

Phosphoserine-rich Protein
from

Phragmatopoma californica

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Herbert Waite

NIH, NSF, NASA

Why Study This Worm?

- ◆ Underwater adhesive
 - ◆ Glue adheres to eggs shells, glass, teeth
 - ◆ Find out HOW adhesion happens under water

Tube Worm



Phylum Annelida

Class Polychaeta

Order Terebellida

Family Sabellariidae

Derivation: Sabell-sand(L); -aria - to dwell "sand dweller"

Distribution: Europe 3, East coast 2, California 1, Indian Ocean 1





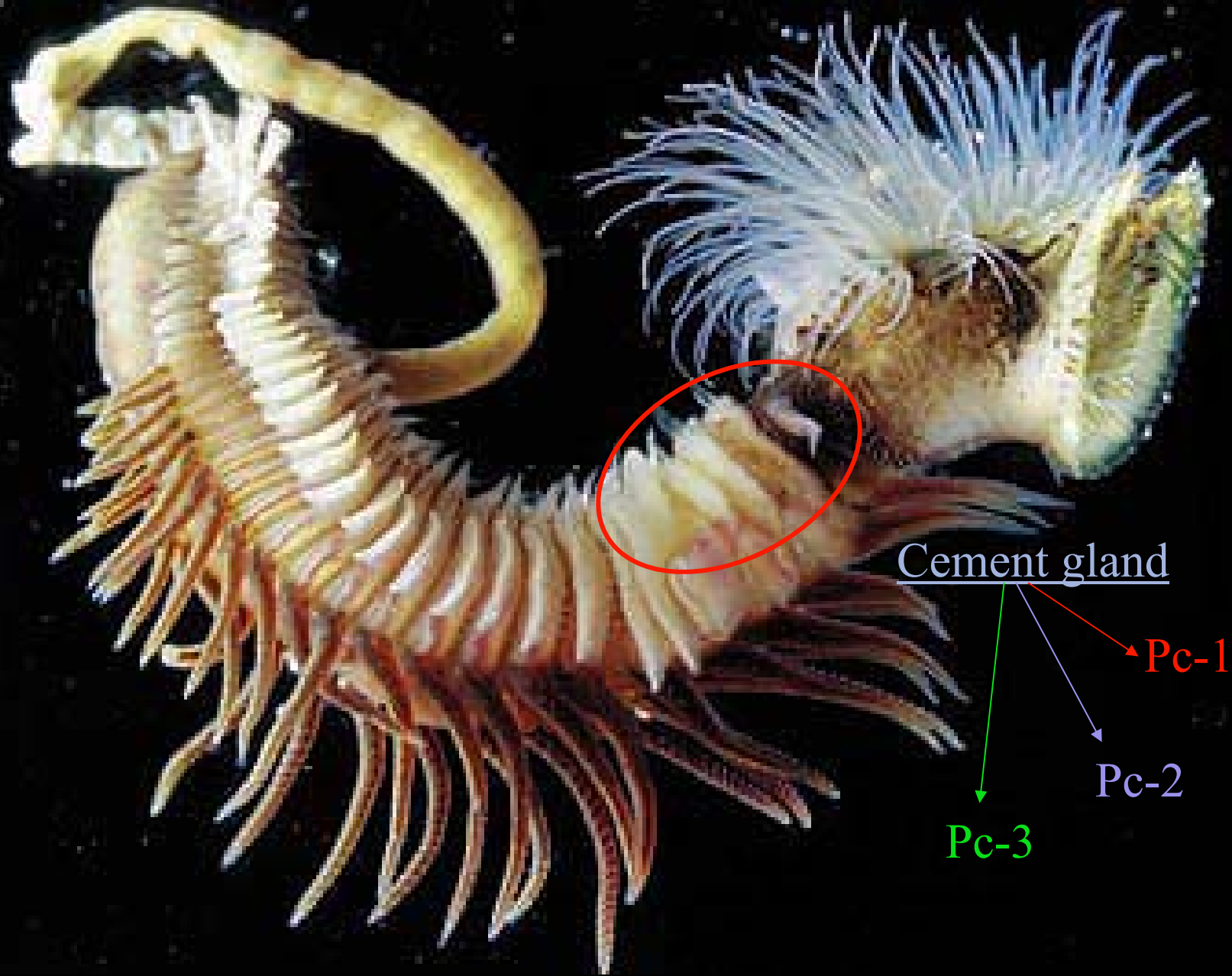
Hold On

Phragmatopoma californica colony





2 mm

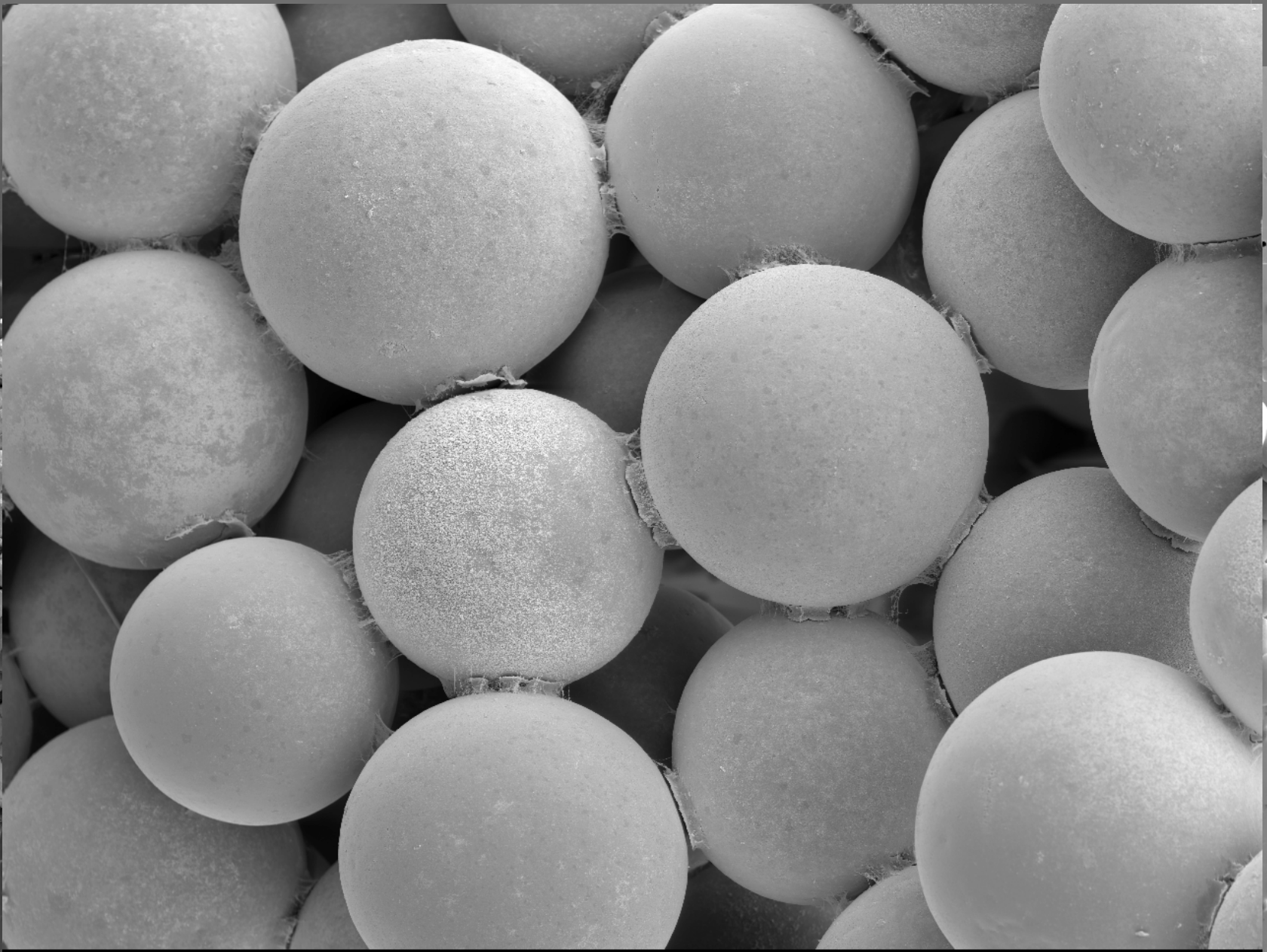


