Table S1: Conditions of modification reactions used in this study. The most reactive amino acid is written in the first position, other potentially reactive amino acids are in the brackets.

Modifier	Modification buffer	Reaction time [min]	Reactive amino acid residues[a]	Mass shift in modified peptides	References
DEP	50 mM potassium phosphate; pH 6.8	60	H (K, C, N-terminal amino acid)	+72; +15	(*18*)
NBS	50 mM potassium phosphate; pH 6.5	5	W (M)	+16; +32	(*19*)
NEM	20 mM Tris-HCl, pH 8.0	30	C	+125	(*5, 20*)
NAI	10 mM potassium phosphate, pH 7.5	60	Y (K, N-terminal amino acid)	+42	(*21*)
NHS	100 mM sodium bicarbonate, pH 8.5	30	K (N-terminal amino acid)	+42	(*22*)
HPG	50 mM sodium bicarbonate, pH 8.0	60	R	+132	(*23*)

Table S2: Enzymatic activities of modified CBS for molar ratio of protein:modifier used in the study. Relative activities are refered to enzymatic activities of unmodifed control in absence of AdoMet; values are expressed as a mean of 3 measurements with standard deviation. Activation of CBS activity by AdoMet in the unmodified control differed for each labelling compound due to variant sample preparation.

			Relative activity of modified protein		Activation of CBS activity by AdoMet	
Modifier	Protein	CBS:modifier molar ratio	-AdoMet	+AdoMet	unmodified control	modified protein
DEP	45CBS	1-5	0.77 ± 0.13	N/A	N/A	N/A
	wtCBS		0.90 ± 0.16	2.45 ± 0.47	3.3	2.7
NBS	45CBS	1-1	0.43 ± 0.05	N/A	N/A	N/A
	wtCBS		1.04 ± 0.20	1.63 ± 0.35	2.3	1.6
NEM	45CBS	1-1	0.98 ± 0.18	N/A	N/A	N/A
	wtCBS		1.36 ± 0.23	3.18 ± 0.33	2.9	2.3
NAI	45CBS	1-250	1.09 ± 0.15	N/A	N/A	N/A
	wtCBS		1.18 ± 0.16	1.67 ± 0.36	3.0	1.3
NHS	45CBS	1-10	0.77 ± 0.17	N/A	N/A	N/A
	wtCBS		0.73 ± 0.13	2.08 ± 0.38	2.7	2.7
HPG	45CBS	1-250	0.69 ± 0.08	N/A	N/A	N/A
	wtCBS	1-500	0.68 ± 0.10	1.26 ± 0.10	3.1	1.9

Table S3: Relative enzymatic activities of modified thermally activated wtCBS. Relative activities are given as ratios of modified enzyme to an unmodifed control; values are expressed as a mean with standard deviation. Heat stimulation shows increase in enzymatic activity upon partial thermal denaturation.

Modifier	Rel. Activity	Heat stimulation
NAI	0.46 ± 0.12	2.1
HPG	0.60 ± 0.07	1.5
NBS	0.56 ± 0.09	1.6
NHS	0.77 ± 0.19	1.9

