

Targeting Protein Therapeutics to the Bone Surface – elucidating key determinants of accumulation, retention, and function

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INTRODUCTION

While biologics have gained popularity as viable therapeutic approaches to treat various musculoskeletal diseases including osteoporosis and rheumatoid arthritis, there are concerns of unintended effects outside of the bone microenvironment, as evidenced by recent clinical trials. Localizing these therapeutics specifically to the bone surface may significantly enhance therapeutic efficacy and broaden the array of potential molecular targets through a precision targeting approach.

The goal of this study is to identify the pharmacologic design parameters which affect the localization of potential protein therapeutics to the bone surface, including affinity, avidity, and circulation time.

METHODS

- mCherry was used as a model biotherapeutic, given the ease of assessing and monitoring its function
- Either a bisphosphonate (alendronate, or ALD) or deca-aspartate (D₁₀) was added to the N- or C-terminus of mCherry
- To enhance circulation time, the Fc-fragment of human IgG₁ was fused to mCherry with D₁₀ on each C-terminus (mCherry-Fc-D₁₀)
- Affinity of targeted proteins was confirmed *in vitro* (bovine bone chip assay) prior to injection in adult (16-20 week old) C57BL/6 mice
- To study avidity and effect of affinity ligands, monovalent D₁₀ and bivalent D₁₀ constructs were added to the C-terminus of human IgG₁
- Bone and blood biodistribution and pharmacokinetics were measured via radiotracing
- mCherry function, localization, and retention within bone tissue were evaluated via confocal imaging

