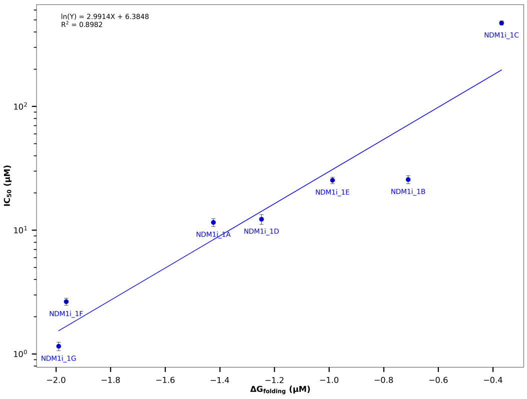
Scientific test: peptide_pnear_vs_ic50

FAILURES

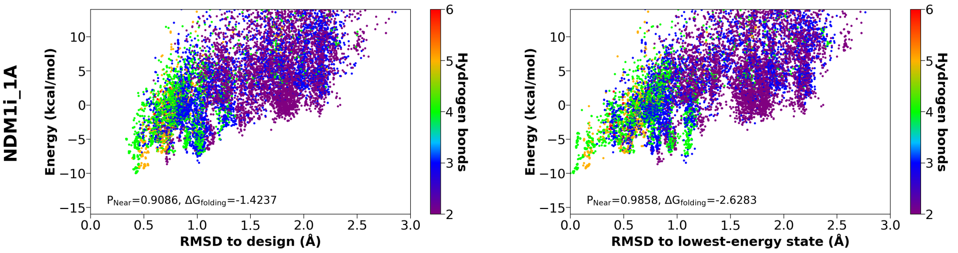
None.

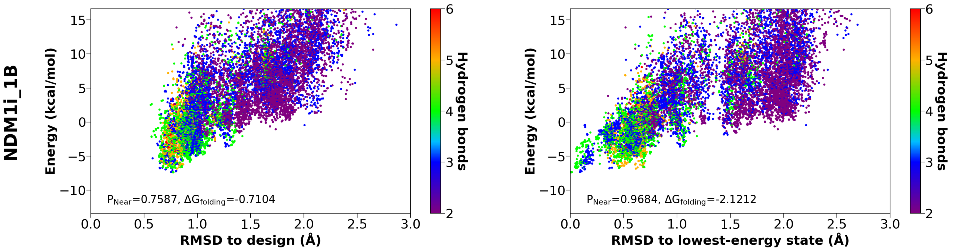
RESULTS SUMMARY

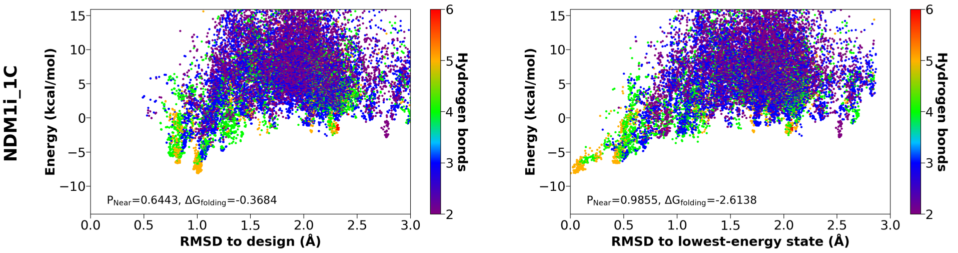
Analysis of correlation of predicted folding propensity with experimentally-measured inhibition values (which should be linear):

