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Supplemental Information

**Structural and Functional Investigations of the N-Terminal Ubiquitin
Binding Region of Usp25**

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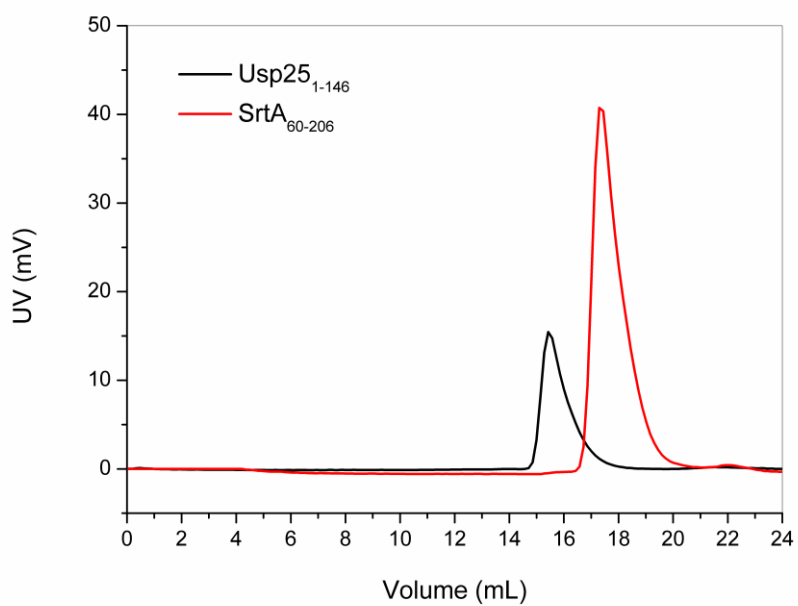


Figure S1. Size-exclusion chromatography profiles of Usp25₁₋₁₄₆ and SrtA₆₀₋₂₀₆. These two proteins have comparable size and both are diluted to the concentration of 0.1 mM. Due to the lack of aromatic amino acid residues, Usp25₁₋₁₄₆ has a lower UV absorbance at 280 nm than SrtA₆₀₋₂₀₆.

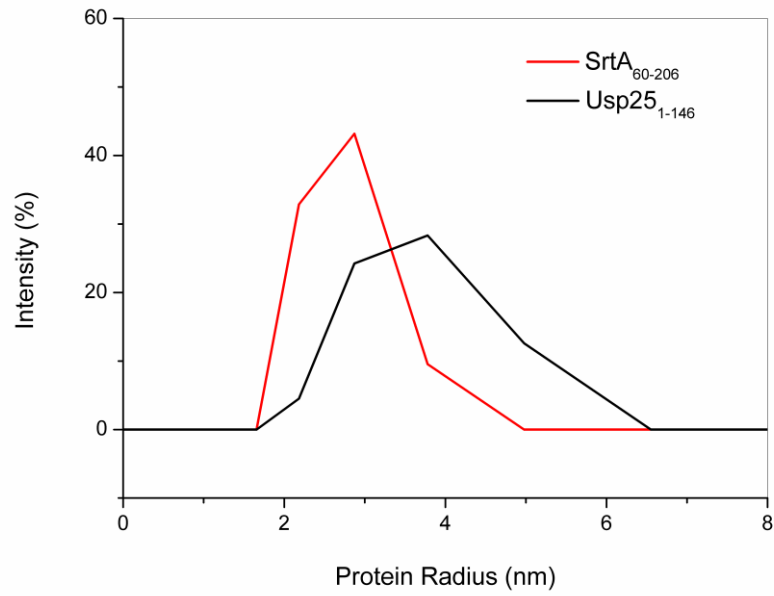


Figure S2. Size distributions of Usp25₁₋₁₄₆ and SrtA₆₀₋₂₀₆ measured by DLS. Both proteins are diluted to the concentration of 1 mg/mL. Usp25₁₋₁₄₆ has a hydrodynamic radius (R_h) of ~3.6 nm, while SrtA₆₀₋₂₀₆ has a R_h of ~2.7 nm.

