US EPA ARCHIVE DOCUMENT

Nanoscale Biopolymers with Customizable Properties for Heavy Metal Remediation

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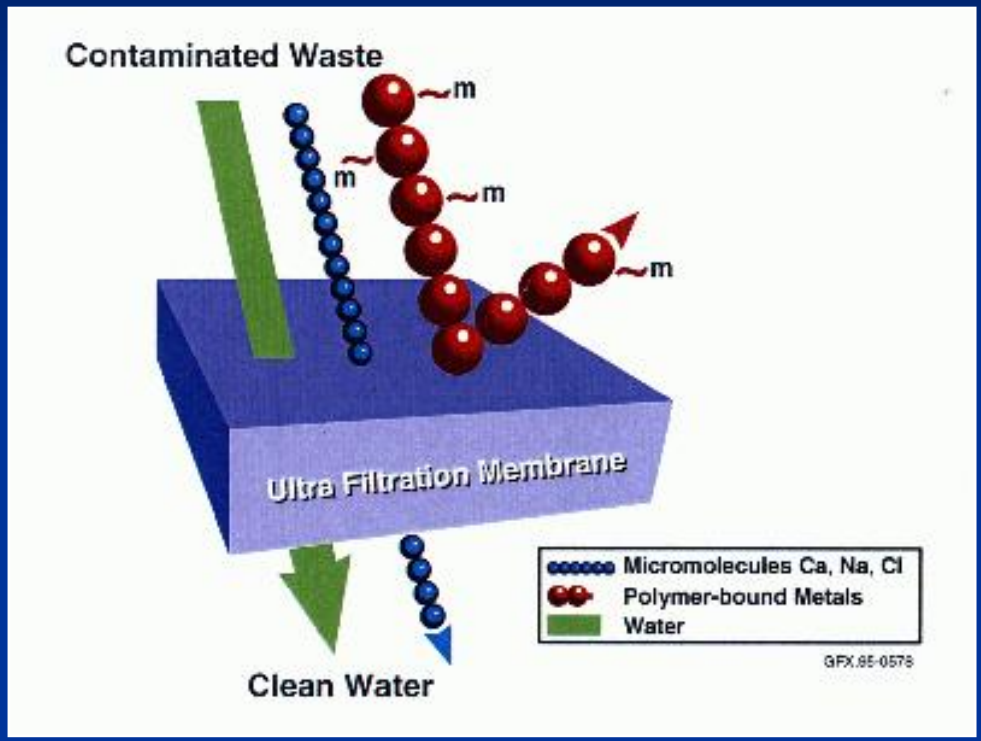
Heavy Metal Contamination

- 2.4 million tons of metal wastes per year from industrial sources
- 2 million tons per year from agriculture and domestic waste
- Pb^{2+}, Hg^{2+} and Cd^{2+} are ranked 2nd, 3rd and 7th, respectively, on the EPA's priority list

Metal Chelating Polymers



- Requires toxic solvents for synthesis
- Ultrafiltration is required
- Membrane clogging

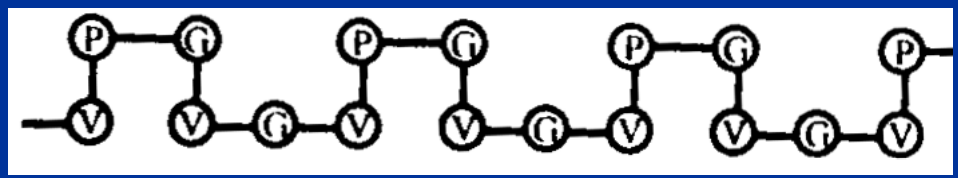
Solution: Develop metal-binding materials that can be recovered by environmental stimuli

Metal Chelating Biopolymers

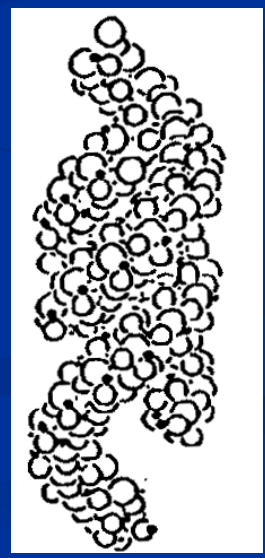
- Based on biological building blocks
- Nanoscale biopolymers that are specially pre-programmed within a DNA template
- Economically produced by bacteria
- Environmentally friendly
- **Tunable properties based on changes in environmental conditions – pH or temperature**

Elastin Biopolymer

- Structurally similar to the repeating elastometric peptide sequence of the mammalian protein, elastin
- VPGVG are the most frequently repeating units
- Undergo a reversible phase transition from water soluble forms into aggregates as the temperature increases

