Supplementary Materials

# Chemical Synthesis and Characterization of Wild-Type and Biotinylated N-Terminal Domain 1-64 of β2-Glycoprotein I †

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**Table S1.** MS data of the proteolytic fragments obtained by proteolysis of the synthetic N-DmI with trypsin (T1-T7) and chymotrypsin (C1-C7) [a]

| Peak Number | Fragment Sequence | Molecular Mass (a.m.u.) |
|---|---|---|
| T1 | $^{20}$TFYEPGEEITYSCKPGYVSR$^{39}$<br>\|<br>$^{60}$CTPR$^{63}$ | 2800.3 ± 0.1<br>(2800.3) [b] |
| T2 | $^{20}$TFYEPGEEITYSCKPGYVSR$^{39}$<br>\|<br>$^{60}$CTPRV$^{64}$ | 2899.3 ± 0.1<br>(2799.3) |
| T3 | $^{20}$TFYEPGEEITYSCK$^{33}$<br>\|<br>$^{60}$CTPRV$^{64}$ | 2238.0 ± 0.2<br>(2238.0) |
| T4 | $^{1}$ GRTCPKPDDLPFSTVVPLK$^{19}$<br>\|<br>$^{44}$KFICPLTGLWPINTLK$^{59}$ | 3912.2 ± 0.1<br>(3912.1) |
| T5 | $^{3}$TCPKPDDLPFSTVVPLK$^{19}$<br>\|<br>$^{44}$KFICPLTGLWPINTLK$^{59}$ | 3699.1 ± 0.3<br>(3999.0) |
| T6 | Uncleaved N-DmI | 7159.4 ± 0.6<br>(7159.4) |
| T7 | $^{1}$GRTCPKPDDLPFSTVVPLK$^{19}$<br>\|<br>$^{45}$FICPLTGLWPINTLK$^{59}$ | 3570.2 ± 0.7<br>(3570.9) |
| C1 | $^{31}$SCKPGY$^{36}$<br>\|<br>$^{59}$KCTPRV$^{64}$ | 1353.6 ± 0.1<br>(1353.6) |
| C2 | $^{31}$ SCKPGYVSRGGM$^{42}$<br>\|<br>$^{59}$KCTPRV$^{64}$ | 1941.0 ± 0.1<br>(1940.9) |
| C3 | $^{22}$YEPGEEITY$^{30}$ | 1100.5 ± 0.4<br>(1100.1) |
| C4 | $^{13}$STVVPL$^{18}$ | 615.0 ± 0.1<br>(614.7) |
| C5 | $^{19}$KTFYEPGEEITY$^{30}$ | 1475.7 ± 0.1<br>(1475.6) |
| C6 | $^{1}$ GRTCPKPDDLPF$^{12}$<br>\|<br>$^{46}$ICPL$^{49}$ | 1786.9 ± 0.2<br>(1786.8) |
| C7 | $^{50}$TGLWPINTL$^{58}$ | 1014.6 ± 0.2<br>(1014.1) |

[a] The peptide fragments eluted in correspondence of the chromatographic peaks T1-T7 and C1-C7 in Fig. 3A, B were lyophilized and subjected to MS analysis. [b] In parenthesis are given the theoretical monoisotopic mass values of the peptide fragments.

