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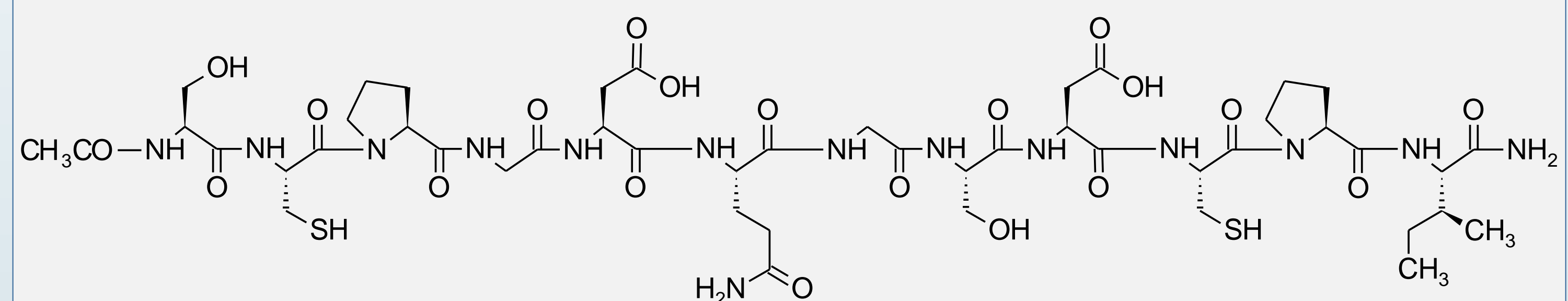
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Introduction

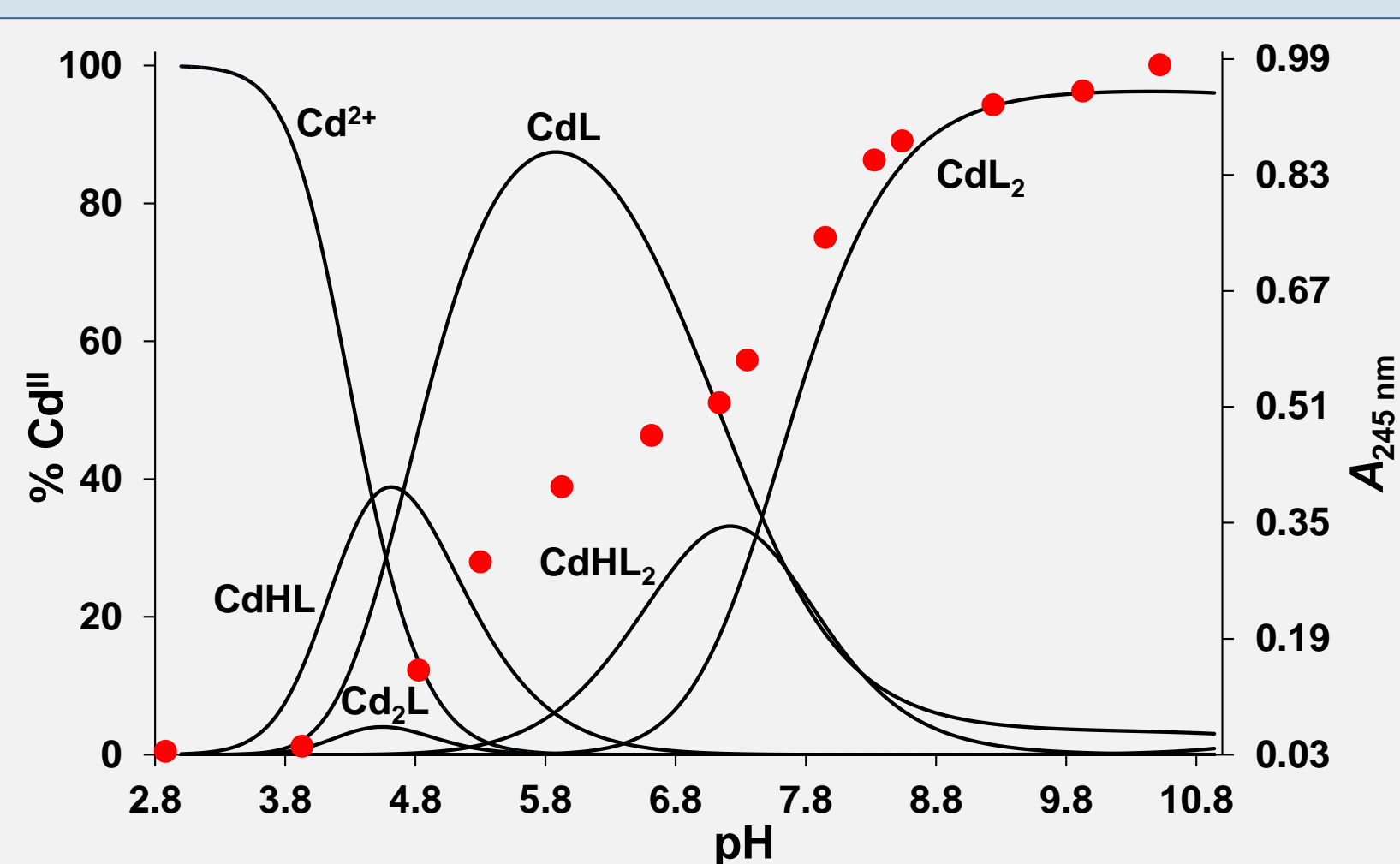
Metalloregulatory proteins of the MerR family are transcriptional activators that sense/control the concentration of various metal ions inside bacteria.¹ The Cu⁺ efflux regulator CueR, similarly to other MerR proteins, possesses a short multiple Cys-containing metal binding loop close to the C-terminus. CueR has a high selectivity for Cu⁺, Ag⁺ and Au⁺, but exhibits no transcriptional activity for the divalent ions Hg²⁺ and Zn²⁺.² The two Cys-residues of the metal binding loop were shown to settle M⁺ ions into a linear coordination environment but other factors may also play a role in the recognition of cognate metal ions.² Nevertheless, it is an interesting question whether the same sequence, when removed from the protein, shows a flexibility to adopt different coordination environments and may efficiently bind metal ions having preferences for larger coordination numbers.

