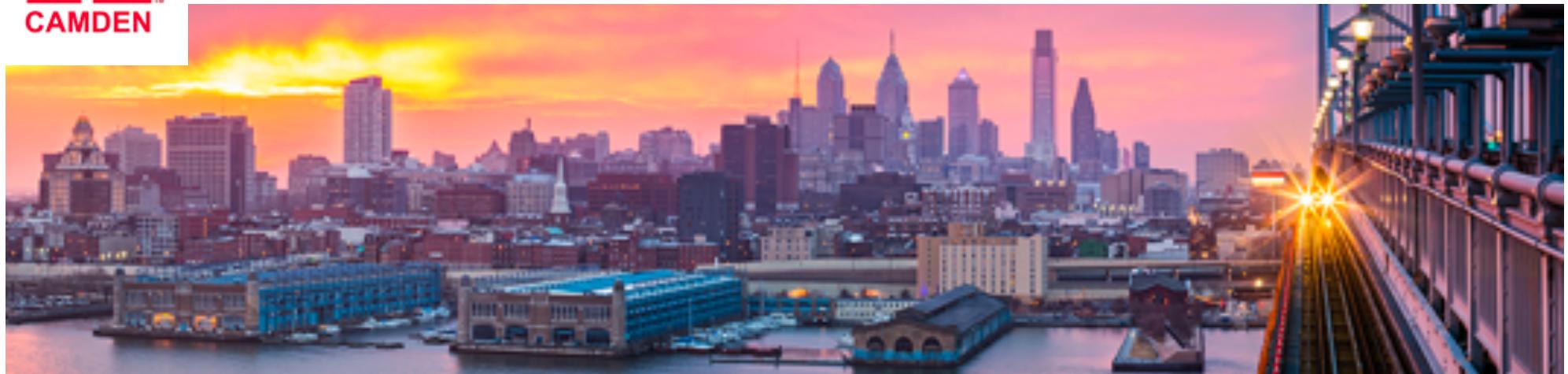


Specificity of Hydrophobic Molecules: Effects on Lipids, Proteins, and Populations

CECAM in honor of Michael L. Klein

Temple Rome, October 28, 2022

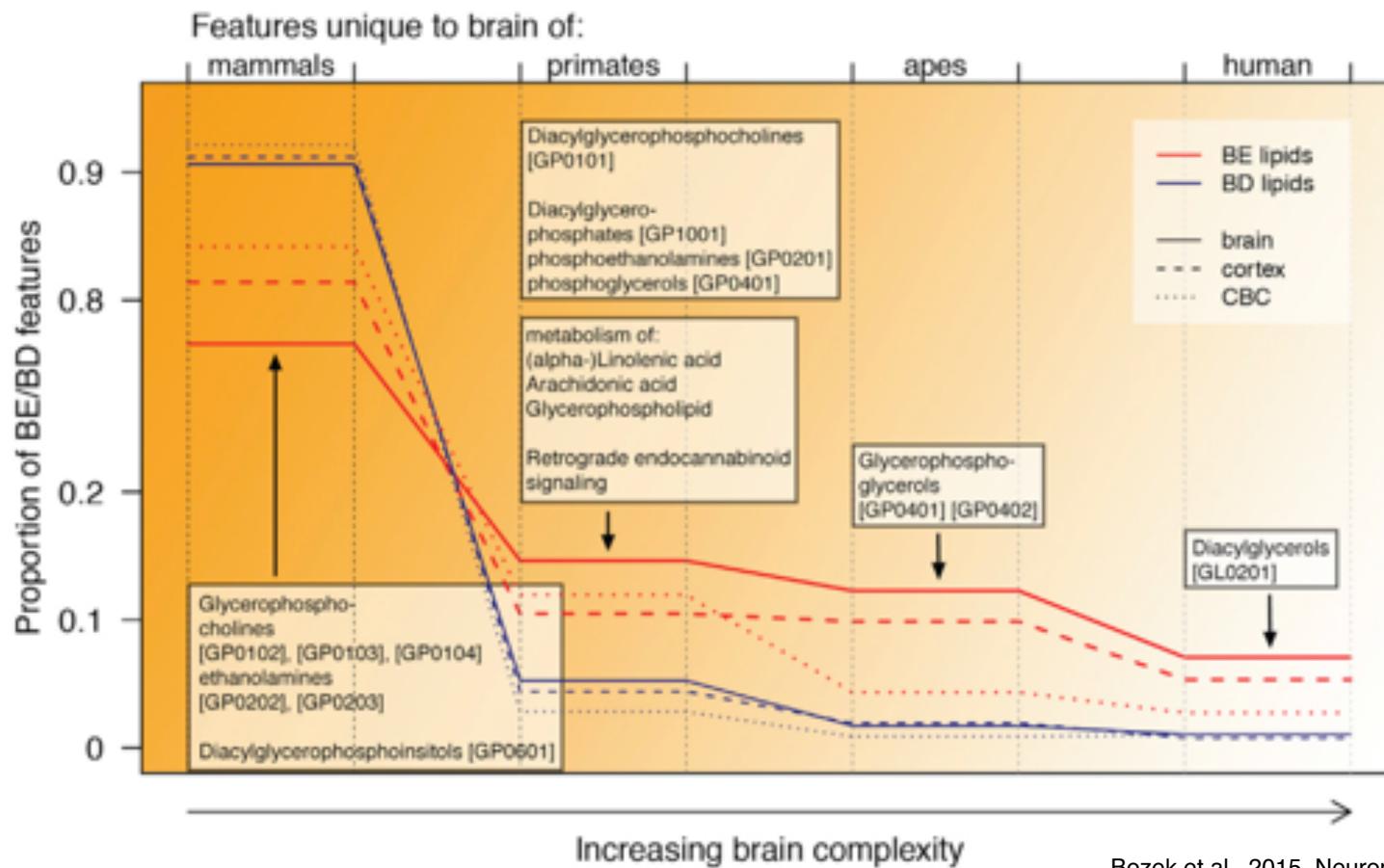
Grace Brannigan
Center for Computational &
Integrative Biology
Rutgers University - Camden



Two stories

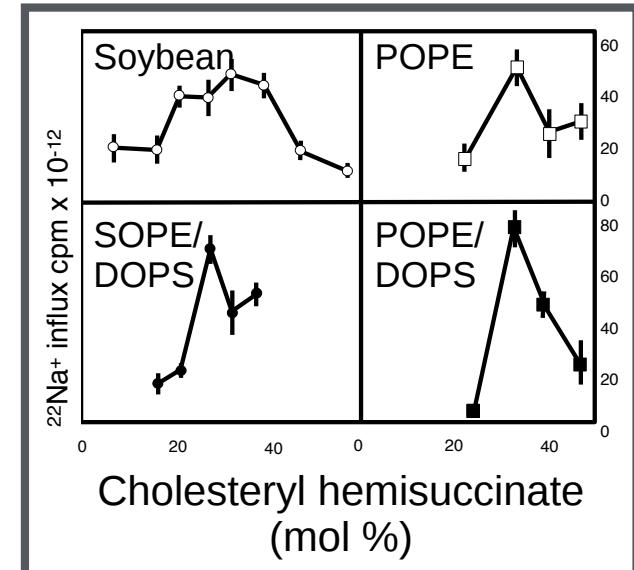
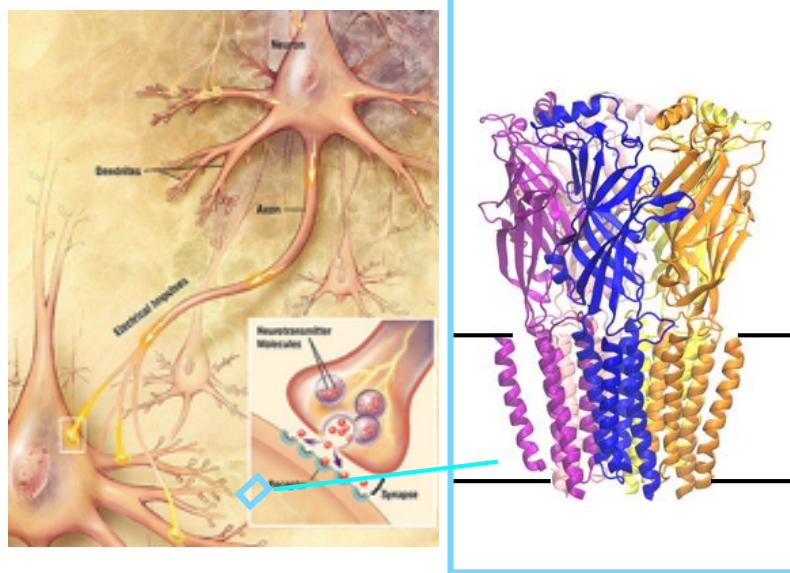
- 1. Catching guilty lipids**
2. Catching guilty amino acids

Lipid diversity -> brain complexity



pLGICs are very picky about lipids

nicotinic acetylcholine receptor, GABA_A receptor, glycine receptor, etc

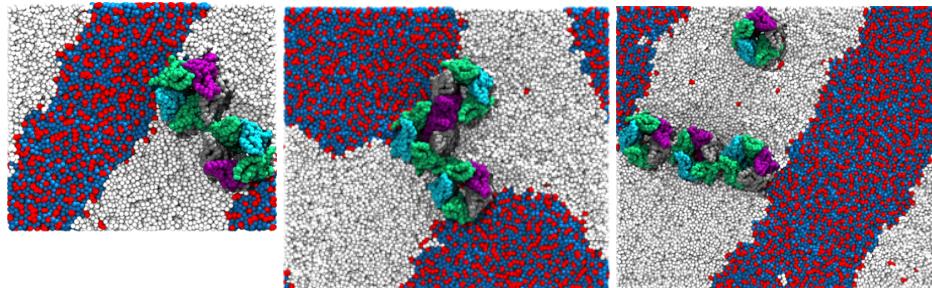
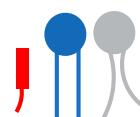


Criado...Barrantes, 1984, J.Biol.Chem.



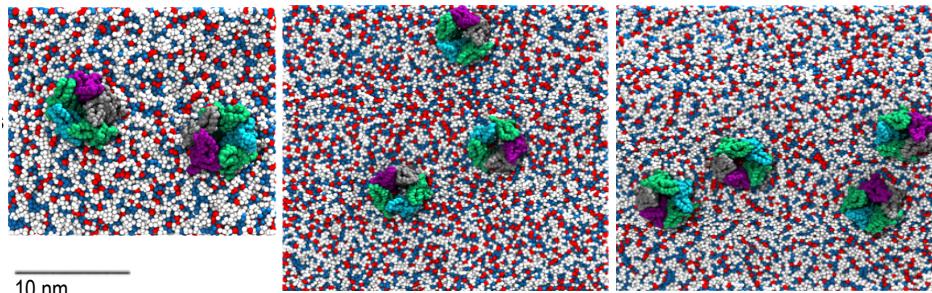
not all model membranes are created equal

Homoacids
+ Chol



which is more
physiologically
relevant?

