SUPPLEMENTARY INFORMATION

The AUDANA algorithm for automated protein 3D structure determination from NMR NOE data

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Supplementary Table S1. Description of the PDBSEQ_DB table in the expanded version of the PACSY database (Lee et al. 2012). [1]

Field	Type	Null	Key	Default	Extra
ID	INT(11)	YES	-	NULL	-
PDB_ID	CHAR(5)	NO	MUL	NULL	-
CHAIN_ID	CHAR(1)	NO	MUL	NULL	-
SEQ_COUNT	INT(11)	NO	MUL	NULL	-
SEQUENCE	TEXT	YES	-	NULL	-

[1] 291,344 protein entries were included as of March 2016. The "genpdbseq_db.py" script available from the NMRFAM software download page (http://pine.nmrfam.wisc.edu/download_packages.html) automatically generates this table. As it can be seen in the Key column, PDB_ID, CHAIN_ID and SEQ_COUNT fields are indexed for performance. Default data for all the fields are set to NULL. Auto increment, time stamp, or other virtual generated data are not set as it can be seen in Extra column.

Supplementary Table S2. AUDANA results for the 14 test proteins.

Targets	PDB ID	BMRB ID	Size	Similar protein (% identity)	Observed regions	Backbone r.m.s.d. [4]	All heavy atom r.m.s.d. [4]	Backbone r.m.s.d. (no database support)[4]
Brazzein [1,7]	2LY5	16215	53	2KYQ (94)	4-51	1.05 (0.68)	1.35 (1.29)	1.00 (0.72)
StT322 [3,5]	2LOJ	18214	63	4YNX (62) 1V1H (35) 2QBP (31)	39-62	1.61 (0.38)	1.96 (0.83)	1.42 (0.42)
HR6470A [2,5]	2L9R	17484	69	1FTT (61) 1VND (61) 3A01 (49)	13-58	1.52 (0.54)	1.89 (1.12)	2.71 (1.73)
HR8254A [3,5]	2M2E	18909	73	2CQR (37) 2YUM (39) 2CJJ (31)	6-60	1.41 (0.49)	1.67 (1.03)	1.60 (0.35)
NS1^{RBD} [2,7]	2N74	25793	73	2Z0A (85)	3-71	1.32 (0.38)	1.66 (1.19)	9.07 (4.71)
Ubiquitin [1,7]	1D3Z	6457	76	5AF6 (98)	2-71	1.16 (0.41)	1.38 (0.94)	2.09 (0.92)
OR135 [2,5]	2LN3	18145	83	2LTA (38) 2LVB (34) 2LND (34)	5-73	1.13 (0.30)	1.47 (0.84)	1.03 (0.19)
HR2876C [3,5]	2M5O	19068	97	1VEH (85)	17-91	1.19 (0.35)	1.43 (0.73)	1.71 (0.53)
HR6430A [2,5]	2LA6	17508	99	2LCW (83)	15-53,64-97	0.71 (0.28)	0.84 (0.81)	0.89 (0.63)
HR2876B [2,5]	2LTM	18489	107	2LTL (33) 2K1H (37) 2VU5 (27)	13-28,38-100	1.90 (0.80)	2.03 (1.19)	1.22 (0.90)
YR313A [2,5]	2LTL	18487	119	2LTM (33) 3L7X (33) 4IWB (28)	19-22,29-34,48-88,94-111	1.59 (0.72)	2.10 (1.01)	1.52 (0.61)
OR36 [2,5]	2LCI	17613	134	2LR0 (98)	3-128	1.75 (0.40)	2.05 (1.01)	2.00 (0.51)
HR5537A [1,6]	2KK1	16349	135	1ZZP (45) 4GE3 (28) 4QYT (28)	39-106,117-134	1.86 (0.72)	2.25 (1.28)	2.80 (3.63)
mThTPase [3,7]	2JMU	15063	224	5A65 (96)	6-66,79-96,106-182,193-213	1.58 (2.76)	2.18 (3.02)	N/A (N/A)[8]

