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

Supplementary Information

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Supplementary Figure S1  
*N*-Glycopeptide analysis of Asn106 in bIFN-γ and tIFN-γ

All signals of $m/z$ corresponding to *N*-glycopeptide and the *N*-glycan structures detected in nanoLC-MS/MS analysis are shown.
### Supplementary Figure S2  C-terminal analysis of tIFN-γ

(a) Sequence coverage map of Lys-C-digested tIFN-γ 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.
Table S1  Site-specific N-glycan structures of bIFN-γ and tIFN-γ and their relative ratios

| Structure | N-Glycosylation site | Expression system | Ratio (%) | bIFN-γ | tIFN-γ |
|-----------|----------------------|------------------|-----------|---------|---------|
|           |                      | Band             | A         | B       | C       | D       | a         | b         | c         | d         |
| Peptide   |                      |                  | Aasn39    | Aasn106 | Aasn39  | Aasn106 | Aasn39   | Aasn106  | Aasn39   | Aasn106  |
| EIEILKEYF^N39ASP DVAK |                  |                  | -         | -       | 1.2     | -       | 8.4      | -         | 56.8     | -         |
| FLY^N106SEK |                  |                  | -         | -       | 4.1     | -       | 60.1     | -         | 96.5     | -         |
|           | HexNAc2             |                  | -         | -       | 1.7     | 5.4     | 0.9      | 5.5       | 2.1      | 5.9       | -         | 3.5       | 27.9     | 29.1     | 96.2     | 67.8     |
|           | HexNAc2             |                  | -         | 8.1     | -       | 7.0     | 0.7      | -         | -        | -         | -         | -         | -        | -        | -        | -        |
|           | HexNAc2             |                  | -         | 2.9     | -       | 1.5     | 2.6      | -         | -        | -         | -         | -         | -        | -        | -        | -        |
|           | HexNAc2             |                  | -         | 8.1     | -       | 7.0     | 0.7      | -         | -        | -         | -         | -         | -        | -        | -        | -        |
|           | HexNAc2             |                  | -         | 2.9     | -       | 1.5     | 2.6      | -         | -        | -         | -         | -         | -        | -        | -        | -        |
| Mannose type |                  |                  | -         | -       | -       | -       | -        | 6.6       | -        | -         | 1.8       | -         | -        | -        | -        | -        |
| N-glycan  | Hex,HexNAc2         | M1               | 2.5       | 5.8     | 2.5     | 5.1     | 2.6      | -         | 6.6       | -         | -         | 1.8       | -         | -        | -        | -        | -        |
|           | Hex,HexNAc2         | M2               | 14.4      | 41.9    | 15.2    | 46.5    | 12.5     | 26.4      | 12.8      | -         | 1.7       | 11.7      | 1.5       | 5.9       | -         | -        | -        |
|           | Hex,HexNAc2         | M3               | -         | 4.2     | 2.1     | 2.4     | 2.6      | -         | -        | -         | 9.8       | 31.6      | 7.7       | 19.5      | -         | -        | -        |
|           | Hex,HexNAc2         | M4               | -         | 2.4     | -       | -       | -        | -         | -        | -         | 1.4       | -         | -        | -         | -         | -        | -        |
|           | Hex,HexNAc2         | M5               | -         | 2.1     | -       | -       | -        | -         | -        | -         | 4.8       | 2.3       | 2.4       | 3.1       | -         | -        | -        |
|           | Hex,DeoxyHex,HexNAc2| MF               | 5.9       | 3.0     | 6.3     | 2.9     | 5.8      | -         | 6.8       | -         | -         | -         | -         | -         | -         | -         | -        |
|           | Hex,DeoxyHex,HexNAc2| M2F              | 68.1      | 21.9    | 69.2    | 25.0    | 59.5     | 7.6       | 17.1      | -         | 1.2       | -         | -         | -         | -         | -         | -        |
|           | Hex,DeoxyHex,HexNAc2| M2FF             | 1.4       | -       | 0.9     | -       | 0.7      | -         | -        | 7.2       | -         | 5.7       | -         | -         | -         | -         | -        |
|           | Hex,DeoxyHex,HexNAc2| M3F              | 6.1       | 2.5     | 1.9     | 2.4     | -        | -         | 0.0       | -         | -         | -         | -         | -         | -         | -         | -        |
| Fucosylated N-glycan |                  |                  | -         | 6.1     | 2.5     | 1.9     | 2.4      | -         | 0.0       | -         | -         | -         | -         | -         | -         | -         | -        |
| Terminal GlcNAc N-glycan |                  |                  | -         | -       | -       | -       | -        | -         | 38.6      | 40.2      | 24.8      | 16.6      | -         | -         | -         | -        |
|           | Hex,HexNAc2         | GNM3             | -         | -       | -       | -       | -        | -         | -         | 38.6      | 40.2      | 24.8      | 16.6      | -         | -         | -        |
|           | Hex,HexNAc2         | GNM4             | -         | -       | -       | -       | -        | -         | 4.6       | -         | -         | 24.8      | -         | -         | -         | -        |
|           | Hex,HexNAc2         | GNM5             | -         | -       | -       | -       | -        | -         | 2.4       | -         | -         | -         | -         | -         | -         | -        |
|           | Hex,DeoxyHex,HexNAc2| GNM3F            | -         | -       | -       | -       | -        | -         | -         | 16.0      | -         | 10.8      | -         | -         | -         | -        |
|           | Hex,DeoxyHex,HexNAc2| GN2M3            | -         | -       | -       | -       | -        | -         | 13.8      | 4.0       | 8.7       | -         | -         | -         | -         | -        |
|           | Hex,DeoxyHex,HexNAc2| GN2M3F           | -         | -       | -       | -       | -        | -         | 6.1       | -         | 3.5       | -         | -         | -         | -         | -        |
| Total     | Mannose-type N-glycan|                  | 16.8      | 56.2    | 19.7    | 53.9    | 17.7     | 26.4      | 19.3      | -         | 16.2      | 48.8      | 11.7      | 28.5      | -         | -        |
|           | Fucosylated N-glycan|                  | 81.5      | 27.4    | 78.2    | 27.9    | 68.5     | 7.6       | 23.9      | -         | 31.3      | 20.1      | -         | -         | -         | -        |
|           | Terminal GlcNAc N-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