Supporting Information

Thiolated polyglycerol sulfate as potential mucolytic for muco-obstructive lung diseases

Justin Arenhoevel,‡[a] Aditi Kuppe,‡[b,c] Annalisa Addante,[b,c] Ling-Fang Wei,[a,f] Nico Boback,[a,f] Cosmin Butnarasu,[a,f] Yinan Zhong,[a] Christine Wong,[b,c] Simon Y. Graeber,[b,c,d] Julia Duerr,[b,c] Michael Gradzielski,[e] Daniel Lauster, *[a,f] Marcus A. Mall, *[b,c,d] and Rainer Haag*[a]

[a]Freie Universität Berlin, Institute of Chemistry and Biochemistry, SupraFAB, Altensteinstr.23a, 14195 Berlin, Germany
[b]Charité - Universitätsmedizin Berlin, Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Augustenburger Platz 1, 13353 Berlin Germany
[c]German Center for Lung Research (DZL), associated partner site, 13353 Berlin, Germany
[d]Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Berlin, Germany, Charitéplatz 1, 10117 Berlin, Germany
[e]Technische Universität Berlin, Institute of Chemistry, Berlin, Germany, Straße des 17. Juni 124, 10623 Berlin, Germany
[f]Freie Universität Berlin, Institute of Pharmacy, Biopharmaceuticals, Kelchstraße 31, 12169 Berlin, Germany
‡ J.A. and A.K. contributed equally

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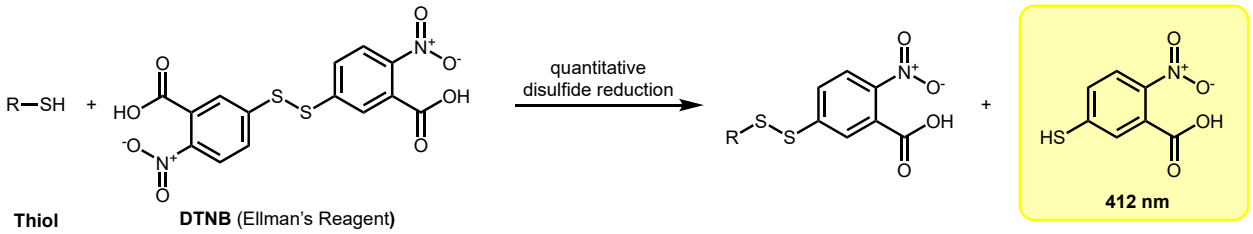
1. Disulfide reduction scheme of the model disulfide DTNB



Scheme S1. Schematic representation of the reduction of the model disulfide DTNB using thiol-containing molecules. Thiol molecules cleave the disulfide followed by a stoichiometric release of TNB^{2-} that absorbs at 412 nm. Spectroscopic quantification of thiol groups for dPGS-SH and dPG-SH was performed as described in the methods section (Ellman's Assay).

2. ^{1}H NMR spectra of dPGS-NH_2, dPGS-SH and dPG-SH

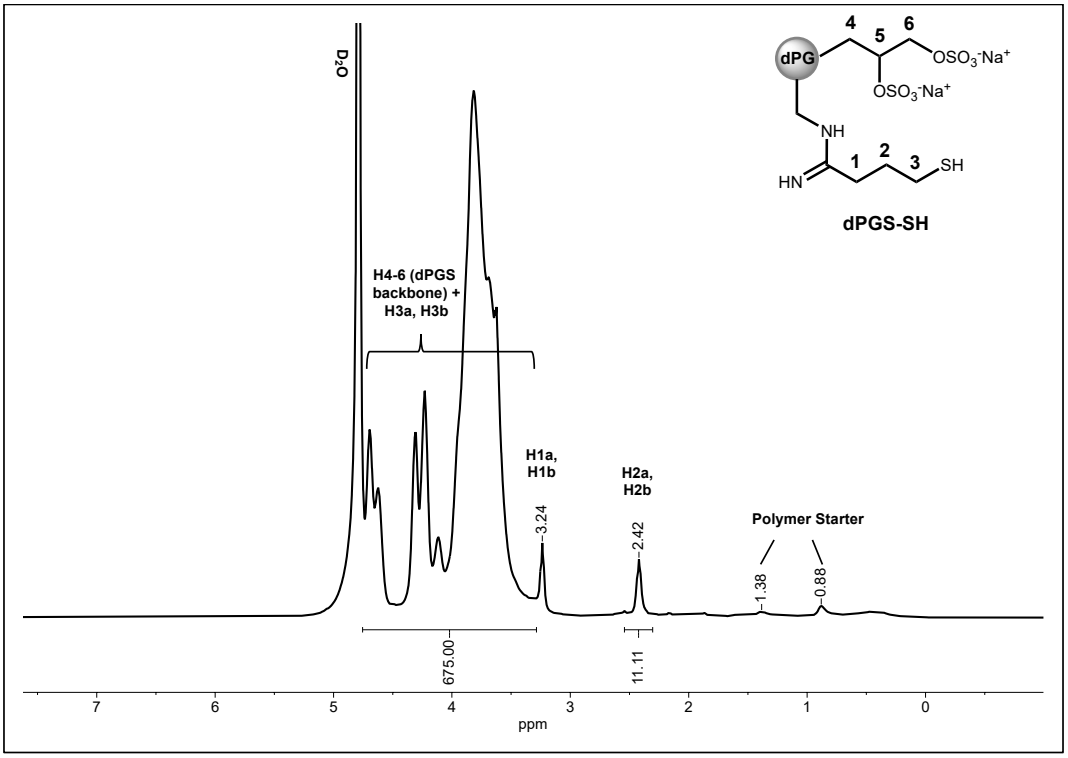


Figure S1. ^{1}H NMR spectrum of dPGS-SH recorded in D_2O. DF was calculated using equation S1 and the integrals of H2a and H2b and the polymer backbone.

