Introduction to Nuvia™ cPrime™: A NEW Hydrophobic Cation Exchange media

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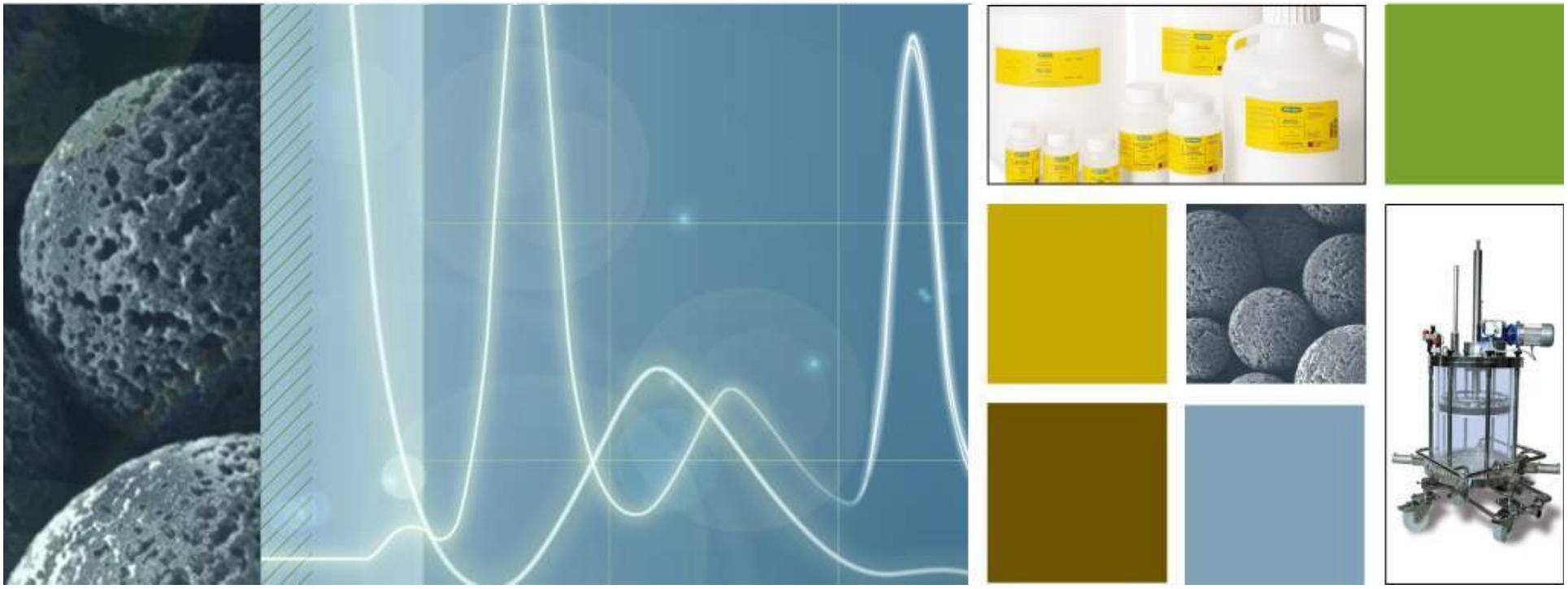
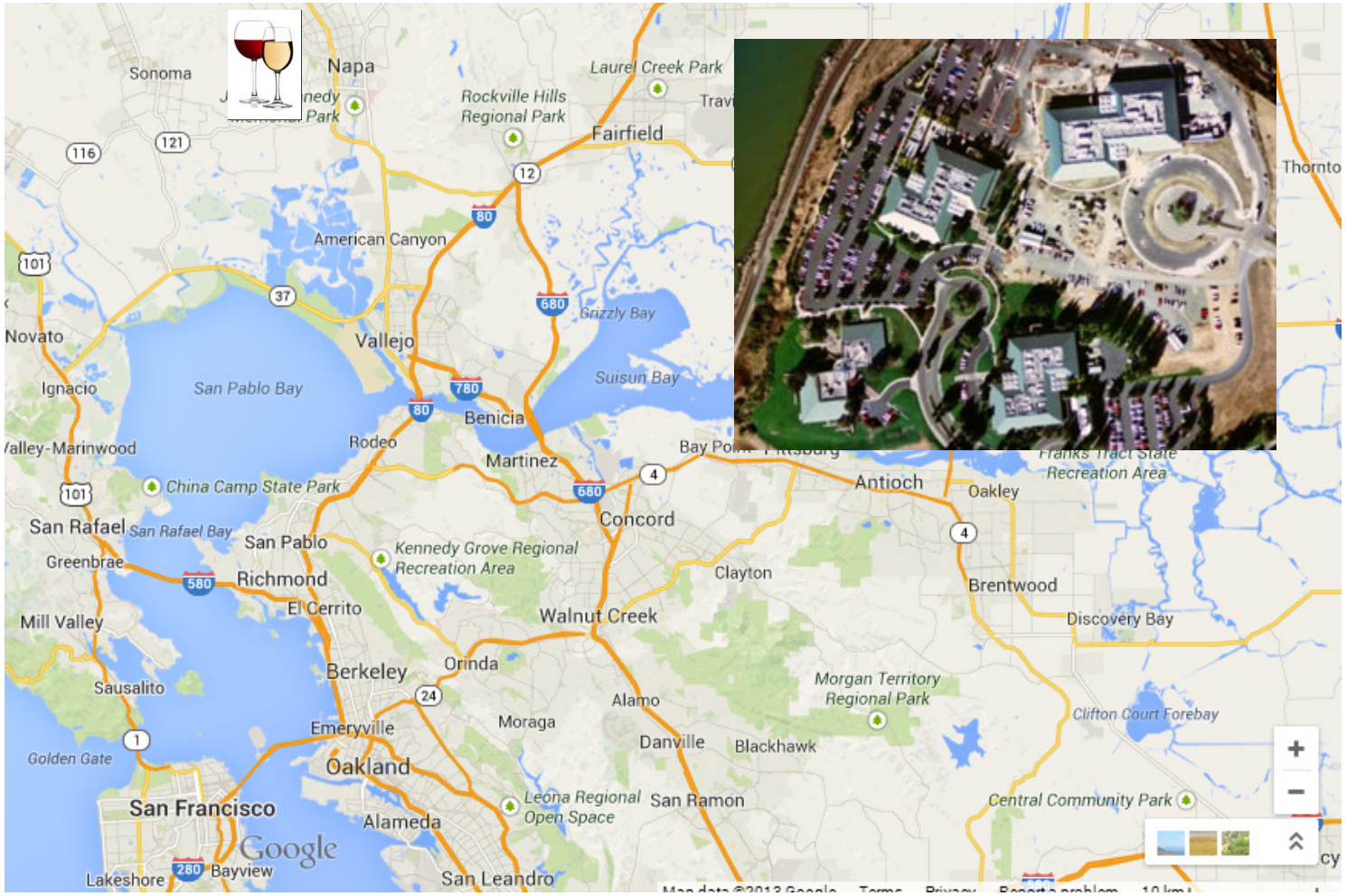
Process R&D Applications Group Manager



