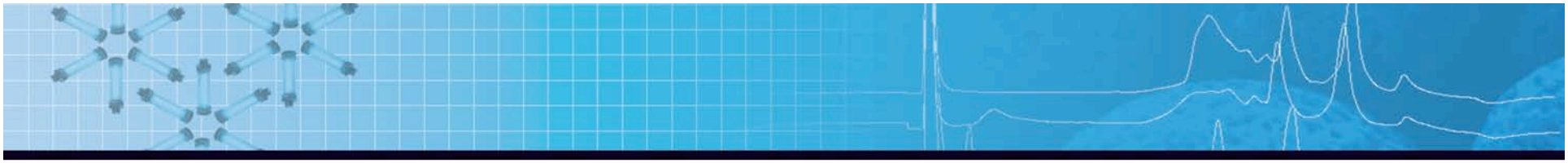

Retention behavior of endotoxin, DNA, and protein A on CHT™ ceramic hydroxyapatite and CFT™ ceramic fluorapatite

Pete Gagnon, Julia Zhen, Paul Ng, Cheryl Aberin,
Jie He, Heather Mekosh, Larry Cummings

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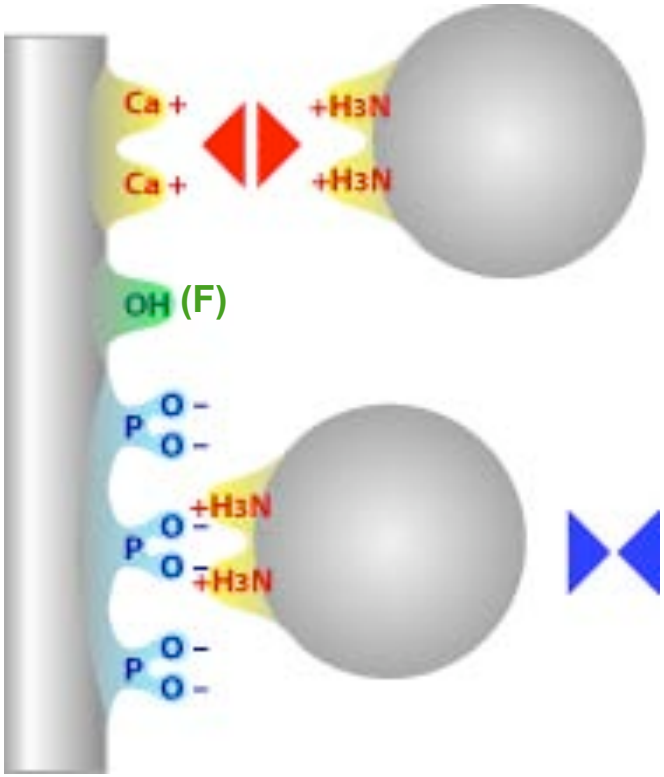
Chemical structure

- **CHT**
- $Ca_{10}(PO_4)_6(OH)_2$

- **CFT**
- $Ca_{10}(PO_4)_6F_2$

Primary retention mechanisms



- **Amino residues**
- Classical cation exchange

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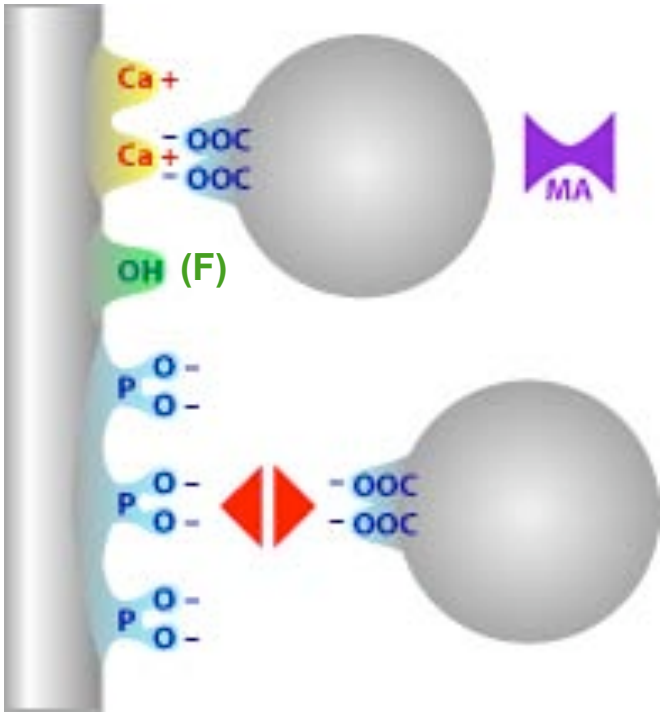
Primary retention mechanisms



