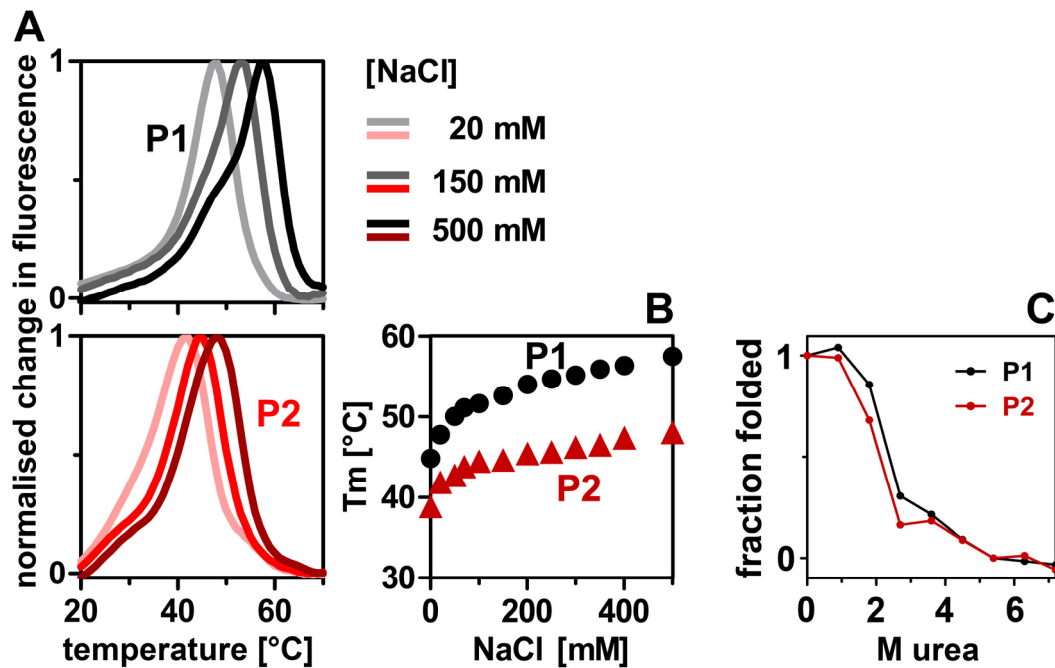


PAPS synthases – naturally fragile enzymes specifically stabilised by nucleotide binding

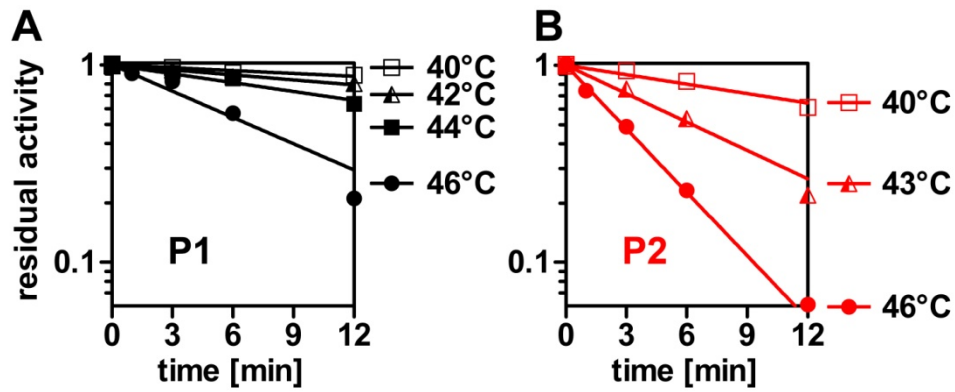
Johannes van den Boom, Dominik Heider, Stephen R. Martin, Annalisa Pastore, and Jonathan W. Mueller

