Pharmaceutically modified subtilisins withstand acidic conditions and effectively degrade gluten *in vivo*

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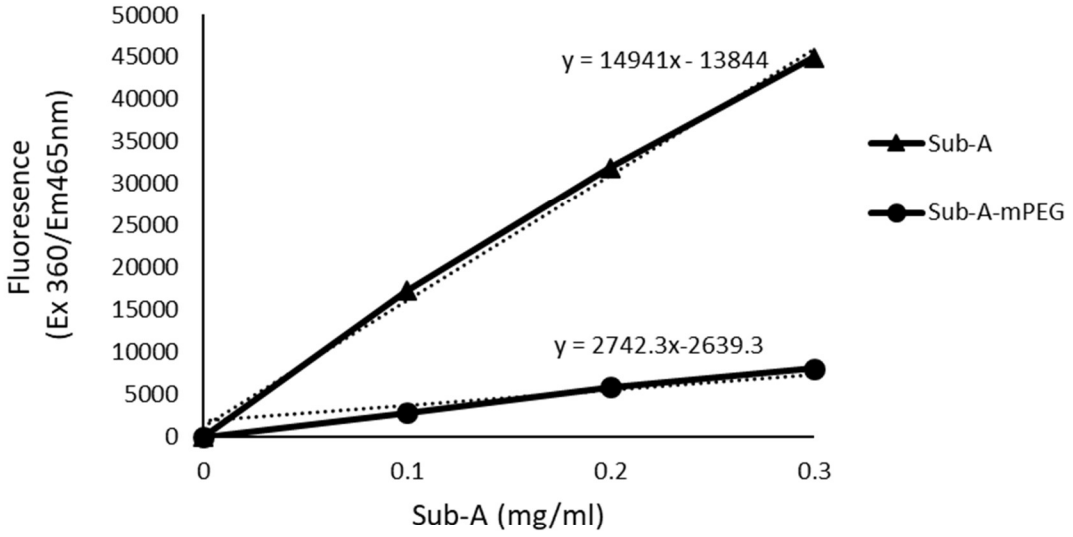
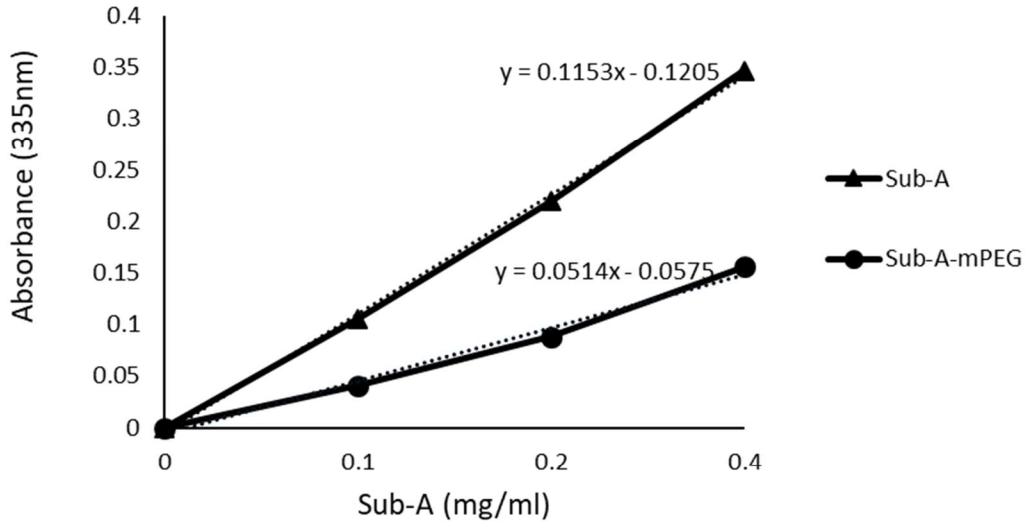
(A) Fluorescamine



(B) TNBSA



Supplemental Figure 1. Assessment of the extent of Sub-A PEGylation by (A) fluorescamine methods and (B) the TNBSA method, n=3.

Supplemental Table 1. Validation of PEGylation of Subtilisin-A (Sub-A) with Two Methods

Methods	**(A) Fluorescamine**	**(B) TNBS**
Target	Primary amine in folded protein	Primary amine in unfolded protein
Measurement	Fluorescent compound	Chromogenic product
Slope of Sub-A	14941	0.1153
Slope of Sub-A-mPEG	2742.3	0.0514
mPEG modification (%)	81.64	55.42
*PEGylated amines	~8	~6

*Number of primary amines in Sub-A is 10.

The data shown are representative of experiments conducted in triplicate.

Supplemental Table 2. Modification of Subtilisin-A (Sub-A)

	Before modification (mg)	After modification (mg)	Yield (%)	Sub-A Conc. (%, w/w)
Sub-A + mPEG	40+120=160	116.6	~73	~34
Sub-A-mPEG + PLGA	10+90=100	43.3	~43	~8

The data shown are representative of experiments conducted in triplicate.