

SUPPLEMENTARY INFORMATION

Probing the interplay between Ca²⁺- π and π - π interactions during Ca²⁺-dependent protein folding

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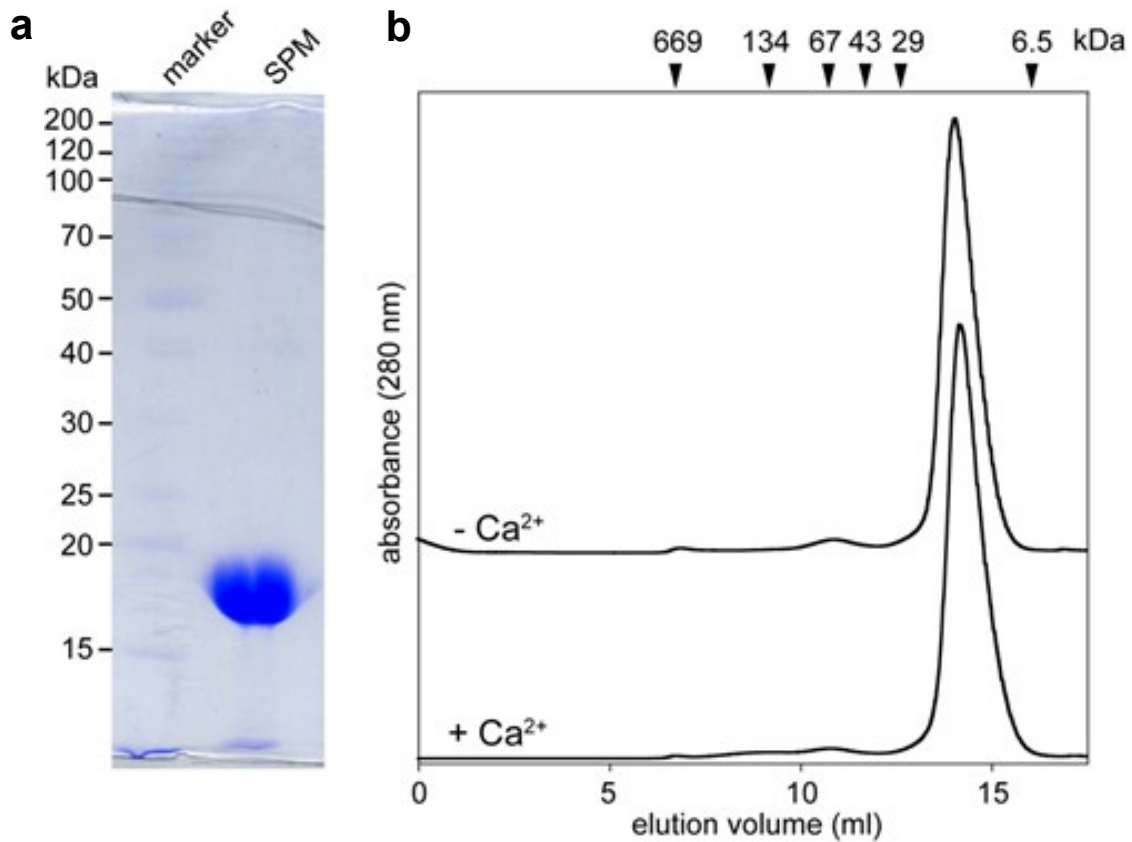


Figure S1.

Biochemical characterization of SPM. (a) SDS-PAGE analysis of SPM. (b) Gel filtration chromatography of SPM in the absence (-Ca²⁺) and the presence (+Ca²⁺) of calcium ions. The calibration of the Superdex 200 HR column is indicated by arrowheads; thyroglobulin (669 kDa), bovine serum albumin (134 and 67 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa) and aprotinin (6.5 kDa).

