Figure S1

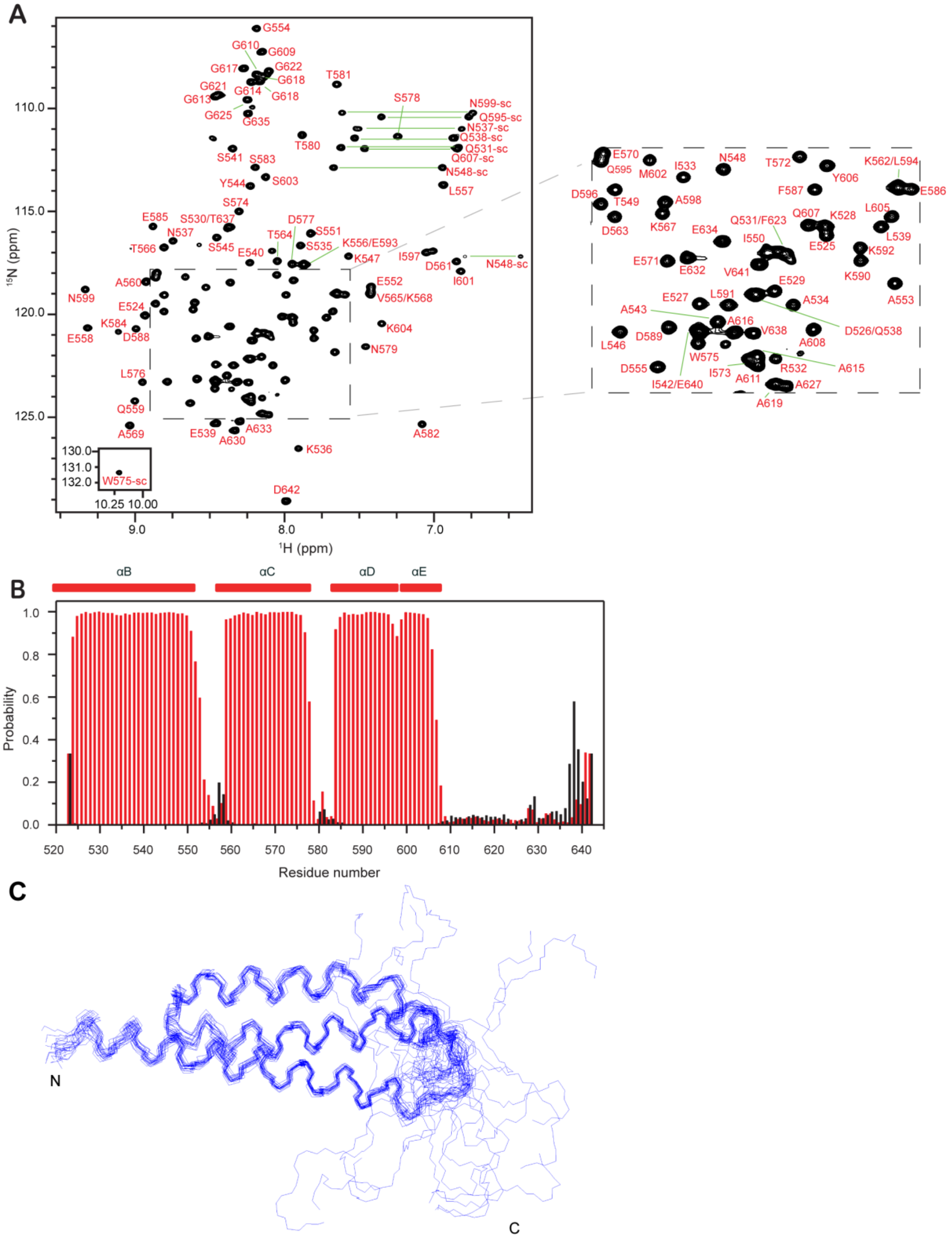


Figure S1. NMR structure for Ssa1(523-642). (A) Backbone assignments for Ssa1(523-642). (B) The secondary structure of Ssa1(523-642) predicted using the software TalosN (54). The predicted α-helix and β-strand probabilities of each residue are plotted in red and black, respectively. The secondary structure elements, indicated at the top of the figure, were obtained using the criterion probability > 0.5 for three or more consecutive residues. (C) Backbone ensemble of 20 structures of Ssa1(523-622).

