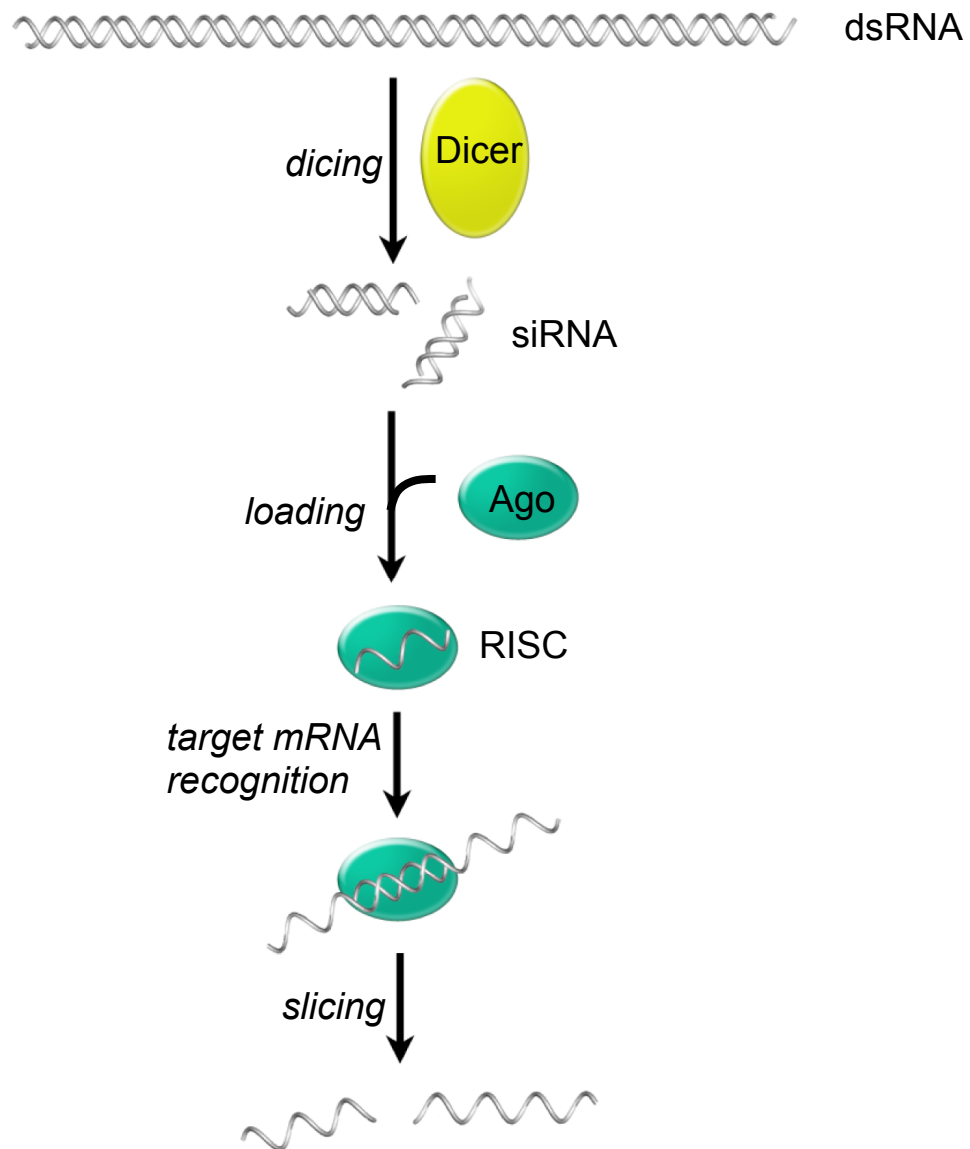


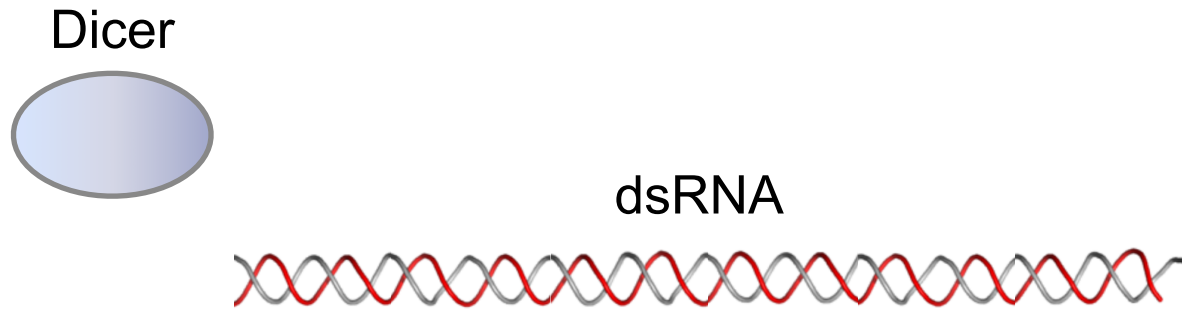
DOLORS: Versatile Strategy for Internal Labeling and Domain Localization in Electron Microscopy

Ian J. MacRae
The Scripps Research Institute
November 2012

dsRNA triggers the RNAi pathway

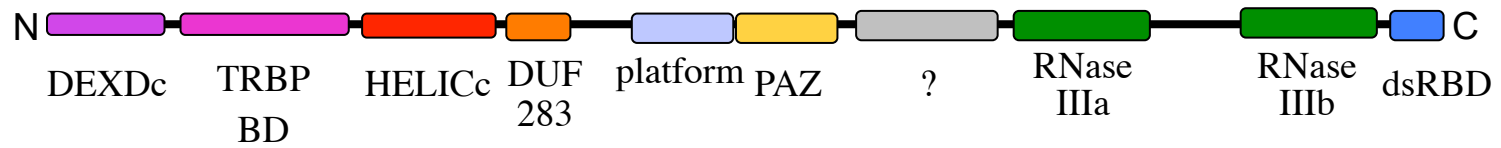


3 steps in dsRNA processing by Dicer

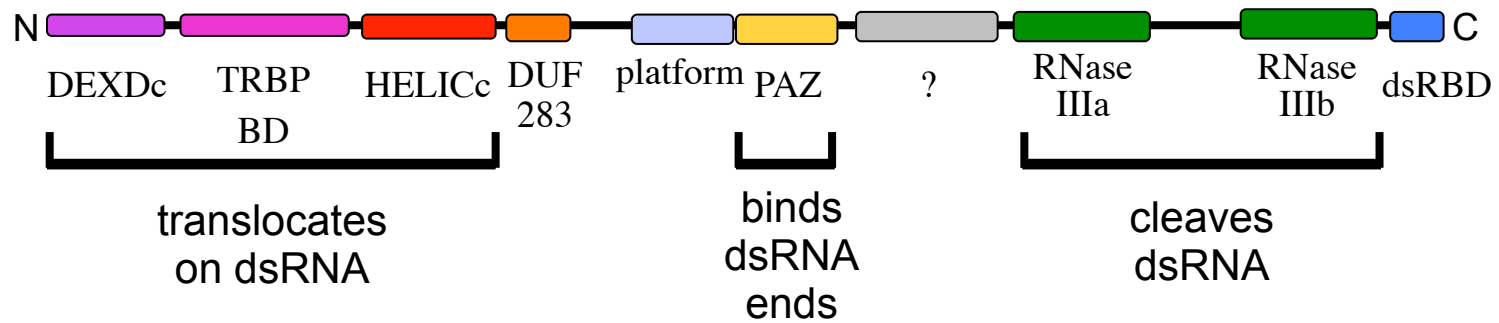


- 1) dsRNA binding
- 2) dsRNA cleavage
- 3) translocation

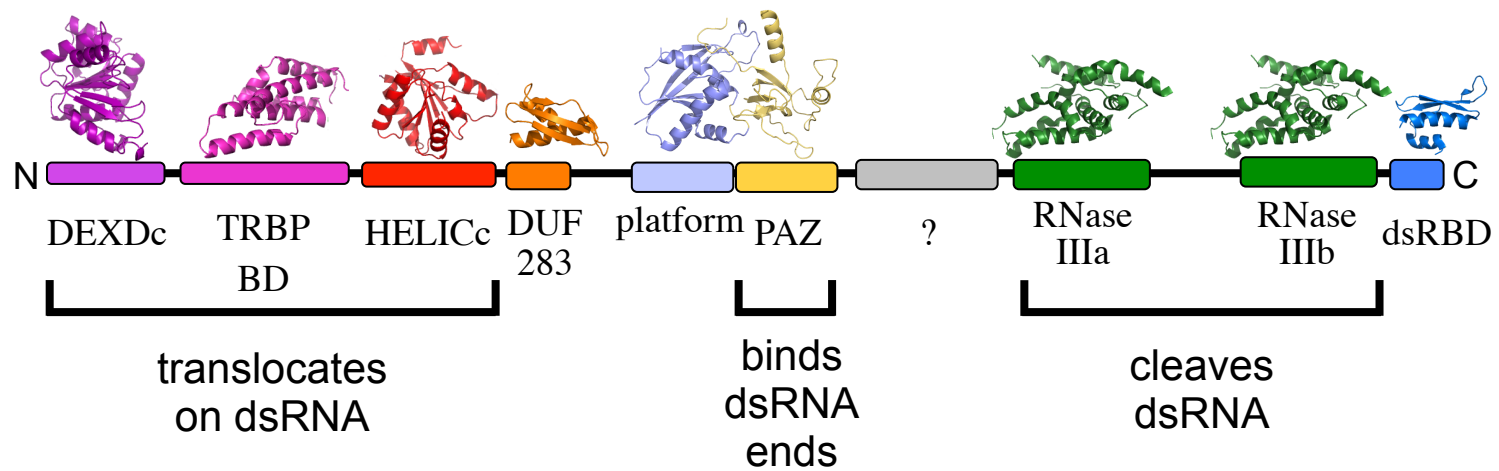
Dicer is a 220 kDa, multi-domain protein



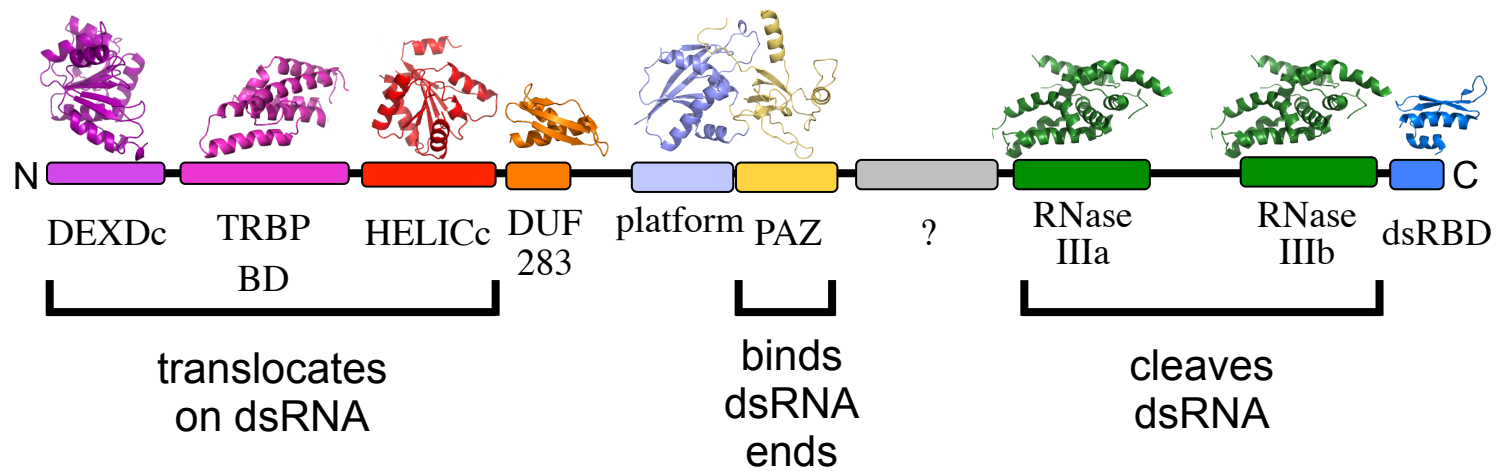
Previous studies have ascribed specific activities to discrete domains



