

Supplemental Data

Site-specific differences in proteasome-dependent degradation of monoubiquitinated α -synuclein

Tharindumala Abeywardana,¹ Yu Hsuan Lin,¹ Ruth Rott,² Simone Engelender,^{2,4} and Matthew R. Pratt^{1,3,4}

¹Department of Chemistry, ³Department of Molecular and Computational Biology
University of Southern California, Los Angeles, CA 90089

²Department of Pharmacology, The B. Rappaport Institute of Medical Research, Technion-Israel
Institute of Technology, Haifa 31096, Israel

⁴Correspondence should be addressed to matthew.pratt@usc.edu or simone@tx.technion.ac.il

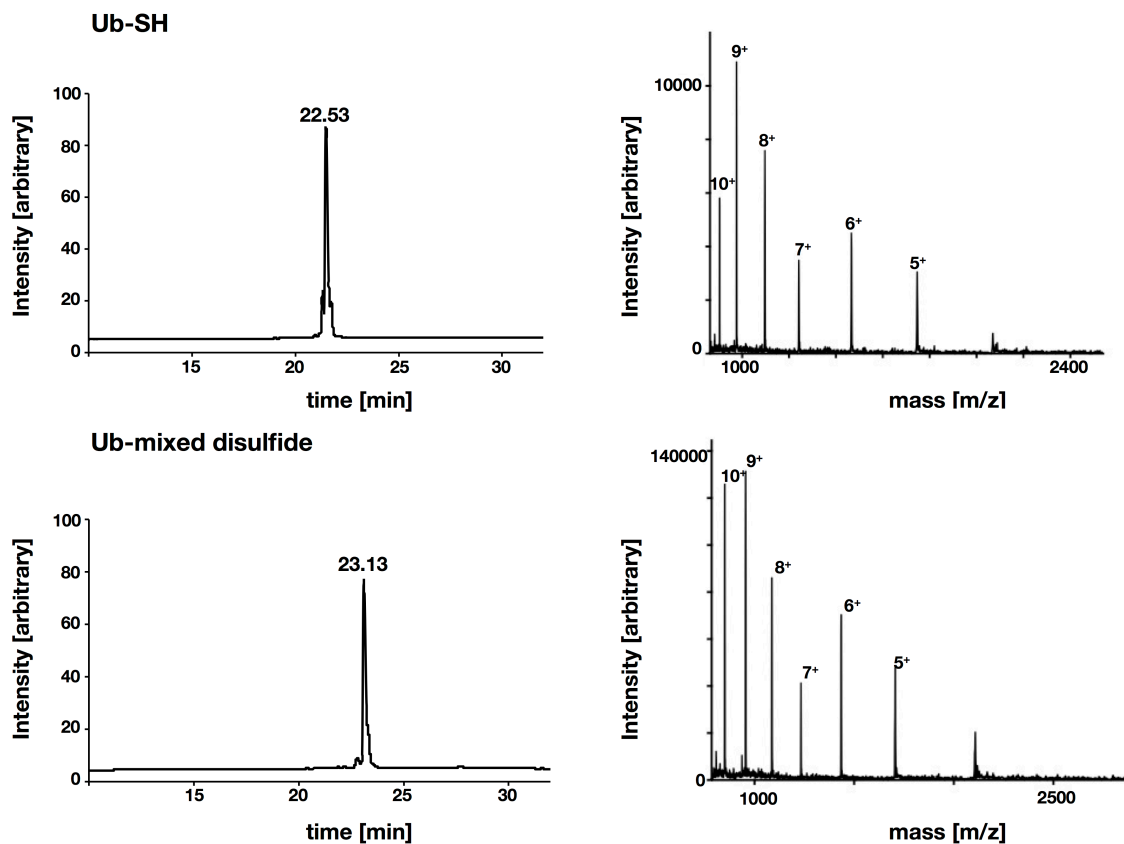


Figure S1. Characterization ubiquitin C-terminal thiol and mixed disulfide, related to Figure 1A.

Ubiquitin C-terminal thiol and the corresponding mixed disulfide were purified by RP-HPLC and characterized by mass spectrometry.

