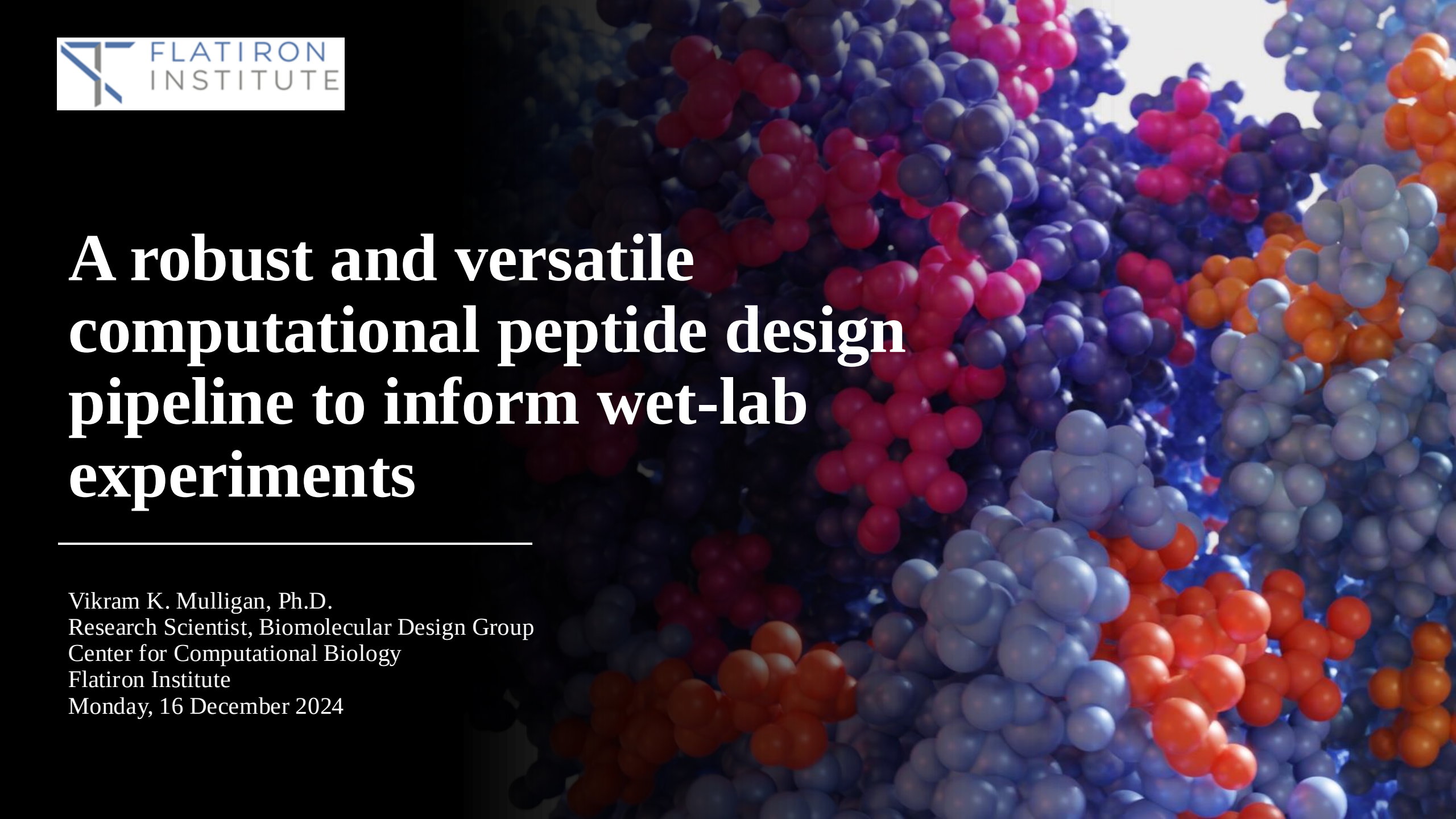


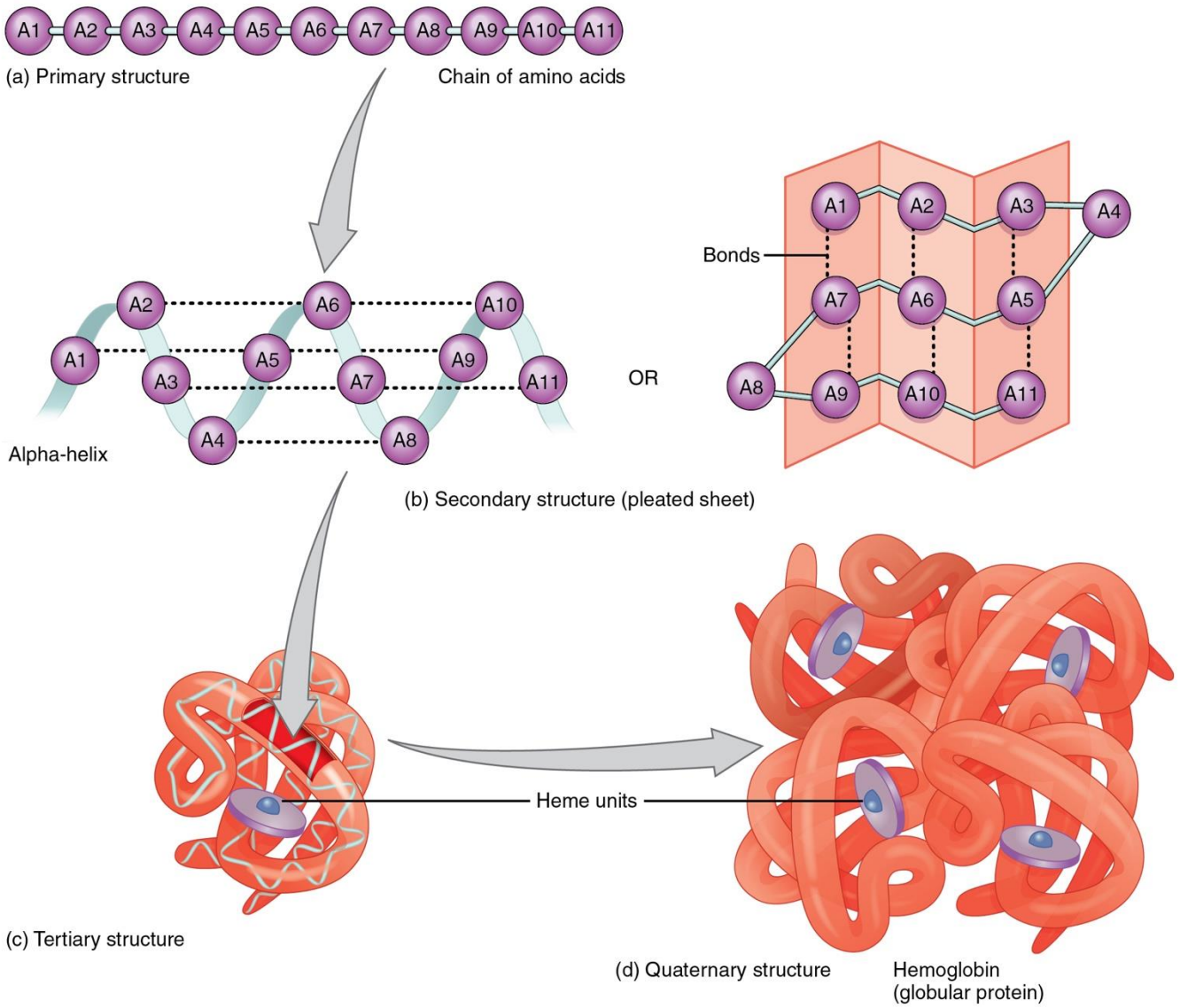
A robust and versatile computational peptide design pipeline to inform wet-lab experiments

Vikram K. Mulligan, Ph.D.
Research Scientist, Biomolecular Design Group
Center for Computational Biology
Flatiron Institute
Monday, 16 December 2024

Outline

1. Peptide and protein drug design on classical computers.
2. Enhancing peptide and protein drug design with quantum chemistry calculations (on classical computers).
3. Enhancing peptide and protein drug design with quantum computers

What do we mean by biomolecule “design”?

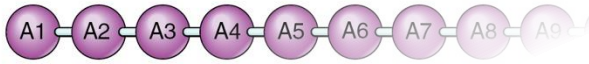


Sequence — Find a sequence of chemical building-blocks...

Fold — ...that folds into a desired structure or fold...

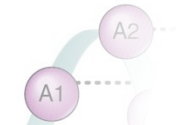
Function — ...that produces a desired function.

What do we mean by biomolecule "design"?



(a) Primary structure

But an *N*-residue peptide made from *B* different building blocks has B^N possible sequences.

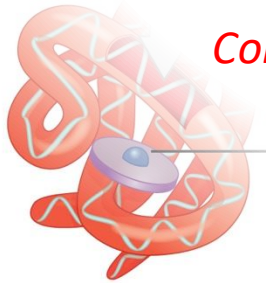


Alpha-helix

For a 10-residue peptide made from the 20 canonical amino acids, there are 20^{10}, or about 10^{13} (ten trillion), possible sequences – already too many to screen using the largest libraries that we can plausibly make.

If we increase the number of possible building-blocks to 1,000, then there are 1000^{10}, or 10^{30}, possible sequences. If you made one molecule of each, your library would weigh roughly 30 tonnes.

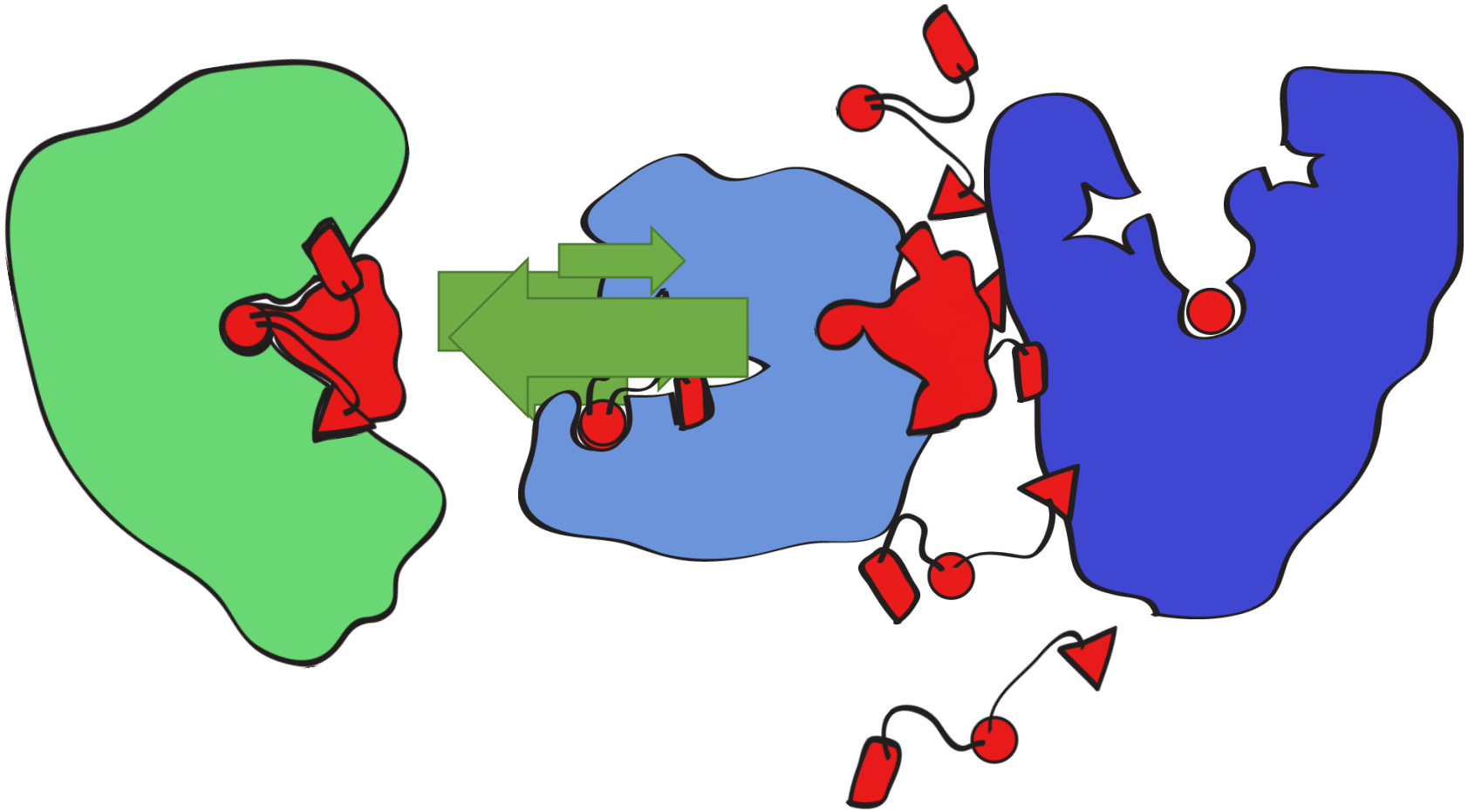
Computational design is therefore an essential complement to library screening.