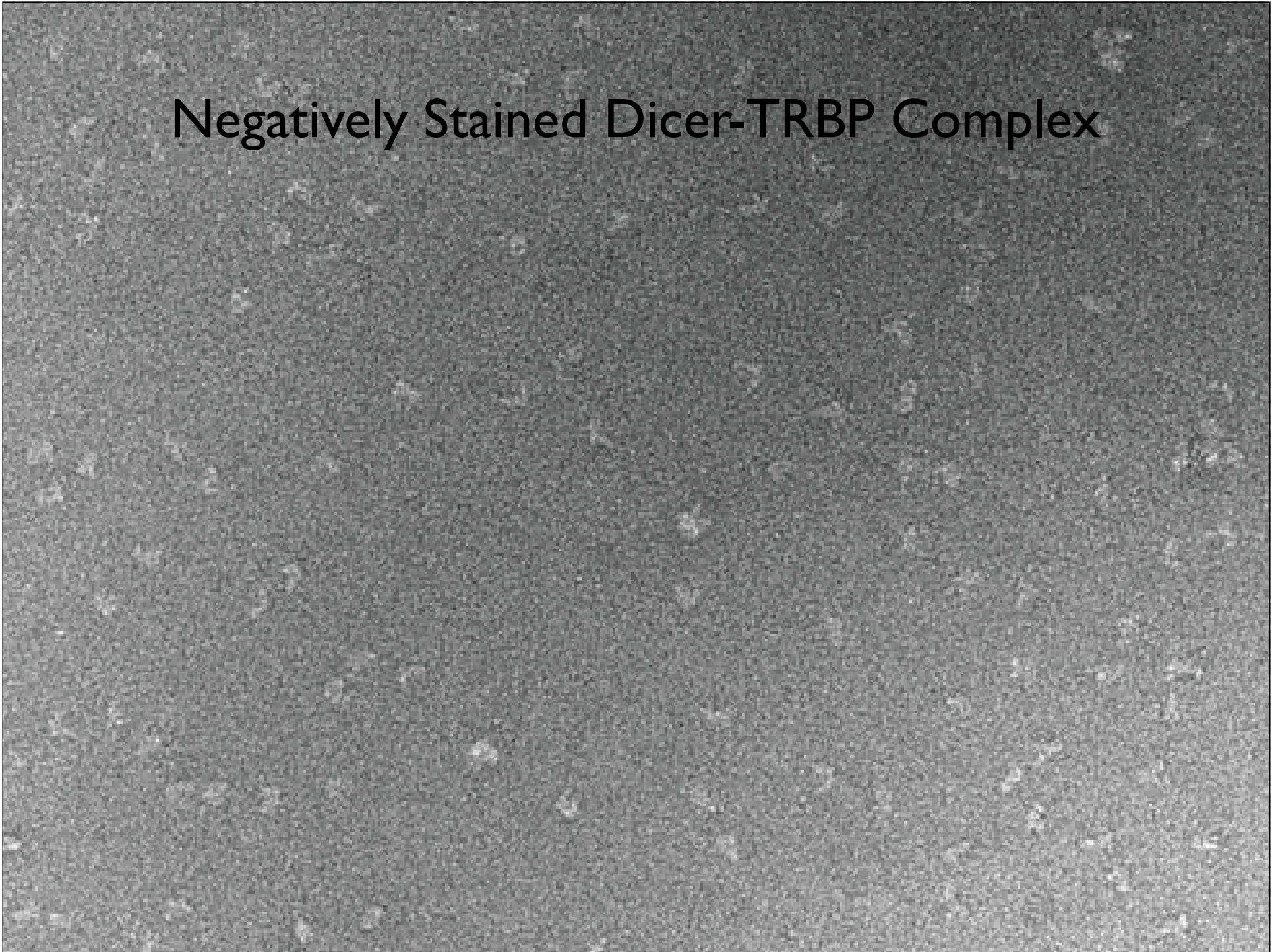
Crystal structures of many domains (or homologous domains) have been established



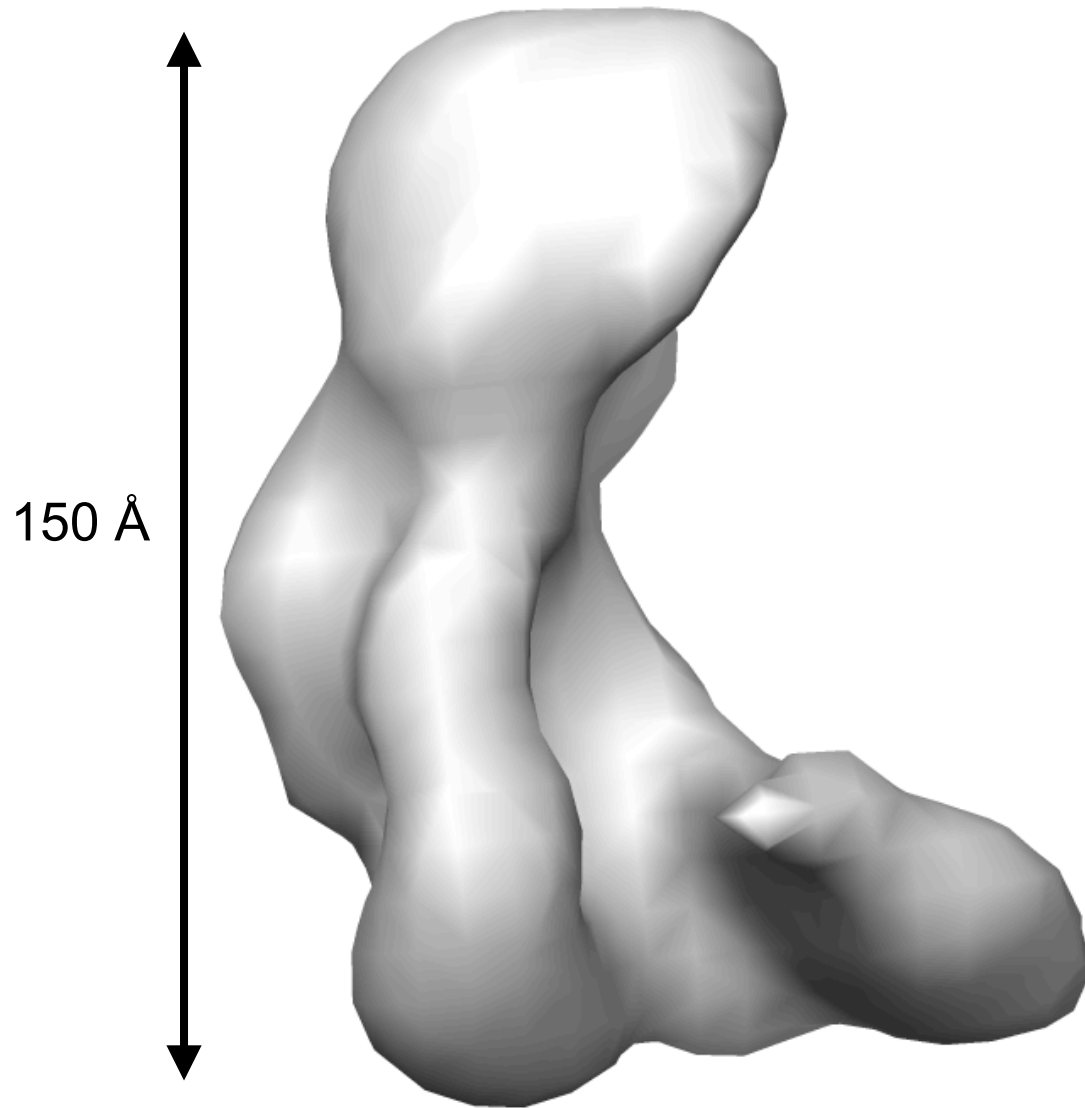
How do these domains assemble in 3D to make a functional dicing machine?



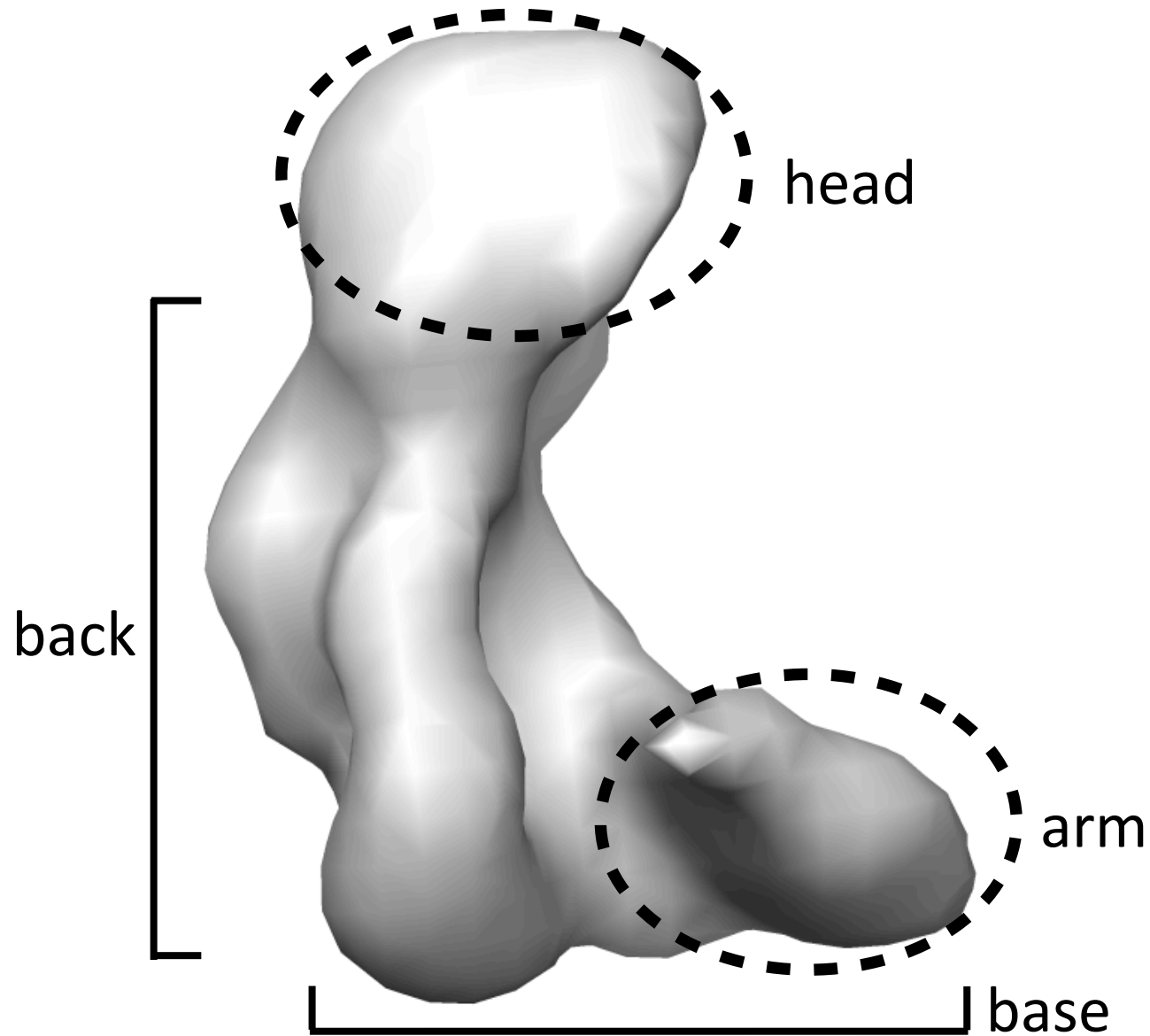
Negatively Stained Dicer-TRBP Complex



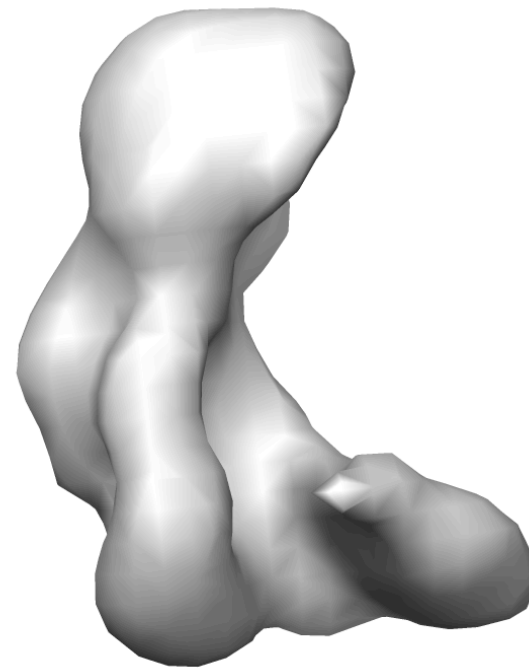
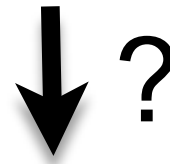
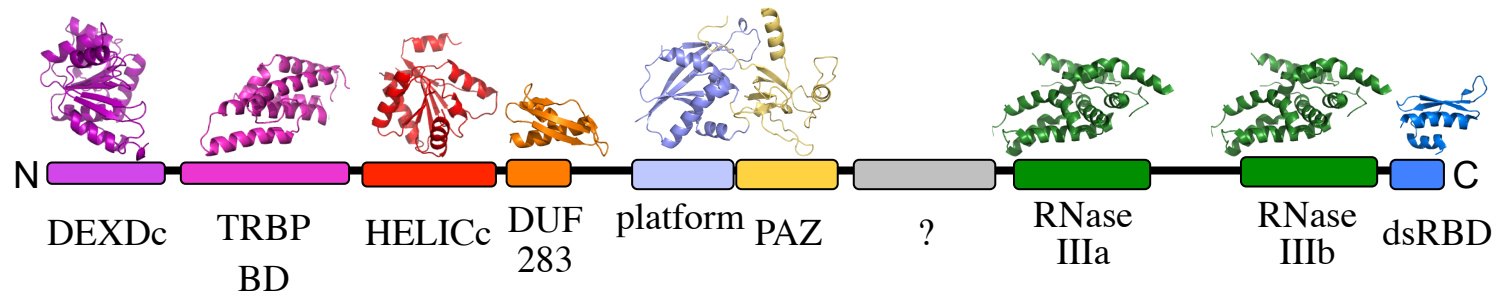
3D reconstruction of Human Dicer-TRBP (20 Å resolution)

