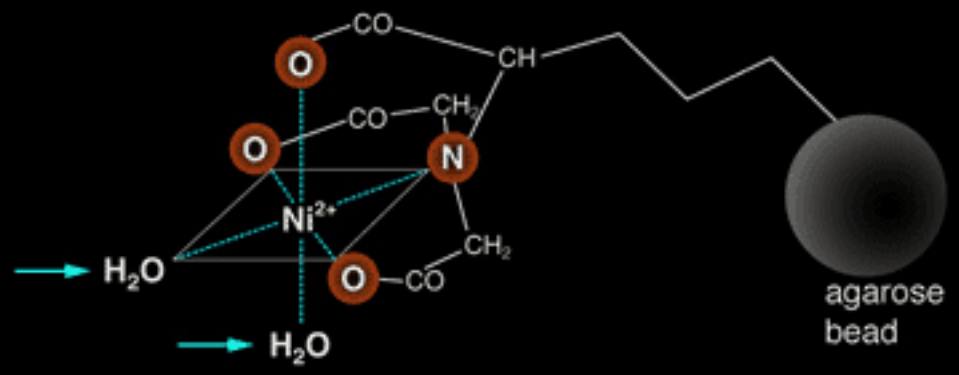

Journal Club

Enabling IMAC purification of low abundance recombinant proteins from *E. coli* lysates

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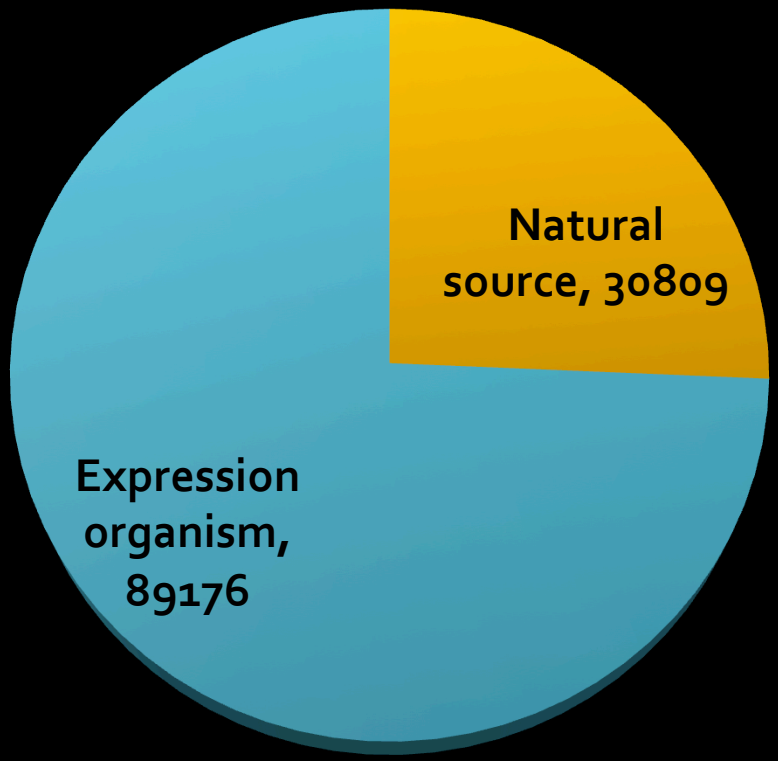
Why this work? (Cont'd)

