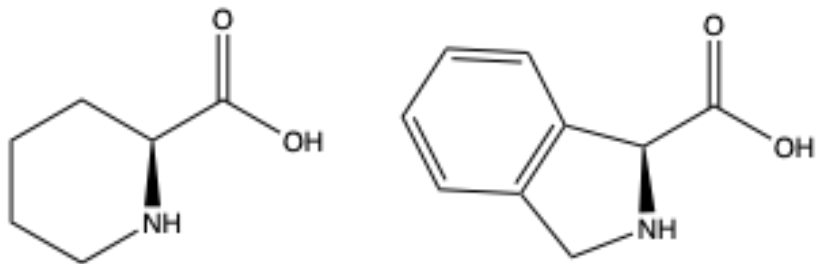


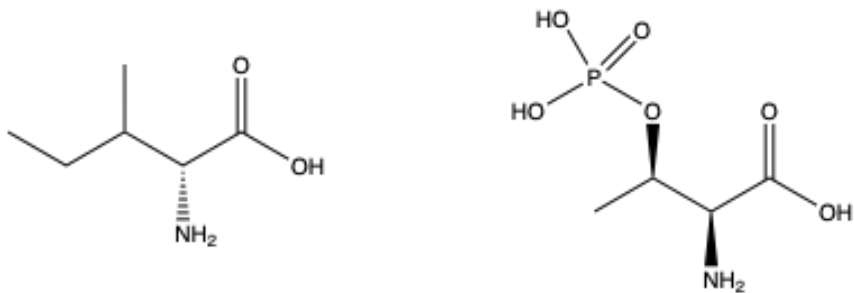
# Non-canonical Peptide and Macrocycle design with Rosetta

Eric Bell, Clay Tydings  
Rosetta Workshop  
11/19/2025

# The magical world of non-canonical amino acids (NCAAs)



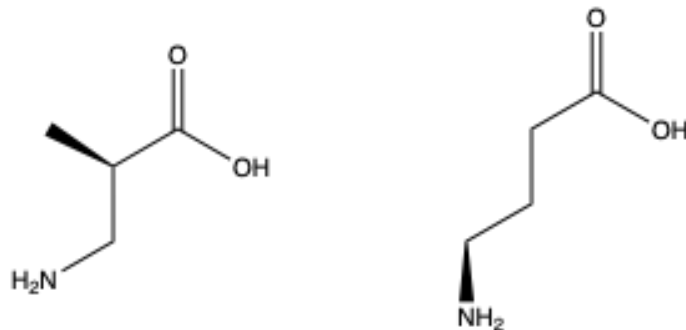
Sidechain conjugation



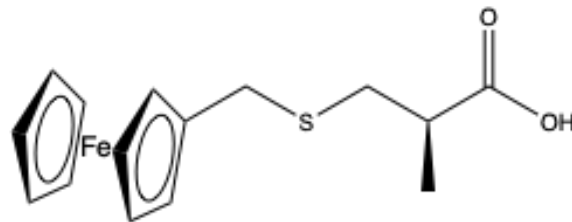
D-AAAs

PTMs

Rosetta can do



Non-canonical backbones



...or just, whatever

Rosetta can't do

ROUND 1	ROUND 2	QUARTER-FINALS	SEMI-FINALS	FINALS	SEMI-FINALS	QUARTER-FINALS	ROUND 2	ROUND 1
N-methylalanine								Hydroxyethylcysteine
<b>Phosphothreonine</b> ✓	<b>Phosphothreonine</b> ✓					<b>Azidothomoalanine</b> ✓		<b>Azidothomoalanine</b> ✓
2-5-fluoroleucine		<b>Phosphothreonine</b> ✓						<b>Dioxyselenocysteine</b> ✓
<b>Histidinol</b> ✓	Histidinol							L-thialysine
<b>3-methylaspartic acid</b> ✓			<b>Phosphothreonine</b> ✓					4-hydroxyphenylglycine
Allo-threonine	3-methylaspartic acid				4-hydroxyproline			Alpha aminoisobutyric acid
Deaminohydroxyvaline		Citrulline				<b>4-hydroxyproline</b> ✓		<b>4-hydroxyproline</b> ✓
<b>Citrulline</b> ✓	<b>Citrulline</b> ✓			Phosphothreonine				Beta-hydroxyasparagine
<b>6-carboxyllysine</b> ✓								<b>Ornithine</b> ✓
Phenylalanyl(methyl)chloride	6-carboxyllysine			<b>Kynurenine</b> ✓			<b>Ornithine</b> ✓	S-palmitoylcysteine
Arginineamide		<b>Selenomethionine</b> ✓						3-fluorotyrosine
<b>Selenomethionine</b> ✓	<b>Selenomethionine</b> ✓							<b>Fluorotryptophan</b> ✓
Benzylcysteine			Selenomethionine		<b>Kynurenine</b> ✓			<b>5-oxoproline</b> ✓
<b>Methionine sulfoxide</b> ✓	<b>Methionine sulfoxide</b> ✓							L-prolinamide
Tyrosinal		Methionine sulfoxide				<b>Kynurenine</b> ✓		2-hydroxypropanal
<b>Norleucine</b> ✓	Norleucine						<b>Kynurenine</b> ✓	<b>Kynurenine</b> ✓