[1] Inputs used: sequence, chemical shifts, ^{13}C-NOESY and ^{15}N-NOESY.
[2] Inputs used: sequence, chemical shifts, ^{13}C-NOESY, ^{15}N-NOESY, aromatic NOESY, and RDC data.
[3] Inputs used: sequence, chemical shifts, ^{13}C-NOESY, ^{15}N-NOESY, and aromatic NOESY.
[4] The numbers outside parentheses are the r.m.s.d. between the PDB deposited model and the best AUDANA model; the numbers in parentheses are the pairwise r.m.s.d. for the 20 structural models.
[5] CASD-NMR 2013 targets (Rosato et al. 2015). Inputs were acquired from CASD-NMR web page (https://www.wenmr.eu/wenmr/casd-nmr-data-sets).
[6] CASD-NMR 2010 targets (Rosato et al. 2009) Inputs were acquired from CASD-NMR web page (https://www.wenmr.eu/wenmr/casd-nmr-data-sets).
[7] NMRFAM internal or collaborative projects (Cornilescu et al. 2013, Jureka et al. 2015, Song et al. 2008).
[8] The *Ponderosa Server* does not proceed to the water refinement if the backbone pairwise r.m.s.d. of the 20 structural models failed to converge under 10 Å as in this case.

Supplementary Table S3. Numbers of distance and angle constraints remaining at the end of phases I-III (Fig. S6 ABC) and used for the final structure calculations. Database-supported constraints were used only in determining endurance scores.

Targets	Intra $i = j$	Sequential $\|i - j\| < 2$	Medium $2 \leq \|i - j\| < 5$	Long $5 \leq \|i - j\|$	Total from NOEs	Angle constraints [1] φ / ψ	Database supported
Brazzein	429	204	226	217	1076	30 / 26	289
StT322	335	146	115	230	826	29 / 31	157
HR6470A	462	212	370	188	1232	40 / 42	259
HR8254A	766	335	496	189	1786	57 / 62	253
$NS1^{RBD}$	200	151	366	104	821	62 / 64	220
Ubiquitin	624	276	284	437	1621	49 / 55	464
OR135	1052	468	452	406	2378	67 / 63	7
HR2876C	869	467	624	518	2478	47 / 52	738
HR6430A	553	340	315	692	1900	57 / 53	747
HR2876B	1143	453	411	420	2427	65 / 75	32
YR313A	1221	496	400	364	2481	70 / 69	46
OR36	1301	619	666	778	3364	103 / 94	1041
HR5537A	497	382	692	87	1658	71 / 80	102
mThTPase	609	437	400	558	2004	127 / 139	571