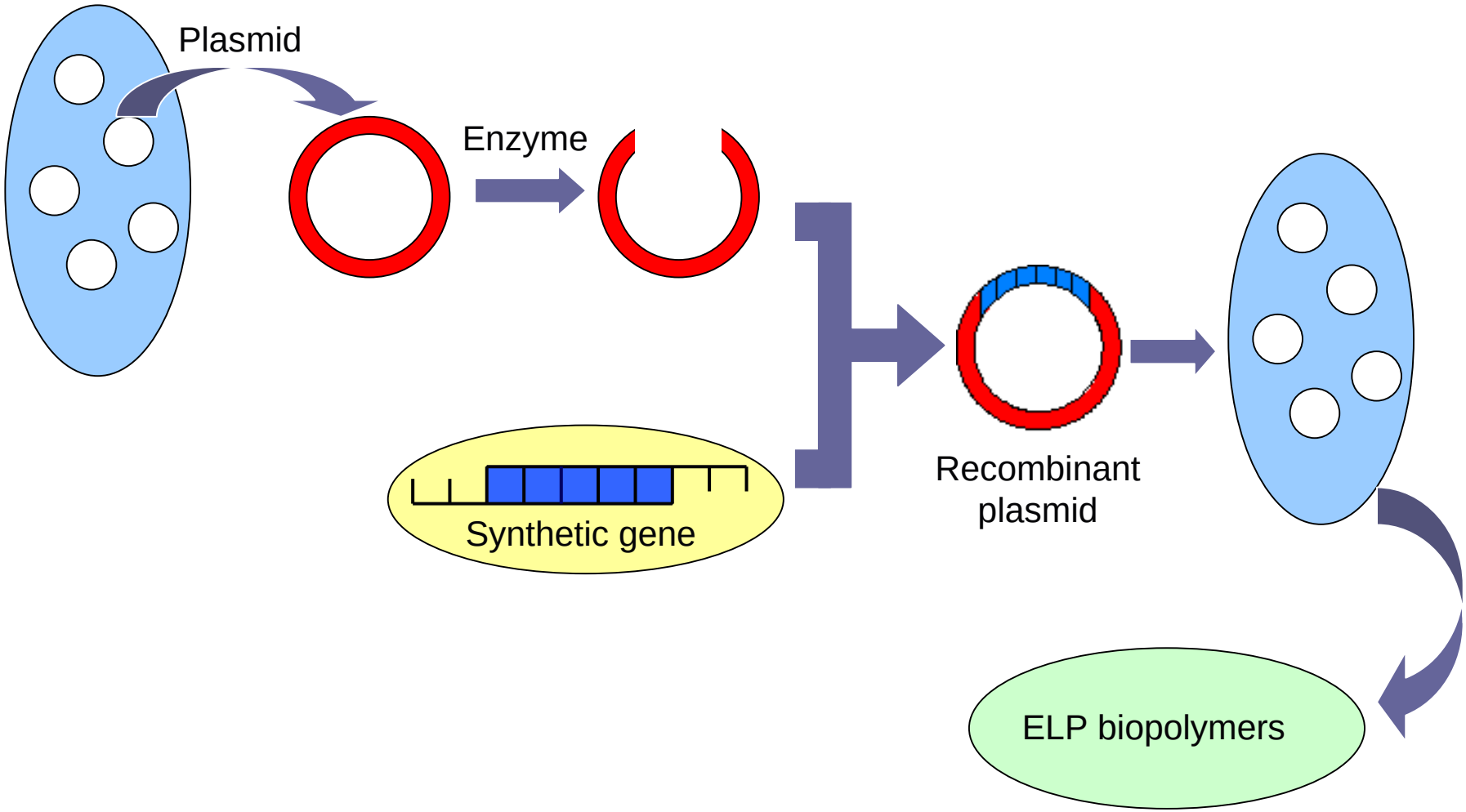


Low temperature: β-turn

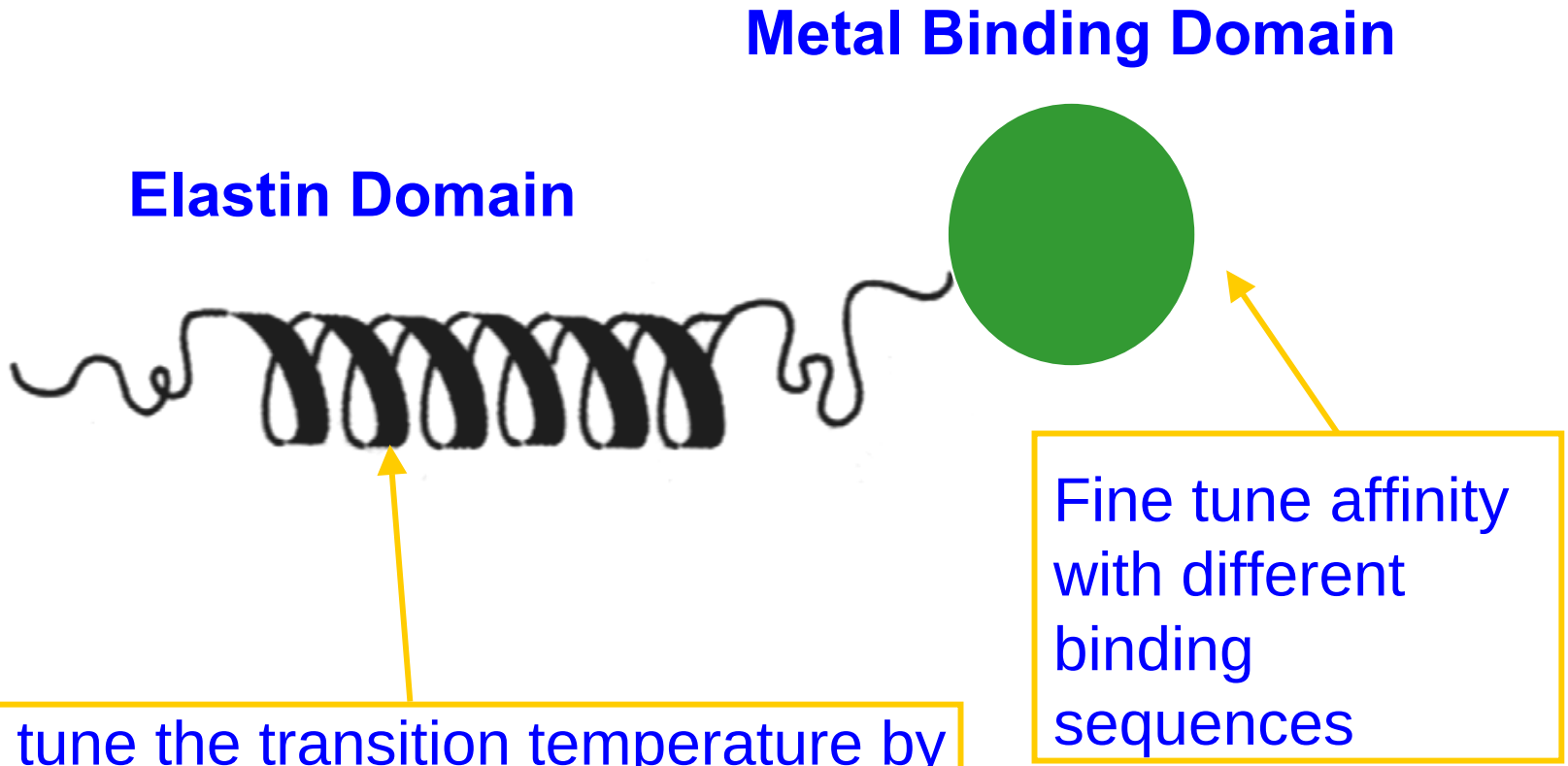


High temperature : twisted filament of β-spirals

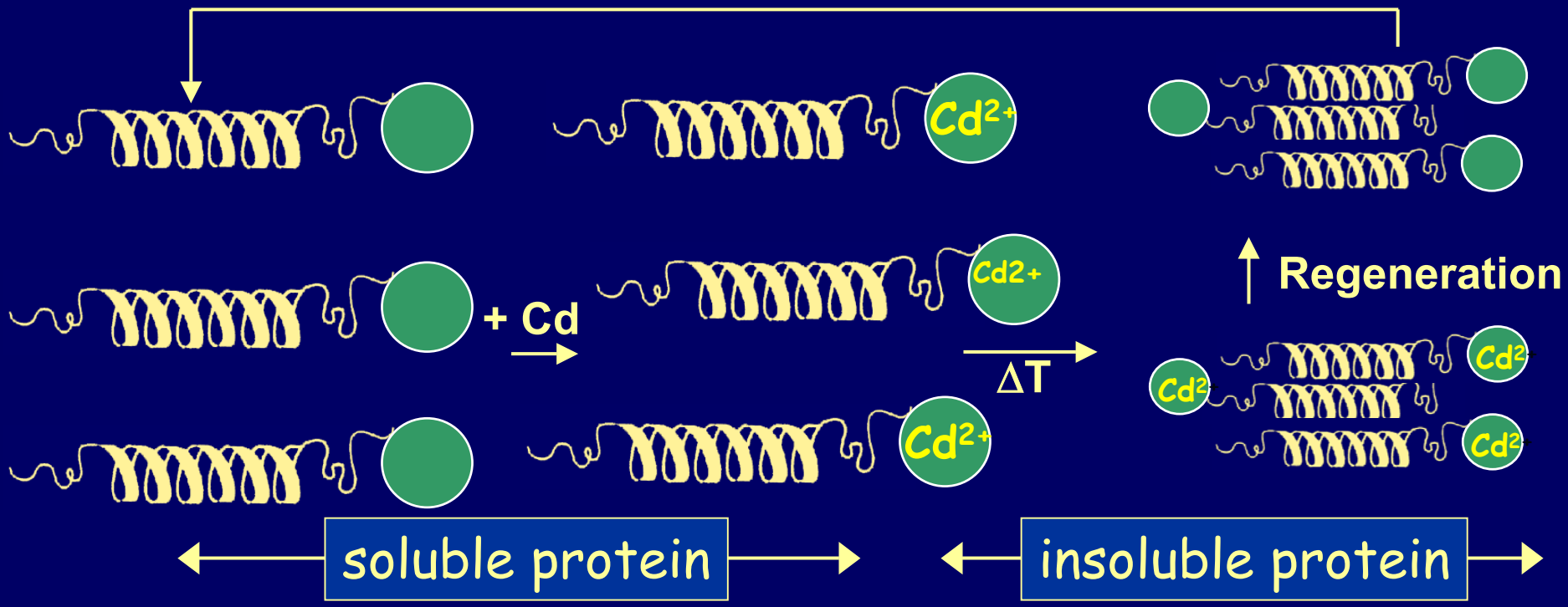
Genetic and Protein Engineering Methodology



Customizable Metal Binding Biopolymers



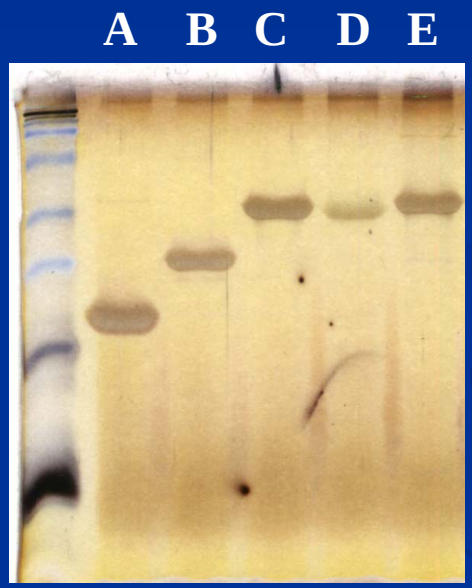
Heavy Metal Removal by Tunable Biopolymer