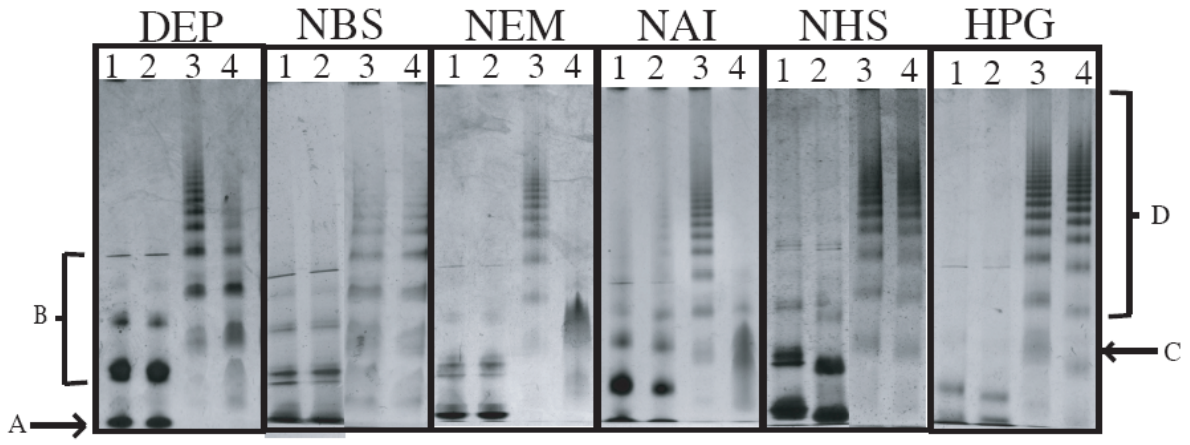


Figure S1: Analysis of labelled CBS by native electrophoresis. Arrows indicate bands corresponding to common oligomeric forms of the unmodified proteins (A – dimeric 45CBS; B – higher order oligomers of 45CBS; C – tetramer of wtCBS; D – higher order oligomers of wtCBS). Molecular weight markers were not used since migration distance of CBS oligomers is not determined by their masses only; the expected migration of oligomers is shown by arrows in accordance with previous reports on behaviour of CBS in native electrophoretic gels (*5*, *47*).

Each labelling experiment is placed in separated box with identical numbering: 1 – unmodified 45CBS; 2 – labelled 45CBS; 3 – unmodified wtCBS; 4 – labelled wtCBS. Proteins shown in this figure were labeled by modifier at the same concentrations used for surface mapping (ratio of protein:modifier are shown in Table S2).

