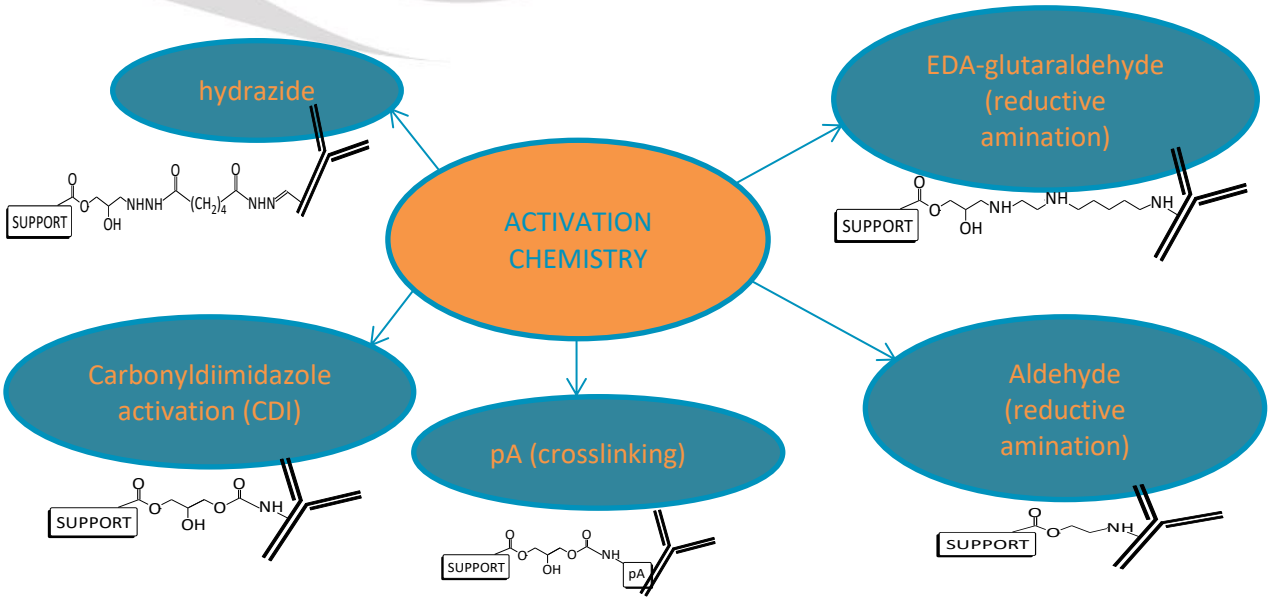
A046 Versatility of CIM® monoliths for covalent immobilization of antibodies

The **CIMac™** antibody immobilization platform enables an elegant immobilization of antibodies, which can be used as immunosorbents in specific diagnostic applications as well as in downstream processes. In this work we show the dependence of the coupling strategy on CIM monolith with the chromatographic efficiency of final immunoaffinity adsorbent. Different activation chemistries (Scheme 1) were tested for the immobilization of two model monoclonal antibodies (mAbs) with subsequent chromatographic characterization of the affinity support.

Scheme 1



Schematic presentation of immobilization of mAbs onto the CIMac platform

Example 1 - Immobilization of mAb antifibrinogen (anti-hFIB)

The first model mAb used was mouse anti-hFIB (IgG2a), specific for the human fibrinogen. Five different immobilization protocols were performed and the achieved density of immobilized mAb was between 4 and 7 mg of anti-hFIB per mL of the chromatographic support used. The prepared immunoaffinity columns were later on tested for their chromatographic binding capacity for its antigen - human fibrinogen (Figure 1). As one can recognise, two immobilization protocols, where oriented immobilization (Fab fragment of mAb extends into the lumen of the pore) was applied (mAb crosslinking to protein A column and covalent linkage of mAb to hydrazide support) were superior to non-oriented linkage protocols.

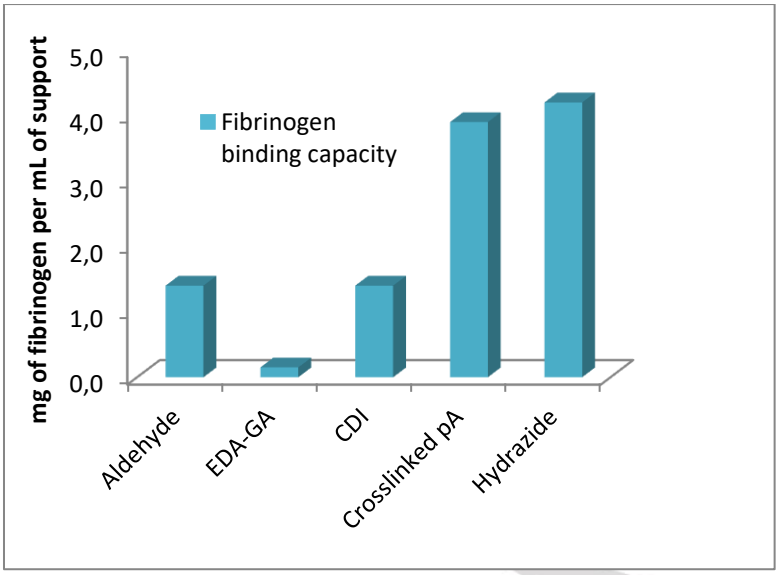


Figure 1

Binding capacities of prepared immunosorbents for human fibrinogen, measured at 50% breakthrough. The experimental conditions are described in Method table.

