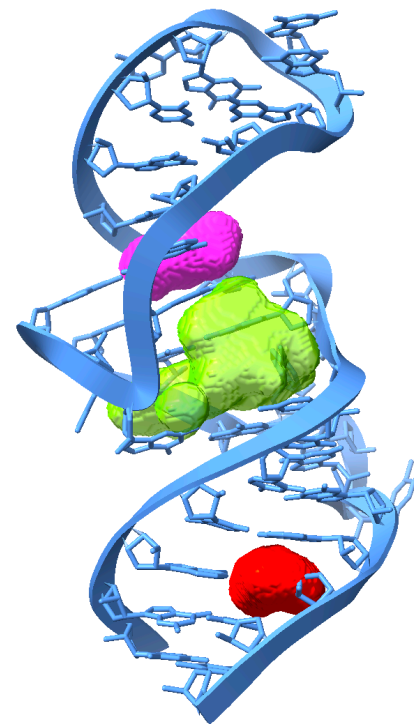
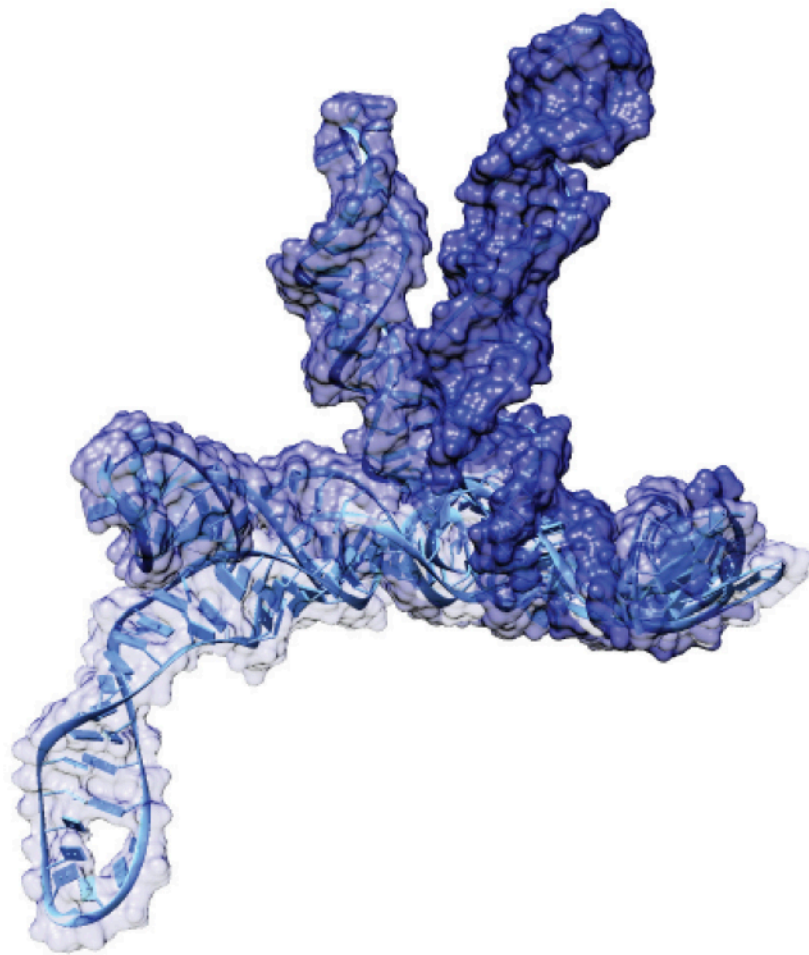


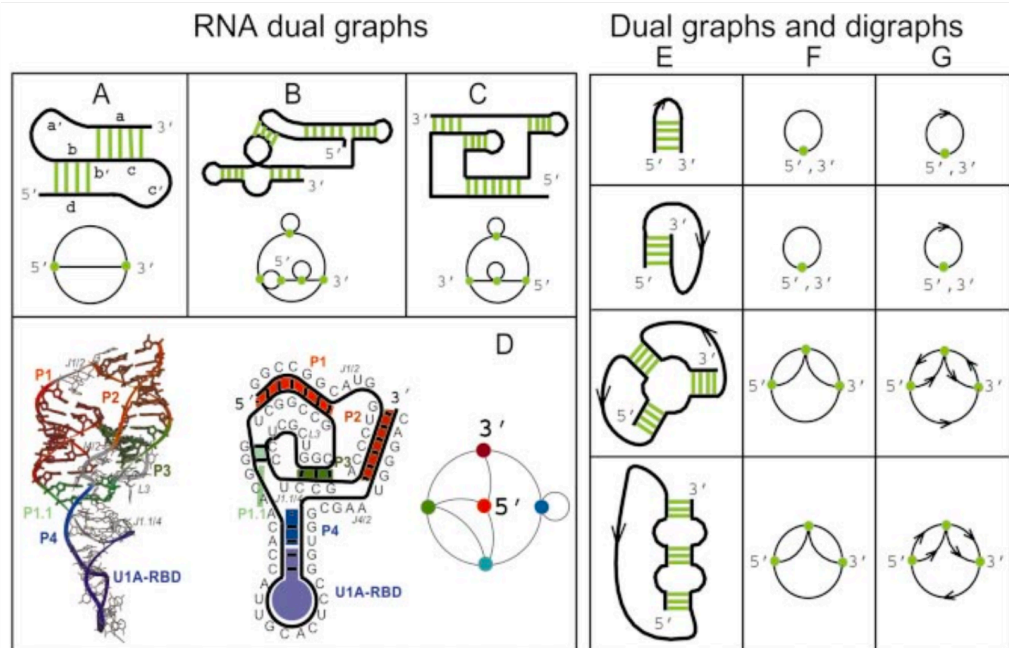
RNA binding pockets characterization, prediction, and dynamics



Samuela Pasquali

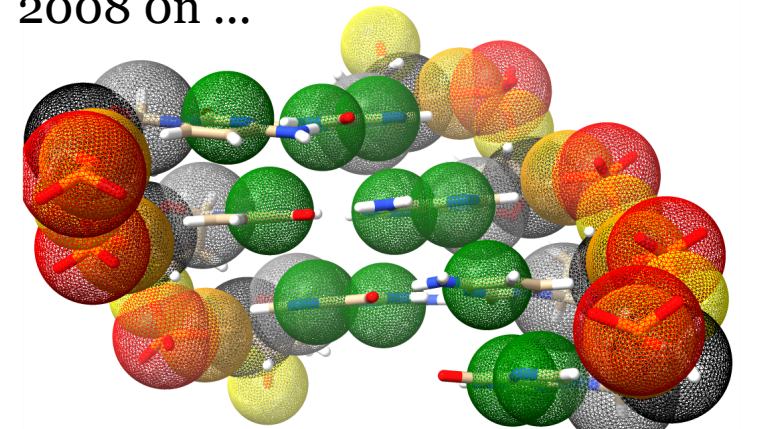
Laboratoire Biologie Fonctionnelle et Adaptative
In Silico Pharmacological Profiling Team

My journey into RNA modeling... (a very top down approach!)

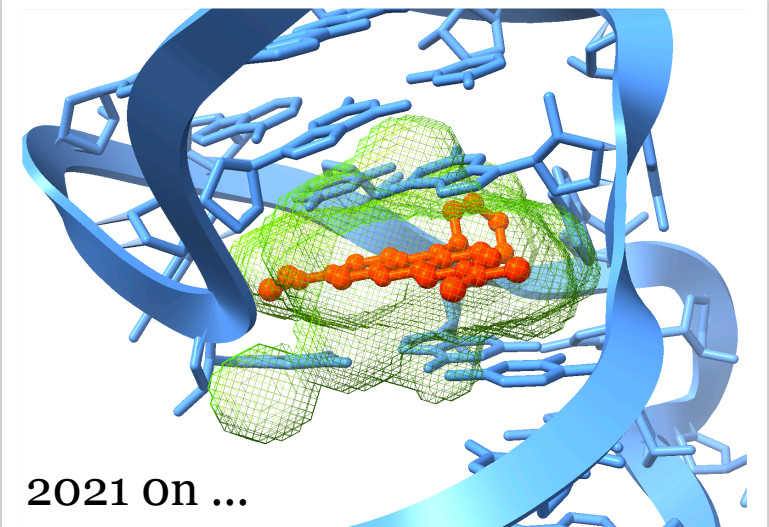
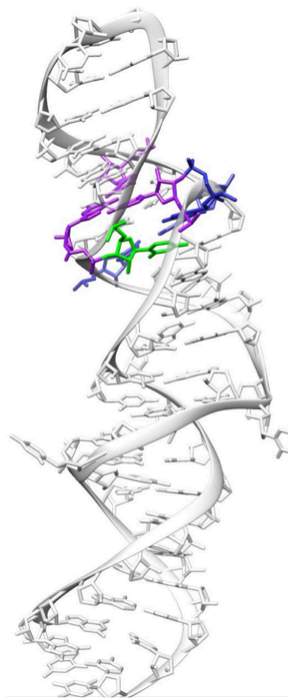


2001-2003

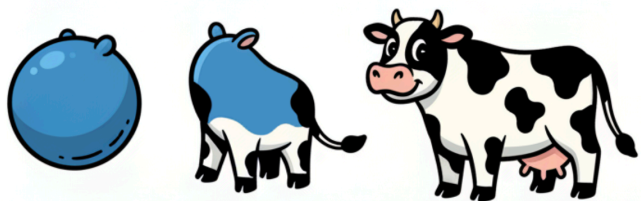
2008 on ...



2015 on ...

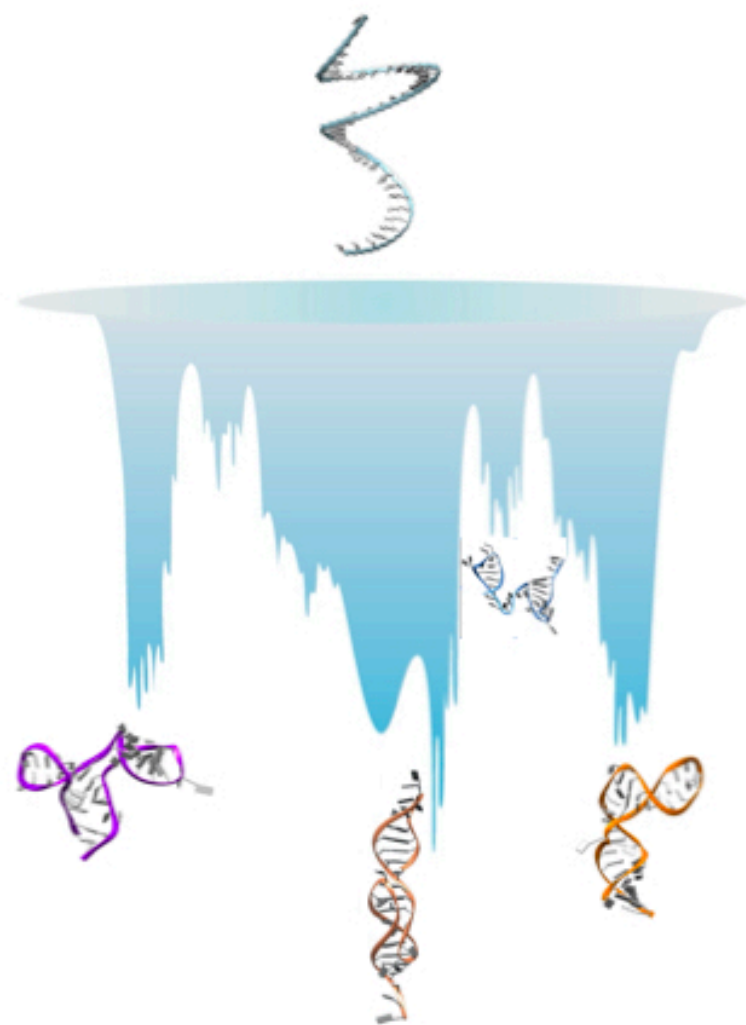


2021 on ...



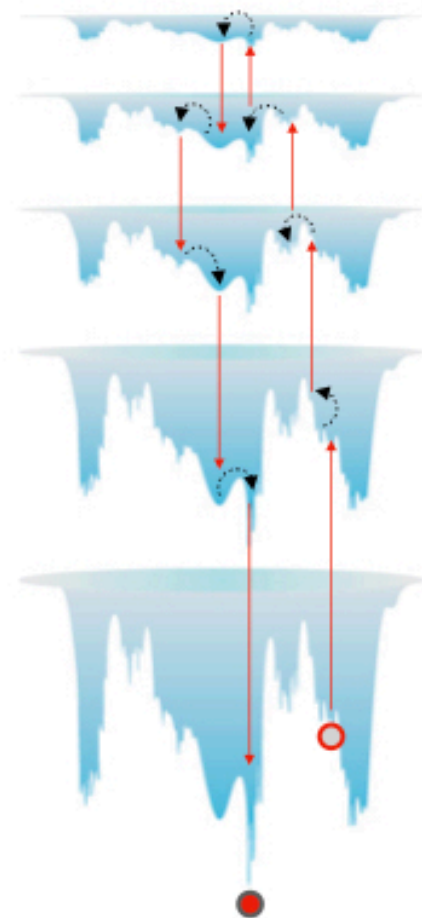
Sampling the 3D RNA energy landscapes

Molecular dynamic simulations & co.