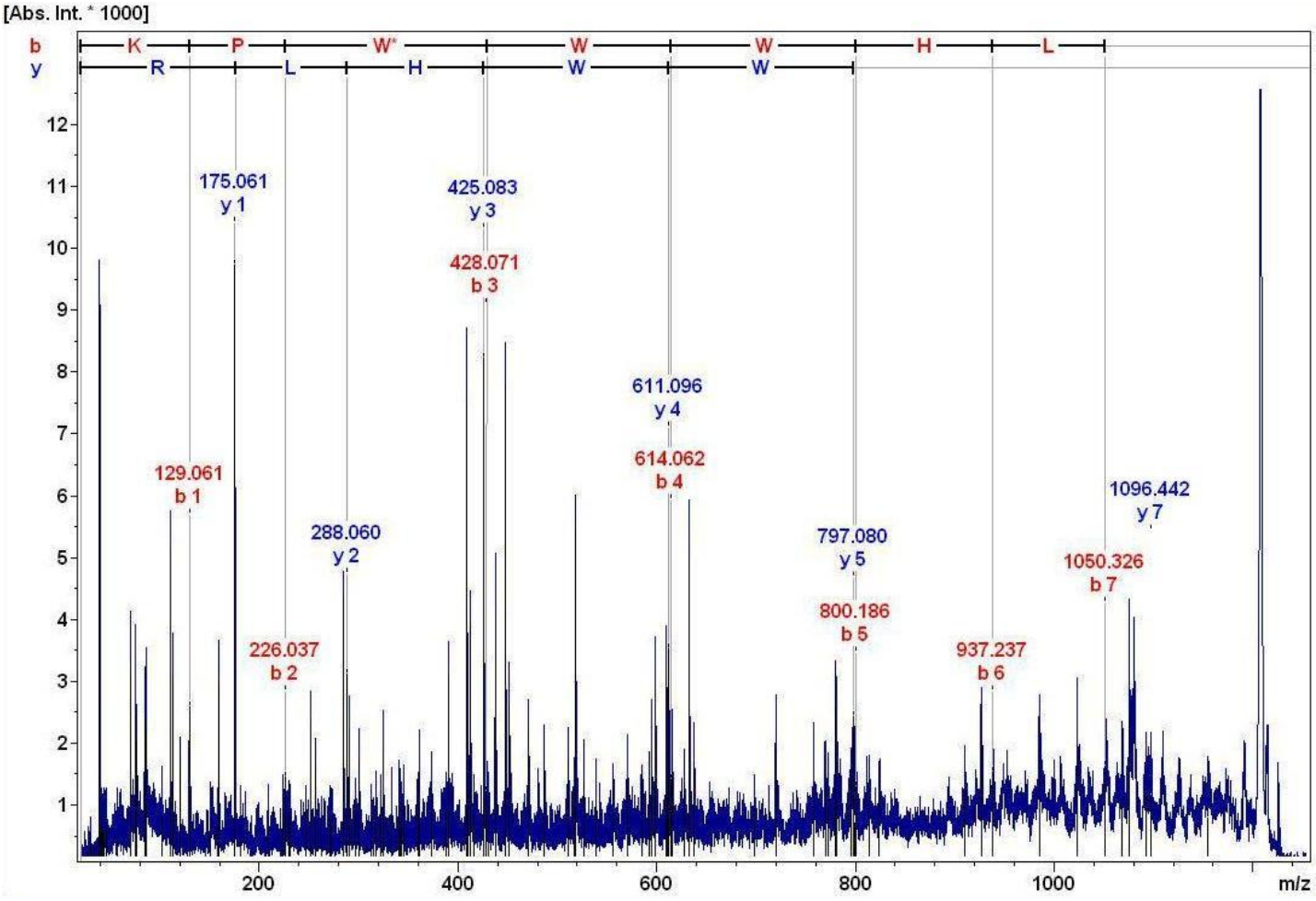


Figure S2: MS/MS spectrum of differentially reactive peptide 406-413 (KPWWWHLR) that contained modified tryptophane cluster W408/409/410. The labelling was perfomed with NBS.

