

Supplementary Information

Cryo-EM structure of the prothrombin-prothrombinase complex

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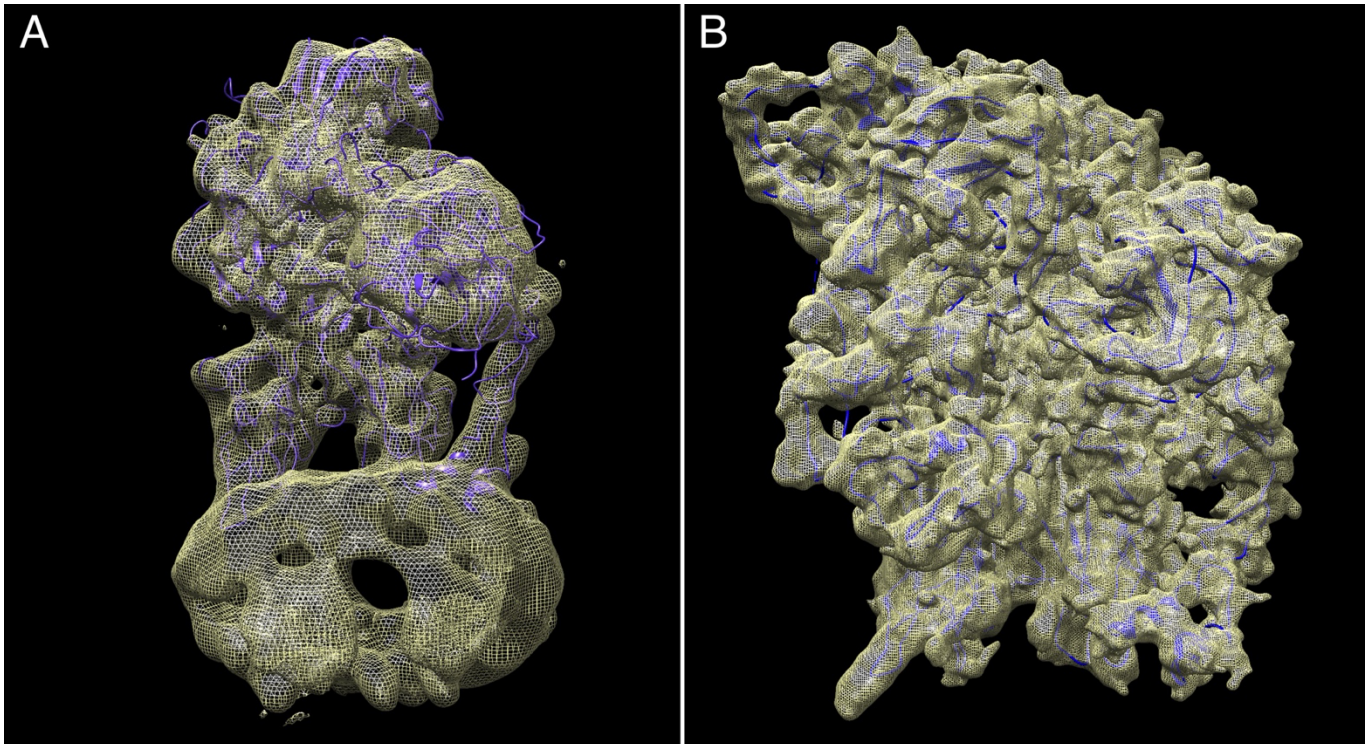
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