Elastin Biopolymers with Metal-Binding Affinity

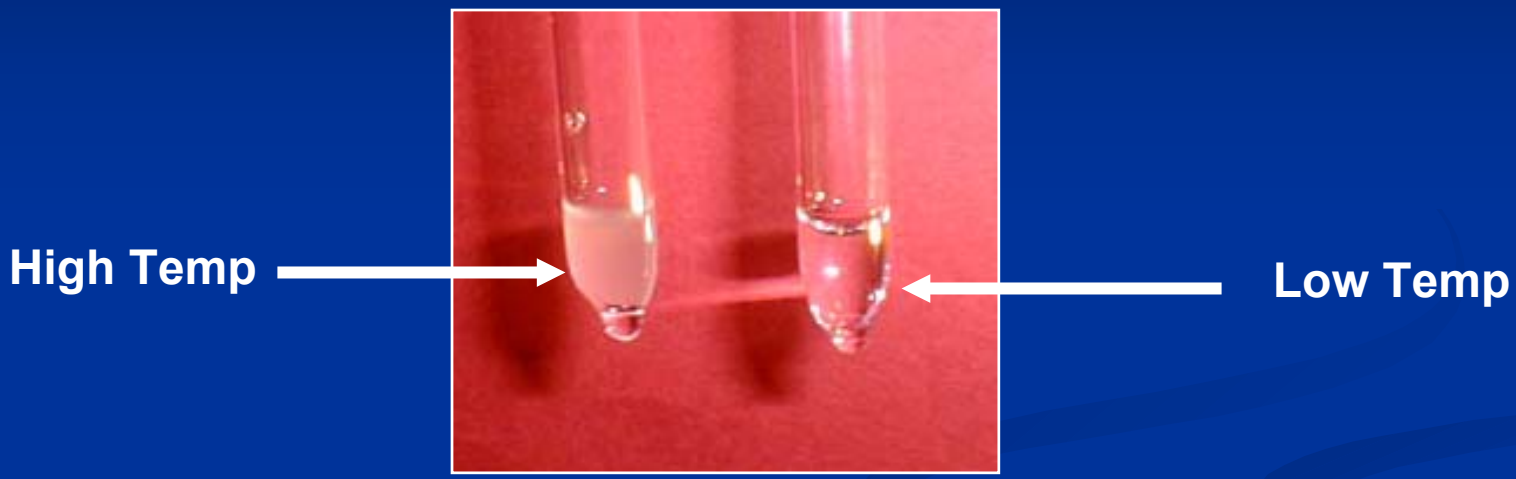
Biopolymer

A. ELP38H6
B. ELP58H6
C. ELP78H6
D. ELP78
E. ELP78H12



Kostal et al. *Macromolecules*, 34, 2257-2261, 2001.

Phase Transition



Transition temperatures can be fine tuned from 20 to 40^{o}C by controlling the chain length and salt concentration

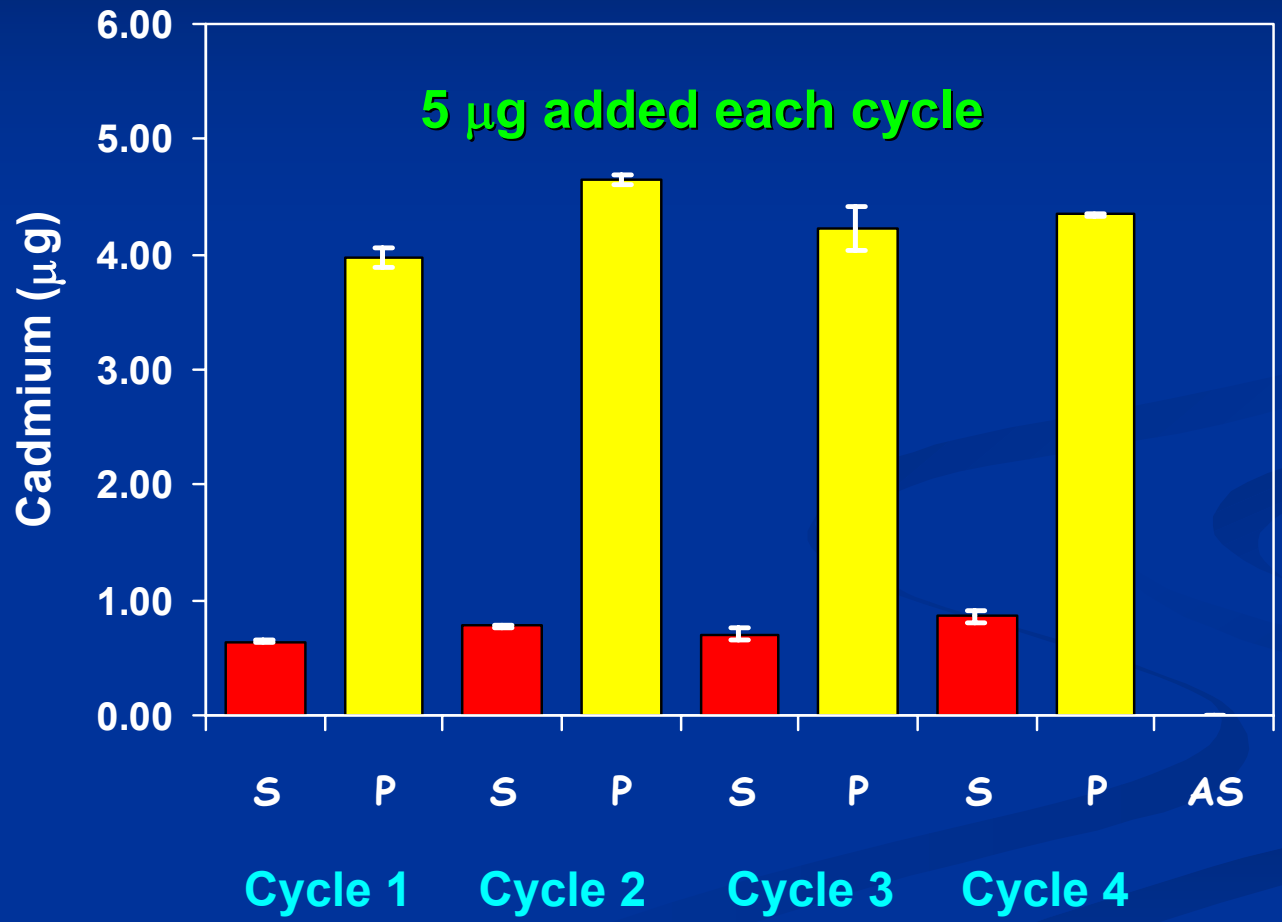
Cadmium Removal

Biopolymer	Cd^{2+} binding (mol protein : mol Cd^{2+} ± SD)
Ela38H6	1 : 1.04 ± 0.04
Ela58H6	1 : 0.97 ± 0.01
Ela78H6	1 : 0.95 ± 0.01
Ela78	1 : 0.01 ± 0.00
Ela78H12	1 : 1.50 ± 0.02

SD - standard deviation

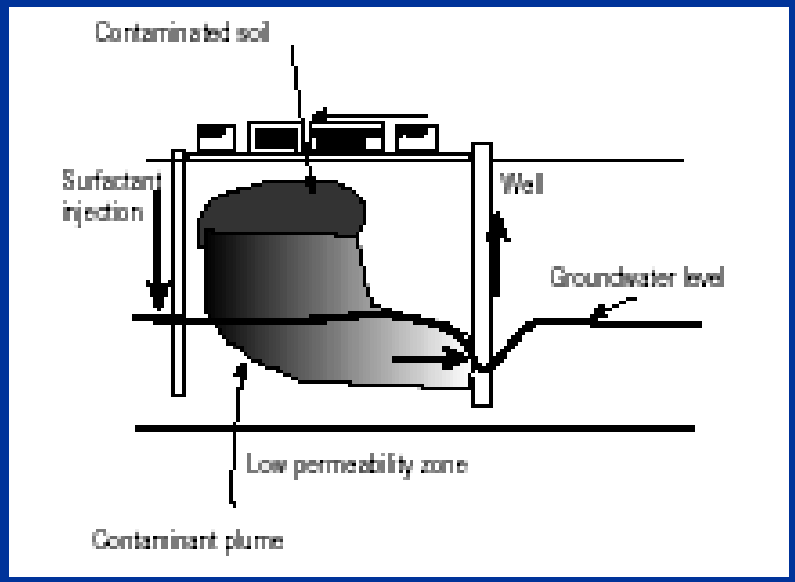
50 nmol of biopolymer were incubated with 100 nmol Cd^{2+}