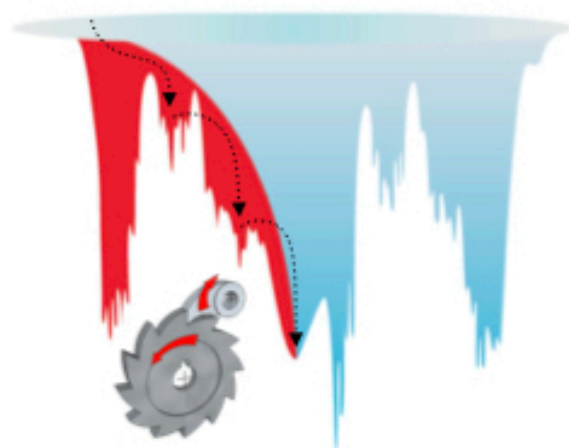


Most of my life is spent here
But it's another section...

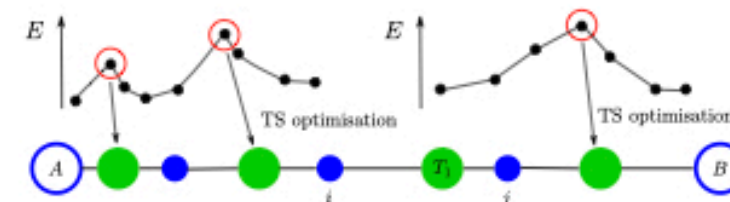
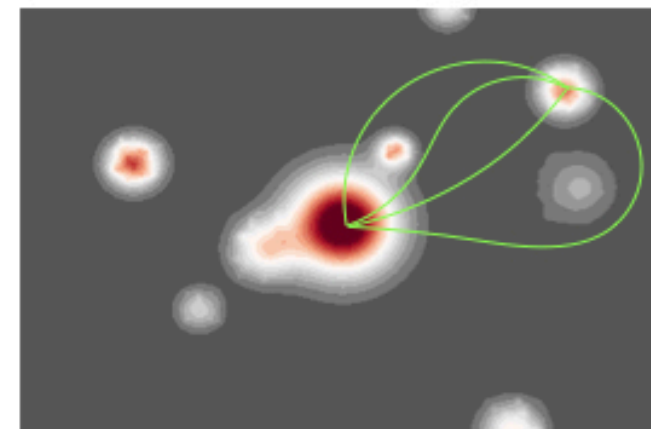
A) Hamiltonian Replica Exchange



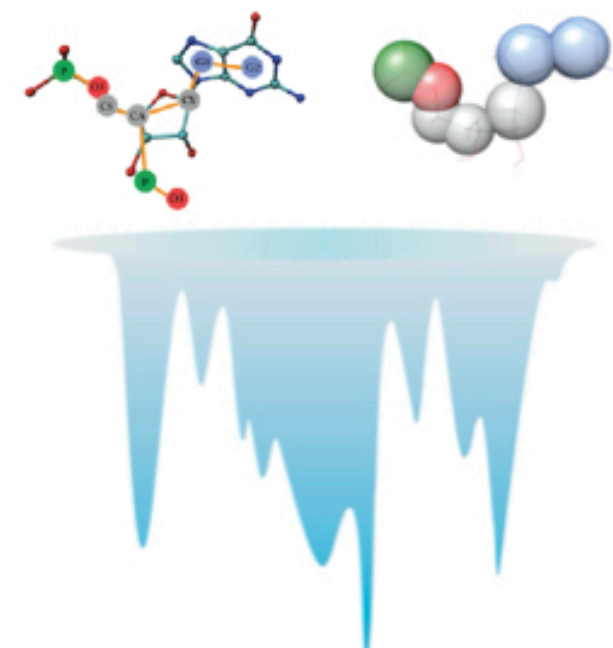
B) Ratchet-and-pawl biased dynamics



C) Path-sampling simulations



D) Coarse-grained models



RNA interactions landscapes

RNA is polymorphic!

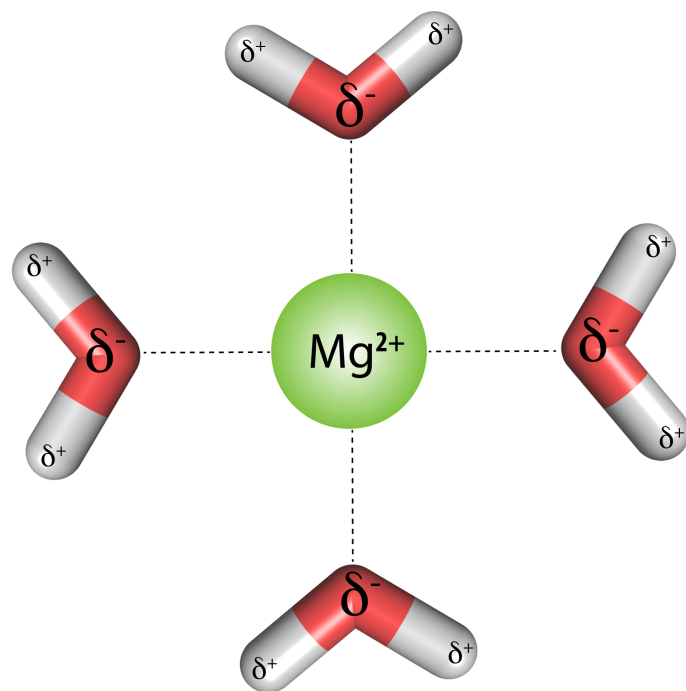


Different structures can have different functional properties:

- different interactions with proteins
- different interactions with small ligands

Physics/chemistry govern these interactions

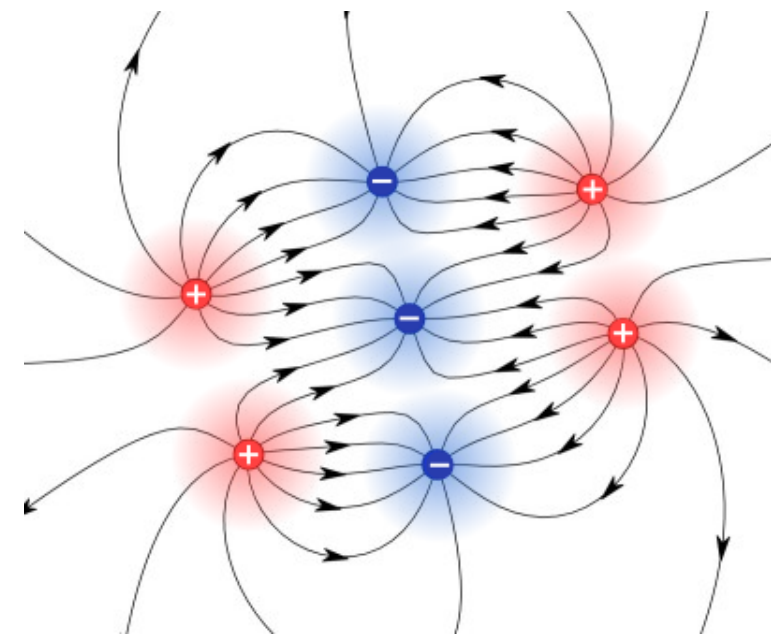
Chemistry



Loren Williams

pairwise interactions

Physics



fields

Statistical Molecular Interaction Fields (SMIFs)

Initially for visualization and to build intuition.

Simple functional forms (Gaussians) parametrized based on known structures to describe molecular interactions:

- Hydrogen bonding

$$\phi_{HB}(\mathbf{x}) = - \sum_i \exp \left[-\frac{(r - r_0)^2}{2\sigma_r^2} \right] \exp \left[-\frac{(\beta - \beta_0)^2}{2\sigma_\beta^2} \right]$$

- Stacking

- Hydrophobic/hydrophilic

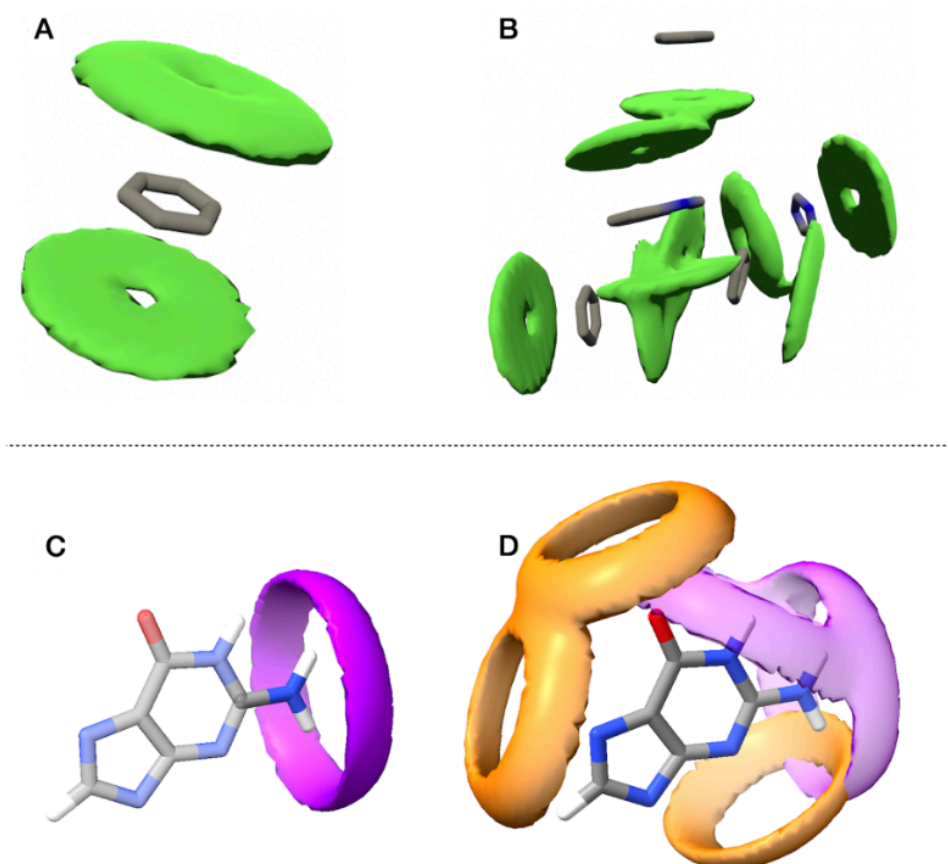
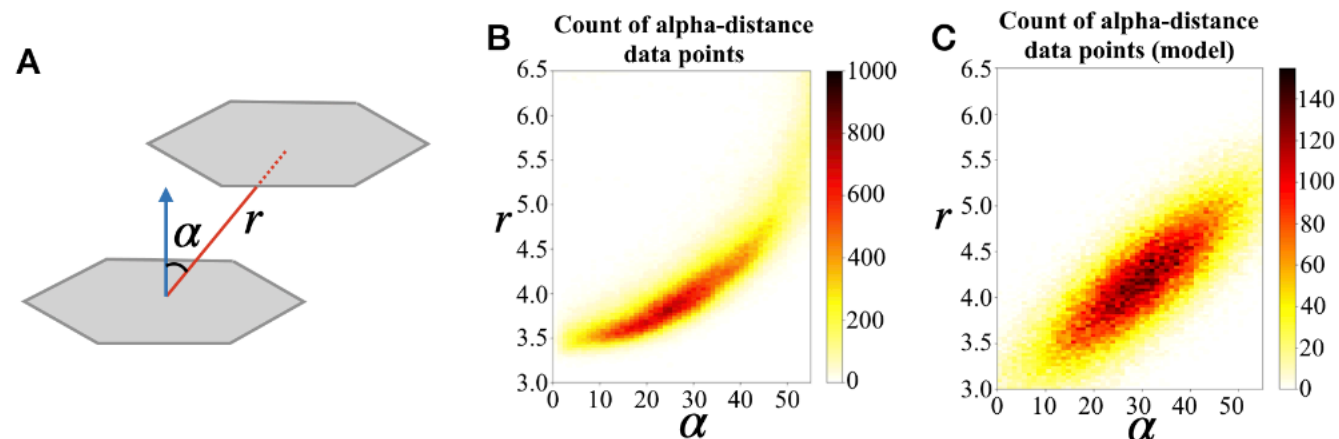
$$\phi_{HP}(\mathbf{x}) = \sum_{i=1}^N K_i \exp \left[-\frac{(r_i - \mu_{HP})^2}{2 \cdot \sigma_{HP}^2} \right]$$

+ Electrostatics (APBS)

$$\phi_{ST}(\mathbf{x}) = - \sum_i \exp \left[-\frac{1}{2}(\mathbf{v}_i - \boldsymbol{\mu}_{ST})^T \mathbf{S}_{ST}^{-1}(\mathbf{v}_i - \boldsymbol{\mu}_{ST}) \right]$$

$$\mathbf{v}_i = \begin{bmatrix} \alpha_i \\ r_i \end{bmatrix} \quad \& \quad \boldsymbol{\mu}_{ST} = \begin{bmatrix} \alpha_0 \\ r_0 \end{bmatrix} \quad \& \quad \mathbf{S}_{ST} = \begin{bmatrix} s_{\alpha,\alpha} & s_{\alpha,r} \\ s_{\alpha,r} & s_{r,r} \end{bmatrix}$$

Large-scale PDB analysis (“statistical”)



