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Supplementary Materials for

A molecular mechanism of chaperone-client recognition

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Supplementary Figures

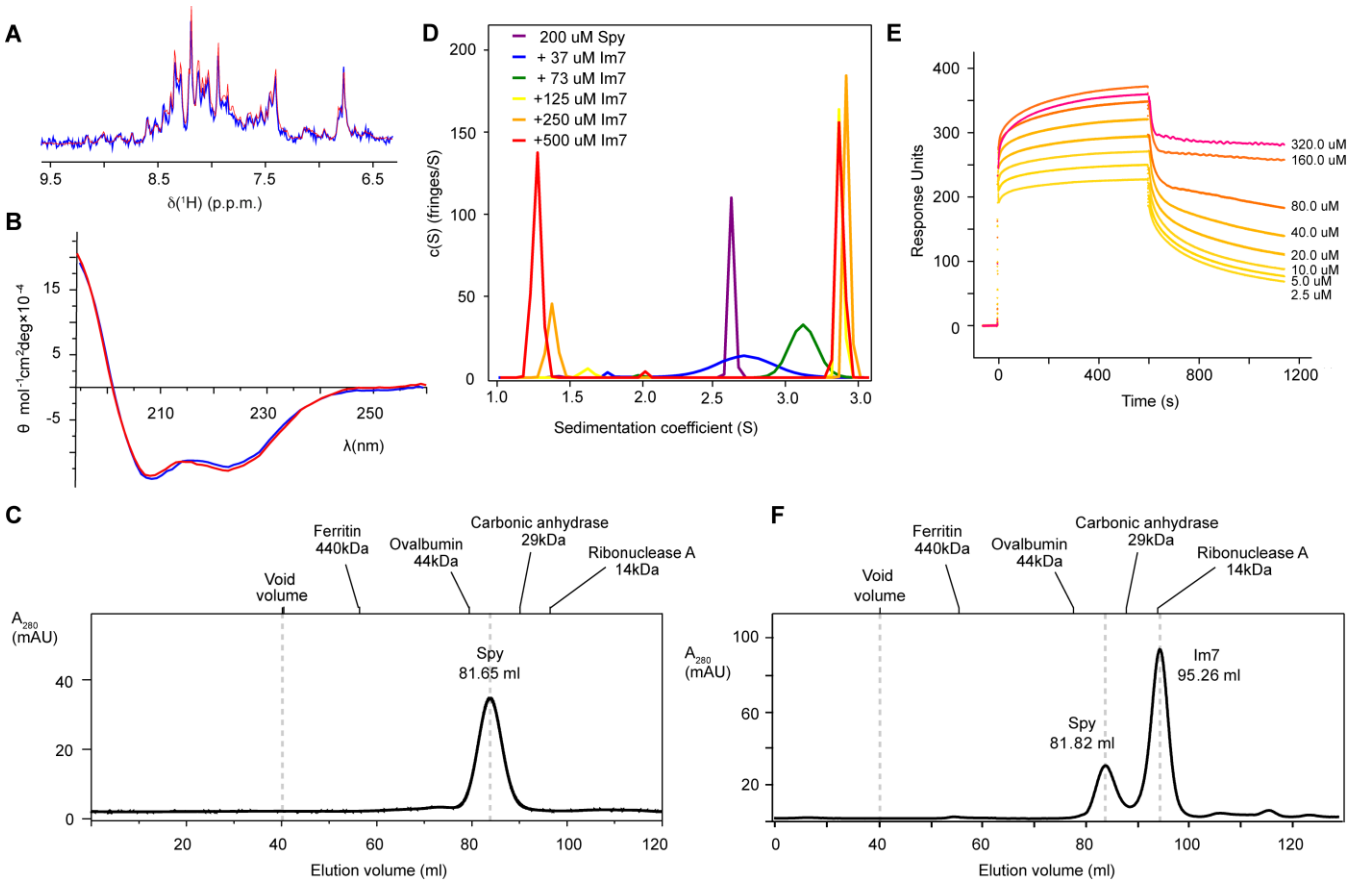

fig. S1. Biophysical characterization of apo Spy and Spy-Im7. (**A**) 1D ^{1}H NMR spectra of 50 μM natively purified (blue) and 200 μM refolded Spy (red) in 20 mM NaH_2PO_4, pH 6.5 acquired on a 700 MHz spectrometer at 25°C. (**B**) Circular dichroism spectra of 3 μM natively purified (blue) and 3 μM refolded Spy (red) in 20 mM phosphate buffer pH 6.5. (**C**) Gel filtration chromatography of apo Spy on a Superdex 200 pg column. Elution positions of a set of reference proteins are indicated on top. (**D**) Analytical ultracentrifugation experiments of apo Spy and Spy in complex with Im7, at the indicated concentrations. Absorbance at 280 nm was analyzed and fitted to c(s) distribution in Sedfit, with baseline and noise correction. (**E**) Surface plasmon resonance sensorgrams of the Spy–Im7 interaction. Im7 was immobilized on the SPR chips. Spy solution with concentrations as indicated was injected for 600 s, followed by 20mM sodium phosphate, pH 6.5 buffer for 1800 s. (**F**) Gel filtration chromatography of a sample of Spy–Im7 complex injected on a Superdex 200 pg column. Elution positions of a set of reference proteins are indicated on top. The elution profile shows two separated peaks corresponding to dimeric Spy and monomeric Im7, respectively.

