Supplemental Information

Proteolytic dynamics of human 20S thymoproteasome

Ulrike Kuckelkorn *et al*.

Table S1	Synthetic substrates investigated by *in vitro* proteasomal digestions
Table S2	AQUA peptides used for the relative quantification of β5 and β5t subunits in the purified C5.5 20S proteasome
Figure S1	Suc-LLVY-MCA degradation and the corresponding model fits
Figure S2	Z-LLE-MCA degradation and the corresponding model fits
Figure S3	Computational model parameters of the 20S proteasome isoform dynamics for the substrate Z-LLE-MCA

Polypeptide substrate	**sequence**	**Antigen**	**Type of MS analysis**
$gp100_{40-52}$	RTKAWNRQLYPEW	$gp100^{PMEL17}$	peptide identification and quantitative kinetics
$gp100_{35-57}$	VSRQLRTKAWNRQLYPEWTEAQR	$gp100^{PMEL17}$	peptide identification and quantitative kinetics
$gp100_{201-230}$	AHSSSAFTITDQVPFSVSVSQLRALDGGNK	$gp100^{PMEL17}$	peptide identification and quantitative kinetics
$MBP_{102-129}$	PSQGKGRGLSLSRFSWGAEGQRPGFGYG	myelin basic protein (MBP) isoform 5	peptide identification and quantitative kinetics
$MAG_{543-576}$	TESPSFSAGDNPPVLFSSDFRISGAPEKYESERR	myelin associated glycoprotein (MAG)	peptide identification
$MAG_{498-528}$	SLELPFQGAHRLMWAKIGPVGAVVAFAILIA	MAG	peptide identification
MOG_{93-122}	EYRGRTELLKDAIGEGKVTLRIRNVRFSDE	myelin oligodendrocytes glycoprotein (MOG)	peptide identification
$MOG_{172-202}$	GLIFLCLQYRLRGKLRAEIENLHRTFDPHFL	MOG	peptide identification and quantitative kinetics

Table S1. Synthetic substrates investigated by *in vitro* proteasomal digestions.

20S proteasome subunit	**position**	**Sequence (upon tryptic digestion)**	***[M+H]+***	***AQUA peptide* sequence**	***[M+H]+ (AQUA-Peptide)***
PSB5 human (β5)	226-239	DAYSGGAVNLYHVR	1521.7	DAYSGGAVN**L**$^{+7}$YHVR	1528.7
PSB11 human (β5t)	216-229	DAYSGGSVDLFHVR	1522.7	DAYSGGSVD**L**$^{+7}$FHVR	1529.7

Table S2. AQUA peptides used for the relative quantification of β5 and β5t subunits in the purified C5.5 20S proteasome. Criteria for selection of peptide sequences: high MS intensity and significant MS/MS ion score in protein identification experiment, no modifiable amino acids, different HPLC retention times between the peptides specific for the two subunits.

