Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Prediction of disulfide dihedral angles using chemical shifts

David A. Armstrong, Quentin Kaas, K. Johan Rosengren

Table S1. Performance of DISH χ1 prediction when φ and ψ angles are removed as inputs

	Stage I MCC	Stage II MCC
DISH Inputs SVM-χ1	0.89	0.70
Chemical shift and secondary structure only inputs SVM-χ1	0.62	0.39

Table S2. Performance of DISH χ2 prediction when χ1 angles are removed as inputs

	Stage I MCC	Stage II MCC
DISH Inputs SVM-χ2	0.85	0.85
Chemical shift and secondary structure only inputs SVM-2	0.62	0.74

Table S3. The Cys residues of Ep-AMP1 and χ1 angles derived from the E.COSY spectrum, χ1 angles predicted by TALOS-N and the χ1 and χ2 angles predicted by DISH as either gauche+ (g+), gauche- (g-) or trans (t).

Residue	**χ1 E.COSY**	**χ1 DISH**	**χ1 TALOS-N**	**χ2 DISH**
1	-	-	-	-
8	-	g- [a]	g+	g-
16	g-	g-	g-	g-
17	g-	g-	-	g-
23	-	t	-	t
33	g-	g-	g-	g-

a Was found to violate in initial structures and removed from final calculations

Table S4. The Cys residues of barrettide A and χ1 angles calculated from the E.COSY spectrum, χ1 angles predicted by TALOS-N and the χ1 and χ2 angles predicted by DISH, either gauche+ (g+), gauche- (g-) or trans (t).

Residue	**χ1 E.COSY**	**χ1 DISH**	**χ1 TALOS-N**	**χ2 DISH**	**χ2 DISH using experimental χ1 as input**
5	t	t	-	g+	-
7	g-	g-	-	g-	-
18	g-	g-	g-	g-	-
23	g+	g-	-	g-	g-

Table S5. Statistics from MolProbity and backbone RMSD of the original Ep-AMP1 structure, and revised structures with additional χ1 and χ2 restraints. All values are averaged over 20 structures ± STDEV.

	Original	**Additional χ1 and χ2**
Clash Score [a]	7.0 ± 2.0	11.9 ± 4.5
Poor Rotamers	0.10 ± 0.3	0.00 ± 0.00
Favoured Rotamers (%)	94.5± 3 .8	93.4 ± 3.3
Ramachandran Outliers	0.25 ± 0.55	0.0 ± 0.0
Ramachandran Favoured (%)	91.5 ± 4.0	94.90 ± 3.7
MolProb. Score [b]	1.9 ± 0.19	1.9 ± 0.20
Percentile (%) [c]	81.2 ± 8.2	81.2 ± 8.23
RMSD (Å)		
Mean Global Backbone	0.86 ± 0.27	0.55 ± 0.11
Mean Global Heavy	1.61 ± 0.29	1.34 ± 0.18

Definition of MolProbity structural statistics.

[a] The number of non-donor-acceptor atoms that overlap by more than 0.4 Å per 1000 atoms

[b] Overall quality of protein statistics. Log weighted combination of the clash score, percentage Ramachandran not favoured and percentage of bad side chain rotamers. Reflects the crystallographic resolution for structures that those values would be expected

[c] 100th percentile is the best among structures of comparable resolution; 0th percentile is the worst.

Table S6. Statistics from MolProbity and backbone RMSD of the original barrettide A structure, and re-evaluated structure with χ2 restraints. Values are averaged over the 20 lowest energy models ± STDEV.

	Original	**Additional χ1 and χ2**
Clash Score [a]	8.7 ± 3.5	9.6 ± 3.2
Poor Rotamers	0.10 ± 0.31	0.11 ± 0.3
Favoured Rotamers (%)	96.7 ± 3.4	95.6 ± 3.9
Ramachandran Outliers	0.2 ± 0.41	0.25 ± 0.4
Ramachandran Favoured (%)	94.7 ± 3.7	92.2 ± 3.3
MolProb. Score [b]	1.8 ± 0.32	2.0 ± 0.18
Percentile (%)[c]	82.9 ± 13.8	75.9 ± 9.9
Residues with bad bonds	0.2 ± 0.41	0.45 ± 0.37
RMSD (Residues 5-23) (Å)		
Mean Global Backbone	0.92 ± 0.34	0.64 ± 0.19
Mean Global Heavy	1.58 ± 0.35	1.34 ± 0.22

Definition of MolProbity structural statistics.