METHOD for Figure 1

Columns:	different CIMac™ columns with immobilized anti-hFIB
Load:	Human fibrinogen (0.1 mg/ml) in phosphate buffered saline (PBS), pH 7.2
Flow rate:	1.0 mL/min
Mobile phases:	Solvent A: phosphate-buffered saline (PBS) Solvent B: 0.1 M glycine, pH 2.0

It was confirmed as well that the prepared immunosorbents specifically recognise human fibrinogen out of the mixture of different proteins applied. An example, where depletion of fibrinogen from human plasma was performed using CIMac protein A column with crosslinked anti-hFIB, is shown in Figure 2.

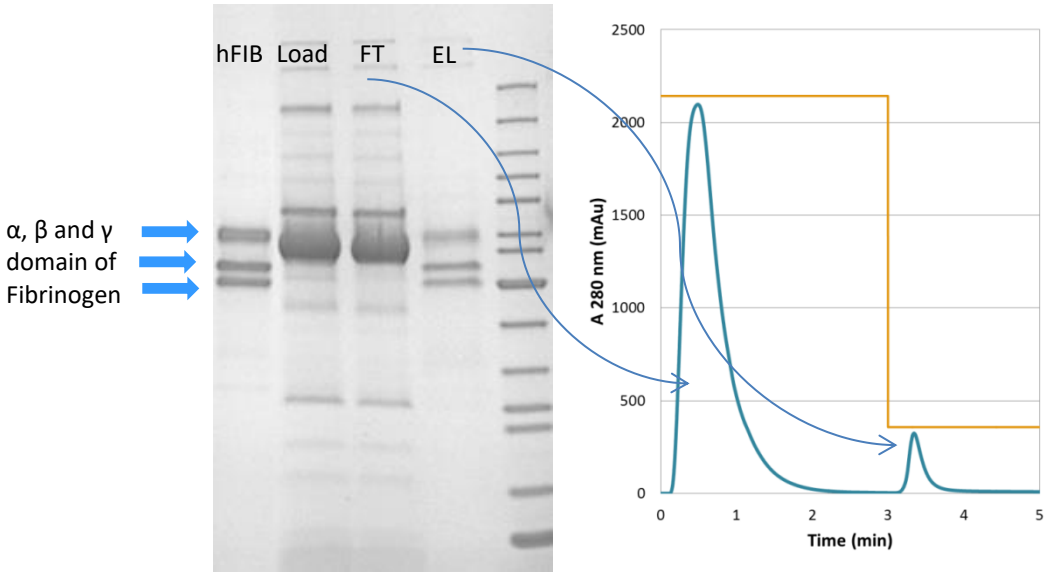


Figure 2

Specificity of immunoaffinity adsorbent for selective binding of the antigen. The chromatographic conditions are described in Method table.

METHOD for Figure 2

Column:	Protein A-based CIMac™ column with immobilized anti-hFib
Load:	100 µL 10 × diluted IgG depleted human plasma
Flow rate:	0.5 mL/min
Mobile phases:	Solvent A (binding buffer): PBS, pH 7.2 Solvent B (elution buffer): 0.1 M glycine, pH 2.0
Gradient elution method:	Solvent A (3 min), solvent B (2 min)

Example 2 – Development of immunosorbent for the recombinant hepatitis B surface antigen (rec-HBsAg) purification

Hepatitis B surface antigen (HBsAg) is one of the molecules purified by immunoaffinity chromatography to develop global human vaccination programs. CIM chromatographic monoliths are elegant solution for efficient and fast purification of such large antigens, such as 20 nm large rec-HbsAg. Therefore an immobilization of HBsAg-specific mouse mAb (CB.Hep-1) onto CIM monoliths was studied.

Three different linkage strategies were followed, two non-oriented (aldehyde and CDI) and one oriented (hydrazide). Different CB.Hep-1 densities on the surface of chromatographic support were used to assess the ligand utilisation. Results of antigen binding capacities and corresponding elution yields are shown in Figure 3. As one can observe, the oriented immobilization resulted in high binding capacities, but we were not able to elute the bound antigen from the column. The elution efficiency was improved with non-oriented immobilization of mAb, however CDI-based immobilization showed remarkably better results than aldehyde-based. Finally, comparing the results of three different CDI-based immobilization strategies, the antigen recovery was drastically improved (to almost 70%) with decrease of amount of immobilized mAb. This results in much higher ligand utilisation as well, what is of great importance in production of immunosorbents due to high cost of mAb production.