Bio-Rad in California



Outline

- Overview of Nuvia™ cPrime™ media
- Salt tolerance
- General approach to method development
- Different than any other resin
- Process operation window
- Case studies of protein therapeutics preparation using Nuvia cPrime
- Summary

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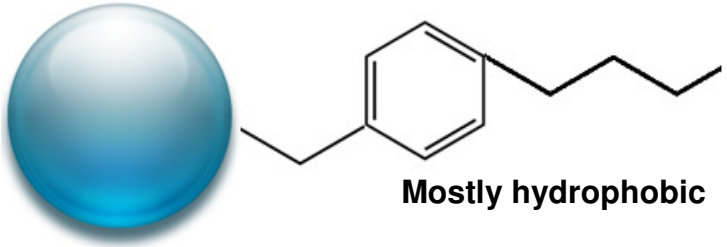
A quick refresher

HIC



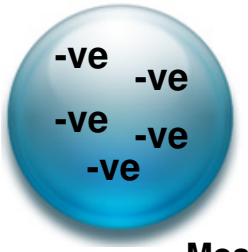
Mostly hydrophobic

HIC variables to manipulate:

Bind: High salt

Elute: Low salt and/or organic solvent (e.g. glycerol, propylene glycol)

CEX



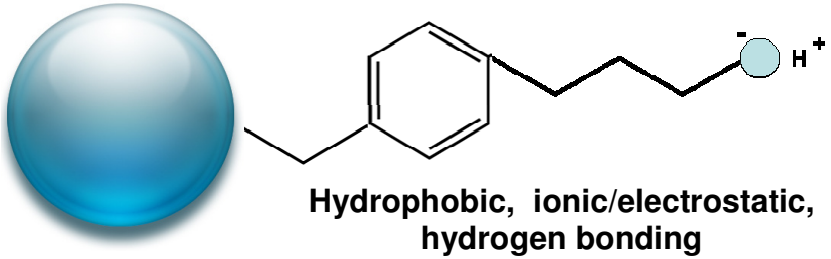
Mostly ionic/electrostatic

CEX variables to manipulate:

Bind: Low salt, low pH

Elute: High salt, high pH

Mixed-Mode

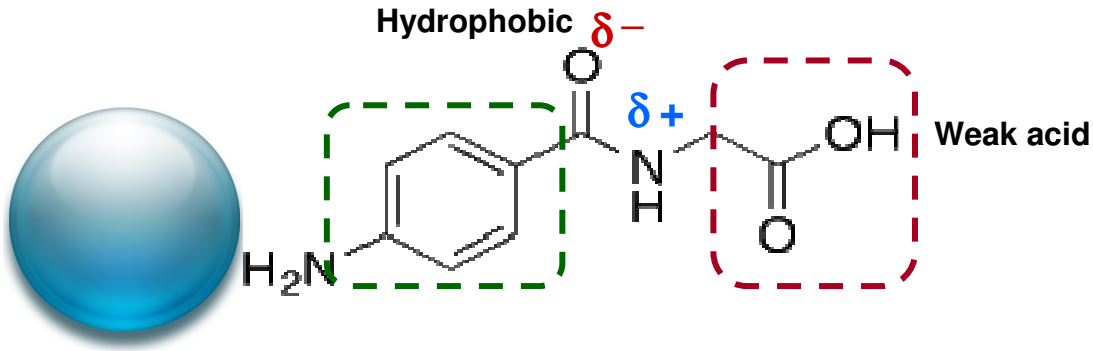


Hydrophobic, ionic/electrostatic, hydrogen bonding

Mixed-mode variables to manipulate:

Salt concentration and/or pH, as well as additives (e.g. arginine)

What is the cPrime ligand?