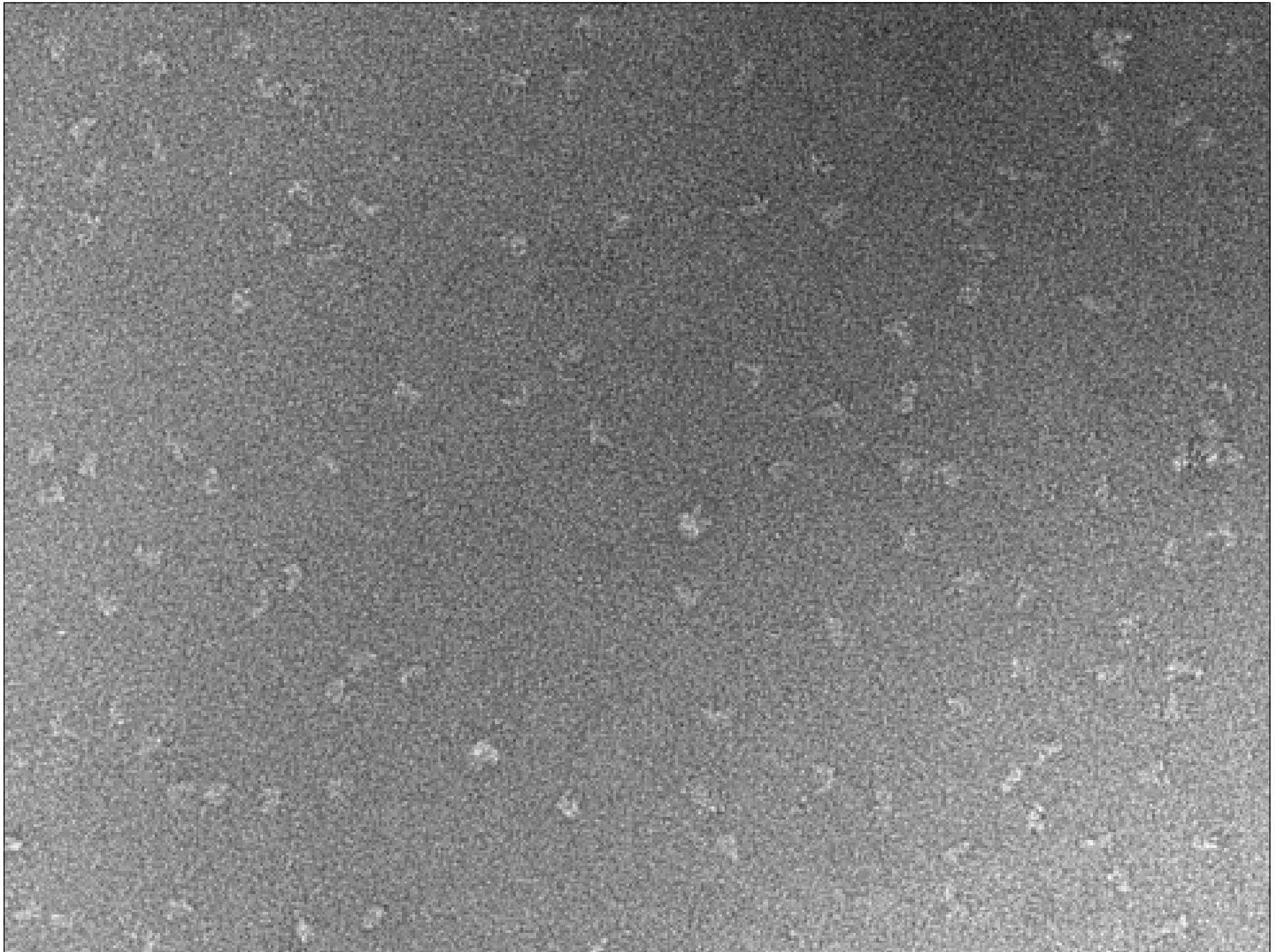


3D reconstruction of Human Dicer-TRBP (20 Å resolution)





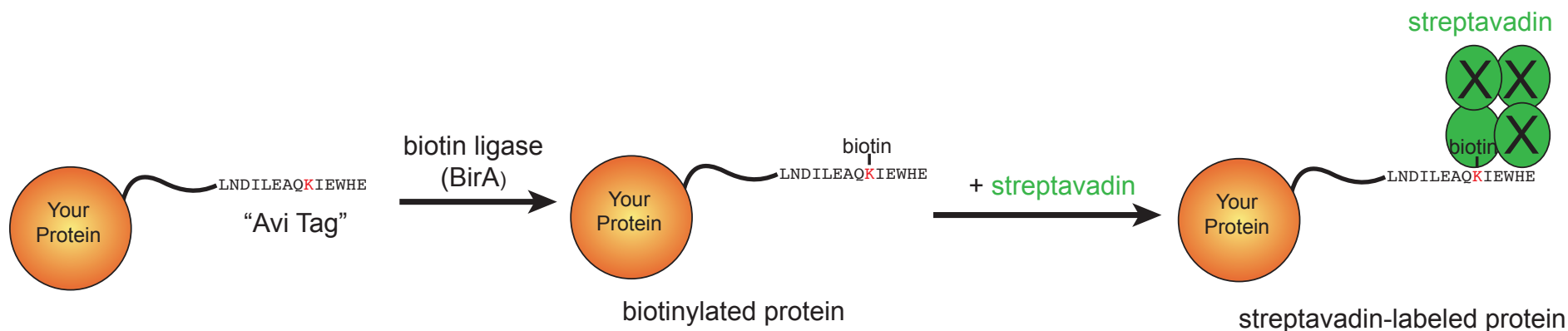




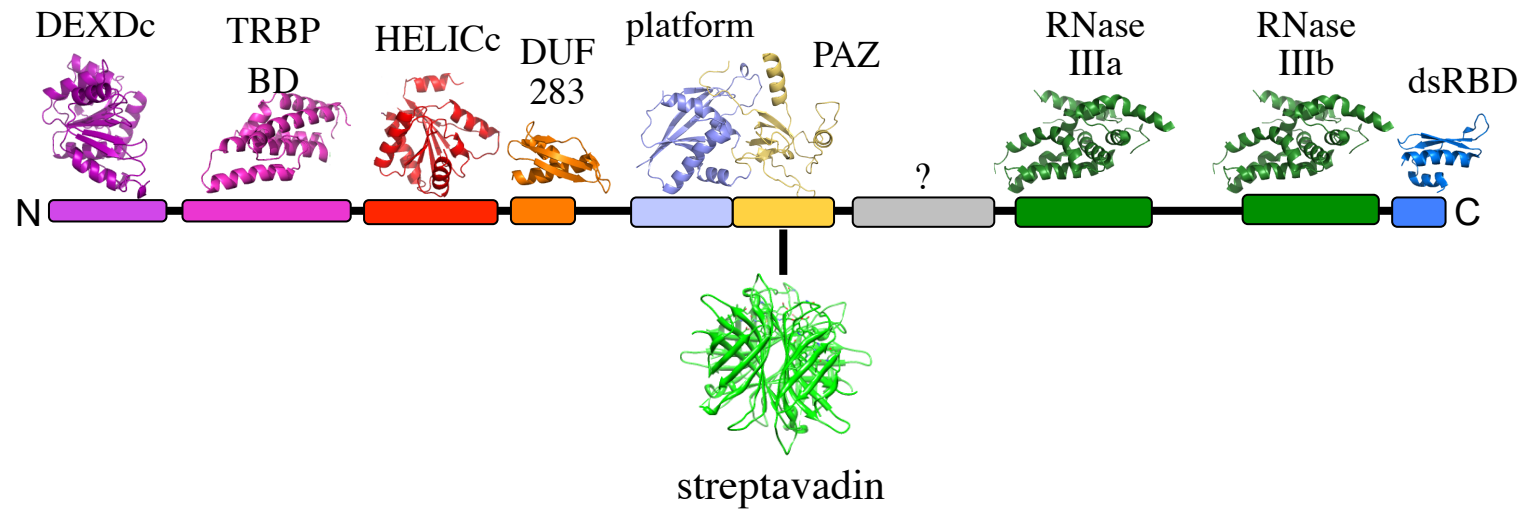
A monovalent streptavidin with a single femtomolar biotin binding site

Mark Howarth¹, Daniel J-F Chinnapen^{1,4}, Kimberly Gerrow^{2,4}, Pieter C Dorrestein³, Melanie R Grandy¹, Neil L Kelleher³, Alaa El-Husseini² & Alice Y Ting¹

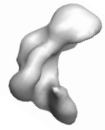
NATURE METHODS | VOL.3 NO.4 | APRIL 2006 |