# Anatomy of a Rosetta amino acid

```
ATOM N Nbb NH1 -0.6046255 -0.350
ATOM CA CAbb CT2 -0.0257287 0.100
ATOM C CObb C 0.6884871 0.550
ATOM O OCbb O -0.6884871 -0.550
ATOM H HNbb H 0.3987955 0.250
ATOM 1HA Hapo HB 0.1157793 0.000
ATOM 2HA Hapo HB 0.1157793 0.000
```

```
ATOM_ALIAS 1HA HA2
ATOM_ALIAS 2HA HA3
```

```
LOWER_CONNECT N
UPPER_CONNECT C
BOND N CA
BOND N H
BOND CA C
BOND CA 1HA
BOND CA 2HA
BOND_TYPE C 0 2
```

**Atom/bond block:**  
Atom names, atom  
types, partial  
charges, atom  
connectivity

```
NAME GLY
IO_STRING GLY G
TYPE POLYMER #residue type
AA GLY
ROTAMER_AA GLY
```

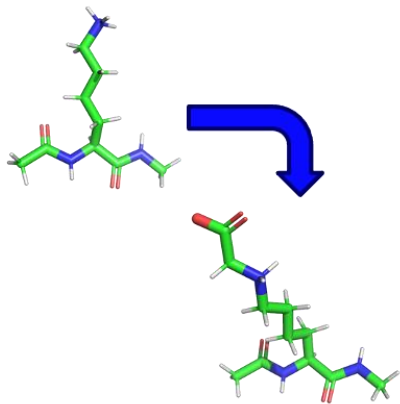
```
PROPERTIES PROTEIN CANONICAL_AA ALPHA_AA METALBINDING ACHIRAL_BACKBONE
METAL_BINDING_ATOMS 0
NBR_ATOM CA
# APL CA to O distance -- not yet measured; default to max CB to O dist
NBR_RADIUS 3.4473
FIRST_SIDECHAIN_ATOM NONE
RAMA_PREPRO_FILENAME all.ramaProb prepro.ramaProb
```

**Property assignment:**  
Rotamers, Ramachandrans, AA name,  
molecular properties, rotamers, etc.

**Internal Coordinates block:** Bond lengths, bond angles, dihedral angles

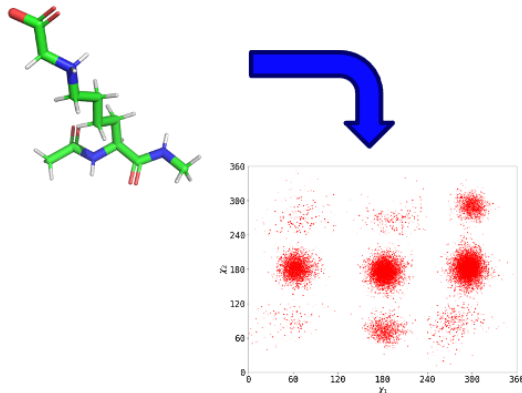
```
ICoor_INTERNAL N 0.000000 0.000000 0.000000 N CA C
ICoor_INTERNAL CA 0.000000 180.000000 1.458001 N CA C
ICoor_INTERNAL C 0.000000 68.799995 1.523259 CA N C
ICoor_INTERNAL UPPER 149.999969 63.800018 1.328685 C CA N
ICoor_INTERNAL O -179.999985 59.200005 1.231015 C CA UPPER
ICoor_INTERNAL 1HA 121.400000 70.500000 1.090168 CA N C
ICoor_INTERNAL 2HA 117.200000 70.500000 1.089353 CA N 1HA
ICoor_INTERNAL LOWER -150.000015 58.300003 1.328685 N CA C
ICoor_INTERNAL H 180.000000 60.850040 1.010000 N CA LOWER
```

# Three methods of NCAA rotamer generation



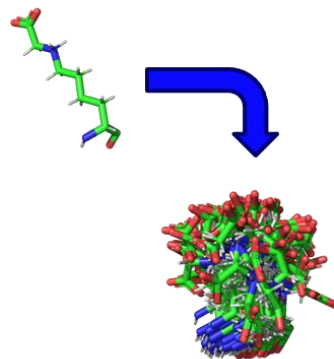
- +Quick and easy
- +Ensures “dunbrack-like” behavior
- Requires the NCAA resemble a CAA

**“Parent” Rotamers**



- +Able to parameterize many NCAA using CHARMM energy
- Longest runtime
- Max chi count of 4
- Best with prior knowledge of chi distributions

