Supplemental Online Material

for the

“Dynamic properties of the native free antithrombin from molecular dynamics simulations;
computational evidence for solvent exposed Arg393 side chain”

by

László Tóth^a, Attila Fekete^a, Gábor Balogh^a, Zsuzsanna Bereczky^a and István Komáromi^{a,b}

^aUniversity of Debrecen, Faculty of Medicine, Department of Laboratory Medicine, Division of Clinical Laboratory Science and ^bResearch Group of Vascular Biology, Haemostasis and Thrombosis, Hungarian Academy of Sciences, Debrecen, Hungary

Supplemental Figures:

Figure S1.: Secondary structure elements of the whole antithrombin protein as a function of time starting from different conformational states, corresponding to 1e04 and 1t1f PDB structures. Simulations were carried out on both the wild type (1t1f-w) and the Val317Cys/Thr401Cys disulfide bridged variant of the 1t1f conformational state. The positions of helices A-I marked by the corresponding capital letters. The position of helix P is marked by red arrow.

Figure S2.: Time dependence of the root mean square deviation of the CA atom positions of antithrombin from the corresponding CA atoms of the starting geometry fitting the dynamics simulation snapshots to the starting structure using their CA atoms only. The simulations were carried out starting from two different conformational states, corresponding to 1e04 and 1t1f PDB structures. Simulations were carried out on both the wild type (1t1f-w) and Val317Cys/Thr401Cys disulfide bridged variant of the 1t1f conformational state.

Figure S3.: Time dependence of the root mean square deviation of the CA positions from the corresponding CA atoms of the starting geometry fitting the dynamics simulation snapshots to the starting structure using their CA atoms only. The extremely flexible N-terminal region and the RCL were excluded from the fitting (see in the text). The simulations were carried out starting from two different conformational states, corresponding to 1e04 and 1t1f PDB structures. Simulations were carried out on both the wild type (1t1f-w) and Val317Cys/Thr401Cys disulfide bridged variant of the 1t1f conformational state.

Figure S4a.: Root mean square atomic fluctuation for the simulations carried out for the antithrombin. The starting geometry corresponded to the 1t1f PDB structure. The blue to red coloring scale was used to indicate the most to least flexible regions, respectively. Fluctuation are shown for both the wild type (1t1f-w) and the Val317Cys/Thr401Cys disulfide bridged variant of the 1t1f conformational state. The usual antithrombin orientation is applied. The flexible N-terminal region is marked by large pink arrow.

Figure S4b.: Root mean square atomic fluctuation for the simulations carried out for the antithrombin. The starting geometry corresponded to the 1t1f PDB structure. The blue to red coloring scale was used to indicate the most to least flexible regions, respectively. Fluctuation are shown for both the wild type (1t1f-w) and the Val317Cys/Thr401Cys disulfide bridged variant of the 1t1f conformational state. The flexible N-terminal region is marked by large pink arrow. The orientation was obtained from the orientation shown on the Fig. S4a by an $\sim 90^\circ$ rotation around the vertical axis.

Figure S5.: Calculated relative Gibbs free energy (in kJ/mol units) surface of the RCL movements as a function of two collective variables, a distance between the Lys236 CA atom and the center of mass of Val388-, Val389- and Ile390 CA atoms (CV1) and an angle defined by the Met251 CA, Glu255 CA and the center of mass of the Ala384-, Ser385-, Thr386-, Ala387-, Val388-, Val389-, and Ile390 CA atoms (CV2). The metadynamics calculation (Barducci, Bonomi et al. 2011) was carried out by means the PLUMED software (Tribello, Bonomi et al. 2014) interfaced to the GROMACS package (Pronk, Pall et al. 2013).

Figure S6.: Generalized correlation (mutual information) matrices for the movements of the CA atoms of antithrombin during the simulations starting from the 1t1f conformational state. The mutual information matrices are shown for both the wild type (1t1f-w) and the Val317Cys/Thr401Cys disulfide bridged variant of the 1t1f conformational state. The positions of helices A-I are marked by the corresponding red capital letters.

Figure S7.: Possible allosteric pathways between the P-helix and the hinge region of antithrombin calculated by the WISP software. The simulation was carried out starting from the conformational state corresponding to the 1t1f PDB structure. The allosteric pathways are depicted for both the wild type (1t1f-w) and the Val317Cys/Thr401Cys disulfide bridged variant of the 1t1f conformational state.