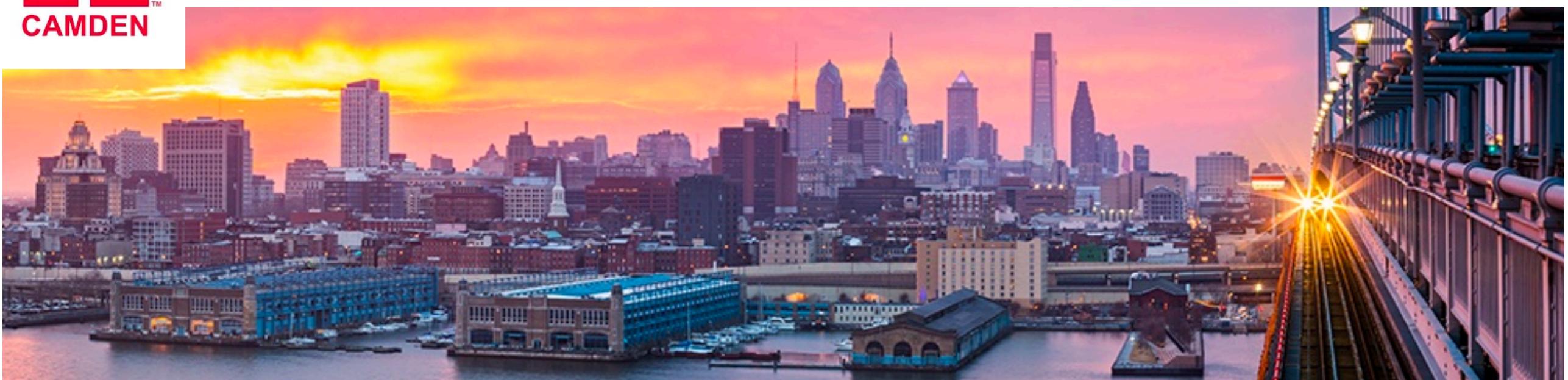


From Data to Information: New approaches for extracting coarse-grained targets from atomistic simulations

Grace Brannigan
Center for Computational &
Integrative Biology
Rutgers University - Camden



Hey look! I measured
a thing no one has
measured before!



Cool! So how
does it compare
to the experimental
quantity?



There is no experimental
measurement. That's why it's
so exciting that I
measured it!



mh

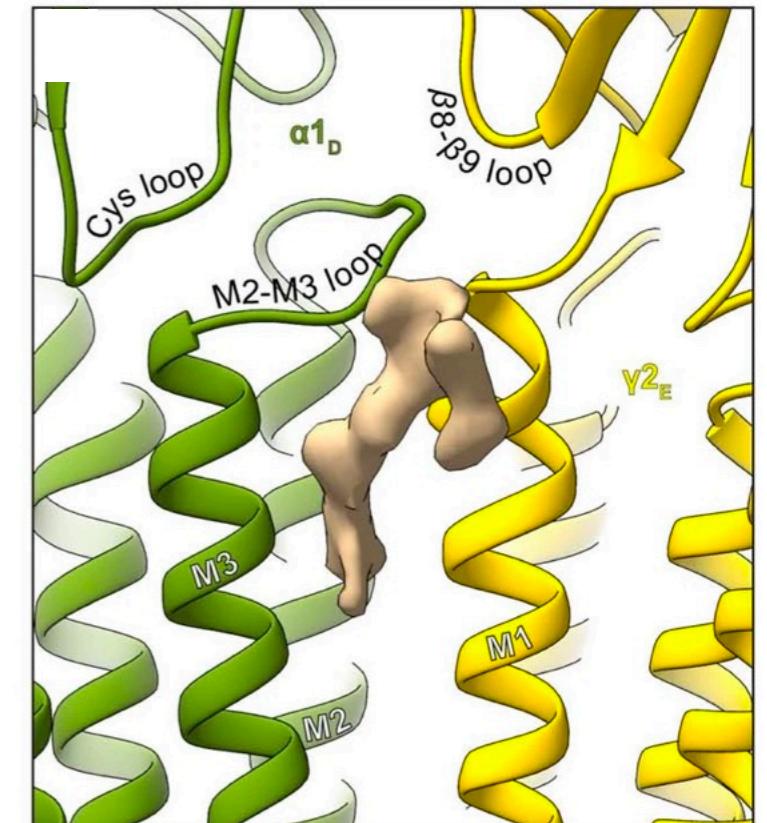
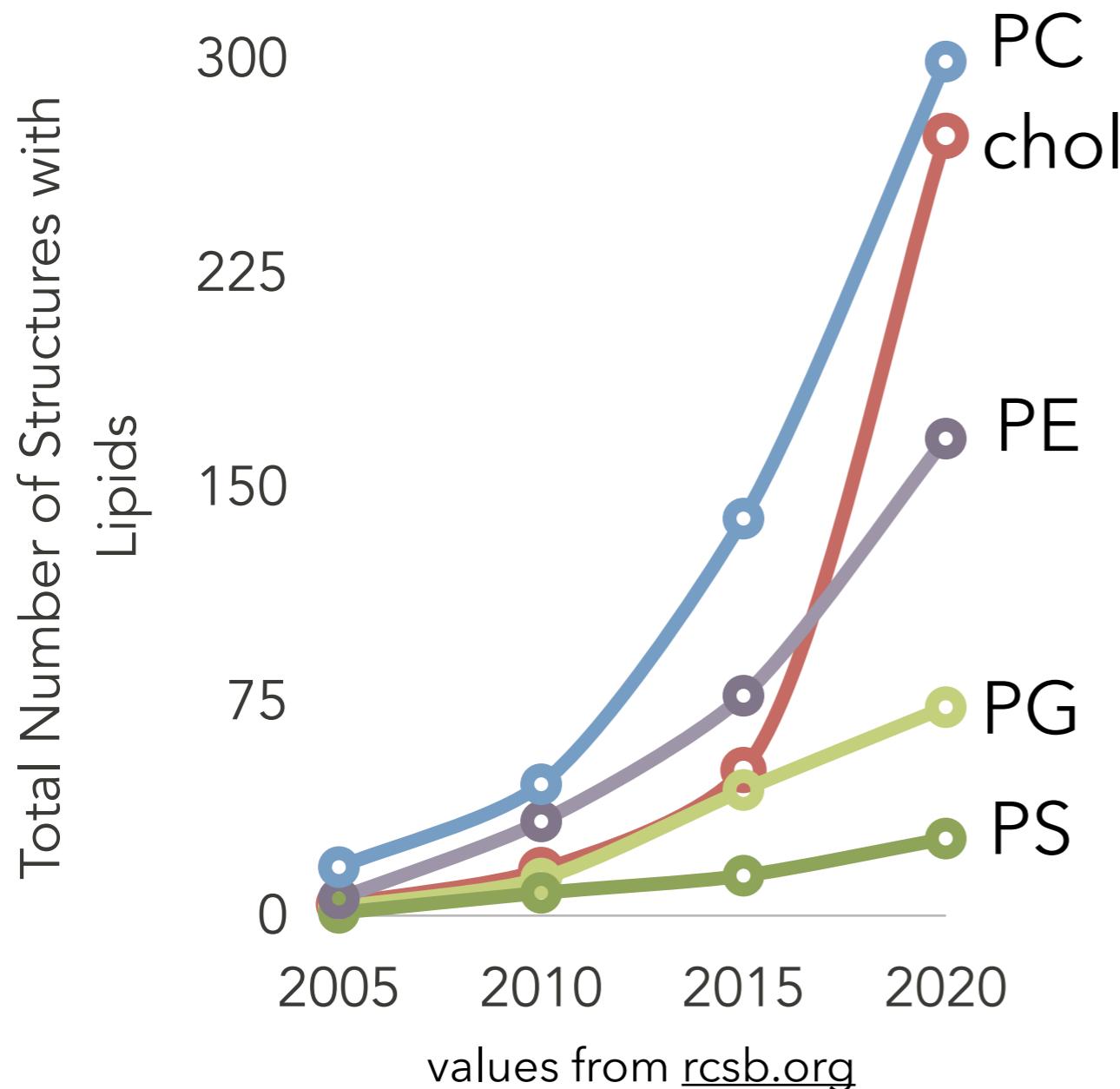
New opportunities for validation

- Data from challenging-but-not-impossible atomistic simulations:
 - Lipid-protein binding affinities
 - Long intrinsically disordered proteins
- Validating coarse-graining approaches using genomics

Quantifying Lipid-Protein Interactions

“Are you my ligand?”

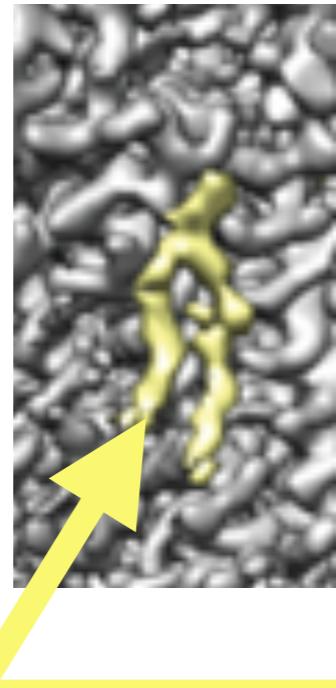
Structures can tell us where lipids bind...



Kim...Hibbs, *Nature*, 2020

...but not (usually) who is binding.

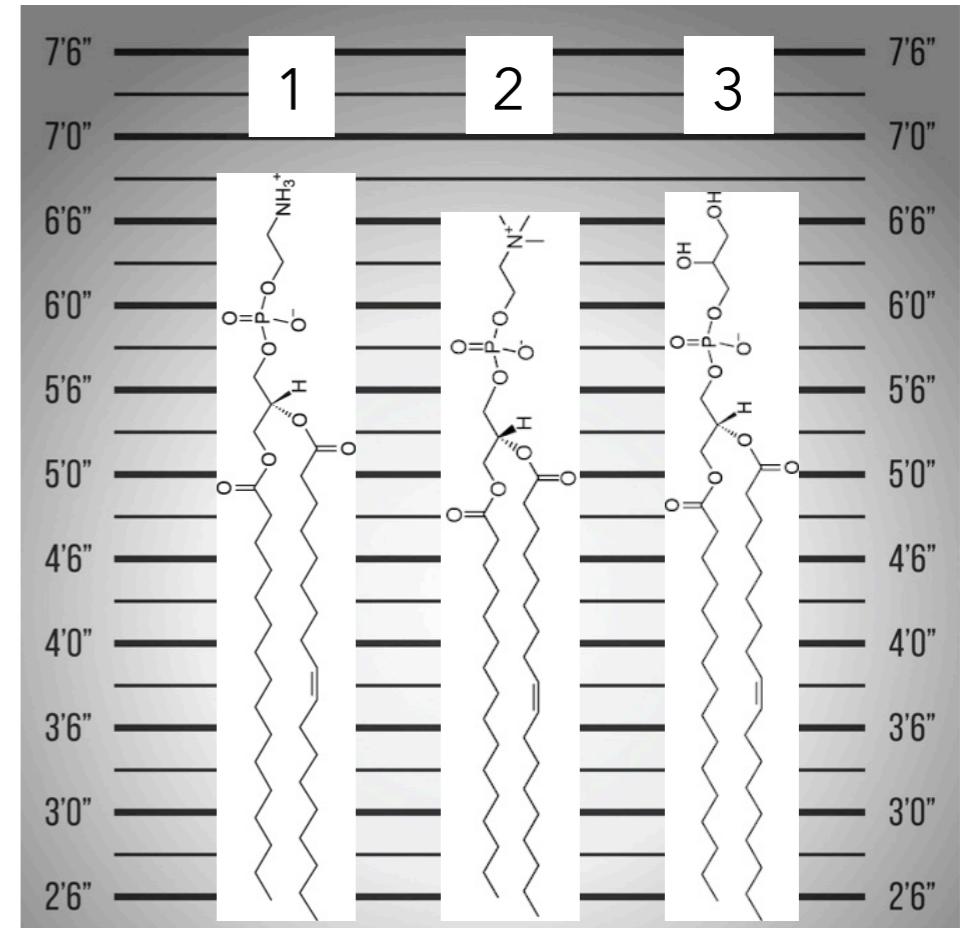
Goal: Fragment identification



Who is this?