Repeated Cycles of Metal Binding

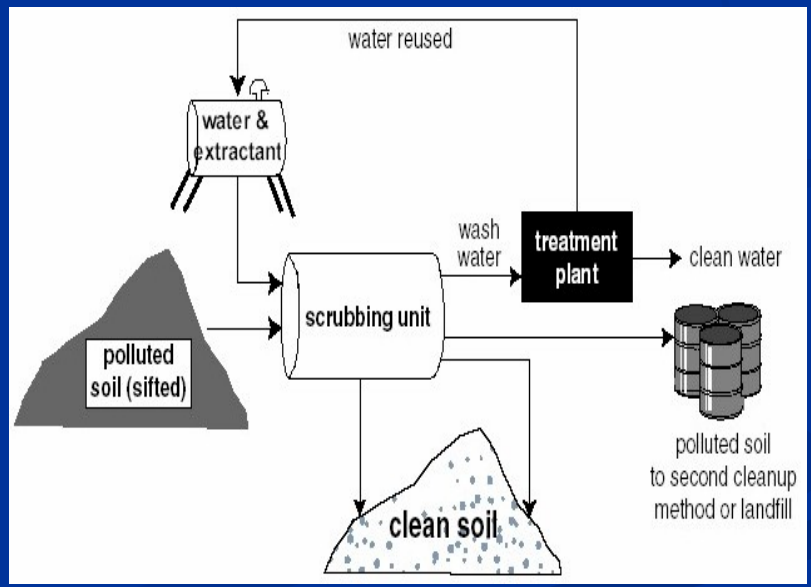


Practical Applications: Soil Flushing/Washing

- Environmental friendly extractant
- Simple separation and recycling



In-situ Soil Flushing

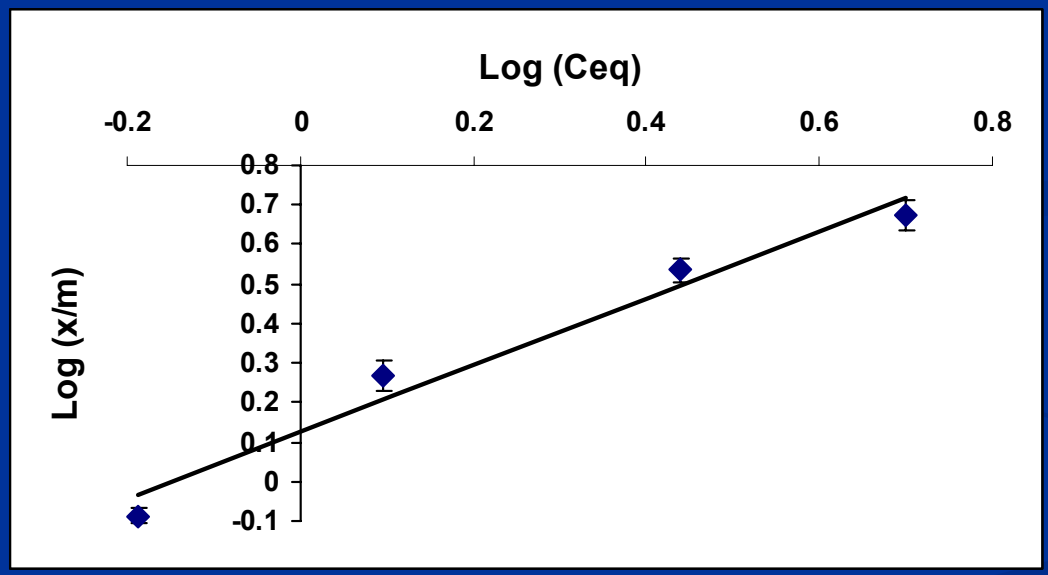


Ex-situ Soil Washing

Soil-Biopolymer Adsorption Characteristics

- **Soil-Biopolymer Sorption Characteristics**
  - Freundlich isotherm

$$\text{Log } x/m = \text{Log } K_f + 1/n \text{ Log Ceq}$$

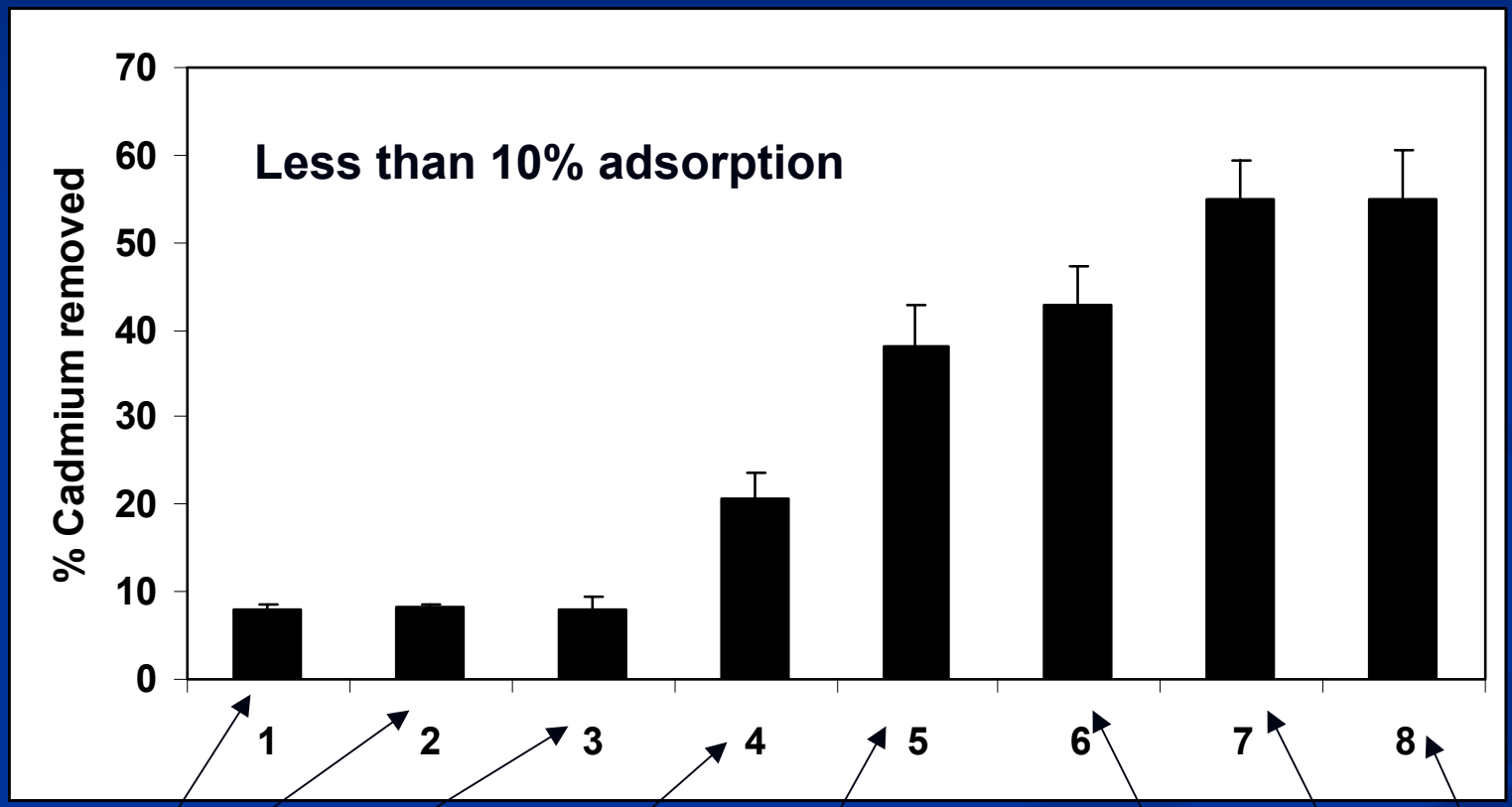


Maximum 19% adsorption

Biosurfactant – typically in the 70% range

Prabhukumar et al. *ES&T*, 38, 3148-3152, 2004.