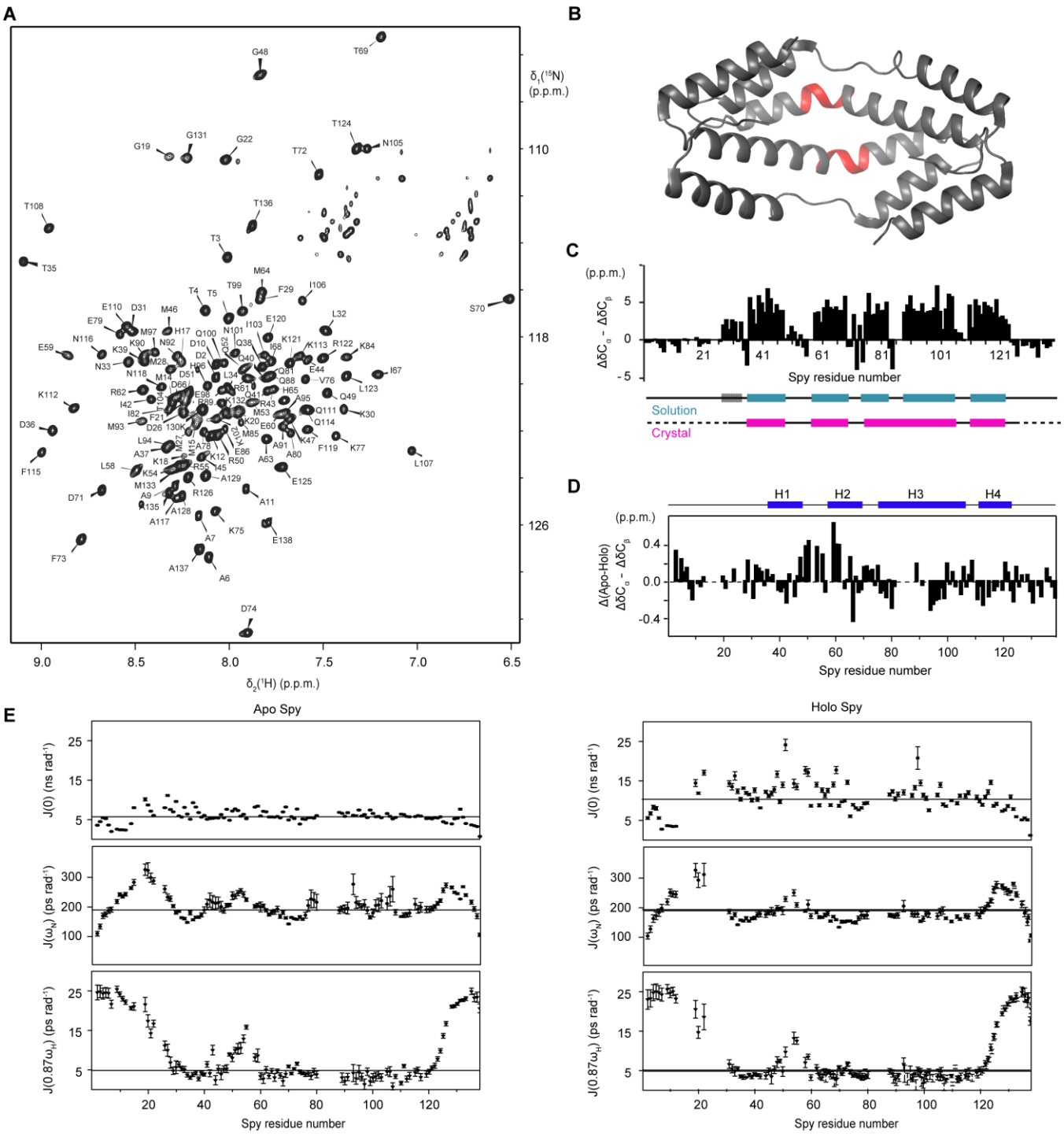

fig. S2. NMR characterization of apo Spy and Spy in the Spy-Im7 complex. (**A**) 2D [^{15}N,^{1}H]-TROSY spectrum of 500 μM Spy in 20 mM NaH_2PO_4, pH 6.5, acquired on a 700 MHz spectrometer. Sequence-specific resonance assignment of the backbone amide groups is indicated. (**B**) Assigned residues are plotted grey and unassigned residues are plotted red in the crystal structure of Spy (PDB code 3O39). (**C**) Secondary chemical shifts of apo Spy in solution, plotted against the residue number.

Positive shifts indicate helical secondary structure propensity. Helical segments in solution and the crystal structure (PDB code 3O39) are displayed below in the plot in blue and magenta, respectively. The short segment of residues 27–32, showing around 50% helical propensity, is indicated by a grey box. Regions unresolved in the crystal structure are indicated by a dashed line. (**D**) Cα-Cβ secondary chemical shift differences between apo and holo Spy. (**E**) Spectral density functions of apo Spy and Spy in the Spy–Im7 complex determined at 25°C. The average value for each plot is indicated by a horizontal reference line.