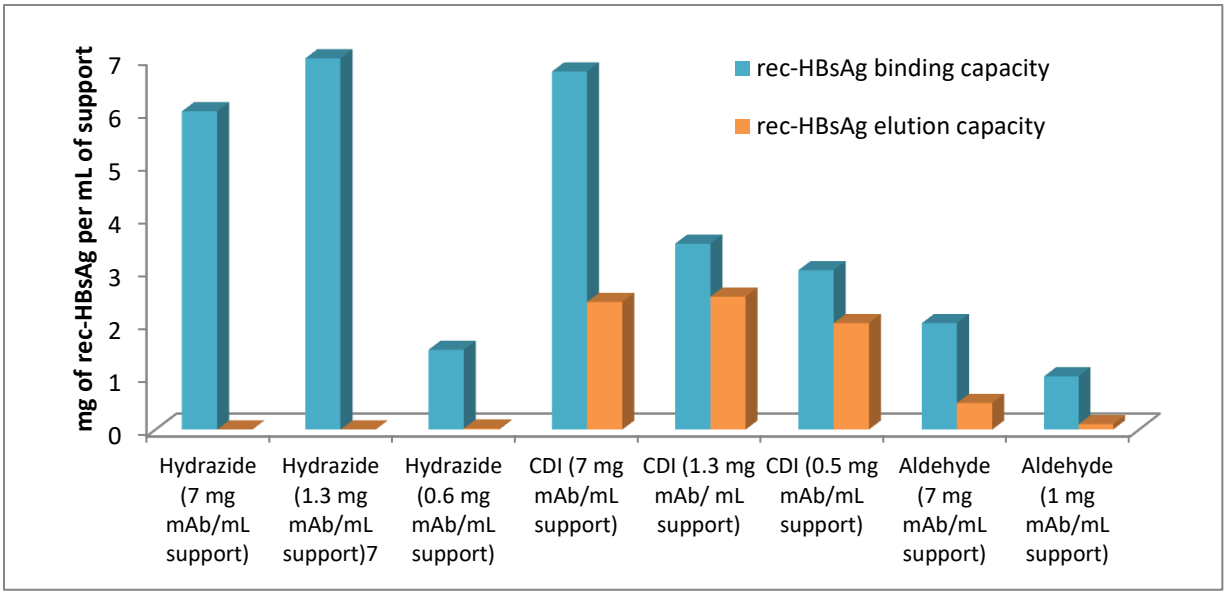


Figure 3

rec-HBsAg binding and elution capacities on different CIM immunosorbents. The experimental conditions are described in corresponding Method table.

METHOD for Figure 3

Column:	different CIMac™ columns with immobilized CB.Hep-1 mAb
Load:	Rec-HBsAg (0.2 mg/mL) in slovent A
Flow rate:	1.0 mL/min
Mobile phases:	Solvent A (binding buffer): 20mM TRIS, 0.5M NaCl, 3mM EDTA, pH 7.4 Solvent B (elution buffer): 3 M KSCN in solvent A

CONCLUSIONS

We were able to covalently bind the model monoclonal antibodies onto a chromatographic monolith using different linkage strategies.

The immobilization of antibodies was proven by measuring the selectivity of the final affinity chromatographic support for the specific antigen as well as with measuring dynamic binding capacities and elution recoveries for the applied specific antigen.

TAKE HOME MESSAGE: The mAb linkage strategy on CIM monolith depends on each application, the column is used for. In order to obtain the most efficient monolithic immunosorbent, an immobilization screening using different linkage strategies is prerequisite.

SEVENTH FRAMEWORK PROGRAMME

HIGH GLYCAN
Methods for high-throughput (HTP) analysis of protein glycosylation

ORDERING INFROMATION

Used product

Catalogue No.	Product description
110.8000-1.3	CIMac™ CDI-0.1 Analytical Column (Carbonyldiimidazole) (Pores 1.3µm)
110.8001-1.3	CIMac™ AE-0.1 Analytical Column (Aldehyde) (Pores 1.3µm)
110.8002-1.3	CIMac™ HDZ-0.1 Analytical Column (Hydrazide) (Pores 1.3µm)
110.5116-1.3	CIMac™ EDA-0.1 Analytical Column (Ethylene diamino) (Pores 1.3µm)
110.1004-2	CIMac™ rpA-0.1 Analytical Column (Recombinant protein A) (Pores 2µm)

Related Products

Catalogue No.	Product description
102.8000-2	CIMmic CDI-0.2 (Carbonyldiimidazole) (Pores 2 um)
102.8001-2	CIMmic AE-0.2 (Aldehyde) (Pores 2 um)
102.8002-2	CIMmic HDZ-0.2 (Hydrazide) (Pores 2 um)

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