Supplementary Materials for

Multimodal binding of collybistin controls gephyrin filament formation in synaptic clustering

Nele Burdina[1], Filip Liebsch[1], Arthur Macha[1], Monika Gunkel[1], Elmar Behrmann[1]*, Guenter Schwarz[1,2]*

[1]Institute of Biochemistry, Department of Chemistry and Biochemistry, University of Cologne, 50674 Cologne, Germany

[2]Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany

*Correspondence: Guenter Schwarz, gschwarz@uni-koeln.de, phone: +49 221 4709 6440; Elmar Behrmann, ebehrman@uni-koeln.de, phone: +49 221 470 76300

Supplementary Materials and Methods

Cryo-EM sample preparation of SEC purified Geph-CB complexes

The Geph-$CB2_{SH3-}$ complex together with Folch was purified by SEC using a Superose 6 increase 5/10 column (3 mL, GE Healthcare). Therefore, Geph and $CB2_{SH3-}$ were mixed at an equimolar ratio using 9 nmol each and additionally 36 nmol Folch lipids in DDM micelles. The samples were adjusted to a volume of 90 µL using EM-buffer (25 mM Hepes/HCl pH 7.5, 250 mM NaCl, 2% (w/v) Sucrose, 50 mM Arginin, 50 mM Glutamate, 10 mM 2-mercapto-ethanol). After centrifugation (17 000g, 10 min, 4 °C) the samples were applied to the SEC column and proteins were separated at 4°C and a flow rate of 0.2 mL/min in EM-buffer. The peak fraction correlating to the Geph-$CB2_{SH3-}$-Folch complex was diluted to 2.5 µM using EM-buffer. 3 µl of the diluted sample were applied to a glow-discharged UltraAuFoil R1.2/1.3 Au300 Holey Au support grid (Quantifoil Micro Tools GmbH) and the grid was blotted for 3.5 s before being flash-frozen in liquid ethane using a Vitrobot Mark IV device (Thermo Fisher Scientific) set to 100% humidity at 22 °C. Grids were stored in liquid nitrogen until usage. Cryo-EM data was acquired using a Titan Krios G3i (Thermo Fisher Scientific) electron microscope operated at 300 kV. Images were collected automatically using EPU (version 3.8.0.7600, Thermo Fisher Scientific) on a Falcon III direct electron detector with a calibrated pixel size of 0.862 Å px^{-1}. Movies were acquired in counting mode with a total dose of 31–34 e^{-} $Å^{-2}$ distributed among 42 frames. Defocus values ranged from −1.0 to −3.0 µm.

SDS-PAGE and Coomassie staining

Samples supplemented with 1x sample buffer (5x: 250 mM Tris/HCl pH 6.8, 30% glycerol, 0.1% Bromophenol blue, 10% SDS, 5% β-mercaptoethanol) were heated for 5 min at 95 °C. Proteins were separated using 12% SDS-polyacrylamide gel electrophoresis and visualized by Coomassie-staining (30% EtOH, 10% acetic acid, 0.25% Coomassie brilliant blue R250).

Visualization and documentation of Coomassie-stained gels was carried out using a ChemiDoc™ Imaging System (Bio-Rad).

Model building using rigid body fitting

To obtain atomic models of the different Geph-$CB2_{SH3-}$ complexes (Fig. 2E, Fig. 4) and the apo-Geph filament (Fig. S3a) we performed correlation optimized rigid-body fitting using available structures of $CB2_{SH3-}$ (PDB 2DFK)[1] and the two GephE dimer structures (PDB 9GW9[2], PDB 2FU3[3]) using UCSF ChimeraX (version 1.8)[4] with some minor modifications described in the following.

The only available structure of $CB2_{SH3-}$ is a co-crystal structure in complex Cdc24, one GEF partner of CB (PDB 2DFK)[1], which was removed prior to the rigid body fit. The crystal structure contains two asymmetric units of $CB2_{SH3-}$ in two different conformations. The relative orientation between the $CB2_{SH3-}$ PH- and DH-domains differed between the two binding sites of the Geph-$CB_{1:1}$ complex and the two conformations observed in the crystal structure. To account for the different orientations of the domains, we separated PH- and DH-domain at Ser200 within a loop region of the crystal structure and performed a rigid body fit for both domains individually.

We utilized the recently published cryo-EM structure of GephE forming filaments (PDB 9GW9)[2] for rigid body fitting as it correlates better with our density maps, particularly in the SDII region, compared to the crystal structure of dimerized GephE (PDB 2FU3)[3]. This cryo-EM structure displays a GephE dimer together with another GephE SDII forming an SDII-SDII´ contact site, while lacking density for the SDII of the other monomer due to the limited resolution in that region[2]. To reconstruct the full-length Geph E-domain dimer, we aligned the complete monomer with the partial Geph E-domain monomer, generating a C2-symmetric GephE dimer. Additionally, the N-terminal end of GephE harboring the CB-binding motif (320 – 329)[5] was not resolved within the GephE cryo-EM structure (PDB 9GW9) but within the GephE crystal structure (PDB 2FU3). We therefore aligned the GephE crystal structure

(PDB 2FU3) to add the loop region containing the CB binding motif to our Geph-$CB_{1:1}$ structure. This resulted in clashes with the DH domain of $CB2_{SH3-}$ for both binding sites (Fig. S2C,E). The loop was manually fitted into free density adjacent to the CB-binding motif followed by a rigid body refinement at both sites (Coot version 0.9.8.95)[6], thereby releasing these clashes (Fig. S2D, F). Notably, rigid body fitting of only $CB2_{SH3-}$ into the density map failed to converge on a consistent solution, as the final fitted positions were highly dependent on the initial starting orientations.

The model of the Geph-$CB_{1:1}$ complex was used for a rigid body fitting of the Geph-$CB_{1:1}$ complex dimer. The 'open' conformation of $CB2_{SH3-}$ in the Geph-$CB_{1:1}$ complex showed better correlation with the Geph-$CB_{4:1}$ complex map compared to the 'closed' conformation, and was therefore used for rigid body fitting.

References

1. Xiang, S. *et al.* The Crystal Structure of Cdc42 in Complex with Collybistin II, a Gephyrin-interacting Guanine Nucleotide Exchange Factor. *J Mol Biol* **359**, 35–46 (2006).
2. Macha, A. *et al.* Gephyrin filaments represent the molecular architecture of inhibitory postsynaptic densities.
3. Kim, E. Y. *et al.* Deciphering the structural framework of glycine receptor anchoring by gephyrin. *EMBO J* **25**, 1385–1395 (2006).
4. Pettersen, E. F. *et al.* UCSF ChimeraX: Structure visualization for researchers, educators, and developers. *Protein Sci* **30**, 70–82 (2021).
5. Harvey, K. *et al.* The GDP-GTP exchange factor collybistin: An essential determinant of neuronal gephyrin clustering. *Journal of Neuroscience* **24**, 5816–5826 (2004).
6. Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. *Acta Crystallogr D Biol Crystallogr* **66**, 486–501 (2010).