

Supplementary figure 1

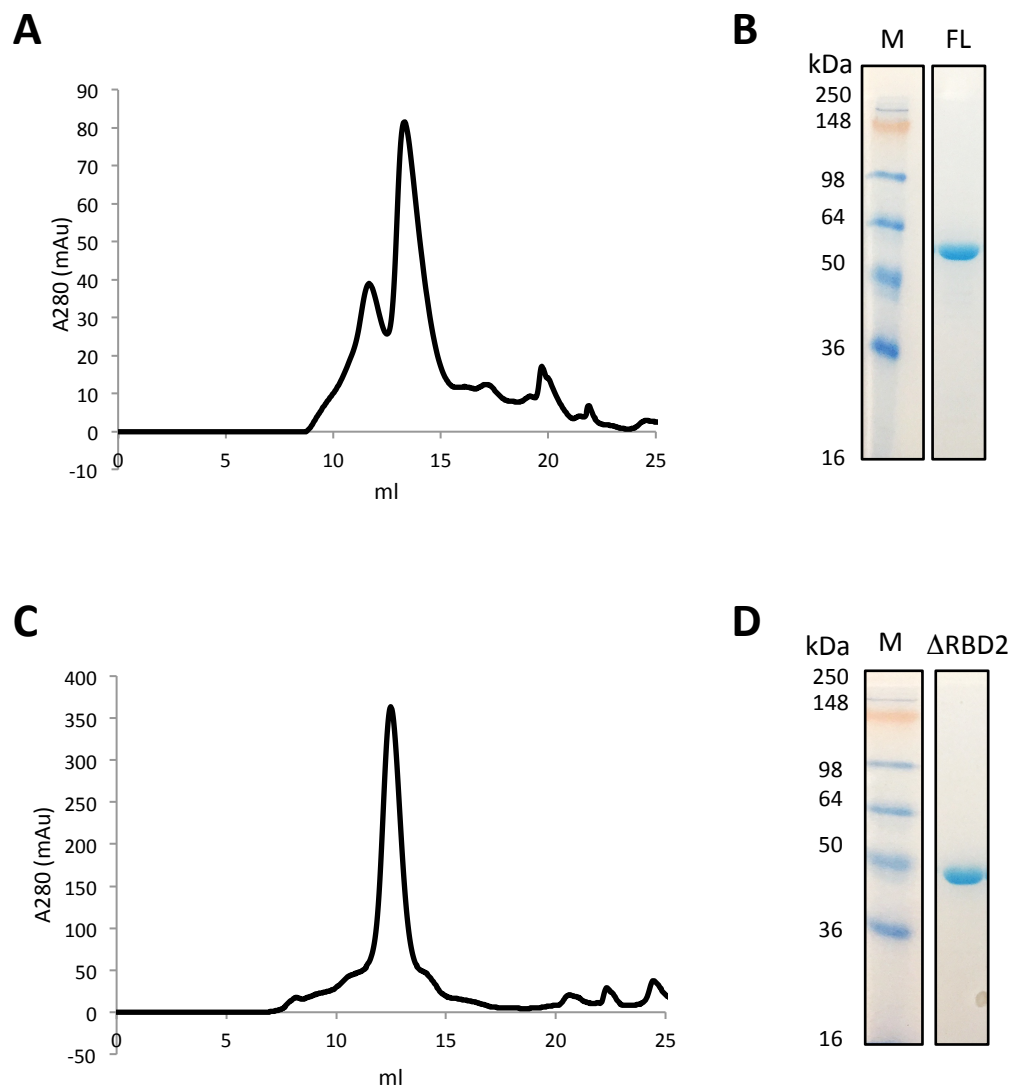


Figure S1: **Purification of hStau1⁵⁵_FL and hStau1⁵⁵_ΔRBD2.** A: Size exclusion chromatography of hStau1⁵⁵_FL in buffer A (25 mM HEPES pH 7.5, 100 mM KCl, 10 mM MgCl₂, 200 mM L-Arg HCl) using a Superdex200 column at 0.5 ml/min flow-rate. B: SimplyBlue staining of 12% SDS-PAGE analysis of purified hStau1⁵⁵_FL. C: Size exclusion chromatography of hStau1⁵⁵_FL in buffer A (25 mM HEPES pH 7.5, 100 mM KCl, 10 mM MgCl₂, 200 mM L-Arg HCl) using a Superdex200 column at 0.5 ml/min flow-rate. D: SimplyBlue staining of 12% SDS-PAGE analysis of purified hStau1⁵⁵_ΔRBD2.

