Supplementary information to Tholstrup *et al.*

Supporting text

Ribosome chase assay.

In order to examine if the stalled protein products were caused by paused ribosomes, we performed a pulse chase experiment. Very slow translation would cause radioactivity to disappear, as a function of time, from the stalled products and eventually to appear in the stop codon terminated protein product. The results from such a pulse chase experiment (Fig. S2) showed that the amount of ^{35}S-Met incorporated during the pulse (20 s) appeared in the final protein spots after a short translation time between the first (10 sec. chase) and the second sampling point (60 sec. chase) and stayed stable for at least 900 sec.

Two-dimensional SDS-PAGE – additional gels

The results in figure 3 are based on two-dimensional SDS-PAGE of proteins from constructs that contain a UAA stop codon immediately upstream from the pseudoknot structure. Peptides visible only in IPTG induced cultures showing the expected molecular weight and pI were used in the quantification frameshift in addition to the stop product and full-length frameshift product. Figure S4 below is an example of such a gel pair (induced and un-induced) for pseudoknot 22/6a. In panel B the in-frame stop product (Stop) is clearly visible as are the frameshift product (FS), GroEL and DnaK. The region containing peptides originating from ribosomes that are stalled within the pseudknot is indicated with a red dashed rectangle.

In addition, Figure S5 shows examples of two dimensional SDS-PAGE representing all the different constructs that were analyzed quantitatively in Figure 3. The 0 construct (Fig. S5) has no pseudoknot sequence inserted and was used as the background control.

Supplementary Materials and Methods

Ribosome chase assay. 5 ml of an exponentially growing culture in MOPS media was induced with 1 mM IPTG (t = 0). At t = 2 min 80 μCi L-[4,5-^{3}H(N)]lysine was added. At t = 10 min 610 μg L-lysine was added as a lysine chase. At t = 15 min 50 μCi L-[^{35}S]-methionine was added. At t = 15 min 20 sec 500 μg L-methionine was added as a methionine chase. At the indicated time points aliquots of the culture were transferred to tubes on ice containing chloramphenicol to a final concentration of 2.5 mg/ml. Cells were harvested and proteins were separated by two-dimensional SDS-PAGE as described in Materials and Methods in the main text. The gels were dried and left to expose an X-ray film (Kodak). Relevant proteins samples were extracted from the gel using a hollow needle and the isotope ratio was determined in a liquid scintillation counter (PerkinElmer, Winspectral 1414).

Protein stability assay. An exponentially growing culture in MOPS media was induced with 1 mM IPTG (final) for 15 min at OD_{436} ~ 0.6. Two 5 ml aliquots of the induced culture were extracted (culture A and B hereafter). Culture A was labeled with 20 μCi L-[^{35}S]-methionine for 30 sec after which 500 μg L-methionine was added as a methionine chase. At the indicated time points aliquots were extracted to tubes at 0°C containing chloramphenicol to a final concentration of 2.5 mg/ml. Culture B was labeled with 200 μCi L-[4,5^{3}H(N)]-lysine for 30 min and transferred to tubes at 0°C containing chloramphenicol to a final concentration of 2.5 mg/ml. Equal amounts of culture B was added to the aliquots of culture A. The mixed aliquots were harvested and proteins were separated on two-dimensional SDS-PAGE as described in Materials and Methods in the main text. The isotope ratio of relevant proteins was investigated as described for the ribosome chase assay. The isotope ratio in the individual protein samples was divided by the isotope ratio of TCA precipitated total protein for each time-point.