(c) Tertiary structure

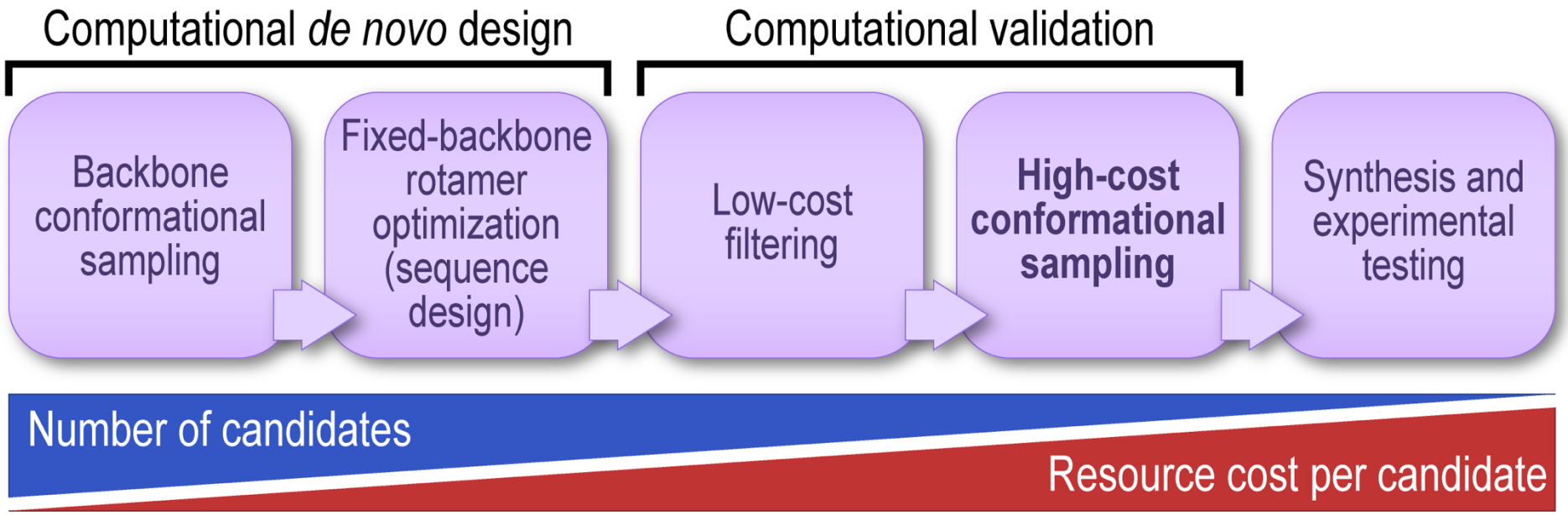
(d) Quaternary structure

Hemoglobin
(globular protein)

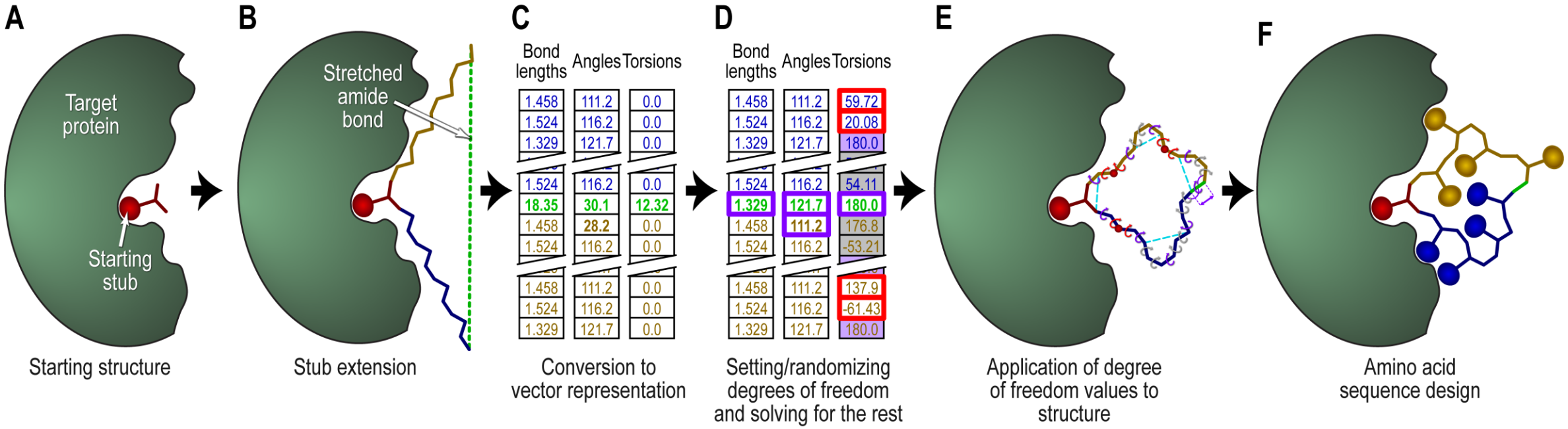
Conformational entropy: The Achilles heel of macrocycles?



Pipeline for designing rigidly-folded peptide drugs

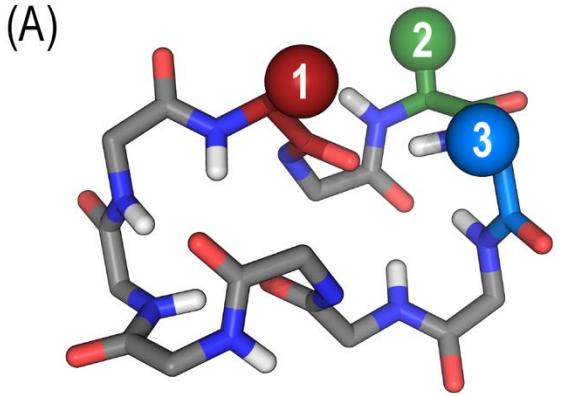


Classical peptide macrocycle design with the Rosetta software suite



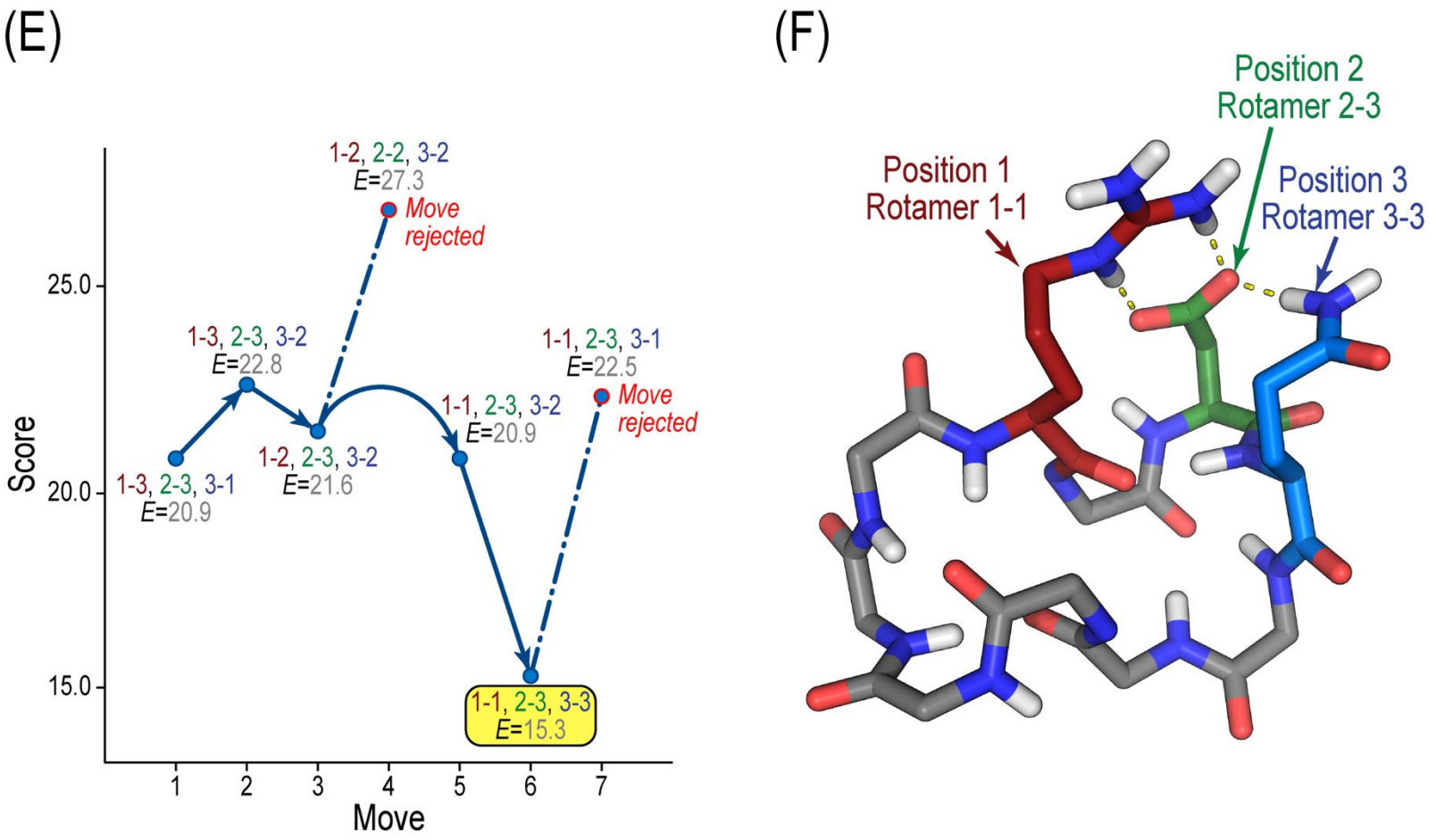
From Mulligan VK. (2022) "Computational methods for peptide macrocycle drug design." Chapter in *Peptide Therapeutics: Fundamentals of Design, Development, and Delivery*, Jois S., ed. Berlin, Germany: Springer. 2022. DOI: 10.1007/978-3-031-04544-8_3.

The rotamer optimization (design) problem



From Mulligan VK and Hosseinzadeh P. (2022) "Computational Design of Peptide-Based Binders to Therapeutic Targets." Chapter in *Approaching the Next Inflection in Peptide Therapeutics: Attaining Cell Permeability and Oral Bioavailability*, Ghodge S. V. *et al.*, eds. Washington DC: American Chemical Society.

The rotamer optimization (design) problem



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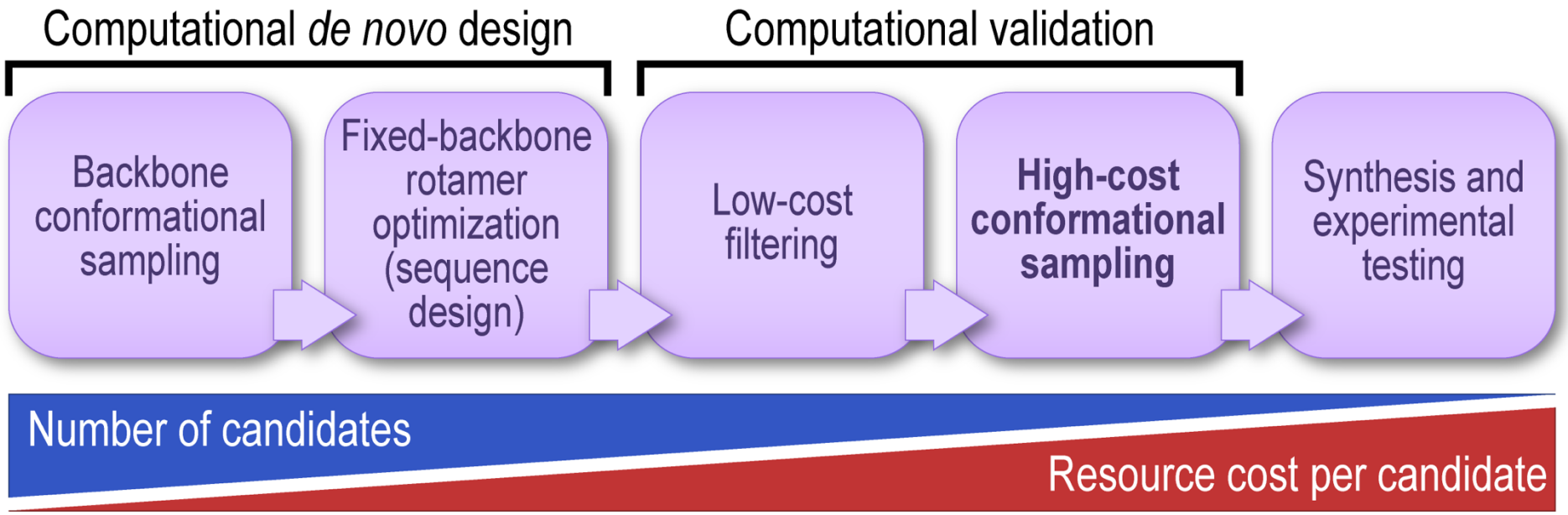
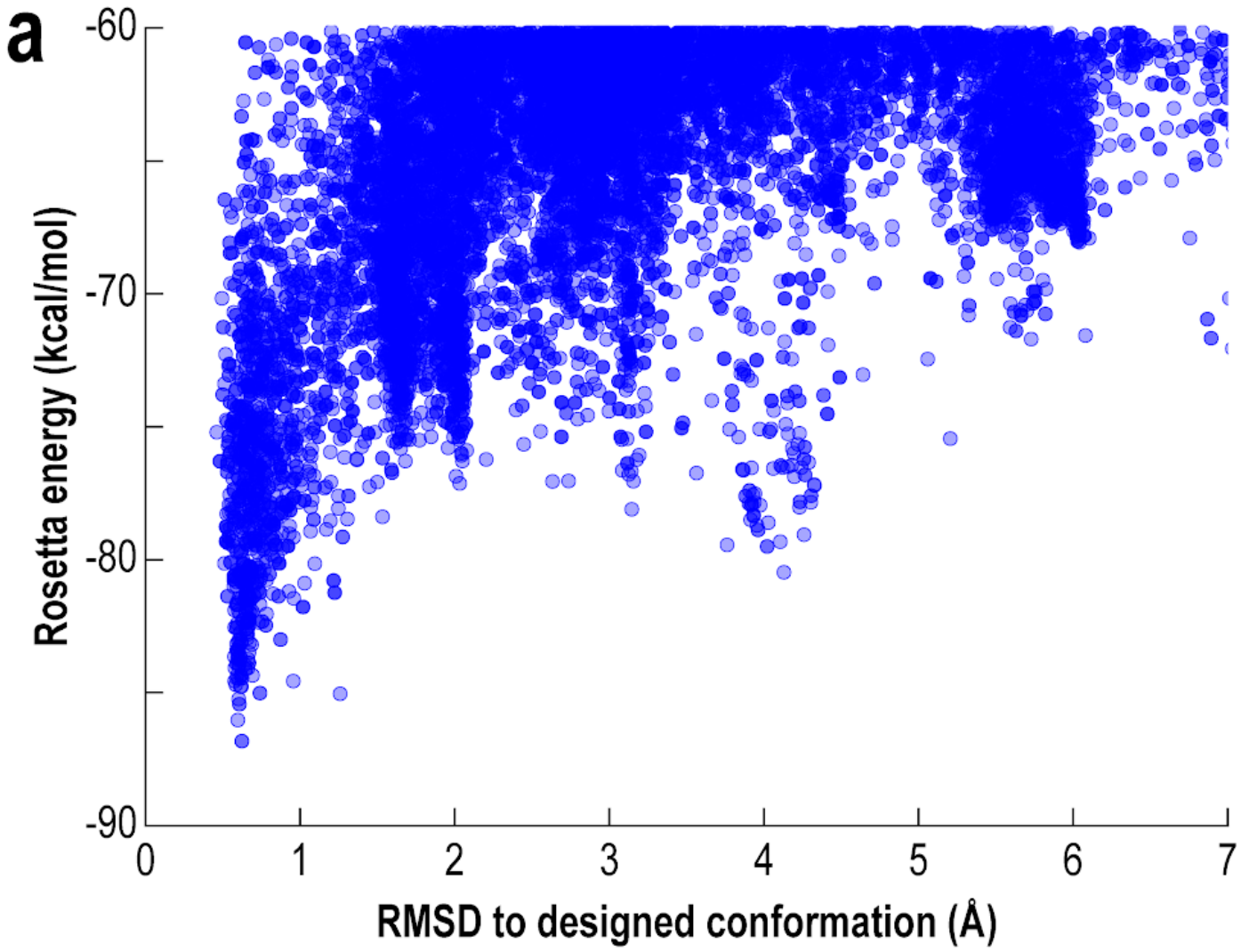
Pipeline for designing rigidly-folded peptide drugs



