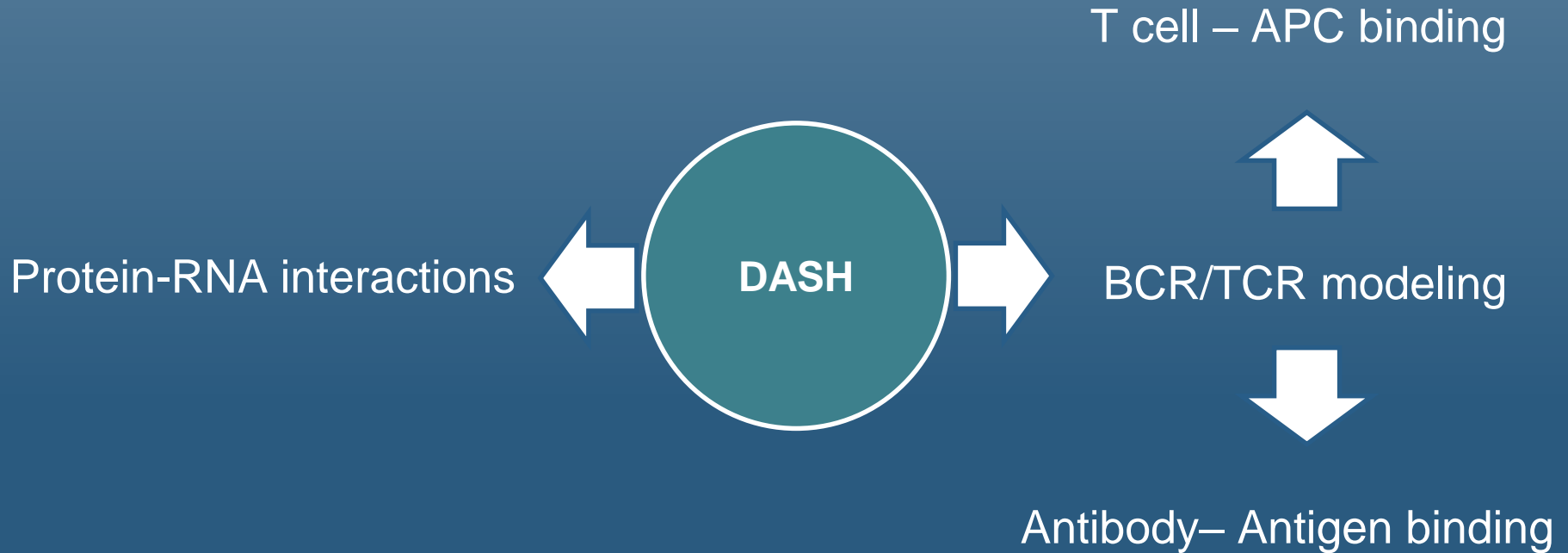
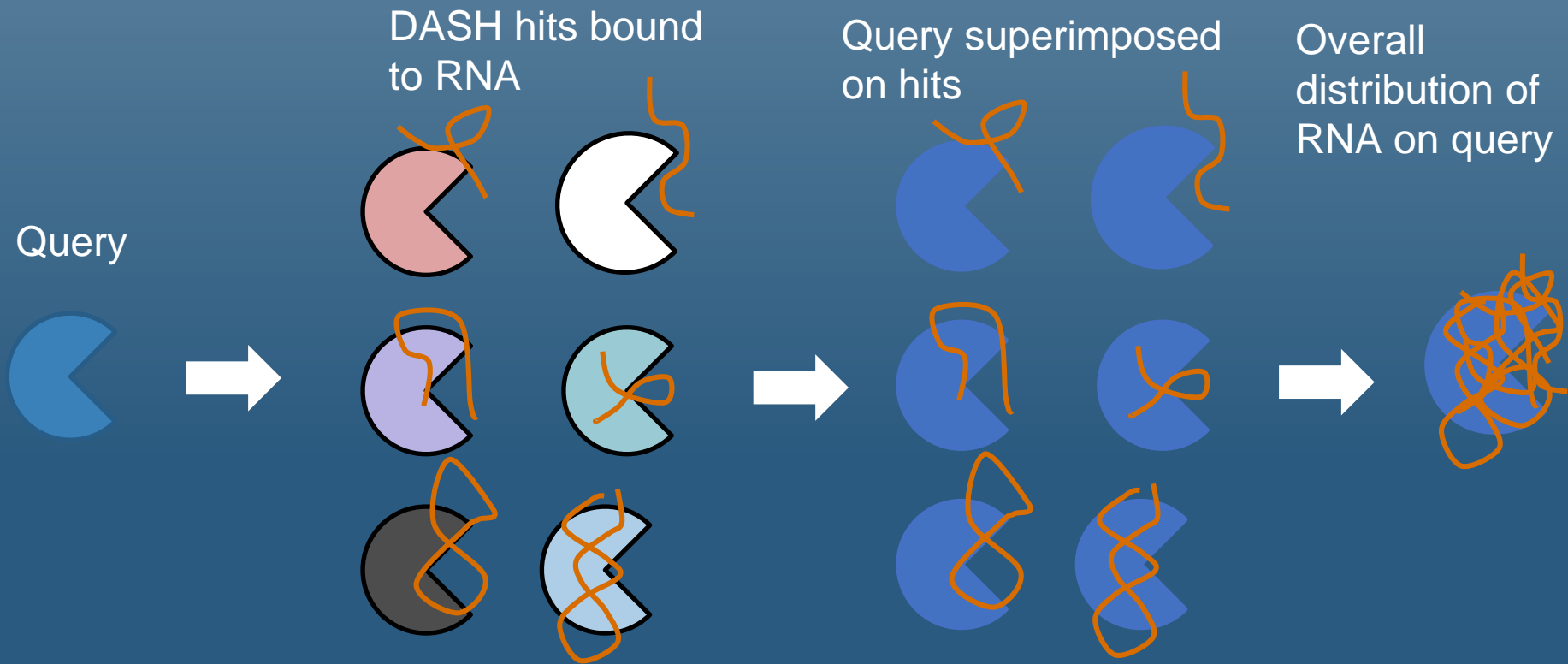


