Supporting Information

ROSETTA v3 options used for *de novo* folding 10,000 T4-lysozyme models with 25 EPR distance restraints scored according to the ROSETTAEPR knowledge-based potential

```
-abinitio::increase_cycles 2.5

-fold_cst::force_minimize
-constraints::cst_file  ./2LZM_dist_w4.cst
-constraints::cst_weight 1.0
-constraints::epr_distance
-constraints::viol
-constraints::viol_level 101

-frags::scoring
-frags::picking::selecting_rule BestTotalScoreSelector

-in::path::database minirosetta_database_r34586
-in::file::native ./2LZM_.pdb
-in::file::fasta ./2LZM_.fasta
-in::file:frag3 ./aa2LZM_03_05.200_v1_3
-in::file::frag9 ./aa2LZM_09_05.200_v1_3

-out::output
-out::prefix 2LZM_
-out::file::silent ./2LZM_.out
-out::file::silent_struct_type binary
-out::file::scorefile ./2LZM_.sc
-out::nstructs 10000
-out::show_accessed_options
```

ROSETTA v3 options used for full-atom refinement of one T4-lysozyme *de novo* folded model with no distance restraints, resulting in ten new models complete with amino acid sidechains

```
-relax::sequence

-in::path::database ./minirsetta_database_r34586
```

```
-in::file::native ./2LZM_.pdb
-in::file::fullatom

-corrections::correct

-out::output
-out::prefix 2LZM_fa_
-out::file::silent ./2LZM_fa.out
-out::file::silent_struct_type binary
-out::file::scorefile ./2LZM_fa.fsc
-out::nstructs 10
-out::show_accessed_options
```

Tables

Table S1: Previously reported EPR distance restraints of T4-lysozyme compared with distances observed in the crystal structure

AA1-AA2[a]	$d_{C\beta}$ (Å)[b]	d_{SL} (Å)[c]	σ_{SL} (Å)[d]	Reference
061-135	37.7	47.2	2.2	Borbat *et al*, 2002
065-135	34.3	46.3	2.2	Borbat *et al*, 2002
061-086	34.5	37.5	2.0	Borbat *et al*, 2002
065-086	28.9	37.4	2.7	Borbat *et al*, 2002
080-135	26.7	36.8	1.0	Borbat *et al*, 2002
061-080	28.7	34.0	2.2	Borbat *et al*, 2002
065-080	22.6	26.5	3.8	Borbat *et al*, 2002
119-131	13.2	25.0	5.0	Alexander *et al*, 2008
123-131	14.6	23.0	5.0	Alexander *et al*, 2008
065-076	16.8	21.4	2.8	Borbat *et al*, 2002
116-131	11.1	19.0	10.0	Alexander *et al*, 2008
119-128	10.4	19.0	4.0	Alexander *et al*, 2008
140-151	15.5	18.0	9.0	Alexander *et al*, 2008
089-093	9.8	16.0	3.0	Alexander *et al*, 2008
086-119	10.0	15.0	3.0	Alexander *et al*, 2008
120-131	10.5	14.0	3.0	Alexander *et al*, 2008
127-151	9.6	14.0	2.4	Alexander *et al*, 2008
140-147	10.1	13.0	7.0	Alexander *et al*, 2008
131-150	8.7	5.7	0.4	Alexander *et al*, 2008
127-154	5.9	7.0	3.0	Alexander *et al*, 2008
131-154	9.5	6.5	4.0	Alexander *et al*, 2008
134-151	10.7	7.0	0.8	Alexander *et al*, 2008
131-151	10.4	9.0	8.0	Alexander *et al*, 2008
088-100	8.9	<6.0	3.0	Alexander *et al*, 2008
089-096	8.4	<6.0	3.0	Alexander *et al*, 2008

[a] Indices of spin labeled amino acids with respect to the crystal structure
[b] Cβ distance as reported in the crystal structure
[c] Spin label distance as observed by EPR
[d] Standard deviation as observed by EPR

Table S2: T4-lysozyme α-helical core domain residues over which model RMSD_{C_α} and sidechain rotamer recovery were computed relative to the crystal structure in order to assess model accuracy

RMSD	Rotamer Recovery
70-80, 82-90, 93-106, 108-113, 115-123, 126-134, 137-141, 143-155	74-75, 78, 84, 87-88, 91, 94-104, 106, 110-111, 113-114, 116-118, 120-121, 125-126, 128-130, 132-134, 136, 138-139, 145-153, 156

Table S3: Benchmarking results of *de novo* folding 10,000 T4-lysozyme models using no EPR distance restraints, 25 distance restraints scored according to the RosettaEPR knowledge-based potential, and 25 distance restraints scored according to a bounded quadratic penalty

Weight	% Models with RMSD_{C_α} < 3.5Å	% Models with RMSD_{C_α} < 7.5Å	% Models with RMSD_{C_α} < 3.5Å	% Models with RMSD_{C_α} < 7.5Å
0	0.03	7.17		
	ROSETTAEPR		Bounded	
1	0.73	21.98	0.89	37.56
2	1.41	31.07	1.18	40.95
3	2.01	37.20	1.58	41.84
4	2.05	42.08	1.62	41.09
5	1.83	45.65	1.43	40.44
6	1.60	47.29	1.40	39.50
7	1.35	49.60	1.40	38.42
8	1.31	51.21	1.62	38.01
9	0.87	50.89	1.59	37.42
10	1.02	52.70	1.57	37.22
20	0.51	54.89	1.44	34.02
30	0.46	53.28	1.22	32.77
40	0.25	49.74	1.27	32.16
50	0.17	47.43	1.12	32.27
60	0.07	43.86	1.01	31.07
70	0.03	43.95	1.29	31.67
80	0.02	43.07	1.34	31.05
90	0.01	40.92	1.39	31.22
100	0.01	41.11	1.12	30.62

