

RBIF-120: Searching for a KLH replacement

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1 Abstract

Should I add this?

2 Introduction

Hemocyanin is the copper-containing, main oxygen carrying protein commonly used in the blood of arthropods, mollusks, and some other invertebrates. Hemocyanin is of interest in biomedical research because of its use as an adjuvant in vaccine development, where it elicits an immunre response to haptens through covalent conjugation. Unfortunately, current production of hemocyanin as an adjuvant is extremely expensive with the harvesting process requiring blood from the limpet *Megathura crenulata*. Key-Hole Limpet hemocyanin (KLH) is challenging to produce with recombinant techniques due to the large size of the protein. The cost of extraction ranges between five thousand to one hundred and fifty thousand dollars per gram of KLH.

Due to the increased demand, vaccine adjuvants may present as a bottleneck for future vaccine development. Recombinant KLH that has been produced with *E. coli*, however, is not used for general medical practice due to unsuitable purity. While the structure of KLH has been resolved to 9Å, with its functional unit resolved to 4Å, potential alternative hemocyanin sources to this protein have not been determined with imaging techniques such as Cryo-EM or X-ray crystallography.

The goal of this work will be two-fold. First, this work aims to understand the significance of adjuvants in relation to KLH, exploring the properties of its medicinal use and trade-offs to alternative conjugate protein adjuvants. Second, this work aims to explore the structure of KLH using computational and bioinformatic techniques in conjunction with analysis found in literature, highlighting potential alternatives to this important protein.

3 Hapten conjugate proteins

3.1 Adjuvant: A broad definition

Since adjuvants were discovered over 100 years ago, numerous substances have been compounded with vaccines in the hopes of an increased immunological response. Derived from the Latin word *adjuvare*, which means "to help"[3], scientists reached for the word "adjuvant" based on its ability to improve the impact of vaccines considering no mechanism of action was understood at the time. Aluminum salts have been successfully deployed in vaccines for decades[1]. Other adjuvants are entering into clinical use, such as the nano oil based mRNA formulations used in COVID-19 vaccinations[2].

Nevertheless, the mechanisms of action for aluminum vs mRNA are drastically different. Thus, a consistent criteria for classification is important as we compare the efficacy of different adjuvants. In all cases, however, an idea antigen meets five interrelated standards[12]:

- Pure:** A chemically consistent adjuvant can be isolated.
- Harmless:** The adjuvant has few (or no) negative side effects.
- Universal:** A common immunogenic response is present in all exposed sub-populations.
- Targeted:** The adjuvant promotes no cross reacting antibodies.
- Predictable:** Variable exposure produces a measurable response, capable of immunomodulation.

With these standards in mind, only hapten conjugate protein adjuvants will be discussed as we explore the efficacy of KLH in comparison to other hemocyanins.

3.2 Common Protein adjuvants

Broadly speaking, haptens are small antigens that elicit a weak response when presented to the immune system[5]. One method to amplify the immonogenic response of a hapten is to covalently attach it to

a large, immunologically active protein. The methods of this attachment depends upon the functional group of hapten and the most commonly used carrier proteins are[11]:

BSA	bovine serum albumin
OVA	ovalbumin
TG	thyroglobulin
CONA	conalbumin
Ig	immunoglobulin
KLH	keyhole limpet hemocyanin

Choosing the correct Hapten-protein formulation depends not only upon the targeted immune response, but also upon the necessary hapten-protein stoichiometry needed to elicit this response[11].

Of the carrier proteins, KLH is the most commonly used and studied[12], often considered a model of its class. This decision to focus on KLH is understandable in respect the criteria for an ideal adjuvant. KLH is stable over a wide rage of temperatures and pHs[10]. Given its large size, it is easy to attach happens to it. Most importantly, even though KLH is xenogeneic to the immune system, it considered both "extremely safe" and effective, outperforming the immunological response of many other carrier proteins[6]. Other than minor side effects, there have been no reports of adverse events related to KLH trials[12].

Nonetheless, KLH is an expensive compound. The cost of extraction and purification ranges between five thousand to one hundred and fifty thousand dollars per gram of KLH. The market for KLH is expected to expand by 2% over the next decade[13], yet the biological capacity of *M. crenulata* may not meet this demand.

In situations where KLH is a highly successful adjuvant, is it theoretically possible that similar immunological activity could be achieved when KLH is substituted by less expensive hemocyanin alternatives?

3.3 Clinical evidence for alternatives to KLH

This is a little bit of a place holder. I am not sure how I want to expand this section yet.