# DASH extensions (Immunology)



# **DASH Extension 1: protein-RNA interactions**

# Example: protein-RNA interactions from DASH?



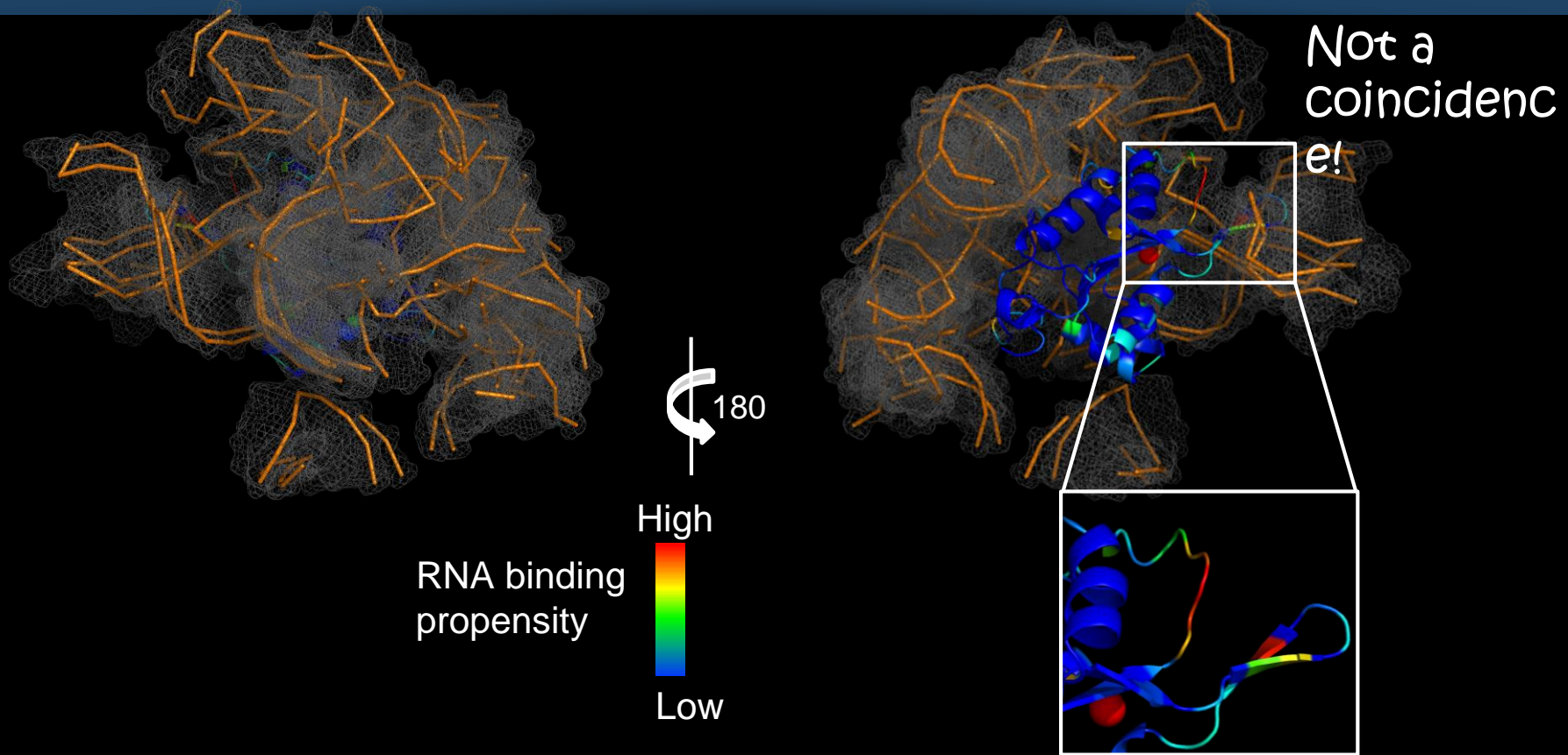
# Overall distribution of nucleotides on Regnase-1

Nucleotide-  
abundant



Nucleotide-scarce

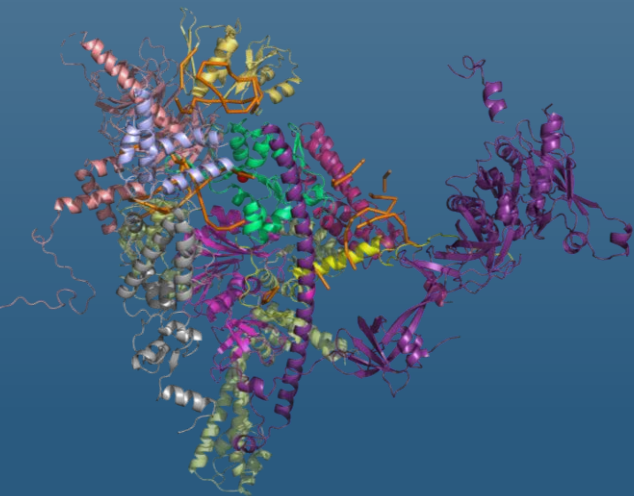
# Is this asymmetric distribution a coincidence?



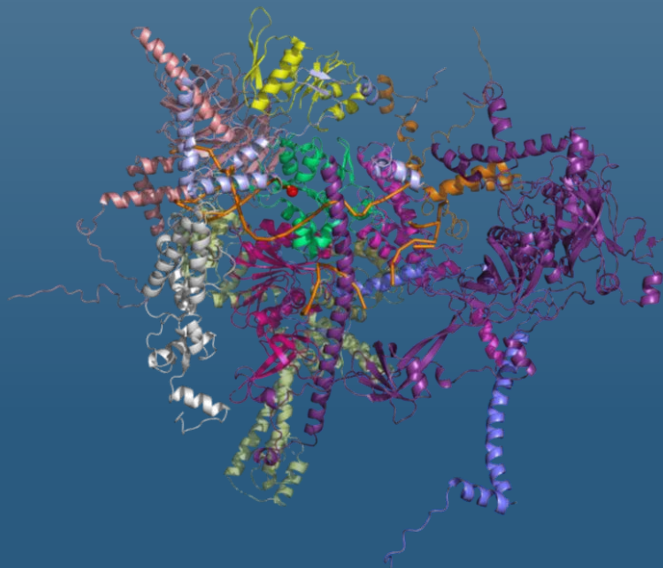
RNA-binding Regnase-1-DASH hits are all ribosome components			
DASH Score	PDB ID	Protein Chains	Complex
37	5OQL_P	C,R,T,Z,a,d,r,w,0	Pre-Ribosome
32	5WLC_SL	L9,LE,LU,NB,NC,NE, SA, SC, SF, SI	Processome
23	4A2I_V	L	Ribosome
20	2YKR_W	K,L	Ribosome

# What are these components?

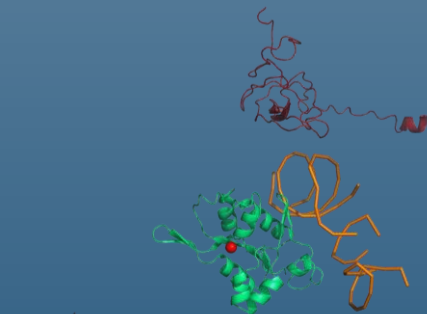
Cleaving rRNA (utp24)



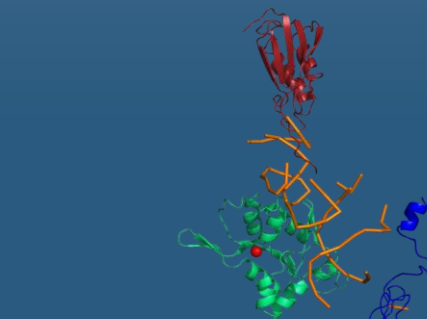
5OQL\_P: Pre-ribosome



5WLC\_SL: Processome



4A2I\_V: ribosome



2YKR\_W: ribosome

# UTP24: a Regnase-1 homolog in ribosomes

The PIN domain endonuclease Utp24 cleaves pre-ribosomal RNA at two coupled sites in yeast and humans

