Multimodal Chromatography Media

多模式层析填料

Properties and applications特点和应用

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Content内容

Introduction介绍

How to work with multimodal media如何使用多模式填料

- Capto™ adhere
- Capto MMC

Multimodal libraries and screening formats

多模式填料家族及其优化模式

Conclusions结论

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Introduction

介绍

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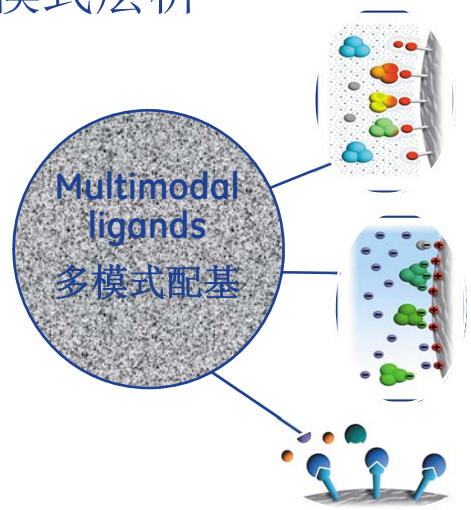
Multimodal chromatography
多模式层析



HIC/RPC (Hydrophobicity)
Phenyl Sepharose™ HP
SOURCE™ 15RPC etc

Ion exchange (Charge)
Q Sepharose Fast Flow
SP Sepharose Fast Flow etc

Affinity (Biorecognition)
MabSelect™
Blue Sepharose etc

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Why multimodal chromatography?
为什么选择多模式层析？

Existing and traditional media "works fine" – in most cases !
目前现有填料和传统填料已经够用-大多数情况！

User needs ?
客户需求

Are there any unsolved purificaton challenges ?
未解决的纯化挑战？

- High salt binding IEX – No need for costly dilution

高盐条件结合的IEX填料-不需要成本昂贵的稀释

- Tailored scavenger for HCP, protein A, ...

除宿主蛋白和Protein A量身定制的填料

- Unique selectivity towards specific targets独一无二的选择性
- Etc ...等等

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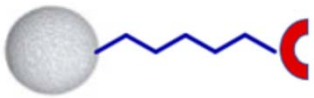
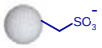
Why multimodal chromatography?
为什么选择多模式层析？

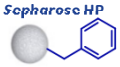
Traditional anion exchange chromatography
传统离子交换层析



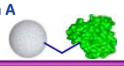
- SP Sepharose™ Fast Flow



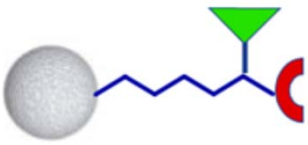
- Phenyl *Sepharose* HP



- Protein A



Multimodal anion exchange chromatography
多模式离子交换层析



Introduction of new interactions on a media with already one primary interaction allow the rapid generation of a library of new media in order to....

在填料已经存在的主要结合作用上加入新结合模式，产生的一系列新填料是为了....

Modulate调节上样条件
Enhance, discriminate增强分辨率
Improved performance提高性能
Generate new selectivities产生新的选择性

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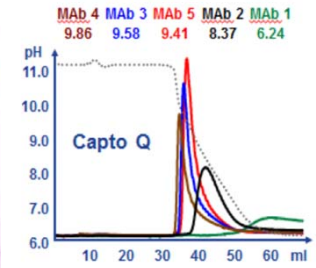
Effect on elution pH洗脱pH的影响

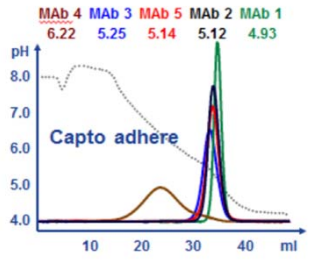
pH gradient elution of 5 MAbs on Capto™ adhere and Capto Q
Capto adhere 和Capto Q纯化5种单克隆抗体时进行pH梯度洗脱的不同表现

