Study on interactions of LL-37 human cathelicidin peptide fragment with Cu(II) ion

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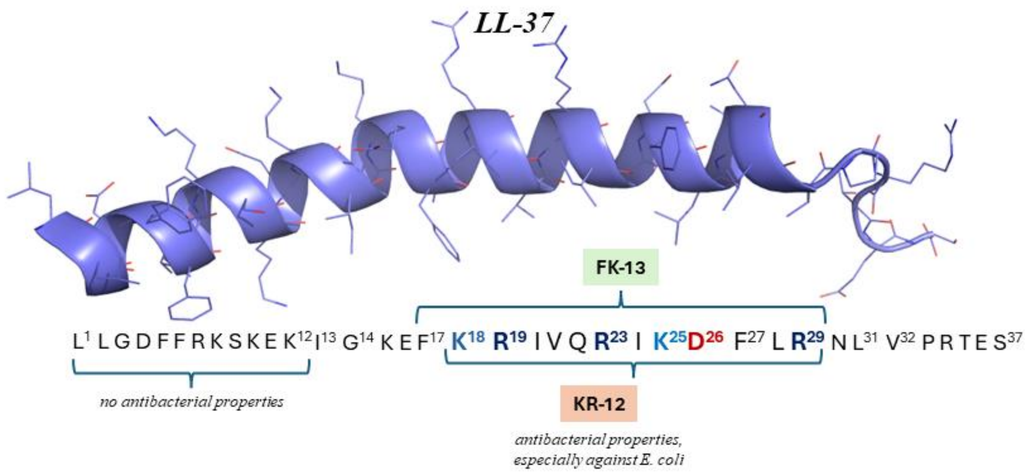
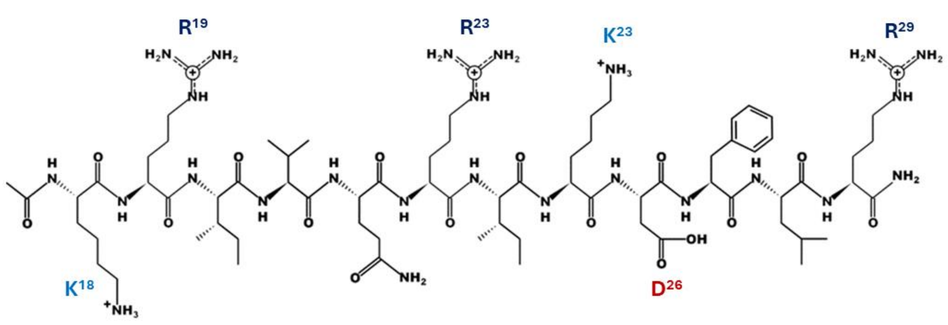
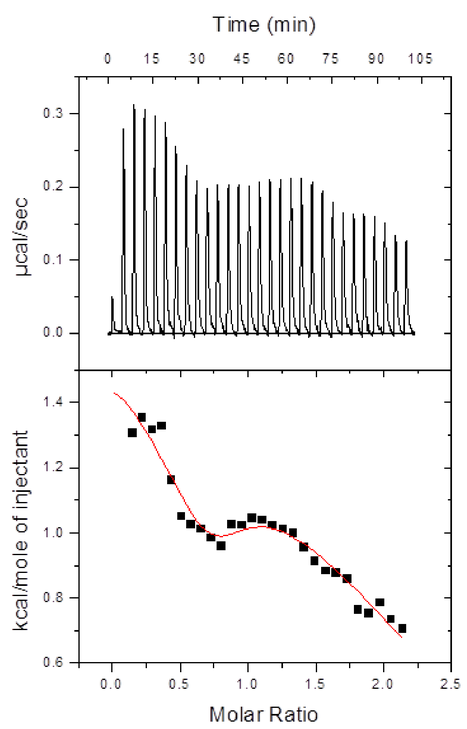
NMR structure of LL-37 in sodium dodecyl sulfate (SDS) micelles and its selected variants.
G. Wang, W. D. Treleaven, R. J. Cushley, *Biochim. Biophys. Acta.*, 1996, 1301, 174–184.





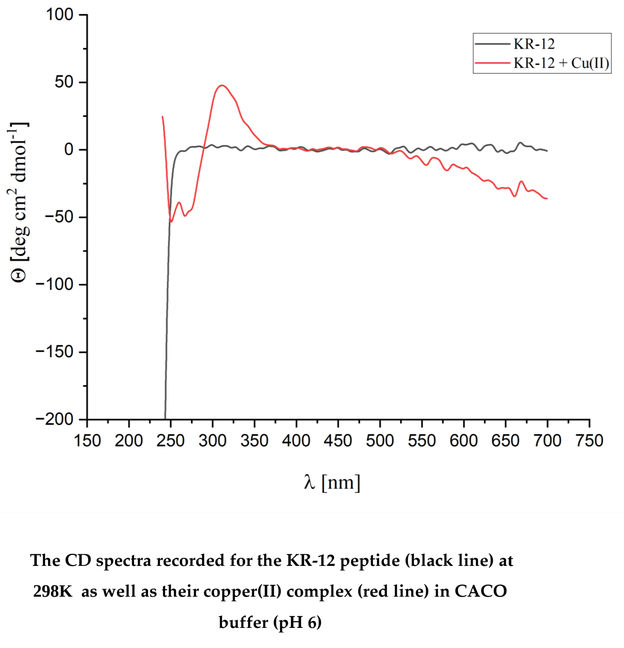
Isothermal titration calorimetry (ITC)



