

**UC Davis**  
**Orthopaedic Surgery**

**Title**

Isolation,of,Binding,Site,of,BMP2,on,CBTerminal,Domain,of,COMP

**Permalink**

<https://escholarship.org/uc/item/0d96b5t7>

**Authors**

Bhatti, Satninderdeep

Fraley, Gabriel

Haudenschild, Dominik

**Publication Date**

2020

**Data Availability**

The data associated with this publication are not available for this reason: N/A

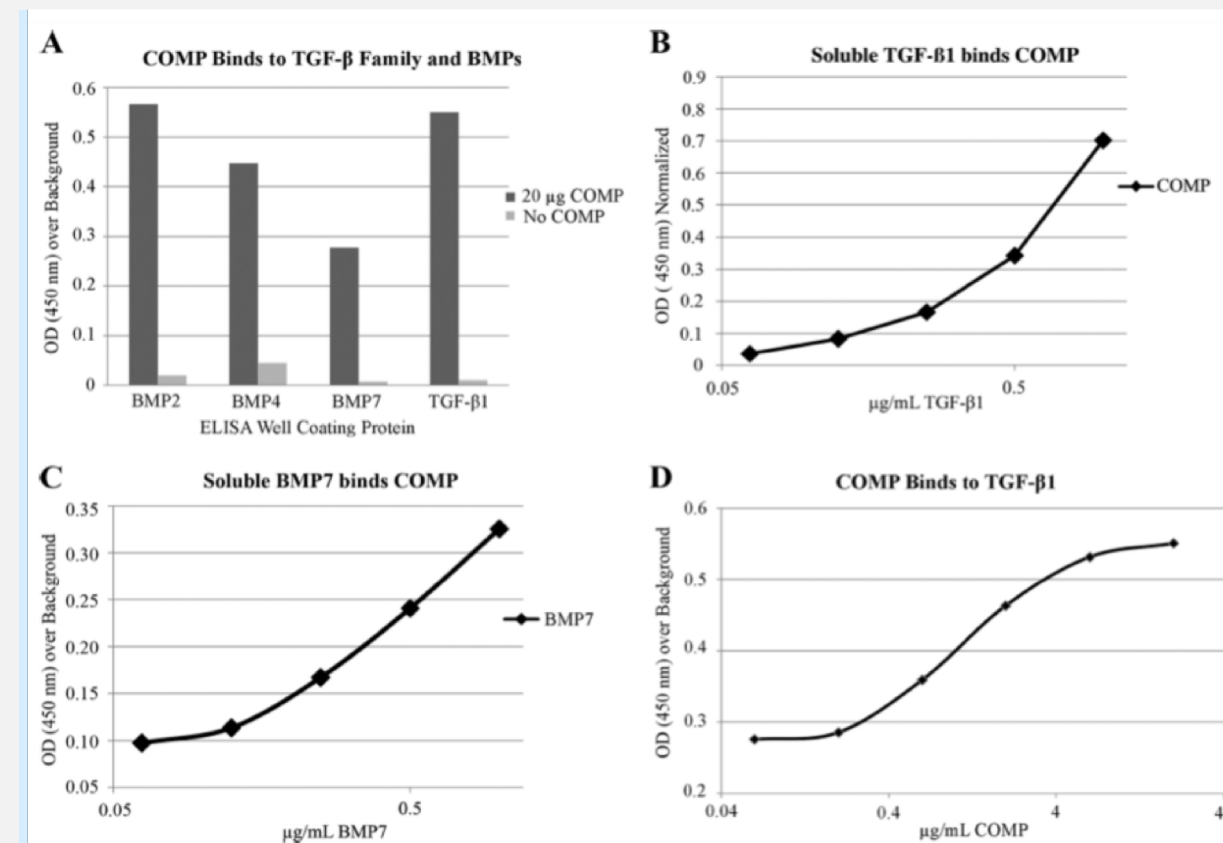
# Isolation of Binding Site of BMP2 on C-Terminal Domain of COMP

Satninderdeep Bhatti BS MPH, Gabriel Fraley PhD, Dominik Haudenschild PhD

University of California, Davis – School of Medicine

## Introduction

- The use of Bone Morphogenetic Protein clinically is supra-physiologic in concentration.
- Bone Morphogenetic Protein 2 (BMP2) is used everyday in orthopaedic surgeries and bone autografts, but it is not used in normal biological amounts.
- Clinical uses are 1 million times more than concentrations found physiologically.
- Cartilage Oligomeric Matrix Protein (COMP) is known to bind proteins from the TGF superfamily and makes the presentation of BMP2 enhanced due to their binding interaction<sup>1,2</sup>.



- Analysis of the interaction between COMP and BMP2 shows that BMP2 binds to the C-Terminal Domain of COMP.
- An understanding of how BMP2 binds allows for more effective presentations of the protein to maximize response and decrease use of super-physiologic concentrations clinically.

## Objective

- Our goal is to identify the BMP2-COMP binding site using a peptide library screening experimental design.