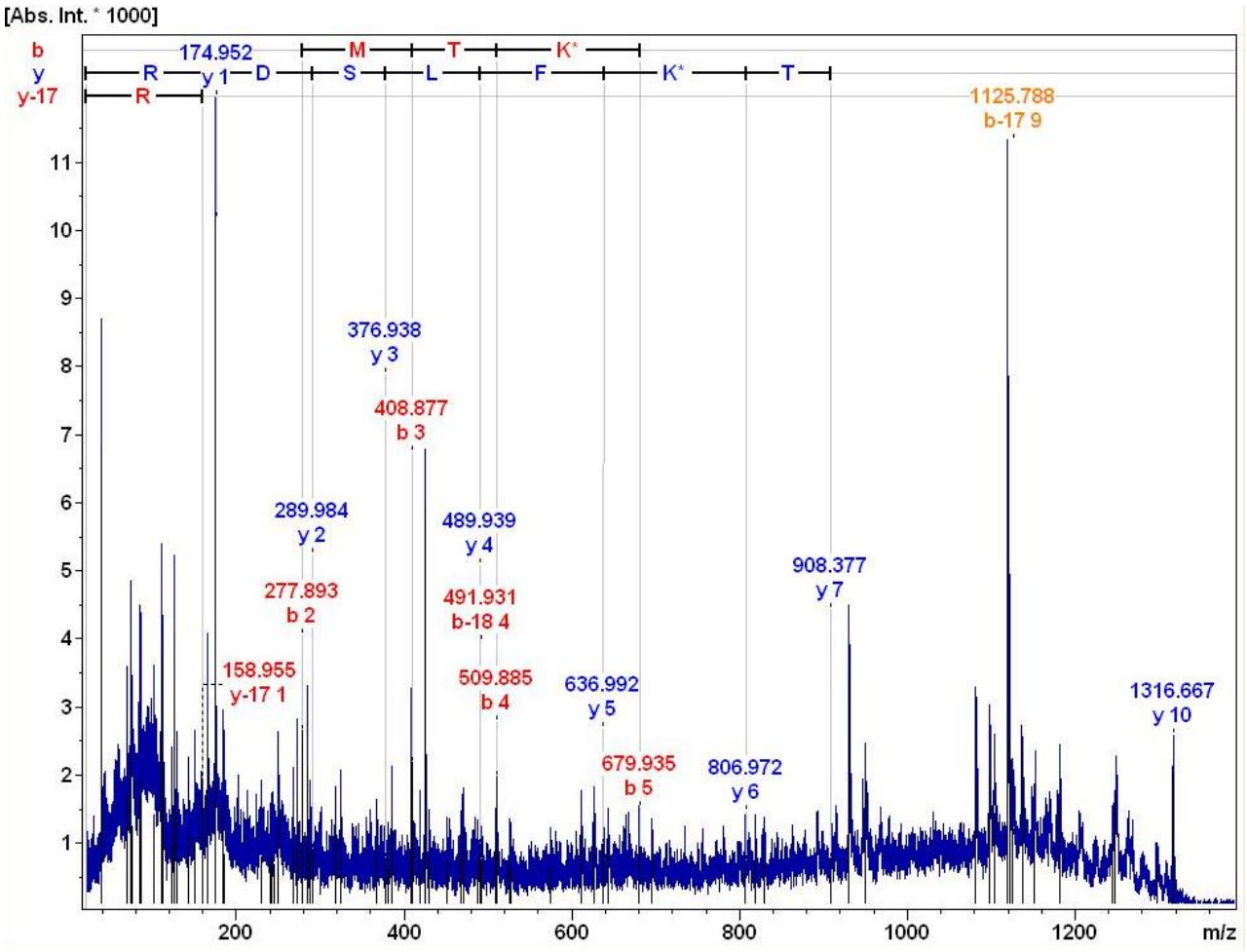


Figure S3: MS/MS spectrum of differentially reactive peptide 380-389 (NYMTKFLSDR) that contained modified K384. The labelling was performed with NAI.