Graeme R. Wells,<sup>1</sup> Franziska Weichmann,<sup>1</sup> David Colvin,<sup>1</sup> Katherine E. Sloan,<sup>1</sup> Grzegorz Kudla,<sup>2</sup>,  
<sup>3</sup> David Tollervey,<sup>2</sup> Nicholas J. Watkins,<sup>1</sup> and Claudia Schneider<sup>1,\*</sup>

Article

Architecture of the 90S Pre-ribosome: A Structural View on the Birth of the Eukaryotic Ribosome

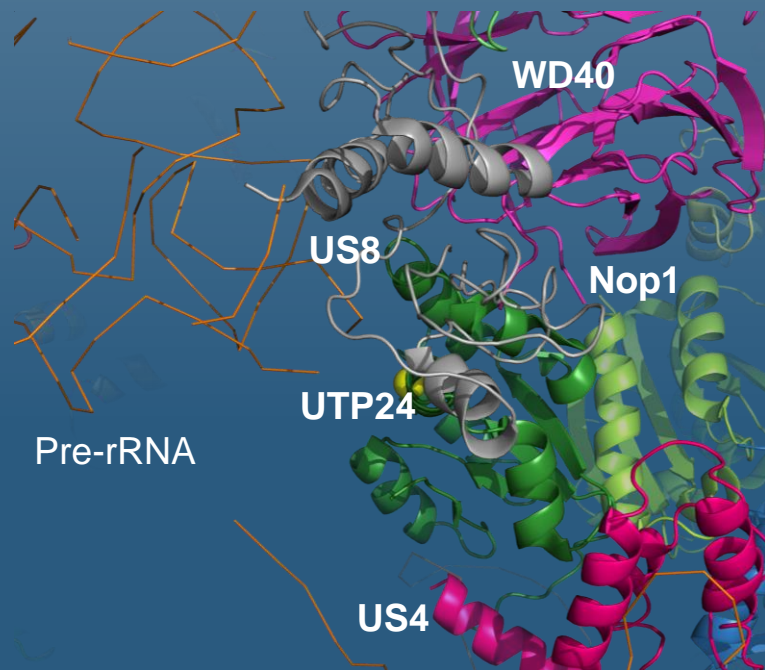
Markus Kornprobst<sup>1,3</sup>, Martin Turk<sup>2,3</sup>, Nikola Kellner<sup>1</sup>, Jingdong Cheng<sup>2</sup>, Dirk Flemming<sup>1</sup>, Isabelle Koš-Braun<sup>1</sup>,  
Martin Koš<sup>1</sup>, Matthias Thoms<sup>1</sup>, Otto Berninghausen<sup>2</sup>, Roland Beckmann<sup>2,4,5</sup>, Ed Hurt<sup>1,4,5</sup> ✉

3.2-Å-resolution structure of the 90S preribosome before A1 pre-rRNA cleavage

Jingdong Cheng, Nikola Kellner, Otto Berninghausen, Ed Hurt ✉ & Roland Beckmann ✉

The complete structure of the small-subunit processome

Jonas Barandun, Malik Chaker-Margot, Mirjam Hunziker, Kelly R Molloy, Brian T Chait & Sebastian Klinge ✉

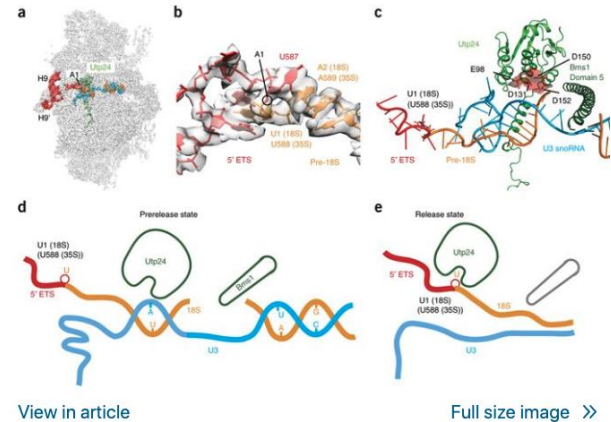




# Helicase may be required for utp24-mediated rRNA cleavage

acidic residues (E98, D131, D150 and D152)<sup>43</sup>. Importantly, we were also able to trace the RNA chain between the 5' end of the pre-18S rRNA and the 3' end of the 5' ETS, visualizing the A1 cleavage site. The 3' end of the 5' ETS 'walks' along the surface of the 90S particle and points to the penultimate H9 of the 5' ETS. Unexpectedly, we found the Utp24 catalytic site close to the box A heteroduplex, which is more than 35 Å away from the A1 cleavage site (U588) (Fig. 7a-c). Such a distance is a clear indication that the 90S preribosome is still not yet in the processome modus regarding A1 cleavage (Fig. 7c,d). We suggest that after the 90S is in a release-competent state, a currently unknown splitting factor/helicase may be activated to unwind the box A/A' helices and unlock Utp24 together with the rRNA, thus resulting in endonucleolytic cleavage at site A1 (Fig. 7e).

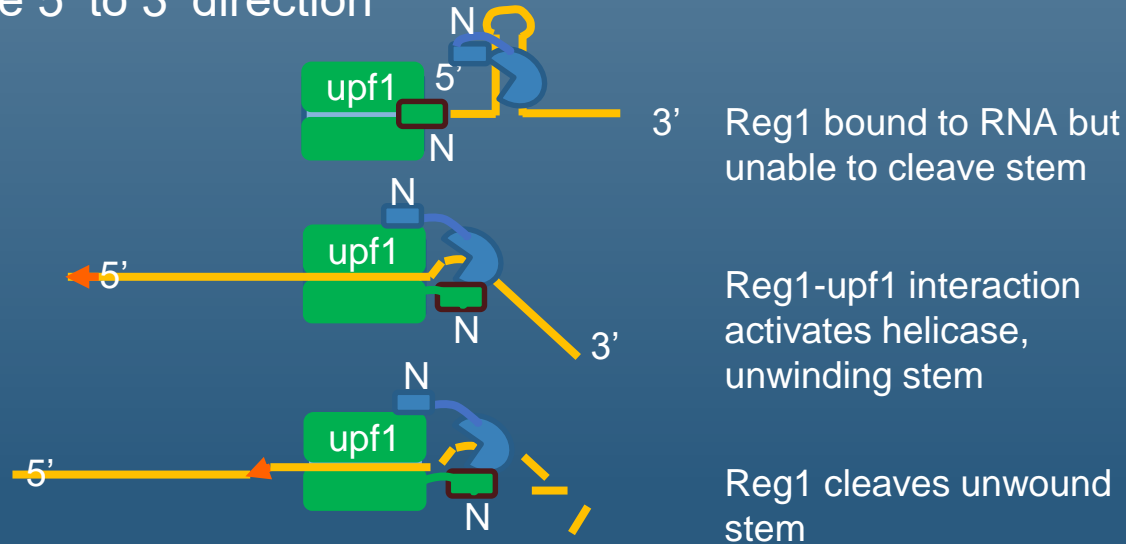
**Figure 7: Prerelease state, A1 cleavage and 90S-preribosome assembly.**