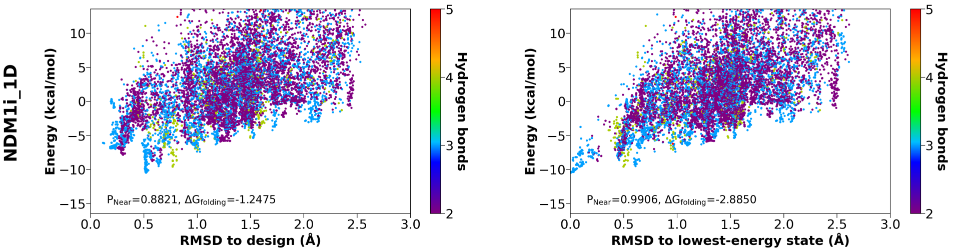


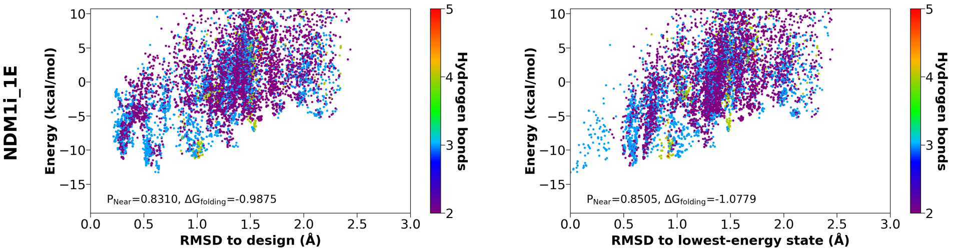
Folding funnels:

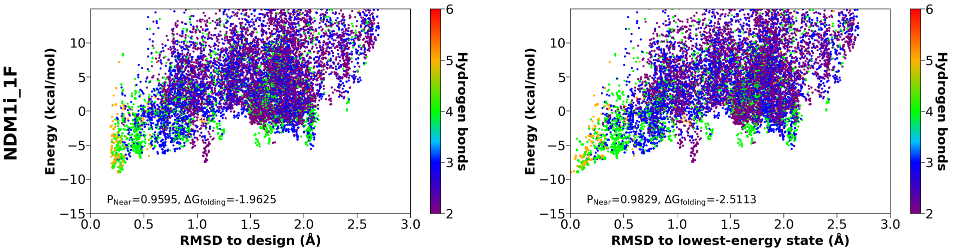

NDM1i_1A
Energy (kcal/mol)
10
5
0
−5
−10
−15
P_{Near}=0.9086, $\Delta G_{folding}$=-1.4237
0.0
0.5
1.0
1.5
2.0
2.5
3.0
RMSD to design (Å)
Hydrogen bonds
6
5
4
3
2
Energy (kcal/mol)
P_{Near}=0.9858, $\Delta G_{folding}$=-2.6283
RMSD to lowest-energy state (Å)
Hydrogen bonds

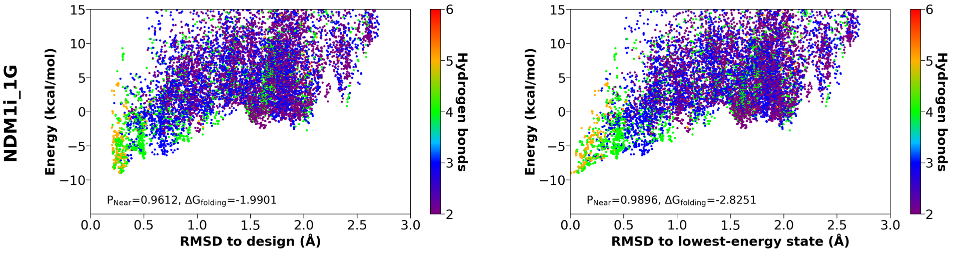

NDM1i_1B
Energy (kcal/mol)
15
10
5
0
−5
−10
P_{Near}=0.7587, $\Delta G_{folding}$=-0.7104
RMSD to design (Å)
Hydrogen bonds
6
5
4
3
2
Energy (kcal/mol)
P_{Near}=0.9684, $\Delta G_{folding}$=-2.1212
RMSD to lowest-energy state (Å)
Hydrogen bonds


NDM1i_1C
Energy (kcal/mol)
15
10
5
0
−5
−10
P_{Near}=0.6443, $\Delta G_{folding}$=-0.3684
RMSD to design (Å)
Hydrogen bonds
6
5
4
3
2
Energy (kcal/mol)
P_{Near}=0.9855, $\Delta G_{folding}$=-2.6138
RMSD to lowest-energy state (Å)
Hydrogen bonds