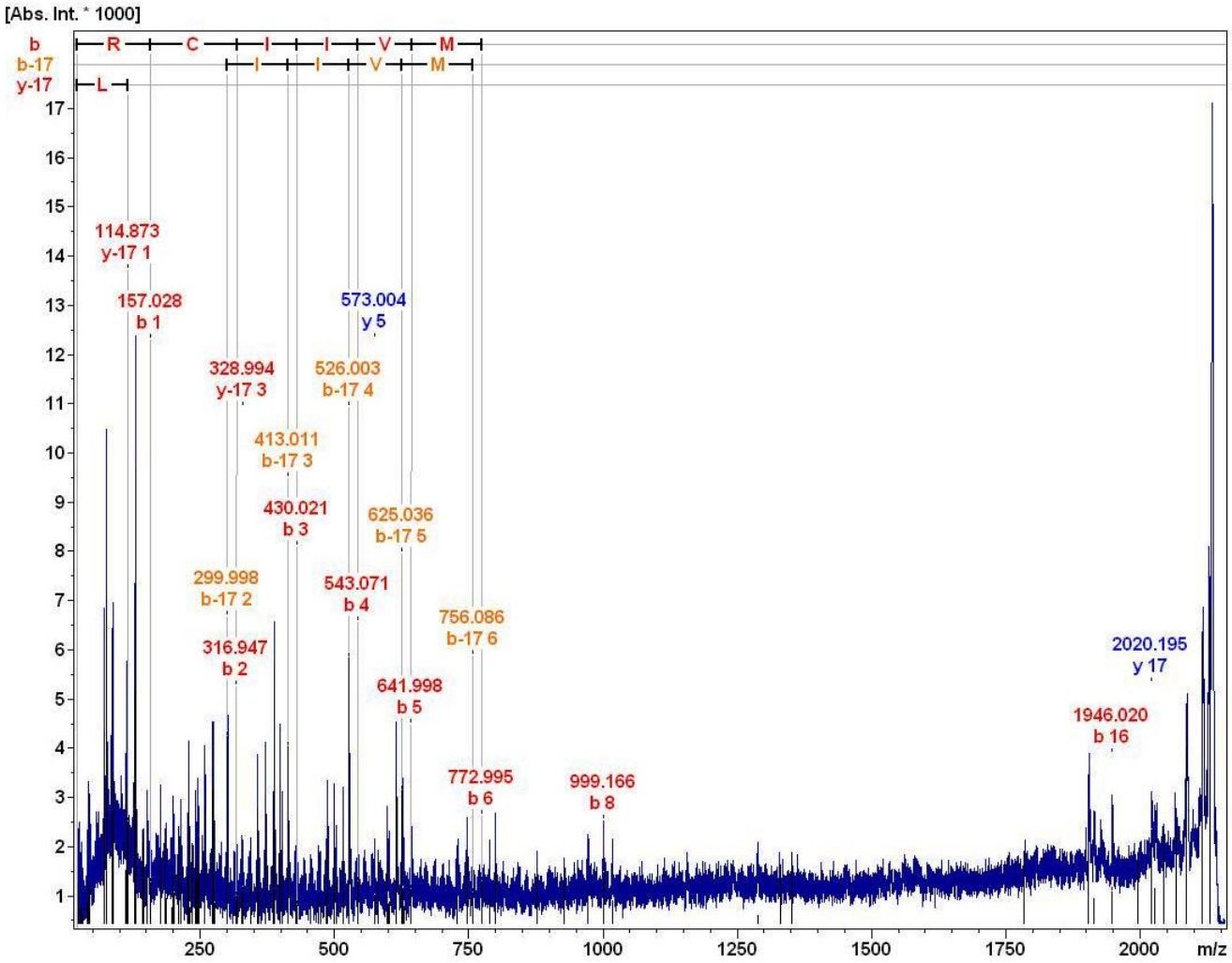


Figure S4: MS/MS spectrum of differentially reactive peptide 164-181 (RCIIVMPEKMSSEKVDVL) that contained modified K172 and/or K177. The labelling was performed with NHS.

Evaluation of the model of the autoinhibitory domain

using statistical coupling analysis and protein sector analysis

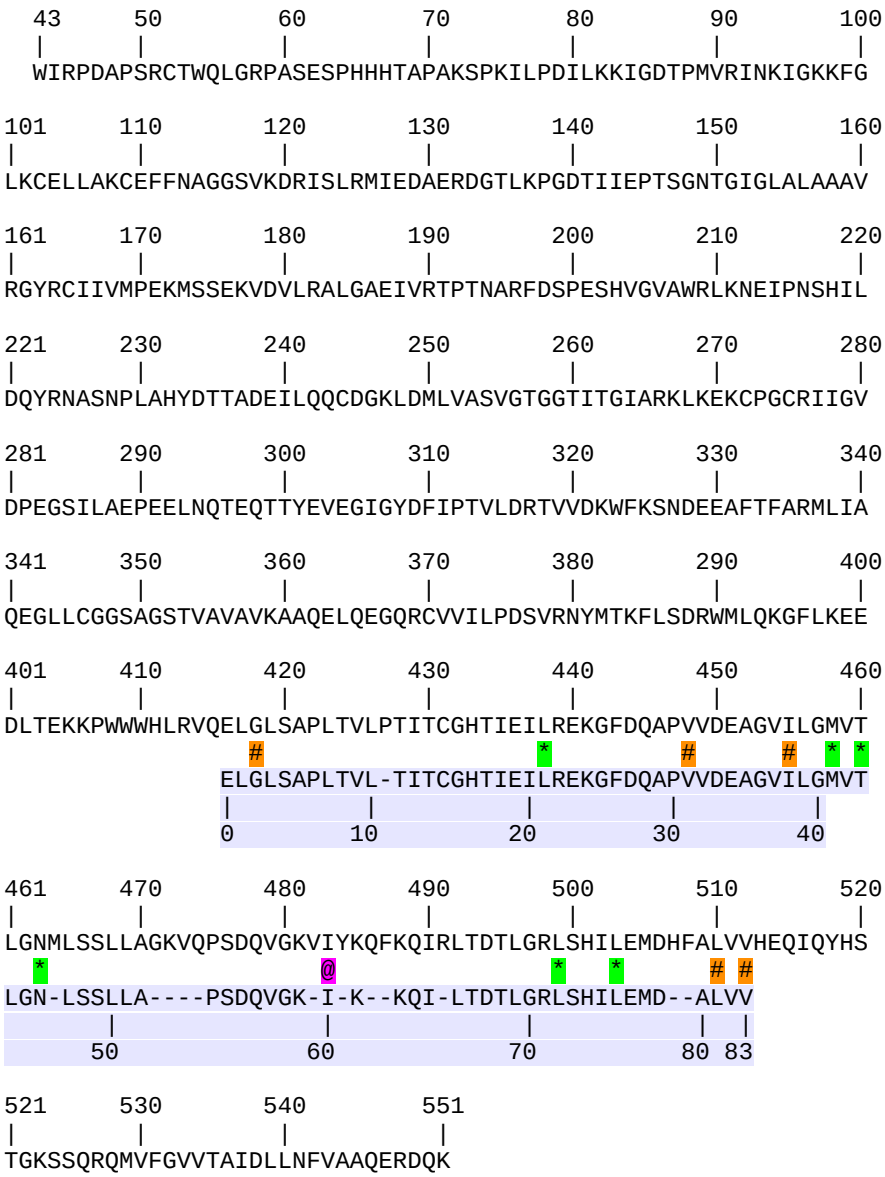
Statistical coupling analysis (SCA) and protein sector identification was performed using our in-house Python script on the basis of procedures introduced by Halabi an co-workers [Halabi 2009]. Data were visualized using Matplotlib library (matplotlib.sourceforge.net). The multiple sequence alignment of CBS domain sequences was obtained from Pfam database [Finn, 2010]. Incomplete sequences were manually removed, resulting to 6,983 sequences. Positions with a high number of gaps (> 20 %) were excluded from the analysis (Scheme SI). The multiple sequence alignment (6,983 x 84) was then converted to a binary form (1 = the prevalent amino acid in the position, 0 = otherwise). Figure S5 shows that application of a binary alignment does not cause a significant loss of information. Figure S6 shows relative entropies (conservation of individual alignment positions, top) and statistical coupling matrix (evolutionary correlation between positions, bottom). High coupling values indicate that a presence of amino acid residue in one position influences the presence of another amino acid residue in another position. Eigenvalues of this matrix are shown as a histogram in Figure S7. The first eigenvector correlates with a net contribution of each position (Figure S8) and it is therefore not used in further analysis. The second, third and fourth eigenvectors show an evolutionary coupling are is illustrated in Figure S9. The weight of the second eigenvector of the position corresponding to Ile483 in human CBS is high as this position is strongly correlated with others. It was therefore identified as a separate sector (magenta). Other two sectors were identified as positions with weights of the third eigenvector lower than –0.12 (green) or higher than 0.17 (orange). Figure 3A illustrates the model of CBS domain with sector residues shown in surface representation. The matrix in Figure 3B was obtained by filtering sector position from the matrix in Figure S6. Detailed explanation of statistical

