



Figure S1: The ssNEDP1 protease can be used for efficient on-column cleavage of ssNEDD8- and bdNEDD8-tagged proteins.

A: A Ni^{2+} chelate resin was loaded with 100 μ M His₁₄-ssNEDD8-MBP and incubated with the indicated concentrations of ssNEDP1 for 1h at 0°C. Eluted proteins were collected by centrifugation. The non-cleaved target protein remaining on the resin was solubilized using SDS sample buffer containing 500mM imidazole. Protein samples were resolved by SDS-PAGE. Efficient on-column cleavage required \approx 300-600nM ssNEDP1 protease.

B: A Ni^{2+} chelate resin pre-loaded with similar amounts of His₁₄-bdSUMO-GFP and His₁₄-bdNEDD8-mCherry was treated with indicated concentrations ssNEDP1 for 1 hour at 0°C essentially as described in Figure 4. Efficient on-column cleavage of the bdNEDD8-tagged target protein required 300-600nM of the orthologous ssNEDP1 protease. Even at a \geq 30-fold higher protease concentration, no significant elution of the bdSUMO-tagged target protein was evident. The elution is therefore specific.