doi:10.1017/S1431927611001516
Microsc. Microanal. 17 (Suppl 2), 2011


# Three-dimensional Structure and Oligomeric State of the N-terminal Peptide of CAP/Srv2 Complex

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CAP - cyclase-associated protein (called Srv2 in yeast) plays a key role in accelerating the turnover of actin filaments by effectively recycling cofilin, profilin and actin [1]. Srv2 is a bifunctional protein with an N-terminal domain that provides a binding site for adenylyl cyclase and a C-terminal domain that has actin binding activity. N-terminal domain of Srv2 is also responsible for the interaction with the actin-cofilin complex, facilitates filament elongation at the barbed end and stimulates ADP-ATP exchange on G-actin, a process that regenerates easily polymerizable G-actin. The C-terminal domain of Srv2 was found to accelerate the depolymerization of F-actin at the pointed end [2] and relieved the inhibition of the nucleotide exchange on G-actin by cofilin. It is thought that Srv2 is present in living cells as a hetero-dodecamer (12'mer), comprised of six Srv2 and six actin molecules [3]. This hypothesis however, still needs to be tested rigorously. One of the possible methods to reveal the oligomeric state of the large molecular complex is single particle electron microscopy. The olygomerization is provided by CC-domain is located on the N-terminus of the whole molecule, therefore we addressed the olygomeric state of the N-terminus

To study the structure of the CAP/Srv2 complex, the N-terminal part of protein, bearing either 6HIS-tag or GST-tag have been expressed in E.Coli and purified using an affinity chromatography. Yields were enough to obtain single particles on carbon coated negative stained EM grid. About 2000 particles were manually collected using the Signature software [4], band-pass filtered (fig.1A) and classified (fig.1B). The 3D reconstruction of the N-terminal part of 6HIS-srv2 has been calculated (fig.1D), using an angular reconstitution method [5]. The 3D structure reveals a cup-shaped structure with six petals (fig.2). The resolution of this 3D structure was estimated using the Fourier Shell Correlation method (FSC) and has been measured at about 30Å. The molecular weight of the N-Srv2 complex with six HIS-tags is about 220 kD. Docking of available crystal structure [6] revealed a six-fold symmetry (fig.2). The penta- or hexameric symmetry of the srv2 oligomer was predicted by previous centrifugation experiments [1]. To obtain a better resolution the GST-tagged N-srv2 protein has been used. We have collected 18000 single particles that allowed us to obtain a 3D structure of GST-N-srv2 with 18Å resolution (fig.3). In this reconstruction the cup-shaped bottom was supplemented by the massive density, apparently descending from the interacting GST tags. The refined structure of the GST-N-srv2 was pentameric, in contrast to the 3D structure of 6HIS-N-srv2. The docking of the crystal structures of N-srv2 monomers into both EM densities (fig.3) was performed using the Situs program. The correlation coefficients were 0,5 for 6HIS-N-srv2, and 0,75 for GST-N-srv2. Docking helps identify the cup-shaped part, as the pentameric N-srv2. The position of affinity tags (6HIS-tag and GSTtag, both with N-terminal location) on the concave surface of the oligomeric part suggests that the N-terminal ends of the monomers are situated on this surface.

References

[1] O.Quinter-Monzon et al, *J Biol Chem*. 284(16) (2009) 10923-10934
[2] K.Moriyama, and I.Yahara. *J Cell Sci* 115 (2002)1591-601
[3] H.I.Balcer, et al *Curr Biol*, 13 (2003) 2159–2169.
[4] G.Z.Chen and N.Grigorieff *J Struct Biol* 157(1) (2007) 168-173

[5] M.van Heel, at al. *J Struct Biol* 116 (1996) 17–24
[6] D.Ksiazek, et al. *Structure* 11(9) (2003) 1171-1178.
[7] This research was supported by the collaborative grant from CRDF under contract #2918 to OS and BLG and grant from RFBR under contract #08-04-91125 to OS.