2:1:1 PC:PE:PG nanodisc

Suspects



Wayland
Cheng

John T. Petroff II, ... Ezry Santiago-McRae, ... Tom Joseph, Jérôme Hénin, Grace Brannigan & Wayland W. L. Cheng, Nature Communications 2022

Spontaneous binding via CG simulation

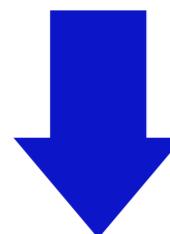
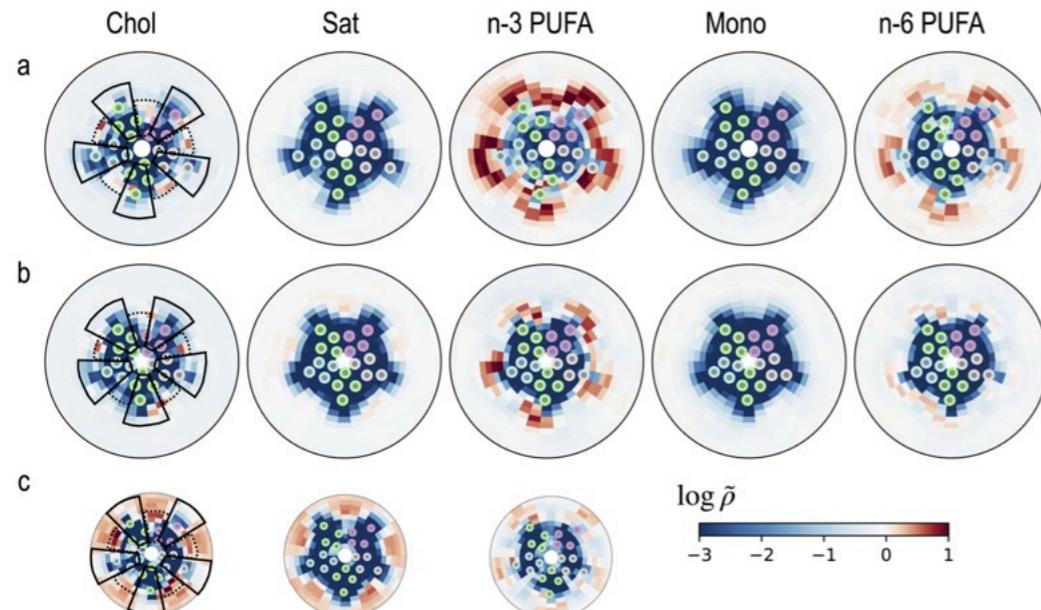


TABLE II. Density-threshold affinities of neutral and anionic lipids for both sites in the inner leaflet, by headgroup. Values are sorted by strength of affinity for inter-subunit sites. Errors are standard errors ($n = 10$ independent replicas).

	Inner inter-subunit sites ΔG (kcal/mol)	Inner M4 sites ΔG (kcal/mol)
PE	0.3 ± 0.2	-0.2 ± 0.1
PI	1.4 ± 0.3	0.3 ± 0.1
PS	1.4 ± 0.2	0.5 ± 0.2
PC	1.3 ± 0.3	0.8 ± 0.1
PIP3	3.1 ± 0.5	2.4 ± 0.4
PIP2	2.4 ± 0.3	1.3 ± 0.4
PIP1	2.2 ± 0.3	1.3 ± 0.4
PA	2.8 ± 0.3	1.9 ± 0.4

Sharp, Brannigan, JCP, 2021



Methods in Enzymology

Available online 4 April 2024

In Press, Corrected Proof



The density-threshold affinity: Calculating lipid binding affinities from unbiased coarse-grained molecular dynamics simulations

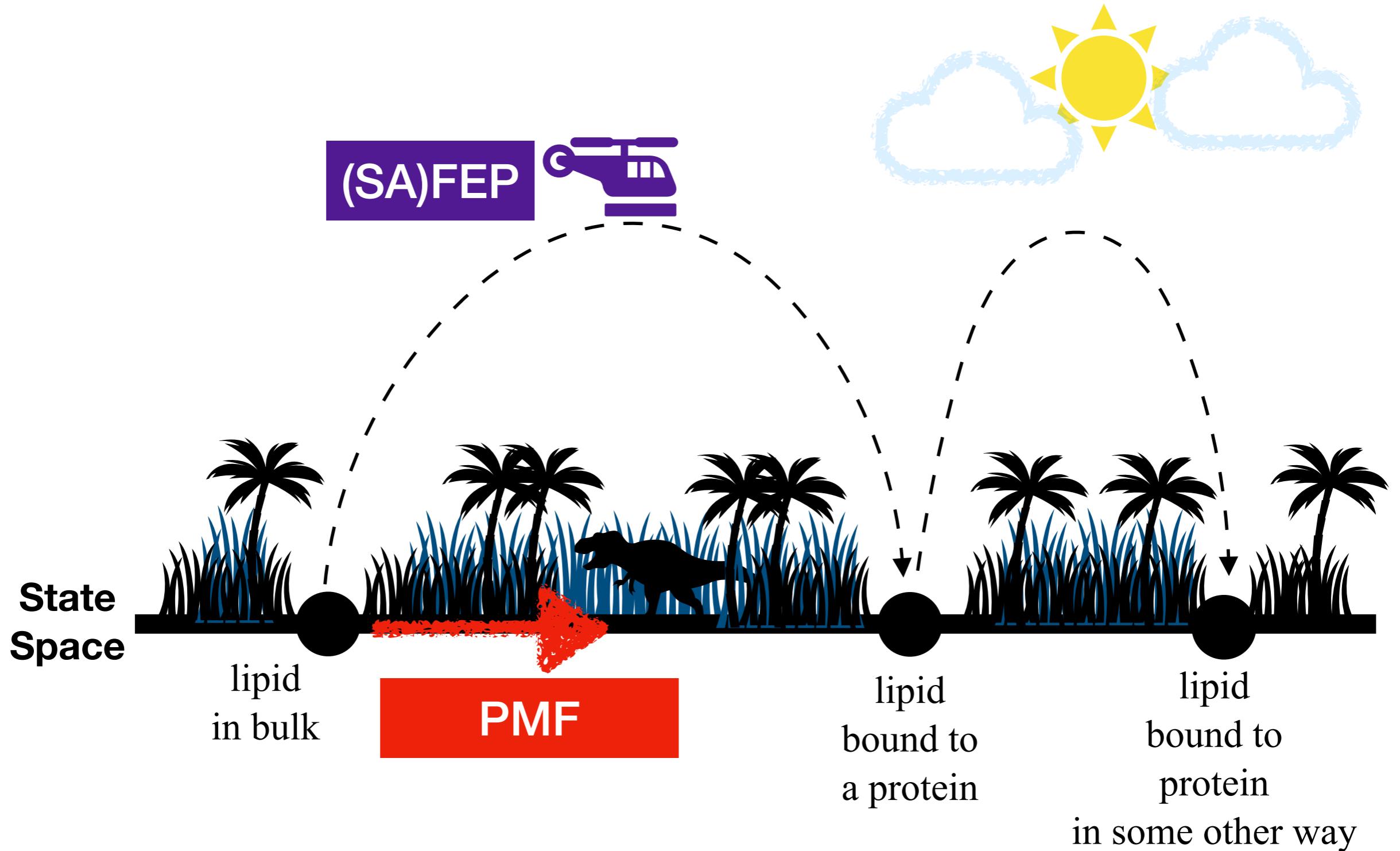
Jesse W. Sandberg^{a,1}, Ezry Santiago-McRae^{a,1}, Jahmal Ennis^{a,1}, Grace Brannigan^{a,b}

Author Link: Free for 50 days Only!!!

<https://authors.elsevier.com/a/1is%7EXHRzCT-YQ>



Traversing the AA Membrane Jungle

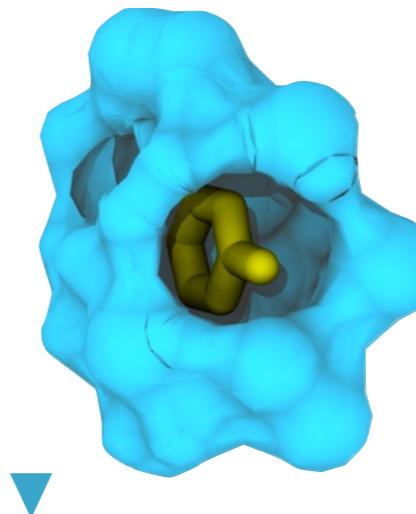


two flavors of alchemical FEP

Absolute

How: Gradually **turn off** the interactions between the ligand and everything else; calculate thermodynamic averages

Output: ΔG of binding



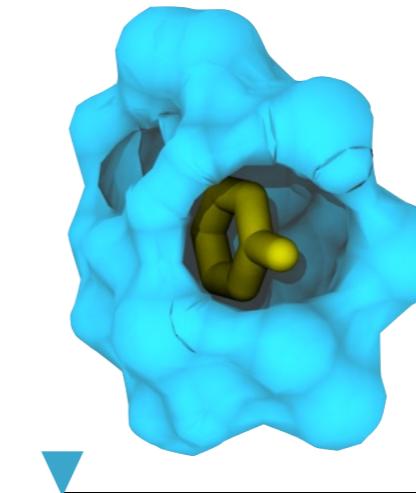
Decoupling Progression

Better for
lipids!

Relative

How: Gradually **transform** one chemical group into another; calculate thermodynamic averages

Output: $\Delta\Delta G$ of binding (compare ligands)



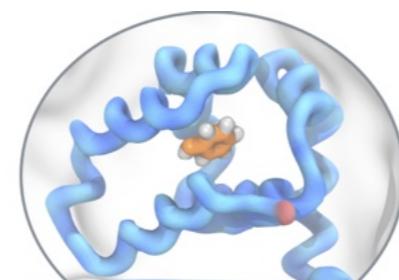
Transformation Progression

SAFEP: a FEP implementation that works in membranes

FEP but in a site-centered reference frame.