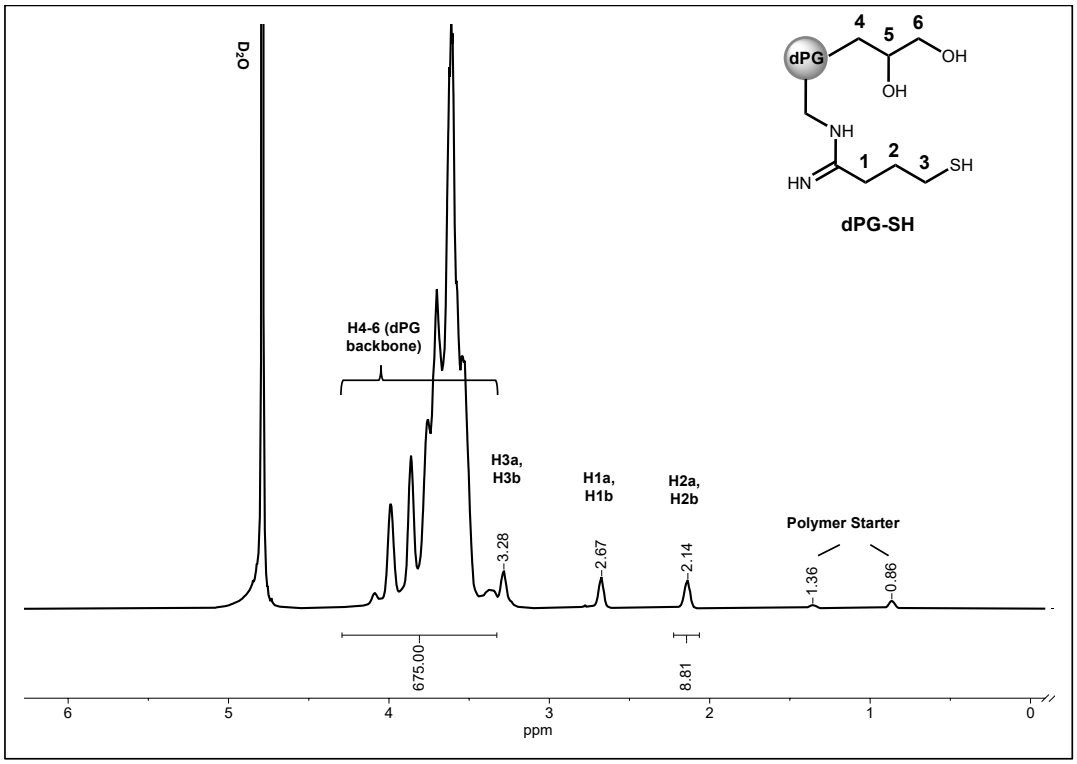


Figure S2. ^{1}H NMR spectrum of dPG-SH recorded in D_2O. DF was calculated using equation S1 and the integrals of H2a and H2b and the polymer backbone.

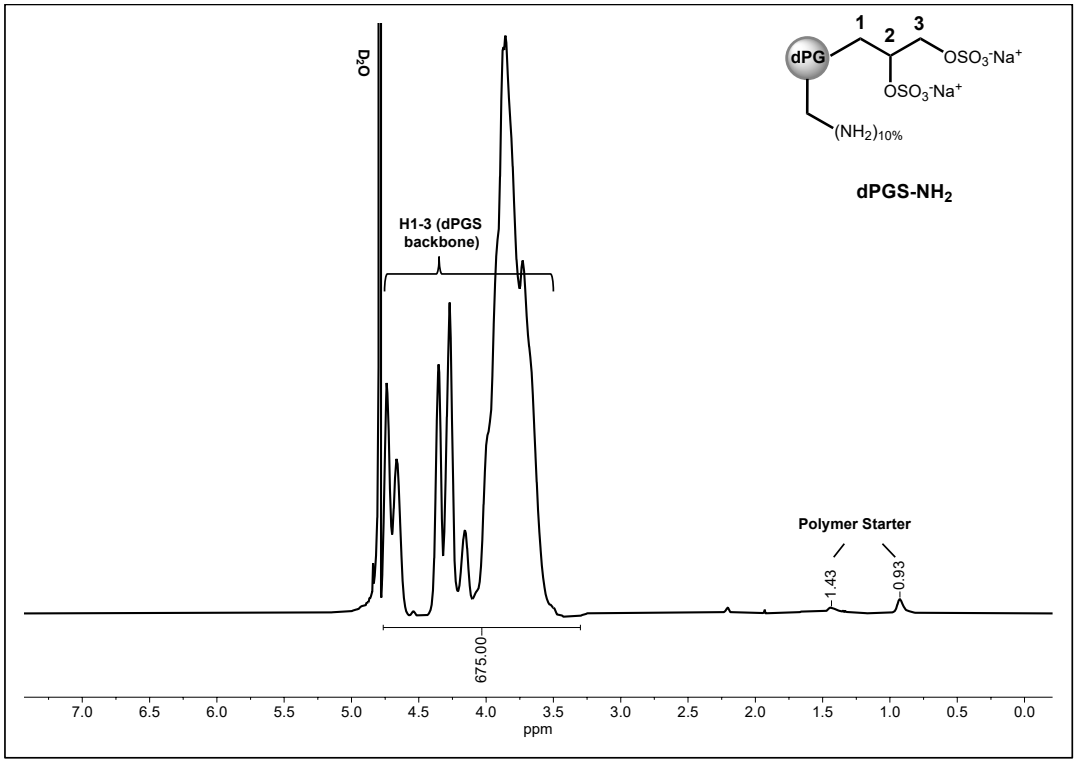


Figure S3. ^{1}H NMR spectrum of dPGS-NH_2 recorded in D_2O. Spectrum was recorded to confirm completed conversion of previous mesylation and azidation steps.

3. Size determination by dynamic light scattering

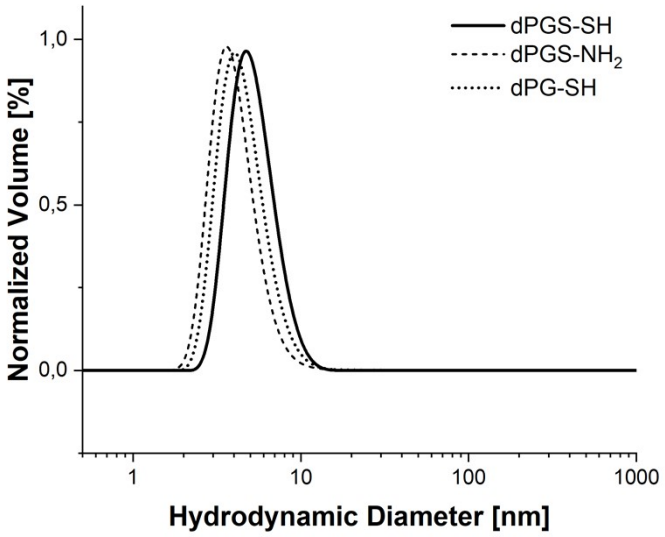


Figure S4. Hydrodynamic diameters of dPGS-SH, dPGS-NH_2 and dPG-SH as determined by dynamic light scattering (DLS). Samples were measured at 25 °C in concentrations of 1.0 mg mL^{-1}.

4. Calculations

Equation S1. The degree of functionalization (DF) for dPGS-SH and dPG-SH was calculated from ^{1}H NMR spectra setting the integral of the polymer backbone to its total number of protons (675 H) assuming 135 glycidol repeating units (RU) with 5 protons each. DF(dPGS-SH) and DF(dPG-SH) was determined by using the integral of peak H2a,b (Figure S1 and Figure S2) with RU = 135. Consequently, a DF of 4.1 and 3.3 for dPGS-SH and dPG-SH, respectively, was determined. Quantification of thiol groups was additionally performed by the Ellman's assay (see Materials and methods section).

$$\text{DF [\%]} = \frac{\text{peak integral}}{\text{number of protons}} * \frac{100}{\text{RU}}$$

Equation S2. The degree of sulfation (DS) was calculated using the results from elemental analysis of dPGS-N_3 (C = 22.88%, S = 14.18%). In relation to a predicted sulfur content of dPGS$_{(100\%)}$-N_3 (C = 24.94%, S = 18.63%) and a normalization to the carbon content, the DS was calculated.

$$\text{DS [\%]} = \frac{\text{carbon content in dPGS(100\%) - N3 [\%]}}{\text{carbon content in dPGS - N3 [\%]}} * \frac{\text{sulfur content in dPGS - N3 [\%]}}{\text{sulfur content in dPGS(100\%) - N3 [\%]}} * 100$$