Cement gland

Pc-1

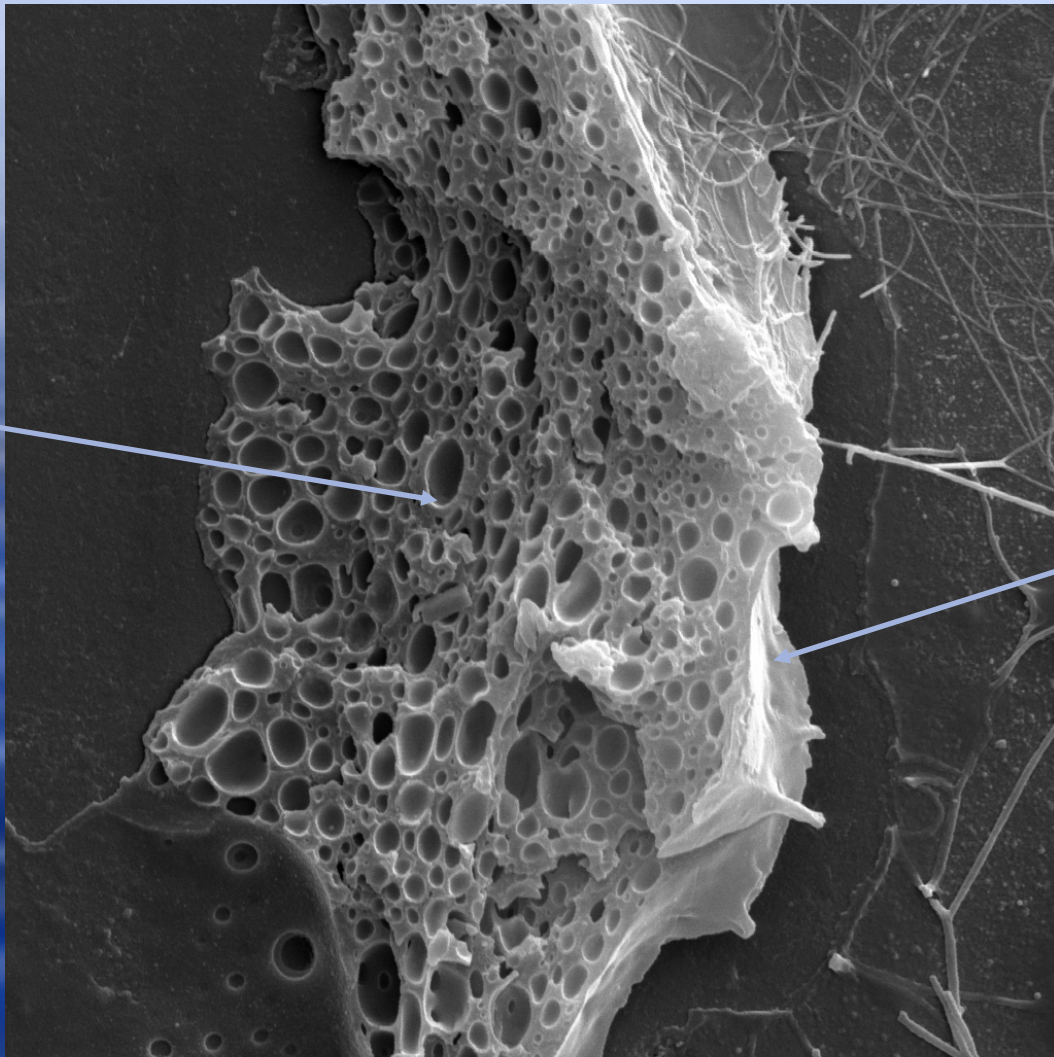
Pc-2

Pc-3

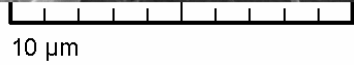


—100 μ m

Foam



Skin

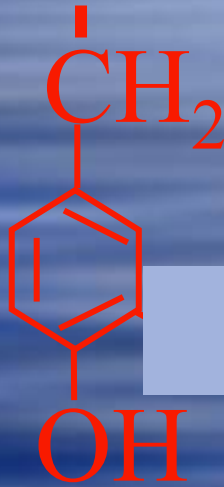


10 μm

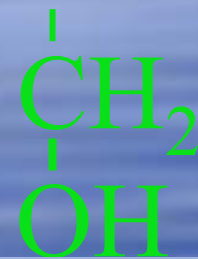
Vega ©Tescan

Proteins Known in Glue

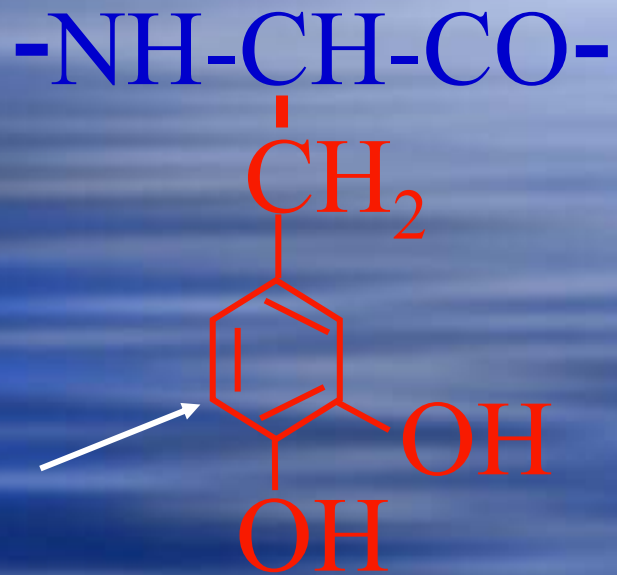
- ◆ Glue has over 25 mol% of serine.
- ◆ Pc-1, Pc-2 both glycine rich. Proteins were purified in the 80's. Post-translational modification: tyrosine to dopa.
- ◆ Pc-3 Serine rich. Deduced from cDNA sequence, protein has not been purified. Post translational modification: serine to phosphoserine.