SAFEP introduces the “distance-from-bound-configuration” (DBC): RMSD of the ligand in the site’s reference frame

A LiveCoMS Tutorial



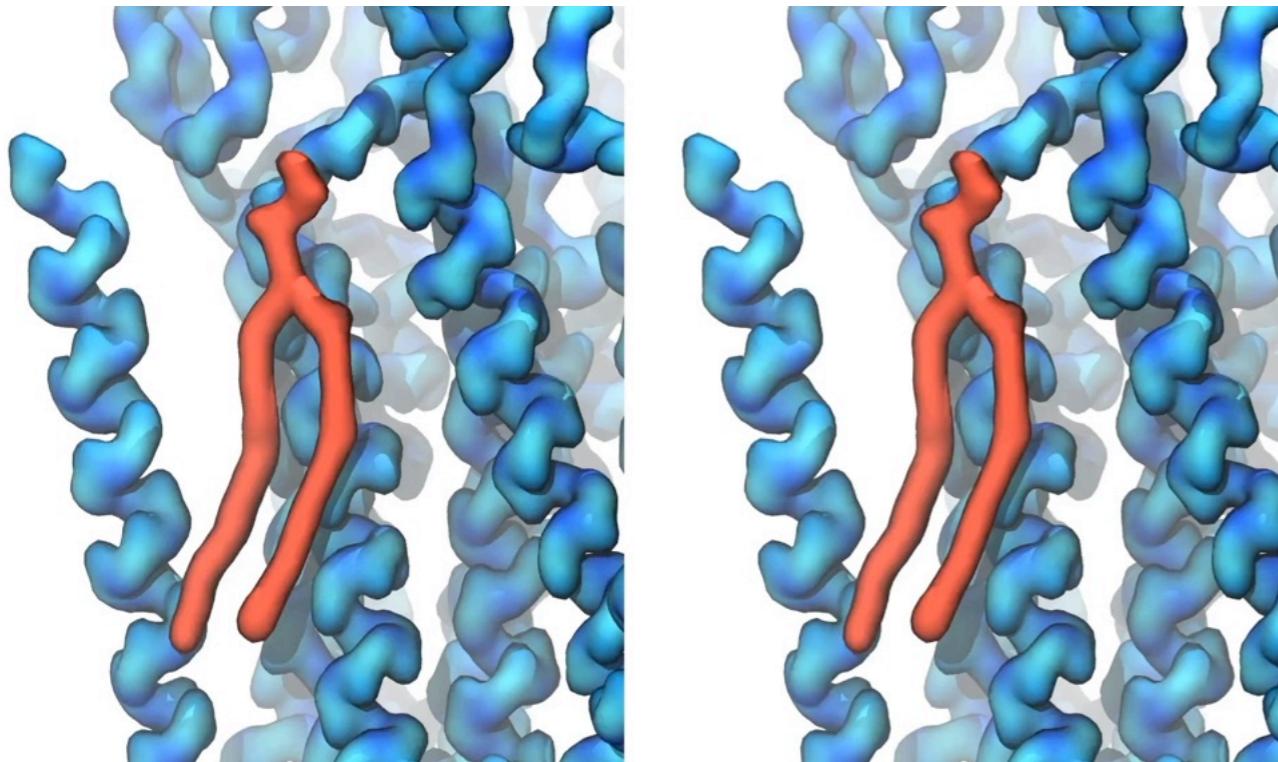
Streamlined
Alchemical
Free
Energy
Perturbation

Computing Absolute Binding Affinities by Streamlined Alchemical Free Energy Perturbation (SAFEP) [Article v1.0]

Ezry Santiago-McRae^{1†}, Mina Ebrahimi^{2,3,4†}, Jesse W. Sandberg¹, Grace Brannigan^{1,5‡}, Jérôme Hénin^{3,4‡}

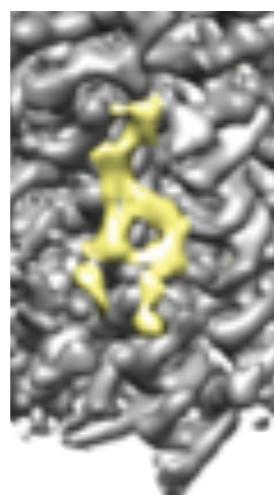
AA: Extracting lipid binding affinities

Decoupling POPG from ELIC (ABFE)
(Audio $\propto \Delta\Delta G$)