Equation S3. The molecular weights (MW) of polymers were calculated based on the molecular weight of dPG-OH (M_n = 10.5 kDa) and the derived number of 135 glycidol RU. First, MW of functionalized RUs was determined, and the polymer MW was obtained from their sum multiplied with the total number of RU (with DF_1 = DF(OH), DF_2 = DF(NH_2), DF_3 = DF(OSO_3Na) and DF_4 = DF(SH).

$$\text{MW} = \left(\sum_{i=1}^{n} \text{DF}_i * \text{MW(functionalized RU)}\right) * \text{RU}$$

Equation S4. The diffusion coefficient (D) was calculated using the diffusion time (τ_D) obtained from the least square fit of the FRAP curve by the Leica Software and the radius of the bleached region of interest region (ω).

$$D = \frac{\omega^2}{4t_D}$$

5. Diffusion studies of dPGS-Cy5 in healthy and CF sputum

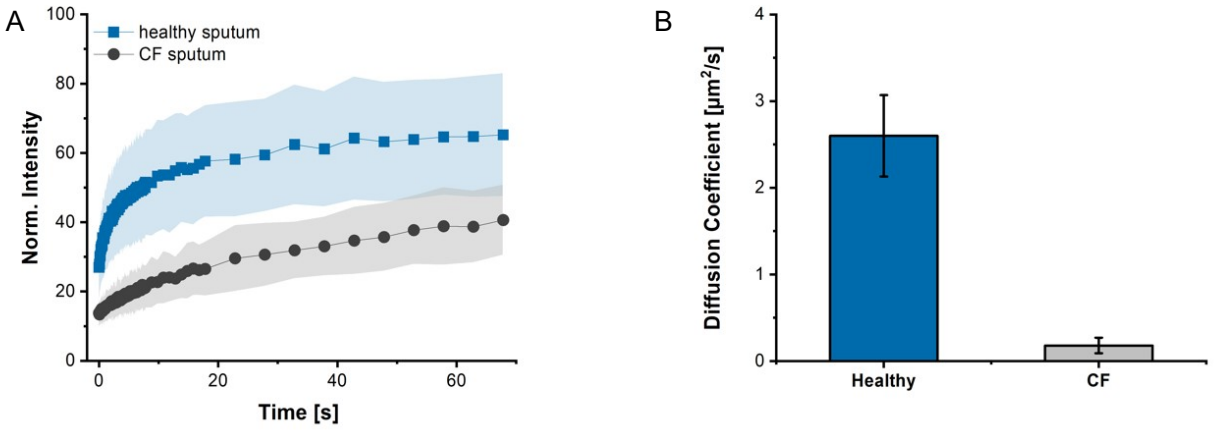


Figure S5. Diffusion studies of dPGS-Cy5 in healthy and CF-patients sputum samples. (A) Normalized recovery profiles of dPGS-Cy5 in healthy and CF sputum samples. (B) Diffusion coefficient of dPGS-Cy5 obtained by FRAP measurements and calculated by equation S4. Data represent the mean value ± standard deviation of n = 4 experiments.

6. Particle size analysis of dPGS-SH oxidation at 37 °C storage temperature

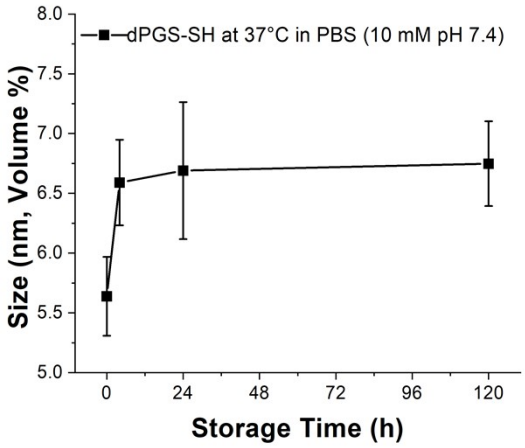


Figure S6. Particle size analysis of dPGS-SH stored at 37 °C in PBS (pH 7.4, 10 mM) determined by dynamic light scattering (DLS). Samples were measured in concentrations of 1 mg mL^{-1} at 37 °C.

7. Demographic and clinical characteristics of patients with cystic fibrosis

Table S1. Demographic and clinical characteristics patients with cystic fibrosis who provided sputum for rheology studies and Western blot analysis.

	CF Patients mean ± SD or n (%)
Number of donors	18
Age, years	28.0 ± 9.2
Sex, female	3 (16.7)
FEV1 (L)	1.9 ± 0.9
FEV1 % predicted	49.5 ± 24.2
CFTR genotype	
F508del/F508del	4 (22.2)
F508del/other	4 (22.2)
Other/other	10 (55.6)
Pseudomonas aeruginosa infection	
Negative	5 (27.8)
Intermittent	3 (16.7)
Chronic	10 (55.6)
Pancreatic insufficiency	17 (94.4)

8. dPGS-SH improves properties of CF sputum at concentrations of 1 and 5 mM

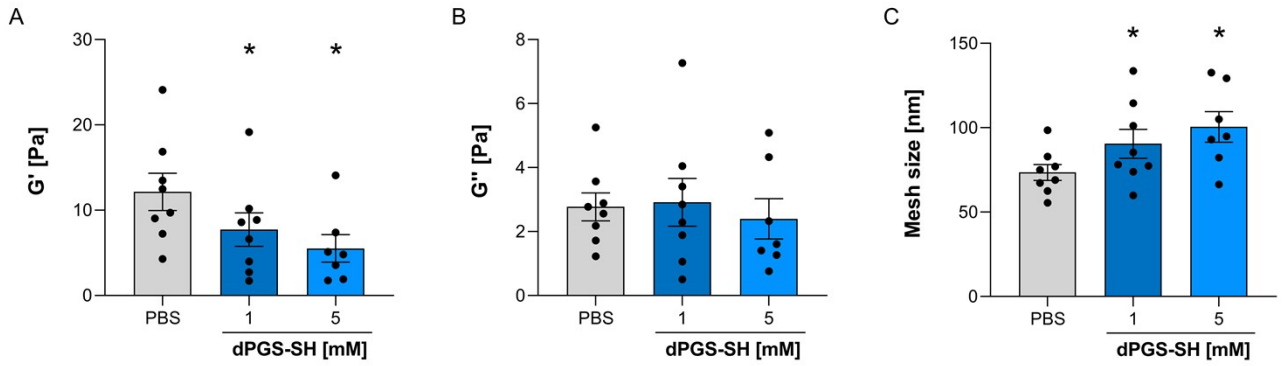


Figure S7: Effect of dPGS-SH on the storage and loss modulus of sputum from patients with CF. Comparison of mucolytic effect of 1 mM and 5 mM solutions of dPGS-SH to PBS-treated control on storage modulus G' (A), loss modulus G'' (B) and mesh size (C). G′ and G″ moduli of CF sputum samples are measured at the linear region of an amplitude sweep from 0.01 – 100 % strain at 1 Hz. Mesh size was calculated from the storage modulus. Data represents the mean value of 6 to 9 samples ± the standard error of the mean. *P < 0.05, compared with PBS treated controls, performed with Wilcoxon test.