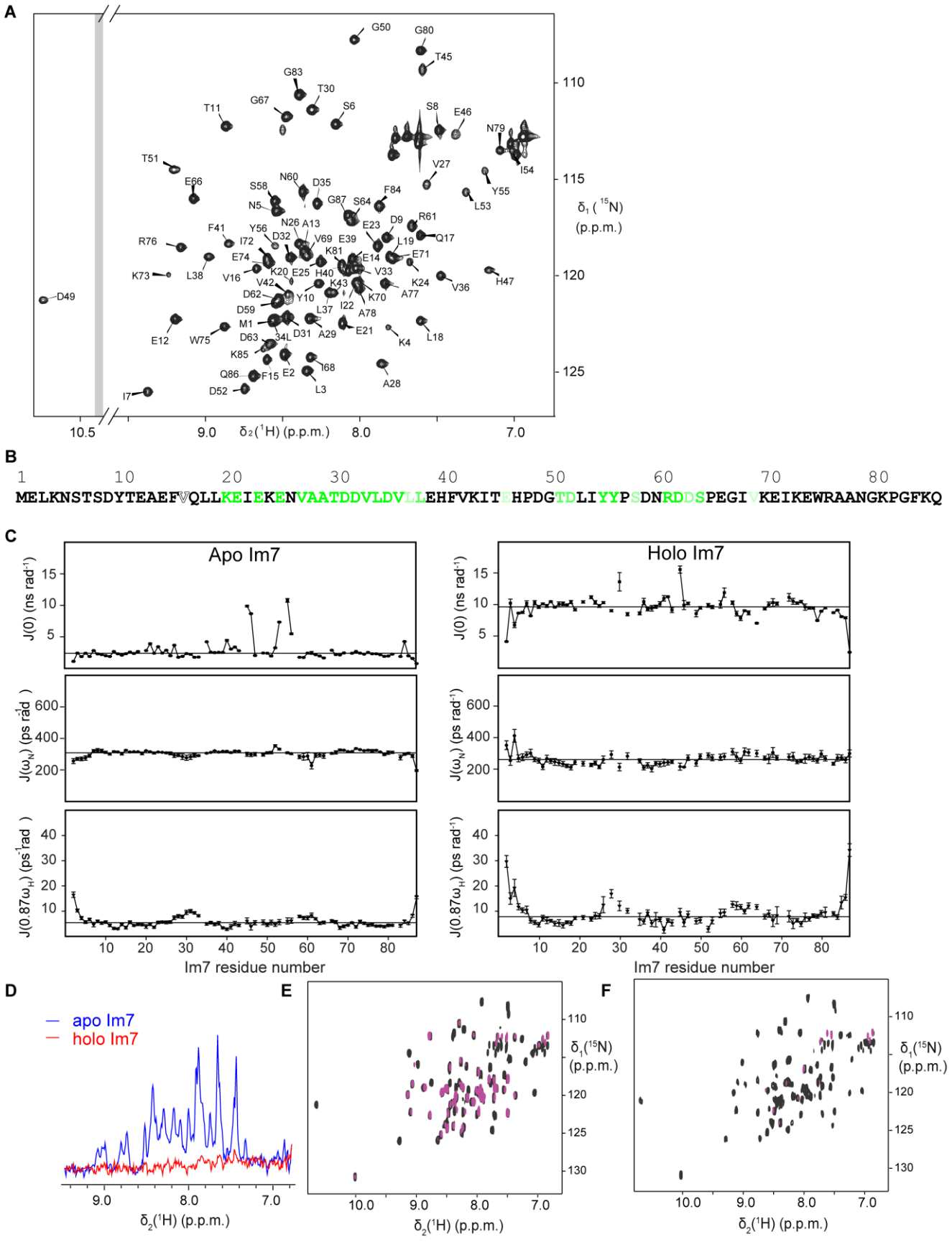
A
B
1 10 20 30 40 50 60 70 80
MELKNSTSDYTEAEFVQLLKEIEKENVAATDDVLDVLLEHFVKITSHPDGTDLIYYPSDNRDDSPEGIVKEIKEWRAANGKPGFKQ
C
Apo Im7
Holo Im7
J(0) (ns rad-1)
J(ωN) (ps rad-1)
J(0.87ωH) (ps-1rad)
Im7 residue number
D
apo Im7
holo Im7
δ2(1H) (p.p.m.)
E
F
δ1(15N) (p.p.m.)
δ2(1H) (p.p.m.)