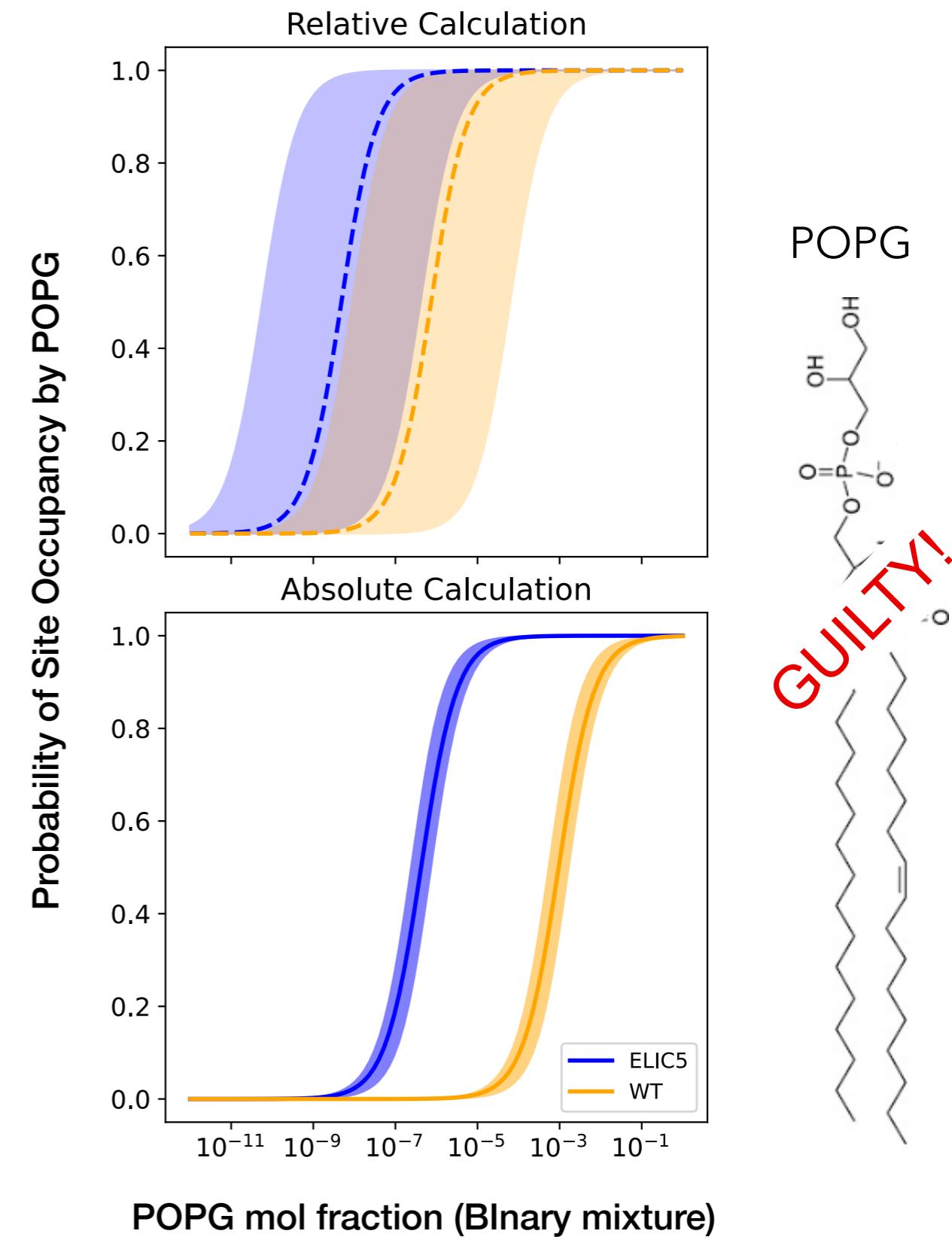


<https://www.youtube.com/watch?v=FS6e38BtUlw>

"Frame of Reference"
-Ezry St. Iago-McRae

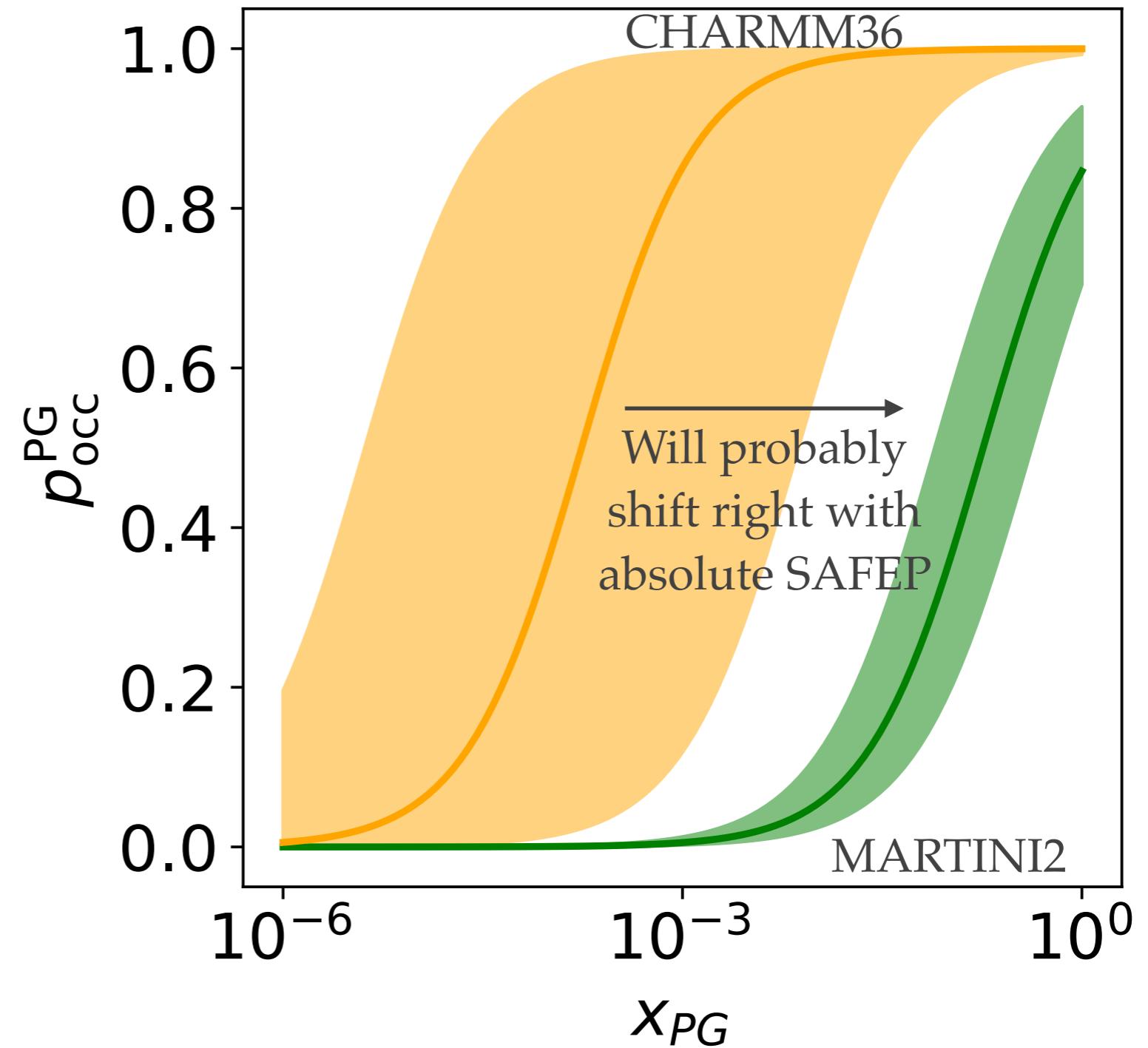


structural pose

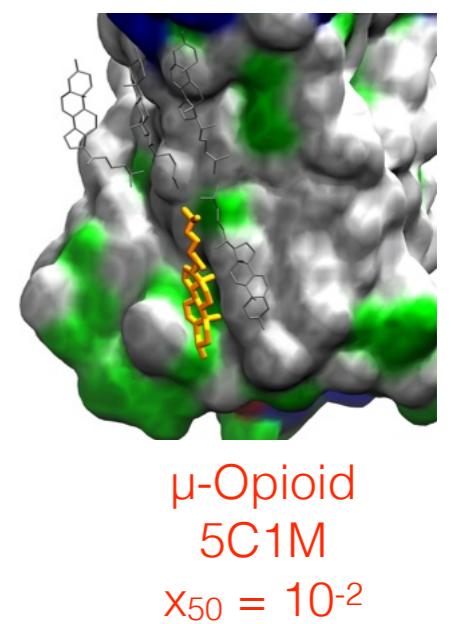
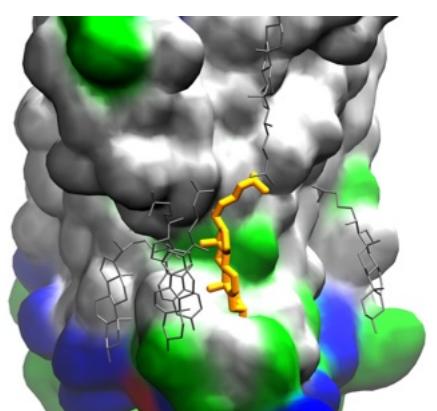
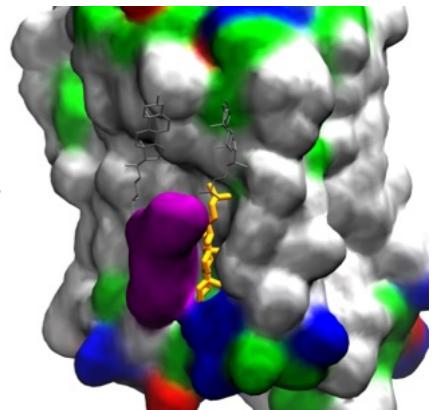
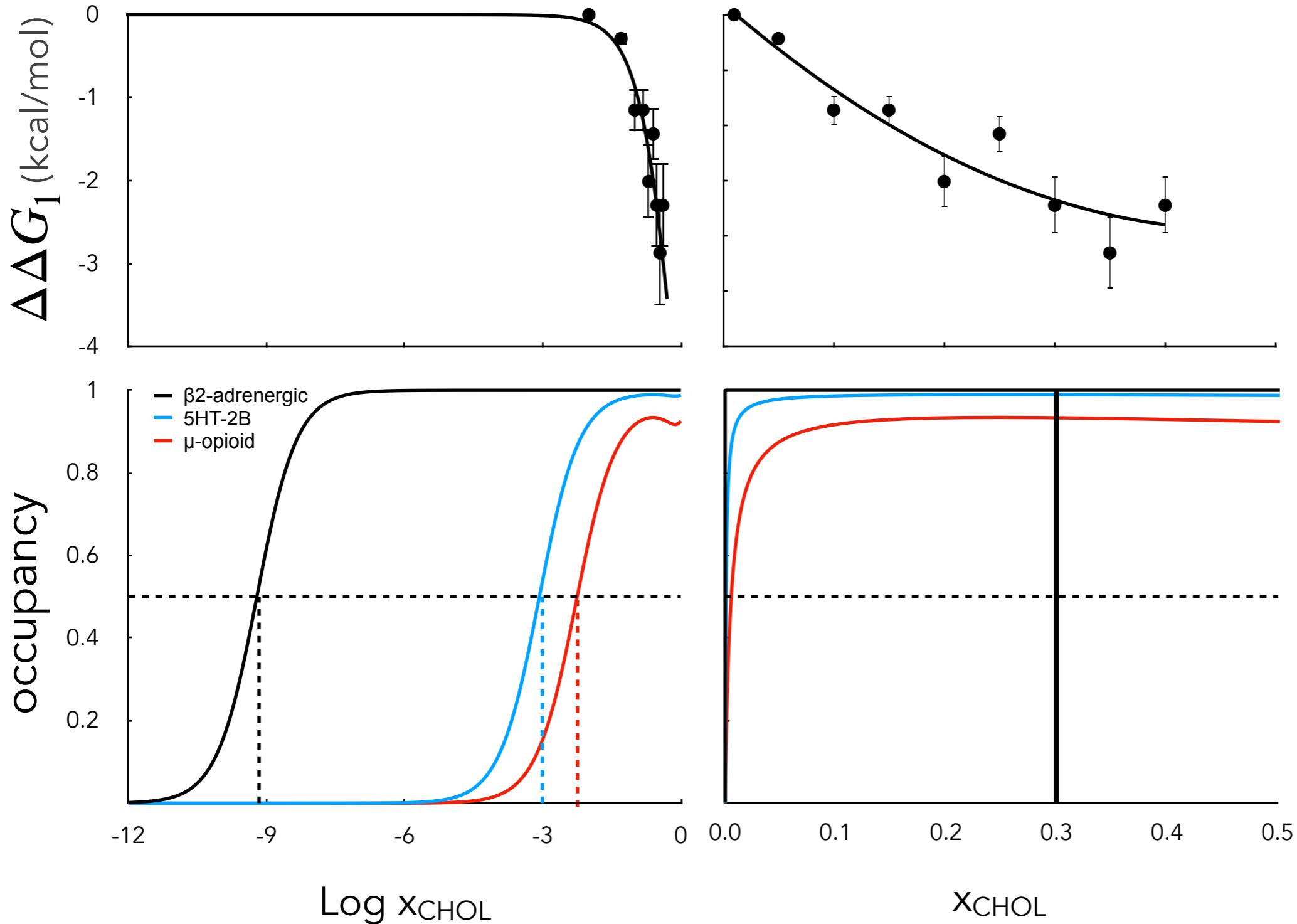


Comparison between CG and AA data

POPG binding to
ELIC: Right now
we only have an
apples-to-apples
comparison for
relative SAFEP :(



SAFEP applied to cholesterol/GPCRs



Lipid-protein binding: takeaways

- Atomistic SAFEP yields converged affinity calculations for lipid-protein binding affinities, suitable for comparison to CG values
- Lipid sorting simulations via Martini 2 probably underestimate specific lipid-protein binding for
 - charged headgroups/ligand-gated ion channels
 - cholesterol/GPCRs

Protein-Protein

“Who is in my neighborhood?”

Prologue

Weird story –

- Hydrophobic to hydrophobic mutation in 92 residue IDP (pro region of BDNF)
- Val66Met
- affects phenotype and secondary-structure far away from the mutation site
- WHY?

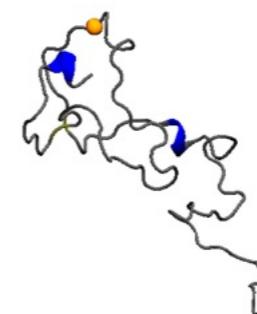
What we did –

Ran outrageously expensive simulations for both Val66 and Met66 form
256μs (2μs x 64replicas)

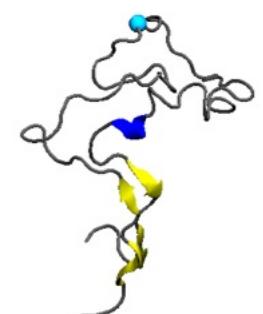
explicit solvent

temperature replica exchange simulation
(300K - 385K) of each prodomain;