Label PAZ domain with streptavidin



Labeling Dicer with Streptavidin



partially purified AviTagged Dicer

Labeling Dicer with Streptavidin

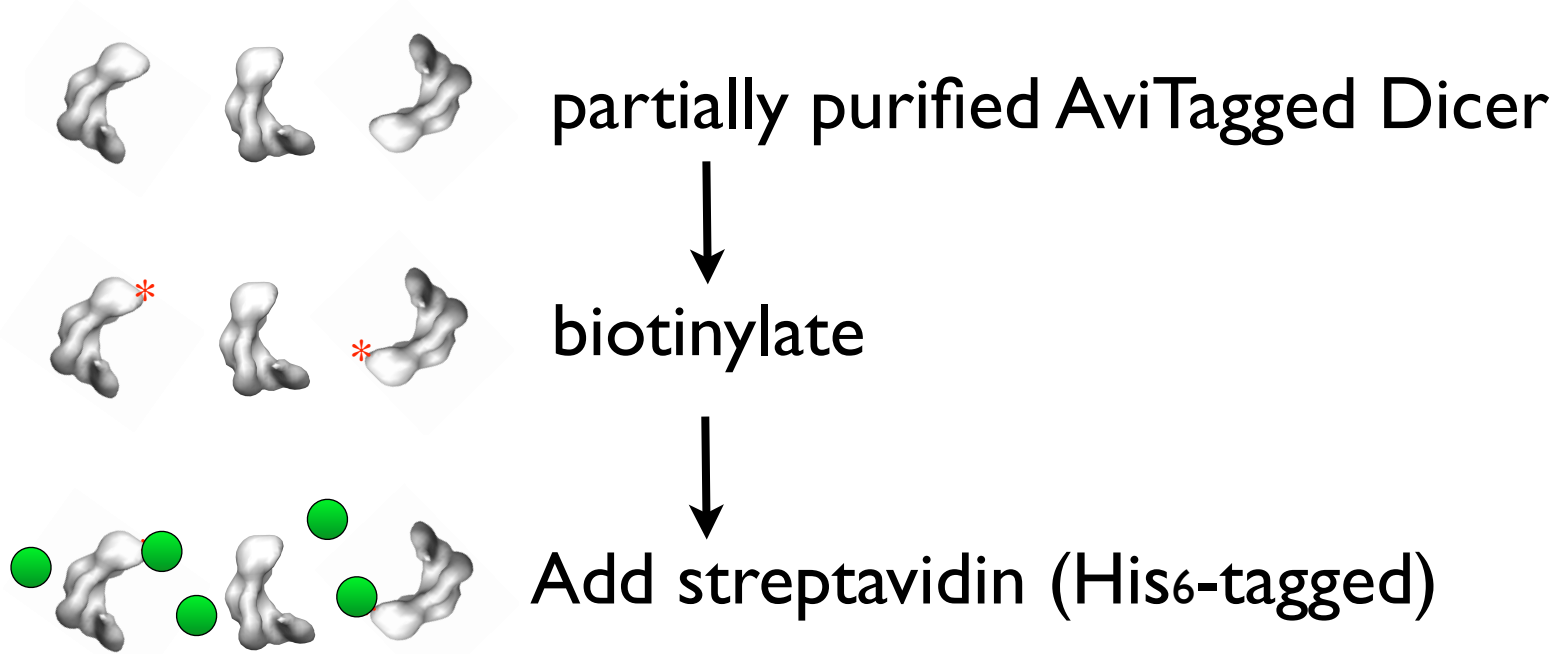


partially purified AviTagged Dicer

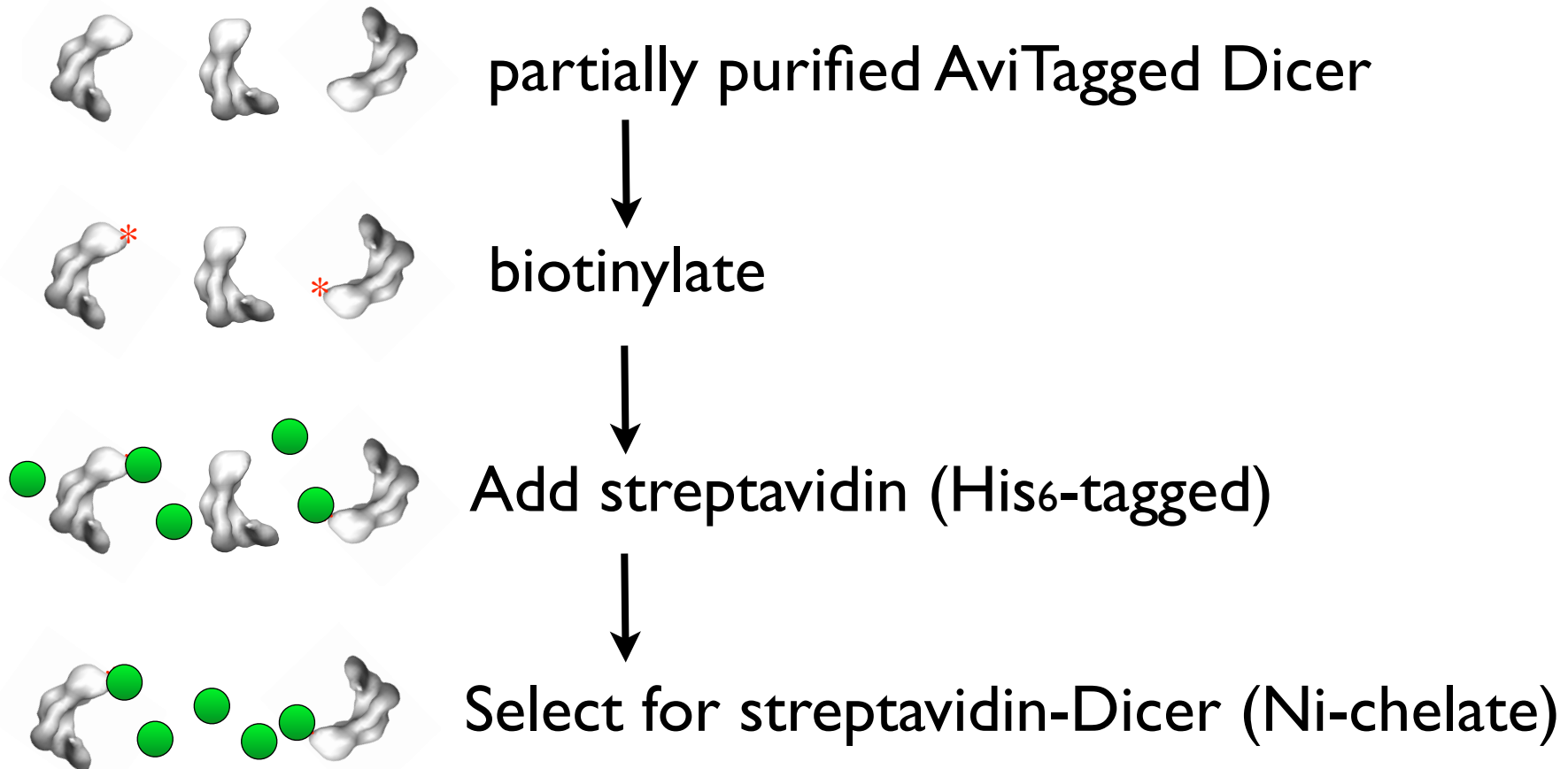


biotinylate

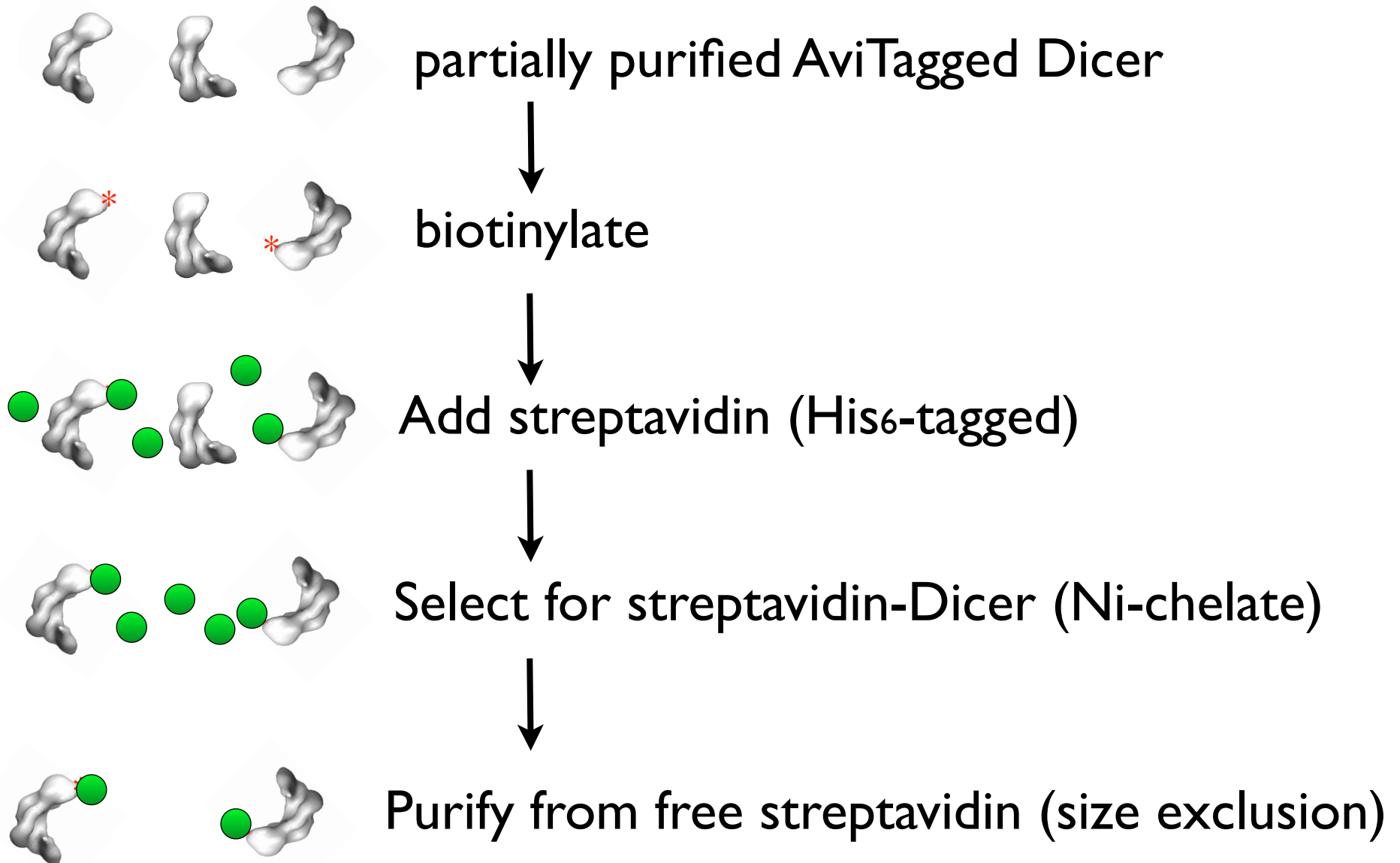
Labeling Dicer with Streptavidin



Labeling Dicer with Streptavidin



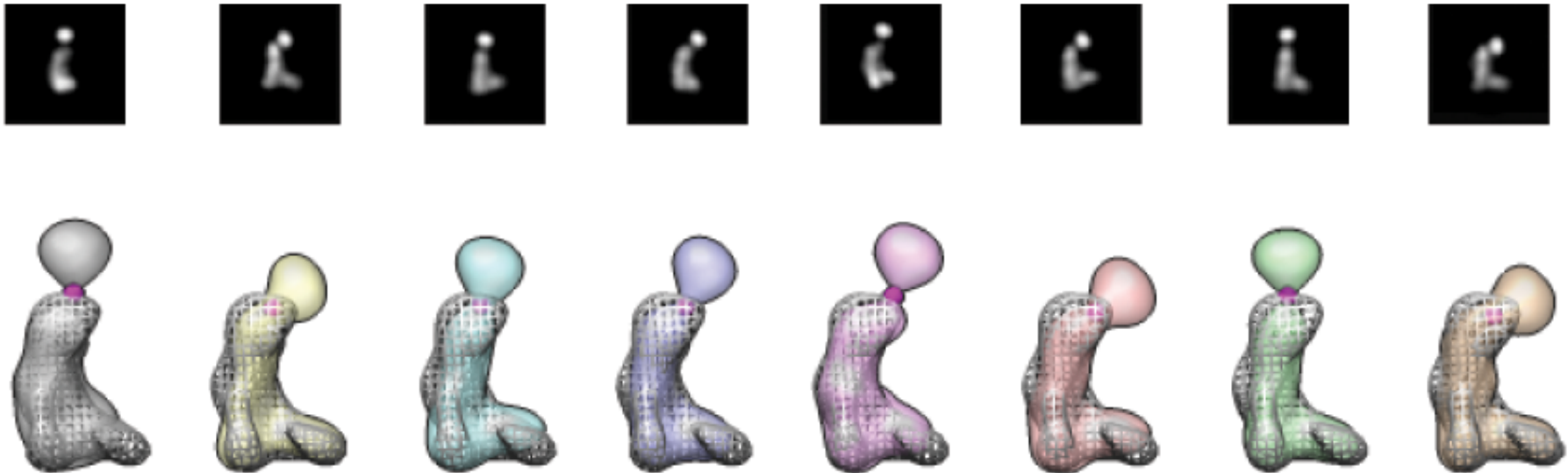
Labeling Dicer with Streptavidin



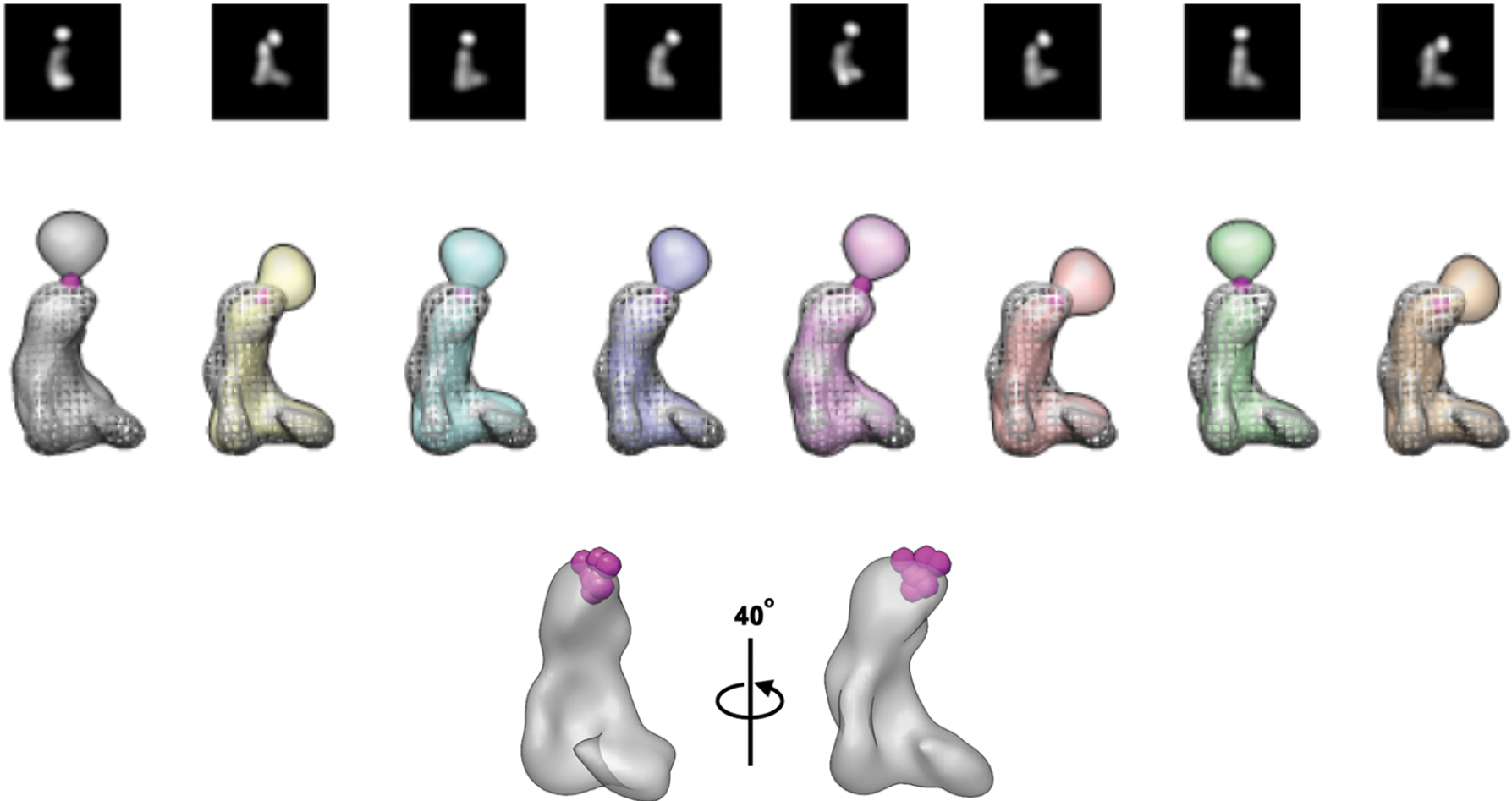
2D Class averages have round density extending from the head