- **Carboxyl clusters**
- Classical chelation

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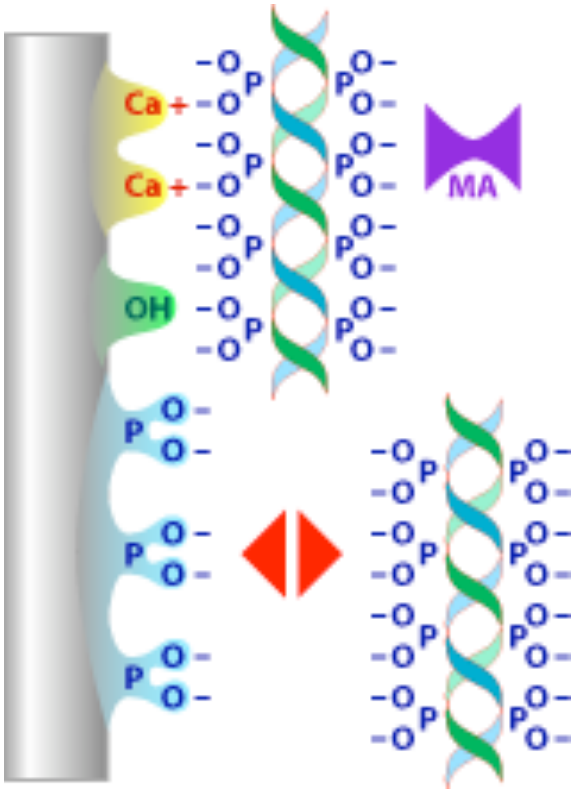
Primary retention mechanisms

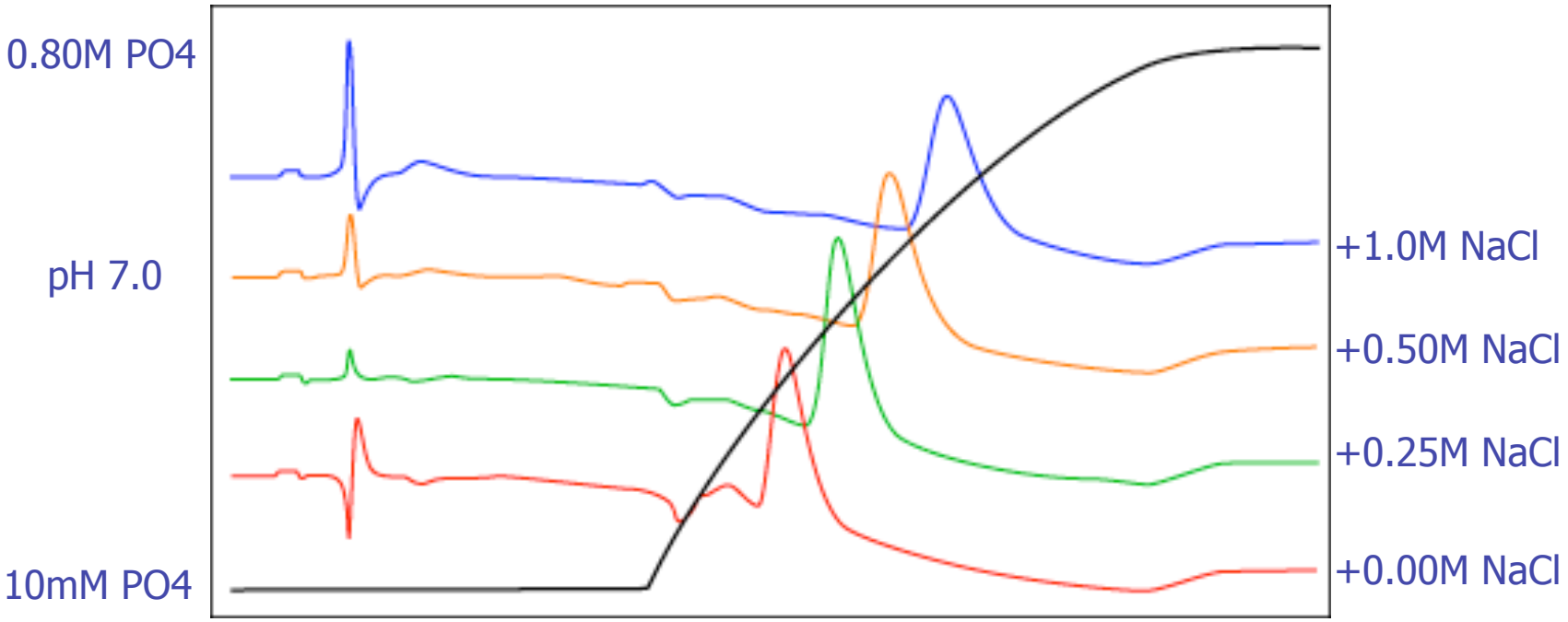


- **Phosphoryl residues**
- Metal coordination

Retention of DNA

- DNA is highly phosphorylated, which should cause it to form strong coordination complexes with CHT calcium.
- Elution in the absence of phosphate should not occur.
- Charge repulsion between DNA phosphates and CHT phosphates may modulate retention behavior. If so, NaCl should have a clear influence.
- Size and rigidity may also affect selectivity.