Supplementary figures



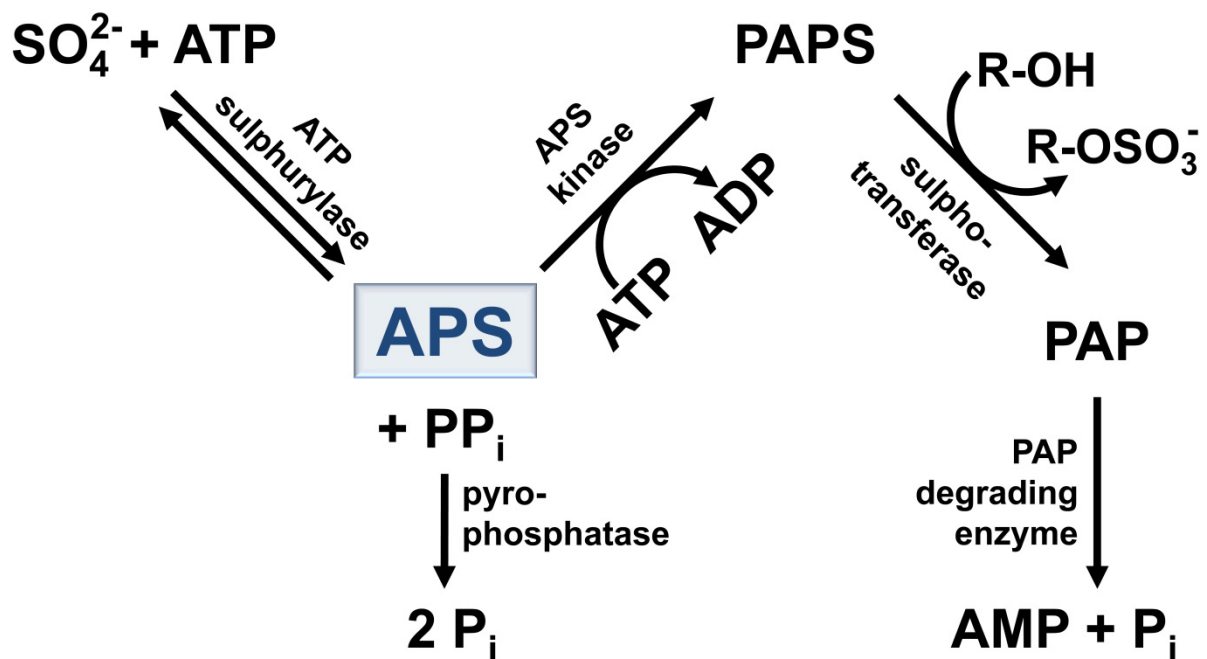
Supplementary figure 1: PAPS synthases show a pronounced salting-in effect.

Human PAPS synthase 1 and 2 were analysed by differential fluorescence spectroscopy (Thermofluor). **A**, change of the fluorescence signal for PAPSS1 (black) and PAPSS2 (red) at three different NaCl concentrations. Unfolding curves were not two-state, but showed one major transition that was assigned T_m. **B**, T_m values for human PAPS synthase proteins at various salt concentrations. The stability of both PAPS synthase proteins increased dramatically up to 100 mM NaCl. **C**, CD data for the chemical denaturation by urea confirm the higher stability of one of the isoforms, human PAPSS1. For this protein, about 0.3 M more urea is required to reach the midpoint of unfolding than for PAPSS2. However, both transitions occur at lower urea concentrations than those in Figure 1B. This may be attributed to the salting-in effect seen in A and B as fluorescence measurements have been carried out in the presence of 150 mM NaCl; for CD only 50 mM were used.

