Purification of C. elegans CHIP and mutants

1. Basic Biochemical Data

	aa	MW	pI
CeCHIP	267	31.1 kDa	6.22
GST-CeCHIP	495	57.6 kDa	6.10
GST-CeCHIP-□TPR	392	45.8 kDa	5.43
GST	240	28.0 kDa	5.99

2. Buffers and Solutions

2 x YT-Medium:	16 g/l Yeast Extract, 10 g/l Tryptone, 5 g/l NaCl
Lysis Buffer:	50 mM Tris-Cl, pH 7.4; 1 mM EDTA, 100 mM $(NH_4)_2SO_4$, 5 mM DTT, 5% glycerol, 1 Tbl./50 ml complete^R Protease Inhibitors
Buffer A:	50 mM Tris-Cl, pH 8.2; 1 mM EDTA, 50 mM $(NH_4)_2SO_4$, 5 mM DTT, 5% glycerol
Buffer B:	50 mM Tris-Cl, pH 8.2; 100 mM NaCl, 1 mM DTT, 5% glycerol
Buffer C:	50 mM Tris-Cl, pH 8.2; 1000 mM NaCl, 1 mM DTT, 5% glycerol
Buffer D:	25 mM Tris-Cl, pH 8,2: 50 mM NaCl.

3. Overexpression and Purification of GST-tagged CeCHIP

Culture and Lysis:
Grow *E. coli* BL21(lDE3) containing CeCHIPpGEX-5X-1 in at least 100 ml LB + Amp at 37°C until OD_{600}=0.6-0.8. Dilute 4 x 25 ml of that culture into 4 x 1 l of 2 X YT + Amp and grow again at 37°C until OD_{600}=0.6-0.8. Induce with IPTG to final concentration of 0.4 mM. Incubate for 4-5 h at 20°C. Harvest bacteria and resuspend in 150 ml ice-cold Lysis Buffer. Sonicate on ice with 30-50 bursts. Centrifuge at 15000 rpm for 20 min in SA-600 rotor. Discard pellets, make supernatants to 150 ml with ice-cold Lysis Buffer.

Ammonium sulfate precipitation:
The protein extract contains 100 mM $(NH_4)_2SO_4$, what corresponds to about 20% saturation at room temperature. Slowly add solid $(NH_4)_2SO_4$, 89 mg/ml while gentle stirring (avoid foaming!) on a magnetic stirrer (cold room!) to obtain 35% saturation. Continue stirring for at least 1 hour. Centrifuge at 15000 rpm for 20 min in SA-600 rotor. Discard pellet. Add solid $(NH_4)_2SO_4$, 128 mg/ml, to obtain 55% saturation. Again, continue stirring and centrifuge. Now, discard supernatant and resuspend pellet in 40 ml

Buffer A. Dissolve pellet while gentle stirring in cold room overnight. The next day, centrifuge at 15000 rpm, 20 min, SA-600.

Affinity separation:
Equilibrate 7.5 ml GSH-Sepharose in ice-cold Buffer A. Apply supernatant to affinity gel in a 50 ml Falcon tube and incubate for at least 1 hour on rotating platform in cold room. Pour the slurry into empty column and let the fluid pass by gravity flow. Wash with 20 column volumes (CV, 150 ml) Buffer B. Elute with 45 ml Buffer B + 10 mM glutathione.

Anion exchange chromatography:
Use MonoQ HR5/5 column at a flow rate of 1 ml/min. Back pressure should not exceed 2.5 MPa. Wash with 5 CV (5 ml) Buffer C and equilibrate with Buffer B until baseline is stable. Apply eluate from the affinity step via superloop. Wash with 5 CV Buffer B. Elute with a gradient of 0-50% Bufffer C over 10 CV. GST-CeCHIP elutes at 23% Buffer C, which corresponds to a total NaCl concentration of 300-310 mM.
Pool the peak fractions. Dialyze against 2 x 2 l Buffer D overnight. Concentrate, freeze Protein in liquid N_2 and store aliquots at –80°C.

4. Overexpression and Purification of GST-tagged CeCHIP-ΔTPR

Culture and Lysis:
Proceed as with wild type. Grow *E. coli* BL21(ΔDE3) containing CeCHIP-ΔTPR-pGEX-5X-1 in at least 100 ml LB + Amp at 37°C until OD_{600}=0.6-0.8. Dilute 4 x 25 ml into 4 x 1 l of 2 X YT + Amp and culture at 37°C until OD_{600}=0.6-0.8. Induce expression with IPTG. Incubate for 4-5 h at 20°C. Harvest bacteria, resuspend in 150 ml ice-cold Lysis Buffer. Sonicate, clarify and make supernatants to 150 ml with ice-cold Lysis Buffer.

Ammonium sulfate precipitation:
The precipitation range of GST-CeCHIP-ΔTPR is between 35% and 60% $(NH_4)_2SO_4$. That corresponds to 89 mg/ml for the preclearing and 163 mg/ml for the precipitation. Dissolve the final precipitate in 40 ml Buffer A overnight and clarify the solution by centrifugation.

Affinity separation:
Equilibrate 7.5 ml GSH-Sepharose in ice-cold Buffer A. Apply supernatant to affinity gel and allow binding for 1 hour. Let the gel settle in an empty column, wash with 20 CV Buffer B. Elute with 45 ml Buffer B + 10 mM glutathione.

Anion exchange chromatography:
Apply eluate from the affinity step via superloop to washed and pre-equilibrated MonoQ HR 5/5. Wash with 5 CV Buffer B. Elute with a gradient of 0-50% Bufffer C over 10 CV. Although the pI is lower than that of the wildtype GST-CeCHIP, GST-CeCHIP-ΔTPR elutes around 23% Buffer C as well. Pool the peak fractions. Dialyze against 2 x 2 l Buffer D overnight. Concentrate, freeze Protein in liquid N_2 and store aliquots at –80°C.