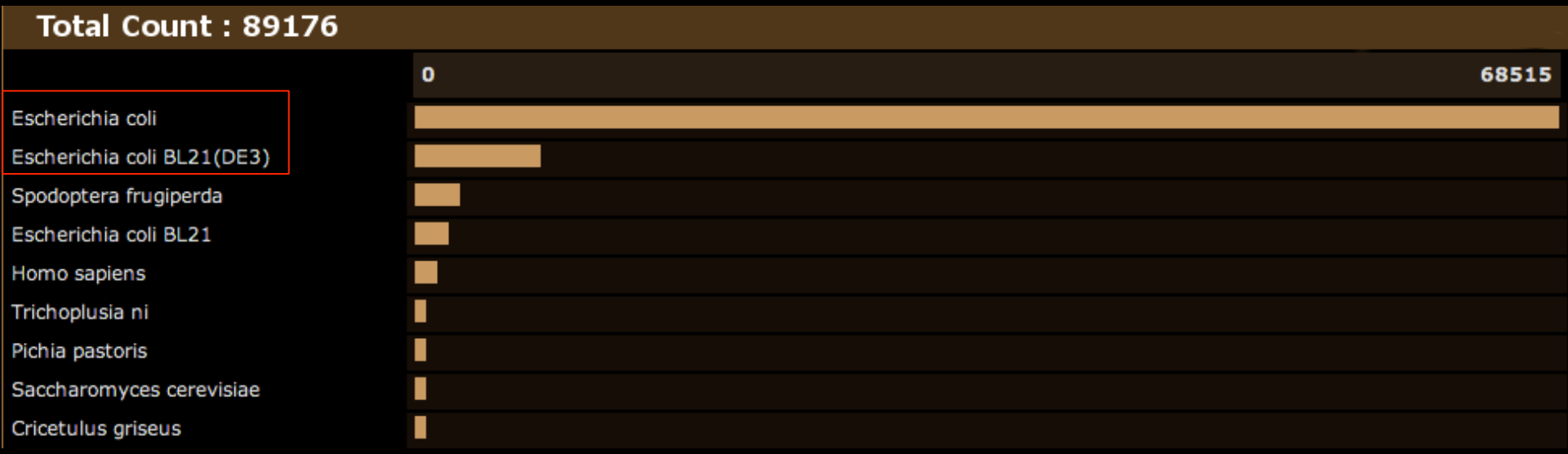
- Recombinant proteins are used throughout biological and biomedical science



Distribution of released structures in the PDB

Why this work? (Cont'd)

- *E.coli* -the most widely used system for protein purification



Source organism histogram

Why this work? (Cont'd)

HOW can we purify
a challenging protein to homogeneity
with sufficient quantities?

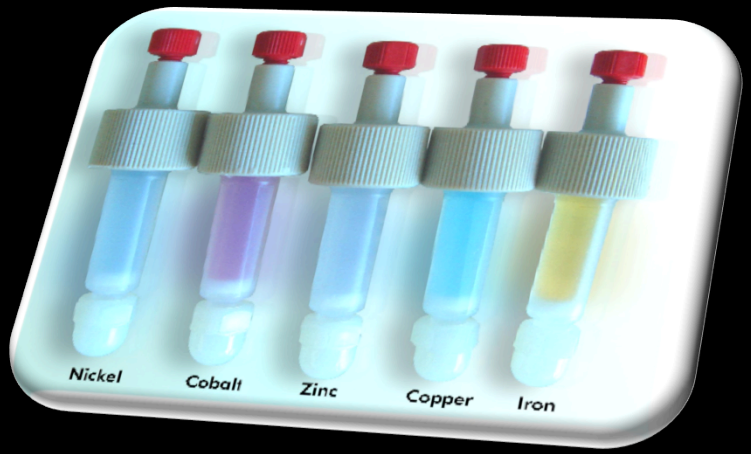
Why this work?

- Immobilized metal affinity chromatography (IMAC)
  -the most broadly used chromatographic methods
  (Block H et al., 2009)

HOW to enable IMAC purification of low abundance recombinant proteins from *E. coli* ?

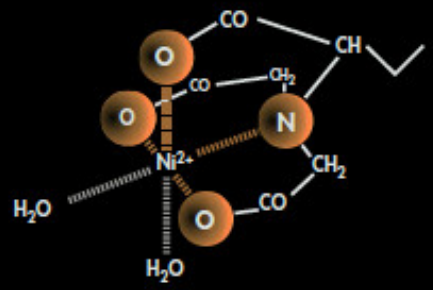
Background (Cont'd)

- First been shown by Porath et al. (1975)
  - affinity of transition metal ion (Zn^{2+}, Cu^{2+}, Ni^{2+}) to His