NDM1i_1D
Energy (kcal/mol)
10
5
0
−5
−10
−15
P_{Near}=0.8821, $\Delta G_{folding}$=-1.2475
RMSD to design (Å)
Hydrogen bonds
5
4
3
2
Energy (kcal/mol)
P_{Near}=0.9906, $\Delta G_{folding}$=-2.8850
RMSD to lowest-energy state (Å)
Hydrogen bonds


NDM1i_1E
Energy (kcal/mol)
10
5
0
−5
−10
−15
P_{Near}=0.8310, $\Delta G_{folding}$=-0.9875
RMSD to design (Å)
Hydrogen bonds
5
4
3
2
Energy (kcal/mol)
P_{Near}=0.8505, $\Delta G_{folding}$=-1.0779
RMSD to lowest-energy state (Å)
Hydrogen bonds


NDM1i_1F
Energy (kcal/mol)
10
5
0
−5
−10
−15
P_{Near}=0.9595, $\Delta G_{folding}$=-1.9625
RMSD to design (Å)
Hydrogen bonds
6
5
4
3
2
Energy (kcal/mol)
P_{Near}=0.9829, $\Delta G_{folding}$=-2.5113
RMSD to lowest-energy state (Å)
Hydrogen bonds


NDM1i_1G
Energy (kcal/mol)
15
10
5
0
−5
−10
P_{Near}=0.9612, $\Delta G_{folding}$=-1.9901
RMSD to design (Å)
Hydrogen bonds
6
5
4
3
2
Energy (kcal/mol)
P_{Near}=0.9896, $\Delta G_{folding}$=-2.8251
RMSD to lowest-energy state (Å)
Hydrogen bonds

```
Peptide NDM1i_1A:
    Total samples = 22868
    Computed PNear = 0.9086
    Computed PNear to lowest E = 0.9858
    Computed DG_folding = -1.4237
    Computed DG_folding to lowest = -2.6283
    Lowest energy = -9.9770 kcal/mol
    RMSD of lowest energy = 0.423 Angstroms
    Lowest RMSD = 0.334 Angstroms
    Highest RMSD = 2.983 Angstroms
    Energy gap (minE>1.5A - minE) = 6.3552 kcal/mol

    More than 18,000 samples? YES
    PNear value over 0.83? YES
    PNear value to lowest E over 0.83? YES
    Lowest energy under 0.45 A RMSD? YES
    Sampling below expected lower threshold RMSD(0.45 A)? YES
    Sampling beyond 1.5 A RMSD? YES
    Sampling beyond 2.5 A RMSD? YES
    3+ kcal/mol energy gap? YES
    OVERALL PASS? YES

Peptide NDM1i_1B:
    Total samples = 26852
    Computed PNear = 0.7587
    Computed PNear to lowest E = 0.9684
    Computed DG_folding = -0.7104
    Computed DG_folding to lowest = -2.1212
    Lowest energy = -7.3795 kcal/mol
    RMSD of lowest energy = 0.772 Angstroms
    Lowest RMSD = 0.562 Angstroms
    Highest RMSD = 2.891 Angstroms
    Energy gap (minE>1.5A - minE) = 6.3277 kcal/mol

    More than 18,000 samples? YES
    PNear value over 0.68? YES
    PNear value to lowest E over 0.68? YES
    Lowest energy under 0.85 A RMSD? YES
    Sampling below expected lower threshold RMSD(0.7 A)? YES
    Sampling beyond 1.5 A RMSD? YES
    Sampling beyond 2.4 A RMSD? YES
    3+ kcal/mol energy gap? YES
    OVERALL PASS? YES

Peptide NDM1i_1C:
    Total samples = 39801
    Computed PNear = 0.6443
    Computed PNear to lowest E = 0.9855
    Computed DG_folding = -0.3684
    Computed DG_folding to lowest = -2.6138
```

```
   Lowest energy = -8.0959 kcal/mol
   RMSD of lowest energy = 1.009 Angstroms
   Lowest RMSD = 0.490 Angstroms
   Highest RMSD = 3.139 Angstroms
   Energy gap (minE>1.5A - minE) = 5.2754 kcal/mol

   More than 18,000 samples? YES
   PNear value over 0.57? YES
   PNear value to lowest E over 0.57? YES
   Lowest energy under 1.05 A RMSD? YES