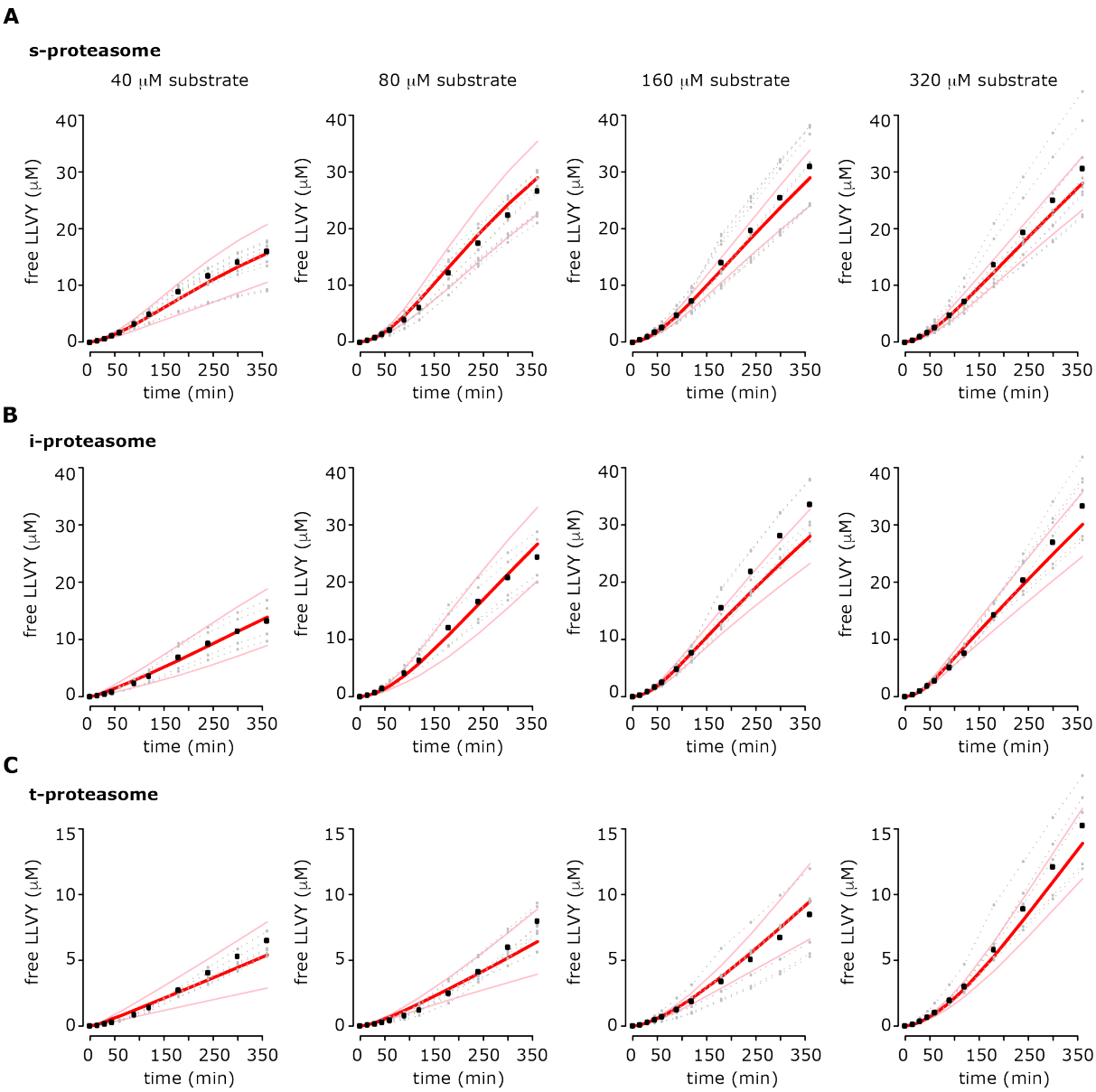

Figure S1. Suc-LLVY-MCA degradation and the corresponding model fits. The experimental data and the corresponding model fits obtained from the degradation of Suc-LLVY-MCA with purified human 20S s- (**A**), i- (**B**) or t-proteasomes (**C**) are depicted. Shown is the time course of 3-5 independent experiments (grey dashed dotted lines), the mean of those independent experiments (black dots), the mean of the obtained model fits (red lines) and the 5% and 95% confidence intervals of the model fits (pink lines).

