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# Criblage de Supports Chromatographiques à Haut Débit

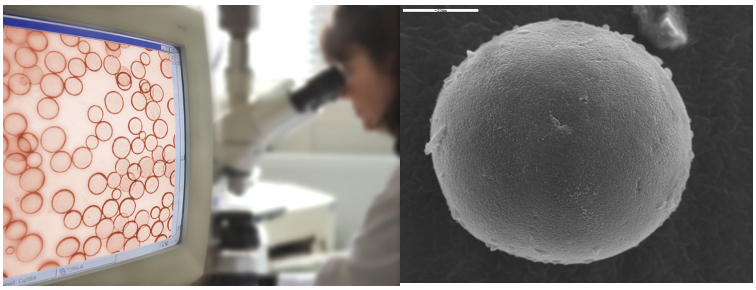
**Innovations-Protéines-Prod**

28-30 Oct 2013

*Dr. Sylvio Bengio, Pall*



## Matrices de base des supports (résines)



*Les supports de chromatographie sont constitués de billes à base d'agarose, d'agarose réticulé, de polymère, de silice, de celluloses, de composites etc… (taille autour de 50-100 µm pour les applications industrielles, <20µm pour l'analytique)*





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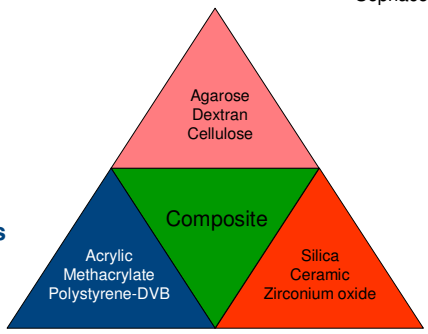
# Supports de base pour la chromatographie

**Polysaccharides**

Sepharose®, Capto™, HyperCel™, Sephacel™ Cellufine™




**Synthetic polymers**

Trisacryl®, UNOsphere™, NuVia™, Eshmuno™ …

Toyopearl® (GigaCap), Fractogel® EMD, Macro-Prep®

Mono®, Source™, Poros®

**Mineral oxides**

Spherodex®, Spherosil®

Ceramic HyperD®

HyperZ®, ZirChrom®



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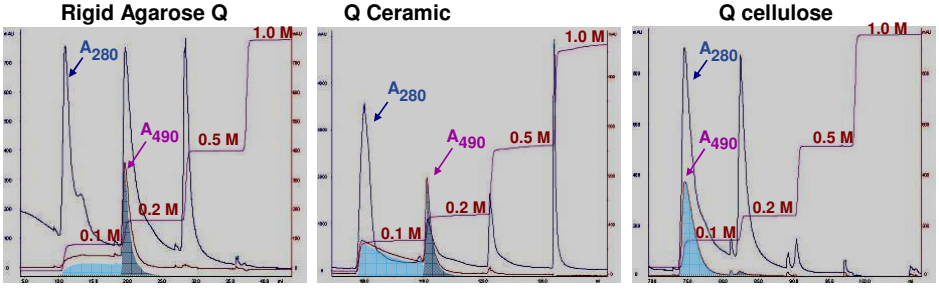
# Impact de la nature chimique du support de base sur la Sélectivité

**Rigid Agarose Q** | **Q Ceramic** | **Q cellulose**




***Purification of rGFP from E.coli lysate on anion exchangers***

50 mM Tris-HCl pH 8.5; step elution at 0.1, 0.2, 0.5 and 1M NaCl; 0.5 cm I.D. x 5 cm at 300 cm/h (1 min RT)

Des différences de comportement chromatographique sont apportées par la nature de la matrice elle-même



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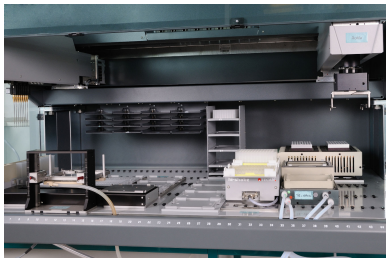
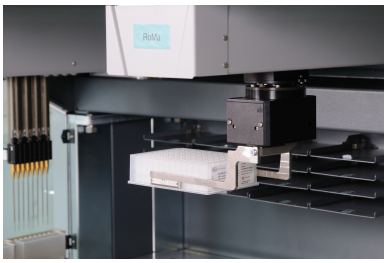
# Introduction de la Robotique





*Janus® BioTx Pro Workstations (PerkinElmer)*



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# Pall ScreenExpert℠ Services

- Service d'aide au développement rapide de procédés de purification
- Différentes supports testés en parallèle: échangeurs d'ions, mixed-mode, affinité,pseudo-affinité, hydroxyapatite, HIC ...
  - Résultats en 2-3 jours grâce à des méthodes analytiques puissantes

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# 2013: Pall ScreenExpert℠ Services Laboratory Cergy (Paris), France