Aims

To address these questions a 12-mer peptide (PP), comprising the metal binding loop of *V. cholerae* CueR, has been synthesized and the Zn²⁺, Cd²⁺, Hg²⁺ and Ag⁺ binding of the ligand has been investigated by various techniques.



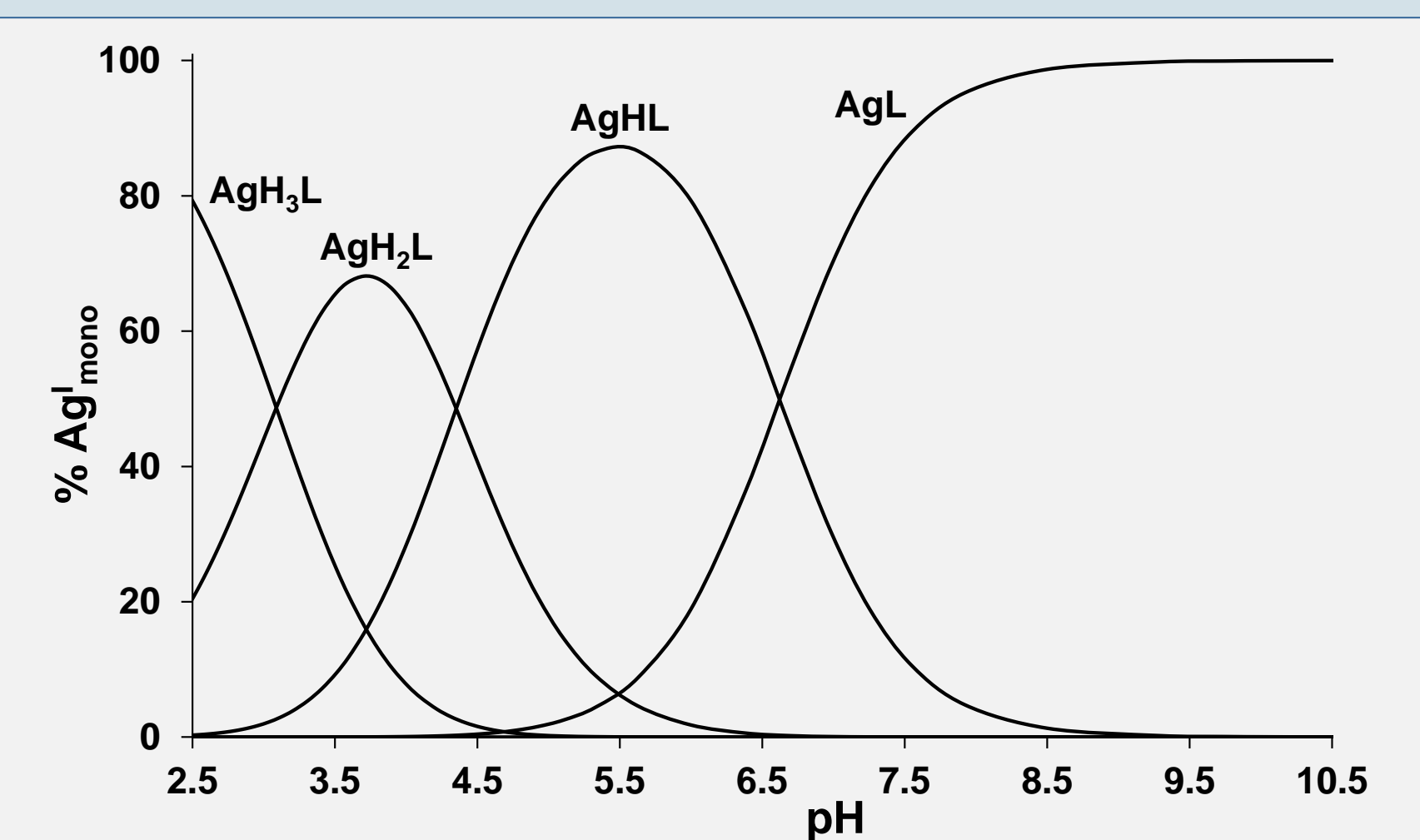
Schematic structure of the studied Ac-SCPGDQGSDCPI-NH₂ (PP) peptide



