DiViNe
Vaccines purification

Affinity chromatography for vaccines purification: EU DiViNe project provides proof of concept

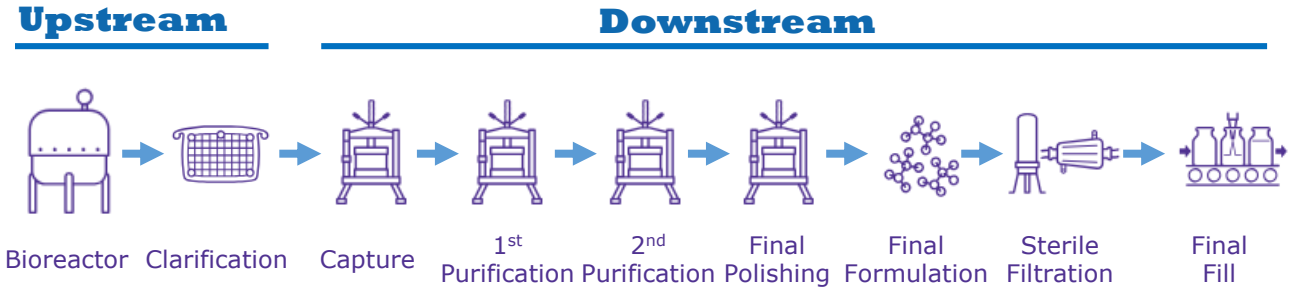
Mikkel Nissum, GSK, Siena, Italy

The DiViNe project has received funding from the European Commission's Horizon 2020 research and innovation programme under Grant Agreement n°635770.

Disclaimer – Conflict of Interest

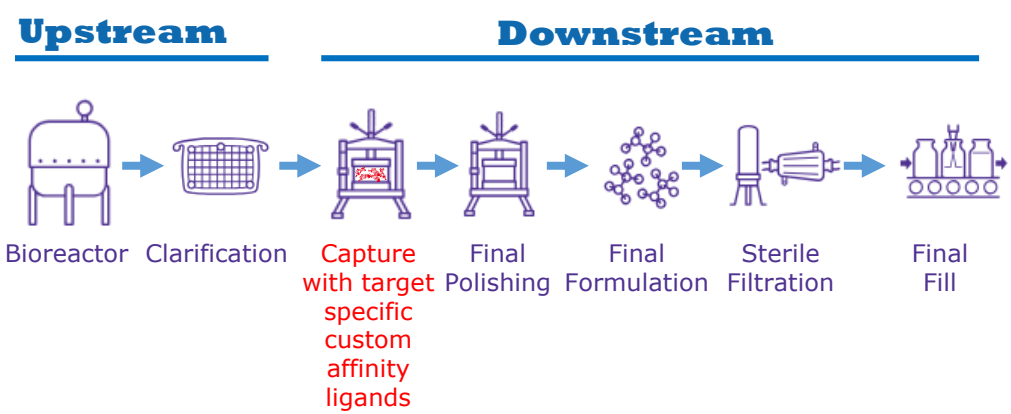
- The work described here was sponsored by GlaxoSmithKline Biologicals SA which was involved in all stages of the study conduct and analysis.
- Mikkel Nissum is an employee of the GSK group of companies.

