SUPPLEMENTARY DATA

Molecular dynamics simulation of GTPase activity in polymers of the cell division protein FtsZ.

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Contents:

1. Materials and Methods

1.1 Normal mode analysis. The analysis was performed using the "*Anisotropic Network Model server*": <http://ignmtest.ccbb.pitt.edu/cgi-bin/anm/anm1.cgi> [1], with a $C\alpha$–$C\alpha$ cut-off of 15 Å and a distance weighting factor of 2.5. Normalized mobility values were calculated as the weighted average of the three main normal modes.

1.2 Analysis of MD trajectories. To ensure that sufficient sampling has been done, a series of analysis of the MD trajectories were performed: temporal profiles of root mean square deviation (RMSD, Fig. S4A), radius of gyration (Fig. S4B) and value of first principal component (Fig. S5B), as well as root mean square fluctuation (RMSF, Fig 5A) profiles of all residues in the FtsZ polymers during MD. In addition, non-weighted covariance matrix of C-alpha atoms of the structure were calculated to obtain cosine content (c_i) [2] of the three first principal components (Table S1). This value ranges between 0 (no cosine) and 1 (perfect cosine). Values close to 1 are representative of random motion, and therefore, of insufficient sampling.

2. Supplementary Figures

Figure S1. Continuous measurement of "d" distance (O_{wat} - P_γ) for all water molecules at the interfaces of the polymers modelled over 8 ns of unrestricted MD. Distances are in angstroms (Å). The range 3.0-3.8 Å, which corresponds to the optimal distance, is indicated (dotted lines). The colour coding of the distance lines in the plots is as follows. Dark green and light green: catalytic water molecules located at less than 3.8 Å and at an angle (see Figure S2) of more than 110º; blue: water molecules at less than 3.8 Å with an inadequate angle; yellow to red: unstable water molecules located in the proximities of the active centre. Interfaces are labelled as in Figures 2 and 4.

Figure S2. Continuous measurement of "α" angle (O_{wat} - P_γ - $O3_\beta$) for all water molecules present in the interfaces of modelled polymers over 8 ns of unrestricted MD. The position of minimum angle (110º) is indicated in each plot (dotted line). Colour codes and labels are as in Figure S1.

Figure S3: Normal mode analysis of modelled FtsZ polymers. The protein trace is coloured according to the normalized mobility values of each residue (blue: limited; red: greater), indicating the putative movements associated with each protein segment in the polymers. Interface labels are coloured according to their predicted behaviour after the MD results: red, inactive; green, active. Normal mode analysis of FtsZ trimer predicted that both interfaces (III_A and III_B) would exhibit limited mobility (green arrows in lane III). In the case of the tetramers and the pentamer, interfaces IV_A, IV_B, V_A, V_B, IV'_B and IV'_C are also predicted to have limited mobility (green arrows in lanes IV, V and IV'). This is probably because the end monomers can adapt their positions relative to the monomers that are in contact with them, thus minimizing the movement of the interface. In contrast, the central interfaces (IV_C, V_C,

V_D, and IV'$_D$) are predicted to experience more movement (red arrows in lane IV and V). Transitions from an active interface (IV$_B$ in lane IV) to an inactive interface (V$_D$ in lane V) and from an inactive interface (V$_C$ in lane V) to an active interface (IV'$_C$ in lane IV') are indicated.

Figure S4: Temporal profiles of Cα root mean square deviation (RMSD, **A**) and radius of gyration (**B**) of FtsZ polymers during MD simulations. Total time for tetramer (IV') is 16 ns as the initial structure was generated from the final structure of pentamer (V) after 8 ns of MD.