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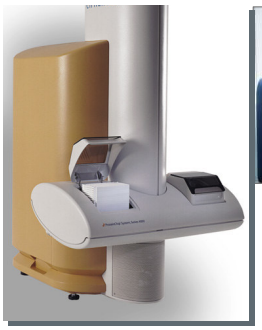
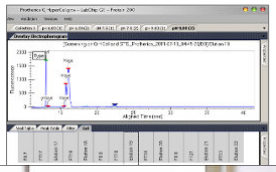
# Les Méthodes d'Analyse à Haut-Débit



SELDI-Mass Spectrometry

Automated microfluidic electrophoresis (LabChip®)



BLI (Bio Layer Interferometry)



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## Mise au point d'un procédé de puriification d'un fragment Fab d'anticorps

- Objectif: mettre au point un process de purification de Fab exprimé dans *Pichia pastoris*
- Transposable à l'échelle industrielle
- Procédé simple, avec supports robustes
- Eviter les ligands d'affinité





## Screening Strategy

**Plan Design**
Select critical parameters (load, wash, elution conditions)

**Sorbent Screening**
AcroPrep ScreenExpert Plates

**High Throughput Analysis**
SELDI-MS, LabChip® GXII electrophoresis, BLI, HPLC…

**Transfer to Prepacked Columns**
Evaluate dynamic binding capacity, optimize residence time

# Criblage de l'étape de Capture du Fab

- **176 conditions sur 9 supports** différents testés en mode « bind/elute » comme candidats potentiels pour la capture du Fab
- Echange d'anions et and cations, Hydroxyapatite, Mixed-Mode, autres
- Resultat : CIEX (CM) et Mixed-mode fonctionnent
  - MEP HyperCel charge à pH 6.0 ; Fab elution à pH 5.0
  - Ou CM Ceramic HyperD, pH 5.0, elution Fab 0.15 M NaCl
  - Le support CM sera retenu en raison d'une plus forte capacité dynamique (> 60 mg/mL)



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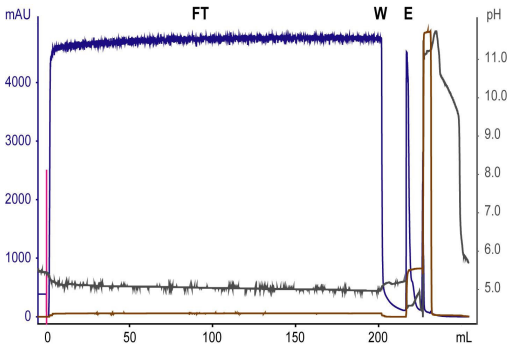
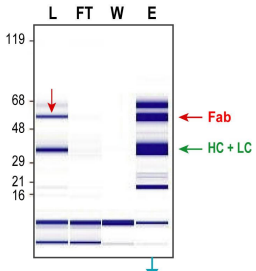
# Capture step by Cation exchange:
## CM Ceramic HyperD® 1 mL PRC Column




**Capture step performance**
- Fab recovery = 96%
- Concentration = 20 times
- Purity = 40%

Equilibration: 50 mM Na acetate pH 5.0
Loading: 200 mL feedstock 1/2 at pH 5.0
Washing: 50 mM Na acetate pH 5.0
Elution: 50 mM Na acetate pH 5.0 + 0.3M NaCl
Flow rate: 150 cm/h – Residence Time = 3 min



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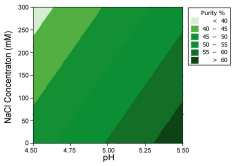
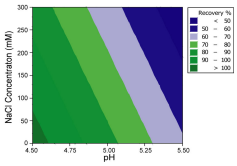
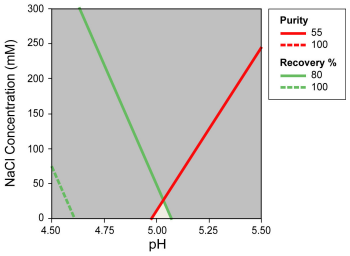
# Intermediate step: Screening on AcroPrep 96 plates (Mixed Mode on MEP HyperCel)

*Fab purity*




*Fab recovery*




Screening of 3 pH (pH 4.5, 5.0, 5.5) and
3 NaCl conditions (0 mM , 150 mM, 300 mM)

Optimal conditions on MEP HyperCel (FT mode):

Binding at pH 5.0 to 5.2, without addition of NaCl



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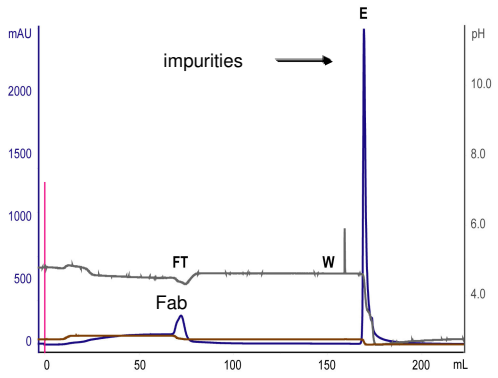
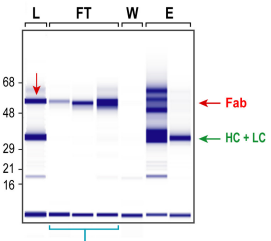
# Etape intermediaire : Mixed Mode
## Transfert sur colonne MEP HyperCel™ 10 mL




