Supplementary Material for

Enhanced Production of the Mical Redox Domain for Enzymology and F-actin Disassembly Assays

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Figure S1. Further analyses of Mical^{Redox} protein purification. (A-B) Unlike the expression and purification strategies we developed that allowed us to obtain high amounts of protein for the RedoxCH portion of the Drosophila and human MICALs [1-4], these strategies did not prove useful for expression and purification of high amounts of the Mical^{Redox} protein. In particular, in this example, similar to our strategy for expressing and purifying the RedoxCH portion of the Drosophila and human MICALs [3, 4], we used bacterial cells engineered to express cold-adapted chaperonin proteins Cpn60 and co-chaperonin Cpn10 from the cryophilic bacterium, Oleispira antarctica [5], and induction temperatures as low as 4-12°C. We also used the pET43.1bNG vector [6], which has a Nus solubility tag and has been used extensively to obtain purified active Mical^{RedoxCH} protein [2, 4, 7-11]. However, in the case of Mical^{Redox} protein, we found that although high levels of solubility-tagged Mical^{Redox} protein was expressed (A, large arrow), we could not obtain appreciable levels of Mical^{Redox} protein (B, arrow) following digestion with a protease to remove the solubility tag. Further, in the case of the Nus solubility-tagged Mical^{Redox} protein, additional analysis indicated that Mical^{Redox} protein became destabilized and precipitated from the solution after removal of the Nus solubility tag from the Mical^{Redox} protein (see Figure 2B). Cpn60 (open arrowhead in A). Nus solubility tag (arrowhead in B). I, input; F, flowthrough; M, protein markers; 3-17 (different fractions eluting from the Nickel-NTA column); U (undigested). D1 (thrombin digested at room temperature); D2 (thrombin digested at 4°C).



Figure S2. Further analyses of Mical^{Redox} protein's catalytic and F-actin disassembly activity. (A-C) NADPH consumption assays as in Figure 4B and where NADPH is present in all conditions. (A) NADPH consumption activity of Mical^{Redox} protein on its own (without its F-actin substrate). Our newly purified Mical^{Redox} protein has enzyme activity (Figure 4B). Furthermore, similar to what we have previously reported for Mical^{RedoxCH} protein (e.g., [4, 7]), Mical^{Redox} protein on its own (without F-actin) has little NADPH consumption activity (2), but Mical's substrate F-actin substantially increases Mical's NADPH consumption activity (e.g., compare the graph in (1) and note that it is at the same timescale as Figure 4B, but with substantially less NADPH consumption). [Mical^{Redox}] = 50 nM as in Figure 4B. [NADPH] = 200 μ M. Time = 180 seconds (s) in (1) and 60 minutes (min) in (2). (B) Rate of NADPH consumption. (1) Mical^{Redox} protein's calculated rate of NADPH consumption. A similar activity for the Mical^{Redox} protein was reported in [11] and corresponds in general to that seen for the Mical^{RedoxCH} protein [4, 7]. (2) The fold difference in the NADPH consumption at 340 nm) for the different conditions versus F-actin only. NADPH present

in all conditions. [Mical^{Redox}] = 50 nM. [NADPH] = 200 μ M. [F-actin] = 18.4 μ M as in Figure 4B. (C) Mical^{Redox} protein dosage-dependent differences in NADPH consumption. Mical^{Redox} protein shows a dosage dependent increase in enzymatic activity such that it consumes more NADPH as it is added in higher concentrations. Note also that as the F-actin concentration is increased, the NADPH consumption of a given [Mical^{Redox}] substantially increases (e.g., compare the blue bars ([50 nM]) in C to B (2)). NADPH present in all conditions. Buffer only and Mical^{Redox} only do not contain F-actin as in A and B. [NADPH] = 200 μ M; [F-actin] = 2.3 μ M. [Mical^{RedoxCH}] is as indicated in each bar of the graph, which is also color-coded to the inset graph (change in absorbance (340 nm) as in A). (D) Our newly purified Mical^{Redox} protein has F-actin disassembly activity (Figure 4D). Furthermore, using Pyrene-actin depolymerizes) and similar to what we have previously reported for Mical^{RedoxCH} protein (e.g., [2, 4, 7]), we find that as the levels of our new Mical^{Redox} protein increases. F-actin disassembly increases. [Mical^{Redox}] as indicated = 50 nM or 100 nM; [NADPH] = 100 μ M; [F-actin] = 4.65 μ M.

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