ESI - Electronic Supplementary Information

An optimized purification process for porcine gastric mucin with preservation of its native functional properties

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Gel formation of 1 % or 2 % (w/v) self-purified mucin only differs in strength of the gel:

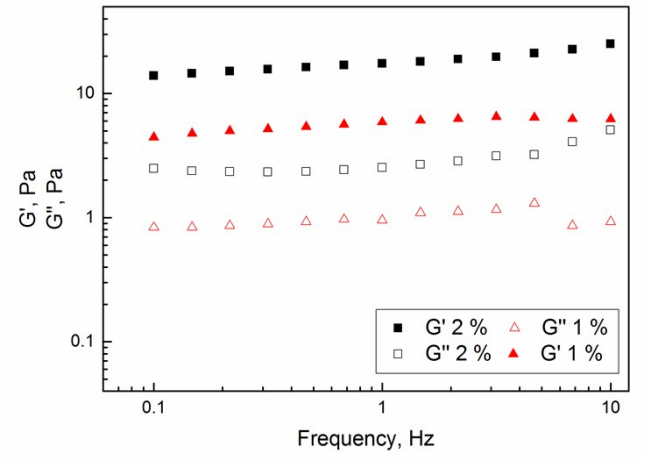

Figure S1. Frequency dependent storage (G') and loss modulus (G'') between 0.1 and 10 Hz for 1 % and 2 % (w/v) purified mucin at pH 2. No qualitative difference is observed in gel-forming behavior; only the strength of the gel is reduced with lower concentration.

Rheological measurement to determine the influence of 10 mM phosphate buffer containing 170 mM NaCl compared to the unbuffered system (200 mM NaCl) (a), and rheological measurements to determine the influence of protease inhibitors during the purification process (b):

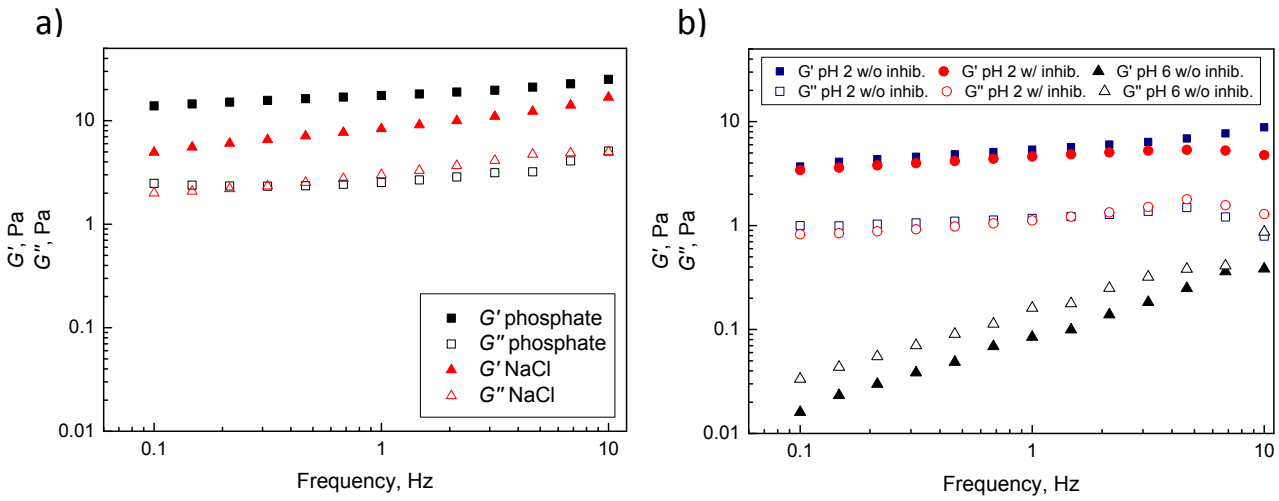

Figure S2. Frequency dependent storage (*G'*) and loss modulus (*G''*) between 0.1 and 10 Hz. a) Rheological measurements of 2 % (w/v) mucin purified in either 200 mM NaCl (NaCl) or 10 mM phosphate buffer containing 170 mM NaCl (phosphate) at pH 2. b) Rheological measurements of 1 % (w/v) mucin purified in 10 mM phosphate buffer and 170 mM NaCl with and without protease inhibitors at pH 2.