   Sampling below expected lower threshold RMSD(0.55 A)? YES
   Sampling beyond 1.5 A RMSD? YES
   Sampling beyond 2.9 A RMSD? YES
   3+ kcal/mol energy gap? YES
   OVERALL PASS? YES

Peptide NDM1i_1D:
   Total samples = 22410
   Computed PNear = 0.8821
   Computed PNear to lowest E = 0.9906
   Computed DG_folding = -1.2475
   Computed DG_folding to lowest = -2.8850
   Lowest energy = -10.4440 kcal/mol
   RMSD of lowest energy = 0.520 Angstroms
   Lowest RMSD = 0.117 Angstroms
   Highest RMSD = 2.800 Angstroms
   Energy gap (minE>1.5A - minE) = 5.0838 kcal/mol

   More than 18,000 samples? YES
   PNear value over 0.8? YES
   PNear value to lowest E over 0.8? YES
   Lowest energy under 0.55 A RMSD? YES
   Sampling below expected lower threshold RMSD(0.25 A)? YES
   Sampling beyond 1.5 A RMSD? YES
   Sampling beyond 2.4 A RMSD? YES
   3+ kcal/mol energy gap? YES
   OVERALL PASS? YES

Peptide NDM1i_1E:
   Total samples = 25786
   Computed PNear = 0.8310
   Computed PNear to lowest E = 0.8505
   Computed DG_folding = -0.9875
   Computed DG_folding to lowest = -1.0779
   Lowest energy = -13.2544 kcal/mol
   RMSD of lowest energy = 0.636 Angstroms
   Lowest RMSD = 0.201 Angstroms
   Highest RMSD = 2.667 Angstroms
   Energy gap (minE>1.5A - minE) = 6.0579 kcal/mol
```

```
    More than 18,000 samples? YES
    PNear value over 0.75? YES
    PNear value to lowest E over 0.75? YES
    Lowest energy under 0.65 A RMSD? YES
    Sampling below expected lower threshold RMSD(0.3 A)? YES
    Sampling beyond 1.5 A RMSD? YES
    Sampling beyond 2.2 A RMSD? YES
    3+ kcal/mol energy gap? YES
    OVERALL PASS? YES

Peptide NDM1i_1F:
    Total samples = 23365
    Computed PNear = 0.9595
    Computed PNear to lowest E = 0.9829
    Computed DG_folding = -1.9625
    Computed DG_folding to lowest = -2.5113
    Lowest energy = -9.0265 kcal/mol
    RMSD of lowest energy = 0.260 Angstroms
    Lowest RMSD = 0.189 Angstroms
    Highest RMSD = 2.802 Angstroms
    Energy gap (minE>1.5A - minE) = 3.4652 kcal/mol

    More than 18,000 samples? YES
    PNear value over 0.88? YES
    PNear value to lowest E over 0.88? YES
    Lowest energy under 0.32 A RMSD? YES
    Sampling below expected lower threshold RMSD(0.22 A)? YES
    Sampling beyond 1.5 A RMSD? YES
    Sampling beyond 2.5 A RMSD? YES
    3+ kcal/mol energy gap? YES
    OVERALL PASS? YES

Peptide NDM1i_1G:
    Total samples = 23675
    Computed PNear = 0.9612
    Computed PNear to lowest E = 0.9896
    Computed DG_folding = -1.9901
    Computed DG_folding to lowest = -2.8251
    Lowest energy = -8.9858 kcal/mol
    RMSD of lowest energy = 0.274 Angstroms
    Lowest RMSD = 0.199 Angstroms
    Highest RMSD = 2.733 Angstroms
    Energy gap (minE>1.5A - minE) = 6.2659 kcal/mol

    More than 18,000 samples? YES
    PNear value over 0.9? YES
    PNear value to lowest E over 0.9? YES
    Lowest energy under 0.32 A RMSD? YES
    Sampling below expected lower threshold RMSD(0.25 A)? YES
    Sampling beyond 1.5 A RMSD? YES
```