Table S4: Enrichment of low-RMSD (< 3.5 Å) model recovery resulting from eight iterations of high-resolution refinement of T4-lyosyzme in ROSETTA v3

Round	Enrichment[a]	RMSD_{C_α} of Best-Scoring Model (Å)	ROSETTA Score of Best-Scoring Model (REU)
1	2.09[b]	5.09	-229.363
2	1.20	2.94	-232.705
3	1.18	3.00	-235.389
4	1.47	1.73	-237.707
5[c]	1.08	1.83	-238.914
6	1.00	1.83	-239.219
7	1.00	1.83	-239.311
8	1.00	1.76	-240.262

[a] *Enrichment = (fraction of low-RMSD models in top 10% by score)*$_{\text{round } N}$ ÷ *(fraction of low-RMSD models in top 10% by score)*$_{\text{round } N\text{-}1}$; *low-RMSD =* $\text{RMSD}_{C_\alpha} < 3.5$ *Å relative to crystal structure*

[b] *Round N-1* is the entire filtered ensemble taken from the 500,000 *de noo* folded models

[c] Only 13,677 models generated in this round

Figure Captions

Figure S1

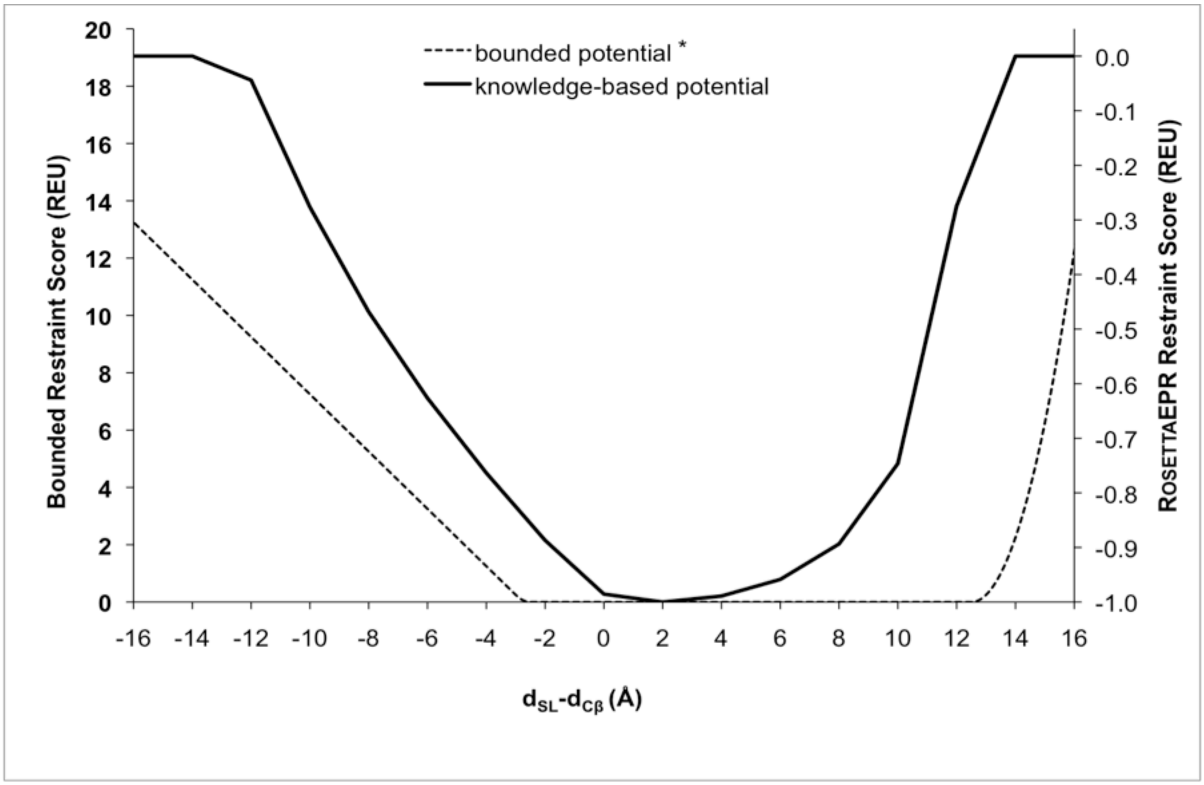
Comparison of the ROSETTAEPR knowledge-based potential (Figure 2E) with the bounded potential. The broader bounded potential against which experimental distance restraint violations are scored is defined according to a quadratic penalty described by the equation reported in the figure, where *ub* = upper bound, *lb* = lower bound, *sd* = standard deviation of 1.0, and *rswitch* = 0.5.

Figure S2

Map of previously reported EPR distance restraints (Table S1) on the T4-lysozyme crystal structure. The 107 C-terminal residues of the T4-lysozyme crystal structure are shown in rainbow with inter-residue distances used as structural restraints depicted as black dotted lines. A full list of experimentally determined EPR distances used in the benchmarking of RosettaEPR for this protein is reported in Table S1.

Figures

Figure S1



*Bounded potential is defined as:

$$f(x)=\begin{cases}\dfrac{(x-lb)^2}{sd} & x<lb\\ 0 & lb\le x\le ub\\ \dfrac{(x-ub)^2}{sd} & ub<x\le ub+rswitch\cdot sd\\ \dfrac{1}{sd}(x-(ub+(rswitch\cdot sd)))+(rswitch\cdot sd)^2 & x>ub+rswitch\cdot sd\end{cases}$$

Figure S2