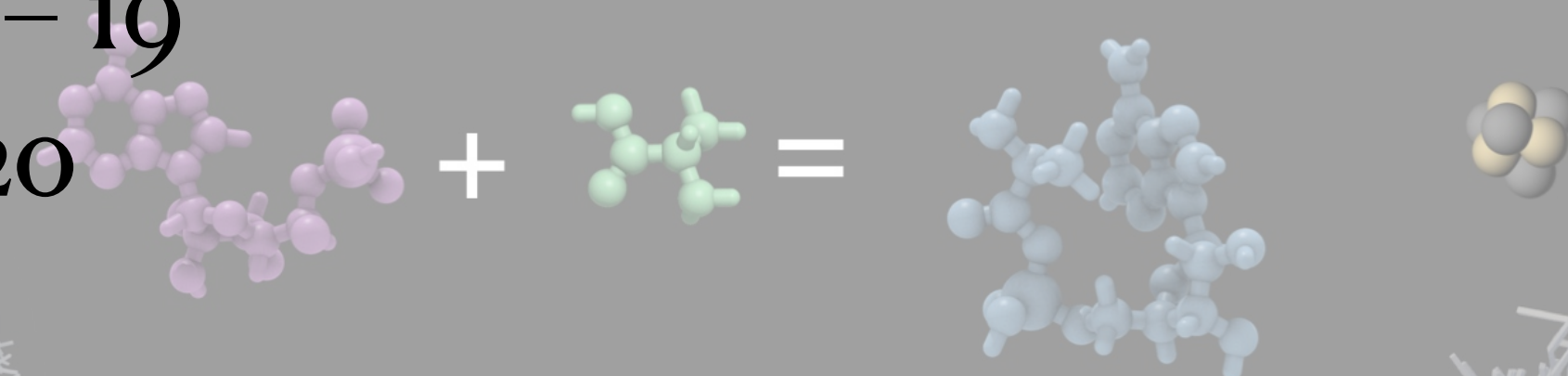




1st Scientific Meeting – 19
and 20 November, 2020
Online

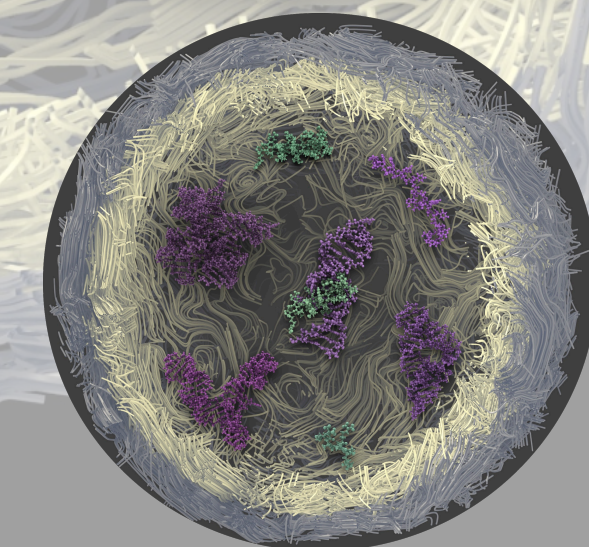


Modified oligonucleotides as RNA-based drugs (biosensors)

prerequisites and opportunities for *in silico* methods within
the framework of fragment-based approaches

Fabrice Leclerc

fabrice.leclerc@i2bc.paris-saclay.fr



RNA Sequence, Structure & Function (SSFA)

I2BC, Dept. Genome Biology

Head: Daniel Gautheret, subgroups:

- RNomics (*bioinformatics*): D. Gautheret & C. Toffano-Nioche - *application to cancer (driver genes)*
- Ribozymes & splicing (*structural biology*): M. Costa - *genetic tools (group II introns as mobile elements)*
- RNA structure & function (*2D & 3D modeling*): F. Leclerc - *application to Alzheimer's disease (BACE1)*

RNA or nucleotide based drugs

Modes of action

- **hybridisation-dependent (RNA target)**

- siRNA (small interfering RNA)
- miR (microRNA)

- ASO (Antisense Oligonucleotides)

- ...

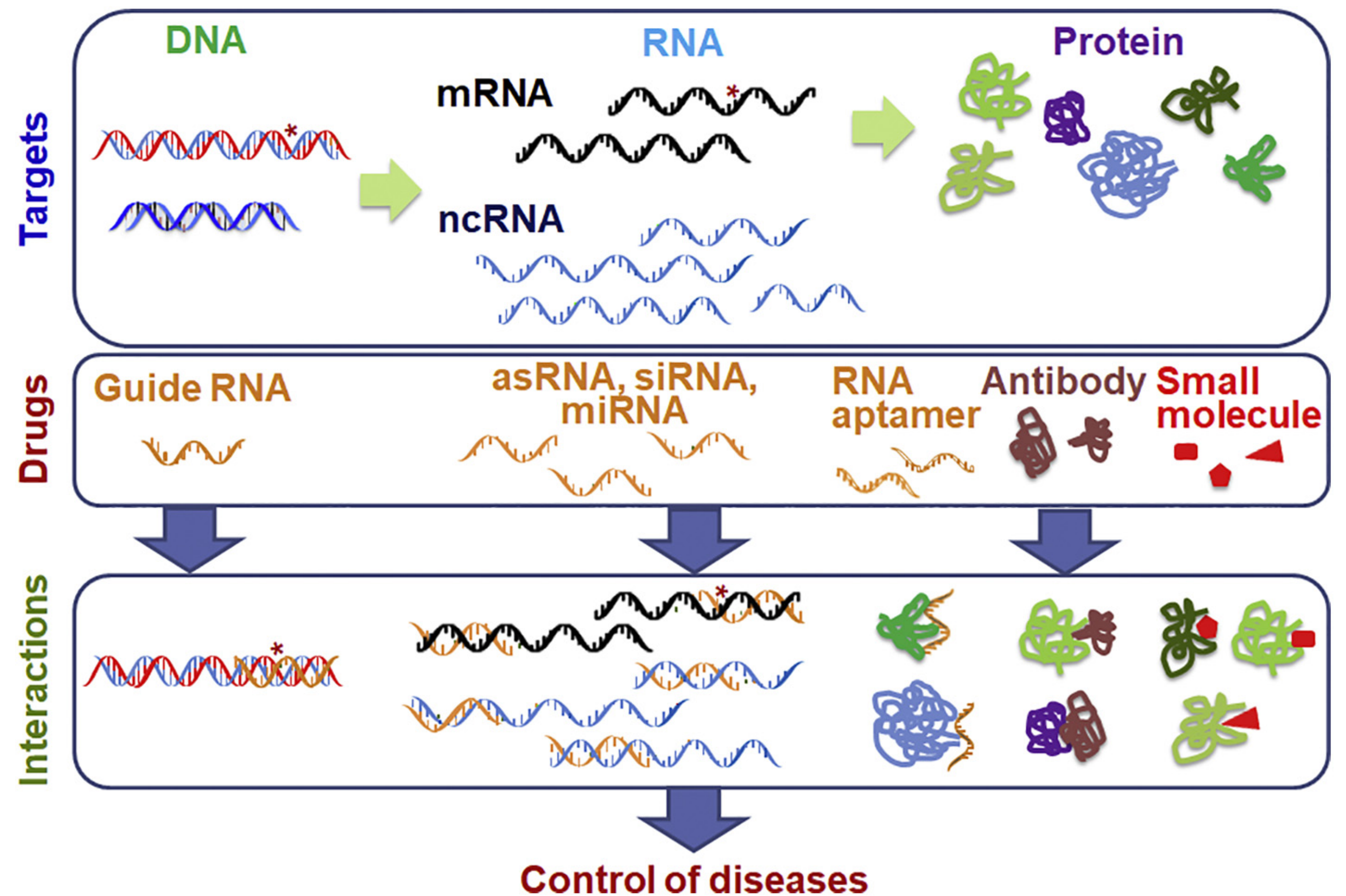
- **hybridisation-independent (targets)**

- aptamers (protein targets, ...)

- immunostimulatory (CpG) oligos

- mRNA

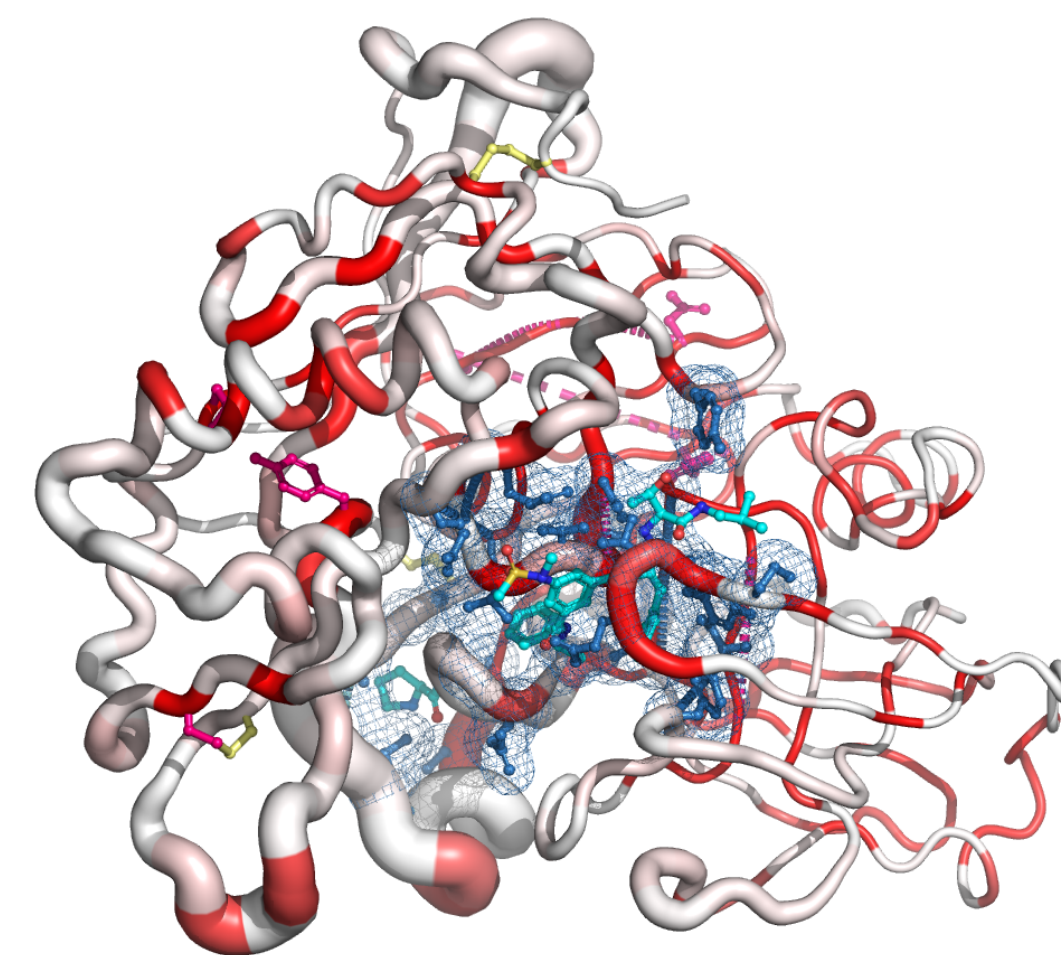
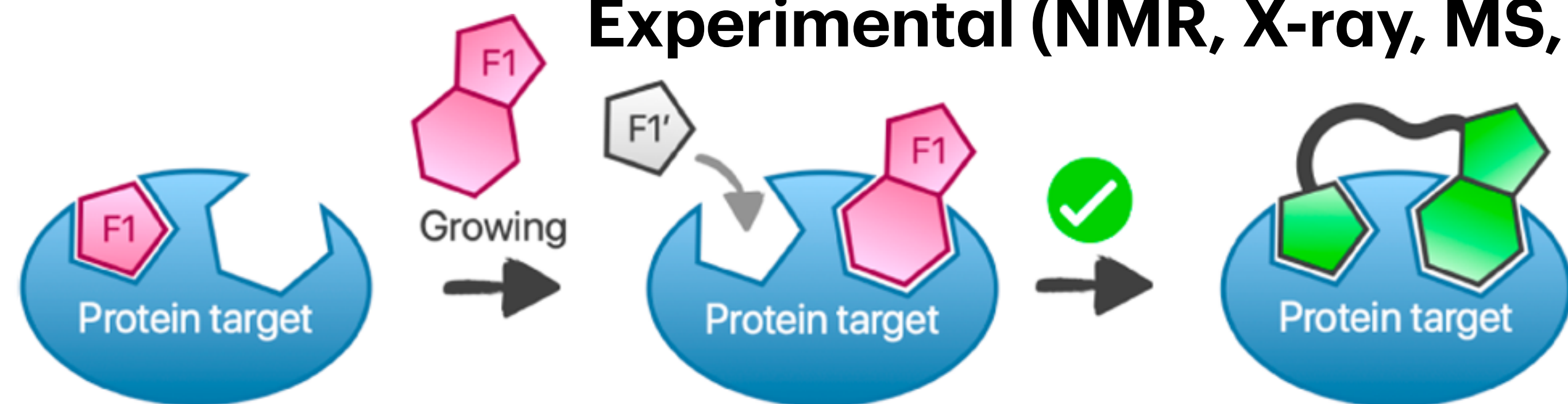
- ...