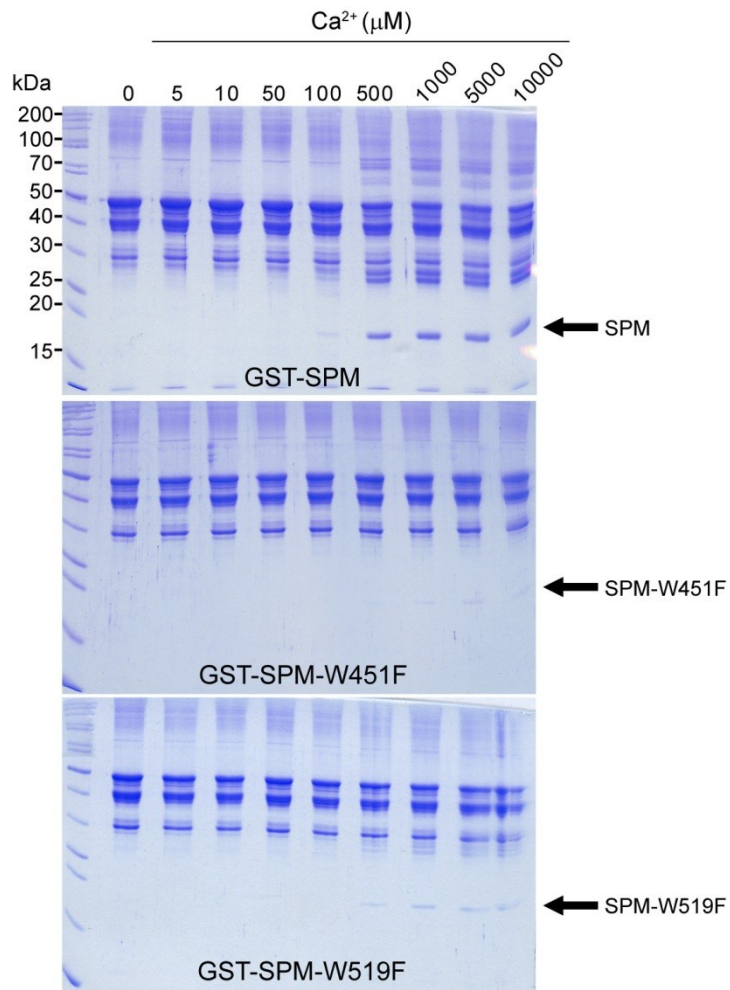


Figure S2.

Single substitutions of Trp415 and Trp519 to phenylalanine inhibit the Ca²⁺-dependent processing of SPM. The GST-SPM fusions (10 μM) were incubated at indicated concentrations of Ca²⁺ ions at 37 °C for 30 min, and the aliquots of the processed proteins were separated by 12% SDS-PAGE gels and stained with Coomassie blue.

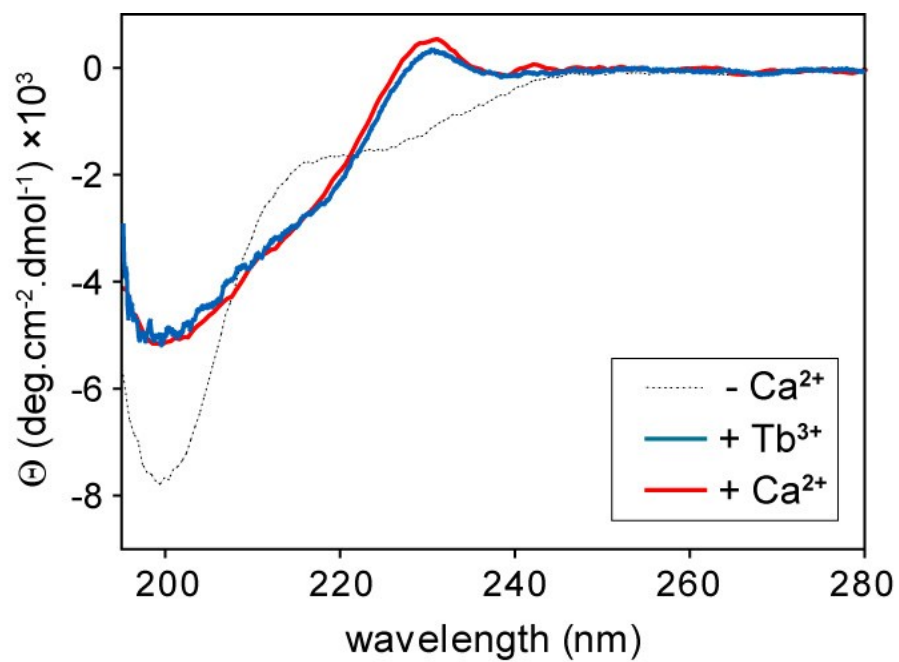


Figure S3.

Far-UV CD spectra of SPM in the absence (dotted line) and in the presence of 200 μ M CaCl₂ (red line) and Tb(NO₃)₃ (blue line), respectively.