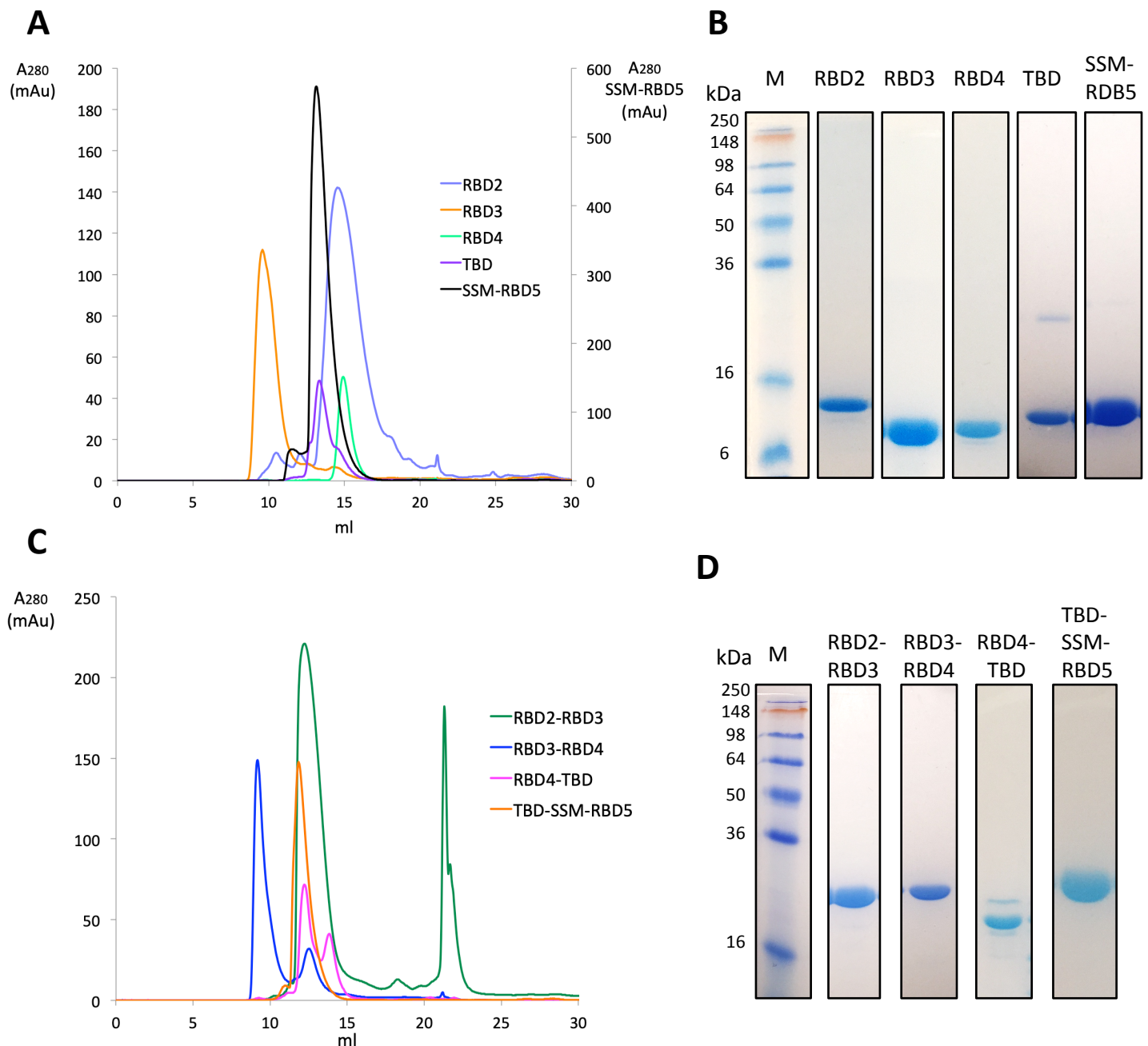


Figure S2: Purification of Staufen constructs. A: Size exclusion chromatography of Staufen individual domains in buffer A (25 mM HEPES pH 7.5, 100 mM KCl, 10 mM MgCl₂, 200 mM L-Arg HCl) using a Superdex75 column at 0.7 ml/min flow-rate. B: SimplyBlue staining of 18% SDS-PAGE analysis of purified Staufen individual domains. C: Size exclusion chromatography of Staufen tandem domains in buffer A (25 mM HEPES pH 7.5, 100 mM KCl, 10 mM MgCl₂, 200 mM L-Arg HCl) using a Superdex75 column at 0.7 ml/min flow-rate. B: SimplyBlue staining of 18% SDS-PAGE analysis of purified Staufen tandem domains.

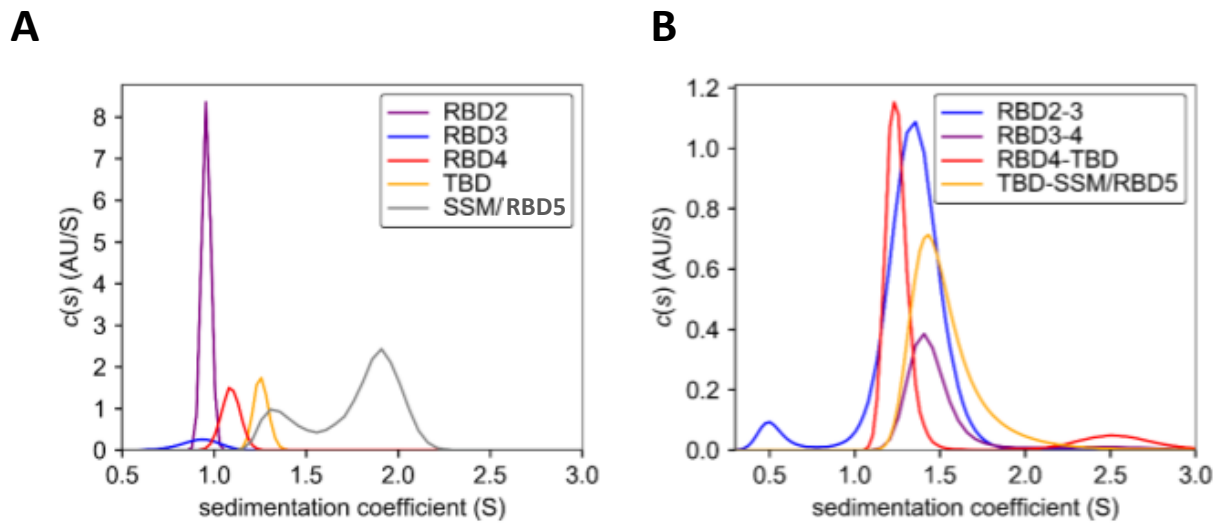


Figure S3: **Analytical ultracentrifugation (AUC) of Staufen constructs.** A: AUC of Staufen individual domains. B: AUC of Staufen tandem domains.

Supplementary figure 4

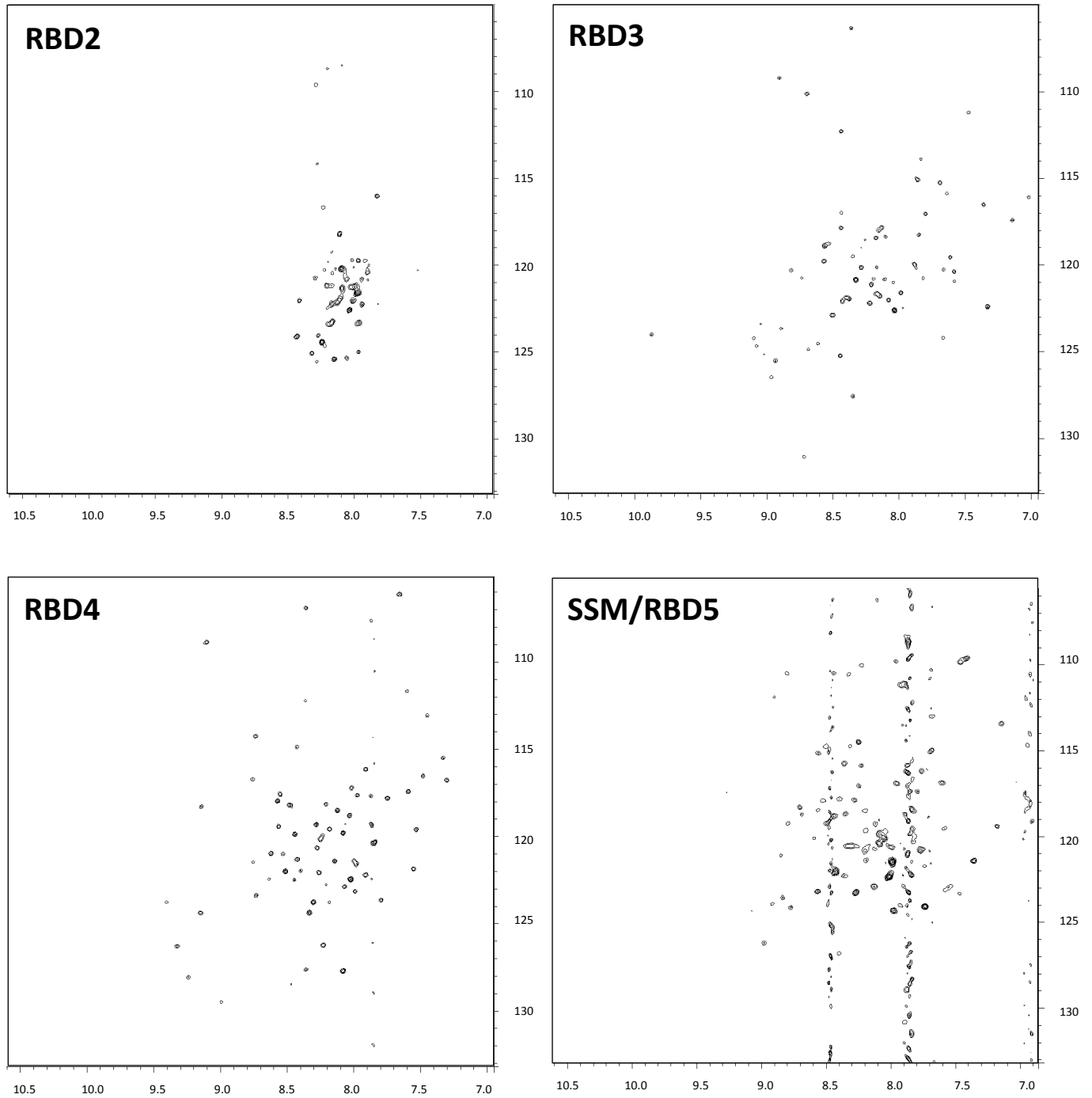


Figure S4: **NMR of Staufen individual domains.** ^{15}N , ^1H -TROSY-HSQC spectra were acquired at 298 K using a Bruker AVANCE IIIHD 600 MHz spectrometer equipped with a 5 mm TCI cryoprobe .