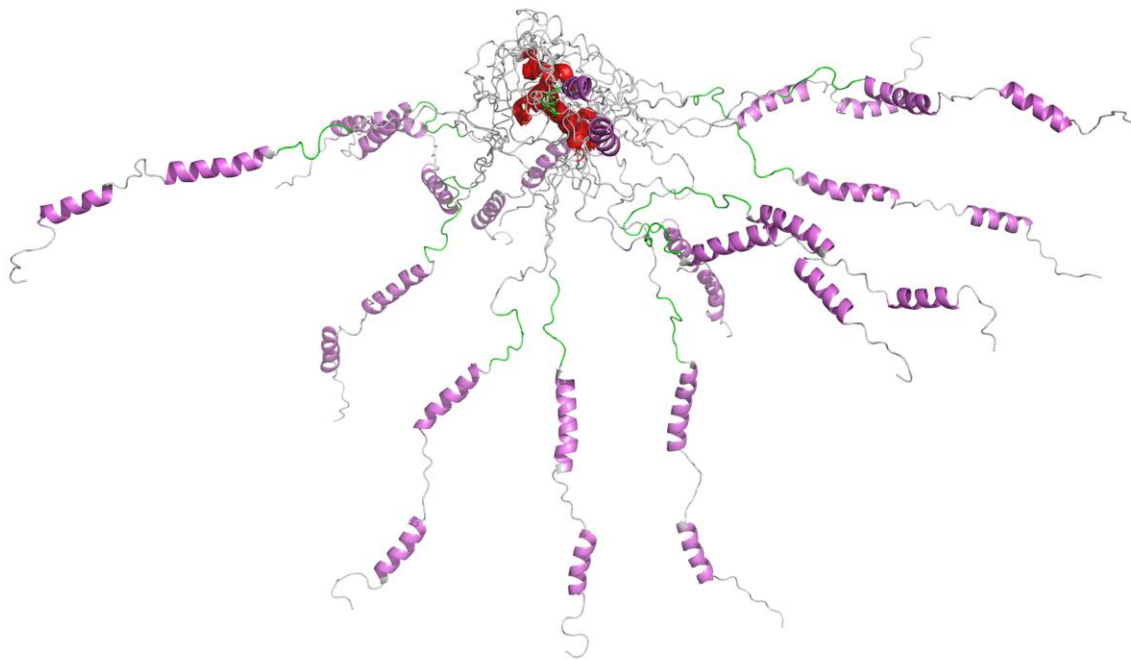


Figure S3. An ensemble of solution structures of Usp25₁₋₁₄₆. UBA, SIM and UIM1/2 are colored in red, green and purple, respectively. UBA domain is aligned as the spatial reference.

