Supplementary Information (SI) for Physical Chemistry Chemical Physics.
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Supplementary Information: Preserving structural integrity: Fold reproducibility in computational design of proteins non-homologous to wild-type sequence

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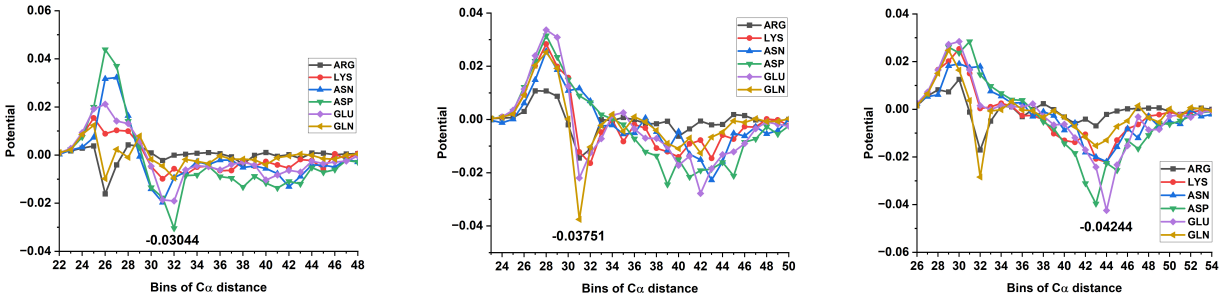
Comparison with RosettaDesign

A comparison of our method with RosettaDesign is carried out and is tabulated in Table S1. From the table, it is clear that our model and developed statistical potential are simple and coarse-grained which significantly reduces computational cost. While RosettaDesign uses Rosetta energy function, a composite energy function that merges physical modeling with statistical preferences. Again Rosetta uses all atom model while our model is coarse-grained. Given the fundamental differences between the two methods, a direct comparison between the sequences generated by our approach and those from RosettaDesign would be inappropriate, as the two methods operate at different levels of design complexity and goal. Nevertheless, we have designed three sequences using RosettaDesign web server (http://rosettadesign.med.unc.edu/) for each three target structures and are represented by RDesign_1, RDesign_2 and RDesign_3. The RMSDs, sequence similarity with the wild-type sequences are also calculated and are provided in Fig. S2, Fig. S3 and Fig. S4. Interestingly, while these sequences achieve 100% recognition of the target secondary structure content, their sequence similarity to the wild-type sequences is significantly higher than that of the sequences designed using our model. The 100% recognition of secondary structure is attributed to the use of the native backbone during RosettaDesign. In contrast, our Cα-based coarse-grained model allows greater sequence diversity, making it better suited for exploring non-homologous yet fold-compatible sequences.

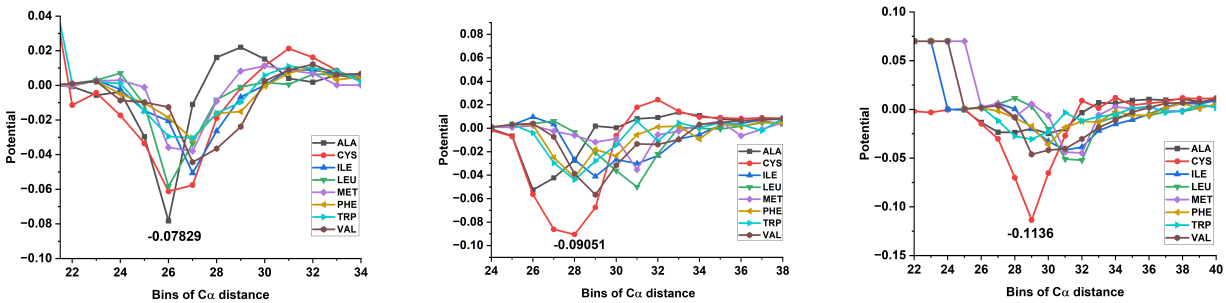
Table S1. Comparison of our design method with RosettaDesign