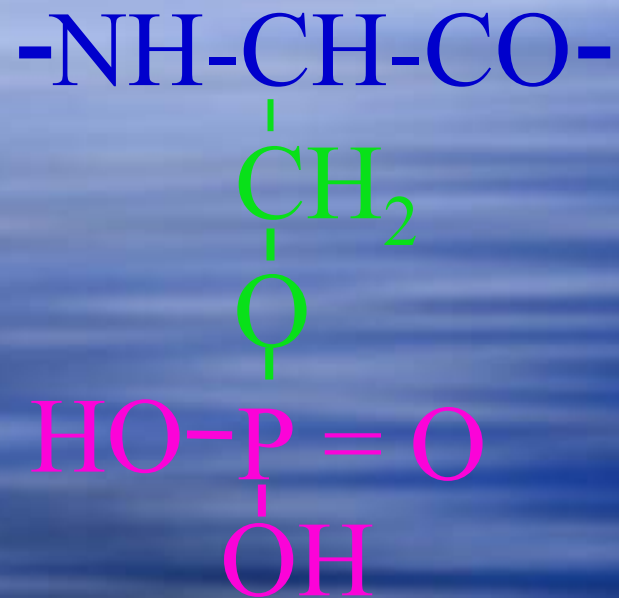
Tyrosine



Serine



Dopa



Phospho-serine

*Dopa: 3,4 dihydroxy-phenylalanine

Worm Glue Protein Extraction

- ◆ **Aim:** To purify pc-3 Serine-rich protein (60-90% serine) or peptide (glue)
- ◆ **Problem:** Not extractable from glue
- ◆ **Try from glue:**
 - ◆ (1) Protein extraction (from previous work, didn't do)
 - ◆ (2) Partial Acid Hydrolysis to break up protein
- ◆ **Try from thorax where all glue proteins were made:**
 - ◆ (1) Protein extraction w/ 5% Acetic acid followed by CaCl_2 precipitation
 - ◆ (2) Protein extraction w/ 0.2M Tris w/ EDTA followed by CaCl_2 precipitation

