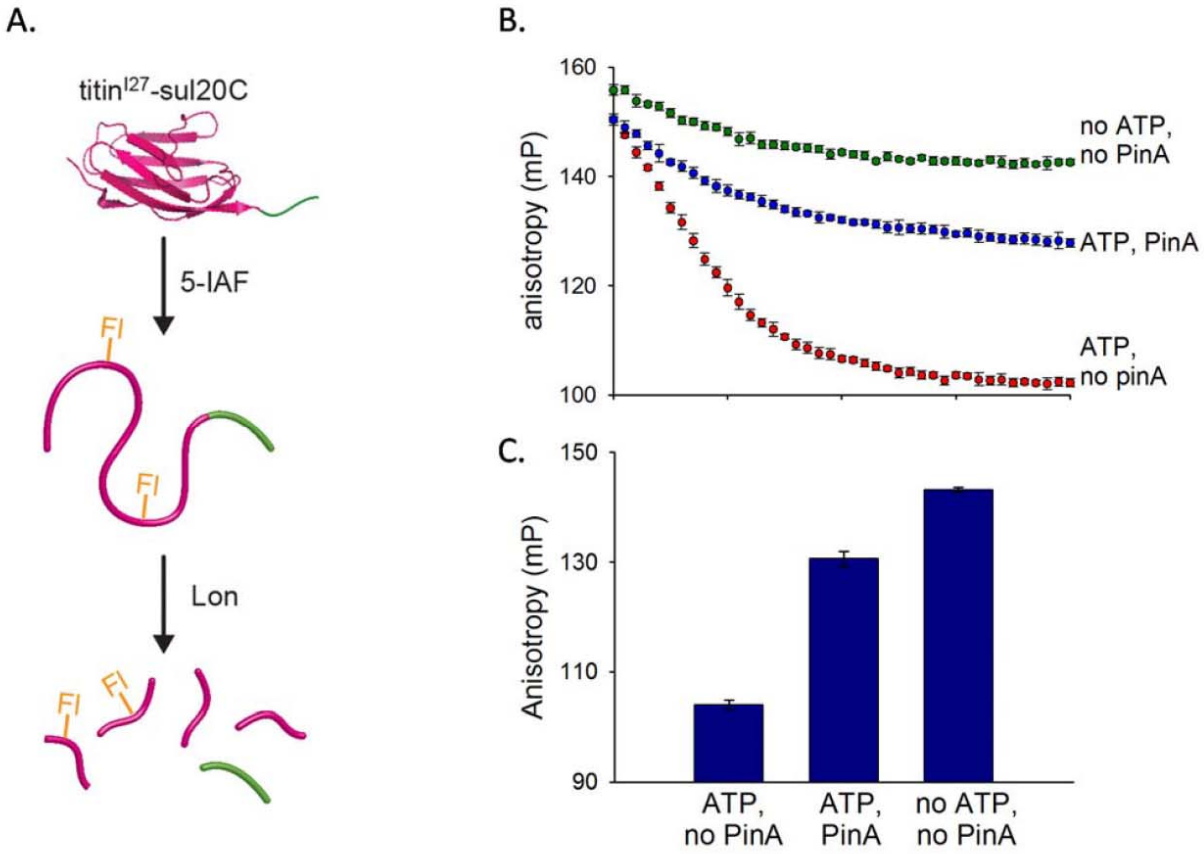

Fig. 1. A high throughput screening assay for Lon activity. (A) Fluorescein-labeling of titnI27-sul20C results in protein unfolding. Degradation of the resulting protein leads to formation of small peptides, some of which are fluorescein labeled. (B) The degradation of the fluorescein-labeled substrate can be assayed by fluorescence anisotropy measurements. The reaction is inhibited in the absence of ATP, or in the presence of PinA, a known Lon inhibitor. (C) As in B, but end-point measurements are presented.