fig. S3. NMR characterization of apo Im7 and Im7 in the Spy-Im7 complex. (**A**) 2D [^{15}N,^{1}H]-HSQC spectrum of 1000 μM Im7 in 20 mM NaH_2PO_4, pH 6.5, acquired on a 700 MHz spectrometer. Sequence-specific resonance assignment of the backbone amide groups are indicated (**B**) Sequence display of the CSPs for Im7 upon interaction with Spy. A grey to green color scale is applied on the sequence of Im7 according to the magnitude of the CSPs for the interaction with Spy. The brightest green indicates the largest chemical shift perturbation. (**C**) Spectral density functions of apo Im7 and holo Im7 bound to Spy, determined at 25°C. The horizontal lines indicate the mean values of each spectral density function. (**D**) 1D-^{15}N-filtered spectra of apo Im7 (blue) and holo Im7 (red) recorded three minutes after exchange from H_2O-based to D_2O-based buffer. (**E**, **F**) 2D [^{15}N, ^{1}H]-HSQC spectra of Im7 before (black) and 3 min after H/D exchange (magenta). Data are shown for apo and holo Im7 in panel E and F, respectively.

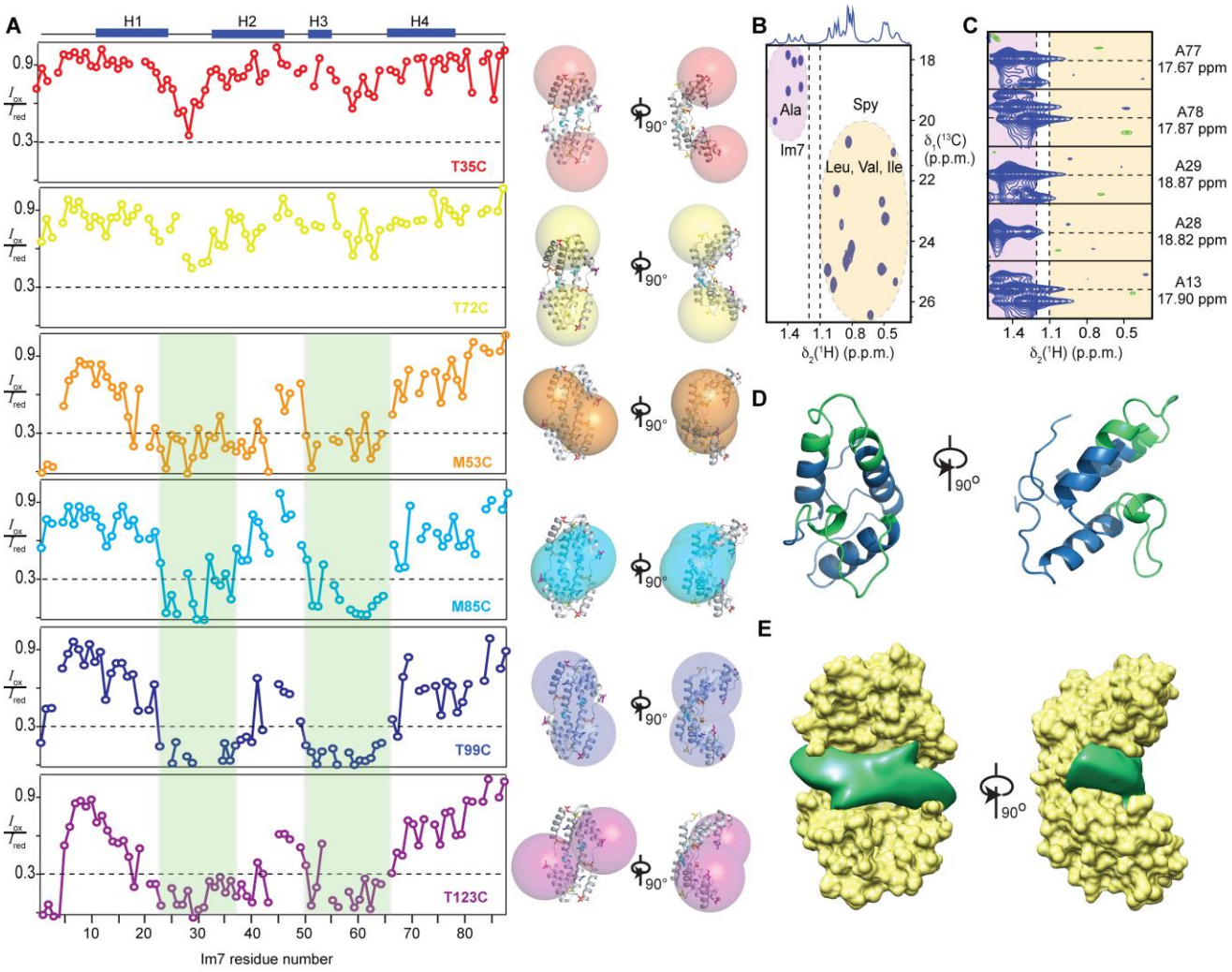

fig. S4. The Spy-Im7 interaction probed by PRE and NOEs. (**A**) Inter-molecular PRE effect plotted as the peak intensity ratio (I_{ox}/I_{red}) against the Im7 sequence. Data of PRE effects of six single amino acid mutants of Spy attached with spin label MTSL are shown: red, Spy T35C, yellow, Spy T72C; orange, Spy M53C; cyan, Spy M85C; blue, Spy T99C; violet, Spy T123C. Residues 23–36 and residues 50–65, which show a strong PRE effect (intensity ratio < 0.3) for the spin labeled M53C, M85C, T99C and T123C mutants, are indicated with green background. On the right hand side, the positions of the spin labels are shown on the structure of Spy by spheres with 20 Å radius around the respective Cys C_β position. (**B**) 2D [^{13}C,^{1}H] HMQC spectrum of the complex of [*U*-^{2}H,^{15}N, Ile-δ_1-$^{13}CH_3$, Leu,Val-$^{13}CH_3$]-Spy (ILV-Spy) with [*U*-^{2}H,3-$^{13}CH_3$-Ala]-Im7. The Ala residues (pink area) and the Ile, Leu, Val residues (orange area) are non-overlapping in the ^{1}H chemical shift dimension. The 1D projection of the spectrum is shown on top in blue. (**C**) Strips from a 3D ^{13}C-edited-[^{1}H,^{1}H]-NOESY spectrum taken at the positions of Im7 alanine residues. The Ala and ILV chemical shift dispersions are shaded pink and orange, respectively. (**D**) Residues 23–36 and 50–65, which have an averaged PRE distance of less than 15 Å from the Spy spin label positions M53C, M85C, T99C and T123C are plotted green on the crystal structure of Im7. (**E**) Spatial volume that is < 20 Å to residues M53C, M85C, T99C and T123C (green), obtained by intersecting the spherical volumes in panel A.