Heteroacids
+ Chol



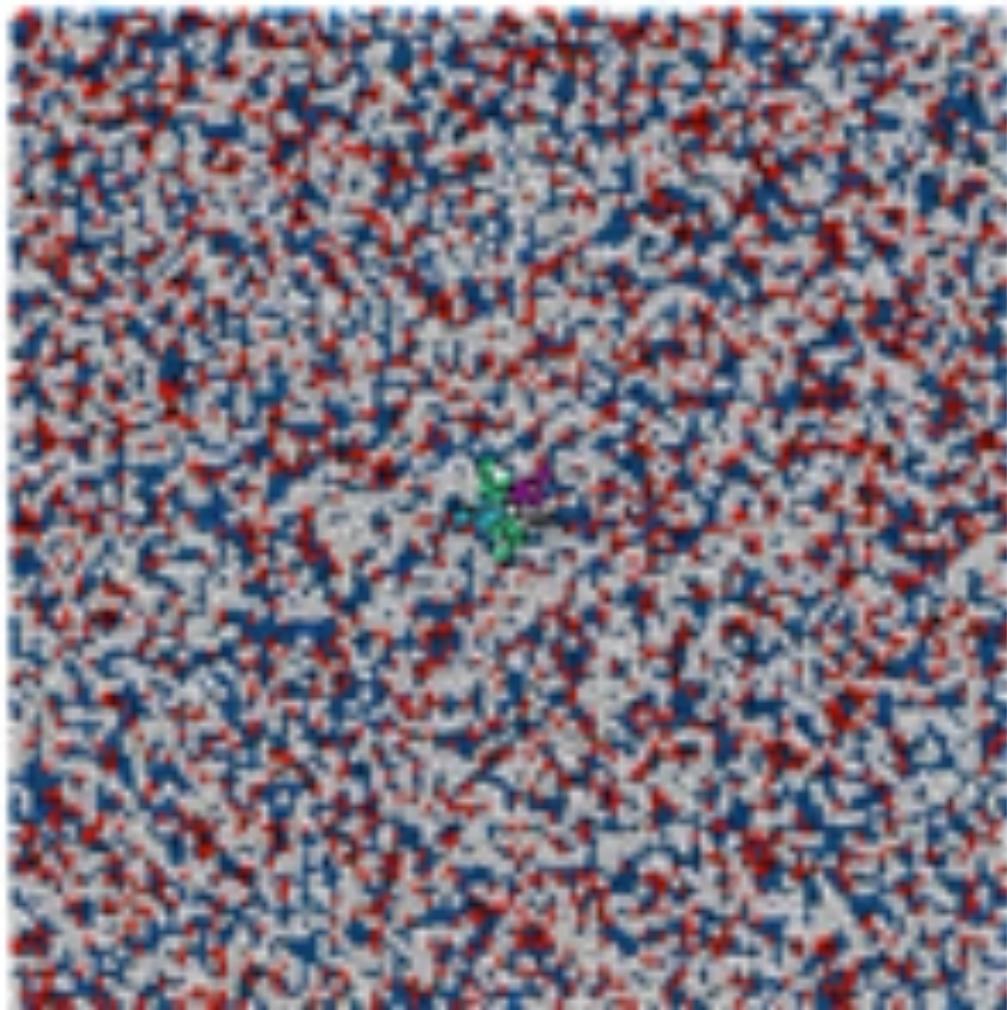
The Journal of Membrane Biology (2019) 252:385–396
<https://doi.org/10.1007/s00232-019-00079-0>



Untangling Direct and Domain-Mediated Interactions Between Nicotinic Acetylcholine Receptors in DHA-Rich Membranes

Kristen Woods¹ · Liam Sharp¹ · Grace Brannigan^{1,2}

🤞 hoping it's homoacids

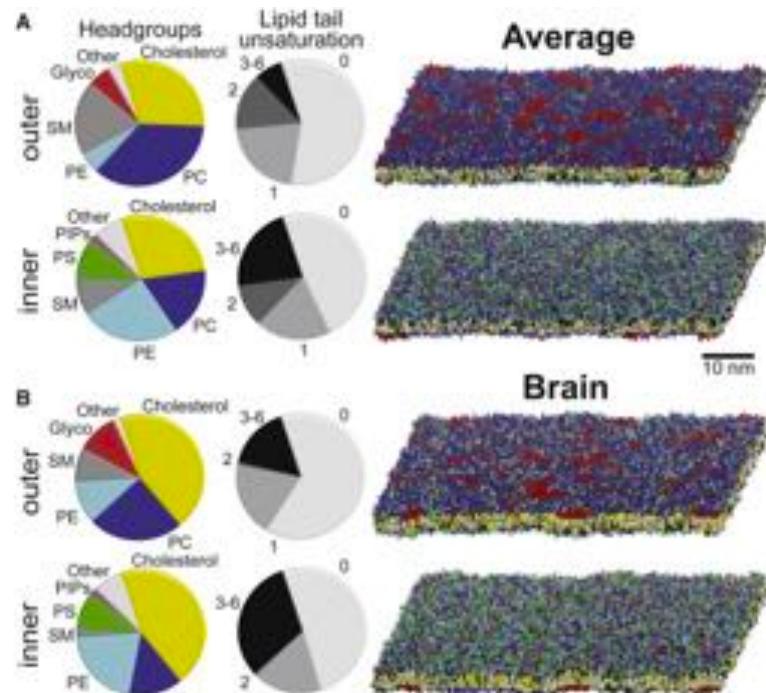


cool result: nAChR binds
cholesterol but partitions to a
cholesterol-poor domain

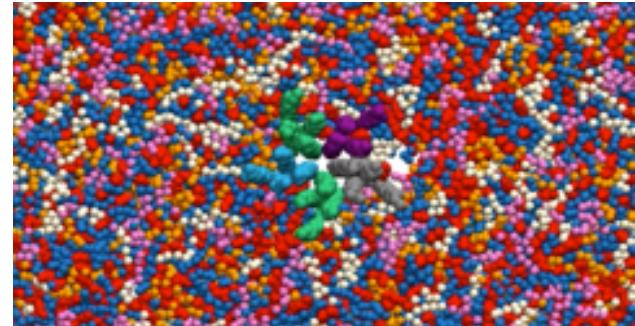
verdict: neuronal lipids are....

Table S1 Lipid composition^a

Lipid tail site-1	Lipid tail site-2	Acronym	Average			Brain			
			Outer leaflet count	Outer leaflet mole fraction	Inner leaflet count	Outer leaflet mole fraction	Inner leaflet count	Outer leaflet mole fraction	
Phosphatidylcholine - PC									
CCCC	CCCC	DPPC	ester/inner ratio		0.69	0.31	ester/inner ratio		
CCCC	CDCC	POPC	1205	0.122	550	0.059	531	0.053	
CDCC	CDCC	DOPC	106	0.011	49	0.005	868	0.087	
CCCC	CDCC	PIP'C	1772	0.179	810	0.087	221	0.022	
CCCC	CDDO	PFPC					118	0.012	
CCCC	CDDC	PEPC	71	0.007	32	0.003			
CCCC	DODC	PAPE	283	0.029	129	0.014	463	0.046	
DODDC	DODDC	DAPE	35	0.004	16	0.002	247	0.026	
CCCC	DODDC	PDPC	71	0.007	32	0.003	169	0.017	
CDCC	CDDC	O1PE					90	0.010	
CDCC	DODDC	O1PC					59	0.006	
Total:			3543	0.357	1618	0.173	2412	0.242	
Phosphatidylethanolamine - PE									
CCCC	CCDC	POPE	135	0.014	569	0.061	127	0.013	
CDCC	CDCC	DOPE	44	0.004	190	0.020	234	0.025	
CCCC	CDCC	P1PE	90	0.009	380	0.041			
CCCC	CDCC	PQPE	22	0.002	95	0.010			
CCCC	DODC	PAPE	124	0.013	522	0.056	312	0.031	
DODDC	DODDC	DAPE	78	0.008	332	0.038	574	0.061	
CCCC	DODDC	PDPE	44	0.004	190	0.020	500	0.050	
DODDC	DODDC	D1PE	22	0.002	95	0.010	922	0.097	
CDCC	CDDC	O1PE					14	0.001	
CDCC	DODC	DAPE					27	0.003	
CDCC	DODDC	O1PE					68	0.007	
Total:			559	0.056	2373	0.254	1093	0.110	
Sphingomyelin - SM									
TCC	CCCC	DPSM	611	0.062	279	0.030	581	0.058	
TCCC	CCCCC	DSM	133	0.013	61	0.007	143	0.015	
TCCOC	CCCCC	DXSM	247	0.025	113	0.012			
TCC	CDCC	DPSM	38	0.004	17	0.002	71	0.007	
TCC	CCDC	PGSM	38	0.004	17	0.002			
TCC	CCCDCC	PSM	381	0.038	174	0.019	132	0.013	
TCC	CCCC	PSM					32	0.003	
TCCC	CCCDCC	BNSM	191	0.019	86	0.009	108	0.011	
TCCOC	CCCDCC	XNSM	287	0.027	121	0.013	27	0.003	
Total:			1906	0.192	868	0.093	892	0.089	
Phosphatidyserine - PS									
CCCC	CCCC	DPPS					219	0.023	
CCCC	CDCC	POPS					46	0.005	
							232	0.025	
								200	0.021



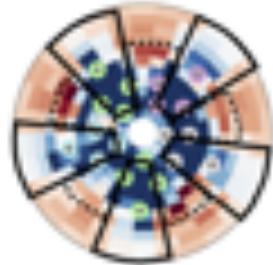
...heteroacids!