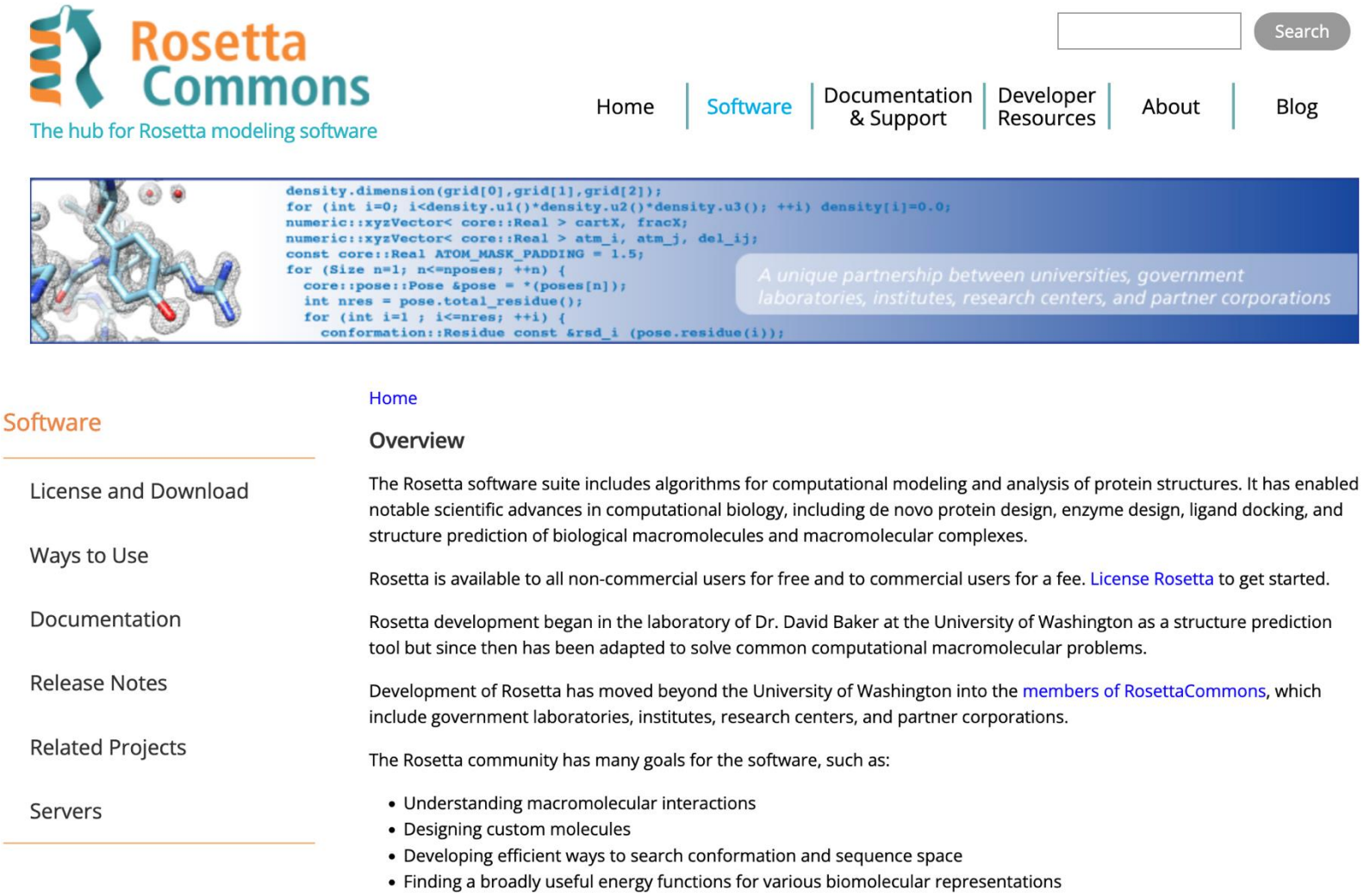
Validating peptide designs with large-scale conformational sampling (Rosetta's simple_cycpep_predict application)



$$P_{near} = \frac{\sum_{i=1}^{N} e^{\frac{-RMSD_i^2}{\lambda^2}} e^{-\frac{E_i}{k_B T}}}{\sum_{j=1}^{N} e^{-\frac{E_j}{k_B T}}}$$

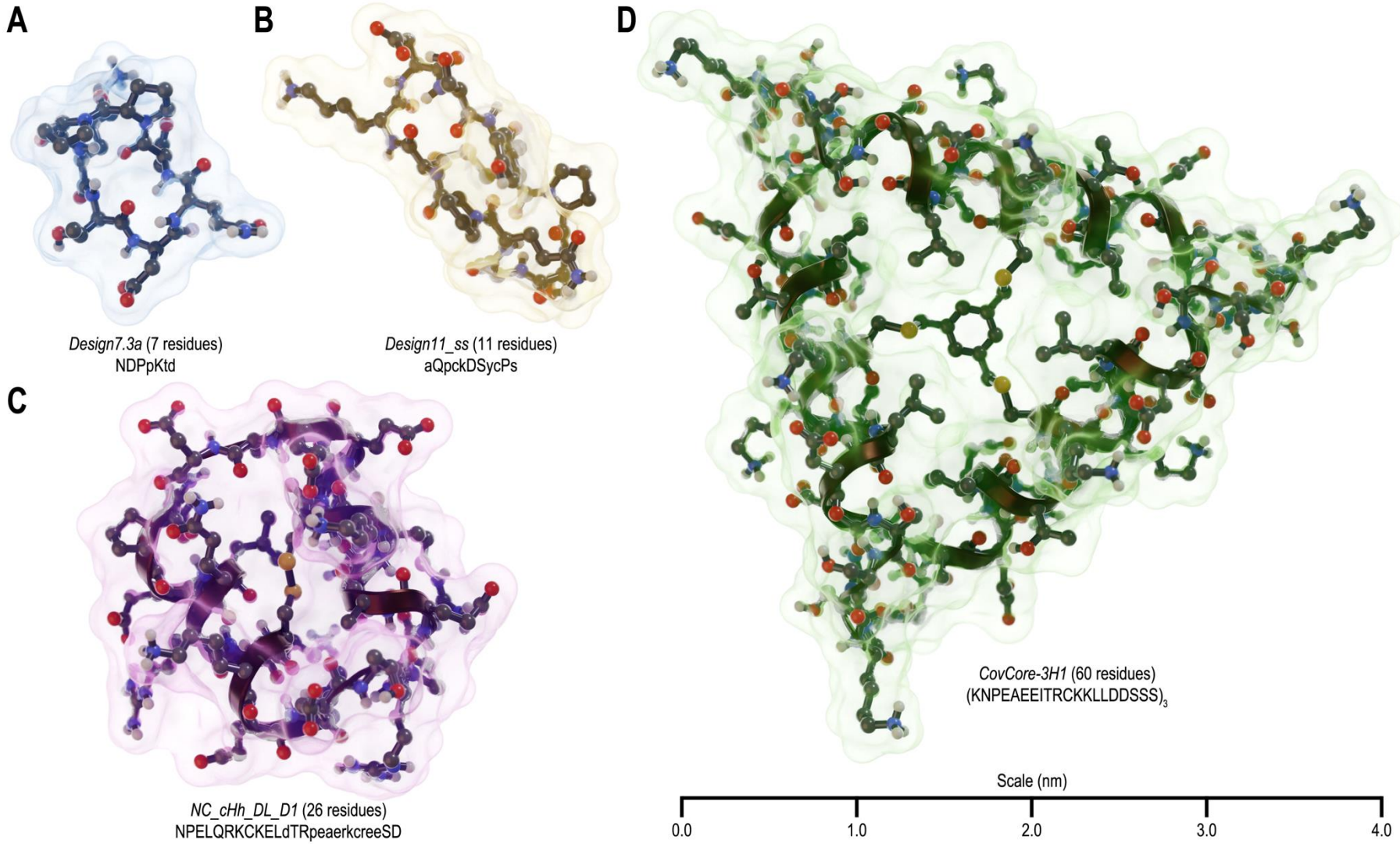
From Bhardwaj, Mulligan, Bahl *et al*. (2016) *Nature* 538(7625):329-35.

The toolkits: The Rosetta software suite



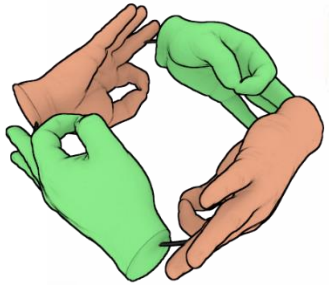
- Rosetta is protein modelling software that has been generalized for more exotic macromolecules.
- The software is free for academics, nonprofits, and governments, and is licenced for a fee for commercial use.
- Originally started in David Baker's lab, Rosetta is now developed and maintained by more than 70 labs in many countries.

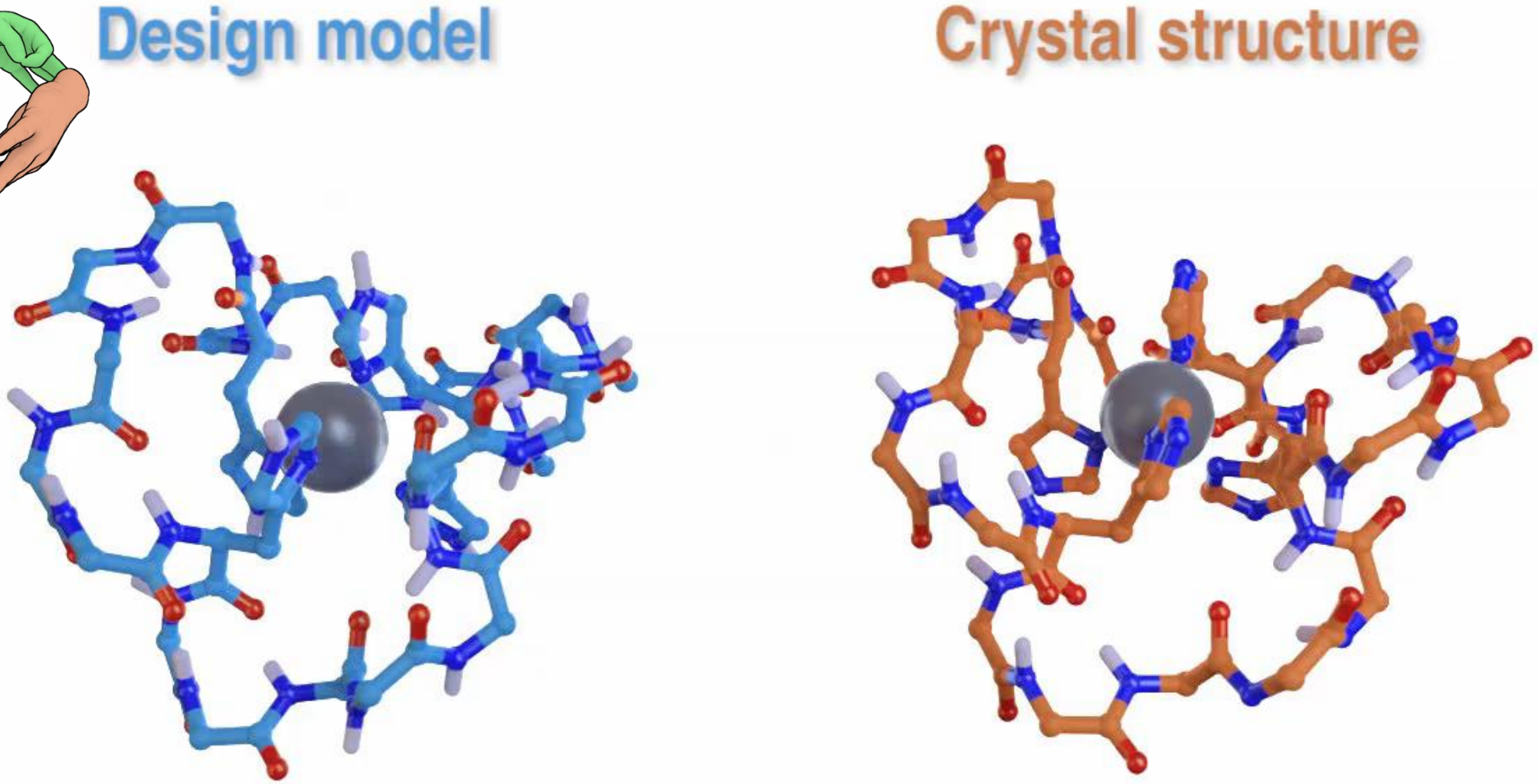
Synthetic peptides designed to fold into rigid structures with the Rosetta software suite



From Bhardwaj, Mulligan, Bahl *et al*. (2016) *Nature* 538(7625):329-35; Hosseinzadeh, Bhardwaj, Mulligan *et al*. (2017). *Science* 358(6369):1461-6; Dang, Wu, Mulligan *et al*. (2017). *Proc Natl Acad Sci USA 114(41):10852-7*.

