Vaccines purification by affinity chromatography with Nanofitin ligands: demonstration with glycoconjugates

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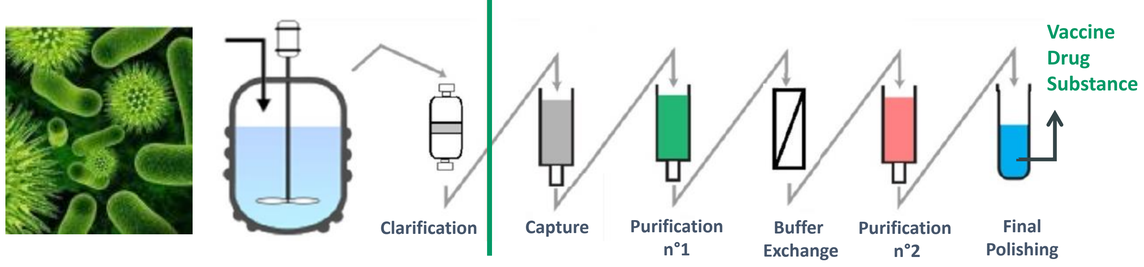
Current vaccine purification process

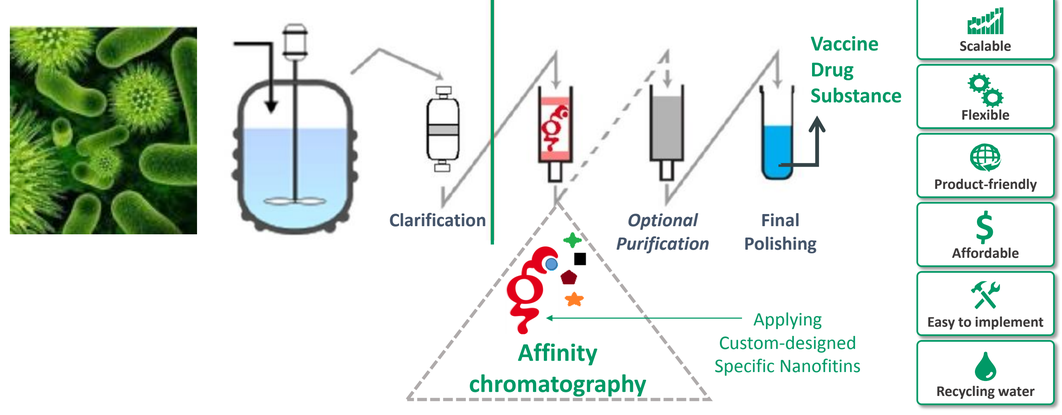


Objectives

REDUCE
Number of unit operations
Product loss

IMPROVE
Process efficiency
Product recovery
Customized development

DiViNe approach



High yield vaccine purification process
- Affinity capture proven extremely successful in purification of monoclonal antibodies
- Development from benchtop to industrial scale to insure a GMP compliant process

Preserved product integrity
- Eluting the product under mild elution conditions not to hamper structure of the product
- Flexibility of the custom-designed Nanofitin-based column

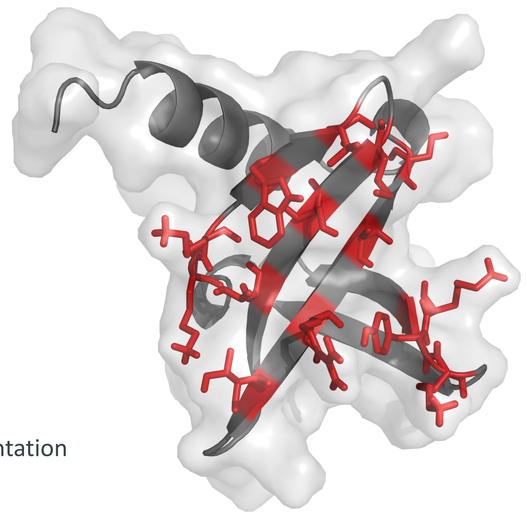
Transfer to other Biologics
- POC on most representative vaccines (glycoconjugates, protein antigens and enveloped viruses)
- Expand to complex recombinant products, gene therapy vectors, blood products...