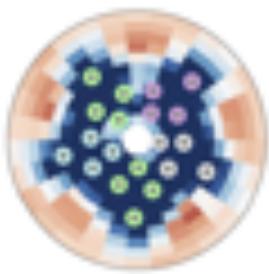
we still see protein-local lipid ordering

chain density

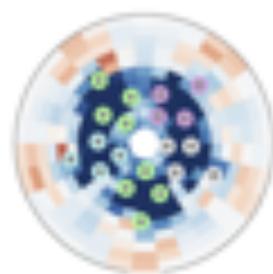
Chol



Sat



PUFA

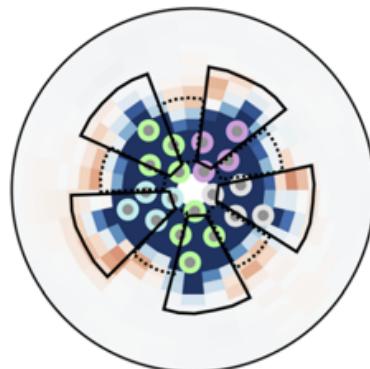


$\log \tilde{\rho}$

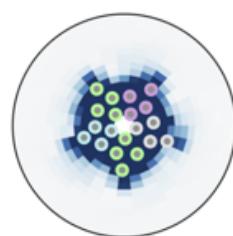


enriched

headgroup density



Anionic



Zwitterionic

we still see protein-local lipid ordering

Spontaneous lipid binding to the nicotinic acetylcholine receptor in a native membrane

Cite as: J. Chem. Phys. 154, 185102 (2021); doi: 10.1063/5.0046333

Submitted: 2 February 2021 • Accepted: 18 April 2021 •

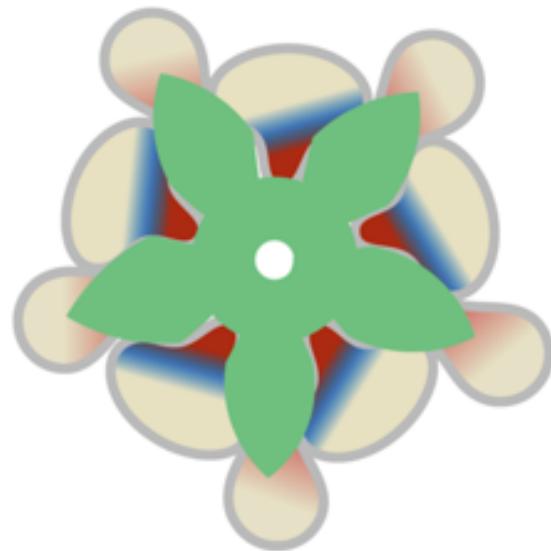
Published Online: 13 May 2021



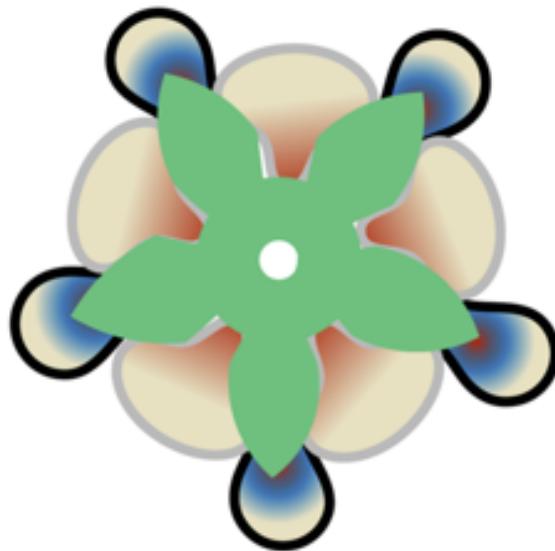
Liam Sharp¹ and Grace Brannigan^{1,2,a)}

new analysis method for **CG lipid binding affinities without kinetic calculations or enhanced sampling.**

Outer Leaflet



Inner Leaflet



PUFA

Saturated

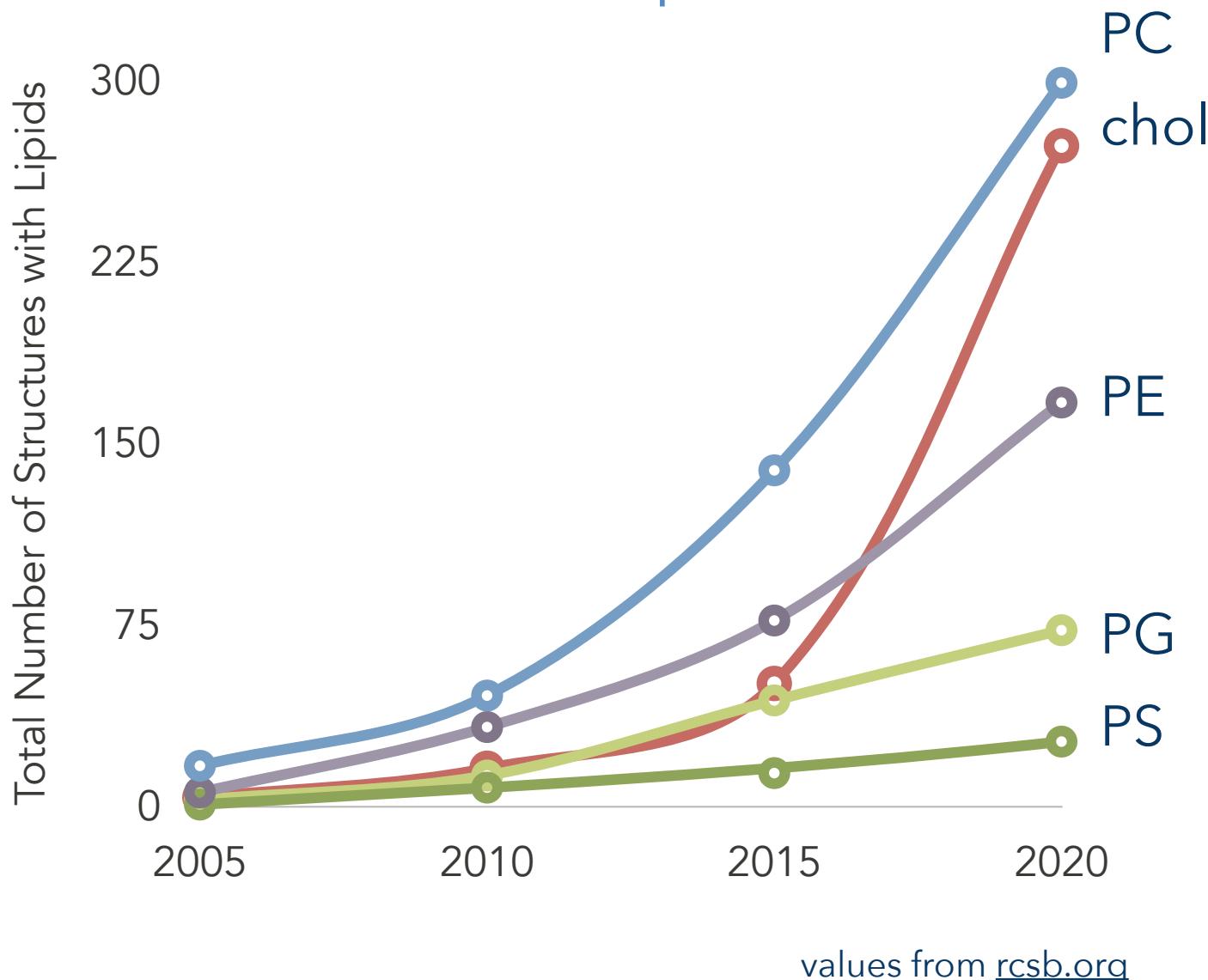
Cholesterol

Neutral

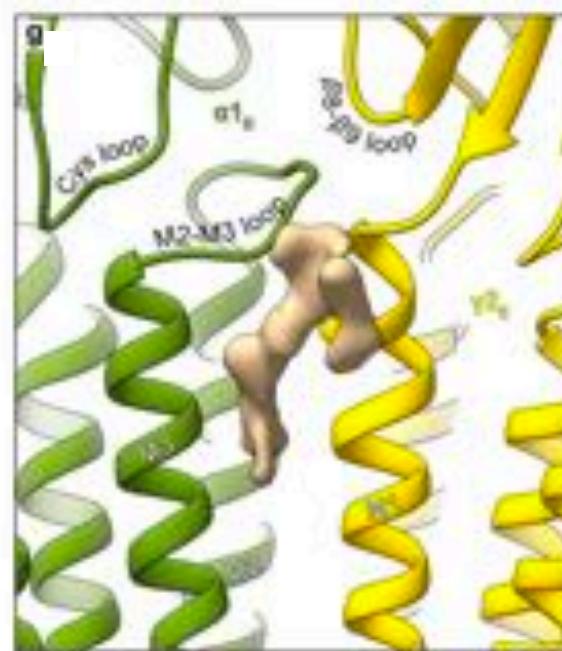
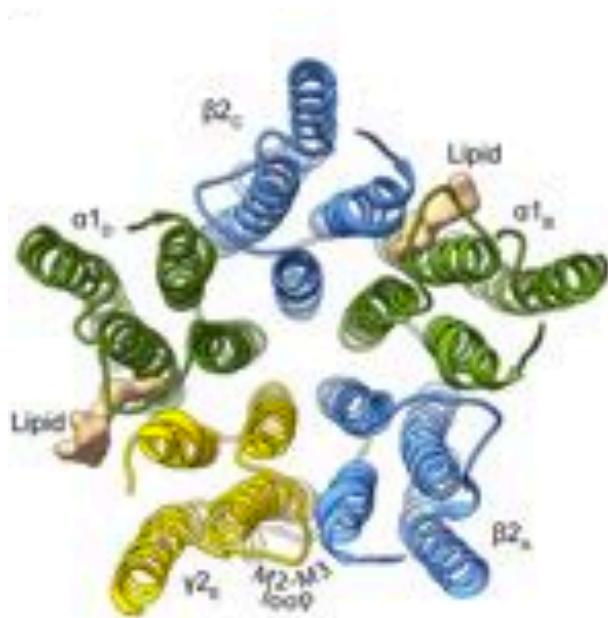
Anionic

Lipid binding with atomistic resolution?

Structures tell us **where** lipids bind...

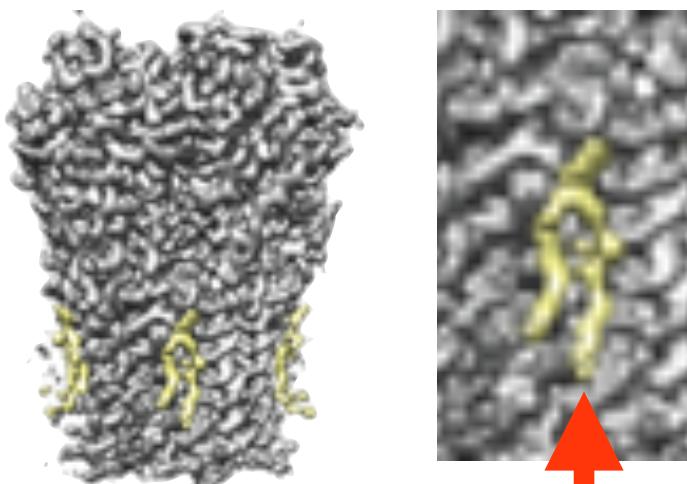
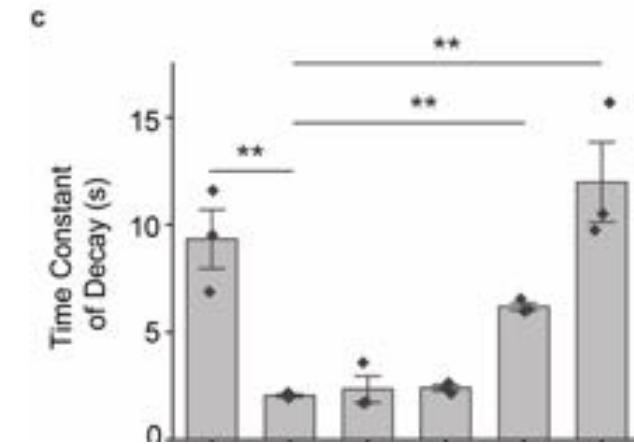


...but not who is binding.