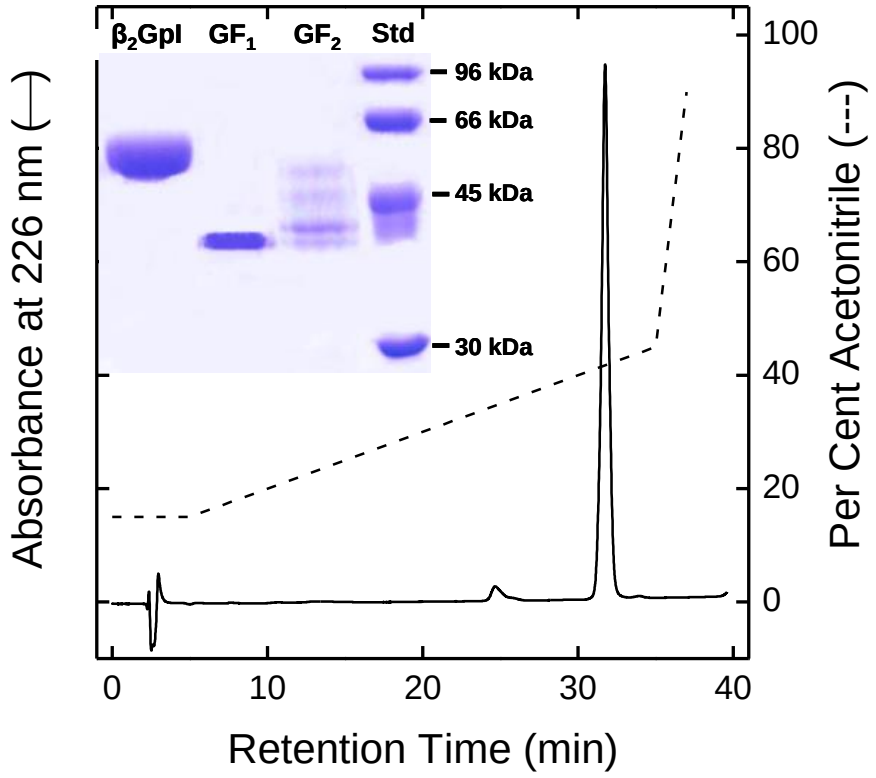


**Figure S1.** Analytical characterization of natural β2GpI. RP-HPLC analysis of β2GpI. An aliquot (30 μg) of purified β2GpI was applied to a Vydac C4 analytical column eluted with a linear acetonitrile-0.1% TFA gradient (---) at a flow rate of 0.8 ml/min. **Inset:** SDS-PAGE (4-12% acrylamide) analysis of β2GpI under reducing conditions. β2GpI, purified β2GpI (4 μg); GF1, an aliquot (10 μl) of the reaction mixture of β2GpI with N-glycanase F conducted under denaturing conditions in buffer A; GF2, an aliquot (10 μl) of the reaction mixture of β2GpI with N-glycanase F under nondenaturing conditions in buffer B; Std, molecular weight protein standards.

Removal of *N*-linked carbohydrate chains was carried out by incubating $\beta_2$GpI (0.5 mg/ml) for 24 h at 37°C with recombinant N-glycanase F (Roche; Mannheim, Germany) with an enzyme:protein ratio of 1:50 (by weight), in 50 mM phosphate buffer, pH 7.8, containing 10 mM EDTA, 0.1% SDS, 0.5% Triton X-100 and 1% β-mercaptoethanol (buffer A). Alternatively, the reaction was conducted for 24 h at 37°C in 20 mM Trs-HCl buffer, pH 7.5, containing 0.15 M NaCl (buffer B).