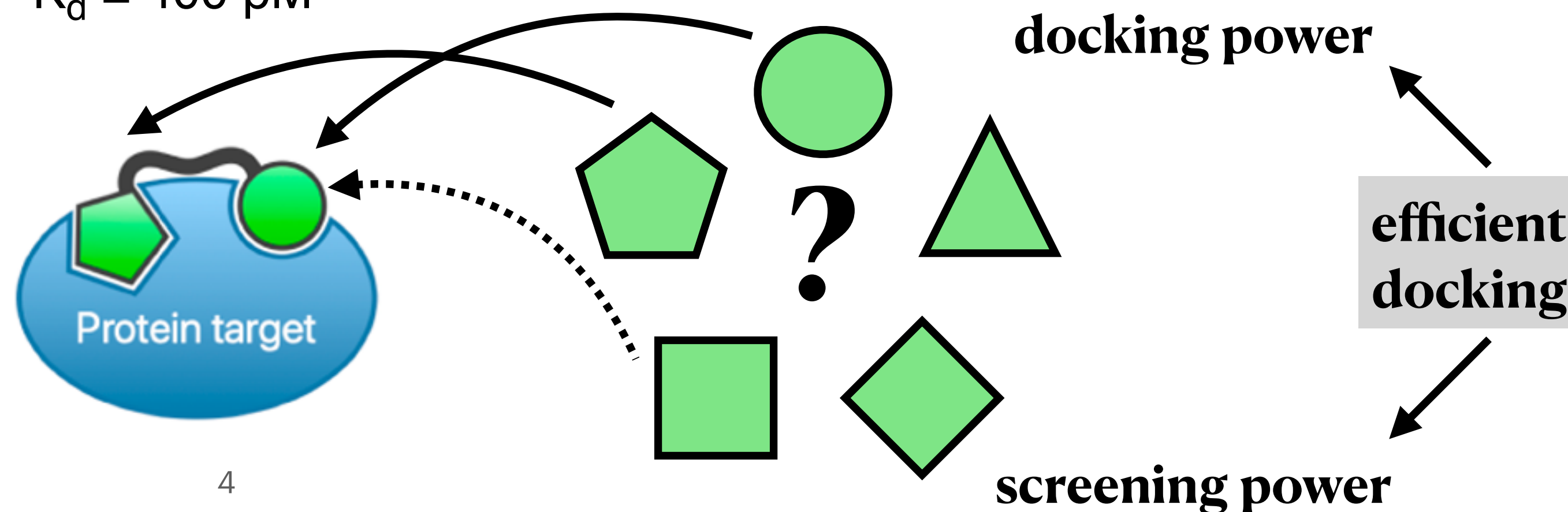
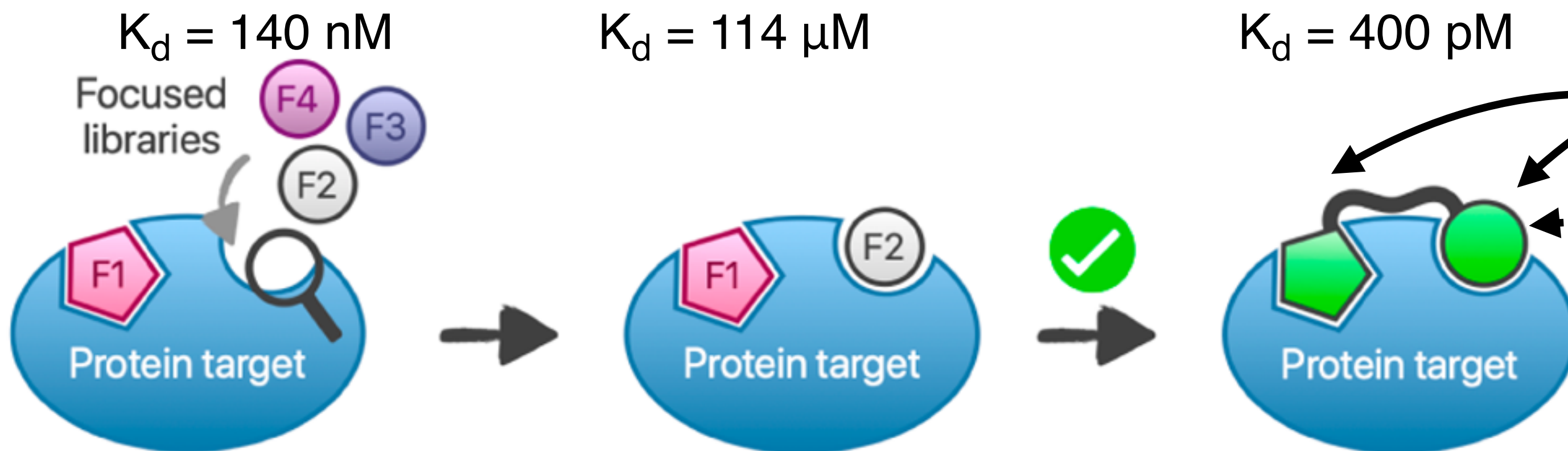
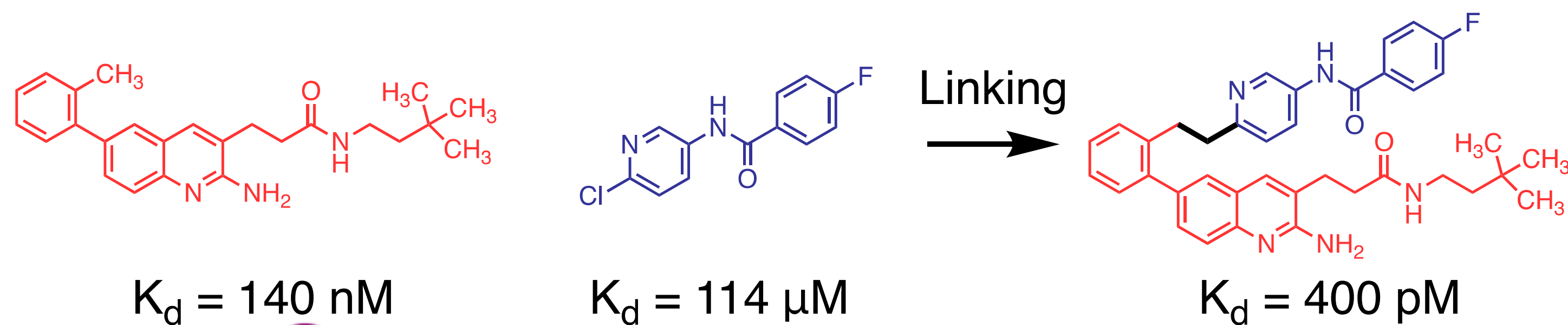
Fragment-based design/discovery (FBD) approaches

Experimental (NMR, X-ray, MS, etc)