coupling analysis and protein sectors can be obtained in the article of Halabi and co-workers [Halabi, 2009].

References:

[Halabi, 2009] Halabi, N., Rivoire, O., Leibler, S., Ranganathan R. (2009) Protein Sectors: Evolutionary Units of Three-Dimensional Structure. *Cell* **138**, 774-786.

[Finn, 2010] Finn, R.D., Mistry, J., Tate, J., Coggill, P., Heger, A., Pollington, J.E., Gavin, O.L., Gunesekaran, P., G. Ceric, G., K. Forslund, K., Holm, L., Sonnhammer, E.L., Eddy, S.R., Bateman A. (2010) The Pfam Protein Families Database. *Nucleic Acids Res.* **38**, D211-D222.



Scheme SI: Amino acid sequence of human CBS. The autoregulatory domain residues analysed by statistical coupling analysis are shown as an alignment with the full sequence (in grey, missing positions were removed prior to analysis because they contained a large number of gaps in the multiple sequence alignment of the family). Identified sectors are highlighted (in green, orange and the residue Ile483 in magenta).

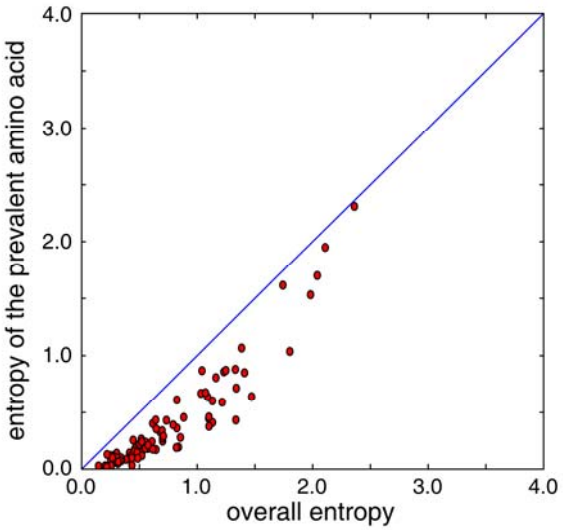


Figure S5: Agreement between the entropy of the prevalent amino acid and the overall relative entropy.