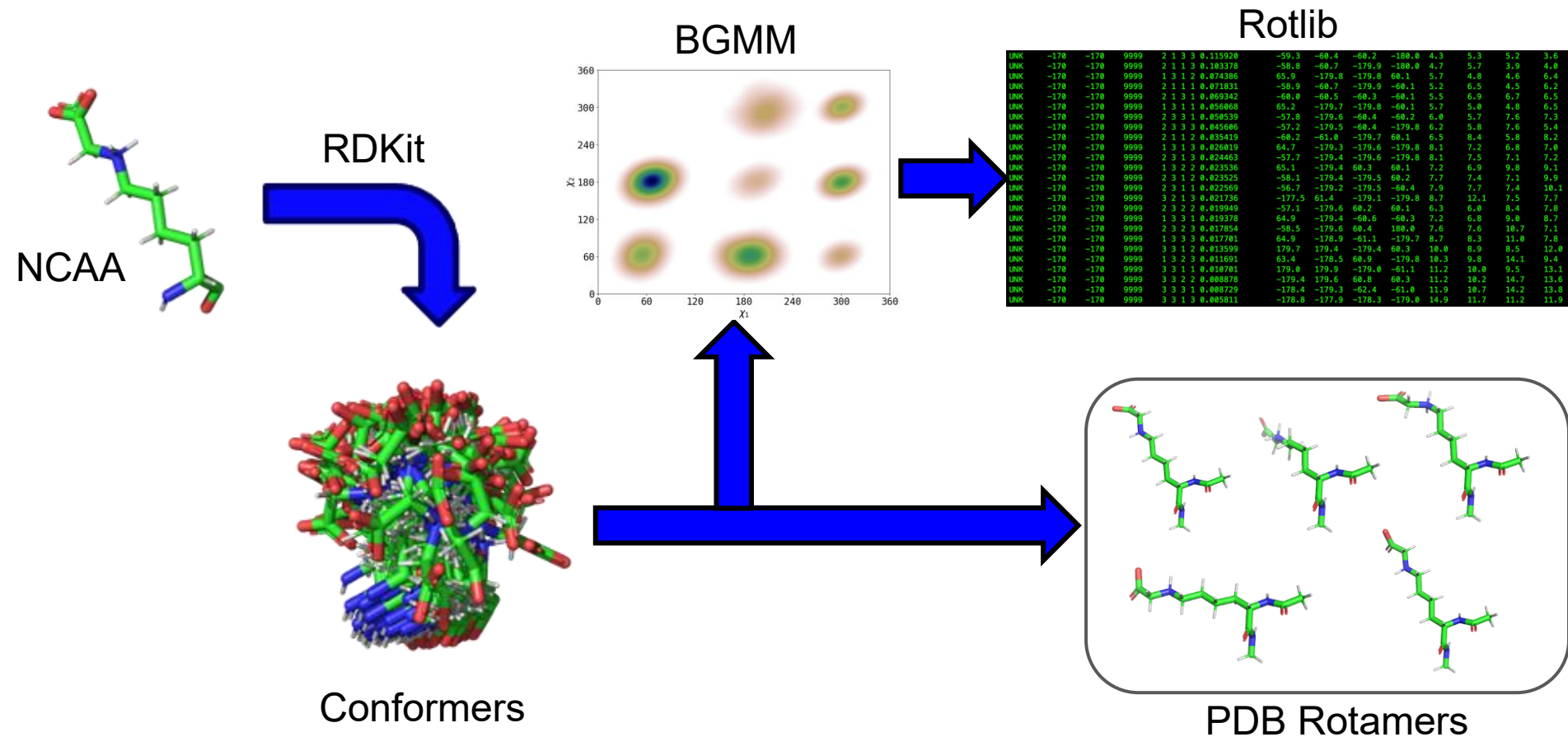
**MakeRotLib**



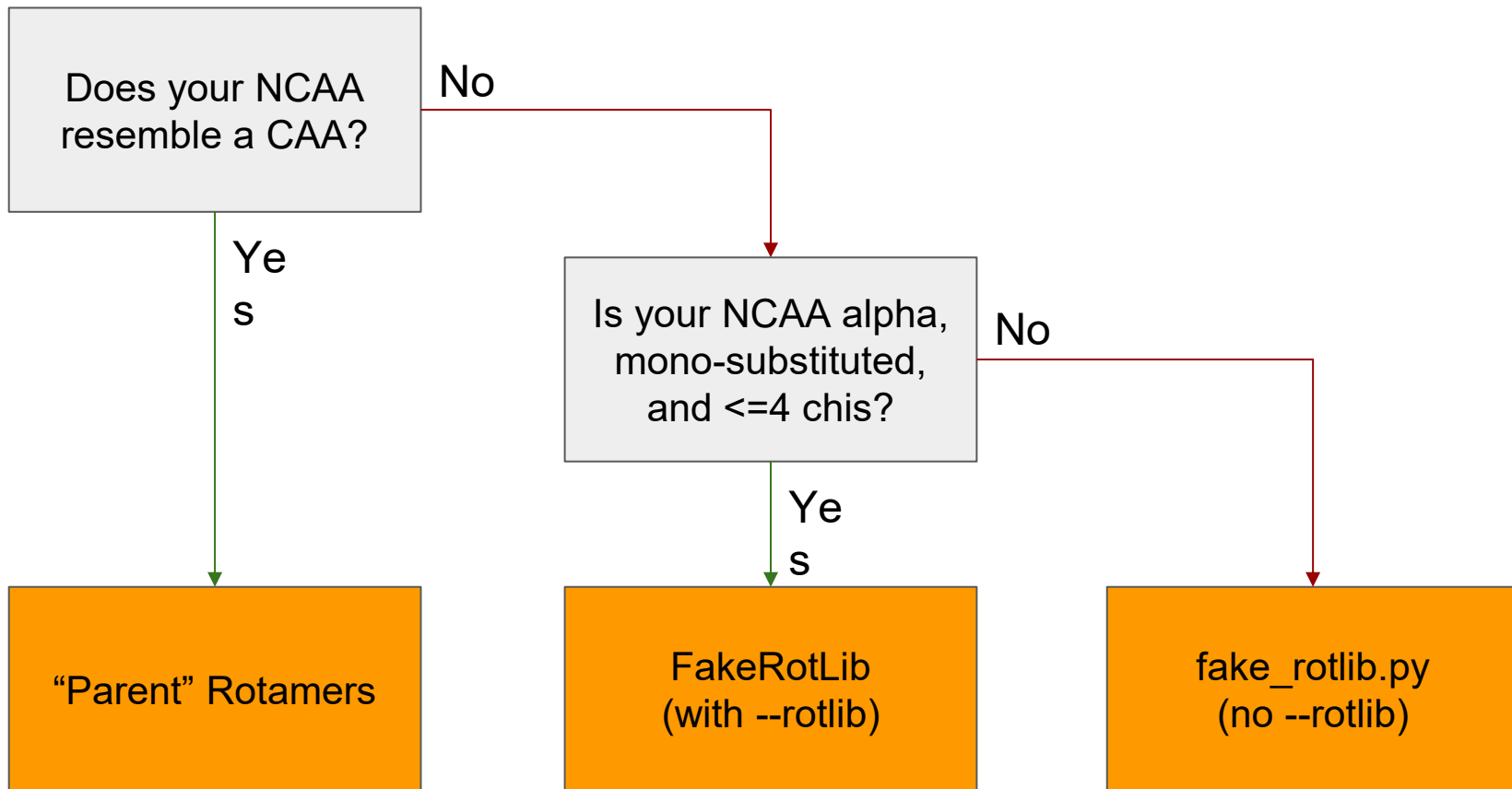
- +Quick because of ligand methods
- +Handles all NCAs
- Lacks “dunbrack-like” behavior
- Worst performing method

