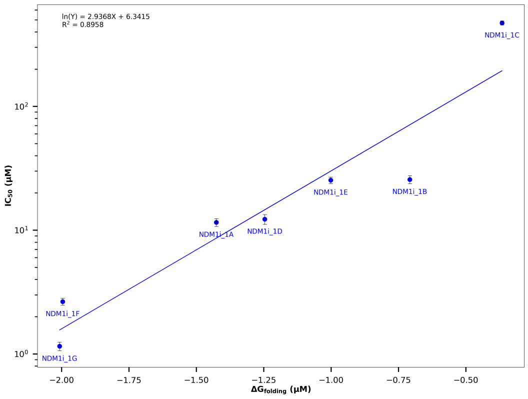
Scientific test: peptide_pnear_vs_ic50

FAILURES

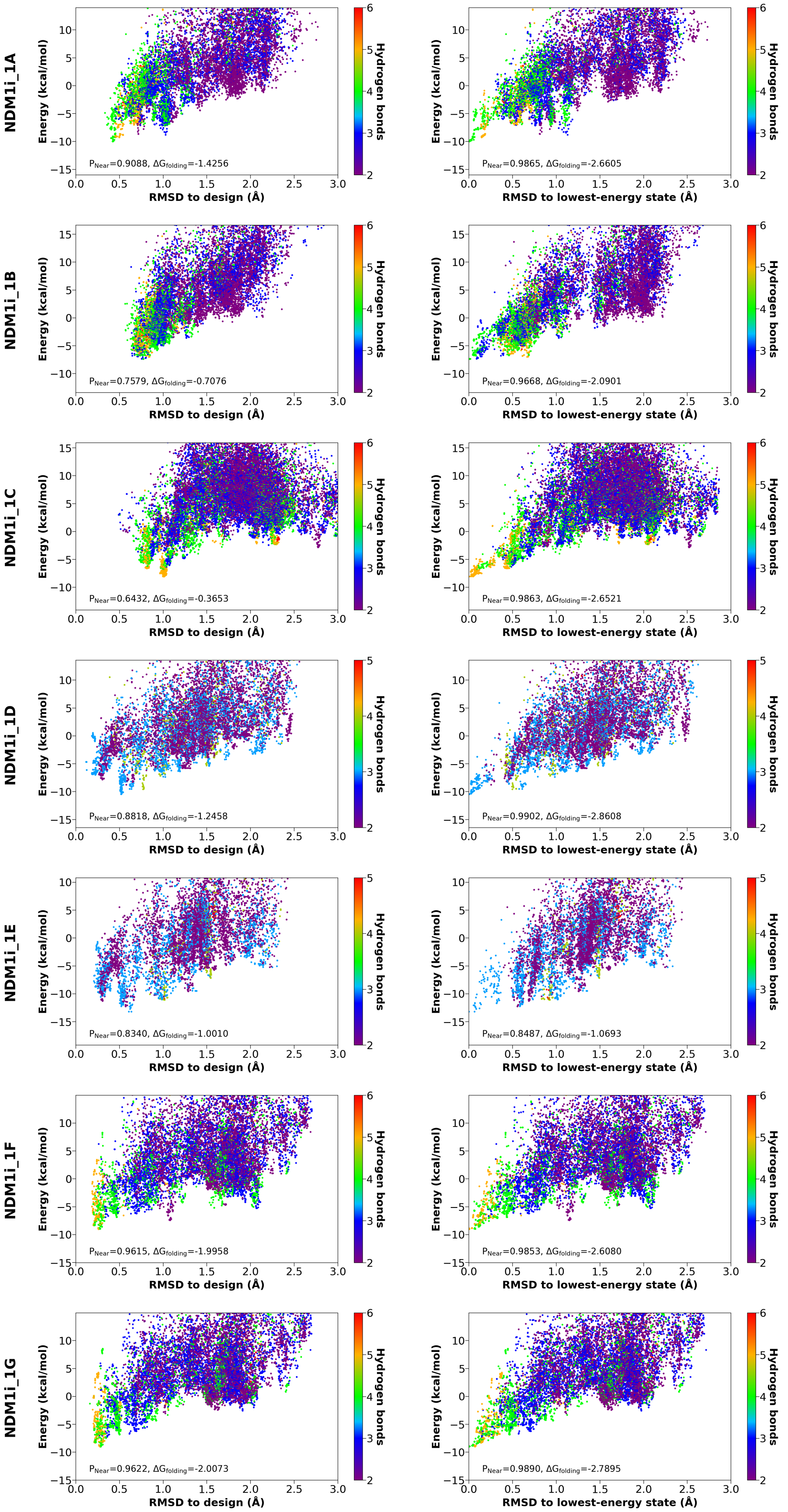
None.