# Functional similarity:

## Regnase-1 also helicase to unwind target RNAs

UPF1 unwinds in the 5' to 3' direction

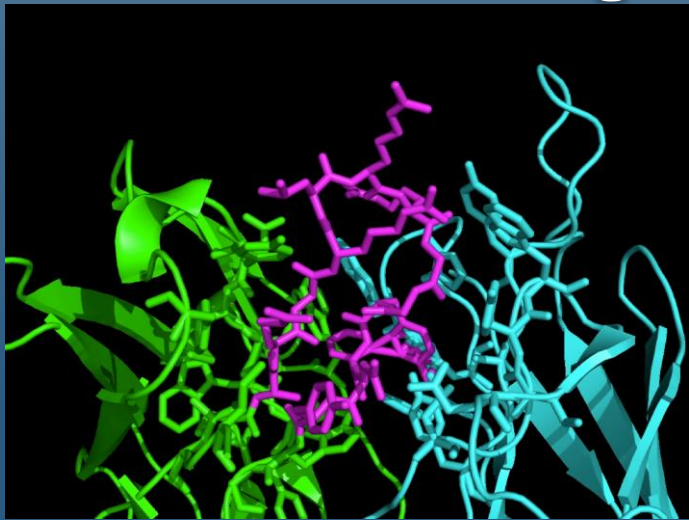


Therefore, Reg1 expected to cleave on 3' side of stem

# DASH Extension 2: BCR/TCR modeling

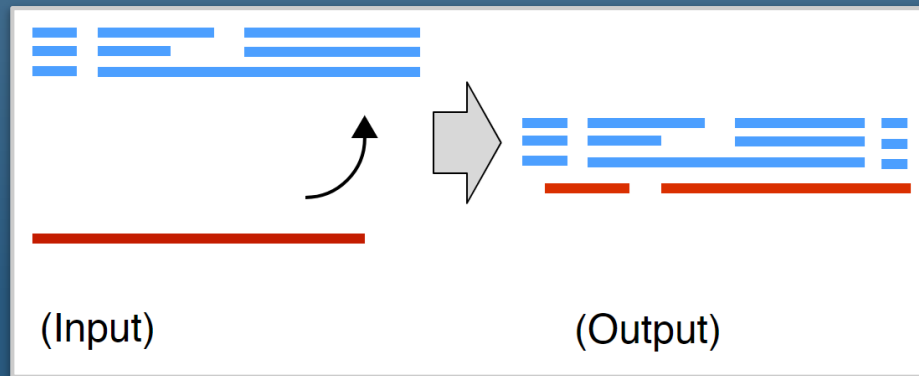
# MSAs encode structural information

**Traditional AB modeling is Slow**



**Approach used by Rosetta,  
Schrodinger, etc. (thousands of hours!)**

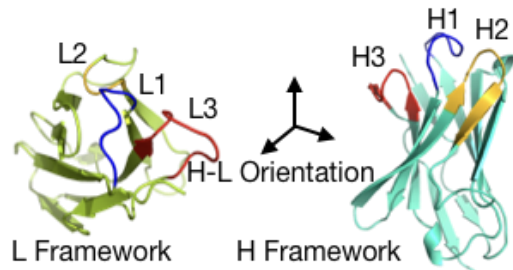
**Adding a sequence to an  
existing MSA is fast (<1 sec)!**



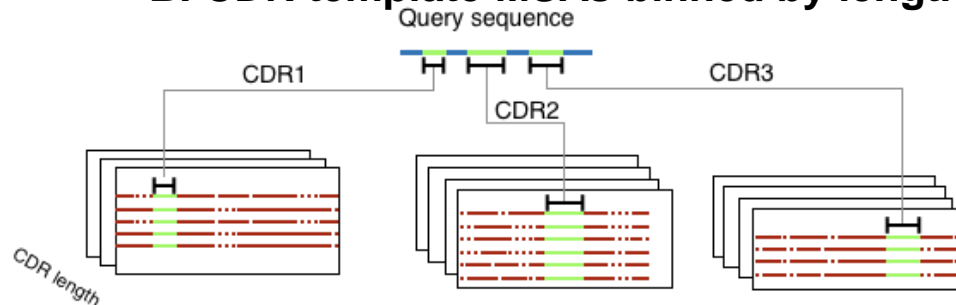
**Repertoire Builder: MAFFT-  
DASH MSA + MAFFT v7  
10,000 models in 30 min!**

