Microfluidic platform to mimic the Golgi-linked N-glycosylation machinery

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Supporting Information

1 Supplementary Tables

Table S1: Composition of enzyme activity buffers that enzymes were stored in with 25% v/v glycerol. Prior to enzymatic assays on chip, enzymes were diluted with their respective buffers.

Buffer name	**Composition**	**Reference**
ERMan I activity buffer	150 mM NaCl, 20 mM MES, 5mM $CaCl_2$, pH 6.7	Aikawa *et al.*[1]
GM I activity buffer	0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, in PBS, pH 7.4	Mathew *et al.*[2]

Table S2: Amino acid sequence of tryptic peptide of PDI glycosylation site 2 and its mass over charge ratio (m/z) depending on the glycan structure present. The charge of the tryptic glycopeptides of site 2 is equal to 3.

Peptide sequence	**Glycan structure**	**m/z**
NSDVNNSIDYEGPR	$Man_9GlcNAc_2$	1148.7865
NSDVNNSIDYEGPR	$Man_8GlcNAc_2$	1094.7689
NSDVNNSIDYEGPR	$Man_7GlcNAc_2$	1040.7494
NSDVNNSIDYEGPR	$Man_6GlcNAc_2$	986.7343
NSDVNNSIDYEGPR	$Man_5GlcNAc_2$	932.7157
NSDVNNSIDYEGPR	$Man_4GlcNAc_2$	878.6966
NSDVNNSIDYEGPR	$Man_3GlcNAc_2$	824.6952

2 Supplementary Figures

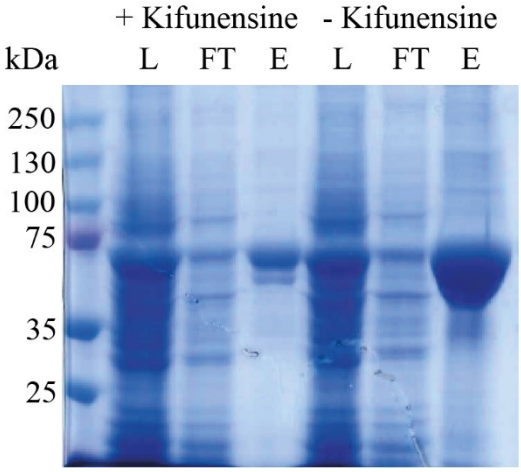

Figure S1: SDS-PAGE of steps of the Ni-NTA purification of PDI co-expressed with and without Kifunensine. The gel shows the lysate (L) of the pelleted High-Five™ cells of the protein expression, flow through (FT) composed of material not binding to the Ni-NTA resin, the elution (E) corresponding to PDI that specifically bonded to Ni-NTA resin *via* its His-tag and eluted from the Ni-NTA resin by treatment with the elution buffer (250 mM imidazole in PBS).

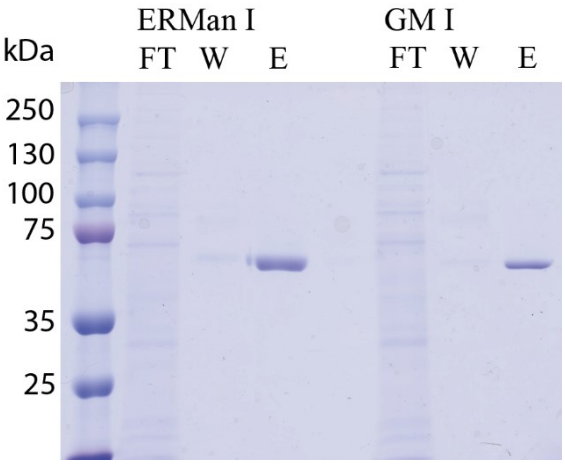

Figure S2: SDS-PAGE of steps of the NI-NTA purification of the glycosylation enzymes ERMan I and GM I. Shown are the flow through (FT) composed of material not binding to the Ni-NTA resin, the wash (W) meaning material eluting from the Ni-NTA resin due to non-specific binding to the Ni-NTA resin during washing of the resin with wash buffer (25 mM imidazole in PBS), the elution (E) corresponding to glycosylation enzymes that specifically bonded to Ni-NTA resin *via* its His-tag and eluted from the Ni-NTA resin by treatment with the elution buffer (250 mM imidazole in PBS).

