Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.


# *Supplementary Information*

## Oligomerization Process of BAX Revealed from Intermediate Structures in Solution

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## S1. Determination of equivalent molecular mass ($M_w$) of the HPLC elution peaks

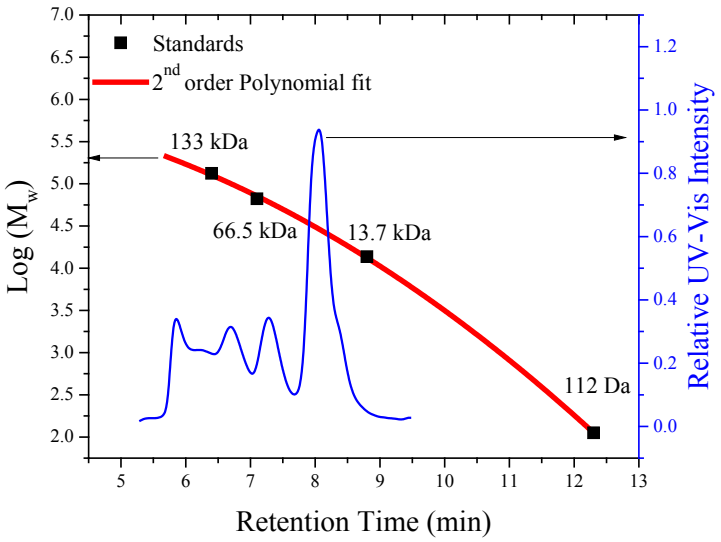


**Figure S1 -** A mass-calibration curve for the HPLC elution peaks (UV-Vis λ = 280 nm) was constructed using four standards of known $M_w$ as indicated. By plotting the molecular mass against retention time the polynomial fit equation (thick red curve) was used to calculate the equivalent molecular mass from the retention times of elution peaks observed from the solution of native BAX after 3 h incubation with BimBH3 peptides (blue curve, same curve as shown in main text Fig. 1b). The calculation results are summarized in Table S1. Standards in use, left to right: BSA dimer, 133 kDa; BSA monomer, 66.5 kDa; Ribonuclease A, 13.7 kDa; Uracil, 112 Da.

## S2.

**Table S1 - Assignments and the corresponding equivalent molecular masses of the elution peaks in main text Fig. 1b**

The difference in the buffer environments for BAX and that of the calibration standards resulted in a systematic error, which could be corrected by aligning the calibrated mass (28.5 kDa) to the BAX monomer mass (21.2 kDa). Note that we have neglected the mass contribution of BimBH3 (ca. 2 kDa) to the oligomers, which should not affect the assignment of BAX oligomers from the elution peaks.

| **Retention Time (min)** | **5.86** | **6.27** | **6.70** | **7.29** | **8.08** |
|---|---|---|---|---|---|
| Equivalent $M_W$ (kDa) | 212.8 | 161.2 | 100.2 | 60.5 | 28.5 |
| (Equivalent $M_W$) / 28.5<br>Normalized $M_W$ | 7.5 | 5.7 | 3.5 | 2.1 | 1.0 |
| Assigned as | octamer | hexamer | tetramer | dimer | monomer |
| Theoretical $M_W$ (kDa) | 169.6 | 127.2 | 84.8 | 42.4 | 21.2 |