Developing a flexible Nanofitin-based platform for vaccines purification

Vaccine Target : CRM_{197} carrier protein

- Cross-Reactive Material CRM_{197}, advantageous carrier protein:
  - ✓ Nontoxic variant of diphtheria toxin
  - ✓ Many lysyl side-chains available for conjugation
  - ✓ Used as a carrier for about 30% of marketed glycoconjugate vaccines
- Current production process:
  - ✓ Based on extraction from cultivated strains (toxin isolated from Corynebacterium diphtheriae C7 (β197) cultures)
  - ✓ Very low yields (below 50 mg/L)

Nanofitins[1,2], 7kDa alternatives to antibodies



Tunable affinity ligands
- 100% *in vitro* selection process in 2 months
- Capture of macromolecules, from peptides to viruses
- With a level of performance comparable to Protein A

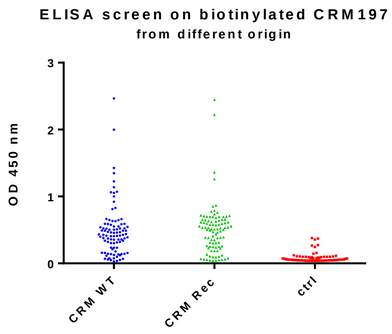
Extremly robust
- Stable to T° (>80°C) and pH (0-12)
- Highly resistant to CIP treatments
- Straightforward and regio-selective conjugation to resins

Affordable custom ligands
- Simple and cost-effective manufacturing by *E. coli* fermentation

Nanofitin Discovery

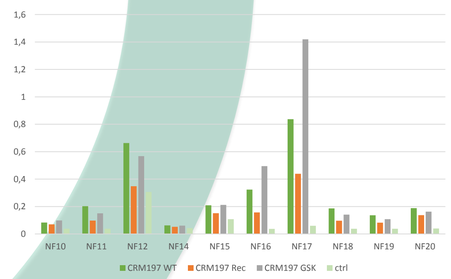
- Selection process in Ribosome Display with Affilogic proprietary libraries:
  - ✓ 4 rounds with increasing washing pressure
  - ✓ Possibility of implementing desired elution parameters to orient selection towards expected specifications
  - ✓ Selection process optimized to reduce discovery time
- Identification of efficient ligands by ELISA screen of clones supernatants

Choice of 12 clones to be characterized based on ELISA signal



Supernatants of selected Nanofitins were tested in ELISA, clones were ranked according to their binding signal and signal to noise ratio

Nanofitins Characterization



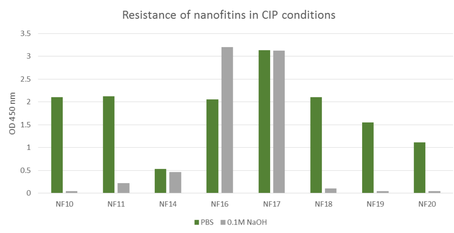
CRM197 targets were coated on ELISA plates and binding of Nanofitins (1 µM) was revealed by anti-RGS HRP antibody

Affinity
- Binding on target from various sources was evaluated on purified clones by ELISA
- Binding kinetics were measured by bio-layer interferometry (OctetRed96)

Chosen Nanofitins present different binding profiles and affinities ranging from sub micromolar to low nanomolar

Stability
- Expected affinity ligands should be stable enough to resist to regeneration cycle
- Nanofitins were tested in CIP conditions

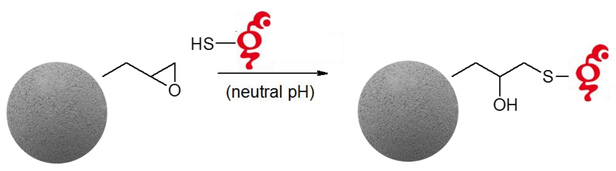
3 of the selected Nanofitins not affected by NaOH treatment



Nanofitins (1 µM) were incubated during 6 hours in NaOH 0.1M or PBS. Affinity for CRM197 was compared by ELISA after treatment.

Resin conjugation

- Conjugation on polymeric chromatography resin
- ✓ Beads inner surface modified with epoxy groups attached to a spacer
- Development of Nanofitins immobilization protocol
- ✓ Nanofitins functionalized with a unique C-terminal cystein
- ✓ Orientation of the Nanofitins on beads
- ✓ Optimization of the buffer coupling conditions



Nanofitins resin conjugation scheme

Affinity, stability and manufacturing yield drive the choice of Nanofitins to be tested as resin ligands

1- Mouratou et al., Proc. Natl. Acad. Sci. USA 2007, 104, 17983–17988
2- Huet et al., PLoS One 2015, 10, e0142304
This work was sponsored by Novartis Vaccines and Diagnostics Srl, now acquired by the GSK group of companies. The authors has declared the following potential conflicts of interest:
Andrea Romagnoli and Mikkel Nissum are employees of the GSK group of companies. Andrea Romagnoli and Mikkel Nissum are listed as inventors on patents owned by the GSK group of companies

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRANT AGREEMENT Nº 635770