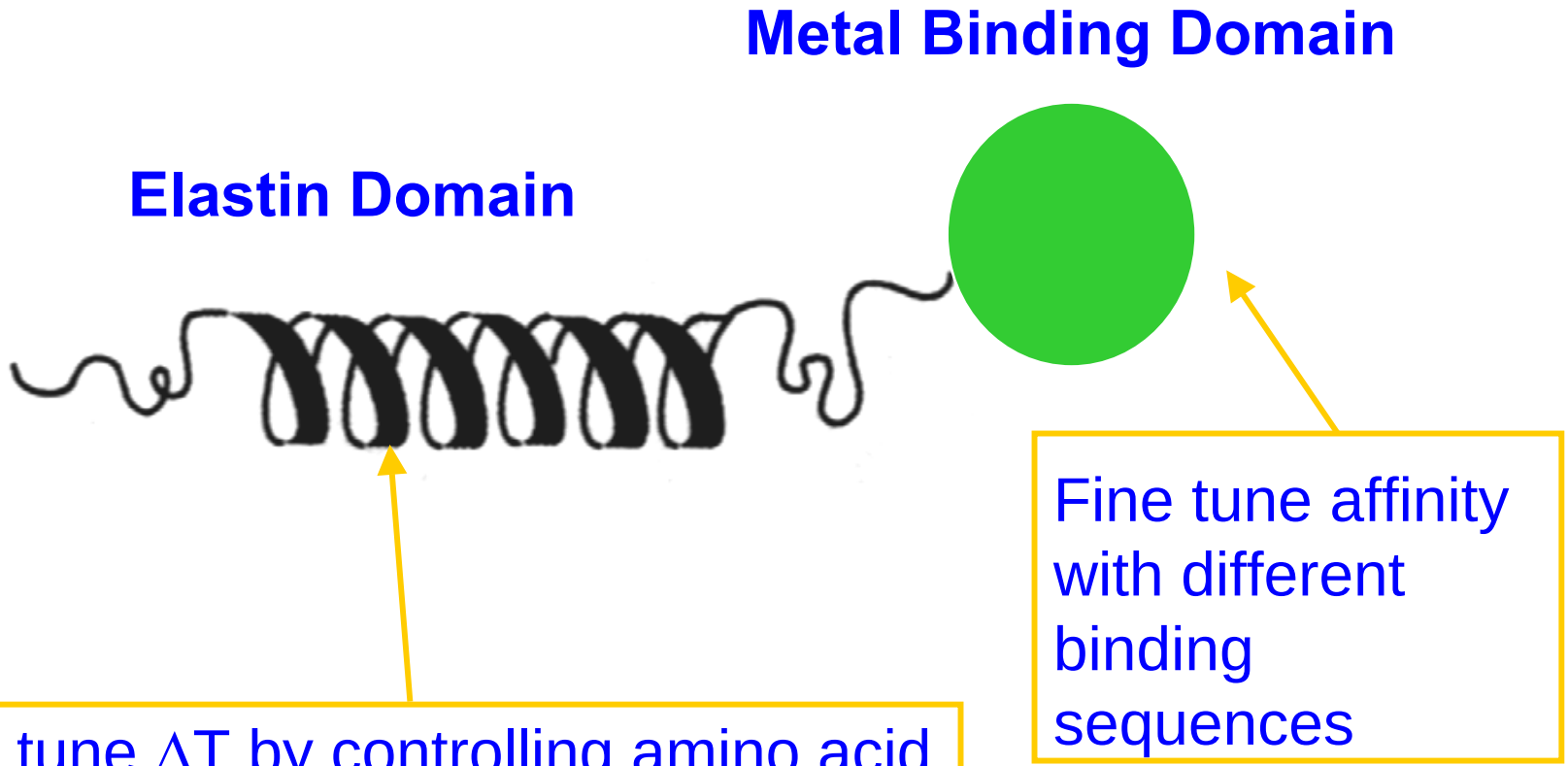
Biopolymer-Cadmium Extraction Studies



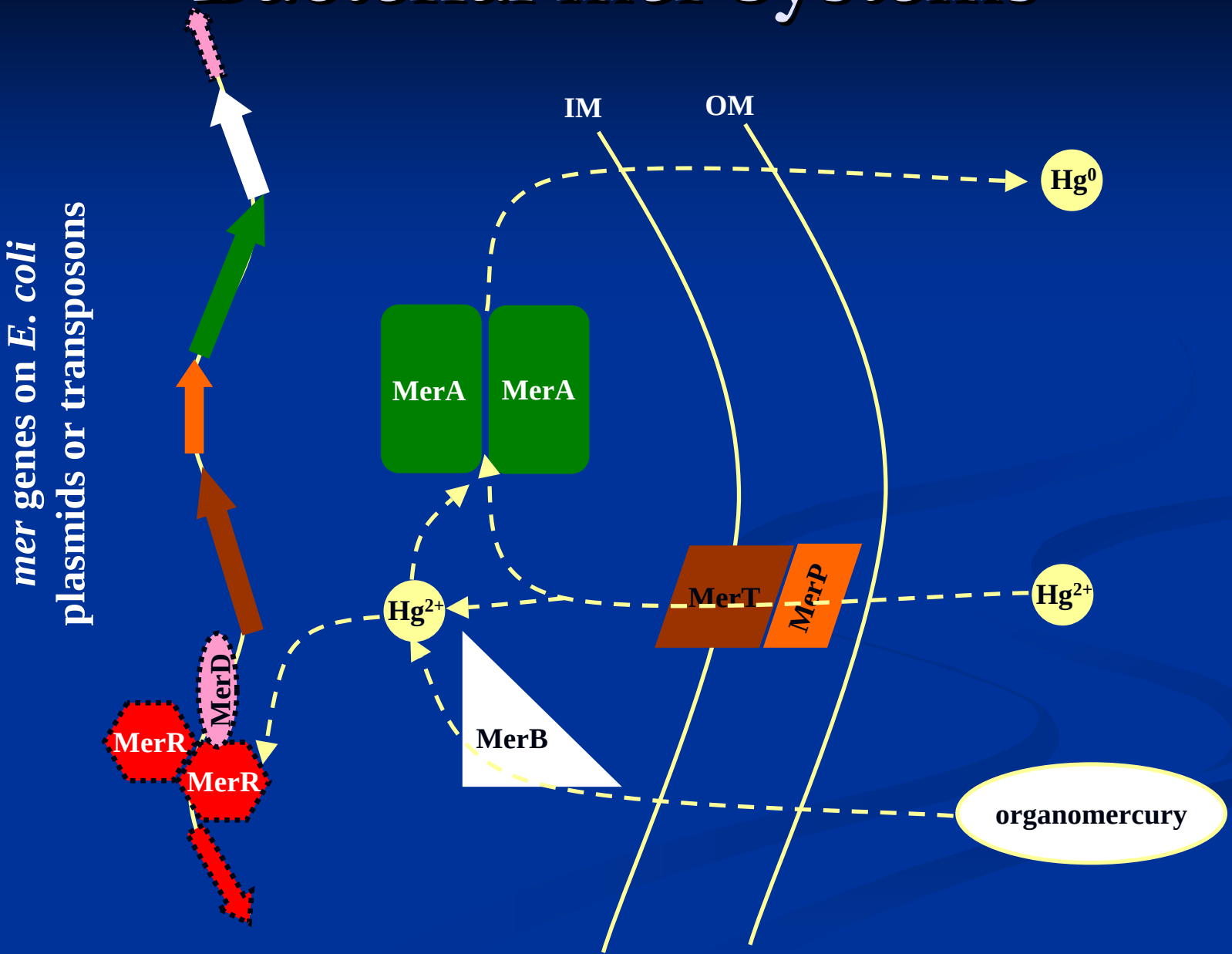
First generation biopolymers

- His_6 or His_{12} tag can serve as a simple metal binding domain
- Both T_t and metal binding capacity can be easily regulated
- Biopolymers can be recycled
- Lack specificity and affinity

