Supplementary figures

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Minor Research Internship Report

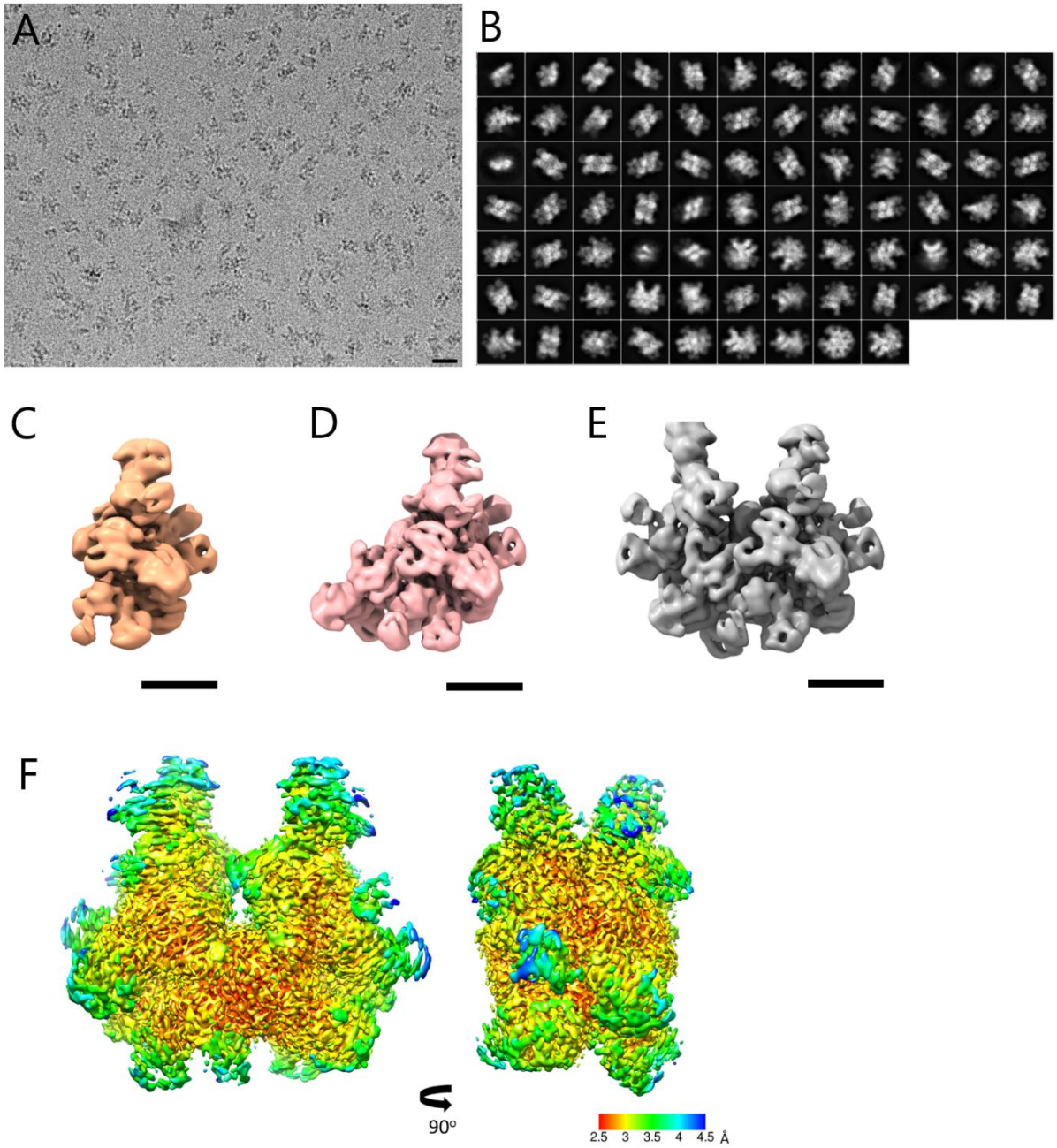

Figure S1: Cryo-EM data collection and processing of *Stanieria* sp. CphA2. A: Example micrograph collected during data collection on a Titan Krios TEM. Scalebar = 20 nm. B: 2D class averages obtained by 2D classification in RELION selected for further processing. C: 3D class average of dimeric *Stanieria* sp. CphA2 obtained by 3D classification in RELION. Scalebar = 50 Å. D: 3D class average of tetrameric *Stanieria* sp. CphA2 obtained by 3D classification in RELION. Scalebar = 50 Å. E: 3D class average of

dimeric *Stanieria* sp. CphA2 obtained by 3D classification in RELION selected for further processing. Scalebar = 50 Å. F: Map obtained after processing colored by local resolution.

