimeric resting states have been described.
Are more diverse forms yet to be found?

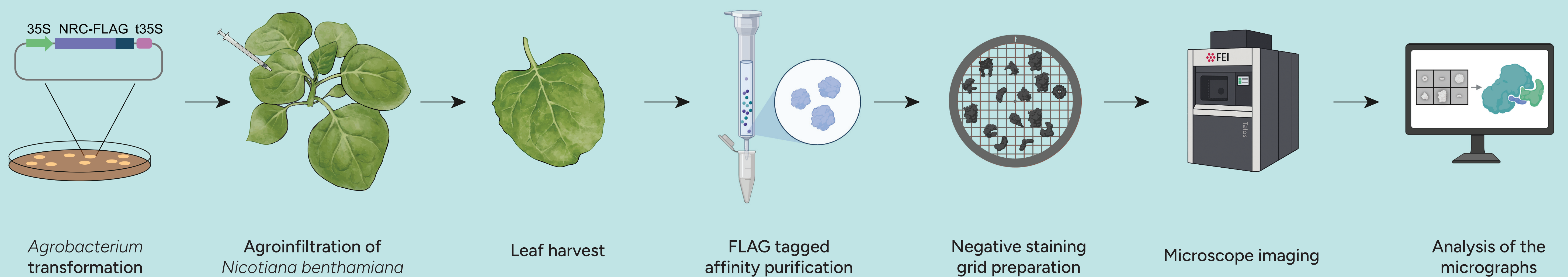
2

AlphaFold 3 mispredicts NRC resting-state compared to activated hexameric resistosomes



3

Immunoprecipitation coupled with electron microscopy for protein purification and structural analysis