Retention of DNA on CHT



Sheared salmon sperm DNA
CHT type I, 40 micron, 600 cm/hr

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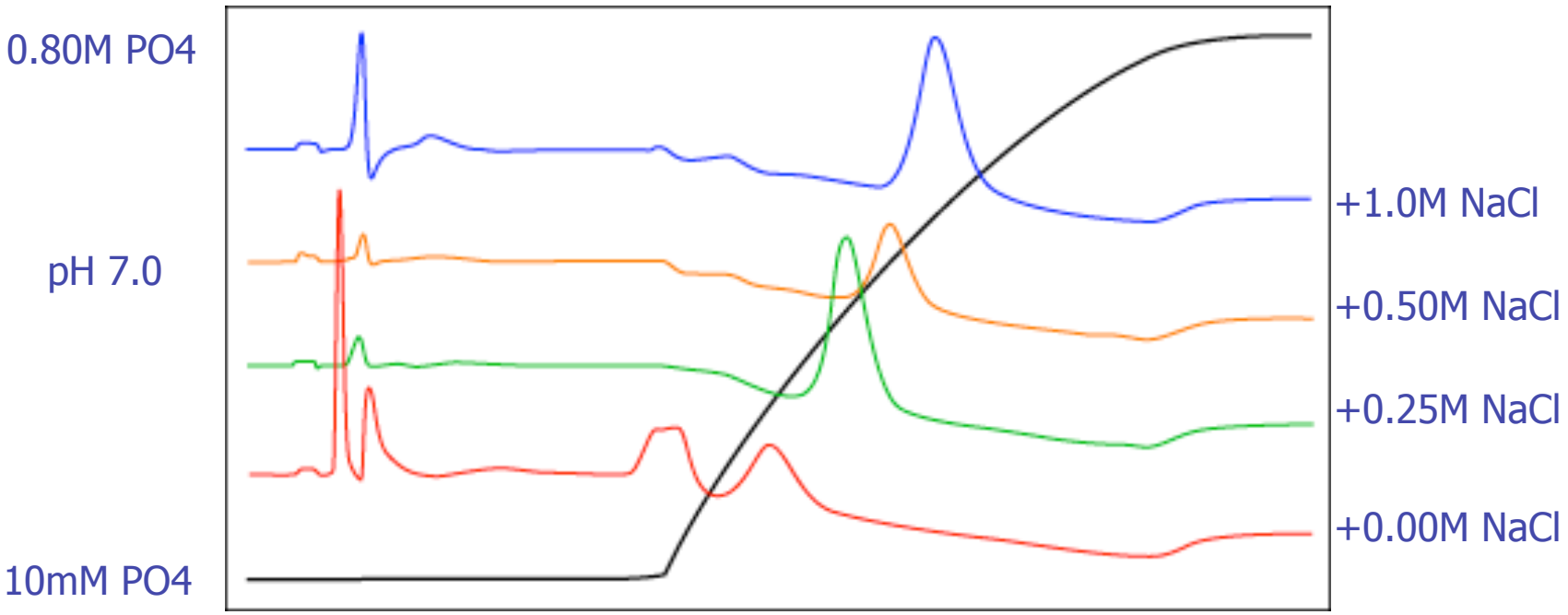
Retention of DNA on CFT



Sheared salmon sperm DNA
CFT type I, 40 micron, 600 cm/hr

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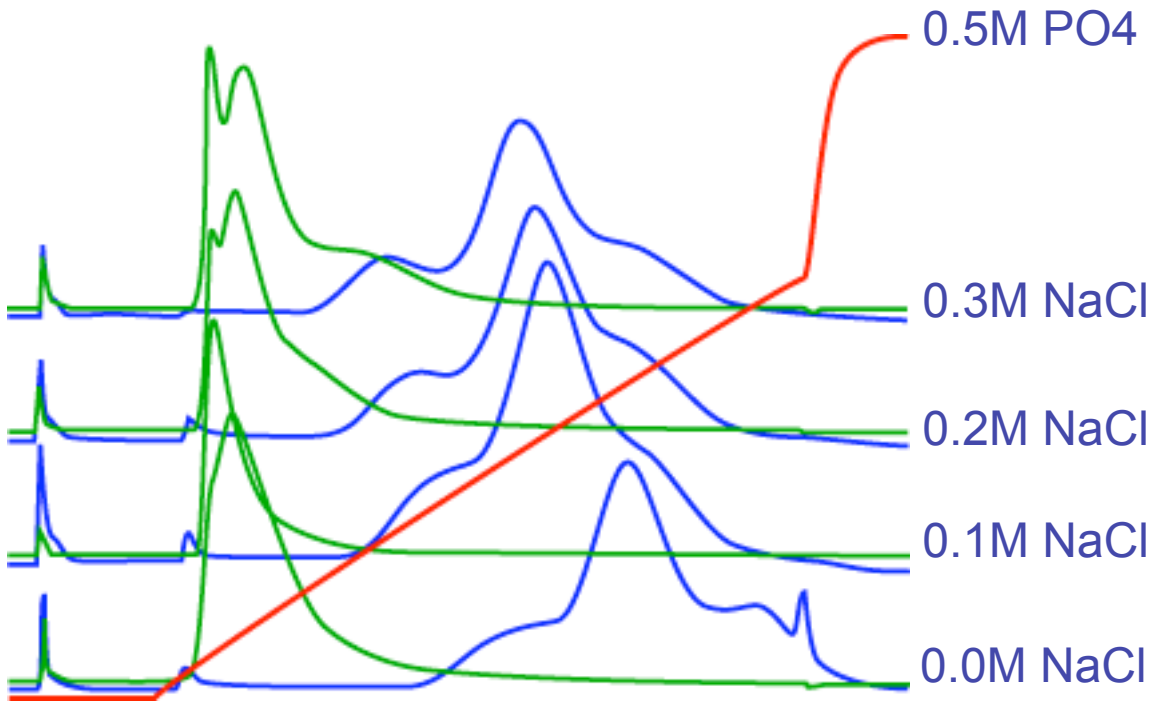
Retention of endotoxins