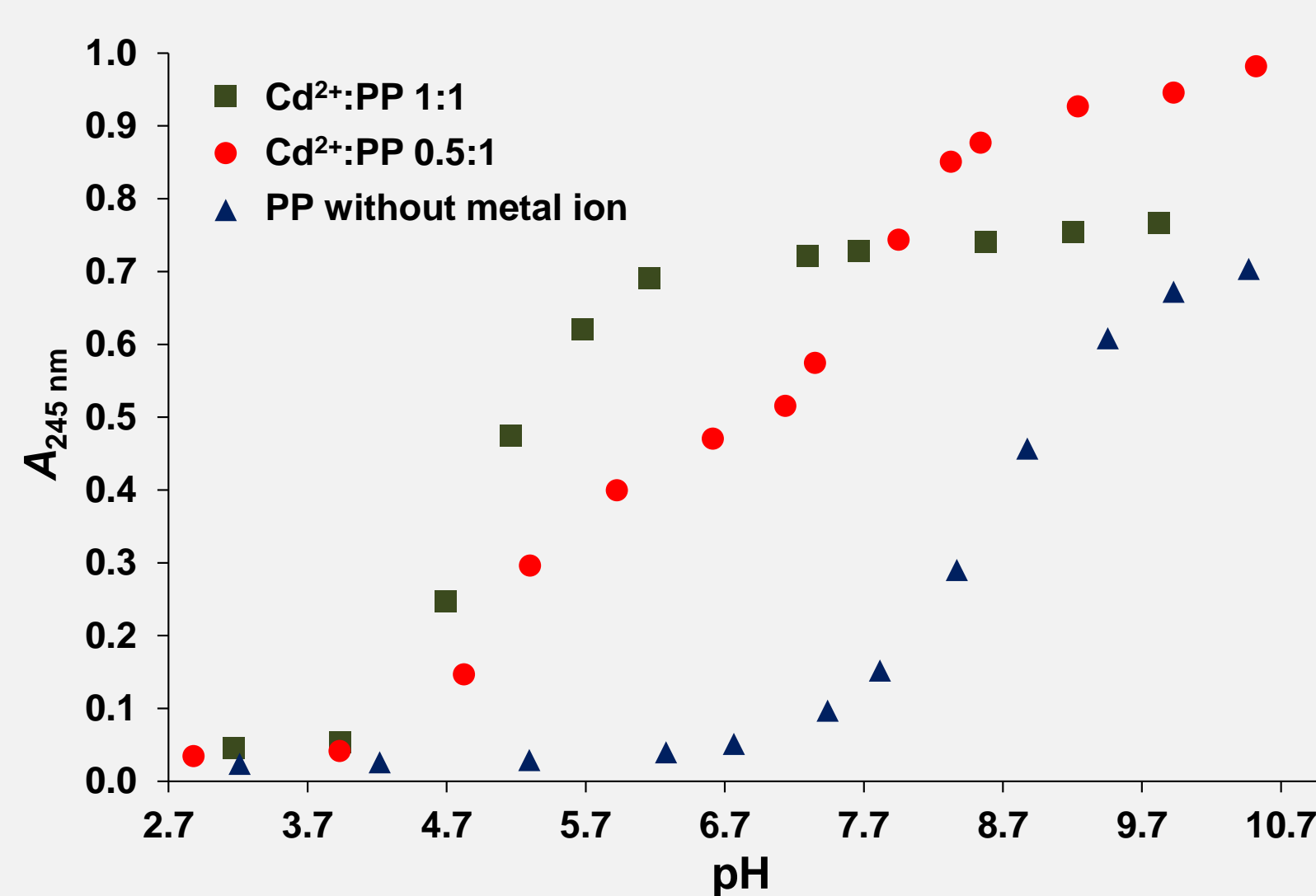
Species distribution curves for the Cd²⁺:PP 0.5:1 system ($C_{PP} = 1 \times 10^{-3}$ M). Red circles represent the recorded absorbances at $\lambda = 245$ nm ($C_{PP} = 2 \times 10^{-4}$ M, $l = 0.5$ cm).

Cd²⁺ - PP and Zn²⁺ - PP systems

LMCT bands emerging below pH ~ 6 on the UV-spectra recorded in the M²⁺:L 1:1 systems refer to the binding of both Cys-S⁻ groups to the metal ions. ¹H NMR spectra measured under similar conditions suggest the participation of Asp-carboxylate(s) in metal ion coordination, besides the Cys-residues, in the monomeric ML species. UV-spectra reveal a tetrahedral {4×S⁻} coordination mode in the ML₂ complexes.