**Intermediate step (negative mode)**
- Fab recovery in FT = 74%
- Purity = 99%

Equilibration: 50 mM Na acetate pH 5.2
Loading: 100 mL feedstock 1/10 at pH 5.2
Washing: 50 mM Na acetate pH 5.2
Elution: 50 mM Na acetate pH 3.0
Flow rate: 150 cm/h – Residence Time = 5 min



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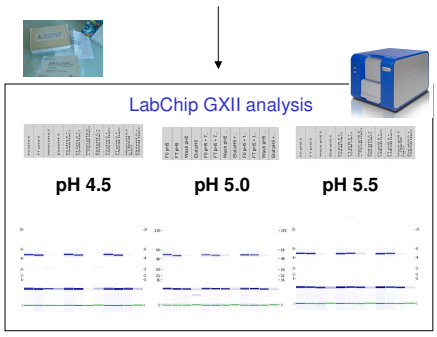
# Fab Polissage (échange anions)
## HyperCel STAR AX

**DoE on AcroPrep ScreenExpert Plates**




| Fractions | Fab recovery in FT % | HCP (ppm) ng/mg Fab |
|---|---|---|
| **Load** | - | **87** |
| **FT pH4,5** | 100 | 13 |
| **FT pH4,5 + 75mM NaCl** | 100 | 27 |
| **FT pH4,5 + 150mM NaCl** | 100 | 19 |
| **FT pH5.0** | 100 | 23 |
| **FT pH5 + 75mM NaCl** | 96 | 32 |
| **FT pH5 + 150mM NaCl** | **100** | **65** |
| **FT pH5,5** | 100 | 51 |
| **FT pH5,5 + 75mM NaCl** | **100** | **44** |
| **FT pH5,5 + 150mM NaCl** | 100 | 90 |

Conditions for acceptable HCP removal (< 30 ppm)
Analysis by anti-HCP ELISA



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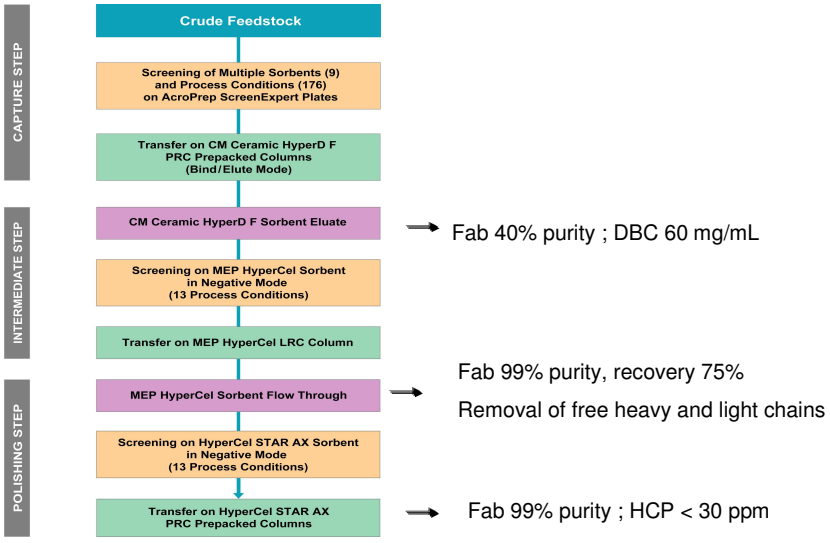
# Résumé du Process



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# Conclusions

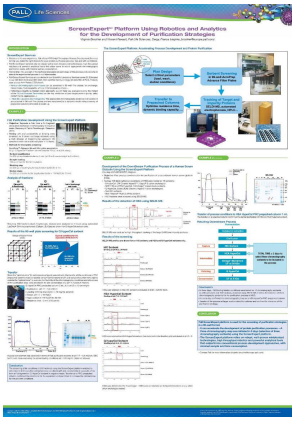
1. Le fragment Fab peut êre purifié en trois étapes simples et transposables à la production (pas de ligand d'affinité)
2. Le criblage en plaques 96 puits accélère considérablement la sélection de supports (176 conditions en 2 jours)
3. Les paramètres optimisés doivent toujours être confirmés sur colonne (capacité dynamique)





# Publications


- [1] High throughput screening of mixed-mode sorbents and optimization using pre-packed lab-scale columns for the purification of the recombinant allergen rBet v 1a. V Brenac Brochier *et al.*, **J. Chromatogr. B (2009).**
- [2] Rapid screening of purification strategies for the capture of a human recombinant F(ab')2 expressed in baculovirus-infected cells using a micro-plate approach and SELDI-MS. J Pezzini *et al.*, **J. Chromatogr. B (2009).**
- [3] Expression optimization and purification process development of an engineered soluble recombinant mouse linker of activation of T cells using SELDI-MS. V Brenac *et al.*, **Prot. Express. and Purif. (2006).**

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