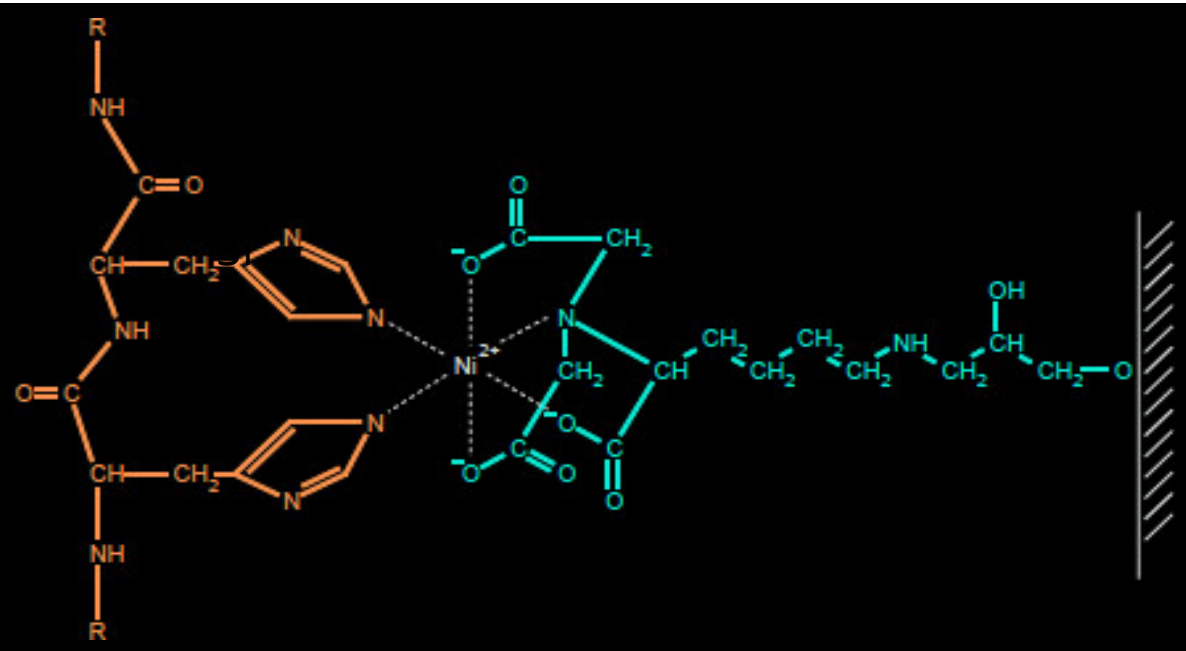
- Use the chelating ligand to fix the metal to agarose

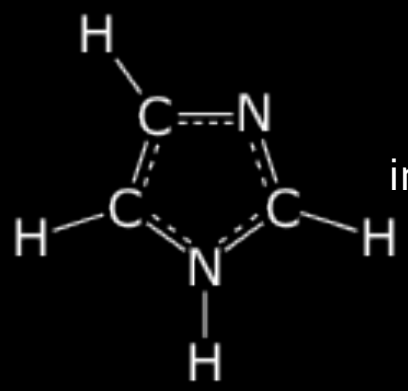
  Nitrilotriacetic acid, a tetradentate chelating ligand



Ni-NTA

Background (Cont'd)





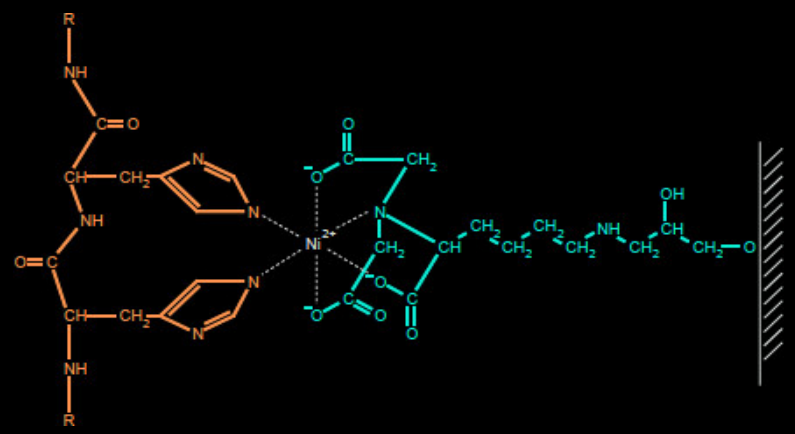
imidazole

- Biomass production
- Simplicity

Hypothesis

- Increasing culture size and thereby increasing tagged proteins do not result in increased yield

- Hypothesis: *E.coli* lysate induced a metal-ion leakage


R
NH
C=O
CH
CH2
N
NH
C=C
Ni2+
O
C
CH2
OH
CH
NH
CH2
CH2
CH2
CH2
CH2
CH2
CH2
CH2
CH
CH2
CH2
CH2
CH2
CH2
CH2
CH2
CH2

E.coli lysate caused extensive migration of His_6-GFP

- 1mL HiTrap Chelating HP column (GE Healthcare) (Ni-NTA with Sepharose)
- Soluble *E.coli* lysate without recombinant protein expression
- His_6-GFP

GFP load | Lysate loading | Wash 1 | Wash 2

Which component of *E.coli* lysate? The endogenous proteins in *E.coli* lysate?