Feature	**RosettaDesign (web server)**	**Our Model**
Objective	Focuses on stabilizing a given protein fold generating sequences compatible with a fixed backbone.	Design non-homologous sequences with low sequence similarity to wild-type, while preserving the native fold.
Input	All-atom or backbone PDB structure.	Cα backbone coordinates of the target protein.
Potential	The energy function consists of (i) a Lennard–Jones potential, (ii) the Lazaridis-Karplus implicit solvation model, (iii) an explicit orientation dependent hydrogen bonding term, (iv) torsion potential, (v) a unique reference value for each amino acid type, (vi) electrostatic interactions.	Cα distance based one body and two body statistical potentials.
Design method	Side-chain rotamer optimization by Dunbrack's backbone dependent rotamer library, energy minimization using Monte Carlo simulated annealing starting from very high temperature to 0°. Starting from a random sequence, single amino acid substitutions are accepted via Metropolis criterion.	Monte Carlo simulation incorporating random point mutations, single amino acid substitutions are accepted via Metropolis criterion.
Sequence variability	Tends to produce sequences with amino acid frequencies comparable to those found in naturally occurring proteins. Yield sequences with moderate similarity to wild type	Specifically aims for non-homologous sequences that fold into native-like structure.

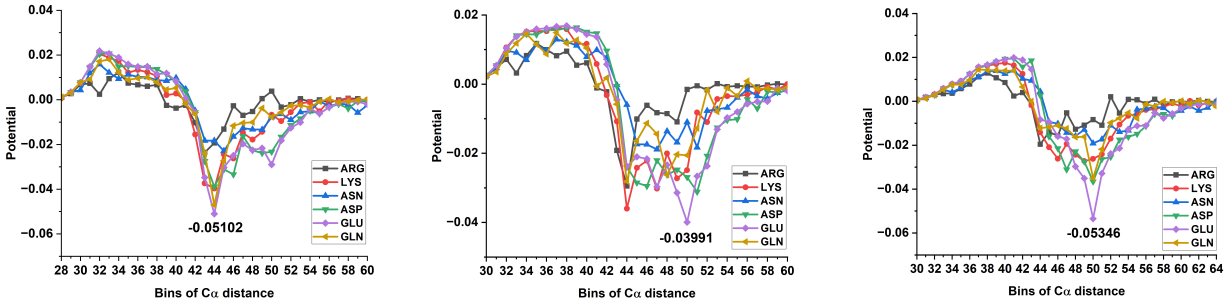
Following are the plots of potential versus bins of Cα distances for different nearest neighbor distances, NN_i, i=2, 3, 4, 6, 7, 8 and 9.



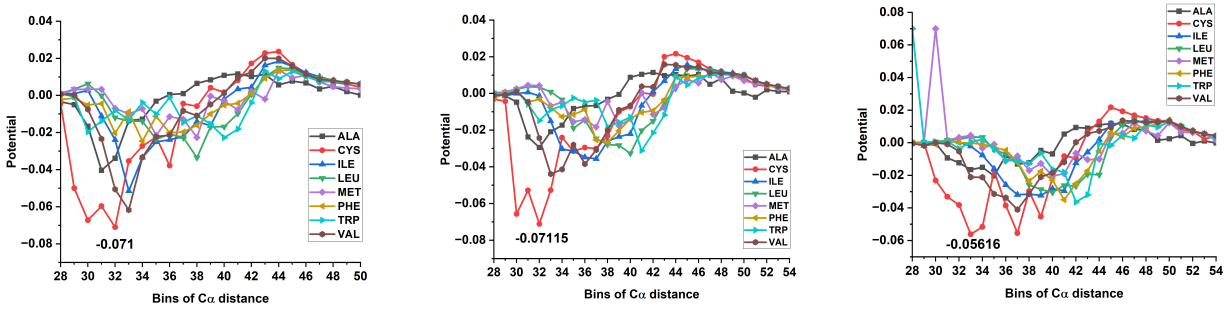
(a) NN_2-hydrophilic (b) NN_3-hydrophilic (c) NN_4-hydrophilic



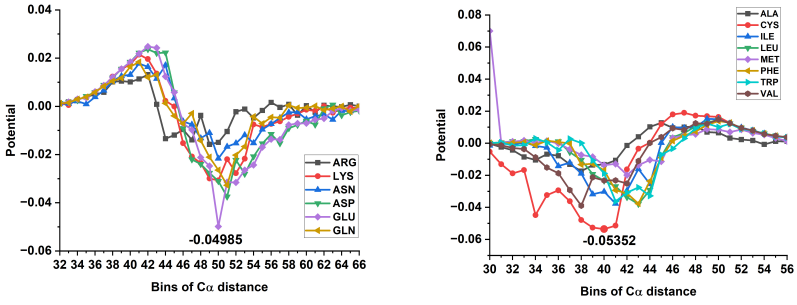
(d) NN_2-hydrophobic (e) NN_3-hydrophobic (f) NN_4-hydrophobic