Methods: Flow Chart 1

Partial acid hydrolysis

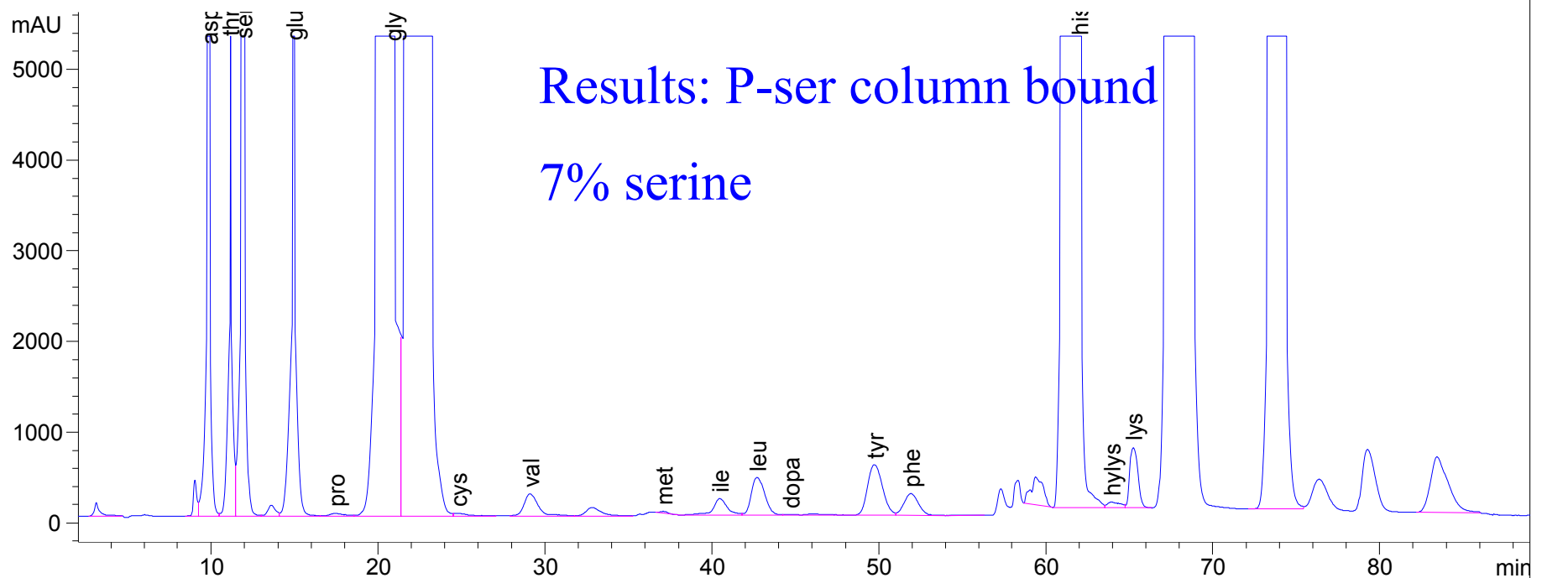
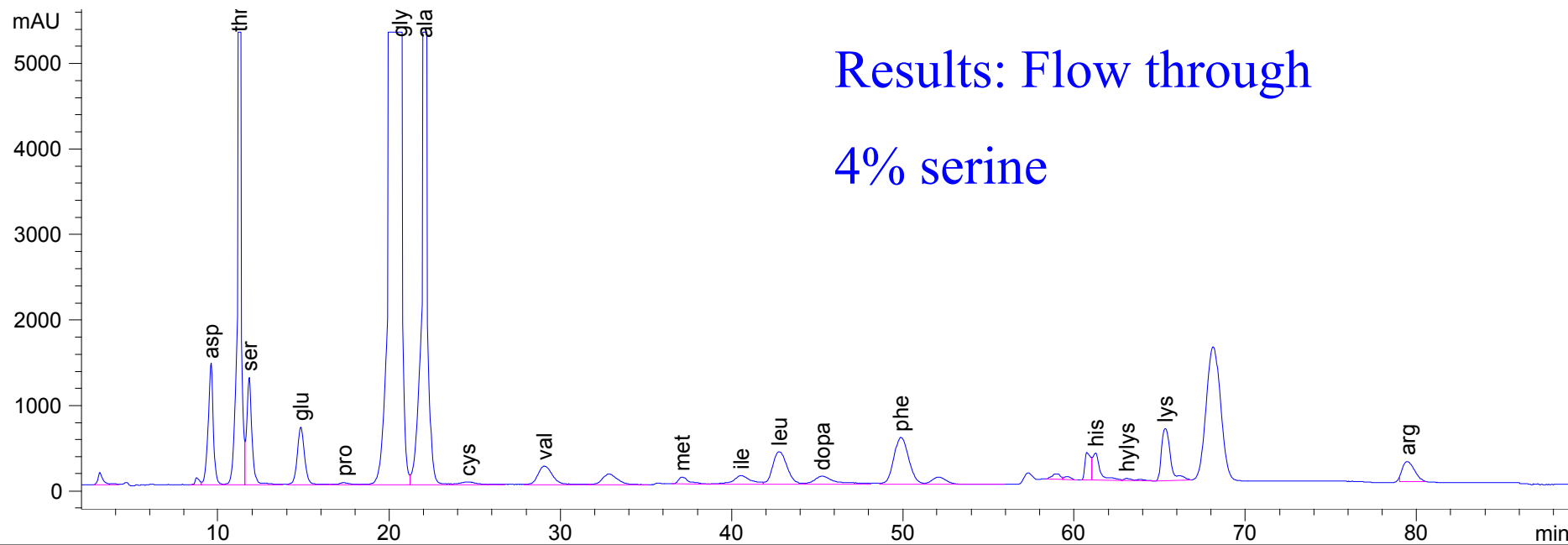
Worm glue & 6M HCl

