AFFINITY CAPTURE OF A (GLYCO)PROTEIN: GETTING YOUR SAMPLE READY FOR THE MASS SPECTROMETER

Valeriia Kuzyk[1,2], Guinevere S.M. Lageveen-Kammeijer[2], Rob Haselberg[1], Manfred Wuhrer[2], Govert W. Somsen[1]
(1) Division of BioAnalytical Chemistry, Amsterdam Institute of Molecules, Medicines and Systems, Vrije Universiteit, Amsterdam,
(2) Center for Proteomics and Metabolomics, Leiden University Medical Centre, Leiden

Introduction

Some details of affinity capturing methods and overall sample handling are often neglected in publications and protocols, however these may improve the yields of a target glycoprotein significantly and thus are important when the target protein amount is very low. In this work we present a roadmap of steps to consider for sample handling for mass spectrometry analysis for obtaining maximum efficiency of the method.

1 Carrier vial choice

Protein LoBind — DNA LoBind

LoBind coated Eppendorfs can be used to limit the vial wall adsorption. Protein LoBind is beneficial for protein samples, however sialylated glycoproteins (carrying a negative charge) show significantly less adsorption when DNA LoBind Eppendorfs are used.

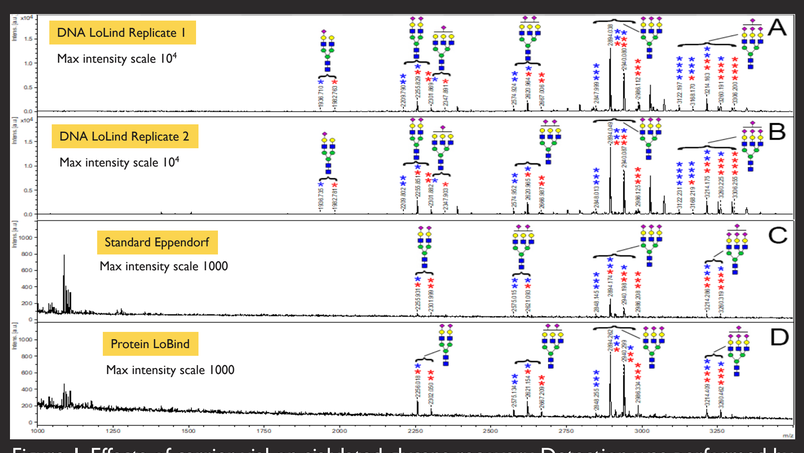
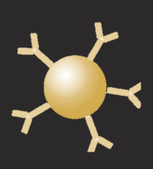


Figure 1. Effects of carrier vial on sialylated glycans recovery. Detection was performed by UltrafleXtreme MALDI TOF/TOF.



2 Solid support choice

Sepharose beads — Magnetic Silica beads

Sepharose beads are used frequently in affinity capturing methods, but their porous surface may trap background and/or target proteins, resulting in lowered yields and high protein background, as well as higher sample loss in the residual liquid. Additionally, to lower the background one may consider preincubating the beads with the matrix.

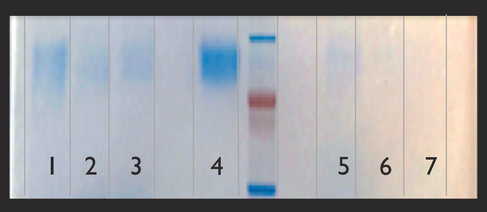
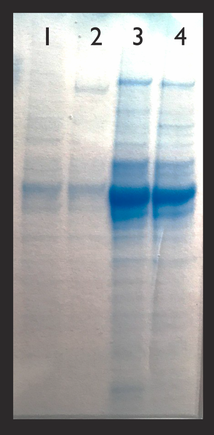


Figure 2. *Upper panel:* target glycoprotein recovery with Bioclone magnetic NHS-terminated beads (lanes1,2,3) and Sigma-Aldrich Sepharose beads (lanes 5,6,7) coated with specific VHS-antibodies. Respective amount of a target protein standard is on lane 4.