## Materials and Methods

- Design a peptide library corresponding to the C-Terminal Domain of COMP to use in epitope mapping to screen for binding sites of BMP2.
- Use a competitive ELISA based assay to pinpoint regions on C-Terminal Domain that BMP2 is binding to via a gradient of concentration ratios between synthetic peptides and human COMP.

### COMP Extraction

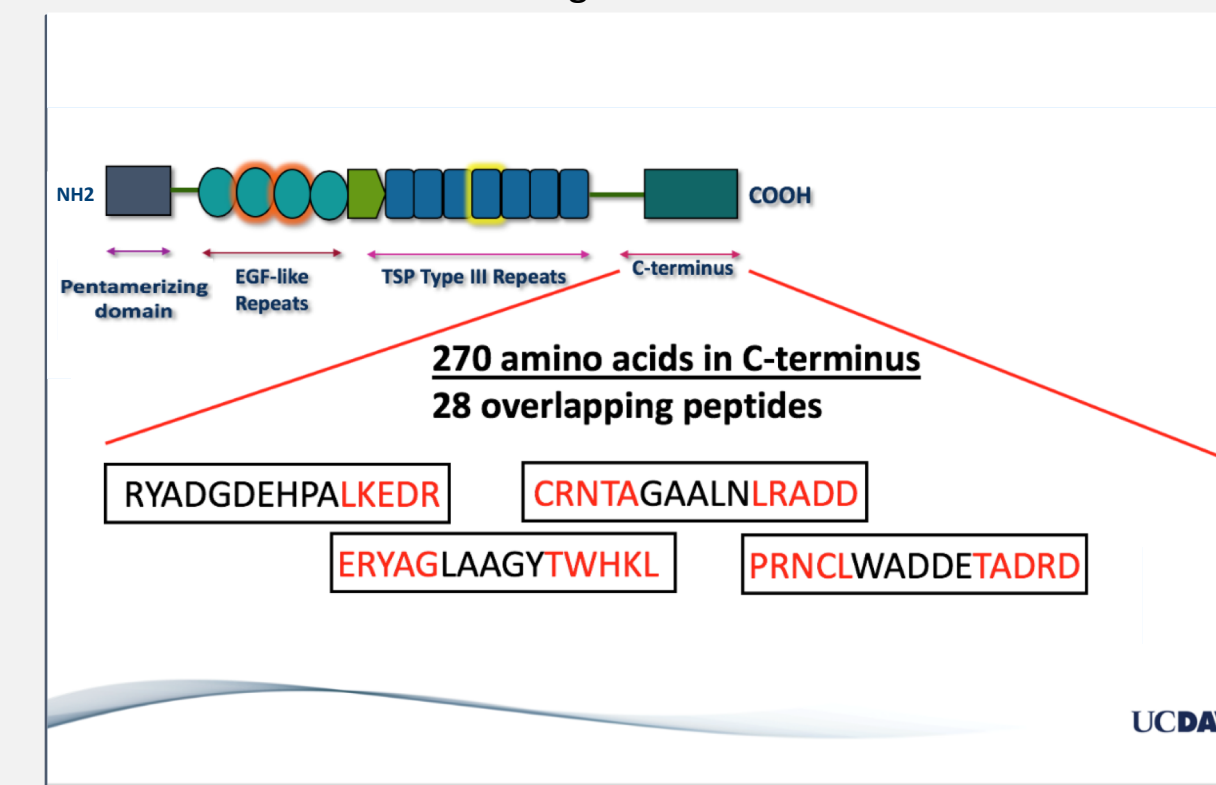
- Use recombinant Human COMP obtained via retroviral transduction in human kidney cells using bacterial plasmids.
- COMP has added Strep Avidin Tags and 8x His tags to the CTD.

### ELISA Based Binding Assay

- Competitive ELISA is used to ensure the ability to selectively bind COMP CTD peptides to BMP2.
- ELISA assay with differing ratios of COMP and synthetic COMP CTD Peptides ranging from 0:1 to 100:1.
- A significant drop in response with increasing concentrations shows that the peptide is successfully competing with COMP to bind to BMP2.

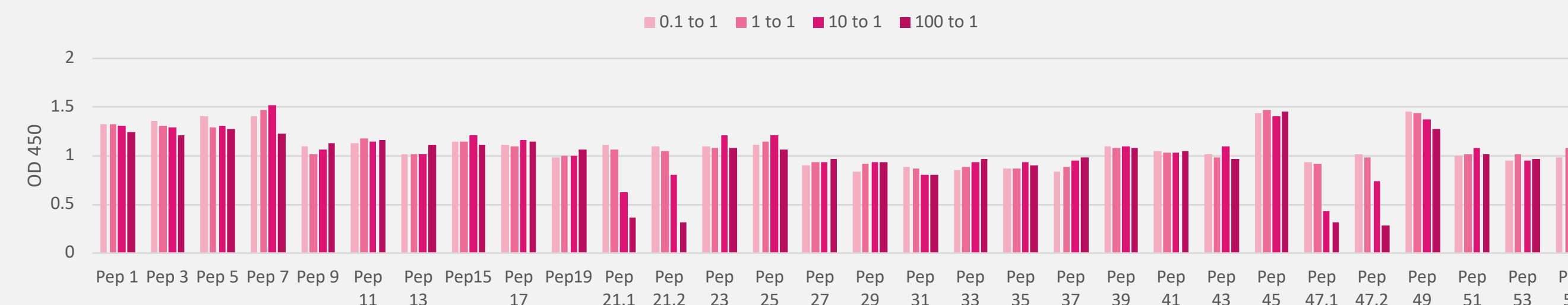
### Peptide Library Design

- Design a series of 28 overlapping peptides corresponding to epitopes of the CTD of COMP starting 270 AA in from the C Terminus.