GROMACS; **Amber99SB*-ILDN with TIP4PD water**



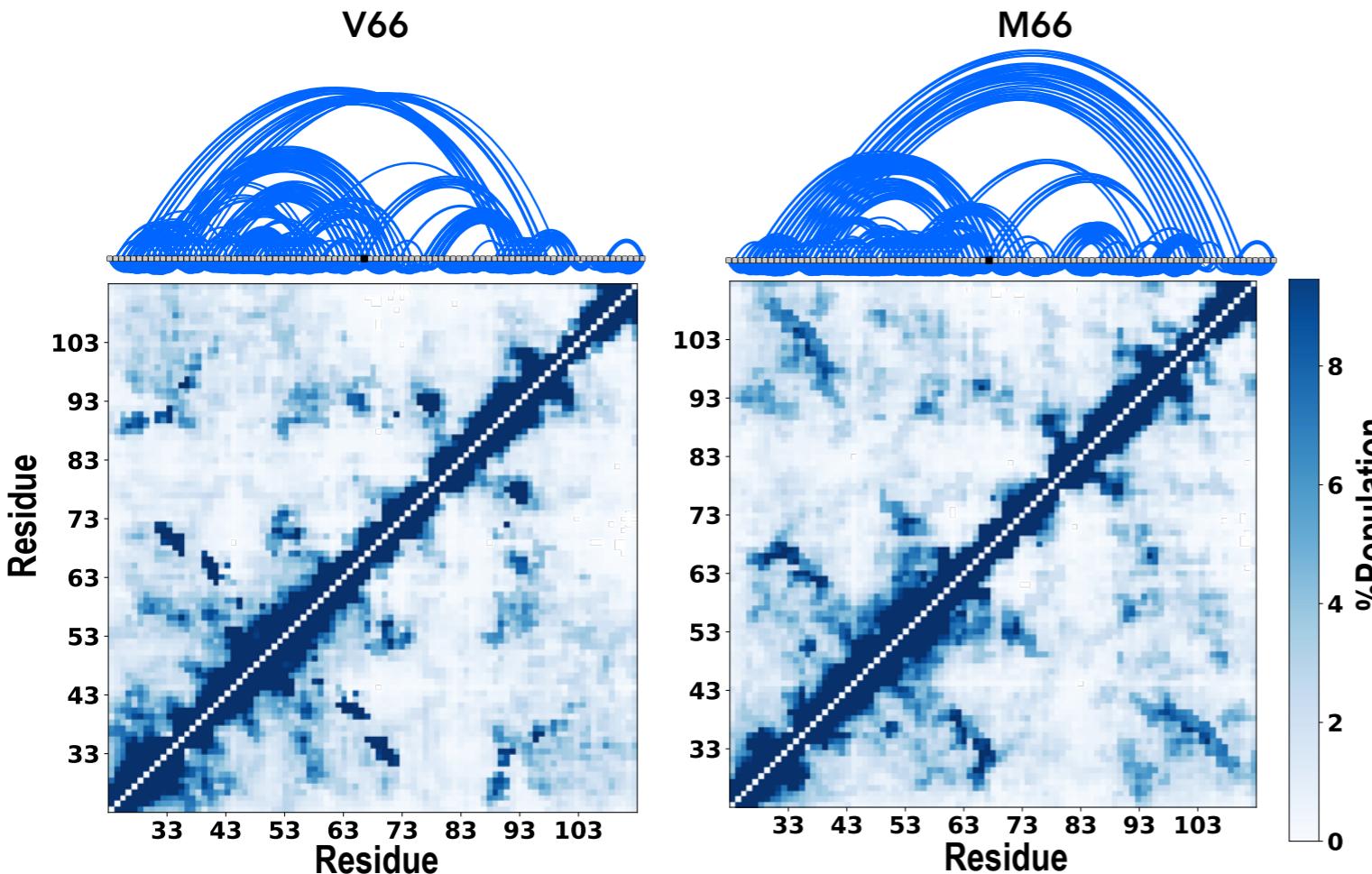
V66



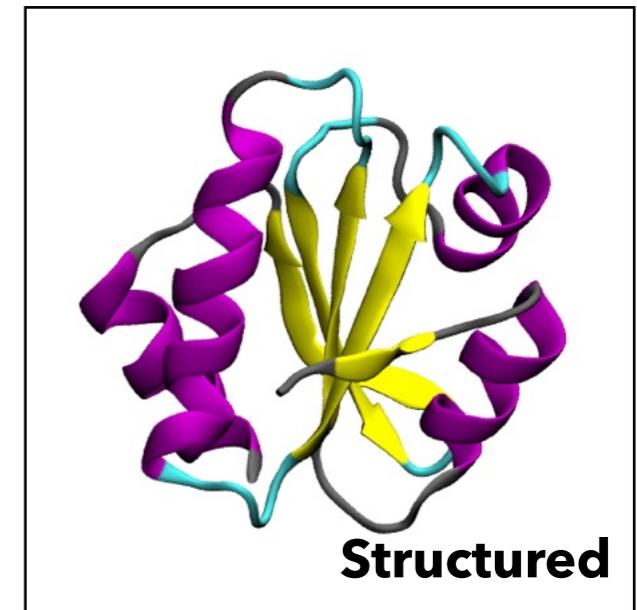
M66

Too much data: How could we analyze this?

try analyzing changes in contacts:

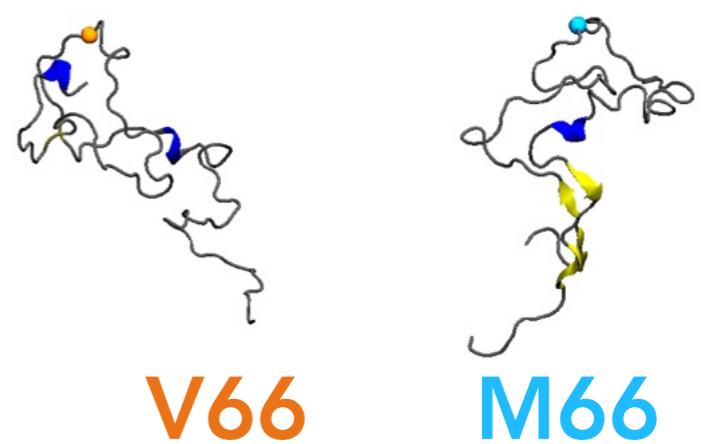


Structured : $O(N)$ Possible contacts



PDBID: 2N5A yeast Thioredoxin

IDP: $O(N^2)$ Possible contacts



This was a statistical nightmare!

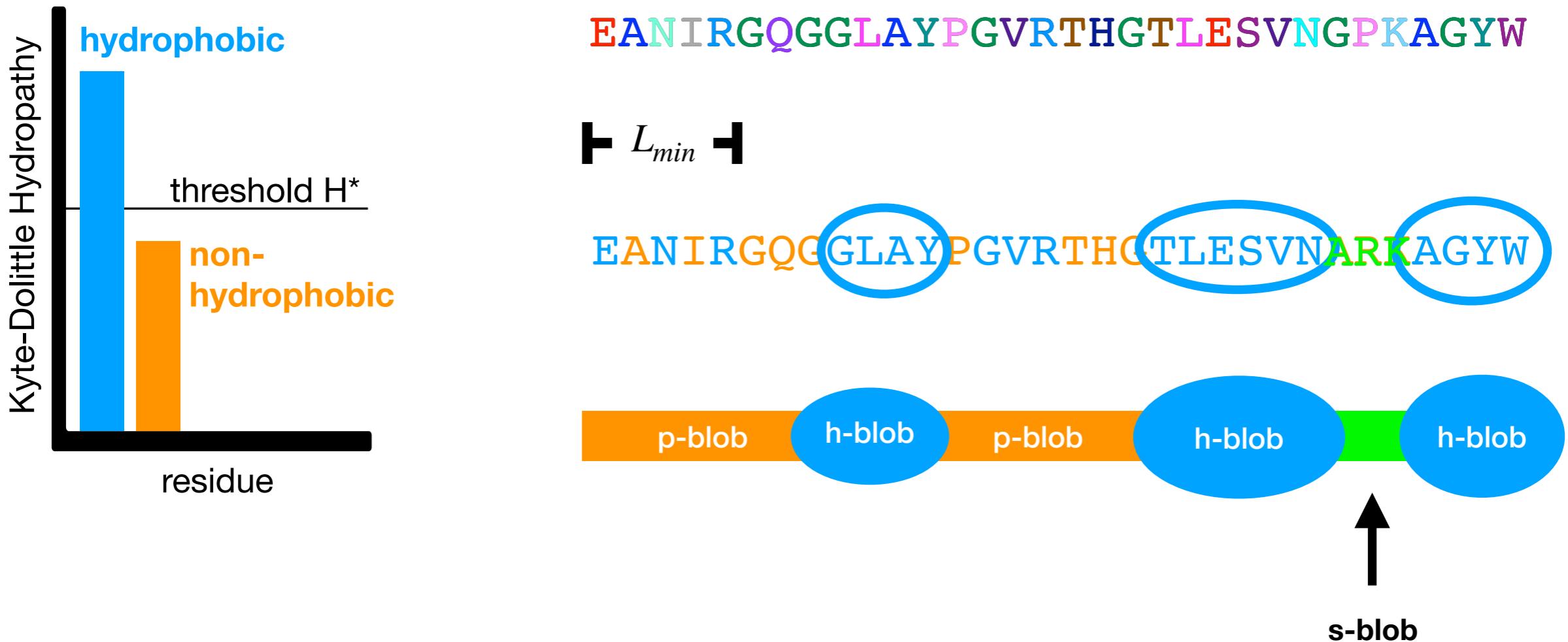
Solution : coarse-grain analysis
of atomistic simulation

We need some sequence organization!

Secondary structure topology

COIL

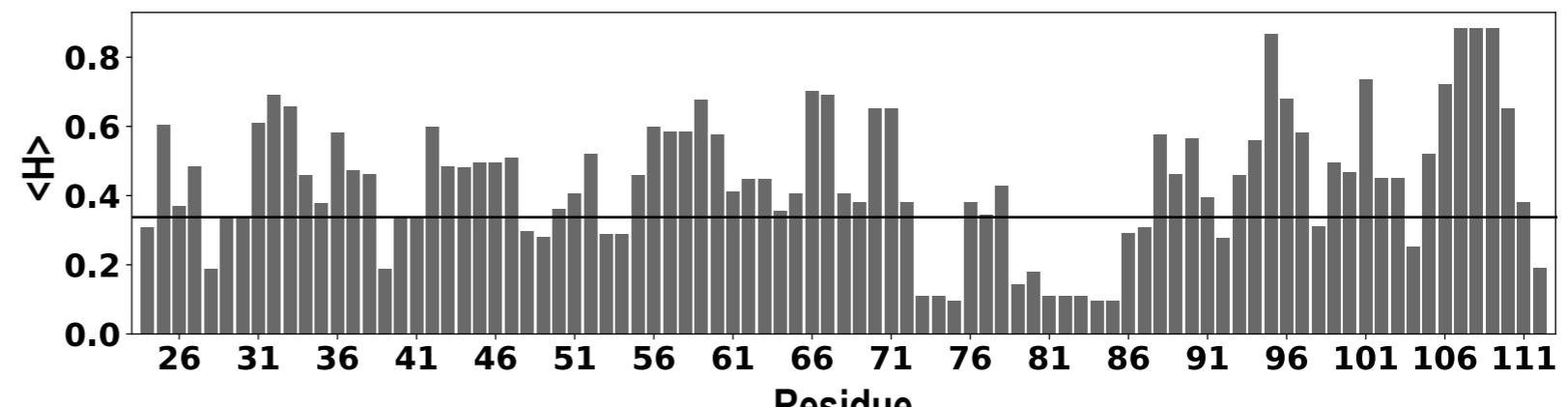
introducing blobulation



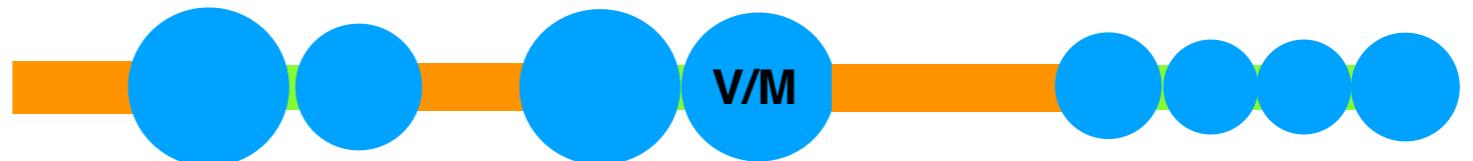
We need some sequence organization!