**Small Molecule approach**

# FakeRotLib: modeling rotamer distributions through BGMM



# NCAA parameterization flowchart

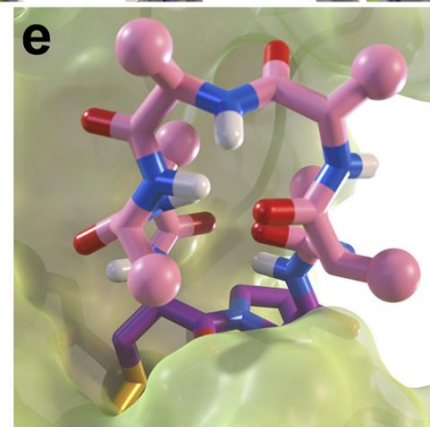
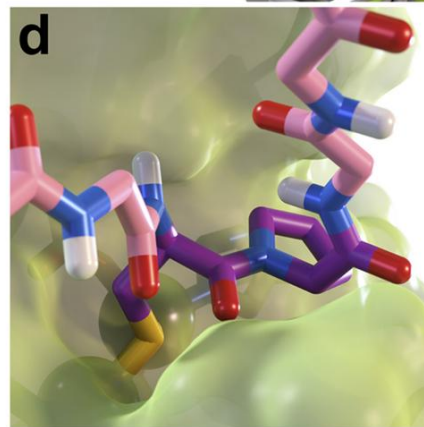
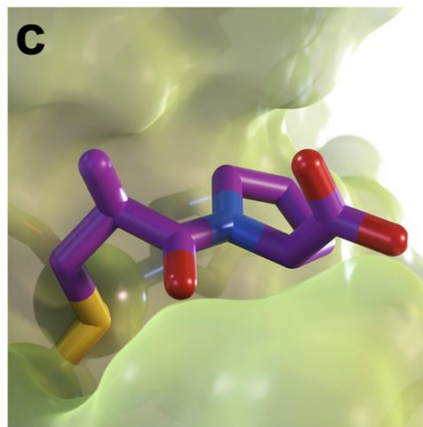
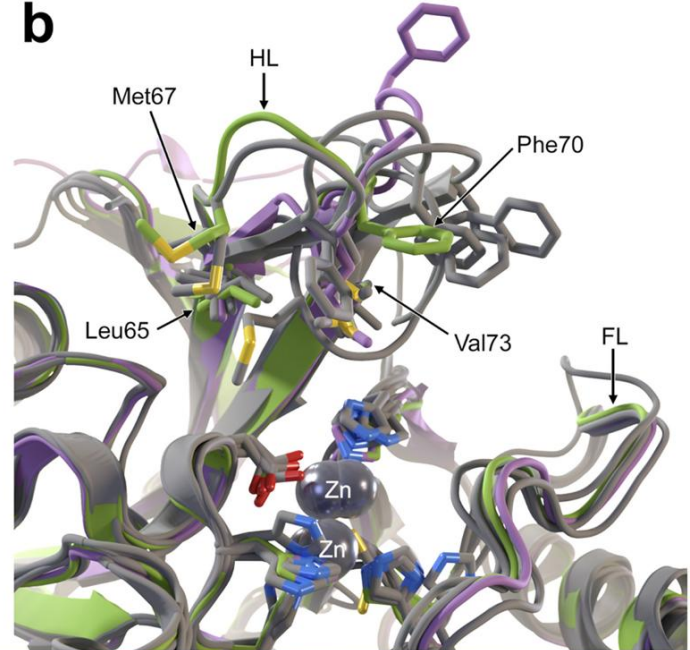
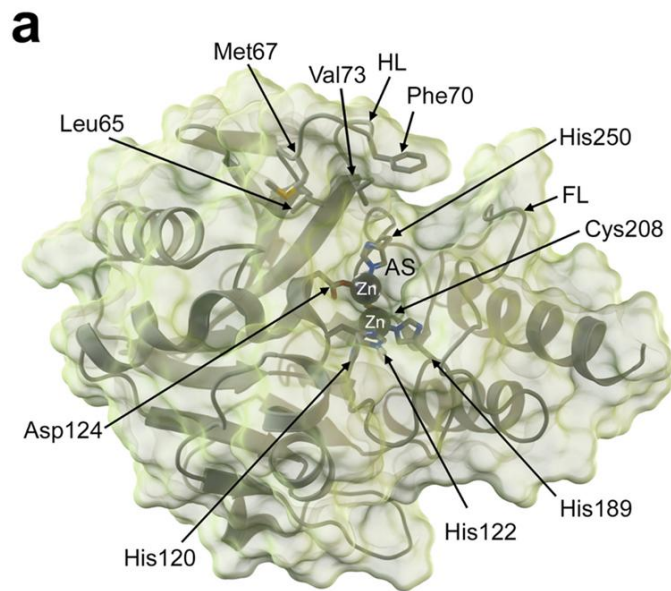