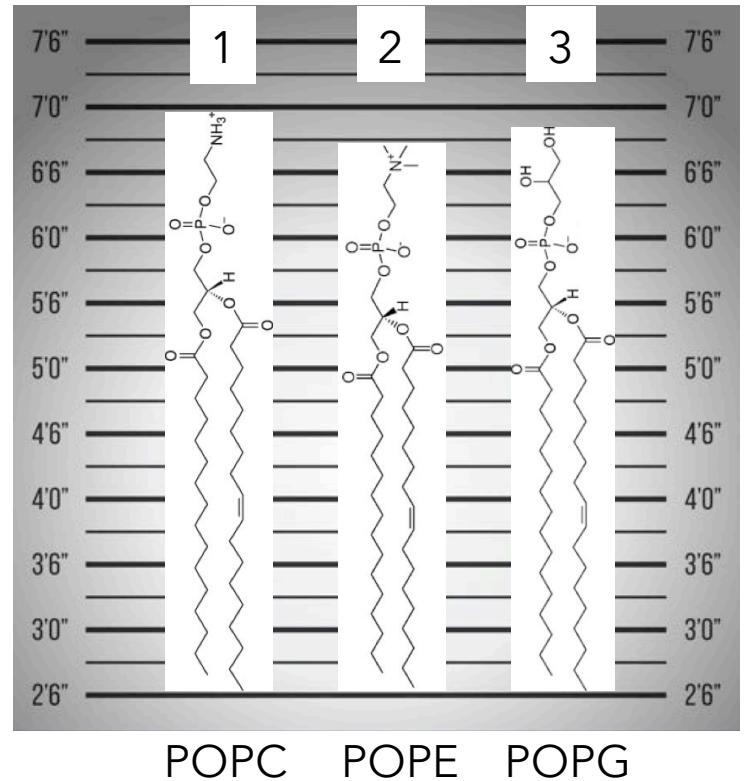
Kim...Hibbs, *Nature*, 2020

Case of the ELIC modulation site



Who is this?

Suspects



Plan: adapt a classic method (FEP) for a new purpose

New FEP Flavor: SAFEP

Streamlined Alchemical Free Energy Perturbation

FEP but in a site-centered reference frame.

Great for superficial sites! (Also buried sites)

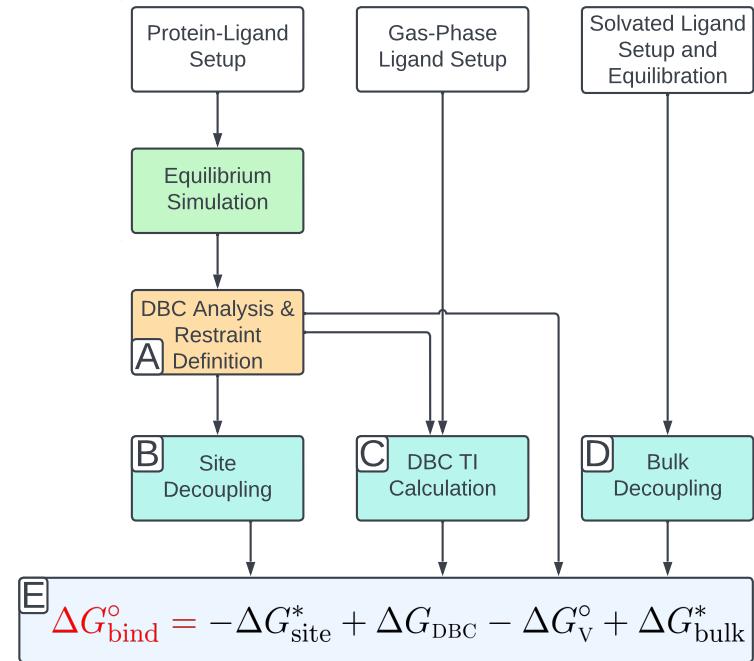
Theory-heavy
and abstract



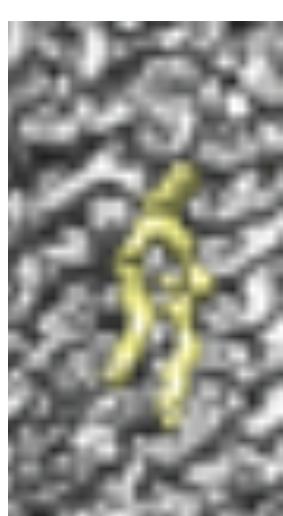
Applied;
includes
tutorial &
protocol

**Computing absolute binding
affinities by Streamlined Alchemical
Free Energy Perturbation**

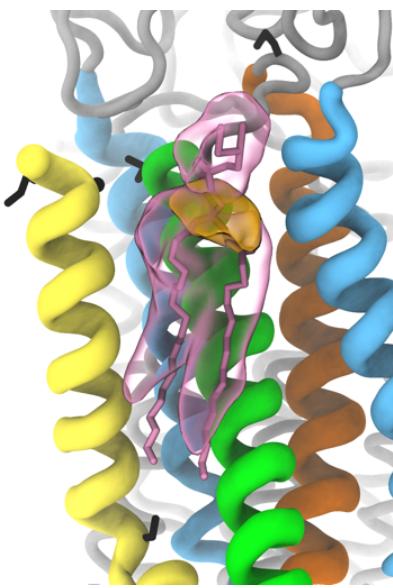
Ezry Santiago-McRae^{1†}, Mina Ebrahimi^{2,3†}, Jesse W. Sandberg¹, Grace Brannigan^{1‡},
Jérôme Hénin^{2,3‡}



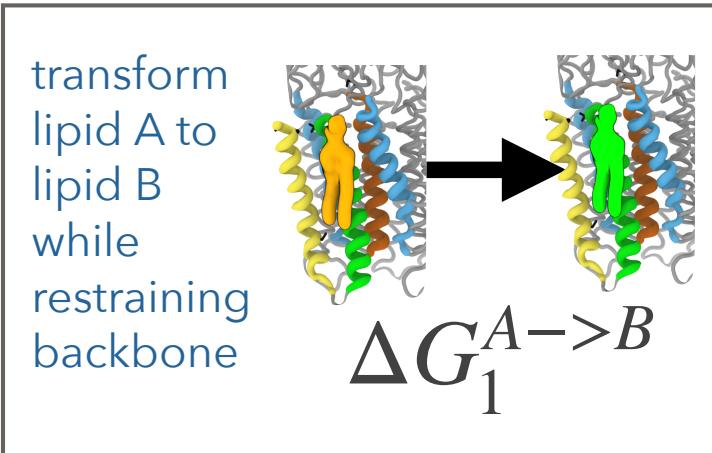
Using SAFEP to catch lipids



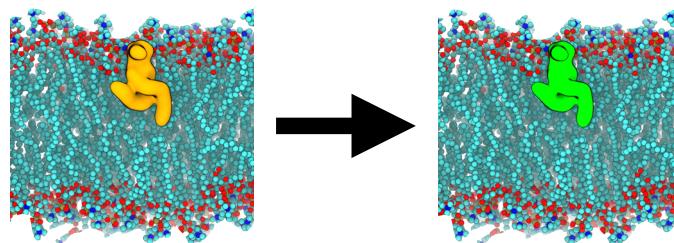
Model each lipid and then simulate with basic MD



Determine allowable deviation of the glycerol backbone



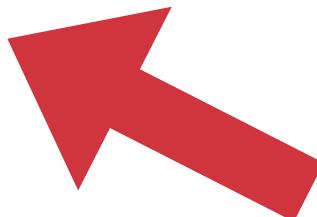
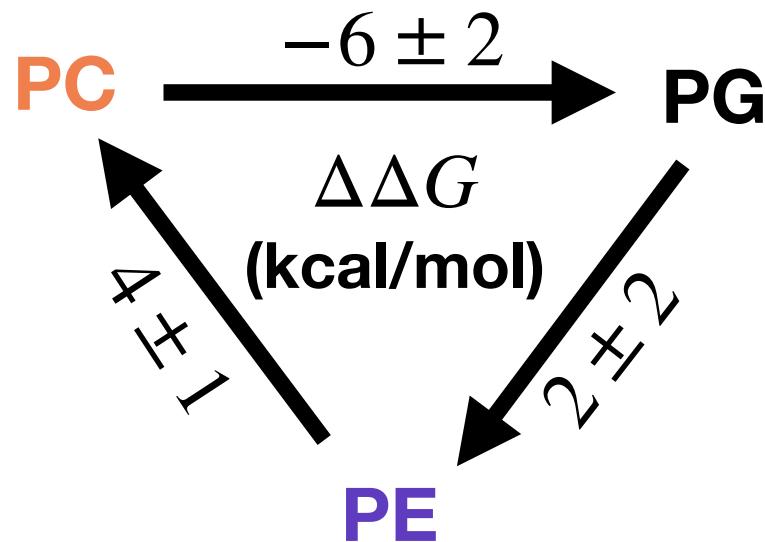
transform lipid A to lipid B while restraining headgroup



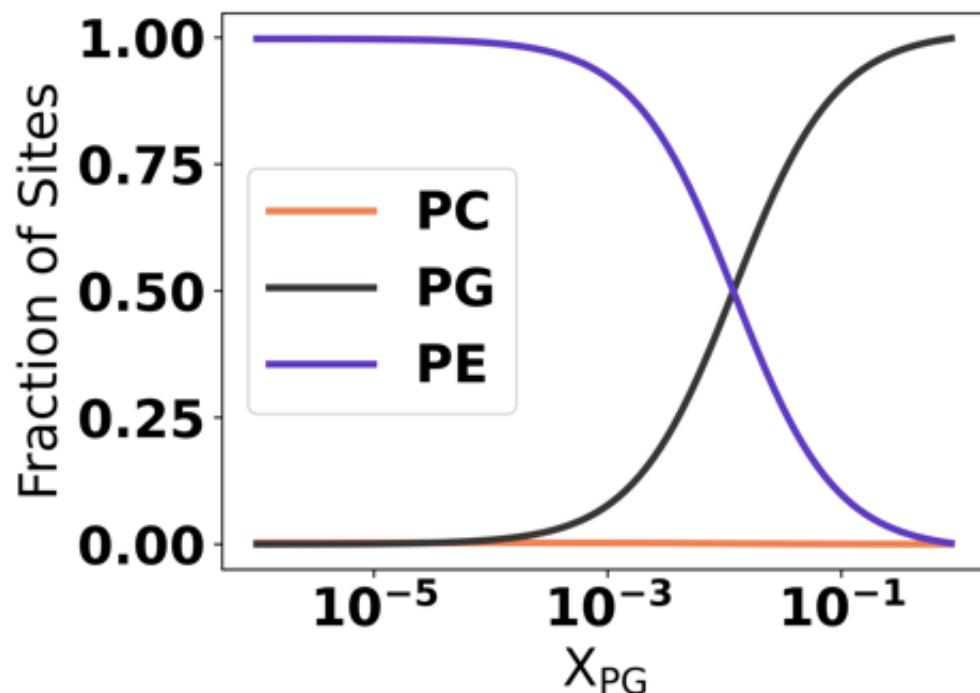
$$\Delta G_2^{A \rightarrow B}$$

If $\Delta G_1^{A \rightarrow B} - \Delta G_2^{A \rightarrow B} < 0$,
then lipid B is more likely!

