Generative Design of Osteocalcin Binder Protein for Point-of-Care Based Sensor

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What is iGEM?

Global Competition

- World's largest synthetic biology competition (350+ teams annually).
- Teams design projects addressing global challenges in health, environment, and technology.
- Integrates lab research, computational modeling, and human practices.
- Emphasizes collaboration, ethics, community engagement, and real-world impact.

UF iGEM History

- Competing at iGEM since 2018.
- **2023**: Silver Medal – developed synthetic biology tools to study sepsis responses in organoids.
- **2024**: Gold Medal – advanced biomedical applications with a focus on organoid models and health innovation.
- **2025**: Designing an osteocalcin-binding protein for bone health monitoring.
- Tradition of strong student leadership, interdisciplinary teamwork, and international collaboration.

Project Description

- **Challenge**: Astronauts lose 1–2% of bone mineral density per month in microgravity, increasing fracture risk. Current diagnostics (DXA, ELISA) are too slow and impractical for spaceflight.
- **Solution**: Design a synthetic protein binder that detects osteocalcin, a key biomarker of bone turnover, using BindCraft (AI-driven protein design).
- **Approach**:
  - Computationally design and screen binders with AlphaFold2 backpropagation.
  - Express and purify top candidates in *E. coli*.
  - Test binding specificity and kinetics via Bio-Layer Interferometry (BLI).
- **Impact**: Develop the foundation for a wearable, non-invasive **biosensor** to monitor astronaut bone health in real time.
- **Broader Applications**: Potential for use in osteoporosis monitoring, dentistry, and personalized medicine on Earth

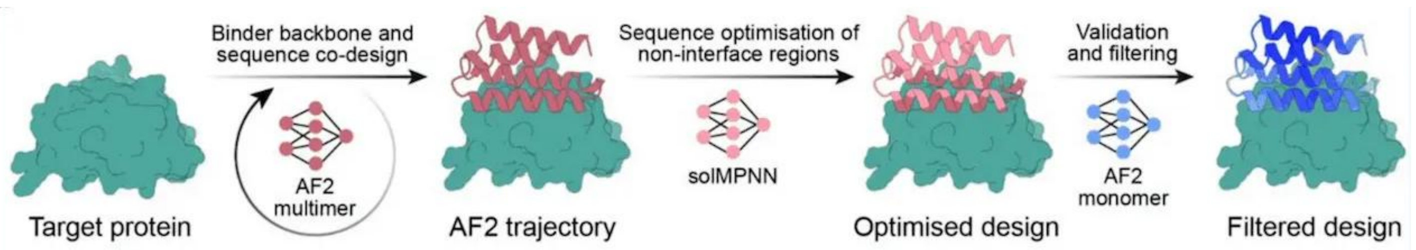
Human Practices

- **Clinical Perspective**: Interviewed orthopedic specialists and space medicine experts on bone health monitoring needs.
- **Ethical Considerations**: Assessed privacy and equity concerns in wearable biosensors for both astronauts and patients on Earth.
- **UF & Local Outreach:** Led STEM workshops and public talks on synthetic biology and bone health awareness.
- **Global iGEM Collaboration:** Shared protocols and feedback with teams at Stanford, QGEM, and KCIS Taipei.
- **Application Expansion:** Explored translation of osteocalcin biosensor for osteoporosis patients and dental implant monitoring.

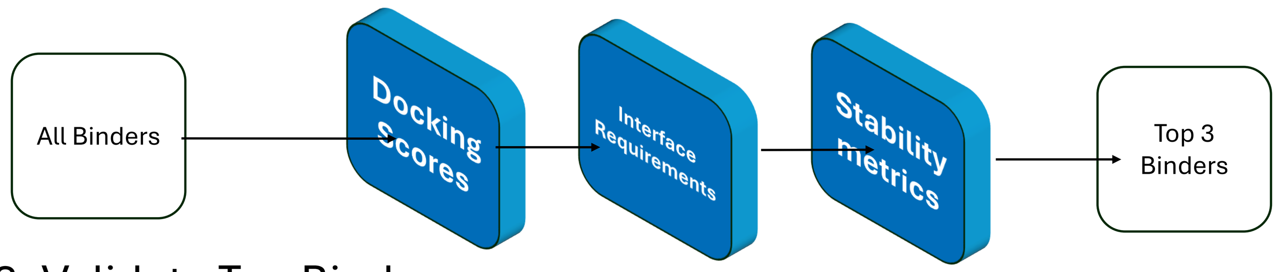
Methods

De novo osteocalcin binders were computationally designed, filtered, and validated for unbiased binding. Top sequences were cloned into engineered plasmids and expressed in *E. coli*. His-tagged proteins were purified with Ni-NTA columns, verified by SDS-PAGE, and quantified by DC assay. Binding kinetics were measured by Bio-Layer Interferometry (BLI) to assess osteocalcin interaction.

