

# From clotting to calcification: Gla-domains as guardians of vascular remodeling

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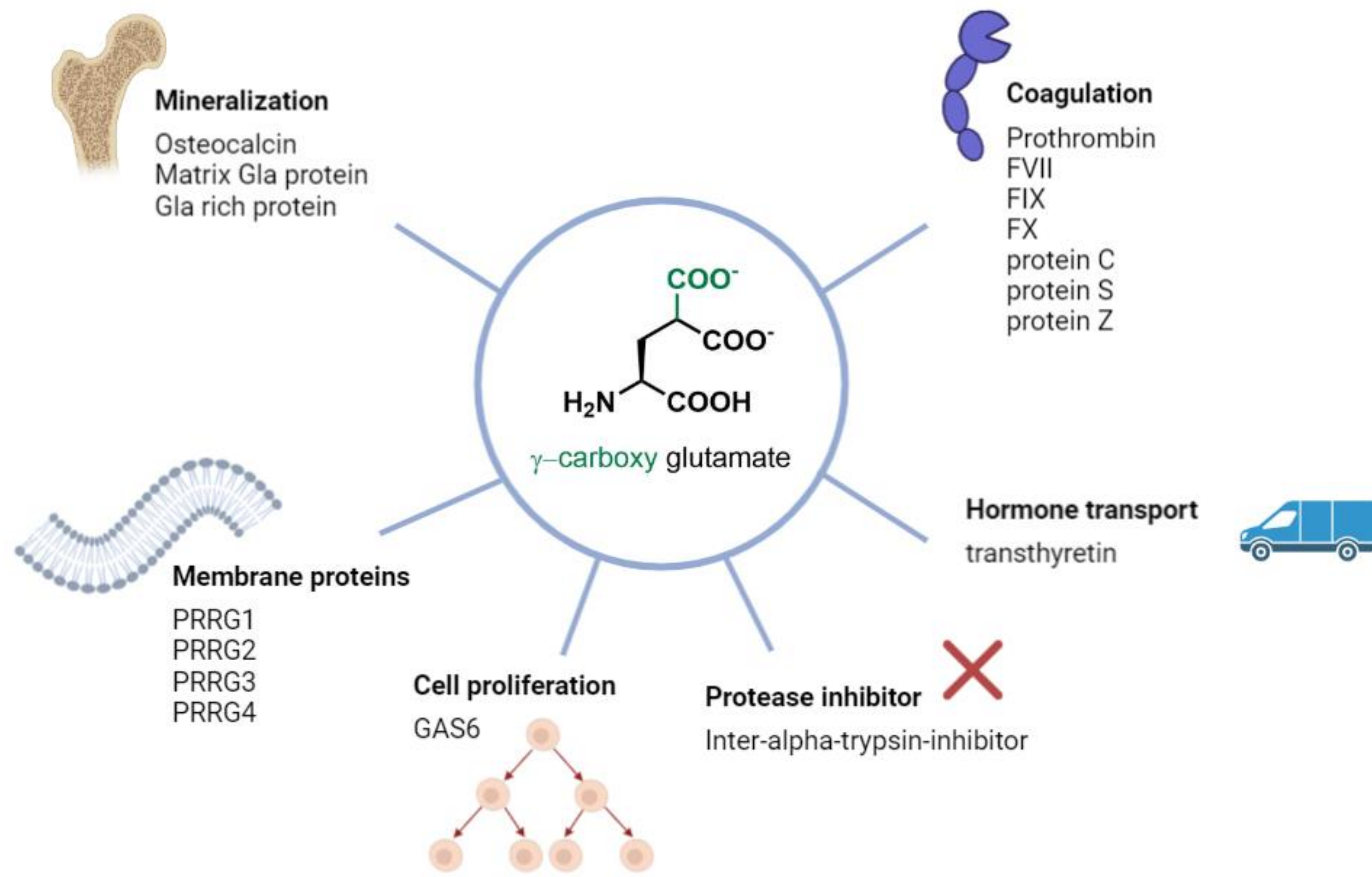
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## Vitamin-K dependent proteins and calcification