computational approaches

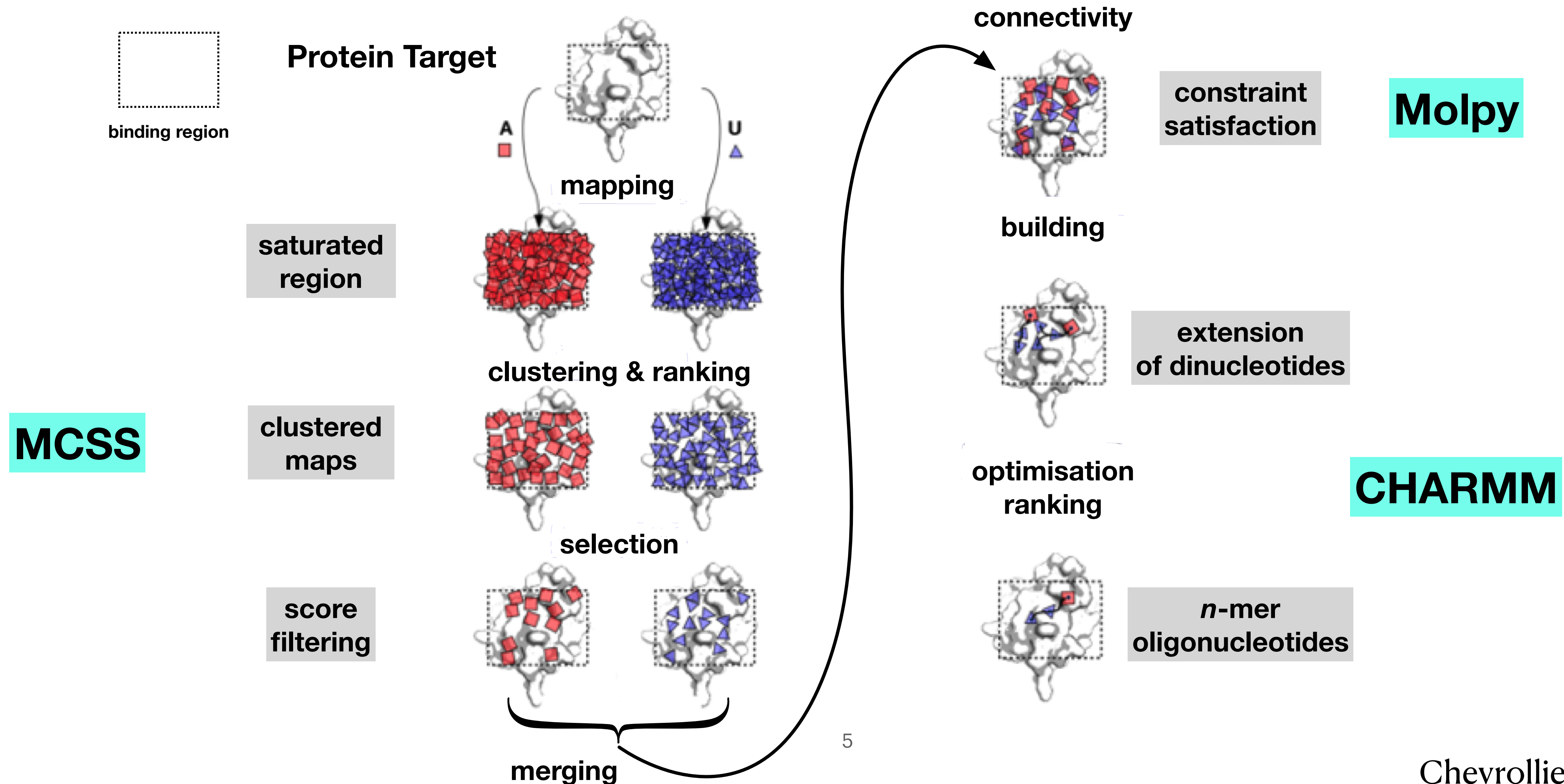


high-resolution structure



MCSS-based workflow for FBD of oligos

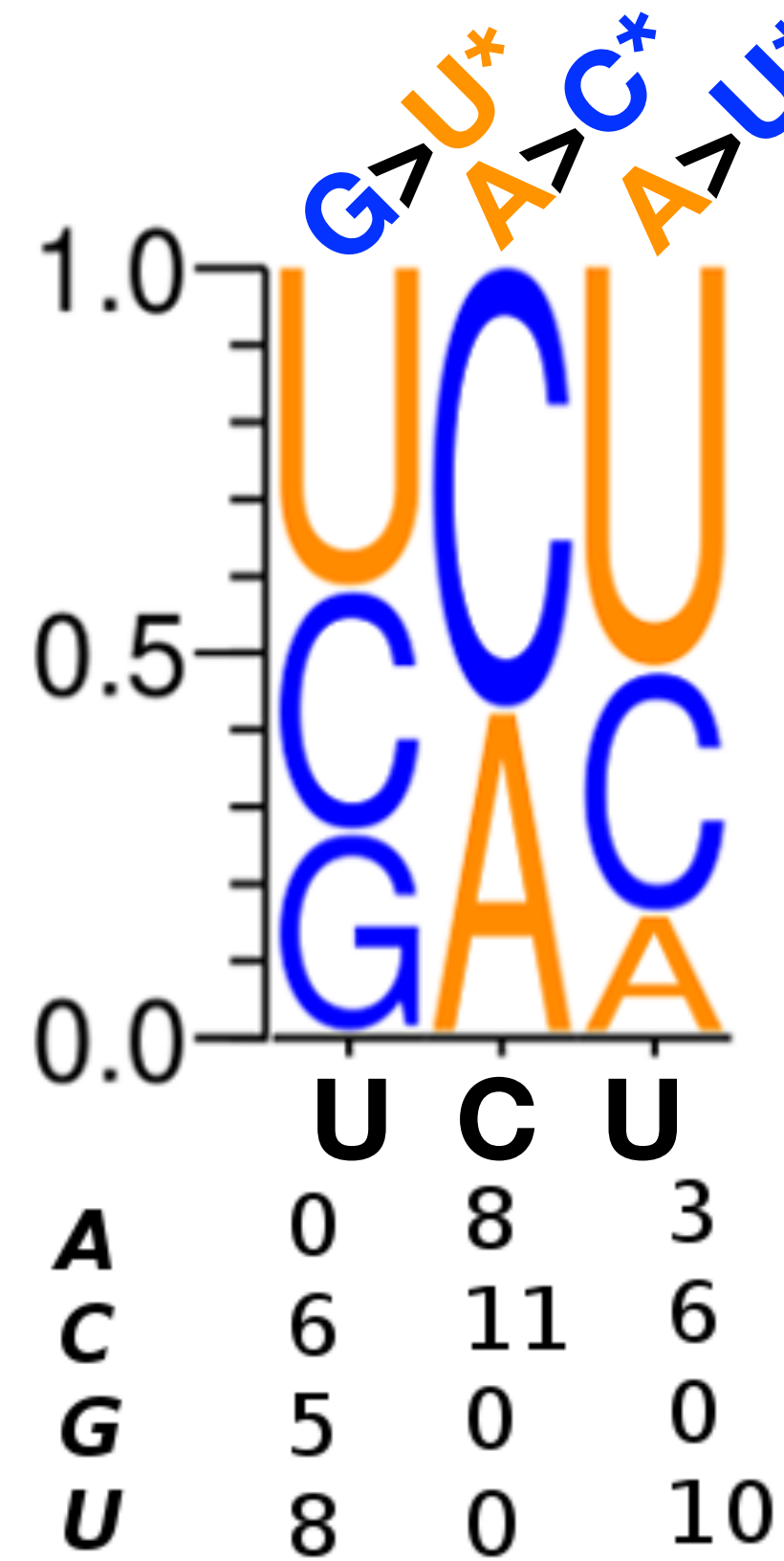
predict oligonucleotide binding mode



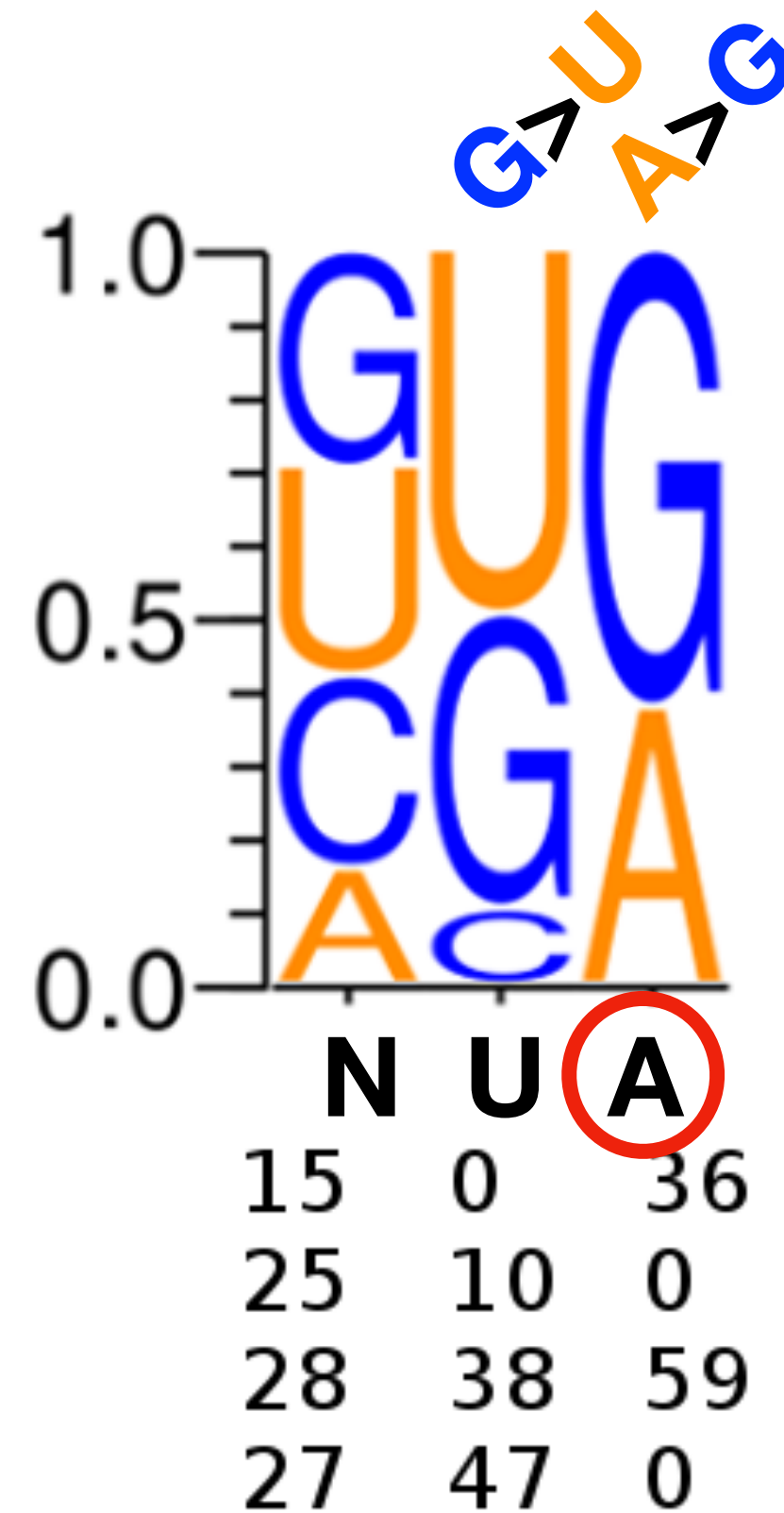
MCSS-based approach to FBD of oligos

predict sequence-specific binding

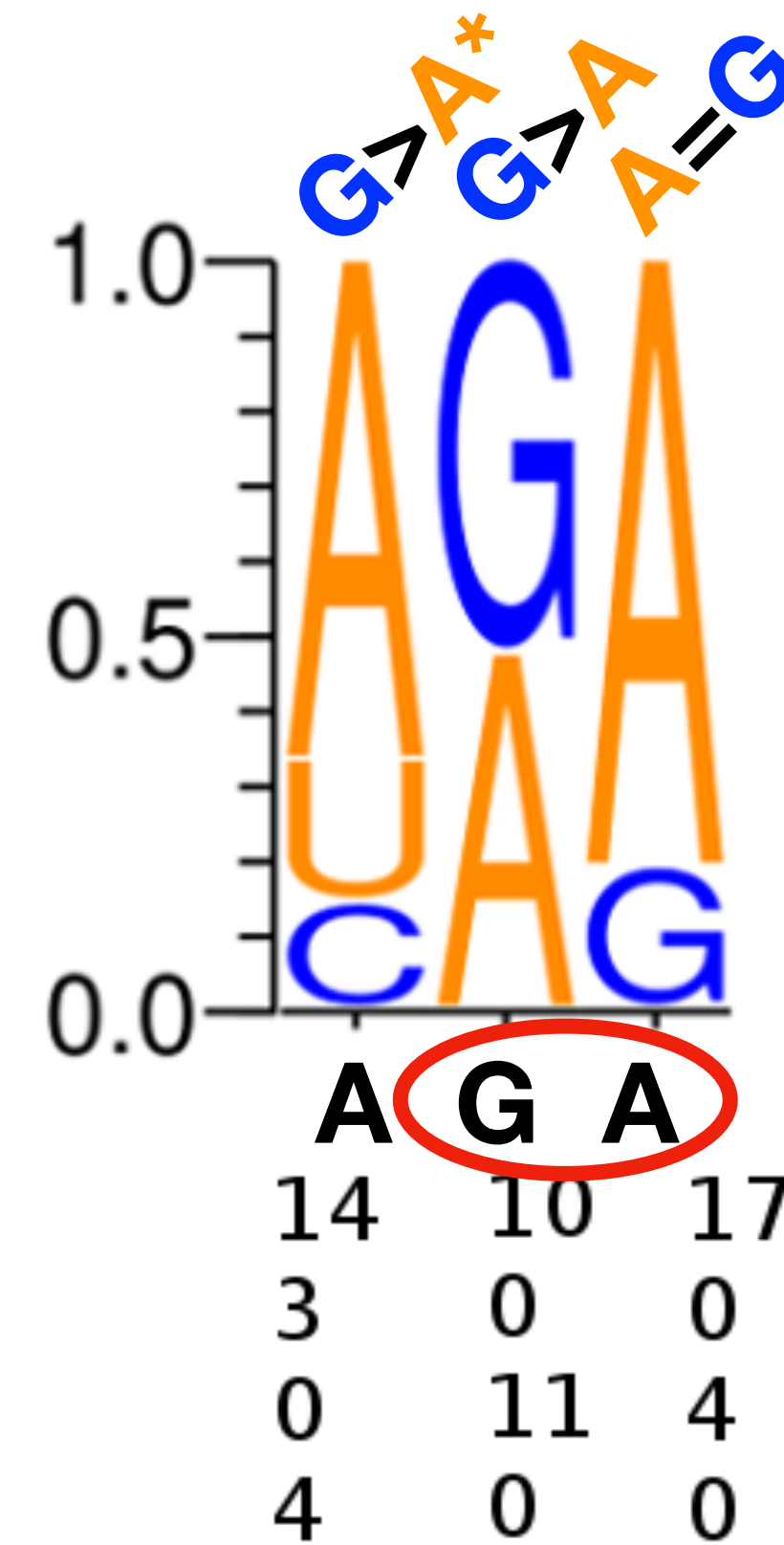
RBPs



**Nab3
(RRM)**



**Unkempt
(CCCH)**



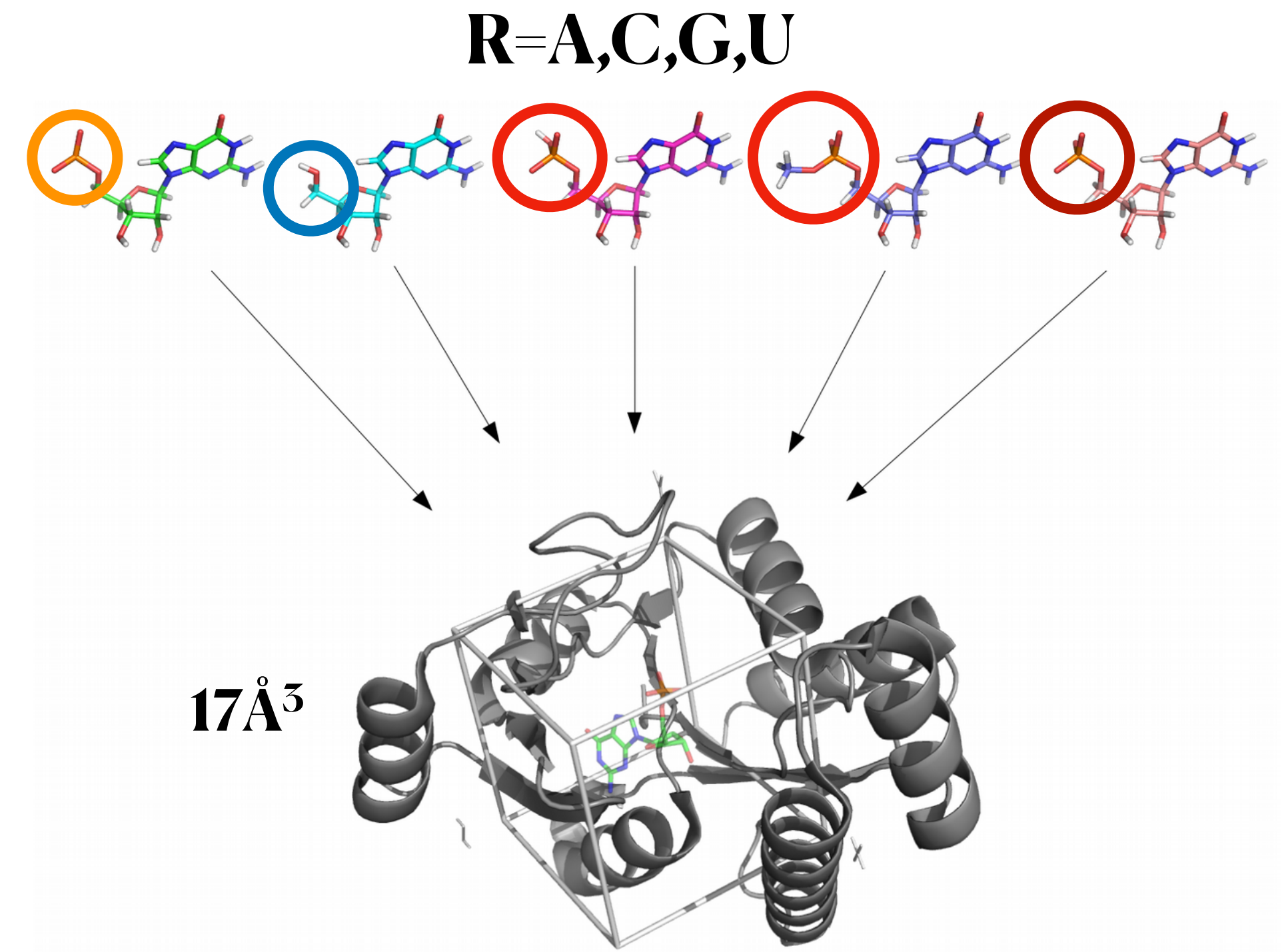
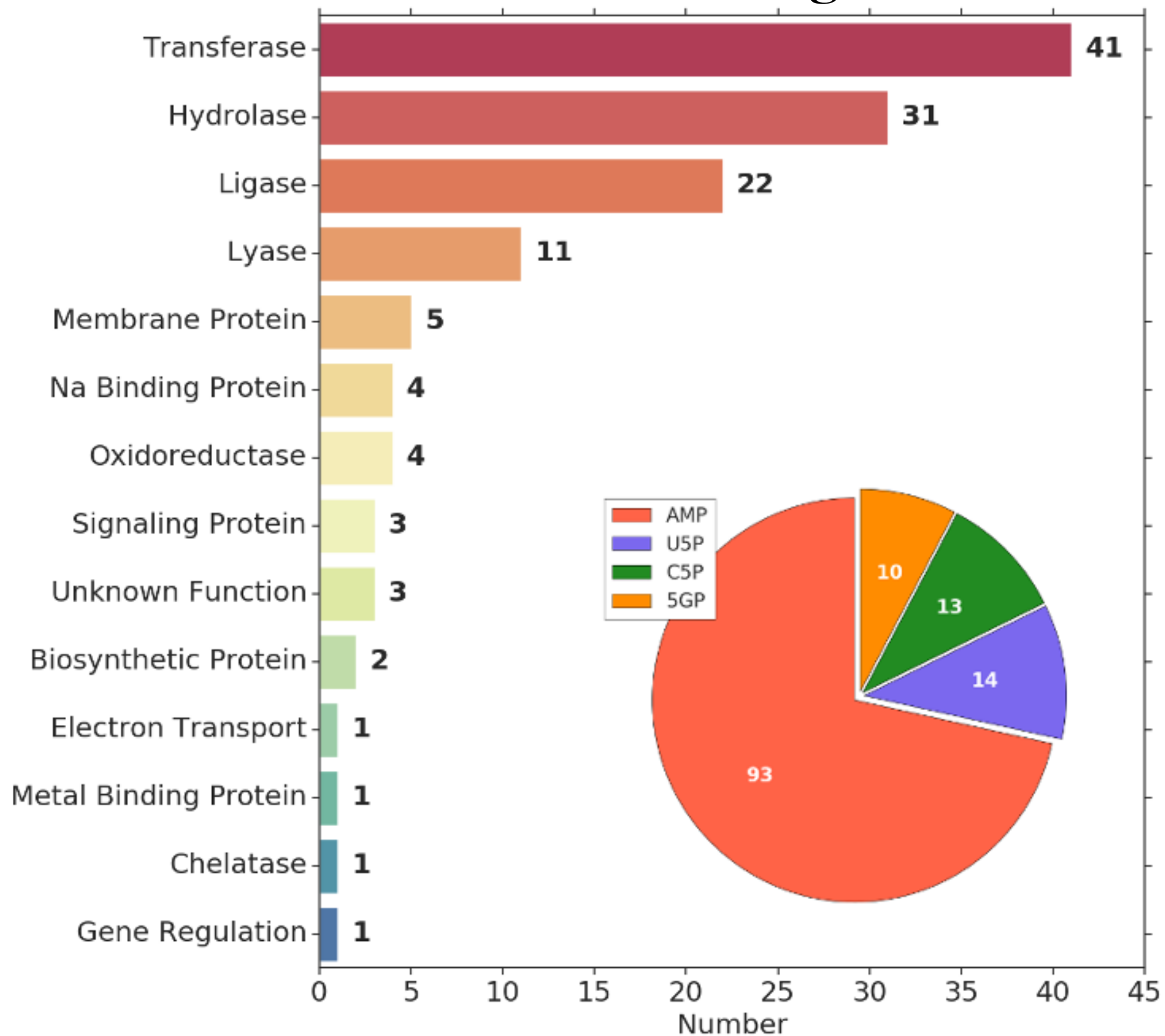
**MEX-3C
(KH2)**