Heat for 5 min @ 100°C

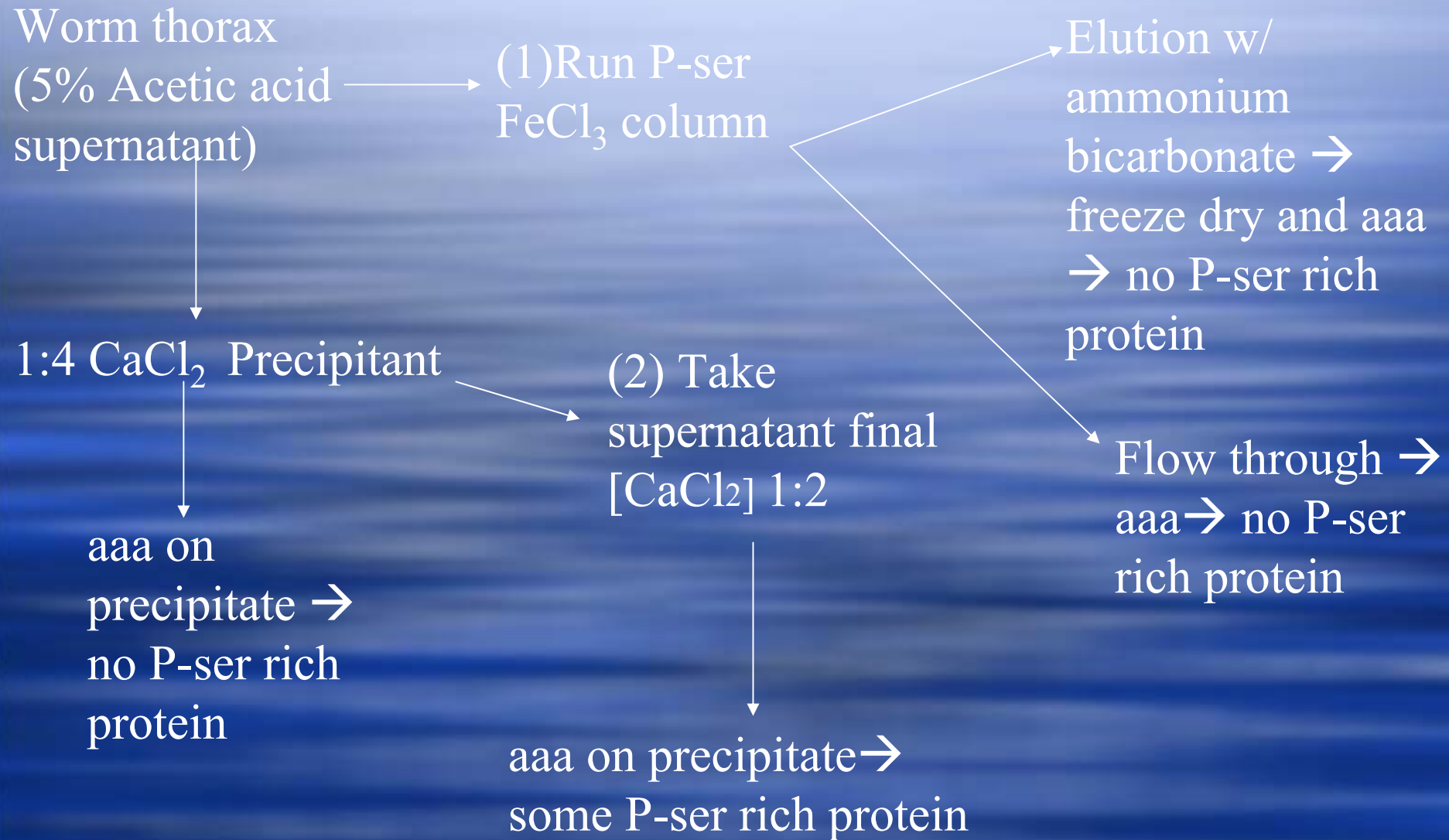
Run P-ser column: gets peptides w/ P-ser b/c will bind to FeCl_3 in column

Elution w/ ammonium bicarbonate buffer and aaa → no P-ser rich protein

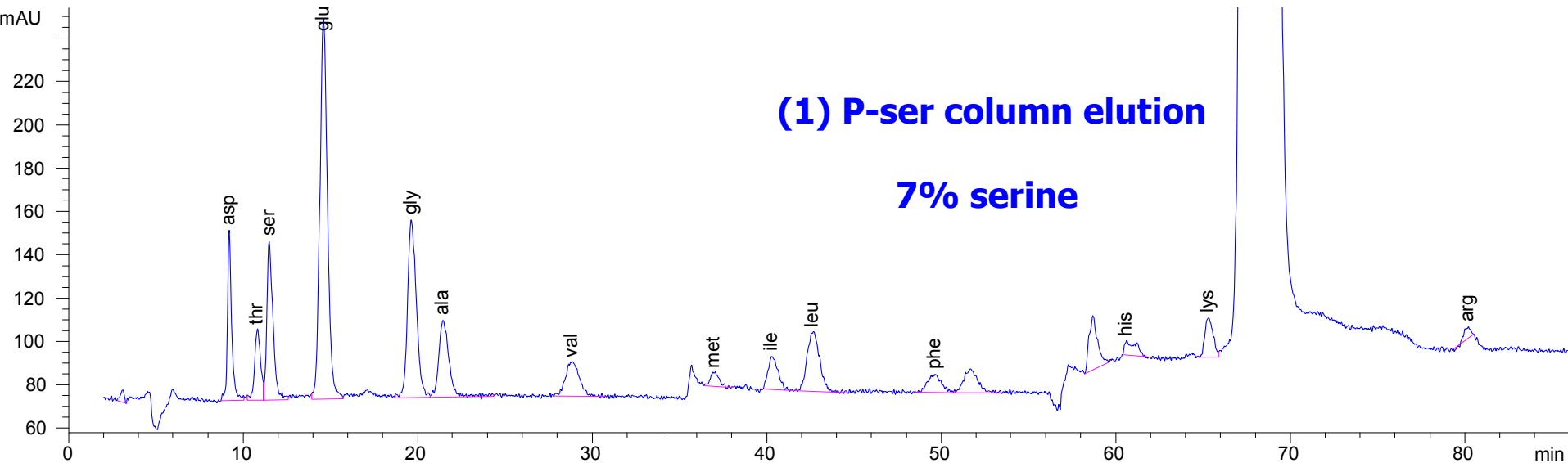
Flow through → aaa → no P-ser rich protein