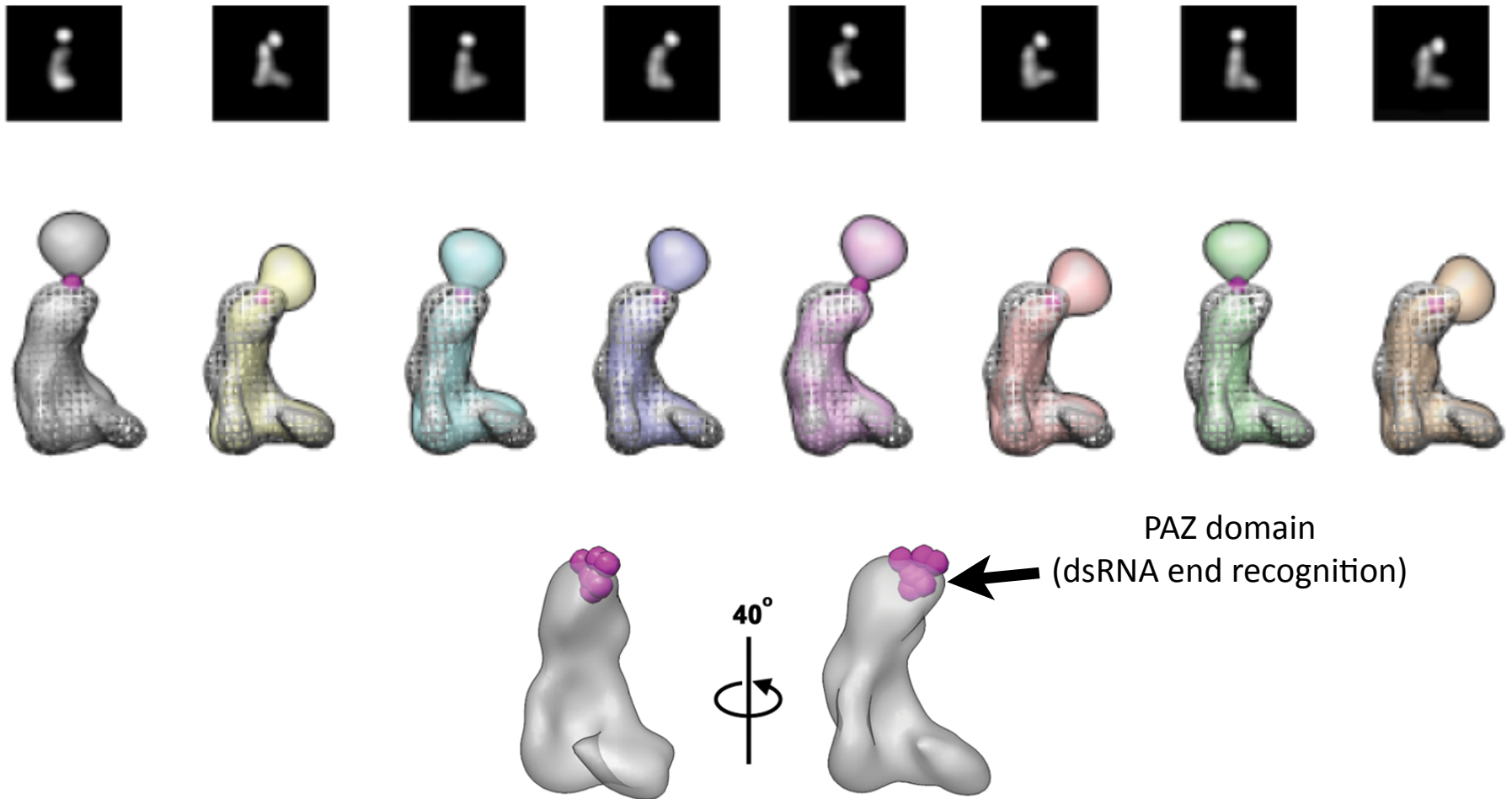
3D reconstructions overlaid on the unlabeled structure



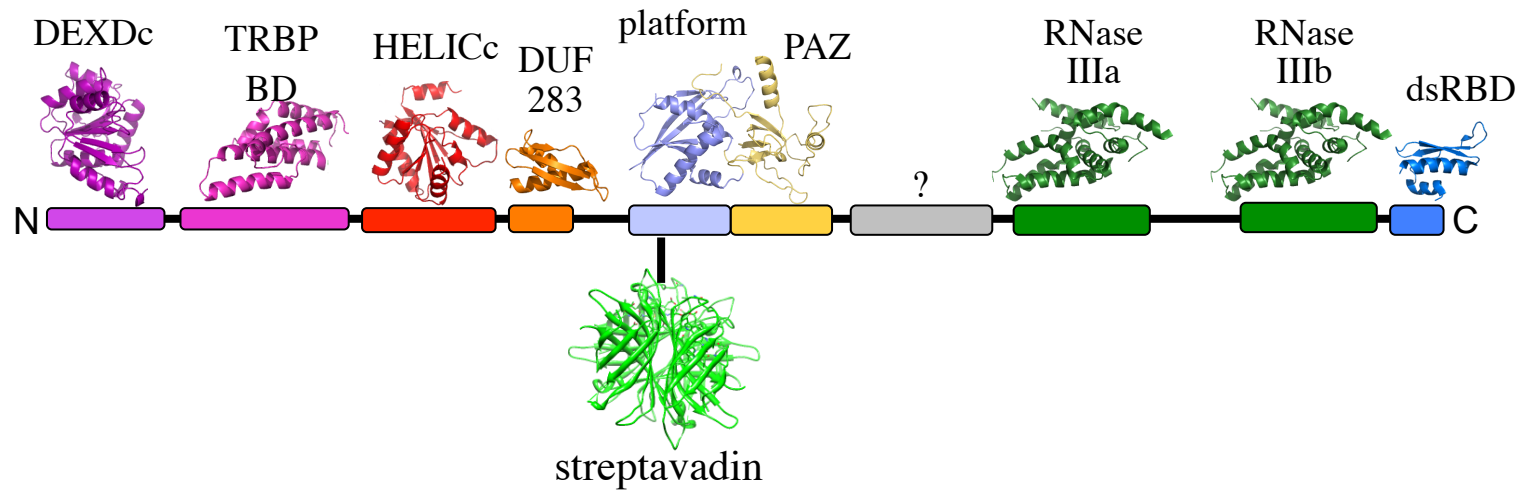
Attachment points map to front of the head



PAZ domain is in the front of the head



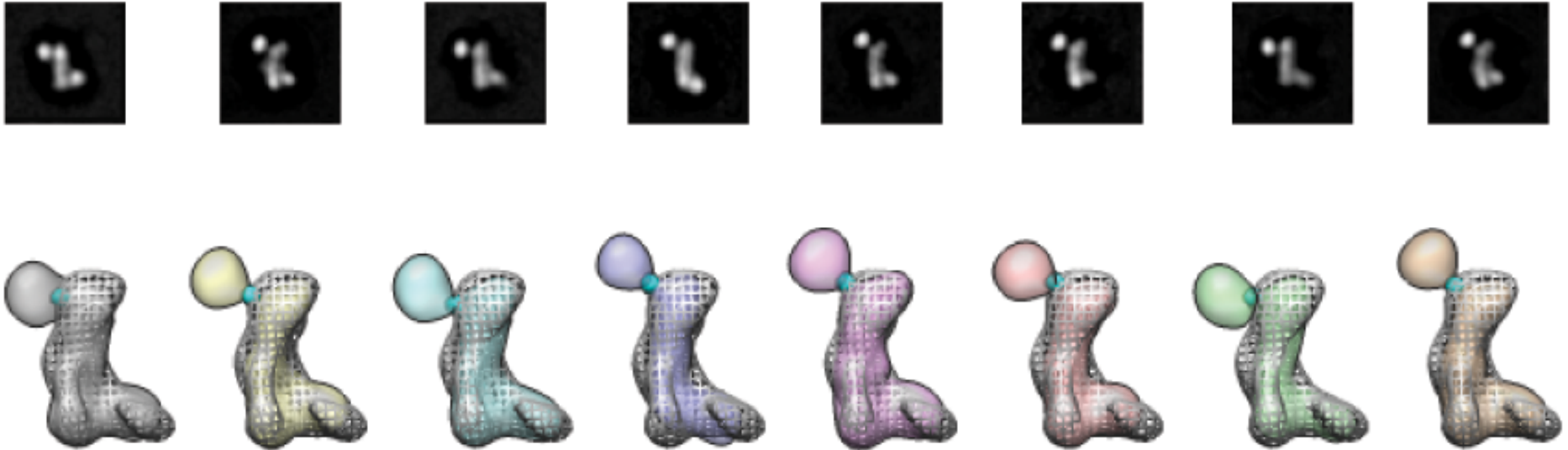
Label platform domain with streptavidin



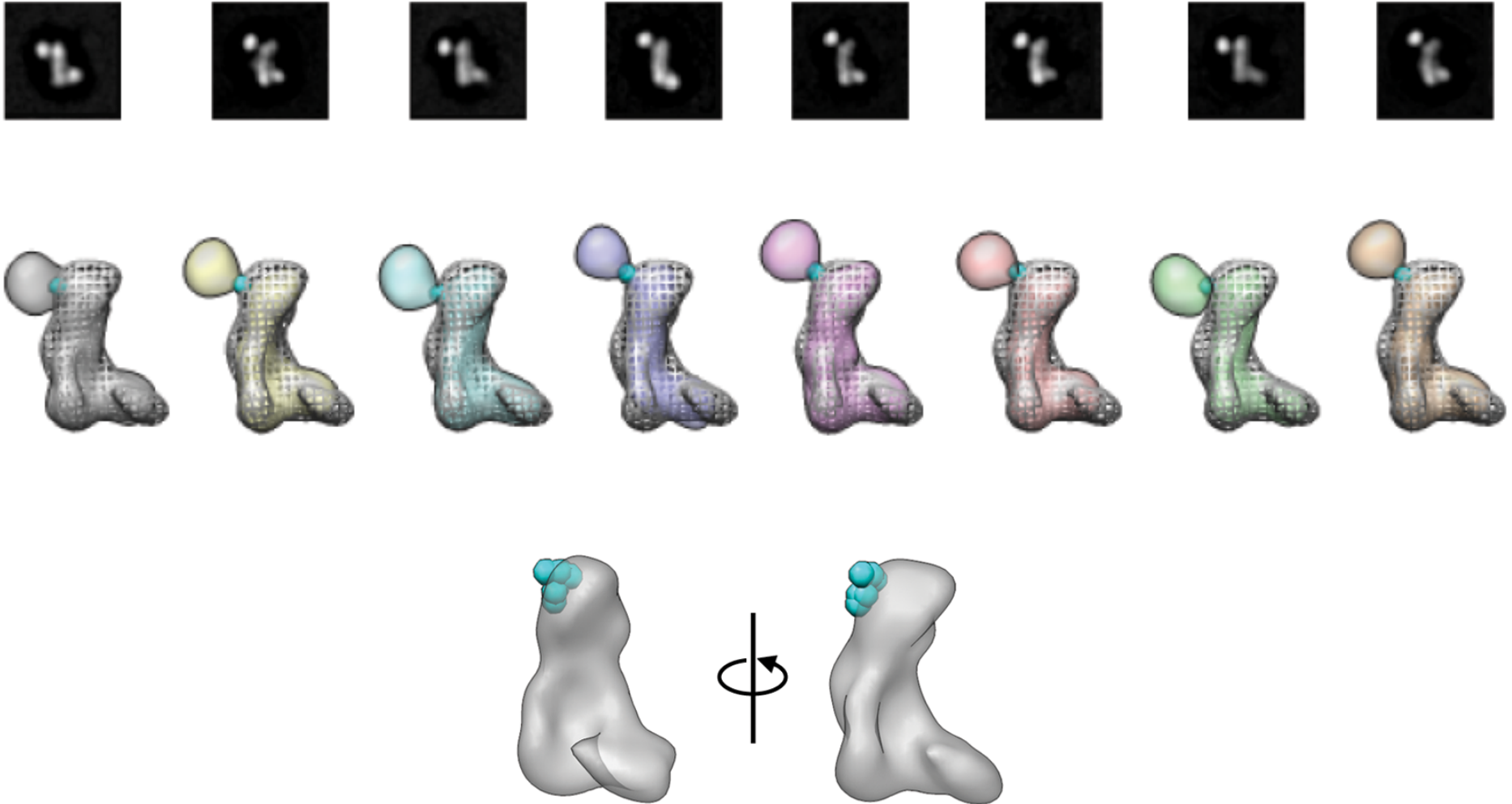
2D Class averages have density
extending from the back of the head