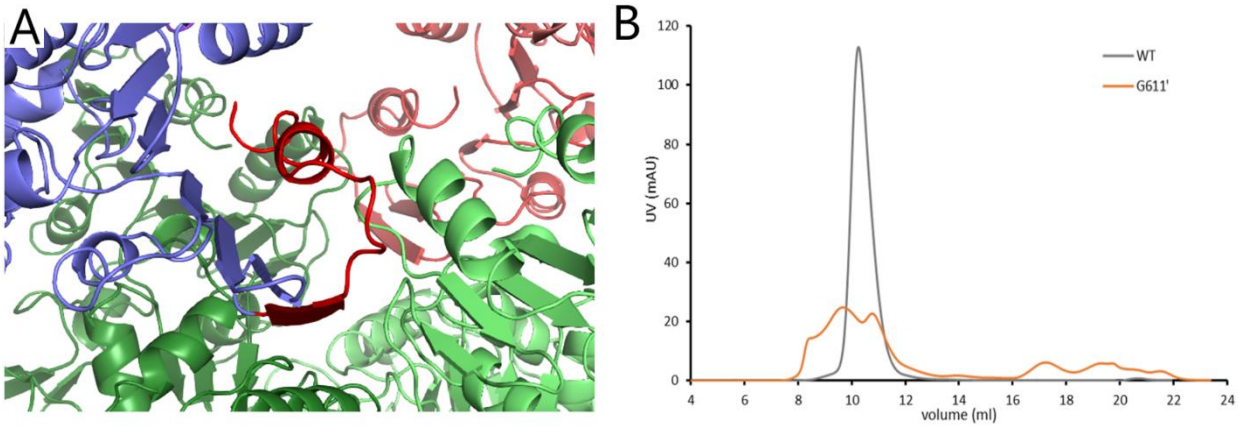

Figure S2: G611' truncation. A: Close-up of dimer-dimer interface framed in Fig. ? with region truncated by G611' mutation in red. B: SEC chromatogram of G611' mutant (orange) compared to WT (grey) *Stanieria* sp. CphA2.