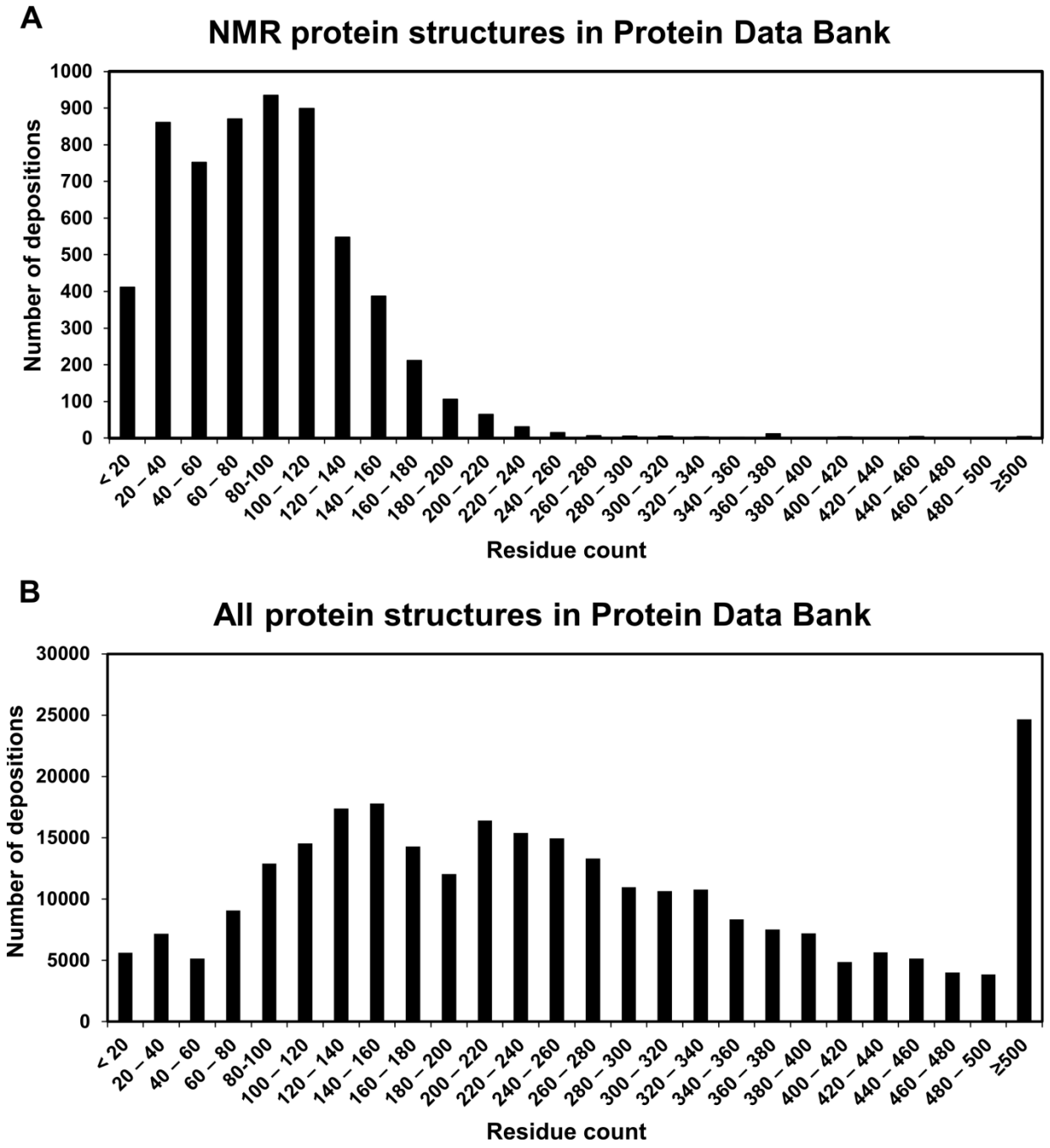
[1] Angle constraints are predicted by *TALOS-N* (Shen and Bax 2013) and optimized by AUDANA.

Supplementary Table S4. Structural quality assessment report from the PSVS package (Bhattacharya et al. 2007).

