Supporting Information

Kolstoe et al. 10.1073/pnas.0902640106

SI Methods

X-Ray Analysis. Synchrotron data from crystals of the SAP/*O*-phospho-L-threonine complex, as described in *Methods*, were processed with MOSFLM (1) and programs from the CCP4 suite (2). Molecular replacement with MOLREP (3) used a previously derived SAP pentamer, 1SAC (4), with the calcium atoms removed from the search model. There was one SAP pentamer in the asymmetric unit with a solvent content of 56%. The structure was initially refined using SHELX (5) and the model built with TURBO-FRODO (6). Refinement was completed using phenix.refine (7) and the model built using COOT (8). Validation was performed using MOLPROBITY (9) with the final model showing no residues within the disallowed region of the Ramachandran plot [supporting information (*SI*) Table S1]. Diagrams in Figs. 3 and 4 in the main text were prepared using PyMol (10).

1. Leslie AGW (1992) Recent changes to the MOSFLM package for processing film and image plate data. *Joint CCP4 + ESF-EAMCB Newsletter on Protein Cyrstallography*, No 26.
2. Collaborative Computational Project, Number 4 (1994) The *CCP*4 suite: Programs for protein crystallography. *Acta Crystallogr D* 50:760–763.
3. Vagin A, Teplyakov A (1997) MOLREP: An automated program for molecular replacement. *J Appl Cryst* 30:1022–1025.
4. Emsley J, et al. (1994) Structure of pentameric human serum amyloid P component. *Nature* 367:338–345.
5. Sheldrick GM, Schneider TR (1997) SHELXL: High-resolution refinement. *Methods Enzymol* 277:319–343.
6. Roussel A, Cambillau C (1991) *TURBO-FRODO: A Tool for Building Structural Models* (Silicon Graphics, Mountain View, CA).
7. Afonine PV, Grosse-Kunstleve RW, Adams PD (2005) The Phenix refinement framework. *CCP4 Newsletter on Protein Crystallography* 42: Contribution 8.
8. Emsley P, Cowtan K (2004) Coot: Model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr* 60:2126–2132.
9. Lovell SC, et al. (2003) Structure validation by Cα geometry: ϕ, ψ and Cβ deviation. *Proteins* 50:437–450.
10. DeLano WL (2005) The PyMOL Molecular Graphics System (DeLano Scientific, San Carlos, CA). Available at http://www.pymol.org.