[Vikram Khipple Mulligan](#)  , [Sean Workman](#) , [Tianjun Sun](#),  [+13](#), and [David Baker](#)  [Authors Info & Affiliations](#)

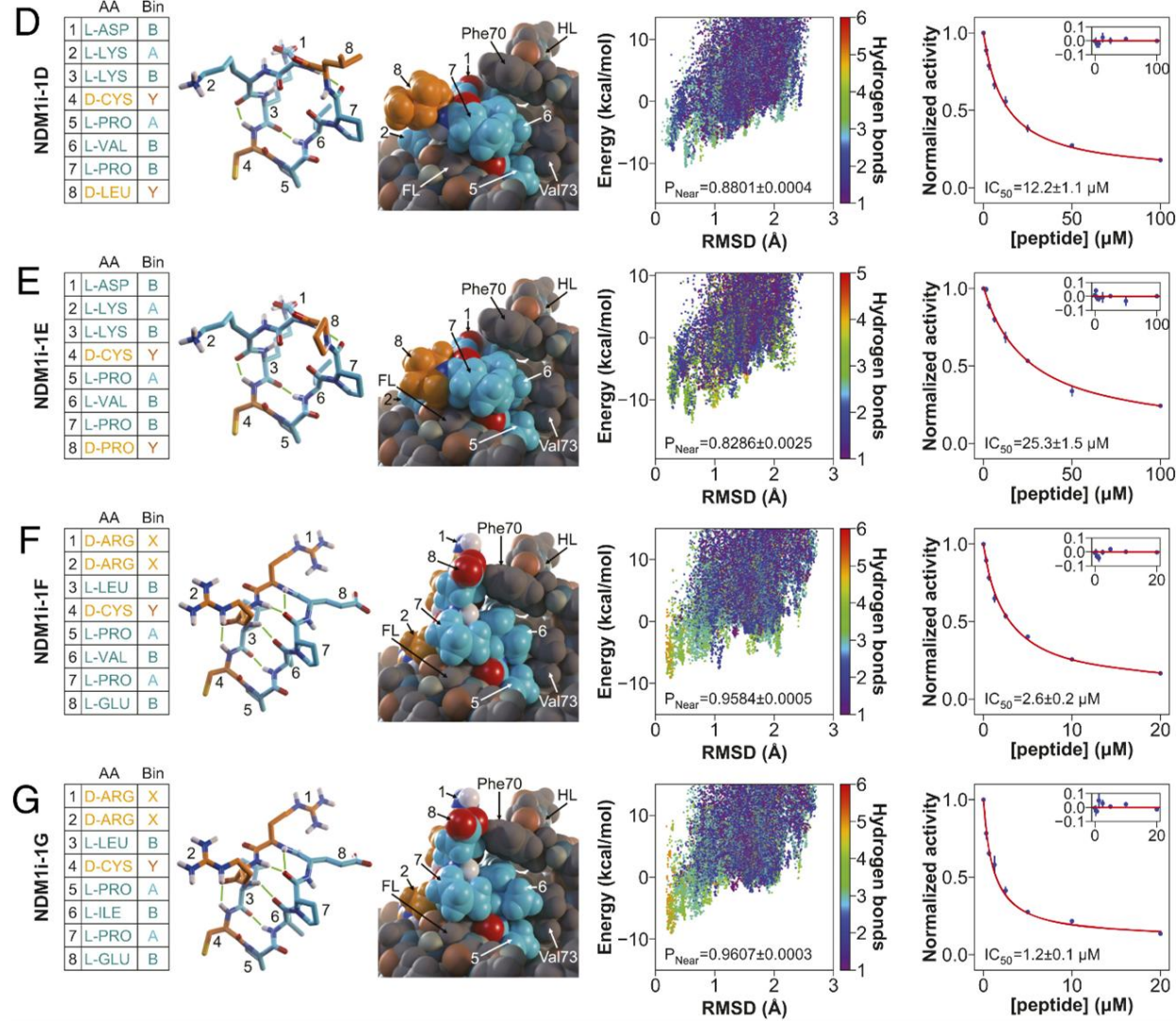
Edited by Susan Marqusee, University of California, Berkeley, CA, and approved February 10, 2021 (received for review June 19, 2020)