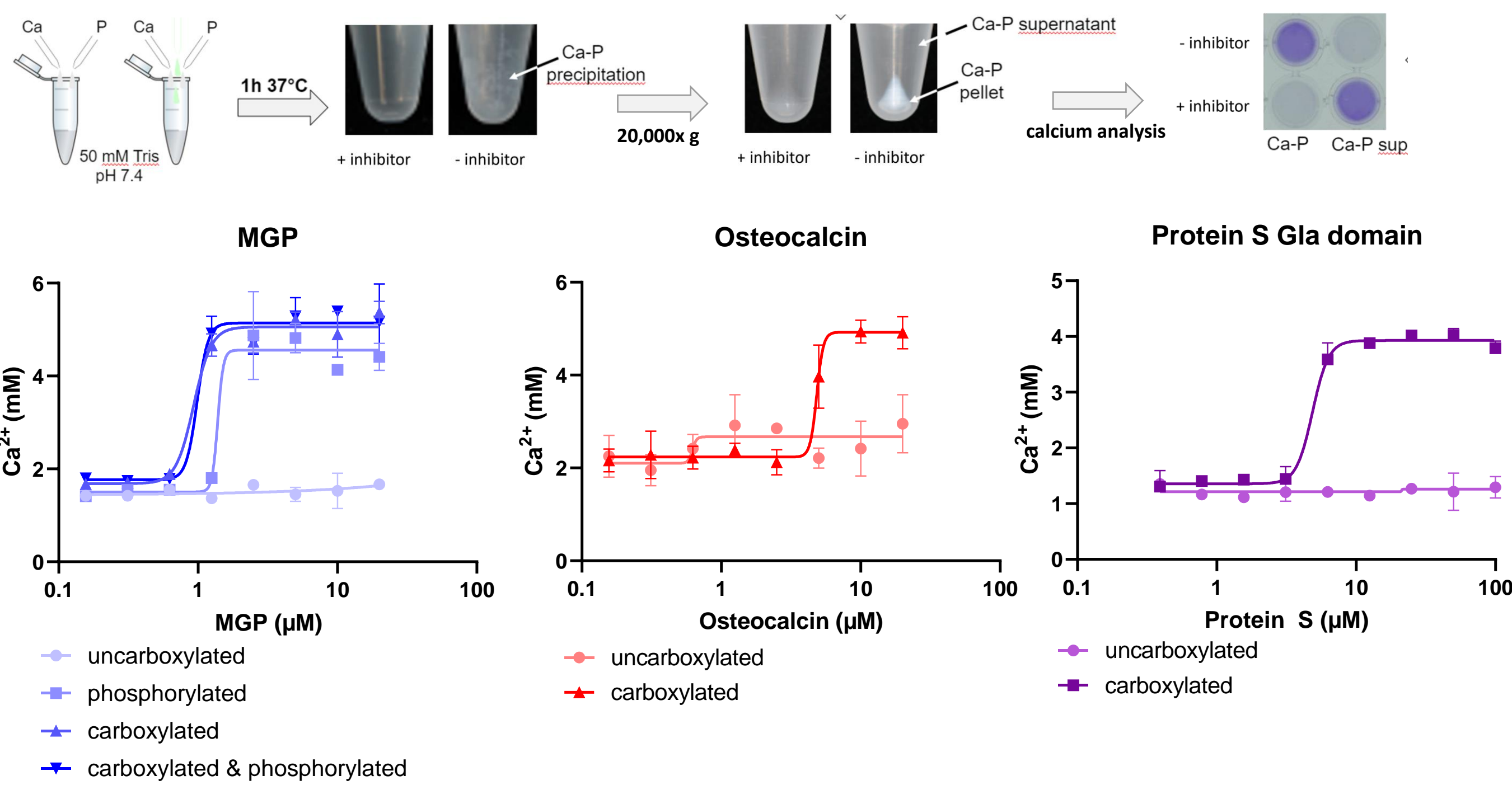
**Coagulation and mineralization** have little in common, proteins from both subfamilies can affect the critical pathological process of **vascular calcification**. Mechanisms by which they modulate calcification, however, are largely unknown. Therapeutic interventions are unavailable, and diagnosis can only be performed at a late stage of disease, hampering possible intervention.



