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MUSCULOSKELETAL INNOVATIONS

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***Integrated in vivo and in vitro  
high-throughput analyses of  
osteocyte-mediated bone remodeling***

Cristal Yee, Ph.D.  
Alliston Lab

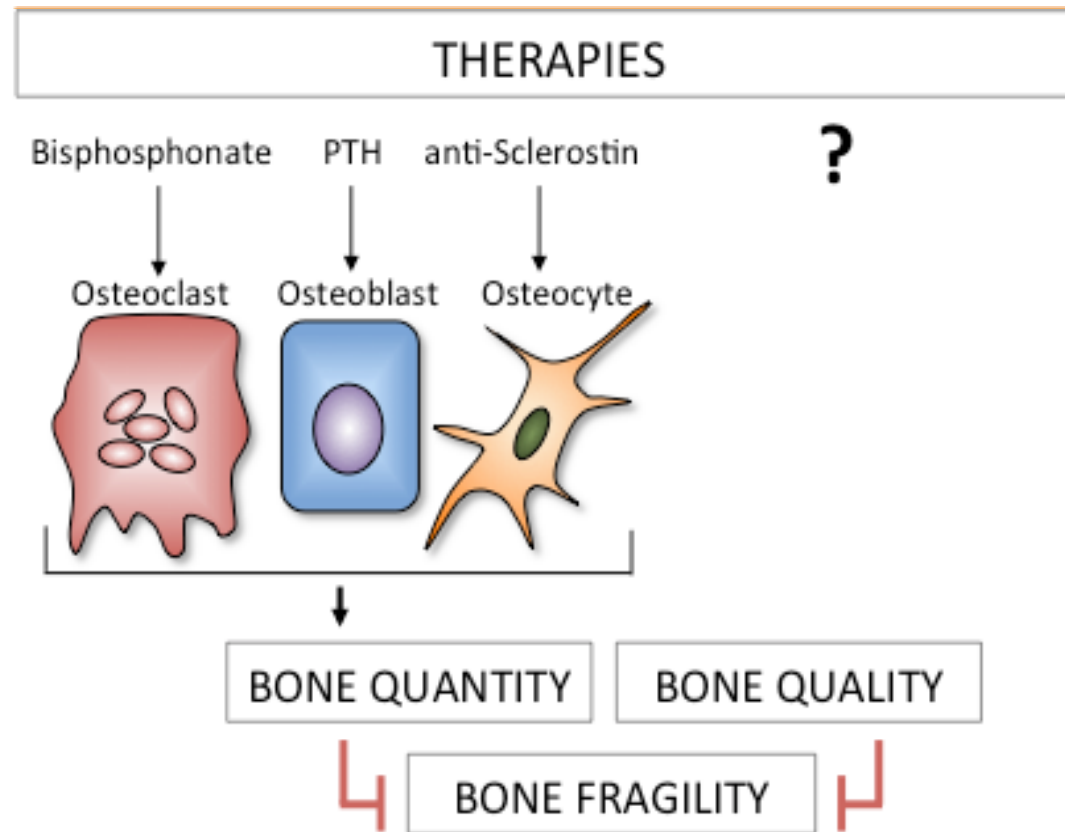
UNIVERSITY OF CALIFORNIA SAN FRANCISCO

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[WWW.NSFCDMI.ORG](http://WWW.NSFCDMI.ORG)