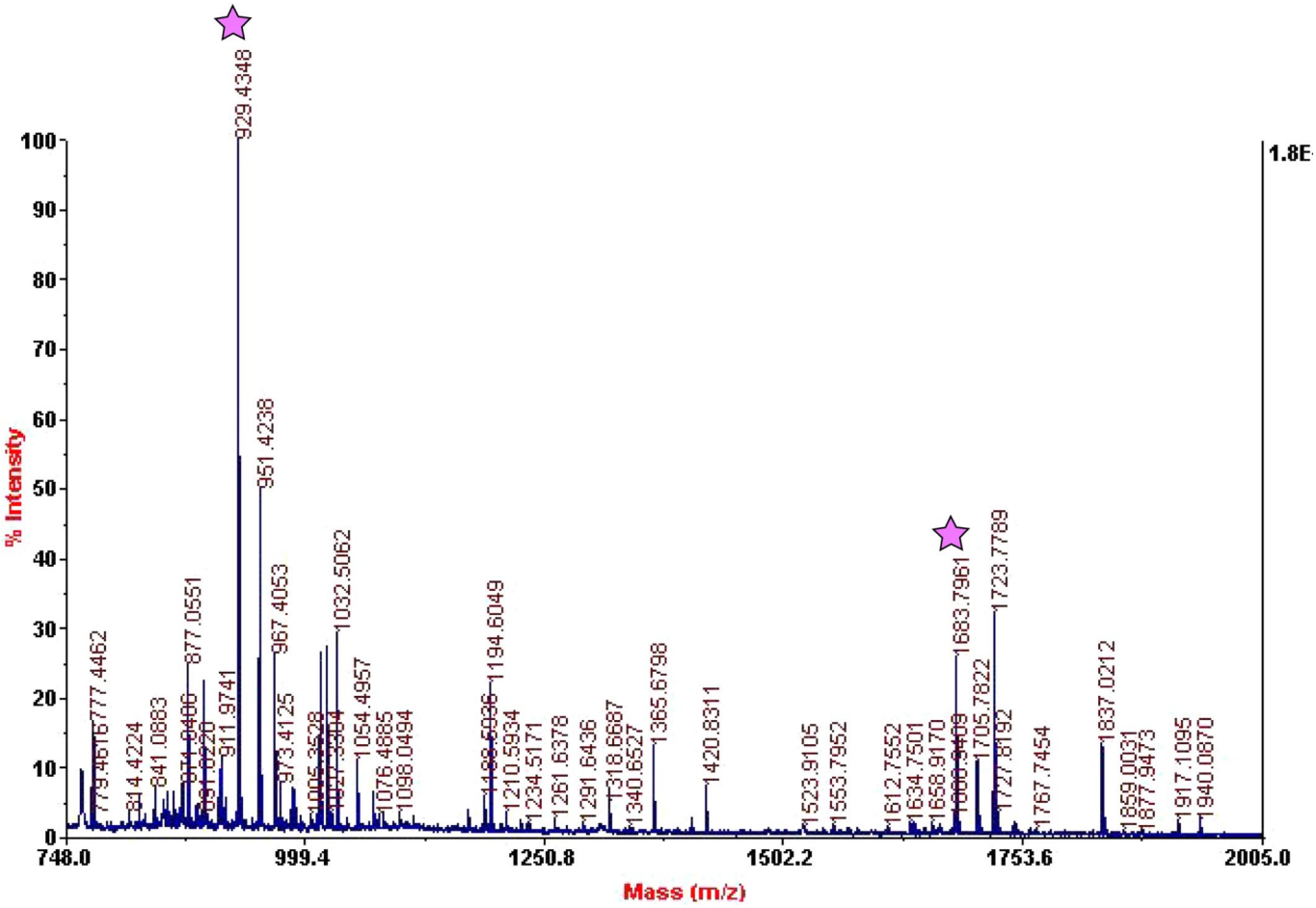

Fig. S1. MALDI mass spectrum of cross-linked SAP after digestion with Asp-N. Two unique peptides are observed after cross-linking that are not observed in unmodified SAP (molecular masses of 929.43 and 1683.79 Da).