Supplementary figure legends

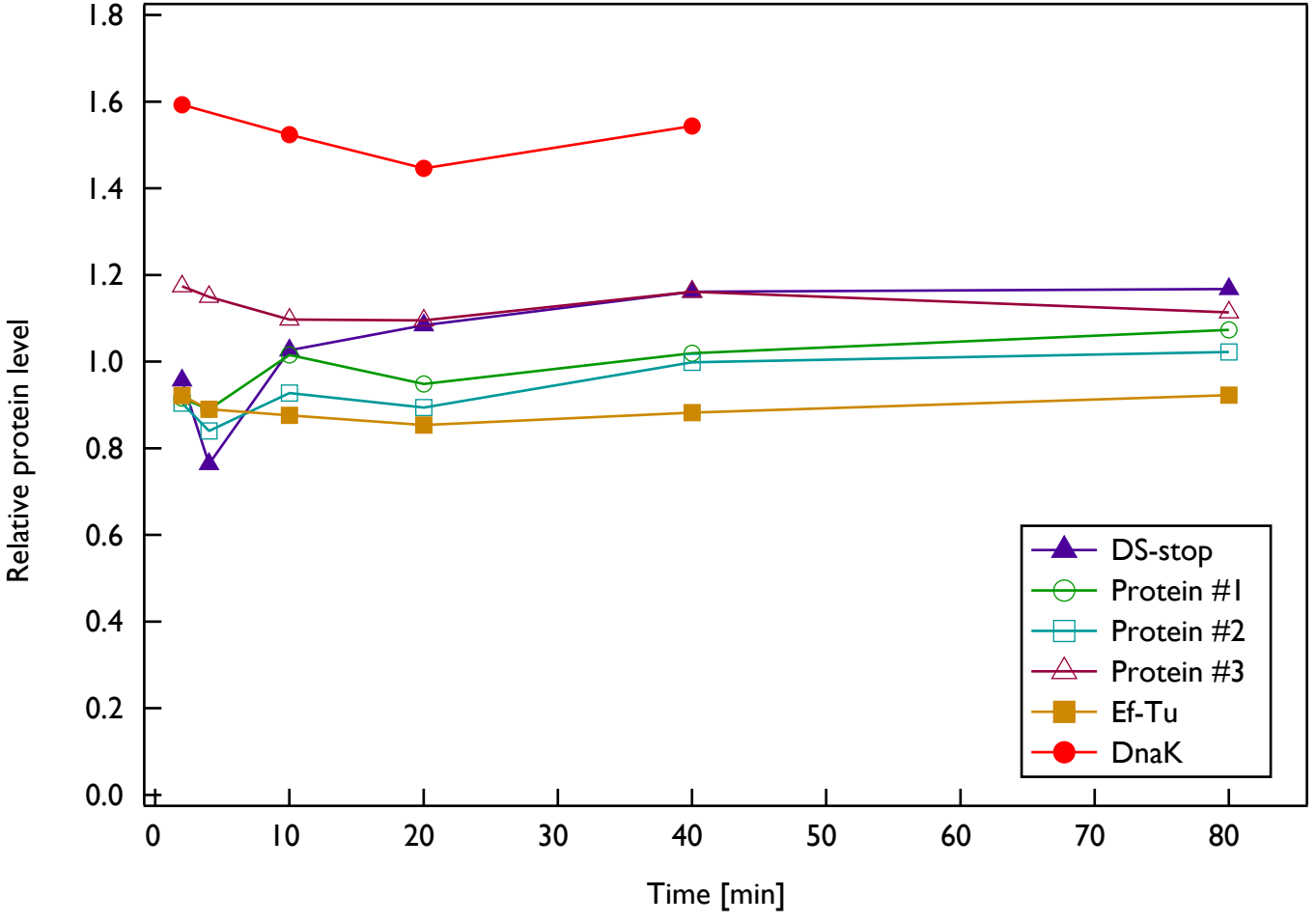
Figure S1: Result from protein stability assay on pseudoknot 22/6a with downstream stop. Relative expression levels of proteins determined as $^{3}H/^{35}S$ in specific protein spots divided by the $^{3}H/^{35}S$ ratio in total protein (TCA precipitabel material) as a function of time in the methionine chase. The specific proteins are the same as those in Figure S2 with the addition of elongation factor Tu (Ef-Tu).

Figure S2: Result from ribosome chase assay on pseudoknot 22/6a with downstream stop. A) Two-dimensional SDS-PAGE of radioactively labeled proteins harvested at time point 980 sec. The proteins selected for analysis are indicated. For each time point a two-dimensional SDS-PAGE was made and the identical proteins were extracted from the gels and the ratio of ^{35}S-methionine to ^{3}H-lysine was determined using a liquid scintillation counter. The selected proteins were DnaK (control), in-frame termination ("Downstream stop") and three popypeptides produced by stalled ribosomes ("Protein#1","Protein #2" and "Protein #3"). **B)** The isotope ratio for the selected proteins indicated in (A) as a function of time in the methionine chase.

