Supporting Information

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SI Text

NMR Spectroscopy. Josephin samples for NMR contained ^{15}N-labeled Josephin (0.3 mM) or ^{13}C, ^{15}N-labeled Josephin (0.5 mM) in TBS. All spectra were recorded at 25 °C on a Varian Inova 600 NMR spectrometer equipped with a cryoprobe, operating at a ^{1}H frequency of 600 MHz.

To investigate the α-Bc binding site on Josephin by chemical shift perturbation, ^{15}N-HSQC spectra were acquired in the presence and absence of unlabeled αB-c at αB-c:Josephin molar ratios of 0, 0.1, 0.25, 0.6, 2, and 5. The spectral dimensions were 2,048 (t2) × 160 (t1) increments with 32 scans per increment and a recycle delay of 1.2 s.

The surface-exposed residues involved in the interaction were probed by using paramagnetic relaxation enhancement. A gadolinium-based paramagnetic relaxation agent, Gd(DTPA-BMA) (1), at a concentration of 5 mM was added to a 0.3 mM ^{15}N-labeled Josephin sample. ^{15}N-HSQC spectra were recorded in the absence and presence of 0.6 mM αB-c.

For assignment purposes 3D HNCO, HNCA, and CBCA(CO)NH spectra (2) were acquired by using a 0.3 mM ^{13}C, ^{15}N-Josephin sample. All data were processed by using nmrPipe (3) with sine squared window functions, zero filling, and linear prediction in the indirect dimensions. Spectra were visualized and analyzed by using Sparky (Goddard and Keller, University of California at San Francisco).

1. Pintacuda G, Otting G (2002) Identification of protein surfaces by NMR measurements with a pramagnetic Gd(III) chelate. *J Am Chem Soc* 124(3):372–373.
2. Sattler M, Schleucher J, Griesinger C (1999) Heteronuclear multidimensional NMR experiments for the structure determination of proteins in solution employing pulsed field gradients. *Prog Nucl Mag Res Spectr* 34(2):93–158.
3. Delaglio F, et al. (1995) NMRPipe: A multidimensional spectral processing system based on UNIX pipes. *J Biomol NMR* 6(3):277–293.

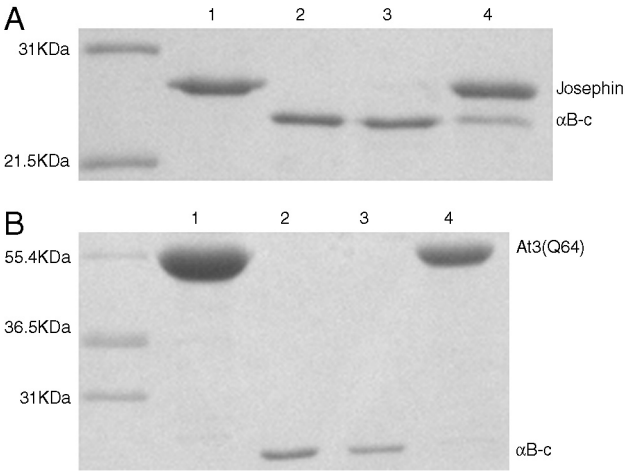

Fig. S1. Qualitative analysis of size-exclusion chromatography (SEC) peaks using SDS-PAGE. Fractions corresponding to the peaks from the Superose 6 column (Fig. 4) were pooled, concentrated, and analyzed by SDS-PAGE. (*A*) Analysis of Josephin:αB-c incubated at a 1:1 ratio for 48 h. Lane 1: Josephin control; lane 2: αB-c control; lane 3: the concentrated fractions eluting from the SEC between 11 and 14 mL (this corresponds to the broadened peak containing multimeric αB-c upon coincubation with Josephin; a small amount of Josephin is visibly coeluting with αB-c in this peak); lane 4: the concentrated fractions eluting from the SEC between 18 and 20 mL. (This corresponds to the Josephin-containing peak. Some αB-c can be seen to coelute with Josephin and this is likely to be a dissociated form of αB-c.) (*B*) Analysis of an at3(Q64):αB-c sample incubated at a 1:1 ratio for 8 h. Lane 1: At3(Q64) only control; lane 2: αB-c only control; lane 3: the concentrated fractions eluting from the SEC between 11 and 13.5 mL [this corresponds to the peak containing multimeric αB-c upon coincubation with at3(Q64)]; lane 4: the concentrated fractions eluting from the SEC between 16.5 and 19 mL corresponding to the at3(Q64)-containing peak. In the left-hand lane of both gels are the molecular weight standards.