- Separate the lysate using a sephadex G-25 column

High molecular weight (HMW) component

A strong peak at the 280nm absorbance spectrum

Low molecular weight (LMW) component

Other fractions after this peak

Low-molecular-weight components reduced the binding capacity

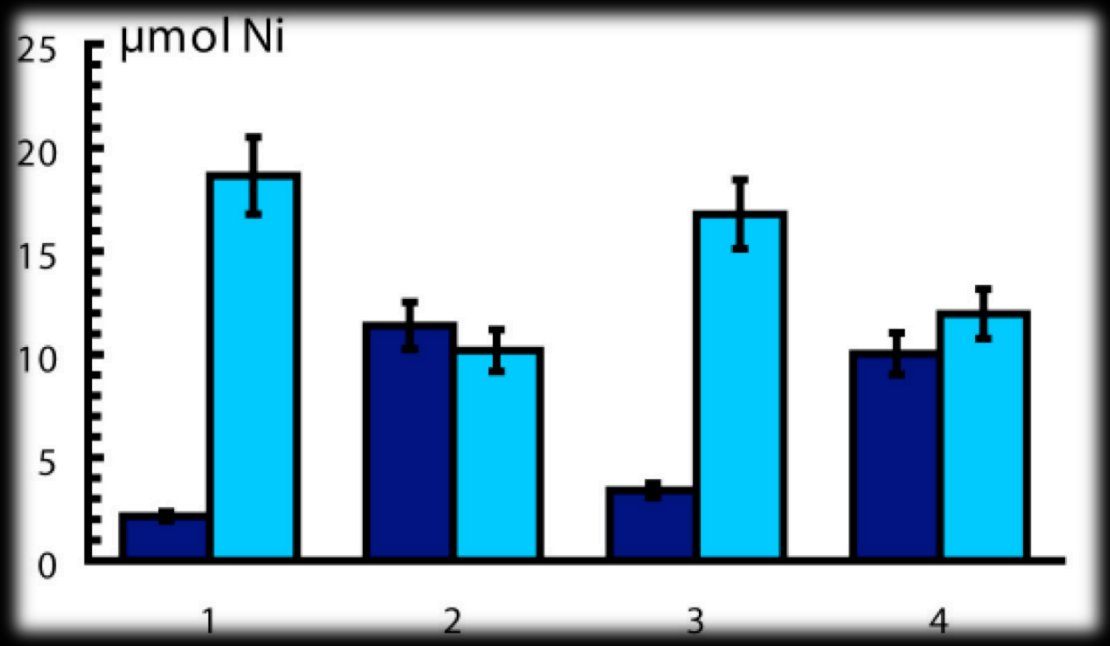
Probably not because of the Competition from the endogenous *E.coli* proteins

1. Wash buffer
2. Lysate
3. HMW components
4. LMW fraction

* Lysate supplemented with 2mM $FeSO_4$ (saturated potential metallophores)

Decrease in binding capacity correlated with loss of immobilized nickel ions

Nickel leakage analysis



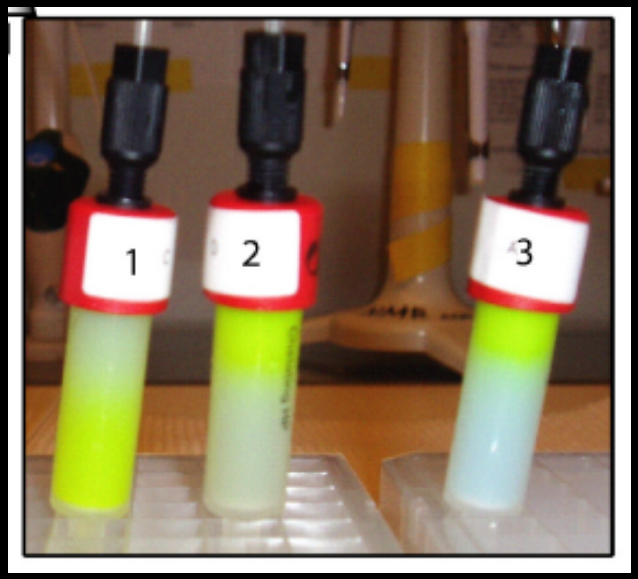
1. Wash buffer
2. Lysate
3. Lysate devoid of LMW components
4. LMW fraction

Dark blue-amount of nickel extracted
Light blue-amount of remaining nickel

The metal chelators in *E.coli* lysate?