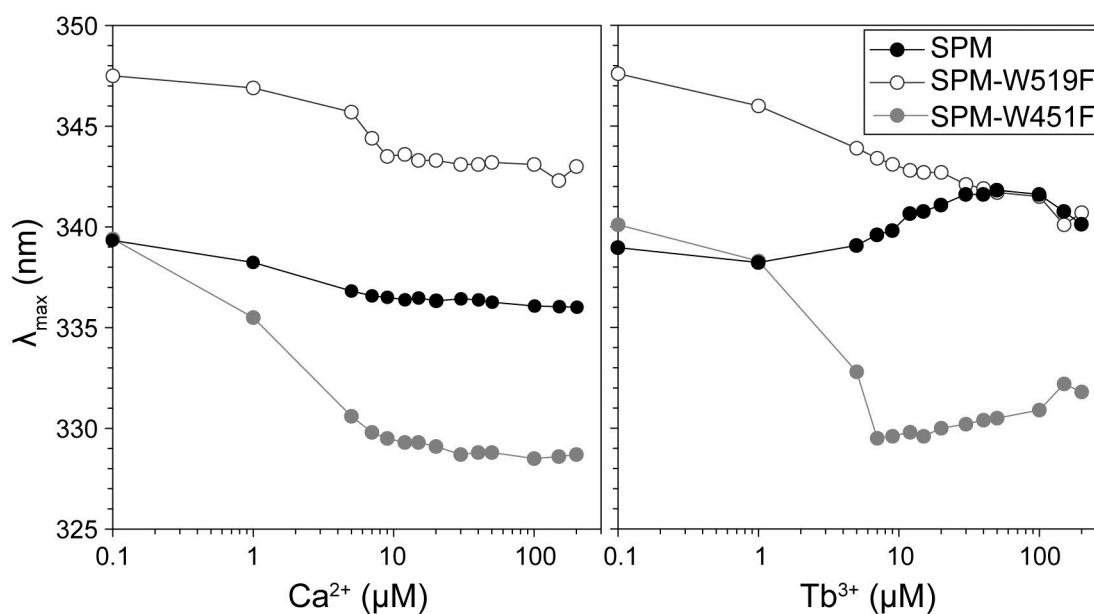


Figure S4.

Changes in the maximum emission wavelength (λ_{\max}) of the fluorescence emission spectra during calcium and terbium titration of SPM proteins. The λ_{\max} values were derived from the tryptophan fluorescence emission spectra (excitation at 295 nm) upon titration of SPM proteins (5 μM) with calcium (left panel) or terbium (right panel) ions and plotted as function of calcium and terbium concentrations, respectively.