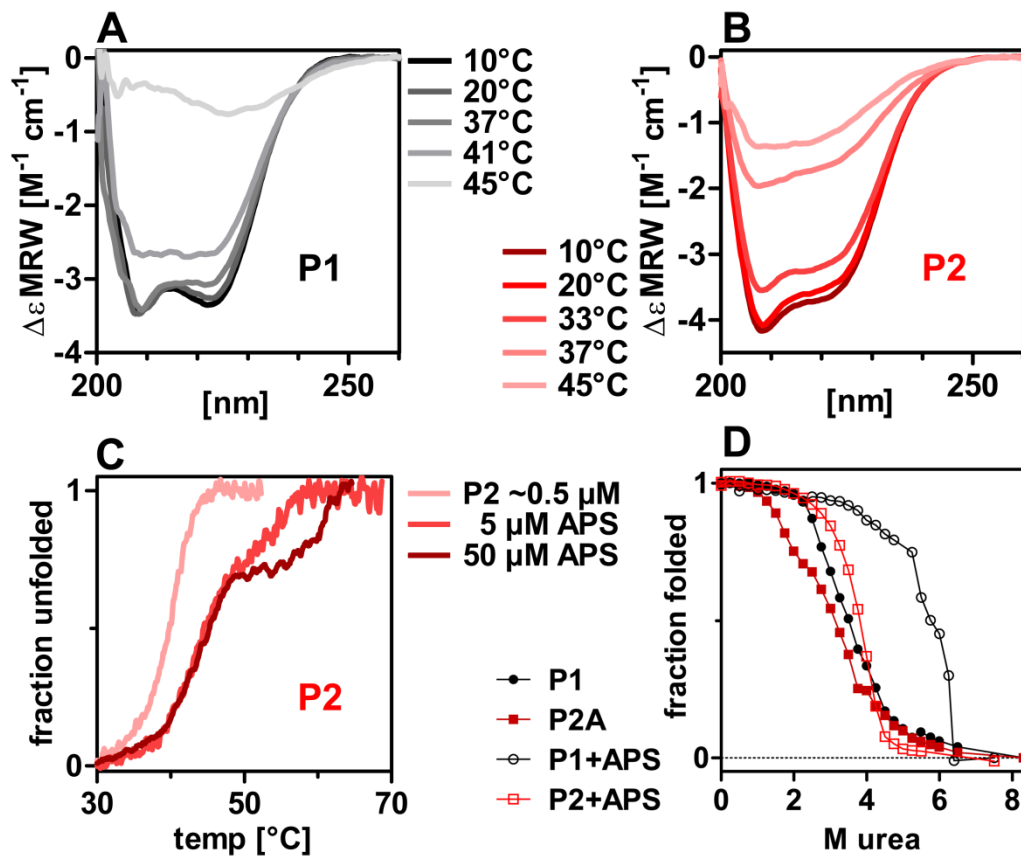


Supplementary figure 2: Inactivation kinetics of the APS kinase activities of human PAPS synthases.

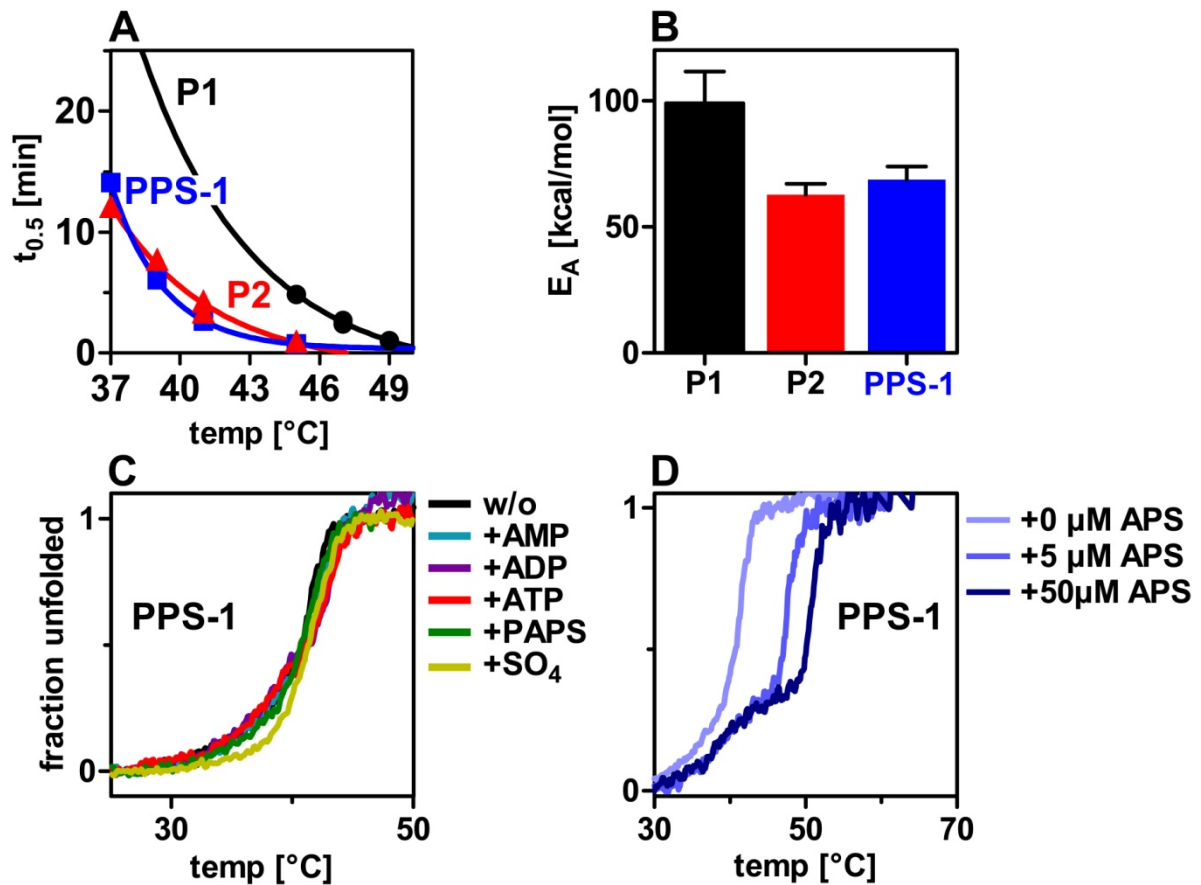
A and B, APS kinase activity was assayed by coupling ADP production via pyruvate kinase and lactate dehydrogenase to NADH consumption which was monitored at 340 nm. The reverse ATP sulphurylase activity was assayed in a similar manner by coupling ATP production via hexokinase and glucose-6-phosphate dehydrogenase to NADPH production (data not shown).