(g) NN_6-hydrophilic (h) NN_7-hydrophilic (i) NN_8-hydrophilic

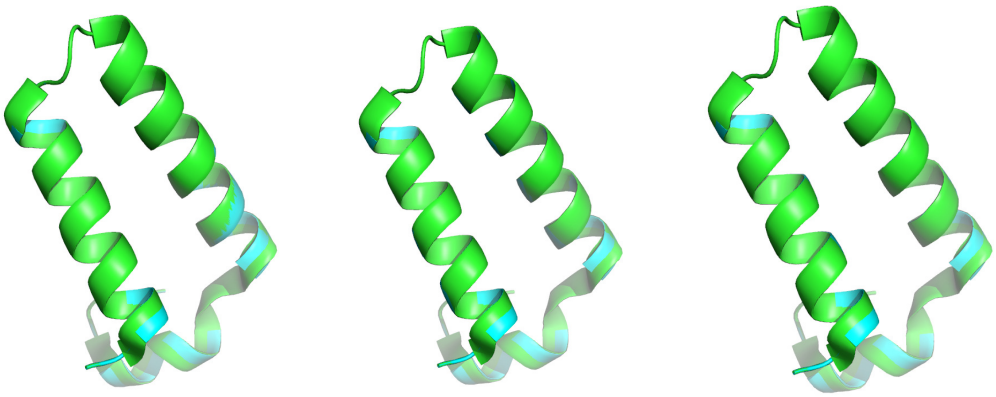


(j) NN_6-hydrophobic (k) NN_7-hydrophobic (l) NN_8-hydrophobic



(m) NN_9-hydrophilic (n) NN_9-hydrophobic

Fig. S1. Plot of potential versus bins of Cα distances (each bin is of 0.2 Å size) for hydrophilic and hydrophobic amino acid residues.

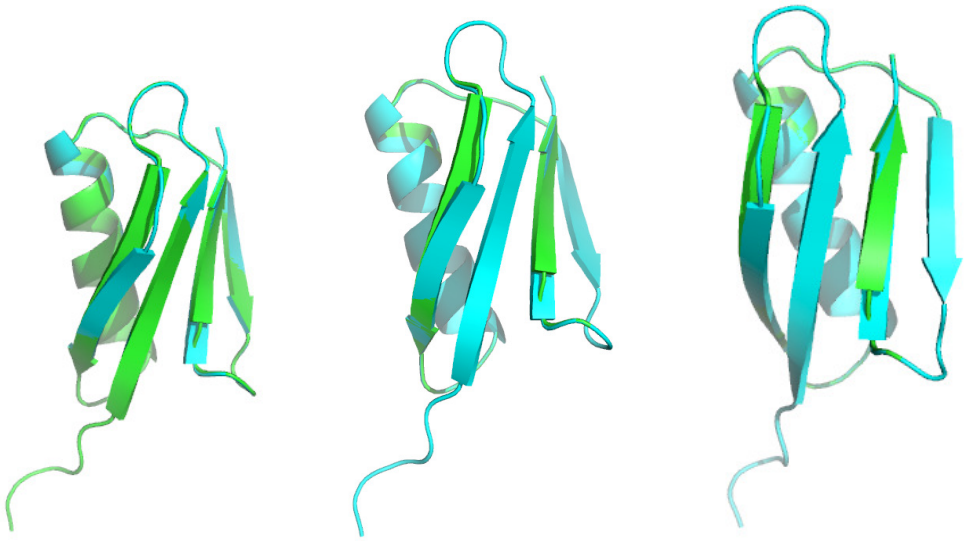


(a) 6FM8, RDesign_1, RMSD=0.007 Å, **Sim=20%**

(b) 6FM8, RDesign_2, RMSD=0.007 Å, **Sim=20%**

(c) 6FM8, RDesign_3, RMSD=0.003 Å, **Sim=16%**

Fig. S2. The three-dimensional structures of the target protein, 6FM8 (colored green) and the structure of the protein designed by RosettaDesign (colored cyan) and their corresponding RMSDs and sequence similarity to the wild-type sequences.