- Endotoxins are highly acidic due to a high content of phosphoryl and carboxyl residues.
- Both should have strong affinity for CHT calcium. Elution in the absence of phosphate should not occur.
- Charge repulsion between protein A carboxyls and CHT phosphates may be a factor.
- Some endotoxins have amino groups which may cation exchange with CHT phosphates at low ionic strength.
- Endotoxins occur in a range of aggregations states, into the millions of Daltons. Most of the charges are internalized because of their association with the hydrophobic lipid A region. Both size and charge shielding may affect retention.
- Endotoxins are frequently complexed with proteins and other cell wall components that may also influence retention

Retention of endotoxin, CHT vs CFT

phosphate gradient elution at different concentrations of NaCl



S. minnesota endotoxin Green CFT Blue CHT

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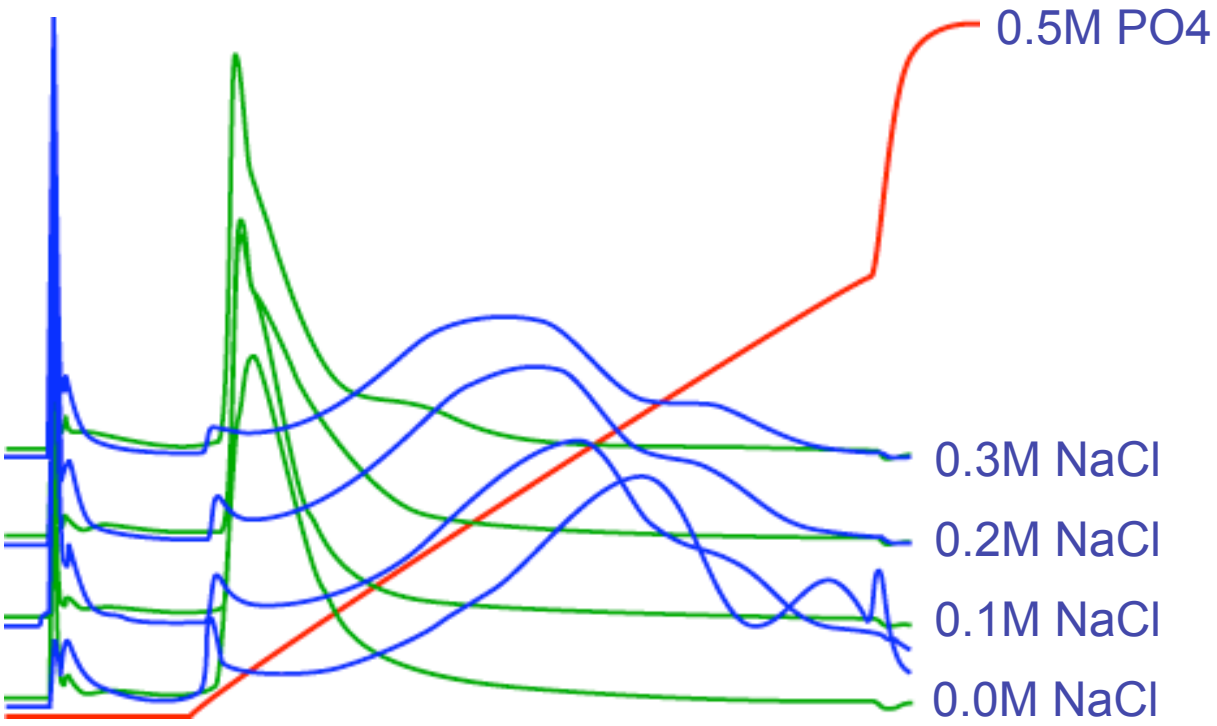
Retention of endotoxin, CHT vs CFT

