Strategies for Complex Protein Capture to Tackle Purification Challenges

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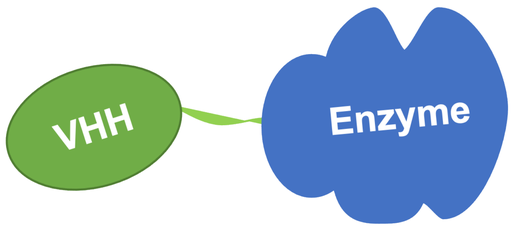
ABSTRACT

As the complexity of molecular design increases, various tagless recombinant proteins and fusion proteins have emerged, such as Fc-fusion proteins, ScFv-fusion proteins, and VHH-fusion proteins. The separation and purification of these proteins have become highly challenging tasks in the field of biotechnology. Among them, protein capture stands as the primary hurdle to overcome. Acid-labile fusion proteins pose a significant challenge to affinity capture, as traditional acid elution leads to protein aggregation or fragmentation. Tagless recombinant proteins also face stability issues, further complicating the capture process due to the absence of tags.

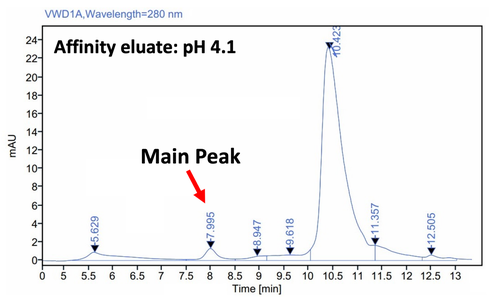
Recently, ProBio has developed the ProBox™ platform, which offers effictive solutions for such molecules. This strategy successfully addresses the instability issue of acid-labile fusion protein affinity capture and introduces an efficient capture process for tagless recombinant proteins.

In summary, we present relevant case studies showcasing the capture strategies for fusion proteins and tagless recombinant proteins, which serve as the crucial first step in protein purification.

CASE STUDY#1: VHH FUSION PROTEIN

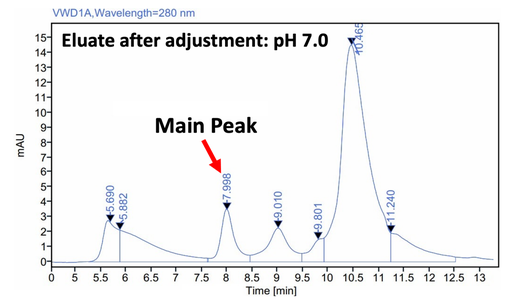


Enzyme+VHH (pI 5.7)



After elution with buffer (50 mM HAc-NaAc, pH 3.5), the eluate pH was 4.1, and precipitation appeared in the eluate. SEC-HPLC characterization showed severe fragmentation of the target protein. This protein is an acid labile protein and needs to be maintained above neutral conditions.

Adjust pH to 7.0



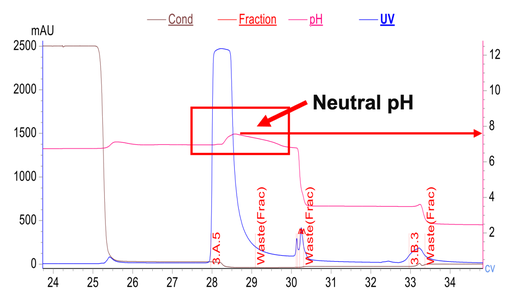
The pH of the eluent was adjusted back to 7.0, but the precipitation phenomenon did not alleviate in any way. SEC-HPLC characterization showed that fragments still dominated the composition while generating more aggregates. The pH callback does not help in quality improvement.

Challenges:

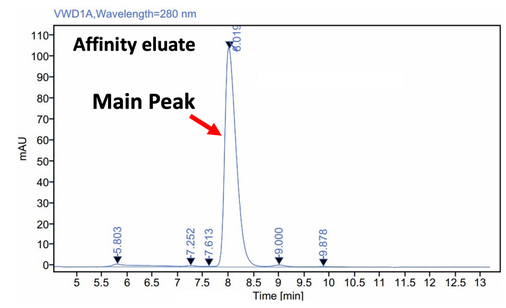
- Extensive precipitation in affinity eluate
- Precipitation still exists after pH adjustment
- The pH adjustment lead to increased aggregates.