Traditional anion exchange chromatography
传统离子交换



→ Higher elution pH

Multimodal anion exchange chromatography
多模式离子交换



→ Lower elution pH

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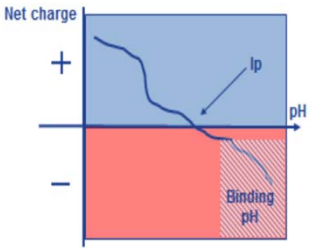
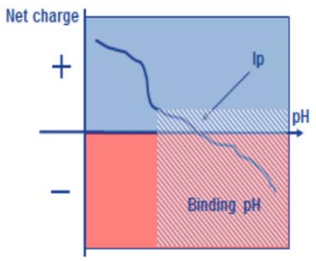
Isoelectric point vs loading pH
等电点 VS上样pH

Traditional anion exchange chromatography



Multimodal anion exchange chromatography
多模式离子交换



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How to work with multimodal media:
Capto™ adhere

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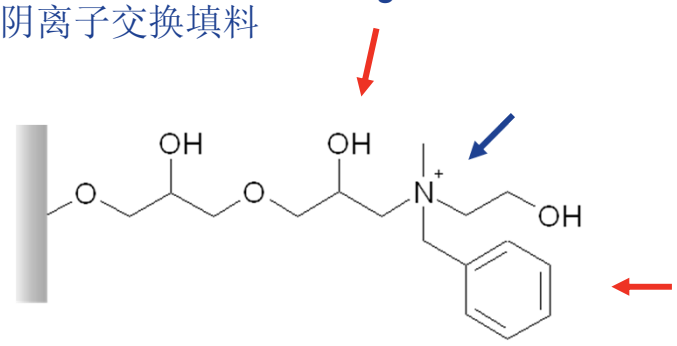
Capto™ adhere

Strong multimodal anion exchanger
强多模式阴离子交换填料



N-Benzyl-N-methylethanolamine

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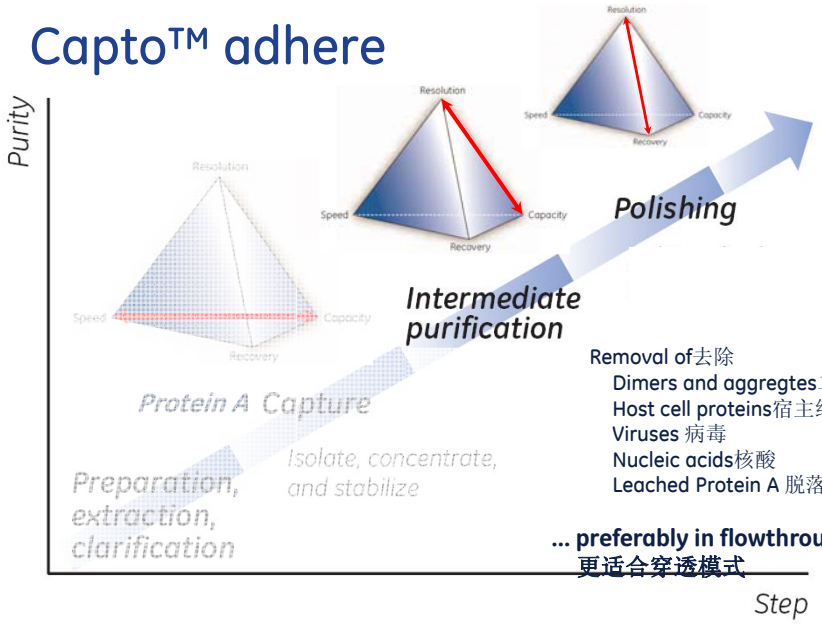
Capto™ adhere



Removal of去除
- Dimers and aggregtes二聚体和聚体
- Host cell proteins宿主细胞蛋白
- Viruses 病毒
- Nucleic acids核酸
- Leached Protein A 脱落蛋白A

... preferably in flowthrough mode
更适合穿透模式

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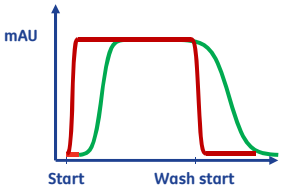
Optimization of loading conditions
优化上样条件