## S3. Deconvolution of HPLC elution peaks

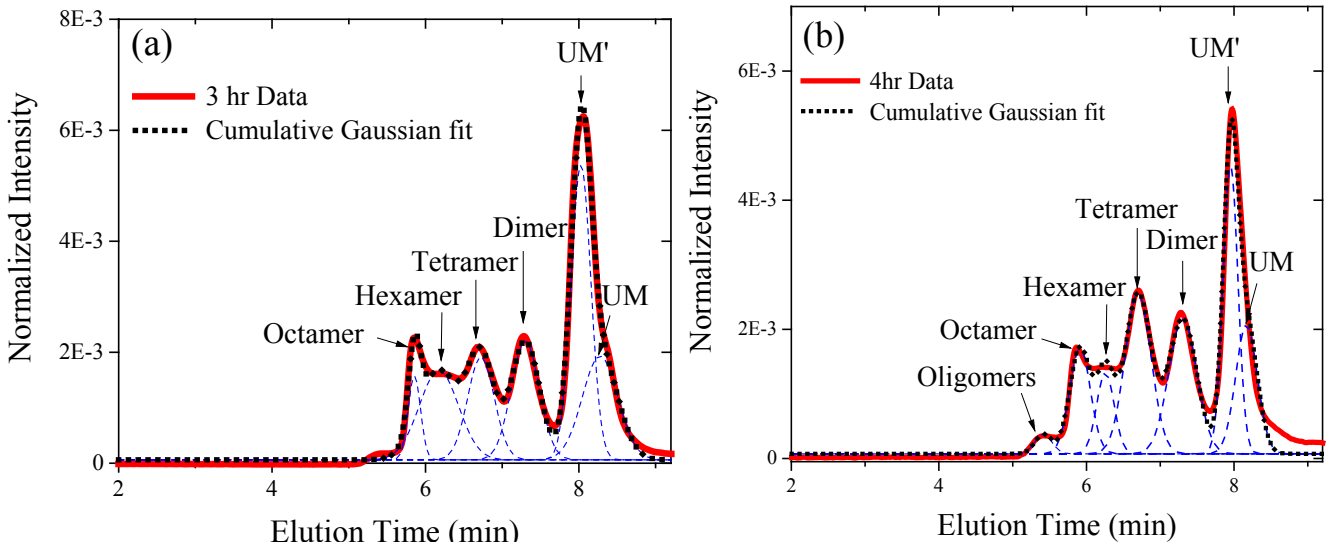


**Figure S2 -** HPLC elution profiles of the BAX solution after (a) 3 h and (b) 4 h incubation with BimBH3, and the results of the Gaussian decomposition using 6 or 7 Gaussian functions. Both figures show the original chromatogram (red curve) superimposed with the reconstruction profile (black dotted curve) from a linear superposition of the individual Gaussian functions (blue dotted curve) marked with corresponding species.

## S4. Structural Analysis of UM' and UM

The identities of UM' and UM were confirmed by the molar masses determined by the volume from a DAMMIN model and the power law of $Q_R$ ($V_c$ calculation). The real space (GNOM) analysis shows that compared to UM', UM has higher values of $R_g$ (by 7.5%) and maximal dimension ($D_{max}$, by 11%) (Table S2). The real space distance distribution functions of UM' and UM shown in Fig. S3a indicate a consistent core profile with minor local structure differences and a more elongated shape for UM. To obtain more information about the global structures of BAX monomers, the GASBOR *ab initio* shape reconstruction program was employed to generate 10 models of UM' and UM with good structural convergence that can fit the corresponding SAXS data (Fig. S3b), as reflected in the low normalized spatial discrepancy values (0.86±0.02 and 0.91±0.02 for UM' and UM, respectively). The two envelopes shown in Fig. S3c depict a relatively longer tail and a cleavage at the head domain for UM, which contribute to the dissimilarity between the $p(r)$ functions in Fig. S3a. By contrast, the head domain of UM' has a more rounded shape. Presumably, the cleavage at UM makes the BH3 domain more accessible for interactions with substrates (BimBH3 in our case) to fill in the cleavage, thereby transforming UM into UM' ready for dimer formation.