Figure S2

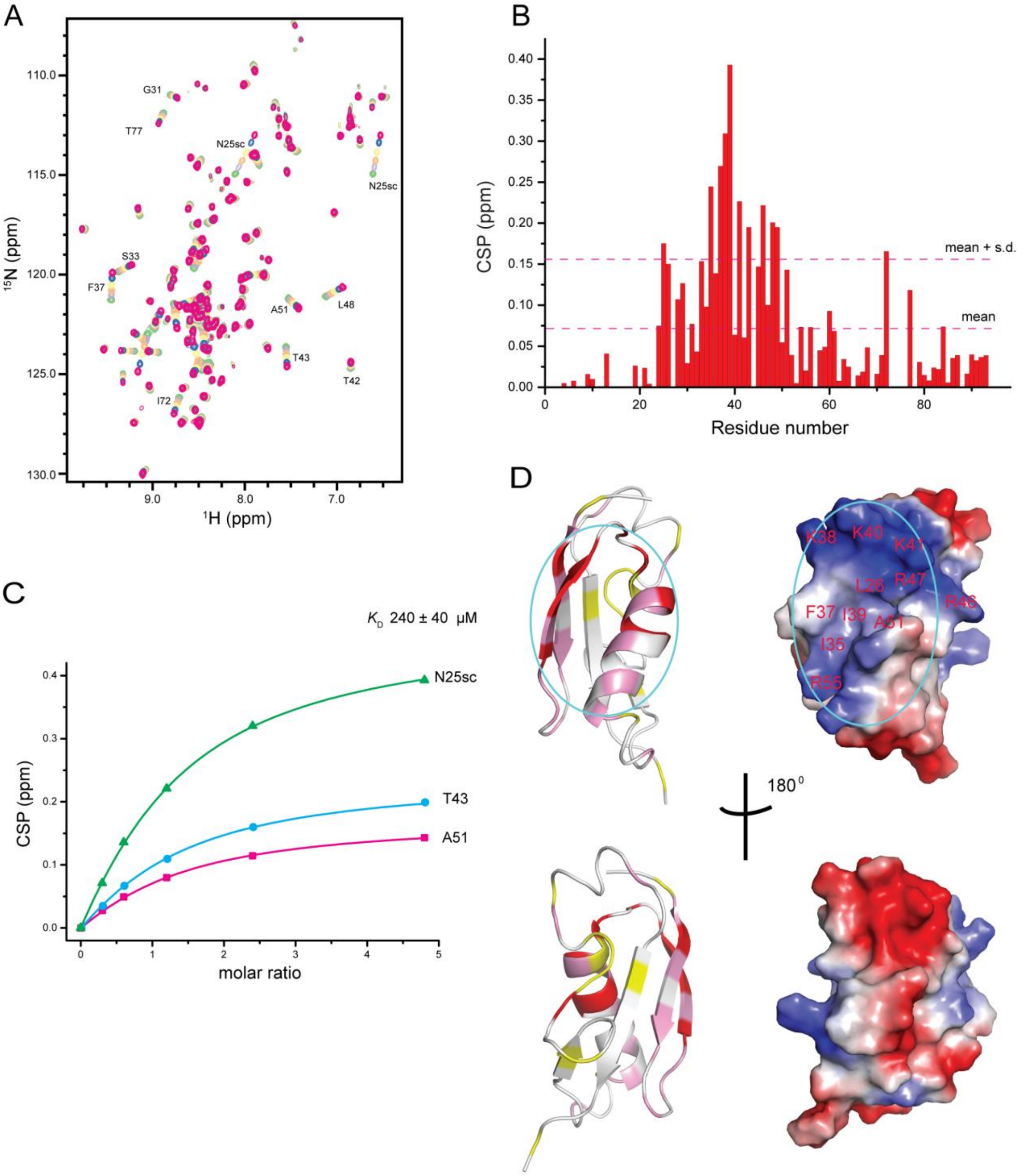