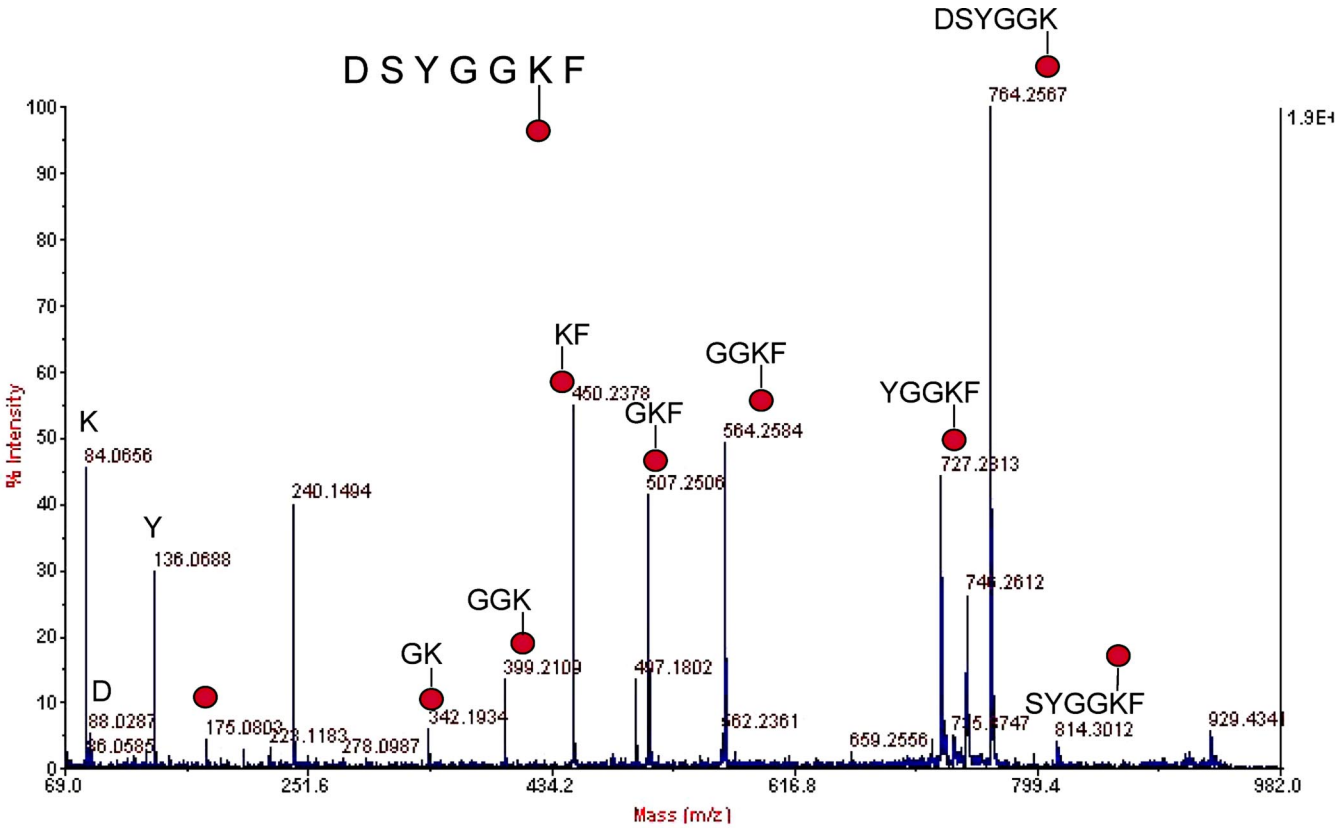

Fig. S2. Tandem mass spectrometry of the 929.43-Da peak identified in Fig. S1. Sequence ions confirm the presence of the cross-linking reagent BS3 attached to residue Lys-143. The BS3 cross-linker is represented by a red circle.