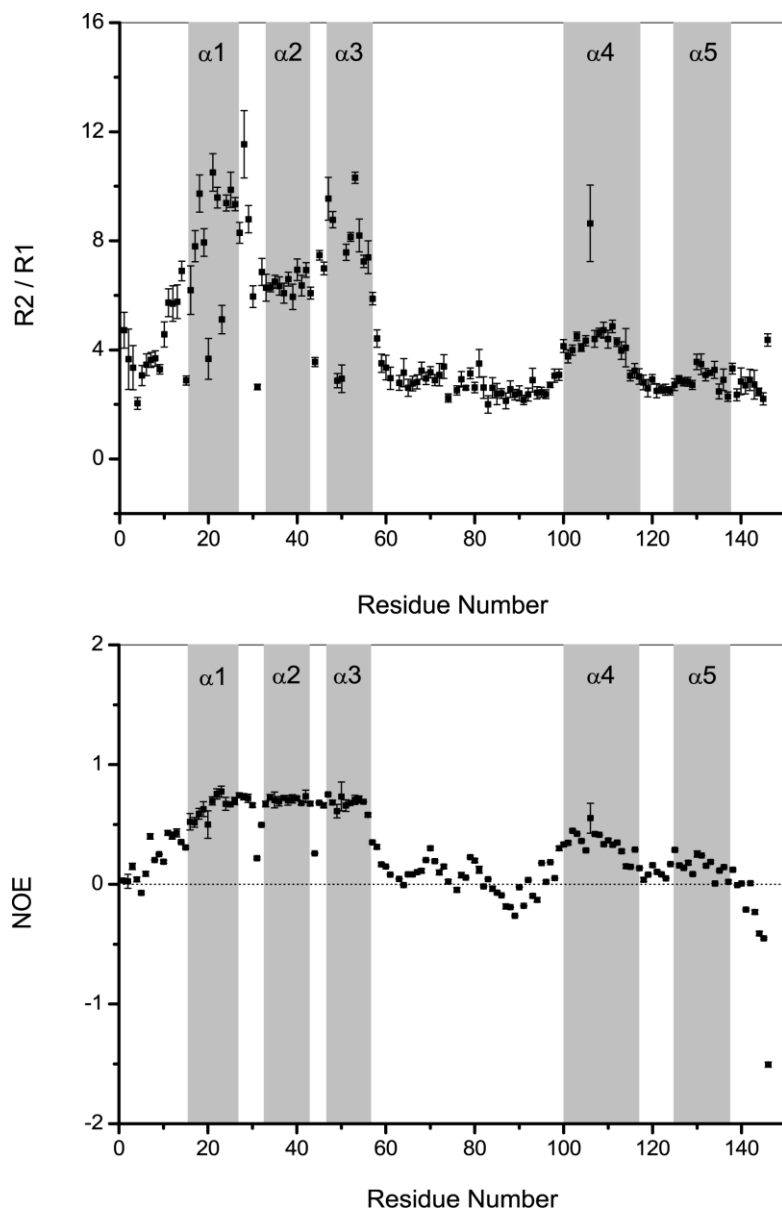


Figure S4. Backbone dynamics of Usp25₁₋₁₄₆. The secondary structure elements are highlighted in grey.

