Preparation of immunoaffinity columns for purification and analytics of MULT-1 and RAE-1

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INTRODUCTION

Immunoaffinity columns using antibodies as ligands against mammalian membrane proteins could be used for different applications in protein expresion control and, if a standard available, for concentration determination. Additionally these columns are ideal for polishing step of Fc fusion proteins of mammalian receptors. Most importantly such columns could extract a significant amount of a pure membrane mammalian protein suitable for structural analyses, such as mass spec analysis of their glycans. Immunoaffinity chromatographic monoliths against MULT-1 transmembrane and RAE-1 GPI anchored glycoproteins were developed as a part of Glycomet project with the main goal to analyze the antigen glycan parts.

RESULTS

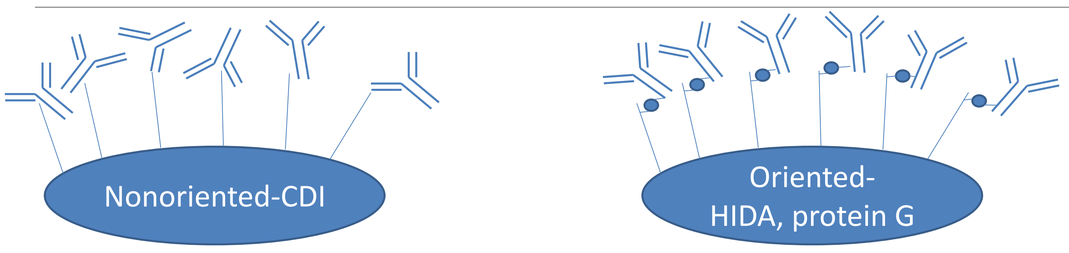


Figure 1: Antibodies immobilized on monoliths in oriented and non-oriented direction. On hydrazide preactivated support (HIDA), monoclonal antibodies (mAbs) are immobilized through oxidized sugars., on carbonyl imidazole preactivated support (CDI), mAbs are immobilized through their amino groups. In case of protein G column, affinity-bound mAbs are additionally covalently crosslinked with the protein G.

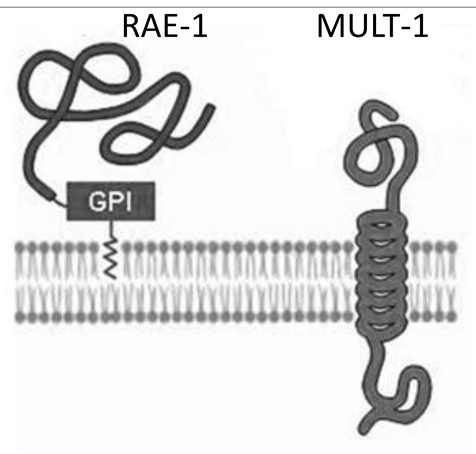


Figure 2: MULT-1 is a transmembrane protein with large hydrophobic transmembrane region. RAE-1 is a GPI anchored protein. To solubilize hydrophobic proteins, detergent is needed before and during separation.

Antibodies

Figure 3: αMULT-1 mAb and αRAE-1 mAb precipitate MULT-1 and RAE-1 from cell lysates of corresponding transfectants

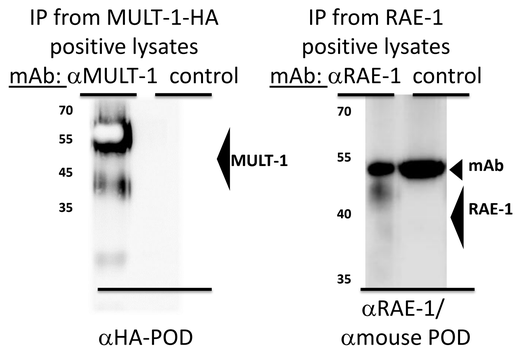
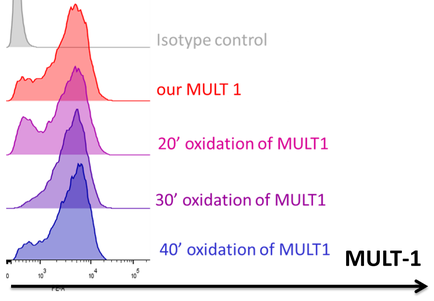


Figure 4: Oxidation does not impact antibody activity (example: MULT-1).



Surface staining of live cells using oxidised αMULT-1 mAb

Cell lysates (Load)

Figure 5: Extraction using 0,1% NP40 is successful. Filtration does not remove proteins from the solution (example: MULT-1).