Hydrophobic, ionic/electrostatic hydrogen bonding

- The hydrophobic/ionic balance of the ligand, in combination with the effect of the bead, provides the unique selectivity
- Hydrogen bonding may also play a role in some cases

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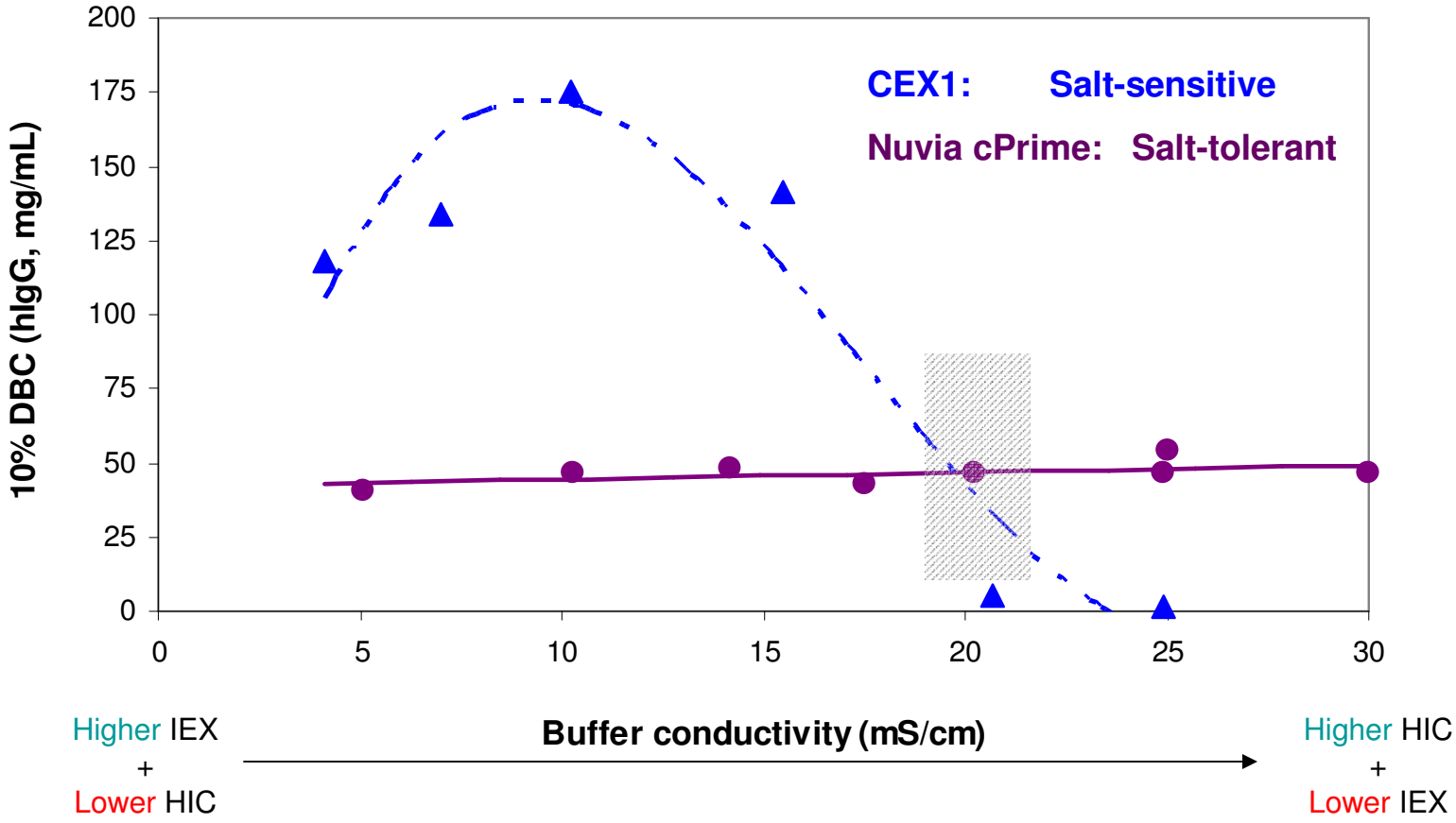
Nuvia cPrime is salt-tolerant



Binding and impurity removal on Nuvia cPrime over a wide range of conductivity

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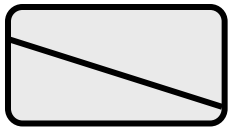
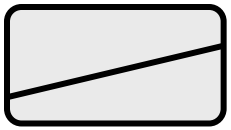
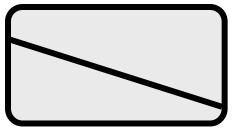
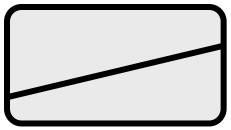
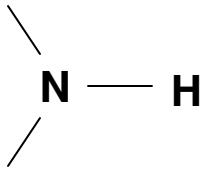
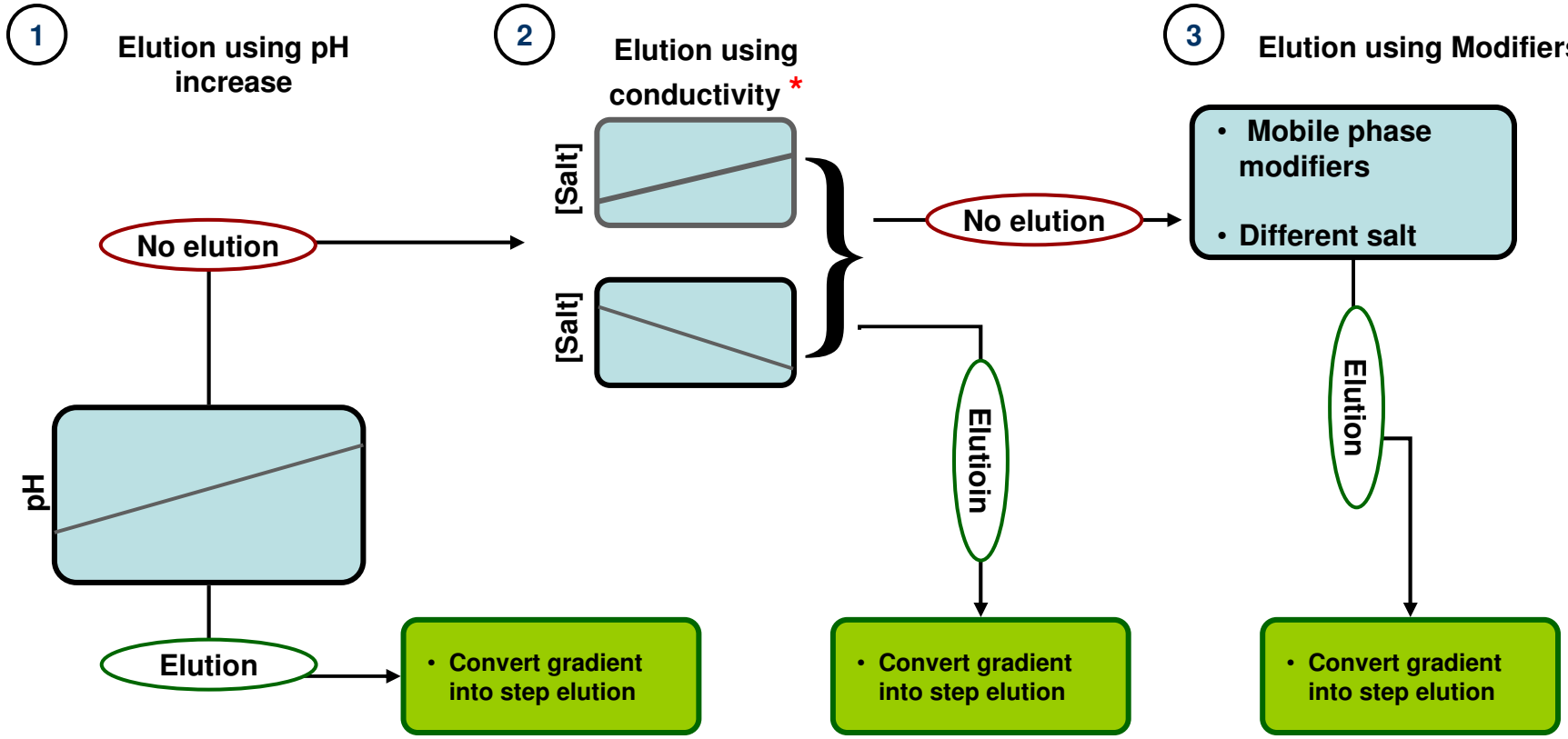
Rationale behind method development

