

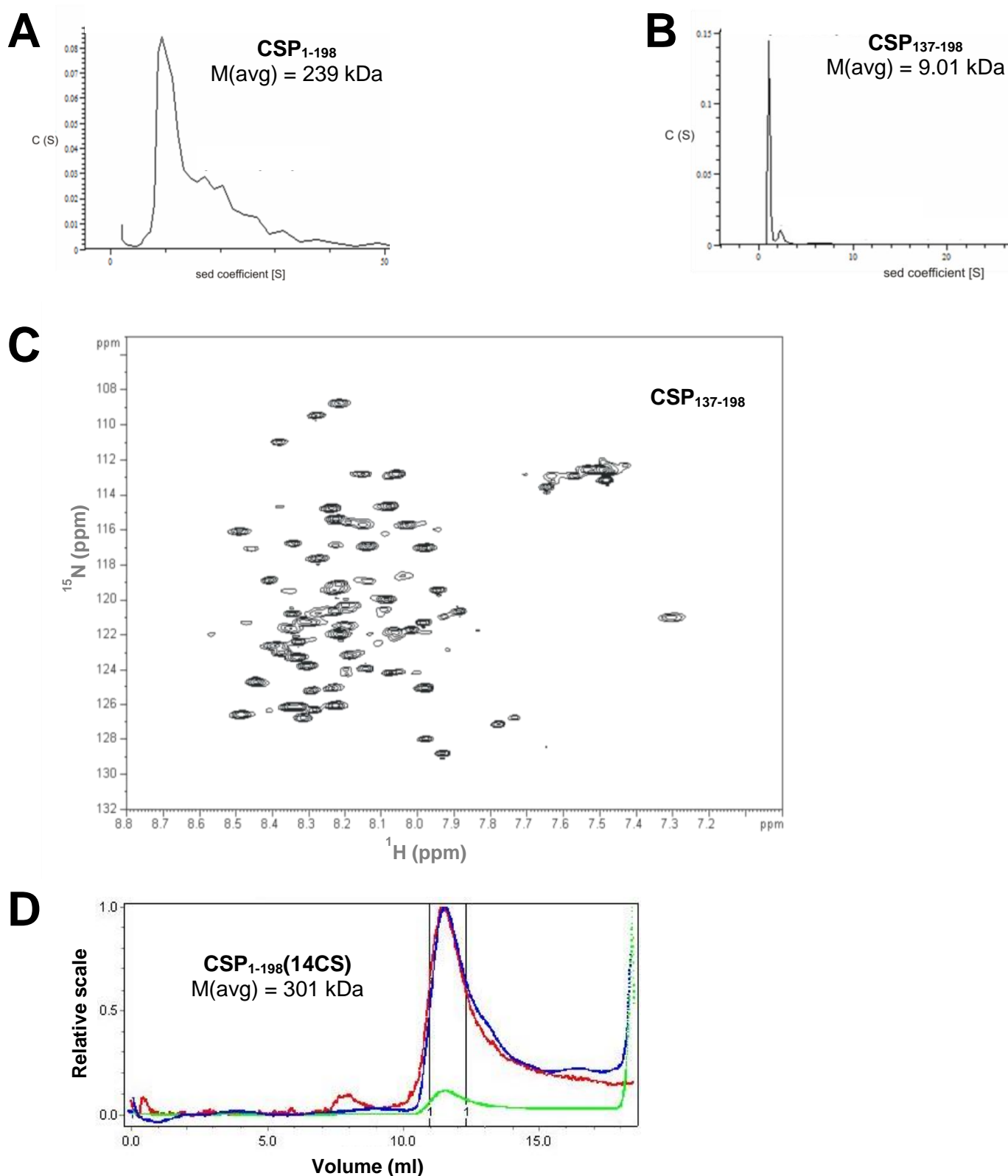
**Structure, Volume 24**

**Supplemental Information**

**Phosphorylation of Cysteine String Protein**

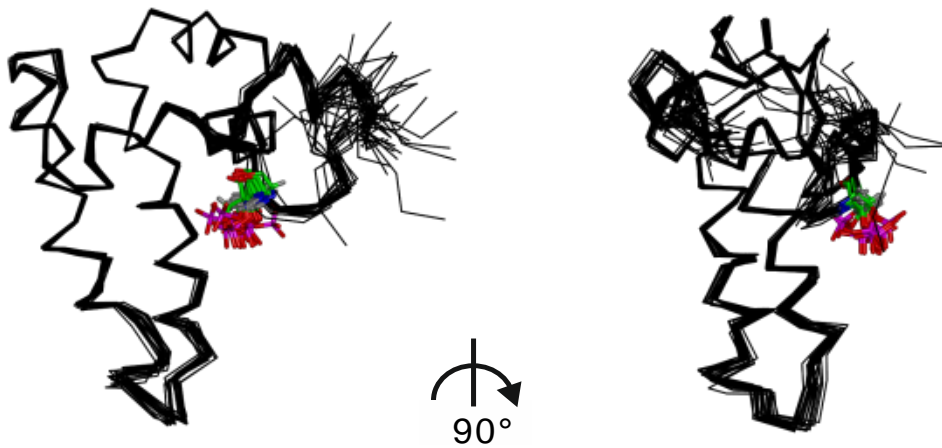
**Triggers a Major Conformational Switch**

**Pryank Patel, Gerald R. Prescott, Robert D. Burgoyne, Lu-Yun Lian, and Alan Morgan**



**Fig. S1, related to Fig. 1: Characterisation of recombinant CSP constructs**

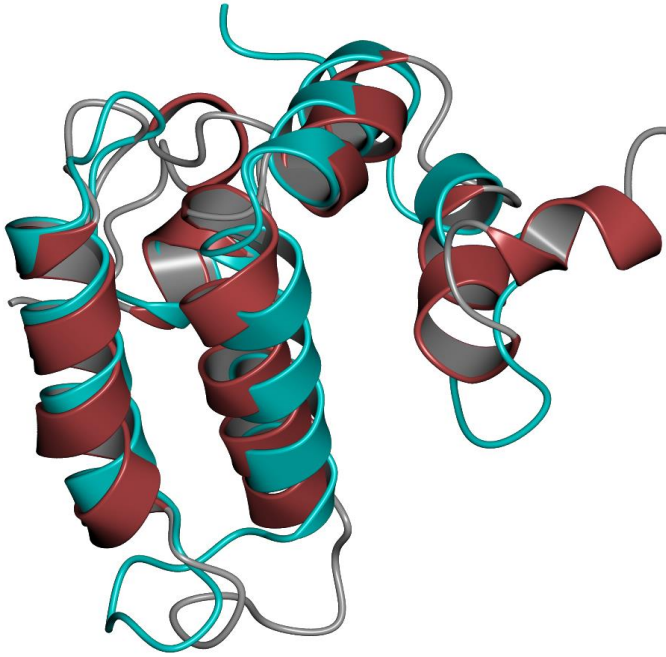
a) Analytical ultracentrifugation of full length CSP<sub>1-198</sub>. b) Analytical ultracentrifugation of CSP<sub>137-198</sub>. c) <sup>1</sup>H-<sup>15</sup>N HSQC spectra of CSP<sub>137-198</sub>. d) Size exclusion chromatography - multiple angle laser light scattering analysis of full length CSP<sub>1-198</sub> with all 14 cysteines mutated to serines (14CS).