Supplementary figure 5

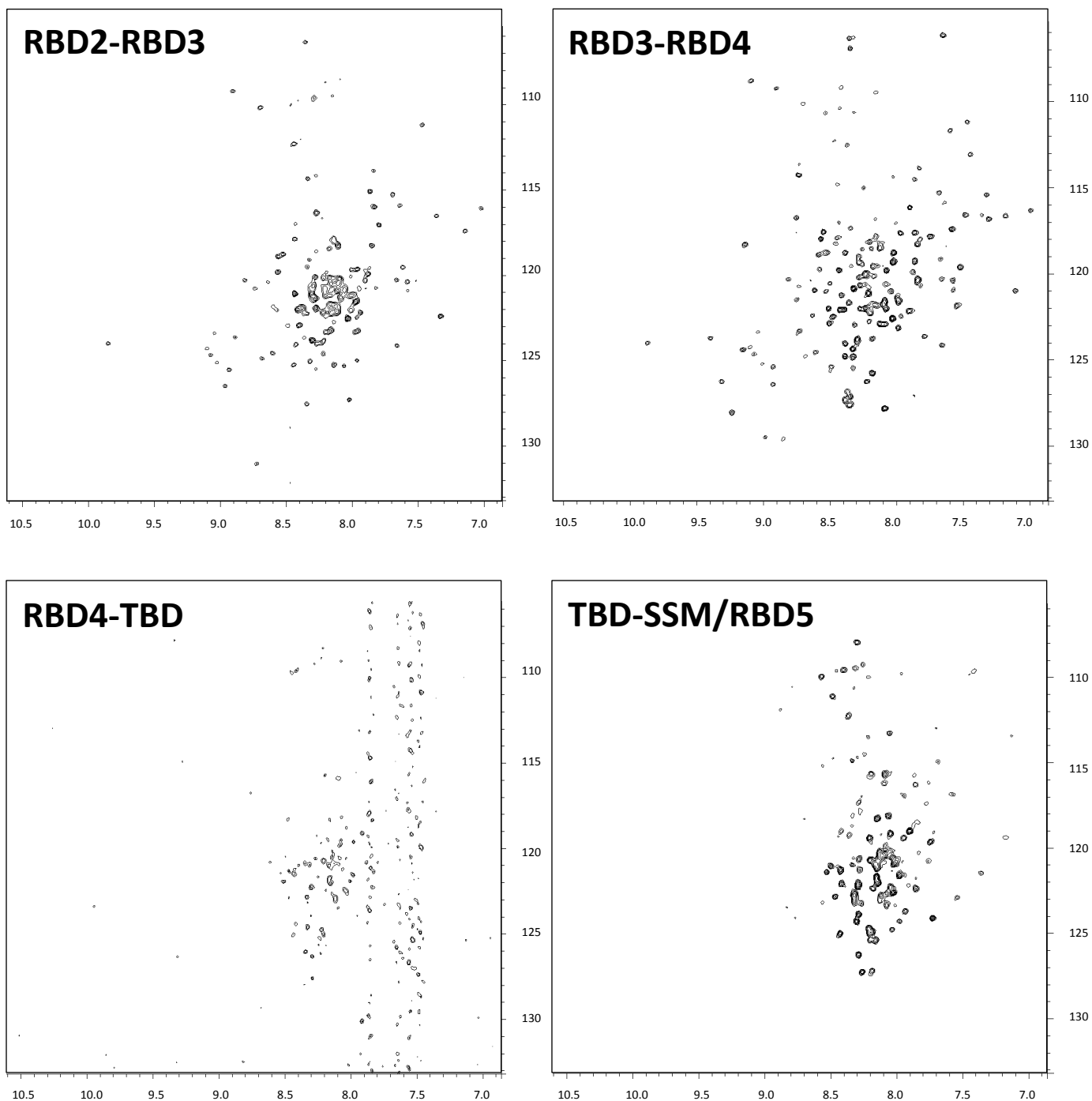


Figure S5: **NMR of Staufen tandem domains.** ^{15}N , ^1H -TROSY-HSQC spectra were acquired at 298 K using a Bruker AVANCE IIIHD 600 MHz spectrometer equipped with a 5 mm TCI cryoprobe .

Supplementary figure 6

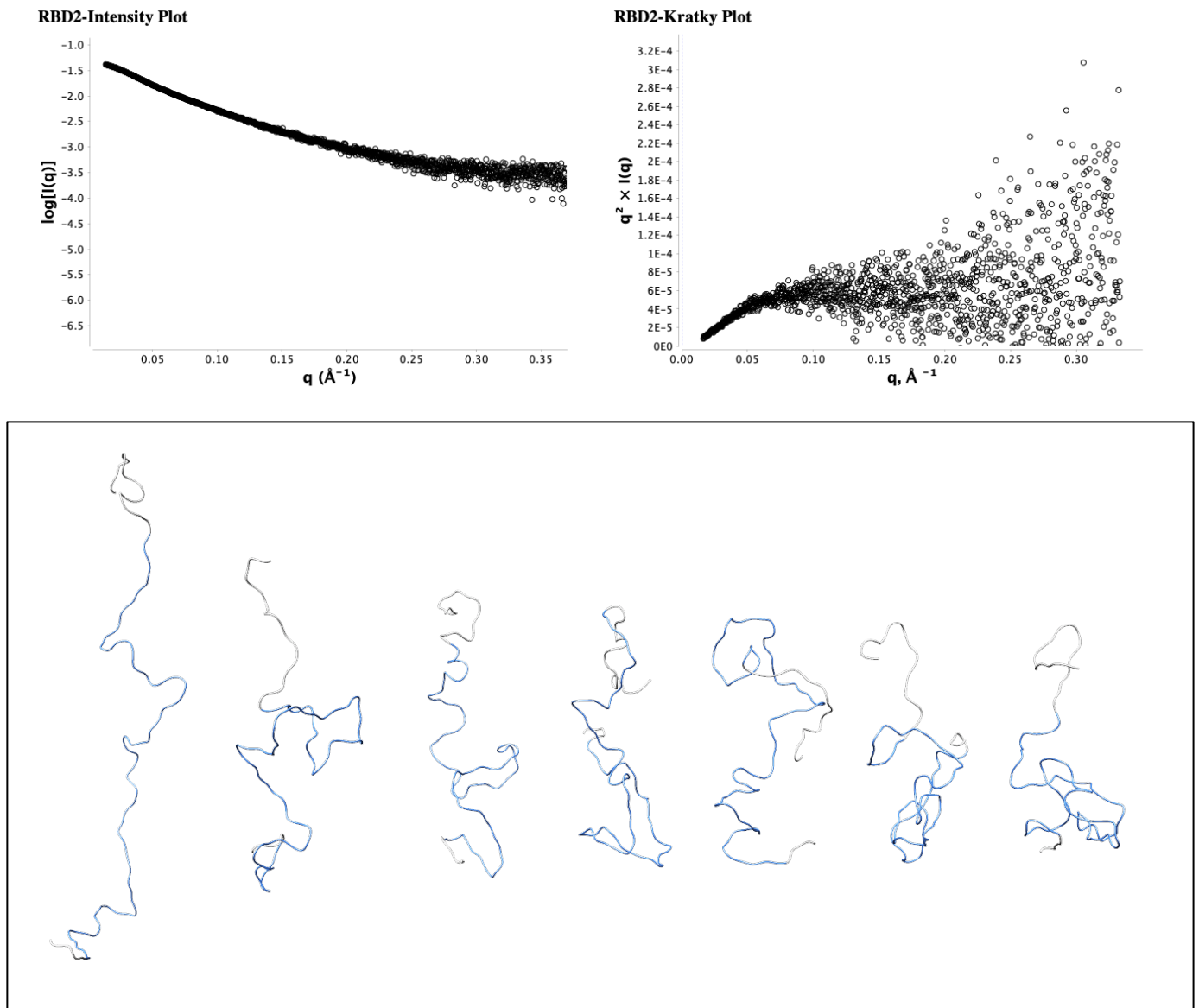


Figure S6: **Small angle X-ray scattering (SAXS) of RBD2.** A: SAXS curve, Kratky analysis and EOM models generated for RBD2. R_g *ensemble* = 37.3 \AA , D_{\max} *ensemble* = 112.64 \AA , $X^2=0.98$, $R_{\text{sigma}} = \sim 85.78\%$ ($\sim 86.70\%$).