Binding enhancement	Interaction	Elution
[Salt] 	Hydrophobic interaction (Changing protein hydration state)	[Salt] 
pH 	Hydrophobic interaction (Changing protein charge state)	pH  Propylene glycol Glycerol
[Salt] 	Ion exchange interaction (Modulating electrostatic interactions)	[Salt] 
pH 	Ion exchange interaction (Changing protein charge state)	pH 
	Hydrogen bonding	0.1 M Arginine Urea 0.1 N Guanidine

BIO-RAD

Nuvia cPrime is easy to use



*at optimum pH, determined from step 1

•Scheme confirmed using several diverse molecules

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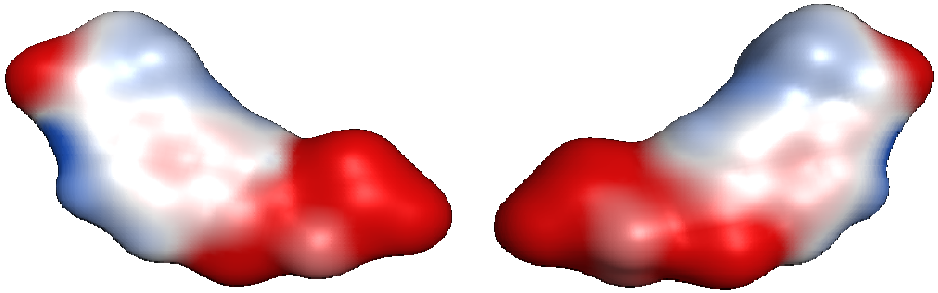
Electrostatic Maps

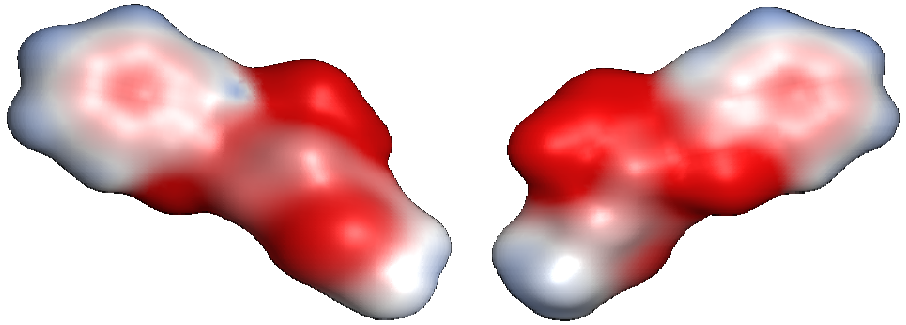
Front **Back**

Nuvia cPrime



Red = Negative EP
Blue = Positive EP

Resin A



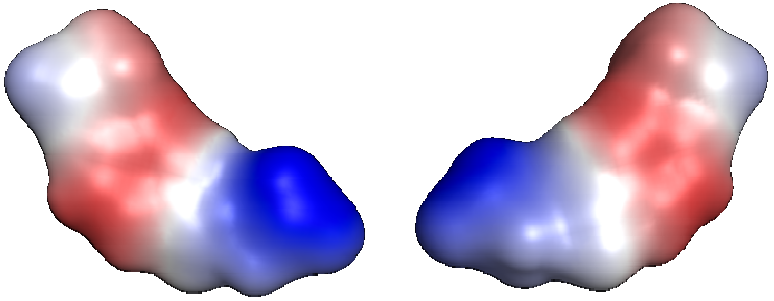
Courtesy S. Cramer

DIFFERENT...