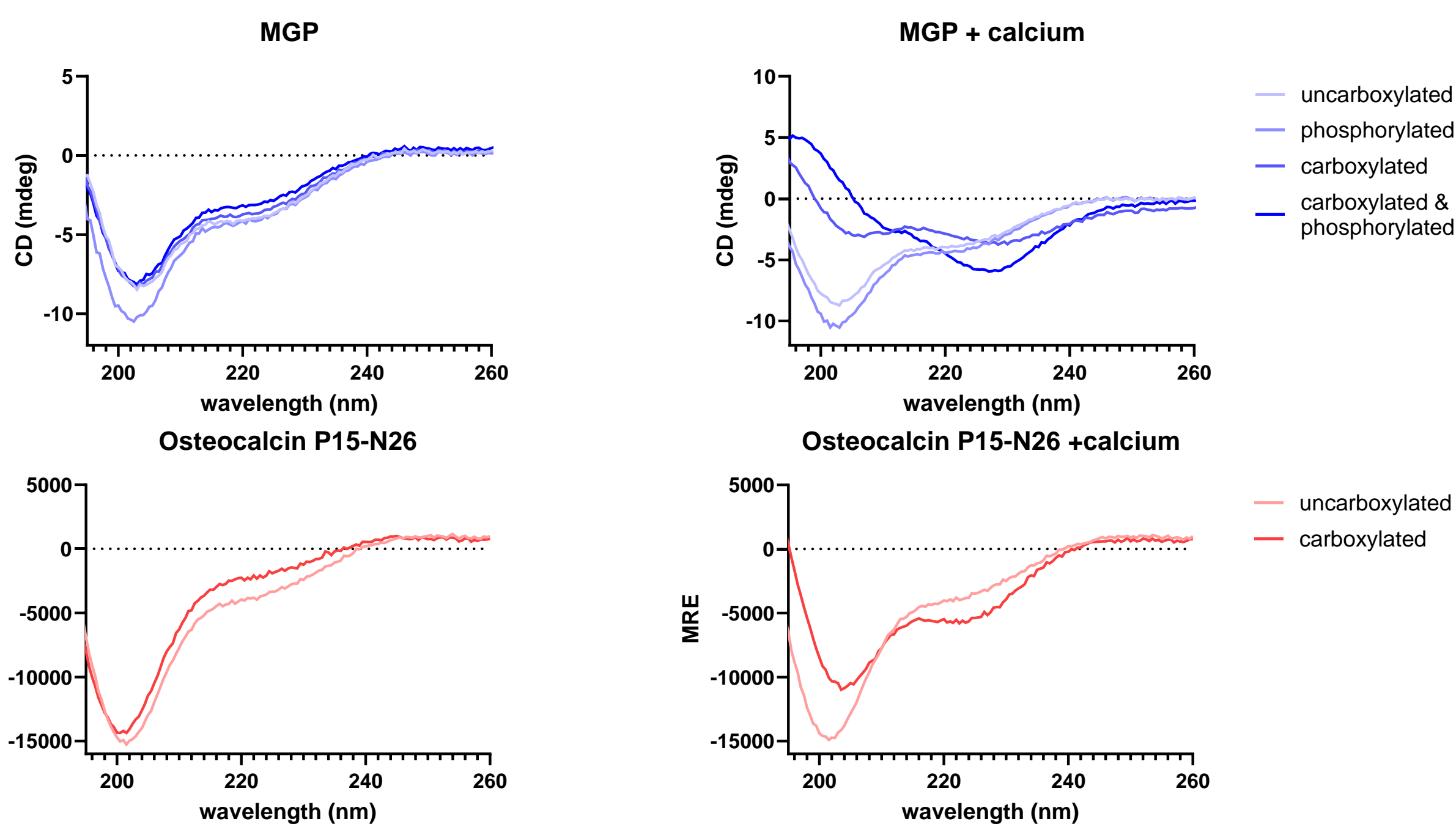
## Aims

- Provide access to post-translationally modified variants of VKD proteins
- Elucidate modulatory mechanism of action of VKD proteins in vascular calcification
- Enable early detection and repair of vascular calcification