phosphate gradient elution at different concentrations of NaCl



P. aeruginosa endotoxin Green CFT Blue CHT

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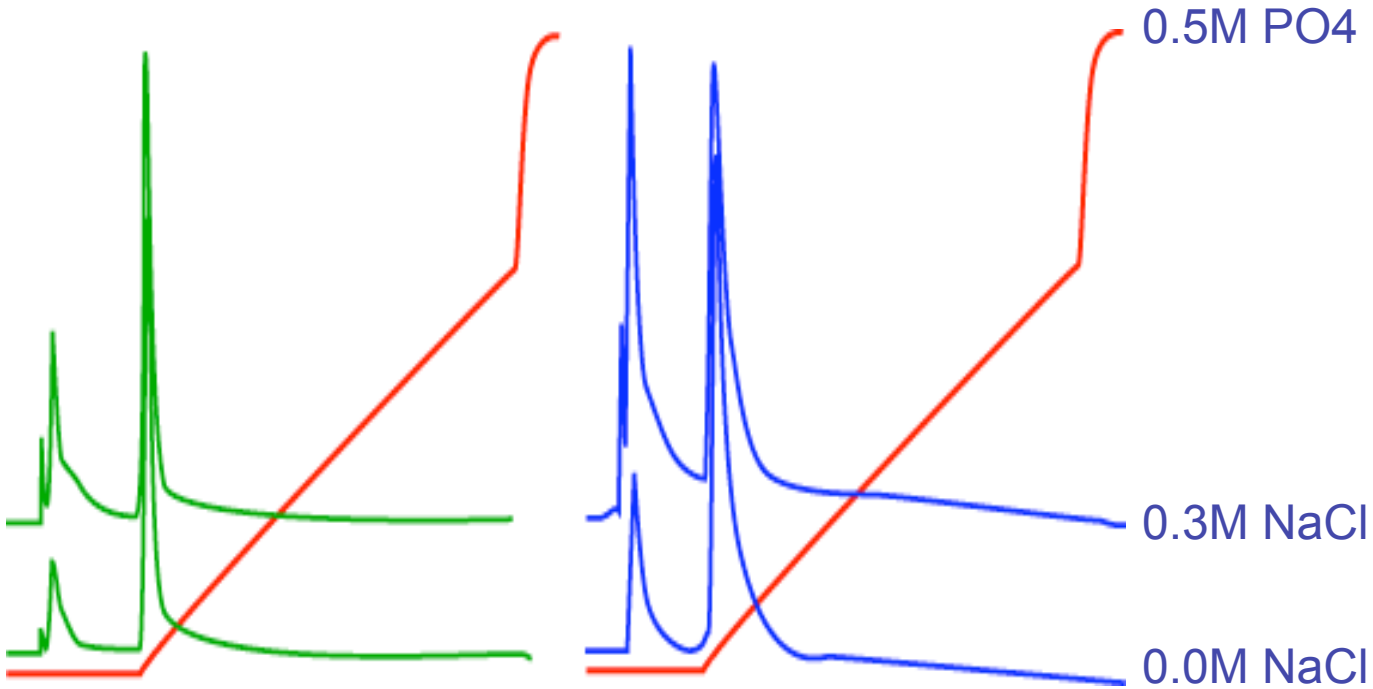
Retention of endotoxin, CHT vs CFT

phosphate gradient elution at different concentrations of NaCl



E.coli endotoxin Green CFT Blue CHT

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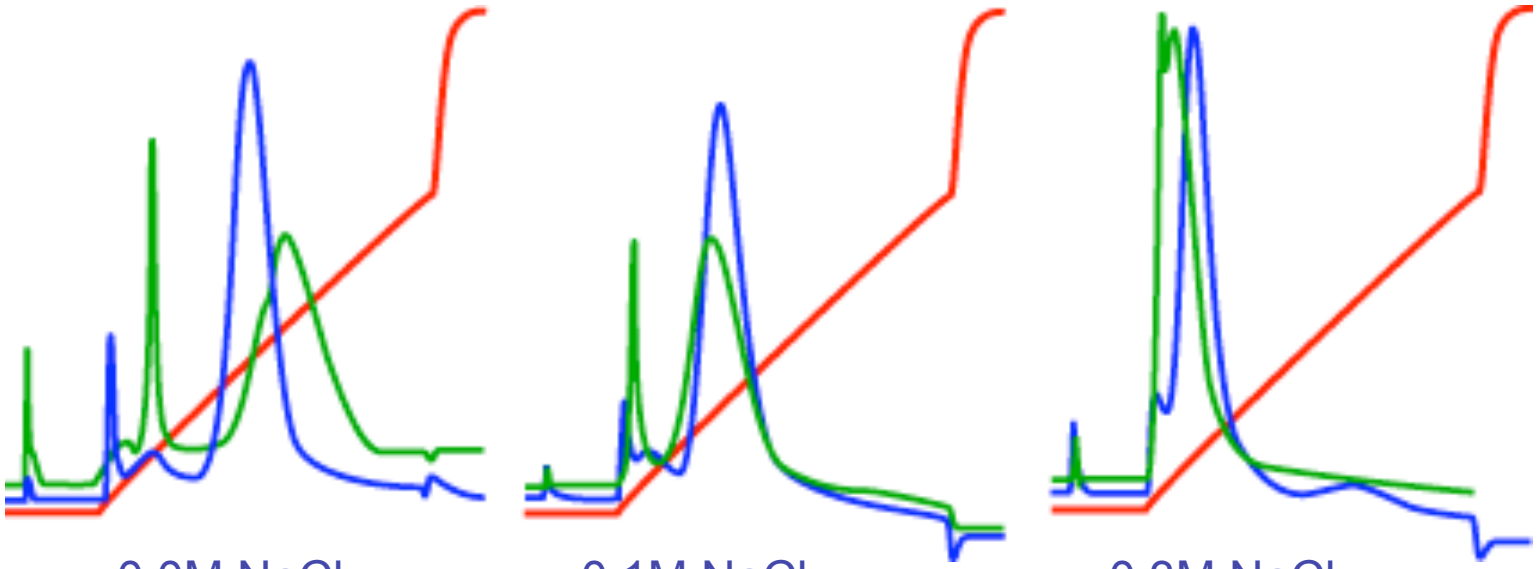
Expected retention of protein A

- Protein A is highly acidic (pI 4.7–5.1), corresponding to its carboxyl-rich composition.
- This should cause strong binding by chelation with CHT calcium. Elution in the absence of phosphate should not occur.
- Charge repulsion between protein A carboxyls and CHT phosphates may be a factor.
- Since protein A binds very weakly to cation exchangers, binding of amino residues to CHT phosphates is not expected to be a major factor.