9. Raw data of rheological measurements

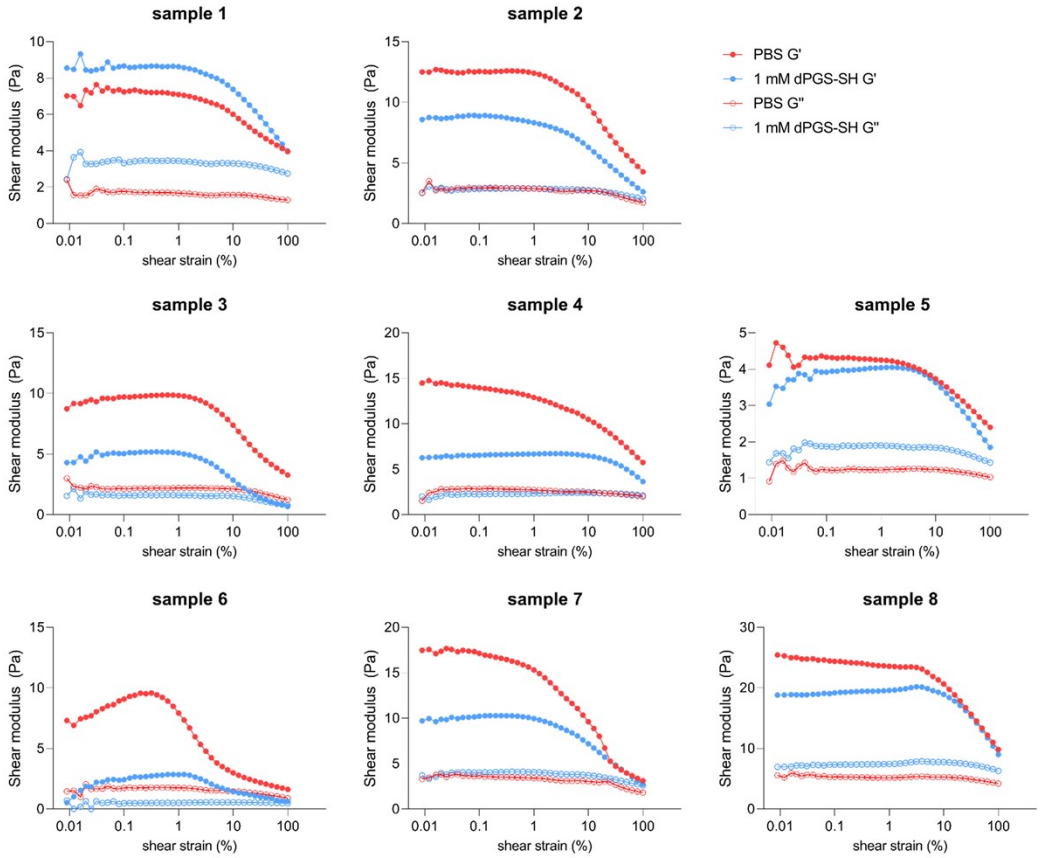


Figure S8: Amplitude sweep plots of rheological measurements comparing storage modulus G' and loss modulus G'' of PBS treated sputum samples with 1 mM dPGS-SH treated sputum samples of CF patients, measured at 1 Hz and from 0.01 to 100 % strain.

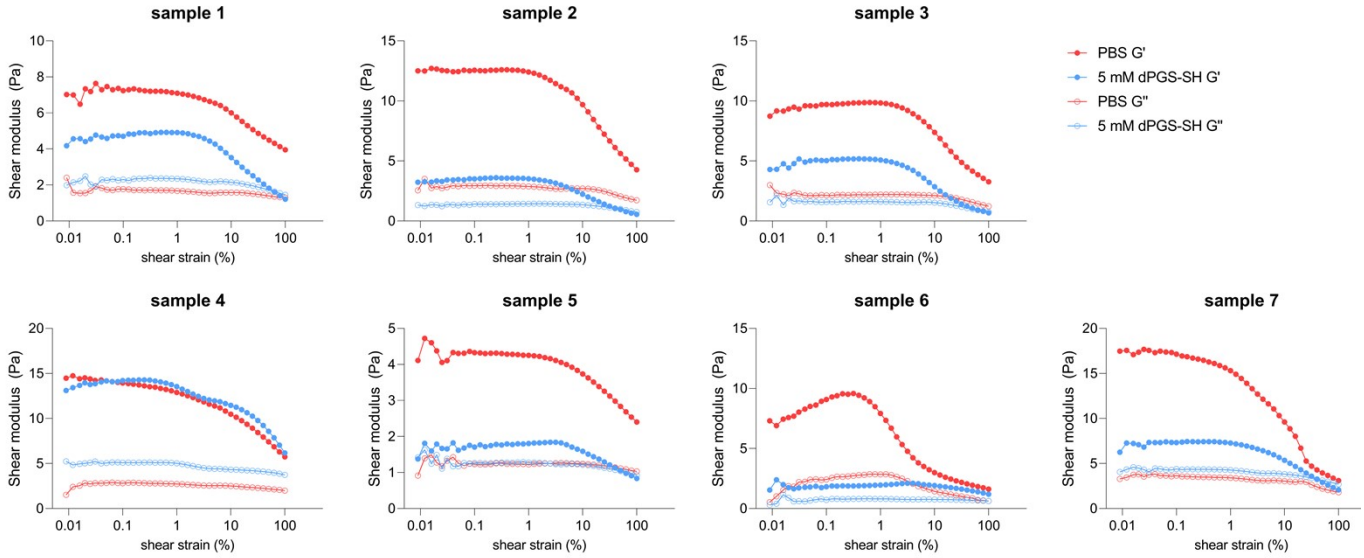


Figure S9: Amplitude sweep plots of rheological measurements comparing storage modulus G' and loss modulus G'' of PBS treated sputum samples with 5 mM dPGS-SH treated sputum samples of CF patients, measured at 1 Hz and from 0.01 to 100 % strain.