SAFEP applied to our ELIC suspects



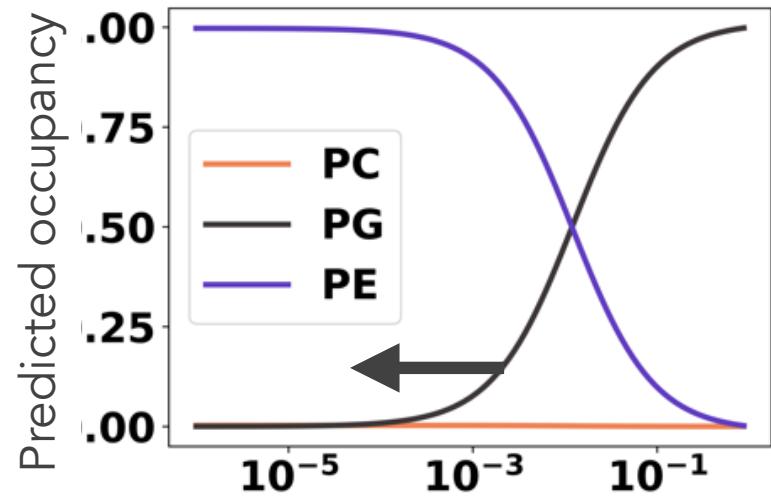
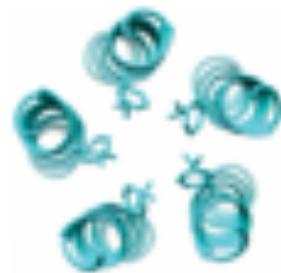
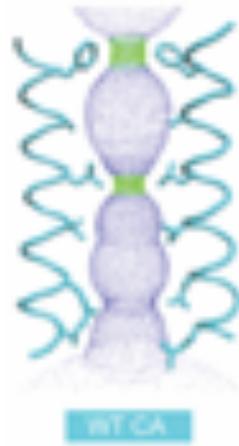
At the
scene!



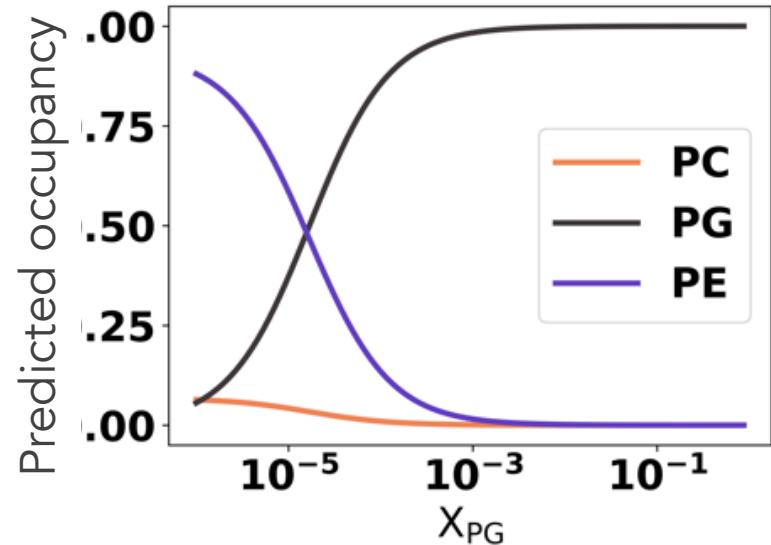
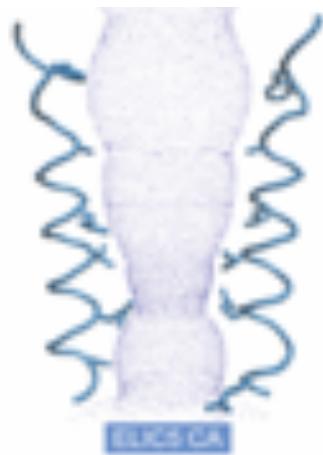
PG was in the outer
TMD binding
site....but did it do
anything?

state dependent lipid binding

pre-active conformation

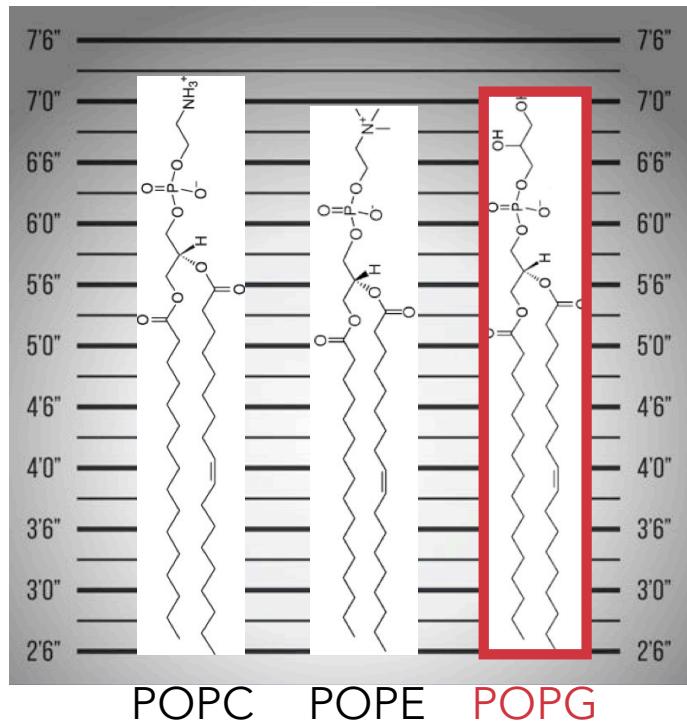


wide-open conformation

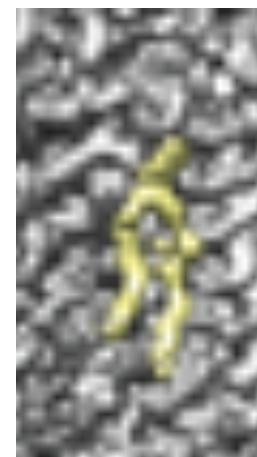
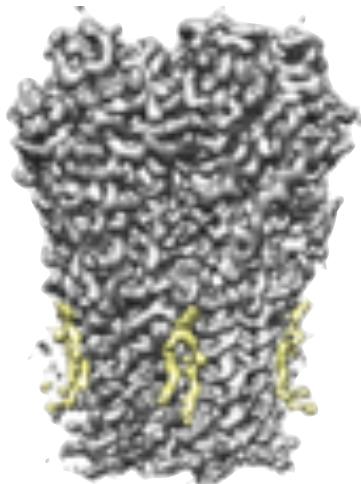


Summary

Who



Where



When

When you have
at least 1-5% PG

Two stories

1. Catching guilty lipids
2. **Catching guilty amino acids**

Brief Communication

The Brain-Derived Neurotrophic Factor Val66Met Polymorphism and Variation in Human Cortical Morphology

Lukas Pezawas, Beth A. Verchinski, Venkata S. Mattay, Michael F. Egan, Andreas Meyer-Lindenberg, and Daniel Weinberger^{1,2}
Genes, Cognition, and Psychosis Program, National Institute of Mental Health, Bethesda, Maryland 20892, USA

Brain-Derived Neurotrophic Factor Val66Met Polymorphism in Psychiatric Disorders: Major Depressive Disorder Studies Confirm Association with Major Psychiatric Disorders, Eating Disorders, and Substance Abuse

Mònica Gratacòs, Juan R. González, Josep M. Martí, and Xavier Estivill¹
¹Department of Genetics, University of Valencia, Valencia, Spain

Cell

Volume 112, Issue 2, 24 January 2003, Pages 257-266

Article
The BDNF val66met Polymorphism Alters Activity-Dependent Secretion of BDNF and Human Memory and Hippocampal Function

Michael F. Egan¹, Masami Kojima^{7, 4, 5, 6}, Joseph H. Callicott^{7, 1}, Terry E. Goldberg^{7, 1}, Bhaskar S. Kolachana¹, Alessandro Bertolino¹, Eugene Zaitsev⁴, Bert Gold³, David Goldman², Michael Dean³, Bai Lu^{4, 6}, Daniel R. Weinberger^{1, 2},  

Molecular Psychiatry (2010) 15, 260–271
© 2010 Nature Publishing Group. All rights reserved 1359-4184/10 \$32.00
www.nature.com/mp

ORIGINAL ARTICLE

Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity

M Verhagen¹, A van der Meij¹, PAM van Deurzen^{1,2}, JGE Janzing¹, A Arias-Vásquez^{1,3,4}, JK Buitelaar^{1,2} and B Franke^{1,3}

A Genetic Variant BDNF Polymorphism Alters Hippocampal Volume in Both

Giuliano Sartori,^{1,2} Rebecca M. Jones,^{1,2} Michael F. Egan,^{1,2} Leah H. Somerville,^{1,2} Daniel R. Weinberger^{1,2}, Theresa Teslovich,^{1,2}  

BDNF Val66Met Polymorphism Alters Hippocampal Volume

Sergio Sandro Ieraci,¹ Tanvir Khan,¹ Mingming Yang,⁵ Bruce S. McEwen,⁶  

(2005) 10, 631–636
© 2005 Nature Publishing Group. All rights reserved 1359-4184/05 \$30.00
DOI: 10.1038/sj.mp.4001130

Val66met polymorphism

and volume of the hippocampal formation

PR Szeszko^{1,2}, R Lipsky³, C Mentschel⁴, D Robinson^{1,2}, H Gunduz-Bruce⁵, S Sevy^{1,2}, M Ashtari⁶, B Napolitano¹, RM Bilder⁷, JM Kane^{1,2}, D Goldman³ and AK Malhotra^{1,2}  

Results: 2,258

(from Web of Science Core Collection)

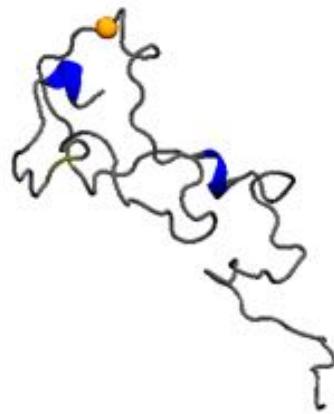
You searched for: TOPIC: (Val66Met)
...More

how?????