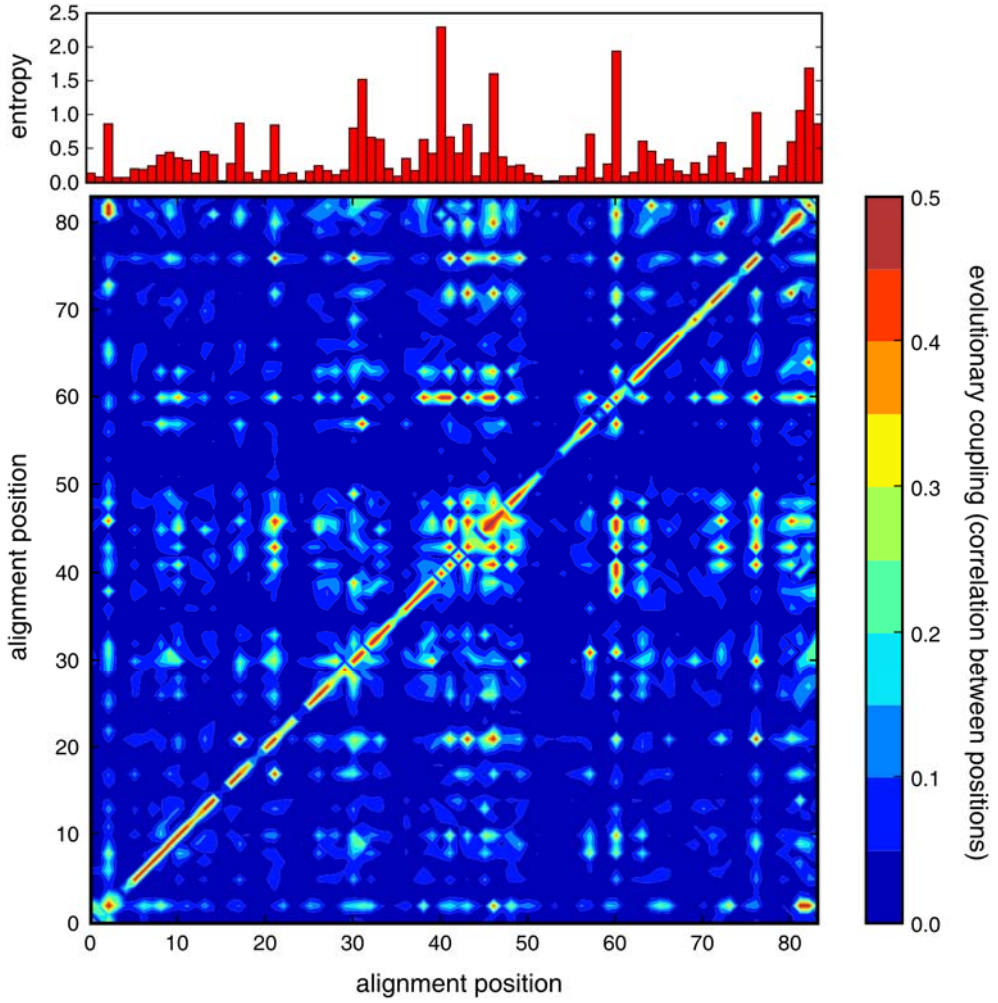


Figure S6: Conservation of individual positions in the multiple sequence alignment of CBS domains, described as relative entropy (top) and SCA matrix (bottom). Alignment positions correspond to Scheme SI.