Clinical information:

- *Helix pomatia* (HPH) [4]
- *Concholepas concholepas* (CCH), *Fissurella latimarginata* (FLH) [10]
- maybe tangential: antimicrobial Hemocyanin [8]
- Should I only show similar to KLH, *Panulirus japonicus* (PjHc) is also interesting as outlier?
[9]

Structural comparisons:

- Molluscan hemocyanin: structure, evolution, and physiology [7]
- Importance to glycans [10]

4 Understanding KLH

Before we can start hunting for replacements for KLH, it is important to understand it's structure.

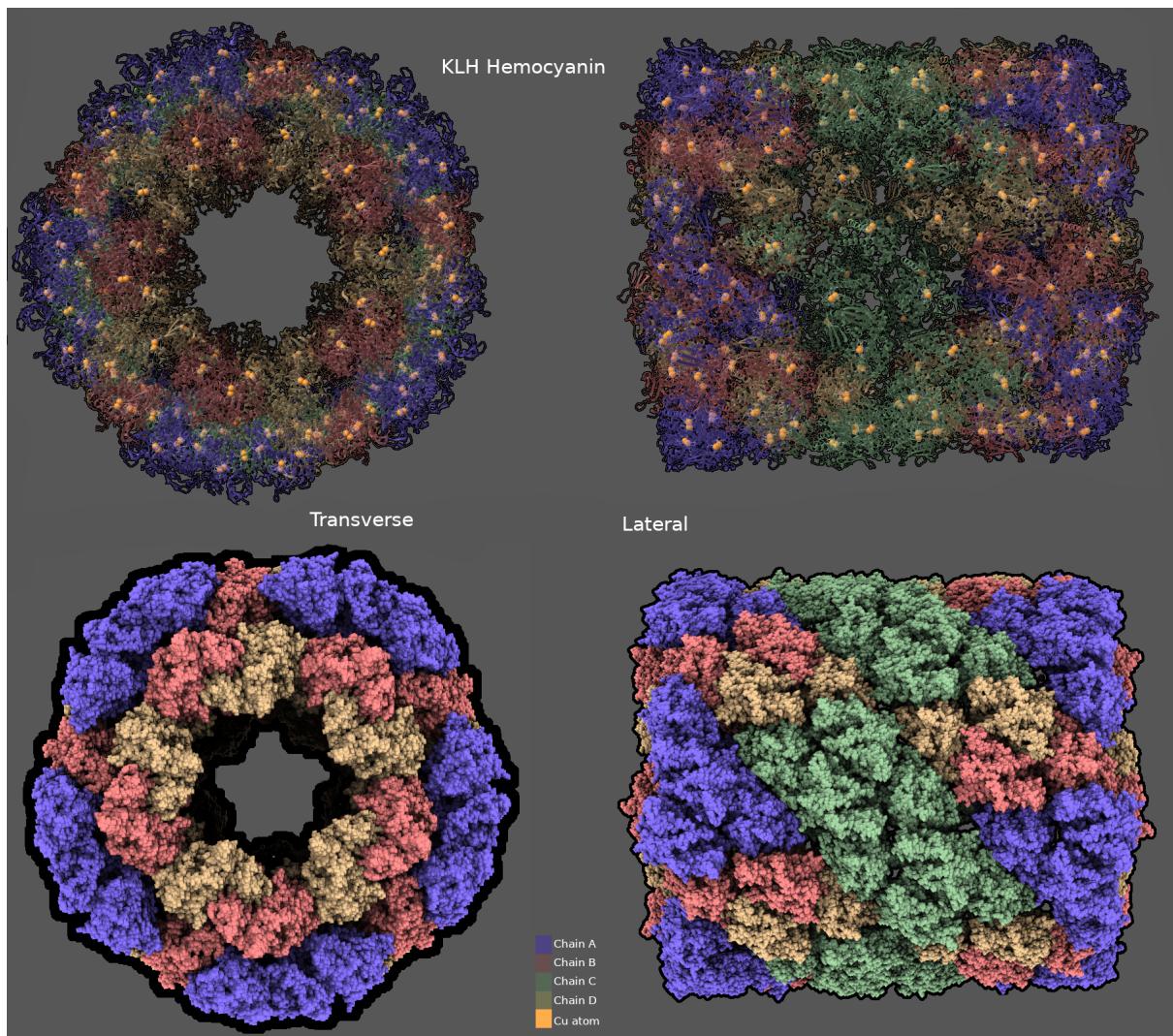


Figure 1: Keyhole Hemocyanin Views

4.1 Arguments in favor

5 Understanding KLH

5.1 Structure

5.2 Phylogenetics

5.3 kmer protein analysis

6 Candidates

7 Homology Modeling

7.1 trRosetta

7.2 Robetta

7.3 Pytorch/AlphaFold clone

8 Conclusion

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