SUPPLEMENTAL DATA

FIGURE LEGENDS

Fig. S1. Size estimation and schematic overview of the product bands in Fig. 2A and B.
The actual molecular mass with # includes the mass of the ($MRGSH_6GS$-) tag, 1.4 kDa.

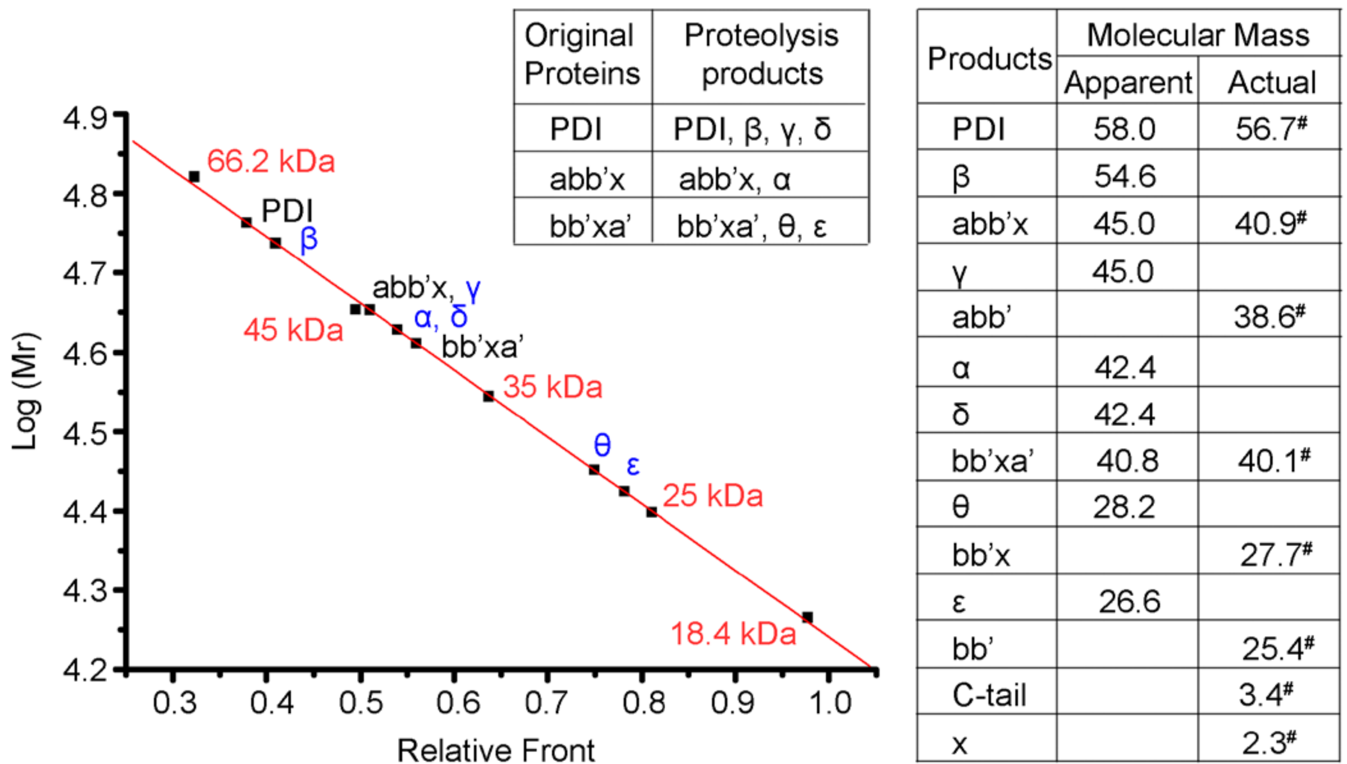
Fig. S2. Proteolysis of alternative $(His)_6$-tagged constructs for accurate product mass determination.
PDI and L343A PDI with N-terminal (MH_6M-) tags at 1 mg/ml (A, B) or 2 mg/ml (C, D) were digested by 0.5 µg/ml proteinase K (A, B) or 0.7 µg/ml chymotrypsin (C, D). Reactions were stopped by 5 mM PMSF at different times as indicated before analysis on SDS-12% PAGE gel. The bands indicated by arrows were cut out and analyzed by tryptic digestion/mass spectrometry.