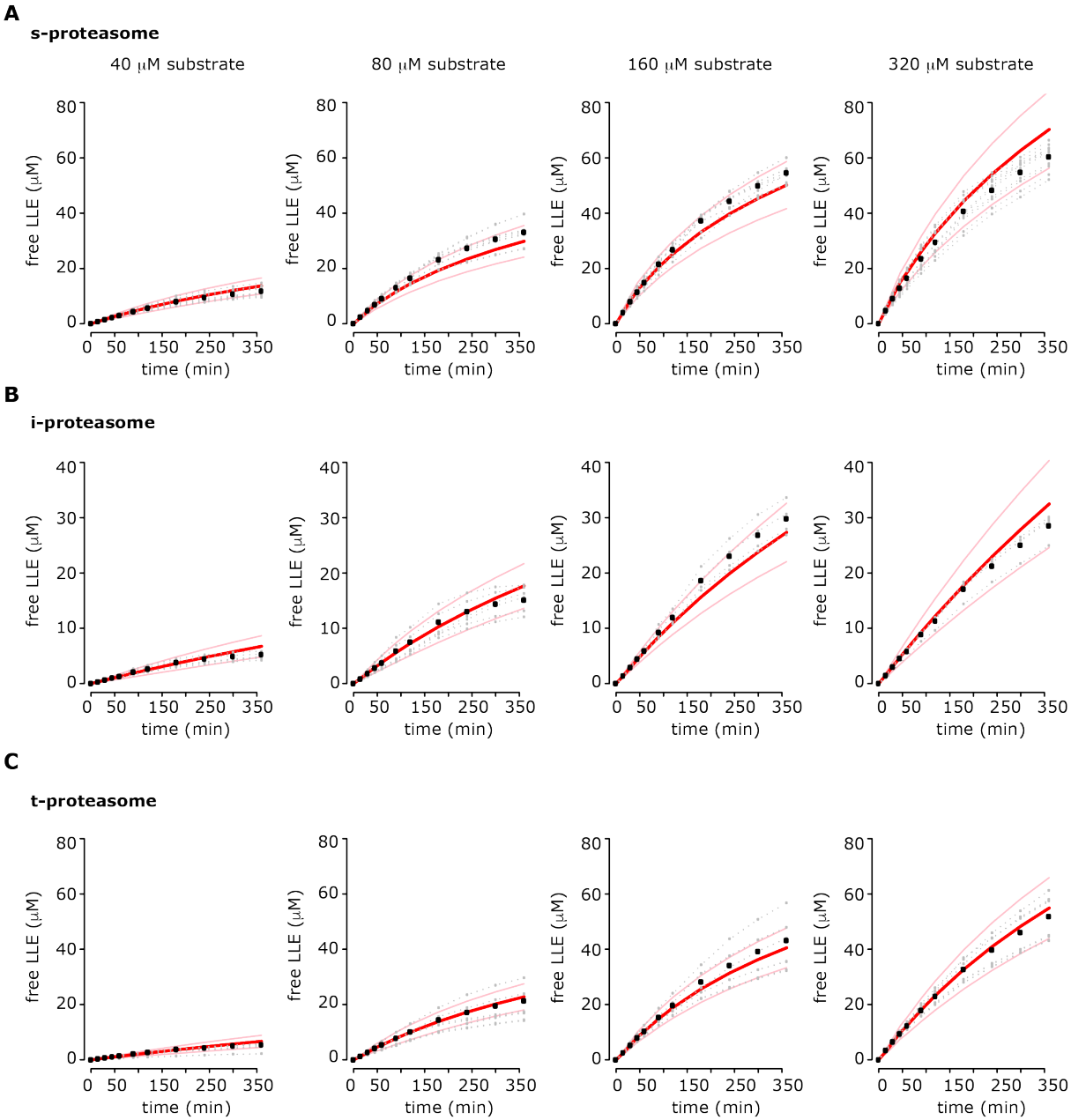

Figure S2. Z-LLE-MCA degradation and the corresponding model fits. The experimental data and the corresponding model fits obtained from the degradation of Z-LLE-MCA with 20S s- (**A**), i- (**B**) and t-proteasomes (**C**) are depicted. Shown is the time course of 3-5 independent experiments (grey dashed dotted lines), the mean of those independent experiments (black dots), the mean of the obtained model fits (red lines) and the 5% and 95% confidence intervals of the model fits (pink lines).