Current Challenges in Vaccine Downstream Purification



Non-templated processes leading to **long timelines** for Process Development

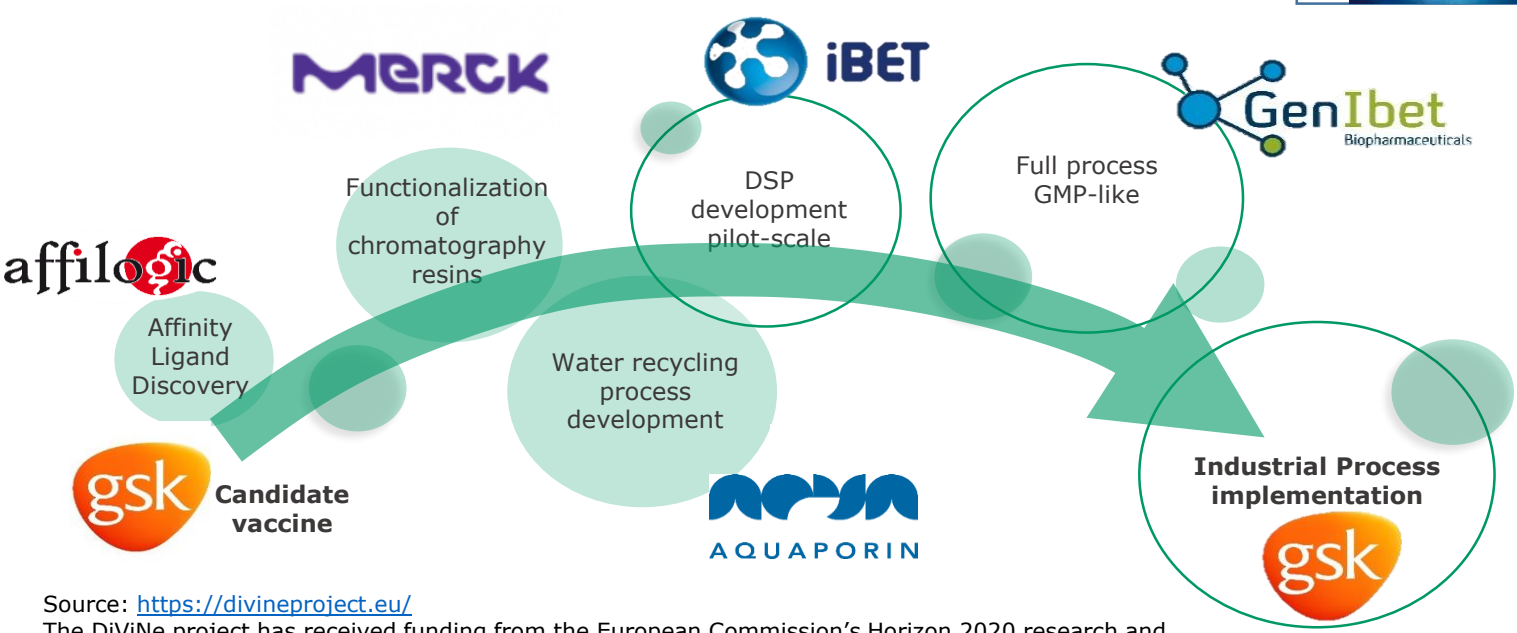
Often have **complicated** purification schemes leading to **high COGS** and **low yields**



Potential to establish a purification **template** with **target specific custom affinity ligands**

Simplifying downstream purification schemes leading to **reduced COGS** and **improved yields**

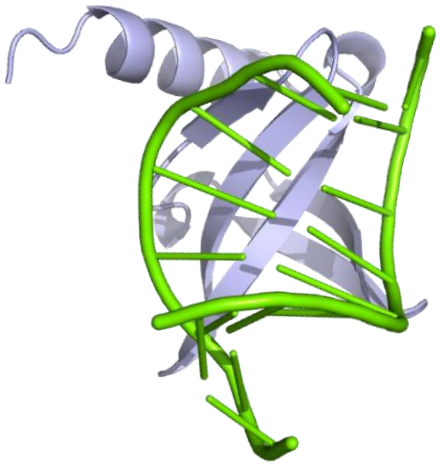
- **The DiViNe consortium is an EU funded project focused on improvements to vaccine purification processes**



Source: https://divineproject.eu/
The DiViNe project has received funding from the European Commission's Horizon 2020 research and innovation programme under Grant Agreement n°635770.

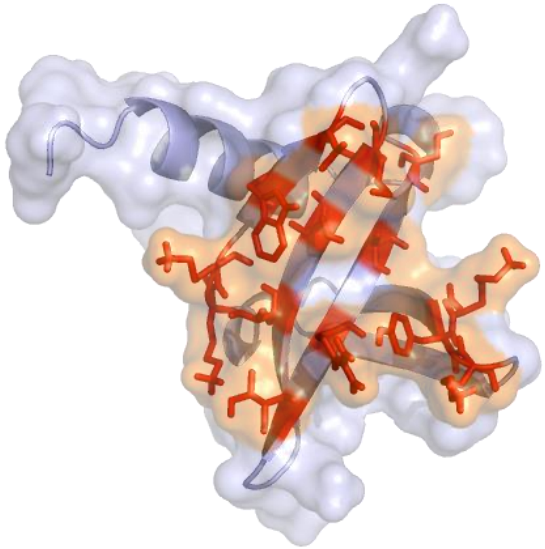
Nanofitins

Nanofitins derive from Sac7d family found in *Sulfolobus acidocaldarius*



Robinson *et al.*, Nature (1998)