10. dPGS-NH_2 does not cleave mucin multimers

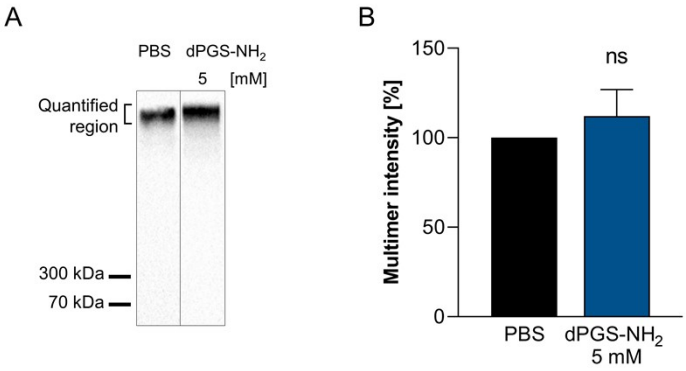
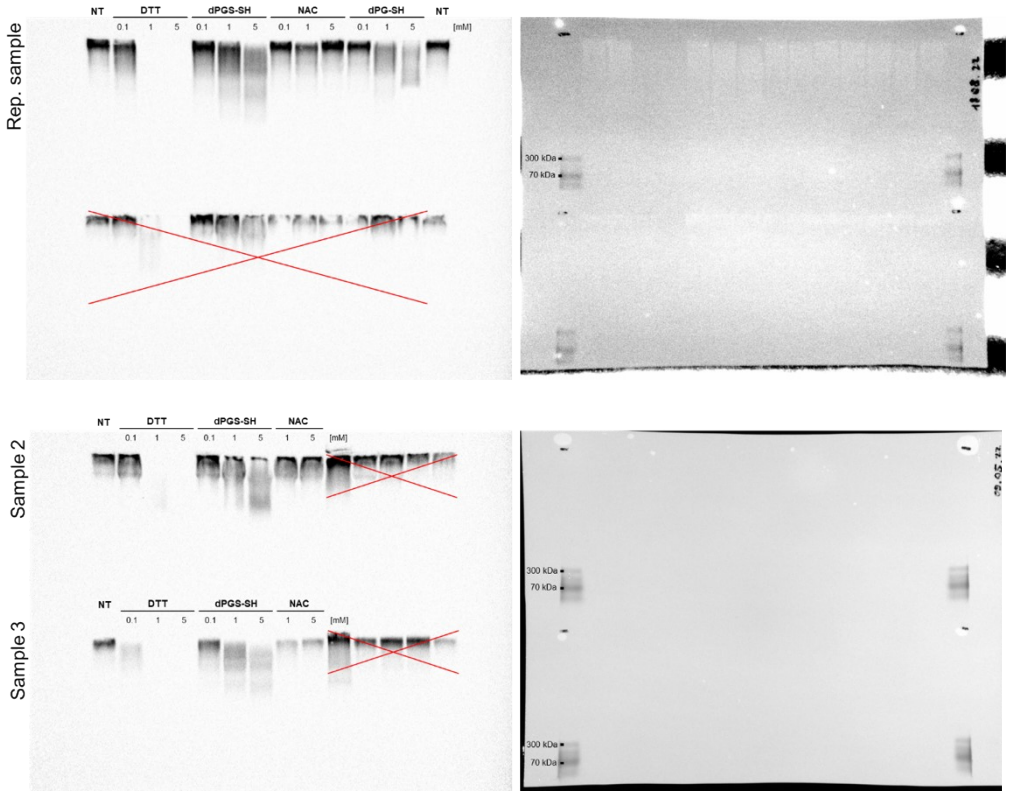


Figure S10: Effect of 5 mM solution of dPGS-NH_2 on the high molecular weight intensity of airway mucins. Representative Western blot images of MUC5B (A). Quantification of multimer intensity of MUC5B Western blots (B), normalized to PBS treated controls. Data represents the mean value of 5 samples ± the standard error of the mean. Source data are provided in chapter 10.

11. Western Blot raw data

Uncropped raw images of Western blot data showing anti-MUC5B (sc-393952) probing with corresponding gel images of molecular mass markers for 10 samples. Unrelated data was crossed out in images. All samples are listed below:



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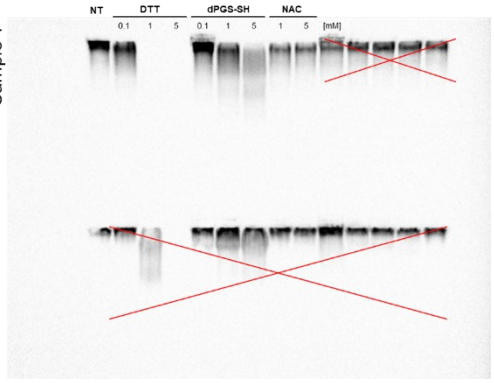

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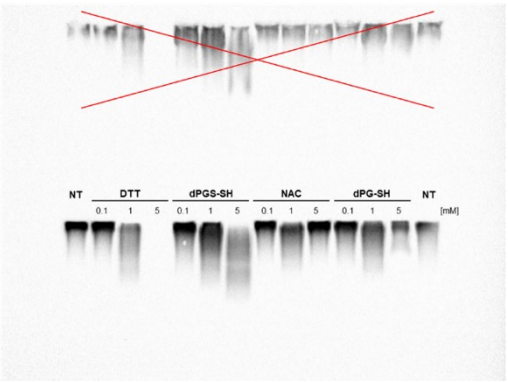
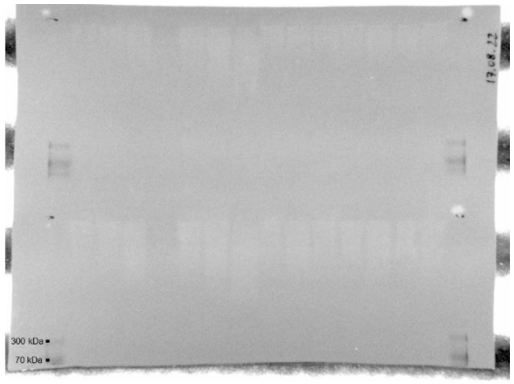
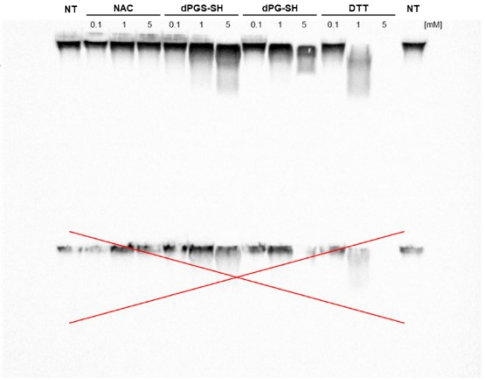
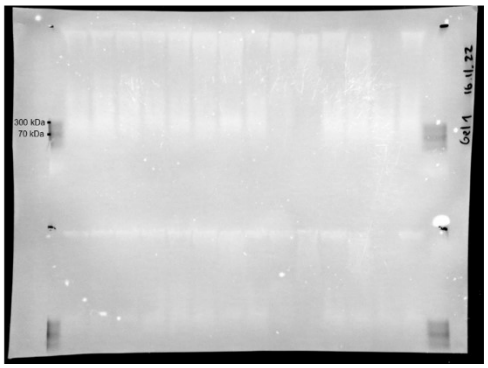




Sample 6





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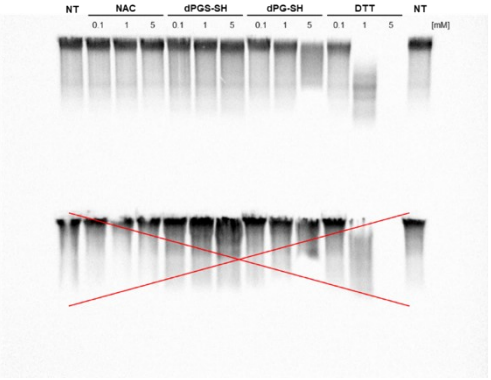
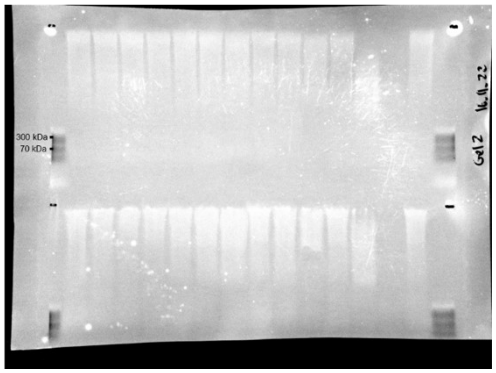