Antibody yield抗体收率
&
Contaminant clearance杂质去除

Yield收率 ⇔ Purity纯度

Optimization优化

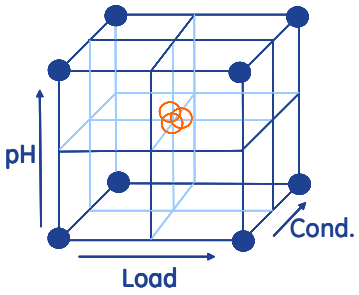


Non-binding不结合
Partial binding部分结合

Design of Experiment实验设计 (DoE)

Factors (inputs)
因子（输入变量）
Load (x_1), pH (x_2), Conductivity (x_3)

Responses (outputs)
响应（输出变量）
Yield (Y_1), Dimer/aggregates (Y_2)
HCP (Y_3), Protein A (Y_4)



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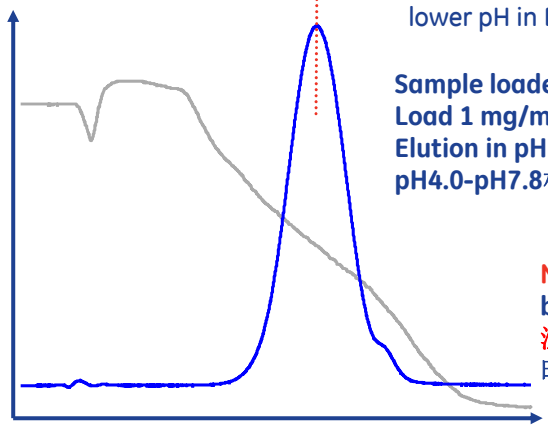
Optimization of loading conditions
优化上样条件

Lower pH limit in the design设计过程中考虑较低pH下限

pH 5.95 ➔



lower pH in DoE: 6.0

Sample loaded at pH 7.8 上样pH为7.8
Load 1 mg/ml 上样浓度 1mg/ml
Elution in pH gradient from 7.8 to 4.0
pH4.0-pH7.8梯度洗脱

Note! A basic antibody, but it elutes at pH 6
注意：碱性抗体，在pH6时被洗脱

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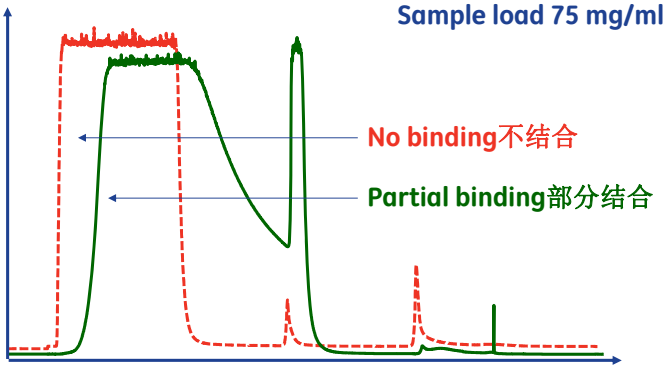
Optimization of loading conditions
优化上样条件
Upper pH limit in the design设计中pH上限



pH 6.0, 2 mS/cm ➔ **Lower pH limit in DoE, 6.0**
pH 8.0, 2 mS/cm ➔ **Upper pH limit in DoE, 8.0**

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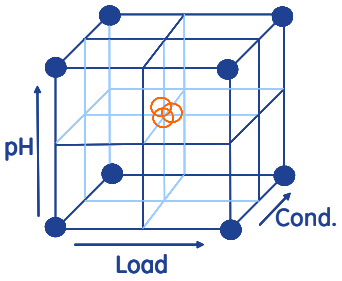
Design of Experiment实验设计

Factor settings:
Load 75 - 187 - 300 mg/ml
pH 6 - 7 - 8
Conductivity 2 - 8.5 - 15 mS/cm

Goals:

Yield	> 90%
D/A	≤ 1%
Pr A	≤ 5 ppm
HCP	≤ 50 ppm



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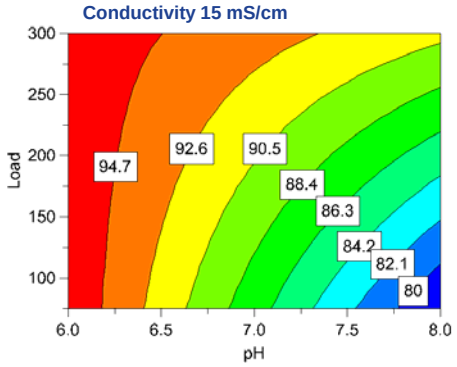
Yield goal > 90%、
目标收率>90%



pH ⇩

Load ⇧

Cond ⇧

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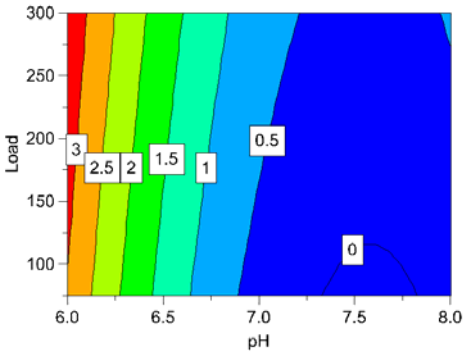
Dimer/aggregate clearance goal ≤ 1%
二聚体/多聚体<1%



pH ⇧

Load ⇧

Cond Not significant

D/A conc in start material: 3.2%

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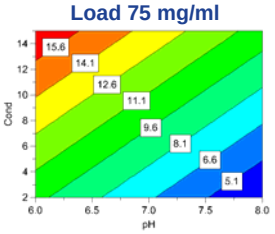
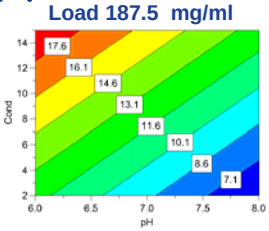
HCP clearance goal ≤ 50ppm
Protein A <5ppm

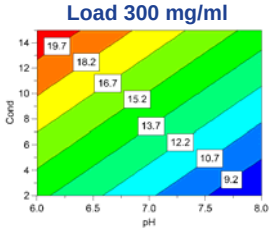
Load 75 mg/ml



Load 187.5 mg/ml



Load 300 mg/ml



HCP conc in start material: 206 ppm

pH ⇧

Load ⇩

Cond ⇩

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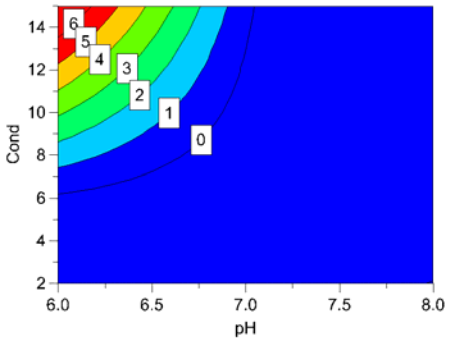
Protein A clearance goal ≤ 5 ppm
宿主蛋白<50ppm



pH ⇧

Load Not significant

Cond ⇩

Protein A conc in start material: 36 ppm

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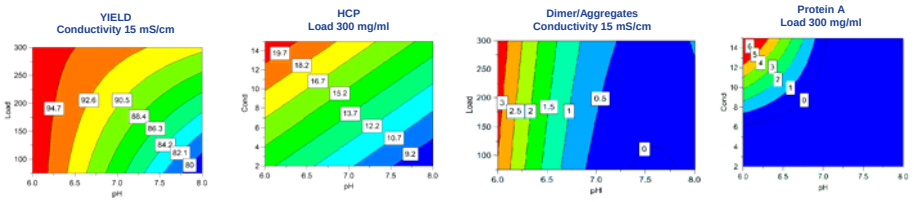
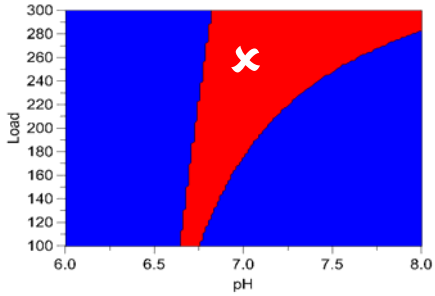
Results – sweet spot analysis结果分析





Sweet spot analysis gives the area (red) where the goals are fulfilled.