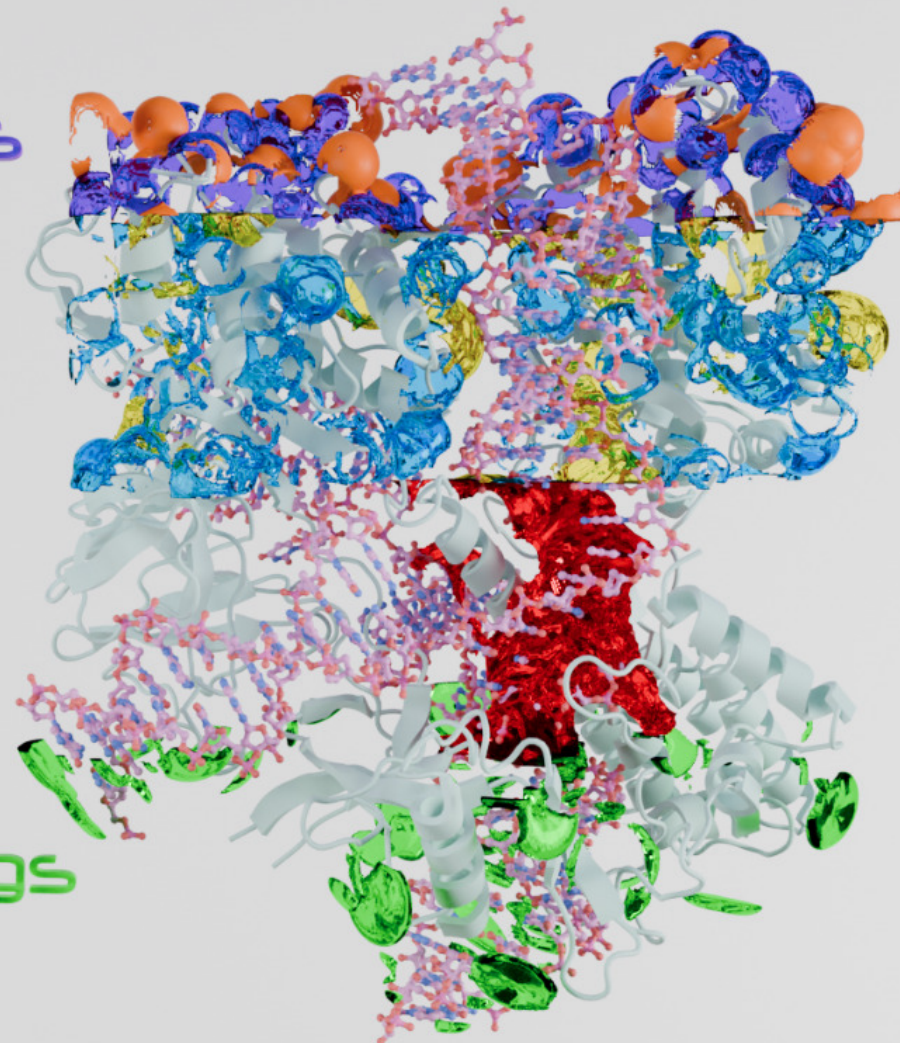
VOLGRIDS

Hbonds Donors
HBonds Acceptors

Hydrophilic
Hydrophobic

Electrostatics

Stackings

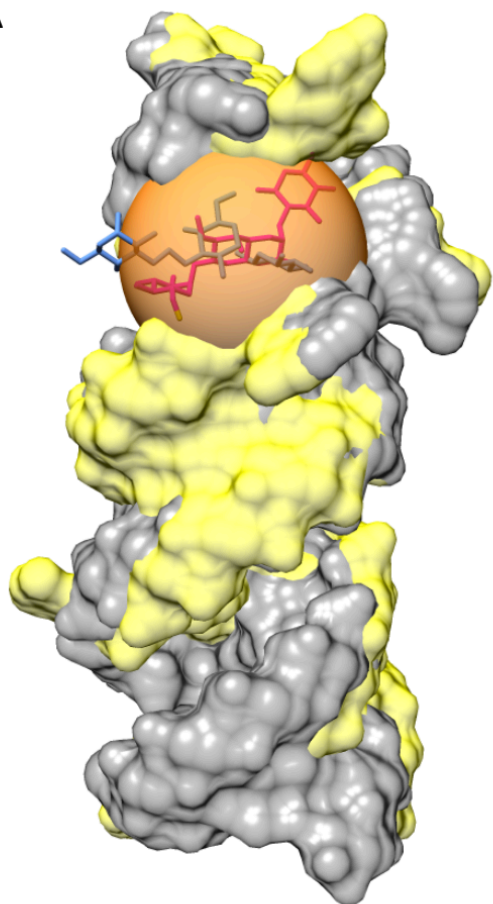


Available on git
repository

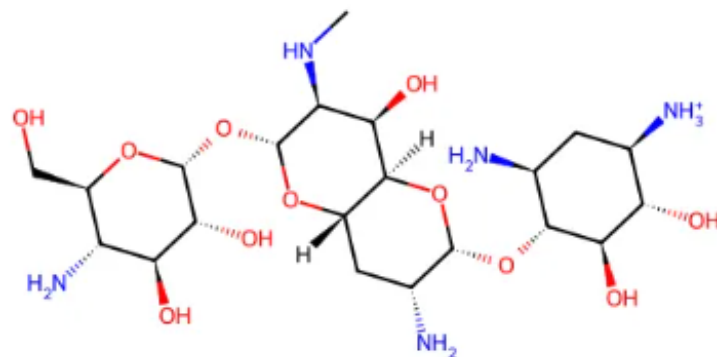


Comparison of the pockets of two drugs

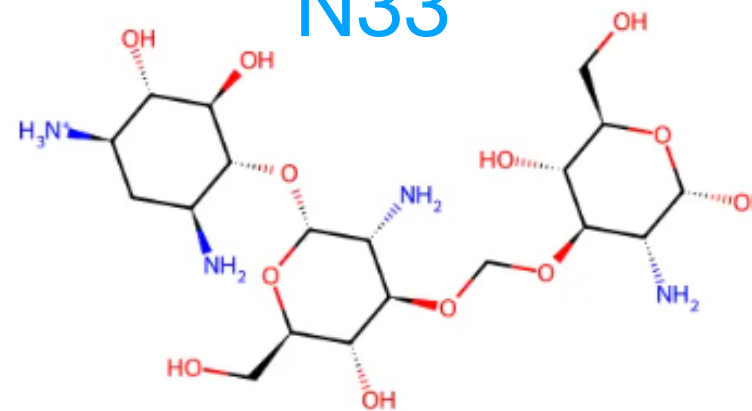
A



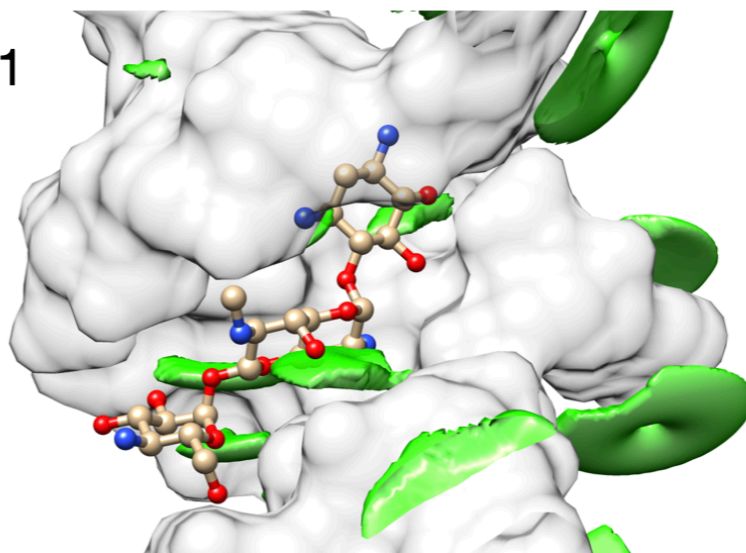
AM2



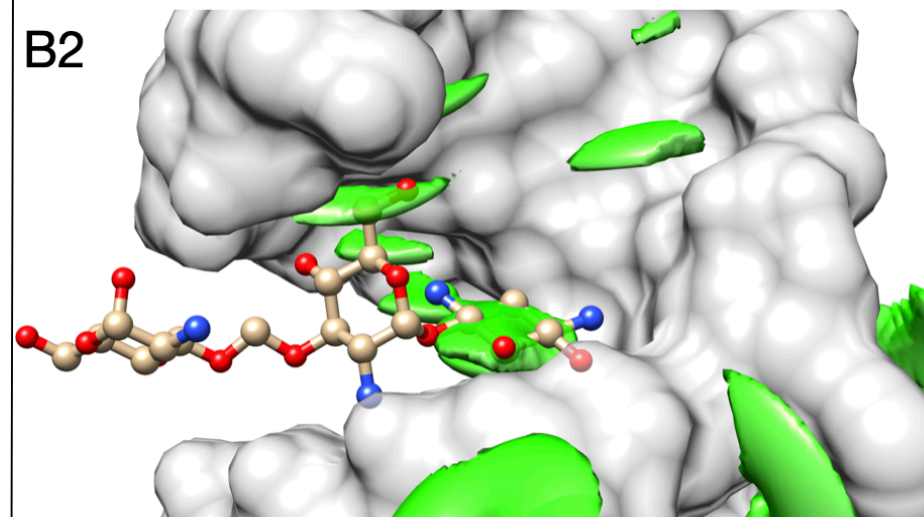
N33



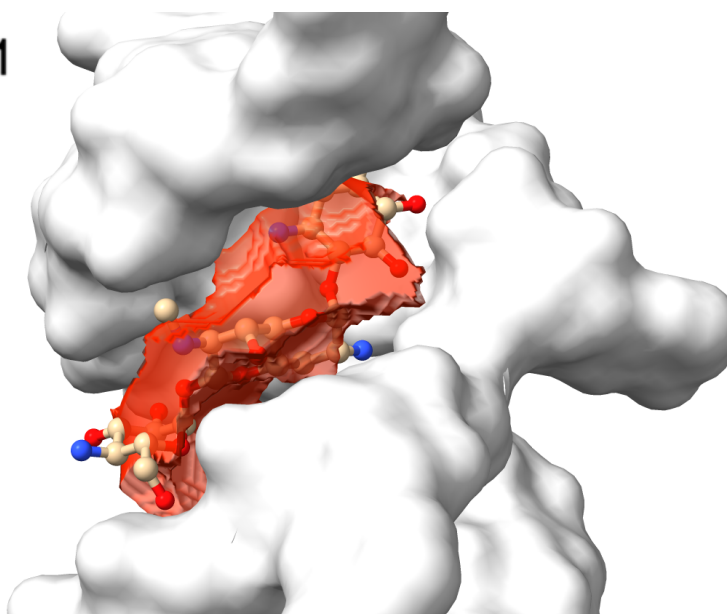
B1



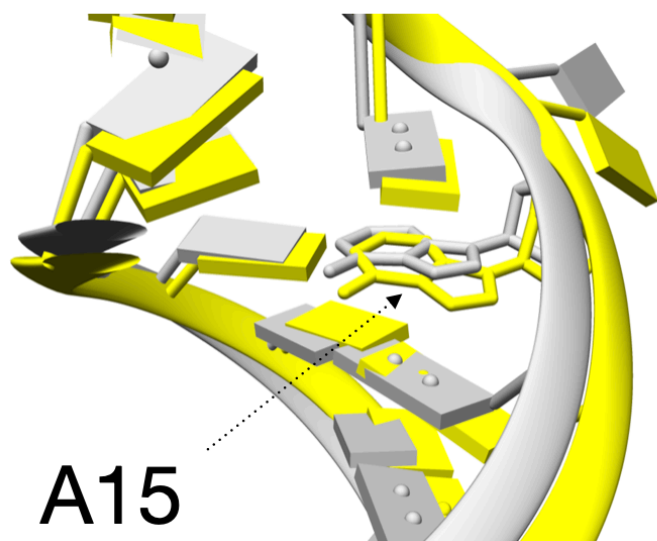
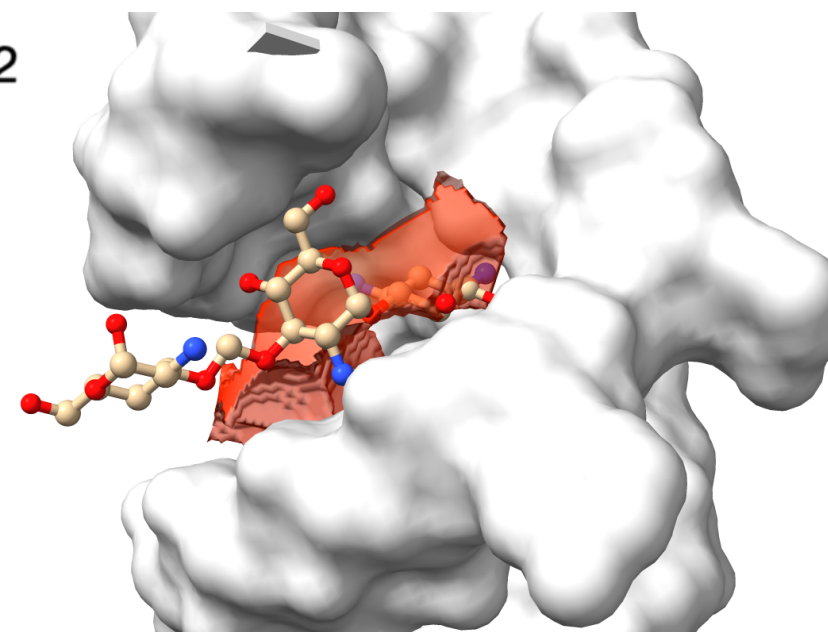
B2