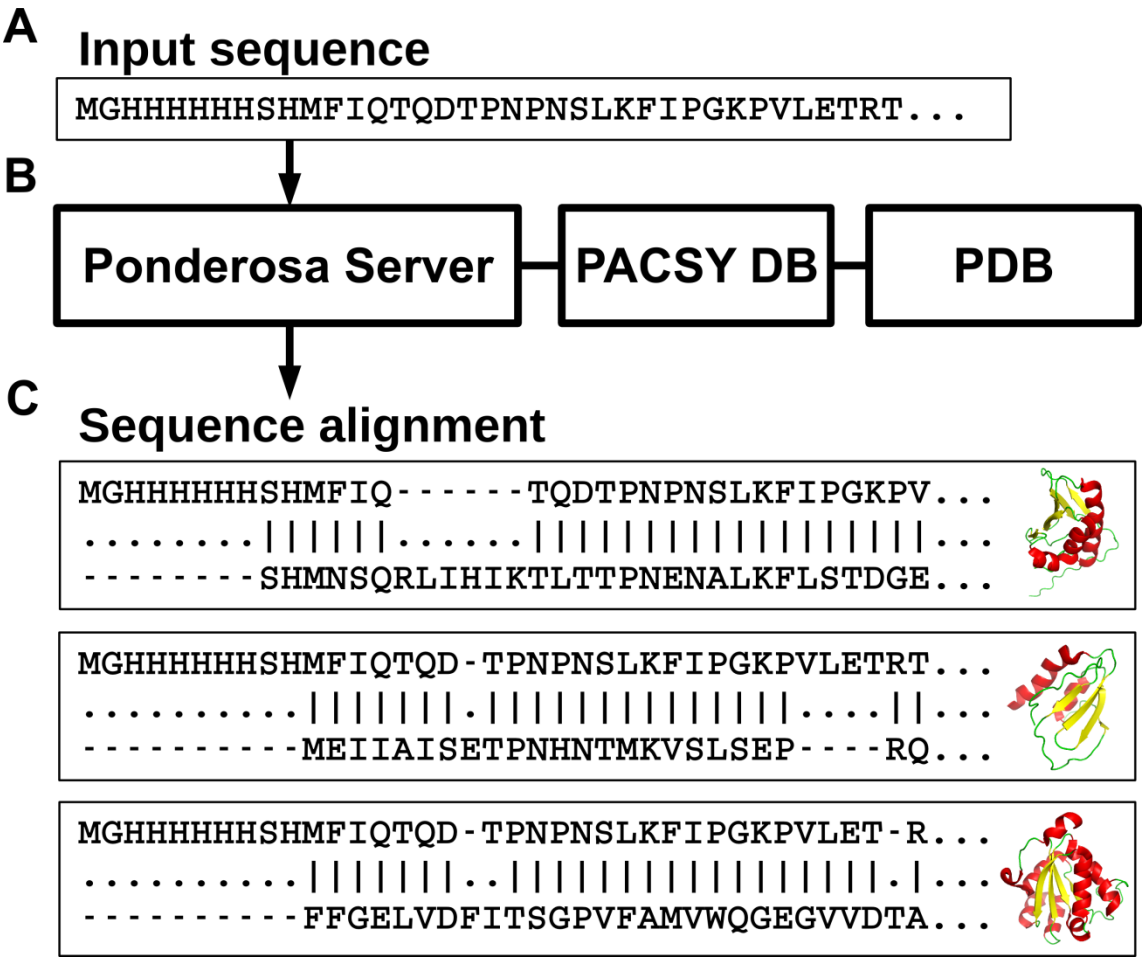
Targets	Procheck Validation					MolProbity Validation				Num. of close contact
	A	B	C	D	Procheck [1,3] φ-ϕ	E	F	G	Clashscore [2,4]	-
Brazzein	77.8	18.3	1.7	2.2	-0.48 (-1.57)	88.0	10.0	2.0	60.31 (-8.82)	9
StT322	84.8	14.1	1.1	0.0	-0.71 (-2.48)	91.7	4.2	4.2	42.45 (-5.76)	4
HR6470A	93.3	6.0	0.4	0.2	0.29 (1.46)	96.1	3.7	0.2	47.52 (-6.63)	15
HR8254A	97.5	2.5	0.0	0.0	0.59 (2.64)	98.4	1.6	0.0	40.44 (-5.41)	14
$NS1^{RBD}$	97.5	2.5	0.0	0.0	0.64 (2.83)	99.9	0.1	0.0	35.72 (-4.60)	5
Ubiquitin	93.4	4.5	2.0	0.1	-0.30 (-0.87)	96.6	1.6	1.9	59.84 (-8.74)	40
OR135	94.2	5.8	0.0	0.0	0.06 (0.55)	98.3	1.7	0.0	66.94 (-9.96)	18
HR2876C	85.8	12.3	1.9	0.0	-0.33 (-0.98)	92.4	6.7	0.9	79.82 (-12.17)	39
HR6430A	93.6	5.0	0.0	1.4	-0.16 (-0.31)	96.8	2.2	1.0	65.45 (-9.71)	7
HR2876B	94.3	4.6	0.7	0.4	-0.14 (-0.24)	97.3	2.4	0.3	46.13 (-6.39)	9
YR313A	89.2	9.6	0.9	0.2	-0.25 (-0.67)	94.2	5.1	0.7	48.87 (-6.86)	25
OR36	94.7	5.3	0.0	0.0	0.07 (0.59)	98.5	1.5	0.0	105.13 (-16.51)	66
HR5537A	97.8	2.1	0.1	0.1	0.48 (2.20)	98.1	1.9	0.0	33.79 (-4.27)	19
mThTPase	90.7	8.3	0.3	0.6	-0.16 (-0.31)	96.2	2.7	1.1	40.89 (-5.49)	35