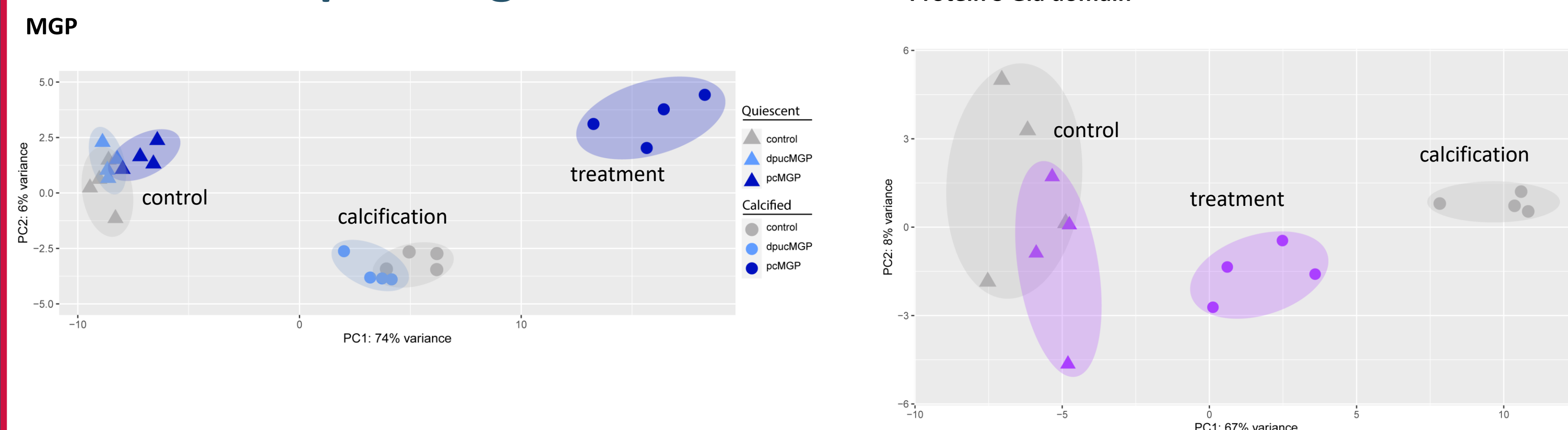
## Calcium phosphate precipitation inhibition



