Bacteriophage T4 capsid as a nanocarrier for Peptide-N-Glycosidase F immobilization through self-assembly

Liang Zhang[1,2], Pei Wang[1], Chang Wang[2], Yike Wu[2], Xiaojun Feng[2], He Huang[3], Lujing Ren[3], Bi-Feng Liu[2], **Song Gao**[1] & Xin Liu[2]

[1] Jiangsu Ocean University (formerly Huaihai Institute of Technology), Lianyungang, 222005, China.
[2] Huazhong University of Science and Technology, Wuhan, 430074, China.
[3] Nanjing Tech University, Nanjing, 211816, China.

Introduction

Enzyme immobilization is widely applied in biocatalysis to improve stability and facilitate recovery and reuse of enzymes. However, high cost of supporting materials and laborious immobilization procedures has limited its industrial application and commercialization[1-2]. In this study, we report a novel self-assembly immobilization system using bacteriophage T4 capsid as a nanocarrier. The system utilizes the binding sites of the small outer capsid protein, Soc, on the T4 capsid. Enzymes as Soc fusions constructed with regular molecular cloning technology expressed at the appropriate time during phage assembly and self-assembled onto the capsids. The proof of principle experiment was carried out by immobilizing β-galactosidase, and the system was successfully applied to the immobilization of an important glycomics enzyme[3-4], Peptide-N-Glycosidase F. Production of Peptide-N-Glycosidase F and simultaneous immobilization was fnished within seven hours. Characterizations of the immobilized Peptide-N-Glycosidase F indicated high retention of activity and well reserved deglycosylation capacity. The immobilized Peptide-N-Glycosidase F was easily recycled by centrifugation and exhibited good stability that sustained five repeated uses. This novel system uses the self-amplifed T4 capsid as the nanoparticle-type of supporting material, and operates with a self-assembly procedure, making it a simple and low-cost enzyme immobilization technology with promising application potentials.

Experimental Section

In this study, a novel self-assembly immobilization system has been developed using the T4 capsid as a nanocarrier. The approach starts with transformation of the immobilization plasmid into E.coli cells. The plasmid contains the PNGase F-Soc fusion protein gene that is under the regulation of Soc promoter and terminator, and when the E. coli cells are infected with Soc⁻ T4 phage, the PNGase F-Soc fusion protein expresses in parallel with other phage proteins. At the late stage of phage assembly, Soc binding sites on the capsid expose, and the PNGase F-Soc fusion self-assembles on the capsid through its Soc-domain. Tail attachment completes the assembly, and the T4 phage particles assembled with PNGase F are released for harvest and isolation. Using this approach, PNGase F is immobilized on phage T4 capsid through self-assembly, and the phage particles are conveniently isolated by centrifugation. Moreover, PNGase F immobilized on the phage particles is ready to perform deglycosylation of glycoproteins. After the reaction, the enzyme is easily separated from reaction substrates and products by centrifugation, and recycled for repeated uses.

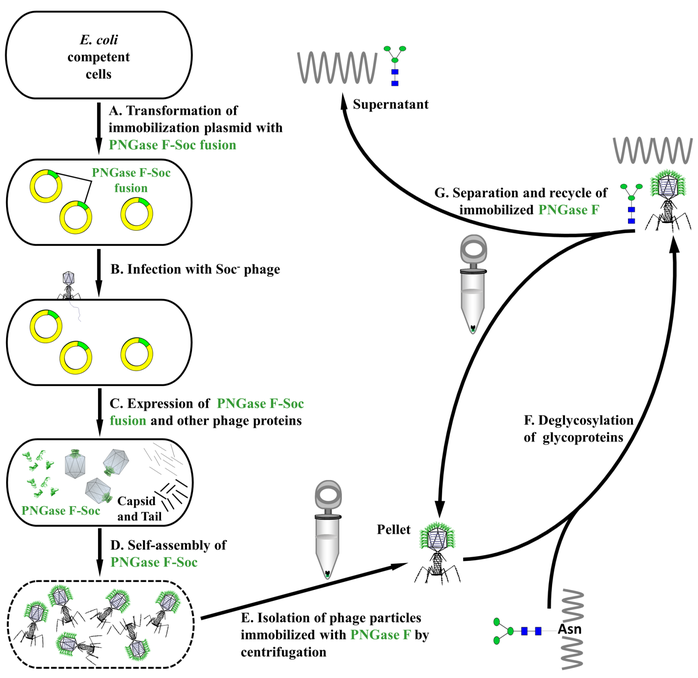

Fig 1. A self-assembly approach to immobilize PNGase F. (**A**) The PNGase F immobilization plasmid was transformed into *E. coli* competent cells. (**B**) *E. coli* cells containing the PNGase F immobilization plasmid were infected by Soc⁻ phage. (**C**) The PNGase F-Soc fusion protein was expressed in parallel with other phage proteins that assembled the capsid and tail. (**D**) The PNGase F-Soc fusion self-assembled on the capsid and immobilized on the Soc binding sites on the capsid. (**E**) Isolation of the phage particles immobilized with PNGase F at 34,500 *g* for 45 min by centrifugation. (**F**) Deglycosylation of glycoproteins with the PNGase F immobilized on the phage particles. (**G**) Separation and recycle of the immobilized PNGase F (pellet) from the product and/or substrate (supernatant) by centrifugation.