分析结果表明，在红色区域内操作，回收率、宿主蛋白残留、二聚体和多聚体、Protein A残留均达到要求

General trends on Capto™ adhere

Purifying MAbscapto adhere

纯化单克隆抗体的一般趋势

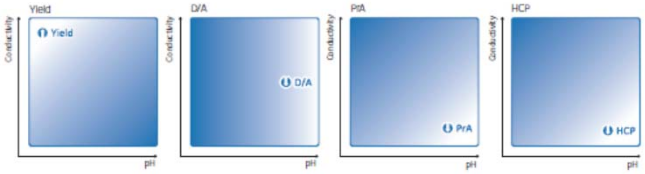


Fig 13. General trends with respect to loading conditions for yield, dimers and aggregates, and Protein A and HCP clearance.

Table 2. Optimal loading conditions for different MAbs with regard to yield and clearance of HCP, Protein A, and dimers and aggregates

MAb	pI	pH	Conductivity (mS/cm)	Yield %	D/A %	Protein A ppm	HCP ppm
1	~ 9	7	8	90	0.5	n.q.	< 15
2	8.3 to 8.9	5.5	3	95	0.6	n.q.	2
3	7.5 to 8.4	6	2	95	0.8	n.q.	9
4	7.7 to 8.0	7	20	91	0.2	n.q.	30
5	6.5 to 9.0	7.5	20	92	< 0.1	n.q.	7.5

Application Note 28-9078-89AA: Optimization of loading conditions on Capto adhere using design of experiments

Virus clearance on Capto™ adhere*
Capto adhere对病毒的去除效果

Virus	Cond (mS/cm)	LRF 95% confidence limit
MVM	10	5.8 ± 0.3
MVM	30	5.9 ± 0.3
MuLV	10	4.5 ± 0.4
MuLV	30	3.6 ± 0.4

Very good log_{10} reduction factors even for conditions where traditional ion exchangers do not work!

对于传统离子交换没有作用的条件有非常好的log_{10}去除效果

* Study performed by NewLab BioQuality AG

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MVM = Minute Virus of Mice
MuLV = Murine Leukemia Virus

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Summary Capto™ adhere小结

- Flowthrough mode is recommended
- 建议应用流穿模式
- Generally, lower pH values should be used than with traditional ion exchangers
- 与传统阴离子交换填料相比，可以使用更低的pH
- The optimal conductivity can vary between applications. Salt tolerance of binding can be expected, but to what extent is protein dependent
- 不同的应用，最佳的上样电导值不同。上样盐浓度可以进行优化，但很大程度上是由蛋白质性质决定的。

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How to work with multimodal media:
Capto™ MMC

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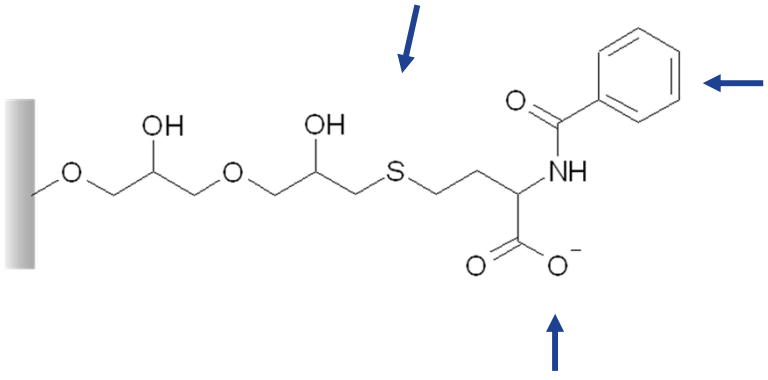
Capto™ MMC
multimodal cation exchanger
多模式阳离子交换层析