A Most favored regions (%)
B Additionally allowed regions (%)
C Generously allowed regions (%)
D Disallowed regions (%)
E Most favored regions (%)
F Allowed regions (%)
G Disallowed regions (%)

[1] (Laskowski et al. 1996)
[2] (Chen et al. 2015)
[3,4] Raw score (Z-score)



Supplementary Fig. S1. Bar graph illustrating 3D structures deposited in Protein Data Bank (Berman et al. 2007) as of October 2015. **A.** NMR structures deposited in PDB as queried from the PACSY DB (Lee et al. 2012). **B.** All structures in PDB regardless of determination method.



Supplementary Fig. S2. AUDANA selects the three most similar proteins by aligning the input sequence from the user to sequences in the PACSY database. **A.** The user sequence is entered. **B.** The PDBSEQ_DB table in PACSY is queried to restrict the pool of sequences from PDB to the size of the input sequence to achieve better performance. **C.** The *Ponderosa Server* aligns sequences by the Smith-Watermann algorithm (Smith and Waterman 1981) to determine the three best matches.

A

$$P_k = \frac{E_{k1} + E_{k2} + E_{k3} + E_{k4}}{\sum (E_{k1} + E_{k2} + E_{k3} + E_{k4})}$$

P_k : Probability of kth inter-proton contact from experimental NOE data.

E_{kn} : Evidence for the nth category for the kth probable inter-proton contact from NOE cross peaks.

B $S_k = C_R \times P_k$

S_k : Endurance score for kth probable inter-proton contact.

C_R : Constant set to 20.

C

$S_{TOT} = S_N + S_C + S_A + S_P$

S_{TOT} : Overall endurance score.

$S_{N,C,A}$: Endurance scores calculated from N-NOESY, C-NOESY, or aromatic NOESY.

S_P : Supportive scores from the overall identity between the tripeptide centered on the residue of interest and the tripeptide from the homologous protein.