Random mutations in binding site



Extremely stable to heat and acid (85°C and pH 2)

Specific, high affinity binders with conserved original features

DiViNe Vaccines purification

Success Criteria: Purification Performance

Criterion	Evaluation	Status
Specificity	NFs demonstrated to be highly specific towards target	✓✓✓
Capacity	High dynamic binding capacity achieved	✓✓✓
Ligand stability	NFs resistant to extreme pH (0-13), 1 M NaOH	✓✓✓
Mild elution conditions	Elution by change in pH and/or conductivity confirmed	✓✓✓
Engineerability	NF binding pocket is engineerable to improve performance (Hydrophobicity, KD)	✓✓✓
Clearance performance	Clearance demonstrated for DNA, HCP, Bioburden, Endotoxins, IPTG	✓✓[1]
Cleaning verification	CIP conditions allow column regeneration	✓✓✓
Ligand leakage	No leakage of NF detected	✓✓[2]
Process duration	Reduction in number of chromatographic steps 3→1	✓✓✓

[1]Endotoxin testing ongoing [2]Below sensitivity with current methods

Criteria adapted from Zhao et. al, Vaccine (2018)

DiViNe Vaccines purification

Success Criteria: Ligand Screening/Development/Production

Criterion	Evaluation	Status
Time required	From target to column: 8 months → aiming at 4 months in 2020	✓✓[1]
Quantity/Purity of target required	Max 1 mg of target, at least 80% purity	✓
Platformability	NF selection, NF engineering (if required), Immobilization, scale-up platformable	✓✓✓
Supply	High expression yields of homogenous NF in *E. coli*	✓✓✓
Column re-use	Cost-effective replacement frequency	✓✓[2]
Cost effectiveness	Biosolve simulations demonstrate cost effectiveness compared to conventional process	✓✓✓
Scalability	Scalability of NF and resin production demonstrated	✓✓✓
Quality requirements	NF affinity resin will be certified animal-free and antibiotics-free (Protein A resin standards to be followed)	✓✓[3]
Carrier selection	NF compatible with resins, membranes, beads	✓✓[4]

[1]End-to-end process optimization ongoing
[2]Expected further improvement in re-use as technology matures
[3]Resin certification not yet performed
[4]Studies with membrane coupling ongoing

Criteria adapted from Zhao et. al, Vaccine (2018)

Nanofitin Affinity Platform: From Target to Small Scale

Target → Nanofitin ligand → Small scale column → Pilot scale → Commercial scale

NF screening

- NF family
- Affinity (5-200 nM)
- Expression yield (> 500 mg/L)
- Stability in NaOH
- Immunogenicity prediction
- No Cys in binding pocket

Purification performance

NF engineering (if needed)

Coupling performance

- NF concentration stability
- Na_2SO_4 behavior
- Chemistry Cys/Lys

- pH screening
- Glycine buffer
- Conductivity

Elution conditions

NF selection

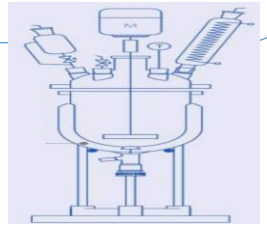
Nanofitin Affinity Platform: From Small to Commercial Scale

Target → Nanofitin ligand → Small scale column → Pilot scale → Commercial scale

- **Scope:**
- 1-5 mL scale
- Wide pH and conductivity window
- Reaction time (e.g. 4h)
- Temperature

Small scale

- **Scope:**
- 100-1000 mL scale
- Inert conditions
- Manufacturing protocol
- Product characteristics