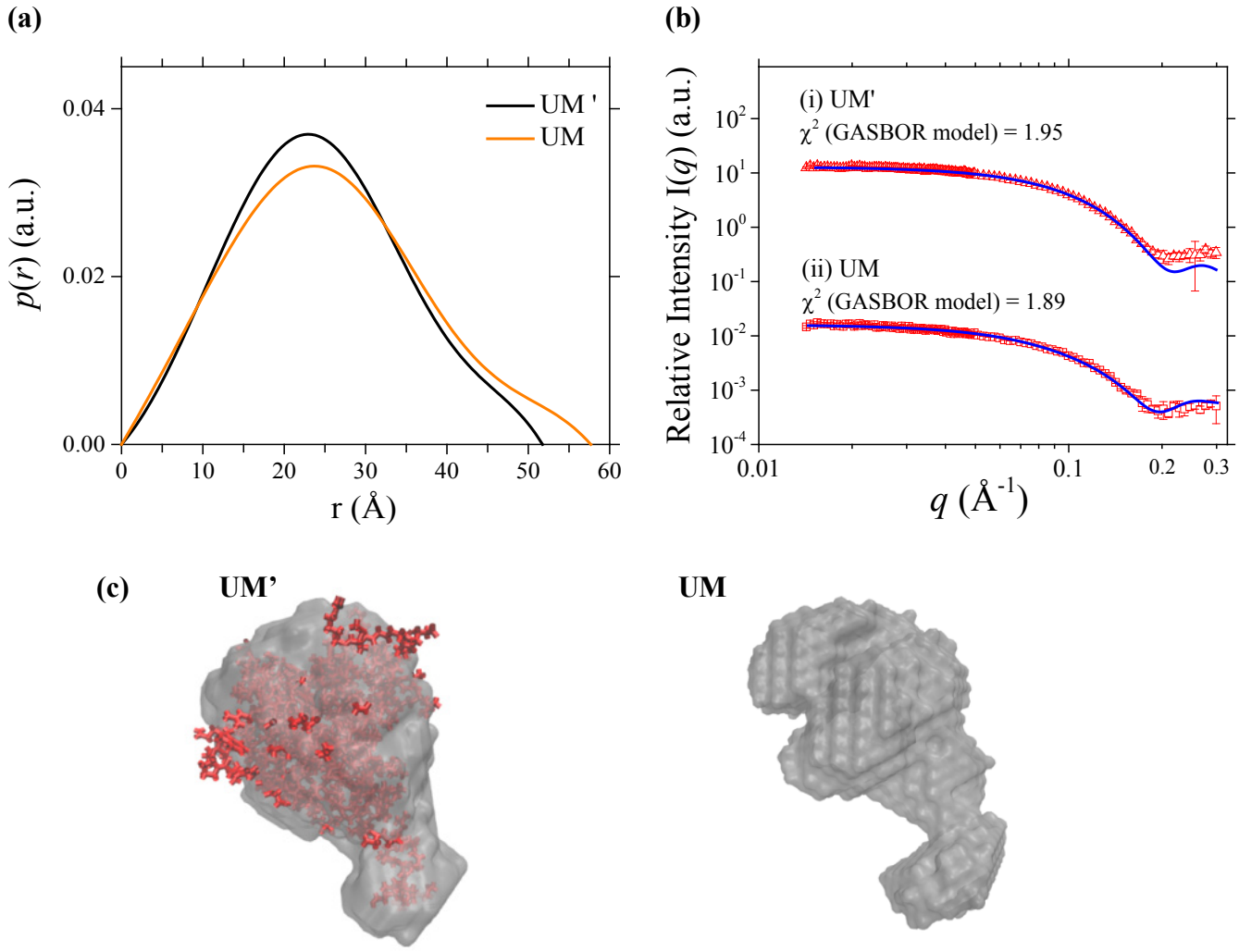


**Figure S3.** (a) Distance distribution functions $p(r)$ of UM' and UM. (b) Experimental scattering curves of UM' and UM (red squares). The blue solid curves denote fits from modeling with GASBOR. (c) Envelopes of UM' and UM, each resulting from superposition of 10 individual *ab initio* (GASBOR) calculations. UM' is overlapped with the crystal structure of BAX (1F16) colored in red to illustrate the more solidifying head domain of UM', due presumably to incorporation of BimBH3 (as revealed from the HPLC elution profiles in main text Fig. 1b).