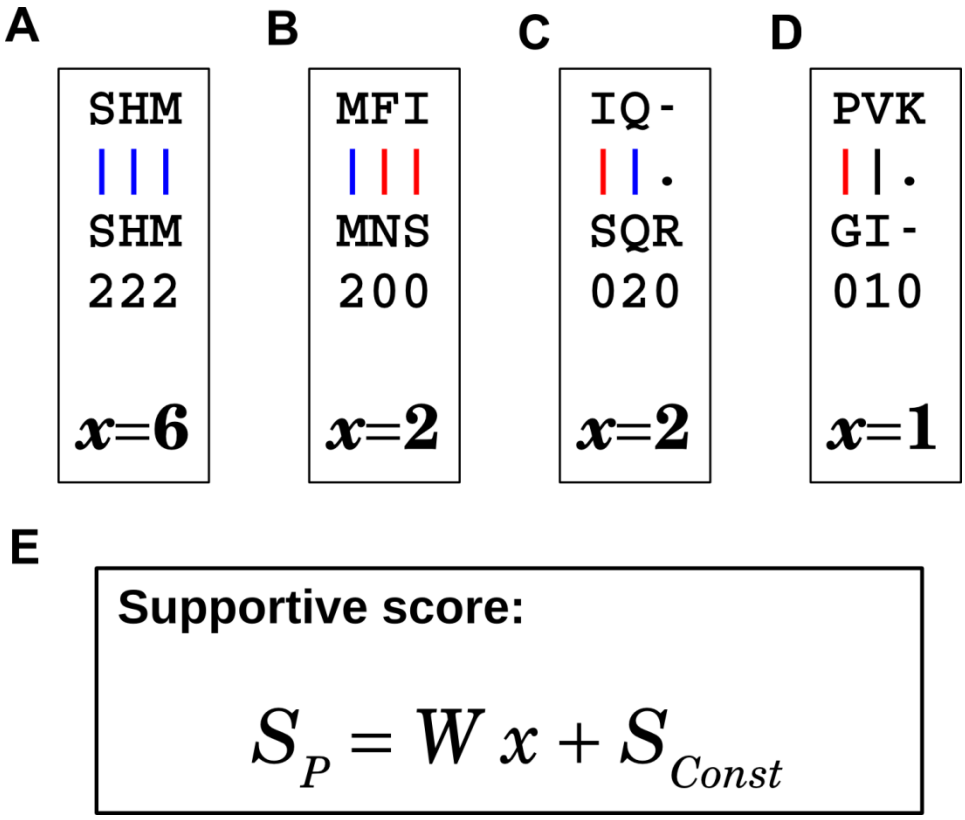
D $S_{NEW} = S_{OLD} - S_{VIOL}$

S_{NEW} : Updated endurance score after a cycle of structure calculation.

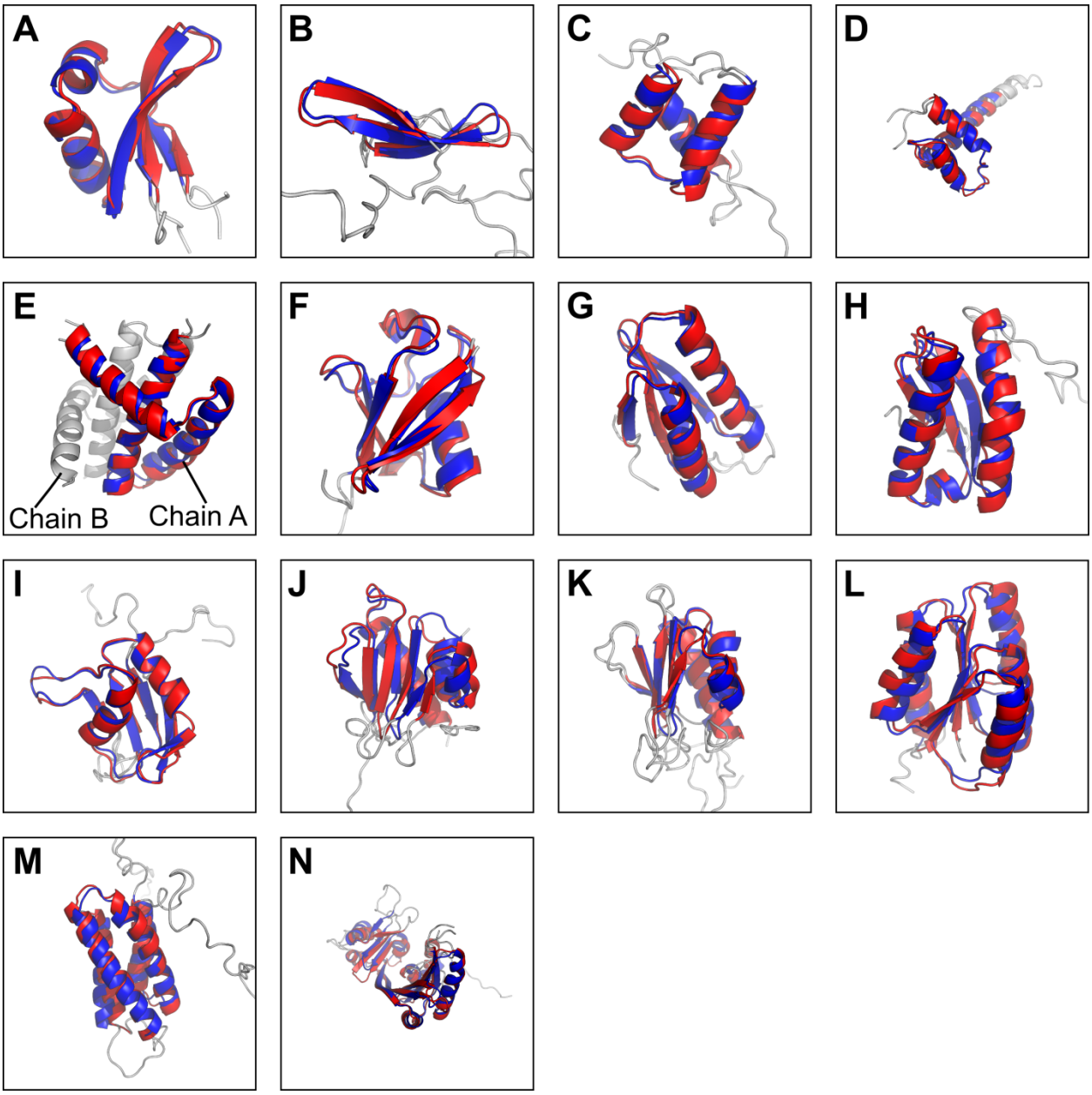
S_{OLD} : The endurance score in the previous cycle.