# Repertoire Builder uses MSA-based approach

## A. Prepare template MSAs



## B. CDR template MSAs binned by length

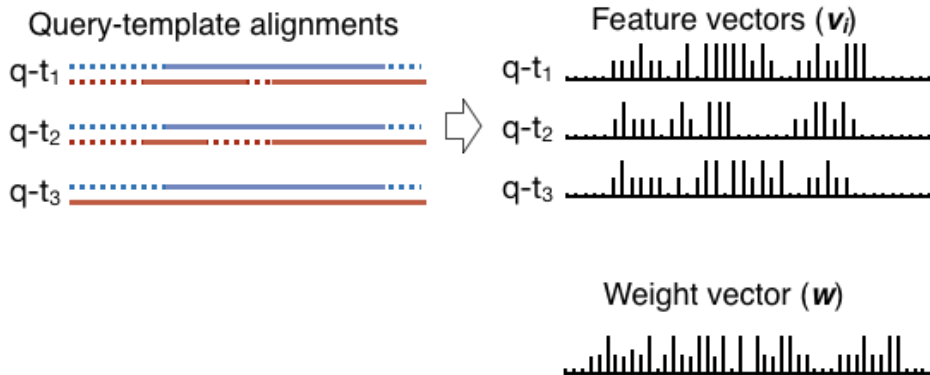


## C. Extend template MSA

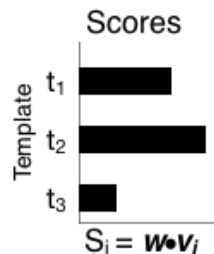


# Repertoire Builder workflow

## D. Rank templates

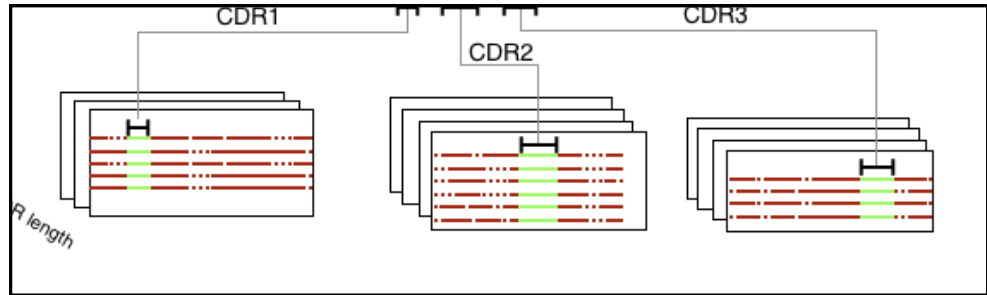


## E. Assemble 3D model



## Repertoire Builder uses MSA-based approach

## How important are MSAs?



# Good MSAs are crucial

## CDR1

## CDR2

## CDR3

## Misalignment

## Misalignment



# Good MSAs are crucial

CDR1

CDR2

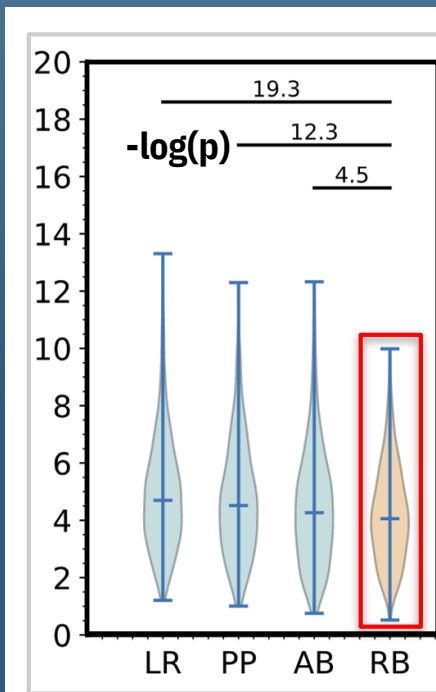
CDR3

No misalignment

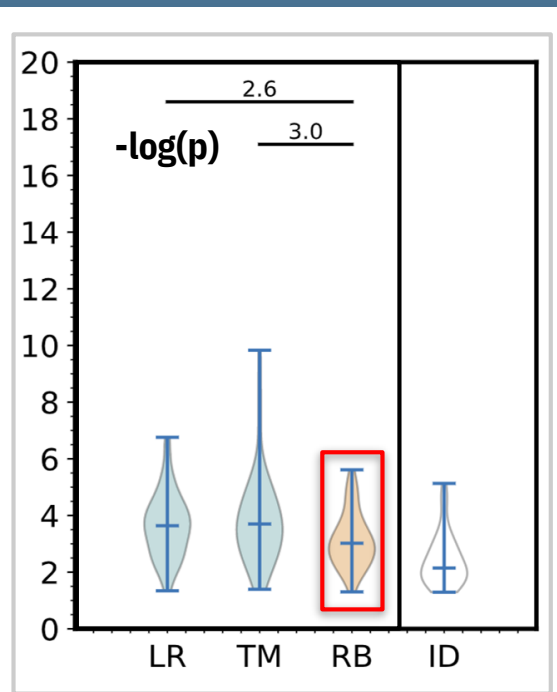
# Repertoire Builder Accuracy

Repertoire Builder error was **lower** than other tested methods.