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)
RMSD to design (Å)
P_{Near}=0.9088, $\Delta G_{folding}$=-1.4256
RMSD to lowest-energy state (Å)
P_{Near}=0.9865, $\Delta G_{folding}$=-2.6605
Hydrogen bonds
NDM1i_1B
P_{Near}=0.7579, $\Delta G_{folding}$=-0.7076
P_{Near}=0.9668, $\Delta G_{folding}$=-2.0901
NDM1i_1C
P_{Near}=0.6432, $\Delta G_{folding}$=-0.3653
P_{Near}=0.9863, $\Delta G_{folding}$=-2.6521
NDM1i_1D
P_{Near}=0.8818, $\Delta G_{folding}$=-1.2458
P_{Near}=0.9902, $\Delta G_{folding}$=-2.8608
NDM1i_1E
P_{Near}=0.8340, $\Delta G_{folding}$=-1.0010
P_{Near}=0.8487, $\Delta G_{folding}$=-1.0693
NDM1i_1F
P_{Near}=0.9615, $\Delta G_{folding}$=-1.9958
P_{Near}=0.9853, $\Delta G_{folding}$=-2.6080
NDM1i_1G
P_{Near}=0.9622, $\Delta G_{folding}$=-2.0073
P_{Near}=0.9890, $\Delta G_{folding}$=-2.7895
0.0 0.5 1.0 1.5 2.0 2.5 3.0
−15 −10 −5 0 5 10 15
2 3 4 5 6

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Peptide NDM1i_1A:
    Total samples = 22671
    Computed PNear = 0.9088
    Computed PNear to lowest E = 0.9865
    Computed DG_folding = -1.4256
    Computed DG_folding to lowest = -2.6605
    Lowest energy = -10.0020 kcal/mol
    RMSD of lowest energy = 0.423 Angstroms
    Lowest RMSD = 0.334 Angstroms
    Highest RMSD = 2.882 Angstroms
    Energy gap (minE>1.5A - minE) = 7.4910 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 = 27142
    Computed PNear = 0.7579
    Computed PNear to lowest E = 0.9668
    Computed DG_folding = -0.7076
    Computed DG_folding to lowest = -2.0901
    Lowest energy = -7.3830 kcal/mol
    RMSD of lowest energy = 0.773 Angstroms
    Lowest RMSD = 0.565 Angstroms
    Highest RMSD = 3.252 Angstroms
    Energy gap (minE>1.5A - minE) = 6.6541 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 = 39739
    Computed PNear = 0.6432
    Computed PNear to lowest E = 0.9863
    Computed DG_folding = -0.3653
    Computed DG_folding to lowest = -2.6521
    Lowest energy = -8.0801 kcal/mol
    RMSD of lowest energy = 1.011 Angstroms
    Lowest RMSD = 0.491 Angstroms
    Highest RMSD = 3.136 Angstroms
    Energy gap (minE>1.5A - minE) = 5.2544 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 = 22542
    Computed PNear = 0.8818
    Computed PNear to lowest E = 0.9902
    Computed DG_folding = -1.2458
    Computed DG_folding to lowest = -2.8608
    Lowest energy = -10.4435 kcal/mol
    RMSD of lowest energy = 0.521 Angstroms
    Lowest RMSD = 0.118 Angstroms
    Highest RMSD = 2.649 Angstroms
    Energy gap (minE>1.5A - minE) = 5.0589 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 = 25885
    Computed PNear = 0.8340
    Computed PNear to lowest E = 0.8487
    Computed DG_folding = -1.0010
    Computed DG_folding to lowest = -1.0693
    Lowest energy = -13.1983 kcal/mol
    RMSD of lowest energy = 0.637 Angstroms
    Lowest RMSD = 0.207 Angstroms
    Highest RMSD = 2.539 Angstroms
    Energy gap (minE>1.5A - minE) = 6.0351 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 = 23366
    Computed PNear = 0.9615
    Computed PNear to lowest E = 0.9853
    Computed DG_folding = -1.9958
    Computed DG_folding to lowest = -2.6080
    Lowest energy = -9.0261 kcal/mol
    RMSD of lowest energy = 0.260 Angstroms
    Lowest RMSD = 0.185 Angstroms
    Highest RMSD = 2.723 Angstroms
    Energy gap (minE>1.5A - minE) = 3.7650 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 = 23504
    Computed PNear = 0.9622
    Computed PNear to lowest E = 0.9890
    Computed DG_folding = -2.0073
    Computed DG_folding to lowest = -2.7895
    Lowest energy = -9.0253 kcal/mol
    RMSD of lowest energy = 0.285 Angstroms
    Lowest RMSD = 0.161 Angstroms
    Highest RMSD = 2.780 Angstroms
    Energy gap (minE>1.5A - minE) = 6.4660 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
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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

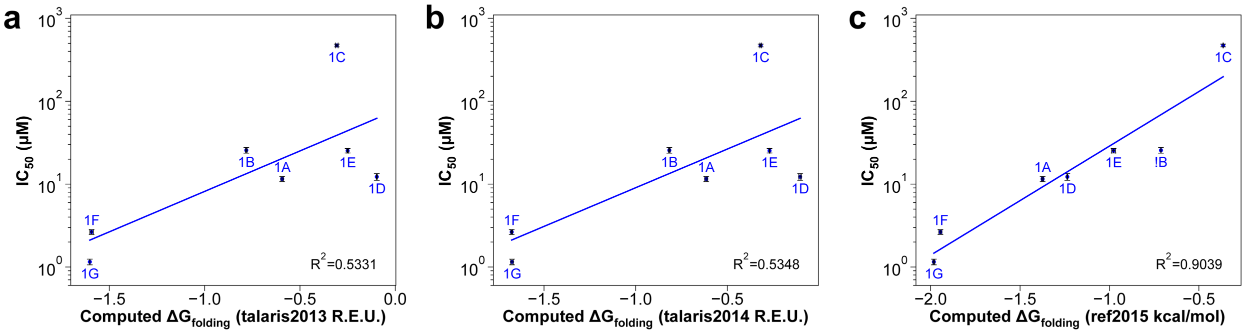
AUTHOR

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.