Electronic Supplementary Information

Spontaneous and site-specific immobilization of PNGase F via spy chemistry

Liang Zhang[1,*], Wenhui Wang[2,*], Yueqin Yang[3], Xiang Liu[2,4], Wenjie Zhu[2], Lingrui Pi[2], Xin Liu[2,**] and Song Wang[1,**]

[1]*Hubei Superior Discipline Group of Exercise and Brain Science from Hubei Provincial, Wuhan Sports University, Wuhan, 430079, China*

[2]*Hubei Bioinformatics & Molecular Imaging Key Laboratory, Systems Biology Theme, Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, 430074, China*

[3]*Exercise Immunology Center, Wuhan Sports University, Wuhan, 430079, China*

[4]*Department of Laboratory Medicine, Wuhan Children's Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430016. China*

* These authors contributed equally to this work.

** Corresponding author

Dr. Xin Liu, Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China
Email: xliu@mail.hust.edu.cn Tel: +86-27-87792203

Dr. Song Wang, Hubei Superior Discipline Group of Exercise and Brain Science from Hubei Provincial, Wuhan Sports University, Wuhan, 430079, China. Email: wangsong@whsu.edu.cn

Table S1. The primers sequence

Primers No.	Sequences
S-F	5′- GTGCCGCGCGGCAGCCATATGATGGCAATGGTTGATACCCTGA-3′
S-R	5′- CTGTCCACCAGTCATGCTAGCGCTACCCCCGCCACCGCT-3′

Table S2. Particle size distributions and zeta potential changes in the reaction procedure. (samples measured were very dilute in DI H_2O @ 25 ° C for DLS measurements. For all experiments, n = 3.)

MNP	**Hydrodynamic diameter (nm)**	**Zeta potential (mV)**
Bare MNP	552.5 ± 8.3	9.8 ± 0.8
MNP@COOH	848.8 ± 77.6	−22.1 ± 0.6
MNP@SpyTag	987.9 ± 62.7	−0.2 ± 0.2

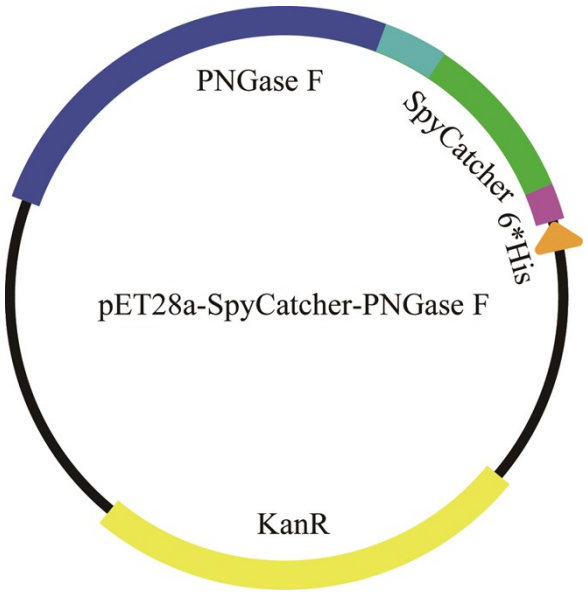

Figure S1. Gene design for pET28a-spycatcher-PNGase F.

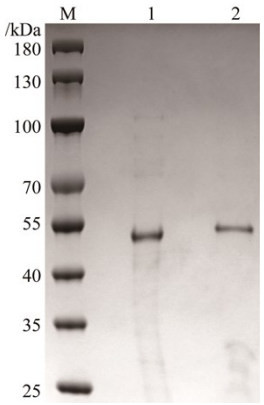

Figure S2. SDS-PAGE analysis of the SpyCatcher-PNGase F and SpyCatcher-PNGase F incubation with SpyTag. Lanes 1: SpyCatcher-PNGase F; Lanes 2: SpyCatcher-PNGase F incubation with SpyTag in mild condition.

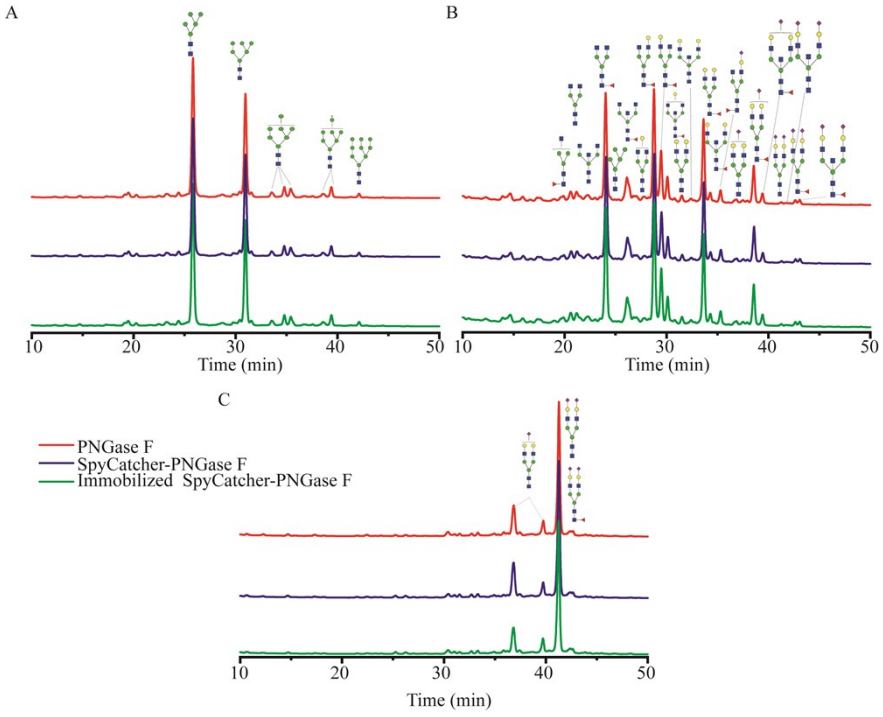

Figure S3. Representative Ultra Performance Liquid Chromatography (UPLC) chromatogram of RNase B (A), IgG (B), and transferrin (C) N-glycan profiles. N-acetyl glucosamine (HexNAc), blue ■; fucose (DeoxyHex), red ▼, mannose (Hex), green ●; galactose (Hex), yellow ●, and sialic acid (Neu5Ac), purple