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Supplemental Information

GraDeR: Membrane Protein Complex Preparation for Single-Particle Cryo-EM

Florian Hauer, Christoph Gerle, Niels Fischer, Atsunori Oshima, Kyoko Shinzawa-Itoh, Satoru Shimada, Ken Yokoyama, Yoshinori Fujiyoshi, and Holger Stark

Supplemental Information

Supplemental Data

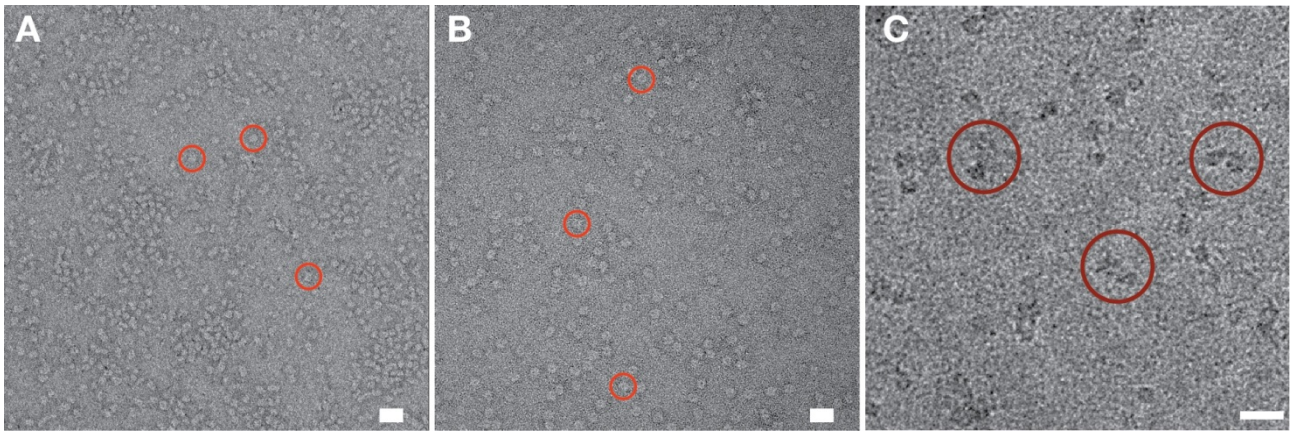

Figure S1, Related to Figure 1 and 2: Appearance of detergent micelles and small protein complexes in EM micrographs and best cryo-EM grid of DDM solubilized V-ATPase; scale bars 20 nm.

A) LMNG (0.02%) micelles in negative stain in the absence of protein. Red circles indicate individual LMNG micelles.

B) Protein complex heme-binding protein S (HbpS, 125 kDa) from *Streptomyces reticuli* in negative stain. Red circles denote exemplary HbpS complexes.

C) Cryo-EM image of the best cryo-grid obtained for DDM-solubilized V-ATPase.

