

## Film-shaped reusable smart polymer to produce lactose-free milk by simple immersion

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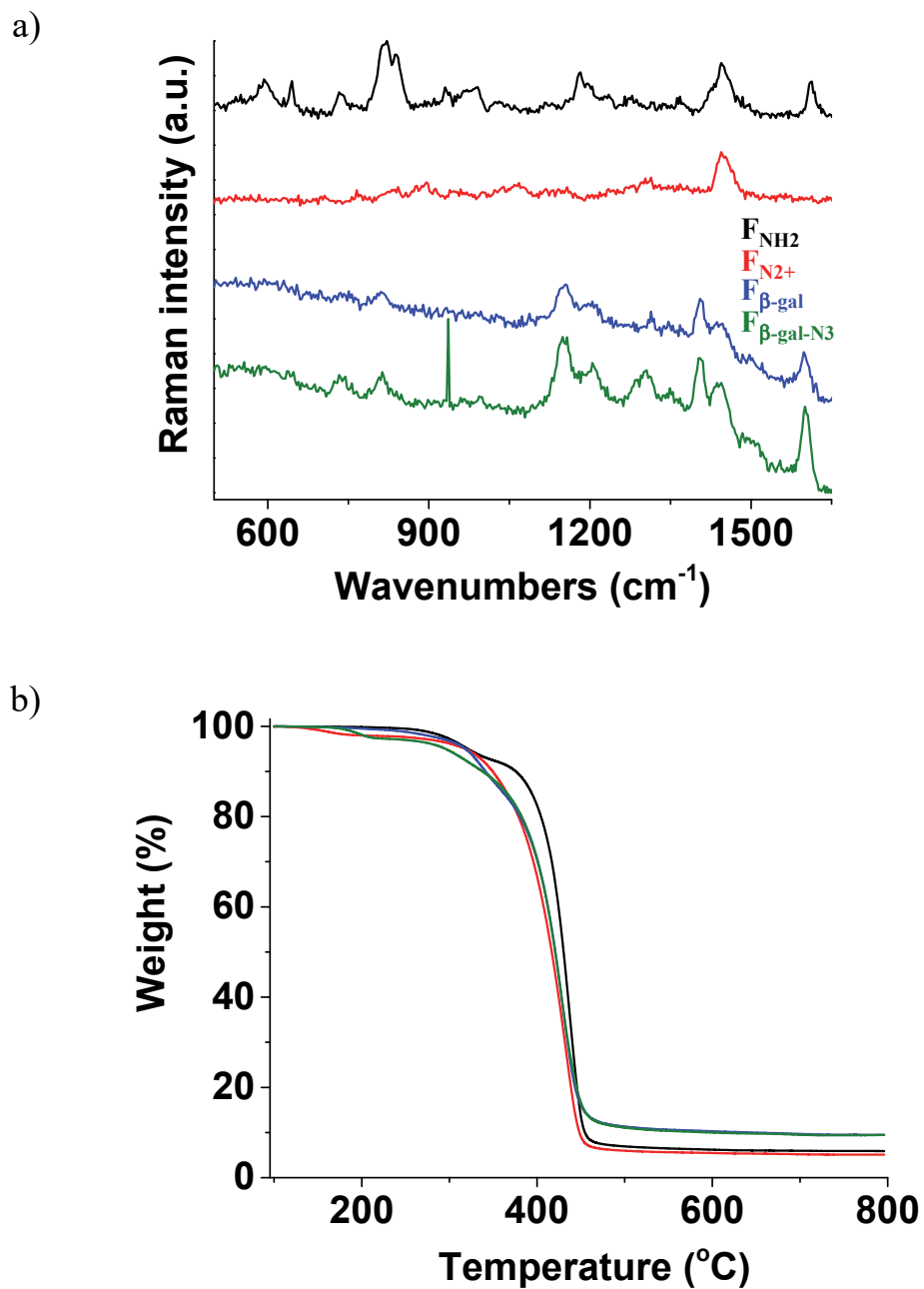
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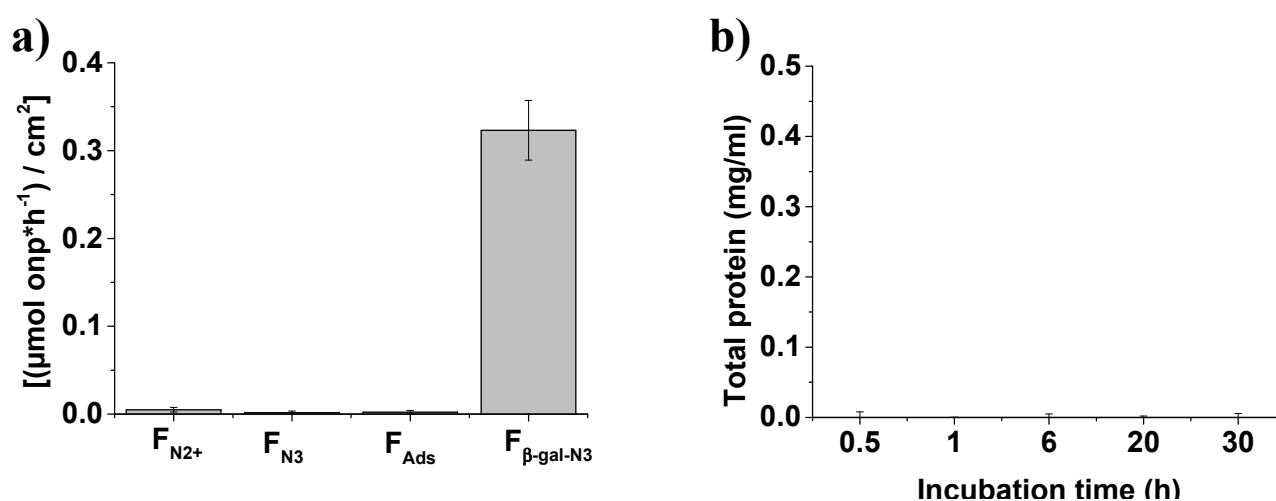
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**S1. Characterization of polymeric films  $F_{NH_2}$ ,  $F_{N_2^+}$ ,  $F_{\beta\text{-gal}}$ , and  $F_{\beta\text{-gal-N}_3}$  by Raman and TGA.**