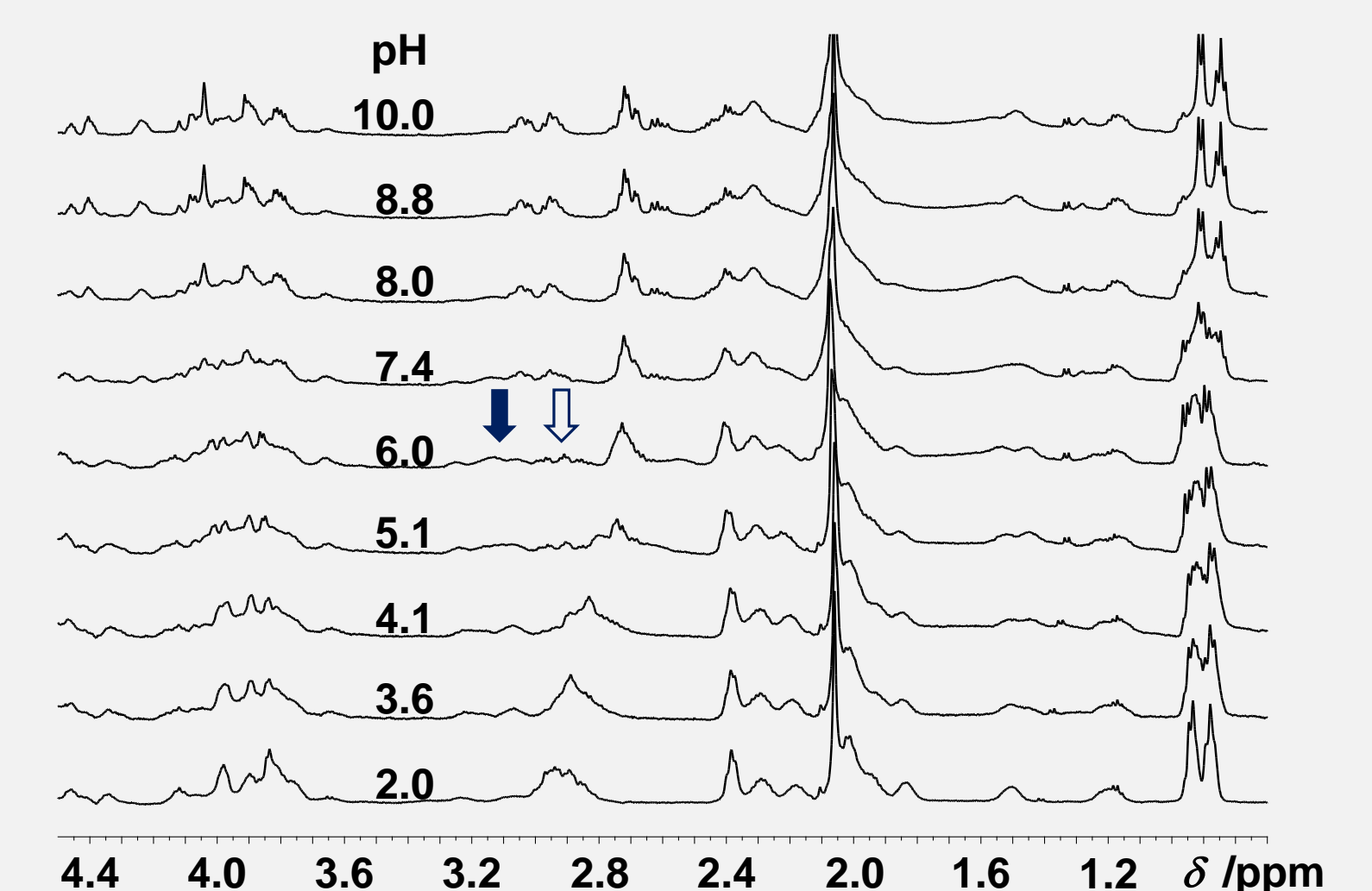
Approximated pH-dependent distribution of monomeric species relative to the total fraction of such complexes in the Ag⁺:PP 1:1 system. ($C_{PP} = 1 \times 10^{-3}$ M).