BCR  
CDR-H3 ERROR

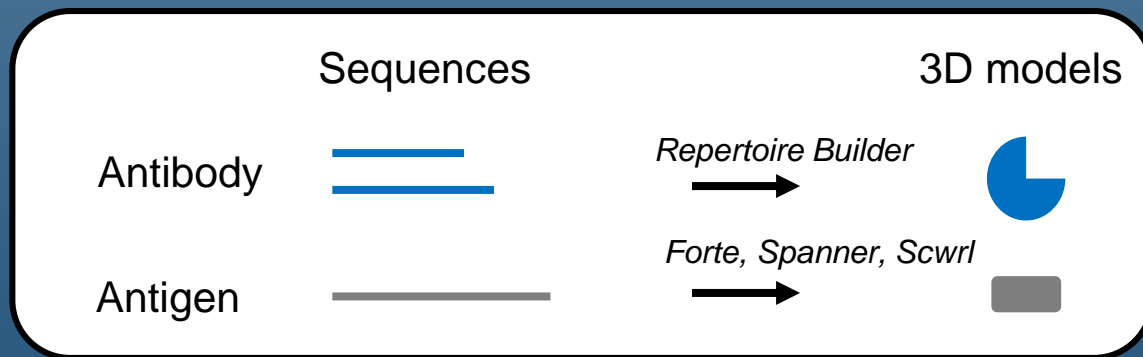


TCR  
CDR-B3 ERROR



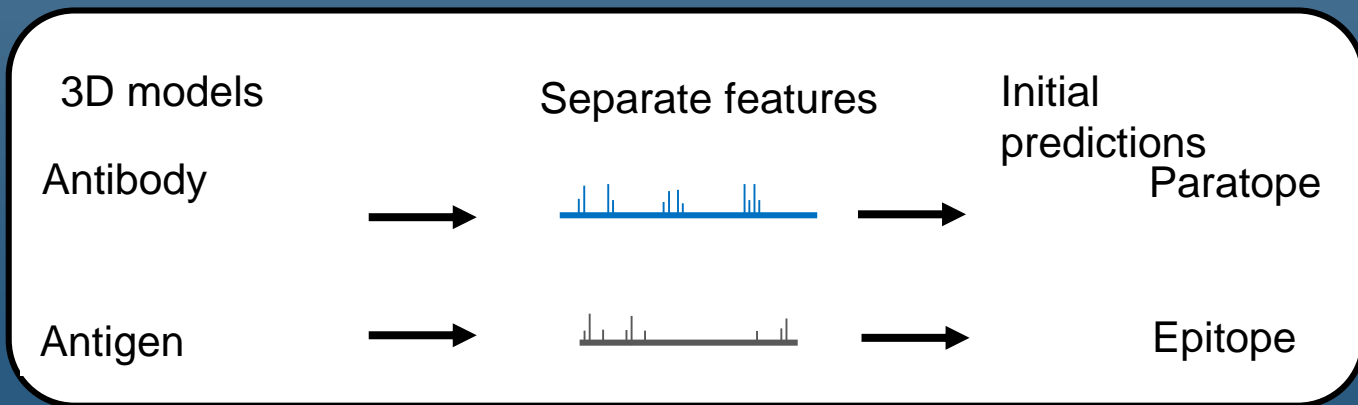
# Extension to Antibody-Antigen docking

## Build 3D models



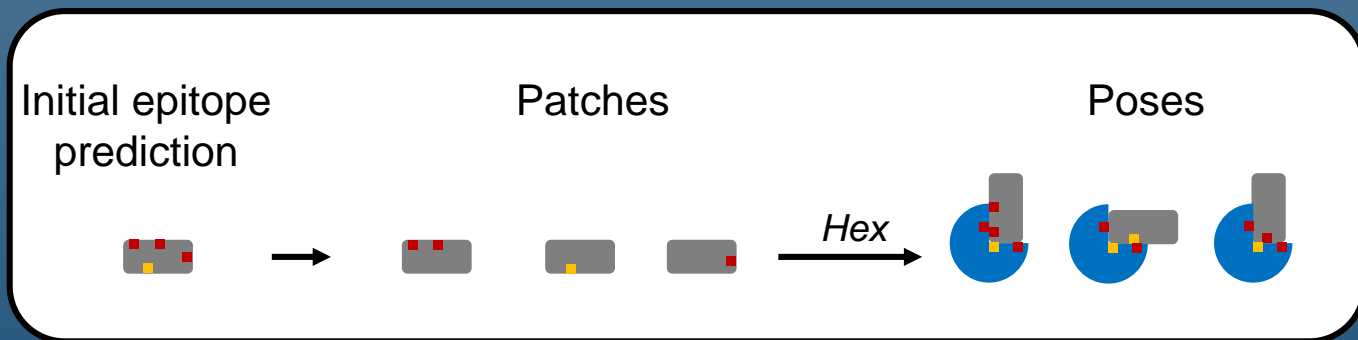
# Extension to Antibody-Antigen docking

Predict initial paratope and epitope



# Extension to Antibody-Antigen docking

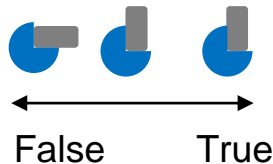
## Dock antibody and antigen



# Extension to Antibody-Antigen docking

Prepare improved features and predict final epitope

Pose classification



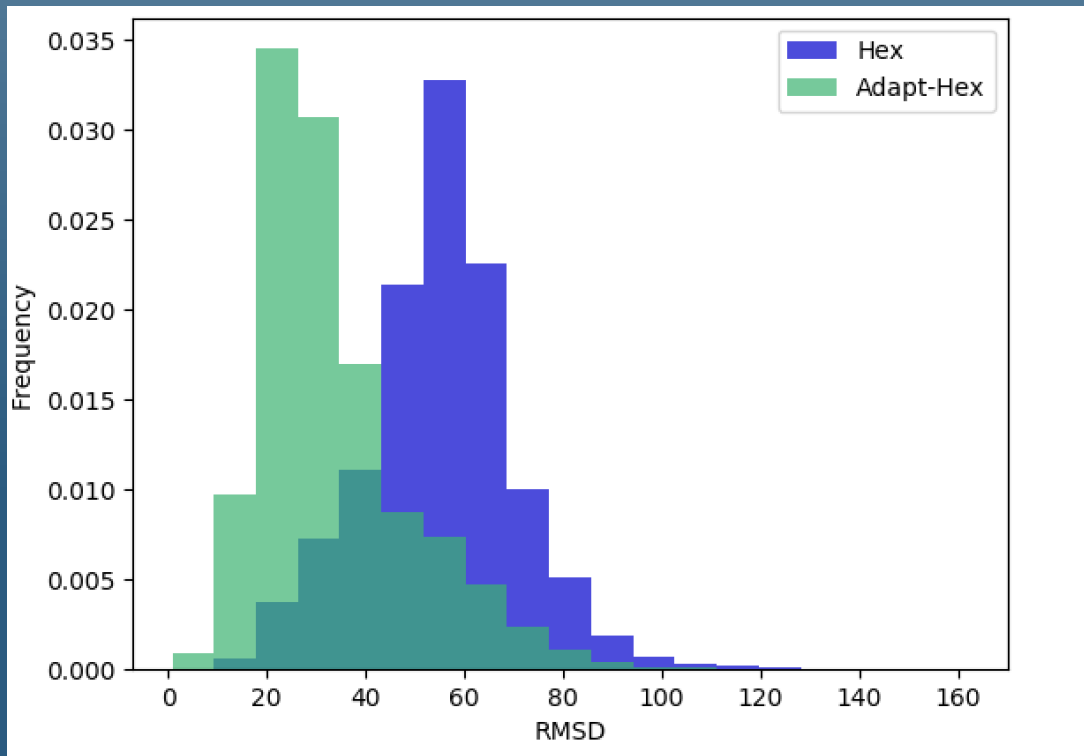
Improved features