## S5. Table S2 - Parameters derived from SAXS data

$I(0)$: zero-angle SAXS absolute intensity (in the scales of $cm^{-1}$ for the absolute scattering cross section per unit volume) $R_g$: radius of gyration obtained from either Guinier approximation (G.A.) or from the pair-distance distribution function $p(r)$. $D_{max}$: maximum dimension

| Parameters | tetramer | e-dimer | dimer | monomer (UM') | monomer (UM) |
|---|---|---|---|---|---|
| **Structural Parameters** | | | | | |
| $I(0)$ from G.A. ($cm^{-1}$) | 0.0215 | 0.0174 | 0.0153 | 0.0205 | 0.0169 |
| $R_g$ from G.A. (Å) | 31.8±0.7 | 34.5±0.8 | 29.7±0.6 | 20.5±0.2 | 23.3±0.4 |
| $I(0)$ from $p(r)$ ($cm^{-1}$) | 0.0238 | 0.0183 | 0.0152 | 0.0198 | 0.0151 |
| $R_g$ from $p(r)$ (Å) | 35.7±0.4 | 36.5±0.2 | 28.5±0.2 | 19.2±0.1 | 20.8±0.2 |
| $D_{max}$ (Å) | 112.6 | 110.8 | 80.4 | 51.8 | 57.7 |
| Porod volume ($Å^3$) | 180880 | 76590 | 73819 | 22397 | 20340 |
| Fitting algorithm | SASREF-DEER | EOM-DEER | CORAL-DEER | GASBOR | GASBOR |
| Fitting $\chi^2$ | 1.2 | 1.3 | 1.1 | 1.9 | 1.9 |
| **Molecular mass (kDa)** | | | | | |
| $M_{W\text{-sequence}}$ | 84.8 | 42.4 | 42.4 | 21.2 | 21.2 |
| From DAMMIN | 85.5 | -- | 39.5 | 16.9 | 15.5 |
| From dry volume $V_c$ | 81.0 | 46.2 | 44.3 | 19.0 | 17.3 |

## S6. Results of size-exclusion fast protein liquid chromatography (SEC-FPLC)

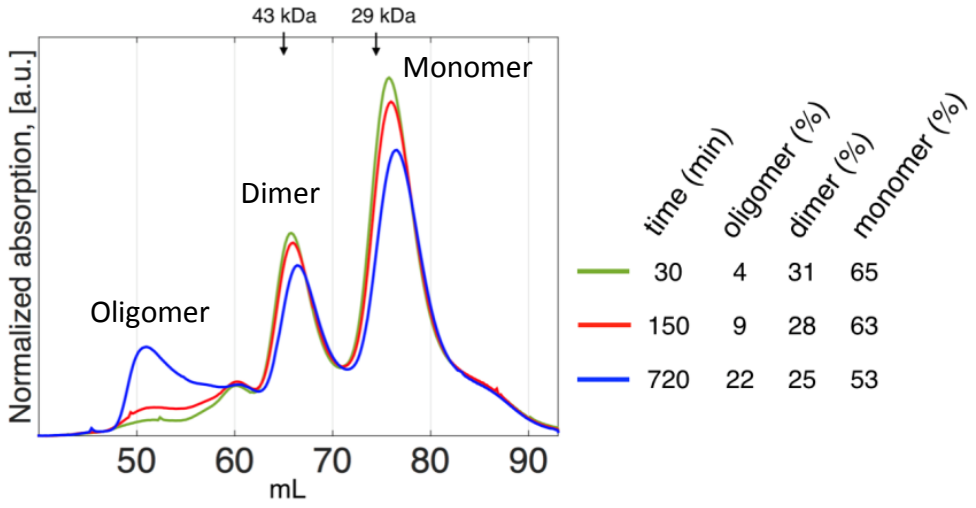


**Figure S4 -** SEC-FPLC results for the mixture of BAX : BimBH3 (1:1) at room temperature at different lengths of incubation time (30, 150, 720 min). Monomer, dimer, and oligomer elution peaks are assigned based on the elution time of the molecular standard markers indicated on the figure. The shown respective populations of BAX monomer, dimer, and oligomer are obtained from deconvolution analysis of the chromatograms. Note that the dimer peak decays as the higher order oligomer peak grows.