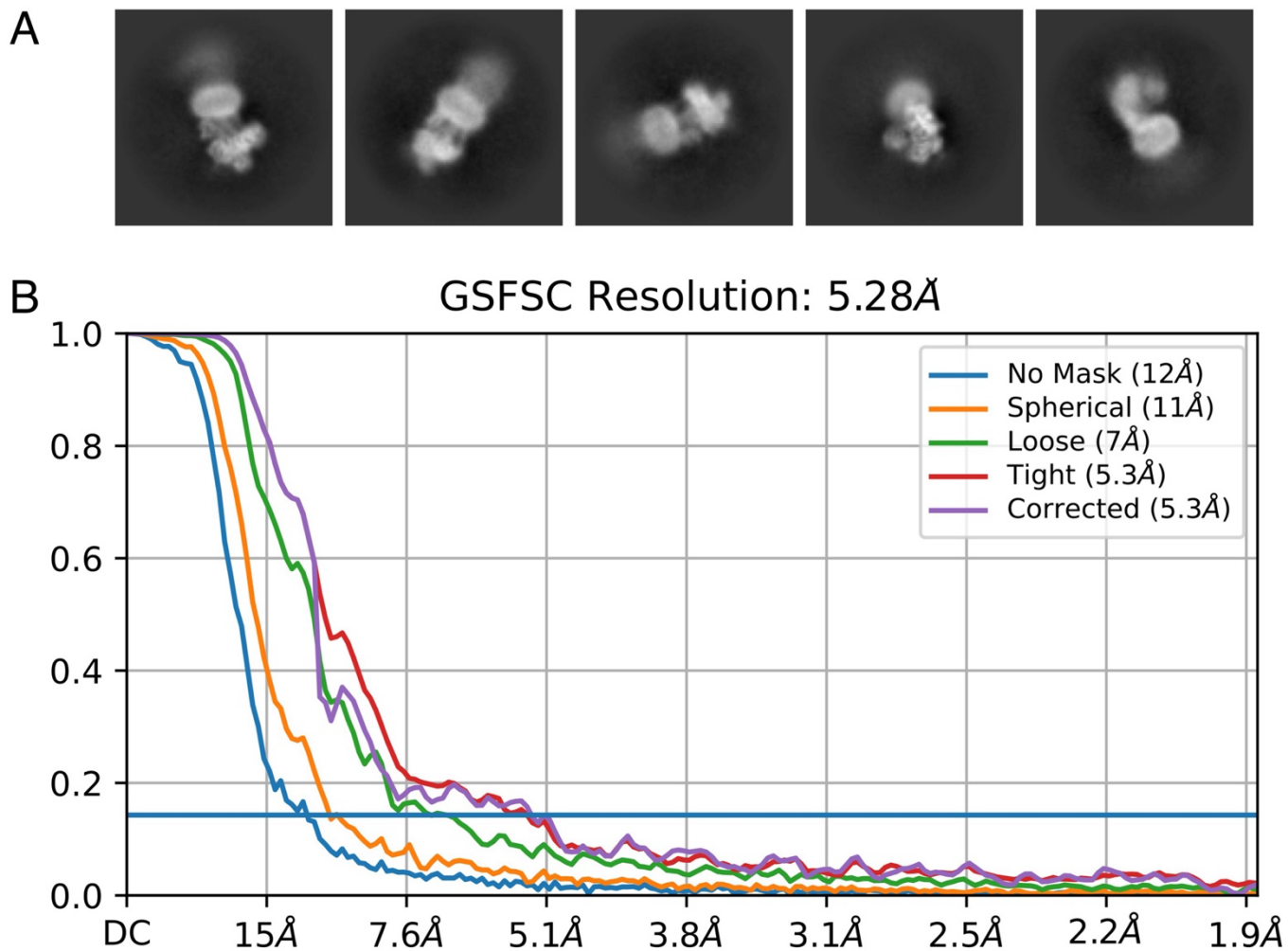
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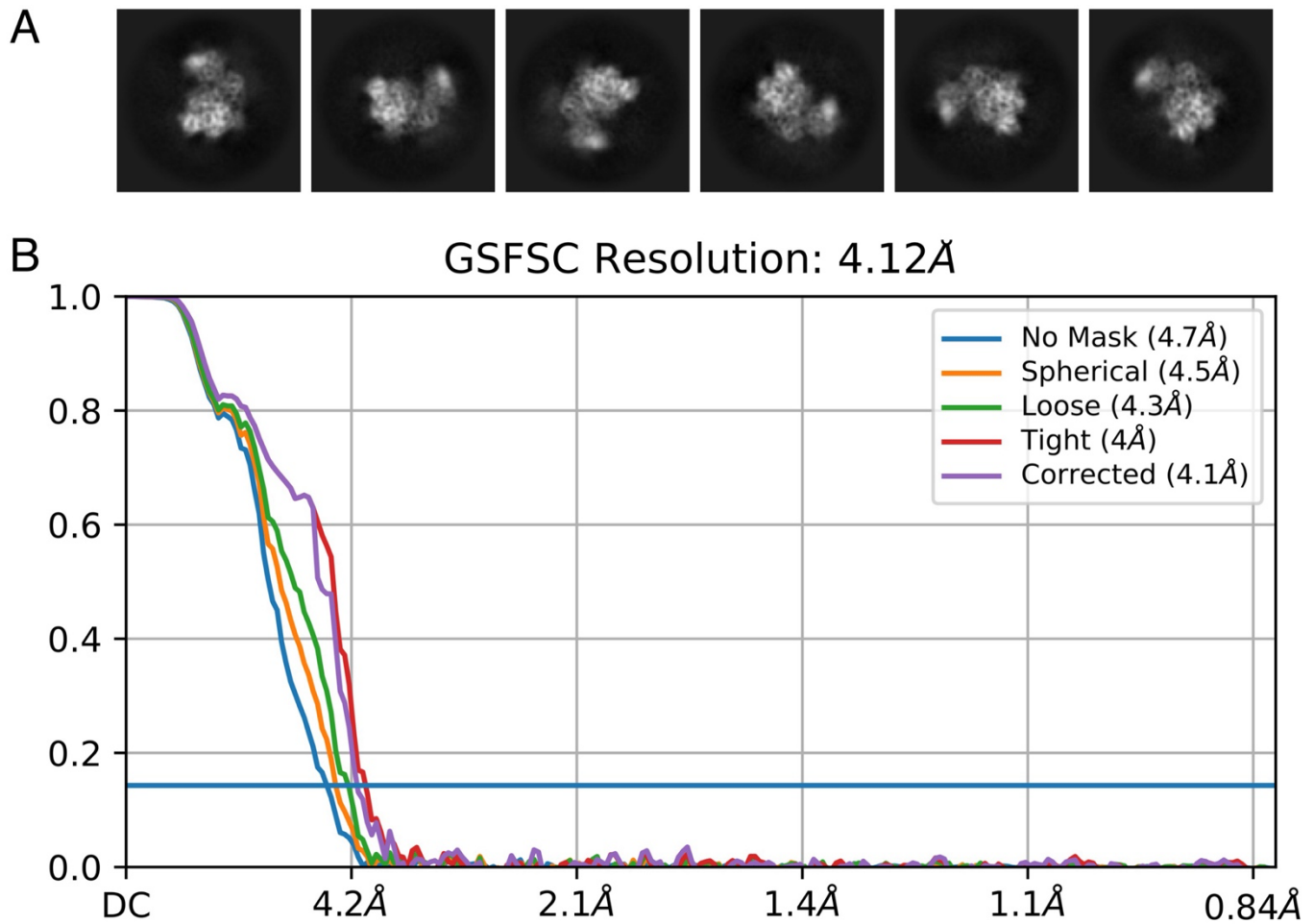
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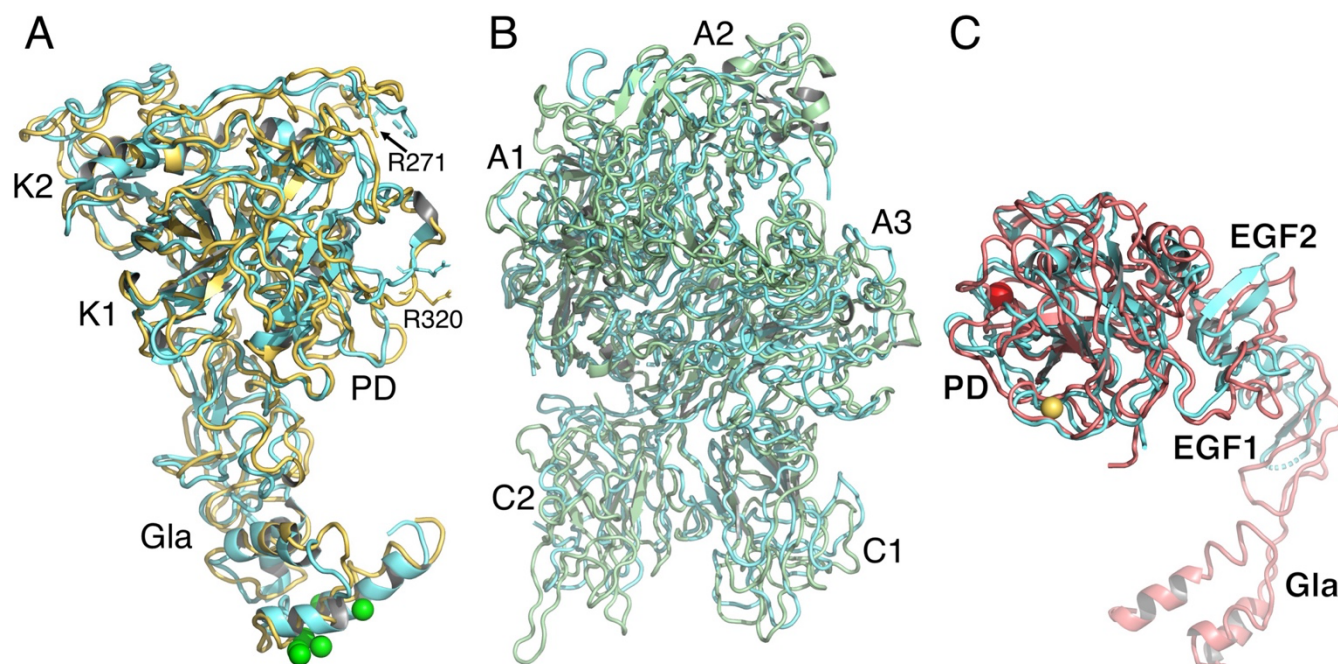
Supplementary Figure 1AB. Map and model of prothrombinase on nanodiscs (A) and of prothrombin bound to fVa and fXa (B). Orientation is similar to that shown in Figure 1AB in the text.



