Expression and Purification of *Dictyostelium* Recombinant Proteins

Dictyostelium cells were grown in HL5 medium (ForMedium^{TM}), at 21 ºC with continuous shaking (120 rpm) and the constructs in pDXA-3H plasmids were electroporated. Protein purification was carried out as follows: cells were harvested with 2700 rpm 7 min in a Beckman J2-MC centrifuge and washed with PBS (140 mM NaCl, 2.7 mM KCl, 10.1 mM $Na_2HPO_4x7H_2O$, 18 mM KH_2PO_4, pH 7.3). Cells were lysated in Lysis buffer (50 mM Tis-HCl pH 8.0, 2 mM EDTA, 0.2 mM EGTA, 3 DTT, 5 mM benzamidine and 40 µg/ml PMSF) with an ultrasound sonicator. After 1 hour incubation on ice the lysate was ultracentrifuged in a Beckman L7-65 Ultracentrifuge with 55,000 rpm 60 min 4°C. Pellets were homogenized in Extraction buffer (50 mM HEPES pH 7.3, 30 mM K-acetate, 10 mM Mg-acetate, 3 mM β-mercaptoethanol, 5 mM benzamidine and 40 µg/ml PMSF) and ultracentrifuged again with 55,000 rpm 45 min 4°C. Pellets were then homogenized in Extraction buffer with additional 10 mM ATP and 10 mM MgCl2 and ultracentrifuged again with 55,000 rpm 60 min 4°C. Supernatant was applied to a Ni-NTA (Qiagen) column and washed with Low salt buffer (50 mM HEPES pH 7.3, 30 mM K-acetate, 3 mM β-mercaptoethanol, 5 mM benzamidine) and High salt buffer (50 mM HEPES pH 7.3, 300 mM K-acetate, 3 mM β-mercaptoethanol, 5 mM benzamidine). After setting back the ionic strength the column was washed with Low salt buffer containing 50 mM imidazol. The elution was carried out with 0.5 M imidazol pH 7.3 with 3 mM β-mercaptoethanol, 5 mM benzamidine. After purification proteins were dialyzed against Assay Buffer and were held on ice or frozen in liquid N_2.