Figure S5: A. Root mean square fluctuation (RMSF) profile of all residues in the FtsZ polymers during MD trajectories. **B.** Temporal profiles of first principal component (PC1) of FtsZ polymers during MD.
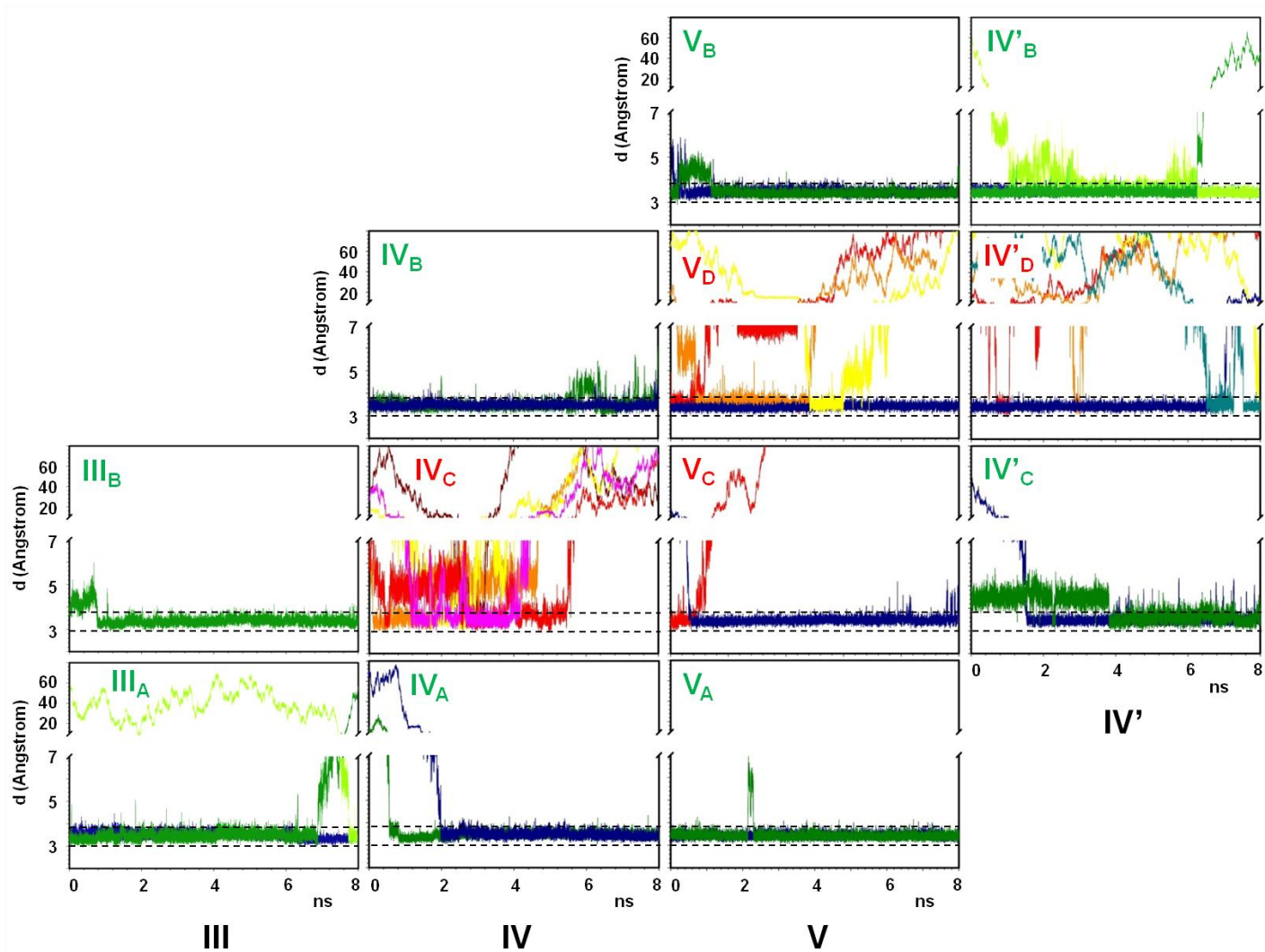
3. Supplementary Tables.

