

Real-time kinetic method to monitor isopeptidase activity of transglutaminase 2 on protein substrate

## SUPPLEMENTARY MATERIAL

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# These authors contributed equally to this study.

## METHODS

### Fluorescence polarisation assay

The fluorescence polarisation assay was used to detect crosslinking activity of TG2 as described before with some modification [18]. 10 nM TG2 and 100 nM FLpepT26 peptide were incubated with various amount of BSA or S100A4(GST) in 20 mM Tris-HCl buffer, pH 7.5, containing 150

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mM NaCl, 5 mM DTT, 0.01% Tween-20 in the presence of 5 mM EDTA or Ca<sup>2+</sup> as negative control or test reactions, respectively. To check the utilisation of GST as an amine donor substrate the [GST] or [S100A4(GST)] were both 20 µM.

#### Dansyl-cadaverine incorporation assay

Dansyl cadaverine incorporation assay was used to study the utilisation of GST as an amine acceptor substrate. The reaction was performed as described before [11] in the presence of 200 µg dimethylated-casein or GST in 100 µl reaction mixture.

#### Peptide-based isopeptidase assay

The effect of S100A4(GST) on isopeptidase activity was measured using A102 Zedira substrate as described before [11] in the presence of different amount of S100A4(GST) protein with some modifications. The TG2 concentration was 150 nM.

TABLE:

Compound	Theoretical molecular mass	Measured molecular mass
FL pepT26	1885.0	1884.9
Cleaved FL pepT26	1886.0	1886.1

Table S1: Results of ESI-MS measurements.

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FIGURES:

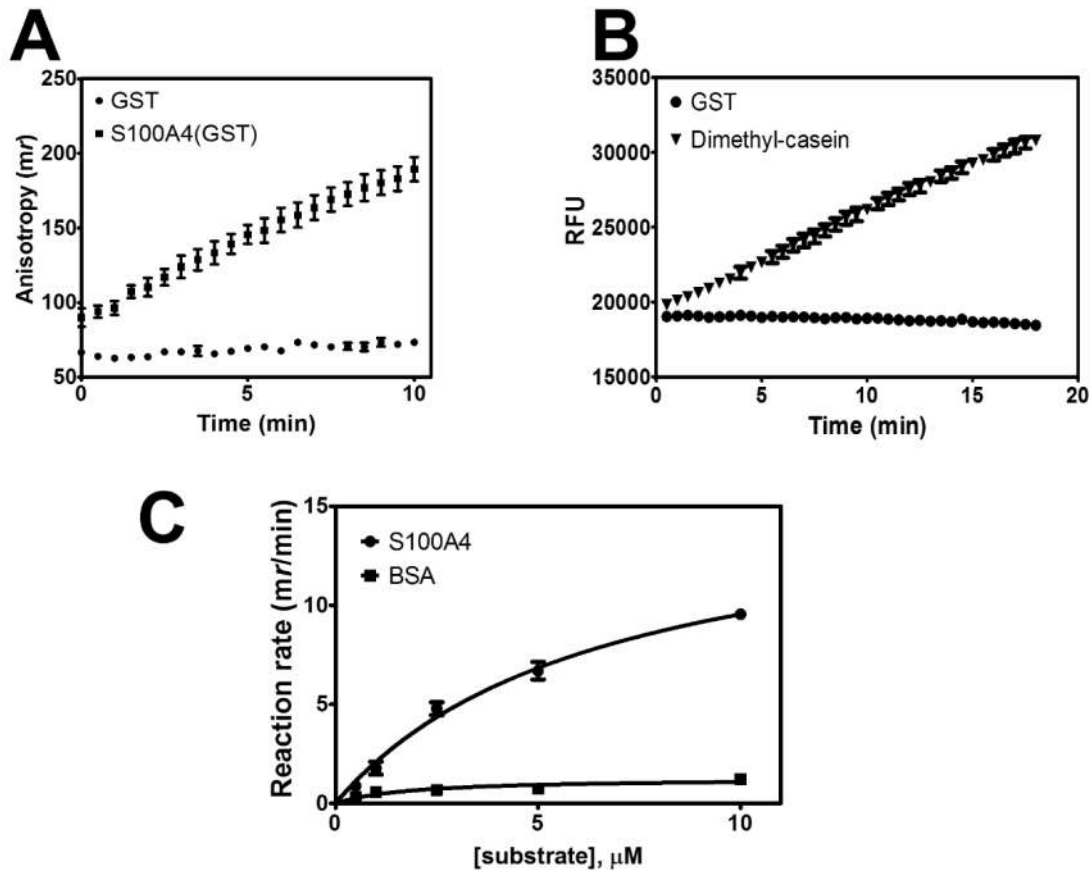


Fig. S1.