1. Create DeNovo Binders

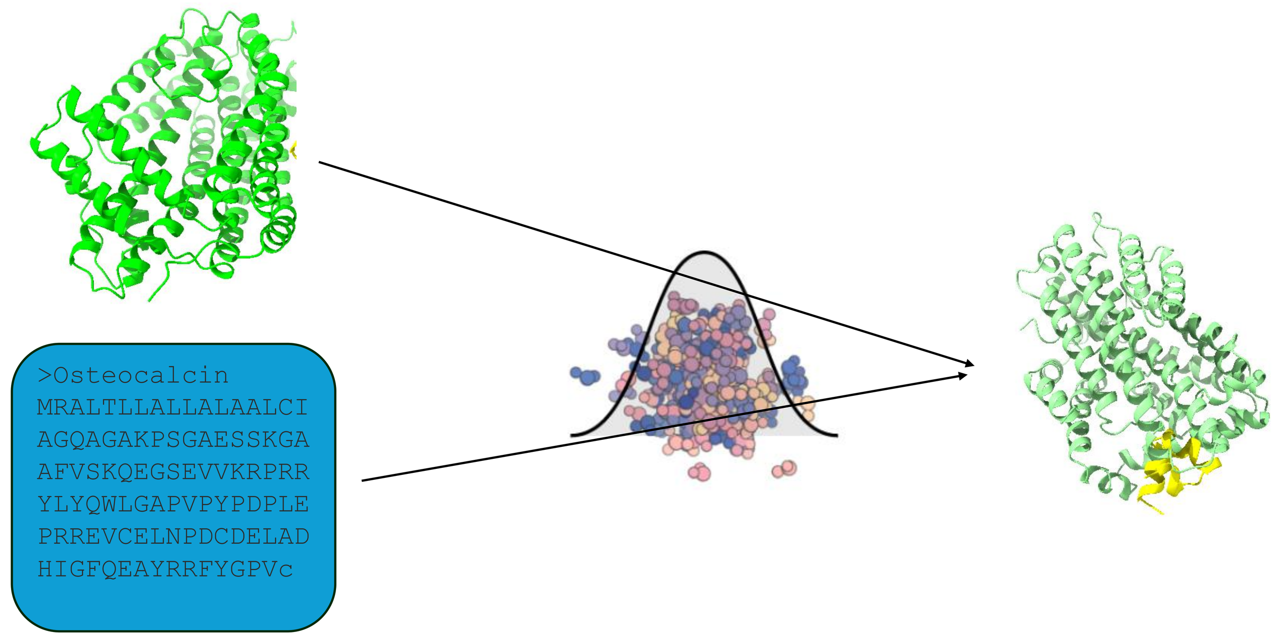


2. Filter all Binders



3. Validate Top Binders

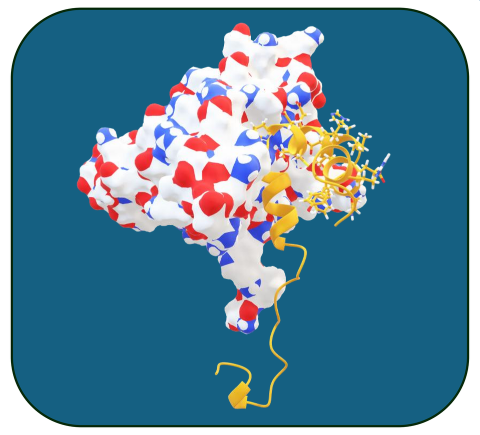
- RF Diffusion used to model all conformations of osteocalcin
- Ensure equal binding to all conformations (no biases)



4. Wet Lab Expression

- Selected three top-ranked binder sequences for cloning into plasmid vectors.
- Engineered plasmid vectors with:
  - 6X His-tag (protein purification)
  - Twin Strep-tag (binder immobilization during evaluation)
  - Linker sequences (reduce steric interference)
  - HRV3C protease cleavage sites (tag removal if needed)
  - T7 promoter and lac operator (inducible expression in *E. coli*)
- Induced protein expression in *E. coli*, followed by cell harvest and lysis.
- Cleared lysates by centrifugation and filtration to remove debris.
- Purified His-tagged proteins using Ni-NTA spin columns.
- Assessed protein purity via SDS-PAGE analysis.
- Quantified protein concentration with a DC protein assay.
- Evaluated binding efficacy by Bio-Layer Interferometry (BLI), immobilizing binders on biosensor tips via the Strep-tag and measuring osteocalcin interaction kinetics.