Sampling beyond 2.6 A RMSD? YES
5+ kcal/mol energy gap? YES
OVERALL PASS? YES

Correlation analysis:
R-squared value from fitting greater than 0.85? YES

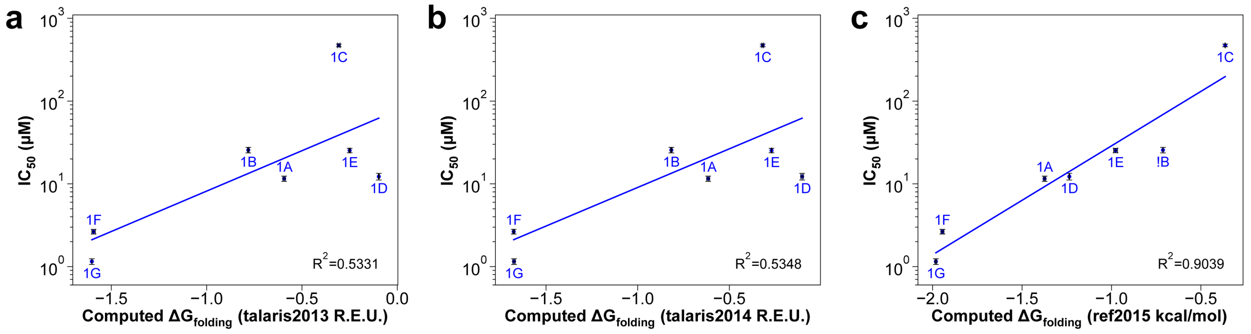
AUTHOR

Vikram K. Mulligan (vmulligan@flatironinstitute.org), Center for Computational Biology, Flatiron Institute, 5 June 2020.

PURPOSE OF THE TEST

When designing peptides to bind to targets, the flexibility of the peptide is a major impediment to binding. This is because there is an entropic cost associated with ordering a disordered molecule on binding. A rigidly-structured peptide that is pre-organized in the binding-competent conformation has a lower entropic cost and can bind more tightly. When interactions between peptide and target have been optimized with Rosetta, peptide rigidity becomes the major determininant of binding affinity. Rosetta predictions of peptide folding propensity, carried out with the simple_cycpep_predict application, correlate strongly with experimentally-measured binding affinity, at least with the ref2015 energy function. This correlation has improved from talaris2013 through talaris2014 to ref2015 due to improved training of the energy function against physical properties of fluids and against fluid MD simulation (see image below). This test exists to ensure that this correlation between prediction and experiment is maintained with future versions of the energy function and with future releases of Rosetta.

Improvements from talaris2013 through ref2015:



What does the benchmark test and why?

This test benchmarks seven peptides that were previously designed to bind to and inhibit the New Delhi metallo-beta-lactamase 1 (NDM-1). (For our purposes, IC50 values for these competitive inhibitors, measured at a constant concentration of the substrate of the reaction being inhibited, are proportional to inhibitor binding affinity.) The test carries out the following steps:

1. Large-scale conformational sampling for each of the seven peptides.

2. From the energy landscape sampled, calculation of the metrics PNear and estimated Delta-G of folding (which are closely-relarted measures of propensity to be rigid in the binding-competent conformation).

3. Plotting of the logarithm of experimentally-measured IC50 values against the computed Delta-G of folding values computed in the previous step.

4. Fitting of these data to a simple linear model, and extraction of the R-squared value for the fit.

The R-squared value should not drop below 0.85.

BENCHMARK DATASET

How many proteins are in the set?

- Seven peptides, named NDM1i-1A through NDM1i-1G. (These were previously called 8res1B, 8res2, 8res3, Holmes, Holmes_l8p, Adler, and Moriarty, respectively). All have been synthesized, the IC50 value for each binding to and inhibiting the NDM-1 enzyme's hydrolysis of nitrocefin have been measured.

What dataset are you using? Is it published? If yes, please add a citation.

- These peptides are described in Mulligan _et al._ (2020). Computationally-designed peptide macrocycle inhibitors of New Delhi metallo-beta-lactamase 1. Manuscript in preparation.