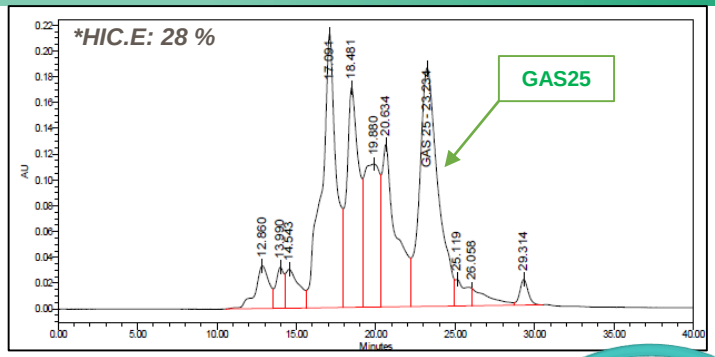


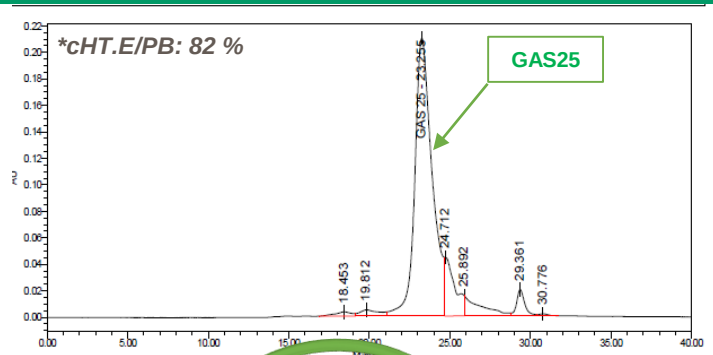
Pilot scale

- **Scope**
- 20-40L scale
- QC
- To be tested

Commercial scale

Case Study I: GAS25 → Current Process 3 Chromatographic Steps





HIC.E

•Hydrophobic chromatography

cHT.E

•Mixed mode chromatography

•Lysis
•Flocculation
•Centrifugation

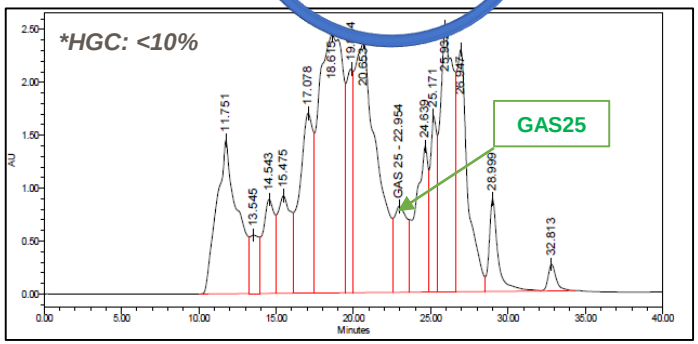
HGC

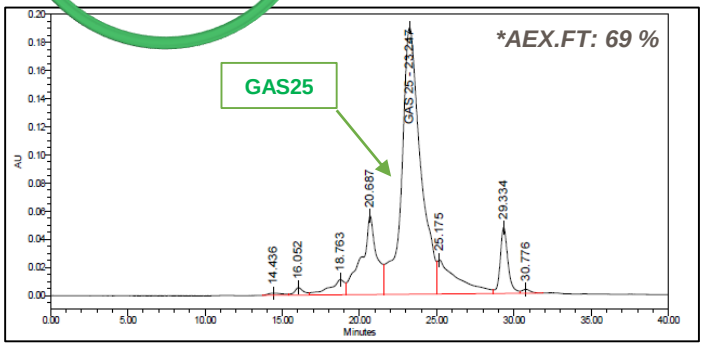
•Anionic exchange chromatography

AEX.FT

•UF/DF 30 kDa