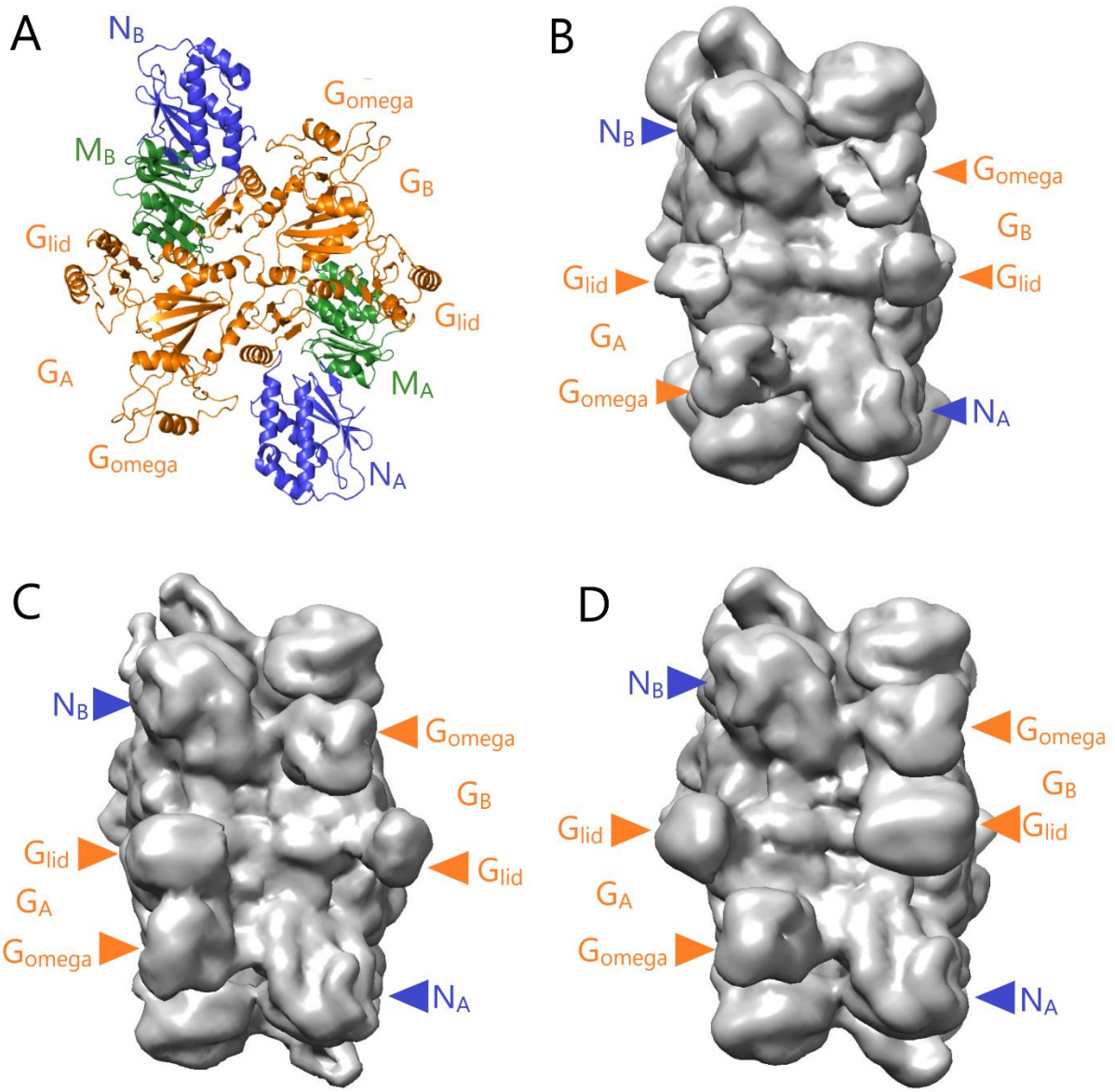

Figure S3: Comparison of (A) *Stanieria* sp. CphA2 structure to first frame of (B) movie 1, 3D variability analysis of *Stanieria* sp. CphA2 dataset, (C) movie 2, 3D variability analysis of *Stanieria* sp. CphA2 with cyanophycin, β-Asp-Arg, ADPCP dataset, (D) movie 3, 3D variability analysis of *Stanieria* sp. CphA2 with cyanophycin, succinyl-Arg, ATP dataset.