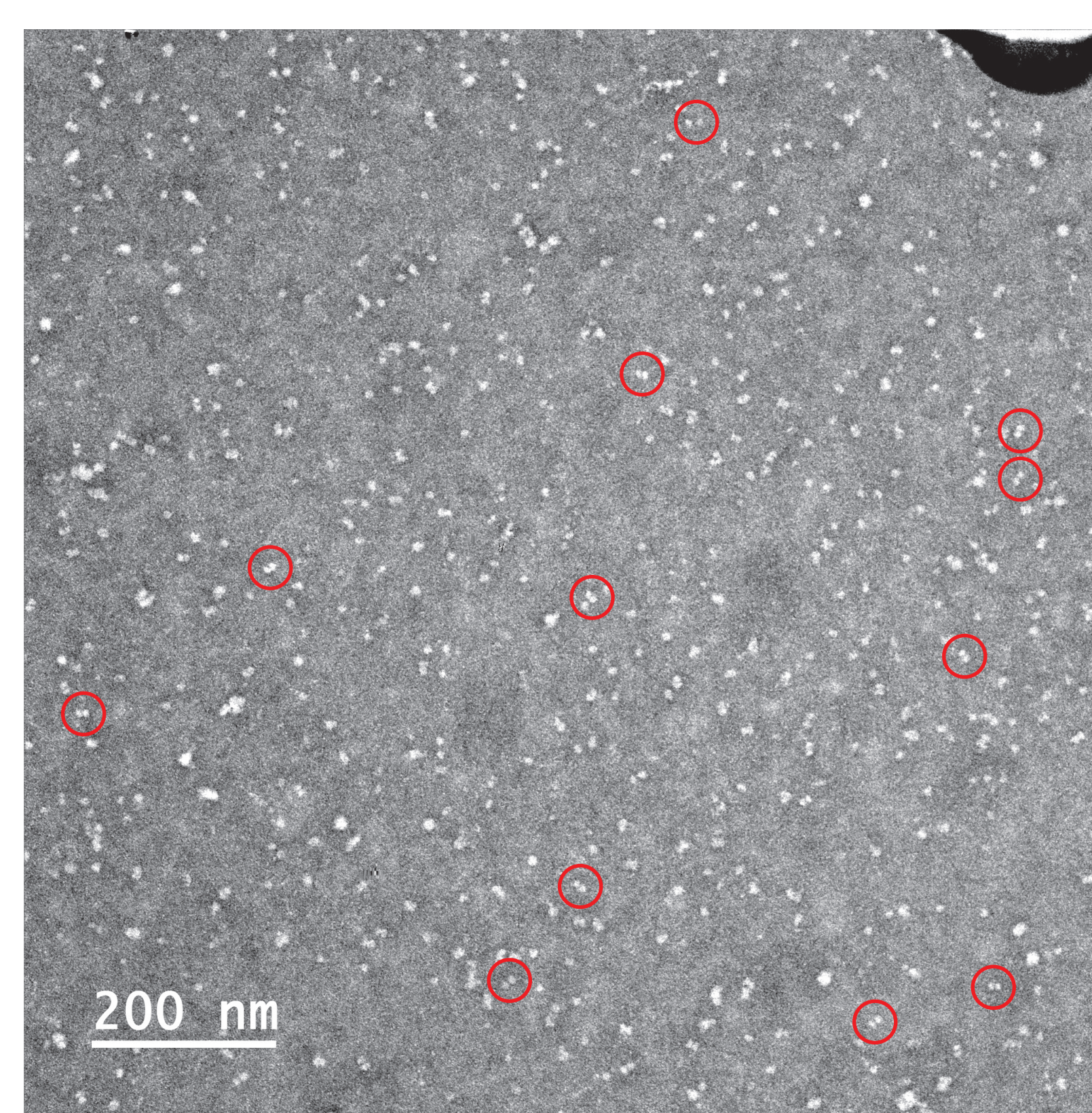
4

IP-EM screening of NRC resting-states

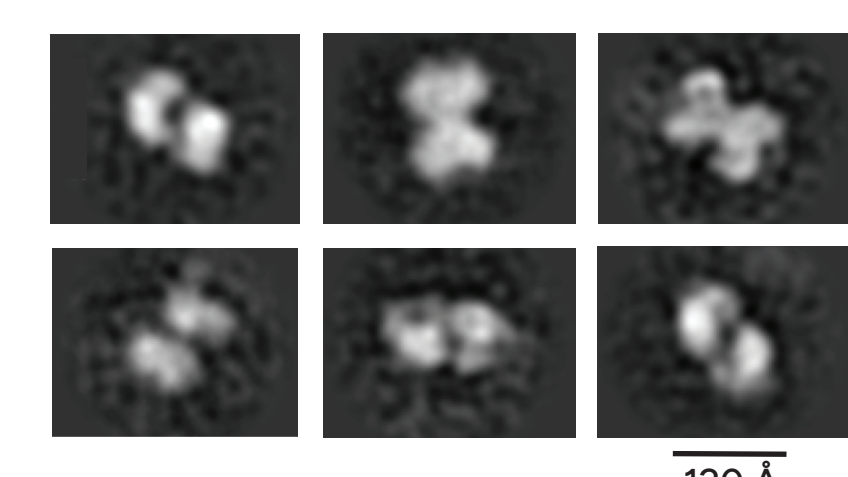
	Purification	Negative Staining
SINRC1	●	●
SINRC2	●	●
NbNRC2a	●	●
NbNRC3	●	●
SINRC3	●	●
SINRC4a	●	●
NbNRC4a	●	●
SINRC4b	●	●
SINRC6	●	●
SINRC7	●	●

5

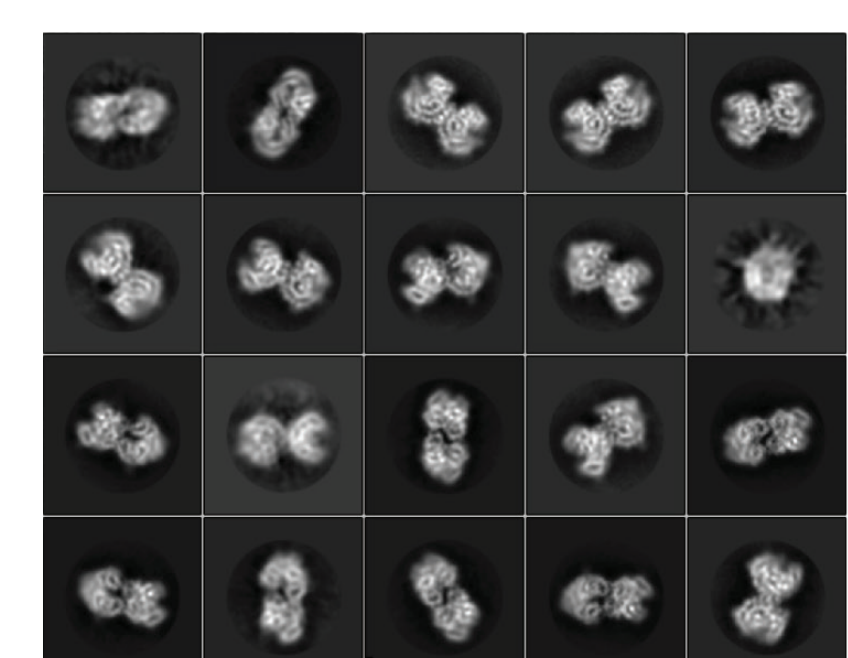
Validation of the IP-EM pipeline with NbNRC2 resting-state homodimer



Negative stain micrograph of NbNRC2 at 45kx magnification



2D classifications from negative staining



2D classifications from Cryo-EM
Selvaraj et al 2024

6

Conclusions

- Resting-state NRCs are difficult to characterize and poorly predicted by AlphaFold3, while active-state models show higher confidence and are more amenable to structural determination.

References

- Selvaraj et al. (2024) PMID: 39423240
- Contreras et al. (2023) - PMID: 37134163
- Madhuprakash et al. (2024) PMID 39504373
- Contreras, Pai et al. (2024) - PMID: 38757730