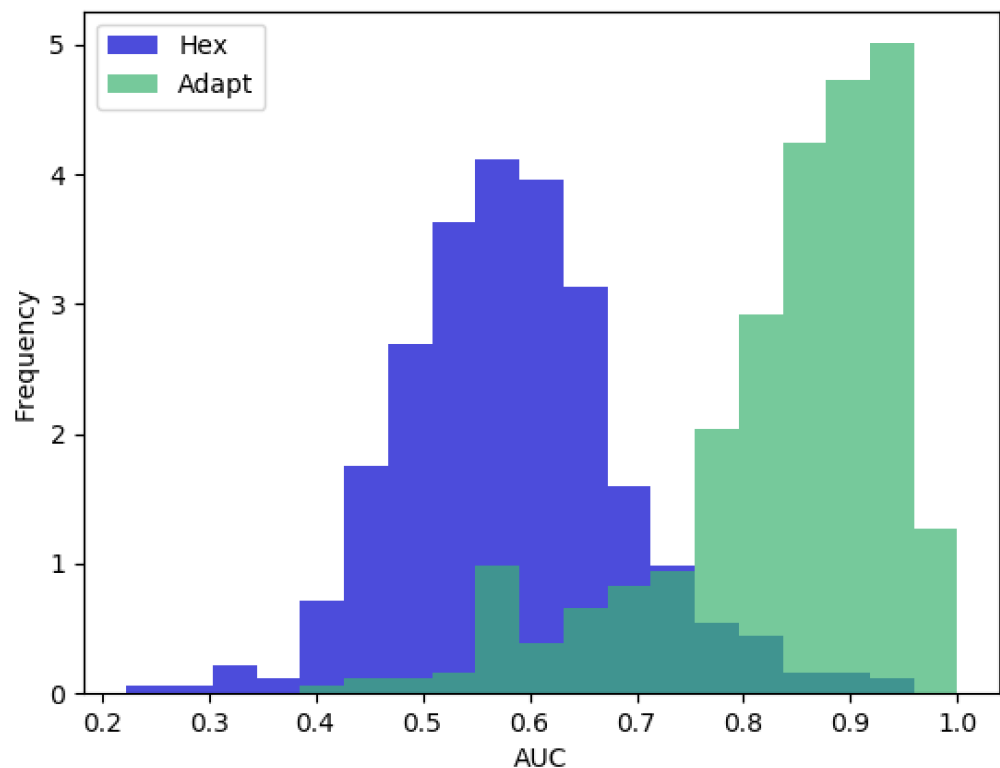
Final epitope



# Significant improvement over Hex sampling

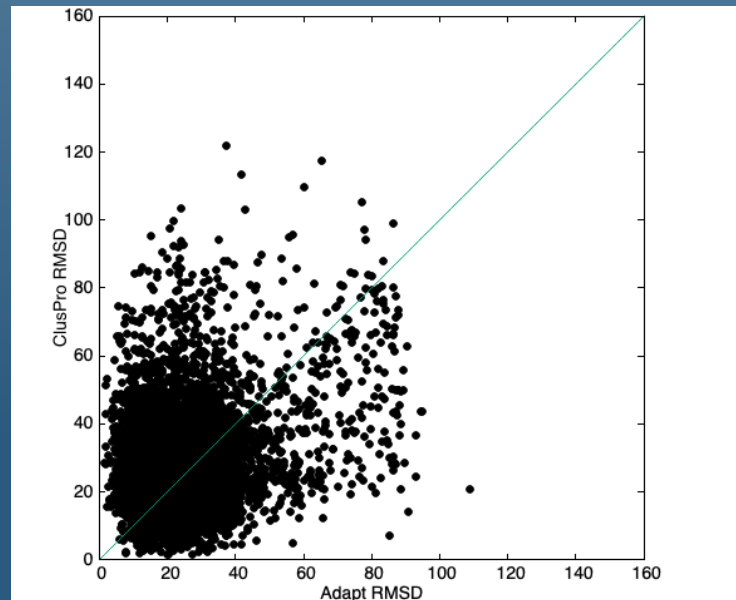
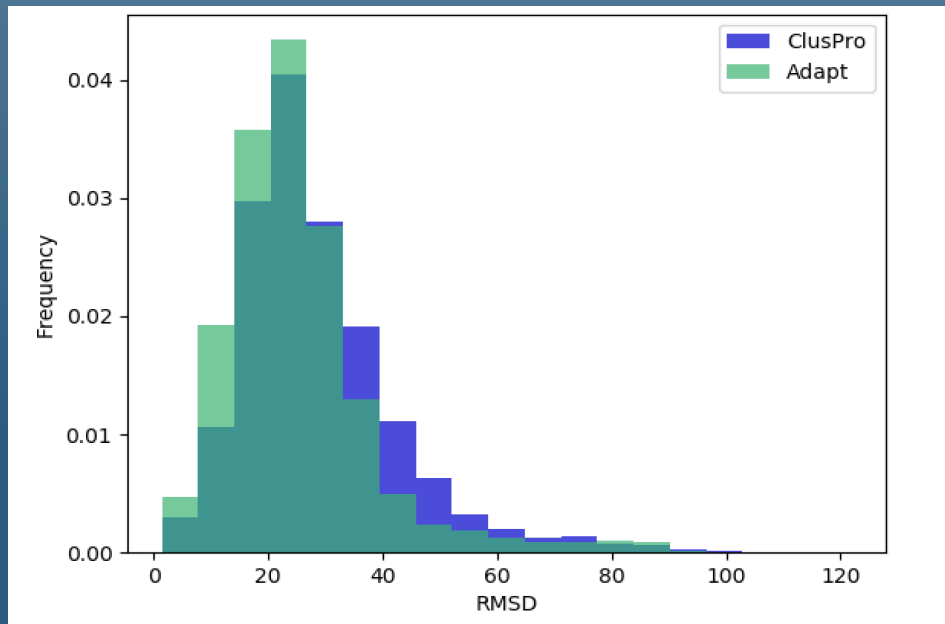


# Significant improvement over Hex scoring





# Quantitative improvement over ClusPro



# Quantitative improvement over ClusPro

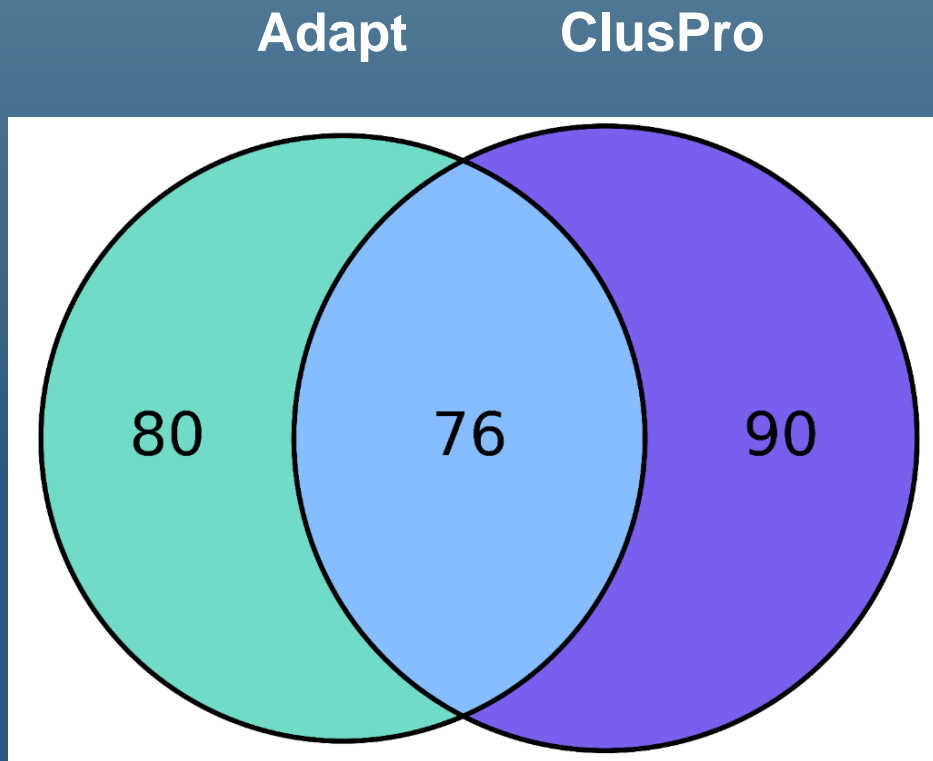
Docking performance comparison between Adapt and ClusPro

	<b>Total query</b>	<b>Successful query</b>	<b>Total models</b>	<b>Successful models</b>
Adapt	430	156 (36.28%)	11218	343 (3.06%)
ClusPro	430	166 (38.60%)	11218	227 (2.02%)

