SUPPORTING INFORMATION

Transient kinetic analysis of USP2-catalyzed deubiquitination reveals a conformational rearrangement in the K48-linked diubiquitin substrate

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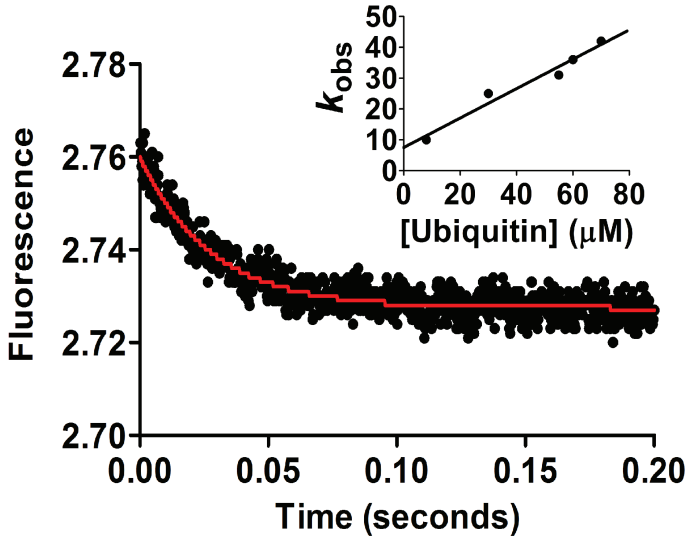

Figure S1. Stopped-flow fluorescence kinetic traces of the binding of ubiquitin to the C276A USP2 catalytic core. Stopped-flow fluorescence traces were obtained by monitoring the C276A USP2 tryptophan fluorescence quenching caused by ubiquitin binding. The data were fit to a single exponential function (solid red line) using GraphPad Prism (GraphPad Software, CA). The fitted rates were plotted against the concentration of ubiquitin (inset). The binding and dissociation rate constants, k_{on} and k_{off}, were determined through linear regression analysis (described in the Experimental Procedure section).

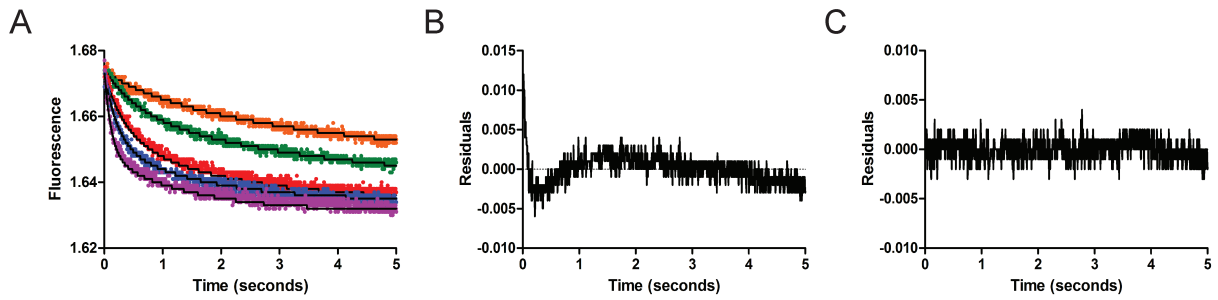

Figure S2. Stopped-flow fluorescence kinetic traces of the binding of native K48-linked diubiquitin to the C276A USP2 catalytic core. Stopped-flow fluorescence spectroscopy was used to follow complex formation over time. (A) USP2 tryptophan fluorescence quenching was monitored over 5 seconds for the native K48-linked diubiquitin binding to C276A USP2. Diubiquitin concentration was varied (orange is 2 μM, green is 4 μM, red is 8 μM, blue is 16 μM, and violet is 32 μM) while C276A USP2 concentration was held constant at 1 μM. The black lines overlaid onto each fluorescence trace are the result of a double exponential fitting. Residuals for the single (B) and double (C) exponential fitting of the representative binding data for 16 μM diubiquitin and 1 μM USP2 are shown. Analyses of the residuals reveal that a double exponential function provides a significantly better fit to the data.