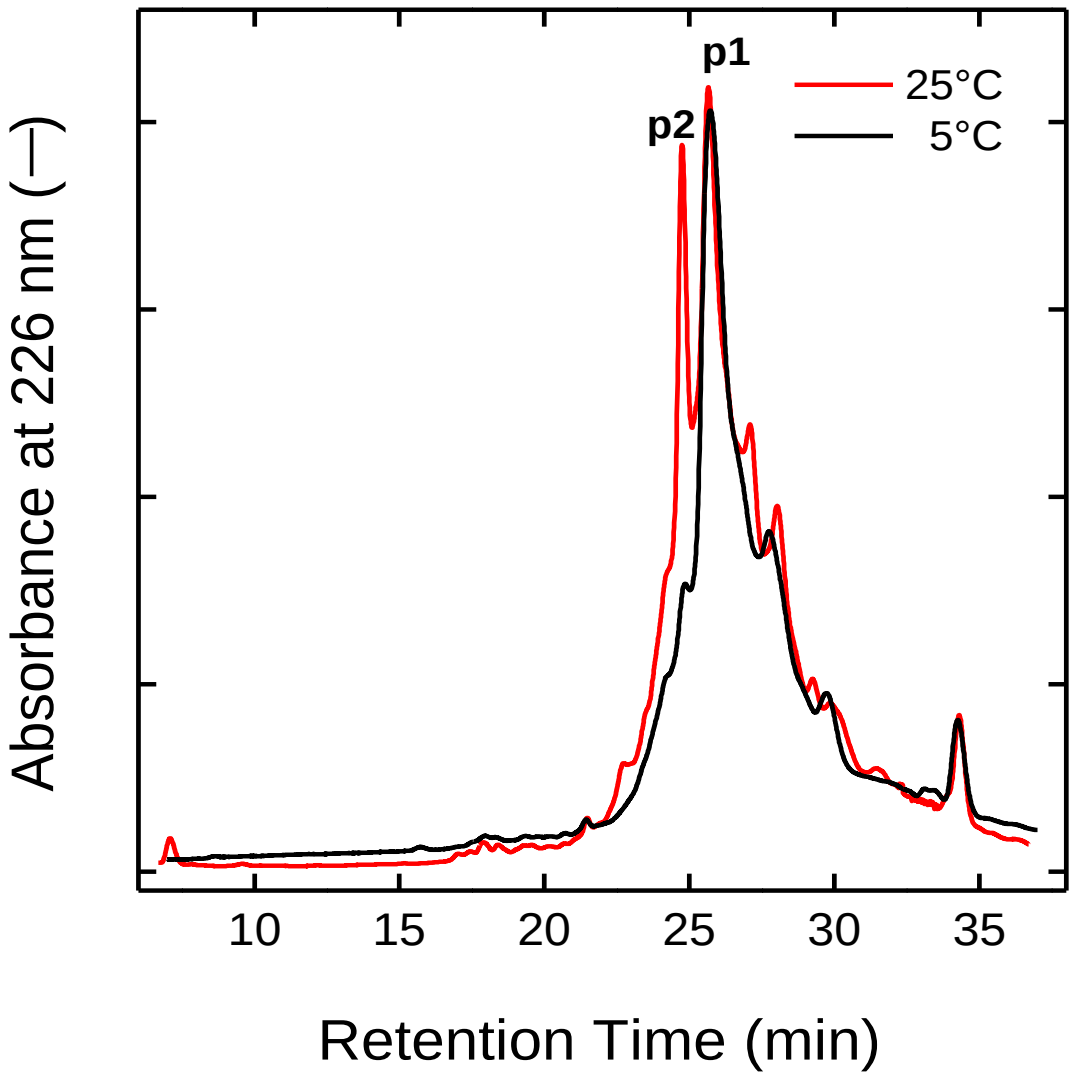


**Figure S2.** RP-HPLC analysis of the crude synthetic DmI in the reduced state (R-DmI) at different temperatures, as indicated. R-DmI peptide was loaded onto a C18 Vydac analytical column and eluted with a linear acetonitrile gradient in 0.1% aqueous TFA (see text). Going from 25 (red) to 5°C (black), the peptide species eluting with p2 are converted to p1 species, thus revealing the nature of the *cis-trans* proline isomerization.

**Table S2.** Refolding Trials on DmI [a]

| Refolding buffer | Time (h) | % Yield |
|---|---|---|
| 0.1M Tris-HCl, pH 8.4 | 24 | 27 |
| 50 mM $NaHCO_3$, pH 8.3, 60 μM β-ME [b] | 24 | 24 |
| 0.1M Tris-HCl, pH 8.4, 60 μM β-ME [b] | 24 | 35 |
| 0.1M Tris-HCl, pH 8.4, 60 μM β-ME [b], 0.5M Arginine | 24 | 35 |
| 0.1M Tris-HCl, pH 8.4, 5 mM GSSG - 2 mM GSH | 24 | 45 |
| 0.1M Tris-HCl, pH 8.4, 4 mM GSSG - 1 mM GSH | 24 | 62 |

[a] A solution of the reduced peptide (R-DmI) in sodium phosphate buffer, pH 5, was added dropwise to different refolding buffers, to a final concentration of 1 mg/ml. The reaction was allowed to proceed for 24 h at r.t. and the approximate yield of the refolded species was determined by integrating the area under the chromatographic peaks, with an average error of ±5%.
[b] ME, β-mercaptoethanol