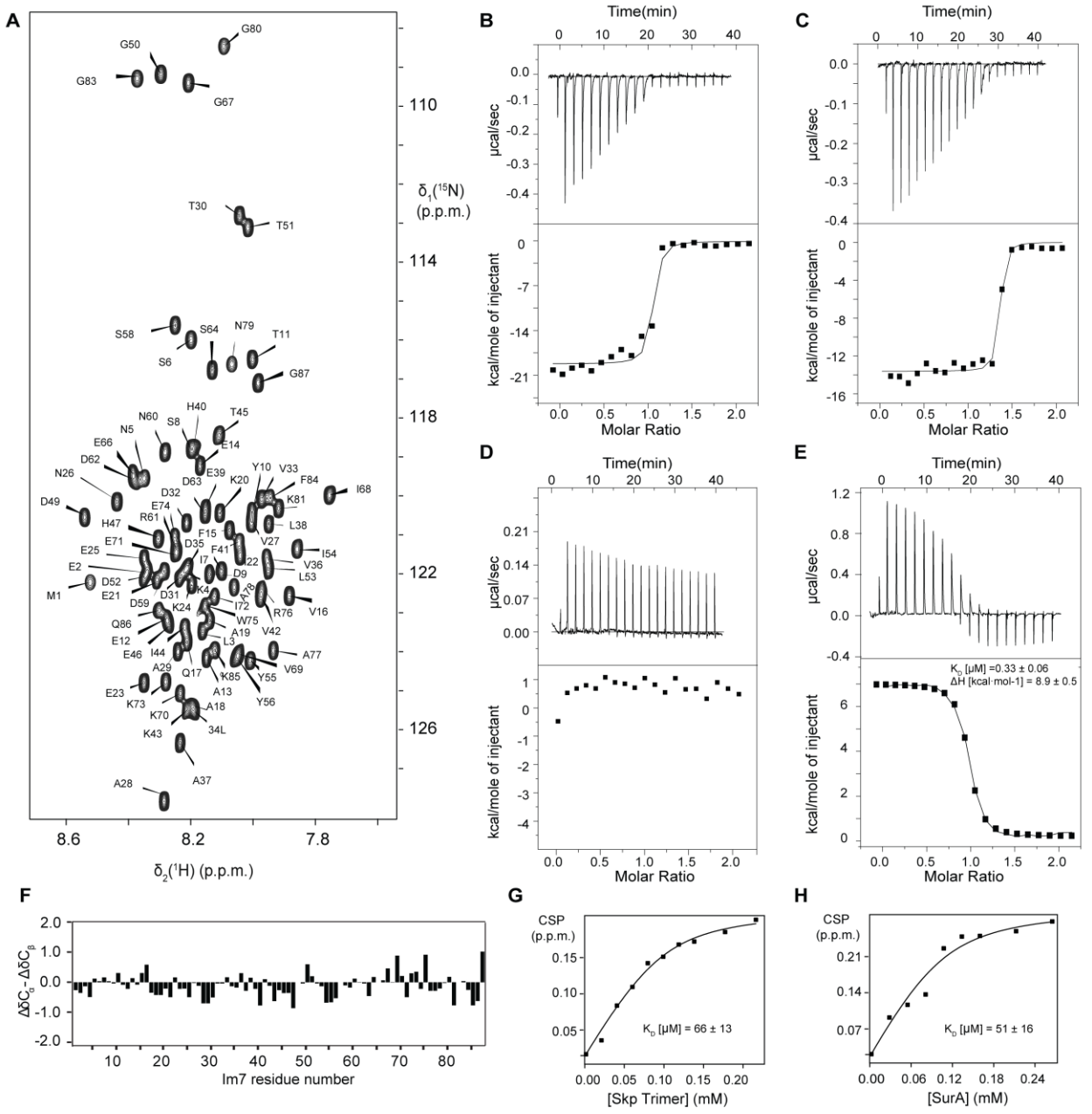

fig. S5. Backbone assignment of Im7$_U$ and ITC. (**A**) 2D [^{15}N, ^{1}H] HSQC spectrum of 1000 µM Im7$_U$ (=Im7(L18A,L19A,L37A)) in 20 mM NaH_2PO_4, pH 6.5, acquired on a 700 MHz spectrometer. Sequence-specific resonance assignment of the backbone amide groups is indicated. (**B**) Titration of 8 µM Im7 in the cell with 80 µM Colicin E7 in the syringe. (**C**) Titration of 8 µM Im7 and 8 µM Spy dimer in the cell with 80 µM Colicin E7 in the syringe. (**D**) Titration of 8 µM Spy dimer in the cell with 80 µM Colicin E7 in the syringe. (**E**) Titration of 80 µM Im7$_U$ in the cell with 800 µM Spy dimer in the syringe. The molar ratio is given relative to dimeric Spy. All titrations were performed in 20 mM NaH_2PO_4, pH 6.5, at 25 °C. (**F**) Secondary chemical shifts of Im7$_U$, plotted against the residue number. No regular secondary structure elements are present. (**G**, **H**) Magnitude of normalized chemical shift perturbation (CSP) for residue E21 of 100 µM Im7 plotted as a function of the concentration of trimeric Skp and SurA and fitted to extract the dissociation constant K_D.