Results

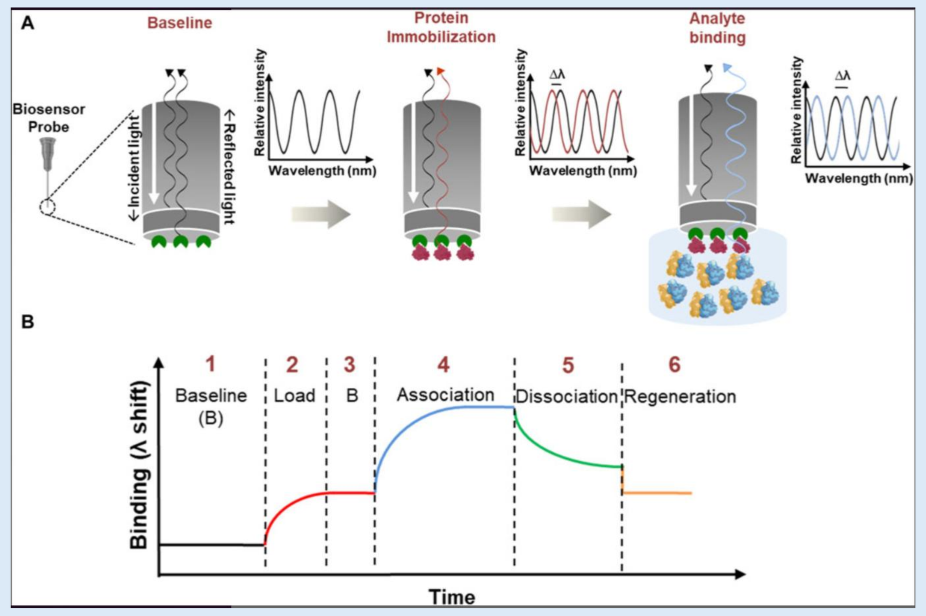


Computational Findings

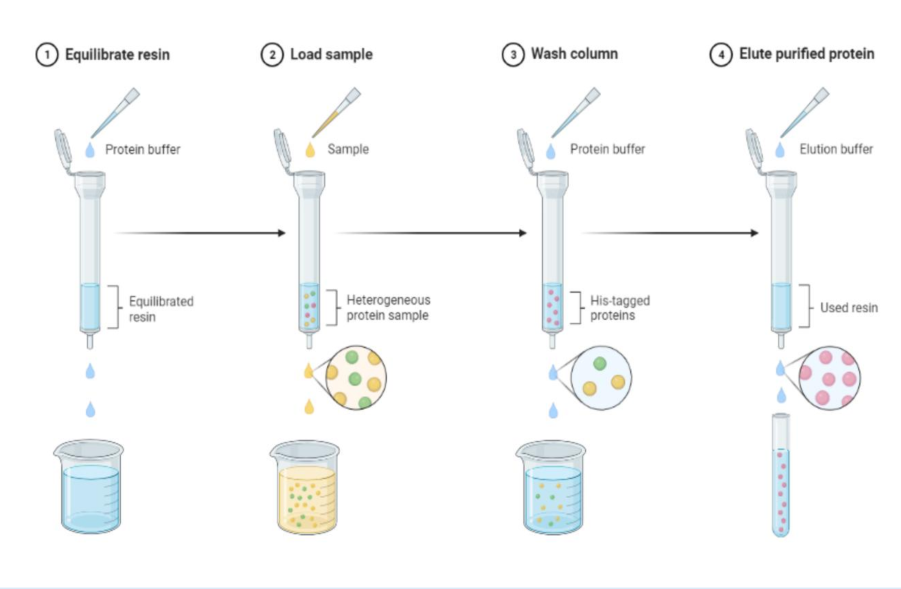
- Higher docking scores correlated with binders containing more hotspot amino acids at the interface.
- Optimal binder length ranged from 85–242 amino acids.

Experimental Findings

- Successful cloning of three candidate binder sequences into plasmid vectors.
- Efficient expression and purification of His-tagged proteins, confirmed by clear SDS-PAGE bands at expected molecular weights.
- Protein concentrations determined, enabling standardization across binding assays.
- BLI analysis produced measurable association and dissociation curves, confirming osteocalcin-binding activity.
- Comparative binding kinetics identified the most promising candidate(s) for further optimization.



Bio-Layer Interferometry (BLI) assay principle. (A) Binders are immobilized on a biosensor tip, and binding of analyte causes a measurable wavelength shift in reflected light. (B) Sensorgram shows baseline, protein loading, association, dissociation, and regeneration phases, enabling quantification of binding kinetics.



Ni-NTA affinity purification workflow for His-tagged proteins. The column resin is equilibrated, the protein sample is loaded, unbound proteins are washed away, and His-tagged proteins are eluted in purified form.

Acknowledgments & Future Directions

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Future Directions

- Optimize binder specificity and affinity under physiological conditions.
- Validate binder performance in complex biological samples.
- Integrate binder into a wearable, non-invasive biosensor platform.
- Expand applications to osteoporosis monitoring, dental implants, and personalized medicine.

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