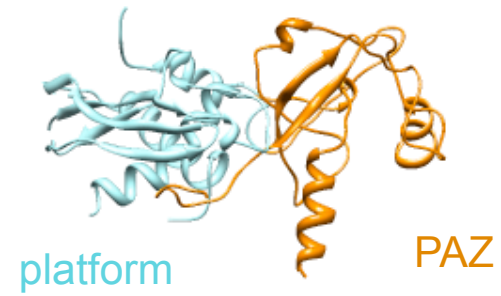
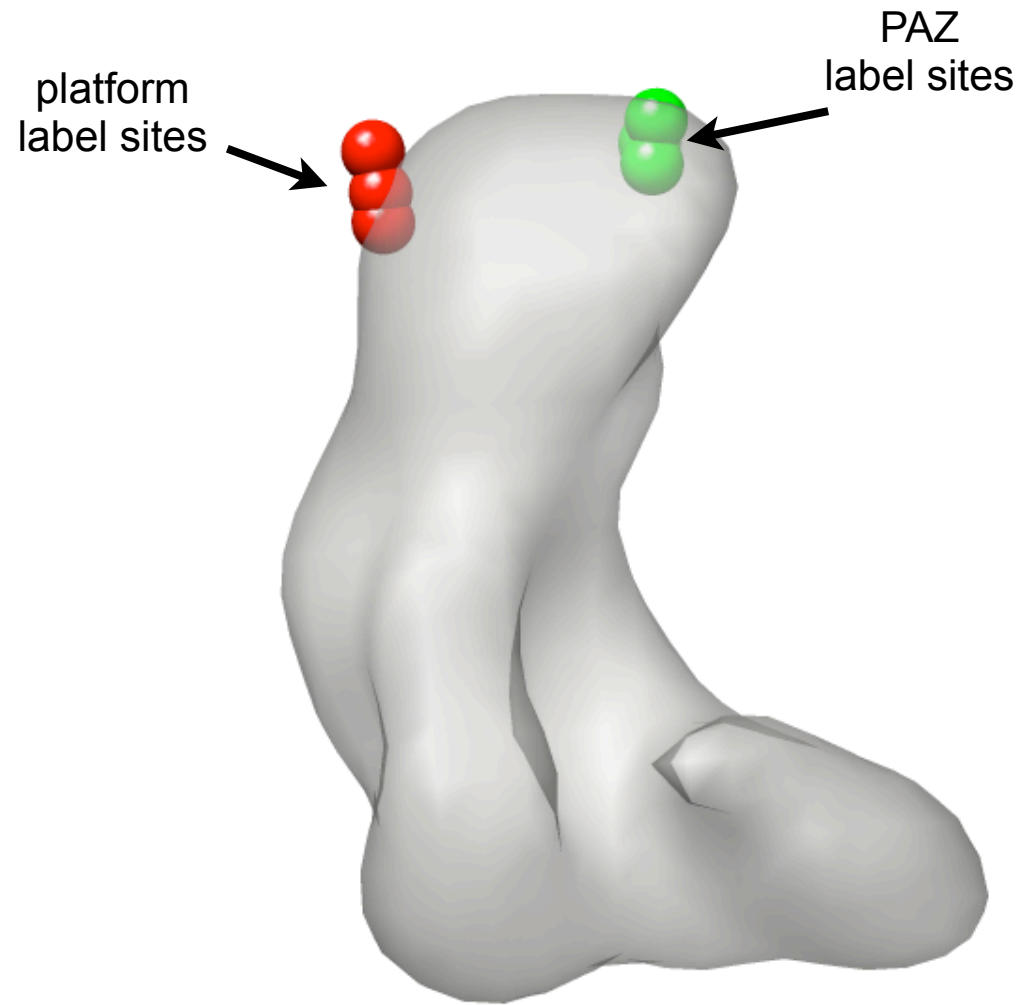
Overlay 3D reconstructions on the unlabeled map



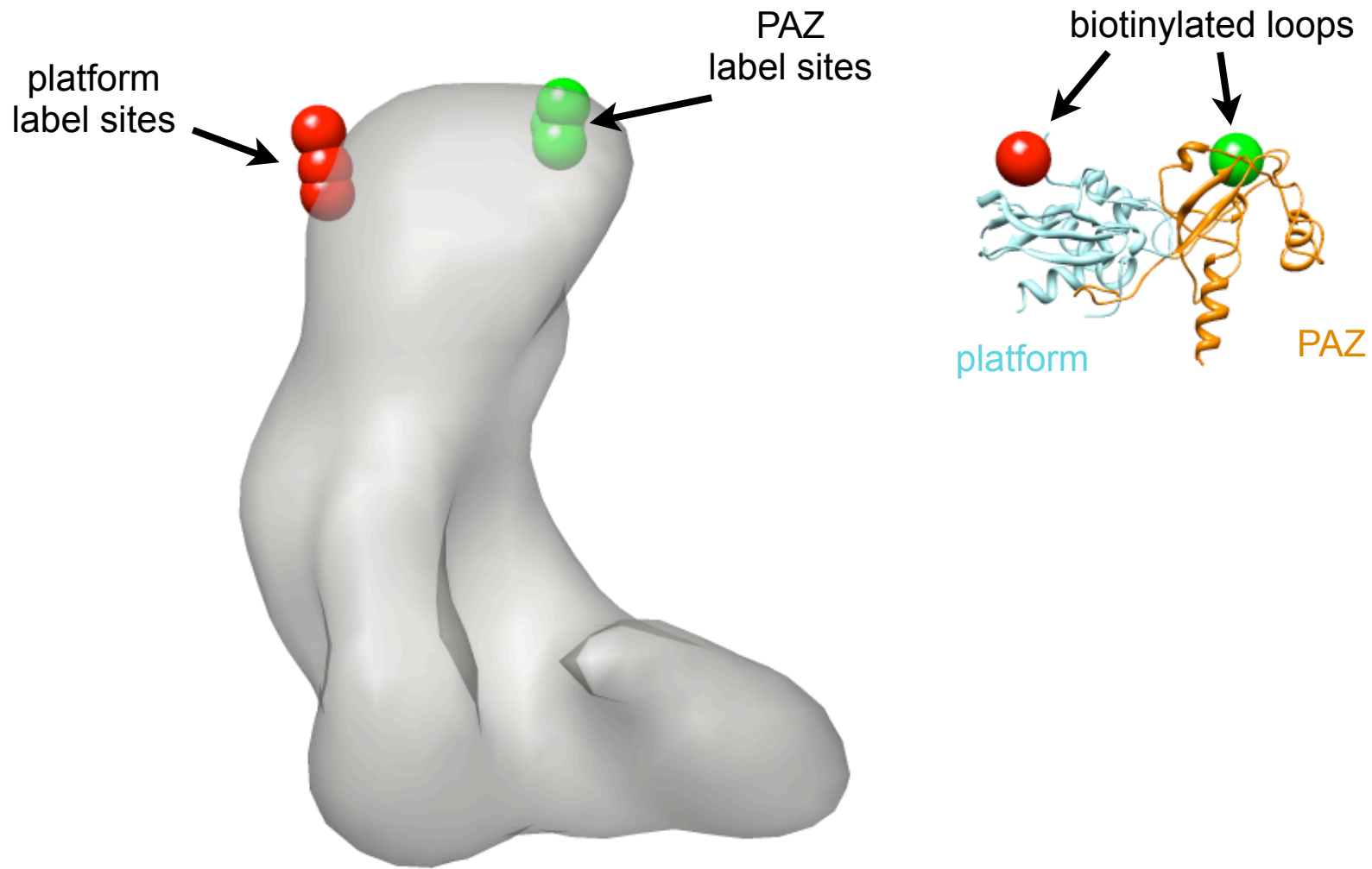
Platform domain maps to the back of the head