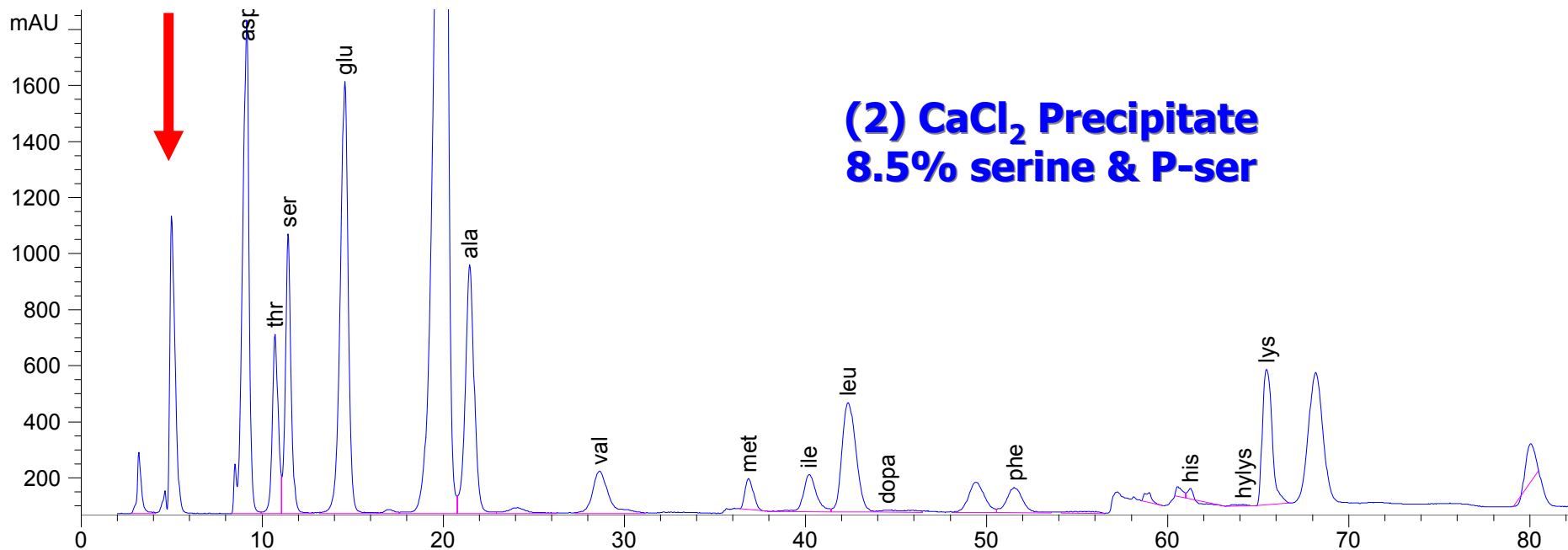
Methods: Flow Chart 2



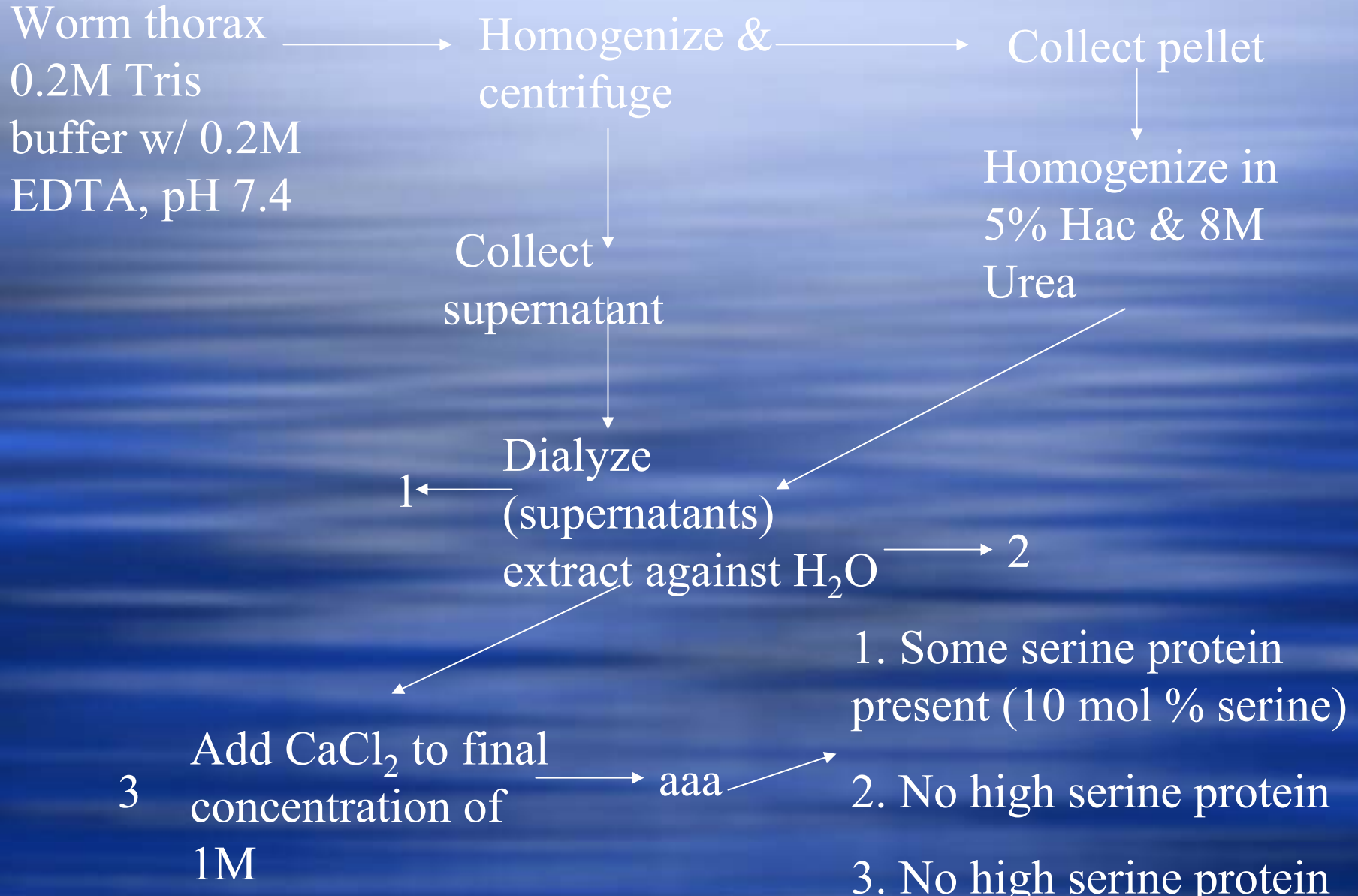
ADC1 A, ADC1 CHANNEL A (030401\TEST5032.D)



ADC1 A, ADC1 CHANNEL A (030401\TEST5012.D)