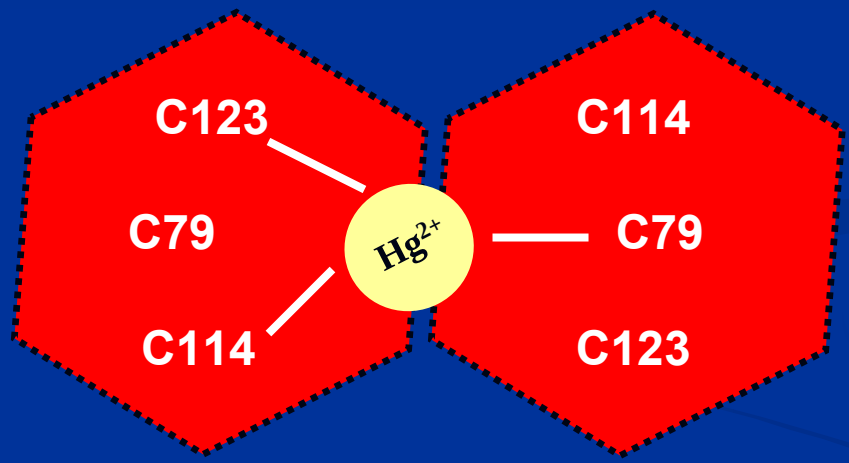
Customizable Metal Binding Biopolymers



Bacterial *mer* Systems

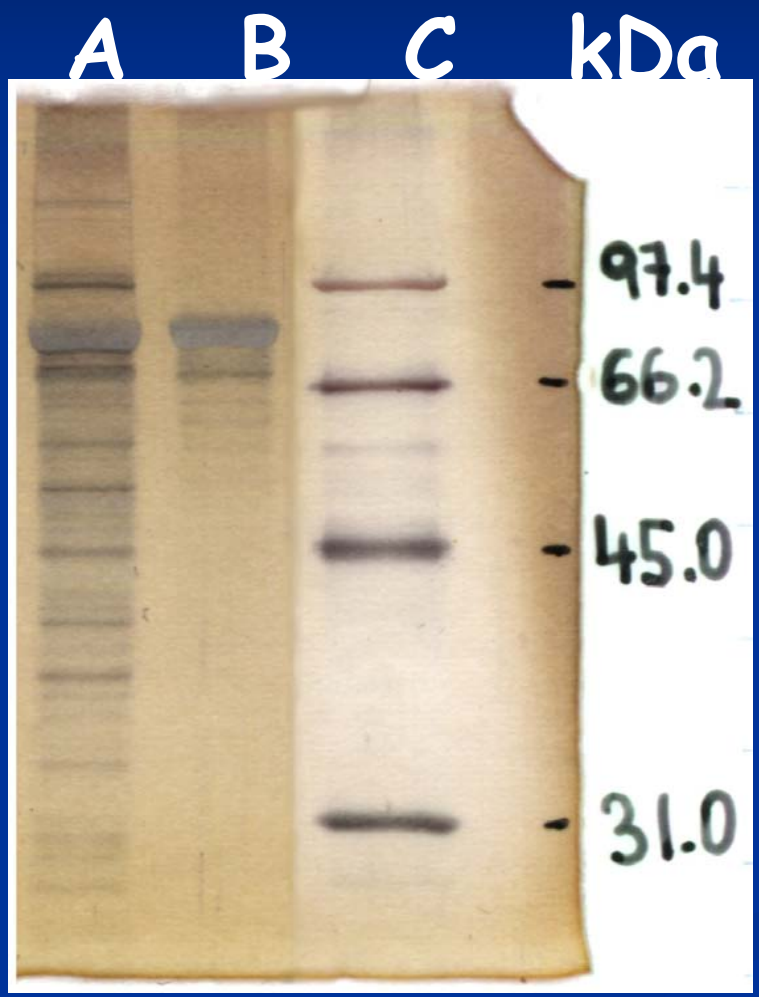


MerR can serve as a specific mercury binding domain



MerR-Hg complex

Production and purification of ELP153-MerR

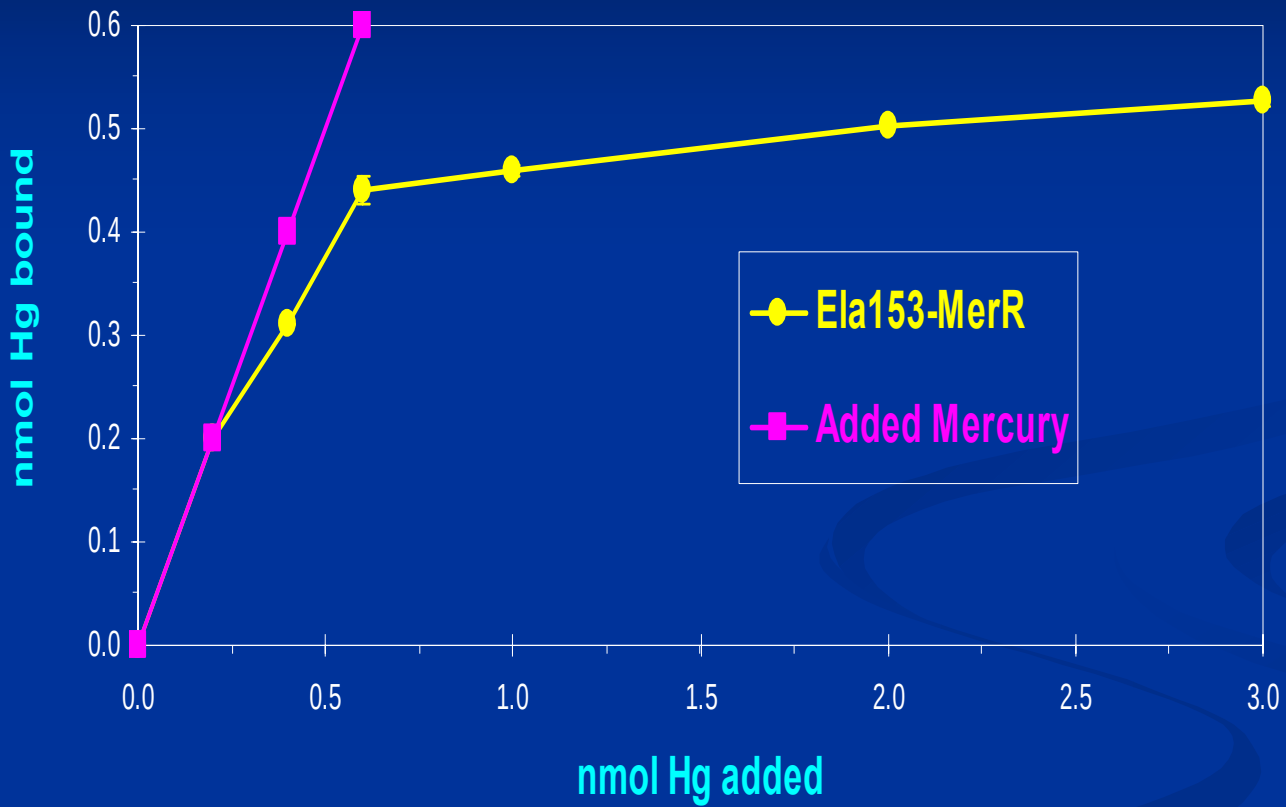


A, cell free extract

B, purified protein (80.1 kDa)

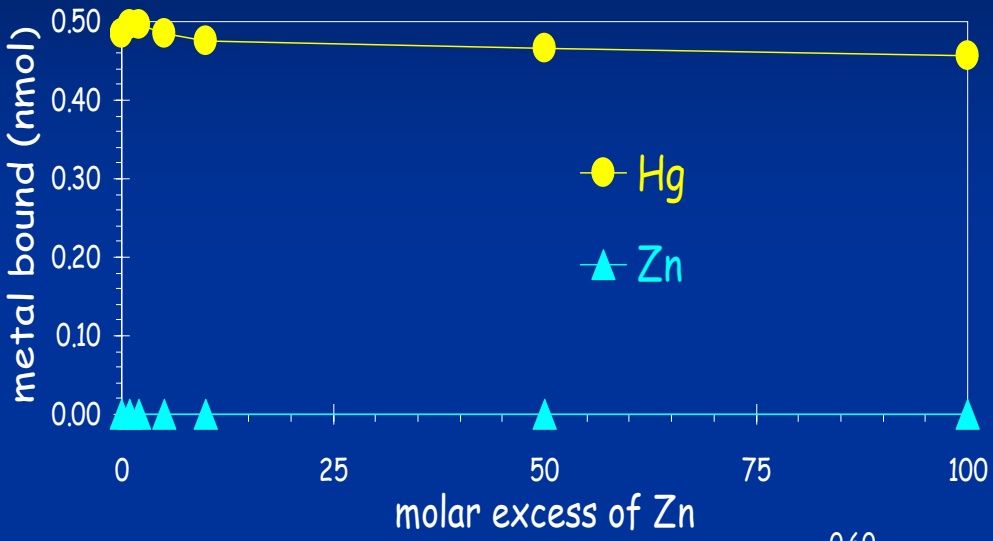
C, size marker

Binding of Mercury to ELP153-MerR



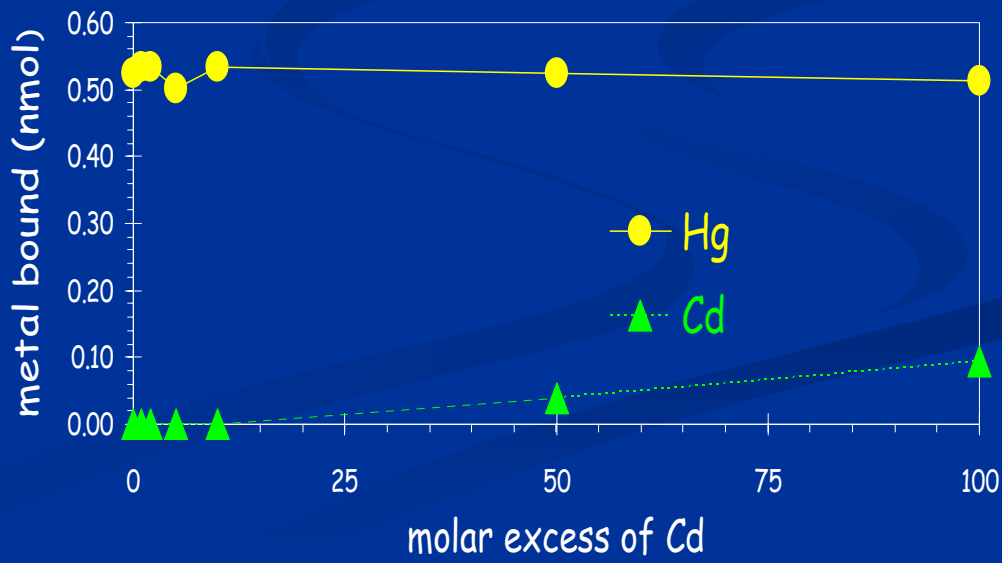
Similar binding from pH 4 to 9

Selective Binding of Mercury by Ela153-MerR Biopolymer



Acidic waste water (pH 4)

Kostal et al. *ES&T*, 37, 4457-4462, 2003.