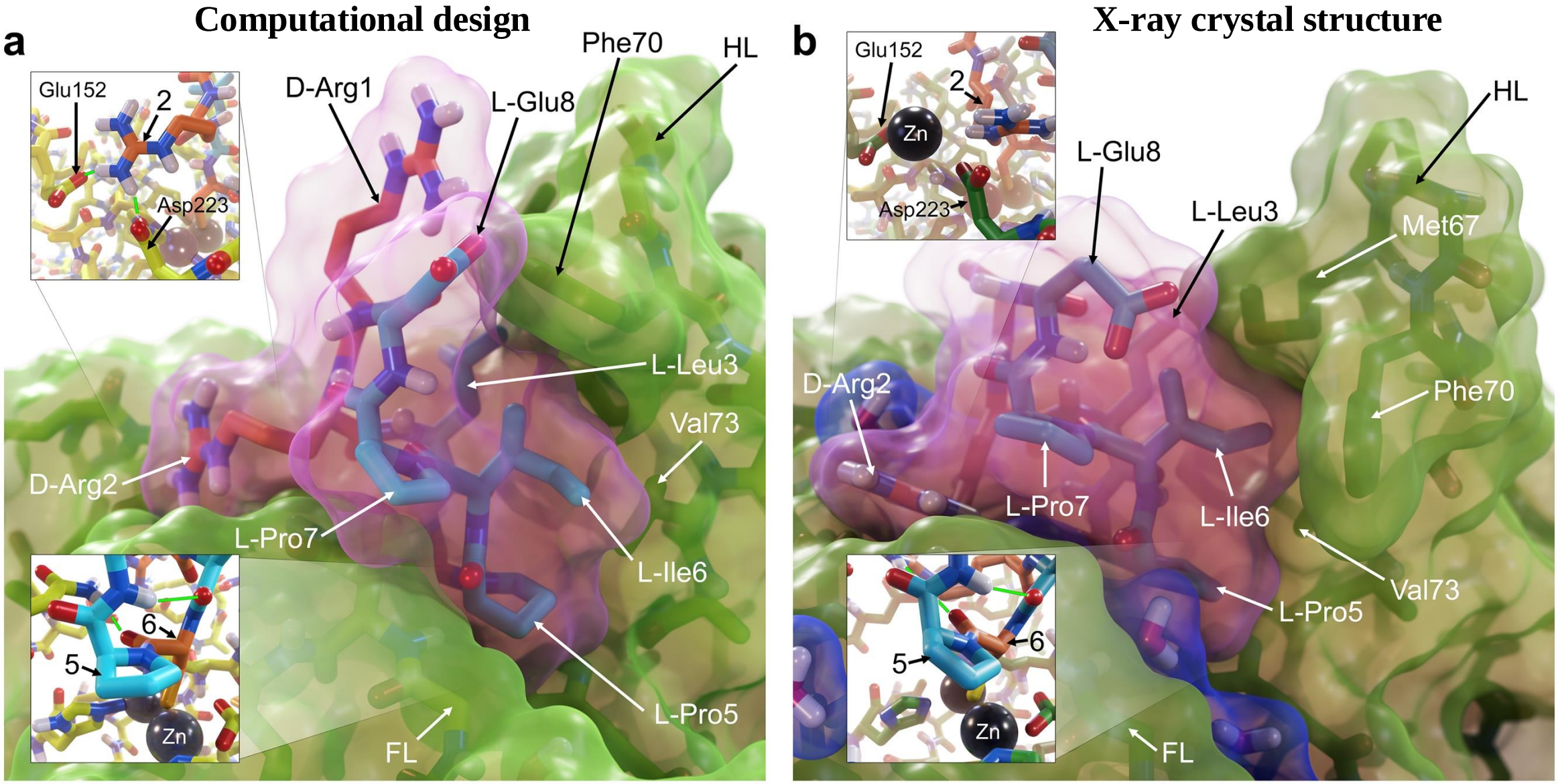
Crystal structure of a designed S4-symmetric mixed-chirality Zn-binding peptide





From Mulligan, Kang, Sawaya *et al*. (2021.) Computational design of mixed chirality peptide macrocycles with internal symmetry. *Protein Sci.* 29(12):2433-45. DOI: 10.1002/pro.3974.

A designed inhibitor of the New Delhi metallo-β-lactamase 1 (NDM-1)

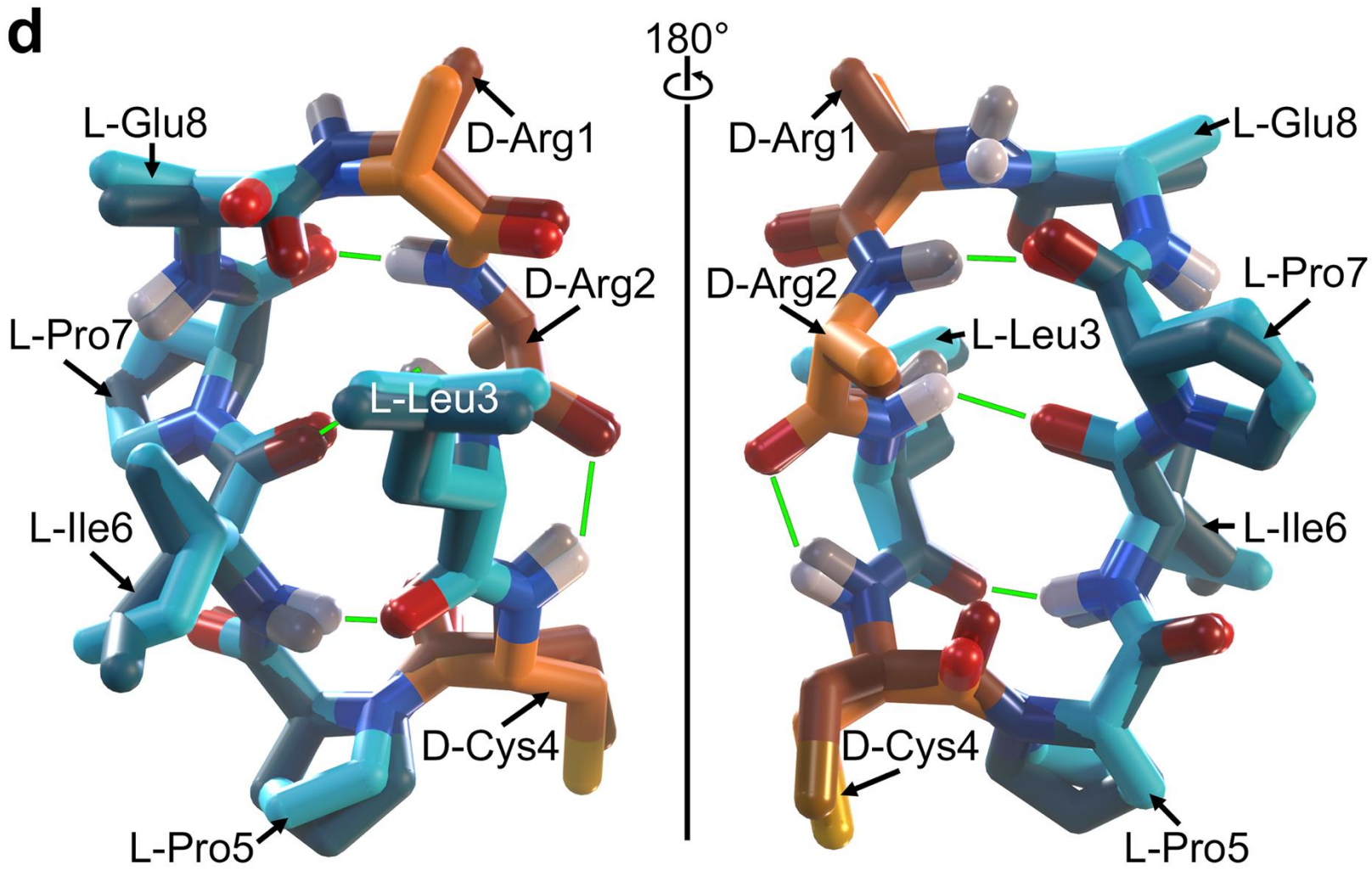


From Mulligan *et al*. (2021). Computationally-designed peptide macrocycle inhibitors of New Delhi metallo-β-lactamase 1. *Proc Natl Acad Sci USA* 118(12):e2012800118. DOI: 10.1073/pnas.2012800118.

A designed inhibitor of the New Delhi metallo-β-lactamase 1 (NDM-1)

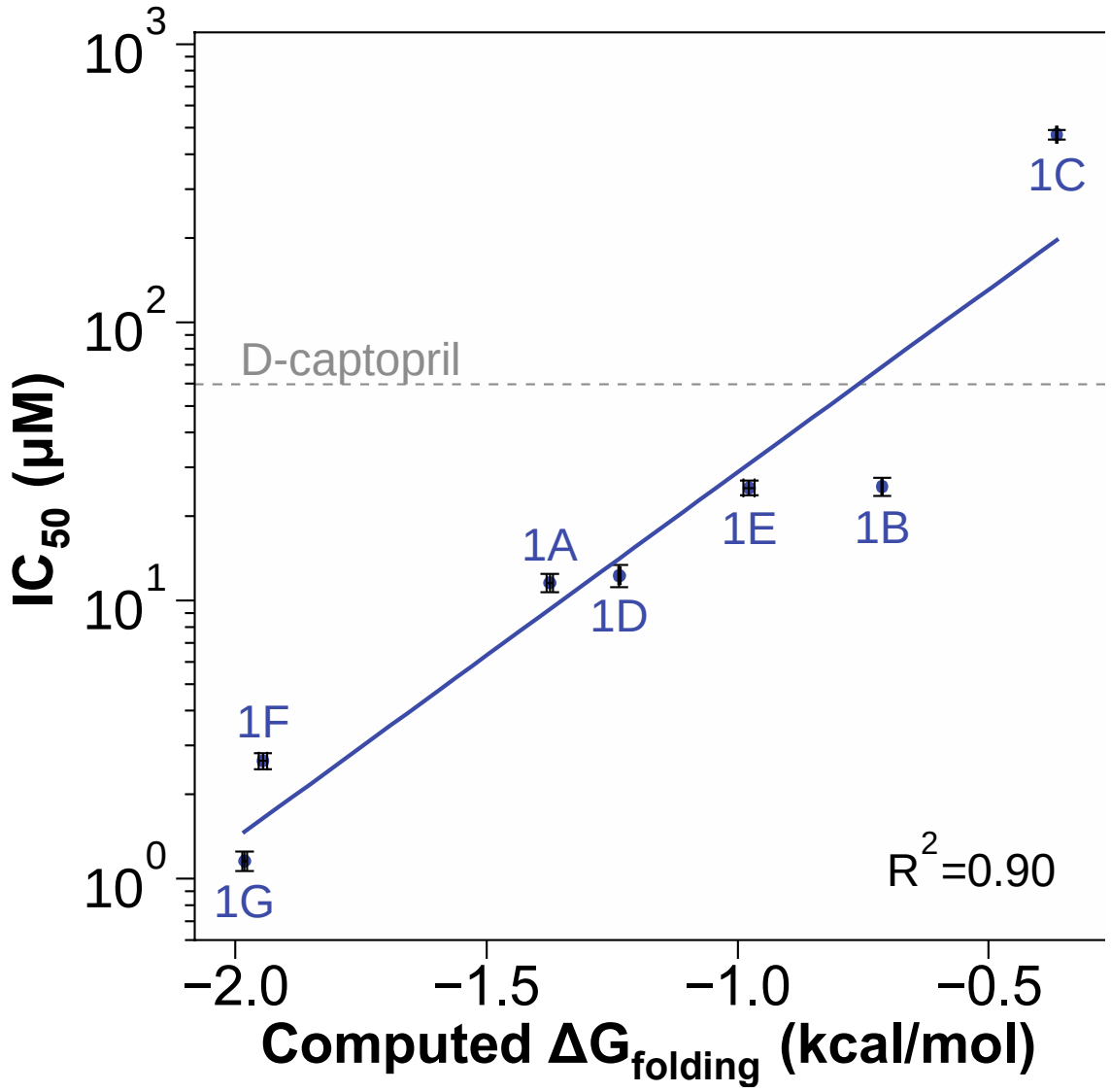
Light: design

Dark: crystal structure



From Mulligan *et al*. (2021). Computationally-designed peptide macrocycle inhibitors of New Delhi metallo-β-lactamase 1. *Proc Natl Acad Sci USA* 118(12):e2012800118. DOI: 10.1073/pnas.2012800118.