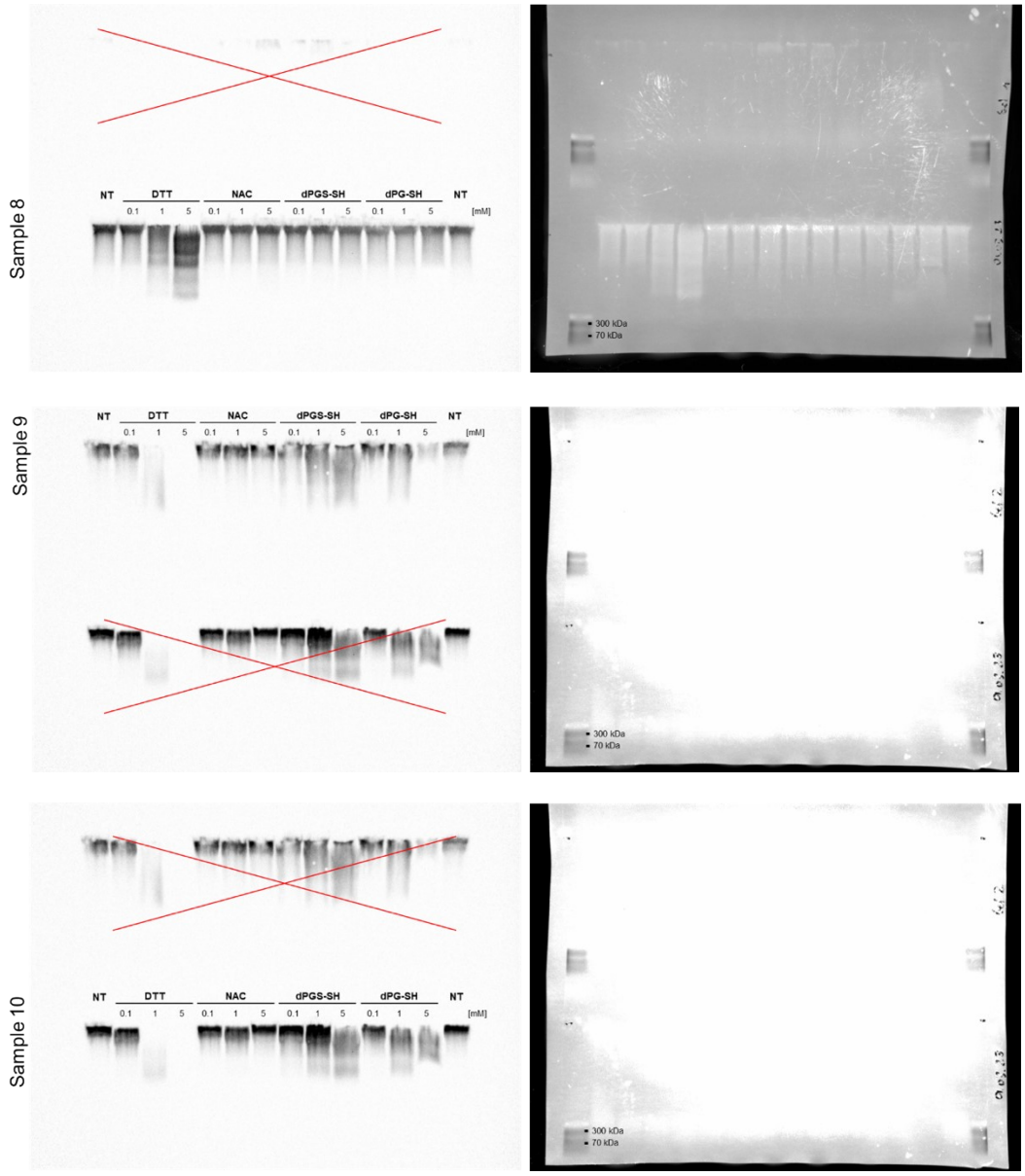
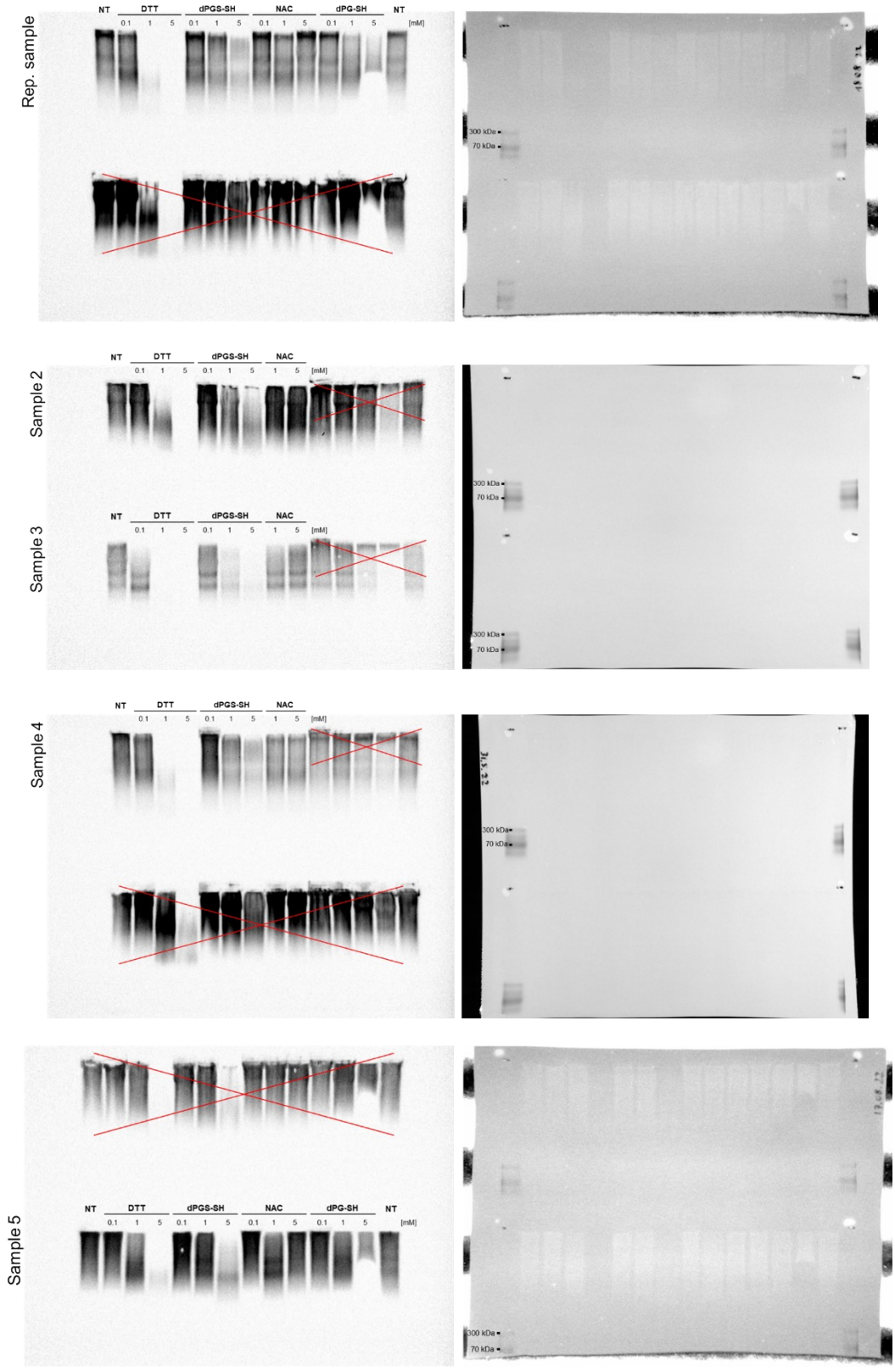


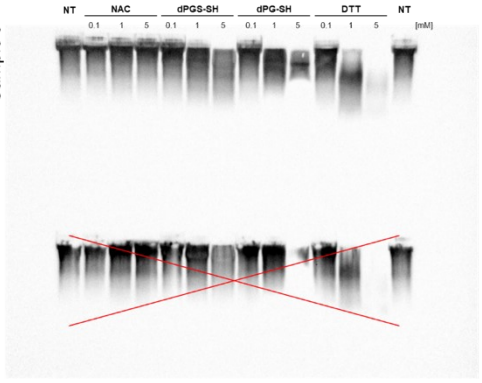
Table S2: Quantification of high molecular weight intensity of MUC5B for all 10 samples, normalized to non-treated samples.