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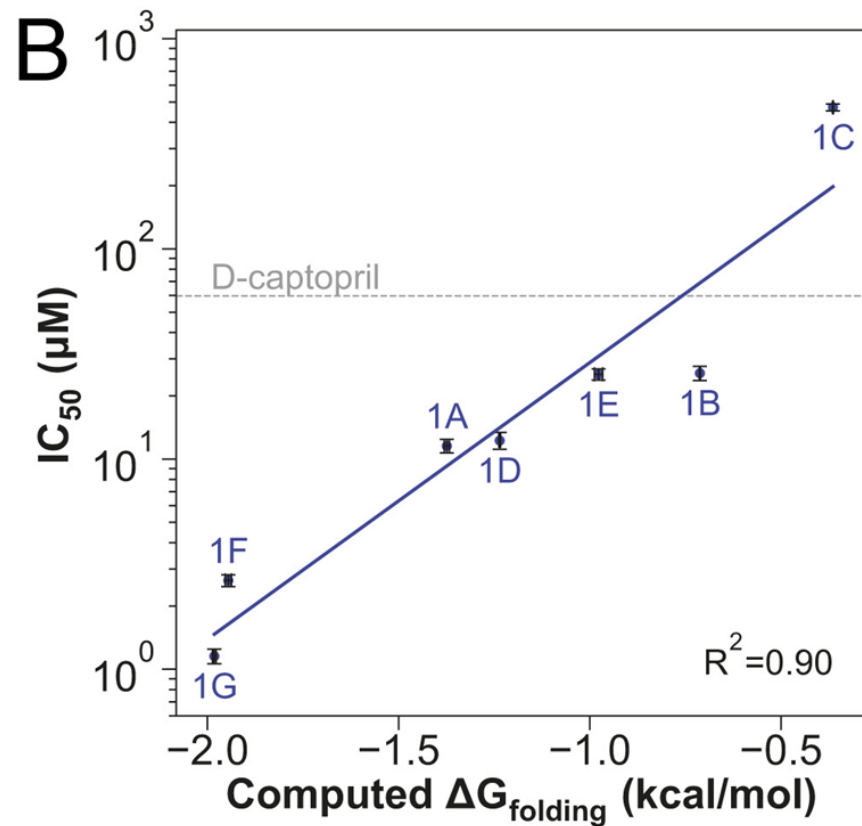
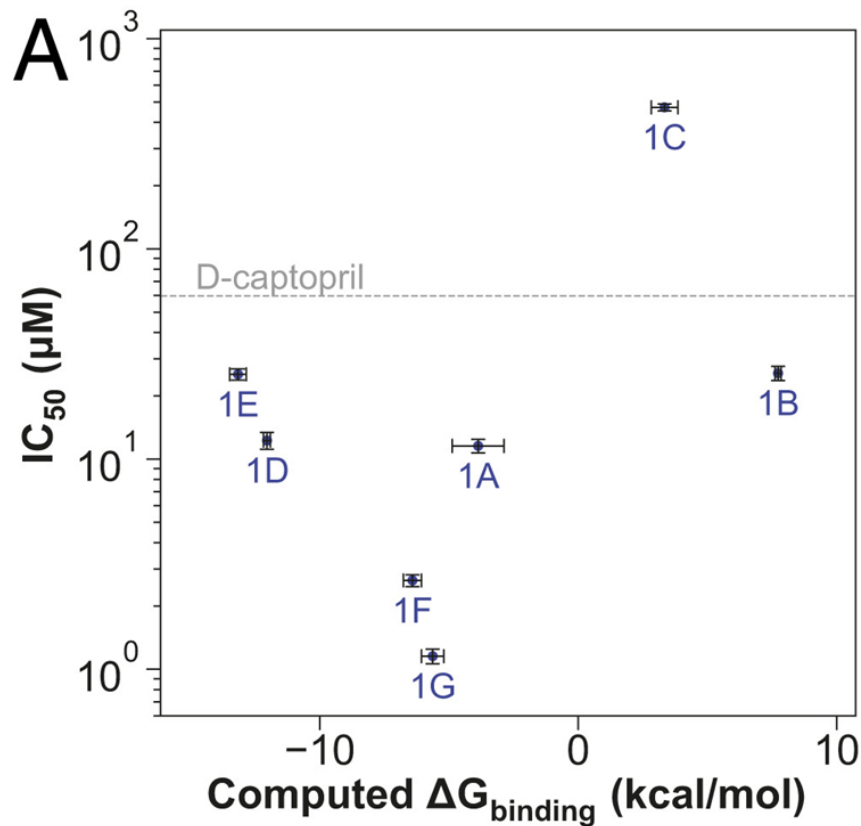
Peptide  
design starts  
from an L-  
Cys D-Pro  
stub