Solutions:

- Choose suitable affinity resin
- Change the elution buffer



Determine the best buffer (100 mM Glycine-HCl, pH 3.5) by optimizing the buffer system and concentration. Under these conditions, the protein eluate can be maintained in neutral conditions (eluate pH 7.2), and the eluate is in a clear state.



SEC-HPLC characterization showed that the target protein maintains a complete structure without obvious aggregation or fragmentation. The SEC purity of the final eluate protein reached 97%.

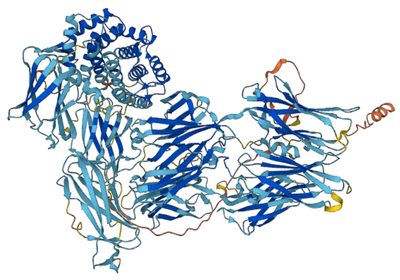
CASE STUDY#2: NO TAG RECOMBINANT PROTEIN

Challenges:

- No tag, difficult to capture.
- Protein is lost by precipitation at Cond. below 10 mS/cm and pH below 5.5.
- Without affinity chromatography, HCP level is high.

Solutions:

- Balance stability and recovery to choose the best loading conditions.
- Investigate the difference in pH and Cond. sensitivity between HCP and target protein, choose pH elution.
- Add wash2 to further reduce HCP.
- Arg is added to the elution buffer to eliminate abnormal pH rises and further reduce HCP.



Tetrameric protein (pI 5.9)

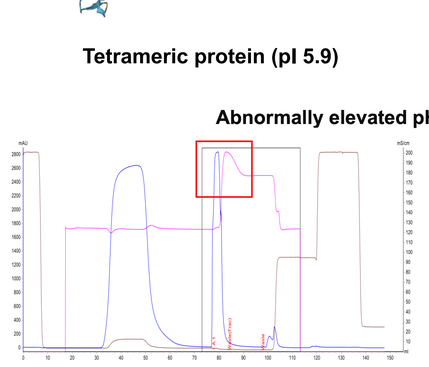
Cond. mS/cm	pH	Rec. %	Appearance
12.54	5.50	94	-
12.78	5.00	**84**	**Turbid and precipitation**
10.04	5.51	96	-
7.81	5.48	95	Turbid
5.84	5.47	**86**	**Turbid and precipitation**
3.93	5.51	**78**	**Turbid and precipitation**

Effects of pH and Conductivity on Protein Solubility

Cond. (mS/cm)	pH	Rec. (%)
10	5.5	121
12	5.5	96
14	5.5	67
11	5.4	122
11	5.6	98
11	5.8	38

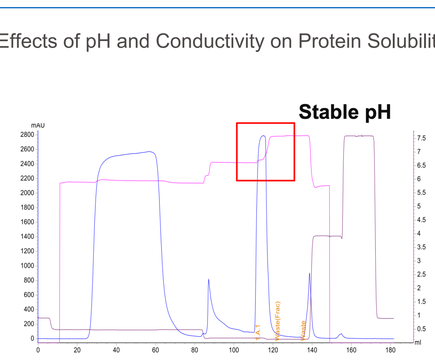
Effects of pH and conductivity on protein binding in CEX

Abnormally elevated pH



Chromatogram of Tris-HCl as elution buffer

Stable pH



Chromatogram of wash2 and Tris-Arg as elution buffer

Wash2	Elution	Rec. (%)	SEC (%)	HCP (ppm)
No	Tris-HAc	114	78	22410
Yes	Tris-HAc	95	85	10007
Yes	Arg	**92**	**88**	**3691**

Effects of wash2 and Arg on SEC and HCP

CONCLUSION

We have demonstrated an efficient affinity capture format for acid-labile fusion proteins. By selecting specific affinity packing materials and elution buffers, the eluent can be maintained at neutrality to stabilize protein quality. For tag-free recombinant proteins, CEX is the optimal capture method. The optimal capture is achieved by balancing the protein stability and CEX binding conditions, and the wash and eluent are optimized to achieve the best elution quality. We have solved the capture problem of two kinds of complex proteins and provided customers with a good CMC process.