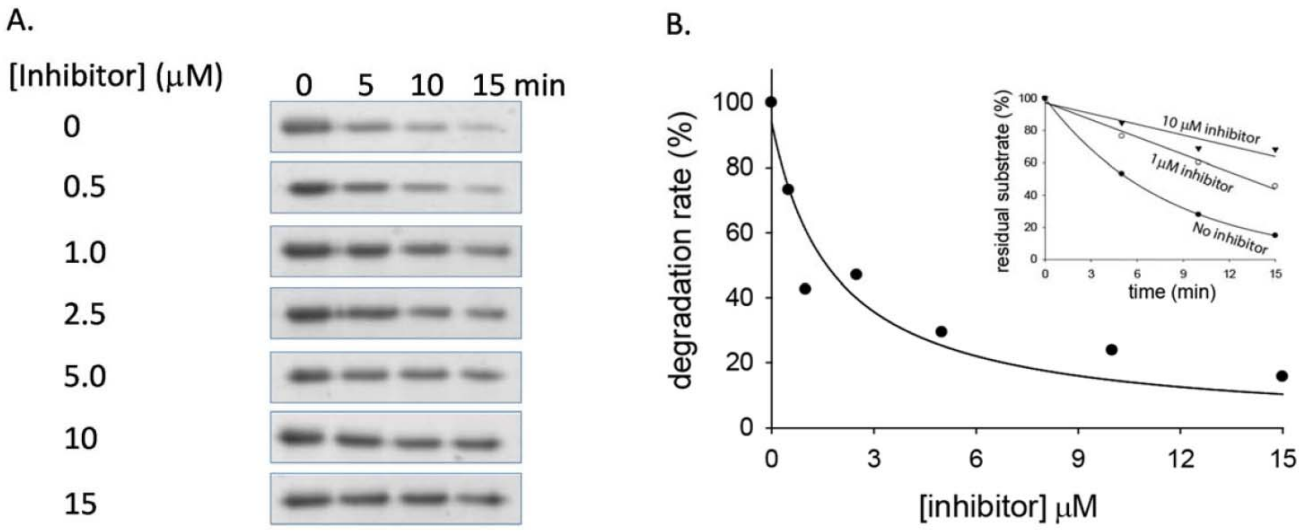

Fig. 2. Identification of a small molecule Lon inhibitor. (A) Degradation of α-casein (5 μM) by Lon (0.3 μM) at increasing inhibitor concentrations. Gels were stained by Coomassie brilliant blue. (C) The results presented in B were quantified by densitometry and the data was fitted to a competitive inhibition equation.

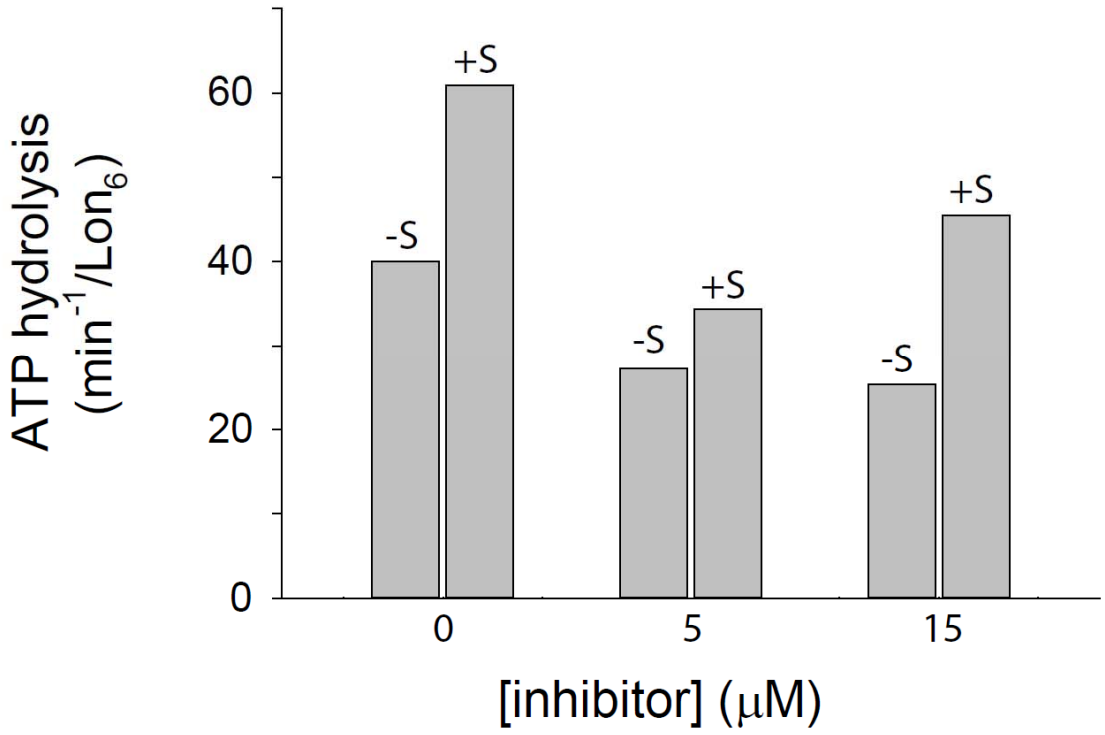

Fig. 3. ATPase inhibition by the identified inhibitor. The ATPase rate of Lon was measured in the presence (+S) and in the absence (-S) of α-casein at the indicated inhibitor concentrations.