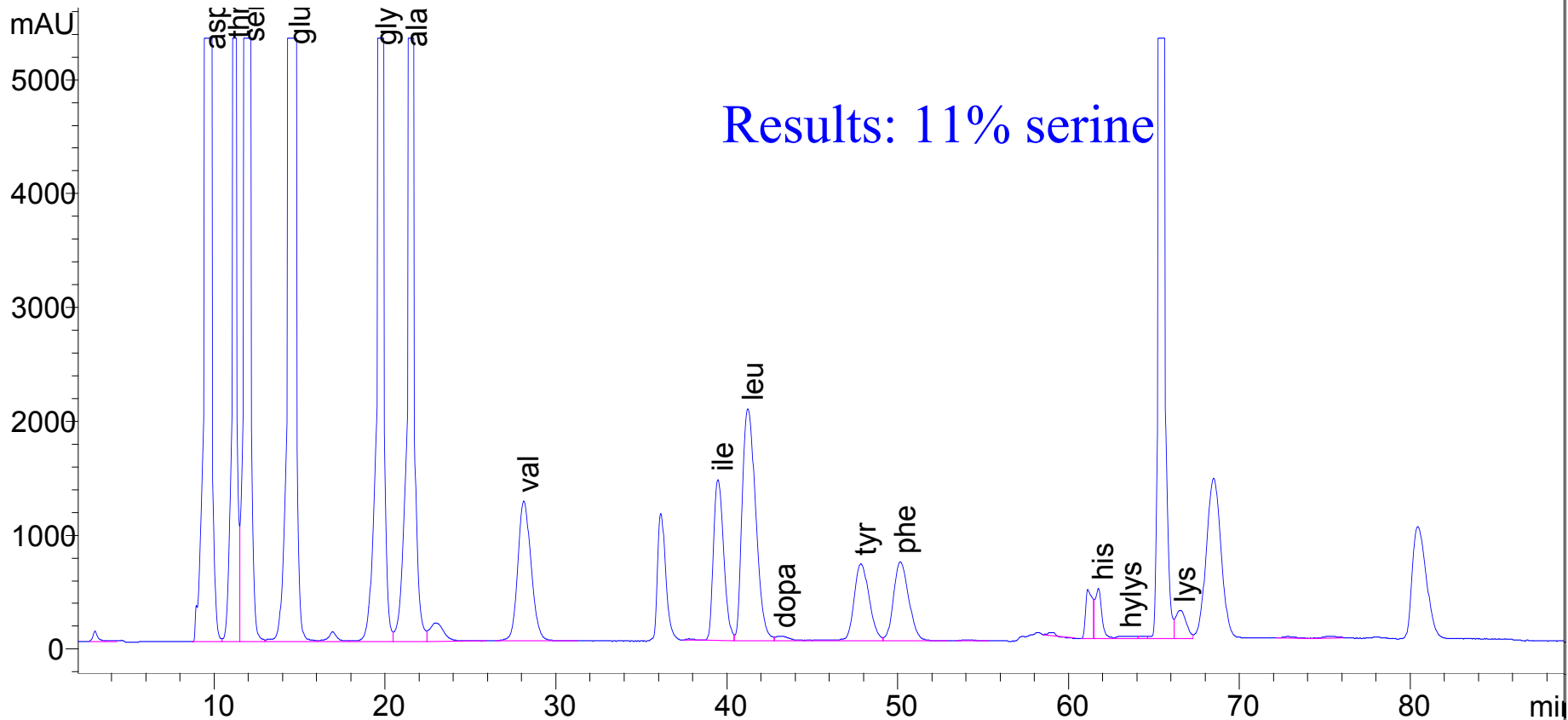


Methods: Flow Chart 3



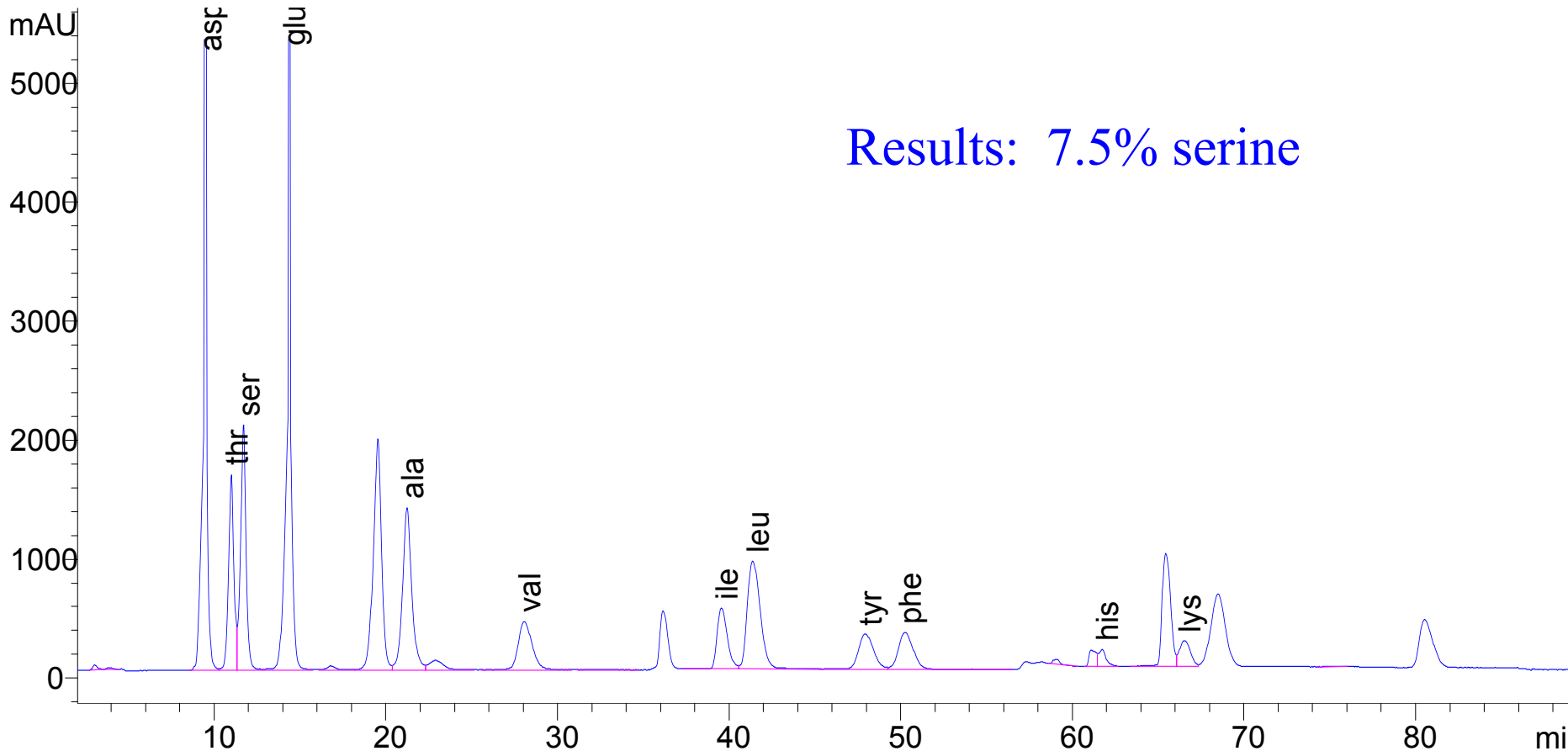
1. Tris/EDTA extract dialyze against H₂O precipitant