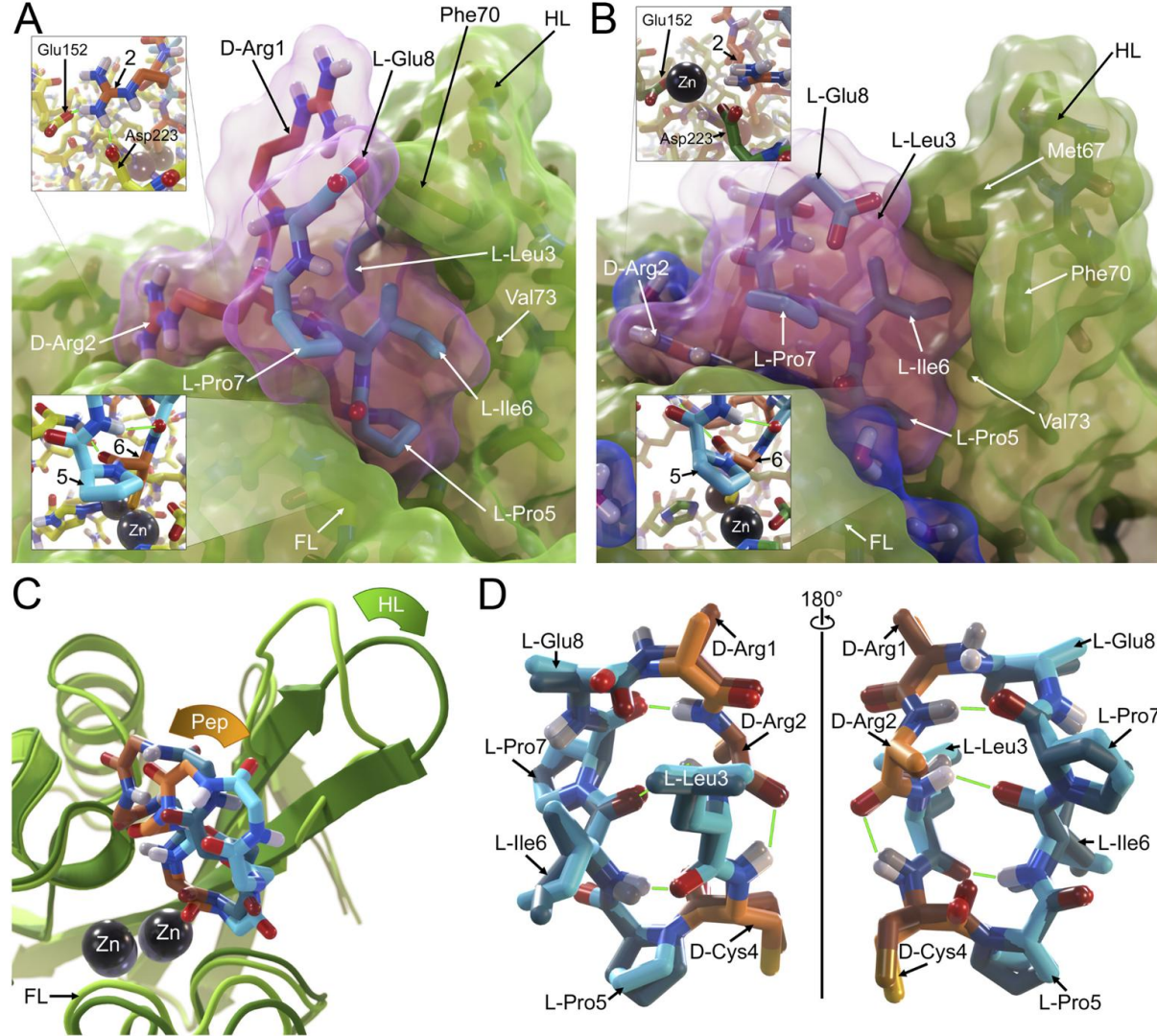
# Macrocycle design produces peptides with varying activities