score-based nucleotide preferences

Back to the basics: nucleotide-protein interactions

Nucleotide Binding Sites

121 benchmark



**Minimized protein
without ligand**

***native pose*: $\text{RMSD}_{(\text{mcSS}/\text{exp})} \leq 2.0\text{\AA}$**

high resolution, non-redundant, etc

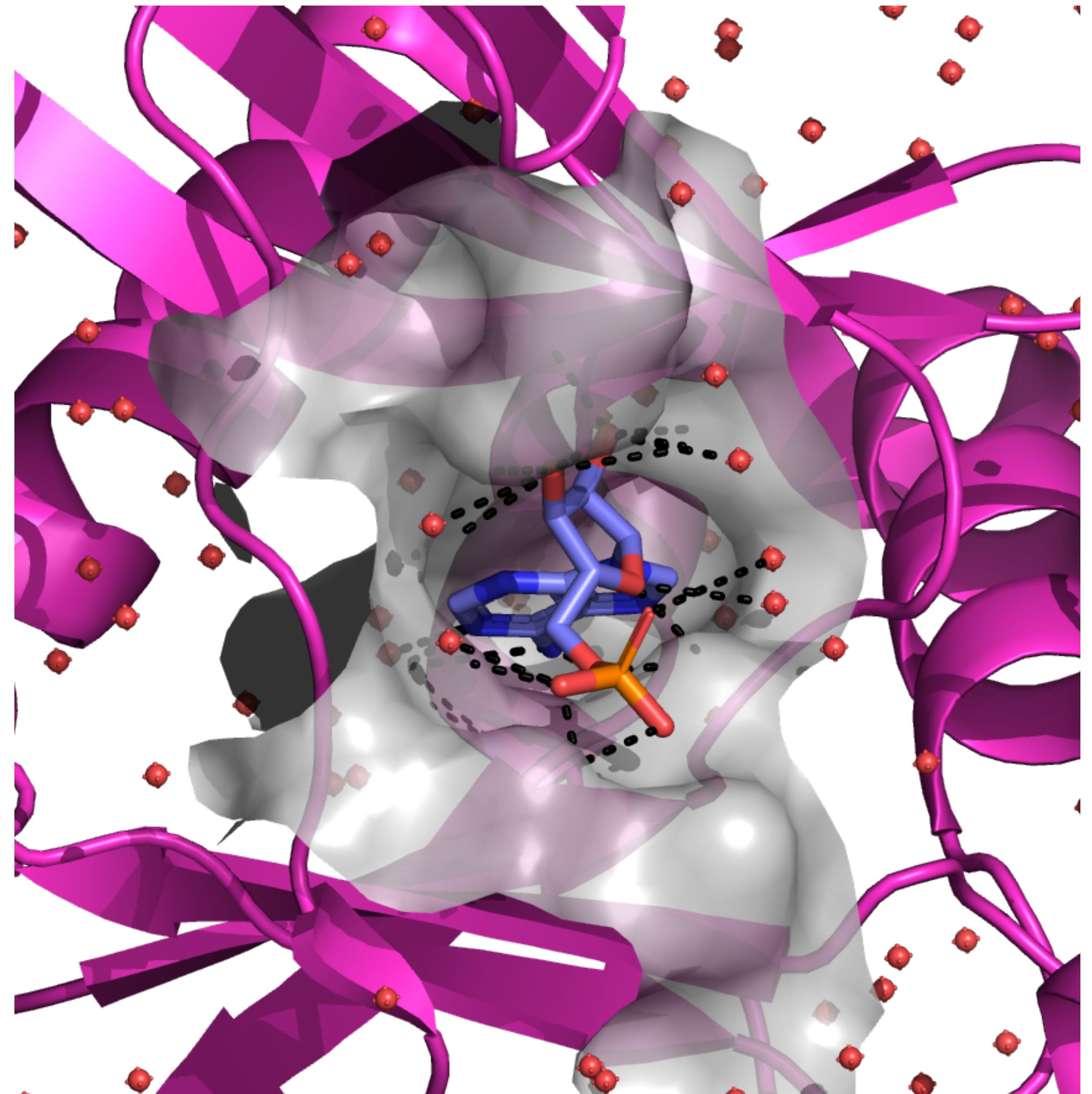
Water molecules

scoring & solvent models

- empirical scoring functions (implicit solvent): Vina, $\Delta_{\text{vinaRF}_{20}}$, etc
- force-field based scoring functions: MCSS-CHARMM (implicit solvent), MCSS-CHARMM (hybrid solvent)

powers	Vina	$\Delta_{\text{vinaRF}_{20}}$	MCSS-imp.	MCSS-hyb.
docking	42 (90)	33 (88)	29	50
screening	- (55)	- (51)	15 (25)	36 (45)

success rates in %; (..) obtained on small organic ligands

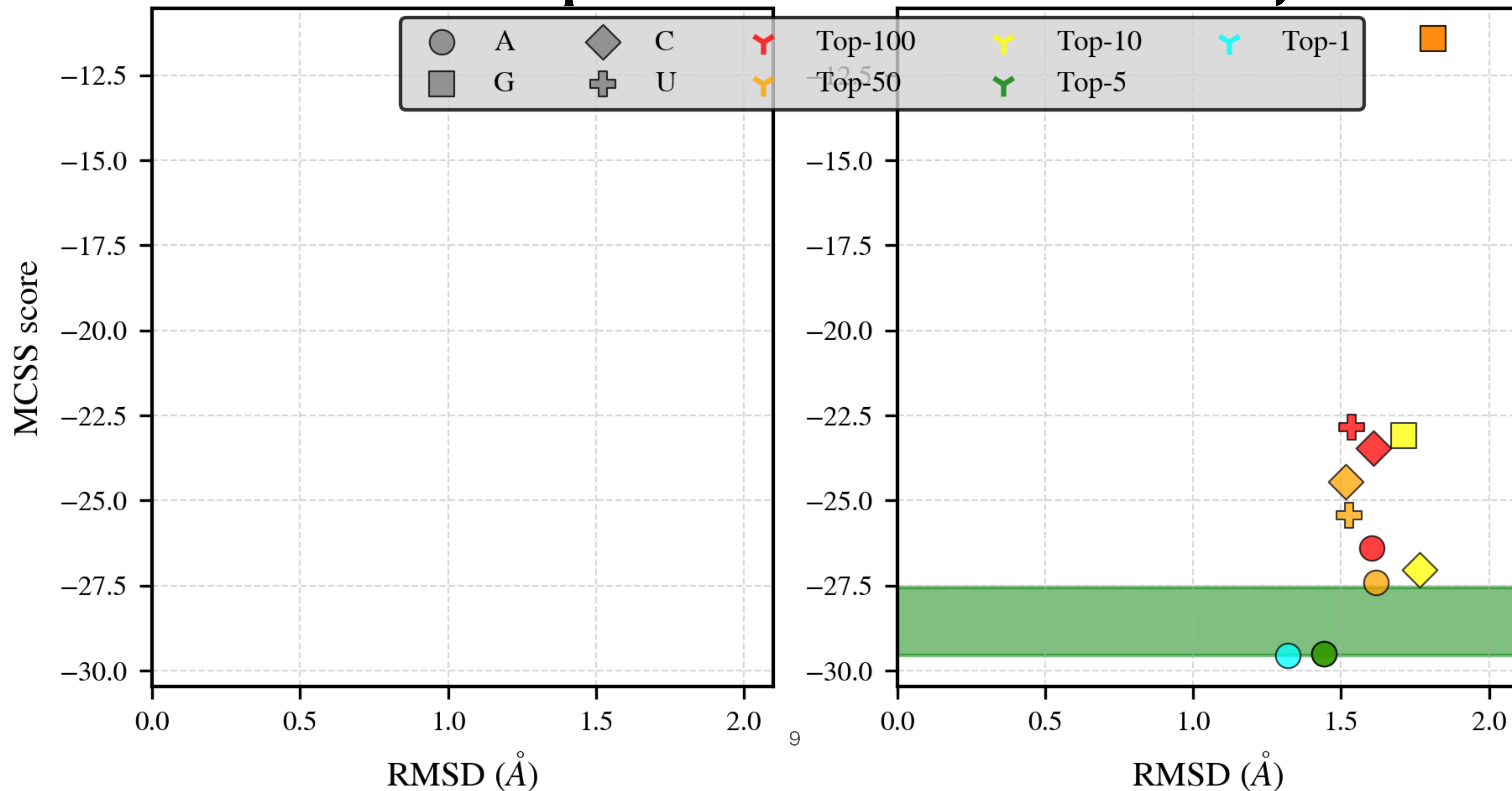


Role of water molecules

Case Study 1S68 (A)

