Targeted protein degradation in lysosome utilizing naturally produced bifunctional antibodies with high levels of mannose 6-phosphate glycans

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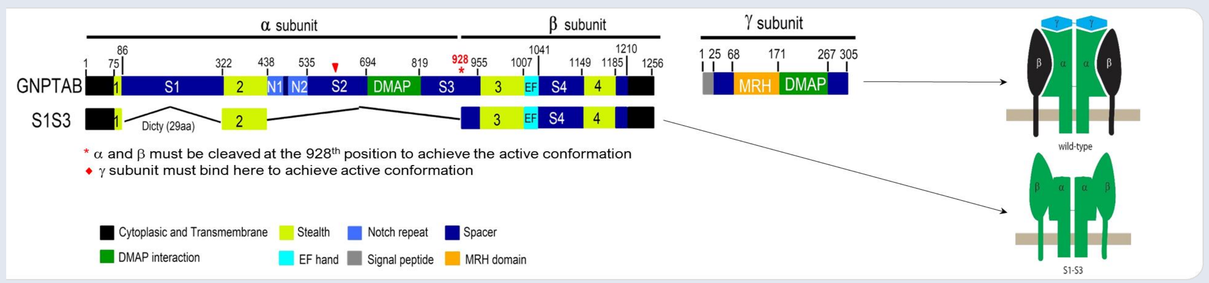
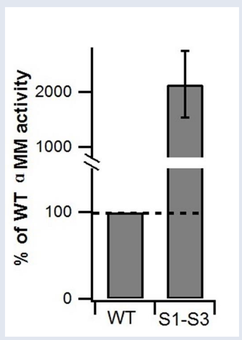
1. Abstract

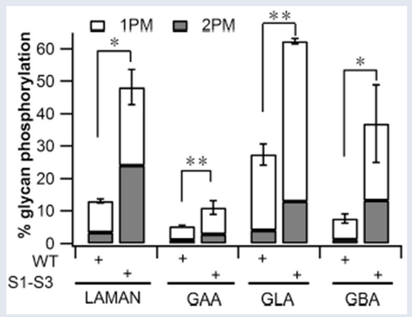
Novel antibodies have been created for targeted degradation of extracellular and membrane proteins in the lysosome. The mechanism of degradation of target proteins for these antibodies has involved either chemical conjugation of synthetic mannose 6-phosphate (M6P) or engineered bispecific antibodies. Currently, recombinant antibodies cannot be produced with naturally phosphorylated N-glycans. Here, we report the development of a novel platform technology for producing bifunctional therapeutic antibodies with high levels of M6P-bearing glycans directly from producing cells. The antibodies designated as phosphorylated N-glycosylated peptide chimeric antibodies (PNCA) maintain their affinity for antigens with concurrent high affinity binding to cell surface cation-independent mannose-6-phosphate receptors that facilitate internalization and delivery of antibody/antigen complexes to lysosomes for efficient degradation of both target extracellular soluble and membrane proteins. This PNCA approach provides a simple, scalable, and viable approach for producing naturally phosphorylated bifunctional antibodies from production cell lines for targeted protein degradation in lysosomes.

2. Introduction

2.1 S1S3 PTase greatly increases the M6P level of glycoproteins.





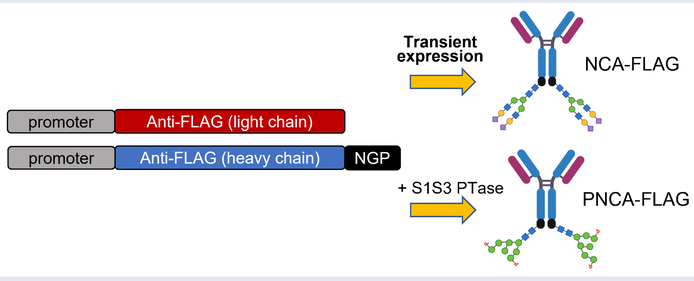


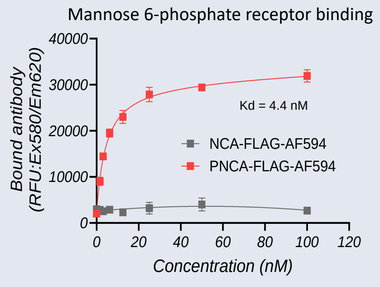
LAMAN: α-mannosidase for Mannosidosis; GAA: α-glucosidase for Pompe disease
GLA: α-galactosidase A for Fabry disease; GBA: β-glucosidase for Gaucher disease