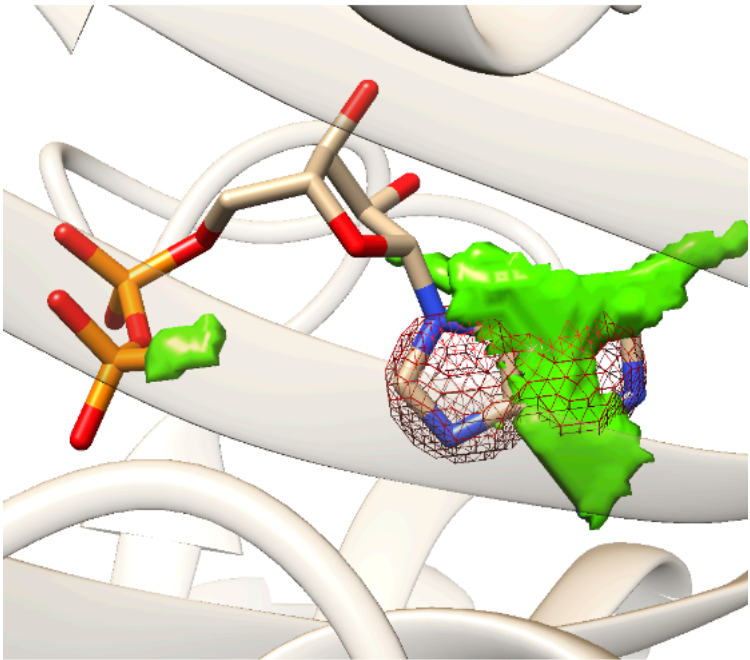
C1



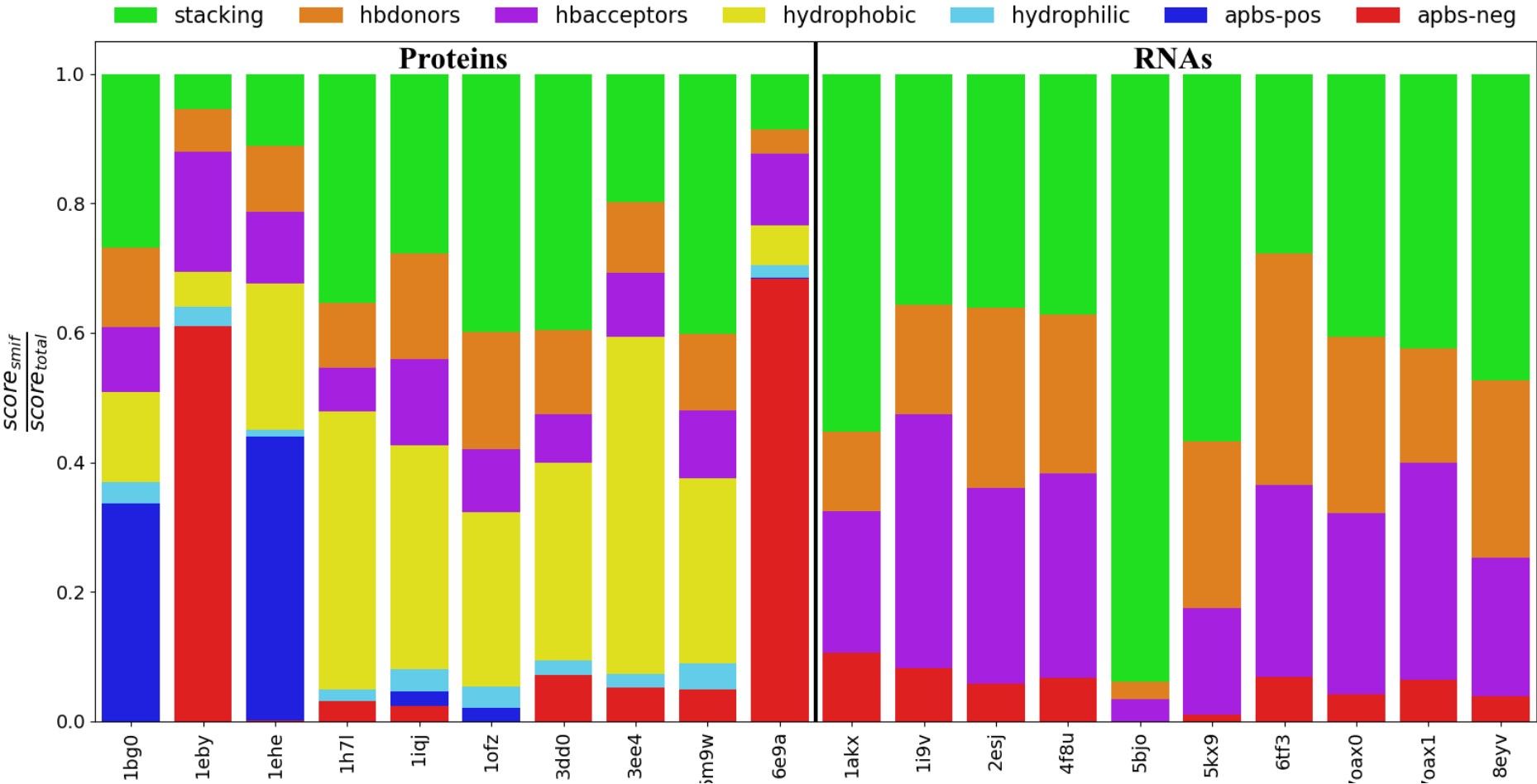
C2



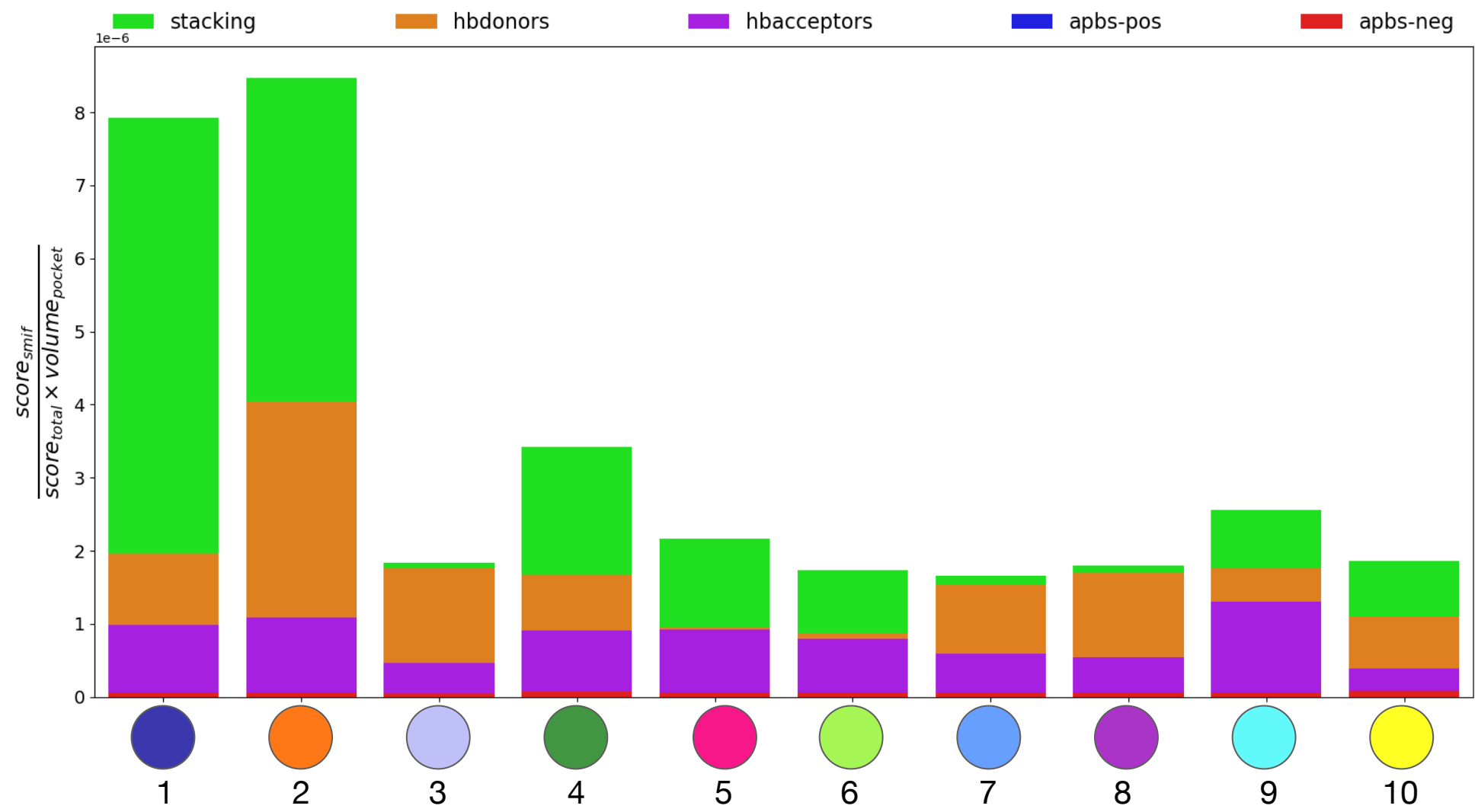
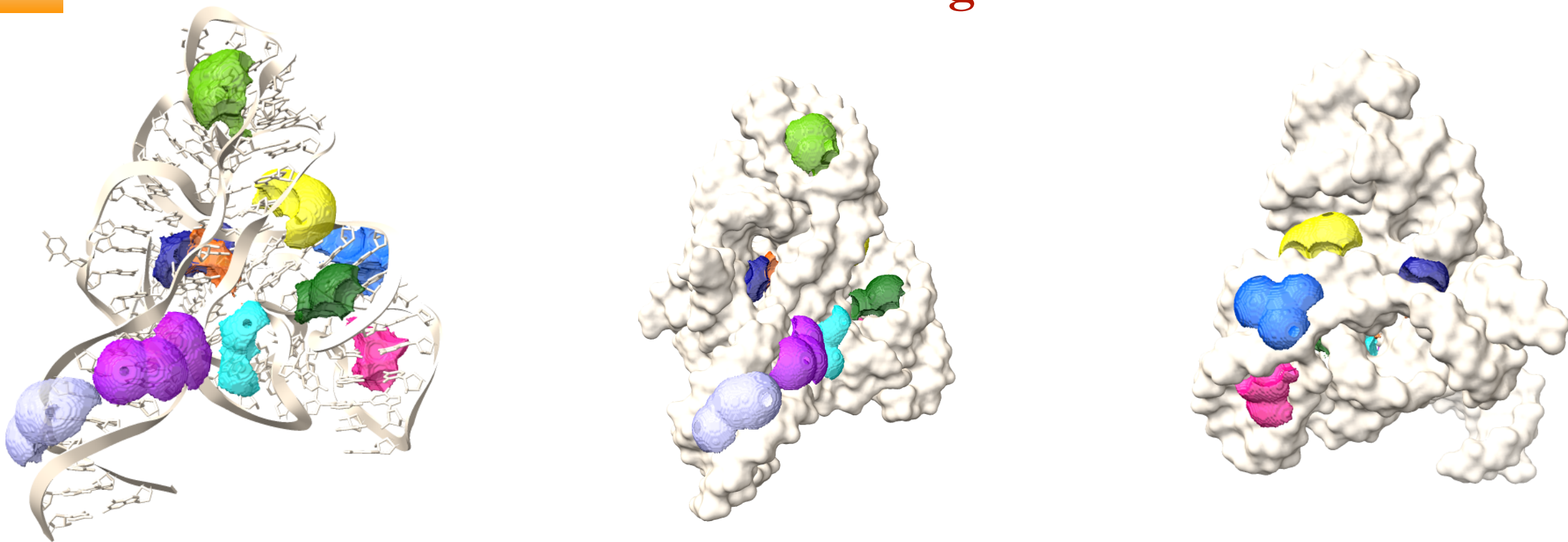
A15



	HBA		HBD		HPH		NEG		POS		STK	
1bg0	8(13)	3	3(3)	2	1(2)	0	2(2)	2	0(0)	0	2(2)	1
1eby	2(8)	2	6(6)	3	4(4)	4	0(0)	0	0(0)	0	0(4)	0
1ehe	4(6)	1	0(0)	0	8(8)	8	2(2)	2	0(0)	0	0(2)	0
1h7l	2(11)	3	1(2)	1	2(2)	2	0(2)	1	0(0)	0	1(1)	0
1iqj	1(5)	0	0(0)	0	4(5)	2	0(0)	0	0(0)	0	1(3)	1
1ofz	2(5)	2	3(4)	1	1(1)	1	0(0)	0	0(0)	0	0(0)	0
3dd0	2(4)	1	1(1)	0	3(3)	1	0(0)	0	0(0)	0	0(2)	0
3ee4	0(2)	0	0(0)	0	5(5)	7	0(1)	0	0(0)	0	0(0)	0
5m9w	3(11)	3	6(8)	2	2(2)	2	0(2)	1	0(0)	0	0(1)	0
6e9a	2(6)	2	3(3)	1	4(5)	7	0(0)	0	0(0)	0	0(2)	0
TOT	14/15		10/10		29/34		4/6		0/0		2/2	
1akx	1(1)	1	2(4)	1	1(1)	1	0(0)	0	1(1)	1	0(0)	0
1i9v	0(19)	0	5(13)	2	1(1)	0	0(0)	0	0(0)	0	0(0)	0
2esj	2(23)	4	9(15)	3	1(1)	0	0(0)	0	0(0)	0	0(0)	0
4f8u	2(12)	3	8(8)	3	3(3)	0	0(0)	0	0(0)	0	0(0)	0
5bjo	2(4)	1	2(2)	1	4(4)	0	0(0)	0	1(1)	1	1(1)	1
5kx9	1(6)	1	0(0)	0	3(3)	0	0(0)	0	1(1)	0	1(1)	1
6tf3	3(12)	2	2(2)	0	2(2)	0	0(2)	0	0(0)	0	2(2)	2
7oax0	0(4)	0	4(4)	1	3(3)	0	0(0)	0	0(0)	0	0(0)	0
7oax1	2(7)	0	2(2)	0	5(5)	0	0(0)	0	0(0)	0	2(2)	2
8eyv	1(4)	0	2(2)	0	4(4)	0	0(0)	0	1(1)	0	1(1)	1
TOT	9/12		11/11		1/1		0/0		2/2		7/7	