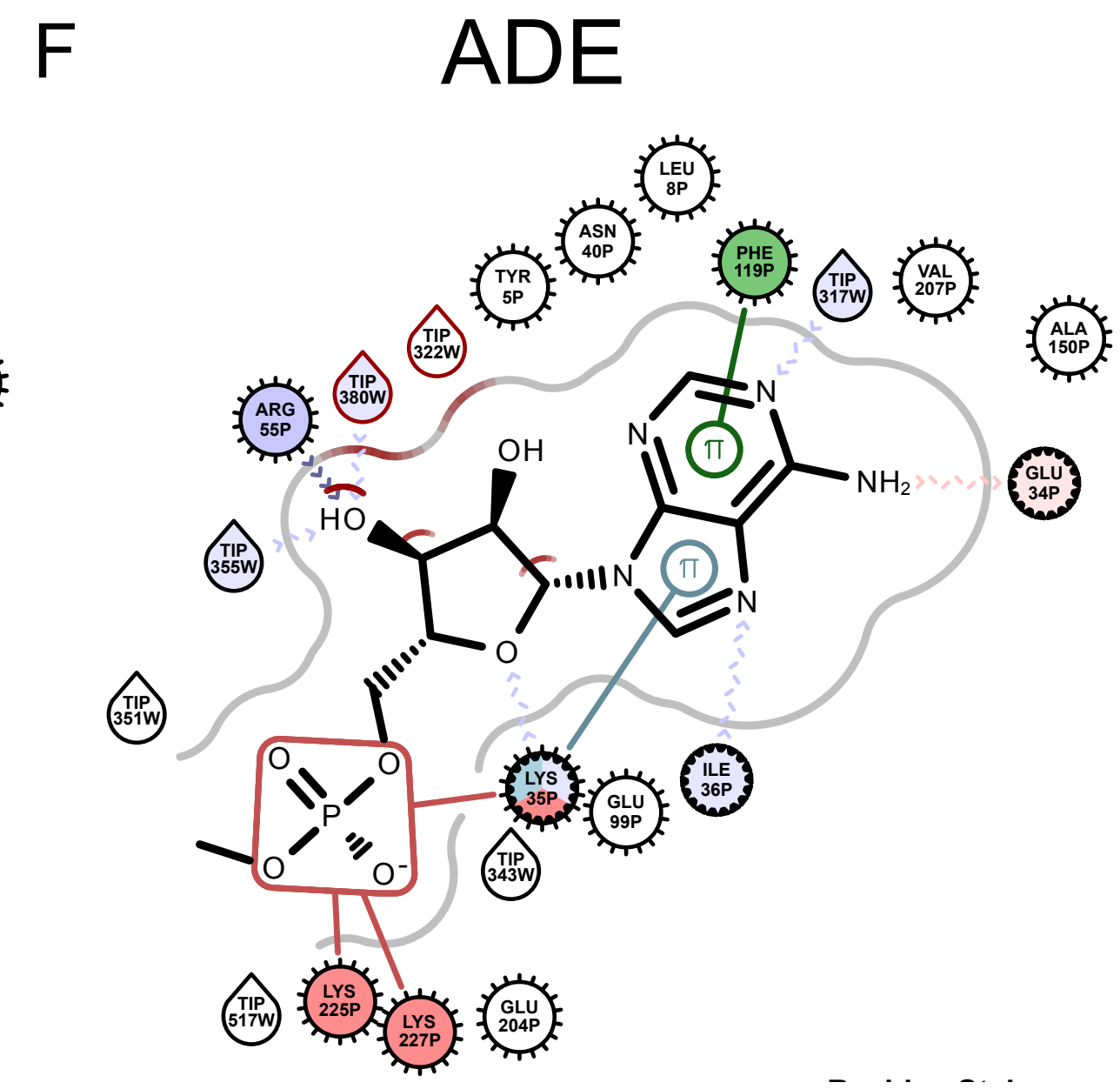
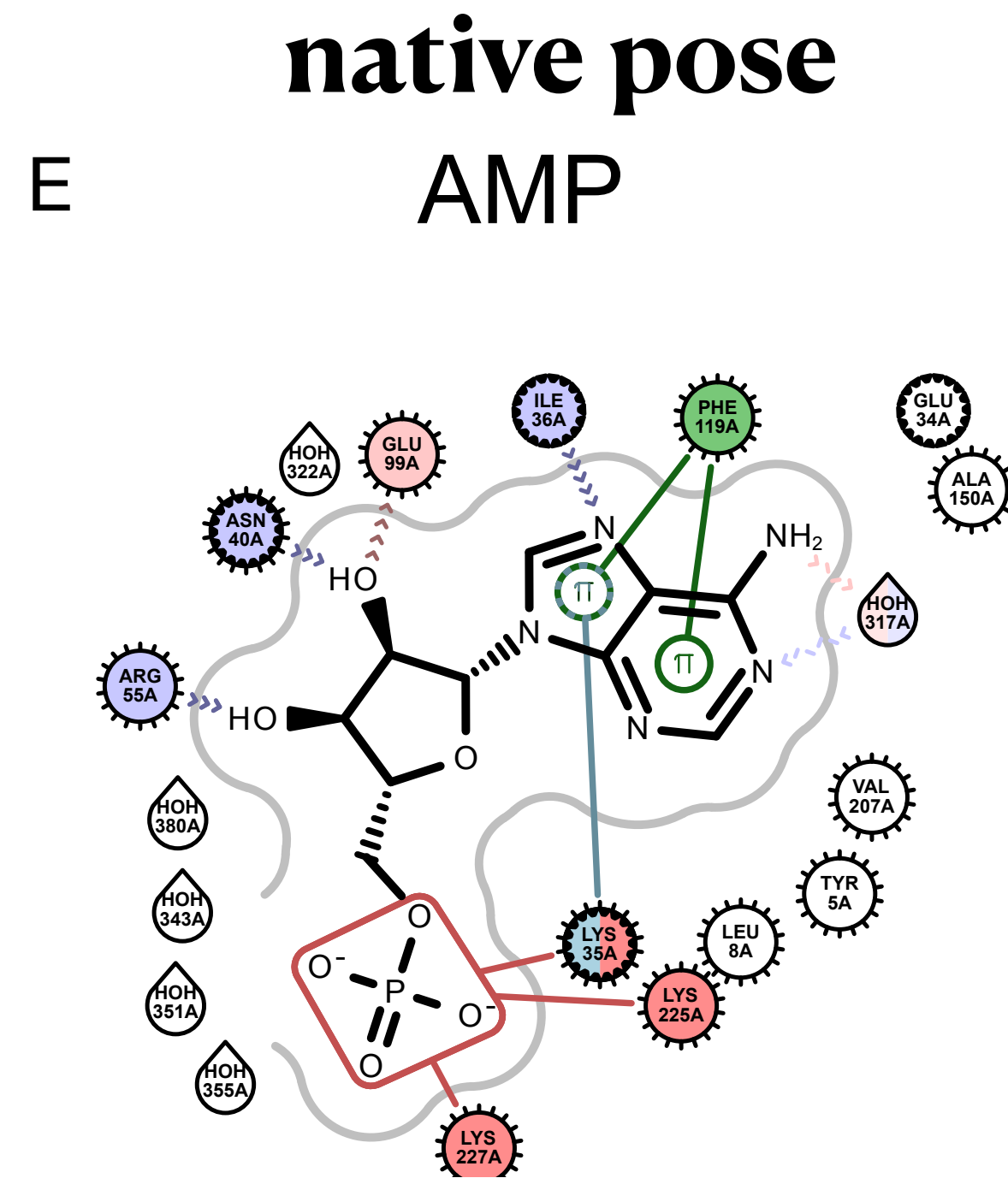
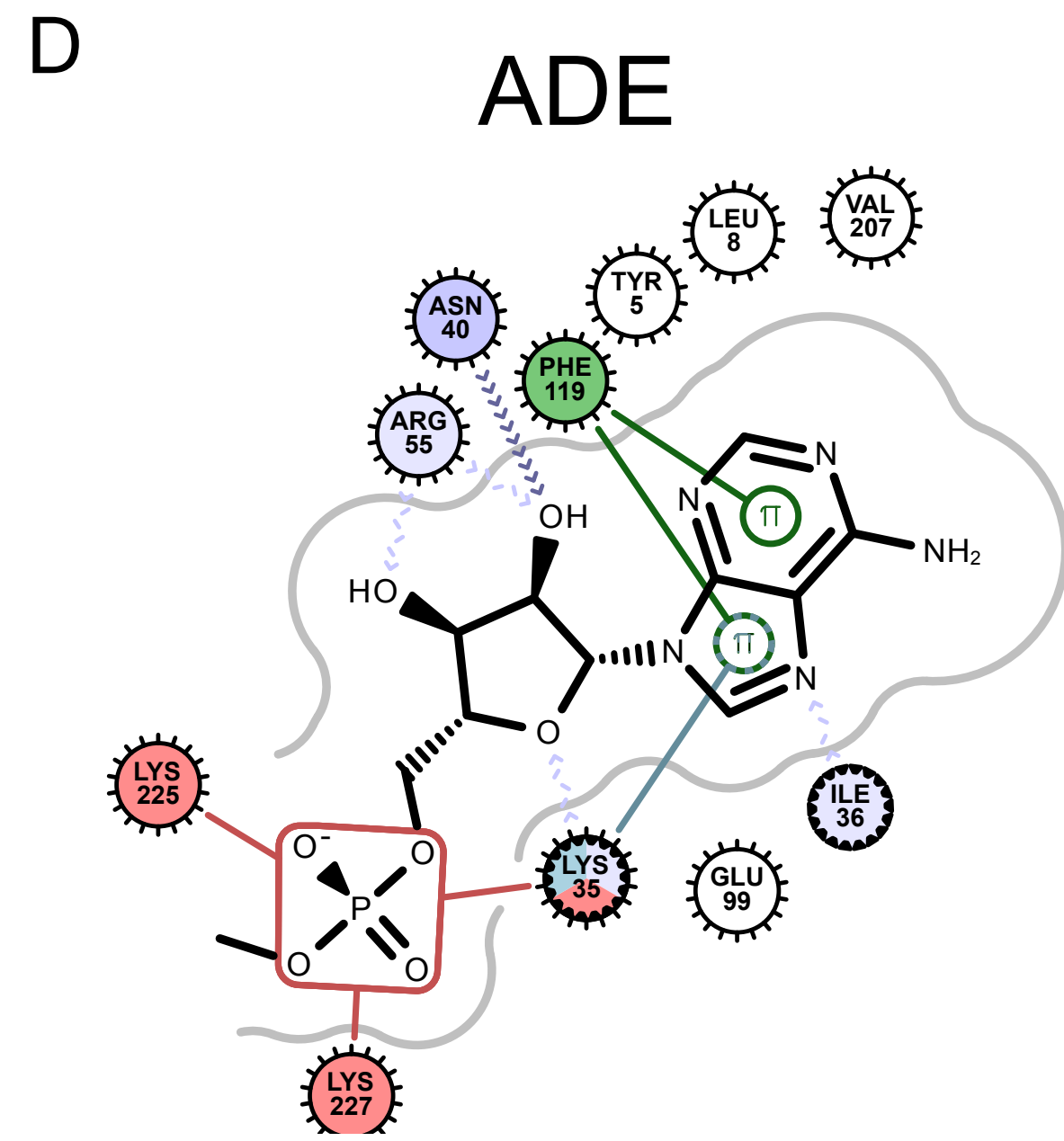
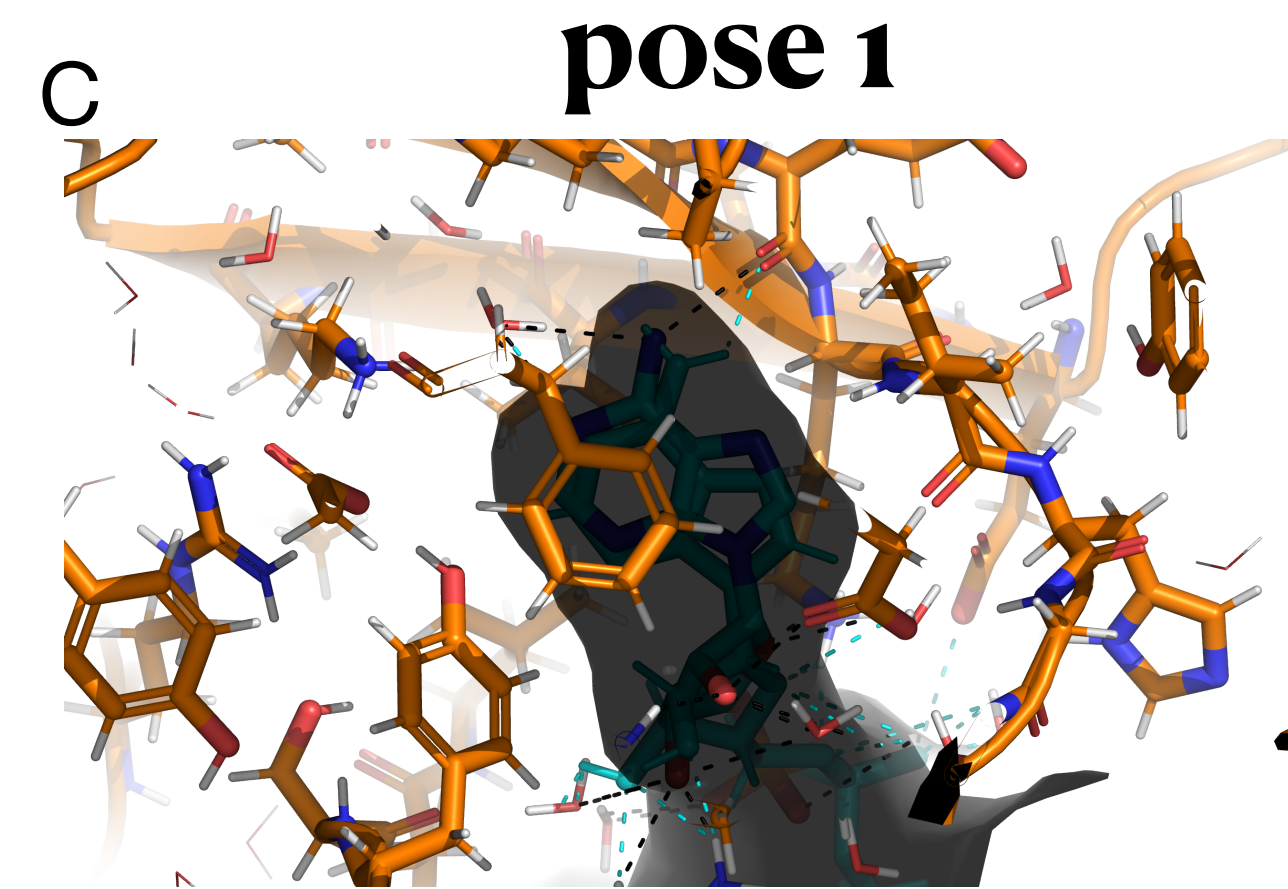
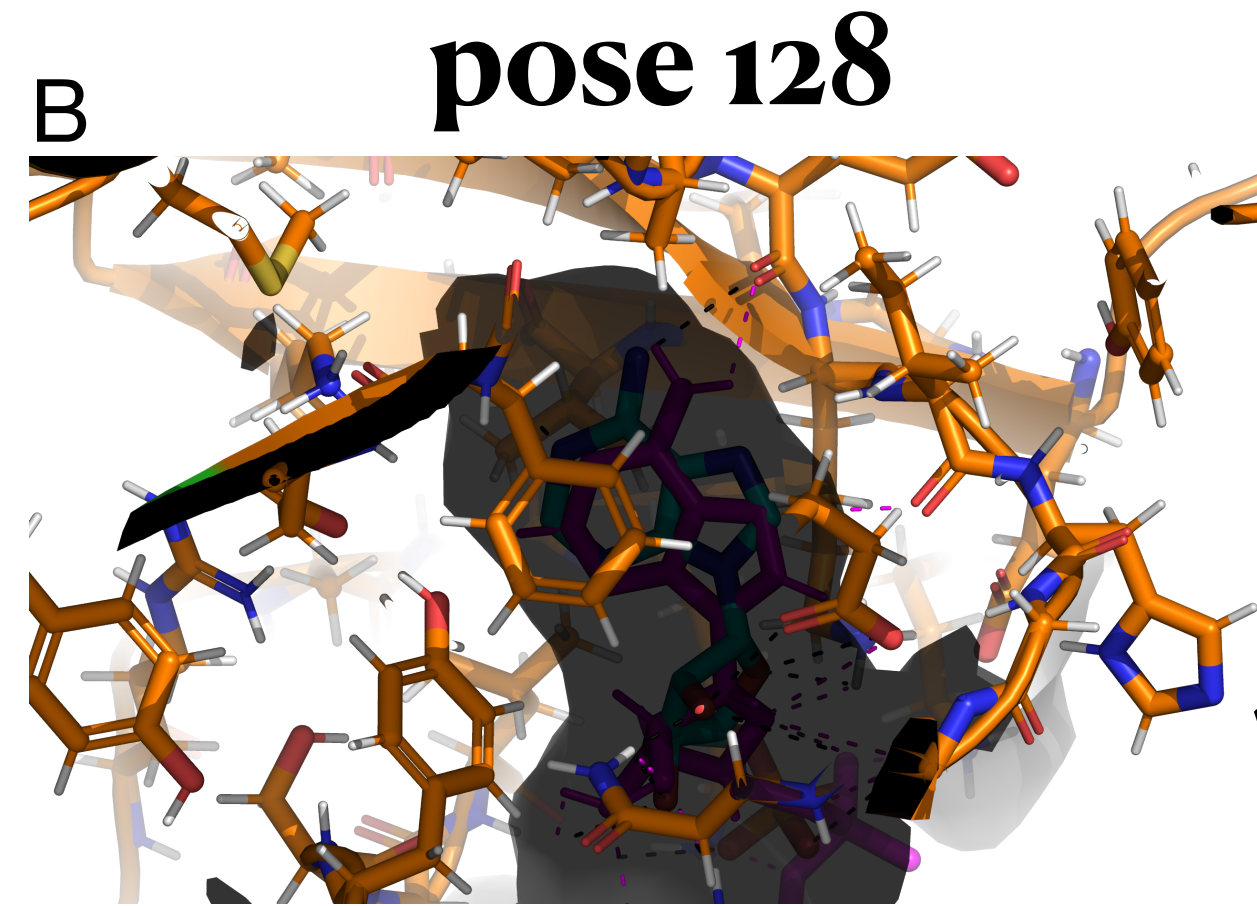
MCSS-imp.