Supplementary figure 7

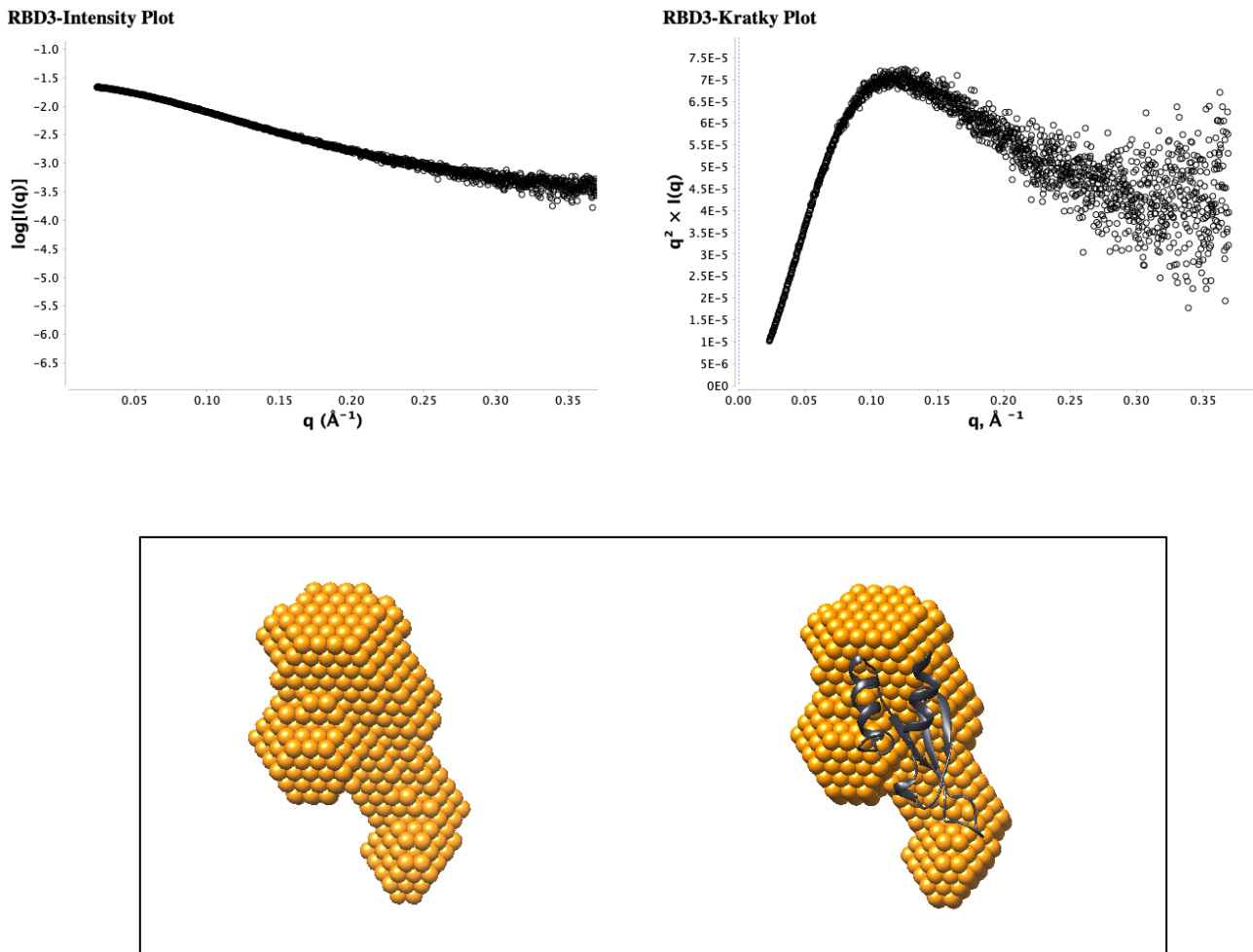


Figure S7. **Small angle X-ray scattering (SAXS) of RBD3.** A: SAXS curve, Kratky analysis and DAMMIN models generated for RBD3. $R_g = 18.74 \text{\AA}$, $D_{\max} = 58.08 \text{\AA}$, $X^2 = 0.835$.