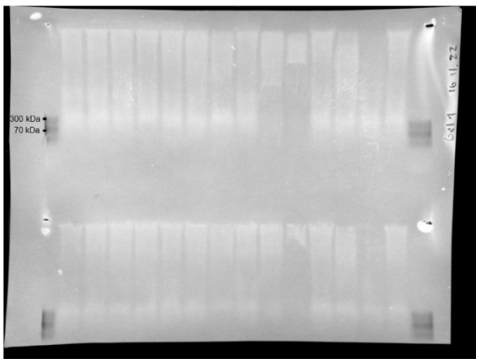
	NT	NAC			dPGS-SH			dPG-SH			DTT		
		0,1 mM	1 mM	5 mM	0,1 mM	1 mM	5 mM	0,1 mM	1 mM	5 mM	0,1 mM	1 mM	5 mM
Rep. sample	100,00	84,52	54,72	100,84	95,60	59,24	17,52	85,92	34,29	13,79	74,09	1,99	2,04
Sample 2	100,00		60,75	63,88	99,27	46,73	27,35				98,51	3,42	3,14
Sample 3	100,00		30,31	45,03	95,65	41,73	10,15				15,61	1,96	1,84
Sample 4	100,00		60,34	65,54	103,32	50,75	12,40				75,60	1,60	1,49
Sample 5	100,00	98,09	58,39	104,53	91,40	73,58	8,87	96,58	65,51	54,75	100,42	35,61	1,99
Sample 6	100,00	86,68	109,21	101,77	102,37	106,86	83,83	98,76	86,04	37,65	80,27	7,73	0,42
Sample 7	100,00	99,19	88,07	92,53	89,06	86,33	78,92	80,98	62,43	32,41	50,64	1,73	1,64
Sample 8	100,00	103,67	95,49	100,43	94,16	93,17	85,42	71,24	71,90	77,95	109,41	86,54	65,17
Sample 9	100,00	103,13	103,79	95,22	72,65	87,23	61,27	110,30	79,44	28,53	83,69	6,42	3,85
Sample 10	100,00	89,59	73,06	100,52	106,54	113,39	26,91	75,86	31,11	16,33	68,57	1,62	1,41

Uncropped raw images of Western blot data showing anti-MUC5AC (MA5-12178) probing with corresponding gel images of molecular mass markers for 10 samples. Unrelated data was crossed out in images. All samples are listed below:

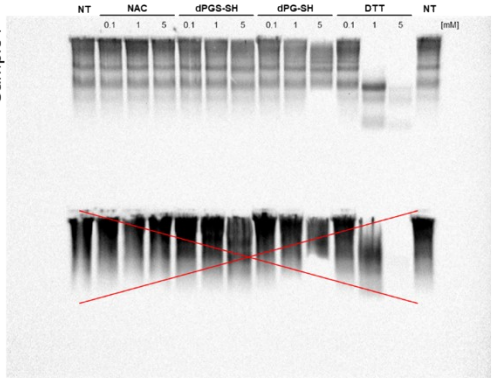


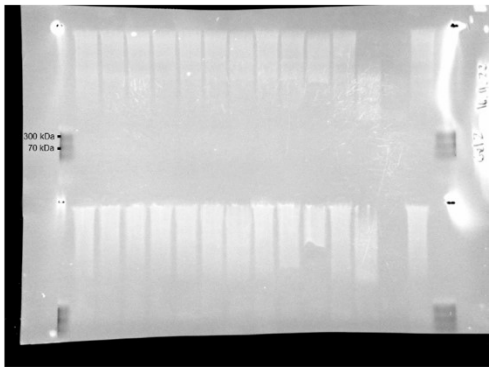
Sample 6





Sample 7





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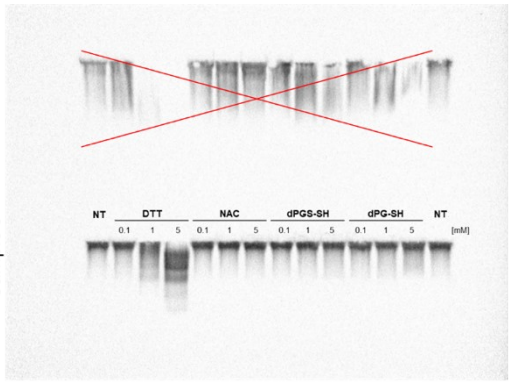

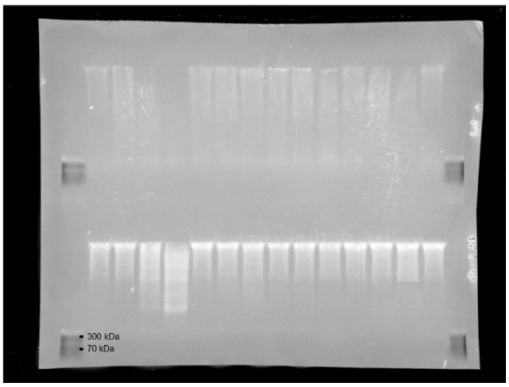

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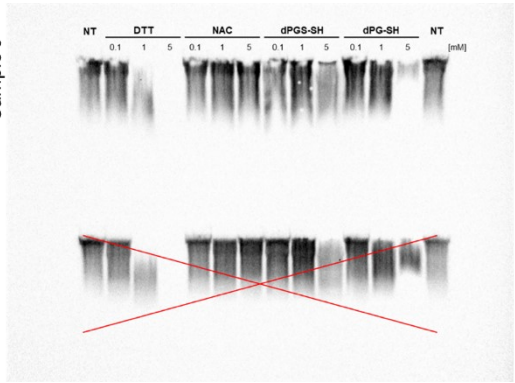
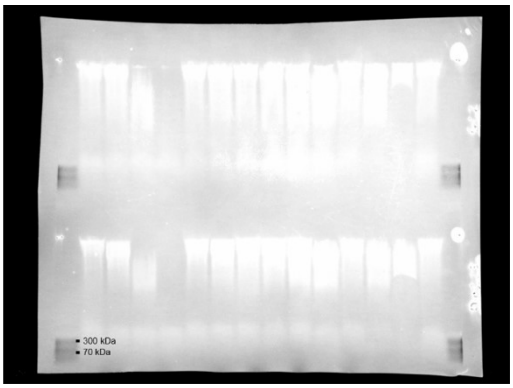