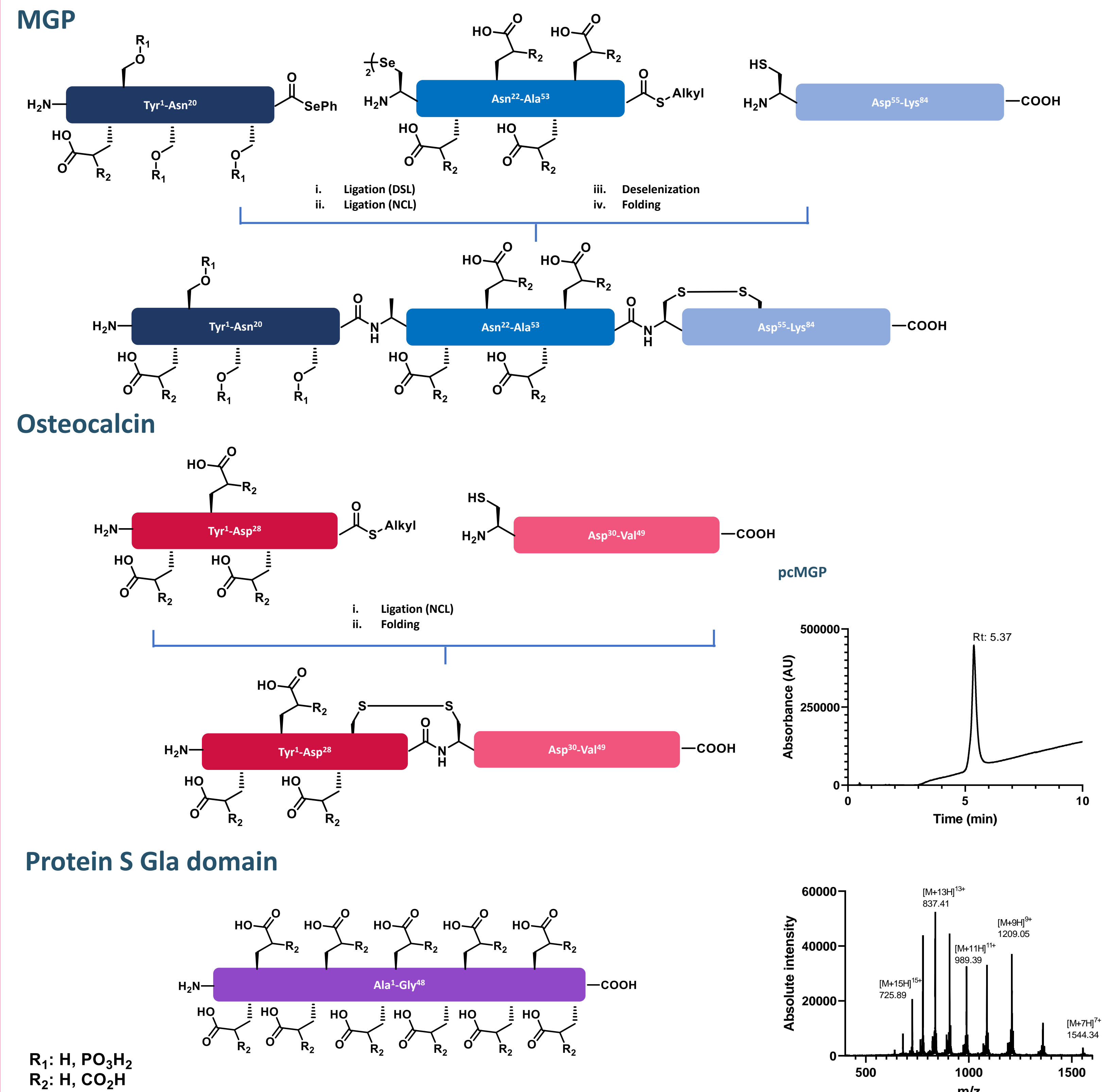
## Protein structure determination using CD