MCSS-hyb.



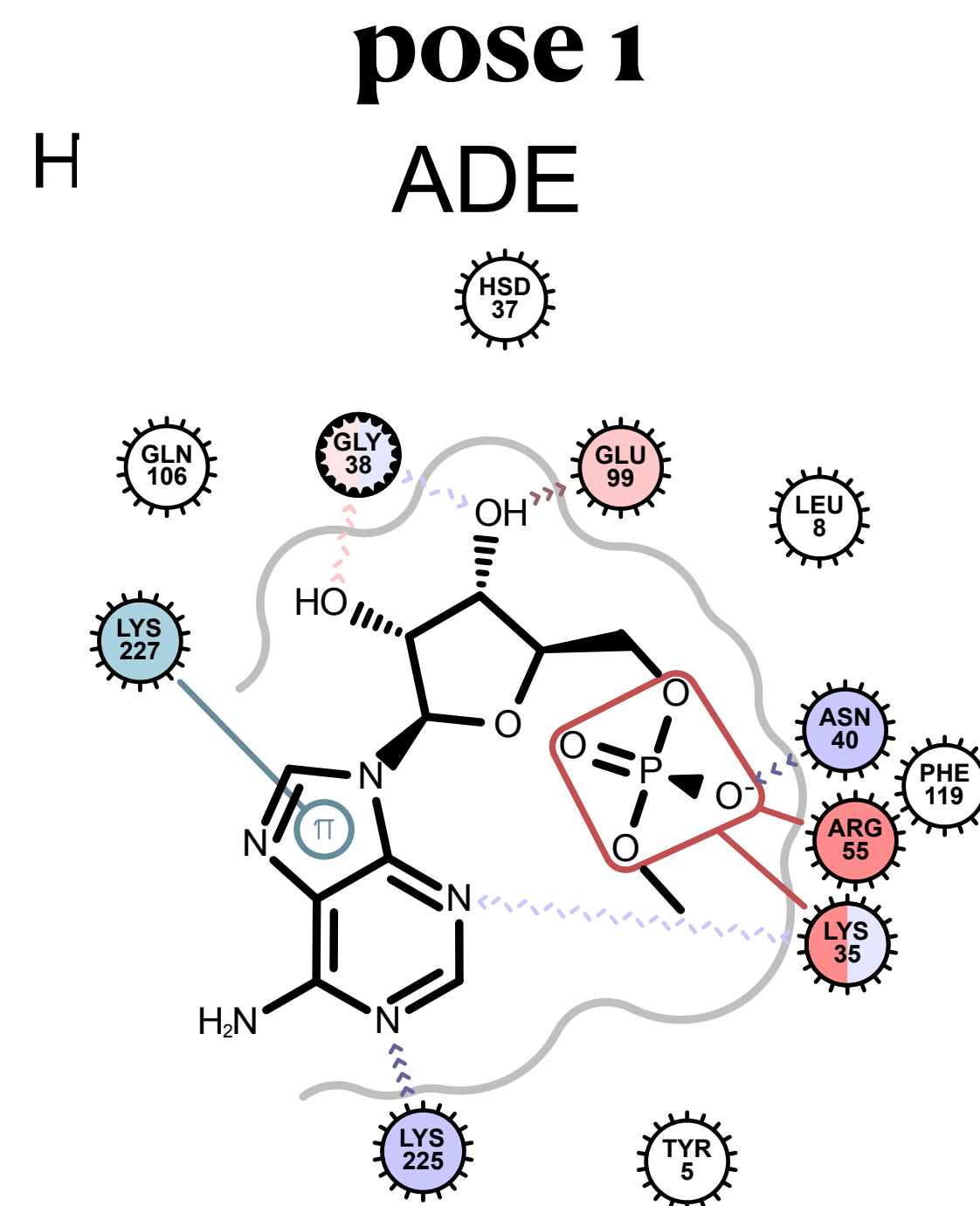
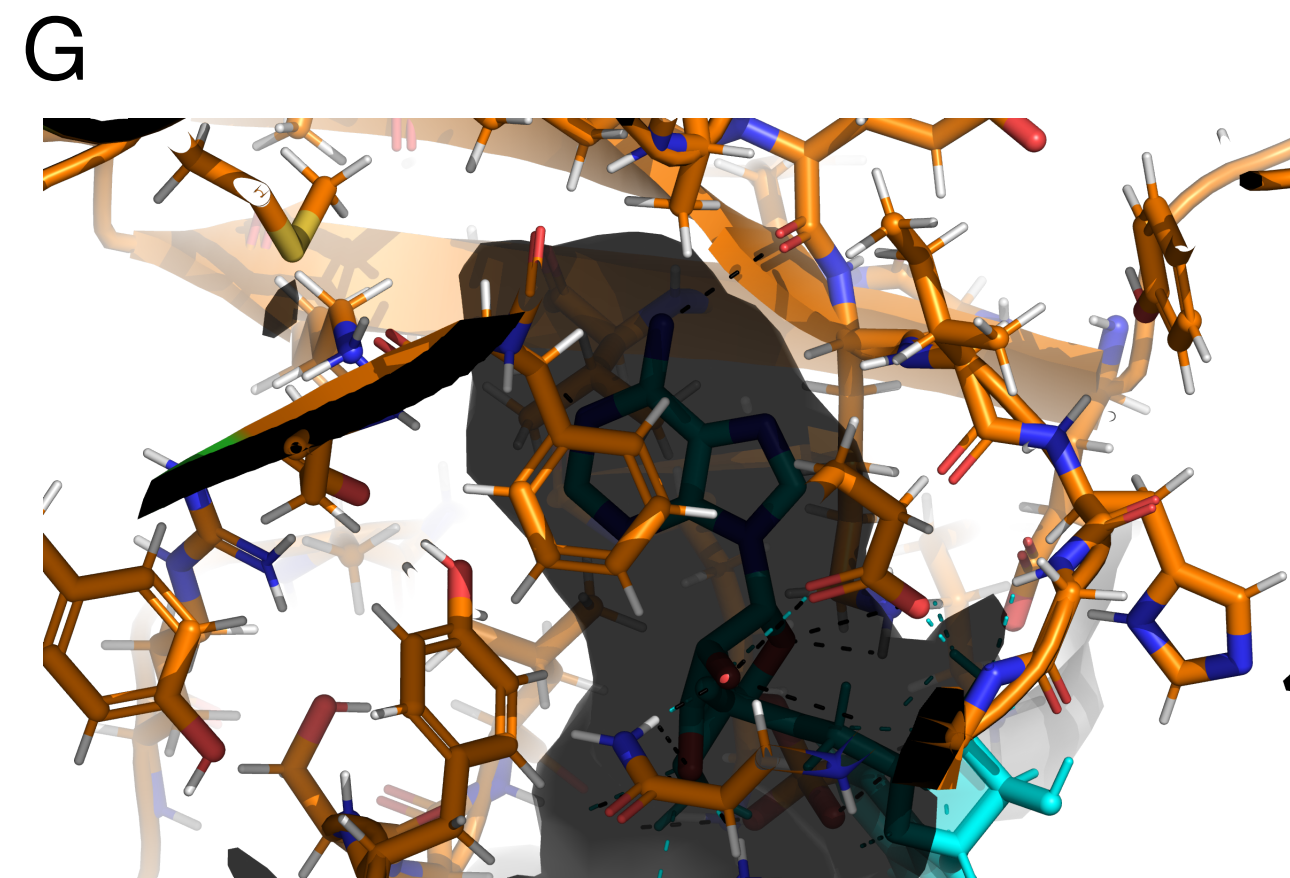
Role of water molecules

Case Study 1S68 (A)

