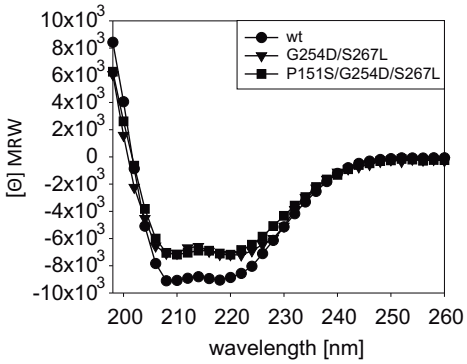
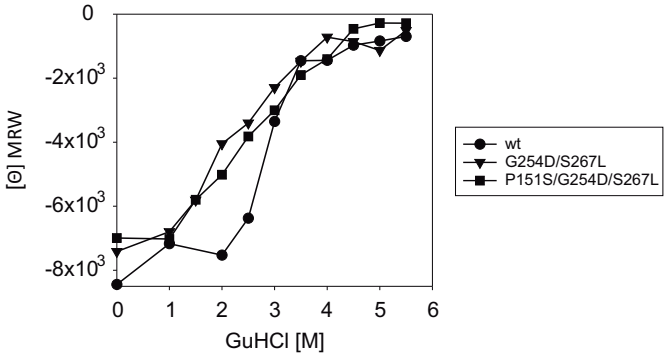
A



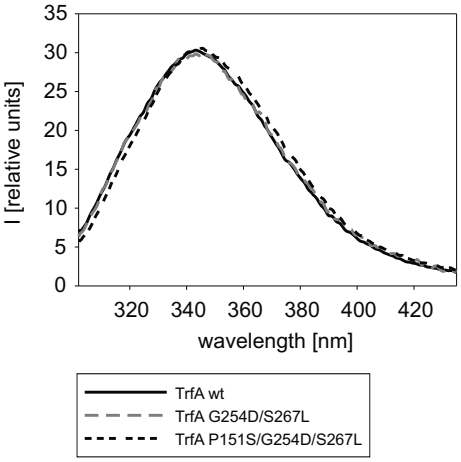
B

TrfA	% content			
	α-helix	β-strand	turn	unstructured
wt	29.2	22.7	19.6	27.7
G254D/S267L	21.2	26.1	22.1	29.7
P151S/G254D/S267L	20.7	27.7	21.3	29.2