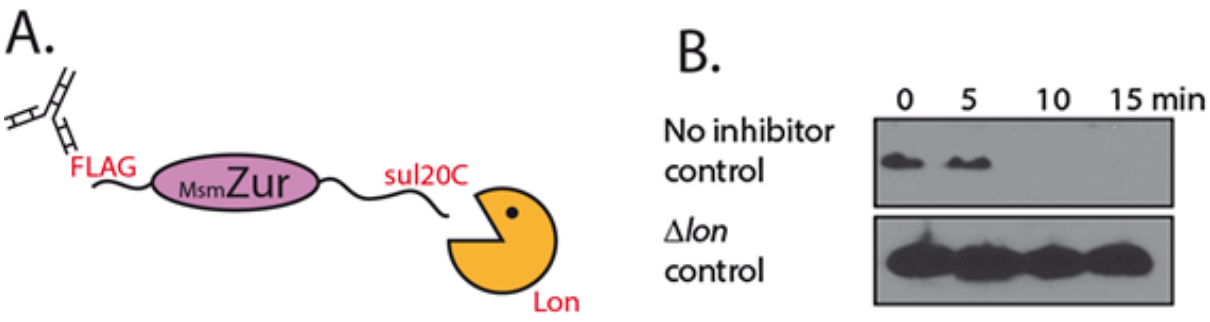

Fig. 4. An *in vivo* Lon degradation assay. (A) Illustration of the Zur fusion used in the *in* vivo assay. (B) Samples were collected as described in the text and Western analysis was performed using antibodies against a FLAG tag.

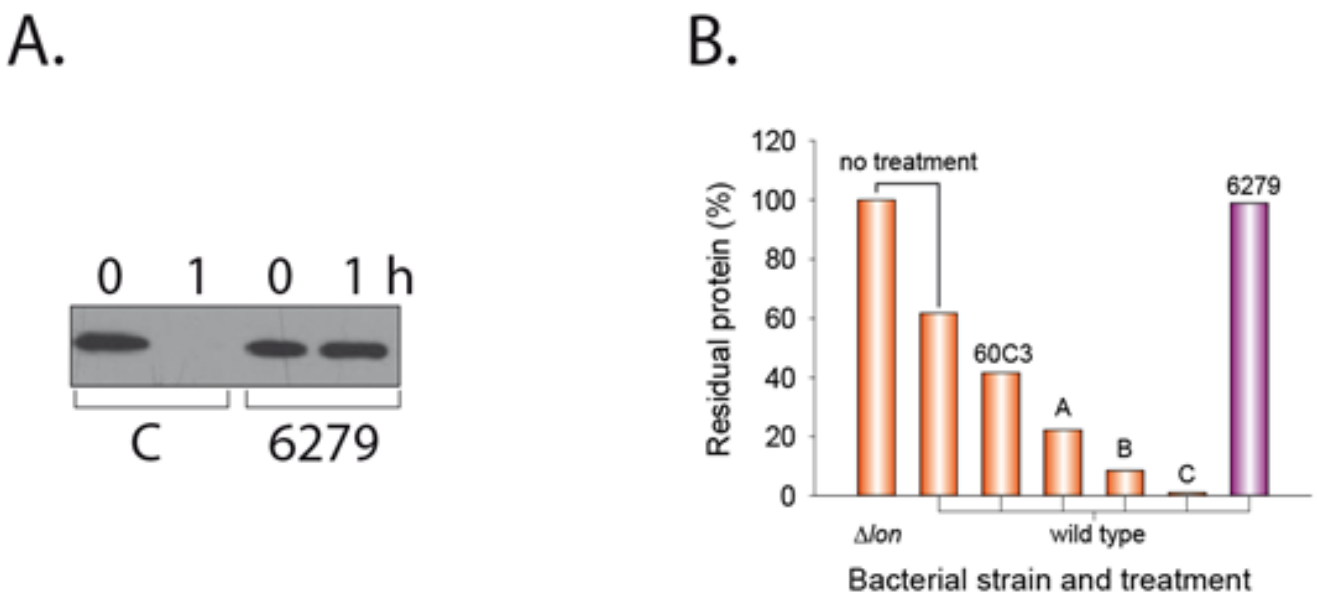

Fig. 5. Identification of an in vivo Lon inhibitor. (A) Cells were grown in minimal media to mid-exponential phase and each tested compound was added to the medium at a final concentration of 0.5 mM. Following 30 min, L-arabinose (2 % (w/v) was added for FLAG-Zur-sul20C induction. Following 30 more min, samples were collected, rifampicin was added and the cultures were incubated for an additional hour before samples were collected again. FLAG-Zur-sul20C levels were analyzed by Western blot using anti-FLAG antibodies. (B) An assay was carried out as described in A, followed by densitometry of the bands. The presented values are normalized according to the levels before rifampicin addition.