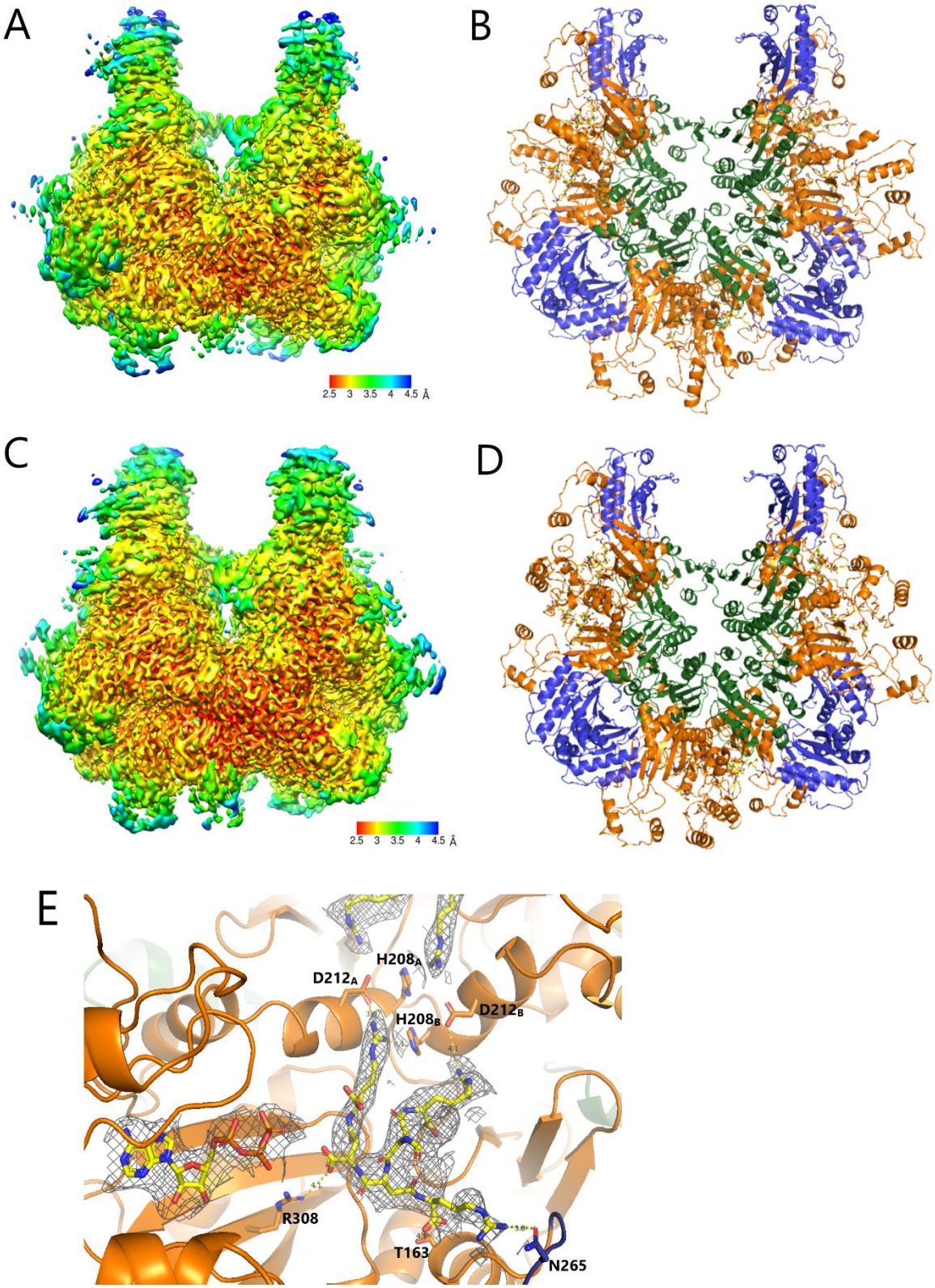

Figure S4: A: Map of *Stanieria* sp. CphA2 with cyanophycin, β-Asp-Arg and ADPCP dataset colored by local resolution. B: Hexameric structure of *Stanieria* sp. CphA2 with cyanophycin, β-Asp-Arg and ADPCP. C: Map of *Stanieria* sp. CphA2 with cyanophycin, succinyl-Arg and ATP dataset colored by local resolution. B: Hexameric structure of *Stanieria* sp. CphA2 with cyanophycin, succinyl-Arg and ATP. E: Structure of the *Stanieria* sp. CphA1 G domain complexed with cyanophycin $(\beta\text{-Asp-Arg})_4$ and ATP at 2.60 Å. The cryo-EM map was carved 2 Å around the substrates at level 2.0.