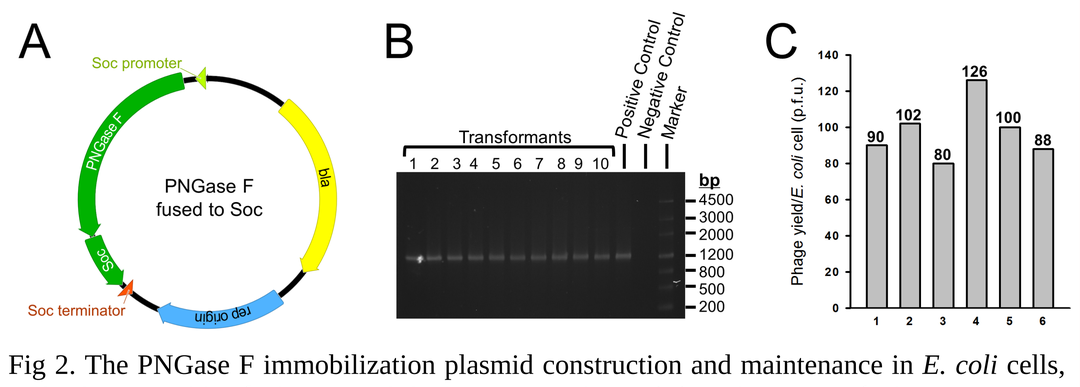

Fig 2. The PNGase F immobilization plasmid construction and maintenance in *E. coli* cells, and phage yield of the immobilization procedure. (**A**) Schematic of the PNGase F immobilization plasmid. (**B**) Screening of the transformants for the presence of the PNGase F-Soc ORF. Transformants 1–10 were subjects for colony PCR with primers specific to the PNGase F-Soc ORF, and the PCR products were analyzed by agarose gel electrophoresis. (**C**) Phage yield of the immobilization by self-assembly procedure. The number of phage particles produced from one *E. coli* cell is presented in plaque forming units (p.f.u.). Phage yields from 6 independent experiments (bars 1–6) are shown. Te exact number of phage yield of each experiment is indicated on top of each bar.

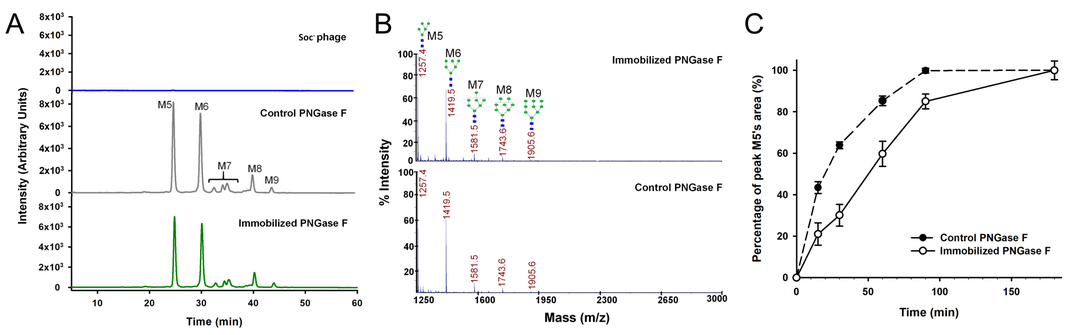

Fig 3. Enzymatic activity of the immobilized PNGase F. (**A**) HPLC profiles of the N-glycans released from RNase B by the immobilized PNGase F (bottom), the control (middle), and the Soc- phage (top). Te peaks of the 5 N-glycans (M5 to M9) are indicated. (**B**) Analysis of N-glycans released from RNase B by the immobilized PNGase F (top) and the control (bottom) with MALDI-TOF MS spectra. Te structures and mass-to-charge ratios of M5 to M9 are indicated. (**C**) Time curves of the percentages of peak M5's areas released from RNase B by the immobilized PNGase F and the control. Error bars represent the standard deviation from 3 independent experiments.

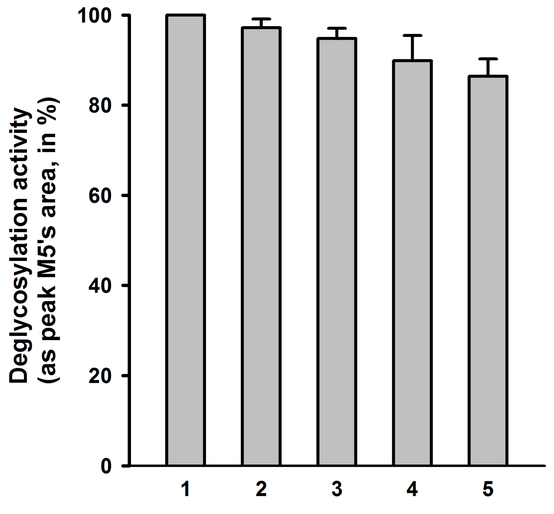

Fig 4. Deglycosylation activities of the immobilized PNGase F in repeated uses. 1.25 × 10^{11} phage particles immobilized with PNGase F (equal to 1 U) were used to digest 10 μg of denatured RNase B at 37 ° C for 1 h. After the reaction, the immobilized PNGase F was recycled in the pellet by centrifugation at 34,500 *g* for 45 min at 4 ° C. The supernatant was used to analyze the deglycosylation activity by quantification of the released M5.

Conclusion

The immobilized Peptide-N-Glycosidase F was easily recycled by centrifugation and exhibited good stability that sustained five repeated uses. This novel system uses the self-amplified T4 capsid as the nanoparticle-type of supporting material, and operates with a self-assembly procedure, making it a simple and low-cost enzyme immobilization technology with promising application potentials.

Acknowledgements

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