Supplementary figure 3: Schematic of overall PAPS production. The AMP moiety of ATP is transferred onto sulphate forming the intermediate adenosine-5'-phosphosulphate (APS). This first reaction step is catalysed by ATP sulphurylase. Then, released pyrophosphate is cleaved to phosphate by ubiquitous pyrophosphatase. Finally, APS is phosphorylated at the 3' OH group of its ribose ring by APS kinase forming the universal sulphate donor 3'-phospho-adenosine-5'-phosphosulphate (PAPS). In metazoans, ATP sulphurylase and APS kinase reside on one polypeptide, the bi-functional PAPS synthase. PAPS is then used for sulphation of hydroxyl groups on a variety of biomolecules catalysed by specialised sulphotransferases. The leftover 3'-phospho-adenosine-5'-phosphate (PAP) is a signalling cue in bacteria and plants. It is finally degraded to AMP and phosphate.

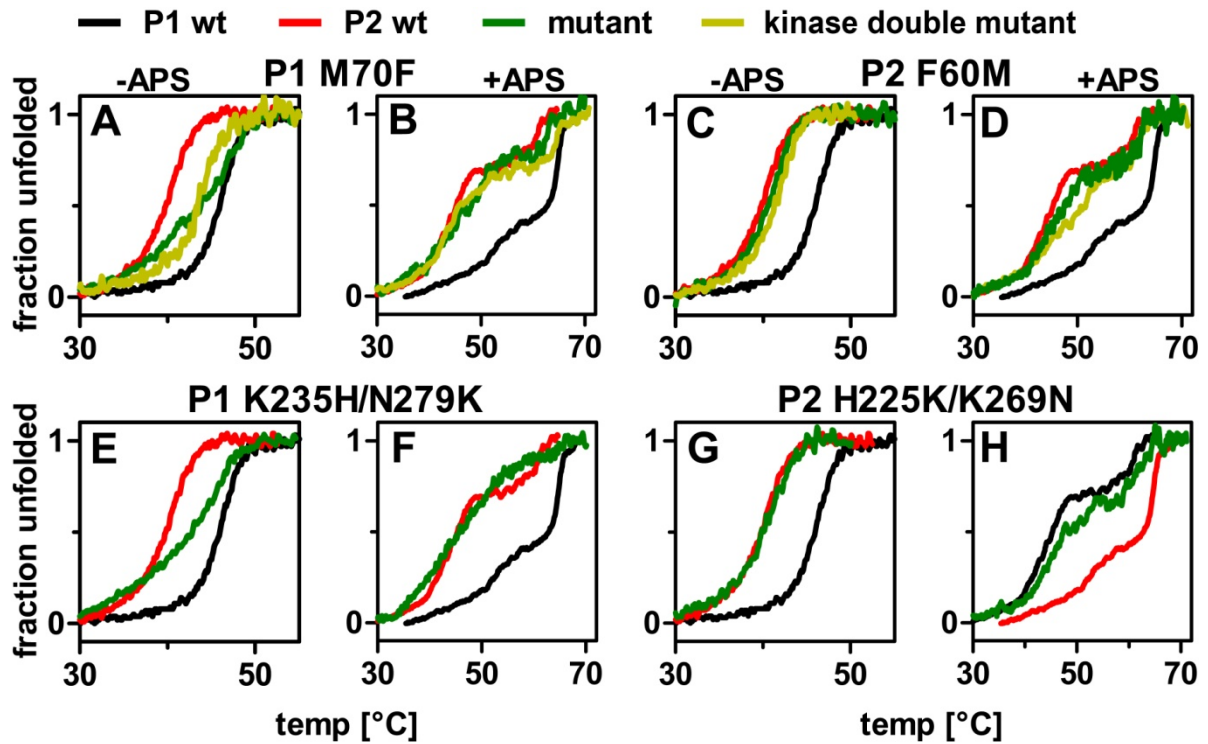


Supplementary figure 4: A and B, CD spectra of human PAPS synthases at increasing temperatures. The CD spectrum of PAPSS1 at 37 °C resembles the one of PAPSS2 at 10, 20 and 33 °C. **C**, thermal unfolding of human PAPSS2 monitored by CD in presence of different concentrations of APS. At 5 μM APS, the overall unfolding transition is shifted towards higher temperatures. Then, at 50 μM APS an unfolding intermediate is notably stabilised. **D**, thermal unfolding occurs with concomitant aggregation. Therefore, the stabilising effect of APS was confirmed using chemical denaturation by urea monitored by fluorescence. Both PAPS synthase isoforms unfold at significantly higher concentrations of the chaotrope in the presence of 50 μM APS.

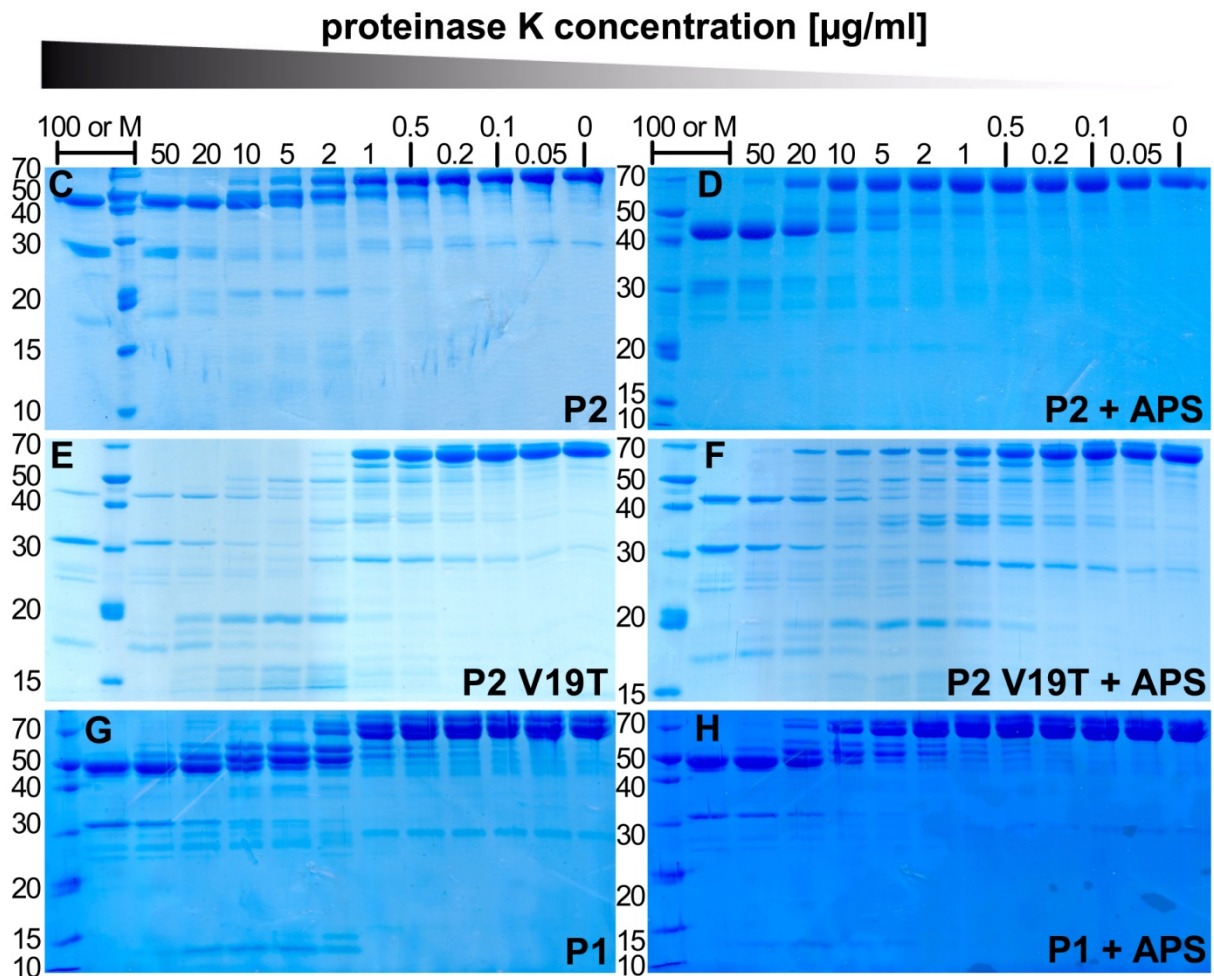
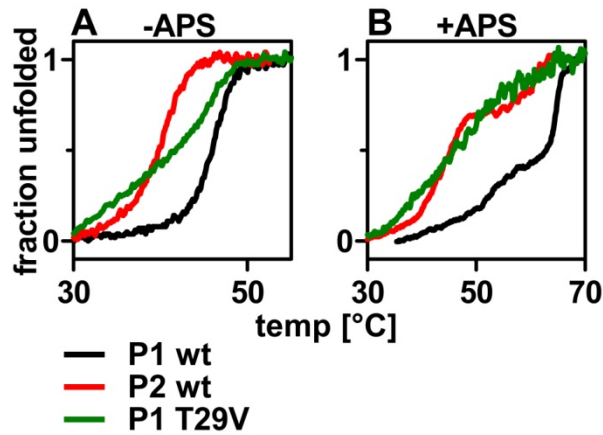