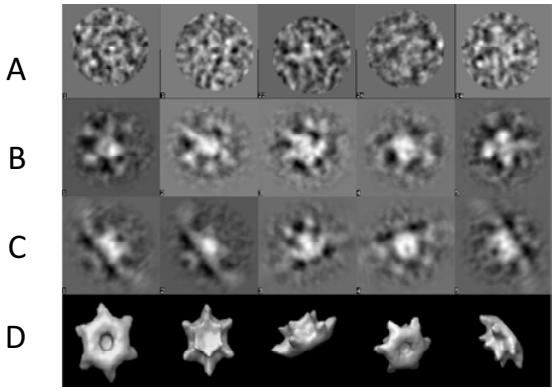


FIG. 1 Structure of N-terminal Srv2 oligomer. (A) Examples of particle classifications; (B) Classification of particles; (C) Reprojections of the 3D structure; (C) The corresponding views of the 3D structure.

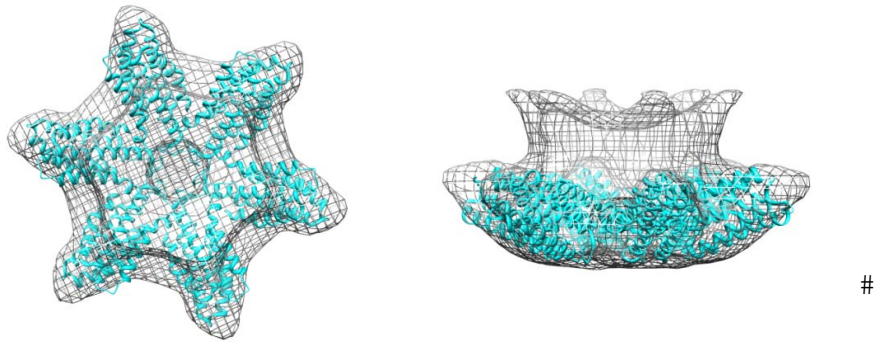

FIG. 2. Docking six Srv2 N-terminal monomers into 3D structure on FIG.1D

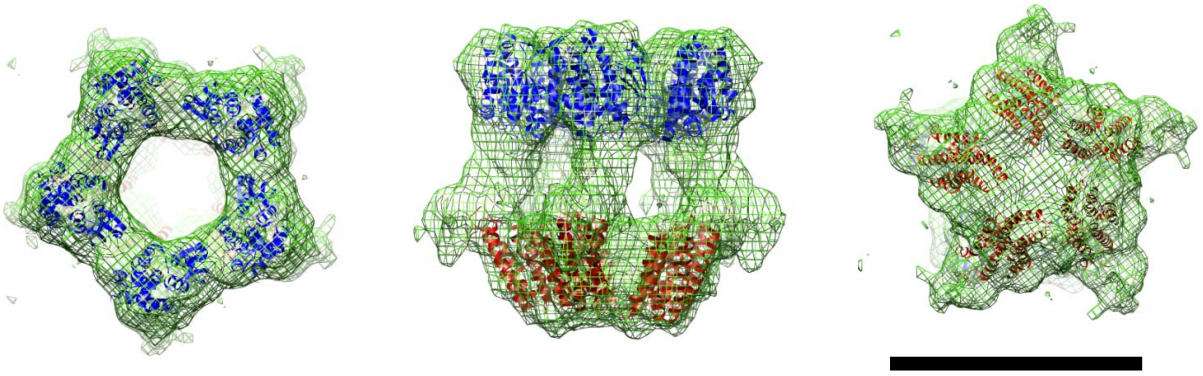

FIG. 3. Docking of crystal structures into the 3D EM structure of N-GST-Srv2. Crystal structures of Srv2 N-terminal monomers are shaded red, and glutathione-S-transferase monomers are shaded blue. Bar size is 10 nm.

https://doi.org/10.1017/S1431927611001516 Published online by Cambridge University Press