Simulations predict success in experiments



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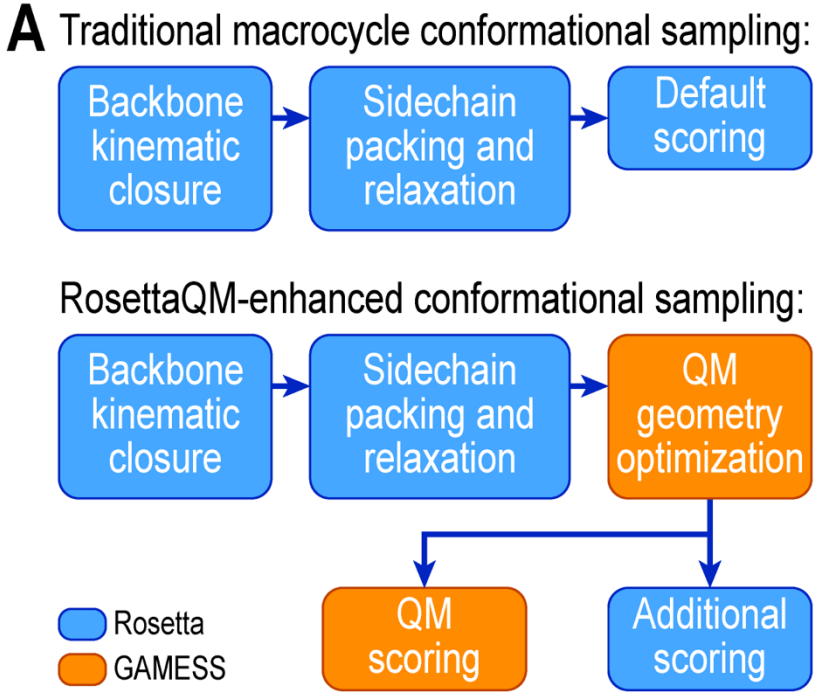
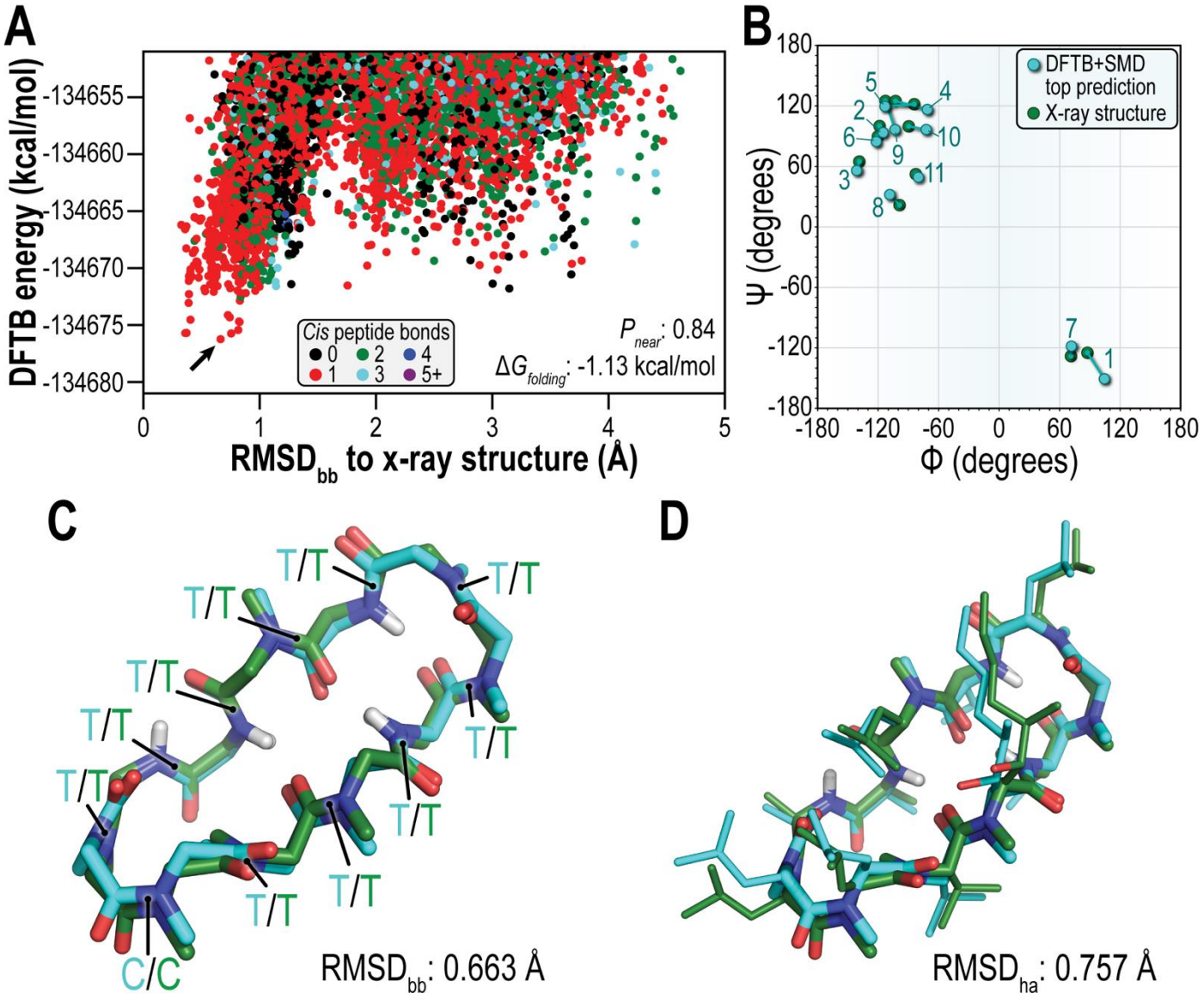
RosettaQM-based prediction of the structure of cyclosporine A in organic solvent



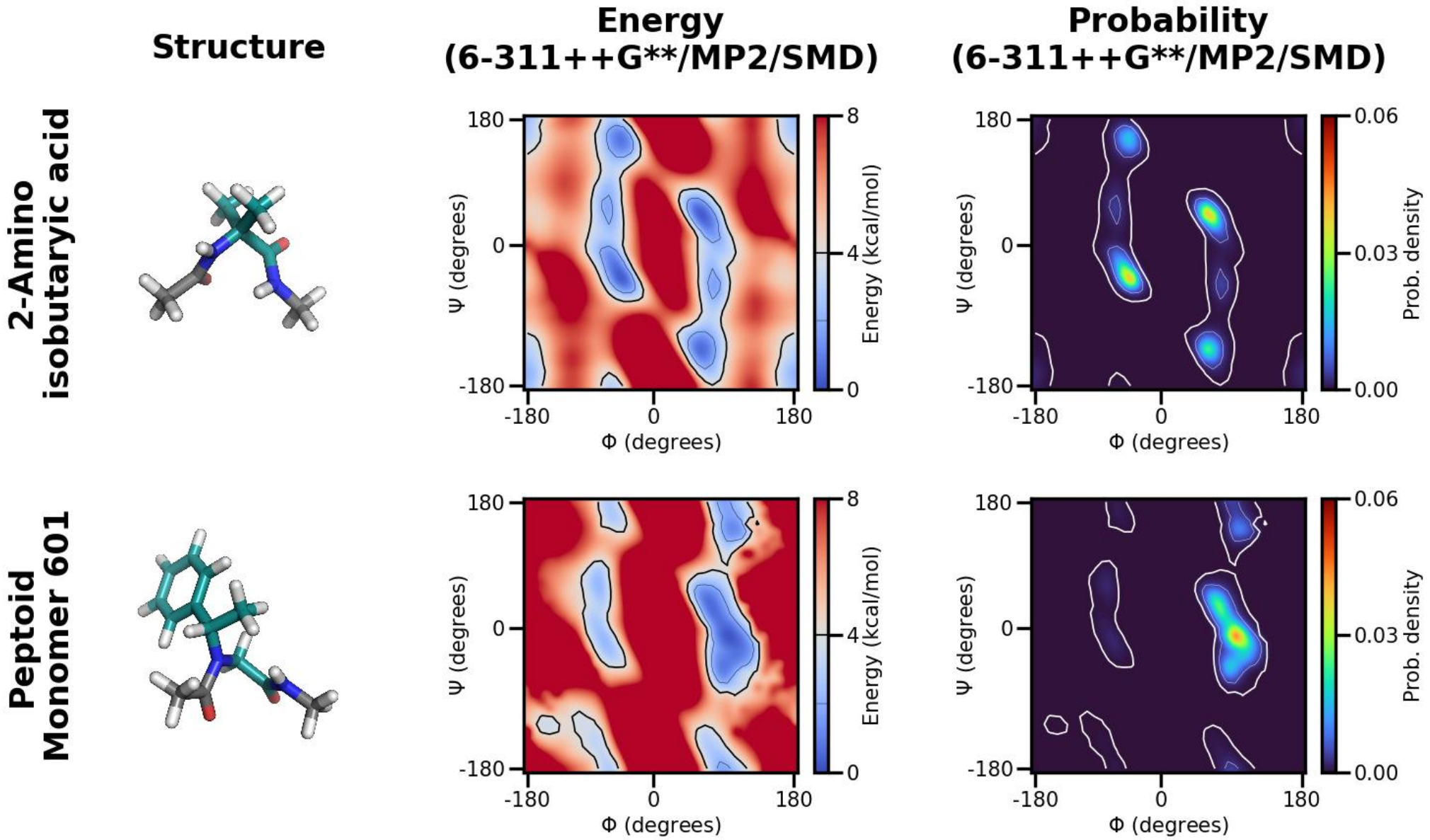
With Benjamin Brown, P. Douglas Renfrew, Chris Jurich, Nancy Hernandez, and Bargeen Turzo.

Improving Accuracy and Generality with QM Energy Calculations



With Benjamin Brown, P. Douglas Renfrew, Chris Jurich, Nancy Hernandez, and Bargeen Turzo.

Prediction of the Ramachandran map: RosettaQM-based parameterization of noncanonical force fields



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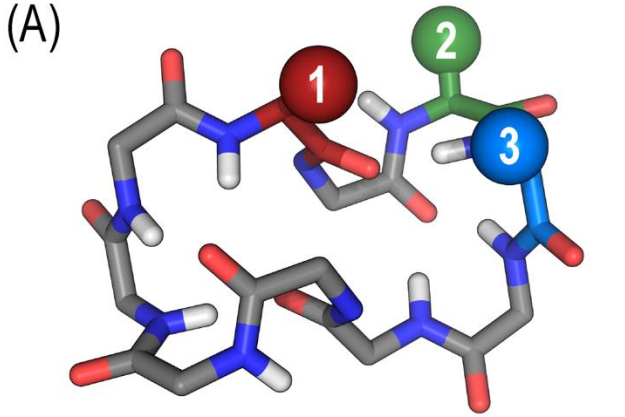
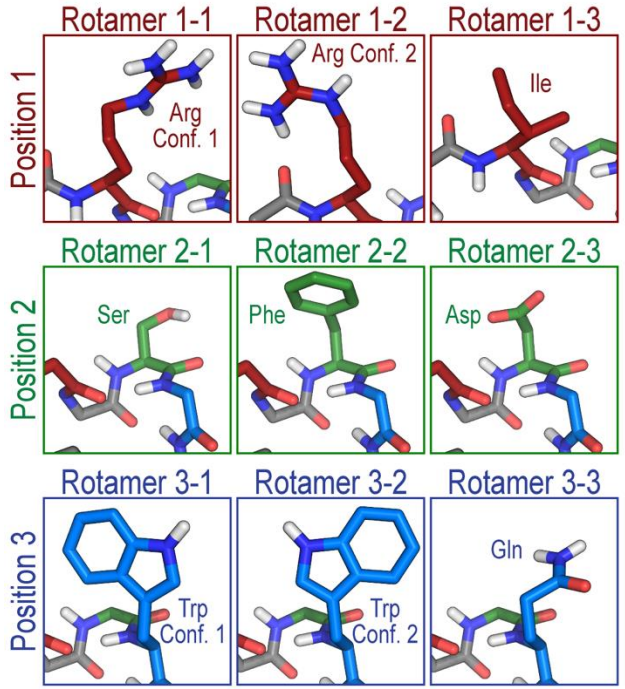
Revisiting the rotamer optimization (design) problem

(A)