Figure S3: Growth curves for strains containing pseudoknots after induction. Exponetially growing cultures were diluted to 0.001 OD_{436} and allowed to grow to $OD_{436} \approx 0.06$ where IPTG was added (time point zero) to induce expression. See insert for construct description.

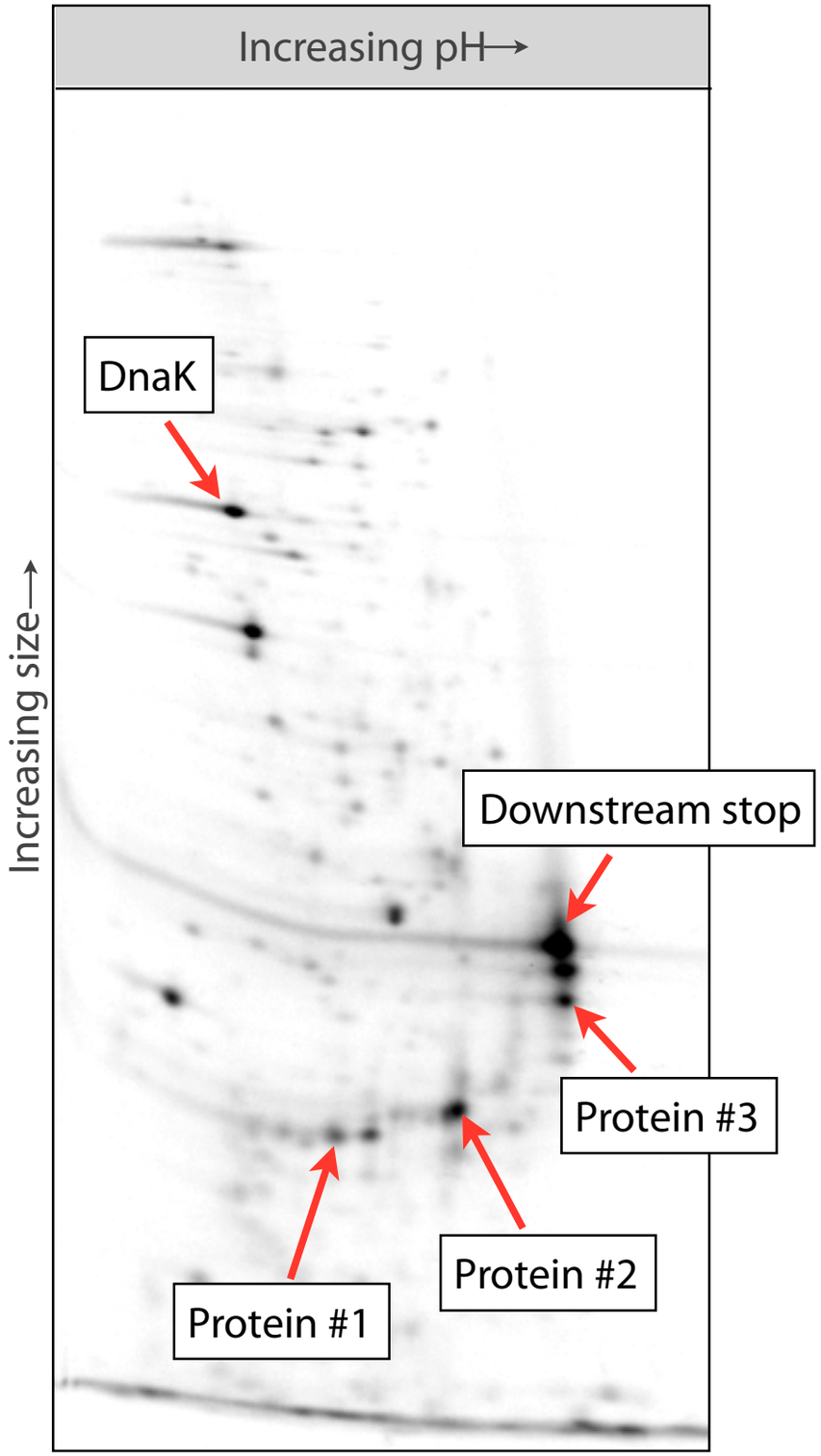
Figure S4: Two-dimensional SDS-PAGE used to estimate true frameshift efficiency. **A:** Theoretical estimation of the molecular weight and pI of peptides produced as translation progresses. GroEL and DnaK is shown as landmarks to ease orientation on experimental gels in panel B and C. **B:** Image of exposed PhosphorImage screen for pseudoknot 22/6a containing a UAA-stop codon immediately upstream from the pseudoknot. GroEL and DnaK is shown to help orientation with panel A, in-frame stop product (Stop) and frameshift product is indicated (FS). Red rectangle indicate region of interest in relation to stalled ribosomes. **C**: Image of PhosphorImage screen for an uninduced culture containing pseudoknot 22/6a. **D**: SDS-PAGE of an uninduced (-) or IPTG induced (+) strain carring PK 22/6a. Red dashed rectangle denote region of interest in relation to stalled ribosomes.