**S7. BH3-in-groove model matches with the core structure determined by ESR**

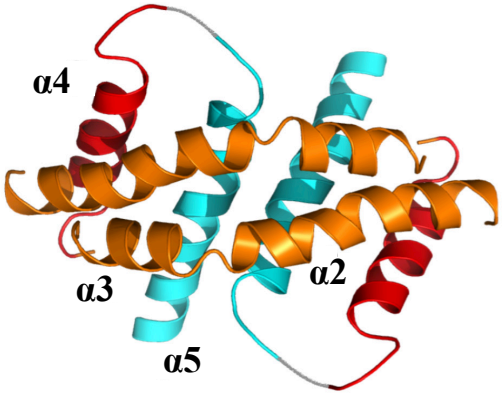


**Figure S5.** The core structure (α2 – α5) of BAX dimer within the oligomer determined by ESR. This BH3-in-groove model is highly similar to the structure obtained from the X-ray crystallographic study (4BDU, ref 19 in main text), as shown in Fig.S6 below.

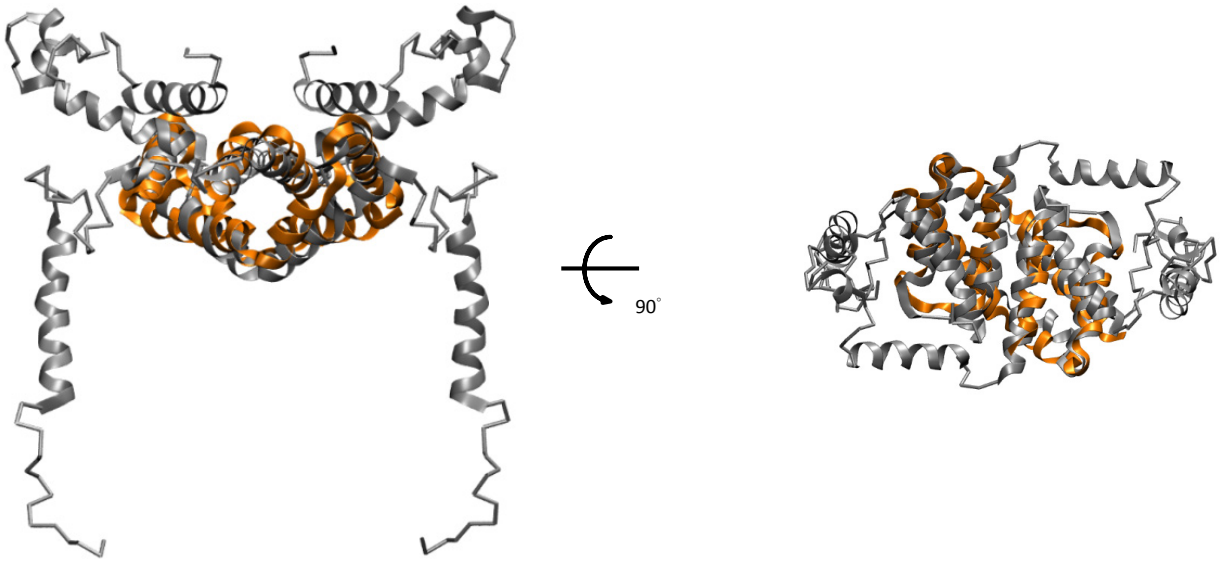


**Figure S6.** Two orthogonal views of the well overlapping of the SAXS-derived dimer structure (Gray) with the BH3-in-groove model (golden yellow) proposed by Czabotar *et al.* (PDB ID: 4BDU).