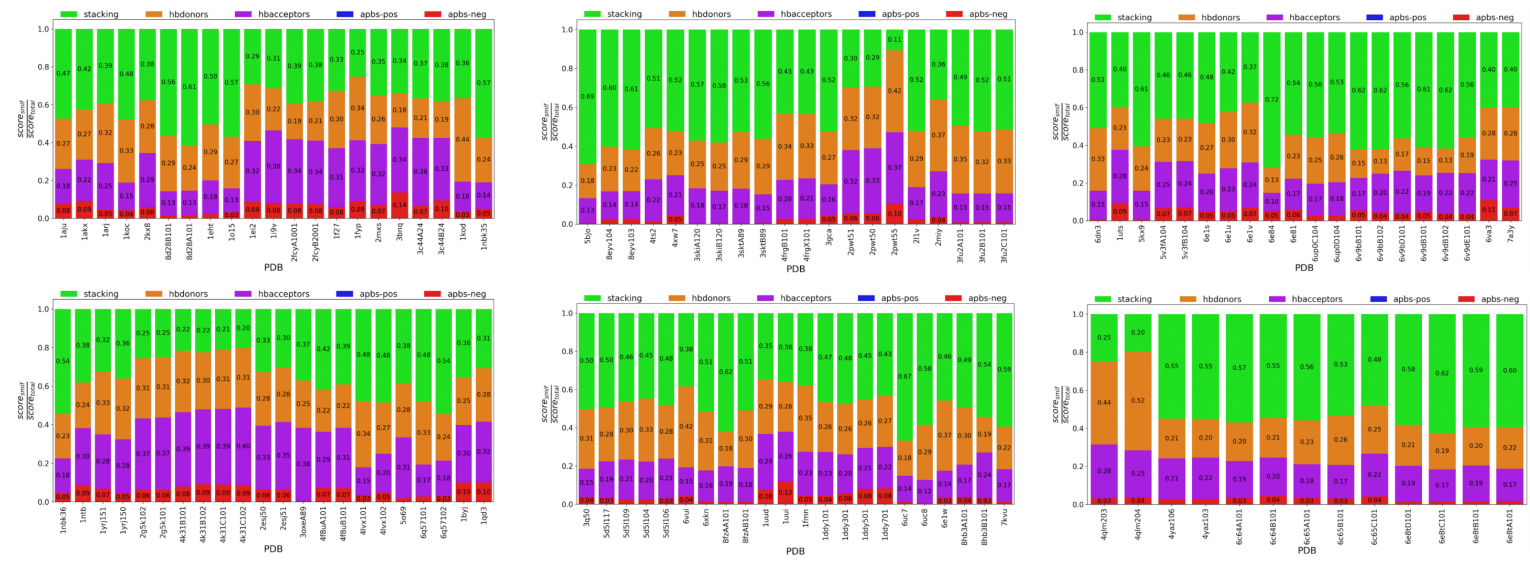
Pockets scoring



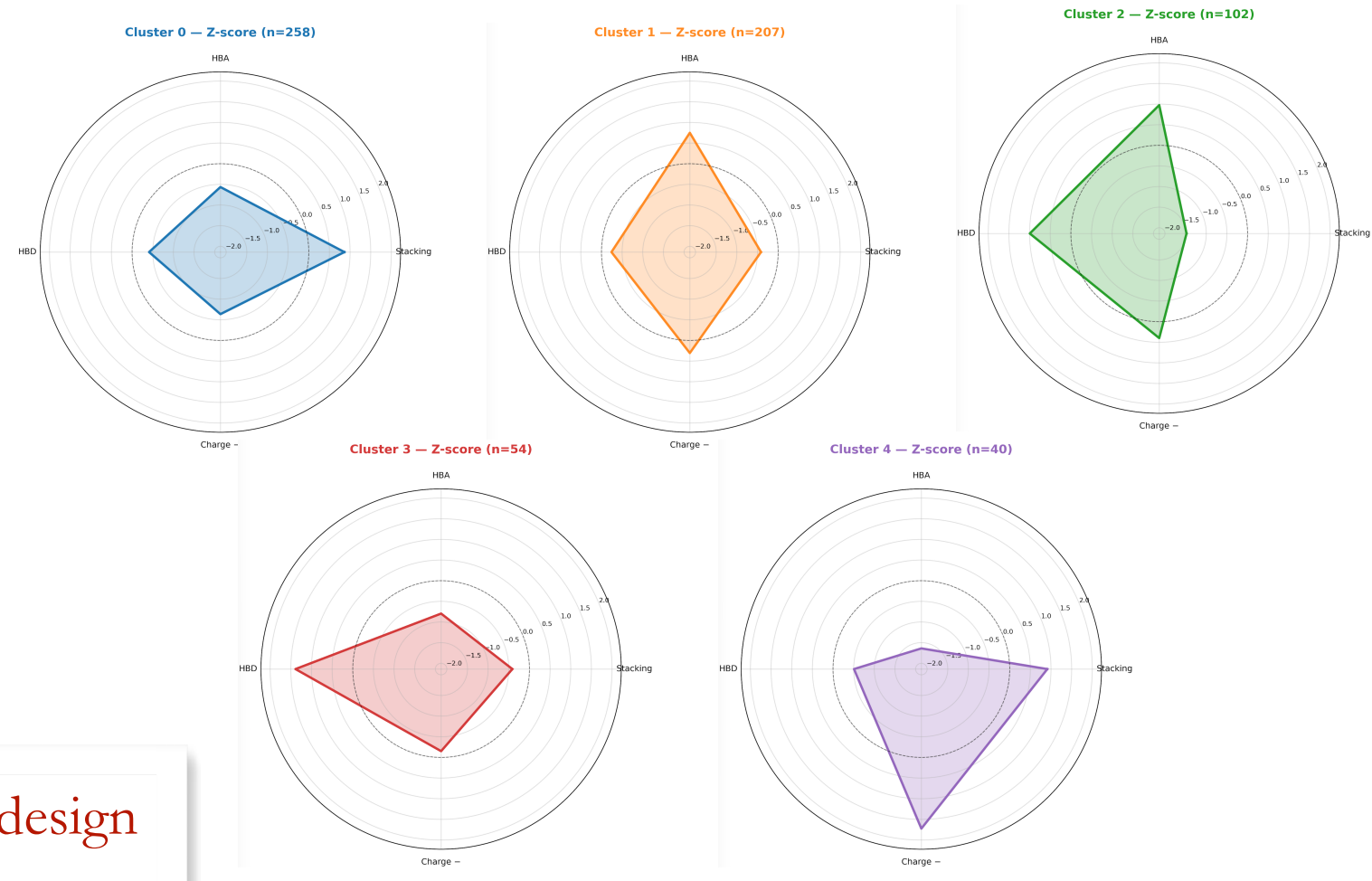
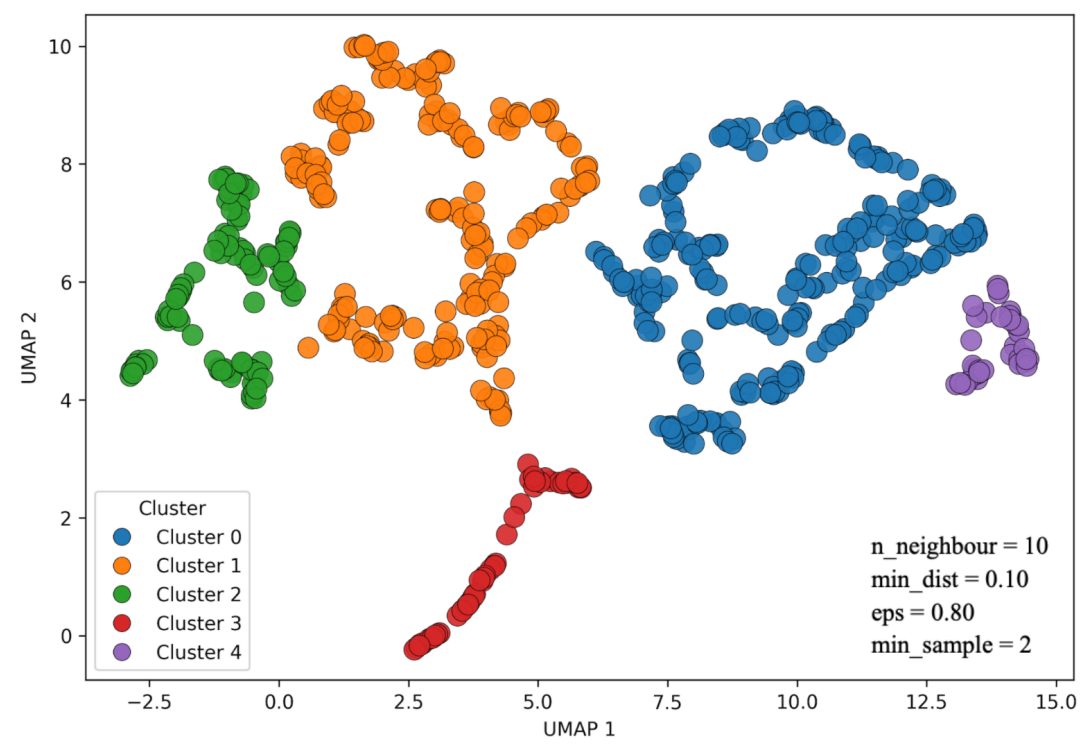


Panei, et al. Bioinformatics 2022

Compute SMIFs for all known pockets (“barcodes”)



Clustering of pockets based on fields values



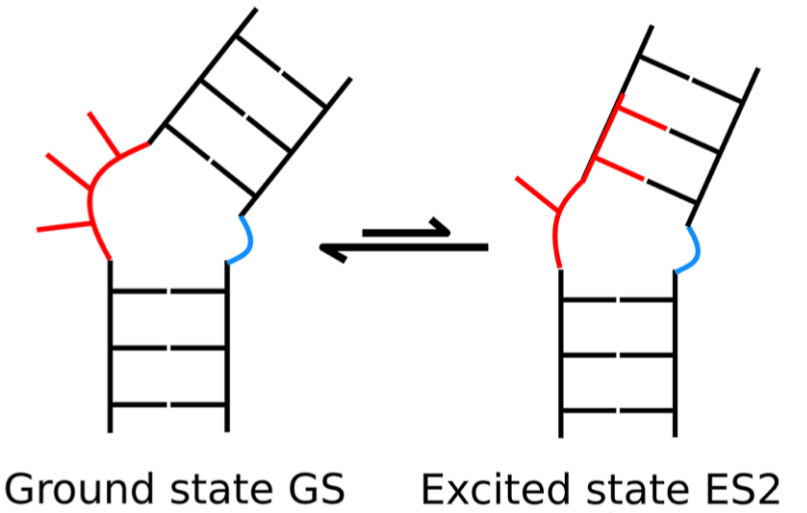
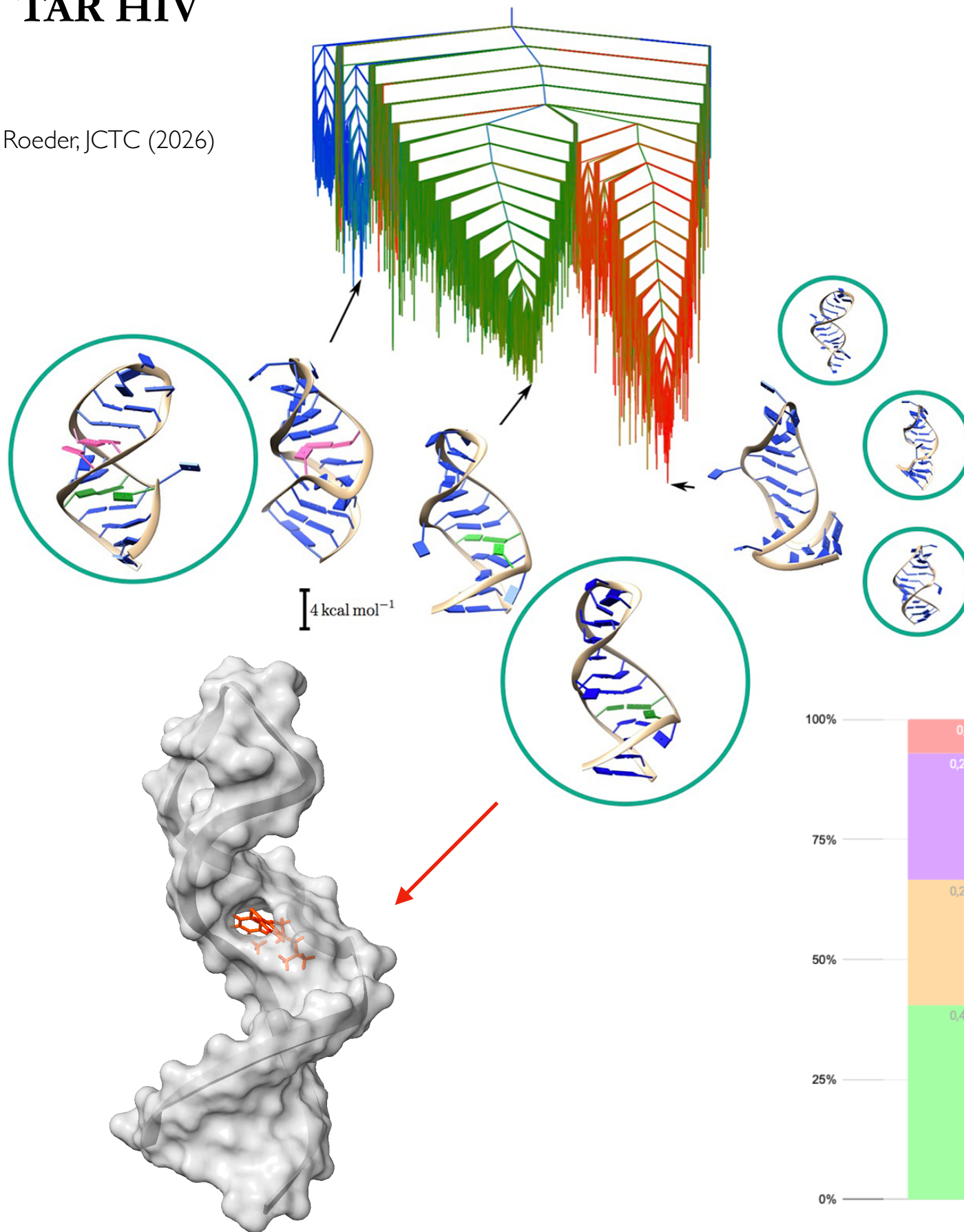
Link clusters and ligand properties for drug design (ML models)