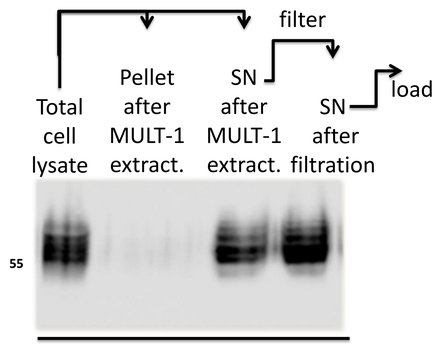
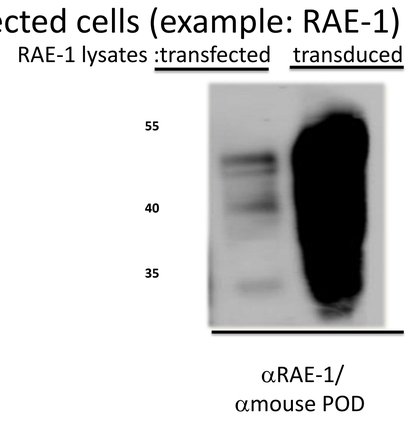
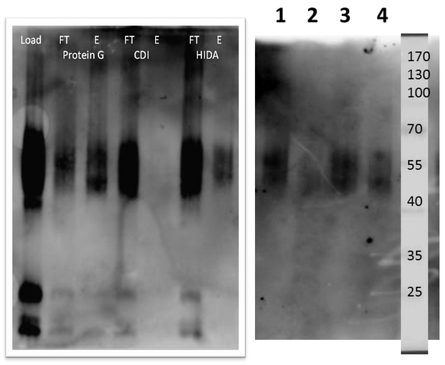


Figure 6: The amount of proteins upon transduction (using a cytomegaloviral vector) is significantly higher than in transfected cells (example: RAE-1)



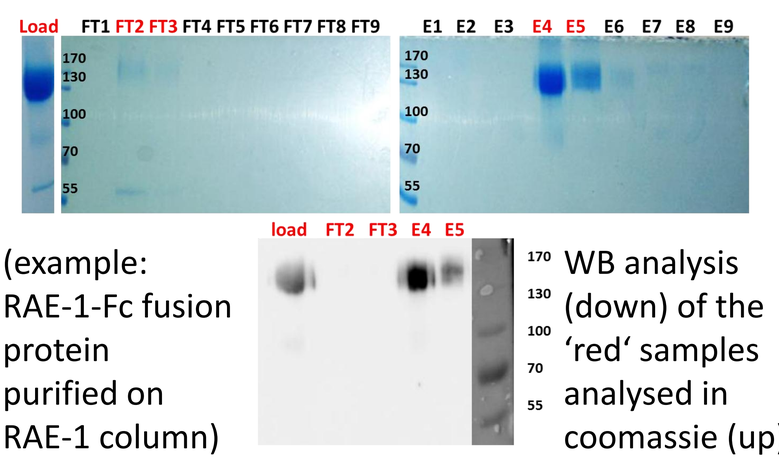
Columns

Figure 7: Left: MULT-1 from lysate of transfected cells was captured with three differently prepared immunoaffinity chromatographic monoliths (based on Protein G, CDI, HIDA). It is observed that part of the MULT-1 remains in the FT fraction. Right: Testing of anti MULT-1 isolation in presence of 0.5% Tween 20 or NP40.



1. E HIDA Tween
2. E CDI Tween
3. E CDI NP40
4. E HIDA NP40

Figure 8: Immunoaffinity columns remove truncated and degraded Fc compounds from a standard (protein A/G purified) Fc-fusion protein. Moreover, Fc-fusion protein is a quantification standard for native mammalian proteins.

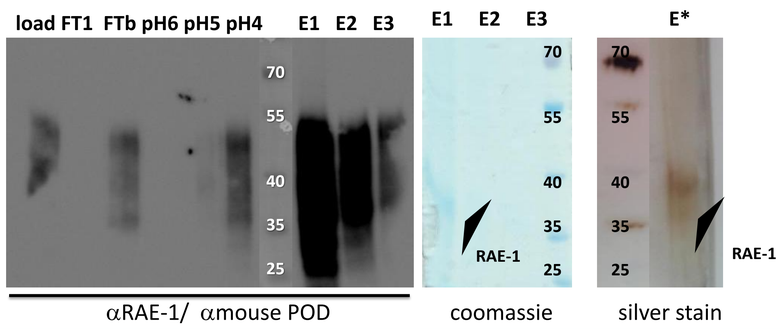


(example: RAE-1-Fc fusion protein purified on RAE-1 column)

WB analysis (down) of the 'red' samples analysed in coomassie (up)

Method: preparative immunoaffinity purification of mammalian membrane glycoproteins – proof of principle

Figure 9: Immunoaffinity columns purify target proteins from lysates of transduced cells. FTb: FT after capacity of the column was reached (cca 20 million cells).
E1-3 eluats/pure target protein (1/5 of the purified protein/round is shown in coomassie).
Estimation: 5ug/round/20 million of transduced cells.
E* eluats from another representative experiment



CONCLUSIONS

- Samples of transduced cells are ideal for massive production of mammalian membrane glycoproteins.
- HIDA columns are ideal support for custom made immunoaffinity supports.
- With anti-MULT-1 column we isolated a small quantity of antigene from lysates of MULT-1 transfected cells, as confirmed by western blot analysis.
- With anti-RAE-1 column we isolated enough antigene from lysate of RAE-1 transfected cells to be visible on SDS-PAGE using silver staining (approximately 5 µg of RAE-1 / round of purification).