How can a hydrophobic to hydrophobic mutation - in a disordered protein - cause such significant functional and conformational effects?

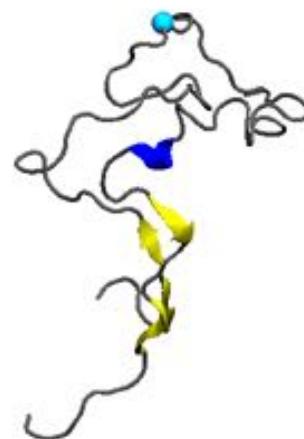
T-REMD; 2 microseconds; 48 replicas; explicit solvent residues 23-113,

signal to noise nightmare!



V66

300K replica



M66

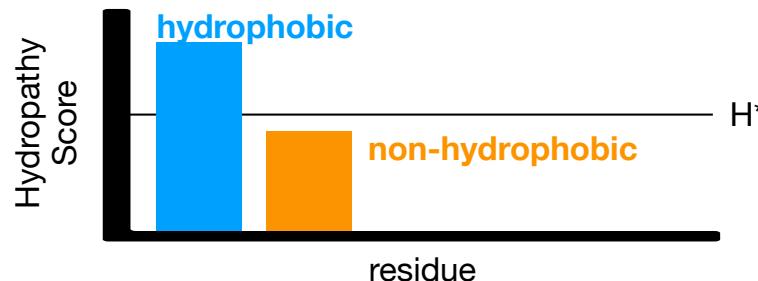
300K replica

$O(91^2)$ possible interactions

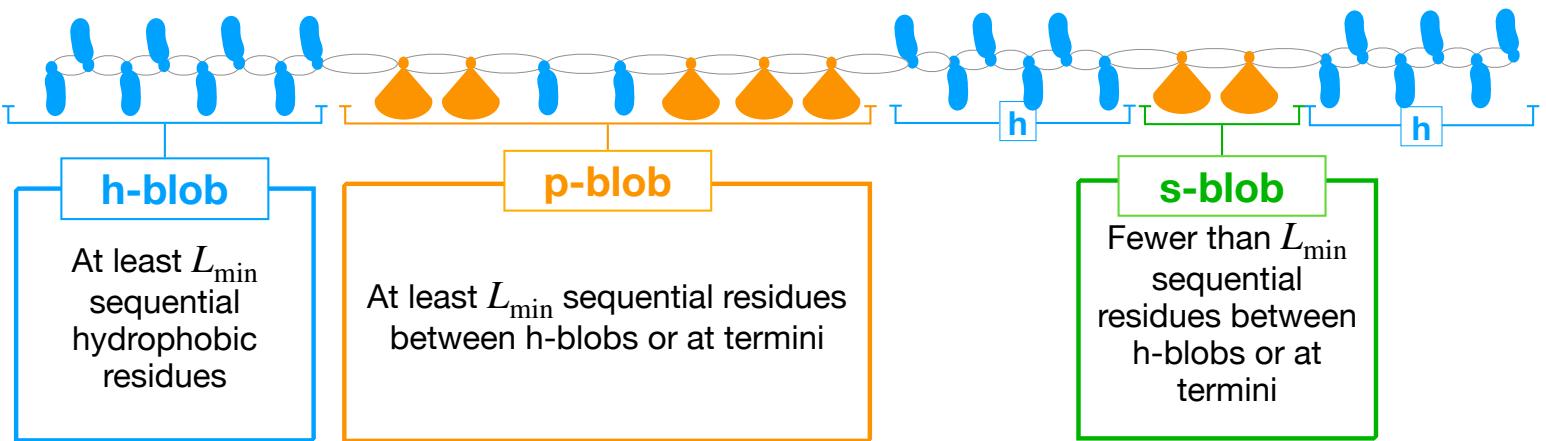
needed to coarse-grain interactions for analysis

introducing blobulation

original sequence



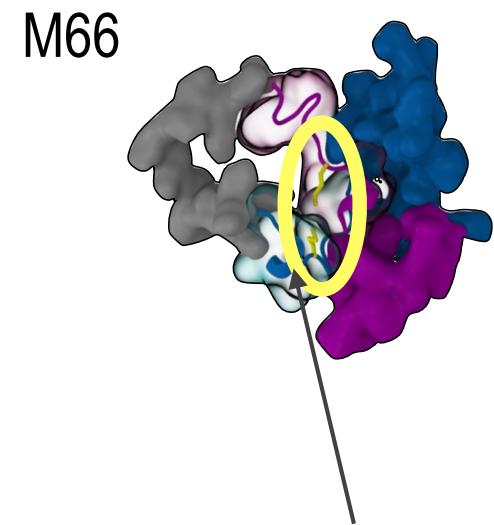
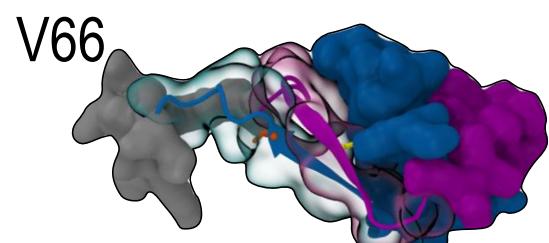
digitized sequence



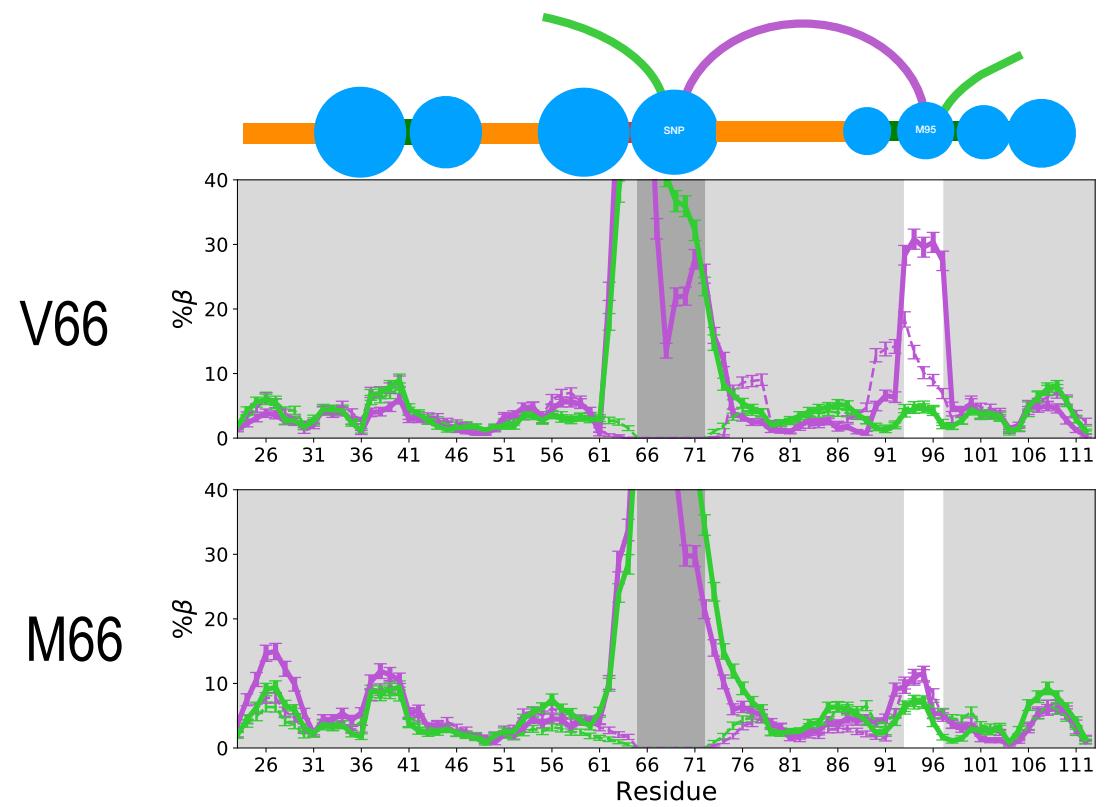
blobulated sequence



proBDNF mechanism: Met-Met interactions



Our mutation has an accomplice! Met-Met Interactions switch backbone-backbone to sidechain-sidechain