Pockets and energy landscapes



TAR HIV

Roeder, JCTC (2026)



Average SMIFs over conformational basins

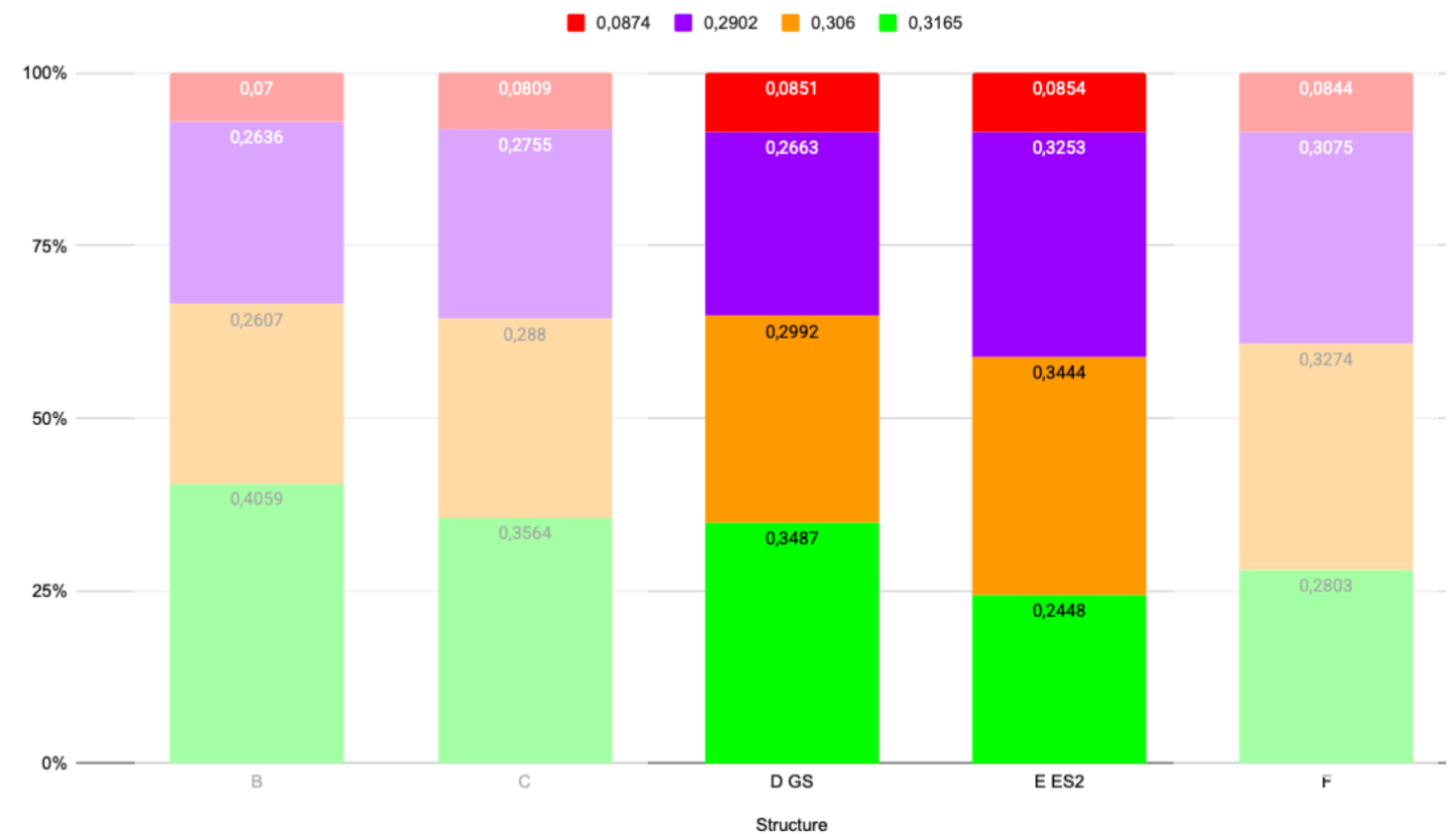


Figure 1 displays multi-frame clusters (max 10) for the tar.stk.graph dataset. The top panel shows the volume (counts) of 10 clusters over 90 frames. The bottom panel shows the base pair coverage for each cluster across the same frames.

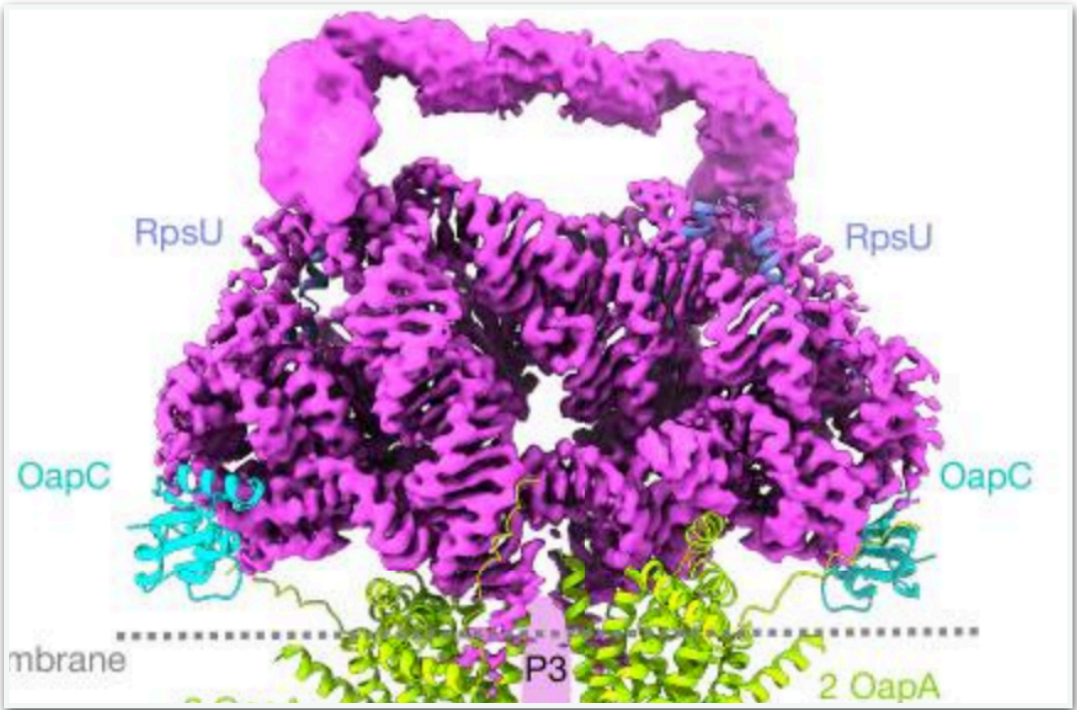
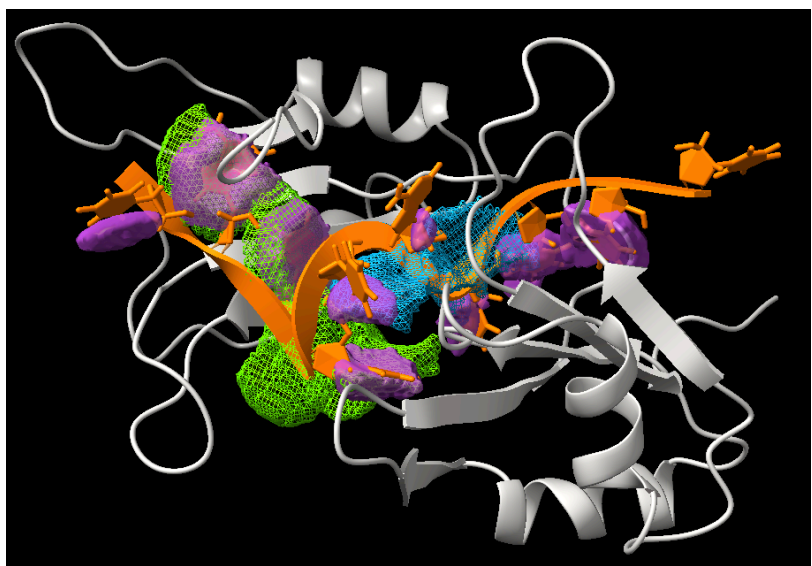
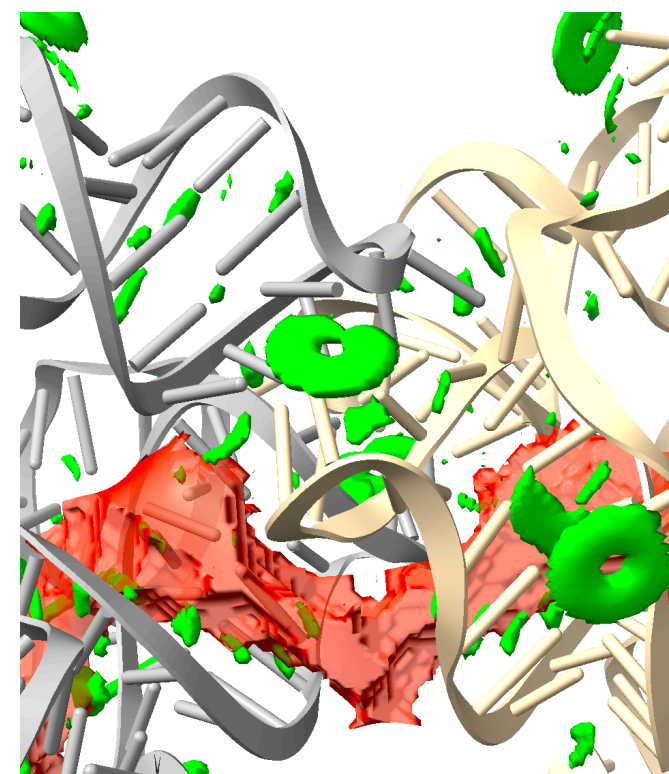
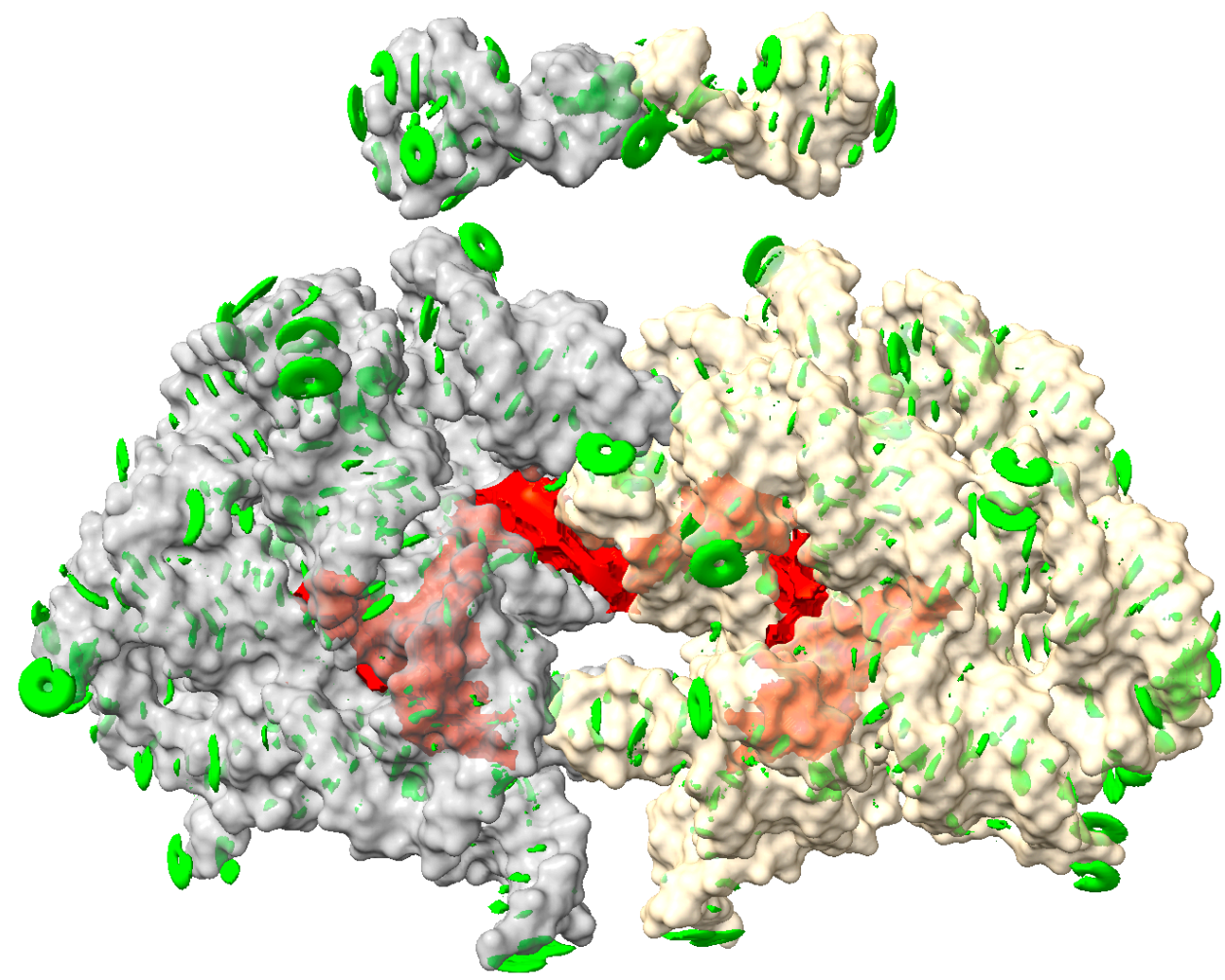
Top Panel: Volume (counts) vs. Frame