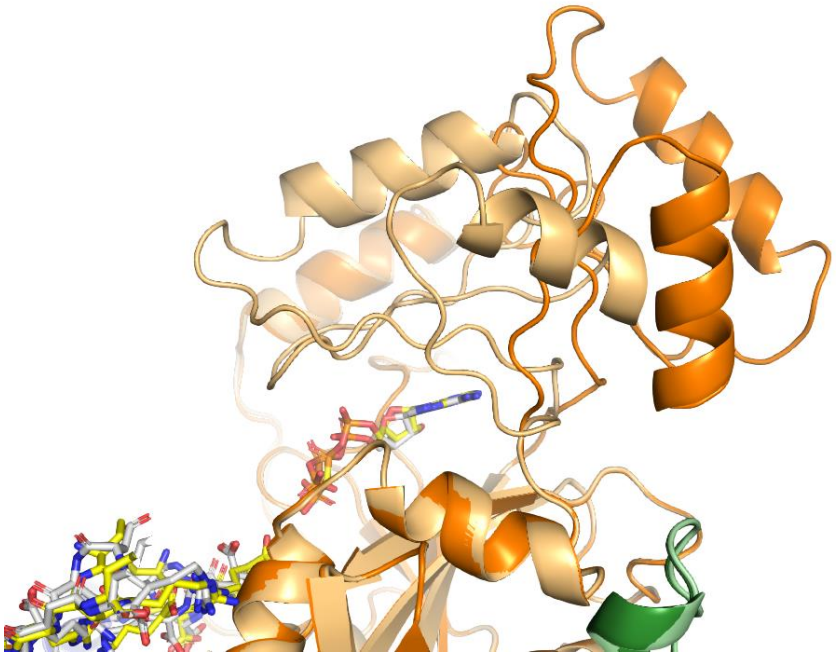

Figure S5: G_{lid} conformations built from dataset 2: *Stanieria* sp. with cyanophycin, β-Asp-Arg, ADPCP (bright orange) and dataset 3: *Stanieria* sp. with cyanophycin, succinyl-Arg, ATP (light orange). G_{lid} from dataset 2 shows the open conformation, G_{lid} from dataset 3 shows the closed conformation. Angle between the open and closed conformation was calculated to be 68 degrees.

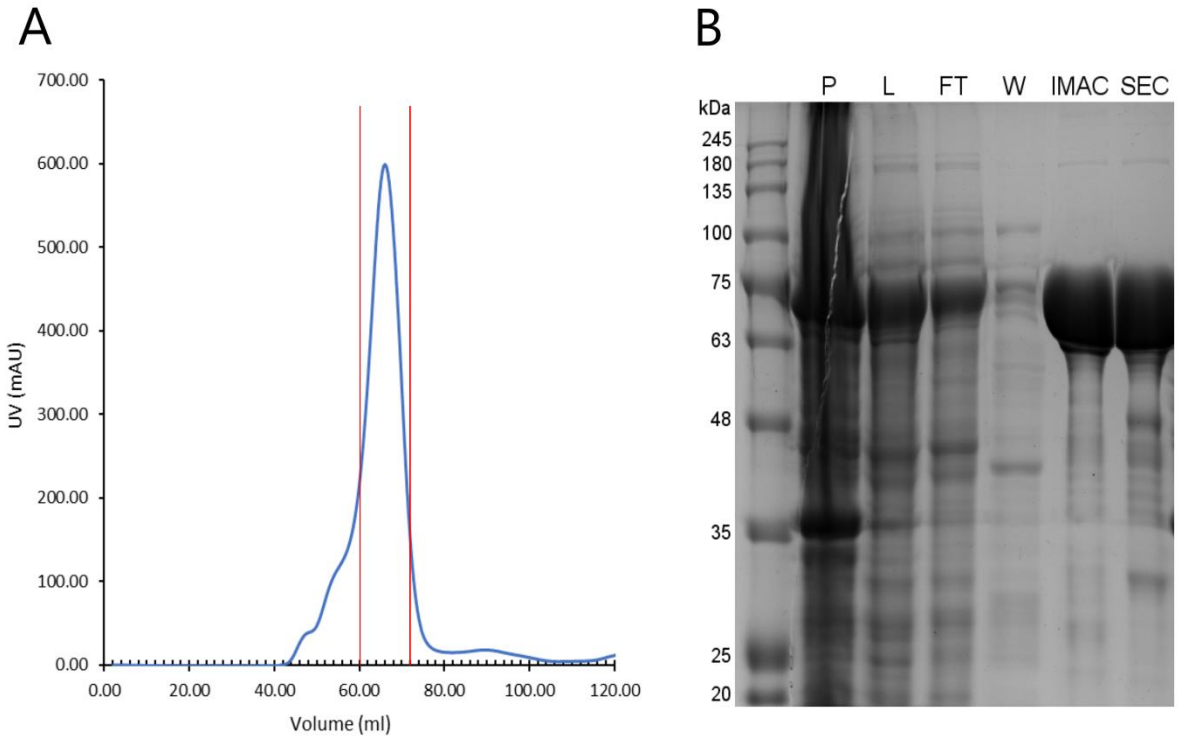

Figure S6: Purification of *Stanieria* sp.. A: SEC chromatogram obtained on a superdex 200 increase 16/60 column. Fractions between red lines were used for experiments. B: 11% SDS-PAGE gel of purification steps. P = pellet after lysis and centrifugation, L = clarified lysate, FT = flowthrough during IMAC, W = wash with 30 mM imidazole during IMAC, IMAC = IMAC elution with 250 mM imidazole, SEC = SEC fractions corresponding to fractions between red lines in A.