**Figure S1.** Characterization of  $F_{NH_2}$ ,  $F_{N_2^+}$ ,  $F_{\beta\text{-gal}}$ , and  $F_{\beta\text{-gal-N}_3}$  by (a) RAMAN and (b) thermogravimetry (curves at  $10^{\circ}\text{C}\cdot\text{min}^{-1}$  under nitrogen atmosphere).

## S2. Enzymatic activity evaluation and protein release study

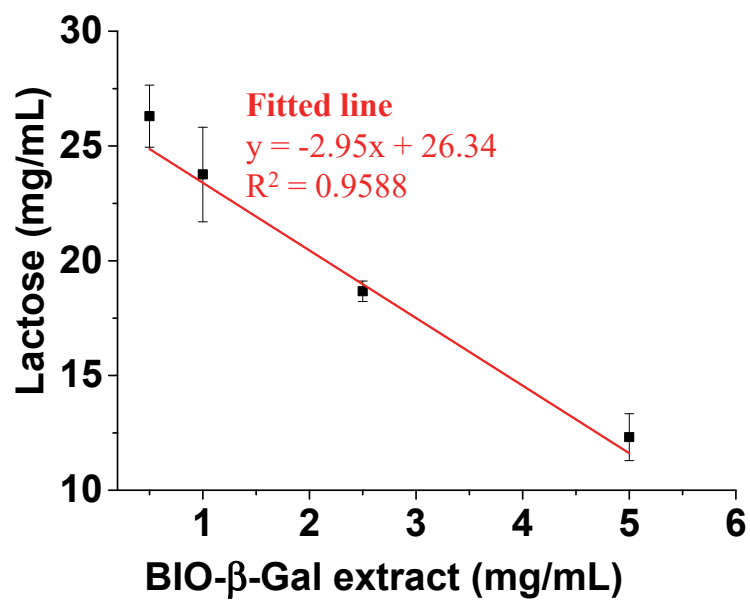


**Figure S2.** (a) Enzymatic activity of  $F_{N2+}$ ,  $F_{N3}$  and  $F_{\beta\text{-gal-N3}}$  discs using ONPG as substrate.  $F_{Ads}$  corresponds to the film incubated with the enzyme solution without the diazotization step. (b) Protein release of  $F_{\beta\text{-gal-N3}}$  discs incubated in citrate-phosphate buffer up to 30 hours. Data are means of three replicates  $\pm$  SE.