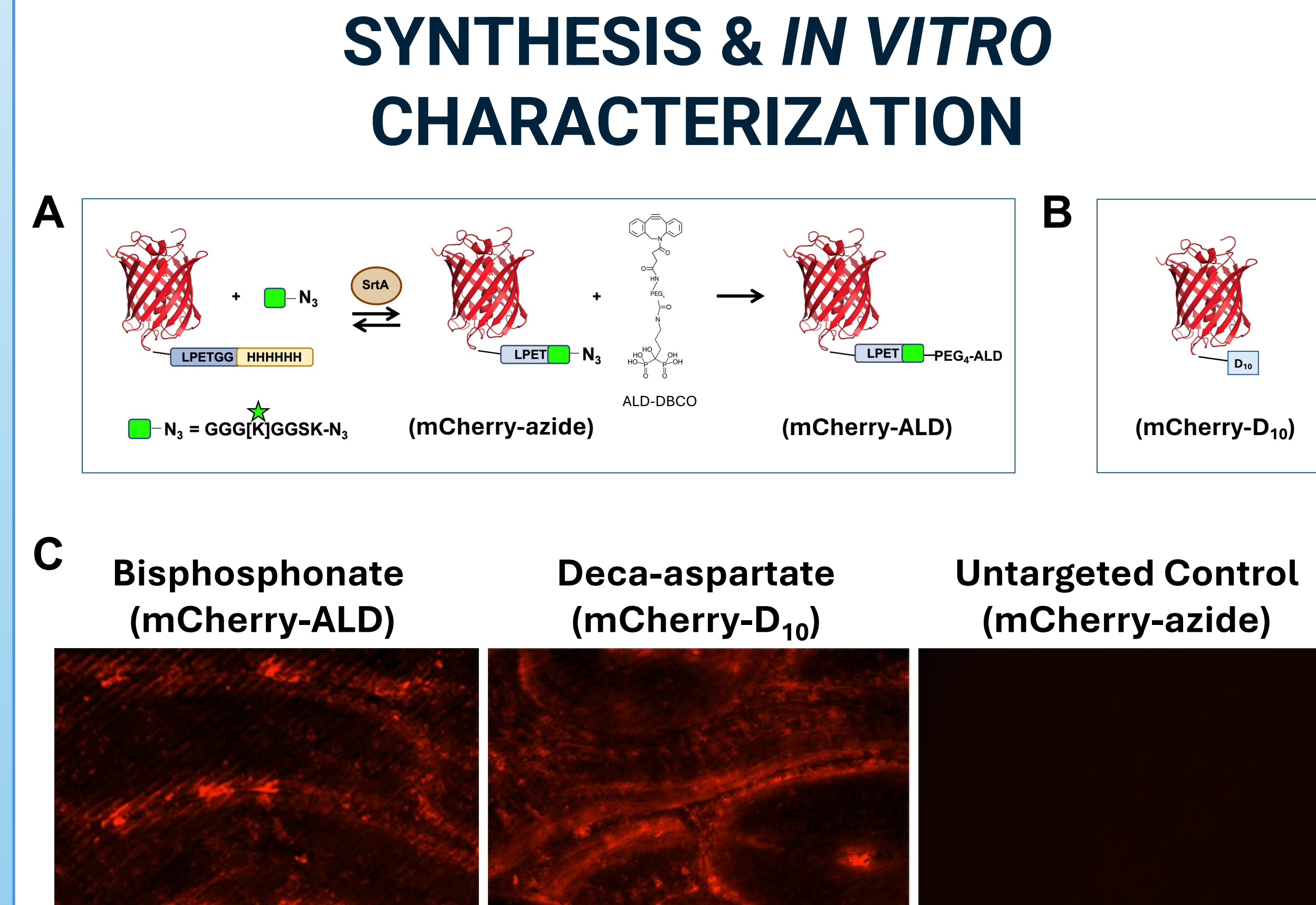


Figure 1. Synthesis and *in vitro* affinity to bone. A. Synthesis of mCherry-azide and mCherry-ALD. The enzyme SortaseA selectively cleaves the peptide bond between threonine and glycine to site-specifically add an azide group (N₃) to the C-terminus of mCherry. ALD-DBCO is then able to react with the azide on mCherry to produce mCherry-ALD. B. mCherry-D₁₀ is synthesized as one continuous peptide. C. Attachment of both bisphosphonate (ALD) or deca-aspartate (D₁₀) was effective in causing localization of mCherry to bone chips compared to the azide (N₃) untargeted control.