(B)

$$E = \sum_{i=1}^{N} \alpha_i + \sum_{j=1}^{N-1} \sum_{k=2}^{N} \beta_{jk}$$

N - Number of designable positions

α_i - Internal energy of selected rotamer at position i

β_{jk} - Interaction energy of selected rotamers at positions j and k

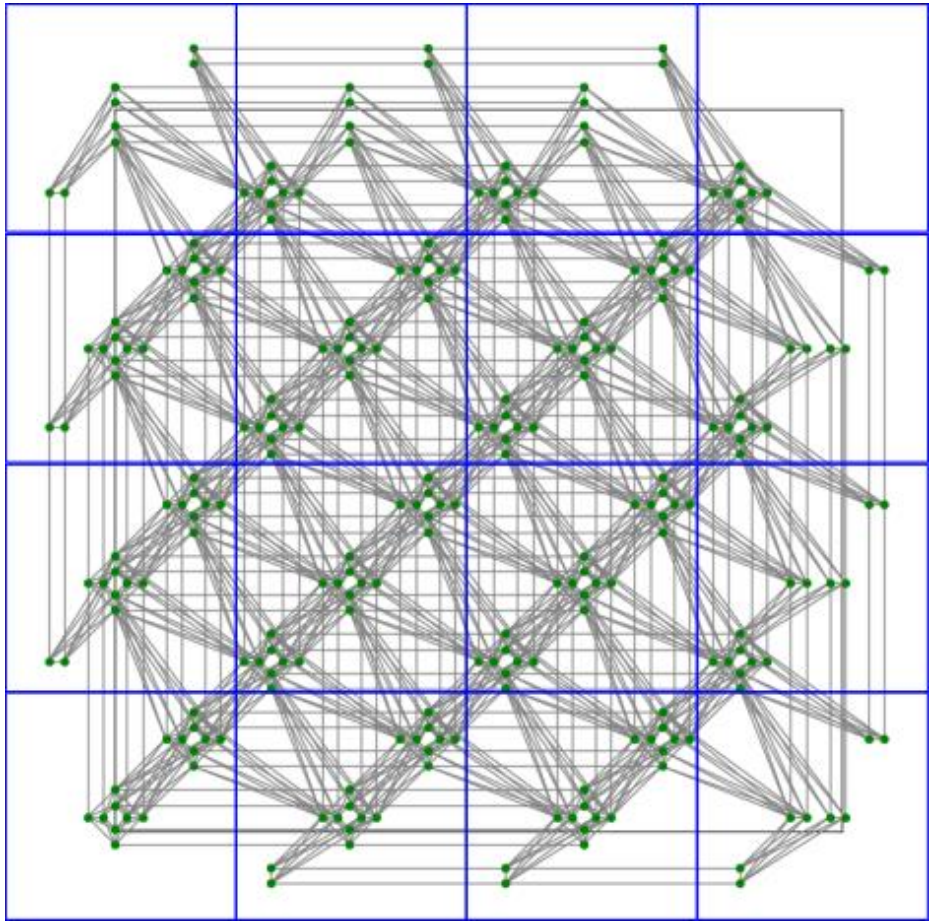
Single rotamer internal energy

Rotamer	
1-1	5.2
1-2	3.3
1-3	7.1
2-1	2.1
2-2	6.8
2-3	3.1
3-1	3.1
3-2	3.5
3-3	1.5

Rotamer pair interaction energy

First rotamer \ Second rotamer	2-1	2-2	2-3	3-1	3-2	3-3
1-1	5.3	1.6	0.7	7.9	4.3	4.1
1-2	5.8	4.8	2.3	1.3	5.3	5.8
1-3	3.1	3.5	3.7	1.4	1.3	1.1
2-1				3.0	3.1	2.9
2-2				3.0	3.6	1.7
2-3				2.5	4.1	0.7

The D-Wave Advantage adiabatic quantum annealer



- The D-Wave Advantage offers about 5,000 sparesely-connected physical qubits. Each is connected to 15 others. This can emulate 177 fully-connected virtual qubits.

- The user provides inputs by setting single-qubit biases (h_i) for each qubit and two-qubit couplings ($J_{i,j}$) for each pair of qubits.

- The total energy of a given state of the computer is:

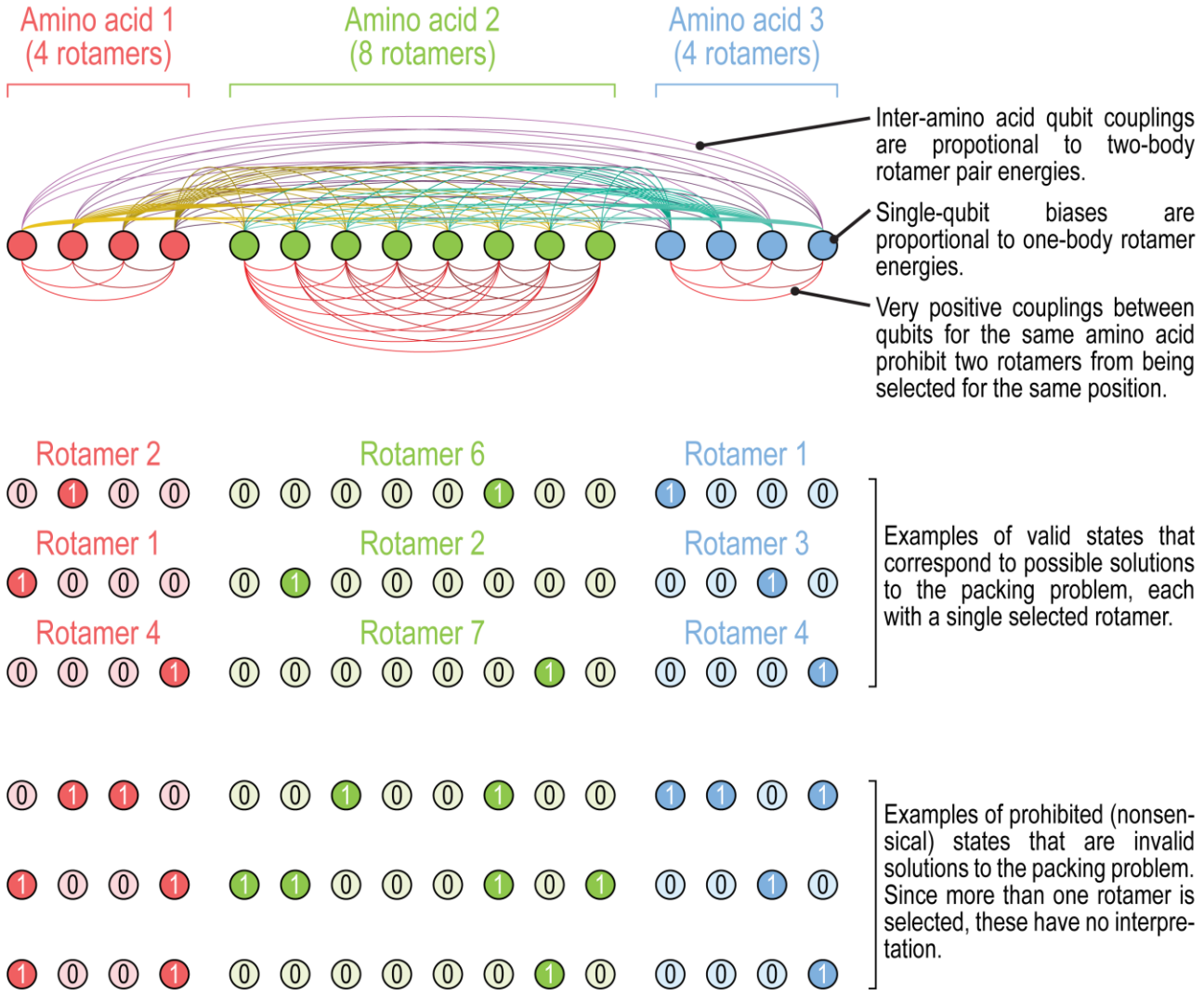
$$E = \sum_{i=1}^{Q} q_i h_i + \sum_{i=2}^{Q} \sum_{j=1}^{i-1} q_i q_j J_{i,j}$$

- In the above, Q is the number of qubits, and q_i and q_j are the value (0 or 1) of the i^{th} and j^{th} qubit (defining the *state*). The annealing process returns as output values q_i for all qubits such that E is a minimum.

Designing peptides using a quantum annealer: QPacker

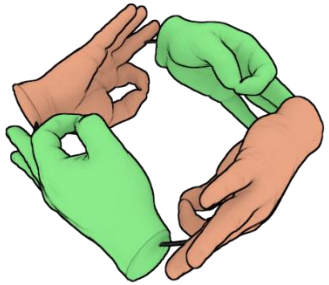
In collaboration with Hans Melo, CEO, Menten AI

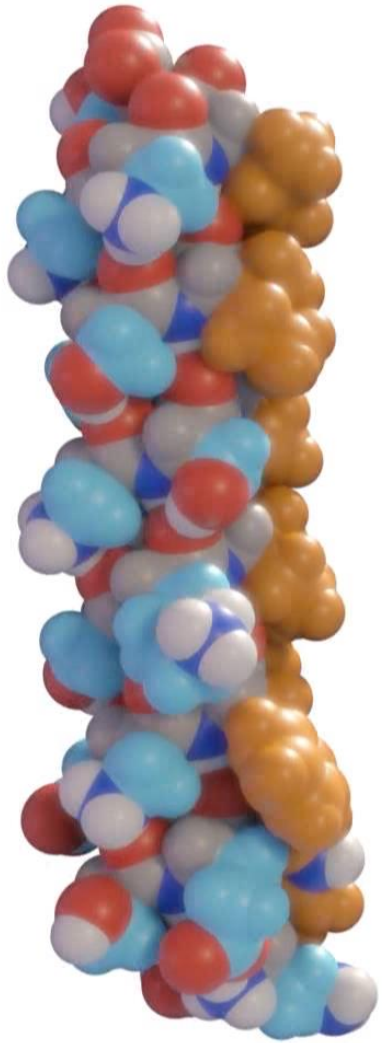
In collaboration with Brian Weitzner, Principal Scientist, Outpace Bio



Described in Mulligan VK, Melo H, Merritt HI *et al.* (2019) Designing peptides on a quantum computer. *bioRxiv* preprint. DOI: 10.1101/752485

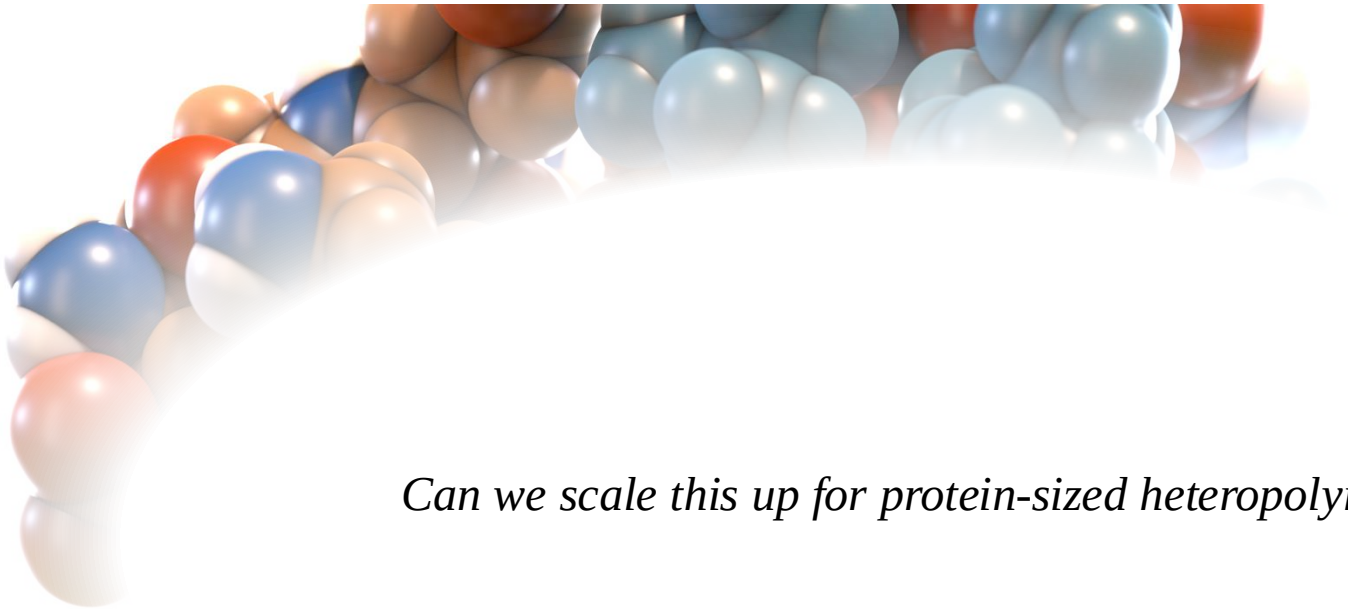
Designing heterochiral helical bundles with QPacker and the D-Wave 2000Q





With Michael Sawaya, Todd Yeates, Parmjit Arora, Haley Irene Merritt, and Hans Melo.