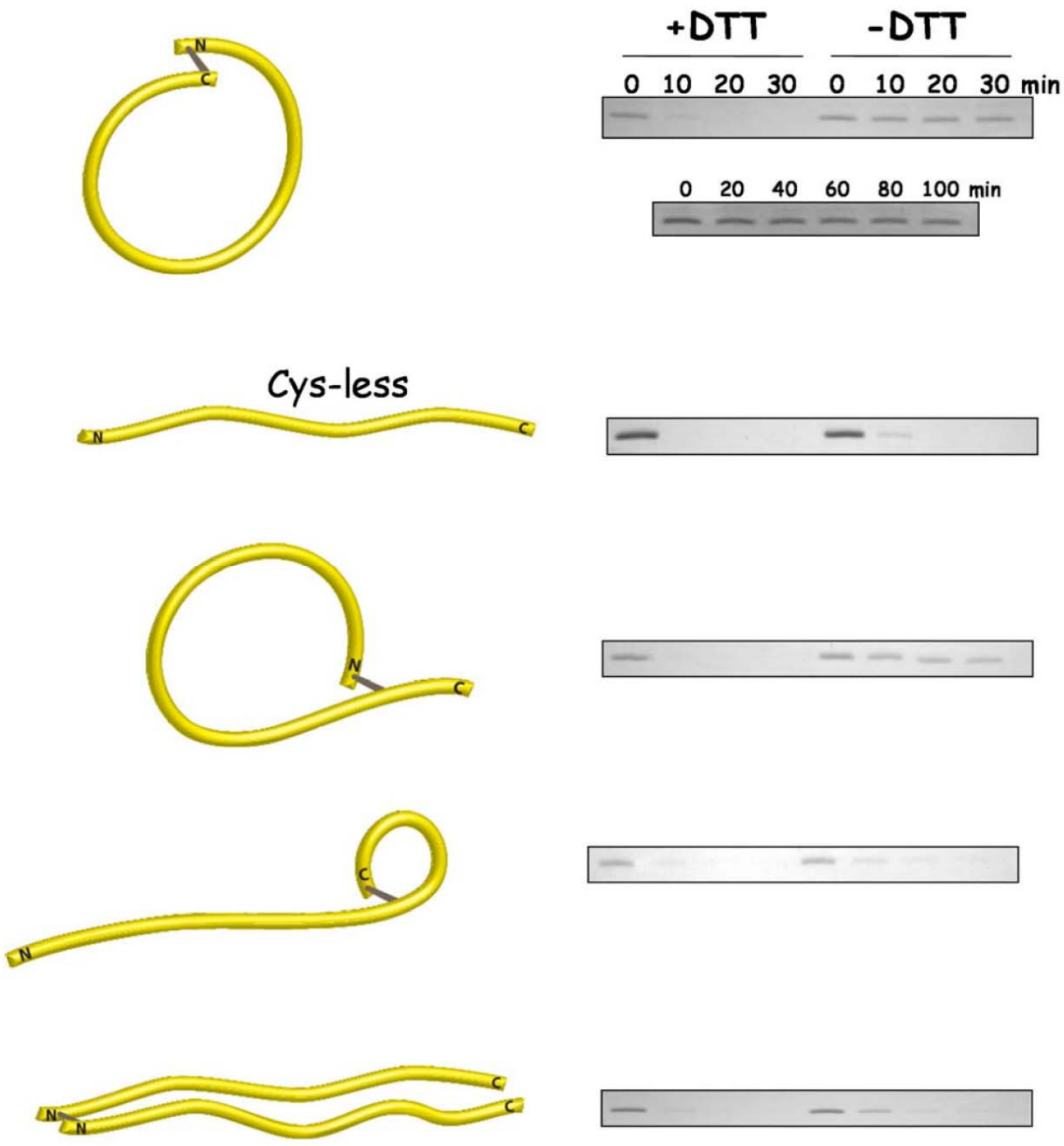

Fig. 6. Degradation of substrate circular permutants. Degradation of the described β-gal3-93 variants (5 μM) by Lon (0.1 μM) was tested in the presence (left) and in the absence (right) of DTT. Gels were stained by Coomassie brilliant blue.