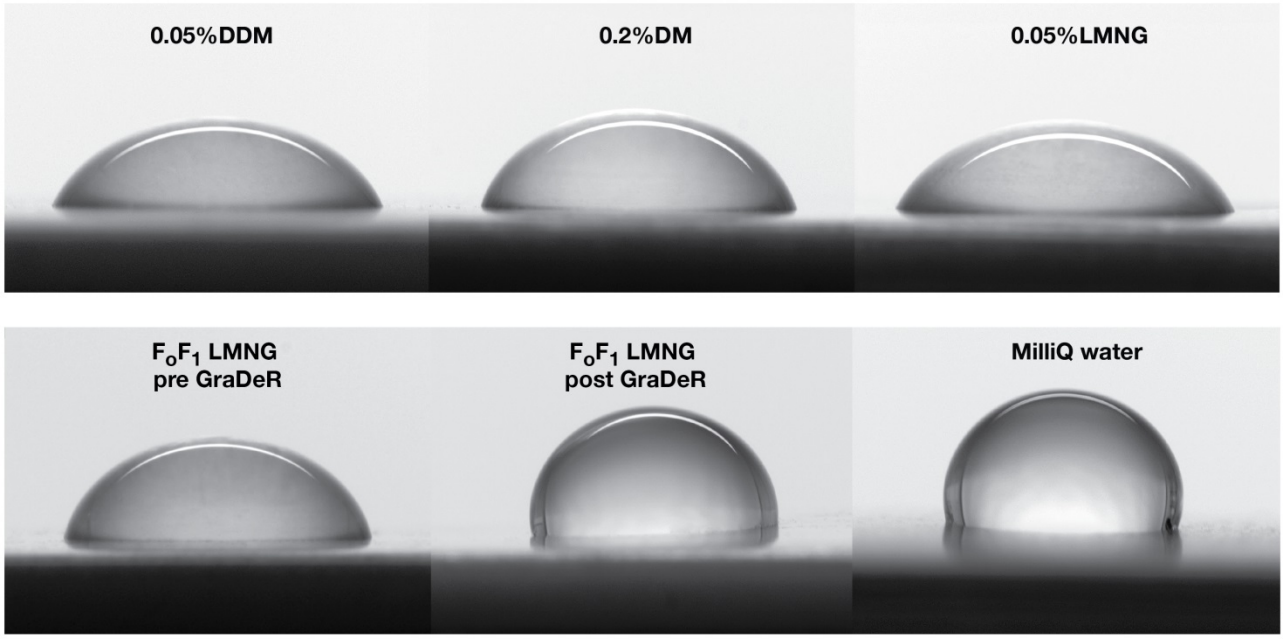

Figure S2, Related to Table 1: GraDeR and surface tension.

Drops of 20 µl buffer/water on freshly peeled Parafilm in the presence of detergent, F_oF_1-ATP synthase before GraDeR or after GraDeR and MilliQ water alone. Greater contact angle of the drop with the surface indicates stronger surface tension (Kaufmann et al., 2006), i.e. a lower concentration of surfactants at the air-water interface, which in turn corresponds to a lower surfactant monomer concentration in the bulk solution.

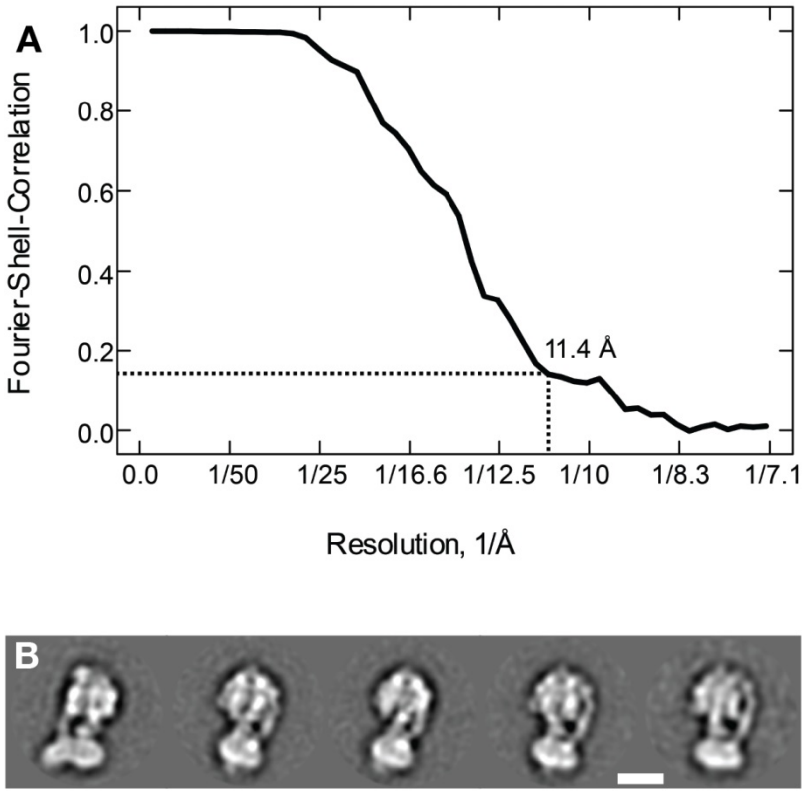

Figure S3, Related to Figure 3: Cryo-EM analysis of GraDeR-prepared bovine F_oF_1 ATP synthase.

A) "Gold-standard" Fourier shell Correlation (FSC) curve for the present cryo-EM map of bovine F_oF_1-ATP synthase indicating a resolution of about 11 Å.

B) Gallery of representative class averages of F_oF_1-ATP synthase; scalebar 10 nm.