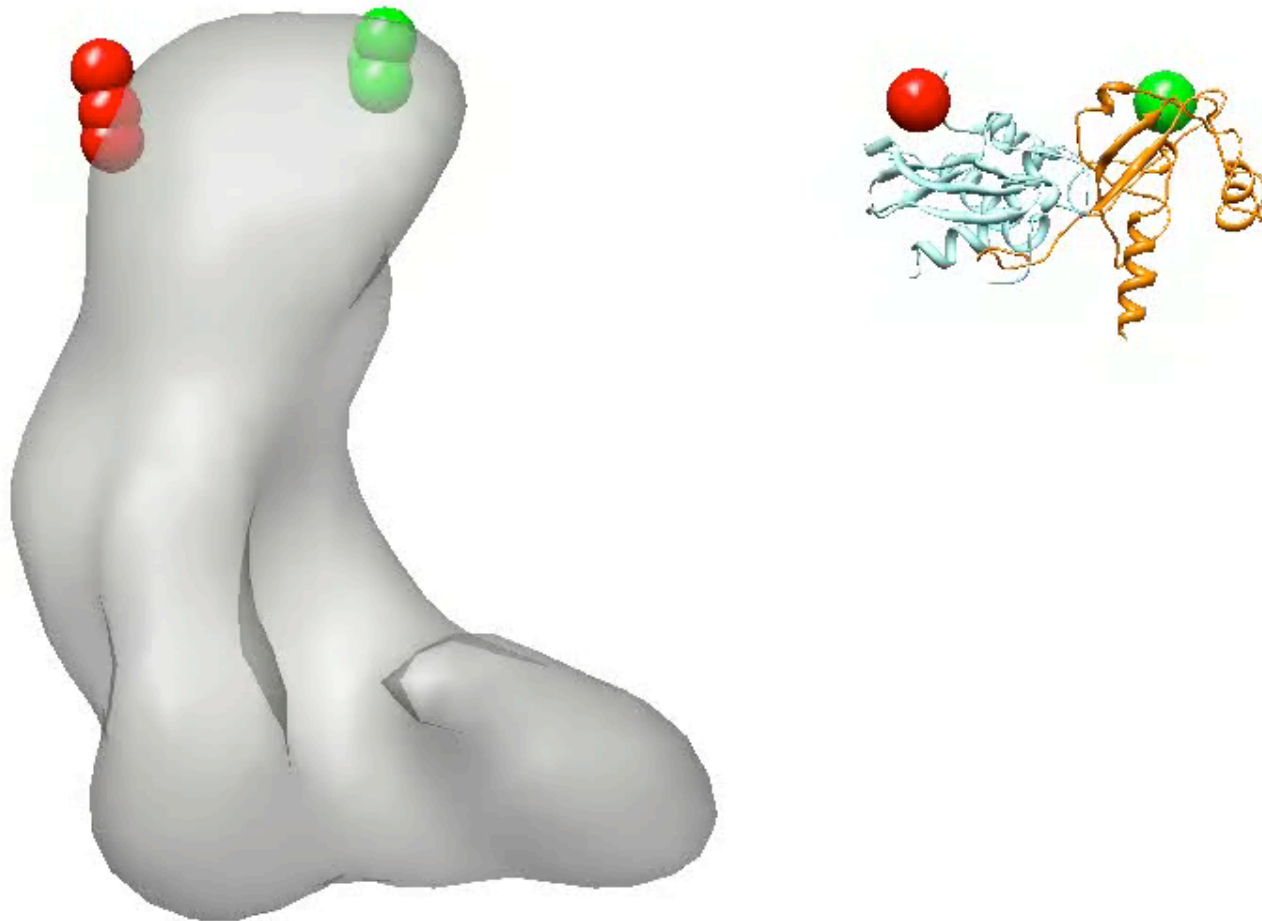
Dock platform-PAZ structure into EM map



Dock platform-PAZ structure into EM map



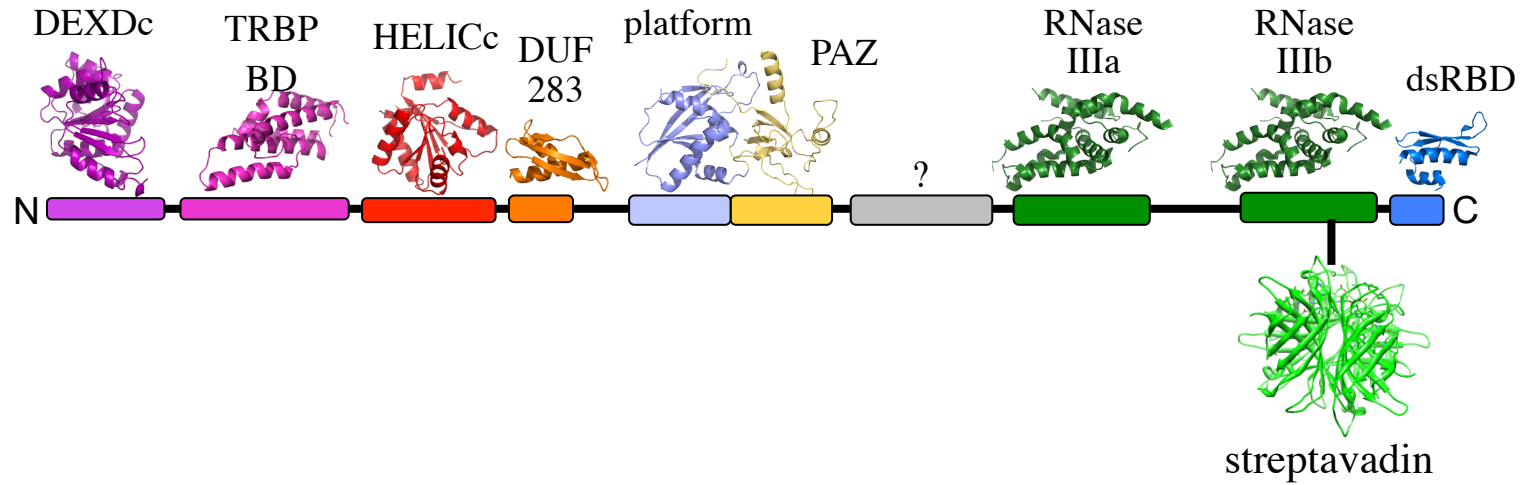
Dock platform-PAZ structure into EM map



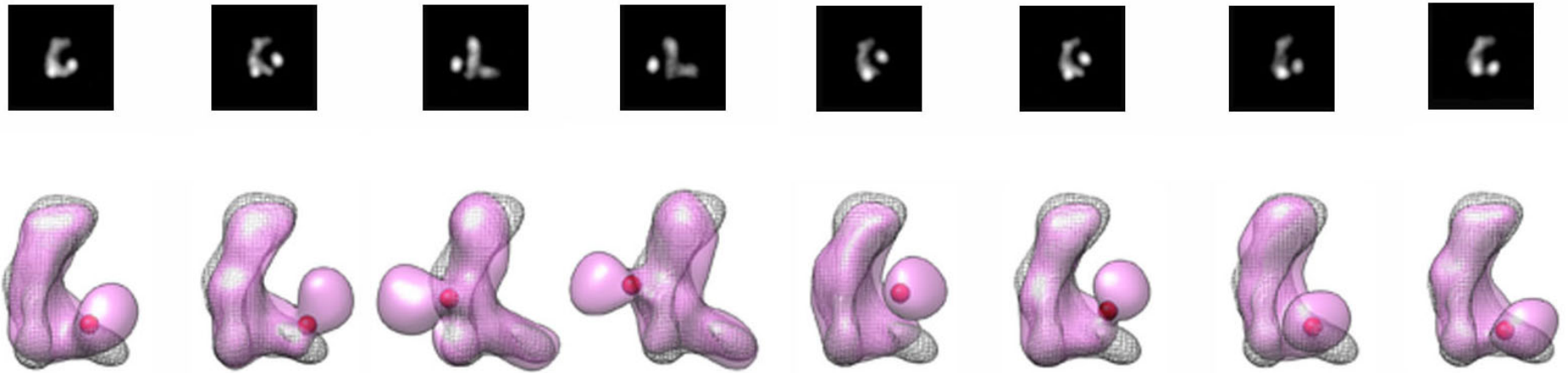
Dock platform-PAZ structure into EM map



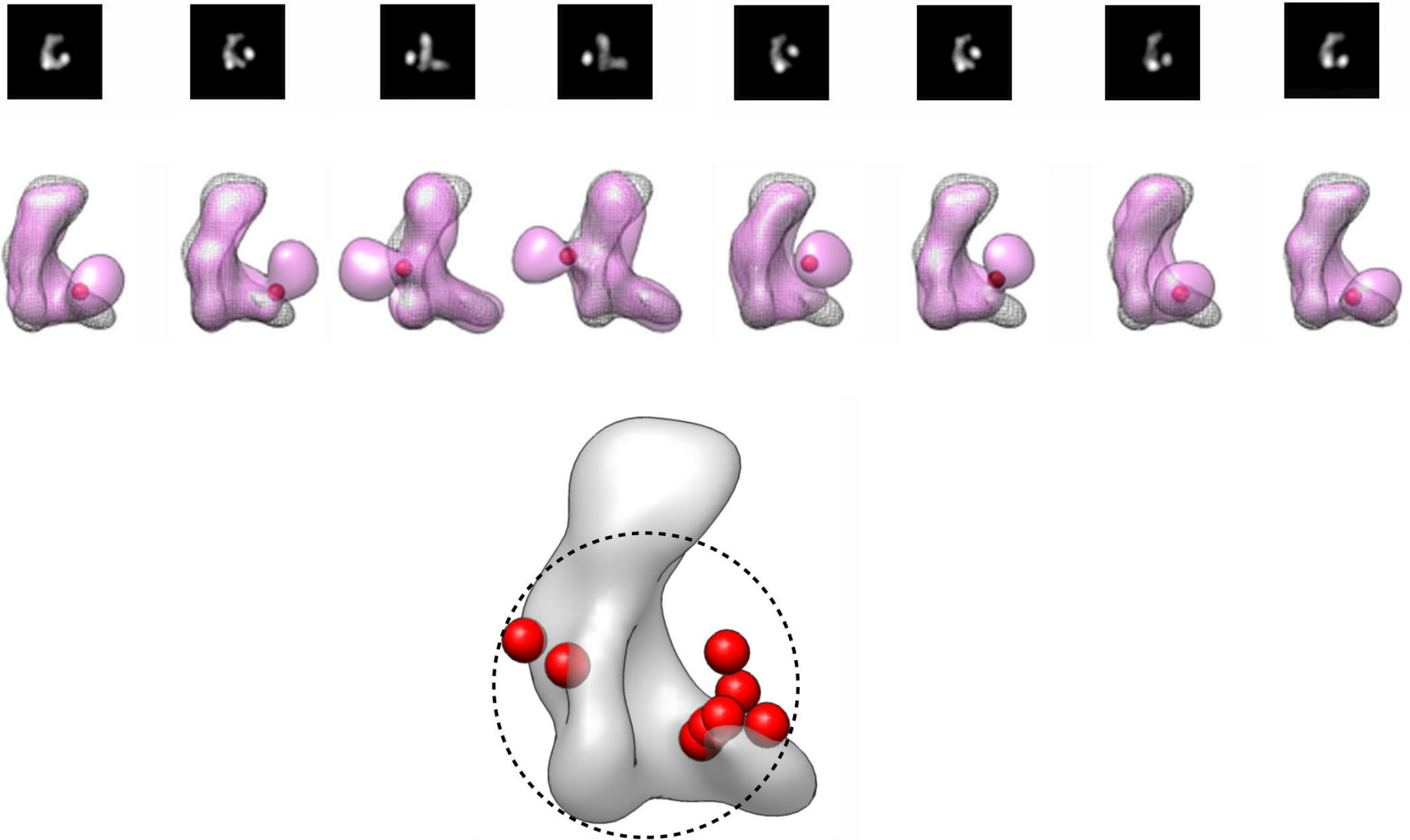
Label RNase IIIb domain with streptavadin



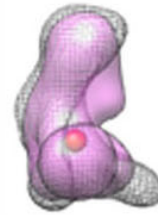
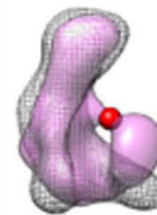
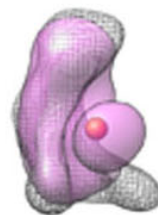
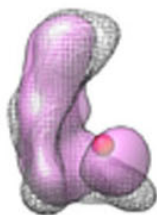
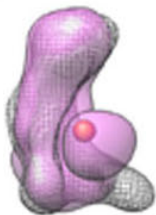
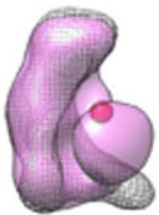
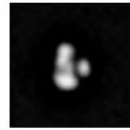
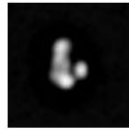
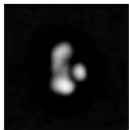
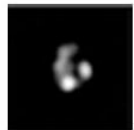
Long loop (45 residue) allows a lot of mobility



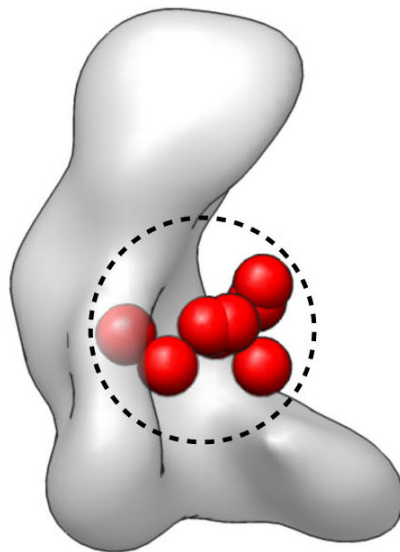
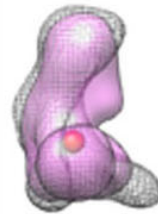
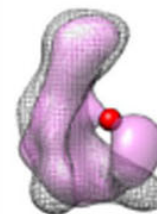
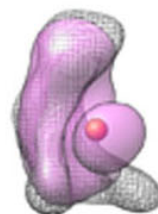
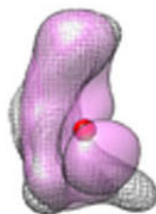
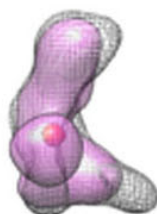
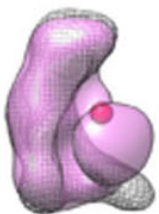
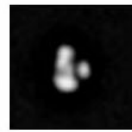
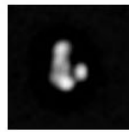
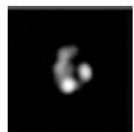
Long loop (45 residue) allows a lot of mobility



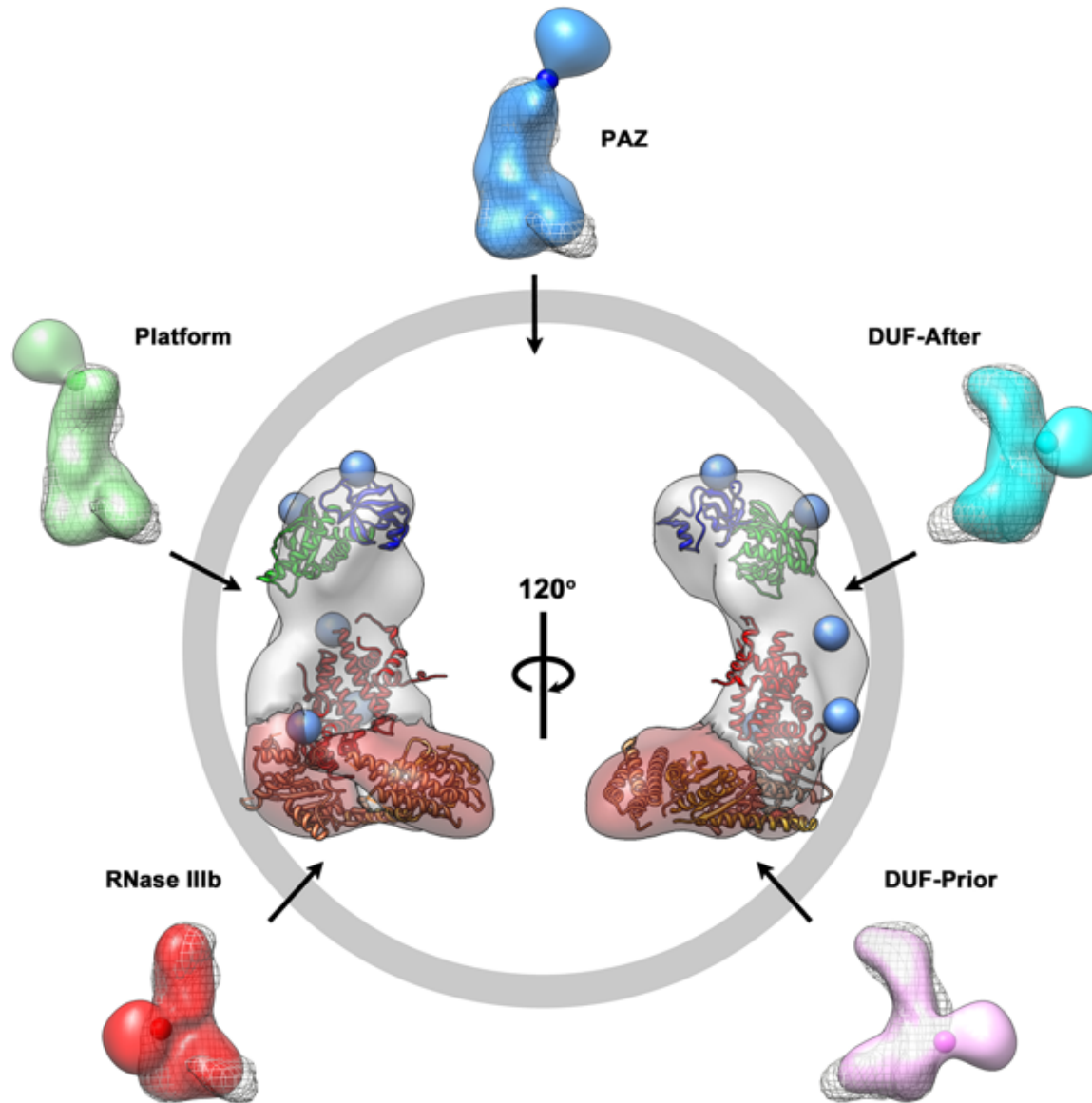
Shorter loop (25 residue) displays less movement