S_{VIOL} : Deducting arisen from violation in the structure calculation.

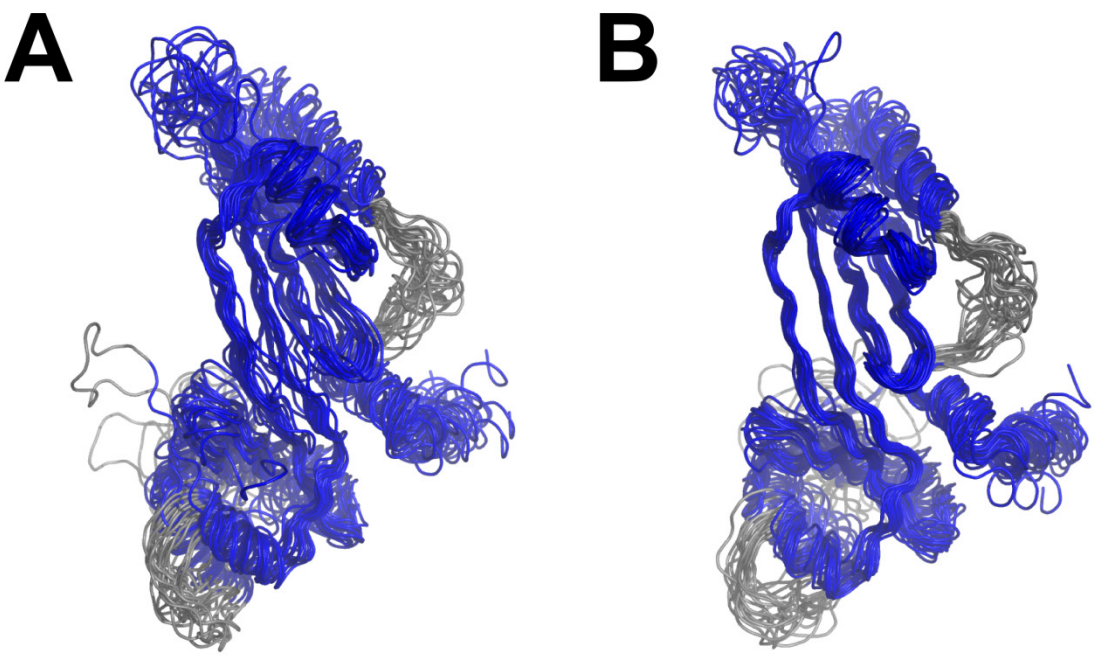
Supplementary Fig. S3. Equations used in determining the endurance score. **A.** Probabilities are calculated for each potential inter-proton constraint corresponding to an NOE peak from: 1) the network of potential interactions involving a pair of residues supported by NOE data (E_{k1}); 2) probability for hydrogen atoms from the particular residue pair being within 5.5 Å (E_{k2}); 3) order parameters as calculated from chemical shifts (Berjanskii and Wishart 2005) (E_{k3}); and 4) agreement between the position of the NOE peak and the assigned chemical shifts (E_{k4}). **B.** The endurance score for the candidate inter-proton constraint from a NOE peak is defined as the product of a constant (C_R) and the probability for the candidate (P_k). The constant 20 was initially picked randomly and other values were optimized depending on the number. Constraints with low probability are vulnerable to being removed in score updates (Supplementary Fig. S4D). **C.** The total endurance score used in a structure calculation is the sum of the scores from different types of experimental data (S_N, S_C and S_A) and the supportive score (S_P, explained in Supplementary Fig. S5). **D.** After each structure calculation cycle, violations are examined, and (S_{VIOL}) is deducted from the endurance score of the completed cycle (S_{OLD}) to yield an updated score (S_{NEW}). The deduction score is dynamically scaled product of the number of violated structures, the violated distances and the phase (Supplementary Fig. S6). If the new score is less than zero, the constraint goes into the recycle bin. If the violated constraint survives, the upper distance is extended elastically. The process is illustrated in Supplementary Fig. S6.



