

**Insights into the quality of recombinant proteins  
produced by two different *Bombyx mori* expression systems**

**Supplementary Information**

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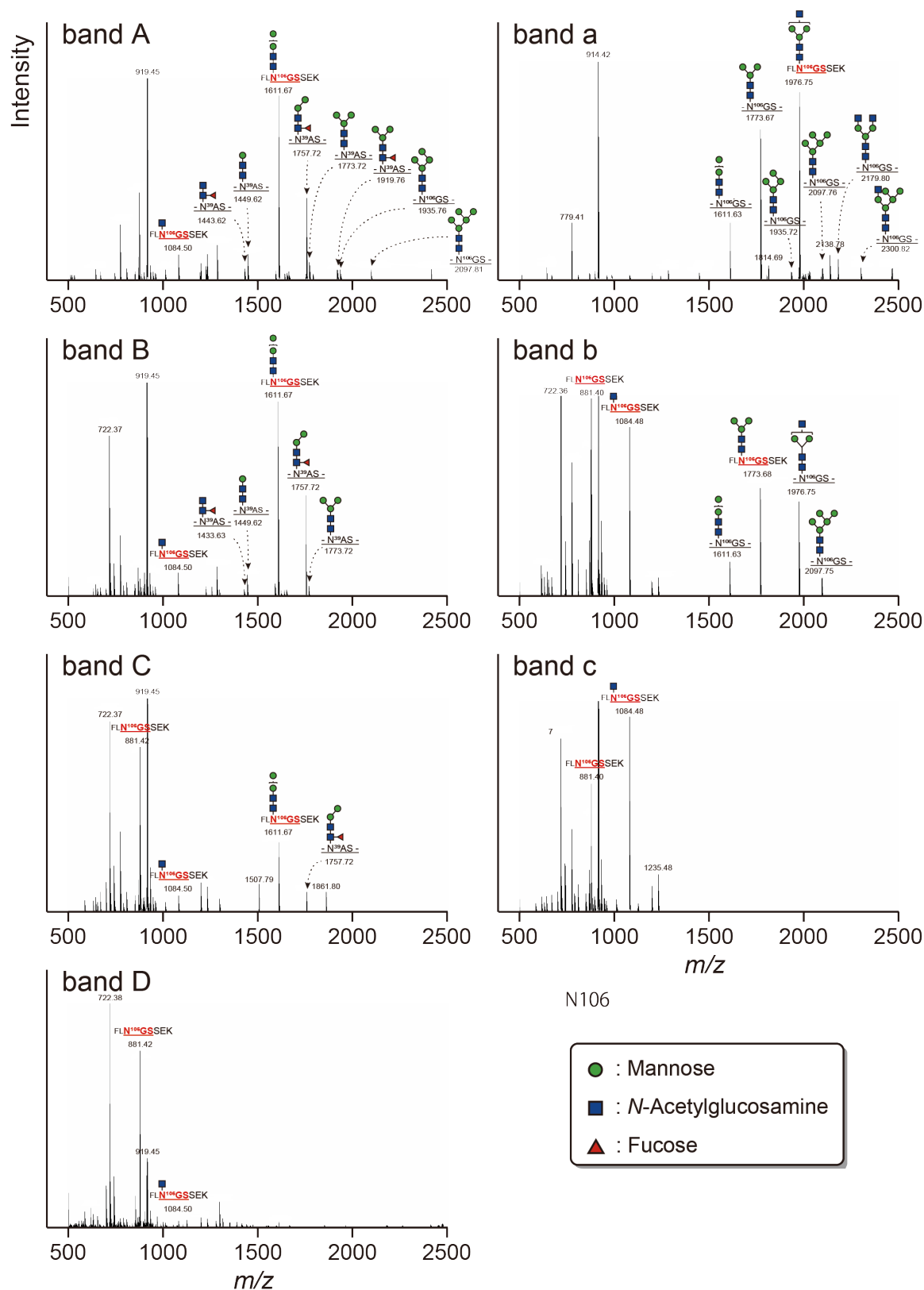
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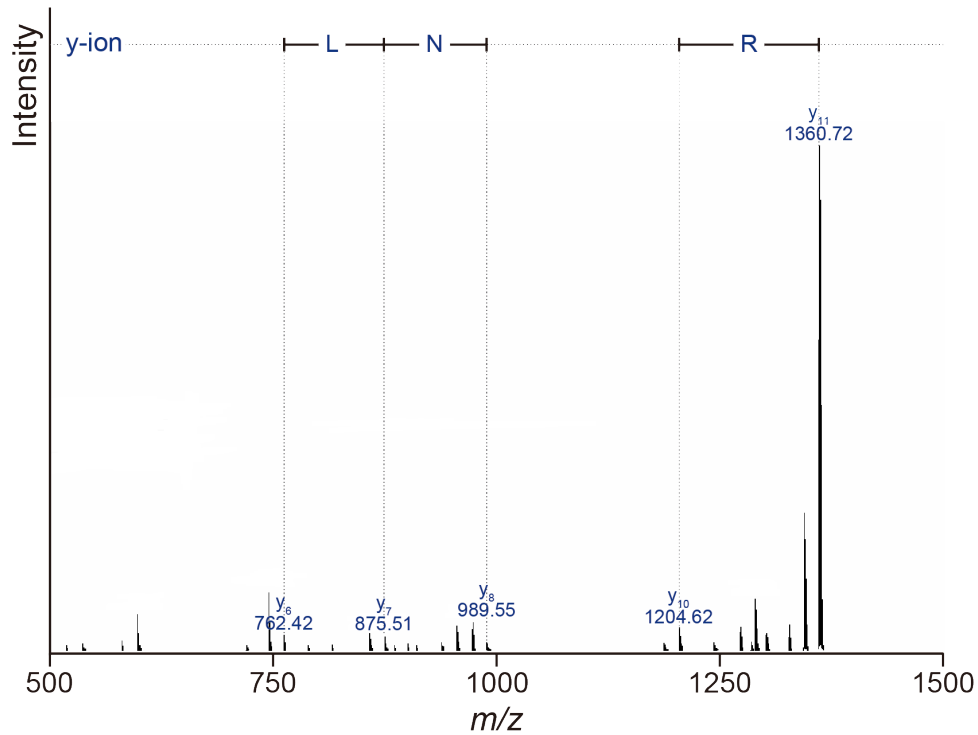
**Supplementary Figure S1** *N*-Glycopeptide analysis of Asn106 in bIFN- $\gamma$  and tIFN- $\gamma$