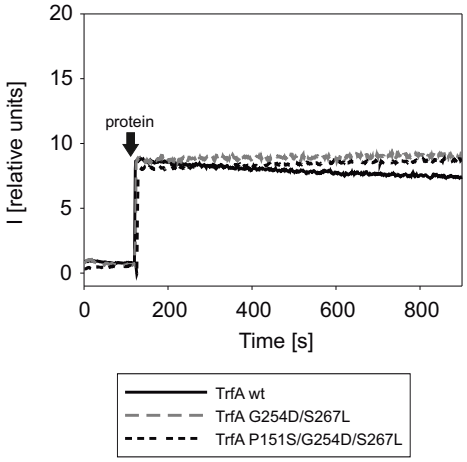
C



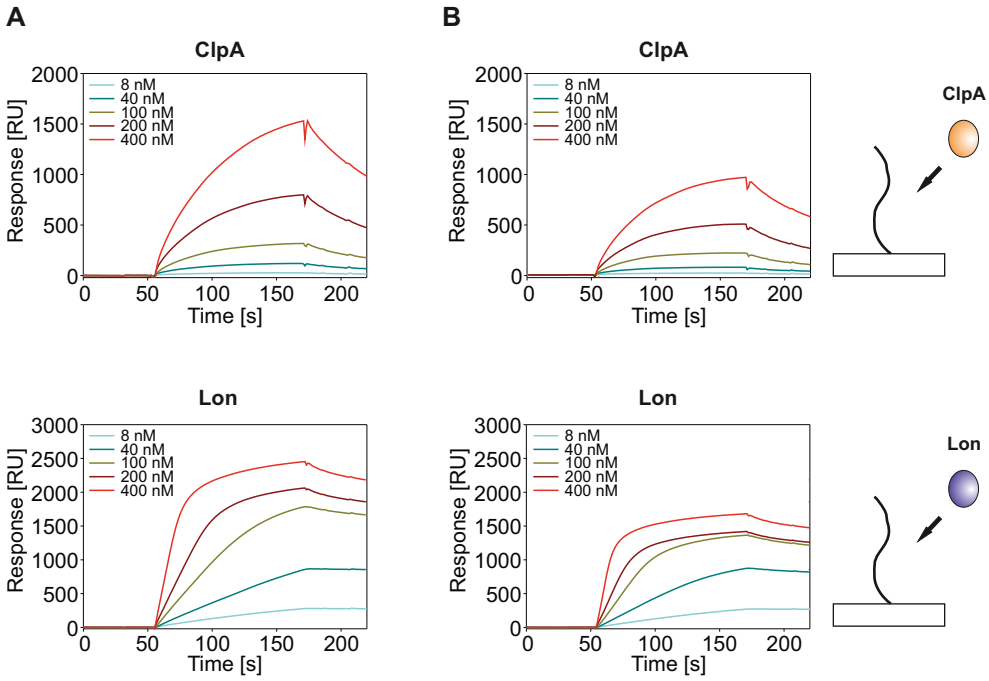
D



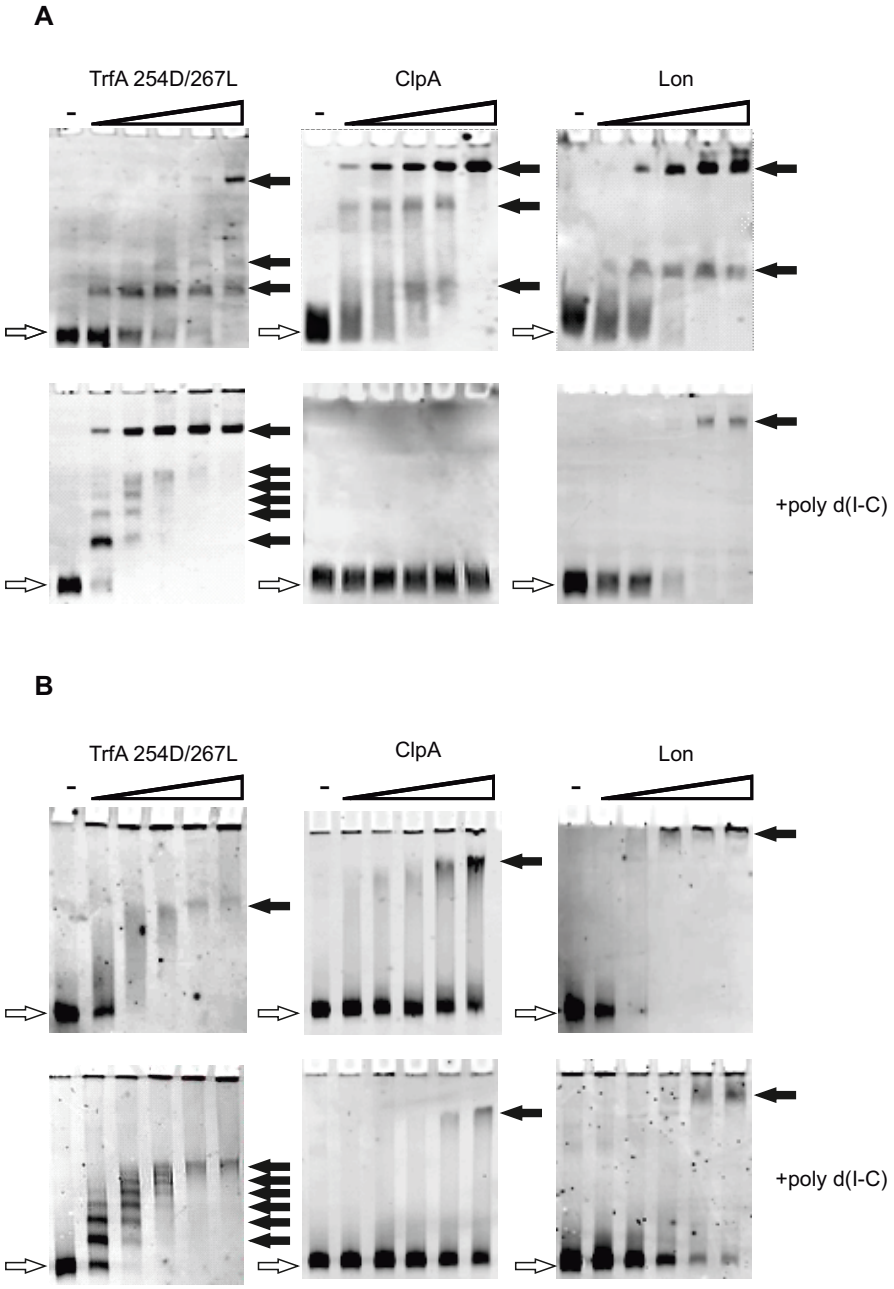
E



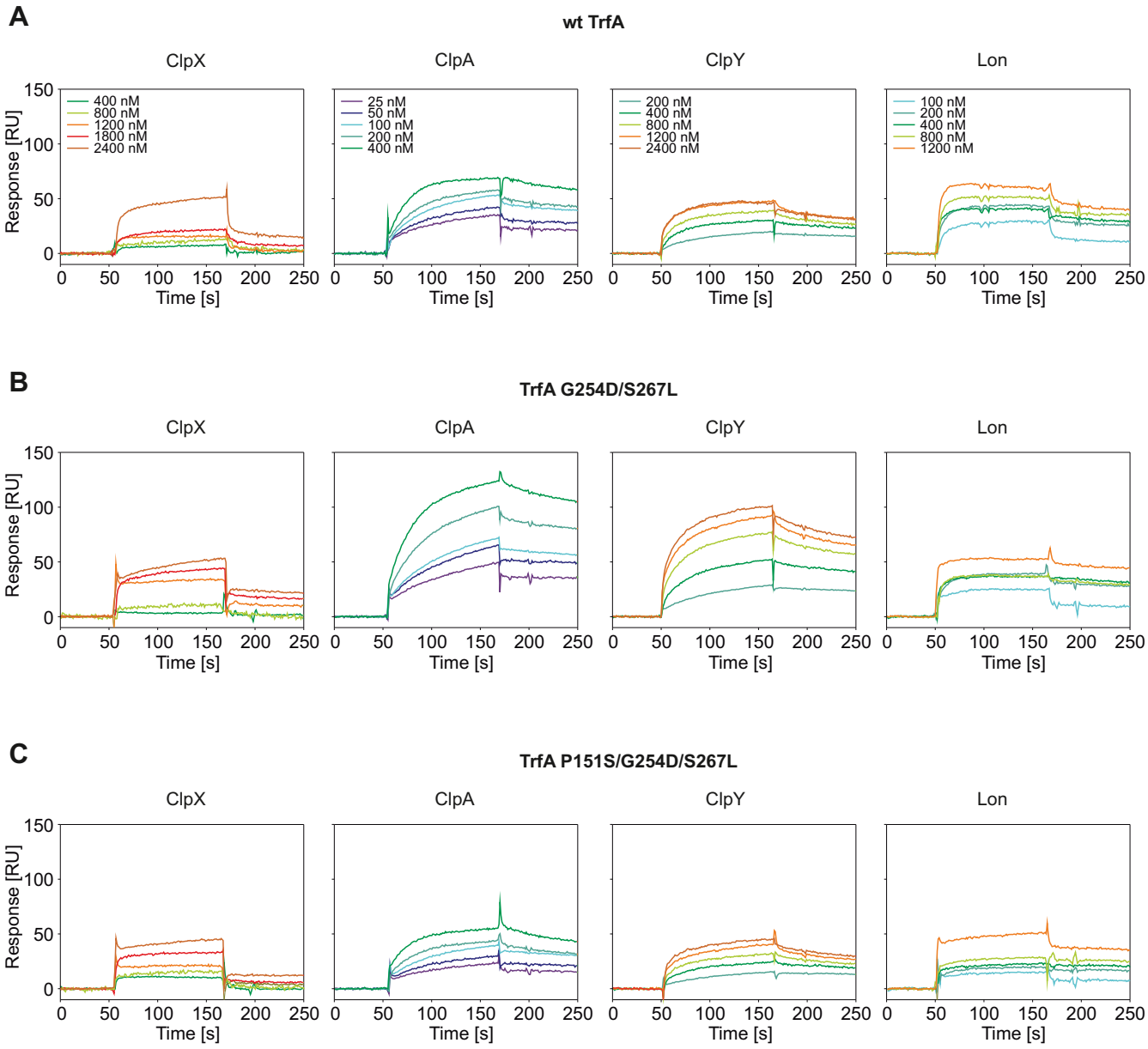
Supplementary Figure 1. **Biophysical comparison of TrfA variants.** (**A**) Graph shows isothermal circular dichroism spectra of the analyzed TrfA variants in a range from 195 to 260 nm, performed as described (Pierechod et al..2009). The content of respective secondary structures for each protein was calculated from the spectra using SELCON3 method from CDPro software (**B**). (**C**) Chemical denaturation profiles of TrfA variants were assayed by monitoring the changes in elipticity at 222 nm in increasing guanidinum chloride concentrations. (**D**) Tryptophan fluorescence spectra of the analyzed TrfA variants (1 μM concentration) excited at 294 nm. Analysis was performed in the proteolysis buffer without the addition of BSA. The excitation and emission slits were set to 5 nm. (**E**) Light scattering of TrfA variants (1 μM). The wavelength was 400 nm and the entrance and emission slits were set to 2.5 nm. All fluorescence measurments were performed in a PerkinElmer Life Sciences LS50B spectrofluorometer.