PLOS COMPUTATIONAL BIOLOGY

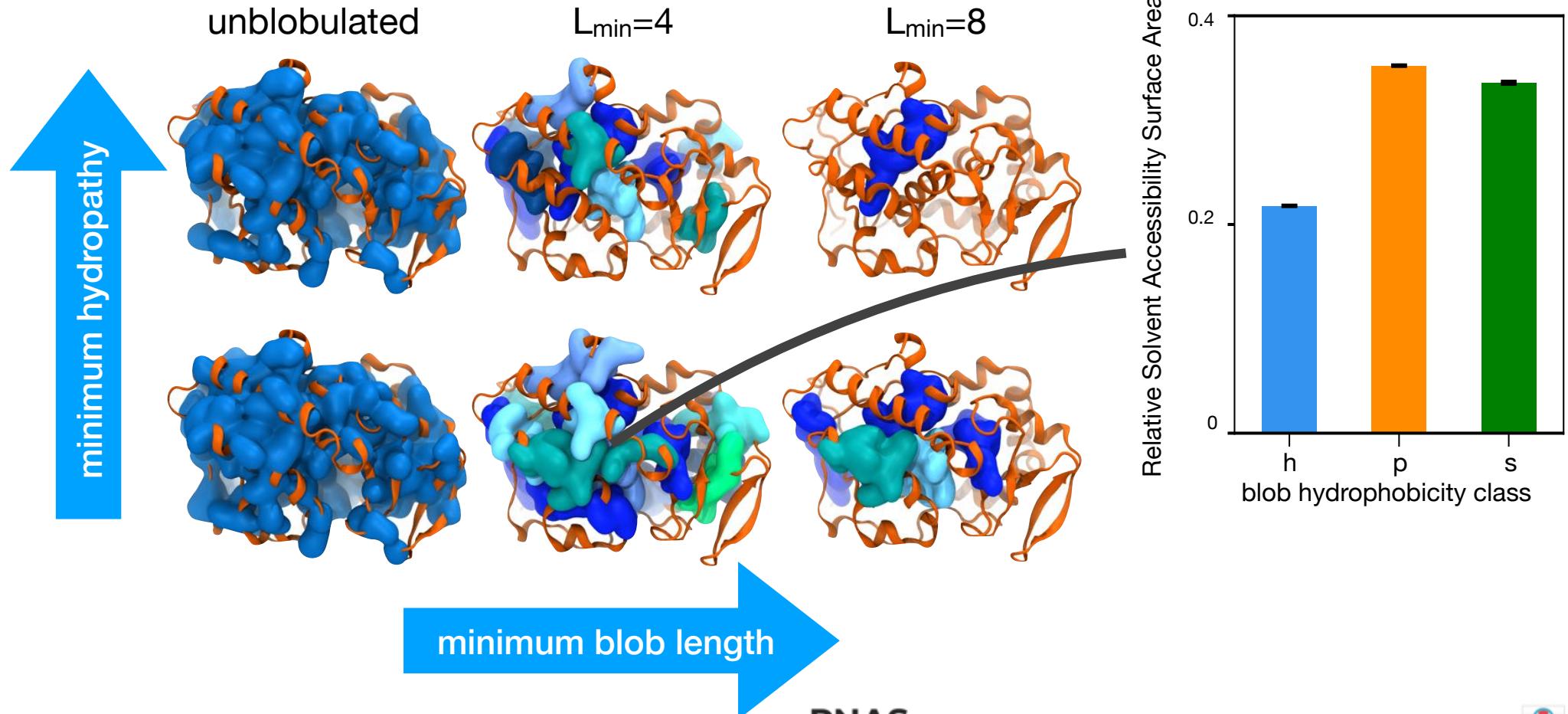
RESEARCH ARTICLE

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*}

is blobulation a meaningful generic sequence-analysis approach?

example: cytochrome C peroxidase



PNAS

RESEARCH ARTICLE

BIOPHYSICS AND COMPUTATIONAL BIOLOGY

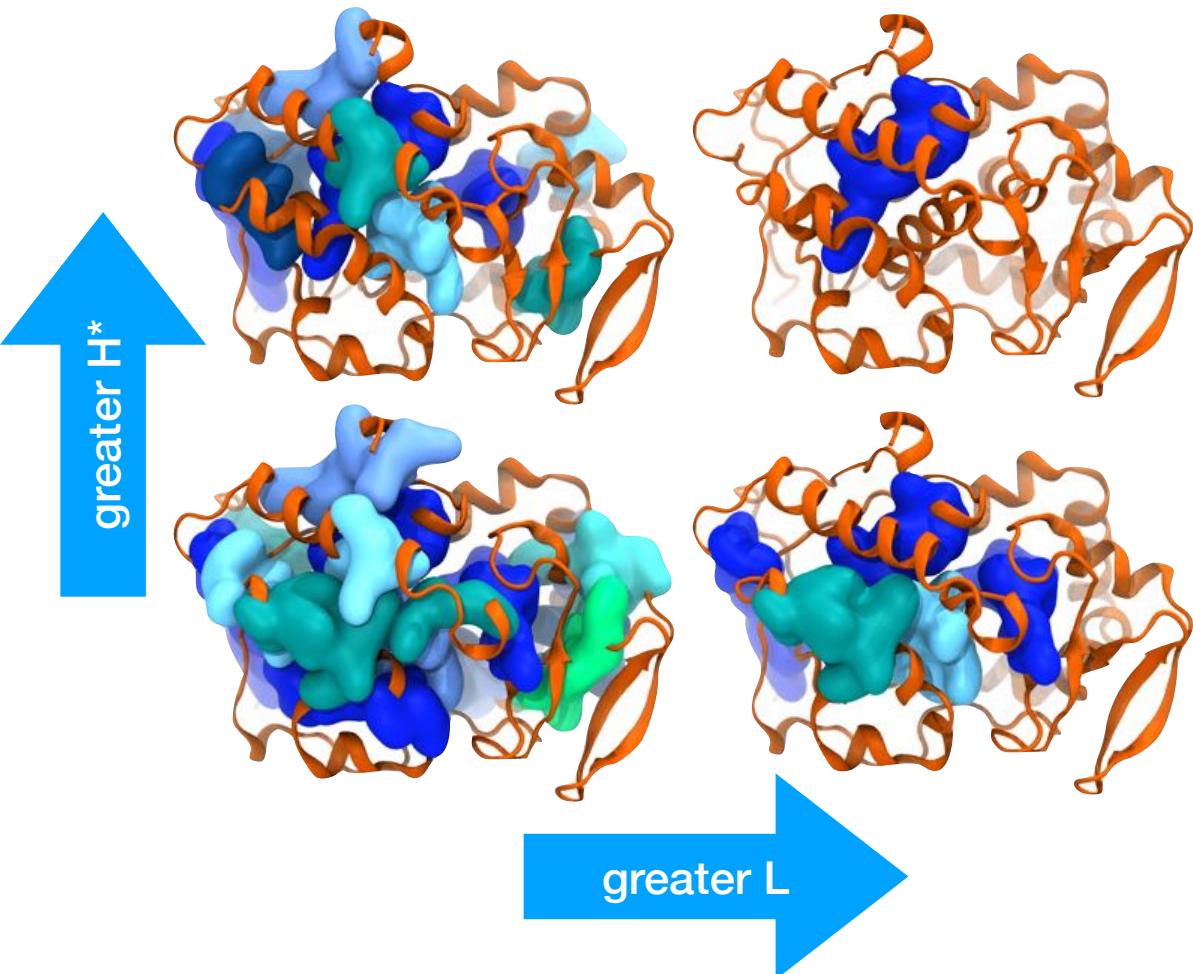


Contiguously hydrophobic sequences are functionally significant throughout the human exome

Ruchi Lohia^{1,2}*, Matthew E. B. Hansen^{1,2}*, and Grace Brannigan^{3,4,12}†

Edited by Ken Dill, Stony Brook University, Stony Brook, NY; received September 12, 2021; accepted February 2, 2022

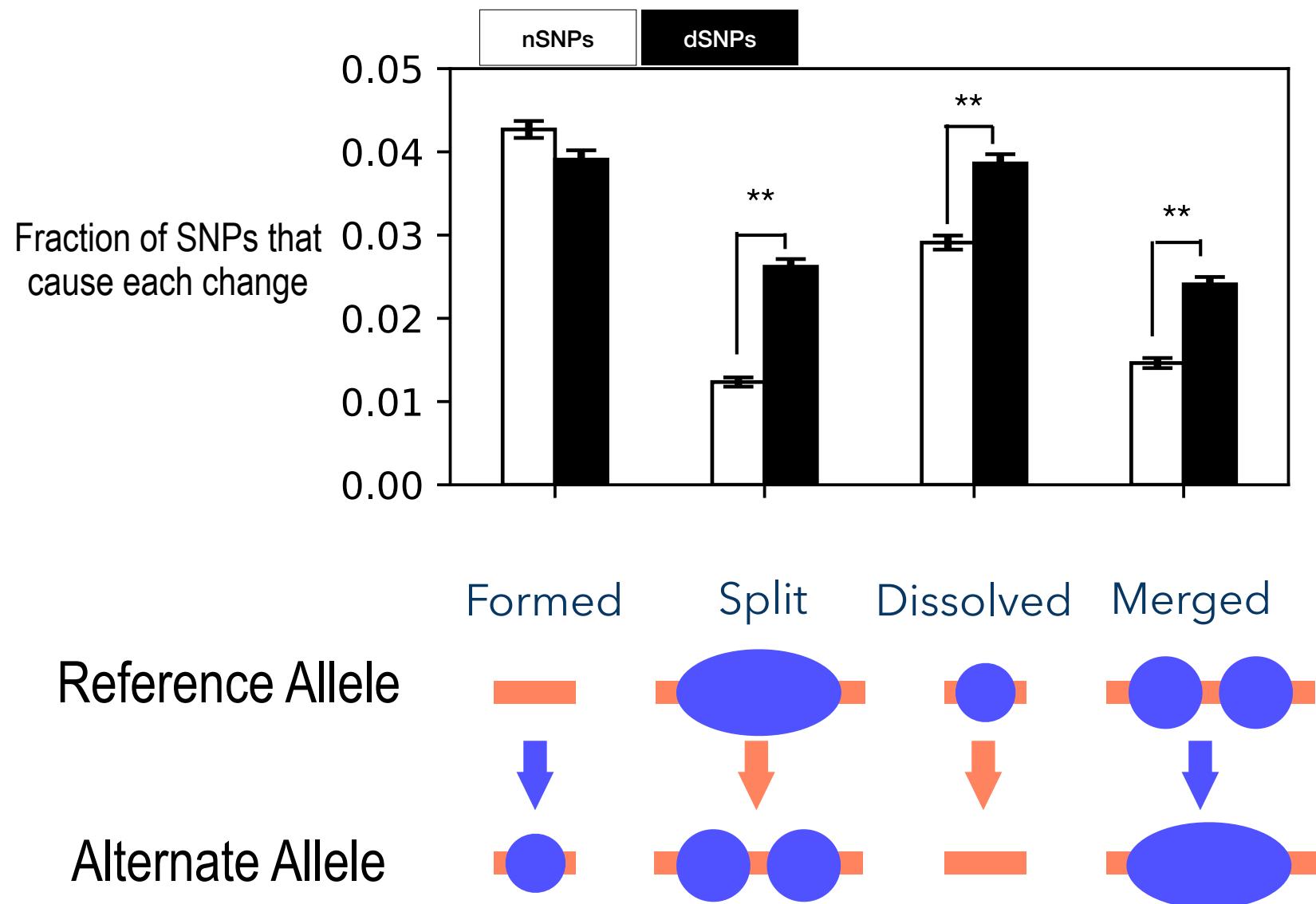
Hypothesis: h-blobs will be more mutation sensitive...