Fig. S3. Limited digestion profiles of PDI with $(His)_6$-tag at its C- terminals.
1 mg/ml PDI with a C-terminal (-H_6) tag was digested by 2 µg/ml chymotrypsin (A) at 25 ^{o}C for different times as indicated. The reactions were terminated by adding PMSF to a final concentration of 0.5 mM, and analyzed by reducing SDS-15% PAGE for Coommassie blue staining. Digestion profiles of PDI with a N-terminal ($MRGSH_6GS$-) tag under the same condition (B) was used for comparison. The major products are indicated by arrows.

Fig. S4. Stern-Volmer analysis of quenching of intrinsic protein fluorescence by iodide anions.
Ksv plots of I272A mutants (○) and L343A mutants (△) in b'x background (■) (A), W111F/W390F background PDI (■) (B) and W111F abb'x background (■) (C).

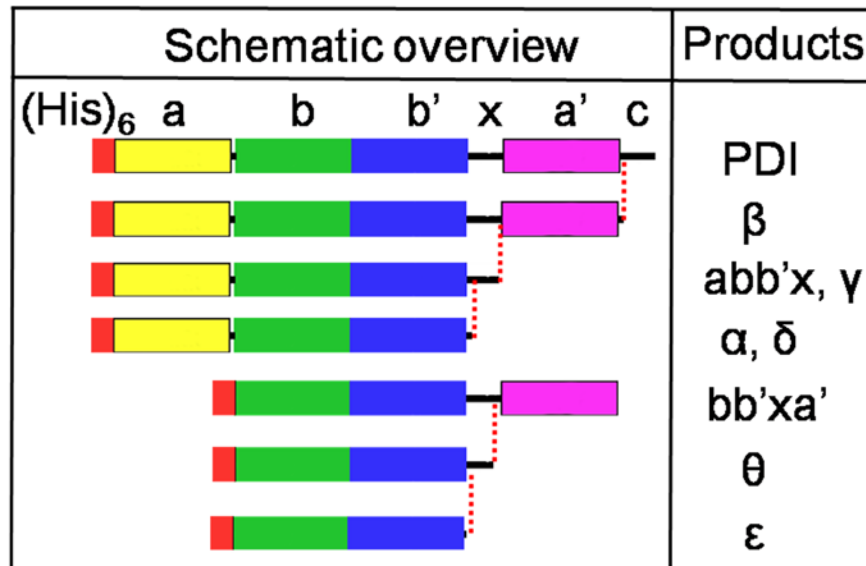
Fig. S5. Far-UV CD analysis of the effects of the mutations on the secondary structure of PDI and abb'x.
Far-UV CD spectra of the mutants in PDI background (A) or **abb'x** background (B) at 2.5 µM in 20 mM sodium phosphate buffer, pH 7.6, at 25 ^{o}C.

