



The **Affinity** Experts

## Use of design of experiments in process robustness studies for Ion Exchange Chromatography

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NBV werkgroependag, 16 april 2009



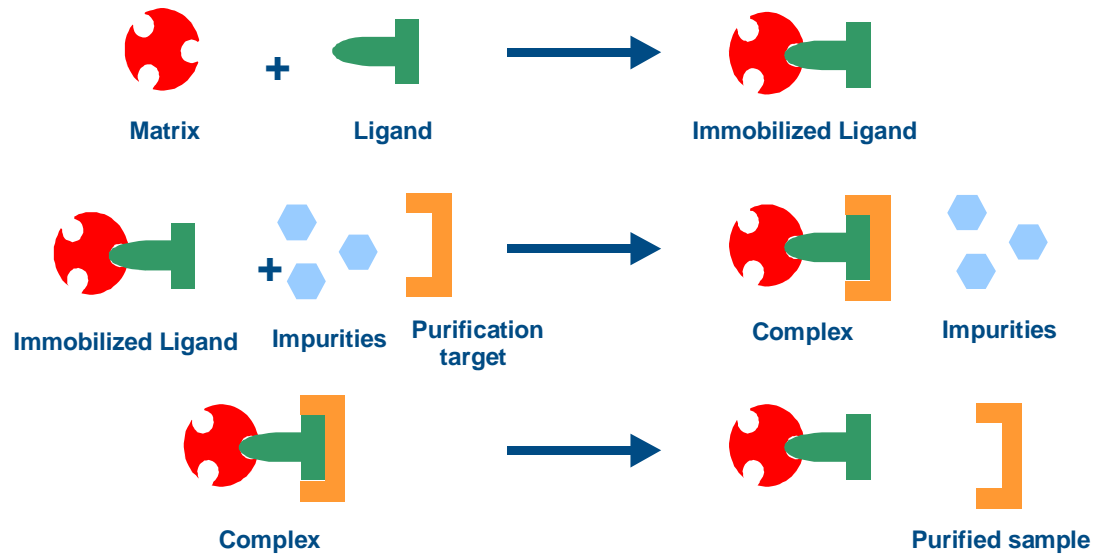
## Affinity products for purification of biopharmaceuticals: CaptureSelect®

- ⌚ 1995 – BAC BV started as subsidiary of Unilever
  - ⌚ 1999 – PoP for use of single domain antibody technology in separation
  - ⌚ 2002 – BAC's spin-off facilitated by Unilever Ventures
  - ⌚ 2005 – Private financing round completed
  - ⌚ 2008 – Secured additional investment for R&D financing
- 
- ⌚ Based in Naarden, NL
    - 37 FTE
    - Dedicated R&D (Leiden, NL)
    - Microbial Biotech manufacturing site (Naarden, NL)