Supplementary figure 8

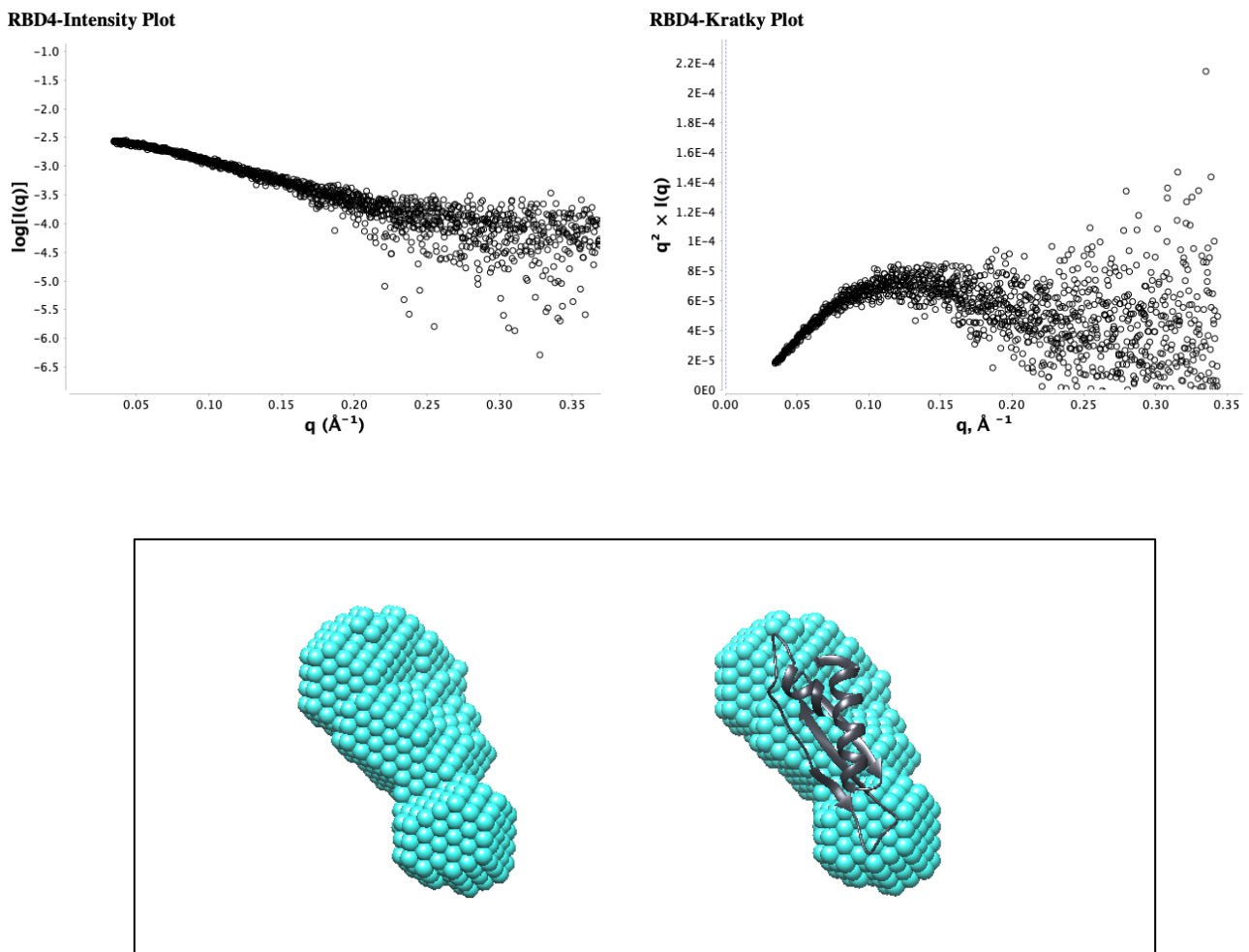


Figure S8. **Small angle X-ray scattering (SAXS) of RBD4.** A: SAXS curve, Kratky analysis and DAMMIN models generated for RBD4. $R_g = 17.15 \text{ \AA}$, $D_{\max} = 51.37 \text{ \AA}$, $X^2 = 0.5314$.

Supplementary figure 9

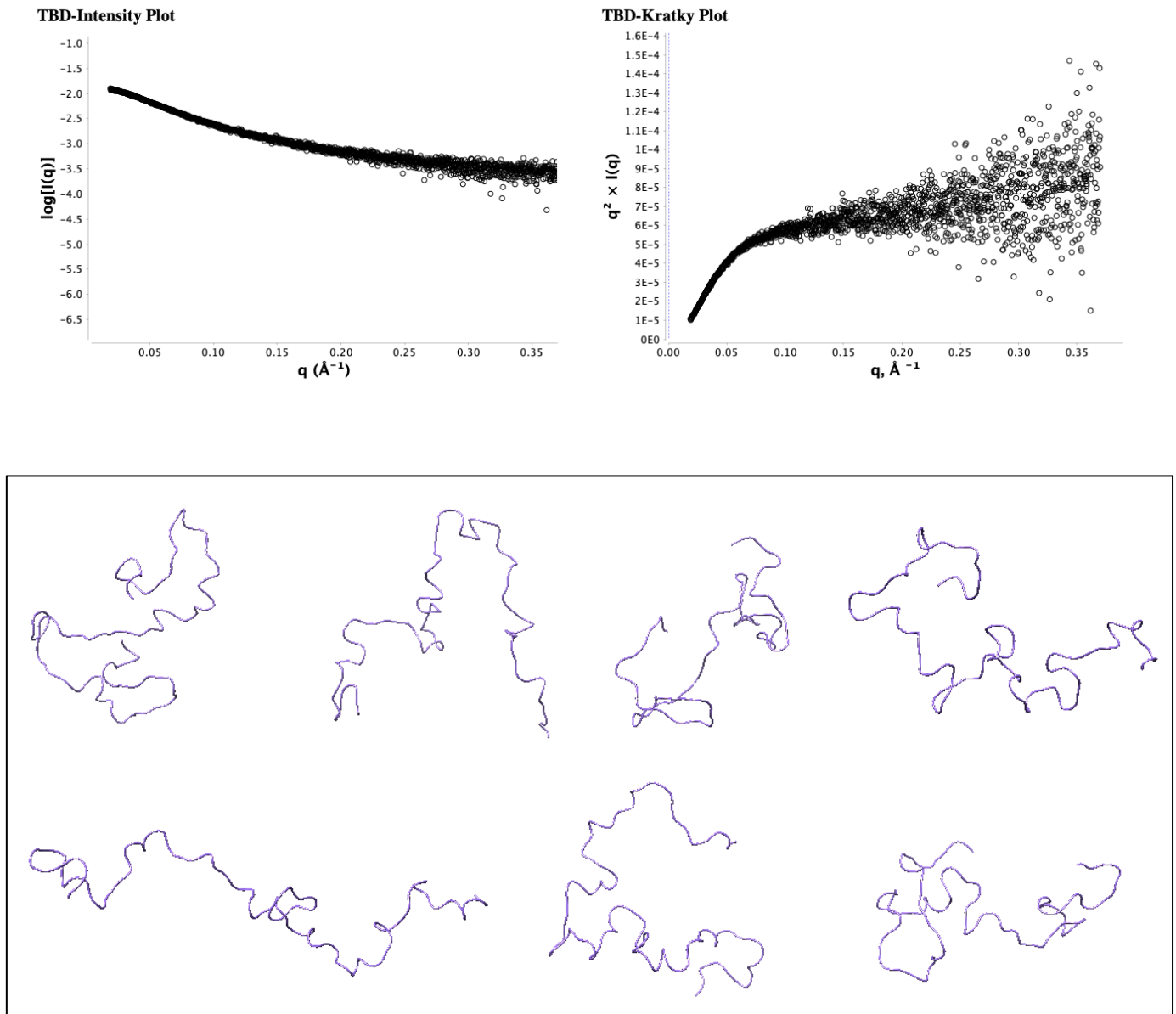


Figure S9. **Small angle X-ray scattering (SAXS) of TBD.** A: SAXS curve, Kratky analysis and EOM models generated for TBD. R_g ensemble = 31.74 \AA , D_{\max} ensemble = 97.24 \AA , $X^2 = 1.29$, $R_{\text{sigma}} = \sim 88.51\%$ ($\sim 86.24\%$).