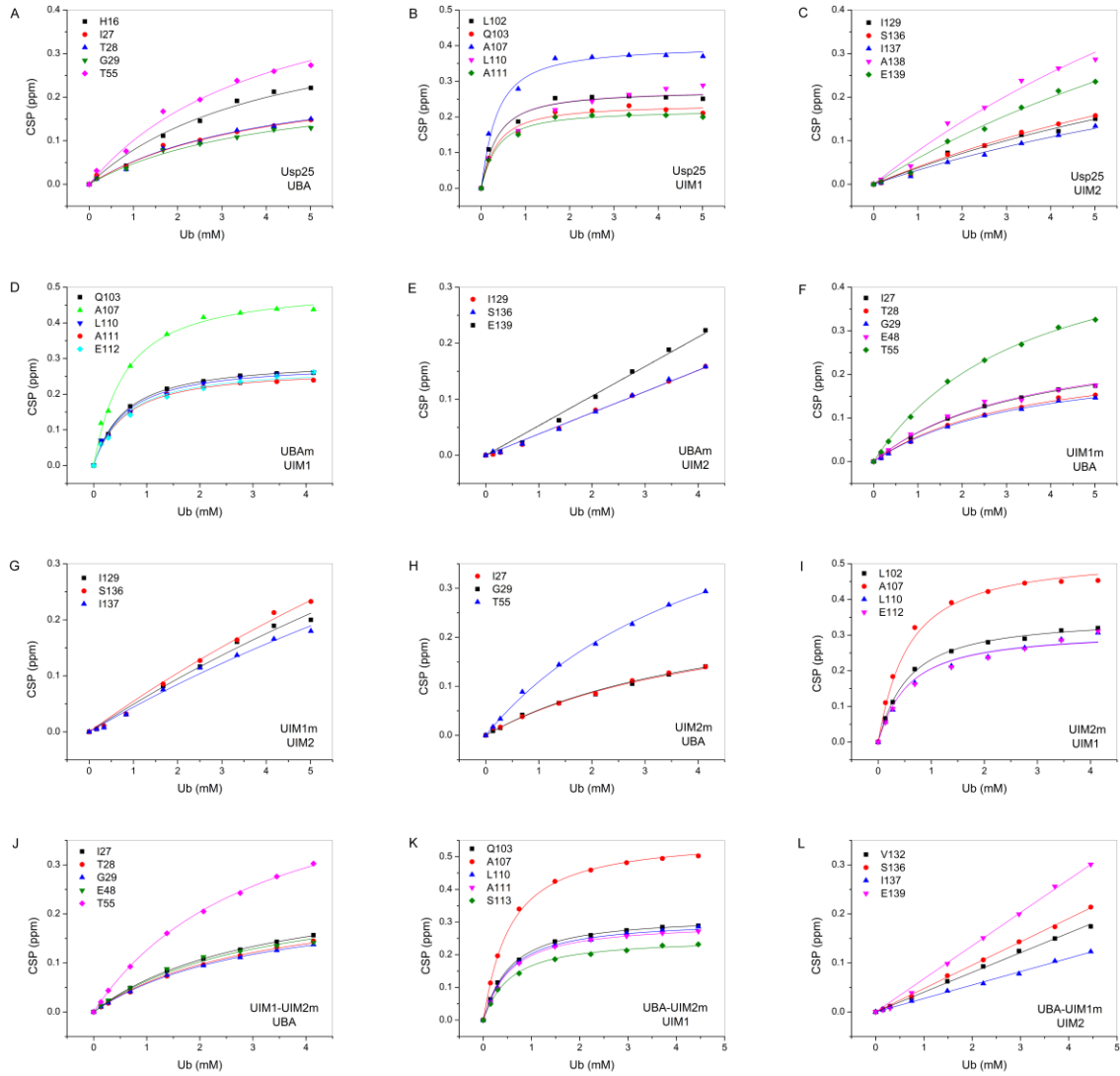


Figure S5. Global fit for the binding of monoubiquitin to each domain/motif of Usp25 UBR or its variants. Determined dissociation constants (K_d) are summarized in Table 2.

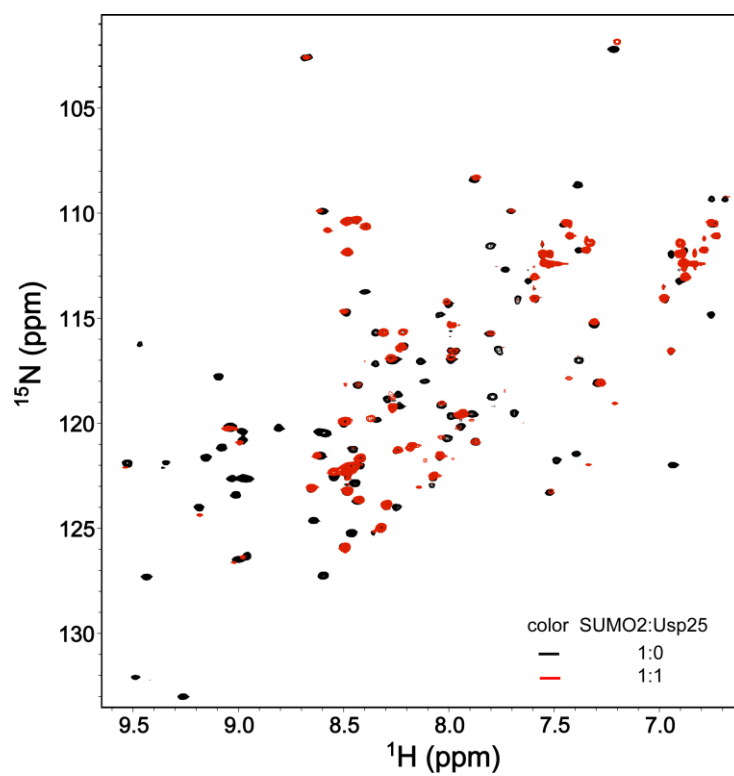


Figure S6. Superimposed ^1H - ^{15}N HSQC spectra of ^{15}N -labeled SUMO2 without (black) and with equal molar of Usp25 UBR (red).