Enzymatic digestion of mucin with pepsin in 10 mM phosphate buffer pH 2/ 5/ 7/ 9: SDS-PAGE was used as qualitative analysis of digested and undigested mucins.

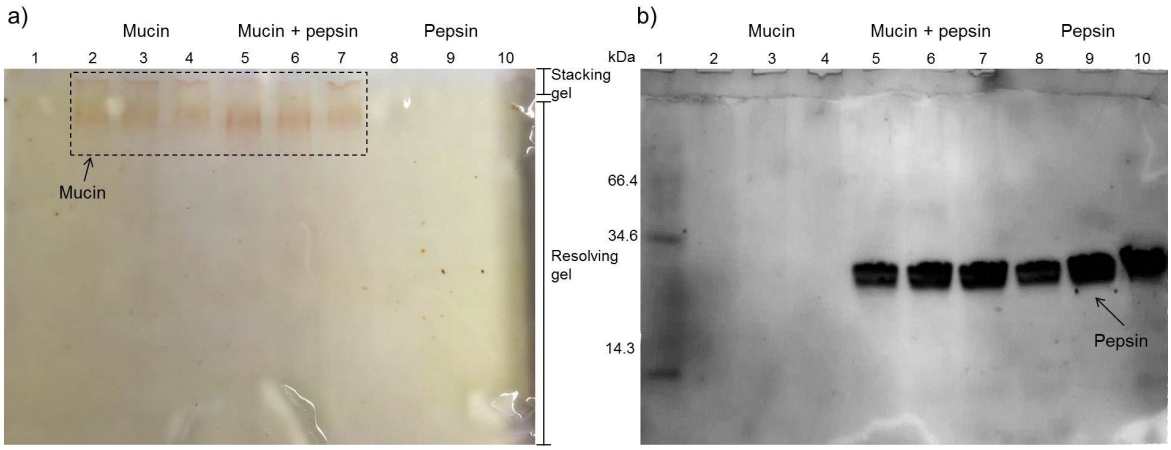

Figure S3. SDS-PAGE of undigested and digested mucin, and pure pepsin at pH 2, 5, and 7. a) Staining of glycoproteins with thymol staining. b) Staining of the same gel with Coomassie Brilliant G250 for detection of all proteins. Lane 1 Marker, Lanes 2-4: pure mucin (13 µg) at pH 2, 5, 7. Lanes 5-7: digested mucin with pepsin (13 µg mucin with 500 µg pepsin) at pH 2, 5, 7. Lanes 8-10: pepsin (500 µg) at pH 2, 5, 7. The stacking and resolving gel are displayed.

Influence of temperature before and after purification:

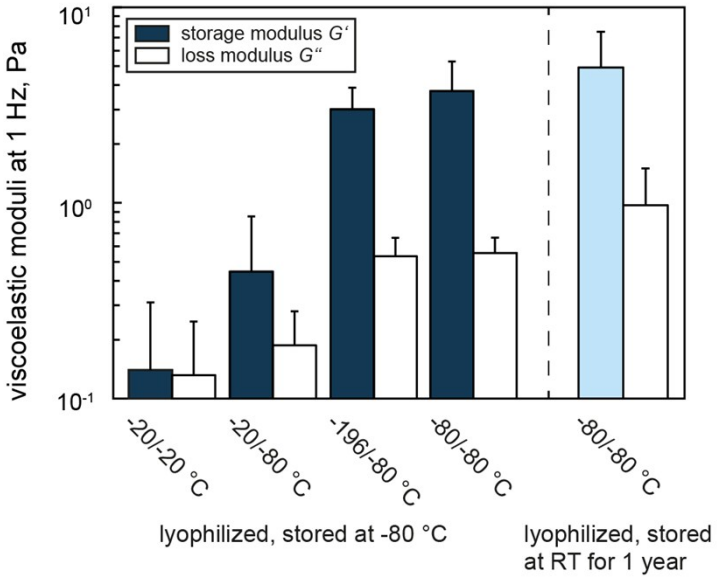

Figure S4. Viscoelastic moduli *G‘* and *G‘‘* of 1 % (w/v) mucin solution at pH 2 and 1 Hz for different storage temperatures of mucus/mucin. Column sets 1-4: Unpurified mucus was frozen at either -20 °C, -80 °C or in liquid nitrogen and long-term stored afterwards at -20 °C or -80 °C. Purified and lyophilized mucin was stored at -80 °C. Column set 5: Unpurified mucus

was frozen and stored at -80 °C, purified, lyophilized and stored at room temperature afterwards for 1 year in an Eppendorf tube. The -20/-20 °C freeze/storage condition did not always form a gel. The error bars denote the s.d. of at least three measurements.

Preliminary studies of crossflow filtration using membrane cassettes with commercially available Mucin type III (Sigma Aldrich):

a) b)

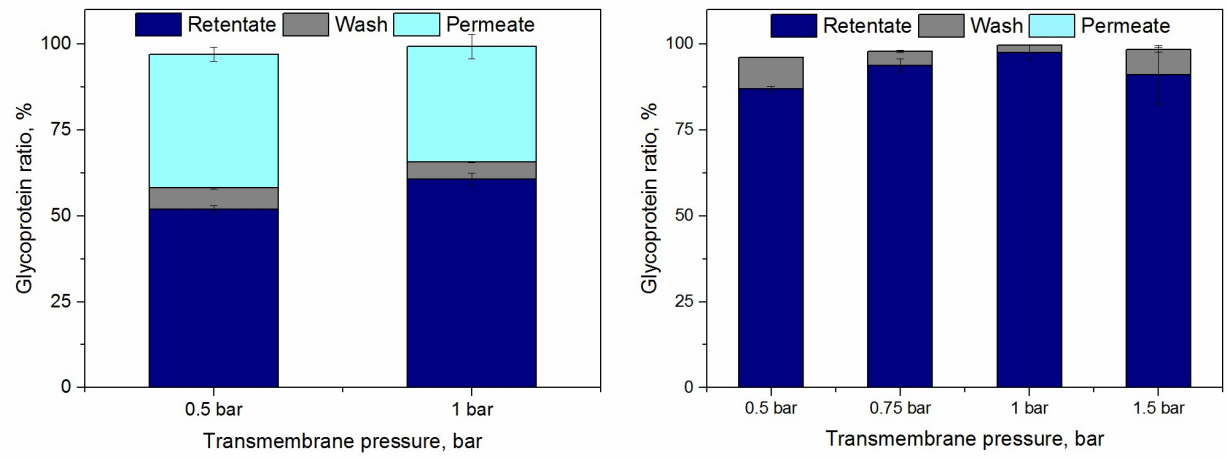

Figure S5. a) Glycoprotein ratio of commercially available Muc type III at different transmembrane pressures 0.5 bar and 1 bar using 300 kDa PESU filtration membrane. b) Glycoprotein ratio of Muc III at different transmembrane pressures 0.5 / 0.75 / 1 / 1.5 bar using 100 kDa regenerated cellulose filtration membrane. The buffer was 10 mM phosphate buffer pH 7 with 170 mM NaCl. Glycoprotein content in the retentate, permeate and washing step were analyzed with PAS Assay. Error bars represent ± s.d. of analytical triplicates.

Comparison of filtration modules regarding their fluid flow:

$$Re = \frac{v \rho d_h}{\eta}$$ Equation S1

$$Sc = \frac{\eta}{\rho D}$$ Equation S2

With v being the velocity in m s^{-1}, ρ the density of fluid (assumption of water: 1000 kg m^{-3}), η the

viscosity of fluid (assumption of water: 0.89 mPa s) and *D* the diffusion coefficient (mean diffusion coefficient of mucins taken from the literature [43]: $4*10^{-8}$ cm^2 s^{-1}).

