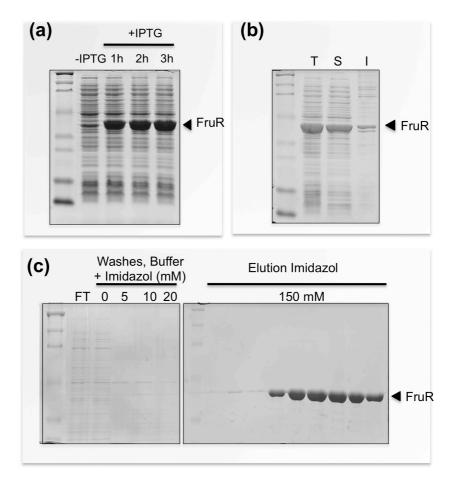
Supplemental Figure S1. Expression and purification of the Cra protein of Pseudomonas putida



(a) Expression test. A high expression level of Cra from the expression vector (explained in the main text of the article) was triggered by addition of 0.5 mM isopropyl-β-D-thiogalactoside (IPTG) followed by induction for a further 3 h. Cells were harvested by centrifugation and the cell pellets frozen at -80 °C. (b) Solubility test. Cell pellets were thawed, resuspended in 50 mM sodium phosphate buffer pH 7.0, 200 mM NaCl (1 mL of culture $OD_{600} = 0.5$ in $50 \mu L$) and mechanically lysed using a French press. Cell debris was removed by centrifugation (20,000 g for 30 min at 4 °C). Note that the majority of Cra protein remains in the soluble phase. **T** = total proteins (before lysis); **S** = soluble phase; **I** = insoluble phase (cell debris). (c) Protein purification. The native His₆-Cra protein isolated from the lysis supernatant was purified using a TALON Metal Affinity Resin (Clontech). The resin was equilibrated with 20 bed volumes of 50 mM sodium phosphate buffer pH 7.0, 200 mM NaCl (equilibration buffer 1X). The clarified extract was passed through the column and then washed successively with 10 bed volumes of equilibration buffer 1X, 10 bed volumes of equilibration buffer 1X plus 5 mM of Imidazol, 10 bed volumes of

- equilibration buffer 1X plus 10 mM of Imidazol and finally with 10 bed volumes of equilibration buffer 1X
- 2 plus 20 mM of Imidazol. The pure protein elution was done with equilibration buffer 1X plus 150 mM of
- 3 Imidazol. **FT** = Flow through.