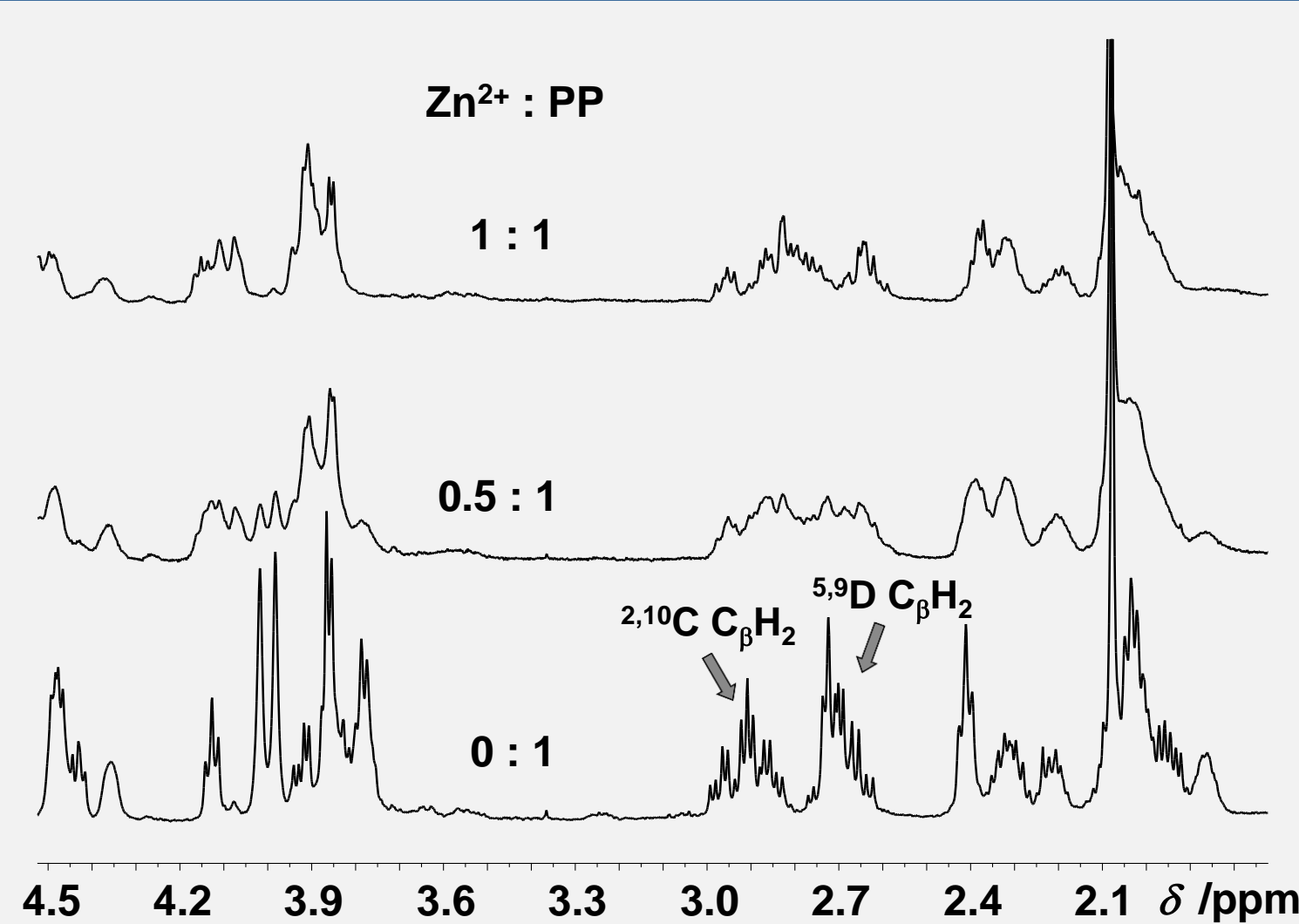
A vs. pH profiles recorded in the Cd²⁺:PP system at $\lambda = 245$ nm ($C_{PP} = 2 \times 10^{-4}$ M, $l = 0.5$ cm).

Hg²⁺ - PP and Ag⁺ - PP systems

The significant downfield shift of the Cys C_βH₂ resonances, relative to those of the unbound PP ligand (data not shown), reflect a tight Hg²⁺ binding through the two distinct Cys-thiolates in a linear coordination fashion already at pH~2. In contrast, PP presumably coordinates Ag⁺ in a monodentate fashion below pH~7 and the second thiolate can be proposed to bind only above neutral pH.



Selected region of the ¹H NMR spectra of the Ag⁺:PP 1:1 system as a function of pH ($C_{PP} = 1 \times 10^{-3}$ M). The filled and open arrows point to the bound and unbound Cys C_βH₂ signals, respectively.



Selected region of the ¹H NMR spectra of PP recorded at pH ~ 7.5 as a function of the Zn²⁺:peptide ratio ($C_{PP} = 1 \times 10^{-3}$ M).

Conclusions

The presented results demonstrate the higher coordination flexibility of the ligand, as compared to the metal binding site of native CueR. Most interestingly, preliminary studies with in the Ag⁺ - PP system suggest that the linear dicoordinate geometry proposed for the metal loaded form of the protein is retained only above neutral pH in the peptidic model.

	ML	ML ₂
Zn ²⁺	{2×S ⁻ , COO ⁻ , H ₂ O}	{4×S ⁻ }
Cd ²⁺	{2×S ⁻ , COO ⁻ , H ₂ O}	{4×S ⁻ }
Hg ²⁺	{2×S ⁻ }	—
Ag ⁺	{2×S ⁻ }	—

Proposed coordination modes of the peptide within the monomeric and metal-bridged structures formed with various metal ions.

Acknowledgements

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References

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