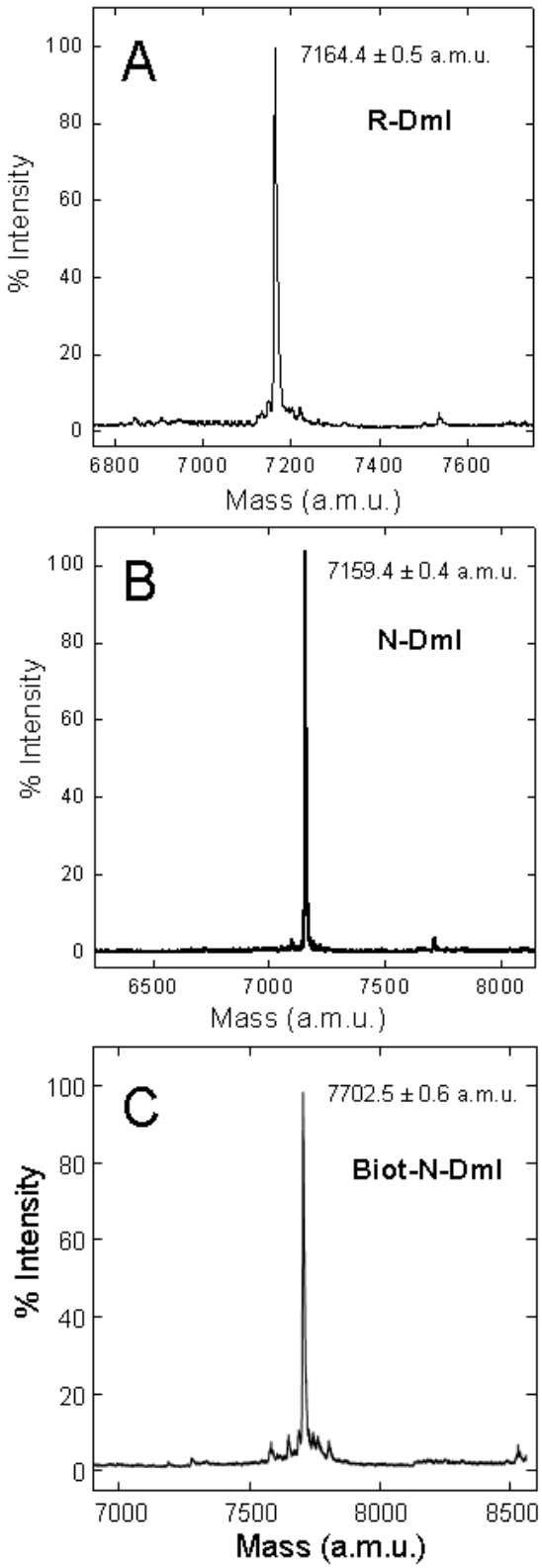


**Figure S3.** MS analysis of RP-HPLC purified DmI species. A: DmI with Cys-residues in the reduced state (R-DmI). B: Disulfide folded DmI (N-DmI). C: biotinylated N-DmI derivative (Biot-N-DmI).

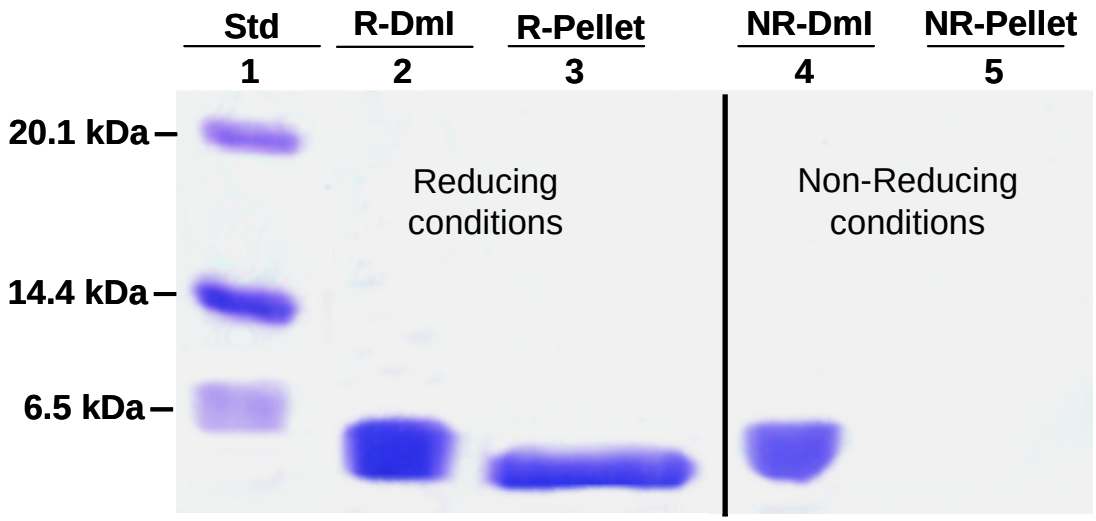


**Figure S4.** SDS-PAGE analysis of the refolding reaction of DmI under reducing and nonreducing conditions. Polyacrylamide gel electrophoresis (4-12% acrylamide) was carried out under reducing (R, lanes 2 and 3) and nonreducing (NR, lanes 4 and 5) conditions. Lane 1, molecular weight protein standards; lane 2, RP-HPLC purified N-DmI (5 μg); lane 3, an aliquot (4-6 μg) of the DmI pellet was dissolved in the sample loading buffer (15 μl), containing 0.1 M DTT; lane 4, RP-HPLC purified N-DmI (5 μg); lane 5, an aliquot (5-10 μg) of the DmI pellet, formed during folding reaction, was added with sample loading buffer (15 μl), without DTT.