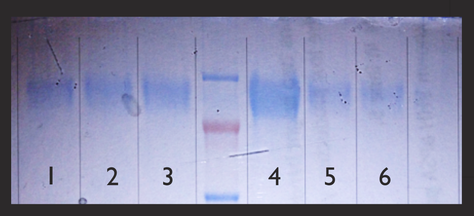
Right panel: serum protein background assessment on Bioclone magnetic NHS-terminated beads (lanes1,2) and Sigma-Aldrich Sepharose beads (lanes 3,4)



3 Concentrating technique

Freeze drying — SpeedVac — Spin-filter

Vacuum centrifuge drying is a common approach to lyophilize and concentrate your sample, however its vacuum doesn't guarantee the lack of turbulence and sample loss. Reduce it by closing the vial and making a hole in its cap. Alternatively, use freeze drying. Spin-filters, also commonly used for concentration, show mediocre results for low sample amounts.



1. Freeze drying 200 µl eluate
2. Freeze drying 100 µl eluate
3. SpeedVac drying, holed tube cap
5. SpeedVac drying, open cap
6. Millipore spin-filter 5kDa MWCO

Reference amount of target glycoprotein (10µg) is on lane 4

Figure 3. Effects of eluate concentration technique on target glycoprotein recovery. Target glycoprotein (10µg spiked) was captured with nanobodies-coated sepharose beads and eluted with 100mM formic acid.

4 Liquid volumes

Low – Less adsorption — High – Better extraction

Most of the protocols recommend high extraction volumes for better submerging/dissolving of the target. However, the higher the extraction volume is, the bigger is the potential adsorption surface. Thus we recommend extraction volume optimization towards the lowest possible value.

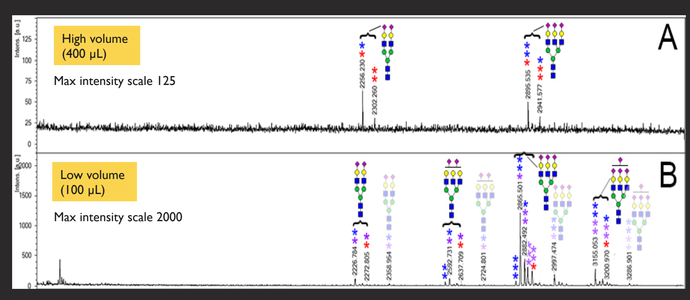


Figure 4. Effects of extraction volume on sialylated glycans recovery. Detection was performed by UltrafleXtreme MALDI TOF/TOF.

0 Nanobody efficiency

VHS-antibodies (or nanobodies) are the single monomeric variable antibody domains. Whilst keeping their specificity, they are very stable in operation. Nanobodies are not glycosylated, that allows bacterial production and lowers the cost (at least 3 times cheaper than monoclonal full-size antibodies).

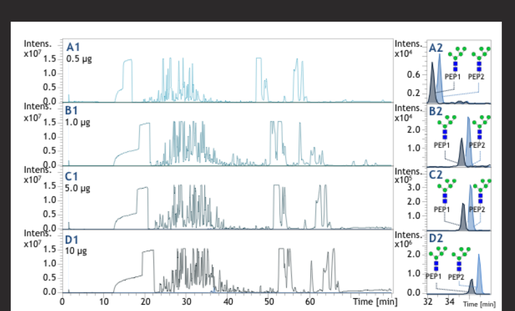


Figure 5. CE-ESI-MS analysis of a target protein (CEA) captured with nanobodies on a solid support. Up to 0.5µg of spiked CEA can be captured, digested and subsequently analyzed with mass spectrometry. *Right panel:* base peak chromatograms of various amounts of captured and digested CEA. Left panel:representative extracted ion chromatocgrams of Man5 glycan with 2 peptide backbones

Conclusions:

1. Variety of minor factors may influence the affinity capturing efficiency thus affecting MS analysis
2. Nanobodies present a potent low-cost alternative to monoclonal antibodies

Contact

Valeriia Kuzyk
VU Amsterdam/LUMC
Email: v.kuzyk@vu.nl
v.kuzyk@limc.nl
Linkedin: /valeriiakuzyk