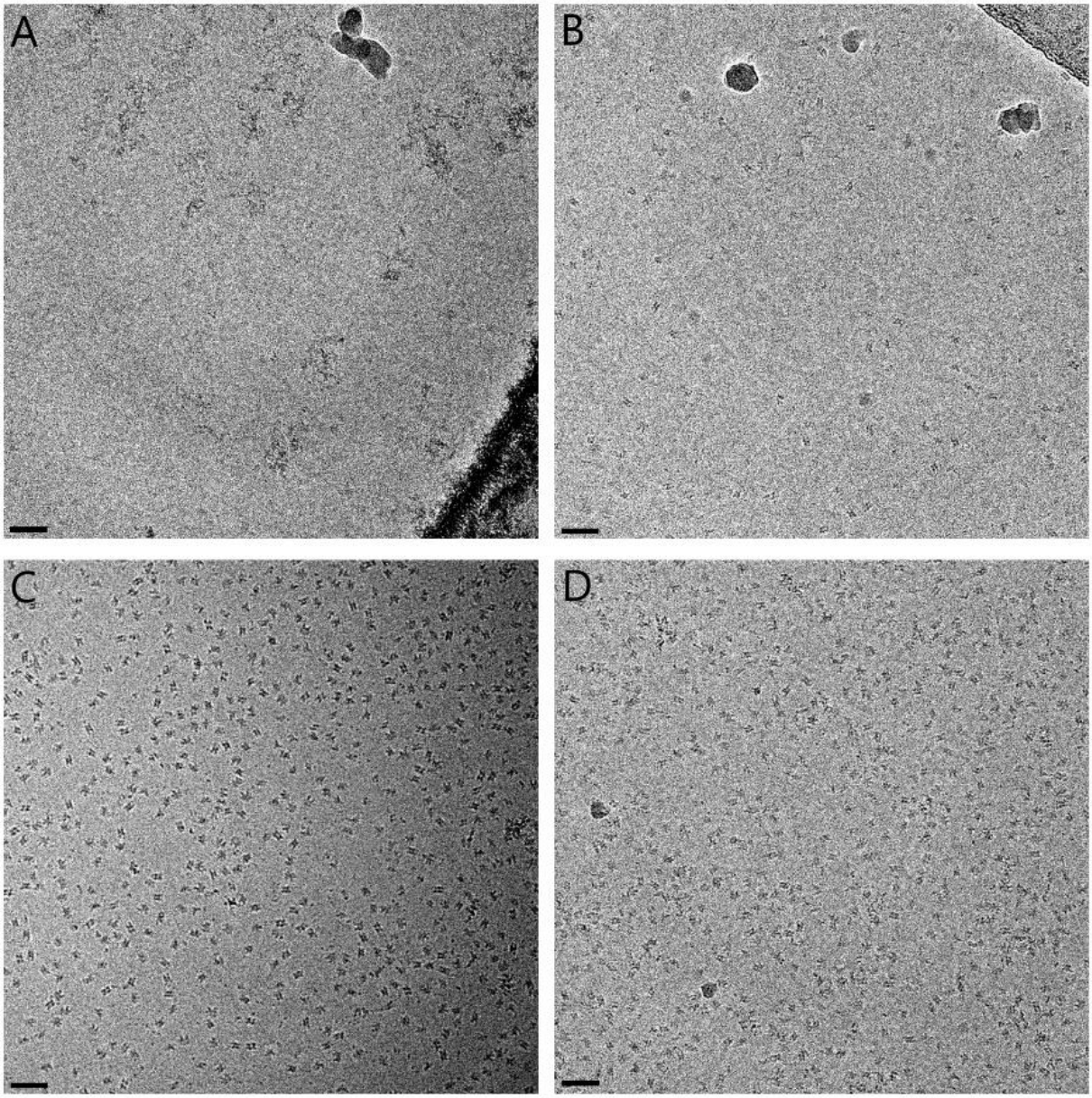

Figure S7: Optimization of sample conditions for cryo-EM. A: 0.3 mg/ml *Stanieria* sp. CphA2 on C-flat 2Cu T-50 grids. B: 0.3 mg/ml *Stanieria* sp. CphA2 on Quantifoil™ 200 Cu mesh grids. C: 1.8 mg/ml *Stanieria* sp. CphA2 on Quantifoil™ 200 Cu mesh grids. D: 2.2 mg/ml *Stanieria* sp. CphA2 on Quantifoil™ 200 Cu mesh grids. Scalebar = 50 nm.