Secondary structure topology

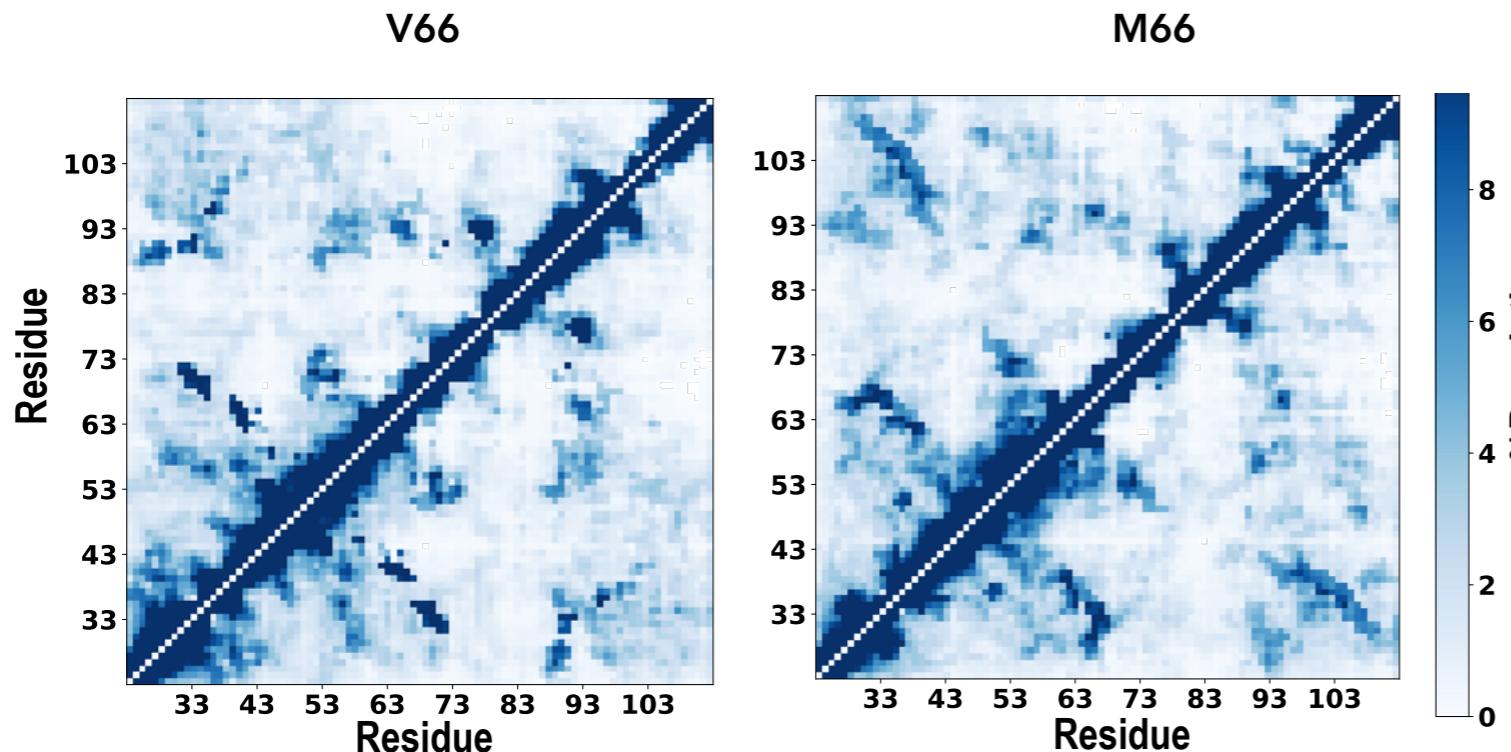
COIL



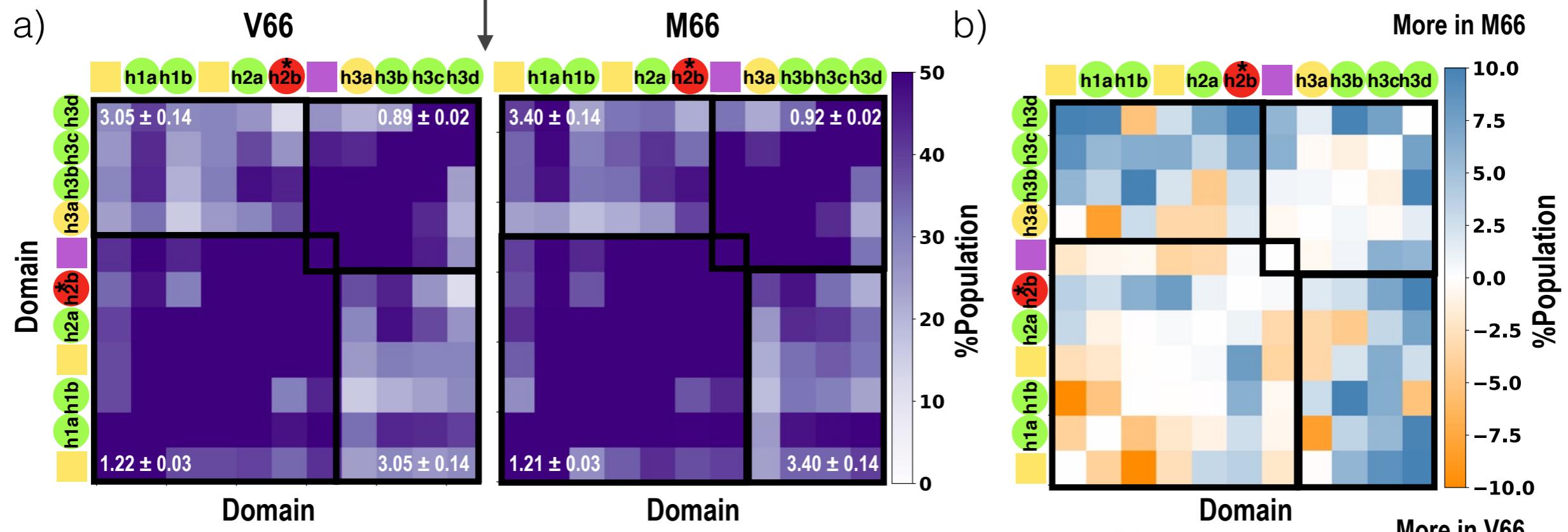
Blob topology



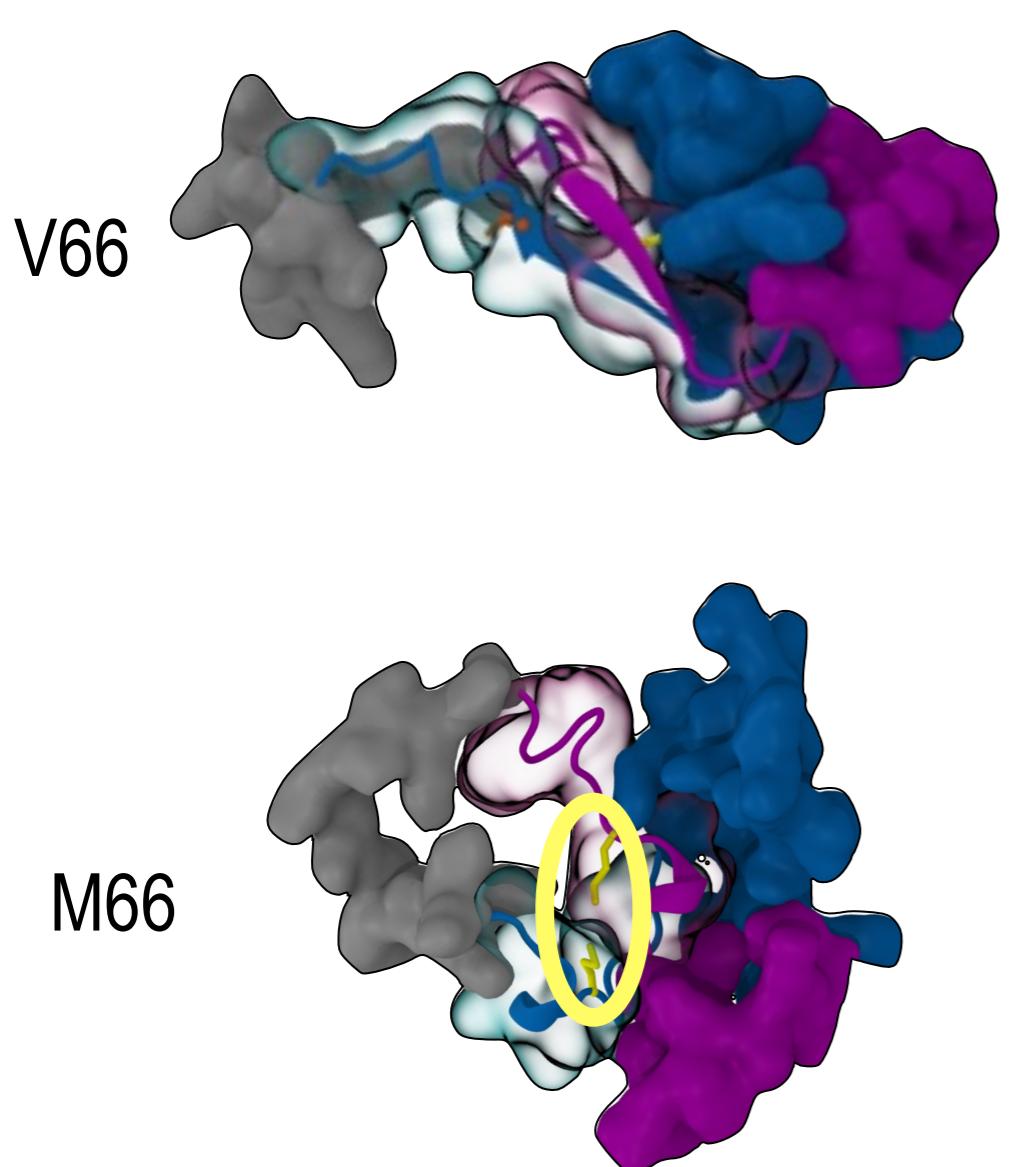
Blob-blob Interactions



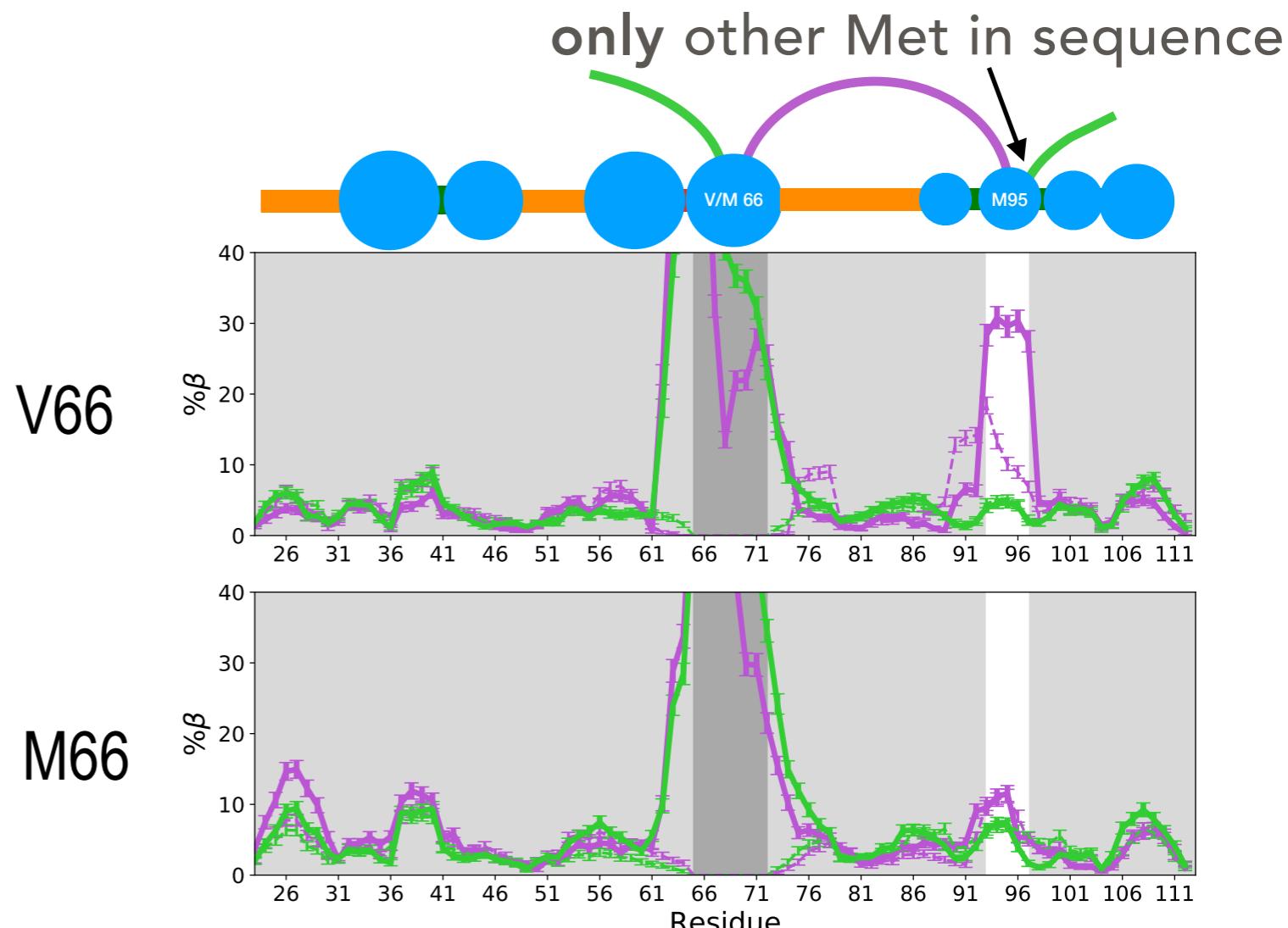
Statistical question: How
“should” blob interactions
depend upon separation
along chain, for an ideal
heteropolymer?