Other Metal-binding Domains

Metallothioneins (MTs)

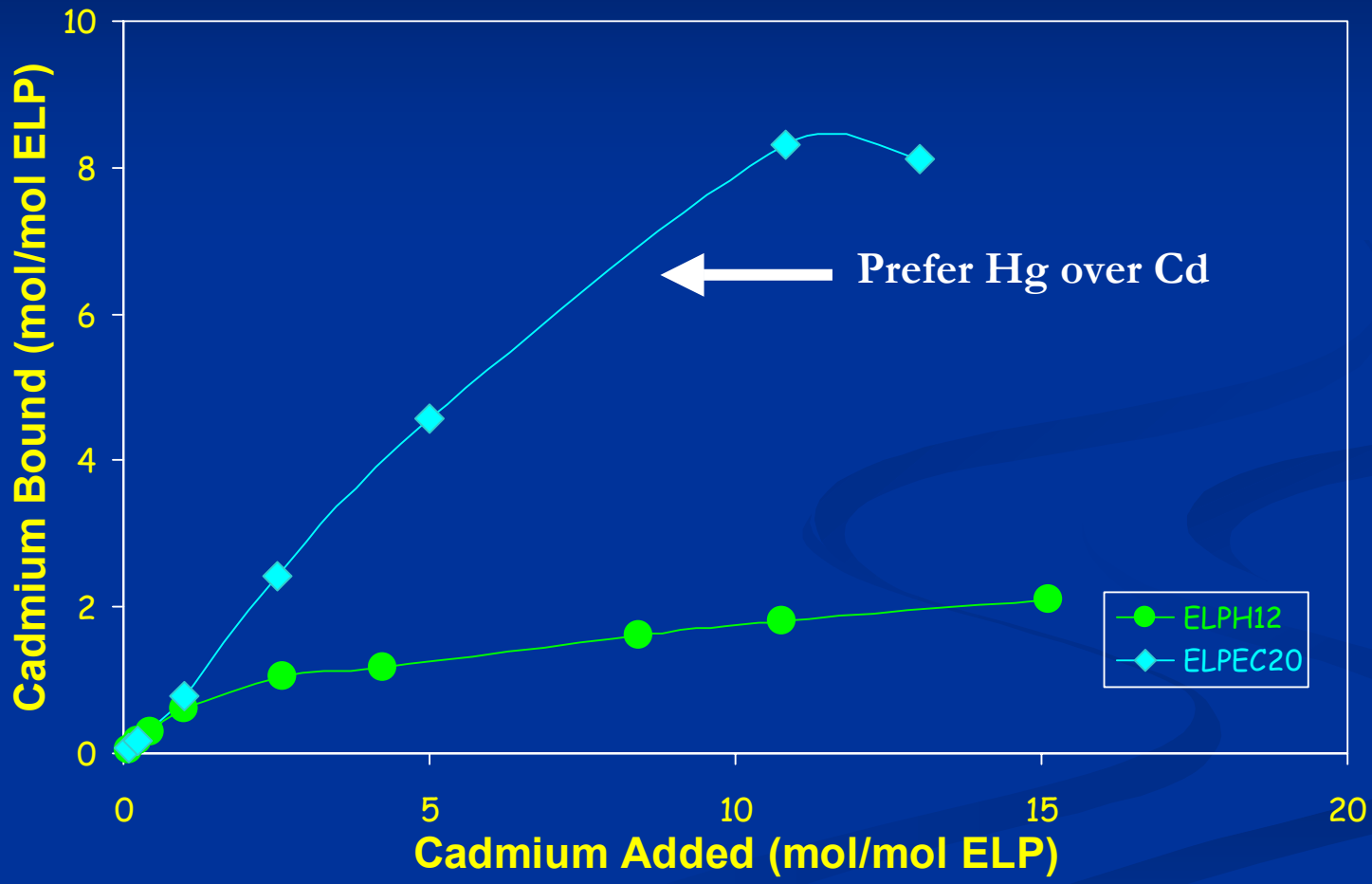
MDRNCSCAACDSCTCAGSCKCKECK
CTSCKKSCCSCCPVGCAKCAQGCICK
GASDKCSCCA

Synthetic Phytochelatins (ECs)

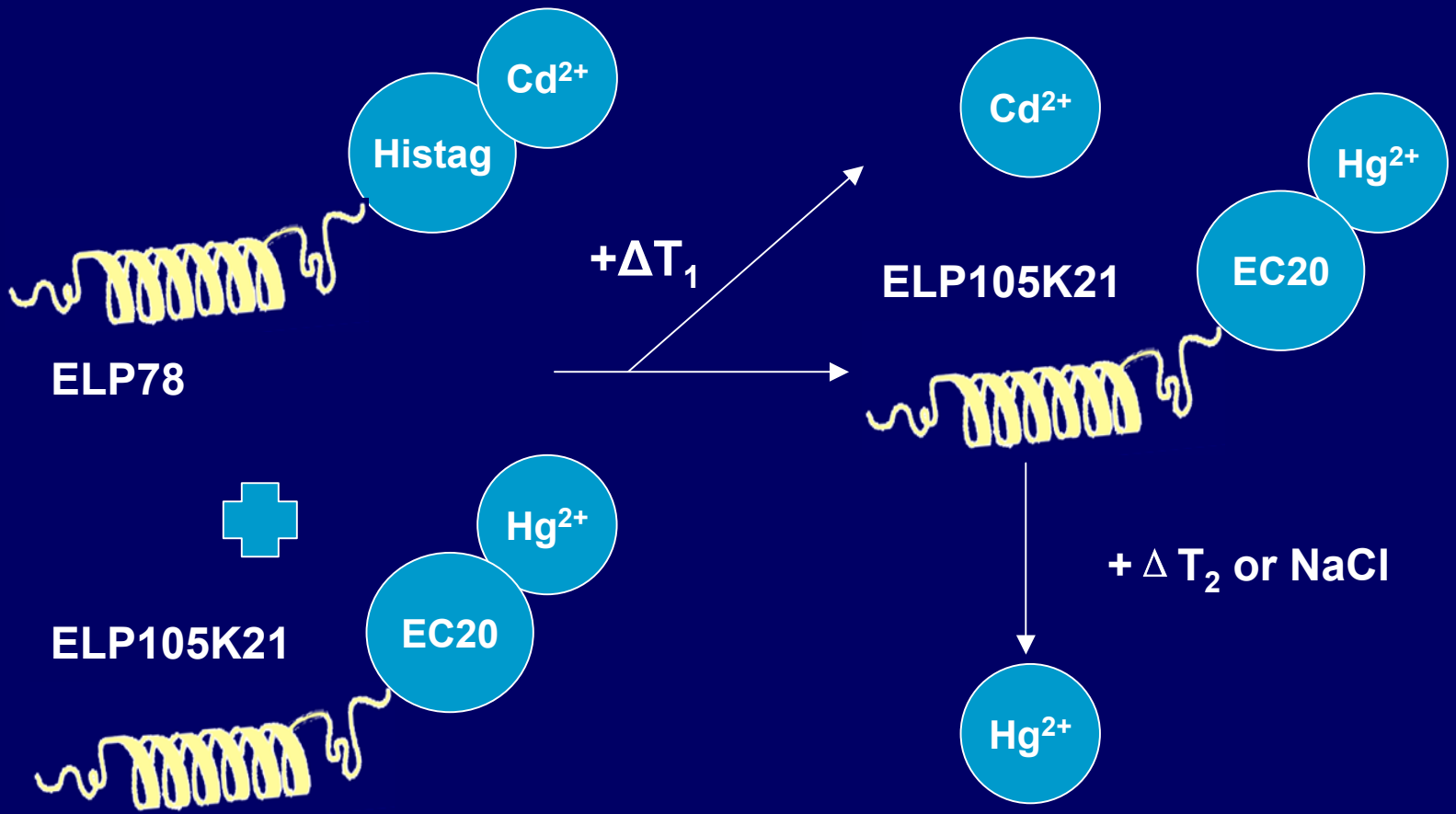
γECECECECECECECECECECECEC
ECECECECECECECG