GST was not utilised neither as an amine donor using crosslinking anisotropy assay with FLpepT26 peptide (A) nor as an amine acceptor using dansyl-cadaverine incorporation assay (B). Representative data from two independent experiments done in triplicate. Comparison of BSA and S100A4(GST) as Lys donor substrates using crosslinking anisotropy assay (C). Data are presented as means with  $\pm$ SEM from two separate experiments done in triplicate.

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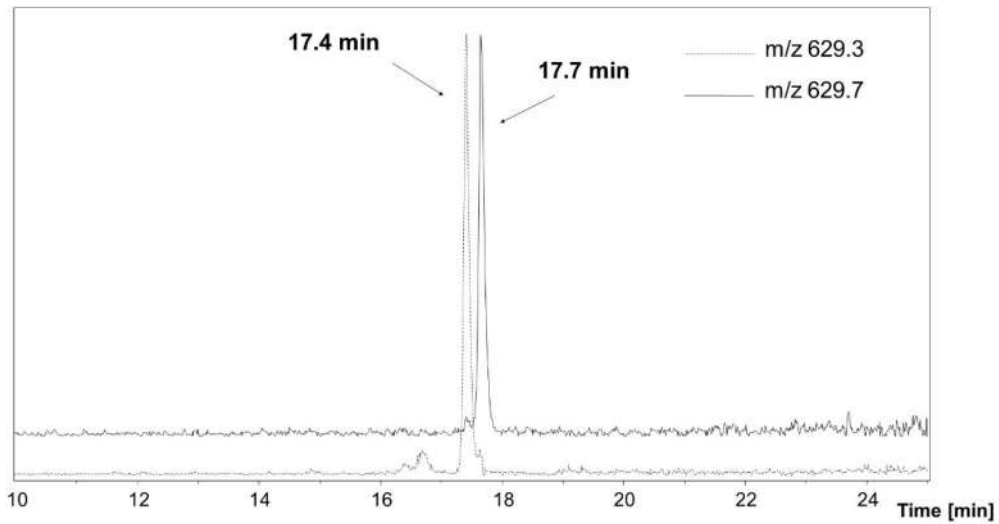


Fig. S2.

Selected ion chromatogram of the triply protonated peptides. Dashed line: original peptide, solid line: cleaved peptide.