Table S1. Comparison of feed stream, hydrodynamic diameter and cross sectional area for calculation of the Reynolds (*Re*), Sherwood (*Sh*) and Peclet (*Pe*) number. The boundary layer was theoretically calculated depending on laminar or turbulent flow.

	Membrane cassettes		**Hollow fiber**
	100 kDa	**300 kDa**	**100 kDa**
Feed stream, mL min^{-1}	200	240	200
Hydrodynamic diameter, d_h, m	0.002[a)]	0.002[a)]	0.0001
Cross-sectional area, m^2	$6.7*10^{-5}$	$6.7*10^{-5}$	$7.9*10^{-9}$
Re	112	134	47687
Sh	102	108	7822
Pe	83	100	8
Boundary layer δ_{BL}, µm	19.6	18.5	0.012

[a)] Estimated gap between the membranes of cassettes: 1 mm. With $b >> d$: $d_{hyd} = 2\,d$

Summary of glycoprotein purification processes (PAS Assay):

- 300 kDa membrane - small scale process:

Table S2. Summary of the overall purification process with the optimized protocol with 300 kDa concentration (membrane cassette), Sepharose 6 *Fast Flow* size exclusion chromatography, 300 kDa diafiltration in terms of yield, concentrations, volumes and mass before and after process units. Determination of glycoprotein concentration was conducted with the PAS Assay. Results are the mean ± s.d. of analytical triplicates.

Downstream process step	**Yield**	**C_{before}, g L^{-1}**	**C_{after} , g L^{-1}**	**V_{before}, L**	**V_{after}, L**	**m $_{before}$, mg**	**m $_{after}$, mg**
Concentration	69%	0.45	0.65	0.2	0.096	90.4	61.9
SEC	82%	0.65	0.42	0.02	0.025	12.9	10.5
Diafiltration	72%	0.42	0.17	0.025	0.044	10.5	7.6
Lyophilization		0.32		0.042			**9.0**
Total	40.3%						

- 100 kDa membrane - small scale process:

Table S3. Summary of the overall purification process with the optimized protocol with 100 kDa concentration (membrane cassette), Sepharose 6 *Fast Flow* size exclusion chromatography, 100 kDa diafiltration in terms of yield, concentrations, volumes and mass before and after process units. Determination of glycoprotein concentration was conducted with the PAS Assay. Results are the mean ± s.d. of analytical triplicates.

Downstream process step	**Yield**	**C_{before}, g L^{-1}**	**C_{after}, g L^{-1}**	**V_{before}, L**	**V_{after}, L**	**m_{before}, mg**	**m_{after}, mg**
Concentration	78%	0.45	0.77	0.196	0.09	88.2	69.0
SEC	83%	0.77	0.51	0.02	0.025	15.3	12.7
Diafiltration	87%	0.51	0.27	0.025	0.042	12.7	11.0
Lyophilization		0.32		0.042			**13.1**
Total	56.3%						

- 100 kDa hollow fiber module – upscaled process:

Table S4. Summary of the overall purification process with the optimized and upscaled protocol with 100 kDa concentration (hollow fiber module), Sepharose 6 *Fast Flow* size exclusion chromatography, 100 kDa diafiltration via hollow fiber module in terms of yield, concentrations, volumes and mass before and after process units. Determination of glycoprotein concentration was conducted with the PAS Assay. Results are the mean ± s.d. of analytical triplicates.

Downstream process step	**Yield**	**C_{before}, g L^{-1}**	**C_{after}, g L^{-1}**	**V_{before}, L**	**V_{after}, L**	**m_{before}, mg**	**m_{after}, mg**
Concentration	79%	1.16	3.58	0.7	0.18	812.0	644.2
SEC	68%	3.58	1.21	0.15	0.3	536.8	363.2
Diafiltration	80%	1.21	1.94	0.3	0.150	363.2	290.3
Lyophilization		0.32					**415.0**
Total	42.9%						