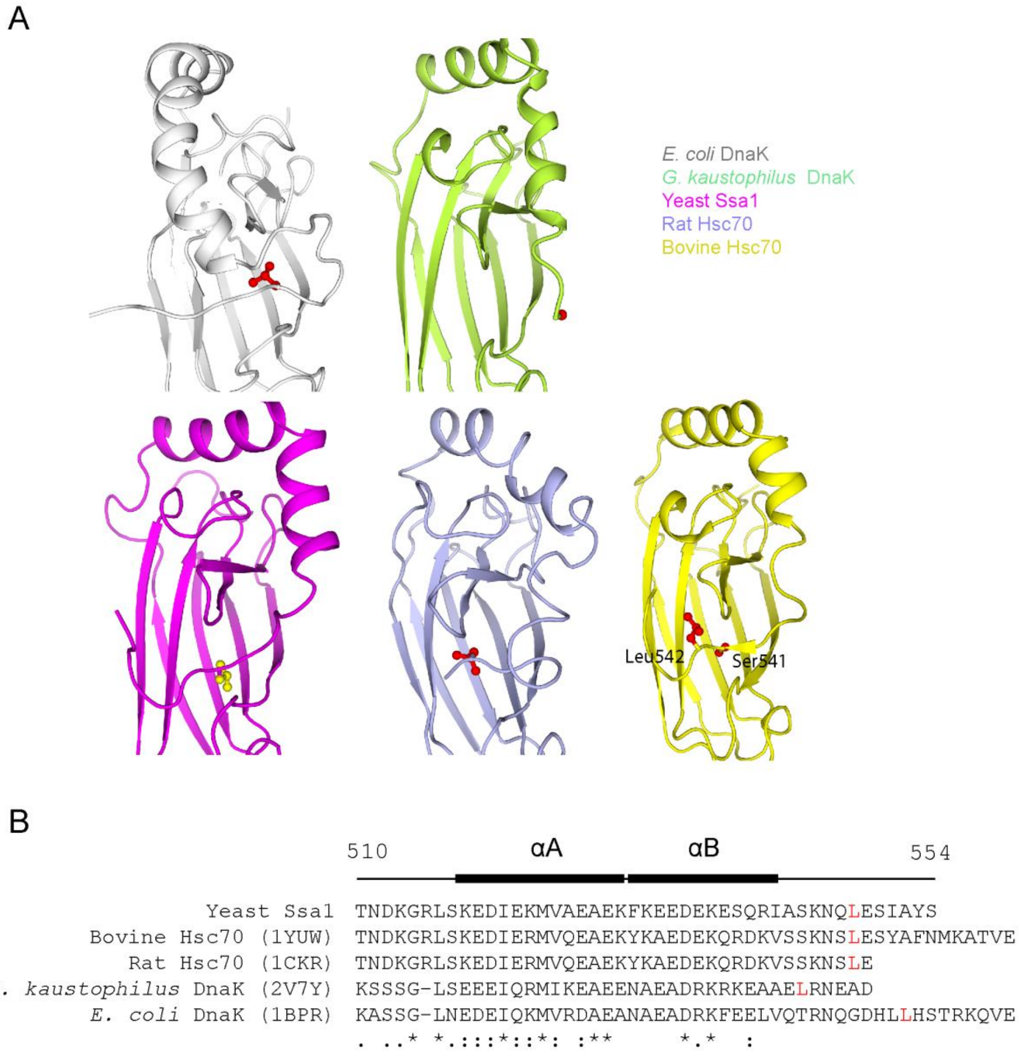