Hydrophobicity Maps

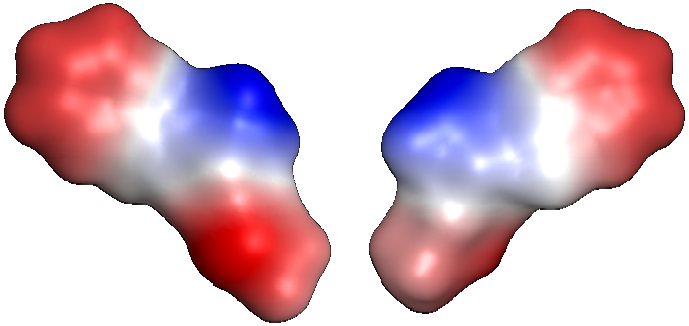
Front **Back**

Nuvia cPrime



Red = Hydrophobic
Blue = Hydrophilic

Resin A

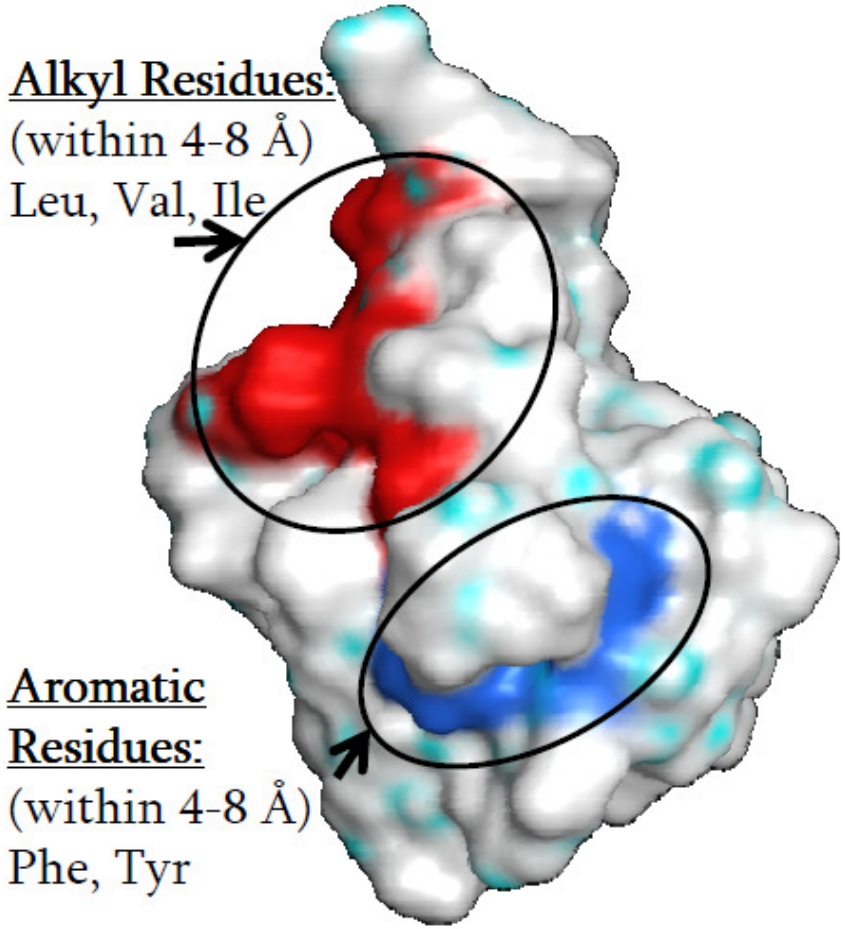


Courtesy S. Cramer

EVEN "HYDROPHOBIC" IS DIFFERENT...