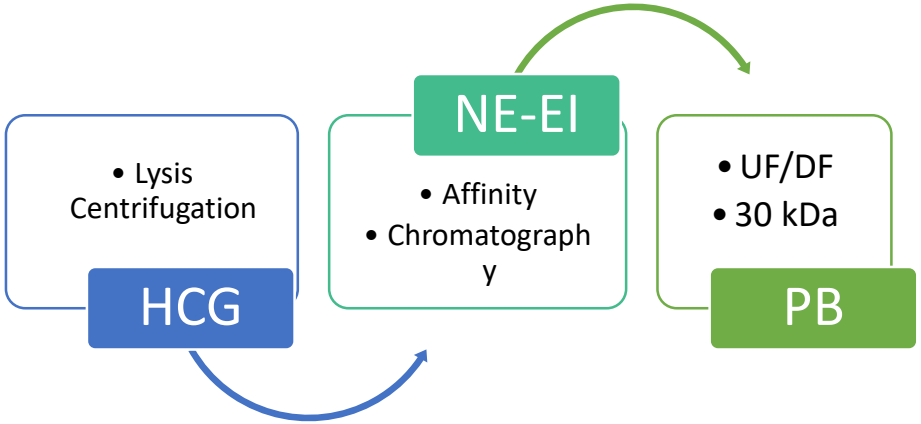
PB





*Purity by SEC

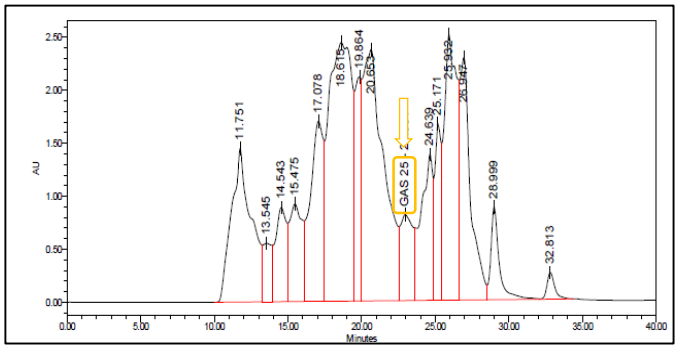
Case Study I: Affinity Purification of GAS25 3→1 Chromatographic Steps



Attributes	NF process*	Standard process
Purity RPC [%]	94	90
Integrity SEC [%]	91	84
Purity SDS-Page [%]	94	84
HCP-WB	Negative	Negative
DNA reduction [log]	4.8	2.5
DNA/protein ratio [ppm]	47	29
Bioburden (plates)	Negative	Negative
Process yield	60%	38%

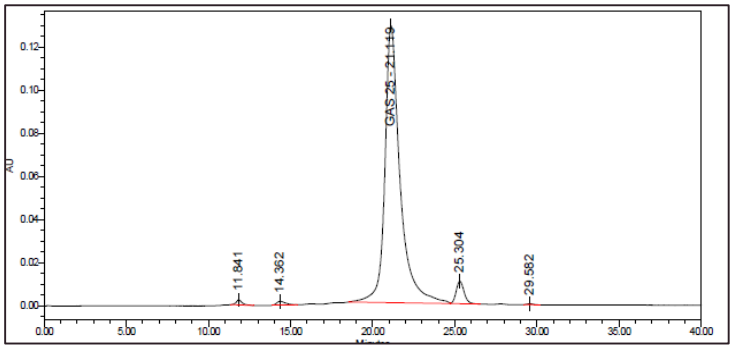
Dynamic binding capacity: 15 mg/mL resin