# Affinity Separation: The Opportunity

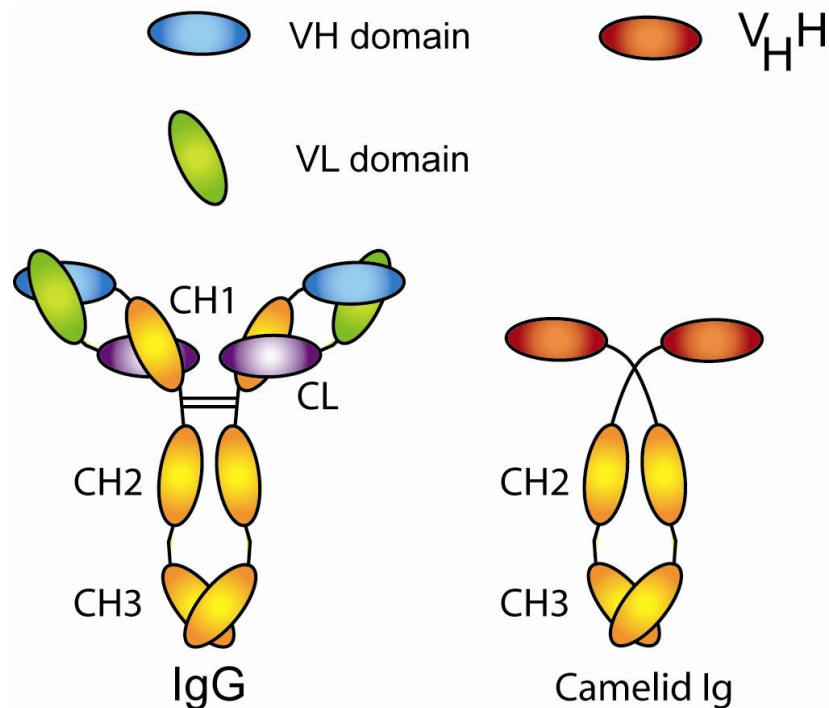


- Biopharmaceuticals a high growth area of therapeutics
  - Production in complex media results in many impurities



- Affinity separation is an excellent protein purification technology
  - Eliminates steps; faster, cheaper, higher yield, high purity

## CaptureSelect® Affinity Ligands use the Uniqueness of VHH antibody fragments



### Advantages:

- Specificity - Broad / Narrow
- Stability - Cleaning Agents
- Screening - Operating Conditions
- Non-Animal Derived
- Quick Discovery and Scale Up

- Custom ligand design
  - A unique platform for the development of ligands for biotherapeutic purification
- CaptureSelect for life science research
  - Providing new specificities and formats for bench-scale purification, proteomics and analysis
- CaptureSelect for Bioprocess
  - Addressing the emerging purification needs of biotherapeutics, such as viruses, antibodies and (blood) proteins, with off-the-shelf affinity products



# Product Development and Supply



## Phase-1

Library construction  
Library Screening  
Lead Identification



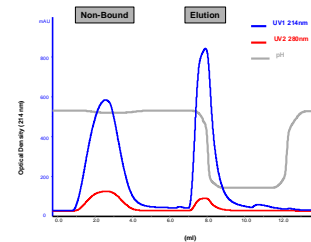
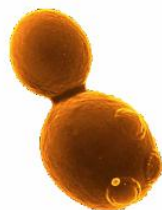
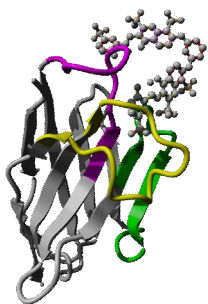
## Phase-2

Product Development

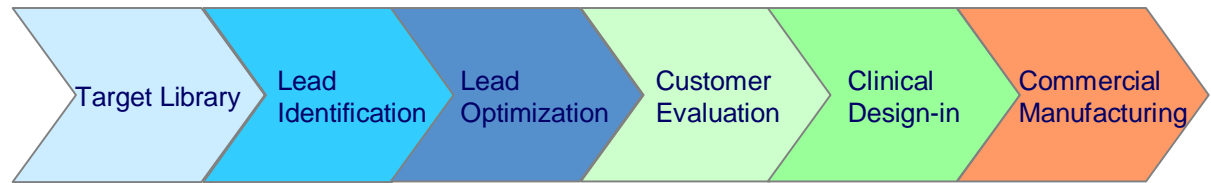


Strategic Partnerships

> 20 Patents and licenses



# BAC's Pipeline



IgG (Fc), AAV, FVIII



AAT, IgA, IgM, IgG4, Fab kappa,  
Fab lambda, ApoA1, Fibrinogen,  
Transferrin, HSA, Multi-IgG,  
Multi-Albumin



Toxins/LPS, Haptoglobin,  $\alpha$  1-acid GP,  
 $\alpha$  -2 Macroglobulin, IgG1



FV, FVII, FIX, FX, FXI, FXII, FXIII,  
FH, Pro-Thrombin,  $\alpha$  -Thrombin III,  
Complement C3/C4/C1q, C1  
inhibitor, HSA-fusion, EPO