Retention of protein A, CHT vs CFT

Phosphate gradient elution at different concentrations of NaCl

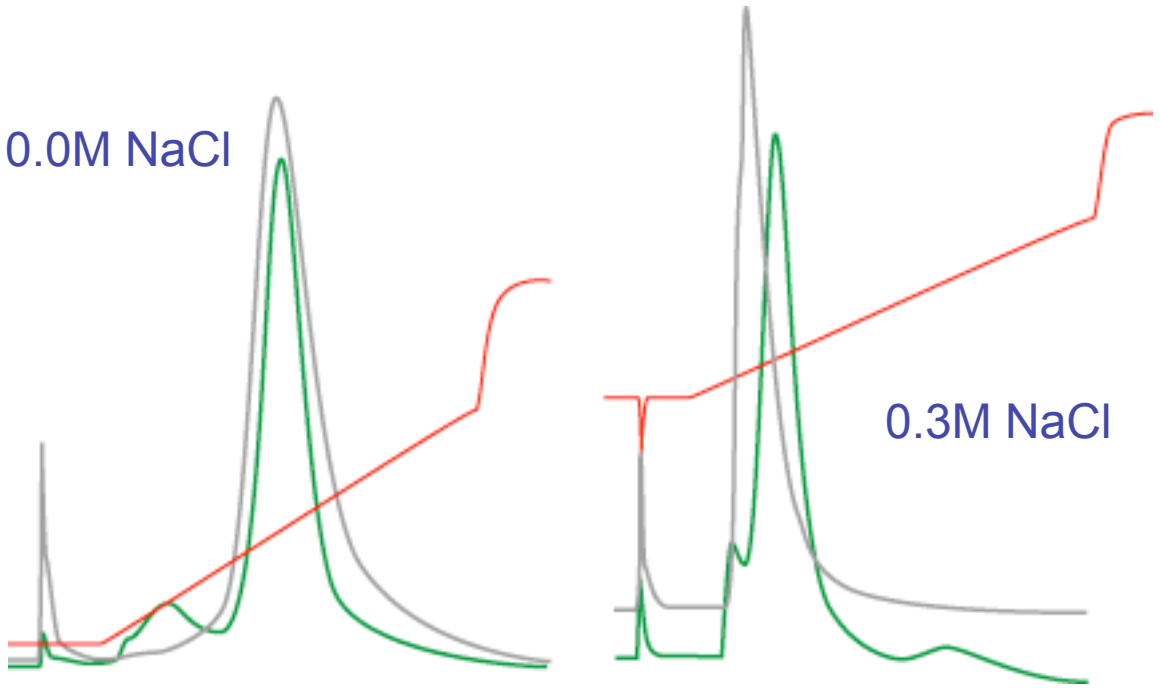


0.0M NaCl 0.1M NaCl 0.3M NaCl

Green CFT type I 40 µm Blue CHT type I 40 µm

Retention of free protein A vs IgG

Phosphate gradient elution at different concentrations of NaCl



CHT, type I, 40µm Green protein A, Gray IgG

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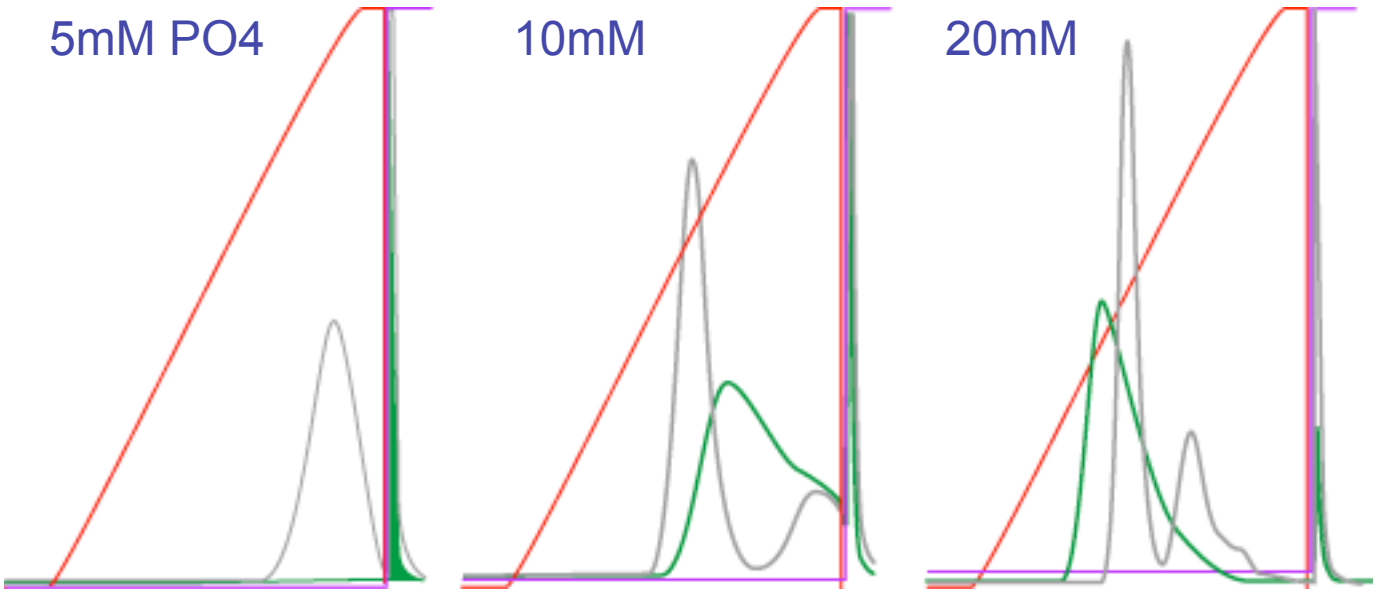
Retention of free protein A vs IgG