Feed: HGC



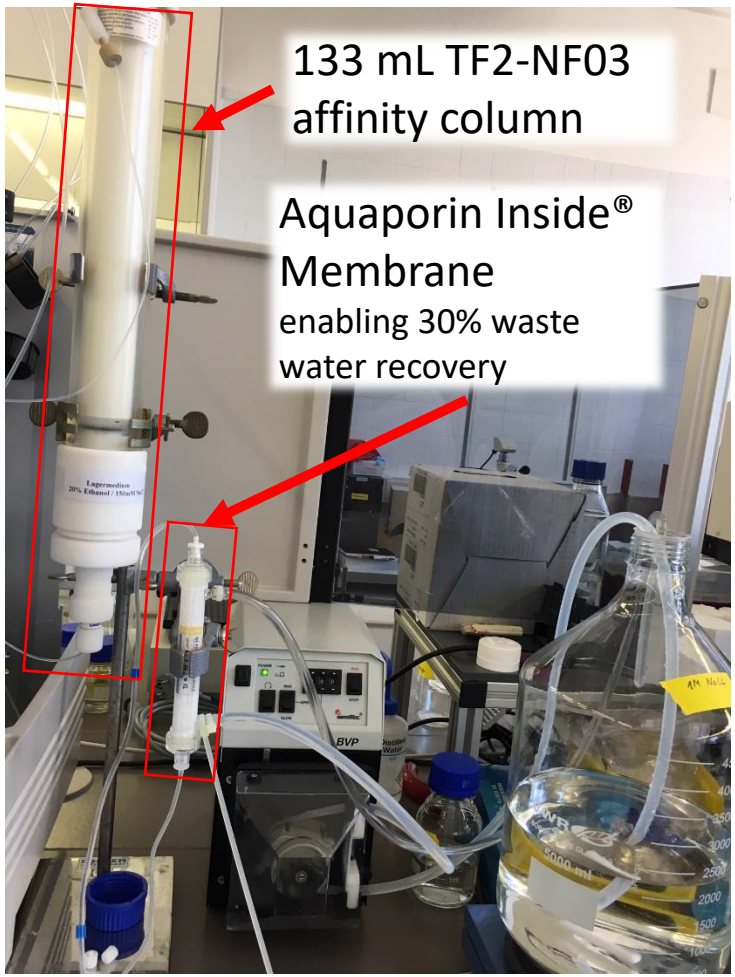
NF-GAS25

Eluted

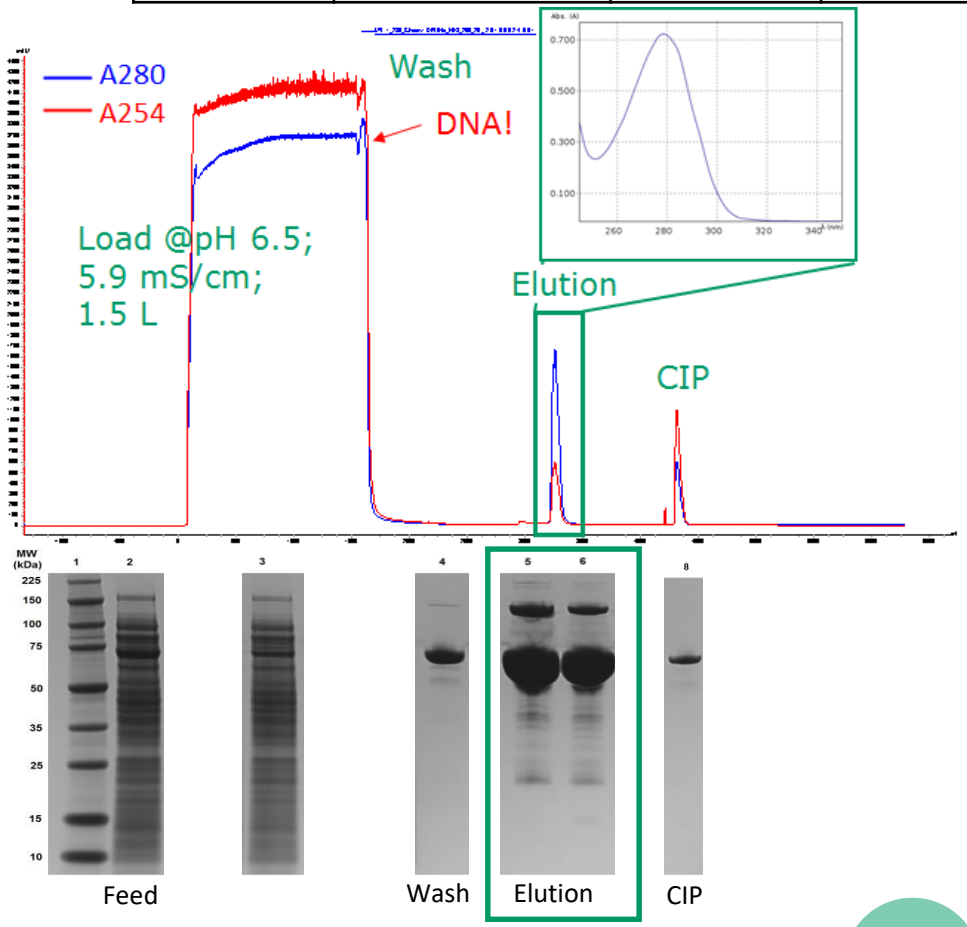


SE-HPLC data

Case Study I: Scale Up to 133 mL Column Including Aquaporin Membrane

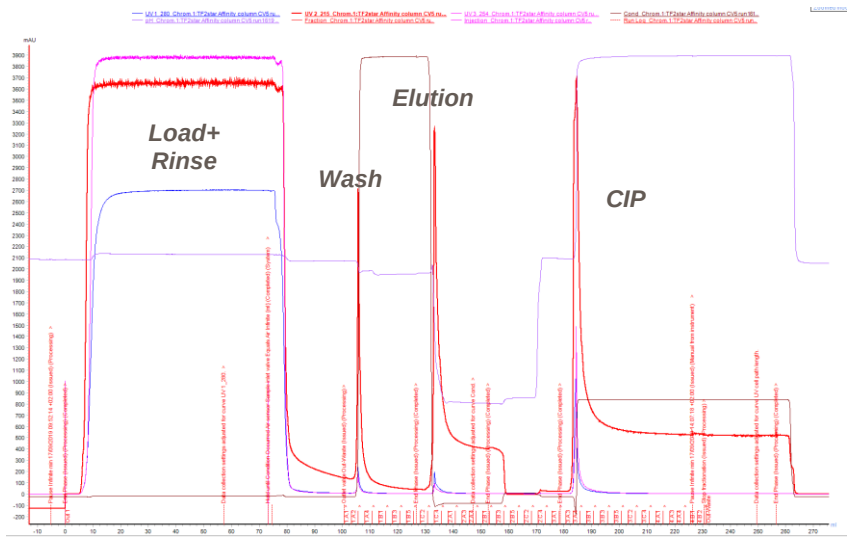


Equilibration	Wash	Elution	CIP
0.5x PBS pH 6.0	0.5x PBS + 1 M NaCl pH 6.0	0.1 M Glycin pH 4.0	0.1 M NaOH