All signals of  $m/z$  corresponding to *N*-glycopeptide and the *N*-glycan structures detected in nanoLC-MS/MS analysis are shown.

(A)

MKYTSYFLALLLCGLLGFSGSYGQGQFFREIENLKEYF**NASSP**  
DVAKGGLFSEILKNWKDESDKKIIQSQIVSFYFKLFENLKDN  
QVIQRSMDIKQDMFQKFL**NGS**SEKLEDFKKLIQIPVDDLQIQ  
RKAINELIKVMNDLSPKSNLRKR**RSQNLFRGRR**<sup>164</sup>ST  
*m/z* 1359.76

(B)



**Supplementary Figure S2 C-terminal analysis of tIFN- $\gamma$**

(a) Sequence coverage map of Lys-C-digested tIFN- $\gamma$  shown as band X in Fig. 5(a). Red, black, shaded in gray, and bold letters are as described in Fig. 3. The calculated *m/z* of Arg154-Gly164, 1359.76, is shown.

(b) MS/MS analysis of the precursor of *m/z* 1359.72. y-type ions are assigned to the signals detected in MS/MS analysis of the precursor of *m/z* 1359.72.

**Supplementary Table S1 Site-specific *N*-glycan structures of bIFN- $\gamma$  and tIFN- $\gamma$  and their relative ratios**