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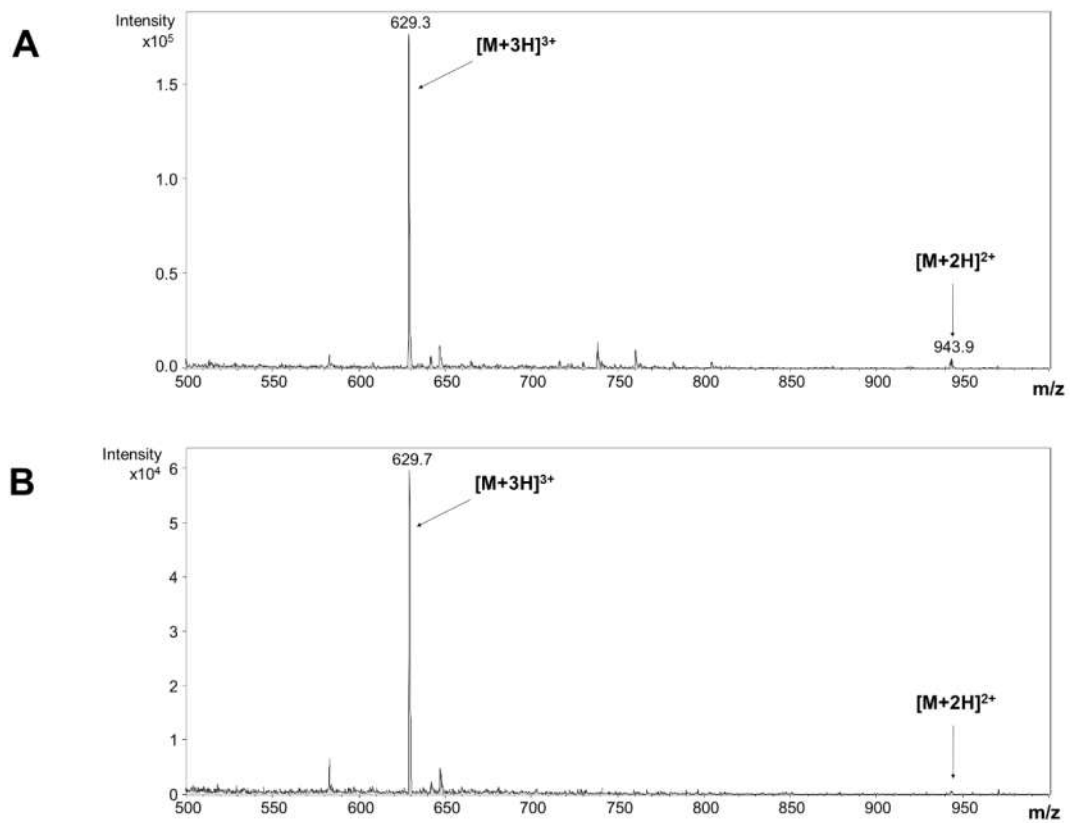


Fig. S3.

ESI-MS spectrum of the original peptide (A) and cleaved peptide (B).



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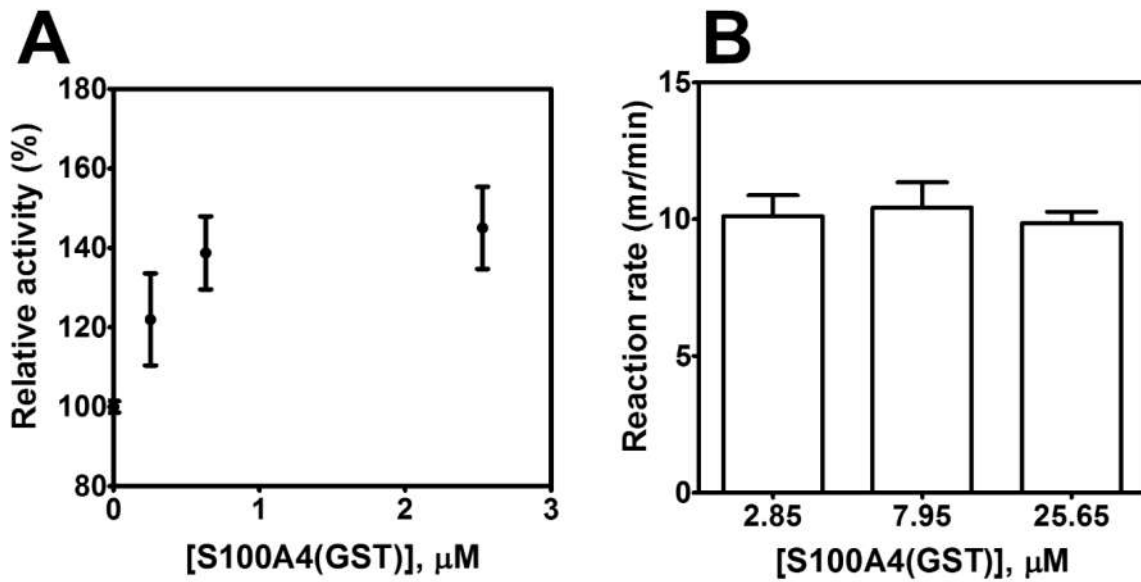


Fig. S5.

Effect of S100A4(GST) on isopeptidase activity using A102 Zedira substrate (A) or FLpepT26-S100A4(GST) peptide based substrate (B). Data are presented as means with  $\pm$ SEM from two separate experiments done in triplicate or duplicate.