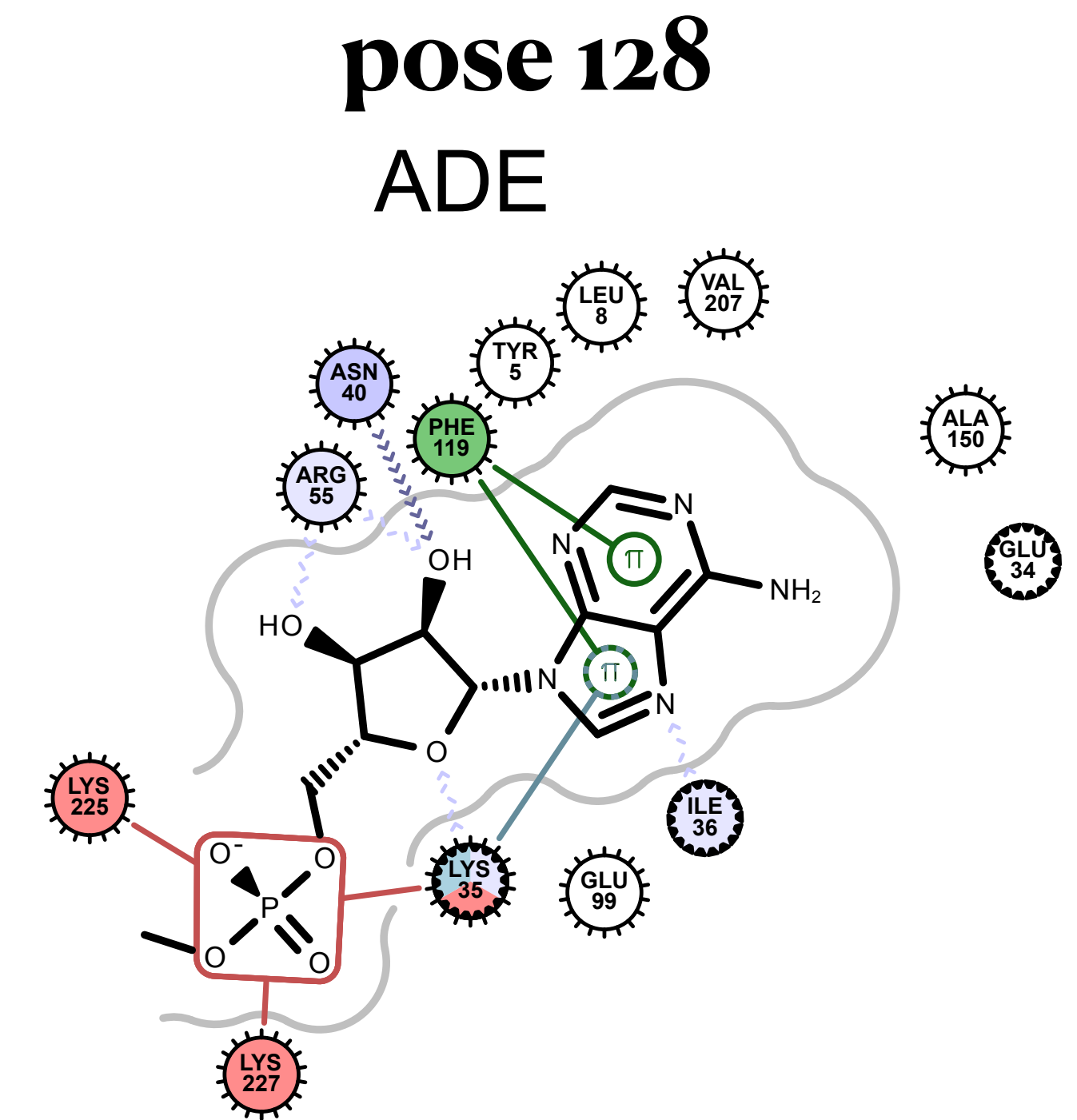


Role of water molecules

Case Study 1S68 (A)



“off-site”



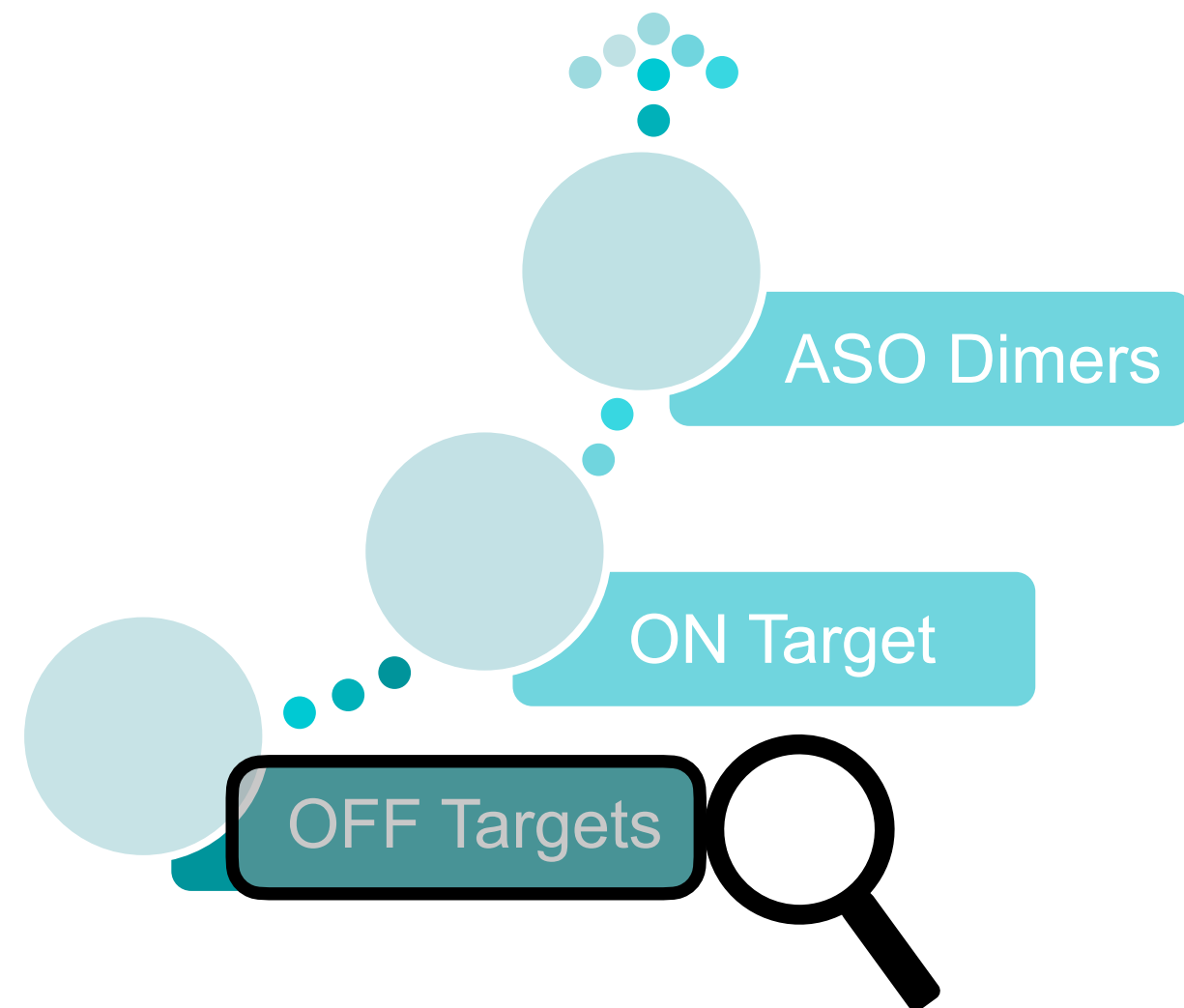
“in-site”

Off-target effects (OTEs)

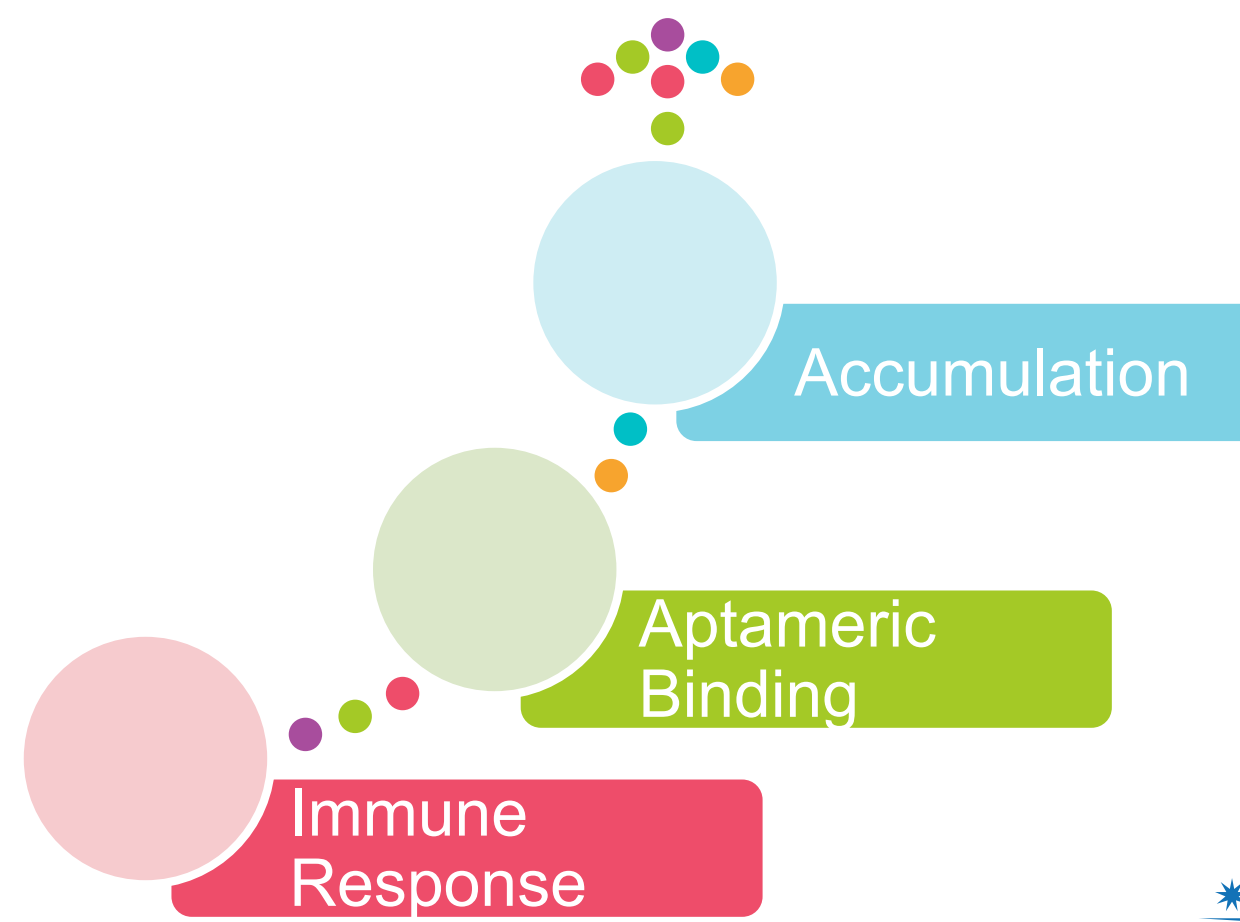
CRISPR-Cas (gRNA), ASOs, RNAi: si/shRNA, microRNA, aptamers, etc

ASO Toxicity

Hybridization **DEPENDENT**
(Watson/Crick base pairing)



Hybridization **INDEPENDENT**



22/03/2020

24



Cas9 off-target effects

- Delivery of Cas9 RNP complex reduces off-target editing, but it is not a total solution
- Other solutions to reduce OTE have significant drawbacks
 - crRNA length reduction (18–19 nt)
 - Chemical modification
- **What about high-fidelity Cas9 proteins?**



B. Sequence and (RNA) hybridization dependent

- On-target safety
- **Off-target (RNA)**



Mitigated by Target Safety Assessment and in silico/RNA seq analysis

C. Sequence, but not RNA hybridization dependent

- Liver toxicity
- Kidney toxicity
- Proinflammatory
 - Injection site reaction
 - Flu-like symptoms
 - Thrombocytopenia
 - ADA



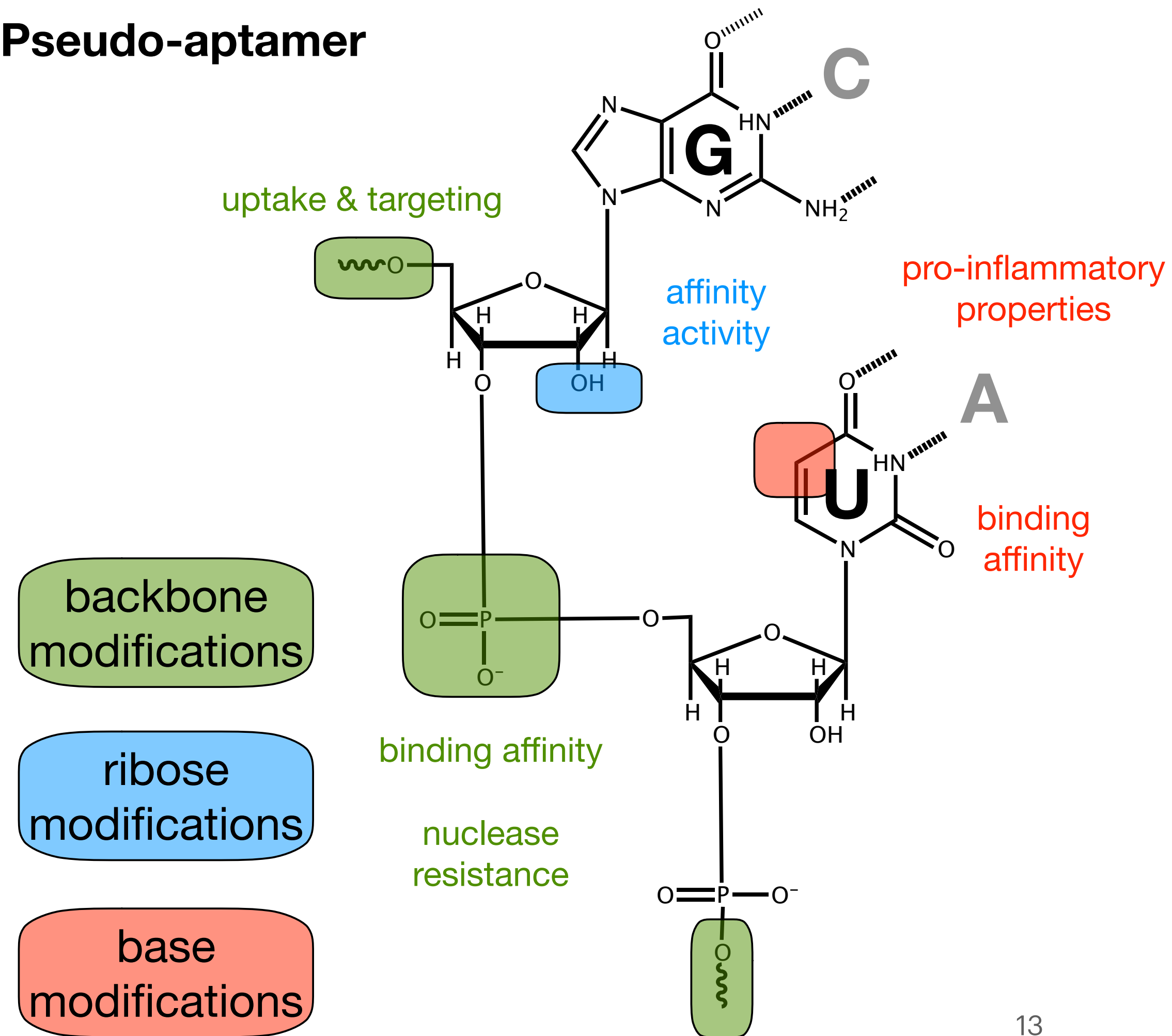
Mitigated by in vitro and in vivo screening