Table S1. Cosine content (c_i) of the first three principal components for modelled FtsZ polymers in complete MD simulations.

|	PC-1	PC-2	PC-3
trimer (III)	0.556	0.308	0.046
tetramer (IV)	0.001	0.067	0.018
pentamer (V)	0.369	0.302	0.074
tetramer (IV')	0.398	0.073	0.001

Cosine content was calculated according to Hess, 2000 [2]. The results, far from a perfect cosine ($c_i = 1$) indicate that the trajectories have reached an overall sufficient sampling.
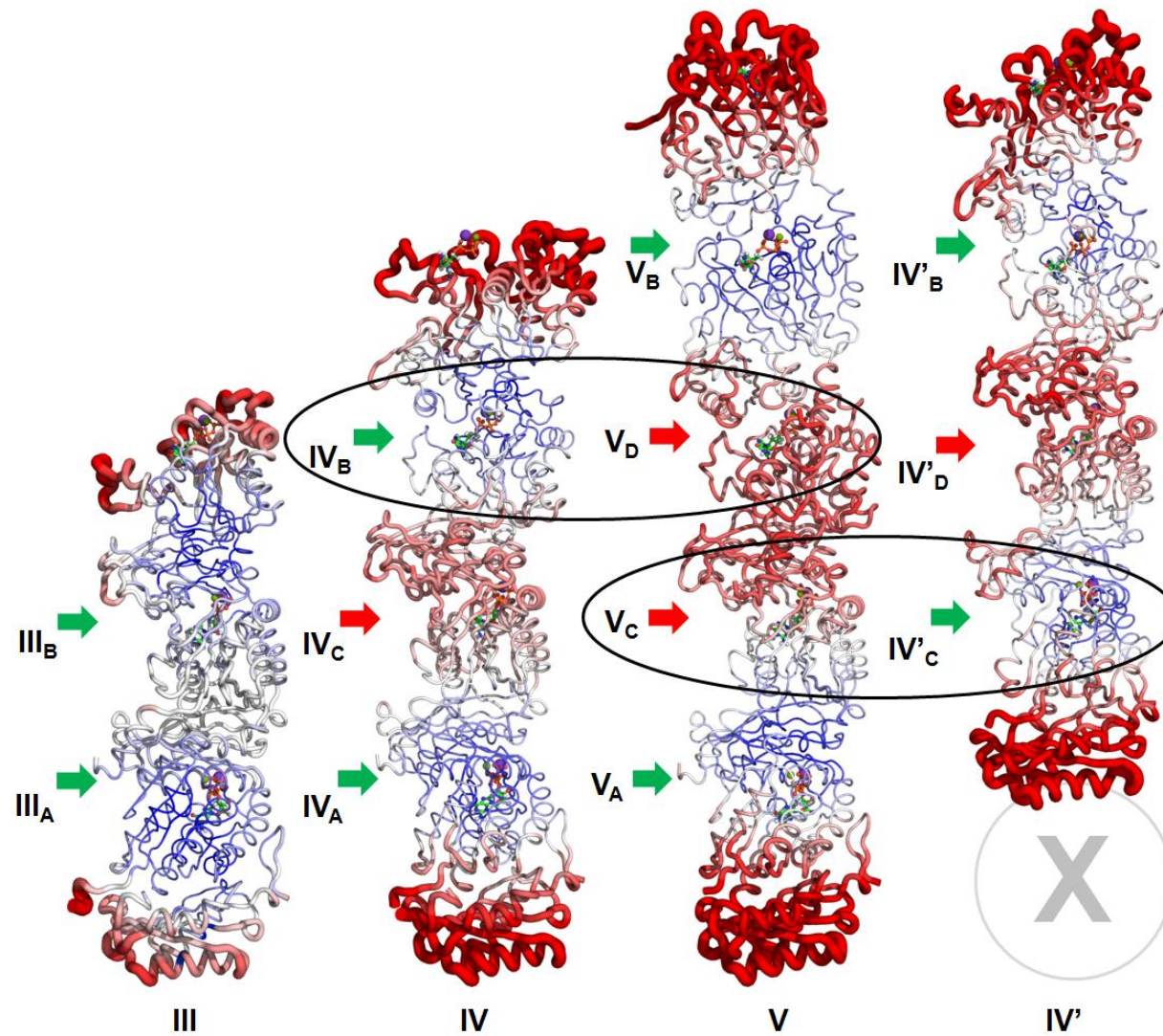
4. References.