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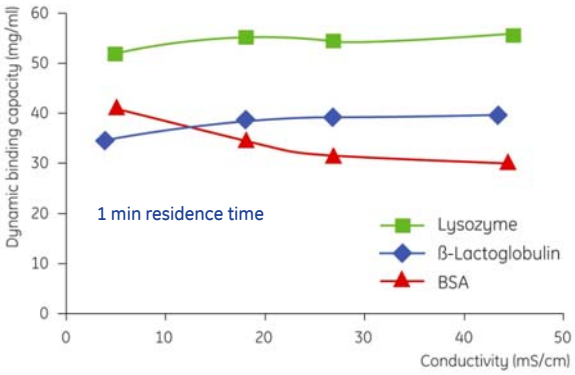
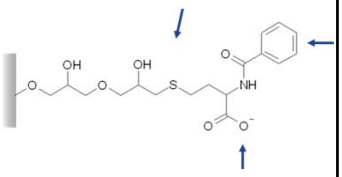
Salt tolerant capacity and selectivity
盐的耐受性和选择性





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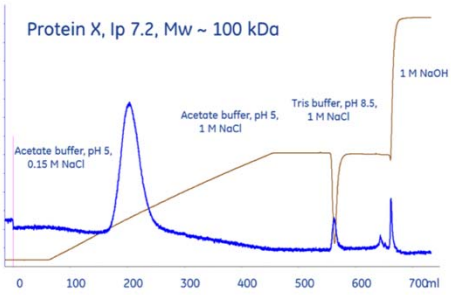
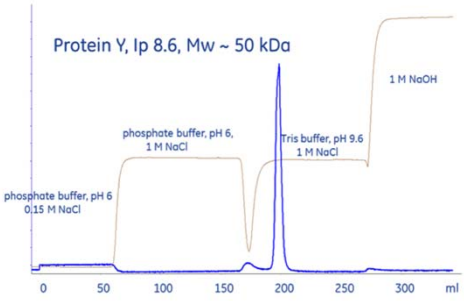
Protein dependent elution
蛋白依赖性的洗脱



Salt elution
盐梯度洗脱



Salt and pH elution
盐和pH梯度洗脱

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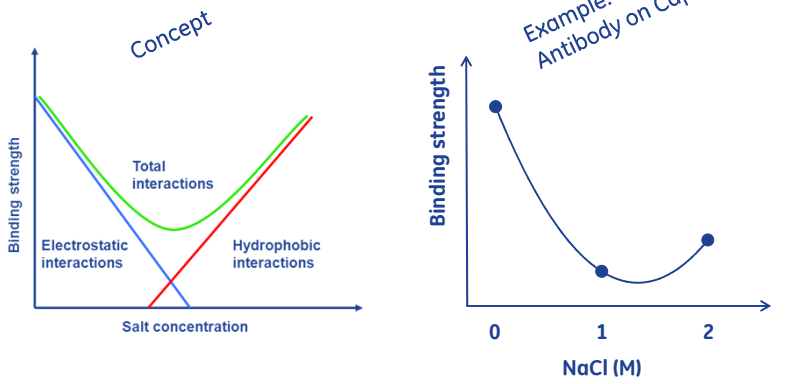
Interaction mode, IEX or HIC?
作用模式，离子交换或者疏水层析?



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Recovery study收率研究

Application Note 11-0035-45

Factors:	
Salt conc	0.25, 1, 1.75 M
Salt type	NaCl or NH4Cl
Buffer ionic strength	0.026 – 0.300
pH	5.75, 6.25, 6.75