Clustering by blob contacts revealed the “answer”: Met-Met interactions



Met-Met Interactions switch
backbone-backbone to
sidechain-sidechain



PLOS COMPUTATIONAL BIOLOGY

RESEARCH ARTICLE

Sequence specificity despite intrinsic disorder:
How a disease-associated Val/Met
polymorphism rearranges tertiary
interactions in a long disordered protein

Ruchi Lohia¹, Reza Salari^{1*}, Grace Brannigan^{1,2*}

Validation

Hi <Experimentalist>! Sorry to bother you again, but we got back reviews of Ruchi's paper, and one of the reviewers is still concerned about a discrepancy with your deposited NMR data from 2013 at R93. I hate to ask but is there any chance something went wrong here? Thanks, Grace

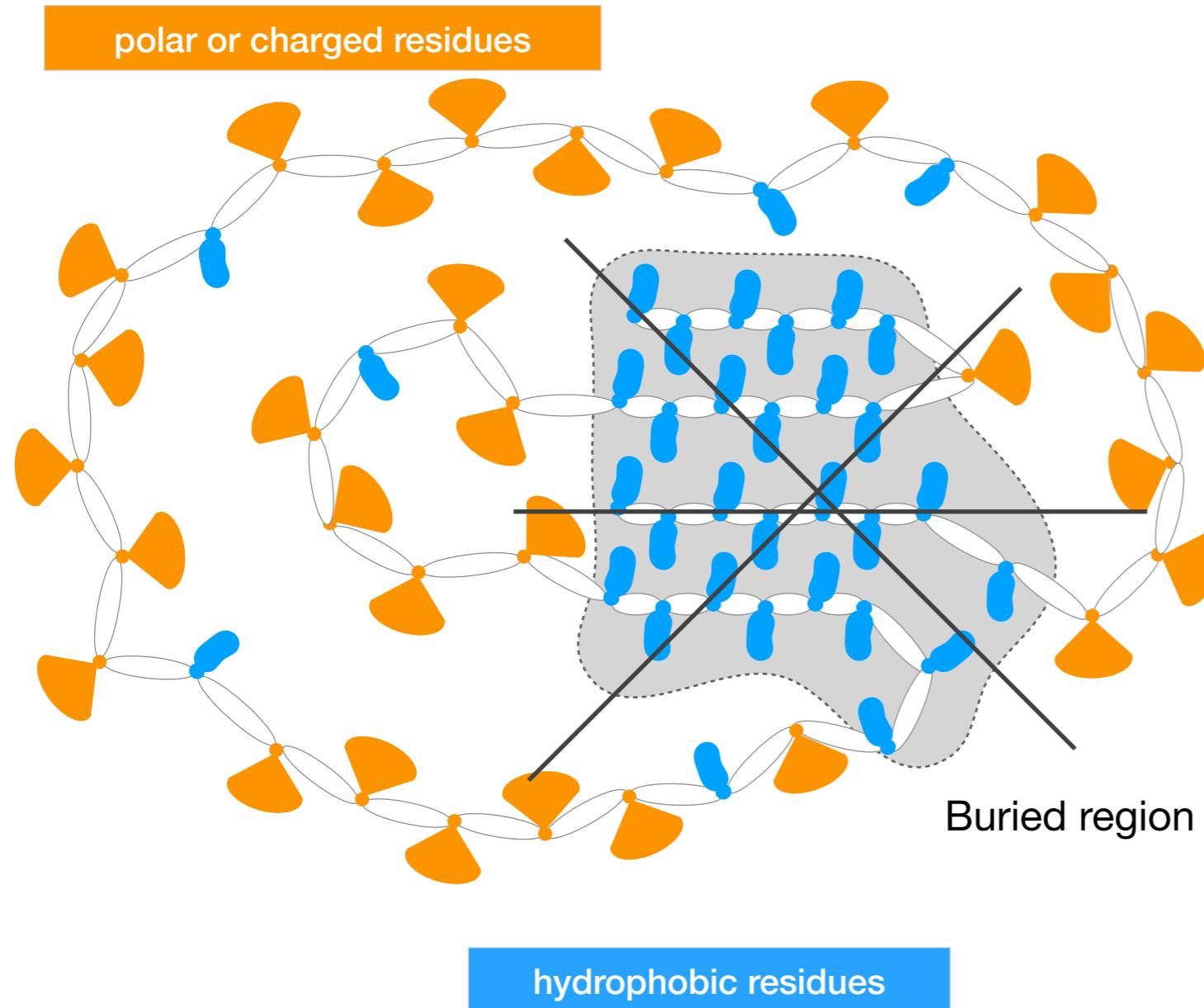
Hi Grace, There is a typo! Thank you for bringing this to my attention! I will get this corrected! Best, <Experimentalist>

Please consider using this dataset! It's available on dryad.
Lohia et al Plos Comp Bio 2019

Blobulation : sequence-based coarse-graining

Useful for more than IDPs?

Contiguous hydrophobicity: Useful for globular proteins?

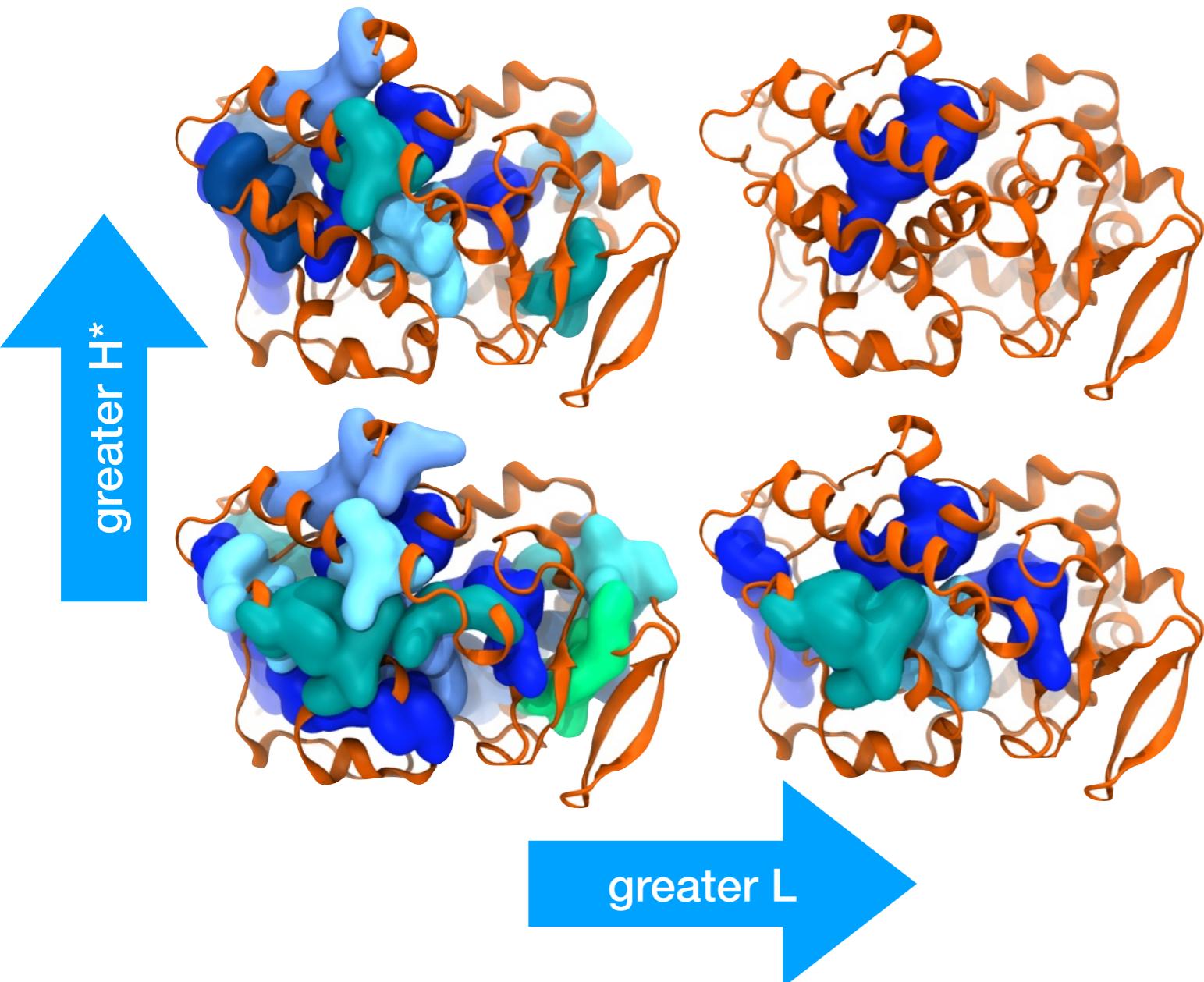


There exist multiple paths through the buried region that connect multiple hydrophobic residues...

...and some of those paths will connect residues along the same peptide chain...

...therefore we should find contiguously hydrophobic regions in the protein core!

Hypothesis: h-blobs will be more mutation sensitive...