[1]   Eyal, E., Yang, L.W. and Bahar, I. (2006). Anisotropic network model: systematic evaluation and a new web interface. Bioinformatics 22, 2619-27.
[2]   Hess, B. (2002). Convergence of sampling in protein simulations. Phys Rev E Stat Nonlin Soft Matter Phys 65, 031910.
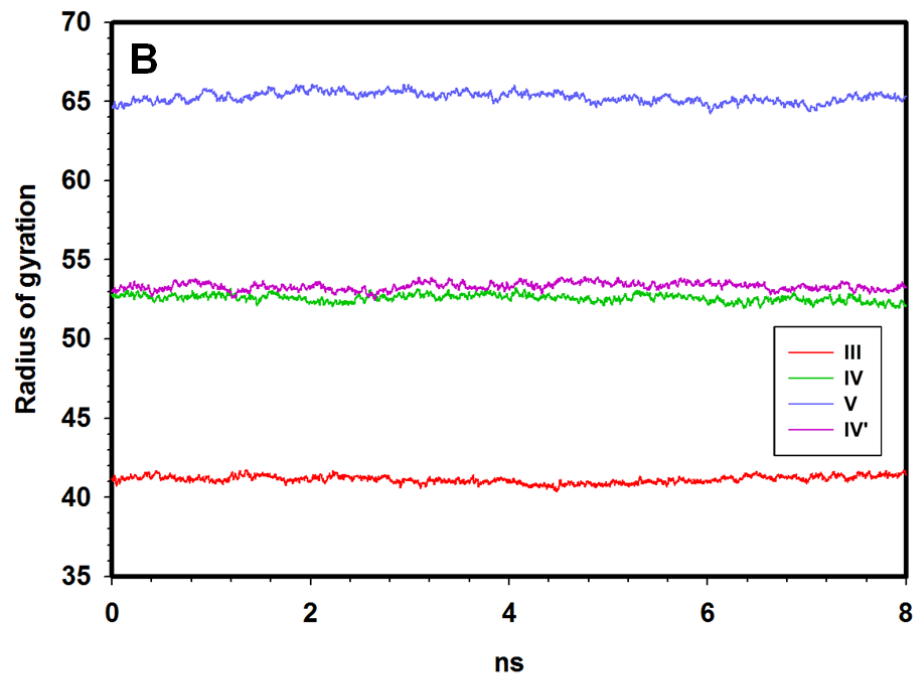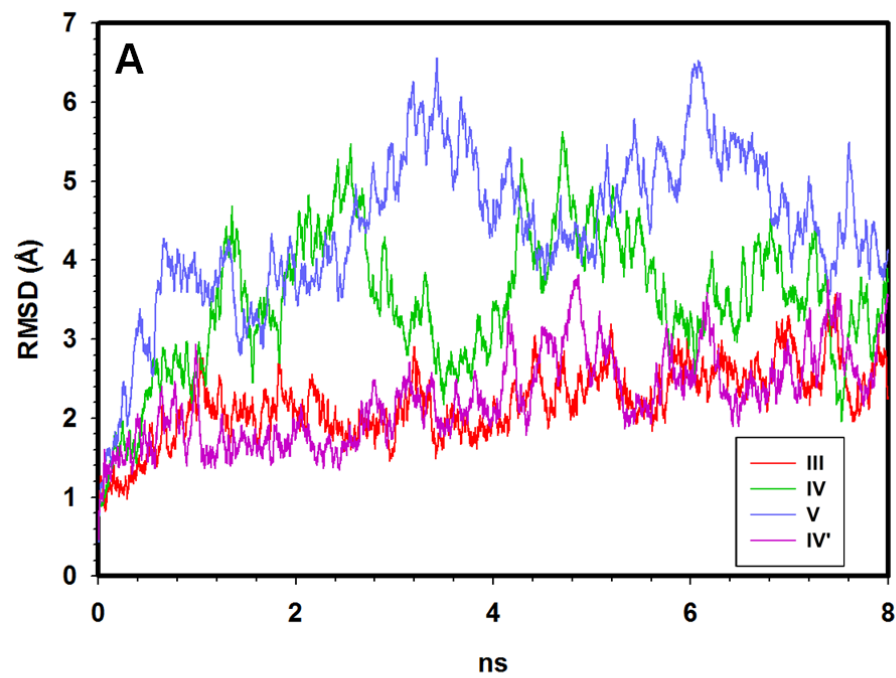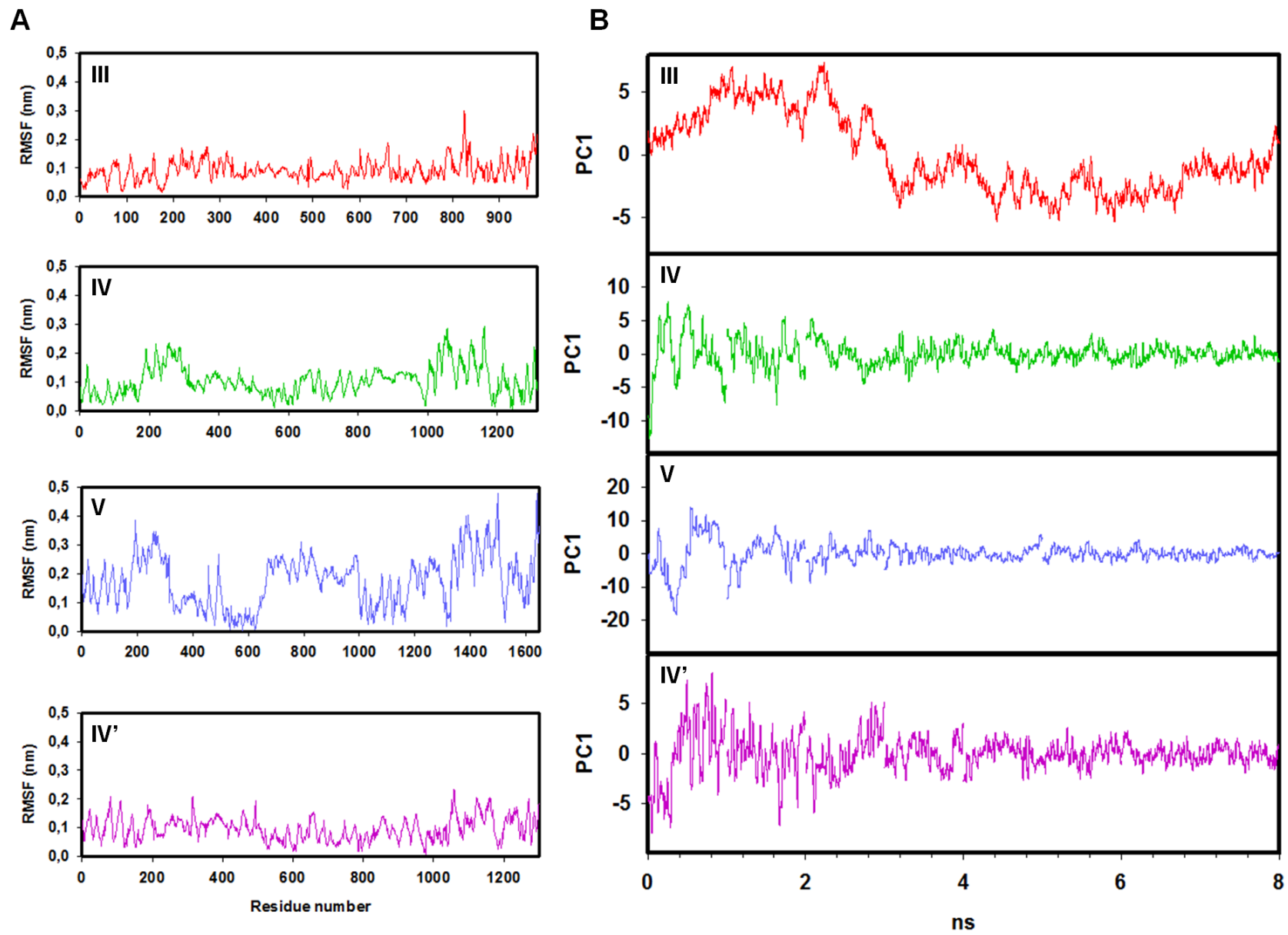