Response:
Recovery (%) of protein in 3 column volumes

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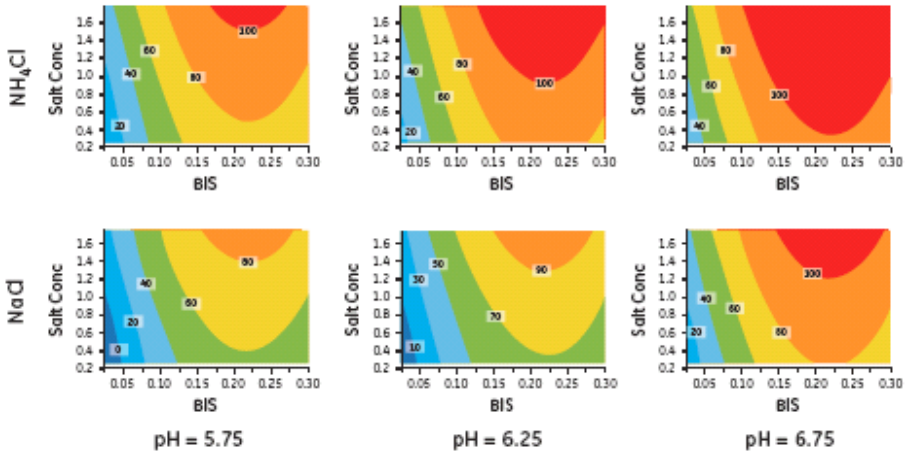
Recovery study收率研究

Application Note 11-0035-45



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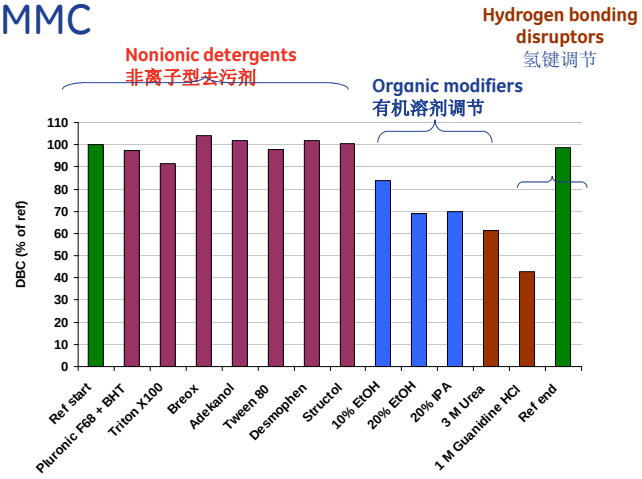
Loading in:
50 mM Na-acetate, pH 4.75, 250 mM NaCl

Effect of additives on BSA capacity
添加剂对BSA载量的影响

Capto™ MMC



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Summary Capto™ MMC小结

Binding at higher pH than with traditional ion exchangers

与传统的阳离子交换填料相比可以在较高的pH值下结合

Salt tolerance greater than for Capto adhere

盐离子的耐受能力比Capto adhere强

Elution may require both pH and conductivity change

洗脱时可能需要pH和电导的双重变化

Mode of interactions (IEX, HIC, H-bond) will vary depending on conditions and protein properties

IEX，HIC，H-bond的作用模式受层析条件和蛋白自身性质影响

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Multimodal libraries and screening formats

多模式填料及筛选形式

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HTPD Formats高通量筛选模式

Rapid parallel screening of chromatography conditions
快速平行

Filter plates
多孔筛选板
PreDictor™ plates

Minicolumns
小型层析柱
PreDictor™RoboColumn™

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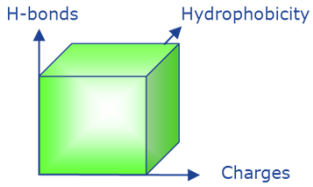
Multimodal libraries多模式家族

- ✓16 Multimodal cation prototypes
- ✓16种阳离子交换多模式原型
- ✓16 Multimodal anion prototypes
- ✓16种阴离子交换多模式原型



Diversity based multimodal libraries
基于多模式家族的多样性

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Summary

Multimodal chromatography media with unique selectivities extend the protein purification toolbox
多模式层析填料拥有独特的选择性，拓宽了蛋白纯化手段

Screen pH and conductivity for optimal running

conditions为最优的运行条件筛选pH值和电导

Use DOE使用DoE

Use parallel screening formats使用高通量筛选模式

imagination at work

Thank you

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