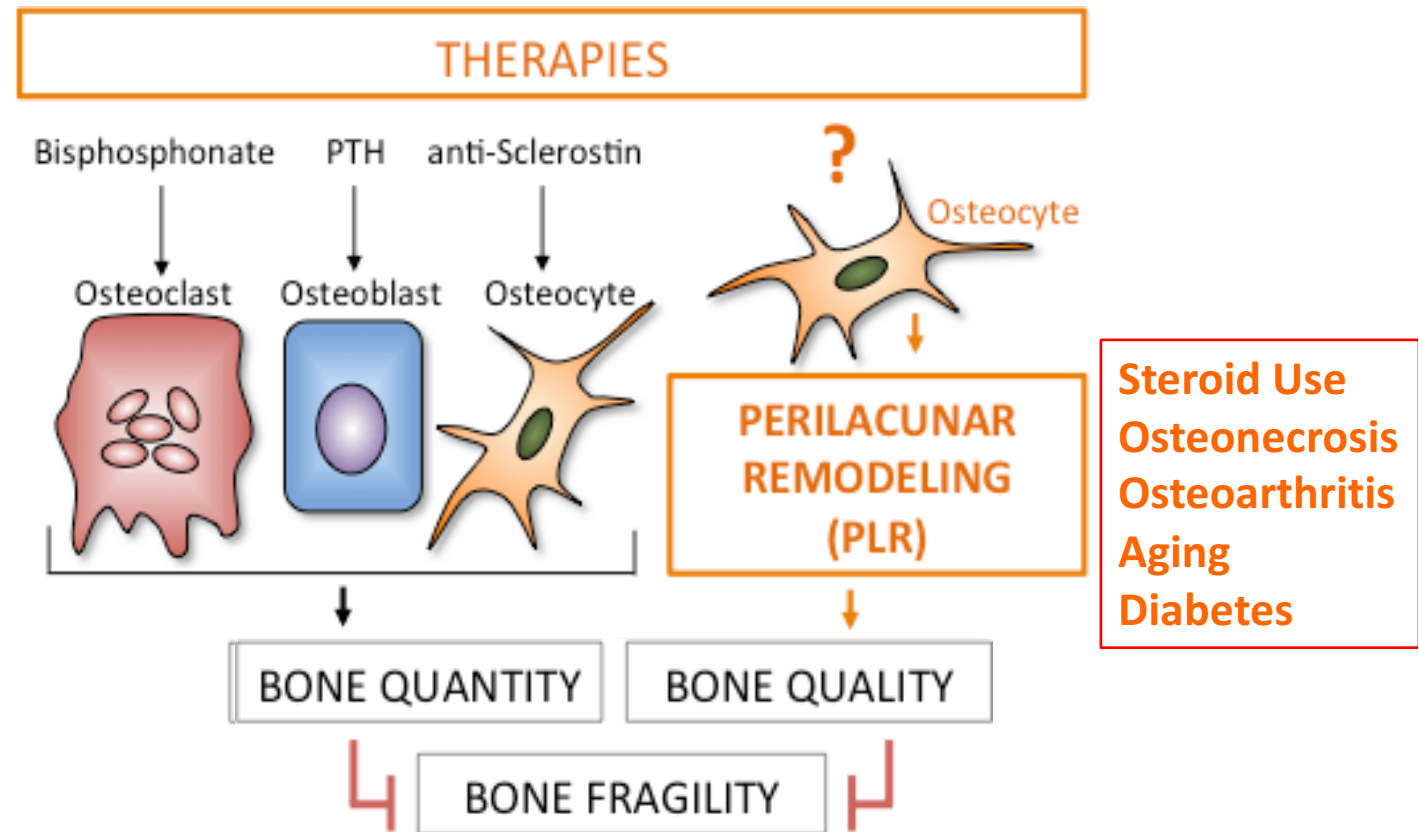
# Clinical Imperative: Treat Bone Quality

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# Clinical Need and Industrial Relevance

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**Knowledge Gap:** role of PLR in bone health or disease & how to study it

## Project Aims

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This project aims to develop a comprehensive approach to evaluate PLR in vivo and in vitro to advance the development of diagnostics and therapies to improve bone quality.

***Aim 1: Develop and validate in vitro measures of PLR function for high throughput screening.***

*- currently, there is no validated in vitro PLR assay*

***Aim 2: Establish the Osteocyte-Mediated Bone Remodeling ECM (OMBRE) Core.***



# Deliverables

## ***OMBRE Protocols I-V*** 1

***I:*** Collagen Organization

***II:*** Lacunocanalicular Analysis

***III:*** PLR Gene Expression

***IV:*** In Vitro Functional pH Assay

***V:*** In Vitro PLR Reporter Assay

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***High Throughput Screen***

## ***CDMI Access to OMBRE Services through the UCSF Skeletal Biology and Biomechanics Core*** 3

## OMBRE Core Services

UCSF Skeletal Biology  
Core Director:  
Tamara Alliston

**1** [Progress Update](#)

OMBRE Recharges  
Proposal Approved  
(March 22, 2017)

**2** [Progress Update](#)

Working on updating  
website: [CCMBM](#)

**3** [Progress Update](#)

UCSF, UC Davis,  
Industry interest for  
services

C D M I



## Core Center for Musculoskeletal Biology and Medicine



### Skeletal Biology Core Services



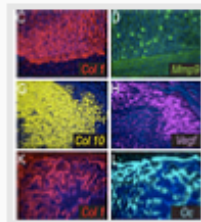
#### Imaging and Histology Sub-Core

Provides imaging services for small animals and tissue specimens using computed X-ray tomography. Offers technical support in tissue extraction and processing for histology, as well as in histomorphometric analysis.