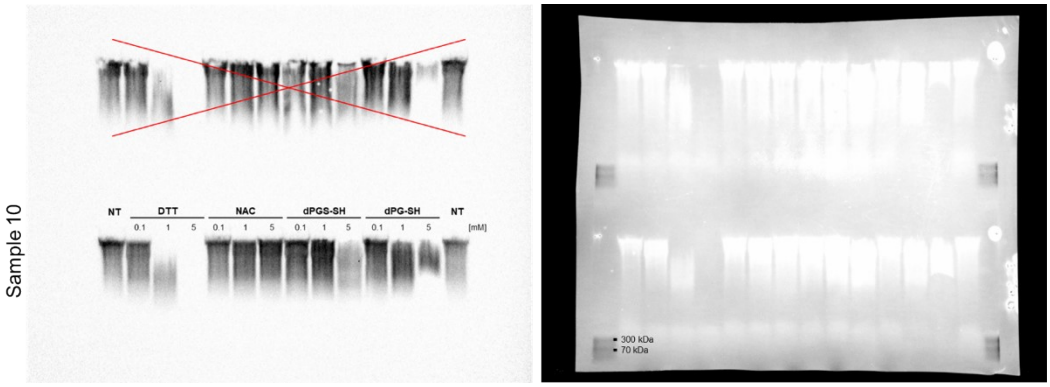
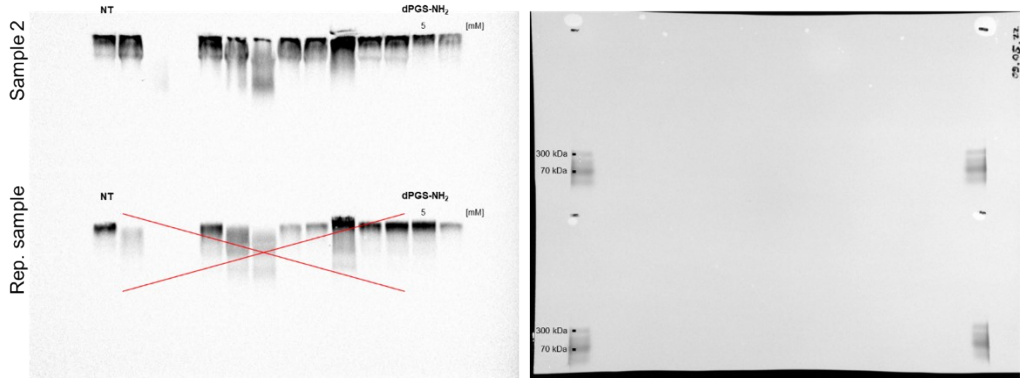


Table S3: Quantification of high molecular weight intensity of MUC5AC for all 10 samples, normalized to non-treated samples.

	NT	NAC			dPGS-SH			dPG-SH			DTT		
		0,1 mM	1 mM	5 mM	0,1 mM	1 mM	5 mM	0,1 mM	1 mM	5 mM	0,1 mM	1 mM	5 mM
Rep. sample	100,00	79,85	47,53	88,06	84,99	48,05	9,97	73,55	20,64	5,50	63,15	2,32	2,23
Sample 2	100,00		62,78	58,49	102,28	32,67	13,56				105,97	4,18	2,37
Sample 3	100,00		31,52	76,83	87,93	10,56	1,51				10,56	1,52	0,81
Sample 4	100,00		53,55	50,42	129,84	42,56	9,30				71,00	1,92	1,27
Sample 5	100,00	120,29	41,92	94,09	98,30	67,14	3,27	113,80	53,29	33,33	107,57	34,72	2,83
Sample 6	100,00	98,60	120,34	117,64	120,33	81,90	33,11	105,76	55,09	17,10	86,63	11,28	0,60
Sample 7	100,00	97,54	93,98	84,19	82,43	65,05	54,80	78,88	49,74	24,23	68,43	2,75	2,63
Sample 8	100,00	106,59	107,11	111,47	105,91	106,92	103,49	99,93	97,63	101,33	100,13	78,68	35,91
Sample 9	100,00	82,73	95,62	73,15	59,59	81,68	34,40	110,61	68,67	19,88	71,93	3,49	1,67
Sample 10	100,00	91,85	72,55	95,54	95,51	92,97	13,78	96,66	36,35	19,02	60,94	2,97	2,51

Uncropped raw images of Western blot data showing anti-MUC5B (sc-393952) probing with corresponding gel images of molecular mass markers for 5 samples. Unrelated data was crossed out in images. All samples are listed below:



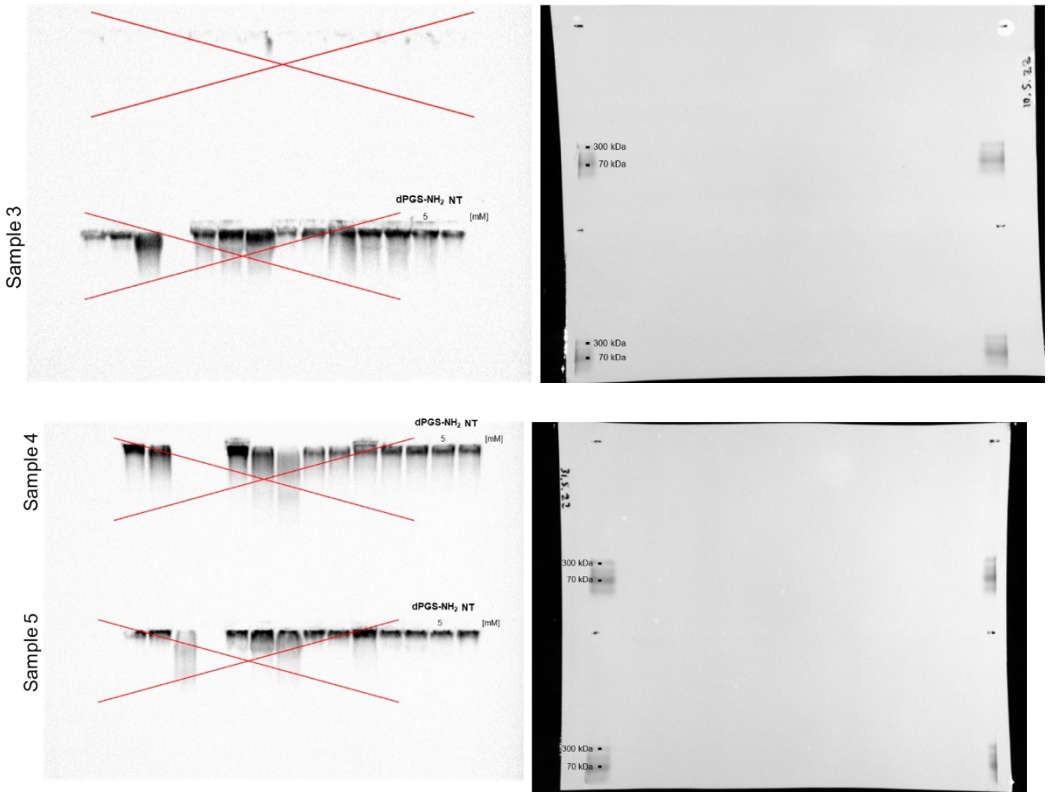


Table S4: Quantification of high molecular weight intensity of MUC5B for all 5 samples, normalized to non-treated samples.

	NT	dPGS-NH_2 5 mM
Rep. sample	100	131,40
Sample 2	100	72,78
Sample 3	100	157,31
Sample 4	100	106,41
Sample 5	100	92,64