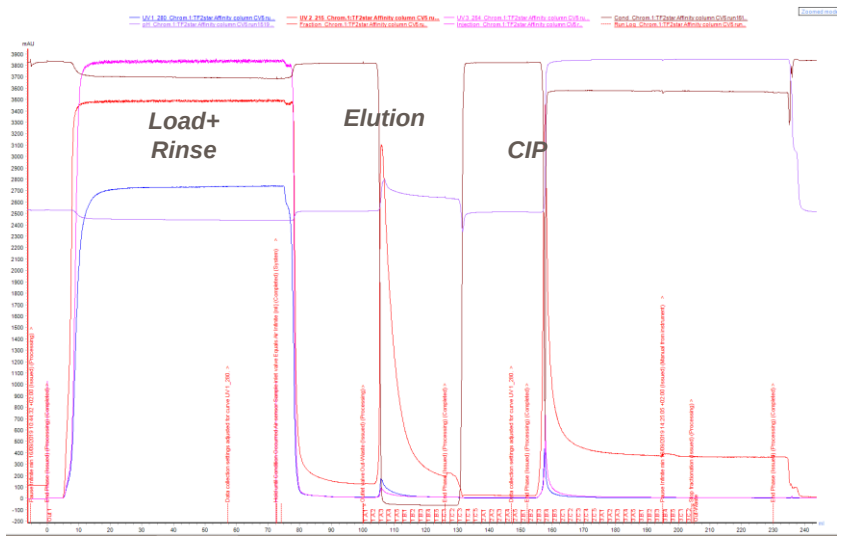
Case Study II: TF2*→ Focus on Elution Conditions

Elution changing pH



- Binding: pH 7.4
- Elution: pH 3

Elution decreasing conductivity



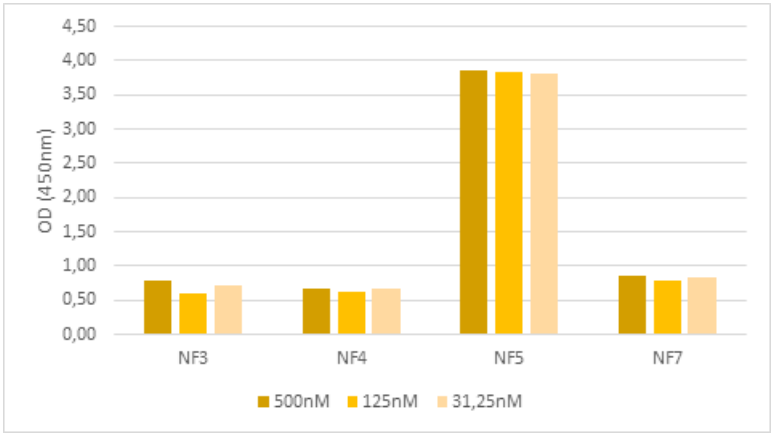
- Binding: 50 mM Tris 150 mM NaCl
- Elution: 50 mM Tris