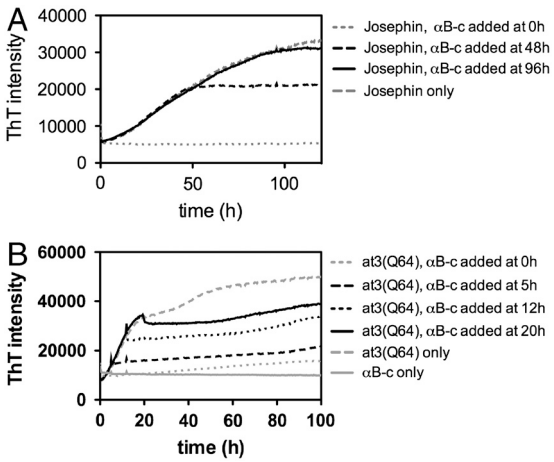

Fig. S2. The effect of αB-c addition after at3 aggregation commencement. αB-c was added to (*A*) Josephin and (*B*) at3(Q64) aggregation reactions at the indicated time points as the reactions were monitored by ThT fluorescence.

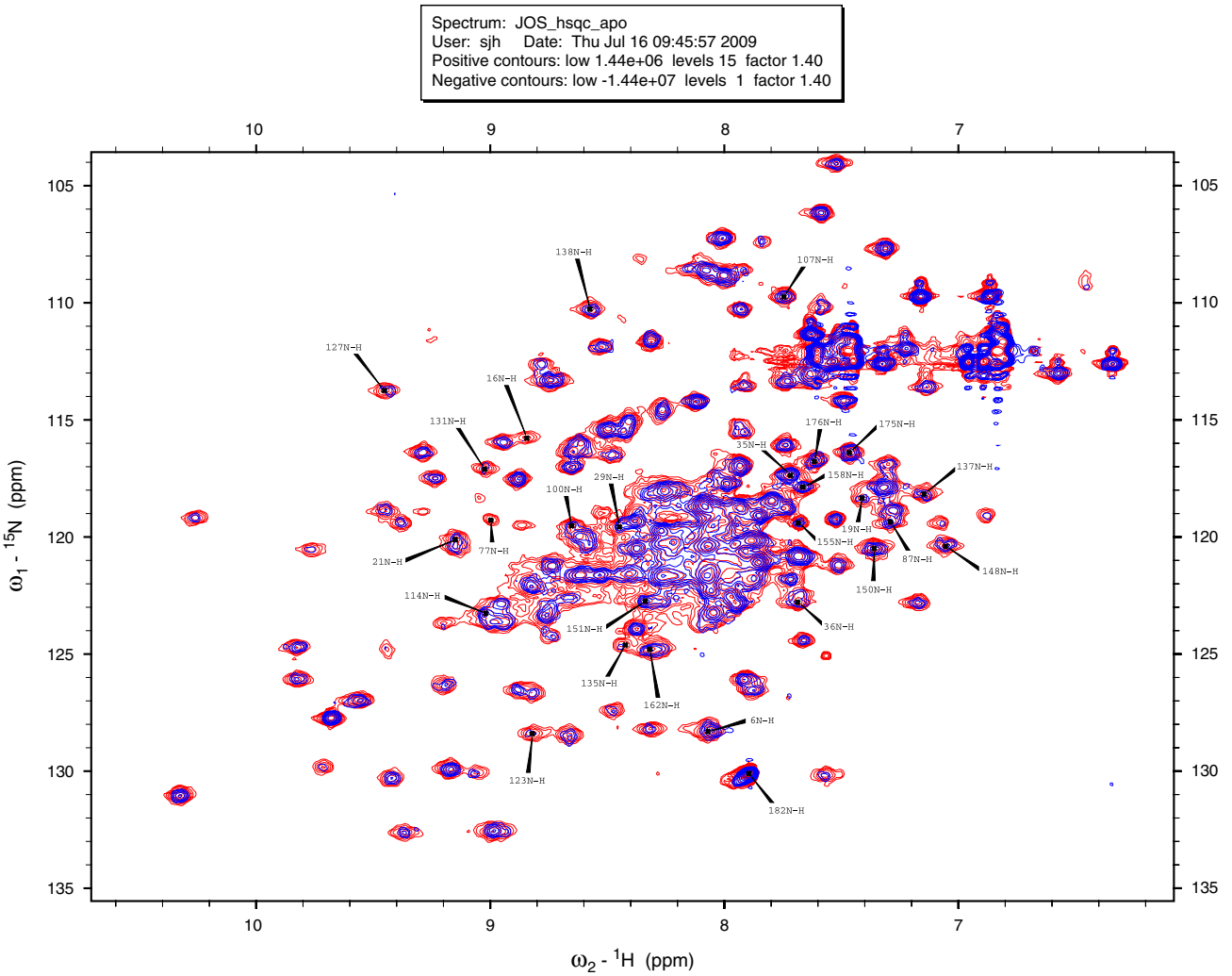

Fig. S3. ^{15}N-HSQC NMR spectra of Josephin in the presence and absence of αB-c. The Josephin spectra in the absence of αB-c is shown in red and Josephin spectra in the presence of a fivefold molar excess of αB-c are shown in purple. Spectra were recorded at 600 MHz and 25 °C.