NaCl gradient elution at different phosphate concentrations



CHT, type I, 20µm Green protein A, Gray IgG

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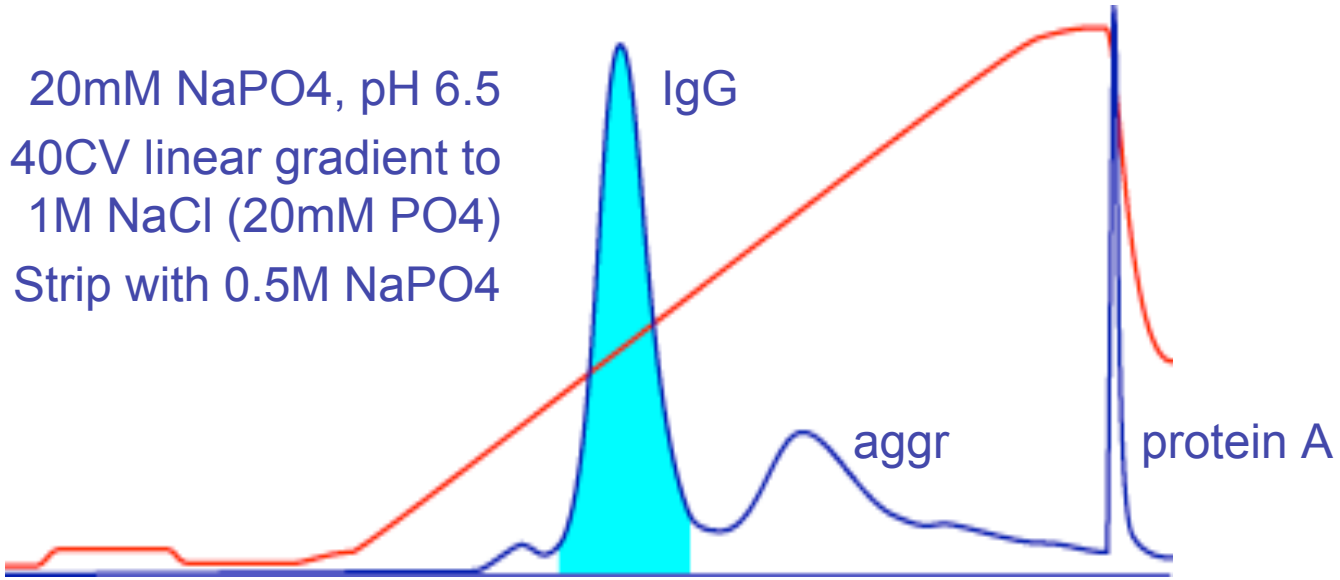
Retention of leached protein A vs IgG



Protein A-purified human IgG1
CHT type I, 20 micron, 300 cm/hr

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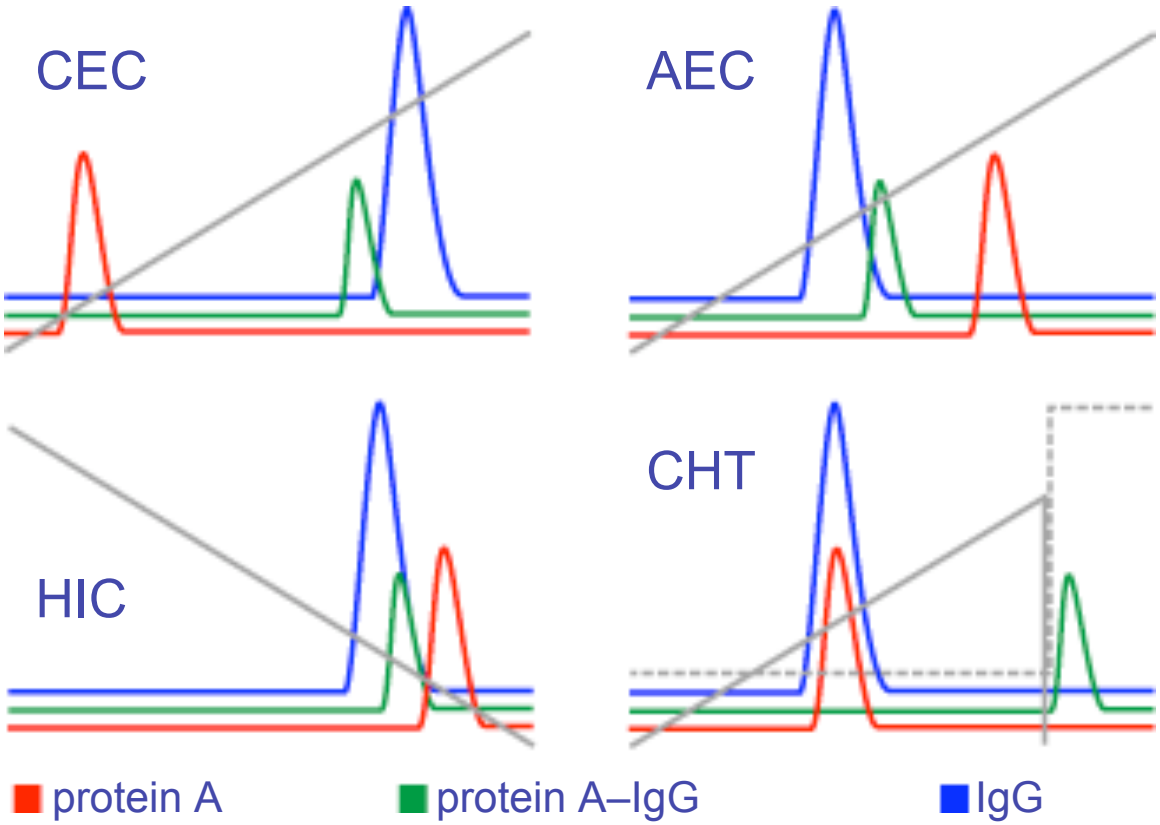
Retention protein A vs IgG