Supplementary figure 5: Stability of the PPS-1 protein from *C. elegans*.

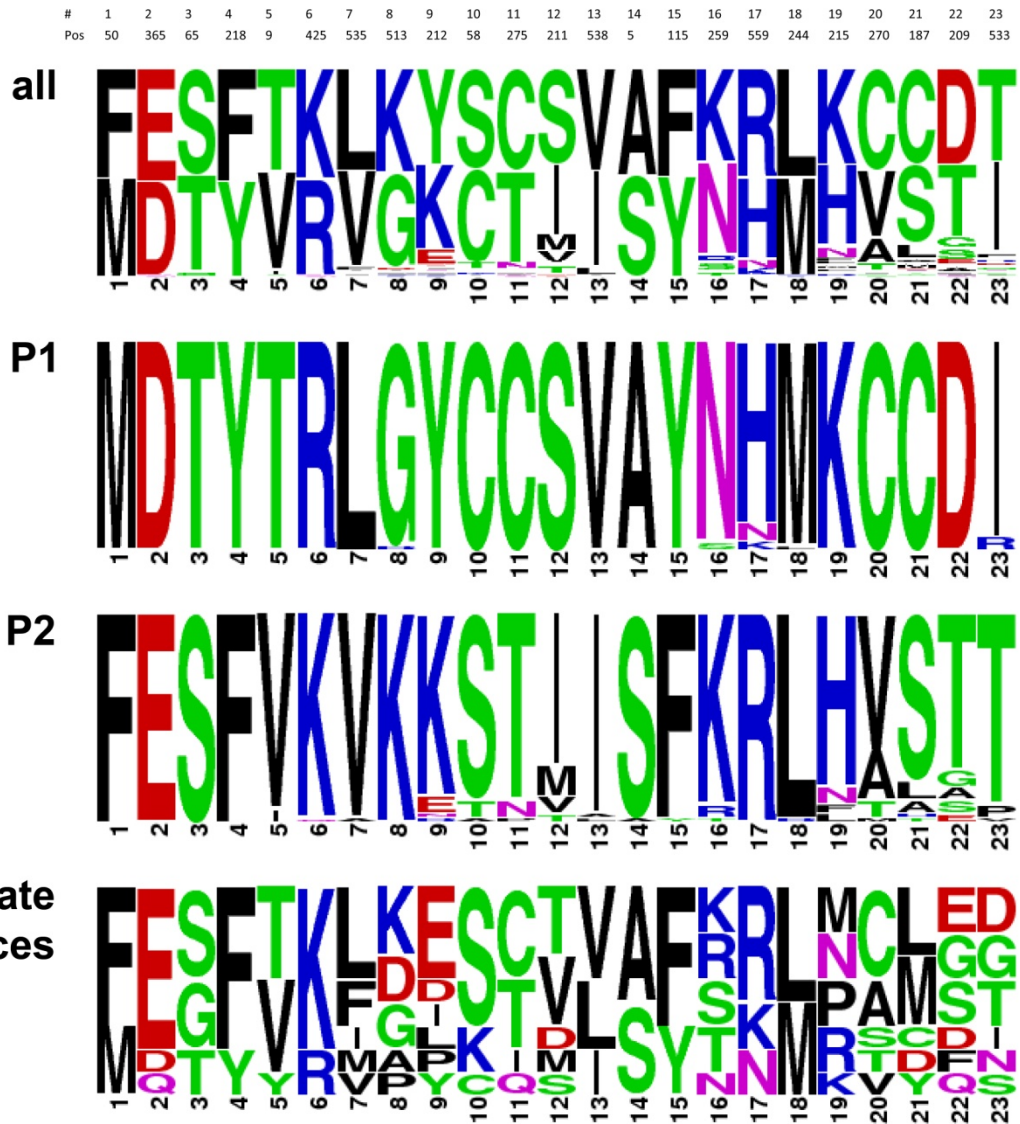
The sole PAPS synthase PPS-1 from the roundworm *C. elegans* behaves like human PAPS synthase 2 with regard to **A**, half life of unfolding at constant temperatures, **B**, activation energies for unfolding, **C** and **D**, stabilisation of its midpoint of thermal unfolding in the presence of APS, but no other nucleotides.