ADC1 A, ADC1 CHANNEL A (030401TEST5060.D)



2. AU extract after Tris-EDTA, dialyze against H₂O precipitant

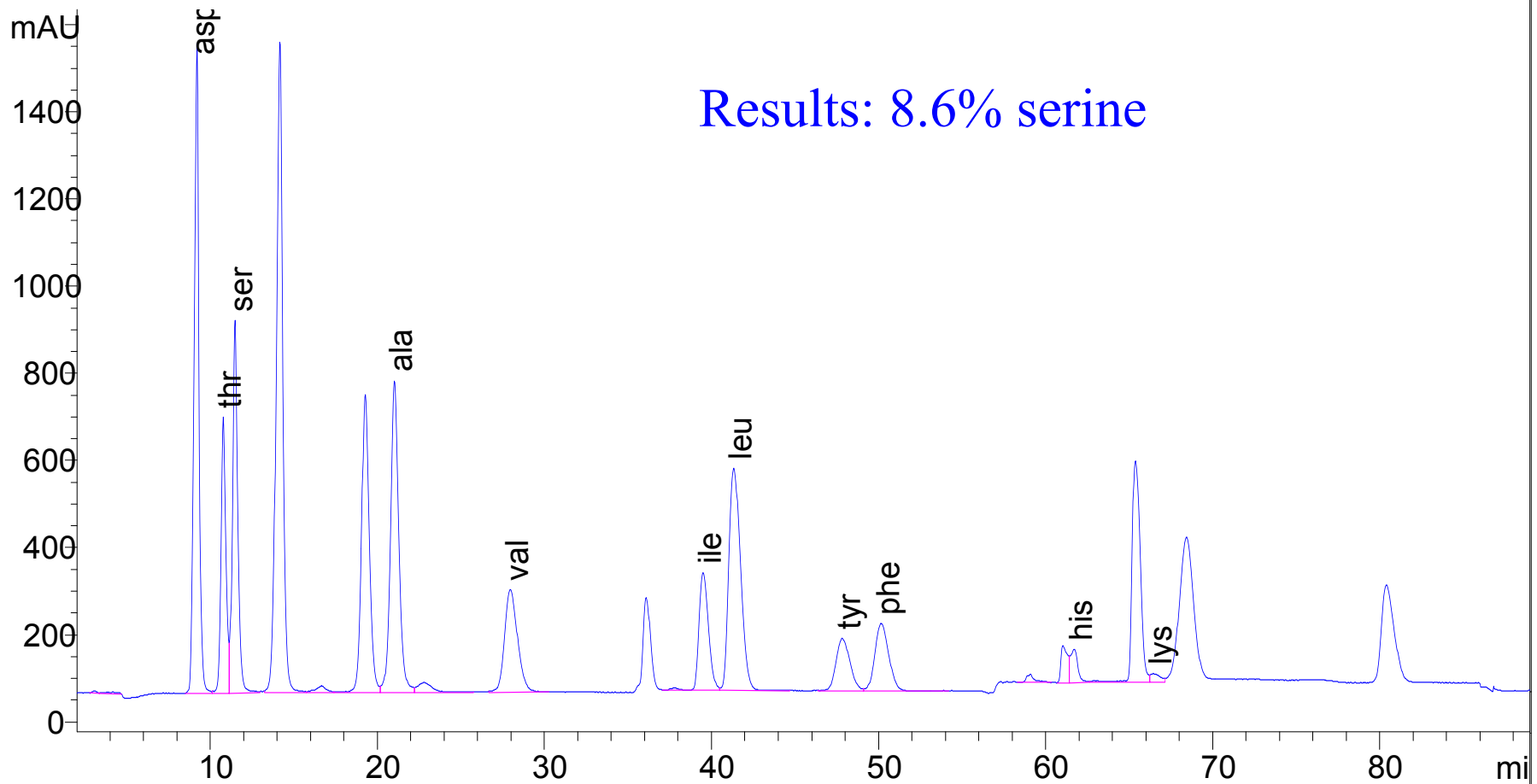
ADC1 A, ADC1 CHANNEL A (030401\TEST5061.D)



Results: 7.5% serine

3. CaCl₂ Precipitate from dialyzed Tris/EDTA extract

ADC1 A, ADC1 CHANNEL A (030401\TEST5046.D)



Next...

- ◆ Keep looking!
- ◆ Redo Tris/EDTA extraction and dialyze against water. Analyze the precipitate.
- ◆ Redo the 5% HAc protein extraction followed by CaCl_2 precipitation and use a higher concentration CaCl_2 .

What I have learned...

- ◆ Save & label everything!
- ◆ Little marine tube worms have perfected synthesis and secretion of an under water adhesive that has taken years to learn about and there is still so much unknown...
- ◆ Research can be so exciting and also so repetitive.

Thank You!