Supplementary figure 10

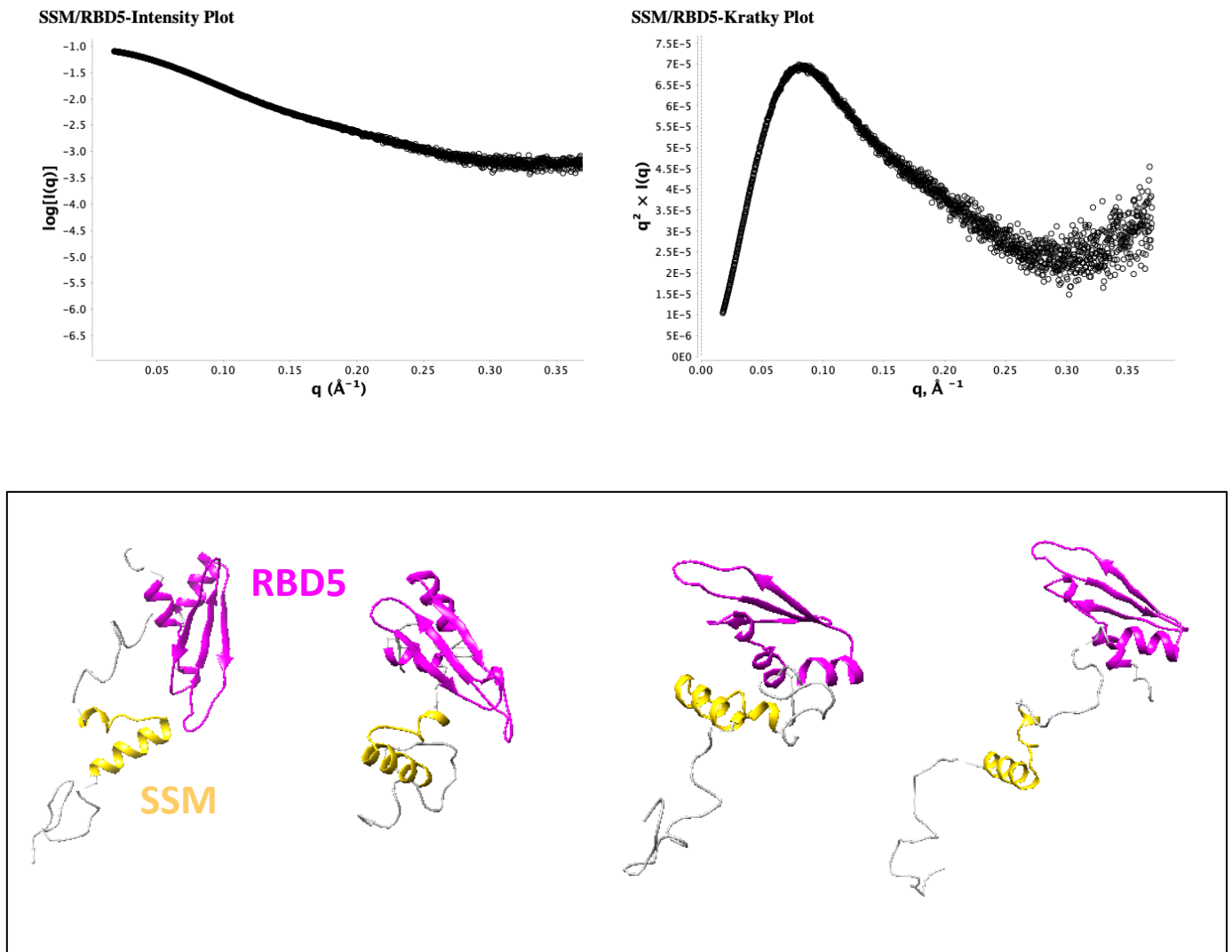


Figure S10. **Small angle X-ray scattering (SAXS) of SSM/RBD5.** A: SAXS curve, Kratky analysis and EOM models generated for SSM/RBD5. R_g *ensemble* = 25.55 \AA , D_{max} *ensemble* = 83.44 \AA , $X^2 = 1.405$, R_{sigma} = ~81.16% (~85.66%).

Supplementary figure 11

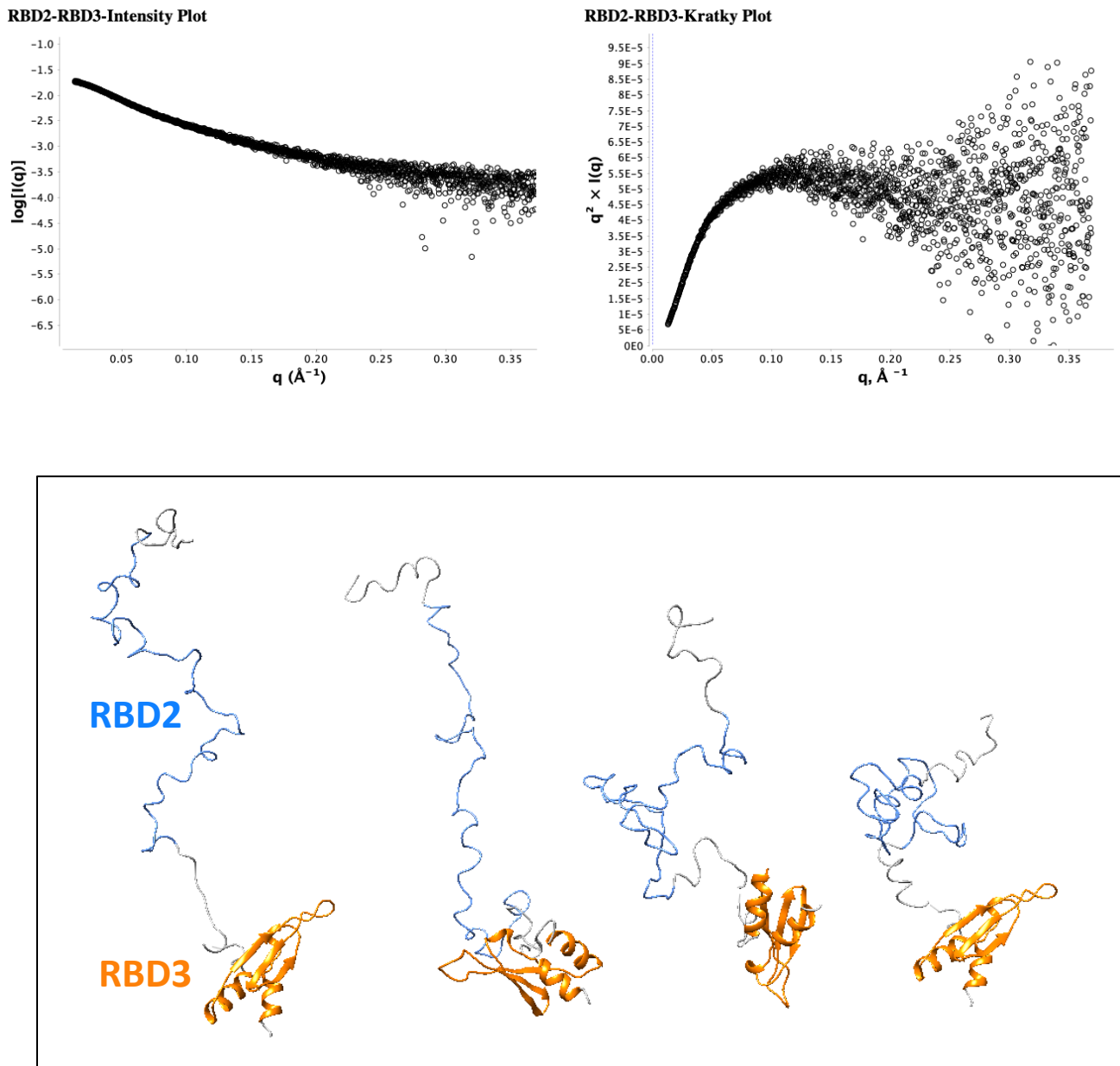


Figure S11. **Small angle X-ray scattering (SAXS) of RBD2-RBD3.** A: SAXS curve, Kratky analysis and EOM models generated for RBD2-RBD3. R_g ensemble = 37.86 \AA , D_{\max} ensemble = 120.74 \AA , $X^2 = 1.16$, $R_{\sigma} = \sim 85\%$ ($\sim 85.85\%$).