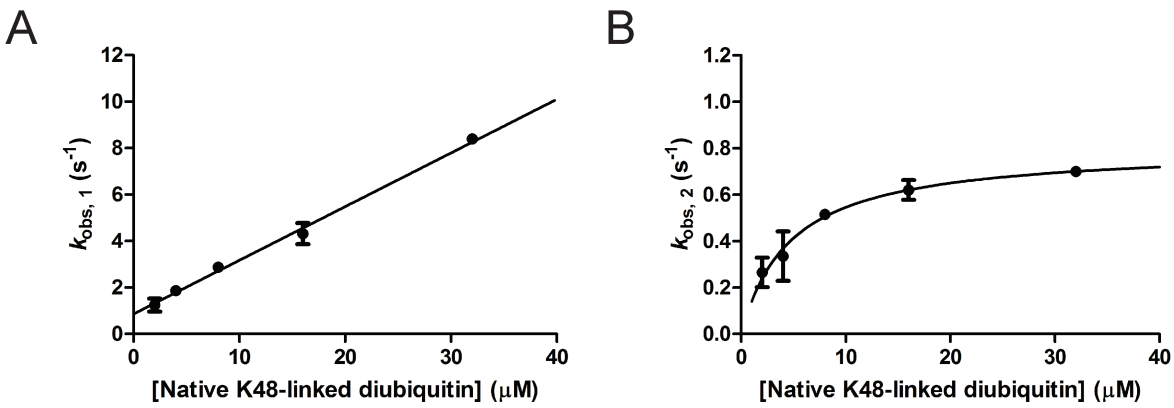

Figure S3. Concentration dependence of observed rates of native K48-linked diubiquitin binding to the C276A USP2 catalytic core. The double exponential rate constants $k_{obs,\ 1}$ and $k_{obs,\ 2}$ determined by fitting the fluorescence data reporting the native K48-linked diubiquitin binding to USP2 (see Figure S2) were plotted against the concentration of diubiquitin.

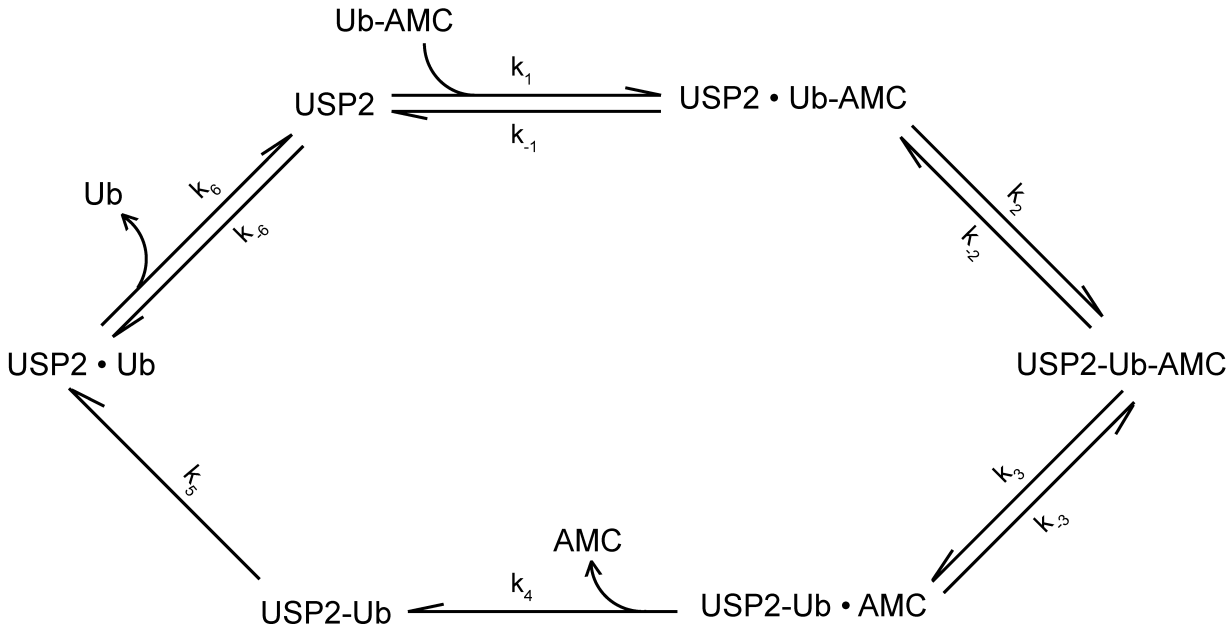

Figure S4. A more complex kinetic mechanism for USP2-catalyzed hydrolysis of Ub-AMC. This model allows for the formation of a tetrahedral intermediate (USP2-Ub-AMC), followed by a chemical step accounting for the collapse of the intermediate which yields the acyl-enzyme intermediate (USP2-Ub • AMC) where AMC is now non-covalently bound to USP2. This is followed by a step accounting for the dissociation of AMC.