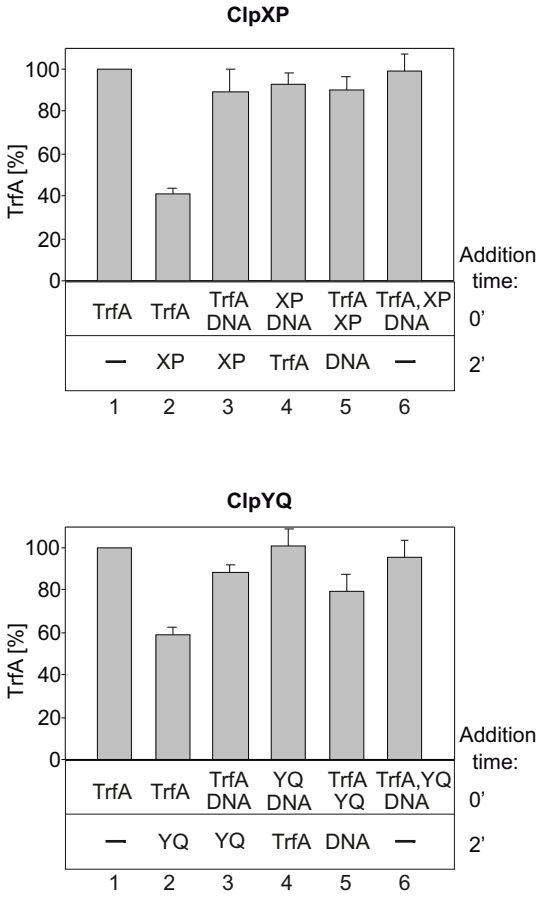
Supplementary Figure 2. **ClpA and Lon interact with DNA.** Binding to DNA of ClpA and Lon was studied by SPR using (**A**) 129 bp-long pUC19 dsDNA fragment (sequence: 5'-AGCTCACAATTCCACACA ACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCAC ATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGT-3') or (**B**) 129 bp-long iterons containing dsDNA fragment (sequence given under Materials and Methods) immobilized on the surface of a sensor chip. Indicated concentrations of ClpA and Lon were injected onto the sensor chip. Buffer used for the analysis was HBS50 (identical to HBS-EP but containing 50 mM NaCl instead of 150 mM) supplemented with 10 mM magnesium acetate and 2 mM ATP.



Supplementary Figure 3. **ClpA and Lon binding to DNA.** 20 µl reactions containing 200 ng of DNA labelled with Alexa Fluor (Invitrogen) and increasing amounts of protein in proteolysis buffer were incubated at 27°C for 20 min. and separated electrophoretically on a 5% TBE-polyacrylamide gel. Reactions in the lower panels additionally contained 50 ng/µl poly d(I-C). Fluorescently labelled DNA was visualized using Molecular Imager FX (Bio-Rad). DNA fragment used in (**A**) was 135 bp long and contained five RK2 iterons. DNA fragment used in (**B**) was about 350 bp long and corresponded to RK2 minimal origin. Both fragments were PCR amplified prior to labelling. The amount of proteins used were 0, 0.25, 0.5, 0.75, 1, 1.5 µg for TrfA and 0, 0.5, 1, 2, 3, 4 µg for ClpA and Lon. Open arrows indicate the position of unbound DNA probe and closed arrows indicate the positions of nucleoprotein complexes.



Supplementary Figure 4. **TrfA binding by Clp and Lon proteins.** Binding of Clp chaperones and Lon to TrfA variants was analyzed by Surface Plasmon Resonance. 1200 RU of wt TrfA, TrfA G254D/S267L or TrfA P151S/G254D/S267L were immobilized on the surface of the CM5 chip through the amine coupling procedure described by the manufacturer. Injections of ClpX, ClpA, ClpY or Lon at given concentrations were performed in HBS-EP supplemented with 10 mM magnesium acetate and 2 mM ATP (or ATPγS in case of Lon). HBS-EP was used as a running buffer.



Supplementary Figure 5. **DNA inhibits TrfA degradation by ClpXP and ClpYQ *in vitro* regardless of reaction components addition order.** Experiments were performed as described in Figure 5 but using ClpXP (a mixture of 1.5 µg ClpX and 1.5 µ ClpP) or ClpYQ (a mixture of 1.5 µg ClpY and 1.5 µ ClpQ) instead of ClpAP and Lon.