Supplementary figure 12

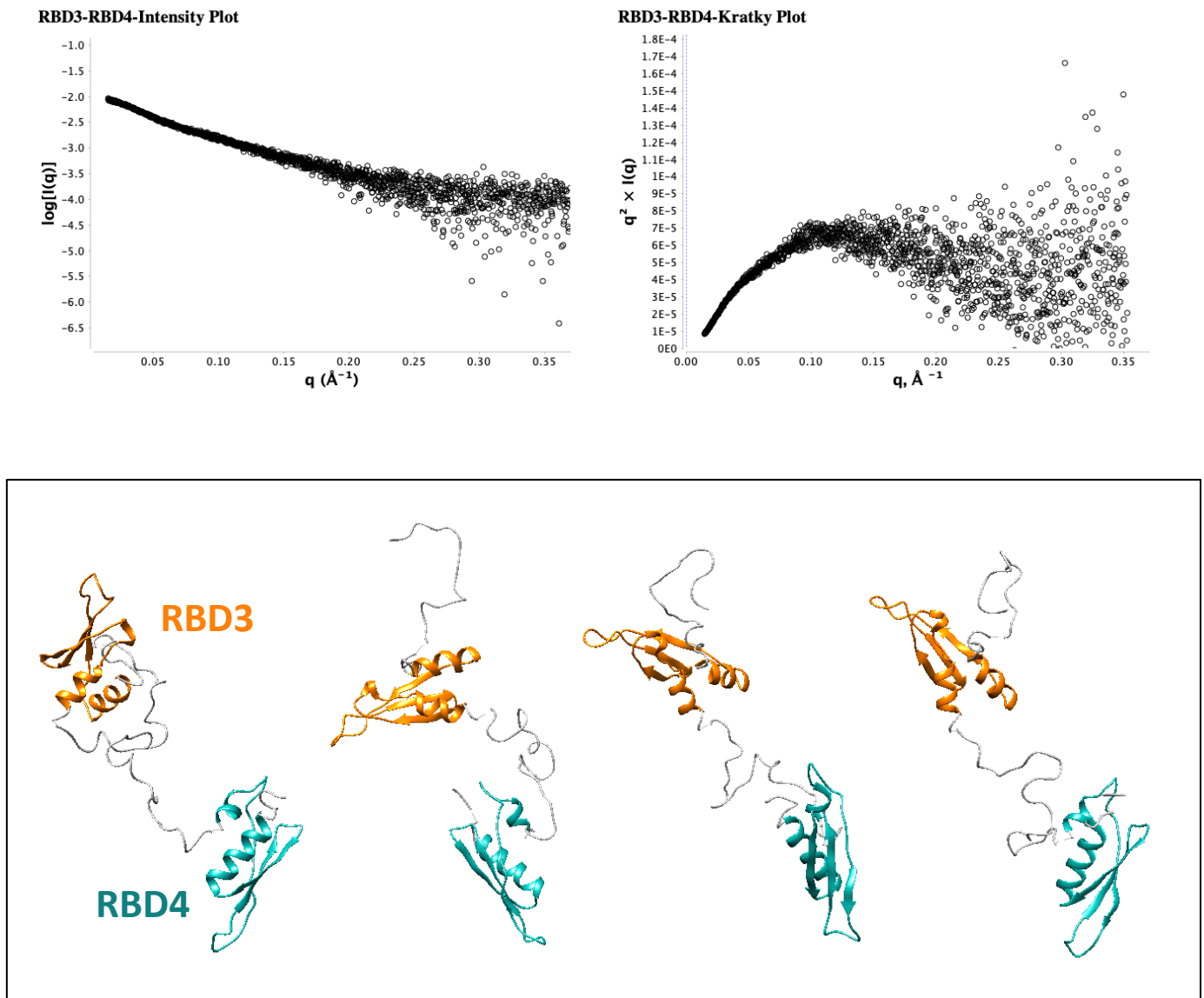
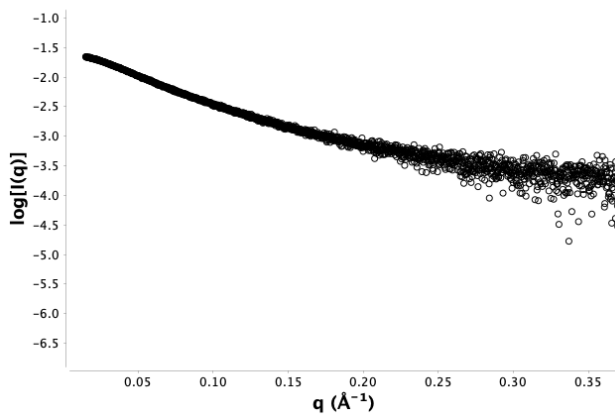


Figure S12. **Small angle X-ray scattering (SAXS) of RBD3-RBD4.** A: SAXS curve, Kratky analysis and EOM models generated for RBD3-RBD4. R_g ensemble = 36.15 \AA , D_{max} ensemble = 114.96 \AA , $X^2 = 1.342$, $R_{\text{sigma}} = \sim 80.28\%$ ($\sim 85.03\%$).

Supplementary figure 13

RBD4-TBD-Intensity Plot



RBD4-TBD-Kratky Plot

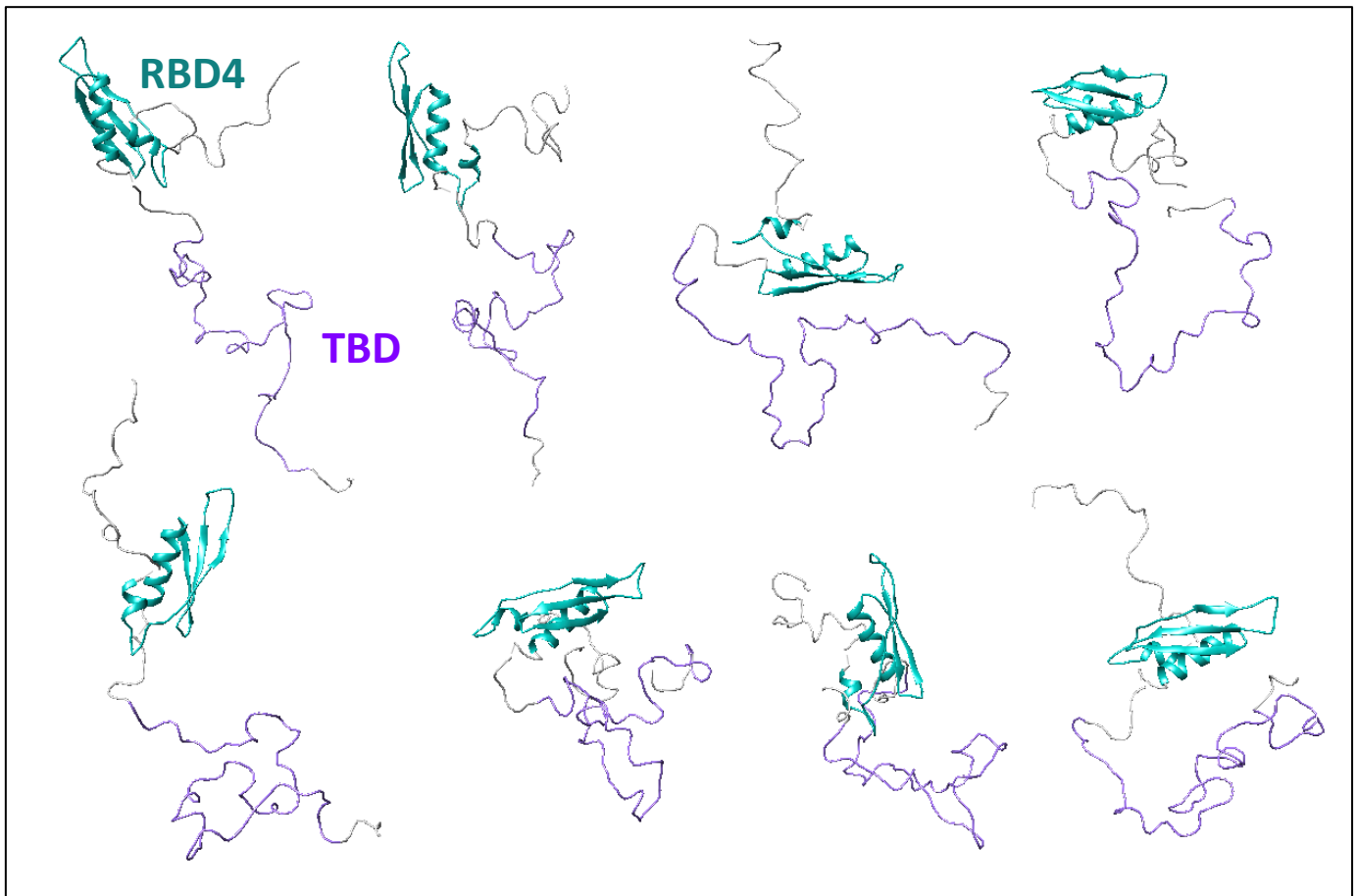
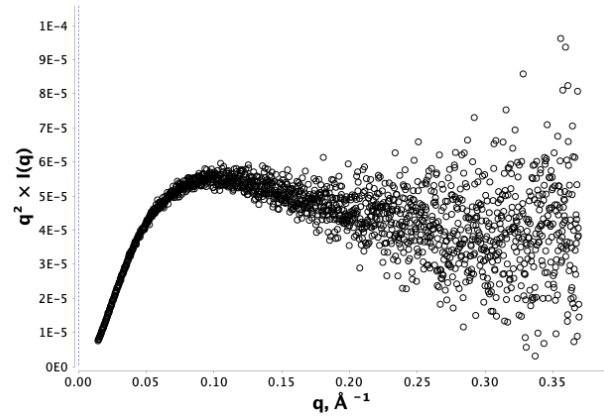


Figure S13. **Small angle X-ray scattering (SAXS)** of TBD-RBD4. A: SAXS curve, Kratky analysis and EOM models generated for TBD-RBD4. R_g *ensemble* = 36.46 \AA , D_{max} *ensemble* = 121.16 \AA , X^2 = 1.253, R_{sigma} = $\sim 88.79\%$ ($\sim 86.24\%$).