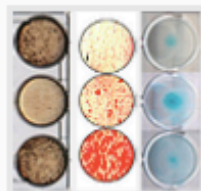
#### Biomechanics Sub-Core

Offers resources and expertise in quantitatively evaluating the mechanical and material properties of skeletal tissue over a range of resolutions and scales.



#### Molecular Biology Sub-Core

Provides expertise in the isolation of RNA and protein from skeletal tissue, the quantitative analysis of gene and protein expression using real-time qPCR and Western blotting, and the qualitative analysis of gene and protein expression through in situ hybridization and immunohistochemistry.



#### Cell Culture Sub-Core

Maintains and supplies a collection of chondrocytic, osteoblastic, and osteoclastic cell lines and provides expertise in preparing primary cultures of murine bone marrow stromal cells and other cell populations. Offers technical support in the use of stains and enzymatic assays to assess bone-related properties in cultures.

Click [HERE](#) for a list of currently available cell lines.

## OMBRE Protocols I-V

**I:** Collagen Organization

**II:** Lacunocanalicular Analysis

**III:** PLR Gene Expression

**IV:** In Vitro Functional pH Assay

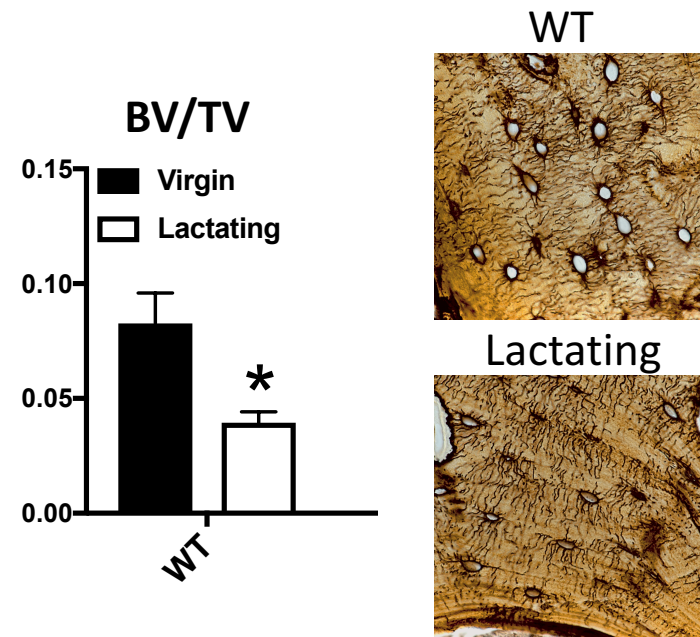
**V:** In Vitro PLR Reporter Assay

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### Progress Update

Harvested tissues for OMBRE validation

- virgin and lactating mice
- N ≥ 7 females per group
- microCT validation complete
- histology underway



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## Progress Update

Harvested tissues for OMBRE validation

- Analysis of PLR Gene Expression
- PLR Gene Expression down regulated in  $T\beta RII^{ocy-/-}$  mice

### OMBRE Protocols I-V

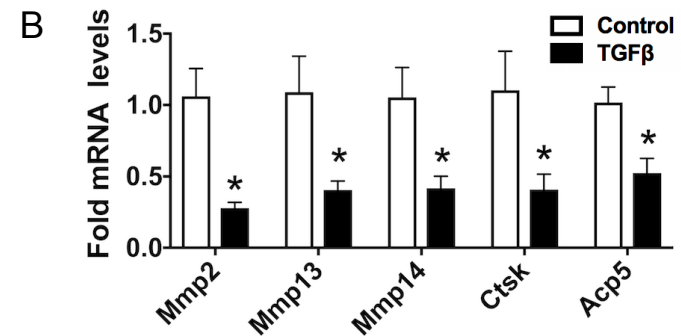
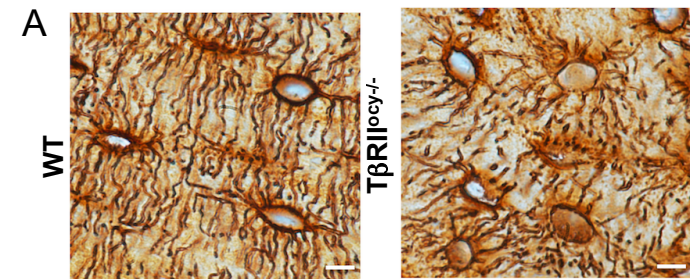
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## OMBRE Protocols I-V