ApoA2, hVWF, IFNa-2b, IgG2/3, IgG  
(CH1), scFv ( $V_H/V_L$ ), AACT,  
Ceruloplasmin, Hemopexin,  
Prealbumin (TTR)



# Ligand Manufacturing



## Production

### Fermentation



## Biomass Removal & Concentration

### Microfiltration & Ultrafiltration



## Purification

### Chromatography



- Microbial production of the ligands using Baker's yeast
- ISO9001 but with increased quality level
- Audited by GE and end-customers

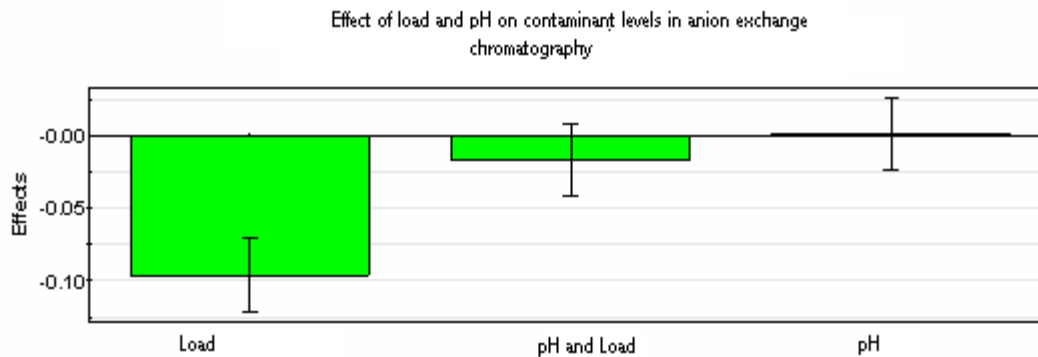
- ~ Fermentation and primary recovery (MF/UF):
  - No product specific development, similar process for all products
  
- ~ Purification (IEX chromatography):
  - Process settings are product specific
  - Definition of critical process parameters
  - Development of optimal process conditions in Anion Exchange Chromatography:
    - Maximal load (dependant on fermentation/primary recovery output)
    - Optimal pH
  - Development of Cationic Exchange Chromatography
    - Optimal pH
    - Optimal load
    - Optimal salt concentrations
  - Worst case testing (critical parameters vs. development result)

- ∞ Definition of critical process input factors and ranges
  - Definition of controllable (pH, load) and uncontrollable factors (starting material, temperature, etc.)
  - Make input ranges as large as possible (operational boundaries, worst case testing)
  - Definition of input factor interactions
  
- ∞ Specification of process responses
  - Quantitative (purity, yield, contamination levels)
  - Qualitative (chromatographic behavior)
  
- ∞ Method selection, experimental design and worksheet creation
  - Selection of optimal model
  - Experimental design by MODDE software by Umetrics

- ⌚ Execution of experiments and sample analysis
  - Execution of small scale IEX chromatography
  - Analysis of samples
  - Completion of worksheet with quantitative and qualitative data
  - First evaluation of data
  
- ⌚ Use of MODDE software for data processing
  - Regression analysis and model interpretation
  - $R^2$ ,  $Q^2$  (estimation of the predictive power), model validity and reproducibility

## Analysis of responses

- Are responses within product specifications?
- Do any of the varied input factors have any significant effect on the responses?
  - Inside specification/significant model
  - Inside specification/non-significant model
  - Outside specification/significant model
  - Outside specification/non-significant model



# Robustness testing (conclusions)



- ⌚ Inside specification/significant model
  - Process is robust for this response
  - Process optimisation might be considered
  
- ⌚ Inside specification/non-significant model
  - Ideal outcome for robustness testing
  
- ⌚ Outside specifications/significant model
  - Use model to predict appropriate input ranges
  - Adapt process input ranges if possible
  
- ⌚ Outside specifications/non-significant model
  - Process development/worst case testing was not sufficient
  - Redesign process



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