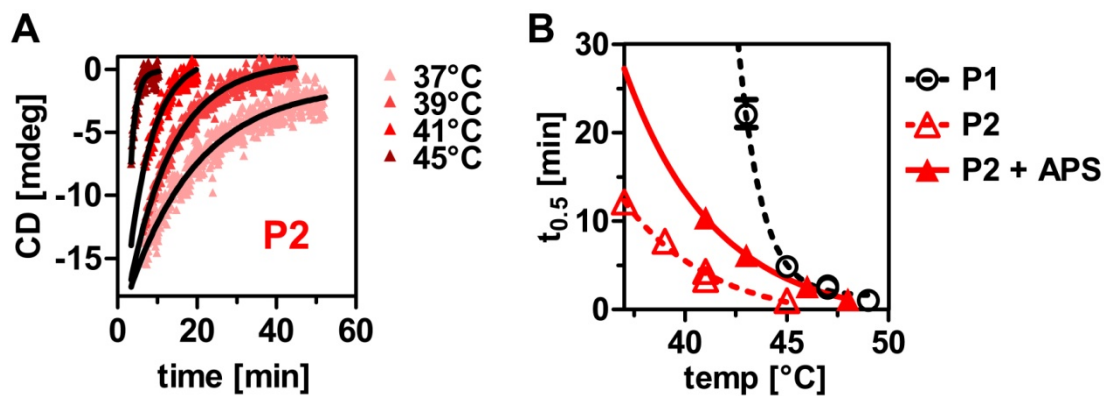
Supplementary figure 6: Other point mutants tested for their thermal unfolding behaviour by CD spectroscopy in the presence and absence of APS.