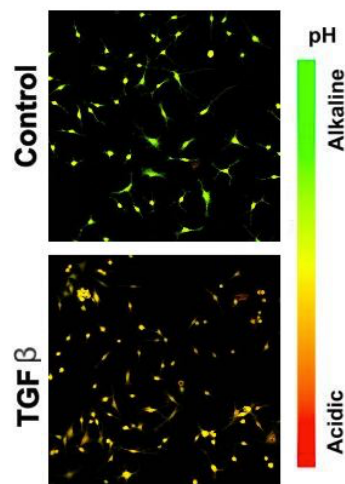
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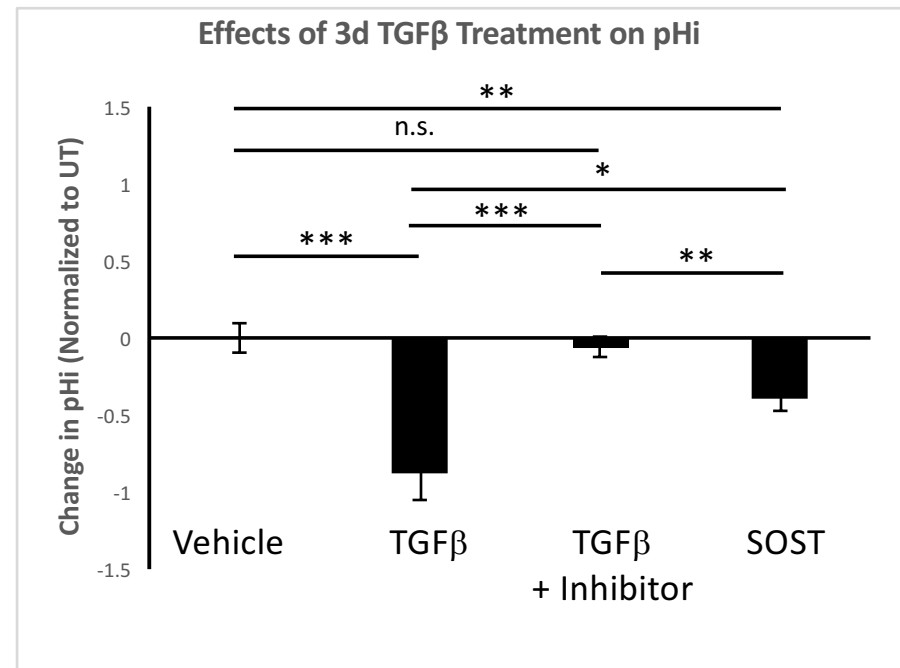


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## Progress Update

Developed & validated in vitro pH assay

- pH regulation matches PLR regulation
- performing viability assay in parallel with pH assay



# Milestones

## In Vitro Assays

- Identify prototypical PLR-inducible gene for 1st in vitro PLR functional outcome
  - Dec 1, 2016
  - **STATUS: New RNAseq data to be analyzed**
- Finish cloning and sequence validation of novel PLR-report construct
  - April 1, 2017
  - **STATUS: waiting for RNAseq data analysis to design desired clone**
- Finish development of 2nd in vitro PLR functional outcome: intracellular pH assay
  - February 1, 2017
  - **STATUS: Assay development and validation complete, writing protocol**

## In Vivo Assays

- Final protocol for OMBRE I: Collagen Organization
  - January 15, 2017
  - **STATUS: Protocol complete, validated in lactating mouse bone**
- Final protocol for OMBRE II: Canalicular Stain
  - March 1, 2017
  - **STATUS: Protocol complete, proceed for quantitative analysis**
- Finish protocol for OMBRE III: PLR Gene Expression
  - May 1, 2017
  - **STATUS: Protocol complete, validated in TBR11<sup>ocy</sup><sup>-/-</sup> mice**

# Clinical Need and Industrial Relevance

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## Contributors

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