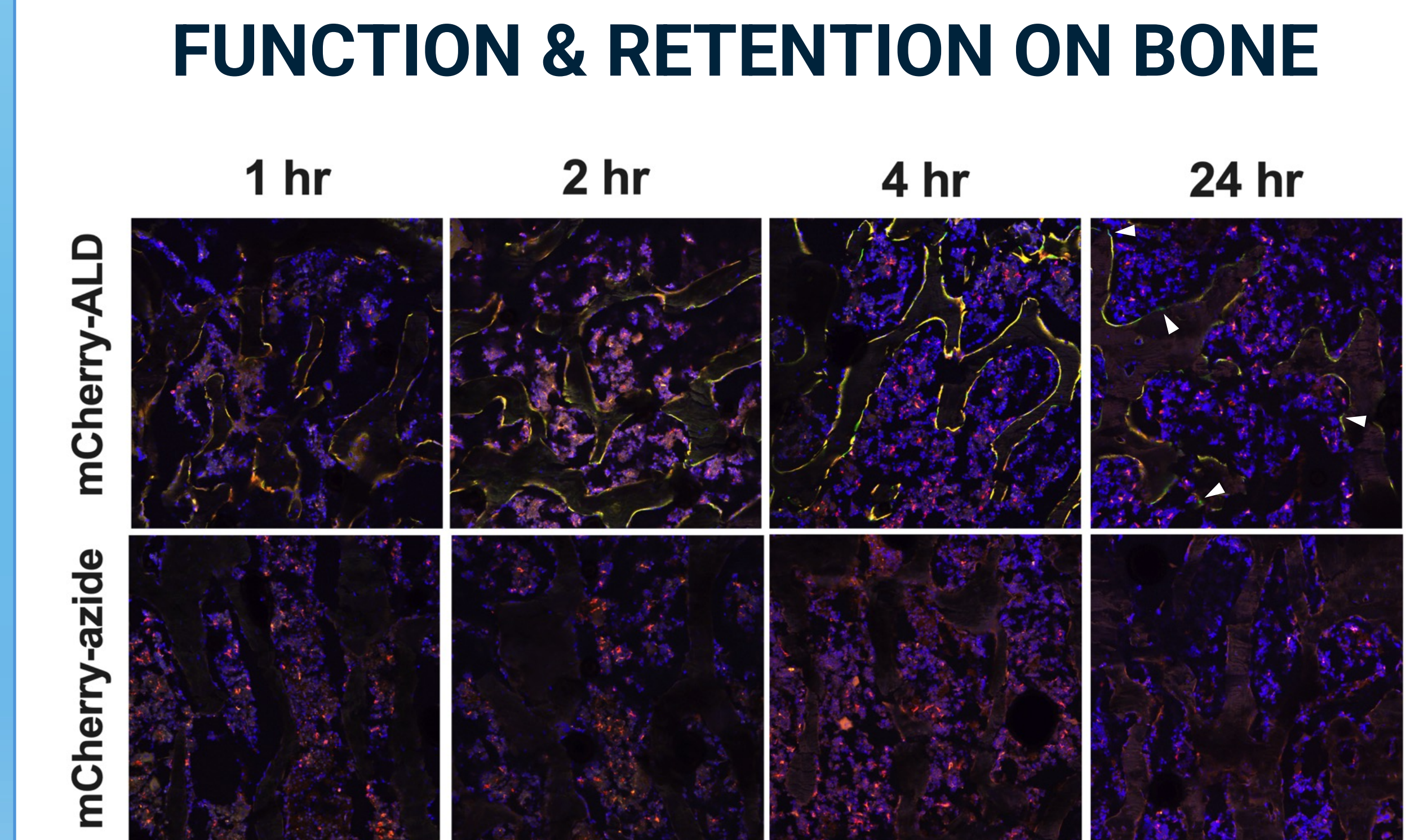


Figure 2. Time course on bone surface. Fluorescence imaging of vertebral bone following injection of 5mg/kg mCherry-ALD or mCherry-azide. Targeted proteins accumulated to a peak at approximately 4 hours post-injection. By 24 hours post-injection, fluorescent signal has decreased and there is appearance of isolated green fluorescence (white arrowheads), suggesting potential inactivation and/or catabolism of bone-anchored mCherry.