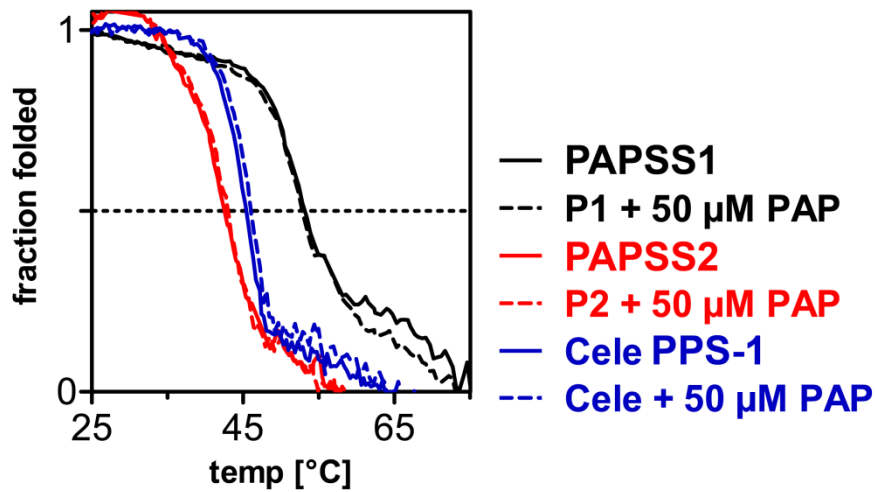
Supplementary figure 7: **A** and **B**, the V19T mutation in PAPS synthase 2 destabilises the protein in the absence of APS. In the presence of APS, this effect is more than rescued. **C-F**, full gels for limited proteolysis of PAPS synthase 2 wild-type and mutant proteins. **G** and **H**, limited proteolysis of PAPSS1 wild type for comparison.



Supplementary figure 8: Logos for 23 highlighted positions from the random forest approach visualising the existence of two classes of PAPS synthases ordered by their average drop-in-performance values. Positions correspond to the numbering within the 604 aa core sequence.



Supplementary figure 9: Kinetics of unfolding of PAPS synthases at constant temperatures. **A**, original traces and **B**, half lives extracted from these data. The presence of 50 μ M APS shifts PAPSS2 stability into the range of the more stable PAPSS1 protein.



Supplementary figure 10: The nucleotide PAP (3'-phospho-adenosine-5'-phosphate) does not influence thermal stability of PAPS synthases. PAP has recently been implicated in stress-signalling in plants. However, it does not stabilise human PAPS synthases or the PPS-1 protein from *C. elegans*.

Supplementary table 1: Primer sequences for site-directed mutagenesis

No.	nucleotide sequence	amino acid
PAPSS1		
845F	5'-gcagagagcaaccaatgtc GT ctaccaagcccatcatgtcag-3'	Thr29Val
846R	3'-cttacgtctctcgttggttacag CA gatgggttcgggtagtacag-5'	
847F	5'-cgggaaagactactgtgagc TtT gccttggaggagtacctg-3'	Met70Phe
848R	3'-gccctttctgatgacactcg AaA cggaacctcctcatggac-5'	
945F	5'-atggtattccatgctaca G tctggatggtgacaatattc-3'	Thr85Ser
946R	3'-gtaccataaggtacgatgt C agacctaccactgttataa-5'	
947F	5'-ggatgcatcttatgaagta CaC gaactatatgtgccagaaaataaac-3'	Lys235His
948R	3'-cacctacgtagaataacttcat GtG cttgatatacacggctcttttat-5'	
949F	5'-tgggcaacccattgaa A ggctttatgagagagaggg-3'	Asn279Lys
950R	3'-caaccggttggggtaactt T ccgaaatactctctctc-5'	
PAPSS2		
913F	5'-ccagcagaaatccaccaatgta AC ctatcaggcccaccatgtgag-3'	Val19Thr
914R	3'-ggtcgtcttttaggtggttacat TG gatagtccgggtggtacactc-5'	
877F	5'-ggtgctggaaaaacaacgataagt AtG gccctggaggagtaccttg-3'	Phe60Met
878R	3'-ccacgacctttttggtgctattca TaC ggggacctcctcatggaac-5'	
951F	5'-tgccatcccttgttac AC cctggatggggacaatg-3'	Ser75Thr
952R	3'-gtacggtagggaacaatg T gggacctaccctggtt-5'	
953F	5'-tatactataatcaaagatata AaA gaactctttgtgccgaaaac-3'	His225Lys
954R	3'-gggatatgatattagtttctatag TtT cttgagaaacacggccttt-5'	
955F	5'-gccactcccctcaa C ggtttcatgcccggagaag-3'	Lys269Asn
956R	3'-cccggtgaggggagtt G ccaaagtacgcctc-5'	
Mutated nucleotides are given as capital letters. Please note that reverse primers are written in 3'-5' direction. Primers were designed according to the QuikChange manual (Stratagene, Agilent Technologies, Waldbronn, Germany).		