- *E.coli* lysate contains chelators
  - dicarboxylic acids
    HOOC-R-COOH
  - metallophores (stressed condition)
    siderophores

- Metallophores would be mainly associated with the periplasmic space of *E.coli*



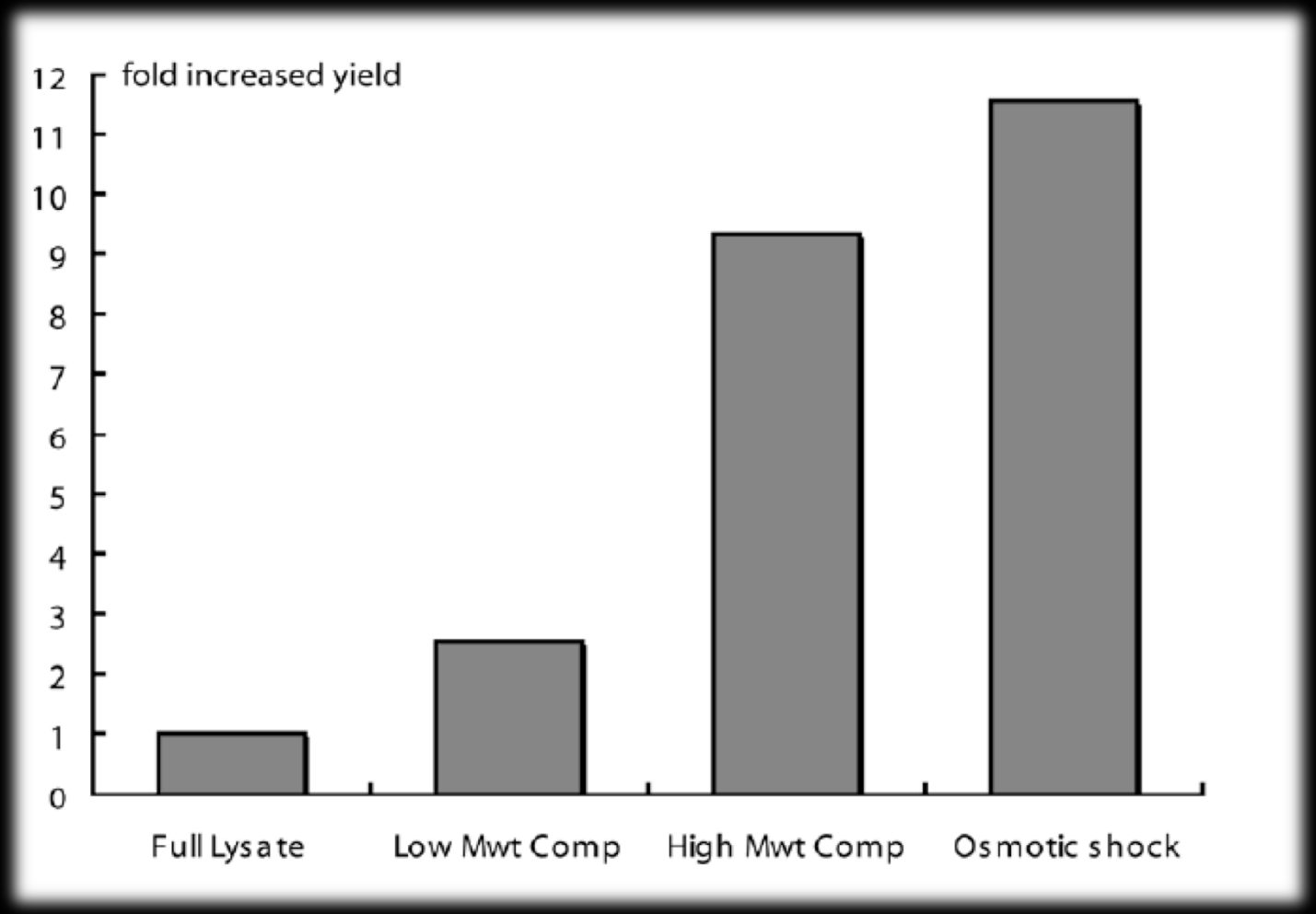
1. *E.coli* lysate
2. Lysate devoid of periplasmic material
3. Wash buffer

The metal chelators in *E.coli* lysate?