Supplementary figure 14

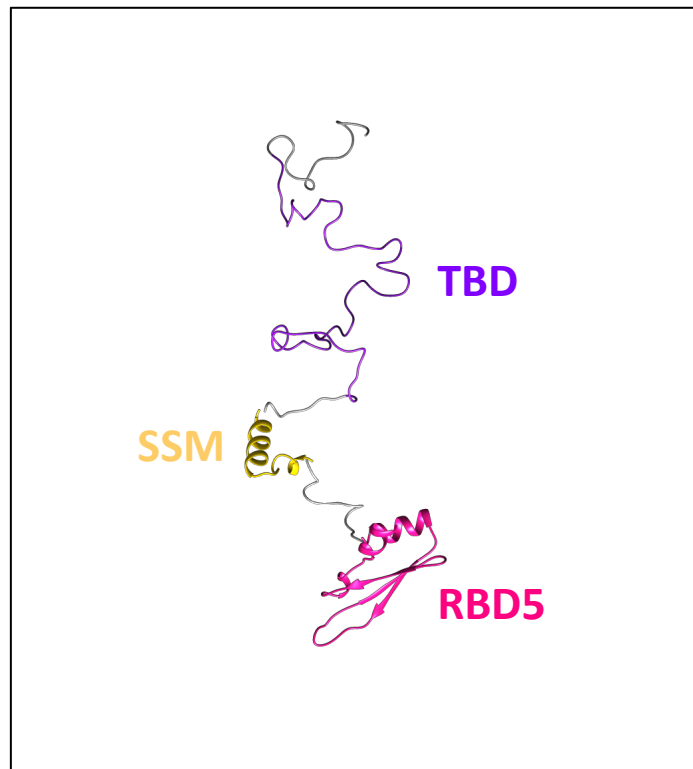
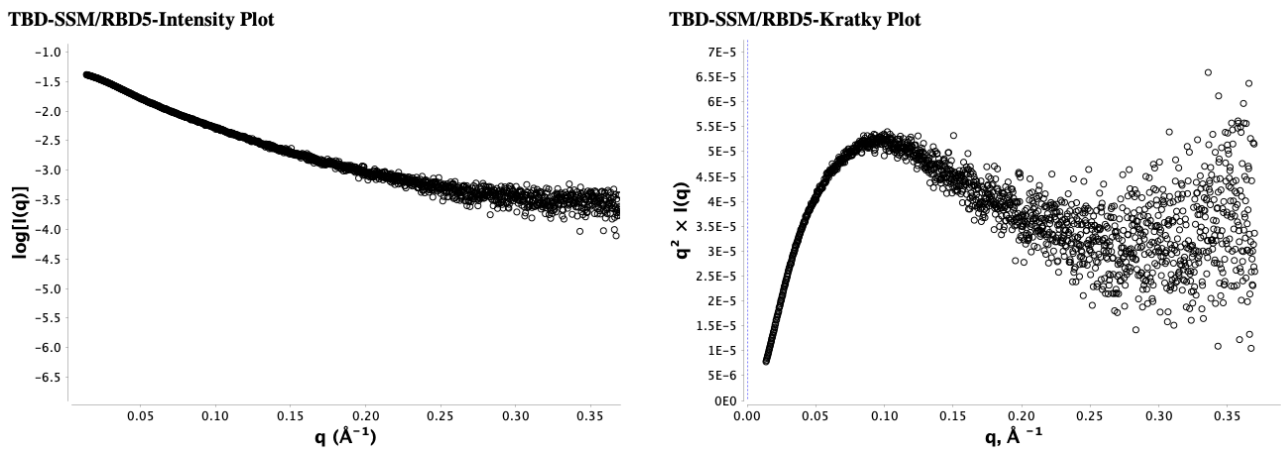


Figure S14. **Small angle X-ray scattering (SAXS) of TBD-SSM/RBD5.** A: SAXS curve, Kratky analysis and BUNCH models generated for TBD-SSM/RBD5. R_g ensemble = 41.24 Å, D_{max} ensemble = 129.65 Å, $X^2 = 1.28$, $R_{sigma} = \sim 89.35\%$ ($\sim 85.53\%$).

Supplementary table 1

Construct	Boundaries (aa)	Oligos
RBD2	1-85	fw: 5'-CCCCGAACATATGATGAAACTTGGAACCAATGTATAA-3' rev: 5'-TGCTTAAAGCTTTCATCATCATGGCAGGGGCTCATTCTGCA-3'
RBD3	95-172	fw: 5'-CCCCGAACATATGTCCGAAGAAGAAAATCTCAATAAAT-3' rev: 5'-TGCTTAAAGCTTTCATCATCACGGTAACTTCTTCAGCTCCT-3'
RBD4	198-275	fw: 5'-CCCCGAACATATGCCAGAATATGGCCAGGGGAT-3' rev: 5'-TGCTTAAAGCTTTCATCATCAGACTTTGAAACCAAGGATCTCCA-3'
TBD	283-366	fw: 5'-CCCCGAACATATGCCCGCACTCAAGTCAGAGGA-3' rev: 5'-TGCTTAAAGCTTTCATCATCAGGCAGGATTCGGAGCTGCC-3'
SSM/RBD5	363-476	fw: 5'-CCCCGAACATATGCCGAATCCTGCCAAGGCCAC-3' rev: 5'-TGCTTAAAGCTTTCATCATCAGTCCAACCTCAGACAGCAACTTTAAG-3'
RBD2-RBD3	1-172	fw: 5'-CCCCGAACATATGATGAAACTTGGAACCAATGTATAA-3' rev: 5'-TGCTTAAAGCTTTCATCATCACGGTAACTTCTTCAGCTCCT-3'
RBD3-RBD4	95-275	fw: 5'-CCCCGAACATATGTCCGAAGAAGAAAATCTCAATAAAT-3' rev: 5'-TGCTTAAAGCTTTCATCATCAGACTTTGAAACCAAGGATCTCCA-3'
RBD4-TBD	198-366	fw: 5'-CCCCGAACATATGCCAGAATATGGCCAGGGGAT-3' rev: 5'-TGCTTAAAGCTTTCATCATCAGGCAGGATTCGGAGCTGCC-3'
TBD-SSM/RBD5	283-476	fw: 5'-CCCCGAACATATGCCCGCACTCAAGTCAGAGGA-3' rev: 5'-TGCTTAAAGCTTTCATCATCAGTCCAACCTCAGACAGCAACTTTAAG-3'

Supplementary table 2

Construct	Concentration for NMR acquisition (μM)
hStau1 ⁵⁵ _FL	66
hStau1 ⁵⁵ _ΔRBD2	400
RBD2	33.3
RBD3	128.44
RBD4	99.1
SSM/RBD5	74.83
RBD2-RBD3	50.69
RBD3-RBD4	22.22
RBD4-TBD	9.57
TBD-SSM/RBD5	42.92

Table S2. **Protein concentration used for NMR experiments.**