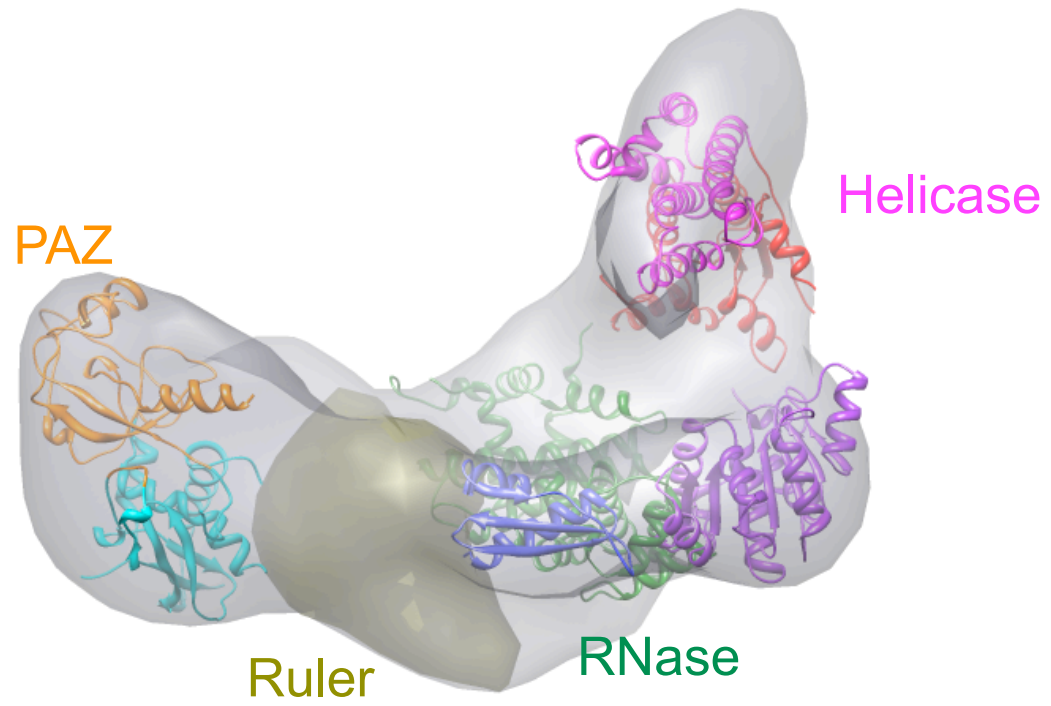


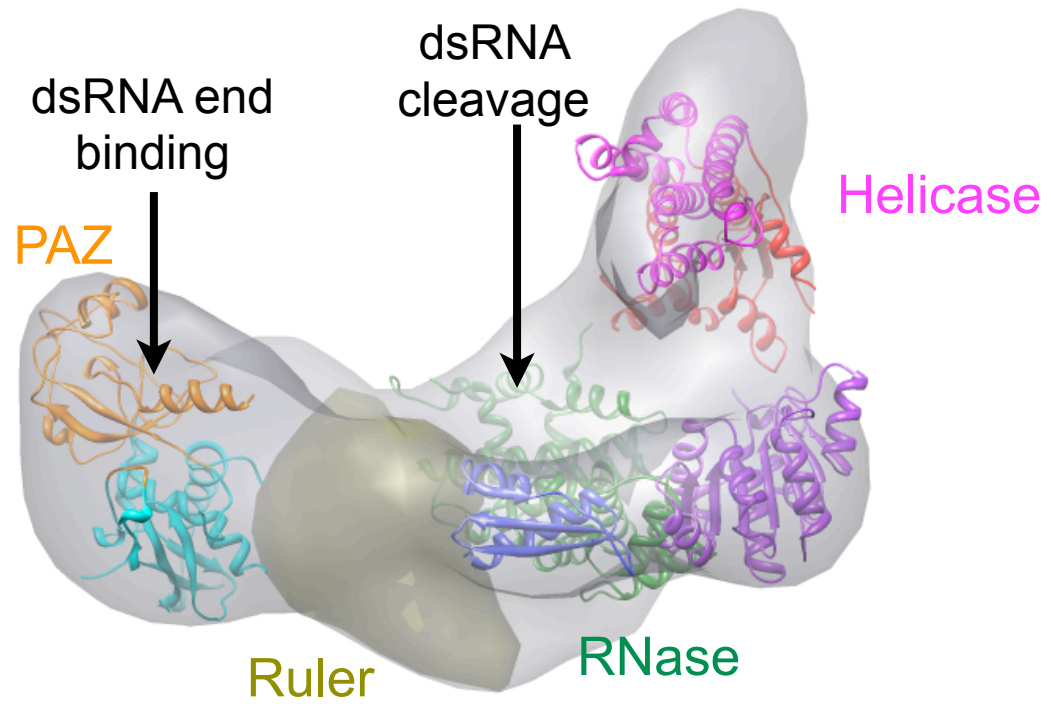
Shorter loop (25 residue) displays less movement

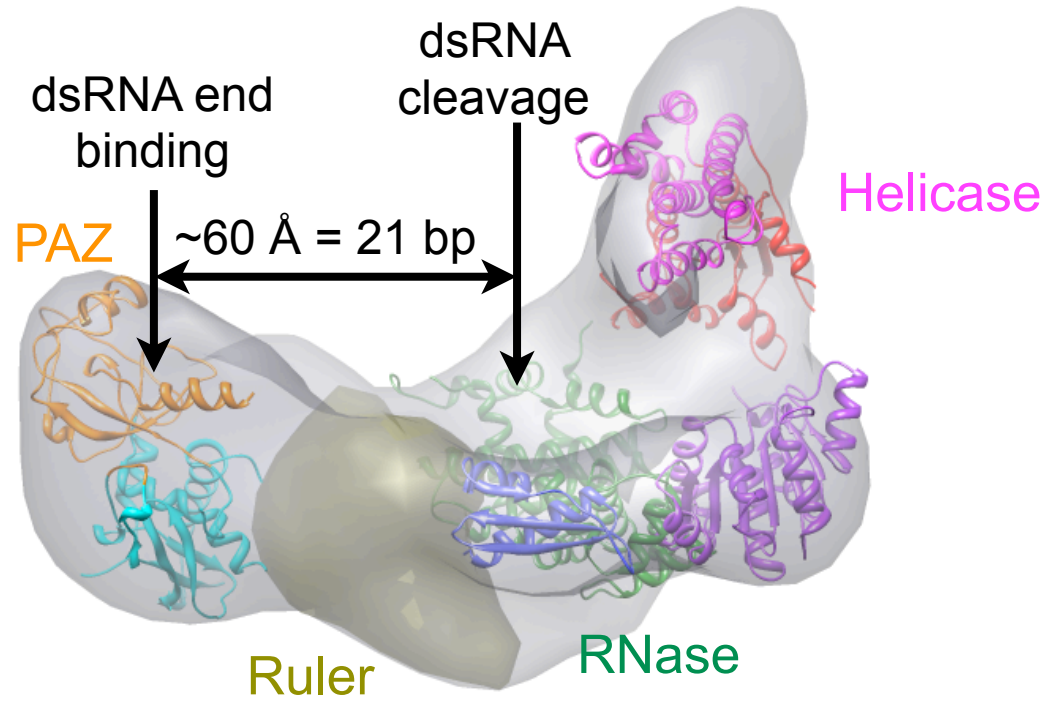


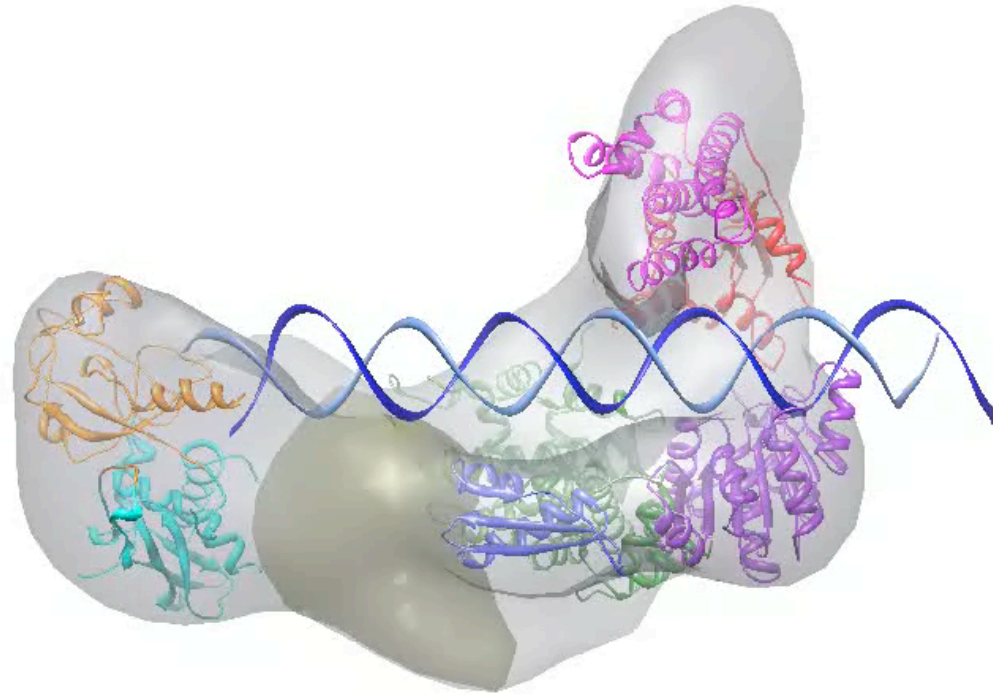
Map many positions

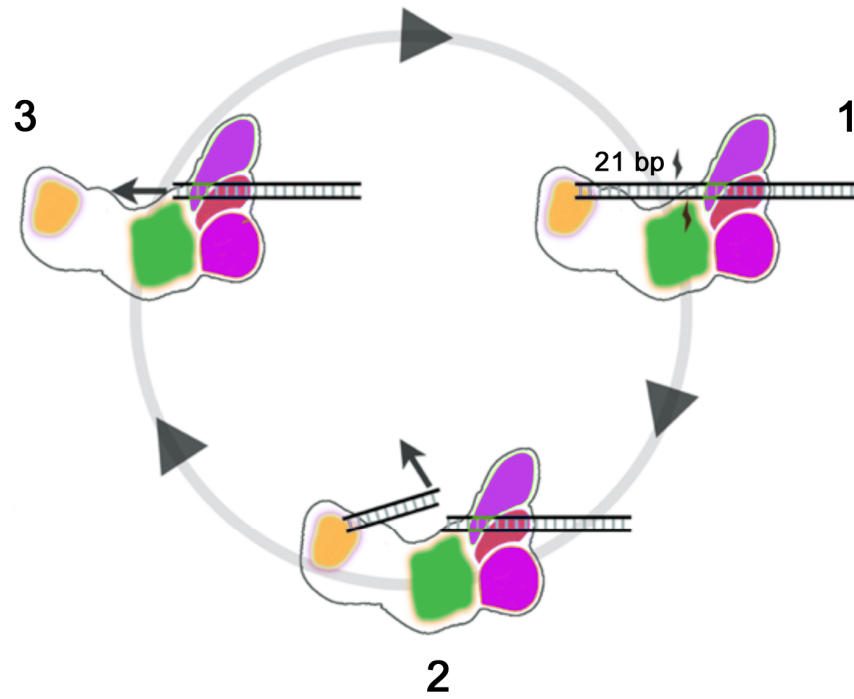
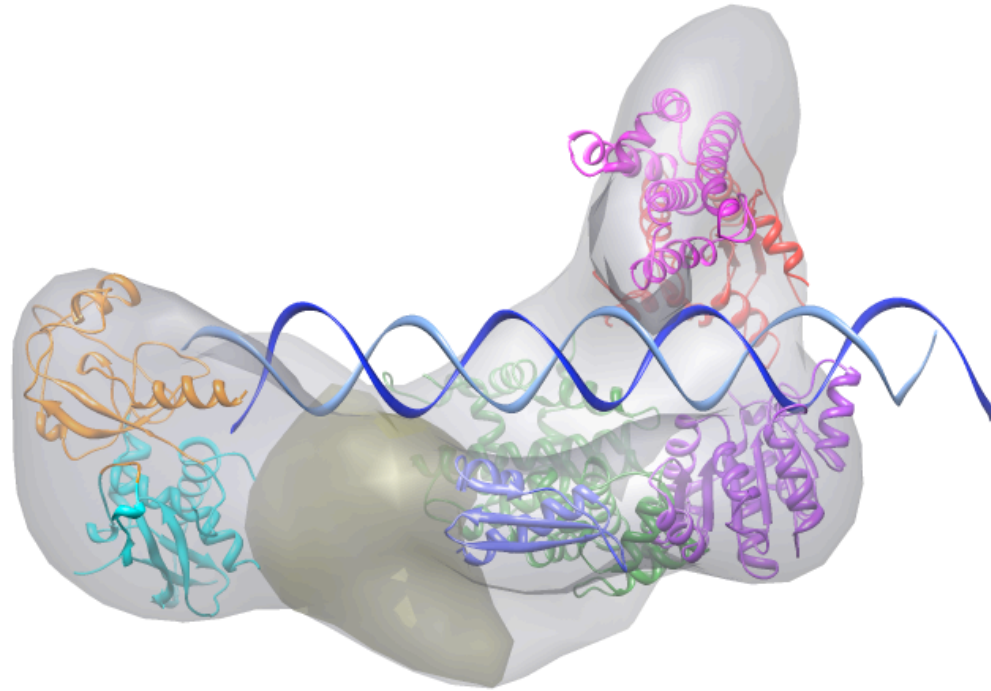












Acknowledgments



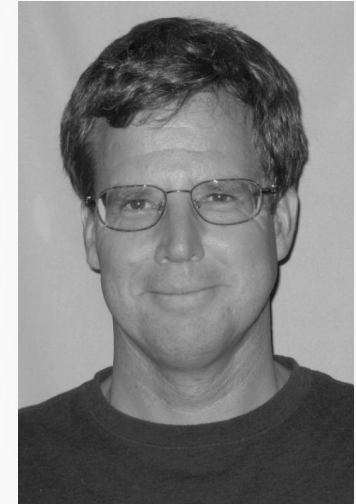
Pick-Wei Lau



Keelan Guiley



Bridget Carragher



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