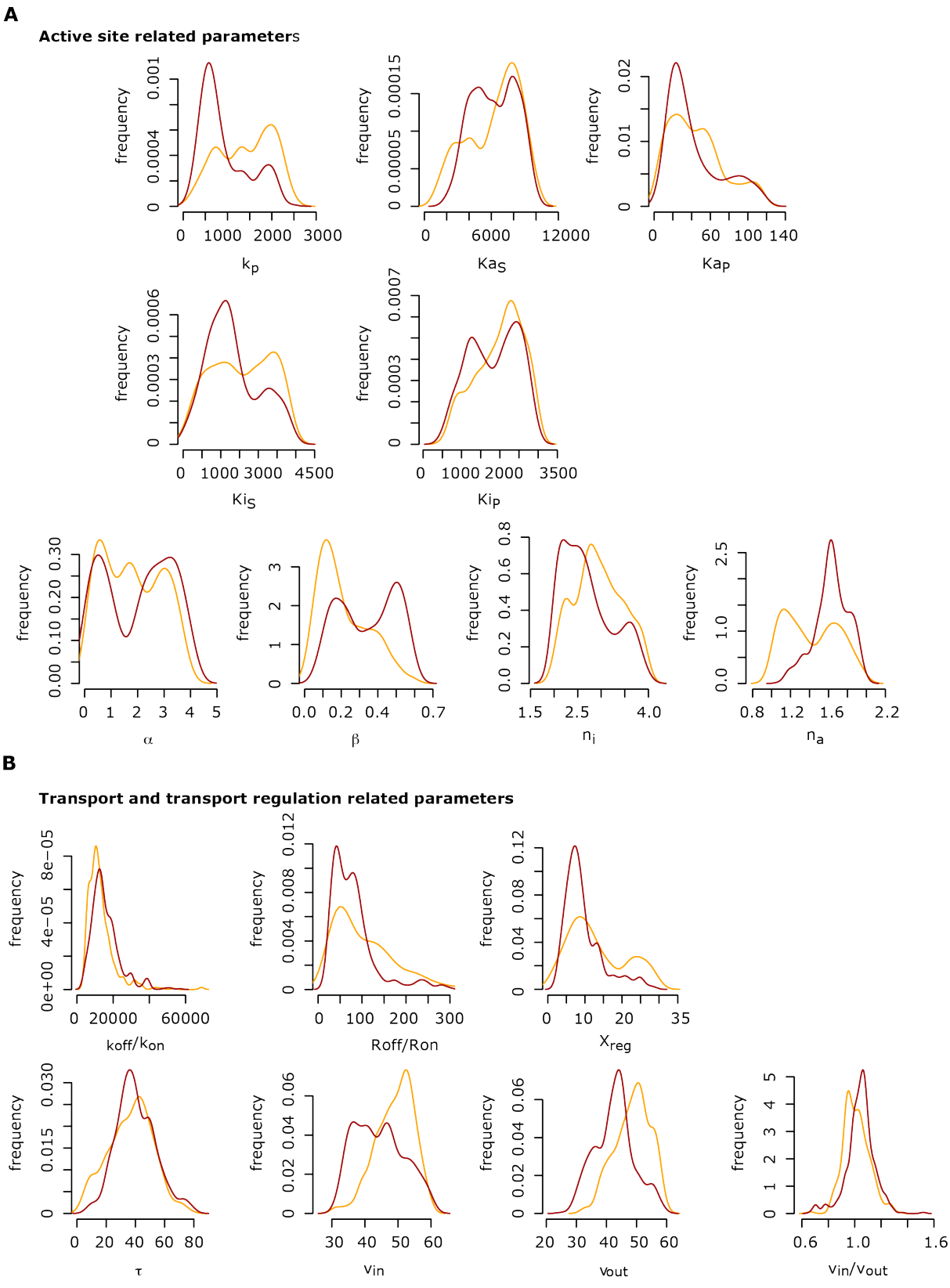

Figure S3. Computational model parameters of the 20S proteasome isoform dynamics for the substrate Z-LLE-MCA. It is shown the marginal posterior parameter distribution obtained from calibrating the proteasome kinetics model against experimental data obtained from the degradation of Z-LLE-MCA (n=3-5) with 20S i- and t-proteasome (yellow and brown, respectively). Parameters are grouped into active site relative parameters (**A**) and transport and transport regulation related parameters (**B**). The meaning of the parameters is explained in **Fig. 2A** and **Table 1**.