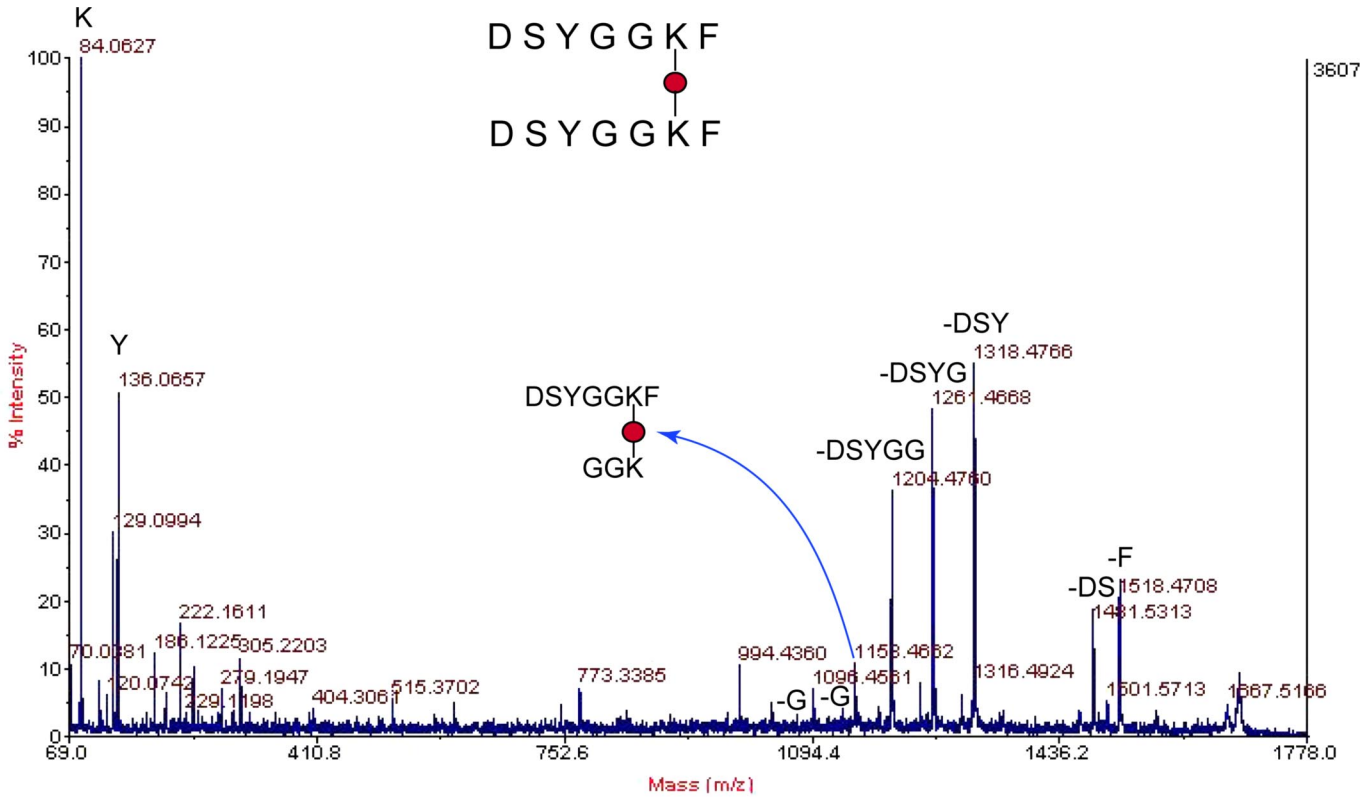

Fig. S3. Tandem mass spectrometry of the 1638.6-Da peptide identified in Fig. S1. Assignment of the sequence ions confirms the identity of the peptide as $(DSYGGKF)_2$BS3 linked by residues Lys-143. The BS3 cross-linker is represented by a red circle.

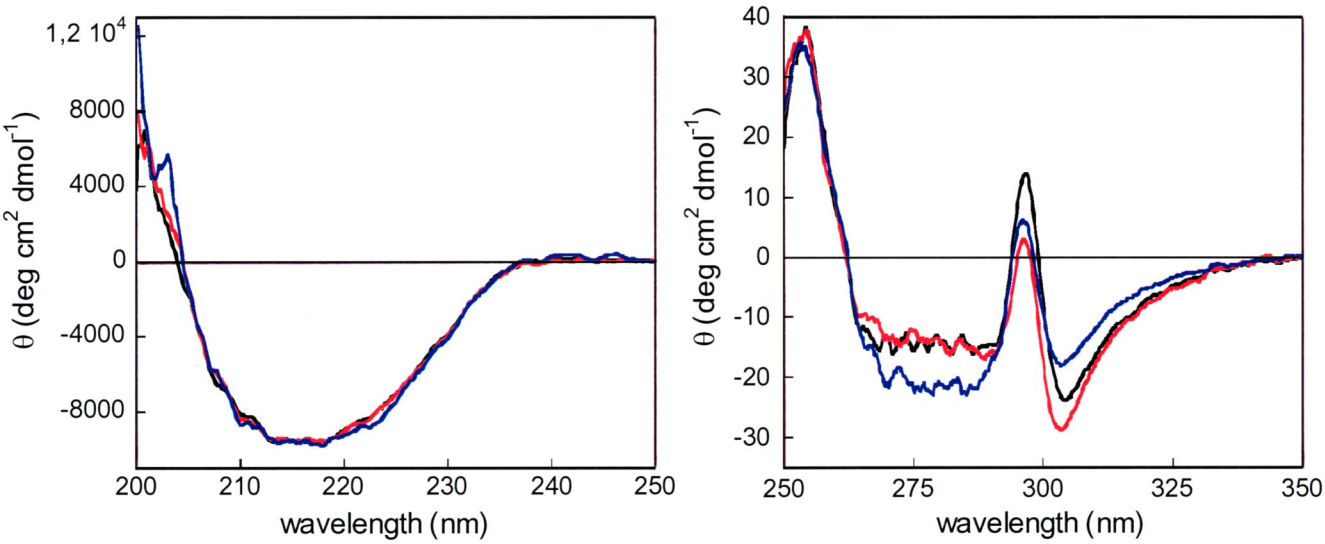

Fig. S4. Far (*Left*) and near (*Right*) UV spectra of SAP alone (black), SAP-CPHPC complex (red), and the SAP-CPHPC complex covalently cross-linked with BS3 (blue), showing no significant differences.

Table S1. Data collection and refinement statistics for the complex of SAP with *O*-phospho-L-threonine

Parameter	Value
Space group	$P2_1$
Unit cell (Å)	$a = 94.77$, $b = 69.43$, $c = 102.06$, $\beta = 97$
Resolution range (Å)	47.6–1.7
Measured reflections	787,858
Unique reflections	140,019
Multiplicity	5.6 (4.0)
Completeness (%)	97 (95.6)
Rmeas (%)	12.1 (38.2)
Mean (*I*)/SD(*I*)	16.7 (9.3)
Solvent content (%)	56
Model Rfactor (%)	16.2
Model Rfree (%)	18.4
Residues in allowed region (%)	100
rms bond lengths (Å)	0.015
rms bond angles (degree)	0.025