**S8. Comparisons of the structures of BAX tetramer**

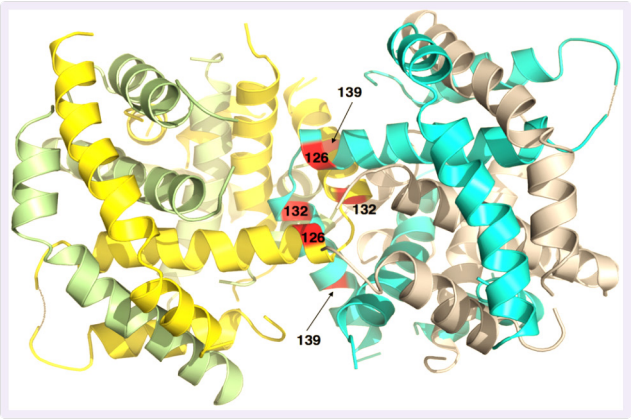


**Figure S7.** Dimer-Dimer interface model of BAX derived from BAX oligomers using ESR techniques. The four monomeric units are colored by pale green, yellow, gray, and cyan. Residues 126, 132, 139 at the dimer-dimer interface are highlighted in red. Based on the ESR-derived model, distances in the denoted residues are used as constraints in the SASREF fitting algorithm.

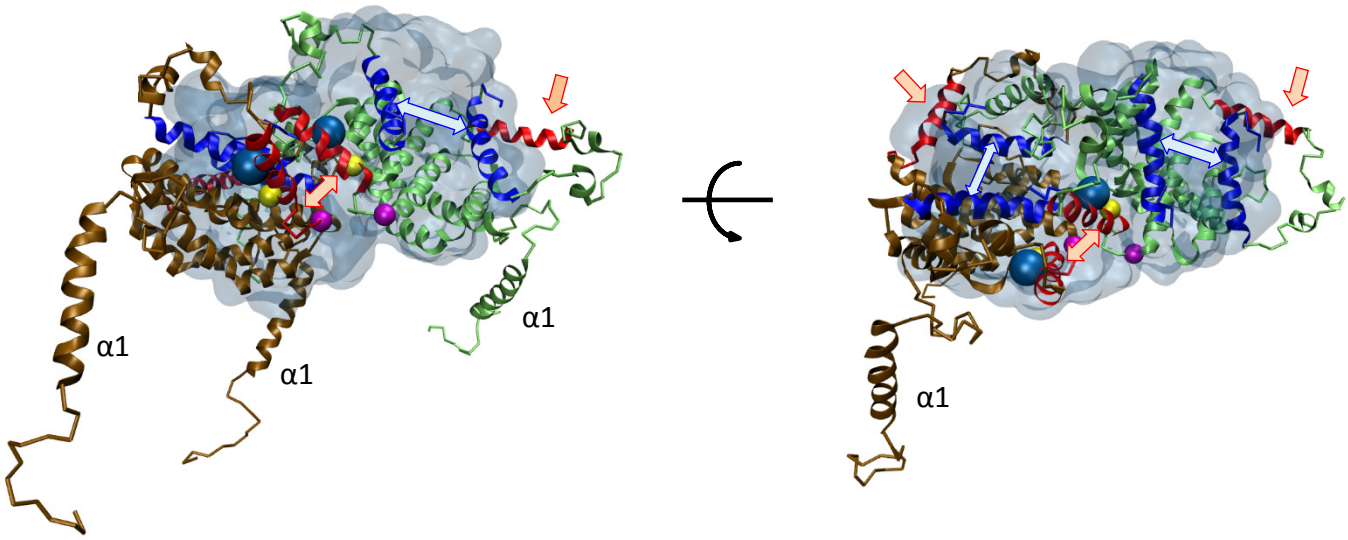


**Figure S8.** Two orthogonal views of the ESR-derived tetramer shown in Fig. S7 (in surface presentation, for clarity, flexible α1 is not shown) with the co-refined HPLC-SAXS/ESR tetramer model. The red arrows indicate α6:α6 interface near the center and the two outreaching ditopic α6 on both sides; the blue arrows indicate the two sets of parallel α9 in each ditopic dimer unit (brown and green).