Load: 60 mg total protein/ml resin

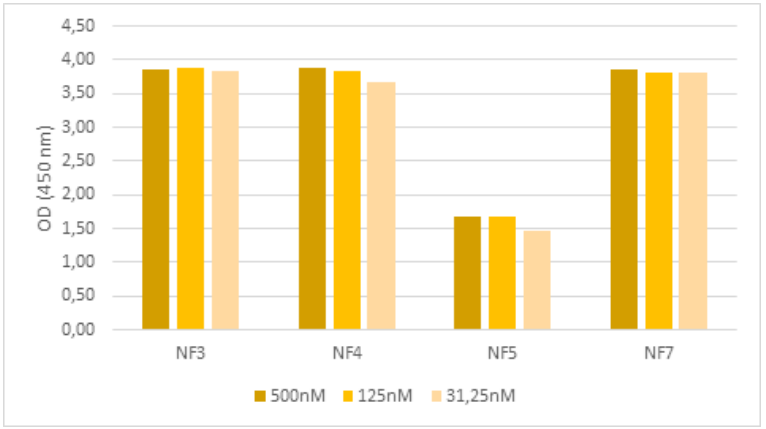
Case Study II: TF2*→ NF Selection based on Sequence Family Repartition

TF2* single domain analysis by ELISA assay

TF2* Domain 2



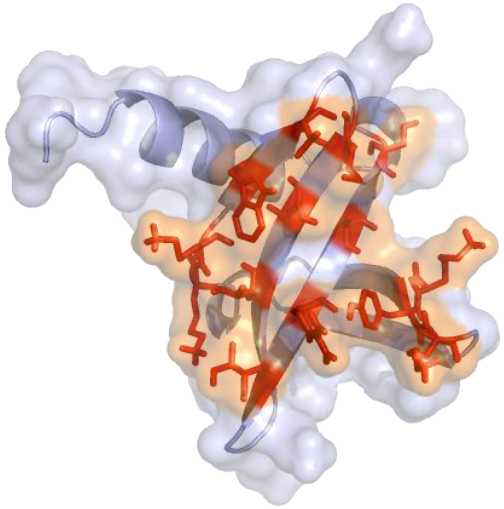
TF2* Domain 3



- ✓NF-03 and NF-04 and NF-07 show higher affinity with **TF2* Domain 3**
- ✓NF-05 shows higher affinity with **TF2* Domain 2**
- ✓Nanofitins from different families recognize different epitope

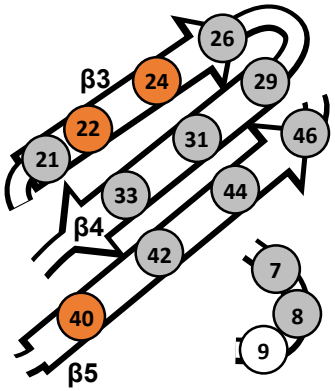
Case Study II: TF2*→ Focus on Ligand Improvement

From active custom binder to improved ligand



Molecular Engineering

Binding site modification
Identification of residues not involved in interaction that help solubility
Screening of variants



Ligand for TF2*

- Affinity ✓✓✓
- Specificity ✓✓✓
- Solubility ✓
- Resin conjugation ✓
- Active in column ✓✓✓

Improved Ligand for TF2*

- Affinity ✓✓✓
- Specificity ✓✓✓
- Solubility ✓✓✓
- Resin conjugation ✓✓✓
- Active in column ✓✓✓

Conserved affinity and specificity

Solubility increased more than 10 times

Equivalent activity on resin

Acknowledgements

- Manuel Carrondo
- Ricardo Silva
- Hugo Soares
- Raquel Fortunato
- Olivier Kitten
- Anne Chevrel
- Nadège Prel
- Achim Schwaemmle
- Jonas Anders
- Romas Skudas
- Maria Salud Camilleri Rumbau
- Joerg Vogel
- Elisa Innocenti