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

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

disease-causing mutations change blob topology

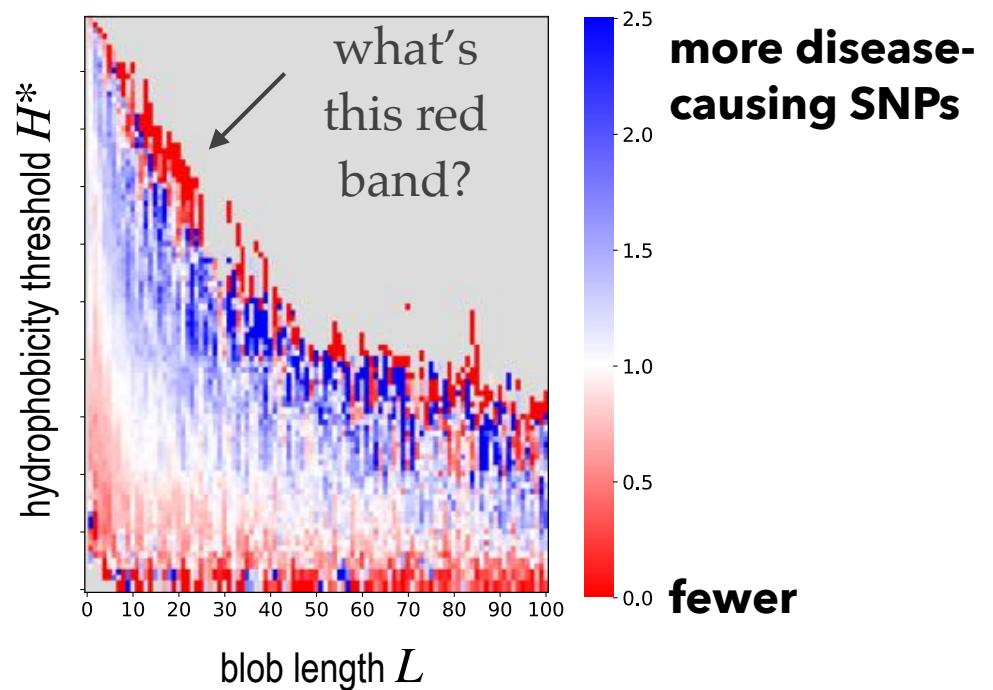


enrichment of disease-associated SNPs (dSNPs)

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)



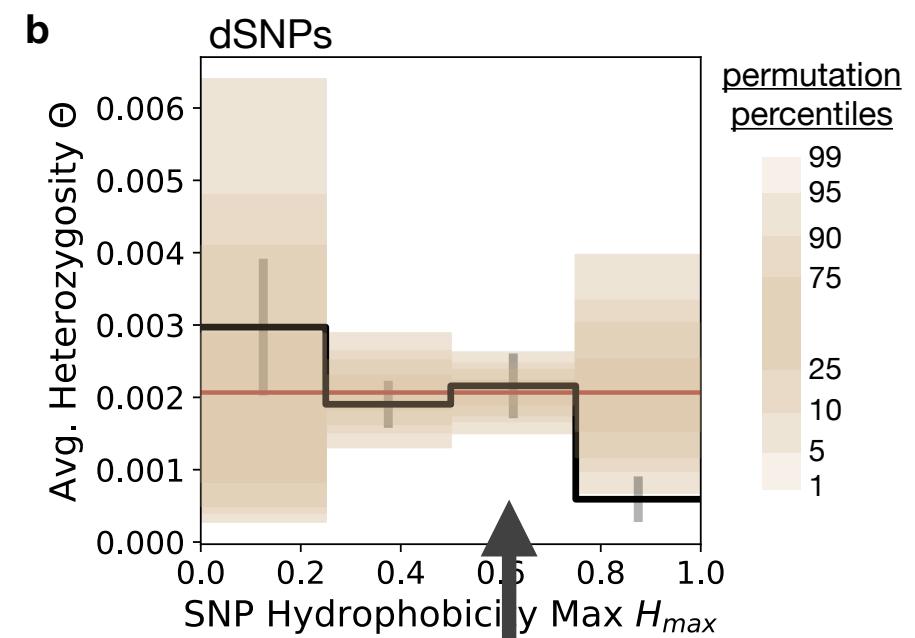
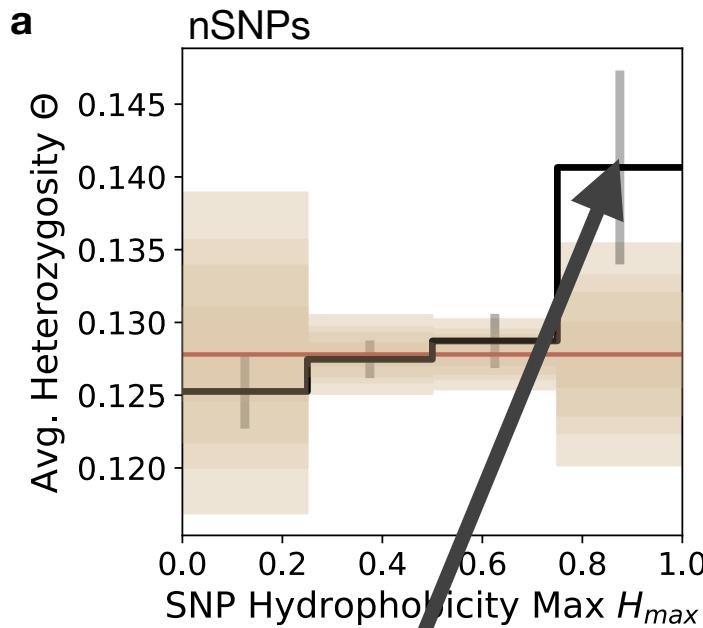
mutations in very long **or** very hydrophobic blobs: most likely to be disease-associated

Why the exception to the trend?

dSNPs/nSNPs plunges for most extreme blobs:

- ✓ Possibility 1: more nSNPs (SNPs are functional, but not deleterious)
- ✓ Possibility 2: fewer dSNPs (so deleterious, they are not observed)

gnomAD : non-Finnish Europeans

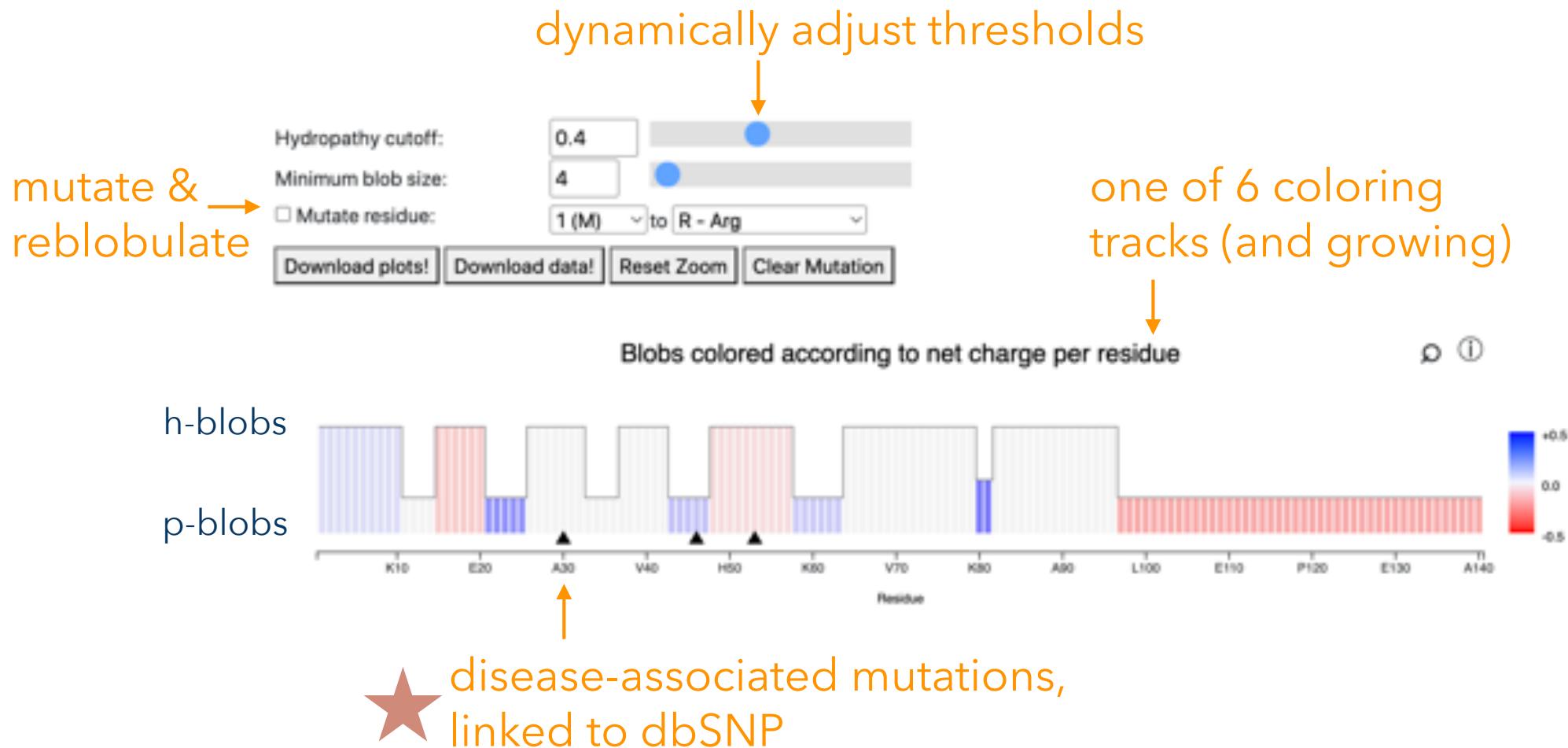


ex: olfactory receptors

ex: transporters

blobulator.branniganlab.org

Detect and visualize blobs in your own sequence

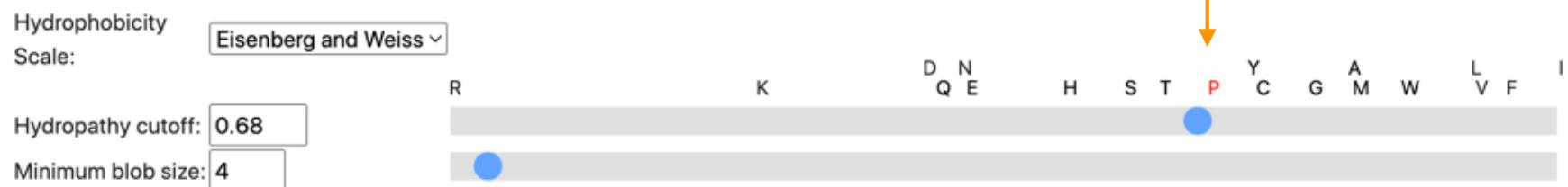


coming soon....

multiple
hydrophobicity
scales



amino-acid based
threshold selection



Summary

- blobulation yields interesting and useful **trends across the human exome** (not just in one protein)
- results are consistent with h-blobs as **physical interaction nodes**
- resulting odds ratios **could be used as priors** in prediction of causal SNPs
- **evidence for selection** on SNPs in most extreme h-blobs
- SNPs that **split h-blobs** are 3 times as likely to be **disease-associated**
- blobulation GUI at **blobulator.branniganlab.org**

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