What are the input files? How were the they created?

- The input for each structure prediction run is the peptide, designed with Rosetta, in PDB format, along with its sequence in ASCII text format.

- The input for the correlation analysis is the set of output Delta-G of folding values from the structure prediction runs, plus an experimentally-measured set of IC50 values.

PROTOCOL

State and briefly describe the protocol.

The simple_cycpep_predict application uses the generalized kinematic closure algorithm (GenKIC) to rapidly sample closed conformations of a heteropolymer macrocycle built from any combination of alpha-amino acids, peptoids, or other related building-blocks. Each closure attempt is relaxed using the FastRelax protocol. For small (~8 to ~10 residue) peptide macrocycles, the application can usually sample close to the native state with less expense than protein _ab initio_.

The simple_cycpep_predict application supports hierarchical MPI-based job distribution and data reduction, as well as multi-threaded parallel job execution within a node. In MPI mode, statistics about the full sampled ensemble, including the funnel quality metric PNear, are computed automatically during the data collection and reduction phase.

Is there a publication that describes the protocol?

The simple_cycpep_predict application is described in the following publications:

1. Bhardwaj G, Mulligan VK, Bahl CD, _et al._ (2016). Accurate de novo design of hyperstable constrained peptides. _Nature_ 538(7625):329-35.

2. Hosseinzadeh P, Bhardwaj G, Mulligan VK, _et al._ (2018). Comprehensive computational design of ordered peptide macrocycles. _Science_ 358(6369):1461-6.

3. Mulligan _et al._ (2020). Computationally-designed peptide macrocycle inhibitors of New Delhi metallo-beta-lactamase 1. Manuscript in preparation.

How many CPU hours does this benchmark take approximately?

Approximately 400 CPU-hours. The test runs on 4 nodes, 20 cores per node, for roughly 5 wall hours.

In debug mode, this test takes approximately 10 CPU-hours (1 node, 20 cores, for 0.5 wall hours).

PERFORMANCE METRICS

What are the performance metrics used and why were they chosen?

All of the following must be true for the test to pass:

- More than 18,000 successful samples (200 in debug mode).

- Lowest-RMSD sample within a threshold RMSD of the design model. (Varies by peptide.)

- Highest-RMSD sample outside of a thrshold RMSD of the design model. (Varies by peptide.)

- Lowest-energy sample within a threshold RMSD of the design model. (Varies by peptide.)

- Energy gap (gap between lowest-energy sample > 1.5 A and overall lowest) bigger than a threshold value. (Varies by peptide.)

- PNear greater than a threshold value. (Varies by peptide.)

- Most importantly, R-squared value for the linear relationship between ln(IC50) and Delta-G of folding is greater than 0.85.

How do you define a pass/fail for this test?

Failure of any of the above results in an overall failure.

How were any cutoffs defined?

These are based on Rosetta performance as of 5 June 2020. The observed R-squared value reported in Mulligan _et al._ (2020) is 0.9.

KEY RESULTS

What is the baseline to compare things to - experimental data or a previous Rosetta protocol?

Past iterations of this test and performance reported in Mulligan _et al._ (2020). (See Figure 3 in that paper.)

Describe outliers in the dataset.

NDM1i-1C should have the lowest PNear value and highest Delta-G of folding; NDM1i-1F and NDM1i-1G should have the highest PNear value and lowest Delta-G of folding. These are the worst and best binders, respectively.

DEFINITIONS AND COMMENTS

State anything you think is important for someone else to replicate your results.

For full reproducibility, see the 92-page supplement to Mulligan _et al._ (2020).

LIMITATIONS

What are the limitations of the benchmark? Consider dataset, quality measures, protocol etc.

The parameters of the correlation are expected to vary from target to target, and for any given target, there are very few data. (Chemically synthesizing a candidate peptide binder and testing its affinity are inherently low-throughput experimental techniques.)

How could the benchmark be improved?

More peptides, and more targets.

What goals should be hit to make this a "good" benchmark?

Well, I think it's pretty good as it is.