| Structure                        |                                                            |                                  | Ratio (%)                 |                              |       |        |       |        |       |        |       |                |       |        |       |        |       |
|----------------------------------|------------------------------------------------------------|----------------------------------|---------------------------|------------------------------|-------|--------|-------|--------|-------|--------|-------|----------------|-------|--------|-------|--------|-------|
|                                  |                                                            |                                  | Expression system<br>Band | bIFN- $\gamma$               |       |        |       |        |       |        |       | tIFN- $\gamma$ |       |        |       |        |       |
|                                  |                                                            |                                  |                           | A                            |       | B      |       | C      |       | D      |       | a              |       | b      |       | c      |       |
|                                  |                                                            |                                  |                           | <i>N</i> -Glycosylation site | Asn39 | Asn106 | Asn39 | Asn106 | Asn39 | Asn106 | Asn39 | Asn106         | Asn39 | Asn106 | Asn39 | Asn106 | Asn39 |
| Peptide                          | EIENLKEYFN <sup>39</sup> ASSPDVAK                          | -                                | -                         | 1.2                          | -     | 8.4    | -     | 56.8   | -     | -      | -     | 6.8            | -     | 3.8    | -     |        |       |
|                                  | FLN <sup>106</sup> GSSEK                                   | -                                | -                         | -                            | 4.1   | -      | 60.1  | -      | 96.5  | -      | -     | -              | 25.8  | -      | 32.2  |        |       |
|                                  | HexNAc <sub>1</sub>                                        | 1.7                              | 5.4                       | 0.9                          | 5.5   | 2.1    | 5.9   | -      | 3.5   | -      | -     | 27.9           | 29.1  | 96.2   | 67.8  |        |       |
|                                  | HexNAc <sub>2</sub>                                        | -                                | 8.1                       | -                            | 7.0   | 0.7    | -     | -      | -     | -      | -     | -              | -     | -      | -     |        |       |
|                                  | DeoxyHex <sub>1</sub> HexNAc <sub>2</sub>                  | -                                | 2.9                       | -                            | 1.5   | 2.6    | -     | -      | -     | -      | -     | -              | -     | -      | -     |        |       |
| Mannose type<br><i>N</i> -glycan | Hex <sub>1</sub> HexNAc <sub>2</sub>                       | M1                               | 2.5                       | 5.8                          | 2.5   | 5.1    | 2.6   | -      | 6.6   | -      | -     | 1.8            | -     | -      | -     | -      |       |
|                                  | Hex <sub>2</sub> HexNAc <sub>2</sub>                       | M2                               | 14.4                      | 41.9                         | 15.2  | 46.5   | 12.5  | 26.4   | 12.8  | -      | 1.7   | 11.7           | 1.5   | 5.9    | -     | -      |       |
|                                  | Hex <sub>3</sub> HexNAc <sub>2</sub>                       | M3                               | -                         | 4.2                          | 2.1   | 2.4    | 2.6   | -      | -     | -      | 9.8   | 31.6           | 7.7   | 19.5   | -     | -      |       |
|                                  | Hex <sub>4</sub> HexNAc <sub>2</sub>                       | M4                               | -                         | 2.4                          | -     | -      | -     | -      | -     | -      | -     | 1.4            | -     | -      | -     | -      |       |
|                                  | Hex <sub>5</sub> HexNAc <sub>2</sub>                       | M5                               | -                         | 2.1                          | -     | -      | -     | -      | -     | -      | 4.8   | 2.3            | 2.4   | 3.1    | -     | -      |       |
| Fucosylated <i>N</i> -glycan     | Hex <sub>1</sub> DeoxyHex <sub>1</sub> HexNAc <sub>2</sub> | MF                               | 5.9                       | 3.0                          | 6.3   | 2.9    | 5.8   | -      | 6.8   | -      | -     | -              | -     | -      | -     | -      |       |
|                                  | Hex <sub>2</sub> DeoxyHex <sub>1</sub> HexNAc <sub>2</sub> | M2F                              | 68.1                      | 21.9                         | 69.2  | 25.0   | 59.5  | 7.6    | 17.1  | -      | 1.2   | -              | -     | -      | -     | -      |       |
|                                  | Hex <sub>2</sub> DeoxyHex <sub>2</sub> HexNAc <sub>2</sub> | M2FF                             | 1.4                       | -                            | 0.9   | -      | 0.7   | -      | -     | -      | 7.2   | -              | 5.7   | -      | -     | -      |       |
|                                  | Hex <sub>3</sub> DeoxyHex <sub>1</sub> HexNAc <sub>2</sub> | M3F                              | 6.1                       | 2.5                          | 1.9   | -      | 2.4   | -      | -     | -      | 0.0   | -              | -     | -      | -     | -      |       |
| Terminal GlcNAc <i>N</i> -glycan | Hex <sub>3</sub> HexNAc <sub>3</sub>                       | GNM3                             | -                         | -                            | -     | -      | -     | -      | -     | -      | 38.6  | 40.2           | 24.8  | 16.6   | -     | -      |       |
|                                  | Hex <sub>4</sub> HexNAc <sub>3</sub>                       | GNM4                             | -                         | -                            | -     | -      | -     | -      | -     | -      | -     | 4.6            | -     | -      | -     | -      |       |
|                                  | Hex <sub>5</sub> HexNAc <sub>3</sub>                       | GNM5                             | -                         | -                            | -     | -      | -     | -      | -     | -      | -     | 2.4            | -     | -      | -     | -      |       |
|                                  | Hex <sub>3</sub> DeoxyHex <sub>1</sub> HexNAc <sub>3</sub> | GNM3F                            | -                         | -                            | -     | -      | -     | -      | -     | -      | 16.9  | -              | 10.8  | -      | -     | -      |       |
|                                  | Hex <sub>3</sub> HexNAc <sub>4</sub>                       | GN2M3                            | -                         | -                            | -     | -      | -     | -      | -     | -      | 13.8  | 4.0            | 8.7   | -      | -     | -      |       |
|                                  | Hex <sub>3</sub> DeoxyHex <sub>1</sub> HexNAc <sub>4</sub> | GN2M3F                           | -                         | -                            | -     | -      | -     | -      | -     | -      | 6.1   | -              | 3.5   | -      | -     | -      |       |
| Total                            |                                                            | Mannose-type <i>N</i> -glycan    | 16.8                      | 56.2                         | 19.7  | 53.9   | 17.7  | 26.4   | 19.3  | -      | 16.2  | 48.8           | 11.7  | 28.5   | -     | -      |       |
|                                  |                                                            | Fucosylated <i>N</i> -glycan     | 81.5                      | 27.4                         | 78.2  | 27.9   | 68.5  | 7.6    | 23.9  | -      | 31.3  | -              | 20.1  | -      | -     | -      |       |
|                                  |                                                            | Terminal GlcNAc <i>N</i> -glycan | -                         | -                            | -     | -      | -     | -      | -     | -      | 75.4  | 51.2           | 47.8  | 16.6   | -     | -      |       |

*N*-Glycan ratios were calculated on the basis of the intensities of signals.

M: Mannose; GN: *N*-Acetylglucosamine; F: Fucose