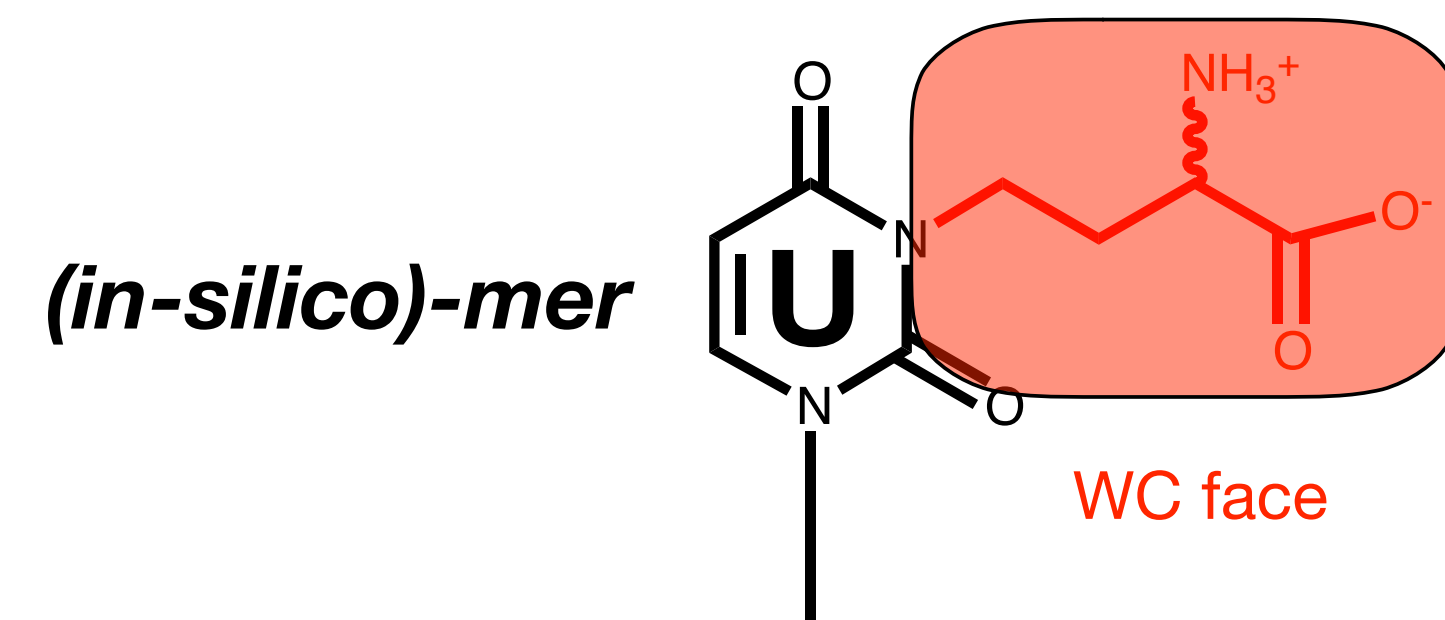
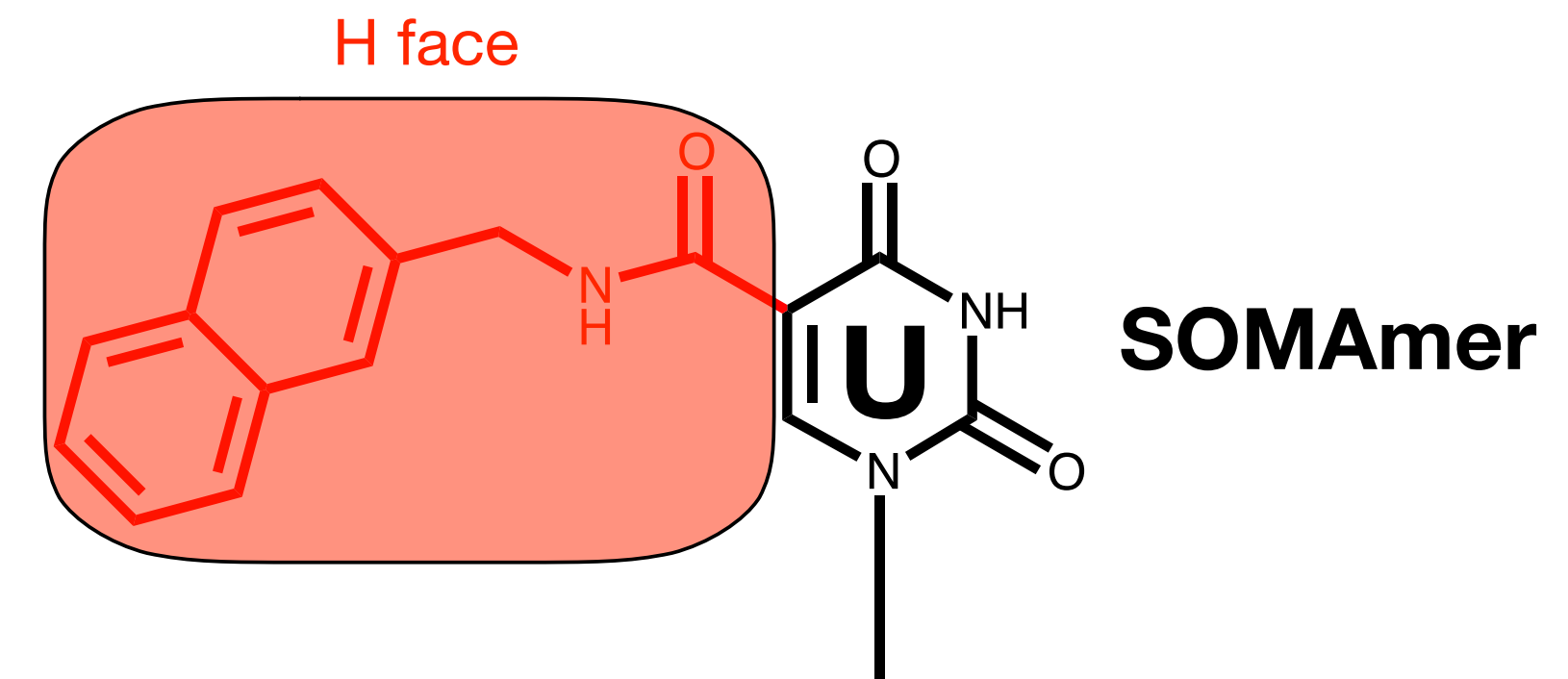
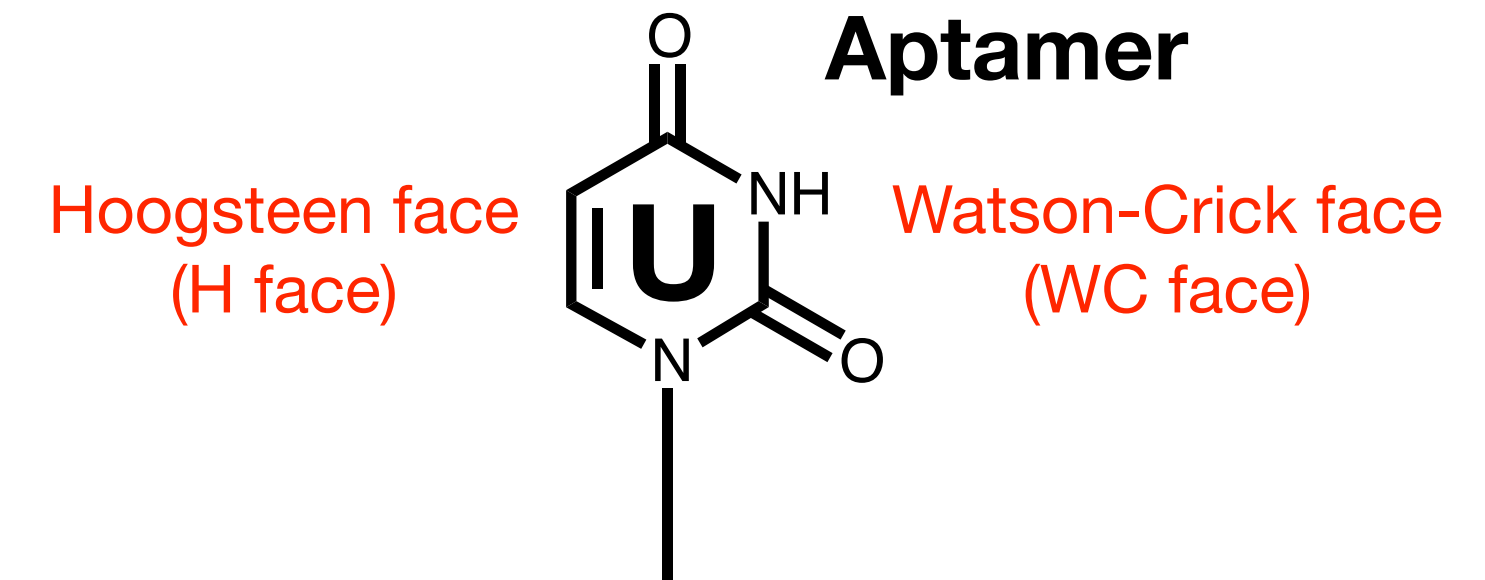
Reduce-Bypass the OTEs

in hybridization-independent strategies

Pseudo-aptamer



Aptamer



Conclusions

perspectives

- **fragment-based** approach applied to single nucleotide binding
- **explicit solvent** improve predictions of nucleotide binding
 - improved docking power (native poses)
 - improved screening power (“true” binders)
- **perspectives**
 - extension to modified nucleotides
 - BACE1 target (Alzheimer’s disease)

Acknowledgments

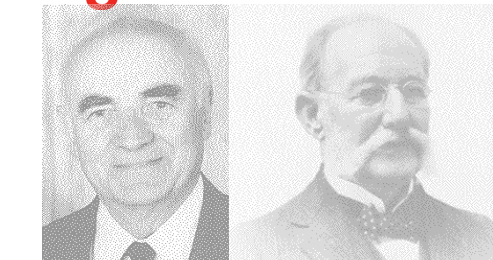
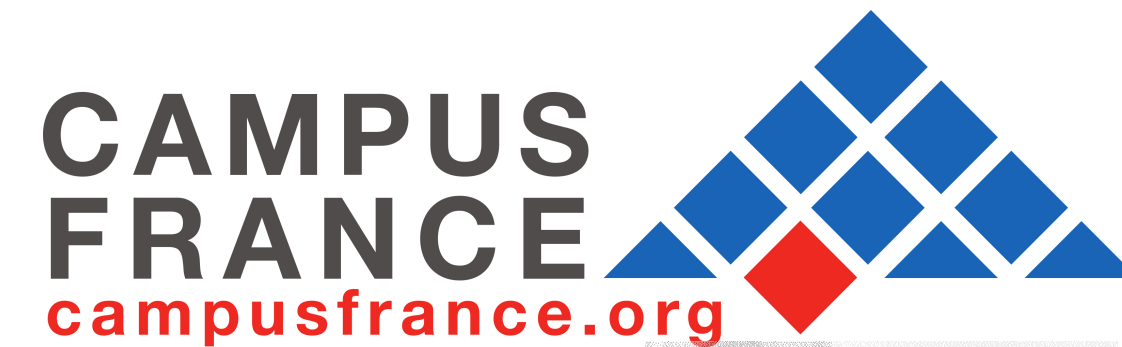
- Nicolas Chevrollier (Ph.D., 2019)
- Roy González Alemán (Ph.D. student, 2019-)
- Taher Yacoub (Ph.D. student, 2020-)
- Yann Ponty (Ph.D.)
- Martin Karplus (Prof. emeritus)
- Luis Montero-Cabrera (Prof. emeritus)
- Yoanna Álvarez Ginarte (Prof.)
- Coralie Rohmer (MSc. 2017)
- Manuel Simoes (Ph.D.)
- Frédéric Checler (Ph.D.)



FONDATION
VAINCRE
ALZHEIMER



Inria
INVENTEURS DU MONDE NUMÉRIQUE



PARTENARIAT
HUBERT CURIEN



université
PARIS-SACLAY



CHARMM
Chemistry at HARvard Macromolecular Mechanics