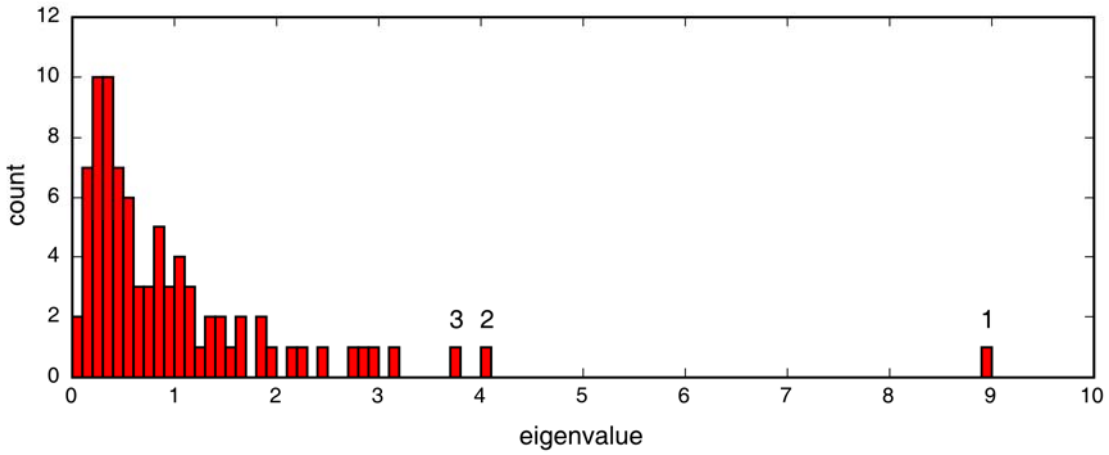


Figure S7: Eigenvalue histogram for the SCA matrix. The first, second and third eigenvalues are indicated.

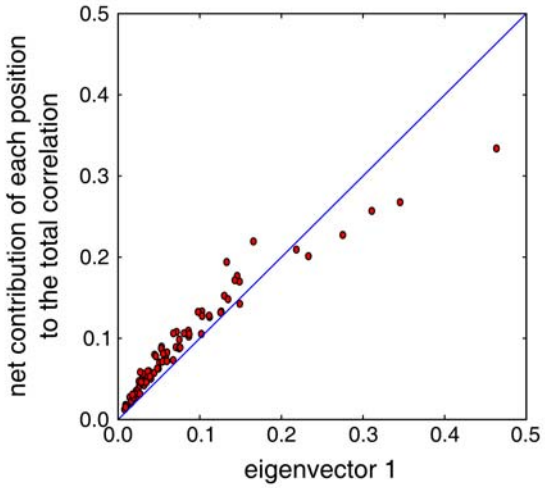


Figure S8: The first eigenvector of SCA matrix against the net contribution of each position to the total correlation.

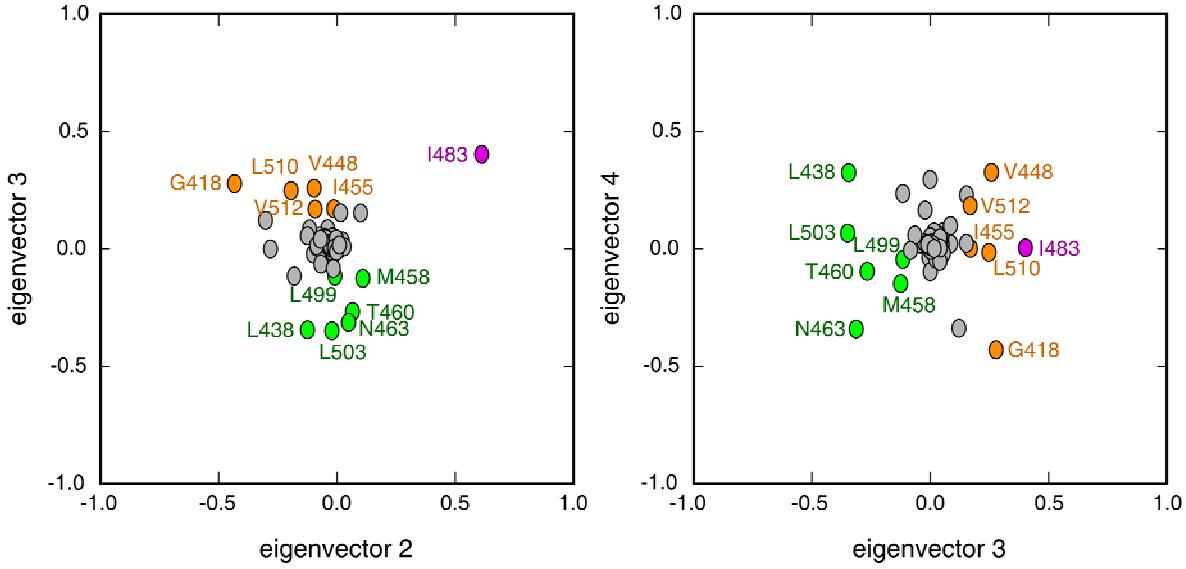


Figure S9: Residue weights along eigenvectors 2, 3 and 4. Sector residues are indicated.