1. Wash buffer
2. Lysate
3. Lysate devoid of LMW components
4. LMW fraction

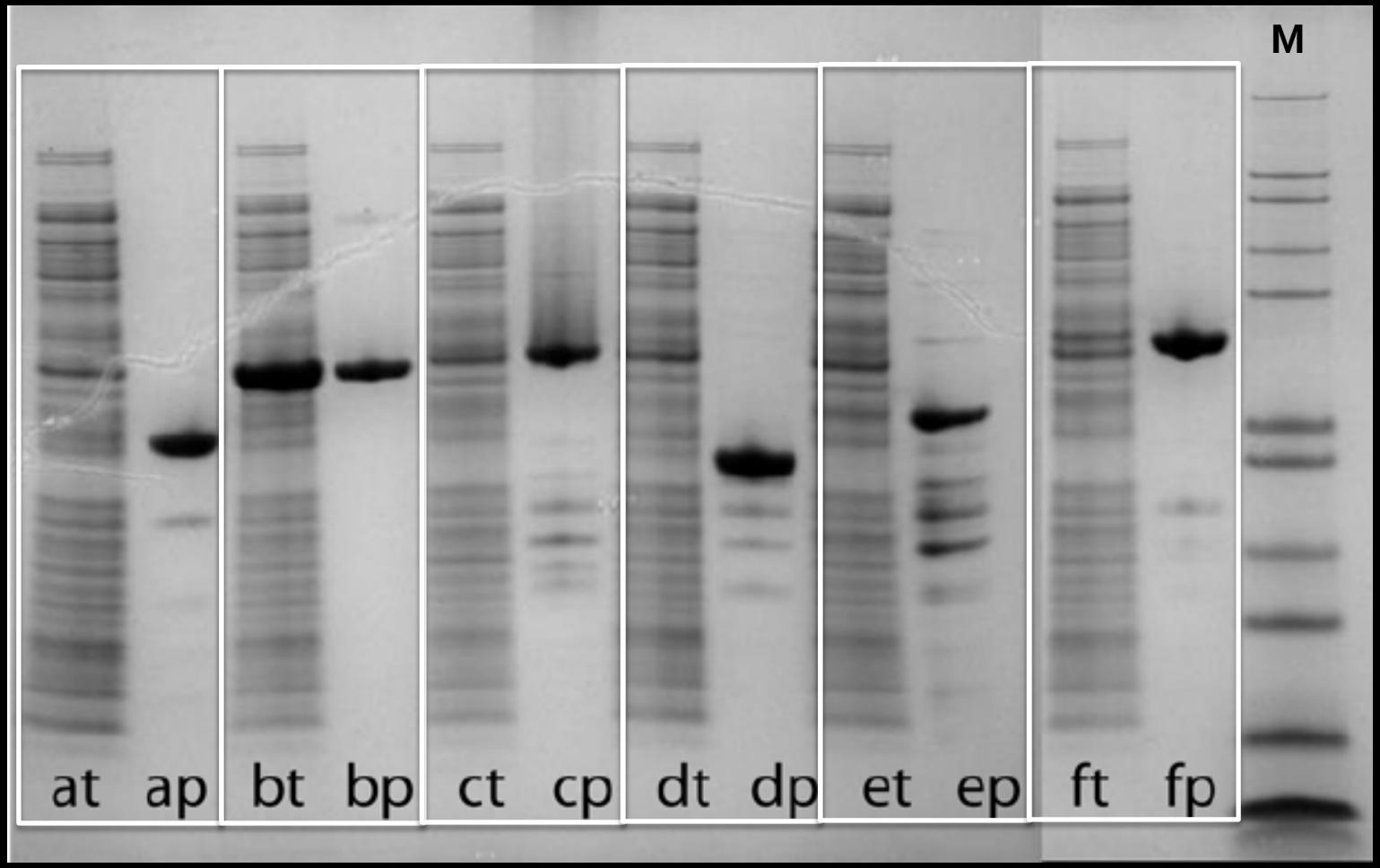
* Lysate supplemented with 2mM $FeSO_4$ (saturated potential metallophores)