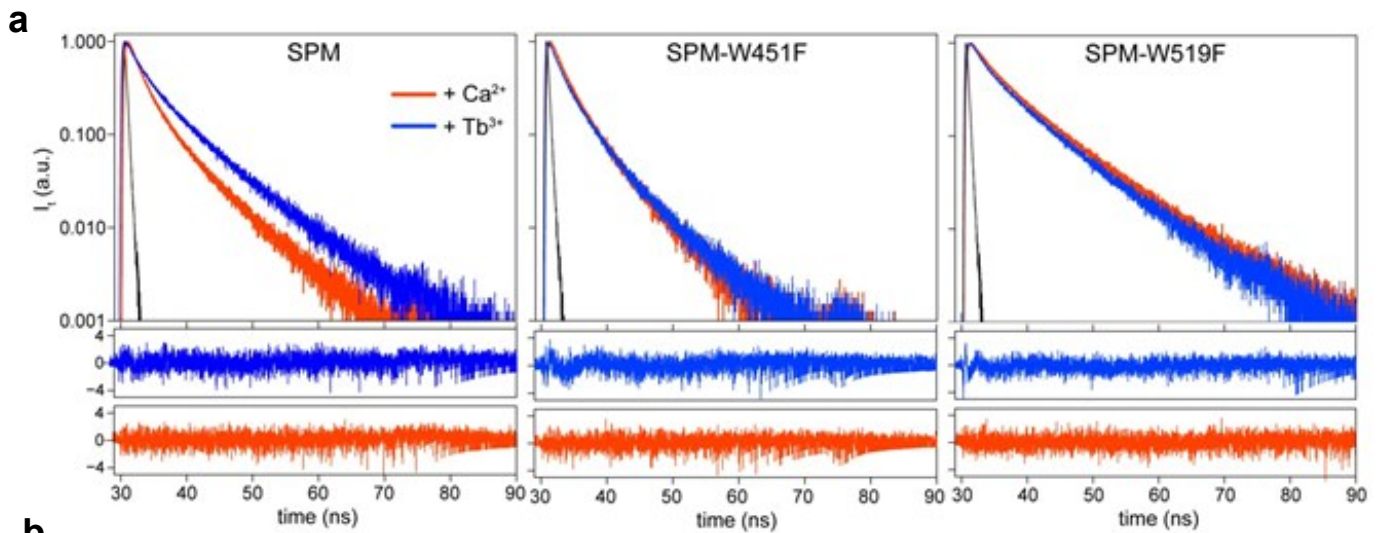


Figure S5.

Time-resolved fluorescence anisotropy of SPM proteins. **(a)** Time-resolved fluorescence anisotropy of SPM (left panel), SPM-W451F (middle panel) and SPM-W519F (right panel) in the presence of Ca²⁺ (orange curve) or Tb³⁺ (blue curve). The proteins (5 μ M) were measured by time correlated single photon counting after 600-ps pulse excitation at 295 nm (emission at 350 nm) at 10 °C. **(b)** Fluorescence lifetimes analysis of the SPM proteins in the presence of Ca²⁺ and Tb³⁺ ions after fitting the data with a triple exponential decay model. Residuals of the fits are indicated above in (a).

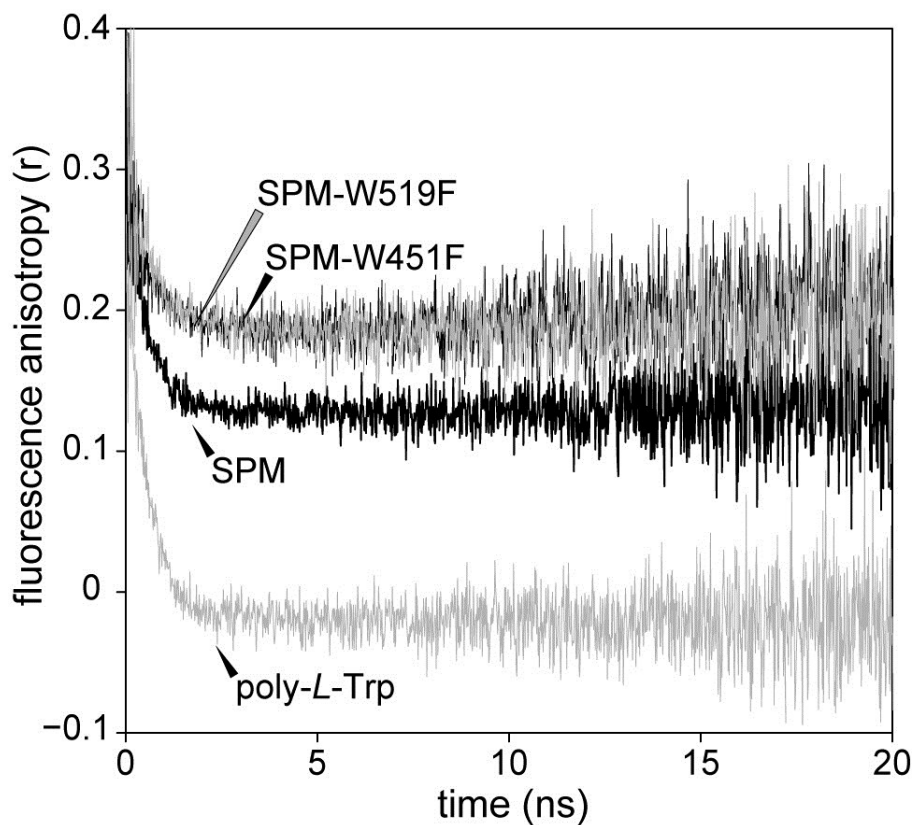


Figure S6.