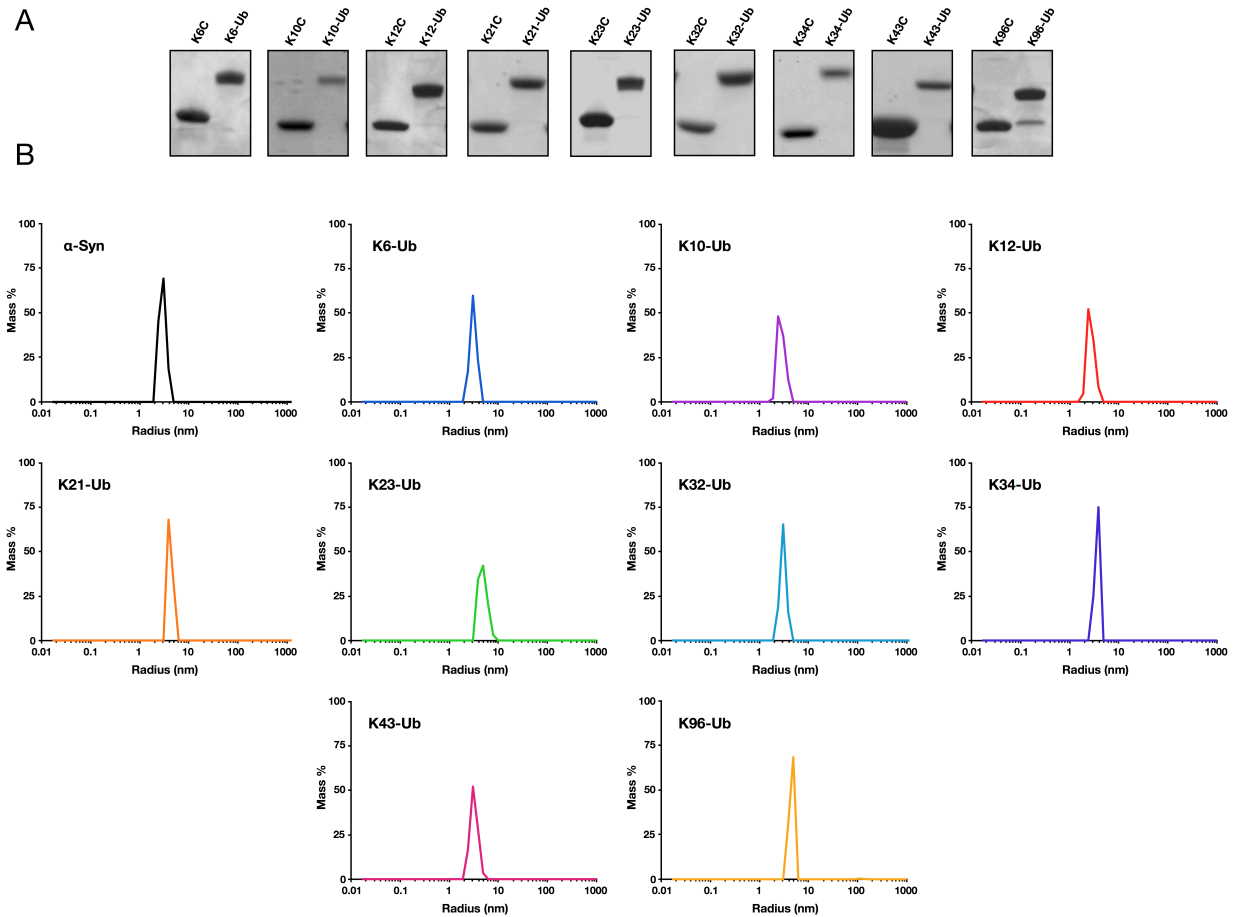


Figure S2. Characterization of unmodified and monoubiquitinated α -synuclein proteins, related to Figure 1.

A) Unmodified α -synuclein and all nine monoubiquitinated analogs were separated by SDS-PAGE and visualized by coomassie blue staining. B) Unmodified α -synuclein and all nine monoubiquitinated analogs were analyzed by light scattering and plotted as mass percentage against Stokes radius.

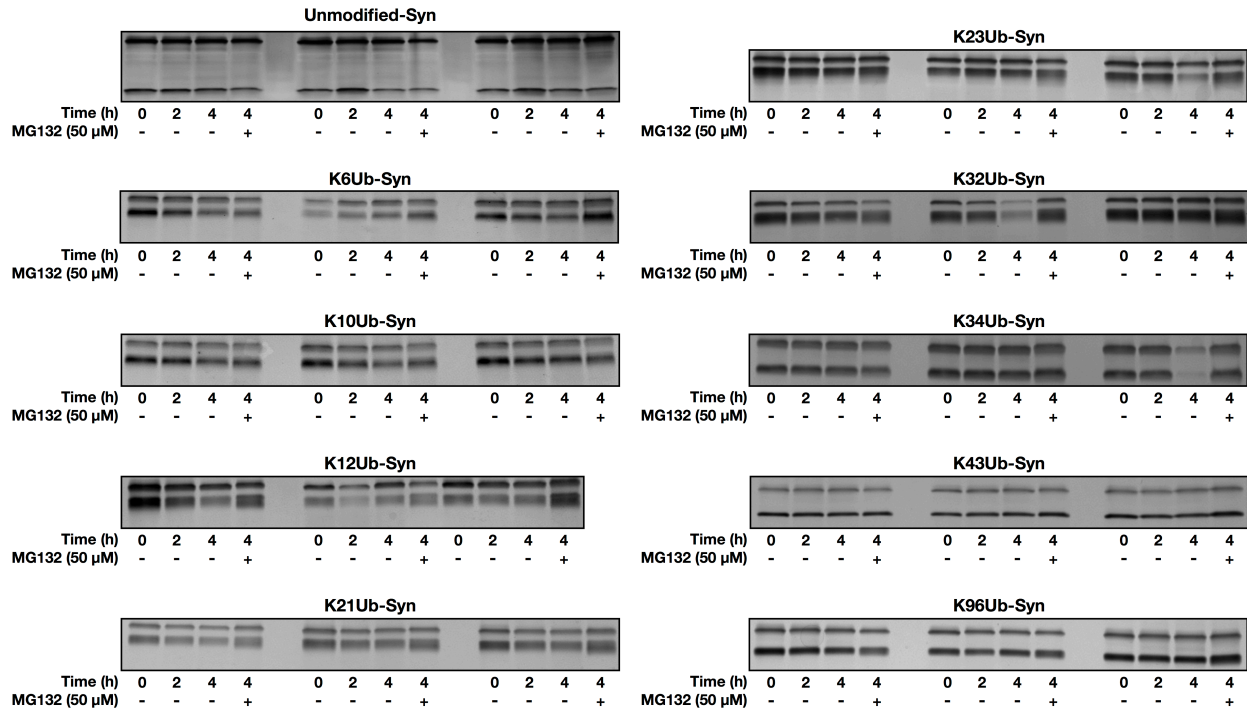


Figure S3. Proteasome-dependent degradation of monoubiquitinated α -synuclein, related to Figure 3.

Unmodified or monoubiquitinated α -synuclein proteins were incubated in triplicate with 26S proteasome for the indicated lengths of time. The proteasome inhibitor MG132 (100 μ M) was added as indicated. The proteins were separated by SDS-PAGE and stained with colloidal silver.