**S9. Representative snapshots of BAX dimer at 350K compare with SAXS-derived extended dimer model**

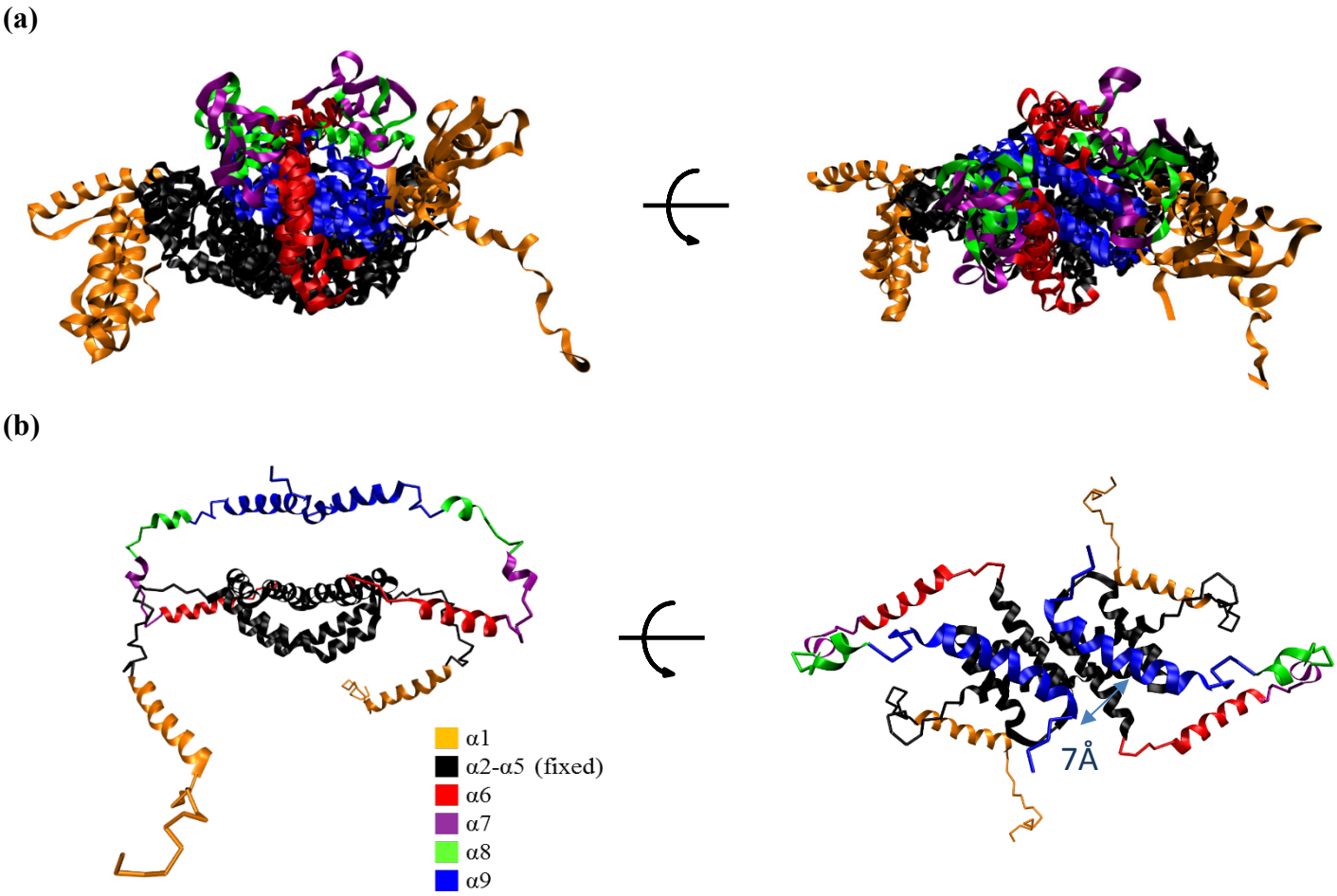


**Figure S9. (a)** Two orthogonal views of four representative snapshots (have similar $R_g$ as that of the extended dimer) during the MD simulation of the compact BAX dimer (Fig. S6) at high temperature of 350K. (b) SAXS-derived extended BAX dimer with the α9-α9 parallel configuration with a spacing of 7 Å persistently observed in the MD simulation trajectory (300K and 350K), as indicated by the blue arrow.