The top panel is a stacked area chart showing the volume (counts) of 10 multi-frame clusters over 90 frames. The y-axis represents Volume (counts) from 0 to 7000. The x-axis represents Frame from 0 to 90. The clusters are color-coded: cluster 1 (blue), cluster 2 (orange), cluster 3 (green), cluster 4 (red), cluster 5 (purple), cluster 6 (brown), cluster 7 (pink), cluster 8 (grey), cluster 9 (yellow-green), and cluster 10 (cyan). The volume peaks around frame 40 (approx. 6000 counts) and frame 85 (approx. 6500 counts).

Bottom Panel: Base pair coverage vs. Frame

The bottom panel is a dot plot showing the base pair coverage for each cluster across the same frames. The y-axis lists the base pairs (e.g., CCG, AAG, UUU, etc.). The x-axis represents Frame from 0 to 90. The dots are color-coded by cluster: blue (cluster 1), orange (cluster 2), green (cluster 3), red (cluster 4), purple (cluster 5), brown (cluster 6), pink (cluster 7), grey (cluster 8), yellow-green (cluster 9), and cyan (cluster 10). The coverage is highest for cluster 1 (blue) and cluster 3 (green).

Correlation of “blobs” HIV IRES with base pairing



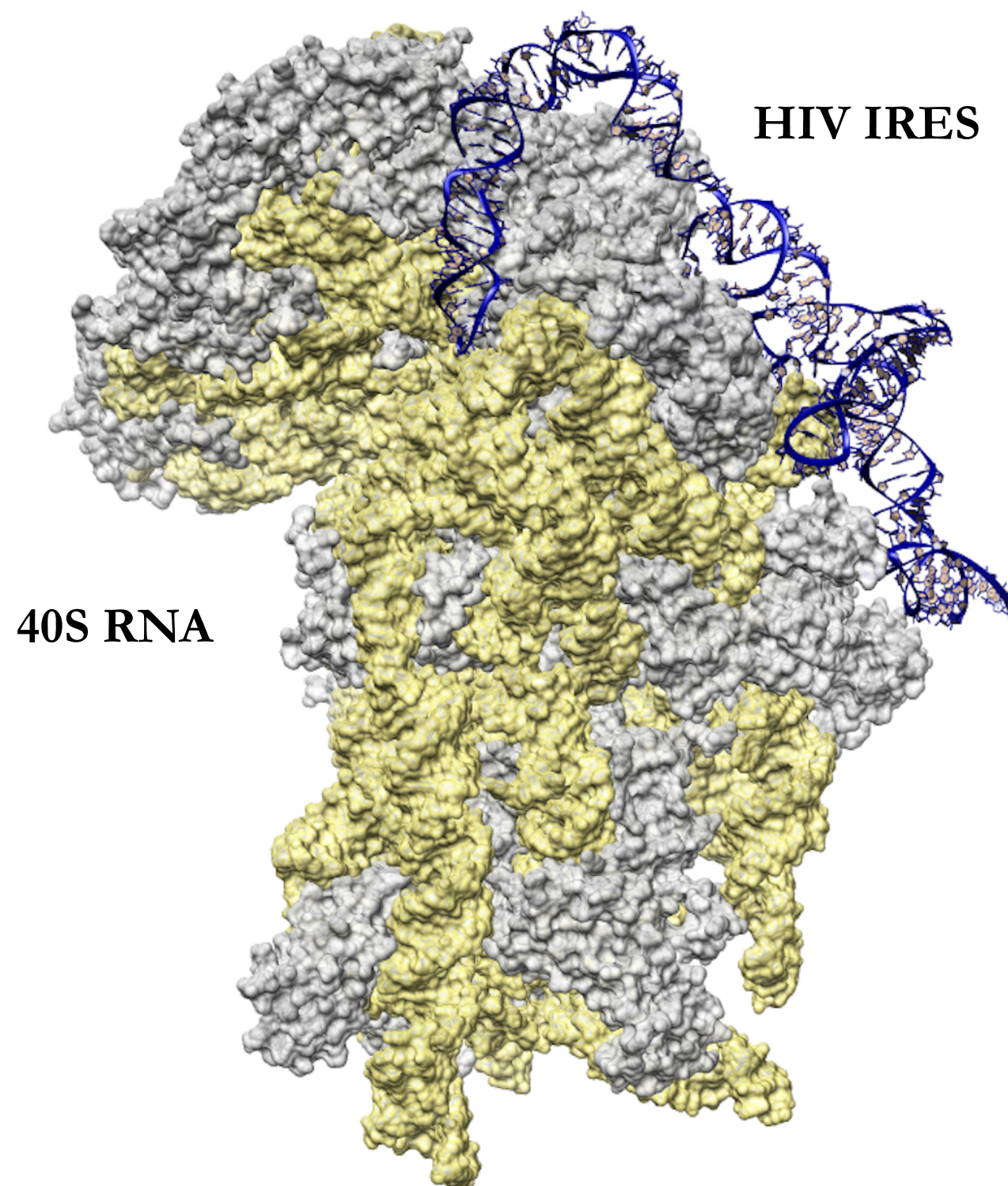
dynamically connected interaction patches

Interaction fields (iSMIFs)

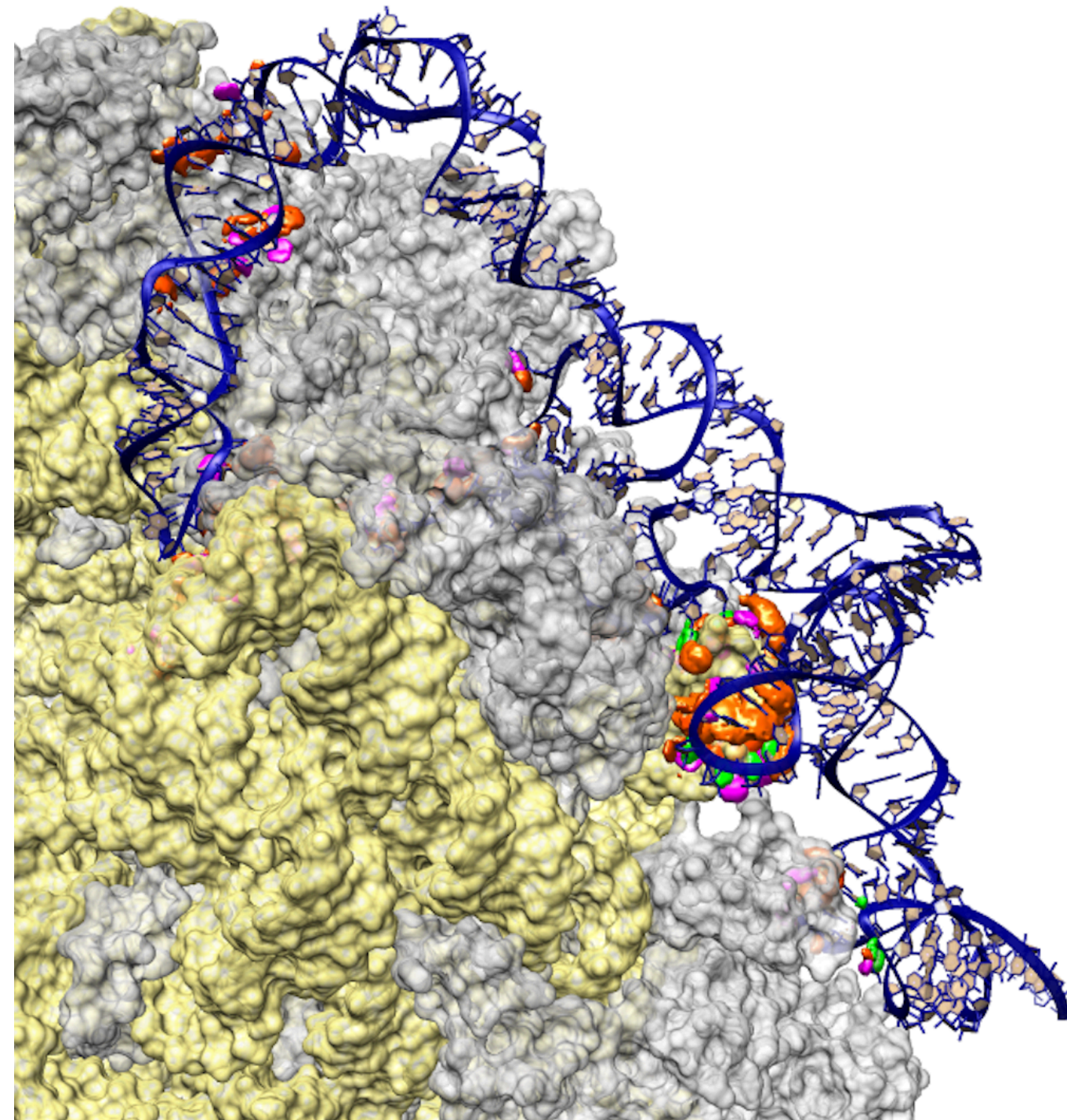
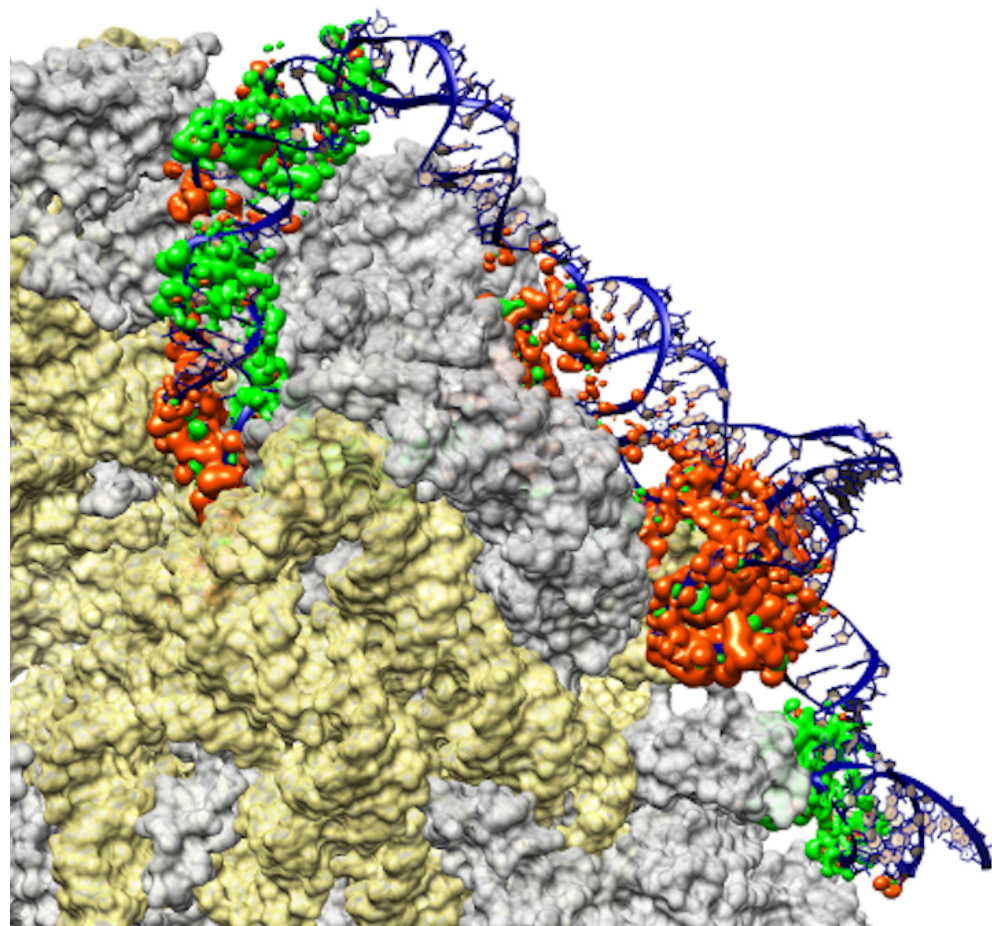
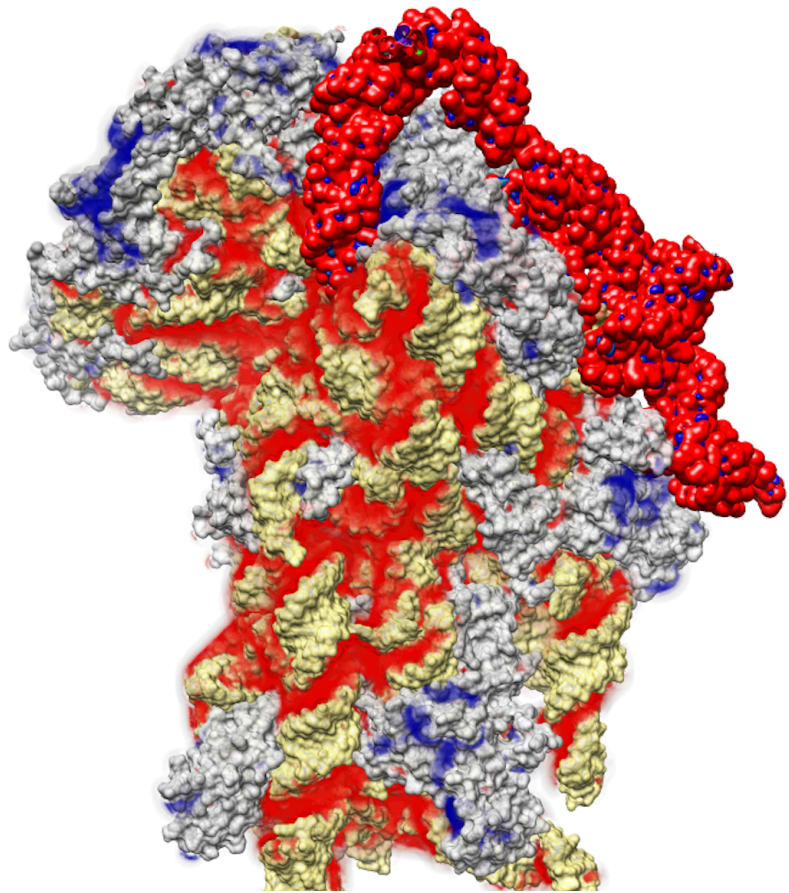
Visualize and rationalize which interactions are “used” in an interface