Supplementary figure S1
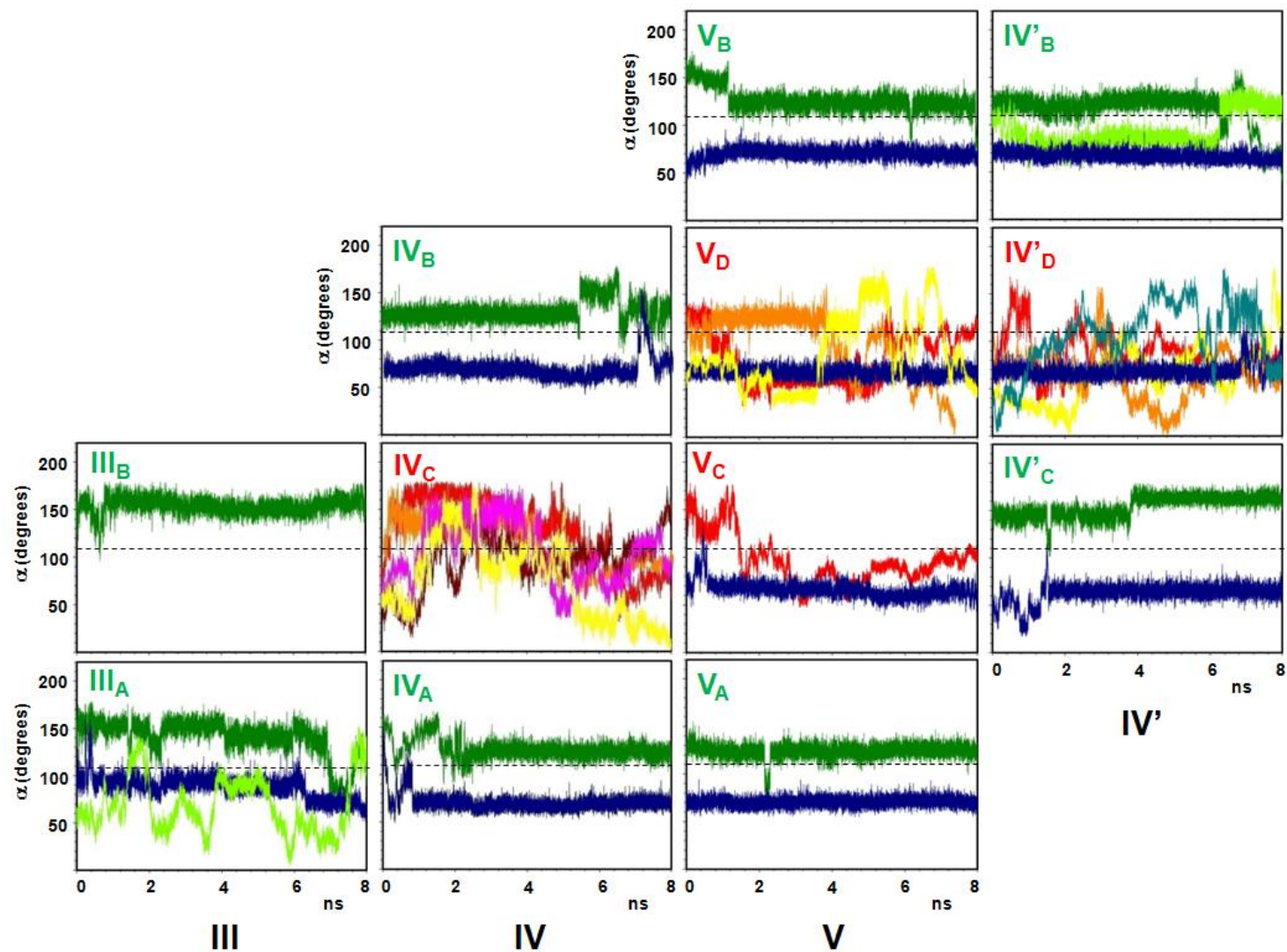
Supplementary figure S2

Supplementary figure S3

Supplementary figure S4

Supplementary figure S5