...and the more hydrophobic the blob, the more sensitive it will be

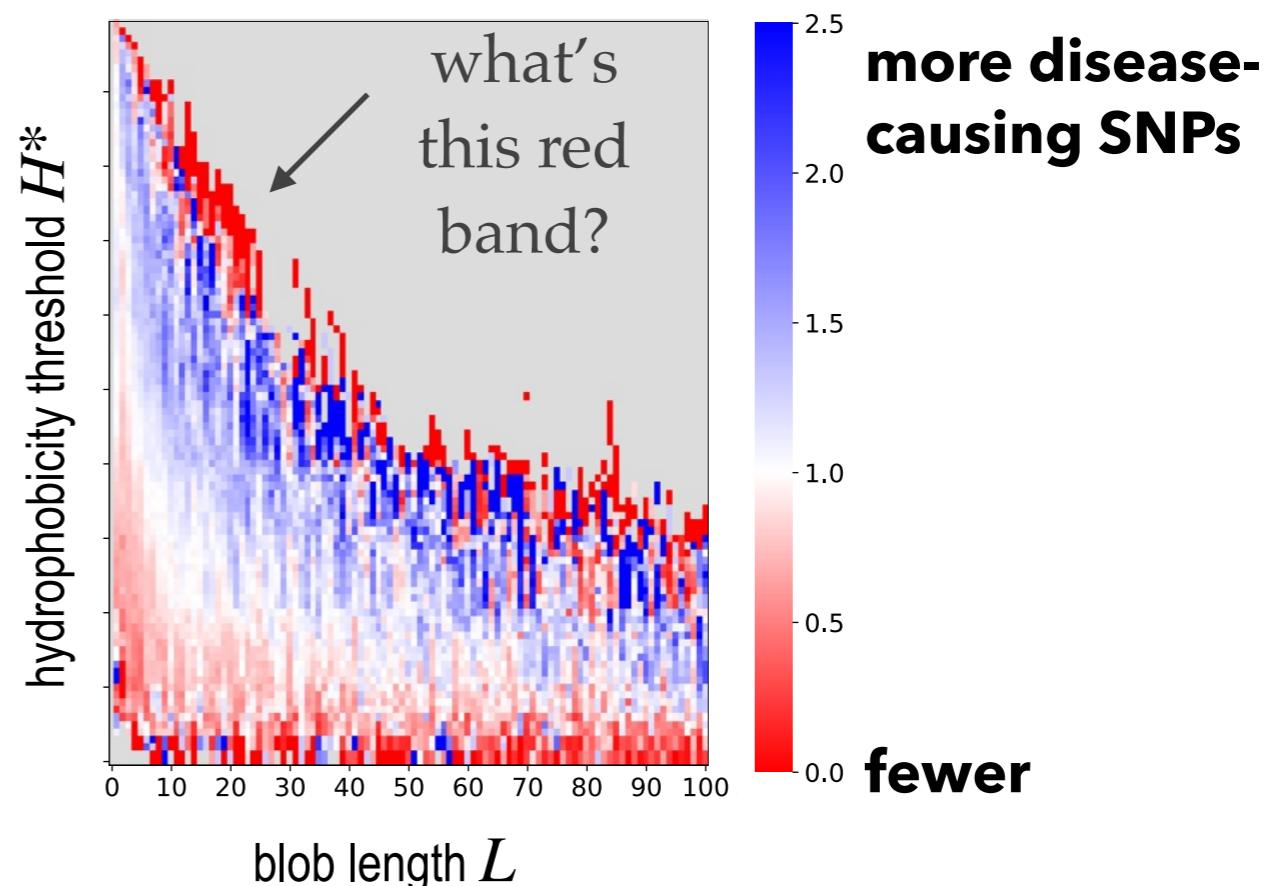
Approach: test for h-blob enrichment of human disease-associated mutations (SNPs)

enrichment of disease-associated SNPs (dSNPs)

For any threshold H^* , how many disease-causing mutations are in h-blobs of length L ? How many non disease-causing mutations?

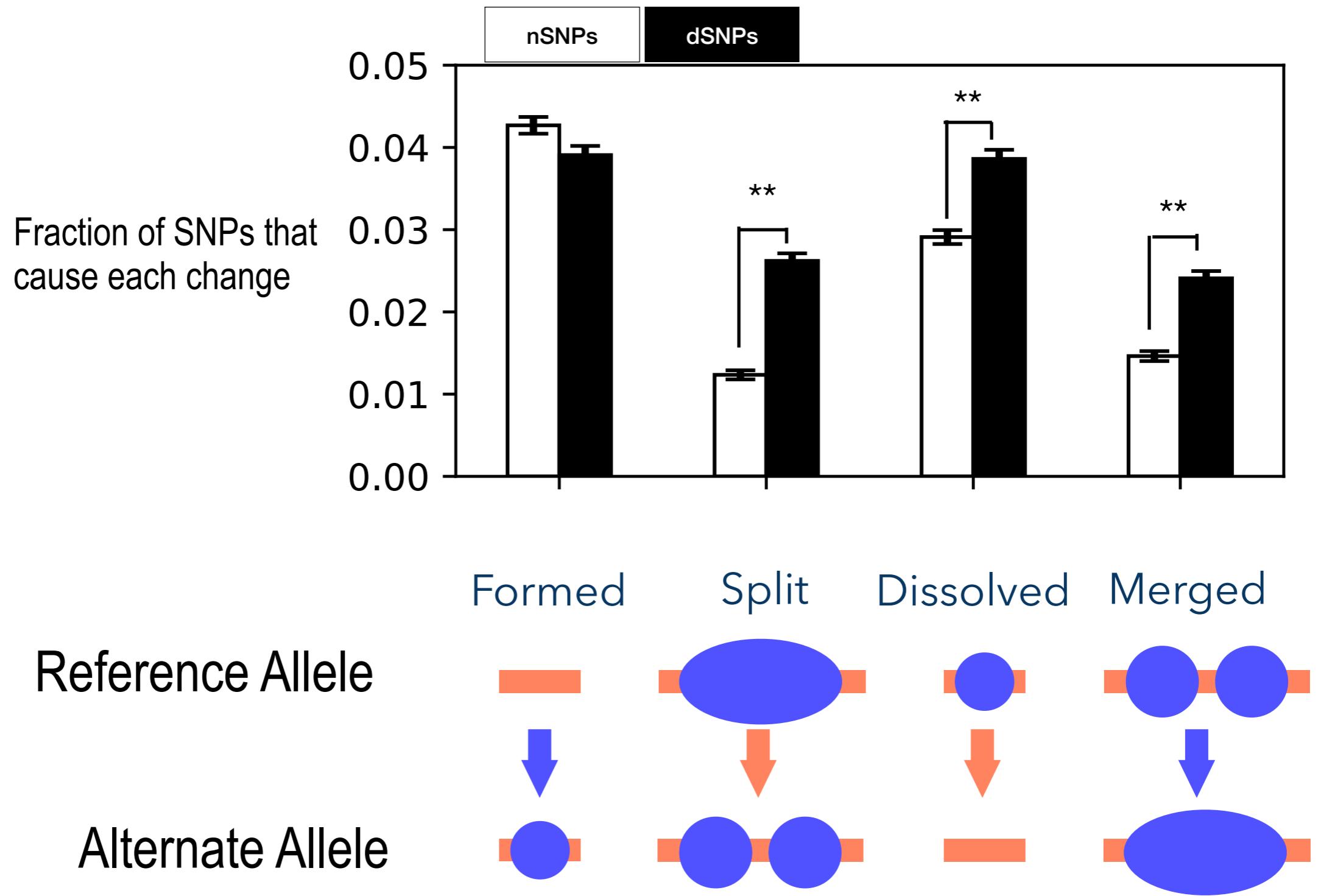
Calculated L for ~70K missense variants (UniProtKB)

- 57% "likely benign or benign" (nSNPs)
- 43% "likely pathogenic or pathogenic" (dSNPs)

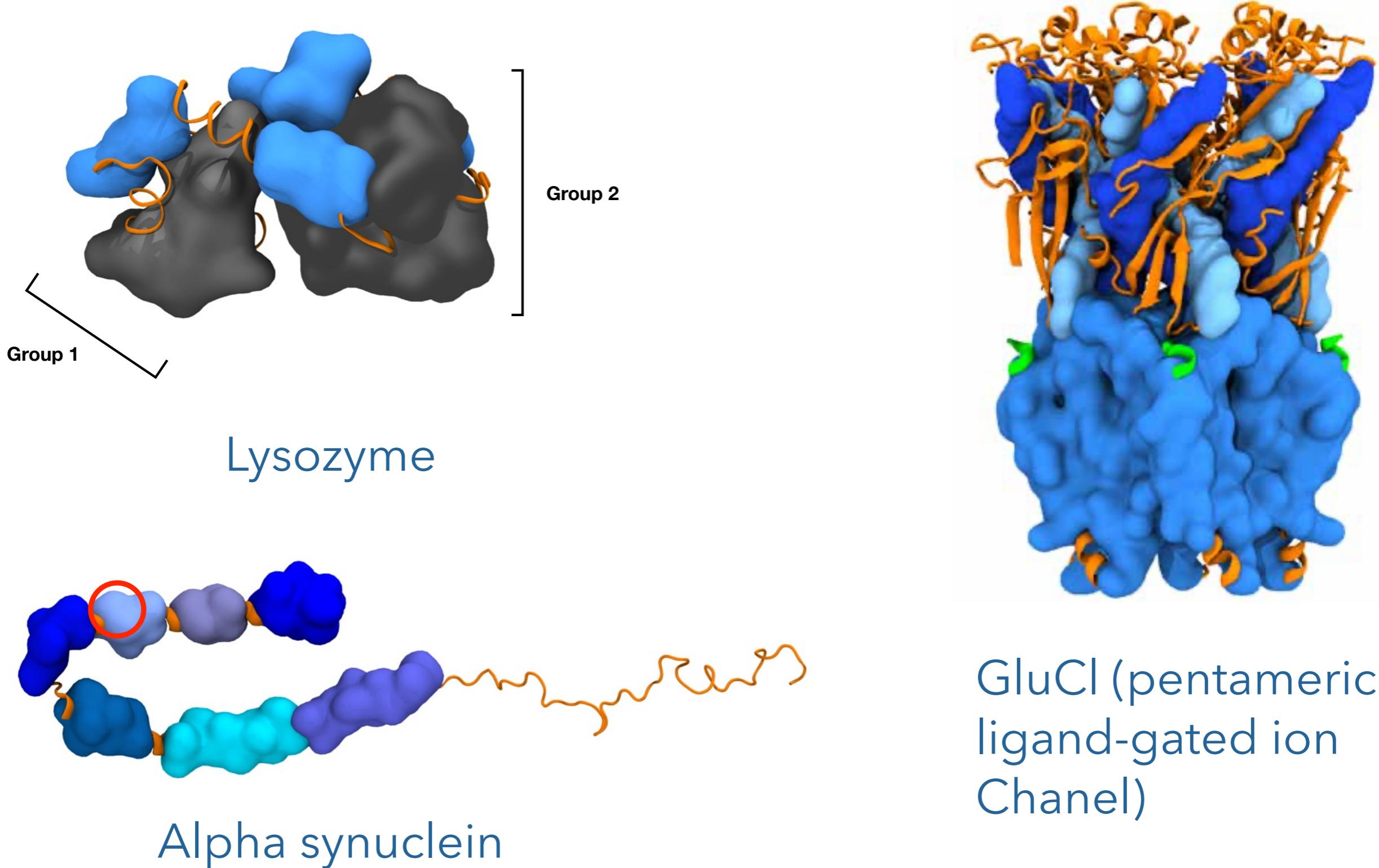


mutations in very long **or** very hydrophobic blobs: most likely to be disease-associated

disease-causing mutations change blob topology



Blobulation Zoo



blobulator.branniganlab.org

Protein-protein: Takeaways

- proBDNF: huge atomistic dataset of a long IDP in explicit solvent
- blobulation yields interesting and useful **trends across the human exome** (not just in one protein, or in IDPs)
- results are consistent with h-blobs as **physical interaction nodes**
- **evidence for selection** against mutations in most extreme h-blobs
- mutations that **split h-blobs** are 3 times as likely to be **disease-associated**
- blobulation GUI at **blobulator.branniganlab.org**

Acknowledgments

Direct contributors (past & present)

Ezry St. Iago-McRae

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Jesse Sandberg

Jahmal Ennis

Liam Sharp (Fairfield University)

Ruchi Lohia (Cold Spring Harbor)

Reza Salari (Washington University - St Louis)



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Matthew Hansen (University of Pennsylvania)

Thomas Joseph (University of Pennsylvania)

Wayland Cheng and Lab
(Washington University - St. Louis)



XSEDE

Extreme Science and Engineering
Discovery Environment



R