**A****B**

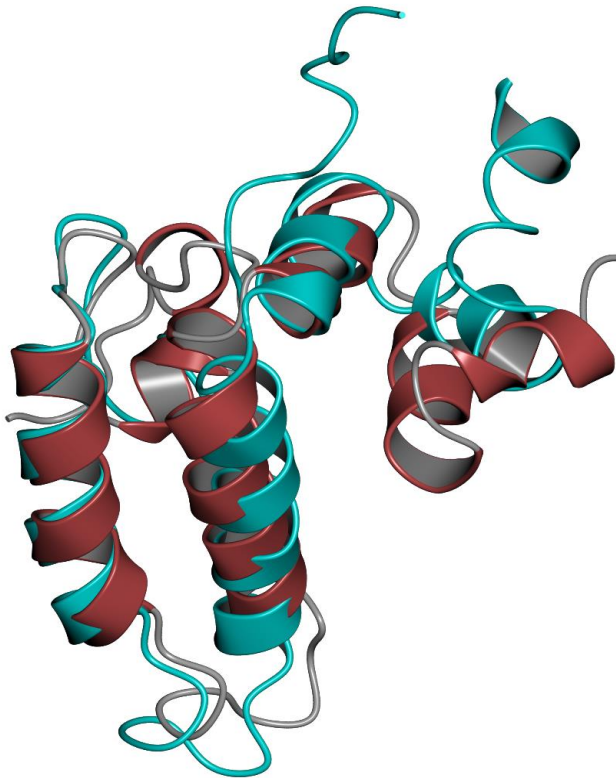
**Fig. S2, related to Fig. 2: Structures of unphosphorylated and Ser10-phosphorylated CSP<sub>1-100</sub>.**

Ensembles of the 20 lowest energy conformers of CSP<sub>1-100</sub> (a) and pCSP<sub>1-100</sub> (b) displayed as C $\alpha$  backbone representations; two different views differing by 90° are shown for each, with Ser10 represented as sticks.

**A**

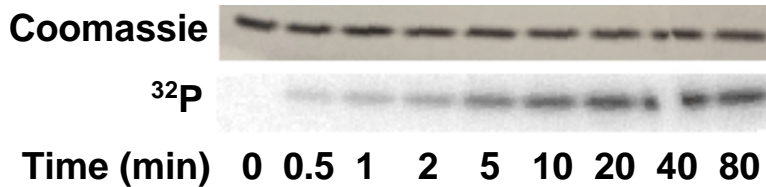


**B**



**Fig. S3, related to Fig. 2: Structural similarities between CSP<sub>1-100</sub> and DnaJ/Hsp40 proteins in the unphosphorylated state.**

a) Overlay of the lowest energy conformer of CSP<sub>1-100</sub> (red) with the J domain of yeast Sis1 protein (PDB code: 4RWU, cyan). b) Overlay of the lowest energy conformer of CSP<sub>1-100</sub> (red) with CSP<sub>5-100</sub> (PDB code: 2CTW, blue).

**A****B**

Sequence	# PSMs	Modifications	IonScore	Exp Value	Charge	MH+ [Da]	ΔM [ppm]	RT [min]	# Missed Cleavages
SLsTSGESLYHVLGLDK	153	S10(Phospho)	63	0.000	2	1885.895	0.31	67.11	0
sLsTSGESLYHVLGLDK	33	N-Term(Acetyl); S10(Phospho)	50	0.000	2	1927.906	0.52	77.20	0
sLsTSGESLYHVLGLDK	33	S8(Phospho); S10(Phospho)	40	0.002	2	1965.861	0.16	76.41	0

**Fig. S4, related to Fig. 3: *in vitro* phosphorylation of CSP<sub>1-100</sub>.**

a) Kinetics of  $\gamma^{32}$ -ATP incorporation following incubation of CSP<sub>1-100</sub> with protein kinase A and radiolabelled MgATP. Samples were run on SDS-PAGE and visualised by Coomassie Blue staining (*Coomassie*, upper panel) and phosphorimaging ( $^{32}$ P, lower panel). Images shown are cropped to remove unnecessary blank space. b) Mass spectrometric identification of phosphorylation sites on CSP<sub>1-100</sub> after incubation with protein kinase A and MgATP.