Supplemental figure 1

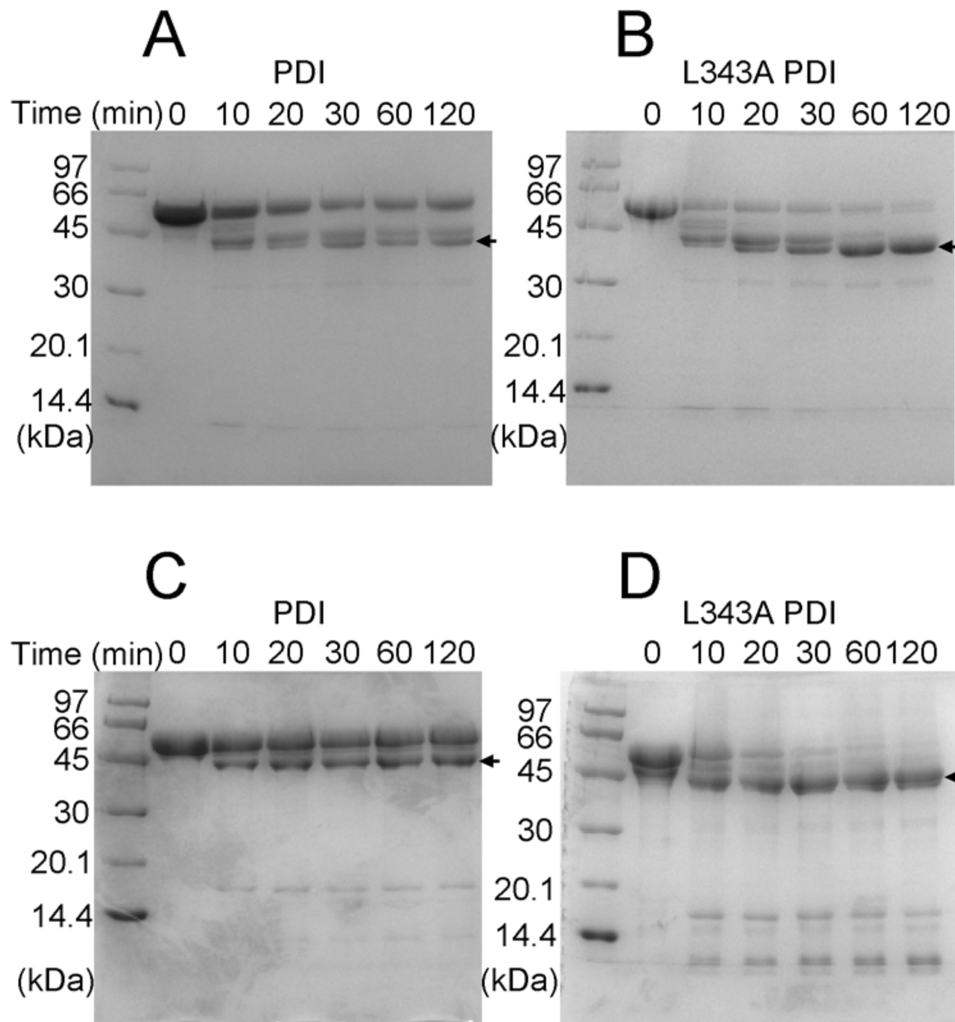


Original Proteins	Proteolysis products
PDI	PDI, β, γ, δ
abb’x	abb’x, α
bb’xa’	bb’xa’, θ, ε

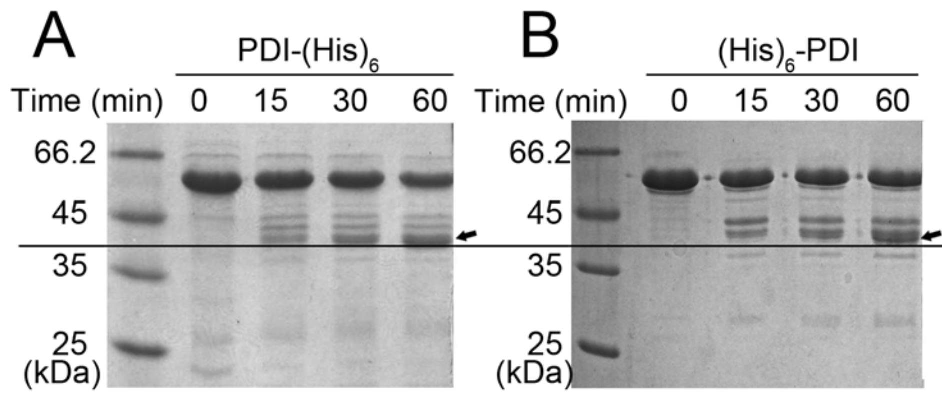
Products	Molecular Mass	
	Apparent	Actual
PDI	58.0	56.7#
β	54.6	
abb’x	45.0	40.9#
γ	45.0	
abb’		38.6#
α	42.4	
δ	42.4	
bb’xa’	40.8	40.1#
θ	28.2	
bb’x		27.7#
ε	26.6	
bb’		25.4#
C-tail		3.4#
x		2.3#