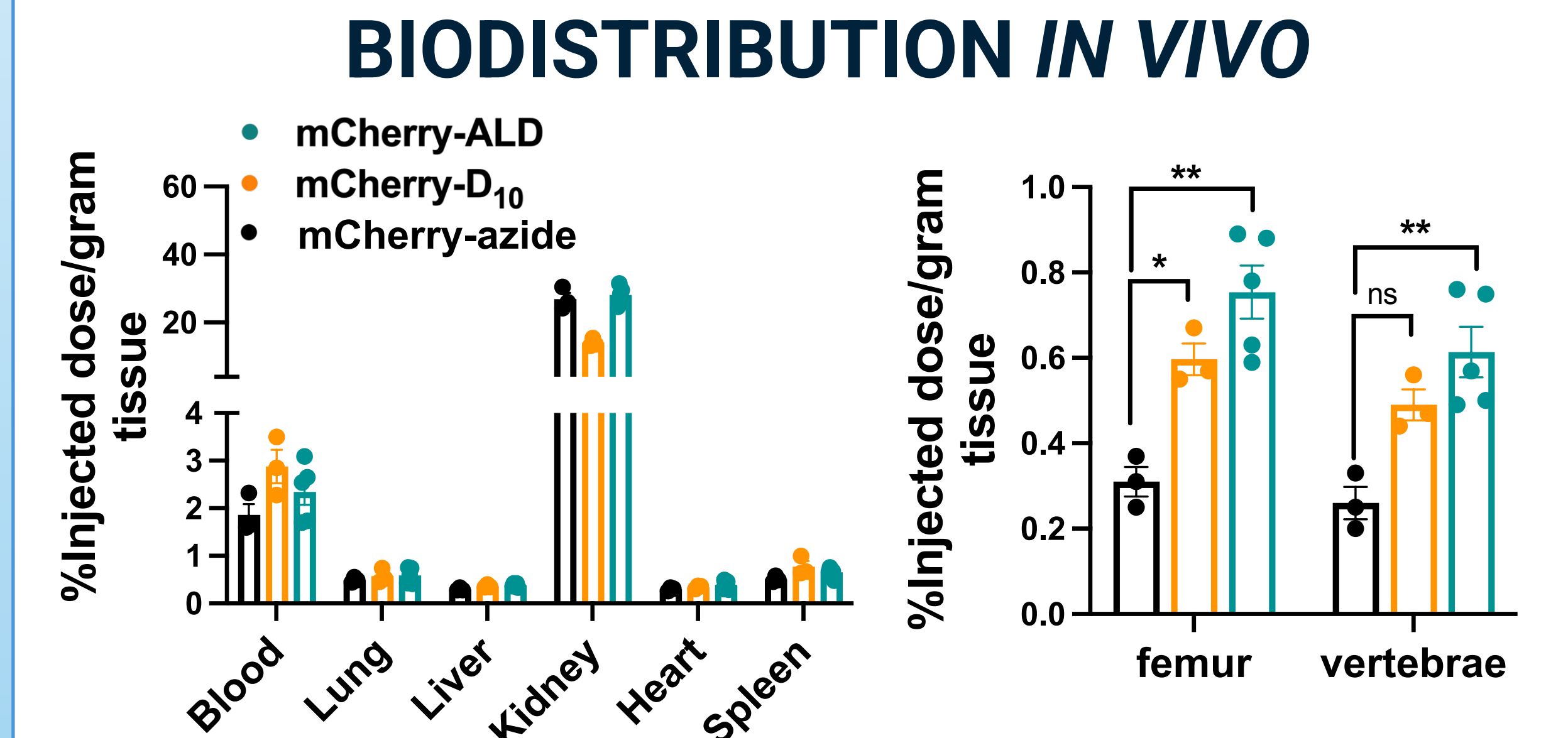


Figure 3. Biodistribution *in vivo*. Mice were injected intravenously with 2mg/kg of ¹²⁵I-labeled mCherry-ALD, mCherry-D₁₀, or mCherry-azide. All proteins cleared from the blood quickly via renal filtration, with only ~2-3% injected dose (ID)/g remaining in the blood and the majority in the kidney and urine. Both bisphosphonate and D₁₀ affinity ligands resulted in selective accumulation on the bone (* = p < 0.05, ** = p < 0.01).