(a) 2IGD, RDesign_1, RMSD=0.008 Å, **Sim=36.06%**

(b) 2IGD, RDesign_2, RMSD=0.008 Å, **Sim=36.06%**

(c) 2IGD, RDesign_3, RMSD=0.008 Å, **Sim=36.06%**

Fig. S3. The three-dimensional structures of the target protein, 2IGD (colored green) and the structure of the protein designed by RosettaDesign (colored cyan) and their corresponding RMSDs and sequence similarity to the wild-type sequences.

The PDB IDs of the 556 proteins which are used to generate non-native conformational ensemble are provided in Table S2 to enhance transparency and reproducibility.

Table S2. PDB IDs of 556 proteins considered for non-native conformation generation

PDB IDs										
1A3H.pdb	1A8Q.pdb	1AAJ.pdb	1ACX.pdb	1AD6.pdb	1AJM.pdb	1AKO.pdb	1ALY.pdb	1ANU.pdb	1ARL.pdb	1BEC.pdb
1BEO.pdb	1BG5.pdb	1BGF.pdb	1BHE.pdb	1BI5.pdb	1BKB.pdb	1BM8.pdb	1BOL.pdb	1BQC.pdb	1BUO.pdb	1BYI.pdb
1BYW.pdb	1C25.pdb	1C3G.pdb	1C44.pdb	1CDY.pdb	1CEM.pdb	1CQY.pdb	1CRZ.pdb	1CWY.pdb	1CZ1.pdb	1D2P.pdb
1DAB.pdb	1DHN.pdb	1DIV.pdb	1DIX.pdb	1DJA.pdb	1DQ0.pdb	1DSL.pdb	1DTZ.pdb	1DUA.pdb	1DUS.pdb	1DVO.pdb
1E5W.pdb	1EDG.pdb	1EDQ.pdb	1EHD.pdb	1EIA.pdb	1EOV.pdb	1EQ6.pdb	1ESR.pdb	1EW4.pdb	1EYH.pdb	1F00.pdb
1F1S.pdb	1FBN.pdb	1FHL.pdb	1FNF.pdb	1FSF.pdb	1FUS.pdb	1FYX.pdb	1G2U.pdb	1G62.pdb	1G8A.pdb	1GBS.pdb
1GH2.pdb	1GLN.pdb	1GQN.pdb	1GQZ.pdb	1GVP.pdb	1GYD.pdb	1GYV.pdb	1H09.pdb	1H2P.pdb	1H3Q.pdb	1H4A.pdb
1HE9.pdb	1HH2.pdb	1HKA.pdb	1HOE.pdb	1I5P.pdb	1I60.pdb	1IAD.pdb	1IFG.pdb	1IJB.pdb	1ILK.pdb	1IO2.pdb
1IS1.pdb	1IV8.pdb	1J2V.pdb	1J7G.pdb	1J7X.pdb	1K40.pdb	1K89.pdb	1KIV.pdb	1KLO.pdb	1KOE.pdb	1KS9.pdb
1KUU.pdb	1LLN.pdb	1LMI.pdb	1LNS.pdb	1LPL.pdb	1LWB.pdb	1LXA.pdb	1MF7.pdb	1MIL.pdb	1MIX.pdb	1MSC.pdb
1MUF.pdb	1MZL.pdb	1N7O.pdb	1N81.pdb	1ND7.pdb	1NG6.pdb	1NI5.pdb	1NOA.pdb	1O5T.pdb	1OJQ.pdb	1OLR.pdb
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1QXX.pdb	1QZM.pdb	1R8N.pdb	1RC9.pdb	1RCB.pdb	1RL0.pdb	1S29.pdb	1S7I.pdb	1SBX.pdb	1SCZ.pdb	1SRV.pdb