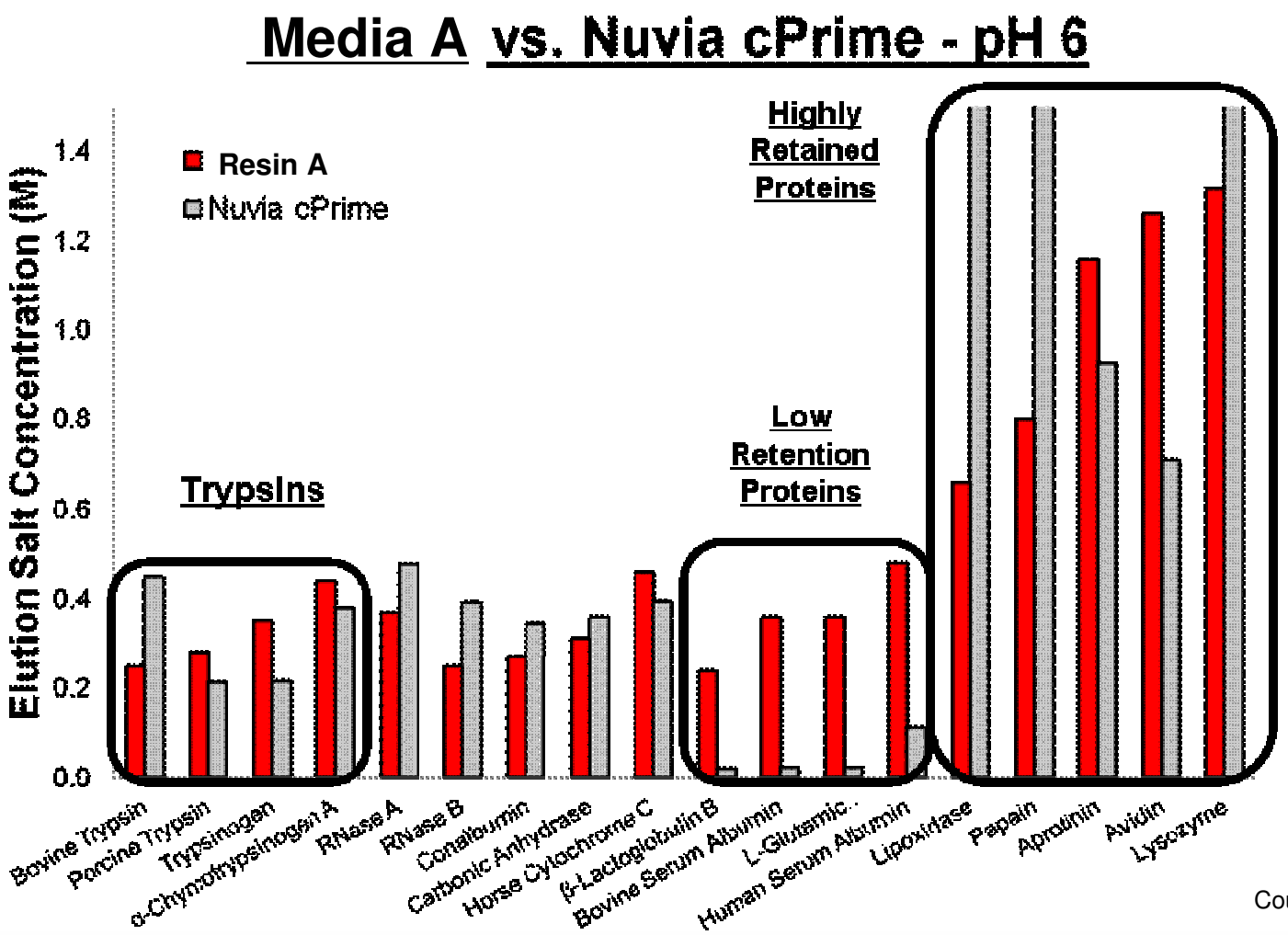
Resin X binds to alkyl groups

Nuvia cPrime (sterically hindered phenyl group) binds to aromatic groups



Courtesy S. Cramer

DIFFERENT!



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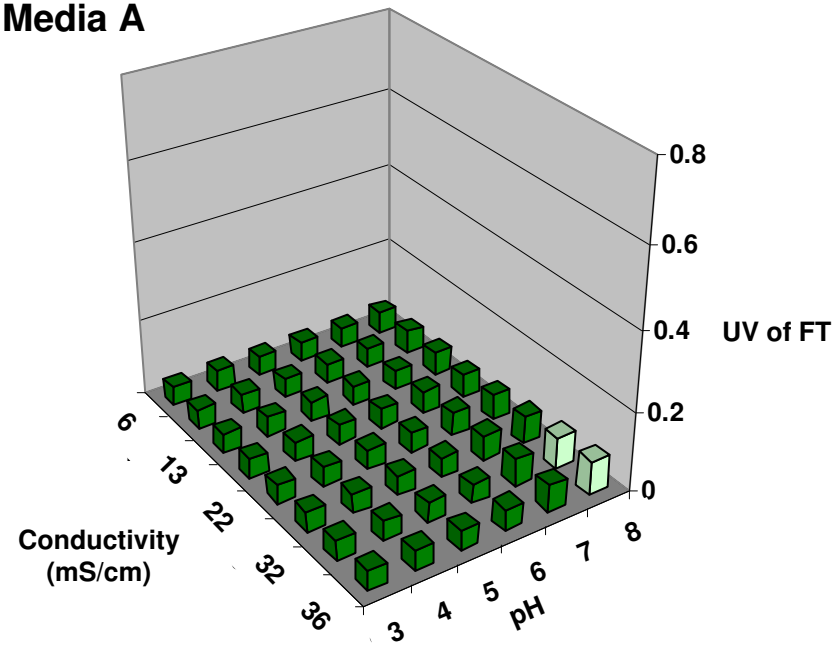
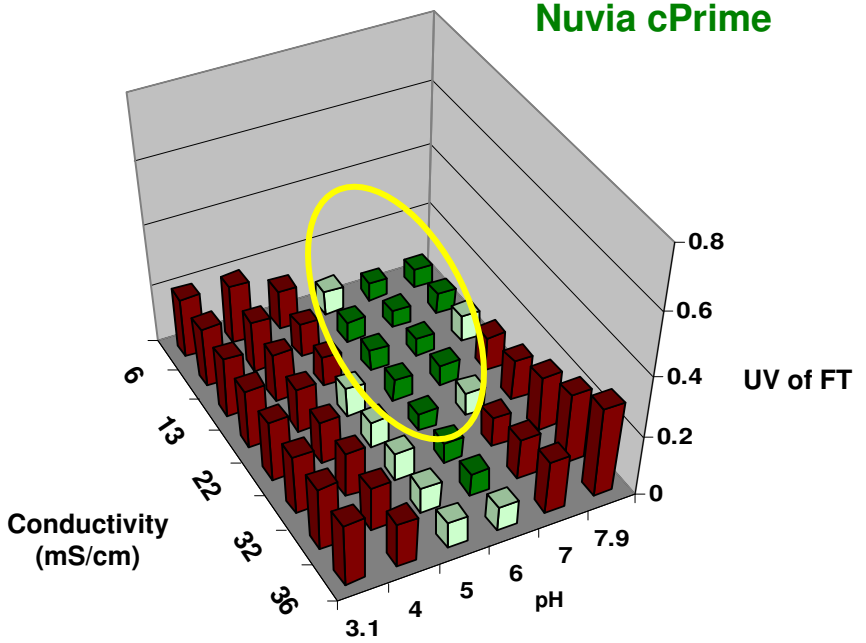
Capture of recombinant protein from culture supernatant