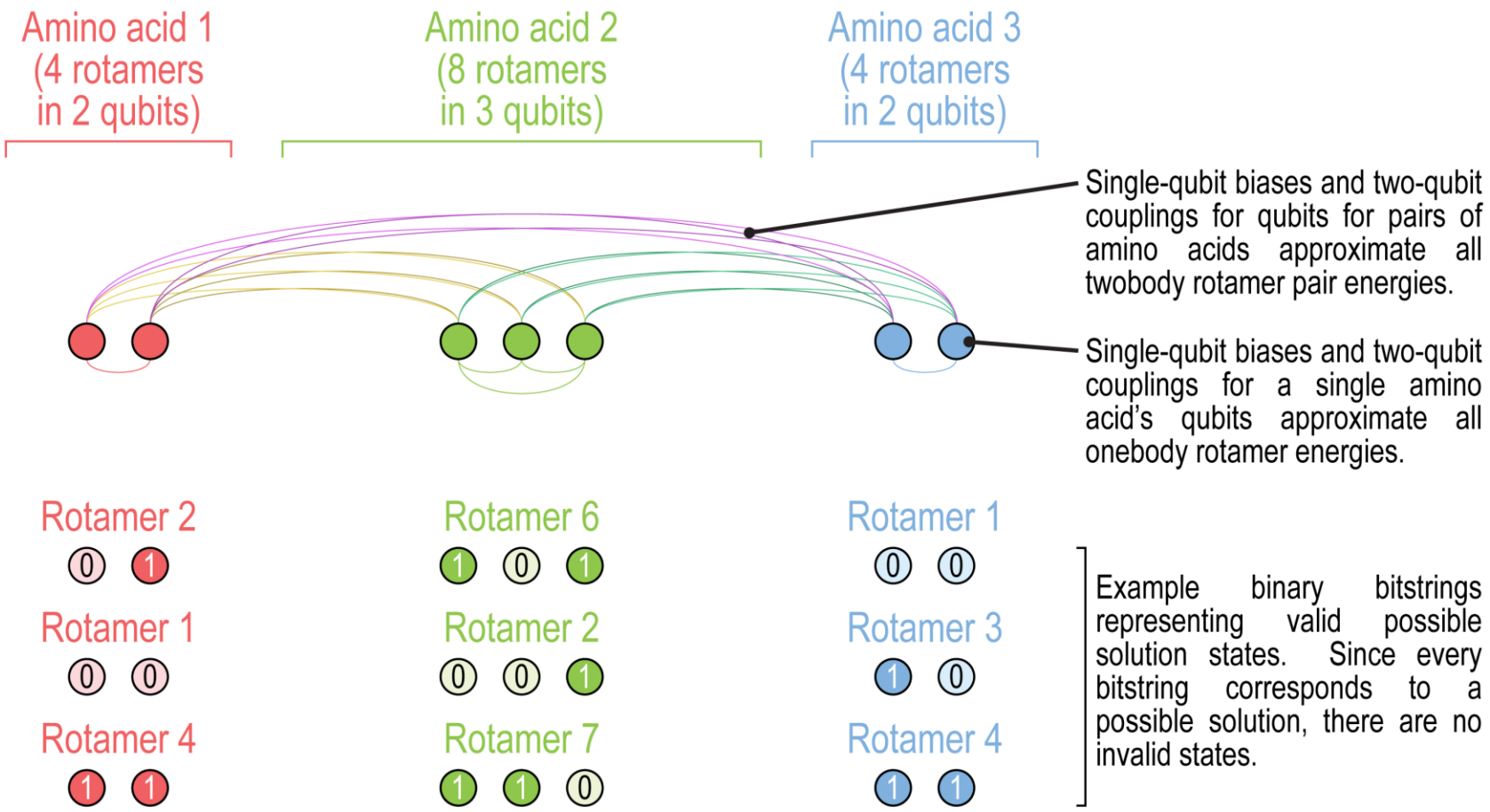
Self-assembling peptides designed with QPacker, with structures confirmed experimentally by x-ray crystallography



Can we scale this up for protein-sized heteropolymers?

With Michael Sawaya, Todd Yeates, Parmjit Arora, Haley Irene Merritt, and Hans Melo.

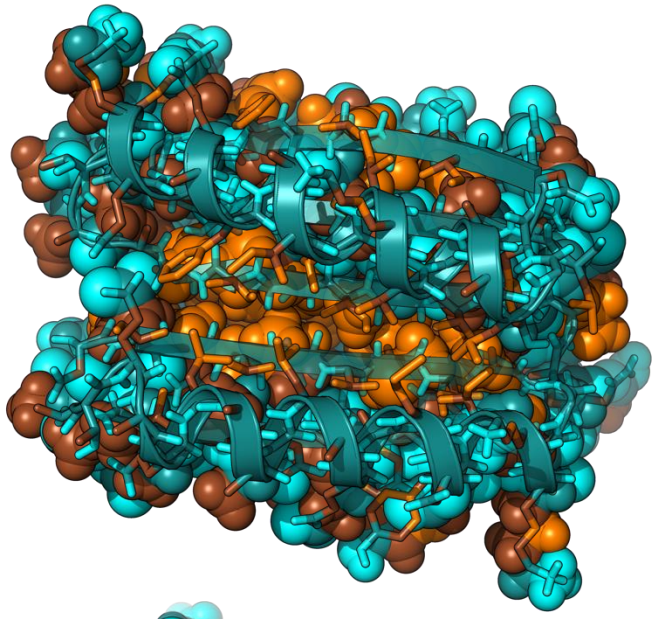
QPacker-B: Compressing problems to use $N\ log_2\ D$ qubits

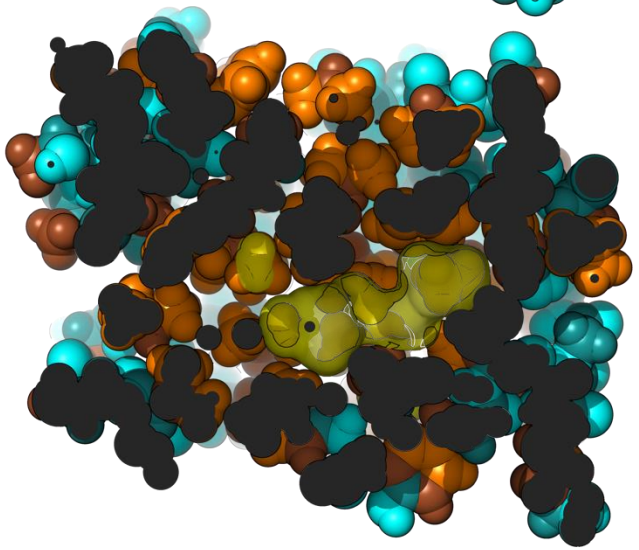


Tristan Zaborniak, U. Victoria

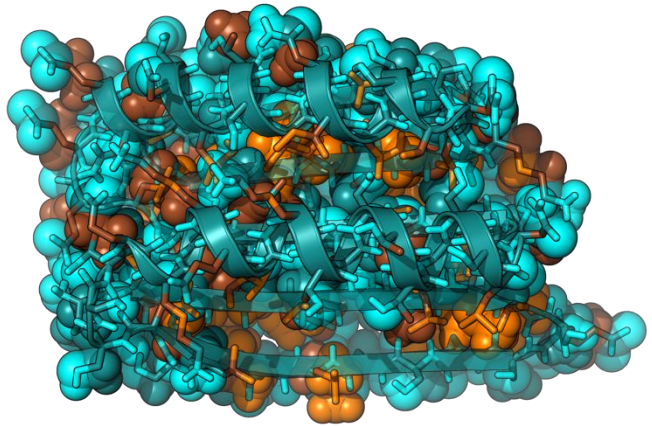
Full proteins designed on the D-Wave Advantage 6.4 Quantum Annealer

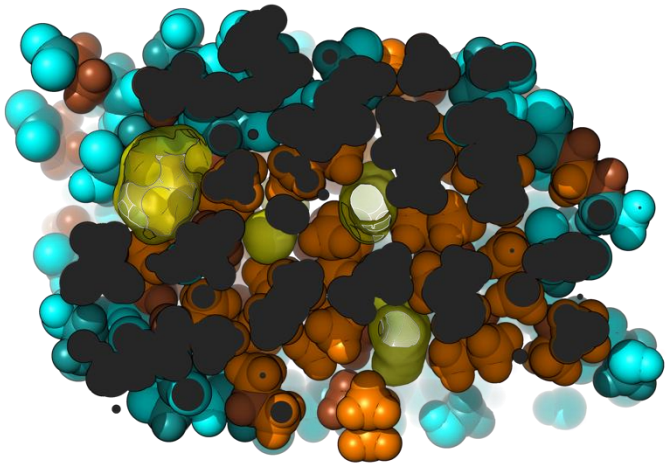
Top7 (classically designed in 2003)



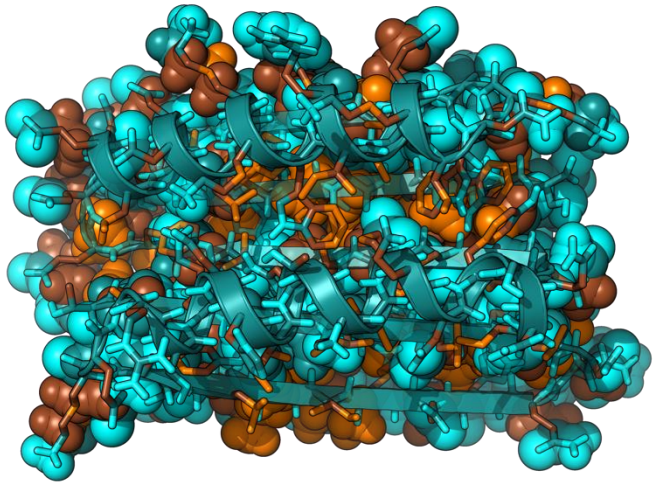


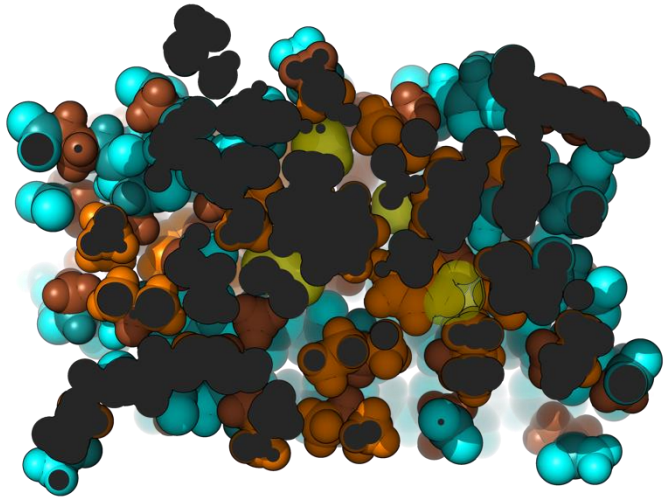
First quantum-designed protein
(15 QPU-seconds, Nov. 2024)





Second quantum-designed protein
(15 QPU-seconds, Nov. 2024)





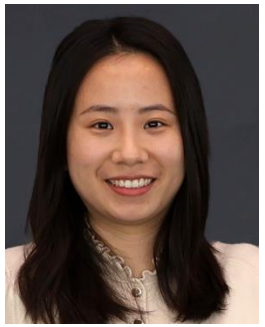
Tristan Zaborniak, U. Victoria

Making enhanced classical and quantum peptide design available for everyone: The Masala software library

- Masala is a free and open-source successor to Rosetta under development at the Flatiron institute.
- It is structured to take full advantage of modern massively-parallel CPU and GPU hardware.
- It has a versatile plugin architecture permitting easy extensibility. Our QPU plugin permits design on quantum computers.
- It is intended to be used as standalone software *or* as a library in other projects. (Rosetta, for instance, can link Masala for high-efficiency design calculations.)
- *To be released shortly.*

P. Douglas Renfrew, CCB

Noora Azadvari, U. Oregon



Qiyao Zhu, CCB

S. M. Bargeen Alam Turzo, CCB

Tristan Zaborniak, U. Victoria

Acknowledgements

Rosetta senior developers:

- Andrew Leaver-Fay
- Sergey Lyskov
- Julia Koehler-Leman
- **P. Douglas Renfrew**
- Andrew M. Watkins
- Jason Labonte
- Jared Adolf-Bryfogle
- Steven M. Lewis
- Rocco Moretti

Biomolecular Design Group:

- **P. Douglas Renfrew**
- Bargeen Turzo
- Qiyao Zhu

Interns and Visiting Scholars:

- Ekaterina Maximova
- **Tristan Zaborniak**
- Noora Azadvari
- Andrew Powers
- Allon Goldberg
- Rutika Patel

RosettaQM collaborators:

- **Benjamin Brown**
- Chris Jurich

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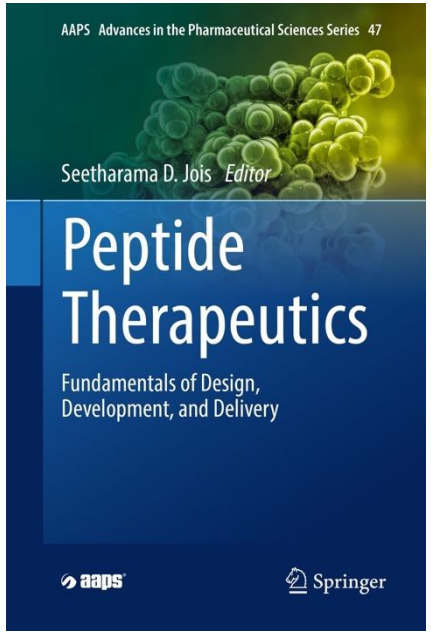
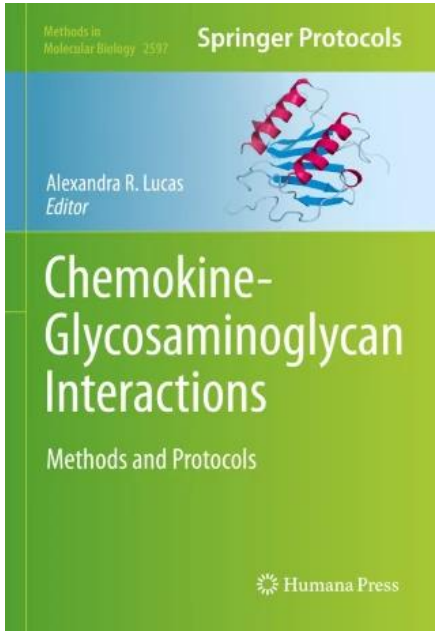
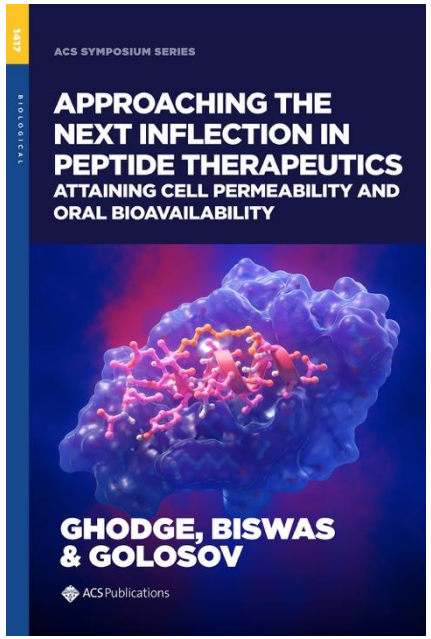
Further reading



V.K. Mulligan. Computational Methods for Peptide Macrocycle Drug Design. Chapter in: S.D. Jois (Ed.), *Peptide Therapeutics: Fundamentals of Design, Development, and Delivery*, Springer International Publishing, New York, 2022: pp. 79–161. https://doi.org/10.1007/978-3-031-04544-8_3.