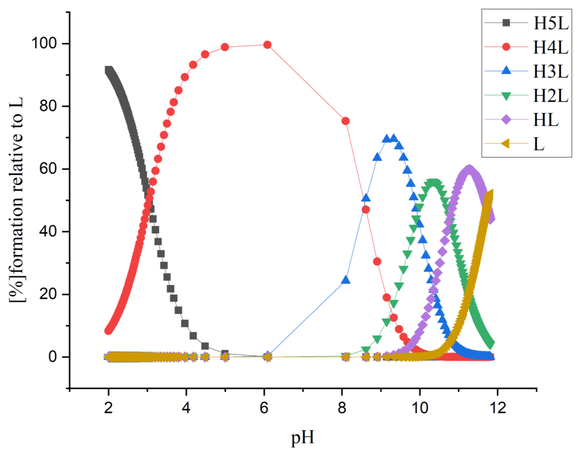
The conditional thermodynamic parameters of Cu^{2+} binding to KR-12 and previously established data for LL-37[1] (grey column) in the 10 mM CACO buffer of pH 6, at 298.15 K.

Parameter	LL-37//Cu^{2+}	KR-12//Cu^{2+}
$\log K_{ITC(1)}$	**4.23** (±0.02)	**5.73** (±0.08)
$\Delta_{ITC}H_{(1)}$ [kcal/mol]	4.08 (±0.03)	1.46 (±0.05)
$T\Delta_{ITC}S_{(1)}$ [kcal/mol]	10.8	9.28
$\Delta_{ITC}G_{(1)}$ [kcal/mol]	-6.72 (±0.03)	-7.82 (±0.11)
$\log K_{ITC(2)}$		**4.20** (±0.18)
$\Delta_{ITC}H_{(2)}$ [kcal/mol]		-5.78 (±2.39)
$T\Delta_{ITC}S_{(2)}$ [kcal/mol]		-0.05
$\Delta_{ITC}G_{(2)}$ [kcal/mol]		-5.73 (±0.25)
$\log K_{ITC(3)}$		**5.16** (±0.19)
$\Delta_{ITC}H_{(3)}$ [kcal/mol]		15.40 (±4.58)
$T\Delta_{ITC}S_{(3)}$ [kcal/mol]		22.44
$\Delta_{ITC}G_{(3)}$ [kcal/mol]		-7.04 (±0.26)
$\log K_{ITC(4)}$		**5.01** (±0.20)
$\Delta_{ITC}H_{(4)}$ [kcalmol]		-10.85 (±2.71)
$T\Delta_{ITC}S_{(4)}$ [kcal/mol]		-4.01
$\Delta_{ITC}G_{(4)}$ [kcal/mol]		-6.84 (±0.28)

[1] J. Makowska, D. Wyrzykowski, E. Kamysz, A. Tesmar, W. Kamysz, L. Chmurzyński, *Journal of Thermal Analysis and Calorimetry*, 2019, **138** (6), 4523-4529



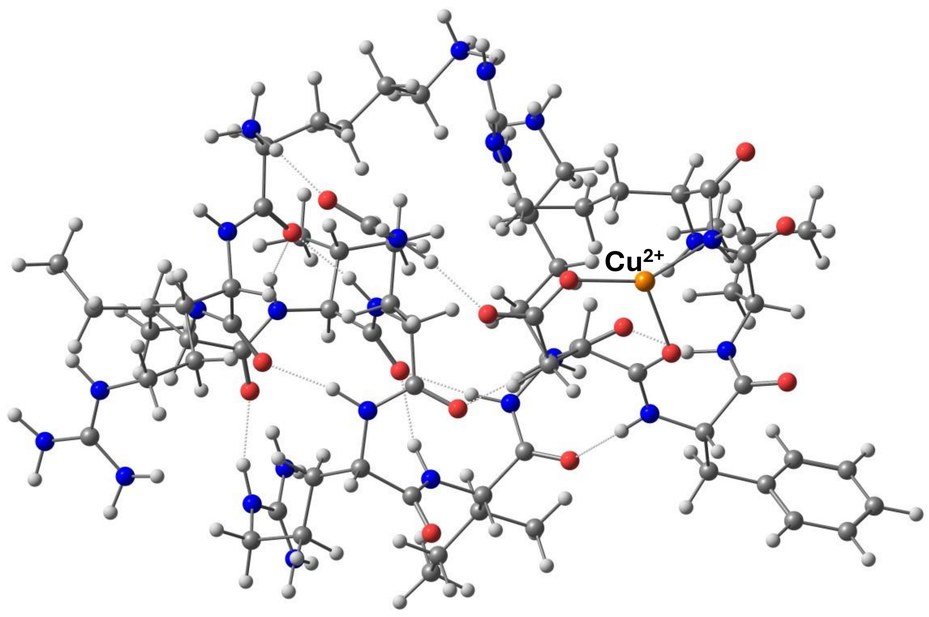
The CD spectra recorded for the KR-12 peptide (black line) at 298K as well as their copper(II) complex (red line) in CACO buffer (pH 6)