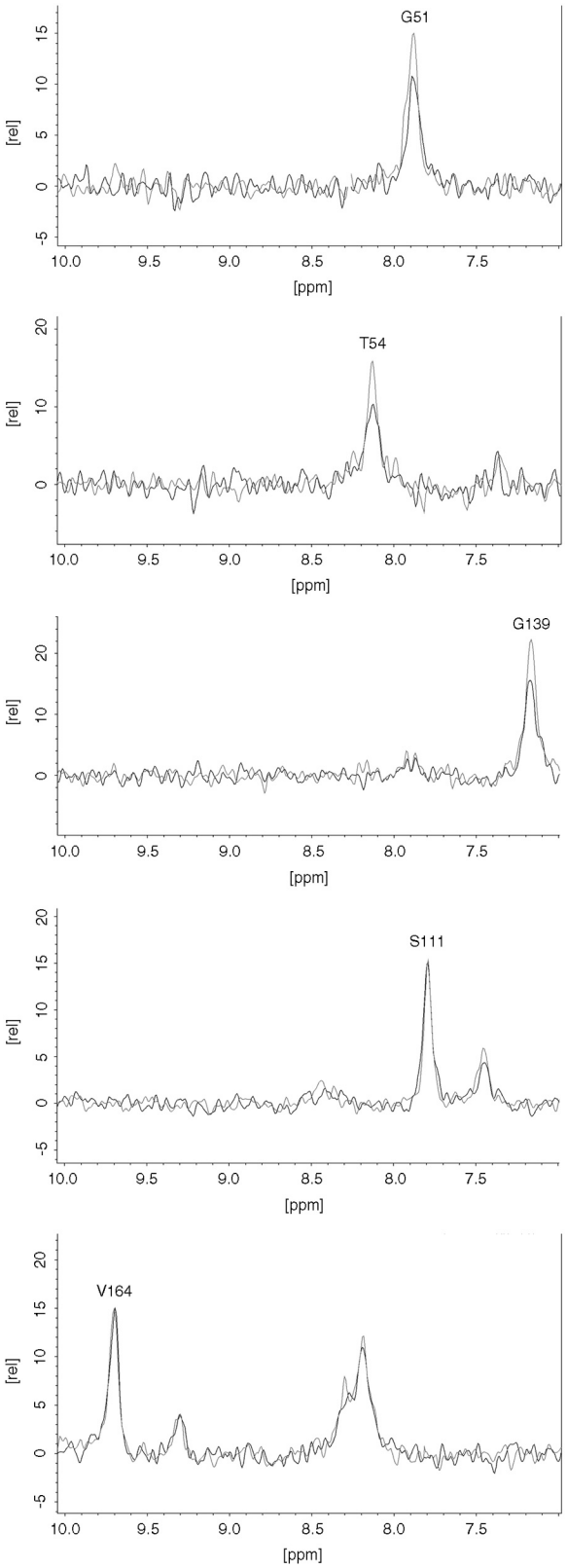

Fig. S4. αB-c partially shields specific residues from paramagnetic relaxation enhancement. ^{1}H slices through ^{15}N-HSQC spectra of ^{15}N-Josephin in buffer containing the paramagnetic relaxation enhancement agent 5 mM Gd(DTPA-BMA). In black is shown apo ^{15}N-josephin and in gray is ^{15}N-Josephin in the presence of equimolar unlabeled αB-c. Residues G51, T54, and G139 are partially shielded from paramagnetic relaxation enhancement by the presence of αB-c, whereas residues S111 and V164 are not.