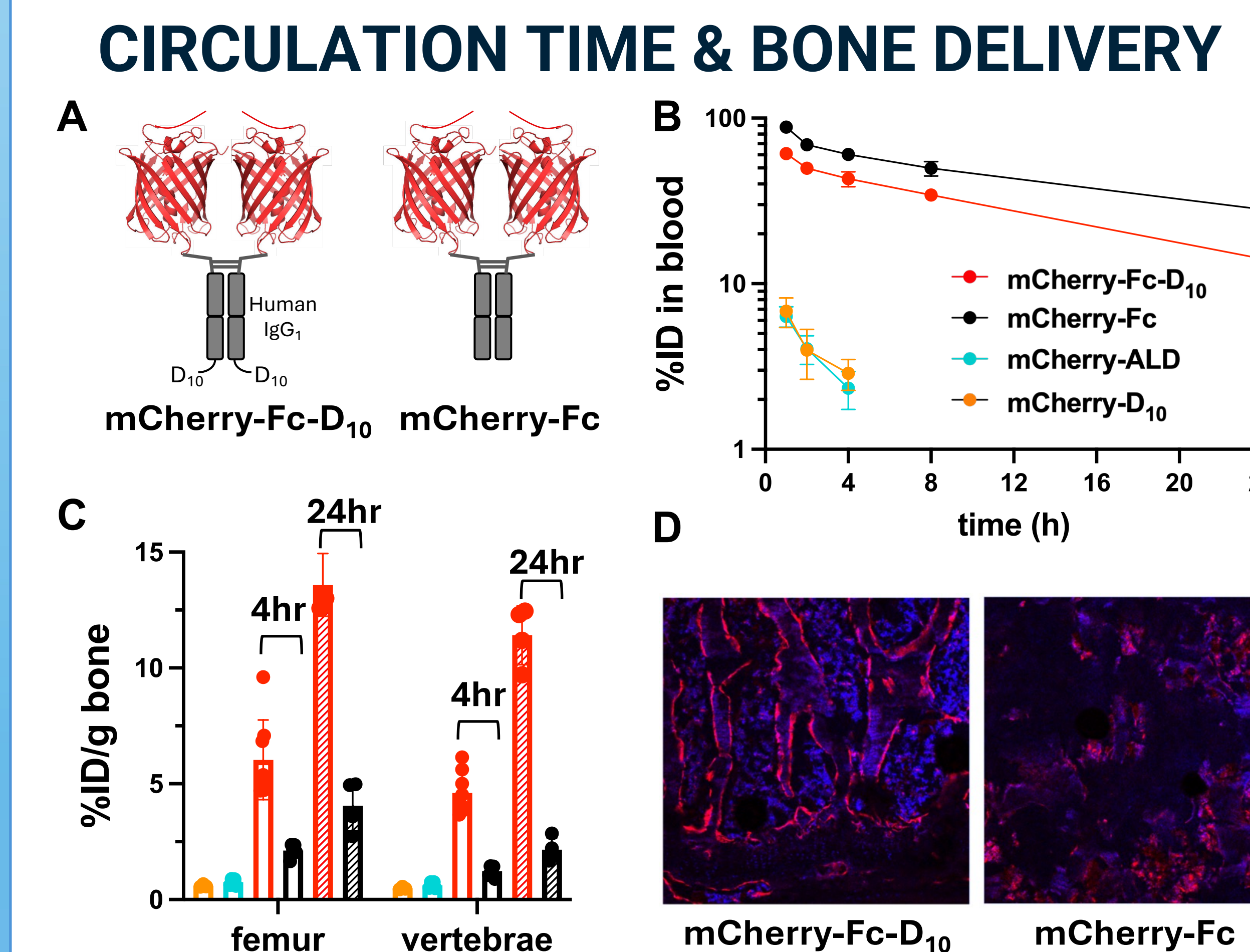


Figure 4. Half-life extension enhances bone accumulation *in vivo*. A. To investigate the role of circulation time, we synthesized a bivalent mCherry-Fc fusion, with the D₁₀ affinity ligand fused to the C-terminus of each heavy chain. Non-targeted mCherry-Fc fusion (i.e., no D₁₀) was used as a control. B. Blood PK shows prolongation of circulation time vs. mCherry-ALD and mCherry-D₁₀. C. mCherry-Fc-D₁₀ shows an order of magnitude increase in %ID/g on bone at 4 hrs vs. mCherry-ALD and mCherry-D₁₀. Given the significant % ID still in the blood at 4 hours, we measured bone accumulation at 24 hrs and found a further 2-3 fold increase in both femur and vertebrae to 13.58±1.36 and 11.41±1.27 %ID/g, respectively (n=4, mean±SD, p < 0.001 vs. all other groups). Interestingly, mCherry-Fc showed selective accumulation in bone as well, although fluorescence imaging (D) suggests that this may be the result of uptake in the marrow, rather than accumulation at the bone surface.