Supplementary Fig. S4. Equations used in determining the supportive score. The supportive score to be added to the endurance score from the experimental data is calculated from the knowledge-based inter-proton contact list (Fig. 2). The score for a residue is determined from the similarity between tri-peptides (with the residue in question as the central residue) from the user sequence and the aligned sequence from PDB. The score for each exactly matched residue is 2; the score for a similar residue is 1; and dissimilar matches or gaps are scored as 0. The scores from the three residues in the tripeptide are summed yield the multiplication factor (x). **A**. Example in which all three aligned residues match; the multiplication factor (x) is 6. **B** and **C.** Examples in which only one residue matches; x=2 in each case. **D.** Example in which two residues do not match, but the third is similar; x=1. **E.** The supportive score for the constraint is calculated as a linear function of the multiplication component (x) with slope W (empirical weighting factor); the x-axis intercept is the minimum supportive score (S_{const}) for the aligned sequence. Currently, W is 2.5 and S_{const} is 15. The individual parameters may be improved as they are empirically driven. However, this setup has worked fairly well so far.



Supplementary Fig. S5. Superimpositions of AUDANA structure (blue) and PDB deposited structure (red) for the 14 protein targets. All the targets calculated by AUDANA were close to those deposited in the PDB (< 2 Å r.m.s.d. for backbone atoms, Supplementary Table S2). **A.** Brazzein. **B.** StT322. **C.** HR6470A. **D.** HR8254A. **E.** $NS1^{RBD}$ (monomeric domain of the symmetric homodimer). **F.** Ubiquitin. **G.** OR135. **H.** HR2876C. **I.** HR6430A. **J.** HR2876B. **K.** YR313A. **L.** OR36. **M.** HR5537A. **N.** mThTPase (25-kDa).



Supplementary Fig. S6. Comparison of the structural ensembles obtained for mThTPase (25 kDa protein) by using the “PONDEROSA-X refinement” and “constraint only for the final step” options. A. Selected the best 20 out of 40 calculated structures from “PONDEROSA-X refinement option”. Pairwise backbone atom r.m.s.d. for the structural models is 2.76 Å. B. Selected the best 20 out of 100 calculated structures from *traditional* “constraint only for the final step”. Pairwise backbone atom r.m.s.d. for the structural models is reduced to 1.81 Å.

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