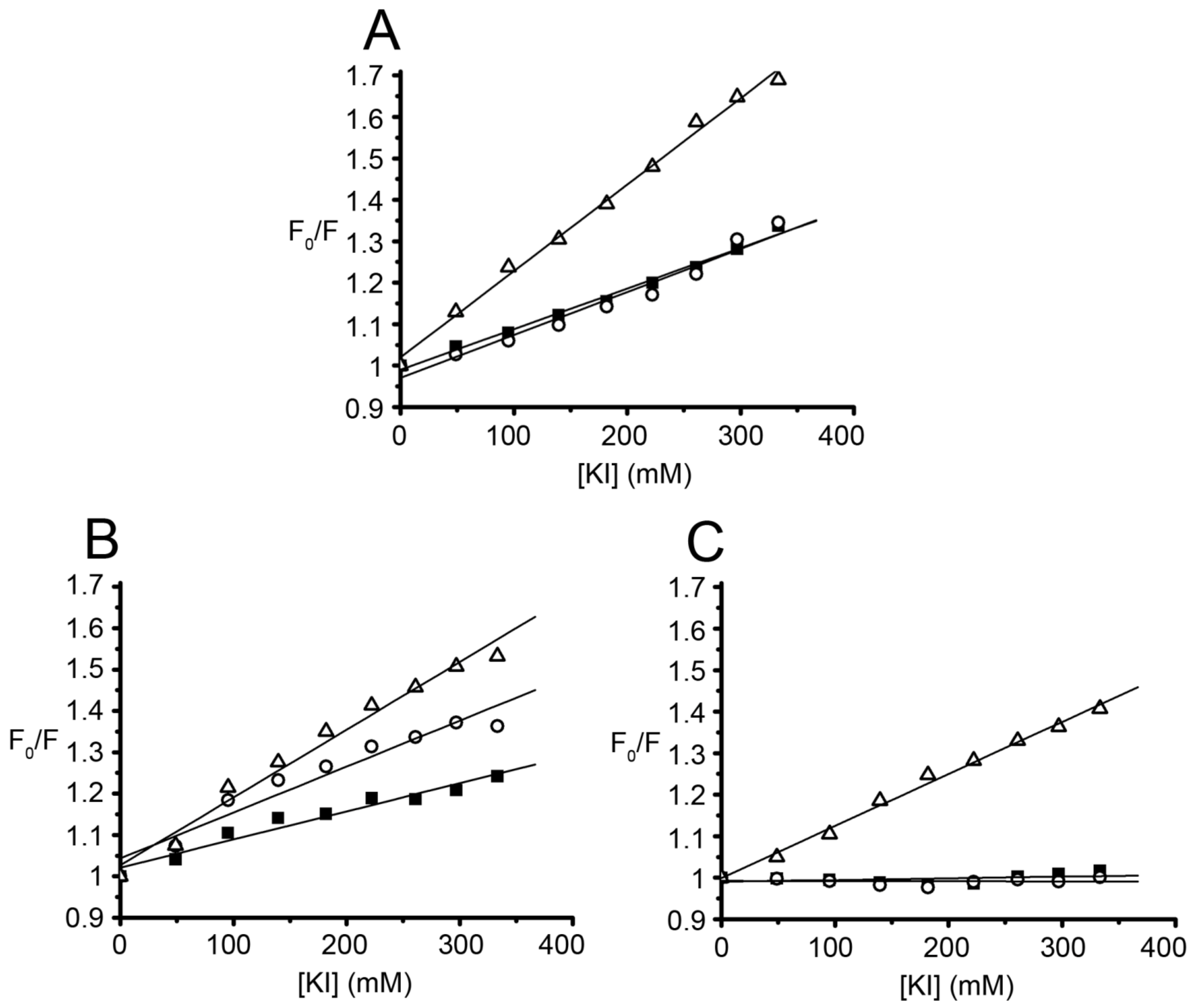
Supplemental figure 2



Supplemental figure 3



Supplemental figure 4



Supplemental figure 5