Higher affinity toward Hg and Cd

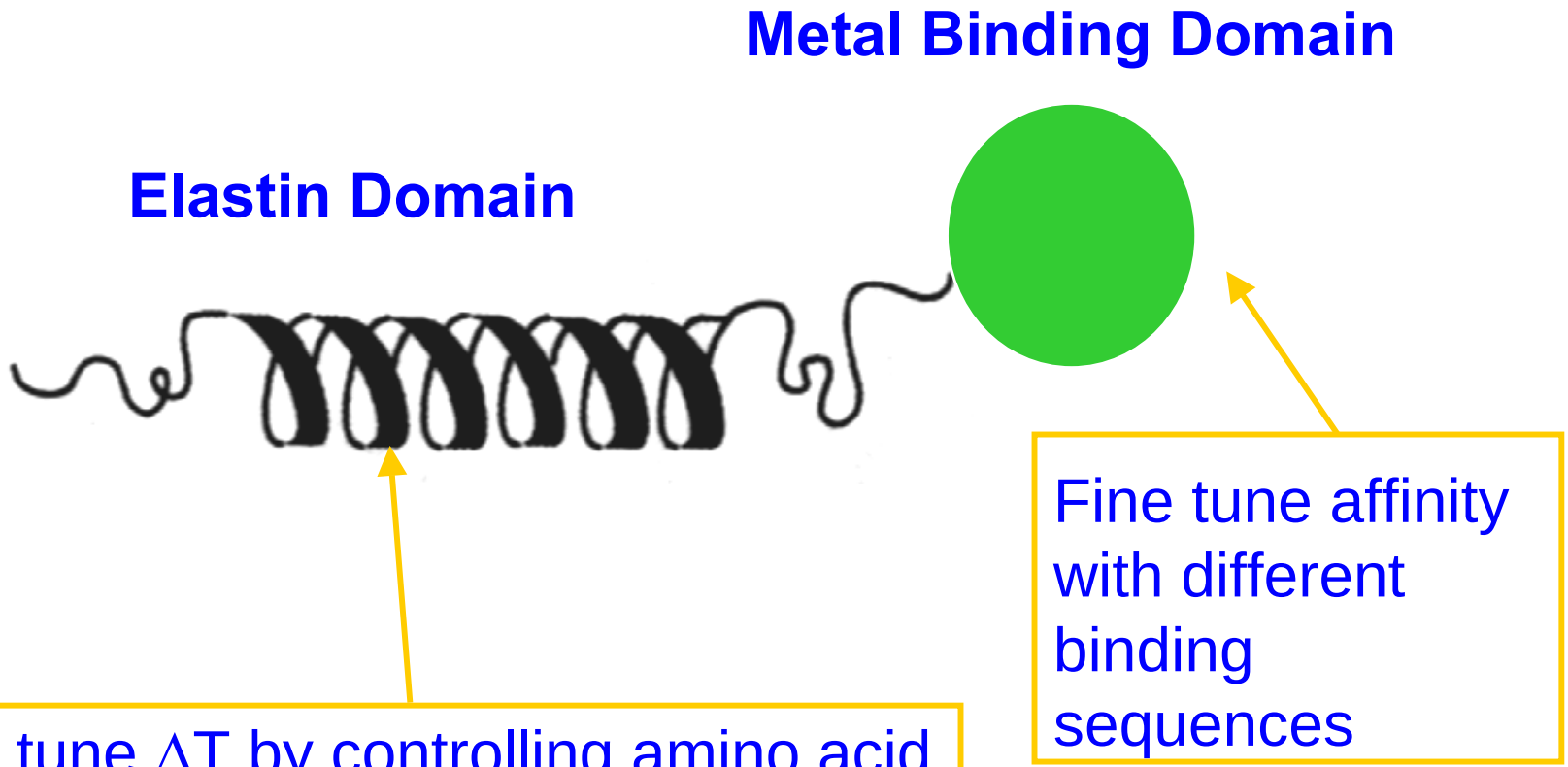
Cadmium Binding



Differential Precipitation and Separation – Strategy

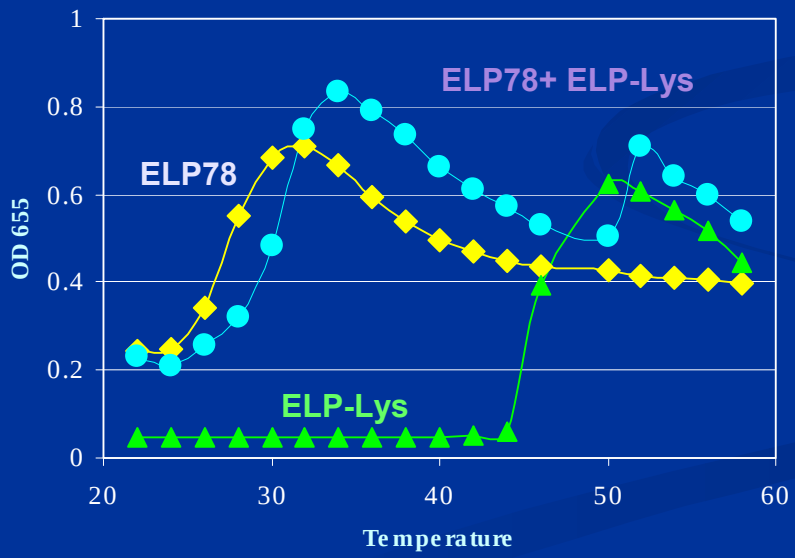


Customizable Metal Binding Biopolymers

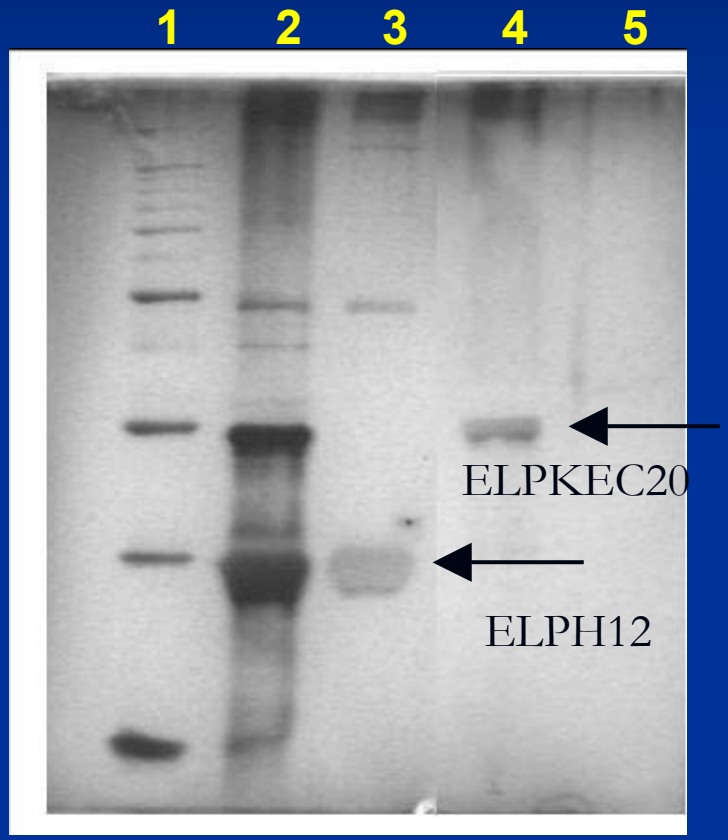


Differential Precipitation and Separation

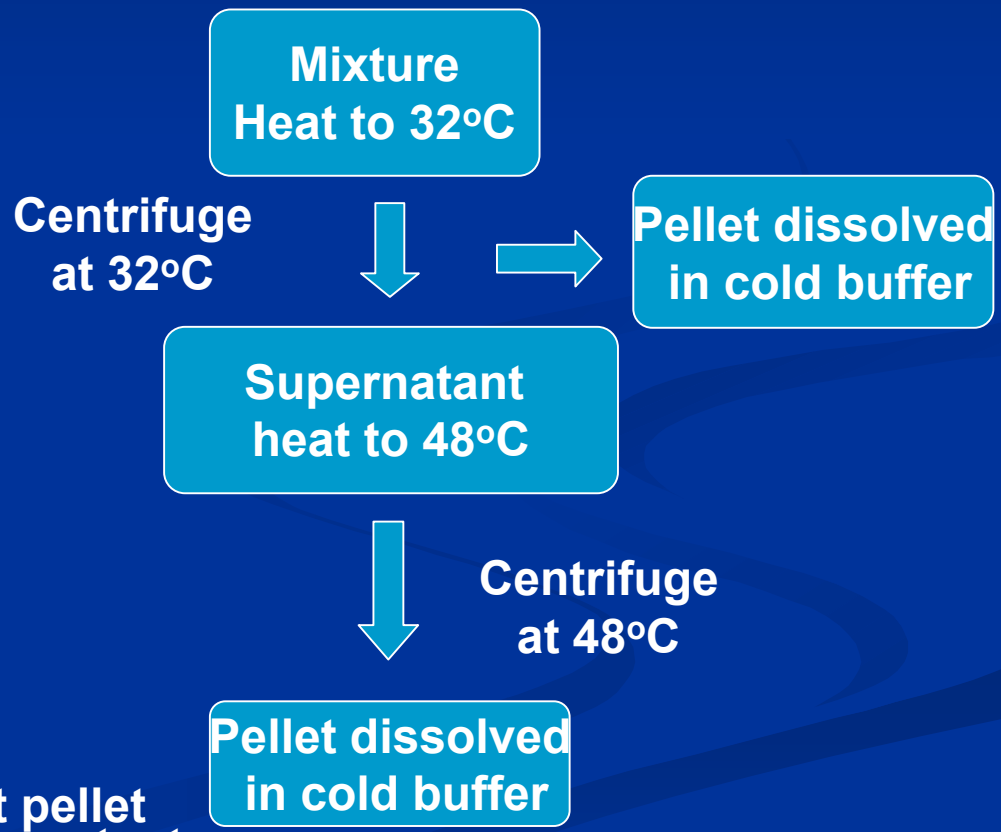
- Differential precipitation and recovery by tuning the elastin composition: Lysine as the guest residues



Differential Precipitation and Separation



1) Marker.
2) Biopolymer mixture
3) 32 °C heat spin pellet
4) 48 °C heat pellet
5) 48 °C supernatant



Conclusions

- Nanoscale ELP biopolymer can be designed with customized properties:
  - Specificity
  - Tunable transition
- Provide a novel, environmental friendly, and green engineering method for removal of heavy metal from water and soil.

Acknowledgement

This research is funded by

U.S. EPA - Science To Achieve Results (STAR) Program

Grant # R829606

Exploratory Research: Nanotechnology