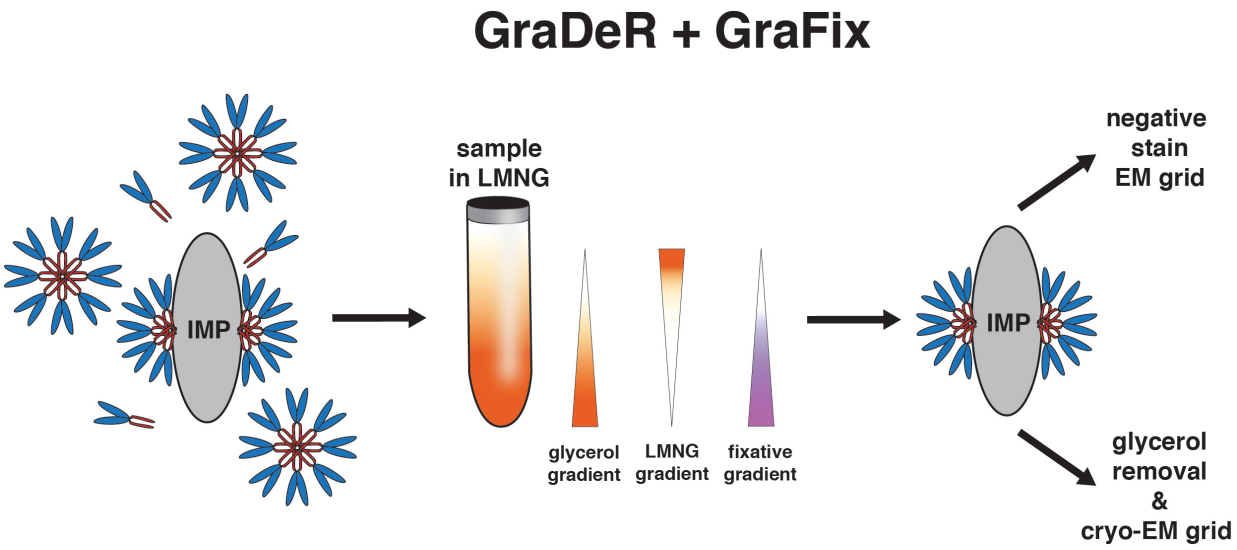

Figure S4, Related to Figure 1: Combination of GraDeR with GraFix.

GraDeR can be combined with GraFix by simply adding a fixative of choice to buffer B (high concentration of crowding agent) before mixing buffer solutions A and B in the GradientMaster for gradient preparation. This would for example result in a triple gradient of 10 - 30% glycerol, 0.003 - 0% LMNG and 0 - 0.05% glutaraldehyde.

Supplemental Experimental Procedures

Model of bovine F_oF_1-ATP synthase

Molecular models of F_oF_1-ATP synthase subcomplexes from X-ray crystallography were docked into the cryo-EM density map using UCSF Chimera (Pettersen et al., 2004), i.e. models were transformed into density maps, filtered to the resolution of the EM map (11 Å) and docked as rigid bodies by optimizing correlation with the EM map. First, the most-complete subcomplex structure, the F_1-c_8 (Watt et al., 2010), entailing the αβ-hexamer, the central stalk, and c_8-ring (pdb 2XND) was docked into the EM map. The fit revealed a significant deviation between the relative orientation of αβ-hexamer and central stalk in the F_1-c_8 subcomplex and the present structure of intact F_oF_1-ATP synthase. For a substantially improved fit, the αβ-hexamer had to be docked separately from central stalk and c_8 ring resulting in a rotation of the central stalk foot region by about 30° in comparison to the crystallographic subcomplex. Such a rotation requires flexibility of the central stalk in line with results from single molecule measurements (Sielaff et al., 2008). The well-defined cryo-EM density for the αβ-hexamer indicated a further re-arrangement within the αβ-hexamer: The α and the β subunit contacting the peripheral stalk in the present complex had to be fitted separately and shifted to describe the cryo-EM map. This shift increases the distance between two β-subunits, which is corroborated by cross-linking data reported previously for the intact bacterial F_oF_1-ATP synthase in the membrane and under proton motive force (pmf) (Masaike et al., 2006). Finally, the crystal structures of the peripheral stalk (pdb 2CLY) (Dickson et al., 2006) and the oligomycin sensitivity conferral protein, “OSCP”, (pdb 2WSS) (Rees et al., 2009) were docked into the EM density map. OSCP was initially placed using the full crystallographic complex including the αβ-hexamer and this fit was refined by docking the isolated OSCP.

Supplemental References

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