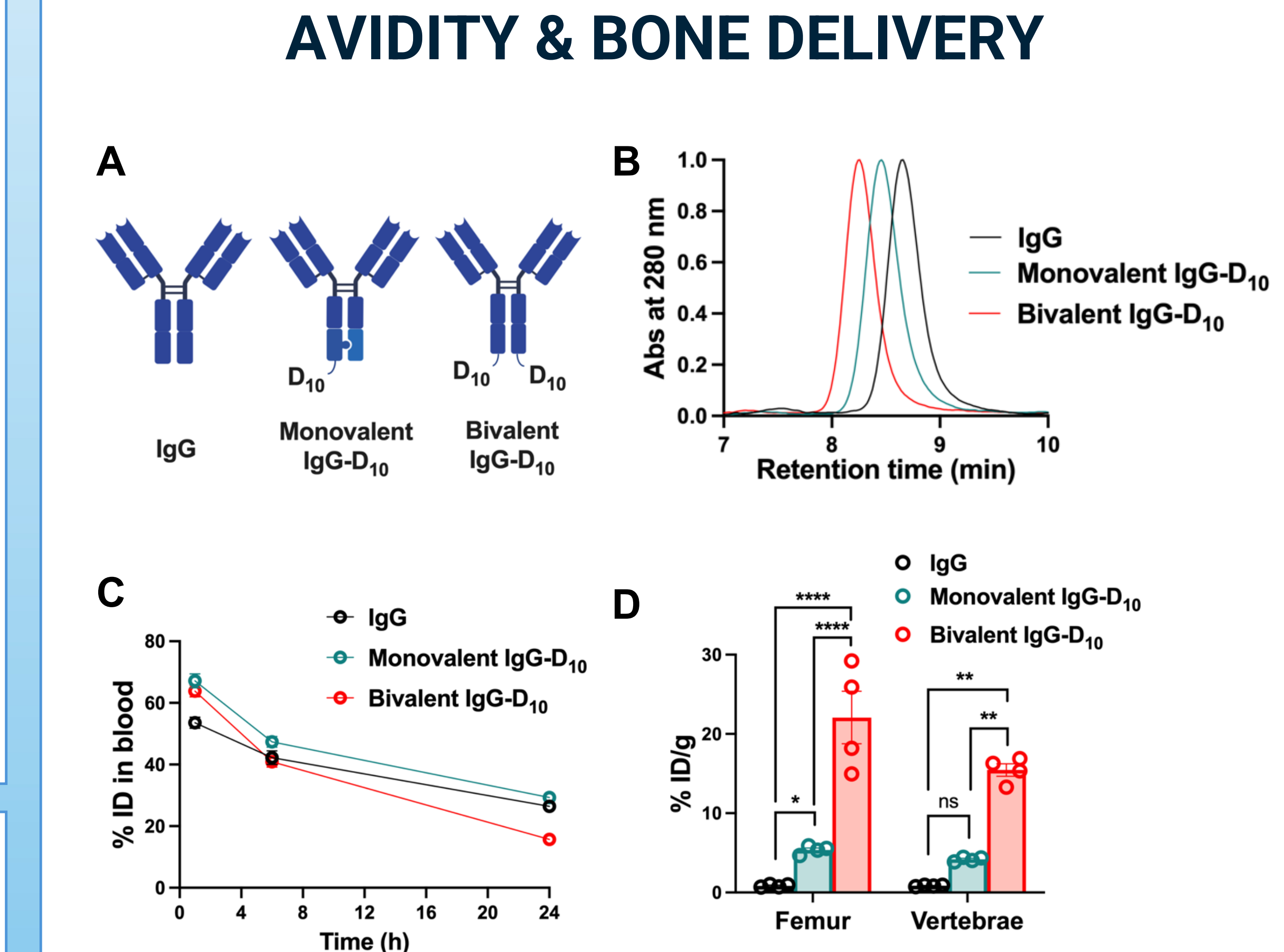


Figure 5. Avidity enhances bone accumulation *in vivo*. A. Schematic of IgG control and IgG with either one or two D₁₀ affinity ligands. B. Size exclusion chromatography indicating an increase in antibody size with the addition of D₁₀ affinity ligands. C. Pharmacokinetics in the blood. D. Bone targeting is increased on the femur with one D₁₀ affinity ligand. Bone targeting is increased in both the femur and vertebrae when two D₁₀ affinity ligands are added to the antibody (* = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001).

CONCLUSIONS

- A modification with either ALD or D₁₀ results in binding of a model fluorescent protein, mCherry, to bone *in vitro* and accumulation on the bone surface *in vivo*
- Addition of the Fc-fragment of human IgG₁ results in increased blood circulation time and an increase in bone accumulation over time
- Bone surface targeting can be modulated by varying avidity – two D₁₀ affinity ligands fused to the C-terminus of an antibody results in more accumulation on the femur and vertebrae than one D₁₀ affinity ligand
- Bone surface targeting may be studied further using a different negatively charged amino acid, glutamate, in an affinity ligand (E₁₀) or longer bone targeting affinity ligands (D₂₀)