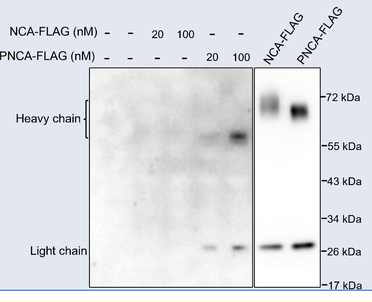
2.2 Novel N-Glycosylated Peptide (NGP) enable natural production of proteins or antibodies with high levels of M6P when co-expressed with S1S3 PTase

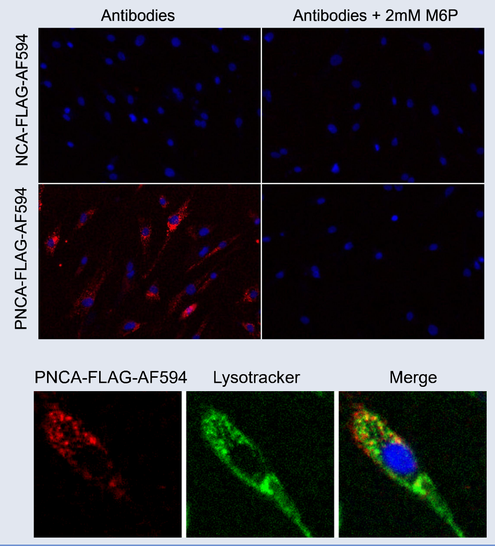
3. Results

3.1. Generation of new N-glycosylated peptide fusion proteins and antibodies with high levels of mannose 6-phosphate (M6P)

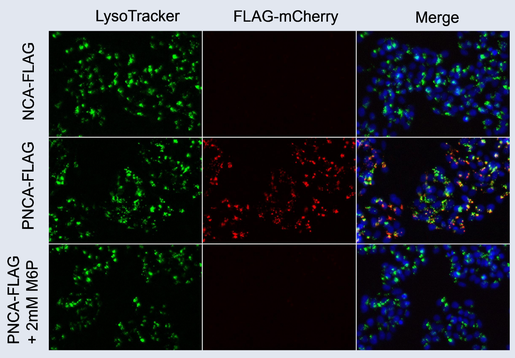


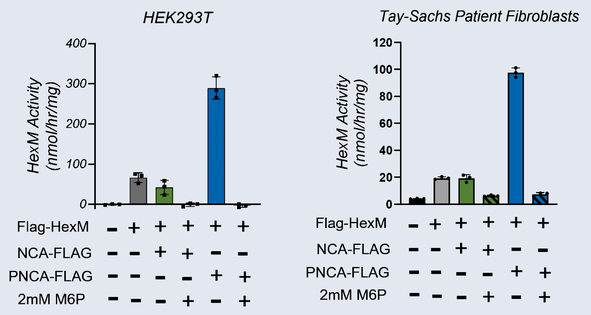


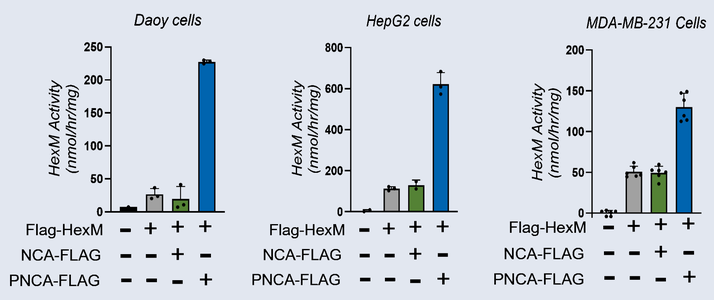




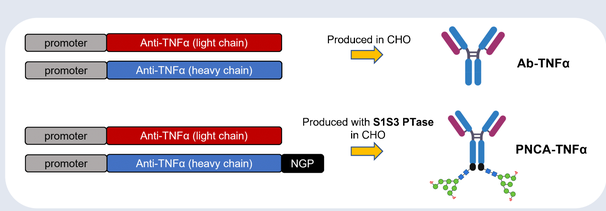
3.2. PNCA-FLAG binds to antigen (FLAG-HexM enzyme) and is internalized and delivered to lysosome with antigen through CI-MPR pathway

