Supplementary Methods for Chappie et al.

Supplementary Methods

Expression of selenomethionine-labeled GG. Selenomethionine labeled protein was expressed in the absence of methionine auxotrophs as described previously[1] with the following modifications. Single colonies from transformations into BL21(DE3) cells were used to inoculate 50 ml of LB per liter of culture, which were grown overnight at 37°C in the presence of ampicillin (100 μg/ml). Starter LB cultures were harvested by centrifugation and each pellet was used to inoculate 1L of M9 minimal media (200 ml 5X M9 salts, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 1% glucose 100 μg/ml ampicillin). Cultures were grown at 37°C until the OD_{600} reached 1.0. Cultures were then shifted to 25°C and the following amino acids were added: 50 mg selenomethionine, 100 mg lysine-HCl, 100 mg threonine, 100 mg phenylalanine, 50 mg leucine, 50 mg isoleucine, and 50 mg valine. The cultures were left shaking at 25°C for 30 minutes and then induced with 0.4 mM IPTG. Induced cultures were grown at 25°C overnight (12-16 hours). Selenomethionine labeled pellets were harvested and purified in the same manner as native GG[2]. The selenomethionine-labeled GG dimer crystallized in the same conditions as the native GG.

X-ray data collection. Native data on a long axis crystal were collected at 95 K on a rotating anode source equipped with multilayer focusing optics using Cu Kα radiation and a Raxis IV image plate detector. Diffraction data on two Se-Met substituted crystals (a short and a long axis one) were collected at APS ID22 at 100K on a MAR300 CCD detector. All data were integrated, scaled internally and zero-dose corrected intensities[3] were estimated using XDS[4].

Structure solution and refinement. Attempts to solve the structures using molecular replacement with rat dynamin as search model (PDB: 2aka) were unsuccessful. Severe radiation

damage prevented the collection of complete data sets at different X-ray energies on a single SeMet substituted crystal and, due to the rather low, 39% solvent content, the single-wavelength anomalous diffraction (SAD) electron density maps were uninterpretable. The long axis form was solved with single isomorphous replacement with anomalous scattering (SIRAS) taking advantage of the Se-S isomorphous differences between native and SeMet substituted long axis crystals that were approximately isomorphous, and using also the anomalous differences from the SeMet substituted long axis crystal in the data set collected above the Se K absorption edge. Fa coefficients[5] were estimated with XPREP[6] and the 12 of the 16 possible Se sites were found with ShelxD[7]. Three additional sites were added, and positional and occupancy parameters of all Se atoms were refined with Sharp 2.2.0[8]. The map was solvent flattened with Solomon[9] and non-crystallographic averaging around the molecular two-fold axis was carried out with DM[9]. The resulting excellent quality map was displayed with O[10] and the GG model was built in manually. Several rounds of cartesian simulated annealing, restrained individual atomic displacement parameter refinement and energy minimization were carried out with CNS 1.1[11]. The refined long axis structure was used as a search model to solve the short axis form with molecular replacement using AMoRe[12], which was also refined with CNS 1.1. The current model for the long axis form contains two protein monomers that include all residues between Met6 and D320 of the G domain, the complete linker sequence between Lys321 and Val328, all GED residues from D726 to Gly743, 613 water molecules, and a GDP.AlF_4^- molecule, Mg^{2+} and Na^+ ion in each monomer. The short axis forms displayed more disorder, especially in some segments linking the core GTPase domain to the bundle signaling element. In this form, electron density wasn't visible between Lys315 and Asp325 in one monomer, while in the other monomer Ala22 to Asn26 and Arg318 to Asp325 are disordered. This structure includes 315 water molecules in addition to one GDP.AlF_4^- molecule, one Mg^{2+} ion and one Na^+ ion per monomer. Neither structure had any residues in the disallowed region of the Ramachandran plot. Buried surface

calculations were performed with MS[13] using a probe radius of 1.8Å. Inter-helix angles were computed using the program interhlx[14]. All molecular figures were made with Pymol[15].

Liposome preparation. For liposomes, a mixture containing 85% 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC; Avanti polar lipids) and 15% porcine brain PIP_2 (Avanti polar lipids) was prepared, dried and rehydrated in buffer (20 mM Hepes pH 7.5, 100 mM NaCl) to a final concentration of 2.5 mM (~2 mg/ml). Liposomes were prepared from this mixture by extrusion through polycarbonate membranes (Whatman) with a pore size of 0.1 μm using an Avanti Mini-Extruder following a series of freeze-thaw cycles. Liposomes were mixed with 0.5 μM dynamin at molar ratio of 1:300 for stimulated GTPase assays prior to adding GTP.

Biochemical Assays for GTPase activity and sedimentation. GTP was purchased from Jena Biosciences GmBH**.** Basal and liposome-stimulated GTP hydrolysis rates of WT and mutant dynamin were monitored as a function of time at 37°C using a malachite green-based colorimetric assay as described previously[16]. GTP was used at a final concentration of 1 mM. Sedimentation assays to examine the assembly properties of the full-length dynamin mutants were performed exactly as previously described[17].

Clathrin-mediated and fluid phase endocytosis assays. tTA-HeLa cells were infected with recombinant adenovirus encoding WT or mutant dynamin-1 and used 16 h after infection. Late events in clathrin-mediated endocytosis were measured as previously described[18] using biotinylated transferrin as ligand and assessing internalization into MesNa-resistant endocytic

compartments. Bulk, fluid phase endocytosis was assessed after incubating cells with horse-radish peroxidase (HRP) and measuring internalized HRP, as previously described[19].

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