## Bulk RNA sequencing

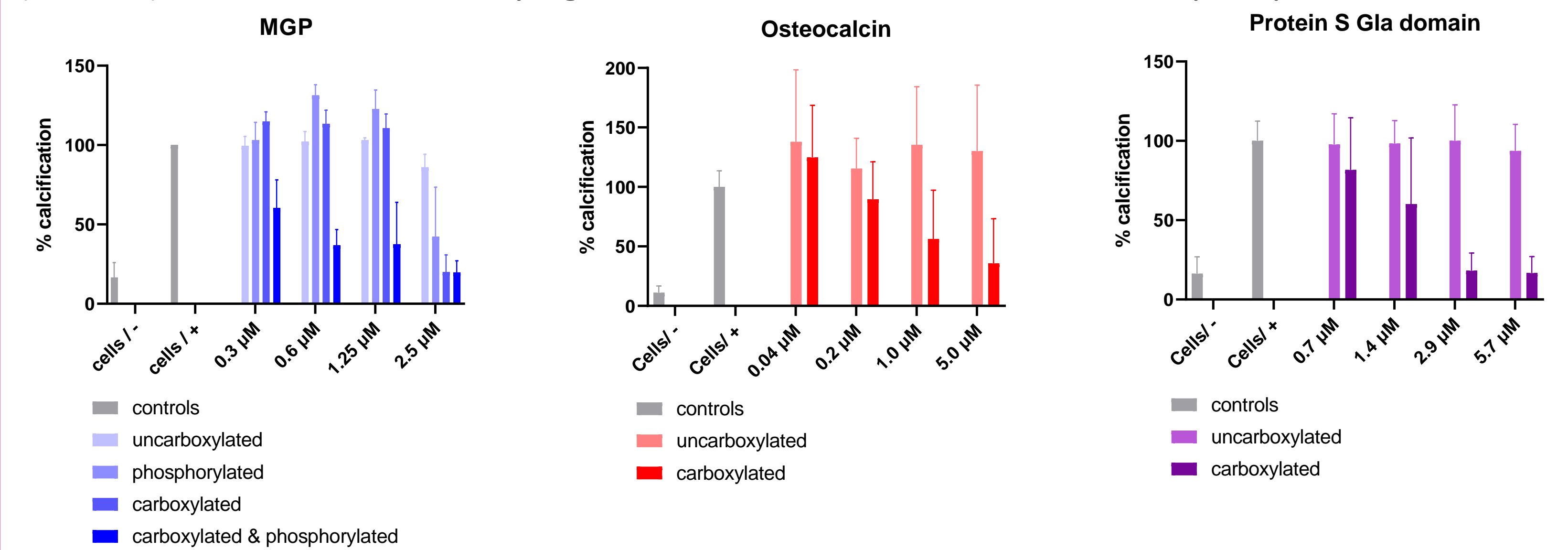


## Chemical protein synthesis



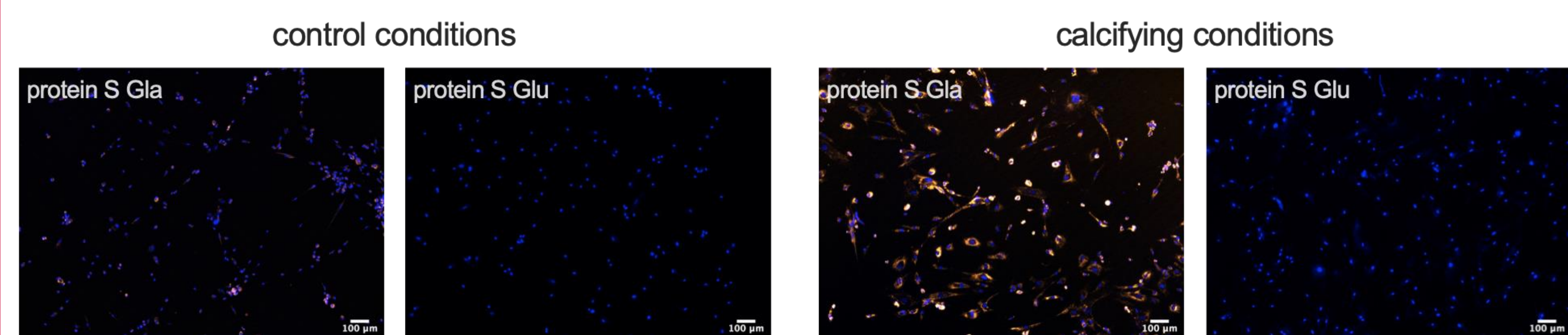
## Calcification modulation of hVSMCs

Cellular model of vascular calcification using primary human vascular smooth muscle cells (hVSMC) cultured under calcifying conditions of increased calcium and phosphate.



## Detection of calcifying cells using protein S Gla domain

Protein S Gla was used to identify calcifying cells; hVSMC were exposed to either stringent calcification conditions or control conditions for 24 hours in the presence of 1.5 µM protein S in uncarboxylated (Glu) or carboxylated (Gla) form.



## Conclusions

- Three different VKD proteins in multiple variants were synthesized
- In vitro calcium phosphate precipitation can be inhibited by all post-translationally modified VKD proteins
- Calcification of human vascular smooth muscle cells was inhibited by phosphorylated and carboxylated variants of VKD proteins
- Structure of carboxylated proteins is influenced by addition of calcium
- RNA sequencing may point to two distinct mechanisms of action of VKD proteins from the mineralization and coagulation group