[a] The number of non-donor-acceptor atoms that overlap by more than 0.4 Å per 1000 atoms

[b] Overall quality of protein statistics. Log weighted combination of the clash score, percentage Ramachandran not favoured and percentage of bad side chain rotamers. Reflects the crystallographic resolution for structures that those values would be expected

[c] 100th percentile is the best among structures of comparable resolution; 0th percentile is the worst.

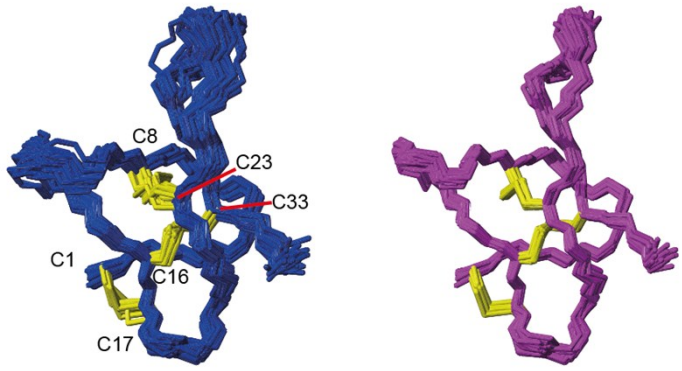

Figure S1. Comparison of the backbone conformation of the 20 lowest energy models of Ep-AMP1 computed using CNS without DISH predictions (PDB 2MFS; in blue) and with DISH predictions (in pink). Cystine side chains are in yellow sticks.

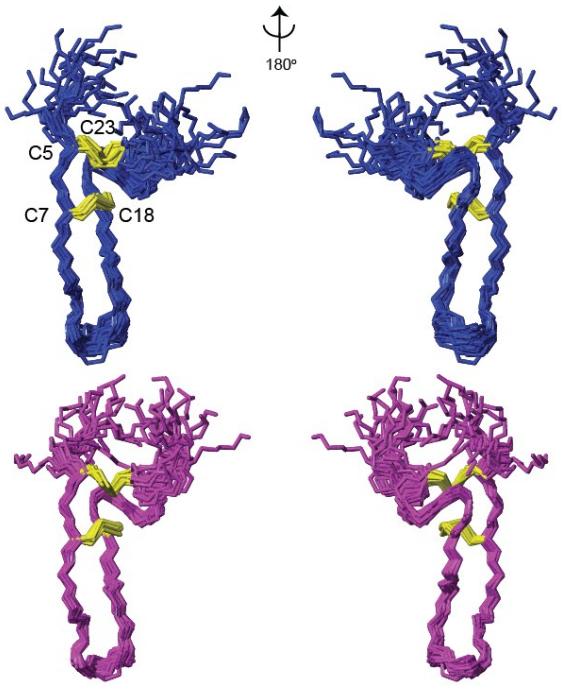

Figure S2. Comparison of the backbone conformation of the 20 lowest energy models of barrettide A computed using CNS without DISH predictions (in blue) and with DISH predictions (in pink). Cystine side chains are in yellow sticks.

Table S7. DISH predictions for Cys residues of hen lysozyme and compared to those extracted from the X-ray crystallography structure (PDB 1iee)

Residue	χ1 X-ray	χ1 DISH	χ2 X-Ray	χ2 DISH
6	g-	g-	g-	g-
30	t	t	g-	g-
64	g+	g+	g+	g+
76	g-	g-	g-	g-
80	g-	g-	g-	g-
94	t	t	g+	g+
115	g-	g-	g-	g-
127	g-	g-	g-	g-

Table S8. Backbone alignment of hen lysozyme NMR structures without DISH predictions and with DISH predictions to the X-ray structure (PDB 1iee).

	No Cys χ1 and χ2	Cys χ1 and χ2
	All Residue Backbone Alignment	
Mean global backbone RMSD (Å)	1.73 ± 0.27	1.55 ± 0.28
Mean global heavy RMSD (Å)	2.49 ± 0.24	2.35 ± 0.32
	Cys Backbone Alignment	
Mean global backbone RMSD (Å)	1.18 ± 0.30	0.82 ± 0.13
Mean global heavy RMSD (Å)	1.32 ± 0.32	0.87 ± 0.18