Supplemental Information

Insights into the mechanism of deubiquitination by JAMM deubiquitinases from co-crystal structures of enzyme with substrate and product

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Supplemental Figure 1: S75 size exclusion chromatography profile and SDS-PAGE gel of the 3-protein complex containing Sst2 Δ^{220} , the UIM-SH3 domain of HseI, and K63-Ub₂.

Supplemental Figure 2: Crystal packing diagram of the Sst2^{Δ220}-K63-Ub₂ structure showing symmetry-related molecules (pink). The proximal ubiquitin moiety (green) makes relatively fewer contacts with neighbors, which may explain the positional disorder we are observing.

Supplemental Figure 3: Crystal structures of Sst2^{cat} and Sst2^{cat}E286A bound to ubiquitin (Ub). (a) Monomer of Sst2^{cat}E286A-Ub structure. The JAMM core, Ins-1 and Ins-2 are shown in white-blue, yellow and green respectively and ubiquitin is shown in orange. (b) Monomer of the Sst2^{cat}-Ub structure. Sst2^{cat} and Ub are shown in limon and dirty violet respectively. Insertion-1 (Ins-1) and insertion-2 (Ins-2) are shown in yellow and green respectively. (c) Superposition of active site zinc coordination of Sst2^{cat}-Ub and Sst2^{cat}E286A-Ub complexes revealing two modes of product binding in the active site.

Supplemental Figure 4: Crystal structures of Sst2^{cat} bound to ubiquitin (Ub). (a) Dimeric structure of Sst2^{cat} bound to Ub in crystals. Monomers are colored limon and orange for Sst2^{cat} and Ub respectively for chain A and B, and teal and orange for Sst2^{cat} and Ub respectively for chain C and D. The inset shows zinc-mediated packing at the interface. (b) Expanded view of zinc coordination at the interface. The electron density is rendered from a $2F_o - F_c$ map contoured at 1.2σ . The coordinating residues are shown as sticks, with carbon shown in limon or teal, oxygen shown in red and, nitrogen shown in blue. Zinc is shown as a gray sphere and chloride as a green sphere.

Supplemental Figure 5: Sequence alignment of the MPN domains for Sst2 (*S. pombe*) and other JAMM metalloproteases: AMSH, AMSH-LP, POH1 (*H. sapiens*), Rpn11 (*S. cerevisiae*), and BRCC36. Residues involved in coordination of the catalytic zinc are absolutely conserved (purple pentagon), while those involved in coordination of the structural zinc (cyan pentagon) from Ins-2 are not conserved. Residues involved in recognition of the distal ubiquitin are largely conserved (orange rectangles) while those involved in proximal ubiquitin recognition (green rectangles) are not.

Supplemental Figure 6: Interaction of Gly76 of ubiquitin with Glu286 of Sst2. (a) Active site of Sst2^{cat} (limon) bound to ubiquitin (orange) showing that Glu286 of Sst2 forms a hydrogen bond with the carboxylate group of Gly76 of ubiquitin. (b) Active site of Sst2^{D354A} (green) bound to ubiquitin (orange) showing that Glu286 of Sst2 in its apo form can also form a hydrogen bond with the carboxylate group of Gly76 of ubiquitin.

Supplemental Figure 7: To study whether dynamics from the active site flap contribute to linkage specificity, two concentrations of wild-type AMSH and a point mutant in the active site flap, F395A were incubated at room temperature with 20 μM Lys48 or Lys63-linked diubiquitin for 5 hours (above) or 23 hours (data not shown). Mutation of Phe395 to alanine did not lead to a change in linkage specificity; like the wild-type enzyme, Phe395Ala was unable to cleave Lys48-linked diub.

AMSH and F395A were purified as described previously(1). Lys48-linked diubiquitin was synthesized enzymatically from ubiquitin using ATP, human E1, and the E2 Cdc34 following previously reported procedures(2), substituting Cdc34 for the E2 complex of Ubc13 and Uev1a. The reaction mixture was incubated at 37 °C for 3.5 hours and was quenched by diluting in 50 mM sodium acetate, pH 4.5. The quenched reaction mixture was subjected to ion-exchange chromatography on a Mono-S column (GE Biosciences), allowing separation of Lys48-diubiquitin.

Supplemental Figure 8: Unlike AMSH, Sst2 appears to cleave Lys48-linked diubiquitin, albeit with significantly lower efficiency than the Lys63-linked chains. After incubating 250 nM Sst2 with 20 μ M diubiquitin, product formation from Lys63-linked diubiquitin is complete within 1 hour, while Sst2 takes 23 hours to produce an appreciable amount of product from Lys48-linked diubiquitin.

References

1. Davies, C. W., Paul, L. N., Kim, M. I., and Das, C. (2011) Structural and Thermodynamic Comparison of the Catalytic Domain of AMSH and AMSH-LP: Nearly Identical Fold but Different Stability, *Journal of Molecular Biology* 413, 416-429.
2. Sato, Y., Yoshikawa, A., Yamagata, A., Mimura, H., Yamashita, M., Ookata, K., Nureki, O., Iwai, K., Komada, M., and Fukai, S. (2008) Structural basis for specific cleavage of Lys 63-linked polyubiquitin chains, *Nature* 455, 358-362.