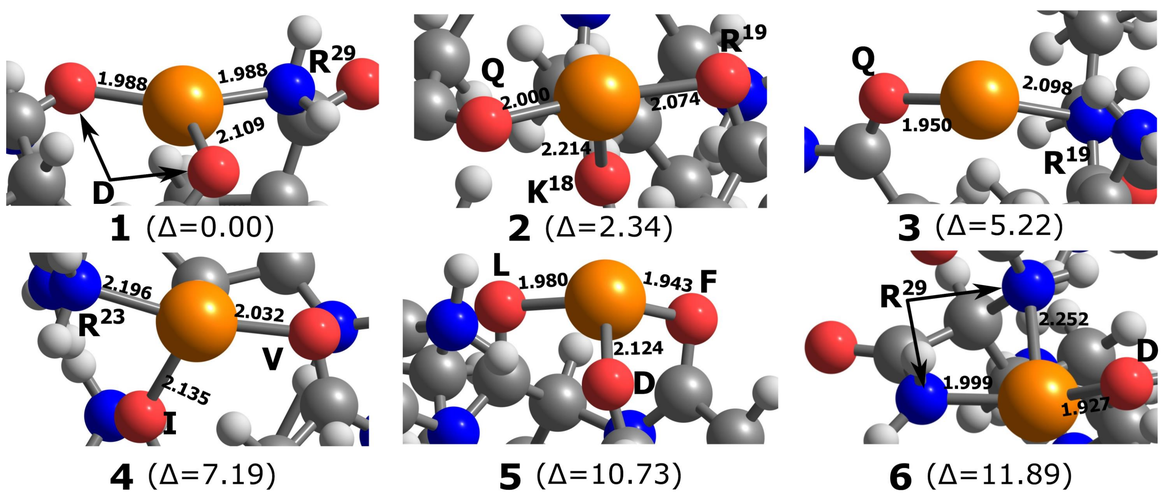
Species distribution diagram of the KR-12 peptide (H_5L) as a function of pH calculated based on the acid dissociation constants obtained from potentiometric titration data.

Biologically essential metal Cu(II) ions could have a two-fold effect on the activity of antimicrobial peptides (AMPs): (a) AMPs bind them, so that microbes do not get enough metals essential for their survival and virulenceor , (b) AMPs require a given metal ion to enhance their antimicrobial activity.

The human Cationic Antimicrobial Protein (hCAP) corresponding to overlapping sequences of hCAP (151-162) named KR-12 peptide is the smallest portion of the only type of human *Cathelicidin*, which has been shown that could be modified into more effective antimicrobials. The Isothermal Titration Calorimetry technique and *in silico* analysis were used to determine potential metal Cu(II) binding sites of KR-12. Based on density functional theory (DFT) calculations, we propose the most likely coordination modes of Cu(II) to peptide as well as to discuss the chemical nature of the interactions. The specific behavior of basic residues presented in the investigated peptide in a solution can govern the process of binding ions to the system. The presented results provide important structural and thermodynamic information to understand the influence of Cu(II) ions on the activity of KR-12 peptide.



The analysis of the presented data at the given theoretical level (GFN2-xTB/ALPB)



The KR-12 interacts with metal ions mostly via the main chain's oxygen atoms, however, the two types of amino acids that are expected to be vital for the interaction of Cu(II) are D and R29. Former due to the overall negative charge arising from the deprotonated carboxylic group within its side chain. Later via O atoms of the main chain, also characterized by significantly nucleophilic character arising from polarization due to the substantial positive character of the guanidine moiety. Additionally, the obtained results indicate that the oxygen atoms of the main peptide chain in KR-12, especially those with positively charged side chains may be crucial when it comes to interaction with electrophiles, such as Cu(II) ions. The most probable and stable dominant structures of the investigated complexes have been selected based on calculations. The presented results provide important structural and thermodynamic information for further study to understand the influence of Cu(II) ions on the activity of the KR-12 peptide.