J. Dodd-O, A.M. Acevedo-Jake, A.-R. Azizogli, V.K. Mulligan, V.A. Kumar, *How to Design Peptides*, Methods Mol Biol 2597 (2023) 187–216. https://doi.org/10.1007/978-1-0716-2835-5_15.



V.K. Mulligan, P. Hosseinzadeh. Computational Design of Peptide-Based Binders to Therapeutic Targets. Chapter in: S.V. Ghodge, K. Biswas, A.A. Golosov (Eds.), *Approaching the Next Inflection in Peptide Therapeutics: Attaining Cell Permeability and Oral Bioavailability*, American Chemical Society, Washington, DC, 2022: pp. 55–102. https://doi.org/10.1021/bk-2022-1417.ch003.

Or e-mail us: the Biomolecular Design Group, in the Flatiron Institute's Center for Computational Biology, is always looking for experimental collaborators.

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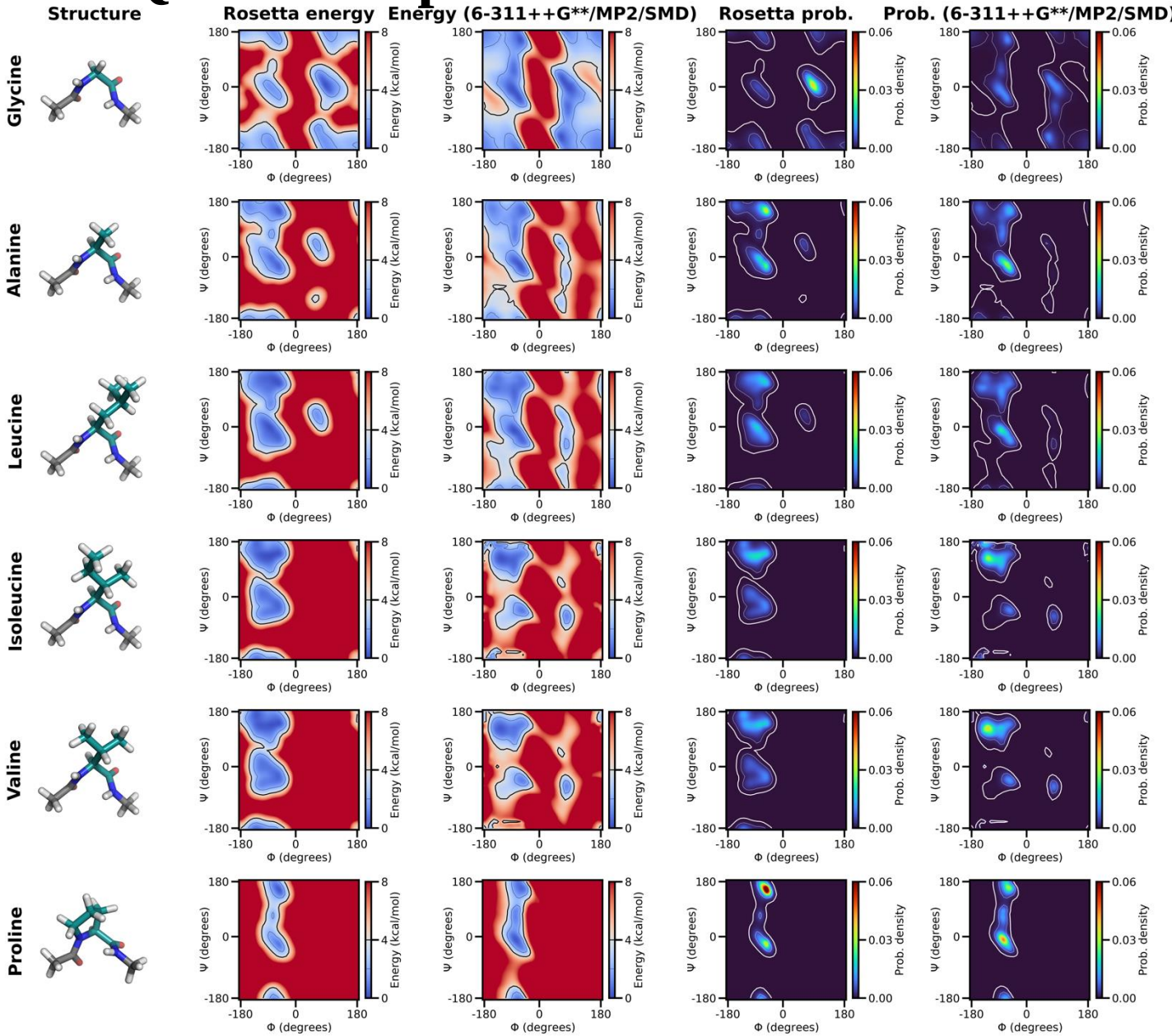
Prediction of the Ramachandran map: RosettaQM-based production of better force fields



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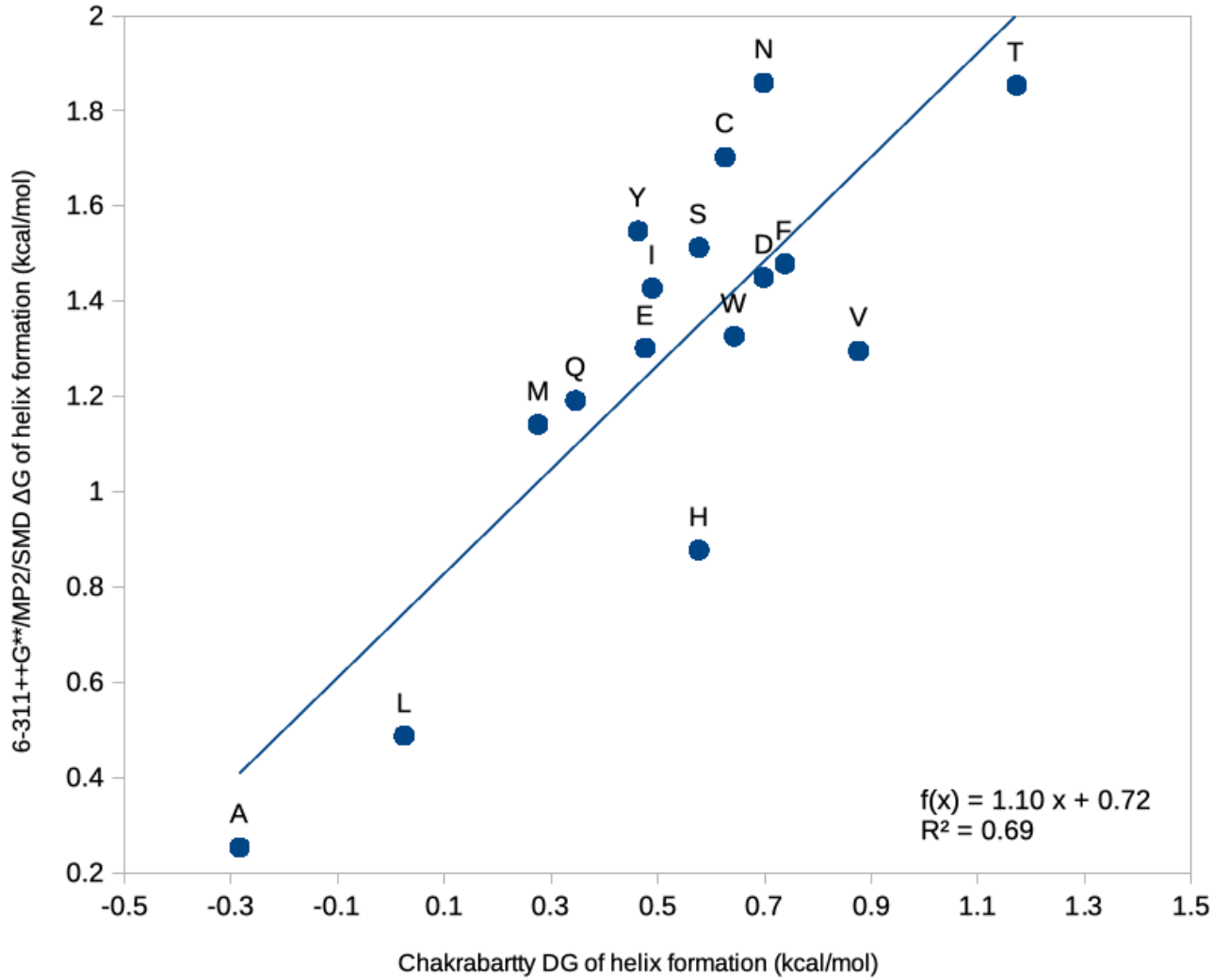


Comparison to Chakrabartty, Kortemme, and Baldwin. (1994). *Protein Sci.* 3(5)843-52. DOI: 10.1002/pro.5560030514.

How many qubits *should* the QPacker use?

- A packing problem with N designable positions and D rotamers per position has D^N possible solutions.

- A register of Q qubits can exist in a superposition of 2^Q states (2^Q bitstrings). If we use qubits efficiently, then each bitstring will map to a unique solution.

- Let $2^Q = D^N$. Then $Q = N \log_2 D$.

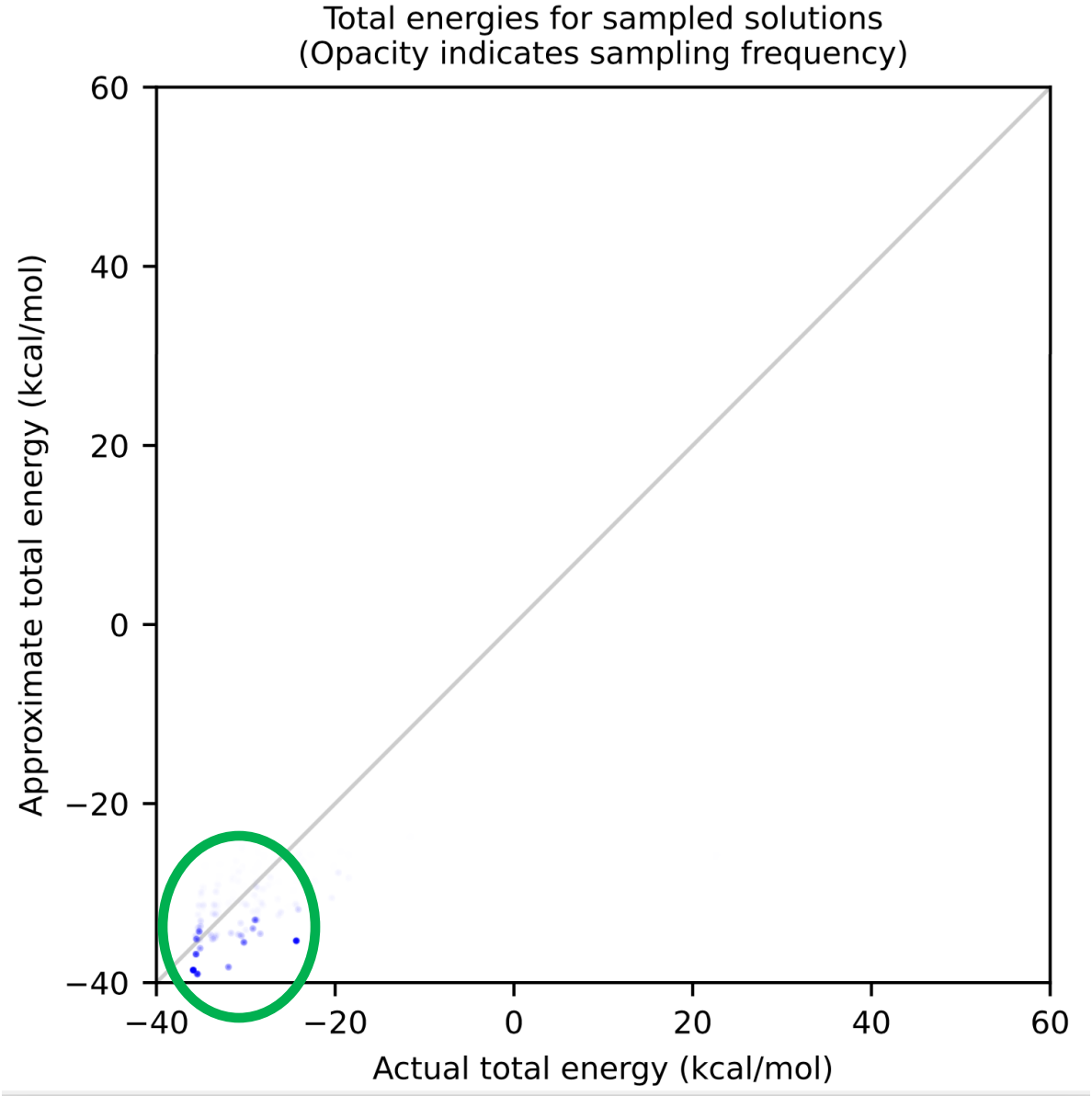
- But we're using ND qubits, not $N \log_2 D$. One-hot encoding of rotamer selections is very inefficient. Can we do better?

QPacker-B: A classical approximation to compress one-body and two-body energies into fewer qubits (16-qubit example)

Actual samples on D-Wave (10,000 attempts)

Blue points: allowed solutions to design problem, each representing a rotamer selection

Red points: duplicates (since *D* is not an exact power of 2). We want to prohibit these.



Tristan Zaborniak