- The elution position of free protein A overlaps to varying degrees with the elution position of free IgG, regardless of whether elution is conducted with phosphate or with NaCl.
- This implies that protein A clearance should be nil, but
- Leached protein A is well separated from IgG when IgG is eluted in NaCl. Most of the protein A is restricted to the 0.5M $NaPO_4$ strip.
- This indicates that the retention properties of both components are additive in the complex, particularly as they relate to their carboxyl interactions with CHT calcium.

Retention of protein A vs IgG



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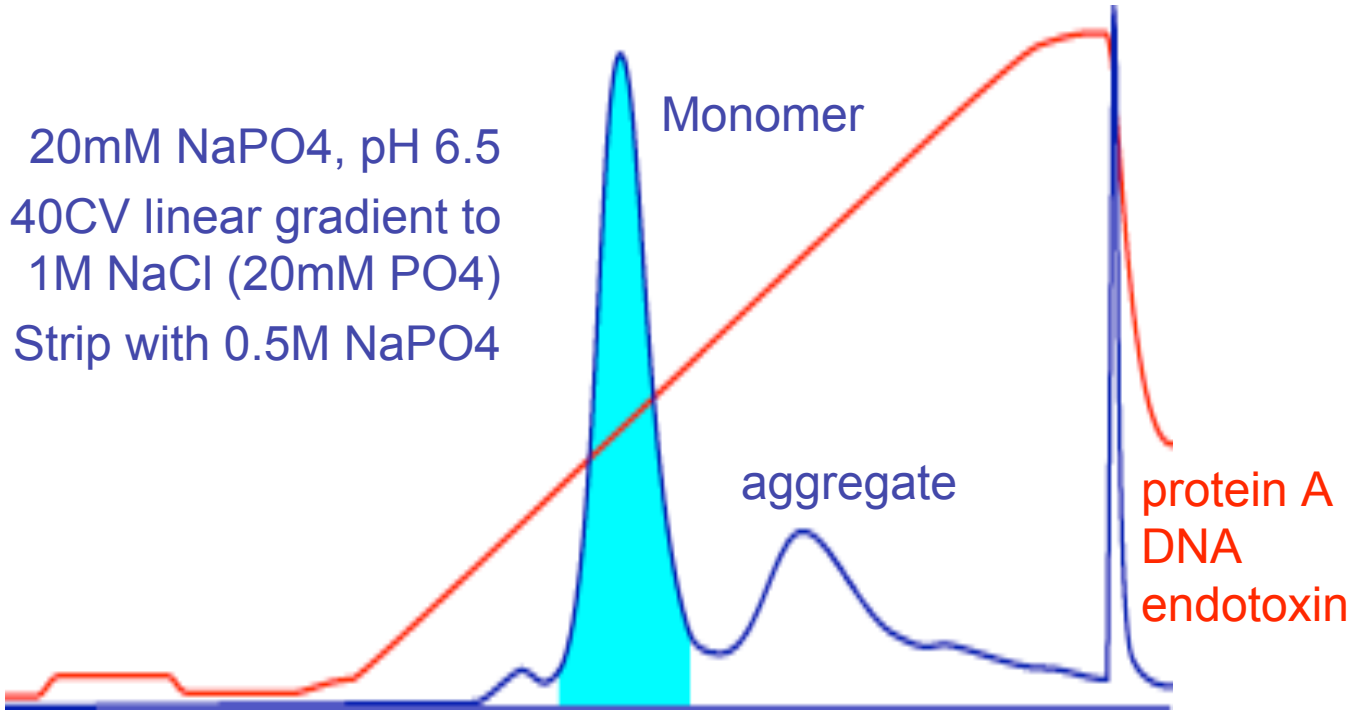
Clearance summary



Protein A-purified human IgG1
CHT type I, 20 micron, 300 cm/hr

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Clearance summary, monomer pool

- **DNA**
  - >10^3 by PCR, to <1ng/mL by Picogreen
- **Endotoxin**
  - 7 x10^4, to 1EU/mL by LAL
- **Leached protein A**
  - 90%, from 55 to 5ng/mL by Cygnus
- **IgG aggregates**
  - >99%, from >40% to <1% by HPSEC

Acknowledgments

- Special thanks to Avid BioServices of Tustin, CA for generously providing antibodies to support this work.

- For a copy of this presentation, please contact <tanis_correa@bio-rad.com>

- For technical questions, you are welcome to contact <peter_gagnon@bio-rad.com>