**Table S2.** Kinetic parameters of free (BIO- $\beta$ -gal) and immobilized ( $F_{\beta\text{-gal-N3}}$ ) enzyme determined by using Lineweaver-Burk plot (ONPG as substrate, pH 5, at 60 °C for 10 minutes). Data are means of three replicates  $\pm$  SE.

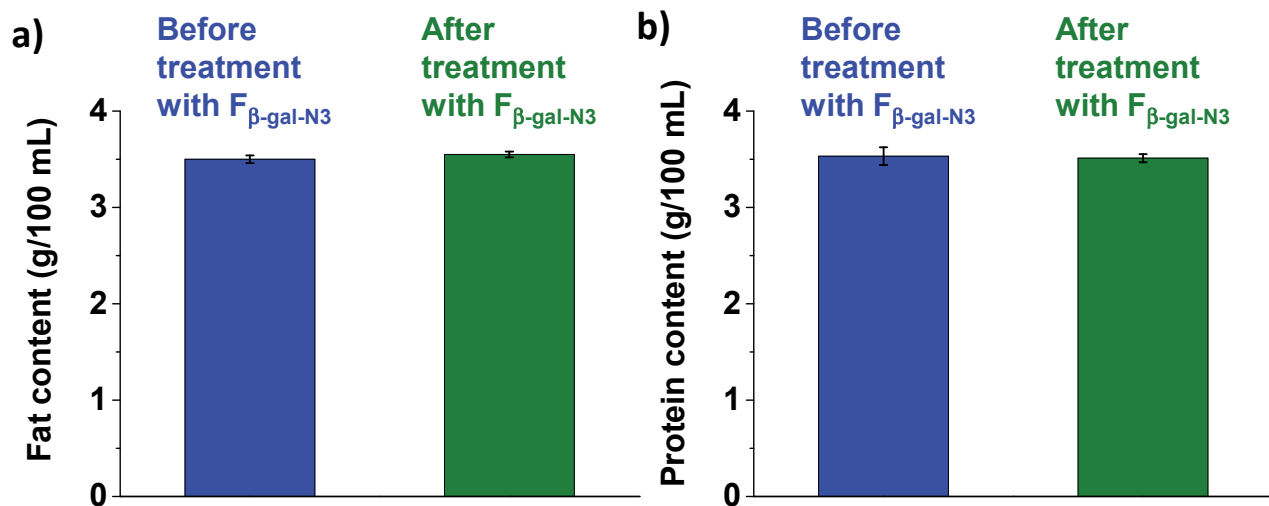
	BIO- $\beta$ -gal	$F_{\beta\text{-gal-N3}}$
$V_{\max}$	( $\mu\text{mol ONP min}^{-1} \text{mg}^{-1}$ of enzyme)	( $\mu\text{mol ONP min}^{-1} \text{mg}^{-1}$ of $F_{\beta\text{-gal-N3}}$ )
	$0.805 \pm 0.012$	$0.013 \pm 0.003$
$K_m$ (mM)	$4.8 \pm 0.1$	$4.9 \pm 1.4$

### S3. Calibration. Equivalence test.



**Figure S3.** Calibration. Experimental conditions: Milk sample volume=1 mL; added amounts of BIOCON β-galactosidase=0.5, 1, 2.5, and 5 mg; temperature=25°C; hydrolysis time=1 hour.

#### S4. Determination of the nutritional content of milk.



**Figure S4.** Determination of the nutritional content of milk samples before and after lactose hydrolysis using  $F_{\beta\text{-gal-N3}}$ . Experimental conditions: Temperature = 4 °C; Time = 30 h. Results are means of three replicates  $\pm$  SE.