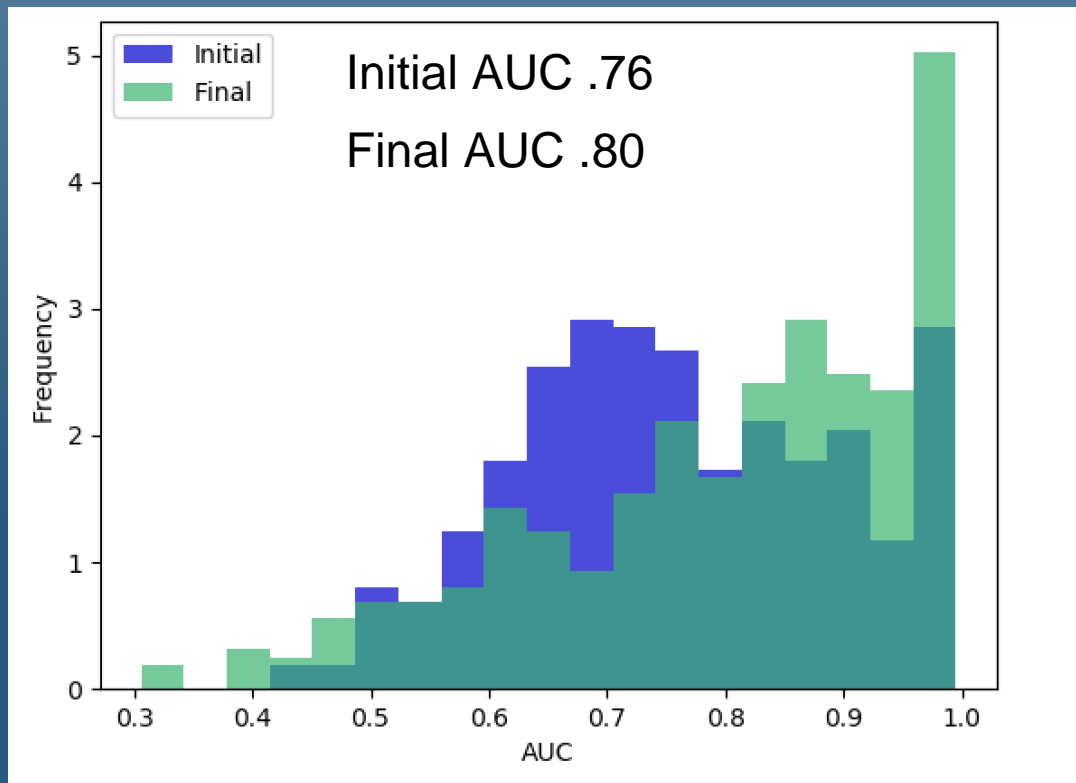
Distribution of three quality classes among successful models

	<b>Successful models</b>	<b>Acceptable</b>	<b>Medium</b>	<b>High</b>
Adapt	343	306/343 (89.21%)	35/343 (10.20%)	2/343 (0.58%)
ClusPro	227	186/227 (81.94%)	39/227 (17.18%)	2/227 (0.88%)

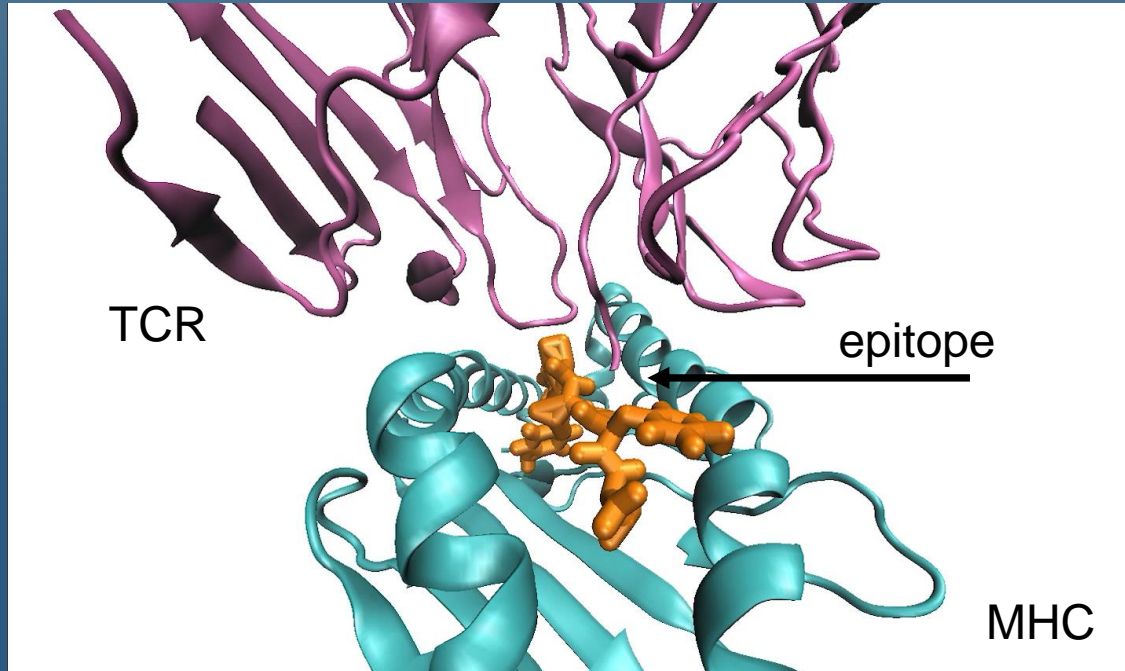
# Adapt poses show low overlap with ClusPro



# Antibody-specific epitope prediction



# Extension to TCR-epitope-MHC modeling



<https://sysimm.org/immune-scape/>



**ImmuneScape**

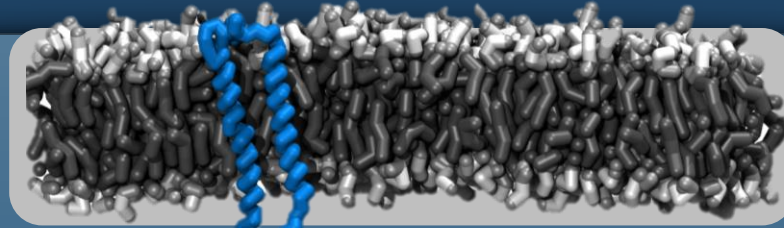
TCR-pMHC modeling  
version 2019.39

ImmuneScape is the first  
automated TCR-epitope-MHC  
modeling server of its kind

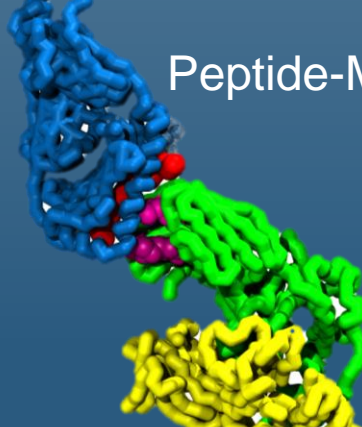
Li et al. . Meth Mol Biol (2019)

# Extension to T cell-APC cell modeling

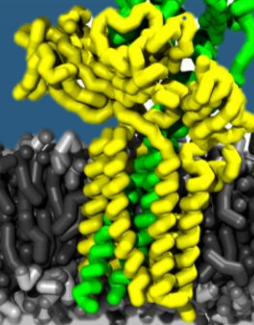
Antigen presenting  
cell (APC)



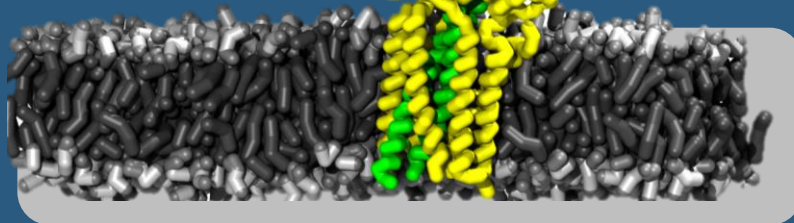
Peptide-MHC



TCR



T cell



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- ◆ Osamu Takeuchi
- ◆ Ai Tachikawa
- ◆ Eliane Piaggio
- ◆ Thomas Boehm