Binding condition screening

Media A



Nuvia cPrime



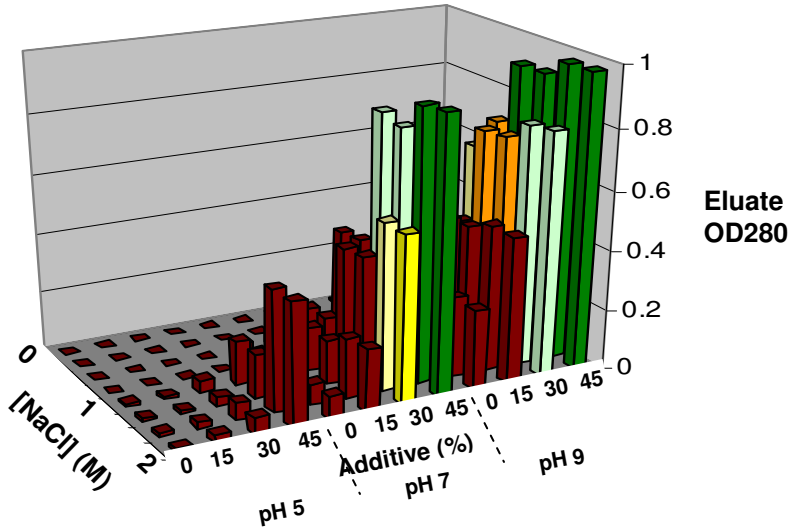
FT = Flow-through
UV in FT: minimal

UV < 0.08
UV = 0.08 ~ 0.1
UV > 0.1

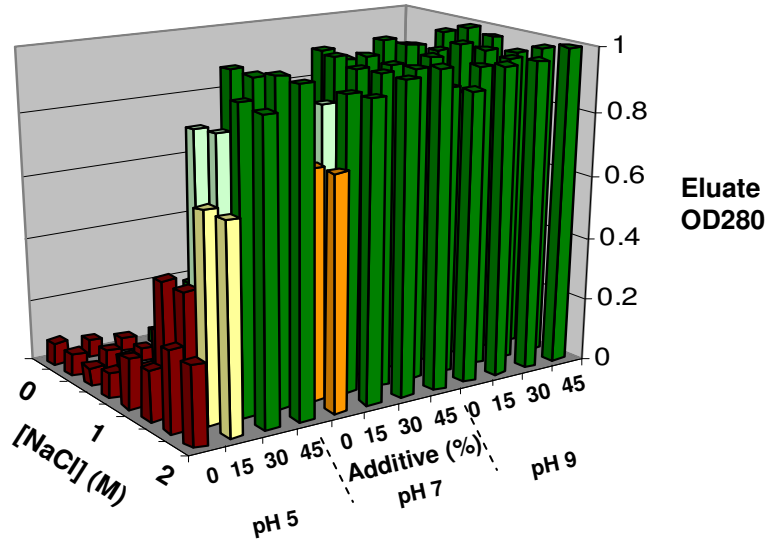
Both Nuvia cPrime and Media A can be used for direct capture from culture supernatant

Elution of bound target protein from column

Media A



Nuvia cPrime



Eluate UV: maximal

- OD280 > 0.9
- OD280 > 0.8
- OD280 > 0.7
- OD280 > 0.6
- OD280 = 0 ~ 0.6

- **Media A:** Needs optimization of [salt], [solvent], and pH
- **Nuvia cPrime:** Wide windows of [salt] and pH, solvent is NOT a must; can choose conditions for easy step transition and product stability

Look beyond binding!

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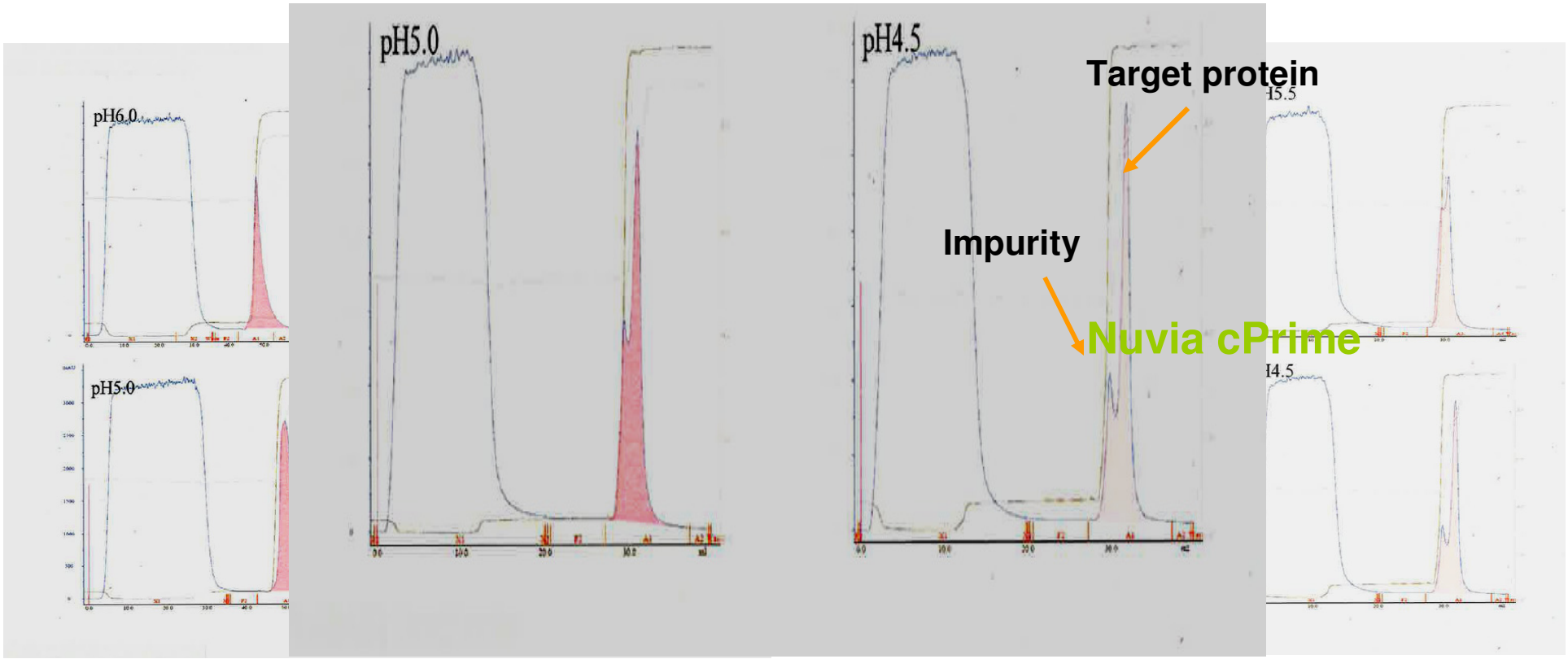
Recombinant enzyme purity