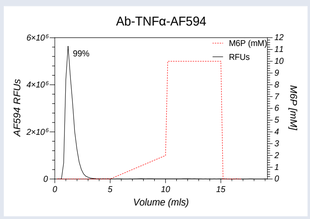


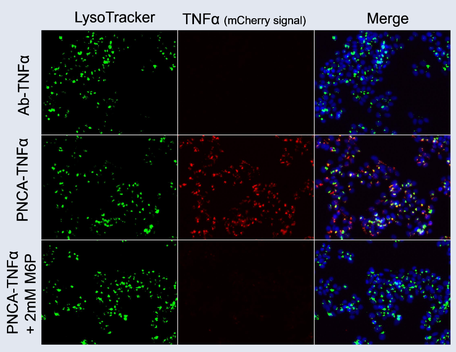


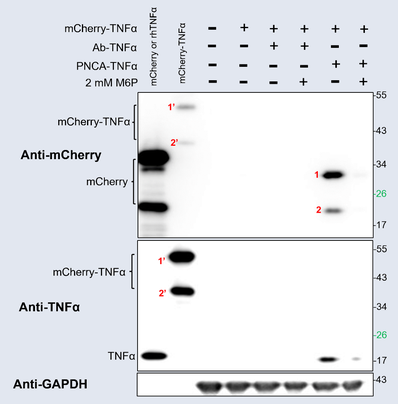


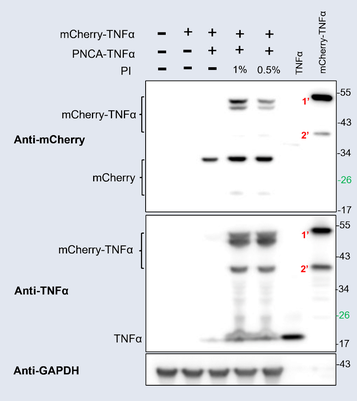
3.3. PNCA-TNFα enables internalization and degradation of extracellular mCherry-TNFα or TNFα in the lysosome



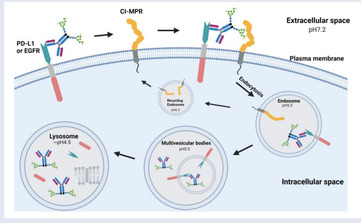


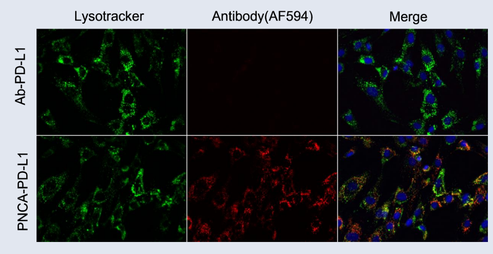


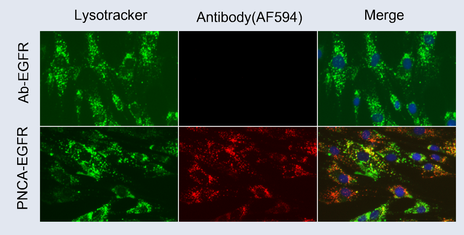


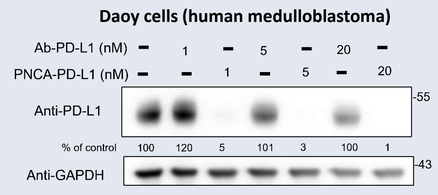


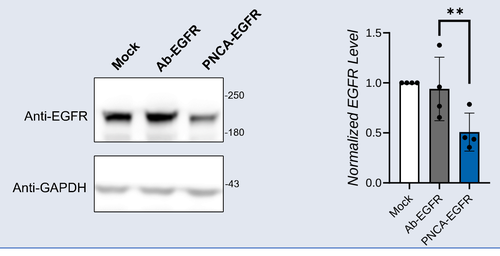
3.4. PNCA antibodies enable degradation of target transmembrane receptors (EGFR and PD-L1)

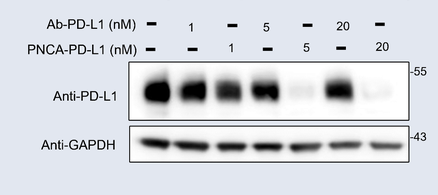












3. Conclusion

In this study, we developed a novel technology to produce bifunctional antibodies with high levels of mannose 6-phosphate glycans directly in CHO cells. The bifunctional antibodies designated as phosphorylated N-glycosylated peptide chimeric antibodies (PNCA). It maintains high binding affinity for antigen recognition as native antibodies, and gains nanomolar binding affinity to cell surface CI-MPR for internalization and delivery to lysosomes for efficient degradation. Four epitopes, including two soluble extracellular proteins (FLAG-tagged protein and TNF-α) and two membrane proteins (PD-L1 and EGFR) were tested in our platform technology. Data shows that the PNCA antibodies enable robust targeted protein degradation in lysosomes for both extracellular soluble and cell surface membrane proteins. The one step production and purification of PNCAs approach could overcome the current limitations such as complex synthesis and bioconjugation.