Figure S5: Two-dimensional SDS-PAGE representing all strains used to estimate frameshift efficiency. All strains contained a UAA-stop codon immediately upstream from the pseudoknot. Images are of exposed PhosphorImage screens for the pseudoknots indicated above each top panel. Each of the lower panels show an enlargement (some are slightly distorted to fit in the panel) of the area indicated by a red dashed rectangle in the top panel right above and denotes the region of interest in relation to stalled ribosomes. The 0 construct ('0 No PK'), which only contain the slippery sequence and the UAA stop codon is indicated by a red rectangle.



Supplementary figure S1; Tholstrup *et al.*

A)



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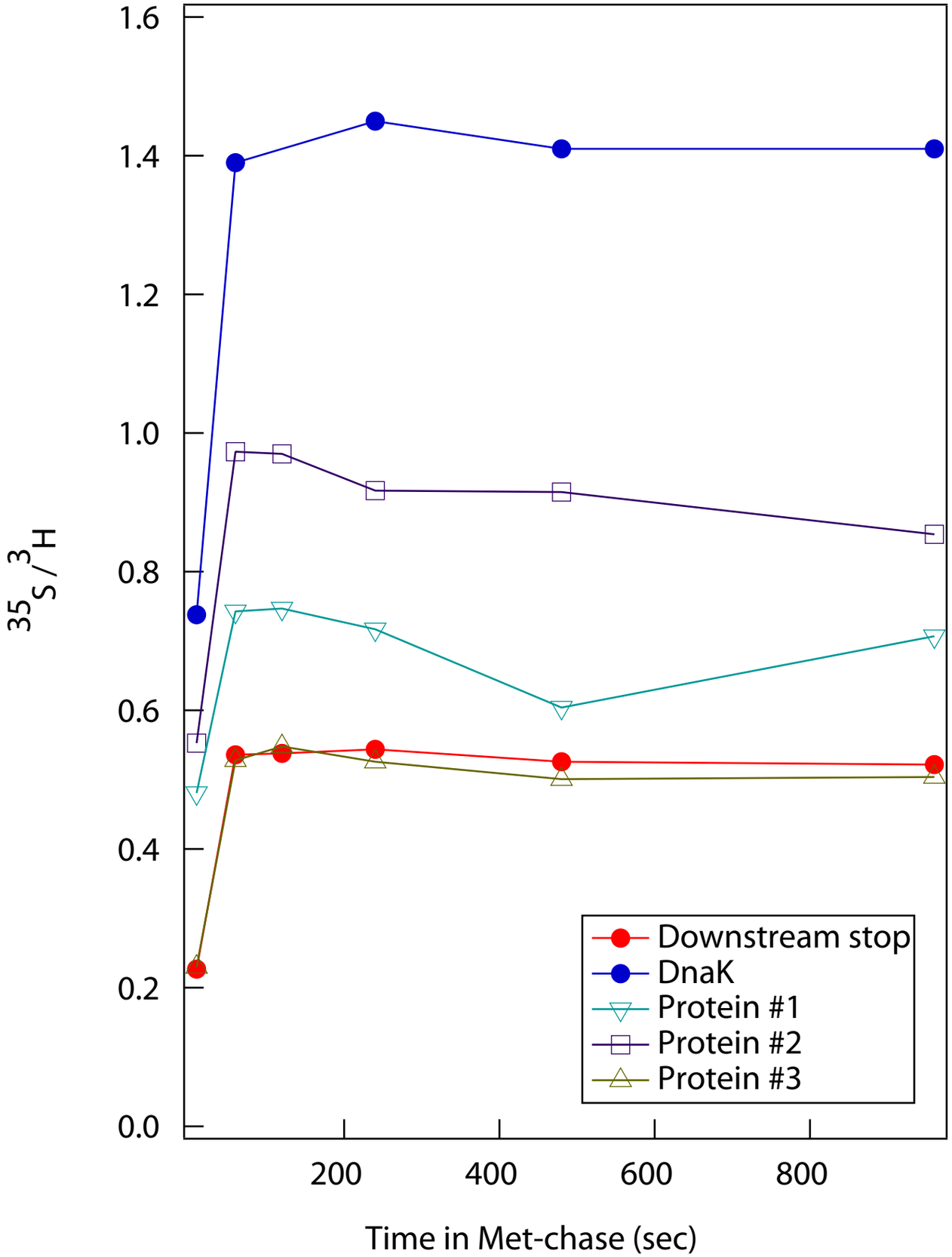
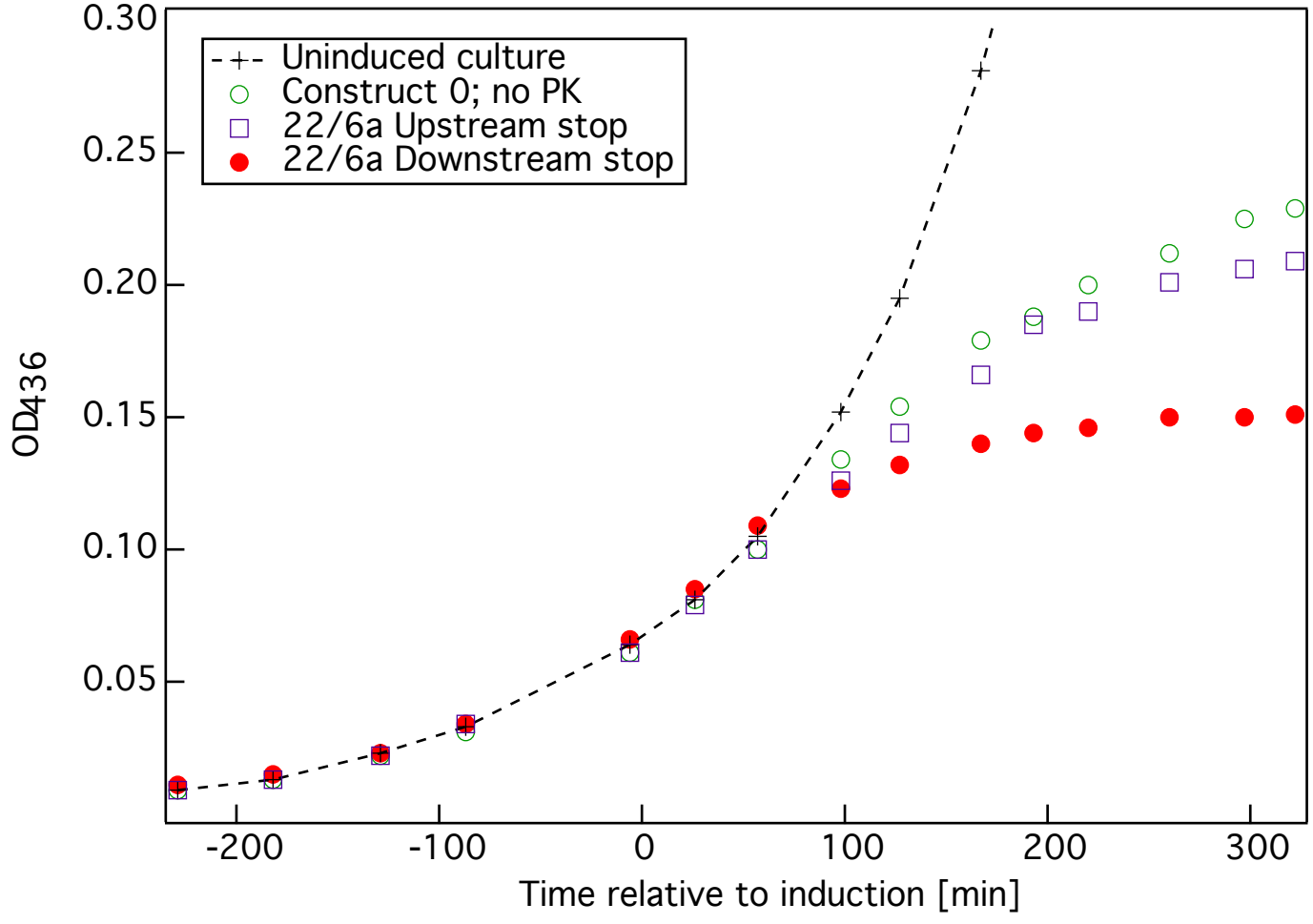
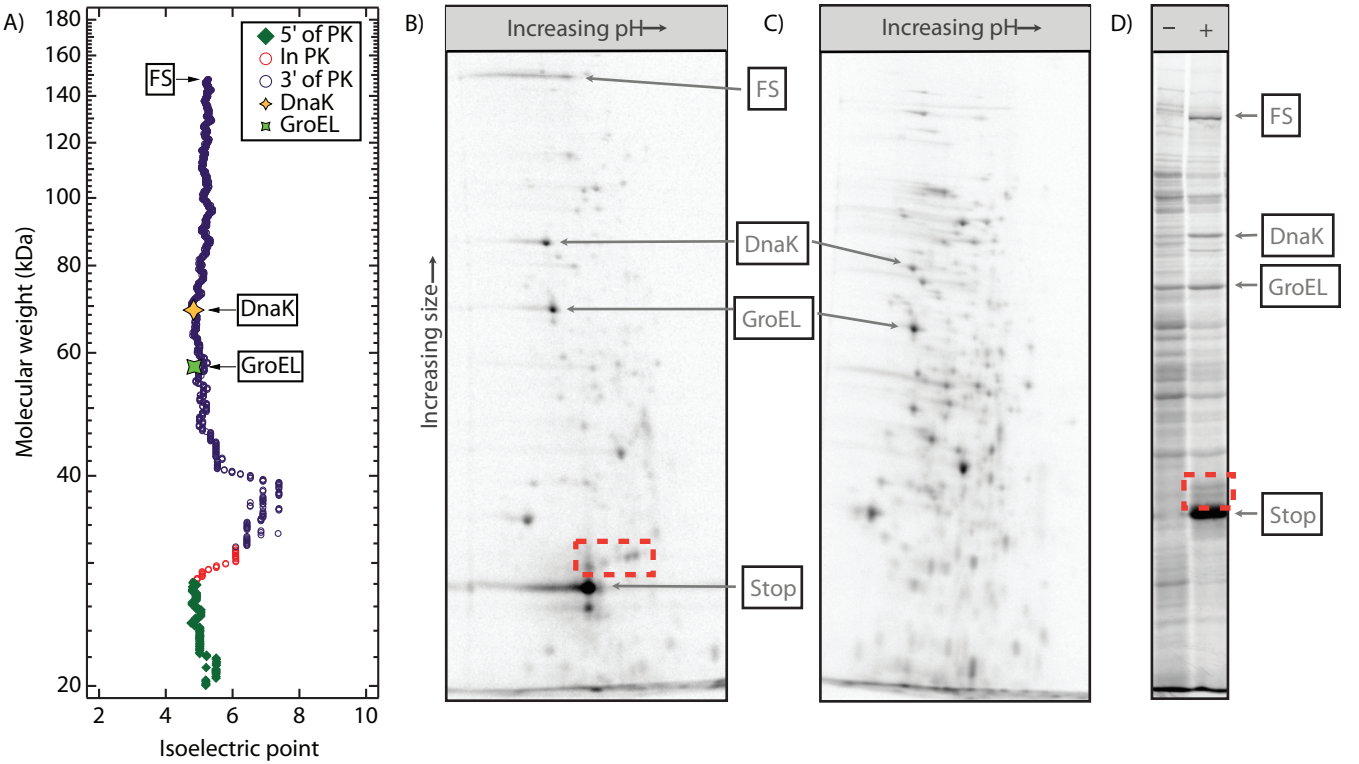