Figure S2. Titration of SMT3 with the Ssa1 C-terminal peptide (PEAEGPTVEEVD). (A) ^{1}H-^{15}N HSQC spectra of SMT3 titrated with the peptide at ratios of 1:0, 1:0.3, 1:0.6, 1:1.2, 1:2.4, and 1:4.8. (B) Bar diagram of CSP versus residue number of SMT3 in (A). (C) Fitting of CSP data using the backbone NH signals of T43 and A51, together with the sidechain NH signal of N25, in order to obtain the K_D. (D) Mapping of the CSP results in (B) onto the SMT3 structure. The binding site with largest CSP values is circled in the ribbon and electrostatic potential surface models (PDB id 1L2N). Red, CSP ≥ mean + s.d. in (B); pink, mean + s.d. > CSP ≥ mean in (B); while, mean > CSP; yellow, proline and unassigned residues.

Figure S3



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                 Yeast Ssa1  TNDKGRLSKEDIEKMVAEAEKFKEEDEKESQRIASKNQLESIAYS
        Bovine Hsc70 (1YUW)  TNDKGRLSKEDIERMVQEAEKYKAEDEKQRDKVSSKNSLESYAFNMKATVE
           Rat Hsc70 (1CKR)  TNDKGRLSKEDIERMVQEAEKYKAEDEKQRDKVSSKNSLE
G. kaustophilus DnaK (2V7Y)  KSSSG-LSEEEIQRMIKEAEENAEADRKRKEAAELRNEAD
         E. coli DnaK (1BPR)  KASSG-LNEDEIQKMVRDAEANAEADRKFEELVQTRNQGDHLLHSTRKQVE
                             . ..* *.:::*::*: :**     *.*  :
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Figure S3. Comparison of structures of different Hsp70 SBDβ truncations (A) and sequence alignment of C-terminal sequences of these constructs (B). Leu542 in yeast Ssa1 (together with other corresponding residues in the homologue proteins) are displayed as ball/stick in (A), and colored red in (B). Secondary structure is labeled according to the structure of yeast Ssa1 SBD (382-554).

Figure S4

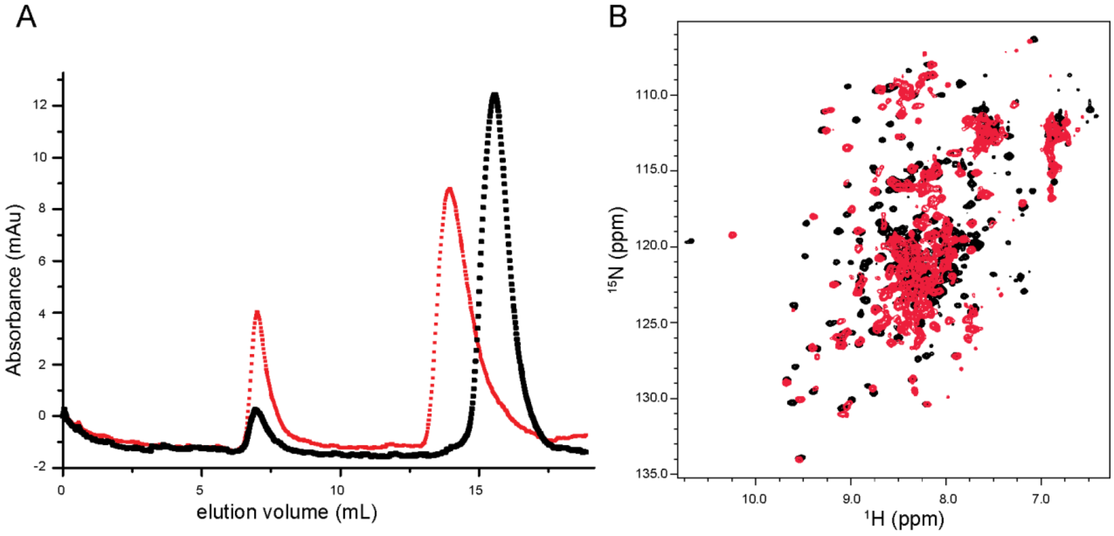

Figure S4. Oligomerization of Ssa1 SBD (382-506). (A) Elution profile of Ssa1 SBD (382-506) (red) and SBD (382-554) (black). (B) ^{1}H-^{15}N spectra of Ssa1 SBD (382-506) (red) and SBD (382-554) (black).