Compute fields for one partner and match with occupation (density) fields of its counterpart (compute the fields overlaps):

- Stacking field with aromatic rings
- Hydrogen bonds acceptor field with hydrogens
Hydrogen bond donor field with hydrogen bond acceptors
- Electrostatic field with charges



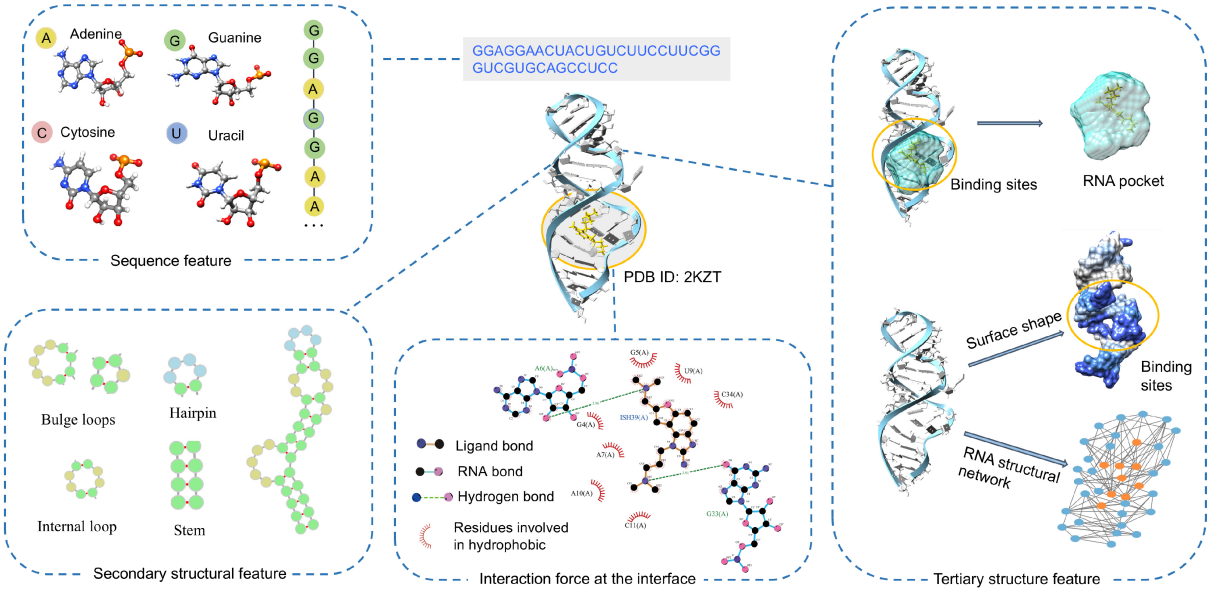
Interaction fields (iSMIFs)



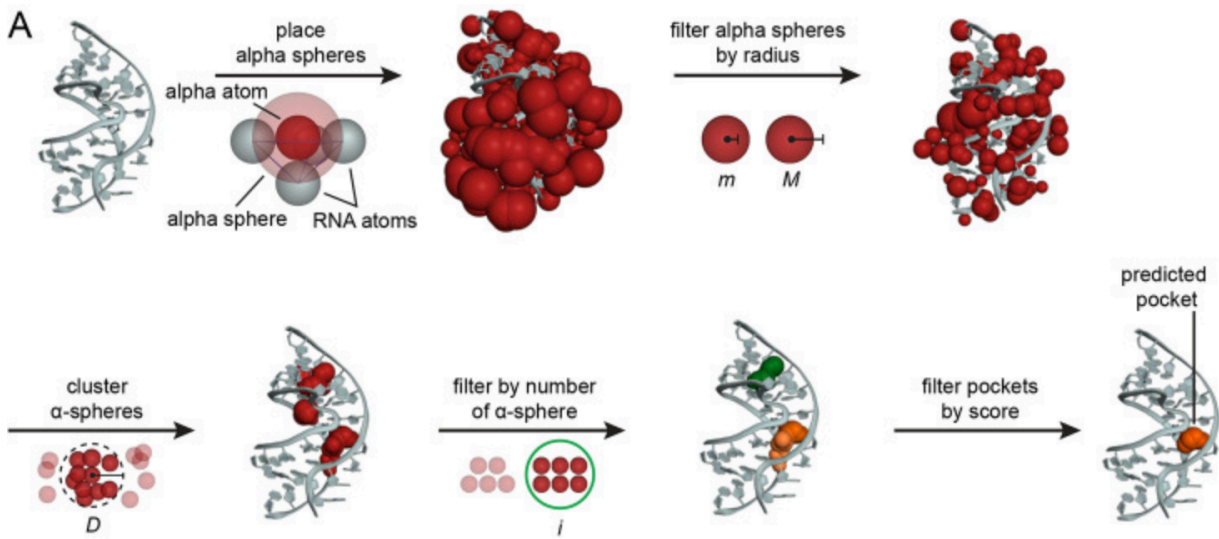
Pockets' prediction



ML methods



Geometry-based methods

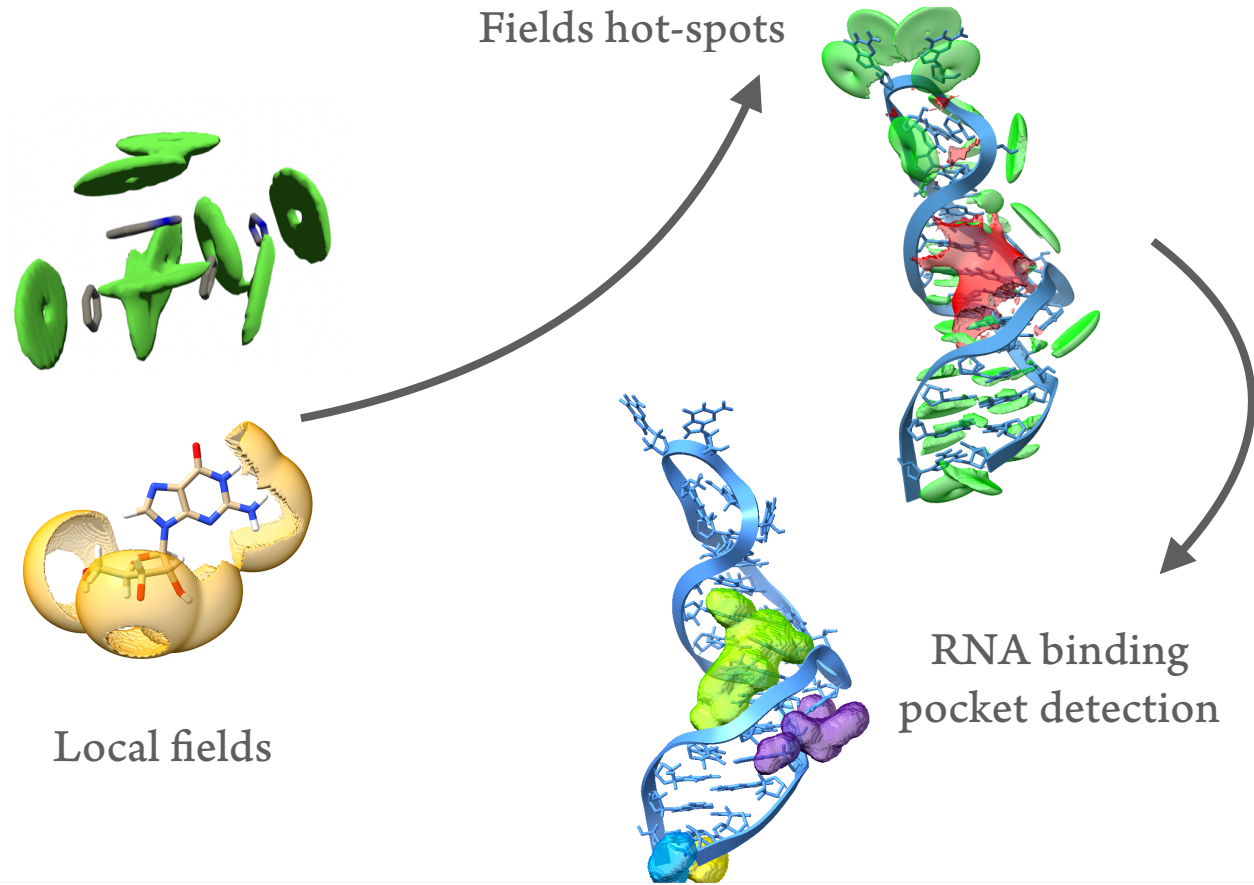


Zhuo et al., Life, 2025

Le Guillaux et al. BMC Bioinformatics 2009
Veenbaas et al, PNAS 2025

SMIFs to detect RNA binding pockets

physics-based



SPockeR



Thanks to...

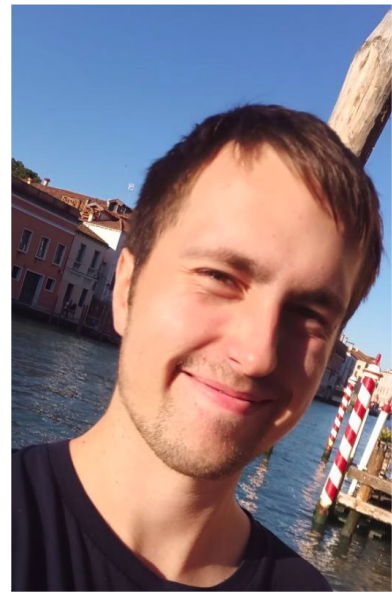
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Pockets



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SMIFs



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(M2 student)
Pockets' classification



Raju Sarkar
(postdoc)
Pockets' predictions

Conclusions: Physics rules!

- 📌 SMIFs help understanding interactions between RNA and small molecules and RNA/protein interfaces.
- 📌 SMIFs hot-spots match known interaction sites.
- 📌 SMIFs provide insightful classification of RNA pockets that can help narrow down possible drug candidates.
- 📌 SMIFs can analyse pockets/interfaces on energy landscapes and dynamics.
- 📌 SMIF-based pocket predictions, SPockeR, performs extremely well without any training, implying totally general applicability to unknown structures, and provide the information of the relevant binding modes characteristics of the pocket.

My two cents...

A simple interaction model (basically a Taylor expansion) provides a clear understanding of the system behavior.

Go simple before making things complex!

Before engaging in involved ML/DL models try to understand what the system does.

Ask the right questions and it will tell you a lot.

Thanks to...

Laboratoire de Biochimie Théorique
Université Paris Cité

Philippe Derreumaux
Marc Baaden, Antoine Taly
(SMIFs)

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University of Cambridge

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King's College London

Konstantin Roeder (EL)

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Università di Milano Bicocca

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