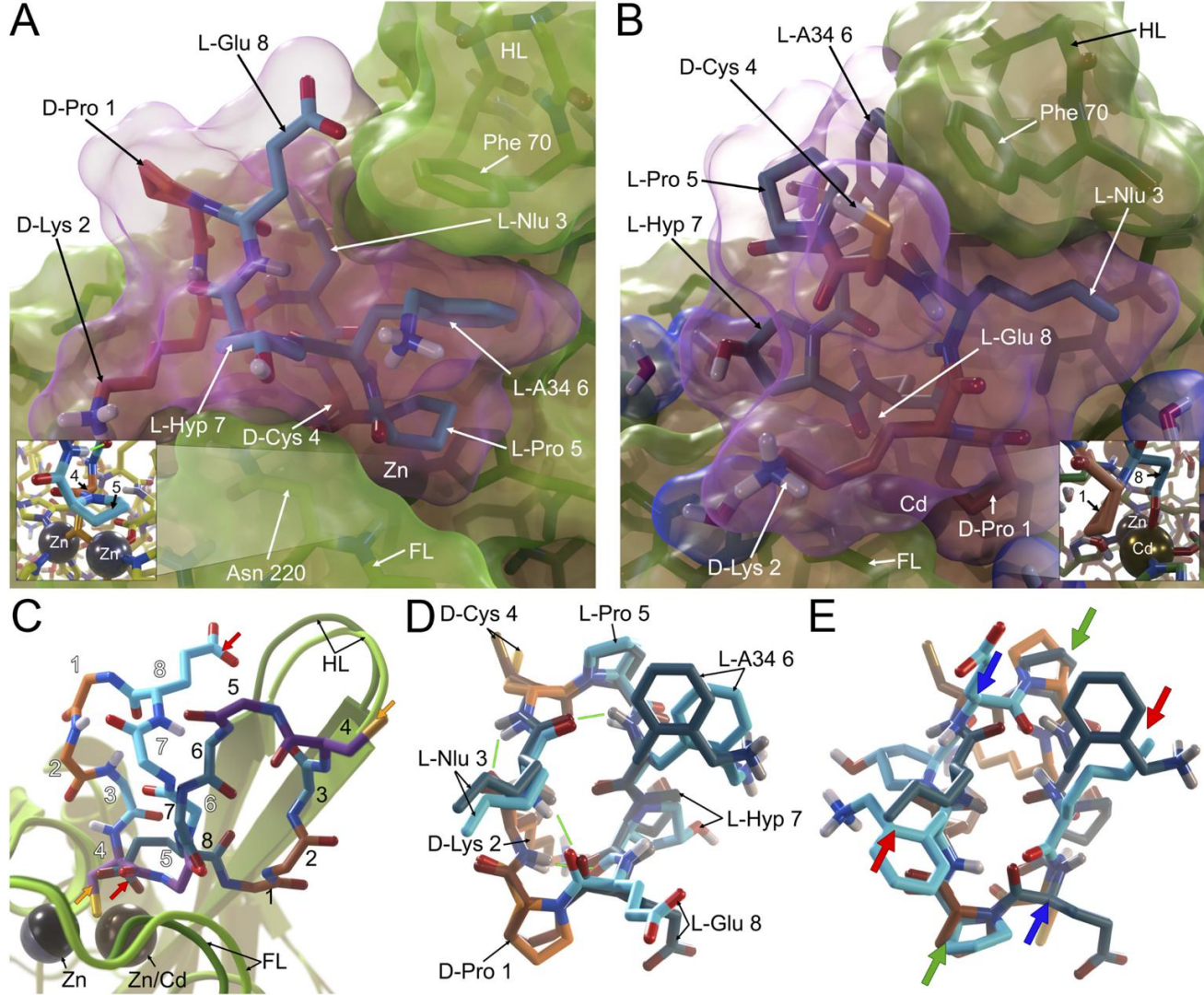
# Peptide folding is more predictive of bioactivity



Designed peptide  
NDM1i-1G binds  
the enzyme  
pocket

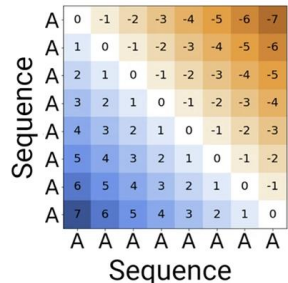


# Designed peptide NDM1i-3D contains NCAA

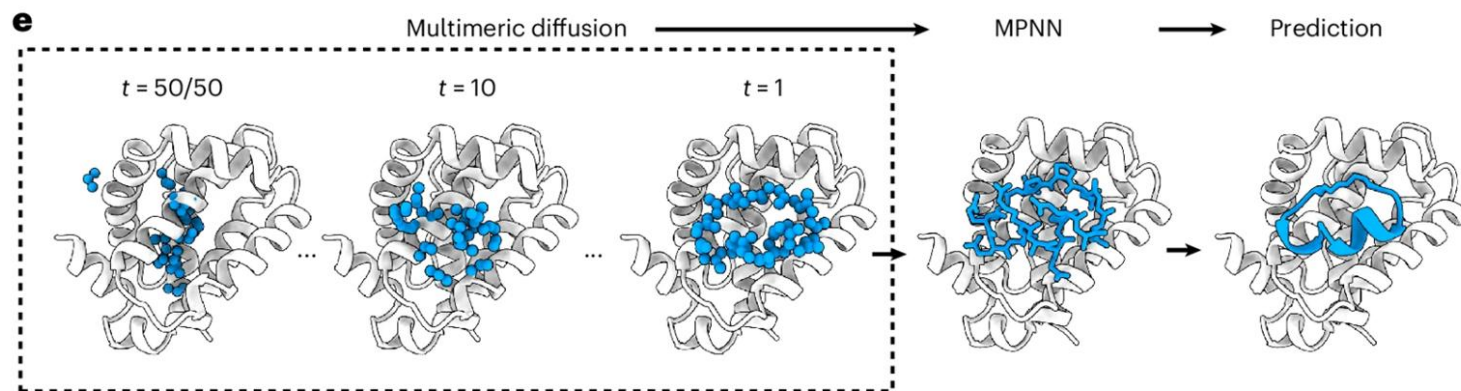
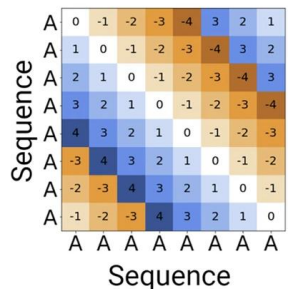


# Cyclic encoding is all you need

Relative positional encoding (Standard)



Relative positional encoding (with cyclic offset)



AfCycDesign - Rettie et al. *Nature Communications* (2025)

RFPeptides - Rettie et al. *Nature Chemical Biology* (2025)