Supplementary Figure 2AB. Data quality of the cryo-EM structure of prothrombinase on nanodiscs shown as (A) representative 2D class averages and (B) gold standard Fourier shell correlation (GSFSC) of masked refinement of the map at 5.28 Å resolution (emd id: 26061).



Supplementary Figure 3AB. Data quality of the cryo-EM structure of prothrombin bound to fVa and fXa shown as (A) representative 2D class averages and (B) Gold standard Fourier Shell Correlation (GSFSC) of masked refinement of the map at 4.12 Å resolution (emd id: 26060).



Supplementary Figure 4ABC. Superpositions of the three components of the prothrombin-fVa-fXa complex with available structures in the free form. Domains are labeled. (A) Prothrombin (yellow) overlaid to the X-ray structure of the closed form PDB ID 6BJR ¹ (cyan), rmsd=2.51 Å over 537 C α atoms. The sites of cleavage at R271 and R320 are shown as sticks. (B) fVa (palegreen) overlaid with the recent cryo-EM structure of fVa PDB ID 7KXY ² (cyan), rmsd=3.29 Å over 1014 C α atoms. (C) fXa (salmon) overlaid with the X-ray structure of Gla-domainless fXa PDB 3K9X ³ (cyan), rmsd=3.04 Å over 284 C α atoms. The bound Ca²⁺ (yellow) and Na⁺ (red) refer to the X-ray structure and could not be located in the cryo-EM structure of the prothrombin-fVa-fXa complex. Residues of the catalytic triad are not visible in this orientation.

References

1. Chinnaraj M, Chen Z, Pelc LA, et al. Structure of prothrombin in the closed form reveals new details on the mechanism of activation. *Sci Rep.* 2018;8(1):2945.
2. Ruben EA, Rau MJ, Fitzpatrick J, Di Cera E. Cryo-EM structures of human coagulation factors V and Va. *Blood.* 2021;137(22):3137-3144.
3. Shi Y, Li C, O'Connor SP, et al. Aroylguanidine-based factor Xa inhibitors: the discovery of BMS-344577. *Bioorg Med Chem Lett.* 2009;19(24):6882-6889.