Periplasmic LMW components of lysate reduce the binding capacity



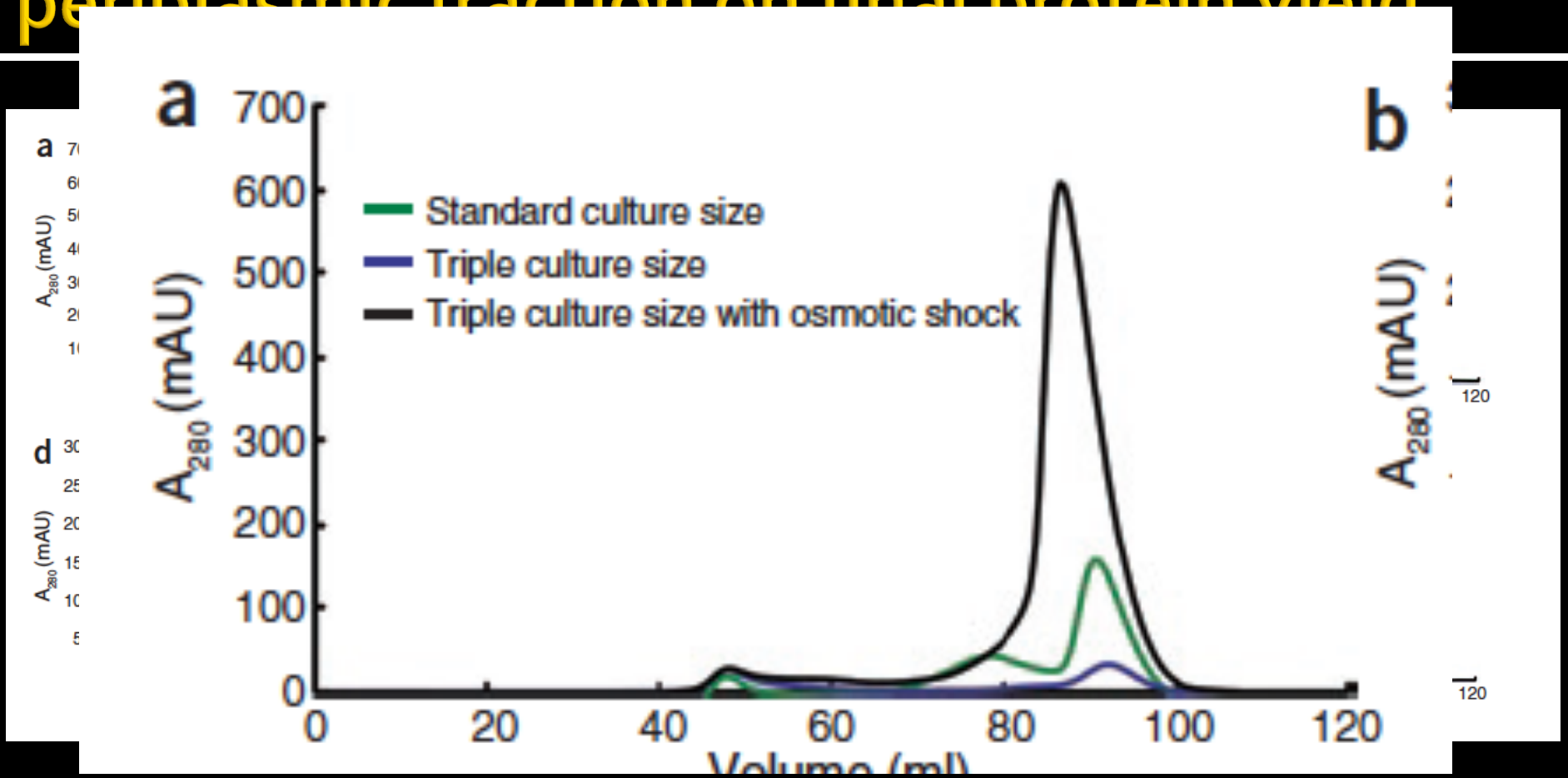
Tenfold increase of His_6-GFP

Improved target protein abundance



A-F: six different target proteins
t: soluble total protein p: purified protein

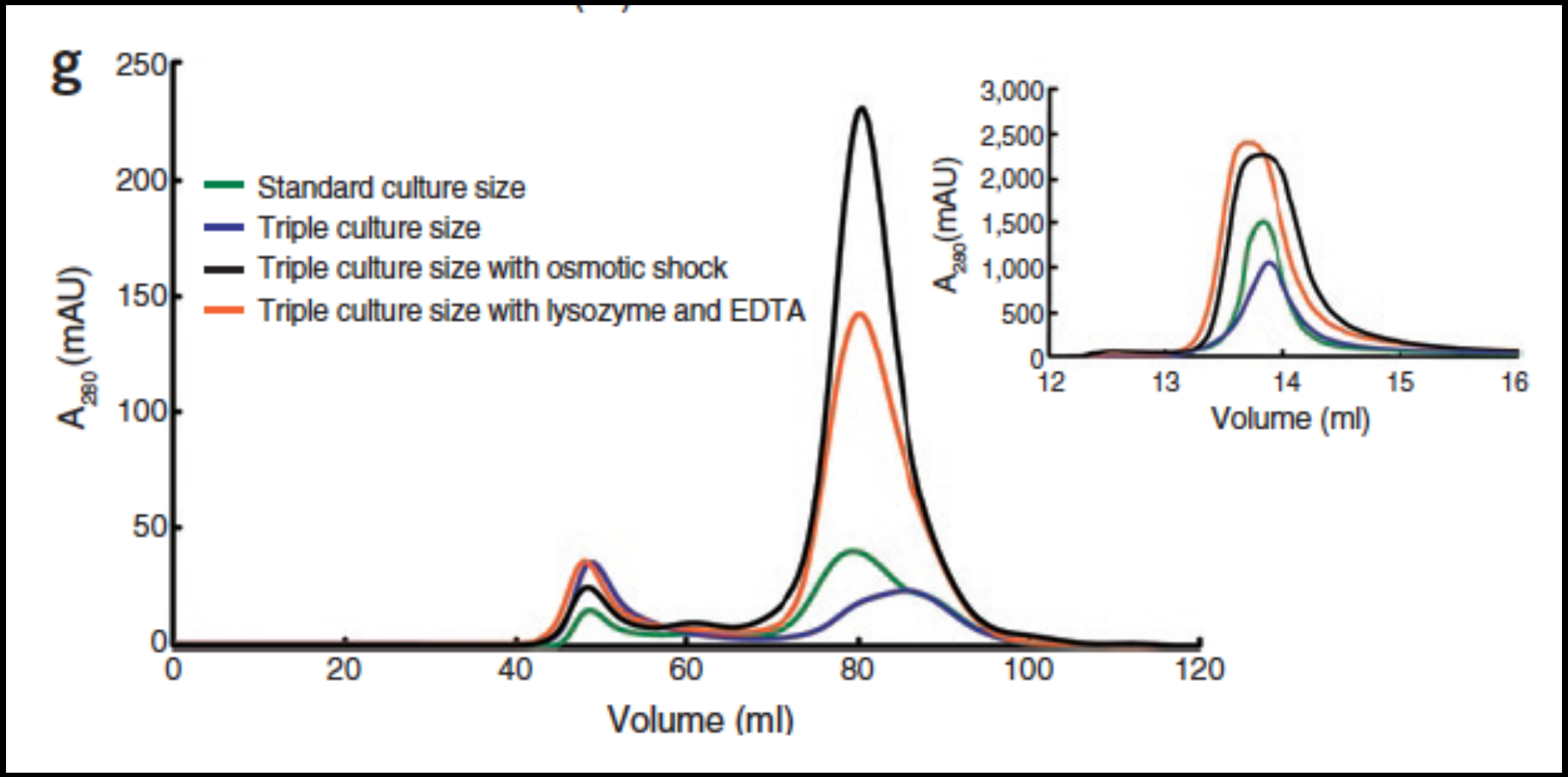
Effect of culture size and removal of periplasmic fraction on final protein yield



a. Glutaminase domain of GLS
b. ATPase domain of HSPA6
c. KH and DEAD domains of GLS
d. Glutaminase domain of CTPS2
e. C-terminal domain of GLE1
f. ACOT1

Chromatography from SEC

Remove the periplasmic fraction by lysozome-EDTA treatment



Kinase domain of FGFR3

Conclusions

- Metal chelator in the *E.coli* lyste caused the metal-ion leakage
- Removing the periplasmic material can increase the protein yield

Take-Home Message

- This method can be used for many proteins
- EDTA may be an inhibitor for certain metalloproteins
- A substantial scale-down of the whole purification setup

Thank You