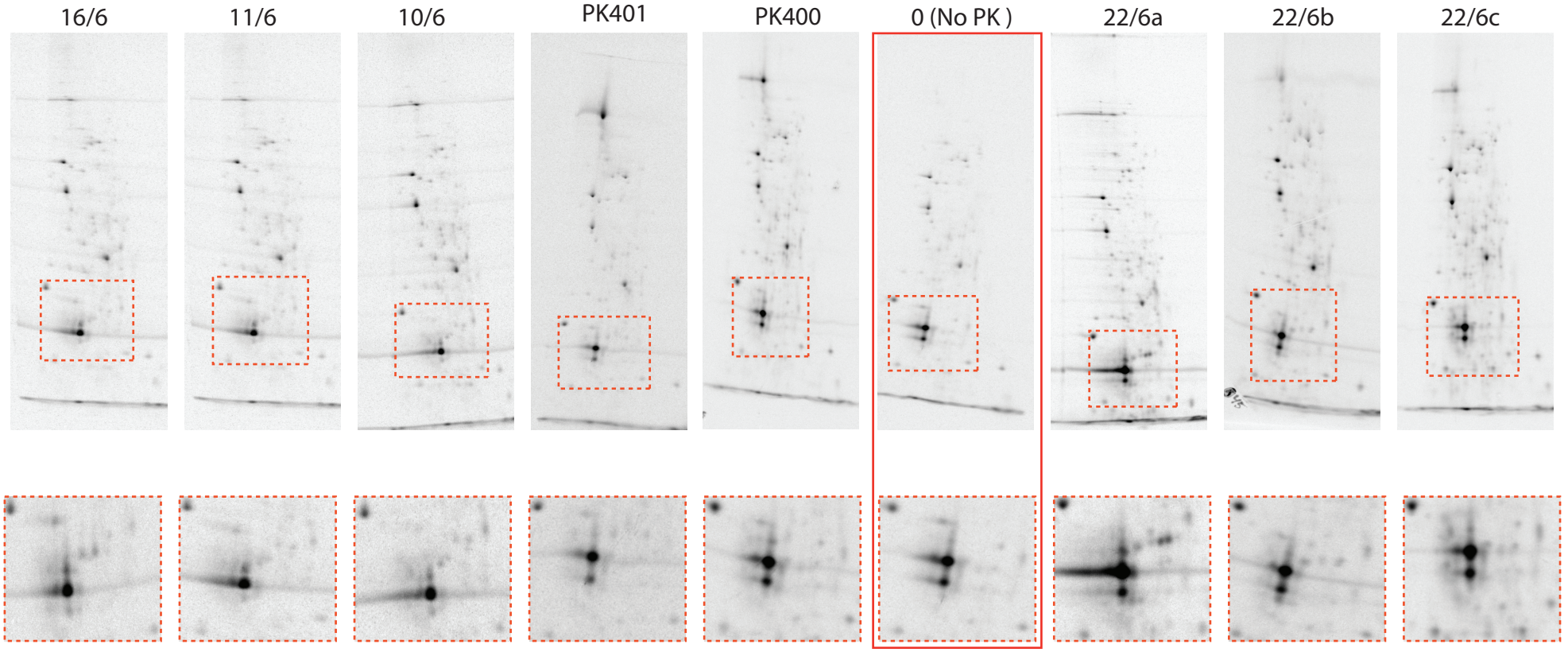
Supplementary figure S2; Tholstrup *et al.*



Supplementary figure S3; Tholstrup *et al*.



Supplementary figure S4; Tholstrup *et al*.



Supplementary figure S5; Tholstrup *et al*.