1SUR.pdb	1T2I.pdb	1TJE.pdb	1TQG.pdb	1UB9.pdb	1UKF.pdb	1ULZ.pdb	1UOK.pdb	1UX5.pdb	1V05.pdb	1VCC.pdb
1VGJ.pdb	1VGP.pdb	1VLS.pdb	1VSM.pdb	1WGB.pdb	1WHI.pdb	1WS6.pdb	1WU3.pdb	1WWB.pdb	1WWI.pdb	1X0M.pdb
1X1H.pdb	1X3O.pdb	1XEU.pdb	1XIX.pdb	1XT0.pdb	1Y2Q.pdb	1YQ8.pdb	1YUW.pdb	1YW5.pdb	1YZF.pdb	1ZEQ.pdb
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2J9V.pdb	2JA4.pdb	2JA9.pdb	2LAO.pdb	2NSC.pdb	2NSN.pdb	2NWD.pdb	2O6Q.pdb	2OF3.pdb	2OHE.pdb	2OKT.pdb
2ORX.pdb	2OV1.pdb	2OZF.pdb	2P25.pdb	2P52.pdb	2PCY.pdb	2PET.pdb	2PKO.pdb	2PLC.pdb	2PLF.pdb	2PND.pdb
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3KVD.pdb	3LD1.pdb	3LIG.pdb	3LJ8.pdb	3M66.pdb	3M70.pdb	3MTV.pdb	3MX7.pdb	3MZZ.pdb	3N0K.pdb	3ND2.pdb
3NSM.pdb	3O5P.pdb	3ONJ.pdb	3OZH.pdb	3PBH.pdb	3PFM.pdb	3PM2.pdb	3PTJ.pdb	3QPA.pdb	3R6D.pdb	3RA2.pdb
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3TOW.pdb	3TUA.pdb	3UAF.pdb	3UFC.pdb	3VFI.pdb	3VOR.pdb	3VPY.pdb	3WA1.pdb	3WFV.pdb	3WP5.pdb	3WY8.pdb
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5Y8E.pdb	5YDN.pdb	5YNS.pdb	5YO6.pdb	5YSX.pdb	5YWZ.pdb	5Z2D.pdb	5Z67.pdb	5Z7Q.pdb	5ZA4.pdb	5ZHG.pdb
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6EXX.pdb	6F2Q.pdb	6F4C.pdb	6F4M.pdb	6FHZ.pdb	6GGP.pdb	6GPM.pdb	6GV5.pdb	6IGV.pdb	6IOJ.pdb	6IY4.pdb
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6ZM8.pdb	7BFY.pdb	7BXY.pdb	7CHR.pdb	7CNB.pdb	7CSN.pdb	7DKR.pdb	7DMF.pdb	7DMS.pdb	7DU0.pdb	7EP8.pdb
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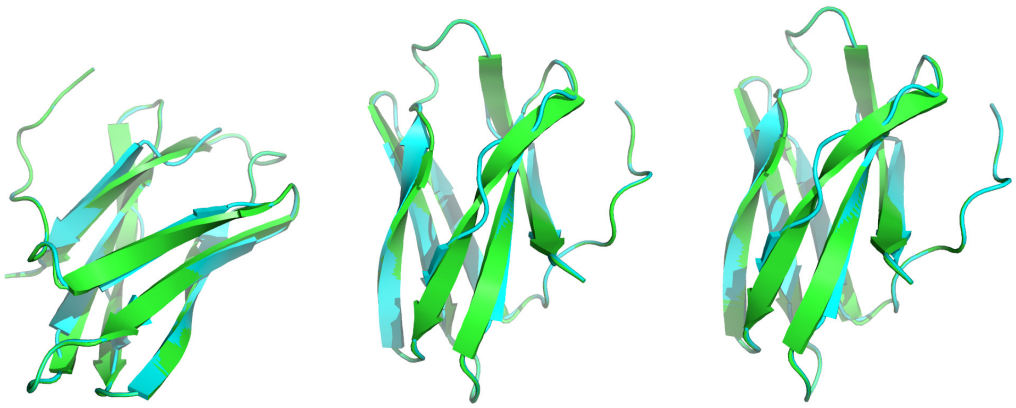

(a) 1HOE, RDesign_1, RMSD=0.004 Å, **Sim=36.48%**

(b) 1HOE, RDesign_2, RMSD=0.006 Å, **Sim=41.89%**

(c) 1HOE, RDesign_3, RMSD=0.004 Å, **Sim=40.54%**

Fig. S4. The three-dimensional structures of the target protein, 1HOE (colored green) and the structure of the protein designed by RosettaDesign (colored cyan) and their corresponding RMSDs and sequence similarity to the wild-type sequences.