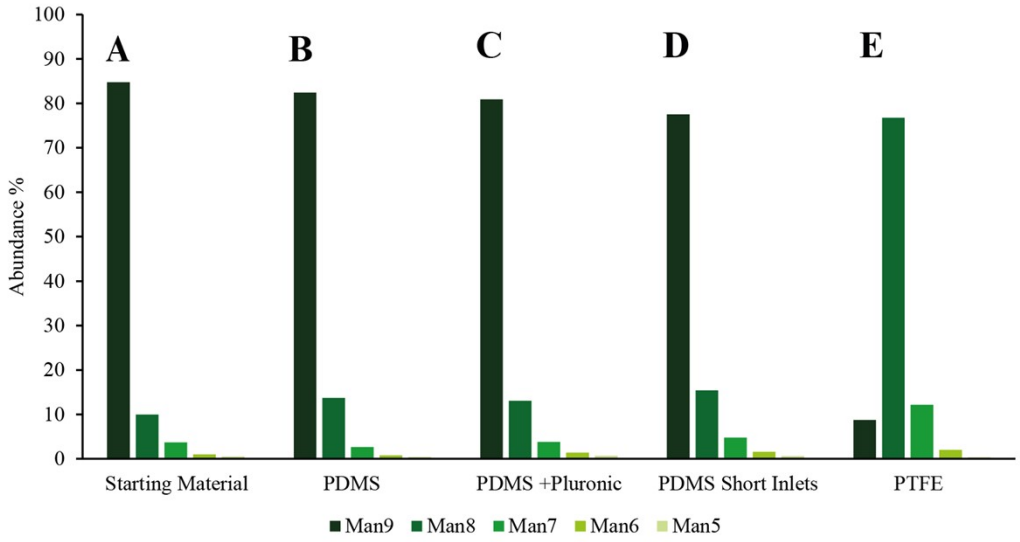

Figure S3: Assessment of PDI and ERMan I co-encapsulation using different microfluidic substrate materials and surface modifications. The disappearance of Man9 peaks and appearance of Man8 and subsequent peaks indicate consumption of the starting material and reaction progression. Modifications of surface chemistry or the use of shorter inlets (and thus shorter contact time between PDMS surfaces and the protein/enzyme substrate) yielded minor effects on reaction progression due to adsorption of the ERMan I prior to droplet formation. Adoption of Teflon® as the substrate material was accompanied by significant consumption of starting material by ERMan I, confirming minimal adsorption of enzyme onto channel surfaces prior to droplet encapsulation. Protein glycosylation patterns: (A) PDI starting material. (B) Bare PDMS microfluidic channels. (C) PDMS microfluidic channels treated with Pluronic® F-127 (D) PDMS microfluidic channels treated with Pluronic® F-127, but with shorter inlets. (E) PTFE microfluidic channels.

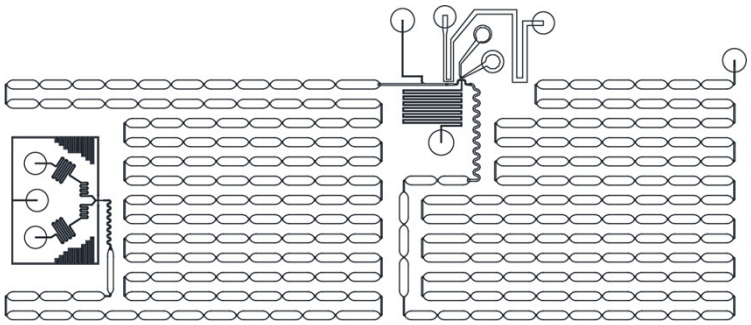

Figure S4: Initial microfluidic chip design, encompassing droplet co-encapsulation, an initial 10-minute droplet incubation, picoinjection and a subsequent 8-minute droplet incubation.

3 References

1. Aikawa J-i, Matsuo I, Ito Y. In vitro mannose trimming property of human ER α-1,2 mannosidase I. Glycoconj J. 2012;29(1):35-45.

2. Mathew C, Weiss RG, Giese C, Lin CW, Losfeld ME, Glockshuber R, et al. Glycan-protein interactions determine kinetics of N-glycan remodeling. RSC Chem Biol. 2021;2(3):917-31.