Time-resolved fluorescence anisotropy decays of Ca^{2+} -loaded SPM proteins measured at -73 °C. SPM proteins ($5 \mu\text{M}$) supplemented with 73 % (v/v) glycerol were vitrified in Optistat DN cryostat to 200 K and depolarization of tryptophan fluorescence was recorded by time correlated single photon counting after 600-ps pulse excitation at 295 nm (emission at 350 nm)

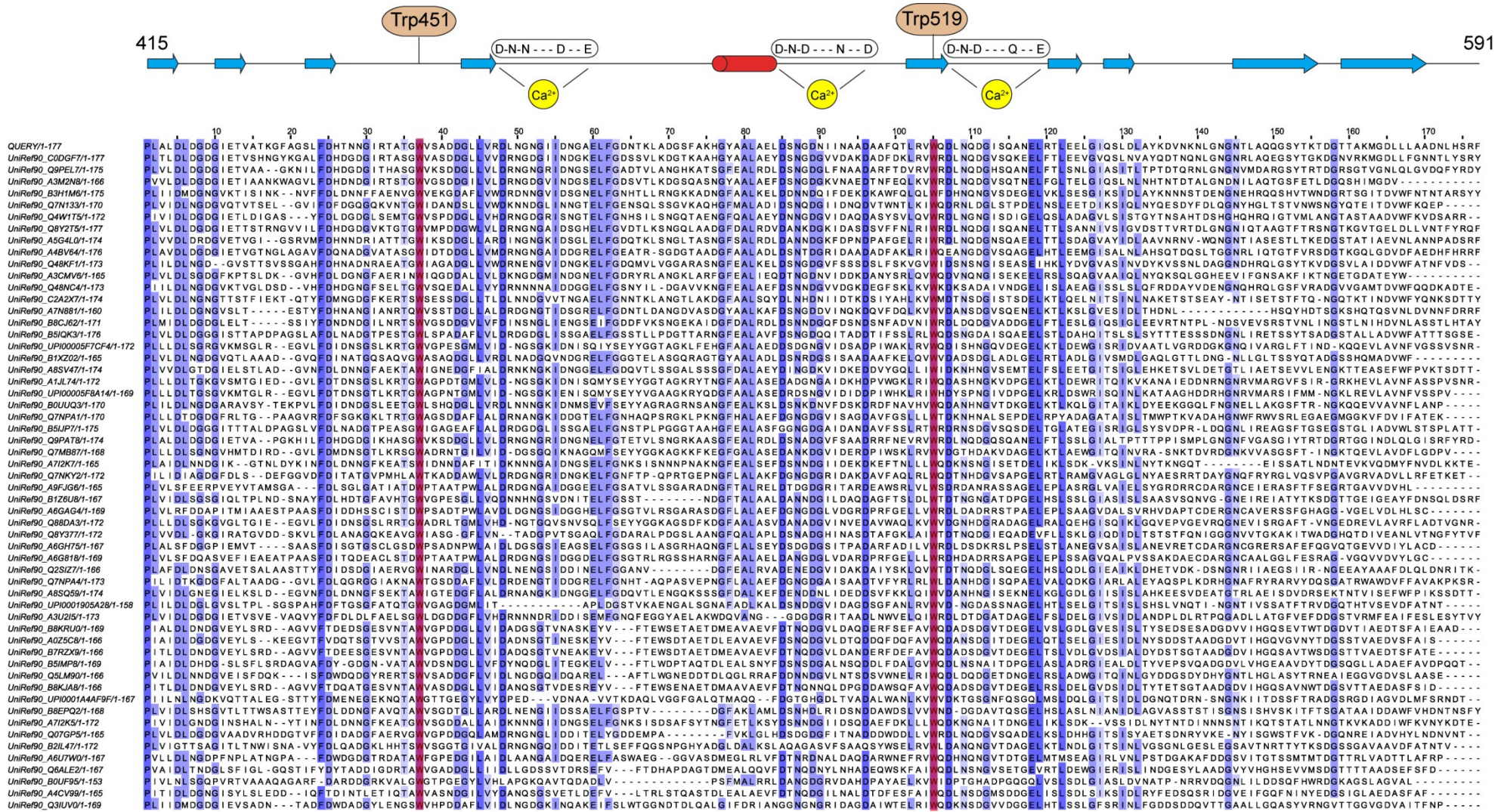


Figure S7.

Multiple sequence alignment of SPM from the homologous sequences as retrieved by Jpred3.⁴⁹ The degree of conservation is represented by color intensity where blue means highly conserved whereas white means no conservation. The conserved Trp residues are indicated by violet boxes. The prediction of secondary structure of SPM is depicted above.