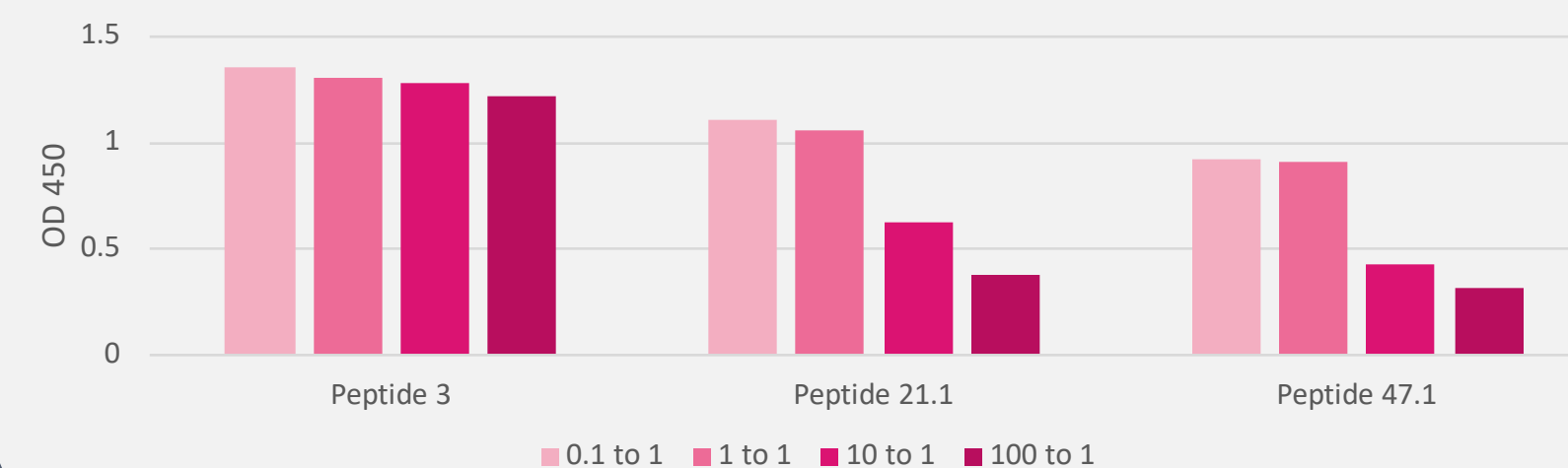


## Results

BMP-2 Solid Phase\_COMP-FL / COMP CTD Peptide Soluble Phase Competitive ELISA



Comparison of Interfering and Non-Interfering Peptides



- There is increased binding of COMP peptide 27 and 47 as the concentration increases, showing successful competitive binding against native COMP.
- Successful ELISA is seen starting at a concentration ratio of 10:1 with a further improvement at 100:1

## Conclusions

- Two positive competitive ELISAs in peptides 21 and 47 show that BMP2 binds to COMP at 2 different sites on the C-terminal Domain.
- These sites on the CTD are the same sites that other growth factors such as TGF-β bind, corroborating previous findings that COMP plays a major role in osteogenesis and chondrogenesis due to its interactions with growth factors<sup>1,2</sup>.

## Future Research

- Further analysis is needed to provide an explanation of how COMP retains the native presentation of BMP2.
- Further testing of the peptides 21 and 47 is necessary to explore whether they alone are enough to amplify the signal of BMP2 or whether the entire COMP molecule is needed for amplification. This could implement the practice of adhering BMP2 onto these peptides before using clinically, allowing a decrease in amount of use.
- Further testing of the amino acid makeup of peptides 21 and 47 via point mutations will show which specific amino acids are involved.

## References

- Ishida, K., Acharya, C., Christiansen BA., Yik, JHN, DiCesare, PE., Haudenschild, D. (2013). Cartilage oligomeric matrix protein enhances osteogenesis by directly binding and activating bone morphogenetic protein-2. *Bone*, 23-25.
- Haudenschild, DR., Hong, E., Yik, JH., Chromy, B., Morgelin, M., Snow, KD., Acharya, C., Takada, Y., Di Cesare, PE. (2011). Enhanced Activity of Transforming Growth Factor β1 (TGF-β1) Bound to Cartilage Oligomeric Matrix Protein. *J Biol Chem*, 43250-8