Nuvia cPrime resolved target protein from impurity, as pH decreased

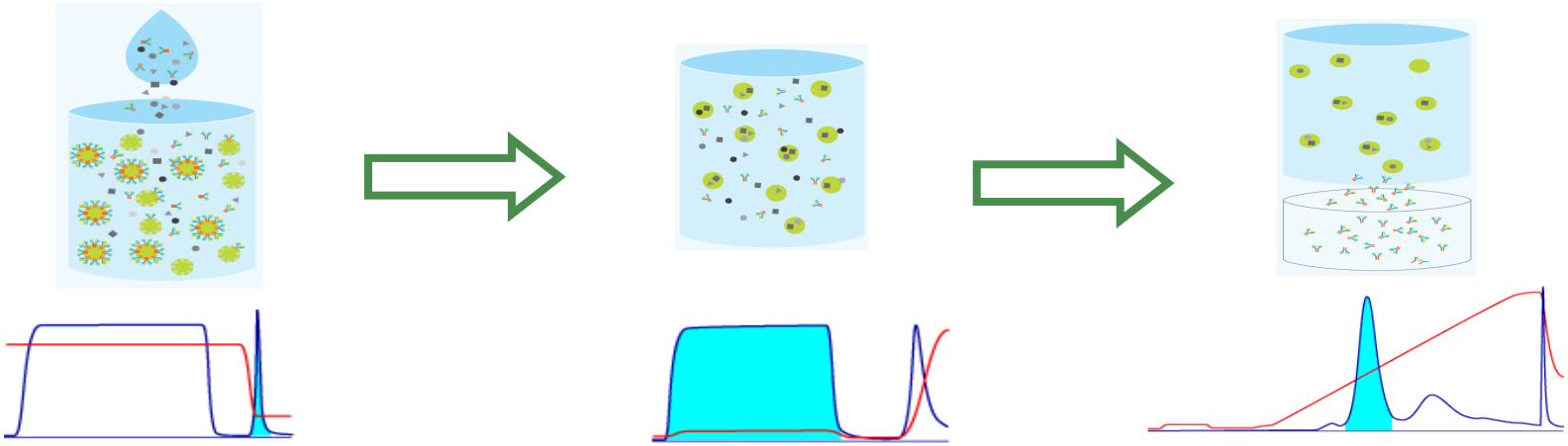
Application 4: A new purification strategy for clinical-grade mAbs

mAb1

Monoclonal IgG expressed in CHO cell culture, 0.754 G/L

CTF also contains significant amount of a L-chain degradation product

Capture	Intermediate purification	Final polishing
Nuvia S	Nuvia Q	Nuvia cPrime



Impurity clearance (non-optimized)

Non-rPA workflow

Sample	Host cell proteins (ng/mg)	Host cell dsDNAs (ng/mg)	Aggregate content (%)
Cell culture supernatant	6.3×10^4	9.3×10^4	Not determined
Nuvia S fraction	2.6×10^3	17	Not determined
Nuvia Q fraction	59	4.1	Not determined
Nuvia cPrime fraction	5.5	Not detected (< 0.008)	• < 0.9 • <0.1% L chain dimers

pH

pH

rPA workflow

Sample	Host cell proteins (ng/mg)	Host cell dsDNAs (ng/mg)	Aggregate content (%)
UNOs SUPrA fraction	1.8×10^2	19	Not determined
UNOs Q fraction	< 3.0	3.5	Not determined
CHT fraction	< 2.0	2.5	• < 0.03 • L chain dimers not determined

Other case studies

- **Case 1: Recombinant protein (pI ~ 7.2) capture from CHO culture**

Impurity	HCP (ng/mg)	DNA (ng/mg)
Load	**6.3×10^4**	**9.3×10^4**
Eluate	**3.9×10^2**	**4.6×10^2**

- **Case 2: Recombinant protein (pI ~ 6, glycosylated) polish (step 2)**

Impurity	HCP (ng/mg)	DNA (ng/mg)
Load	**1.6×10^3**	**12**
Eluate	**1.6×10^2**	**2.0**

- **Case 3: Recombinant protein (pI ~ 7.2) polish (step 3)**

Impurity	HCP (ng/mg)	DNA (ng/mg)
Load	**59**	**4.1**
Eluate	**5.5**	**<0.008**

Summary

A hydrophobic cation exchange mixed-mode media:

New and unique selectivity	Balance of electrostatic and hydrophobic character	Permits powerful ways to separate proteins and clear impurities
Salt tolerance	Afforded by the hydrophobic character	Reduces the need for dilution and extensive manipulation
Wide design space	Ligand and media design	Broadly effective binding and elution spaces; robustness
High recovery	Ligand and matrix design; balance between modes of reversible interactions	No need to compromise between recovery and selectivity
Intuitive method development	Simple design	Fast method development and predictable behavior

An effective, easy-to-use media without the compromise common to other mixed modes