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Evaluation of different spin labels for the study of protein-peptide interaction in cellular extract using Electron Paramagnetic Resonance spectroscopy techniques

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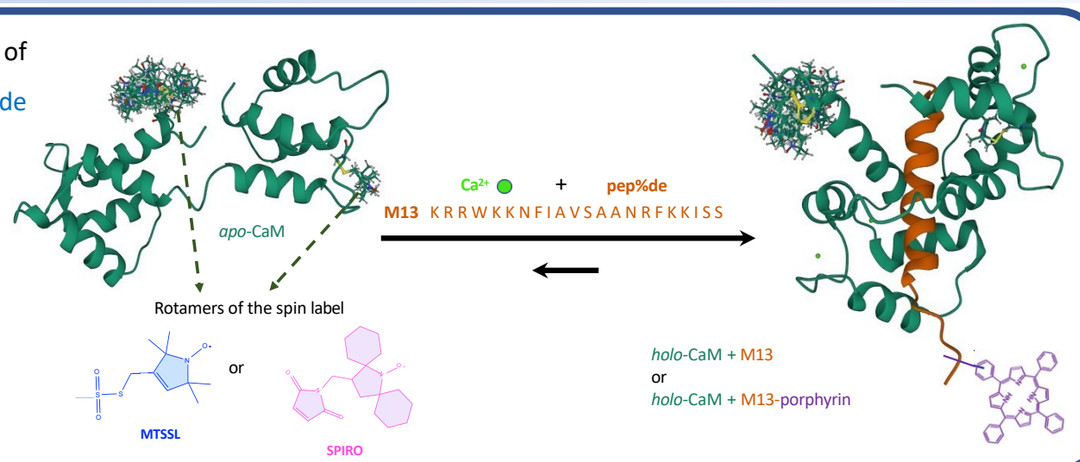
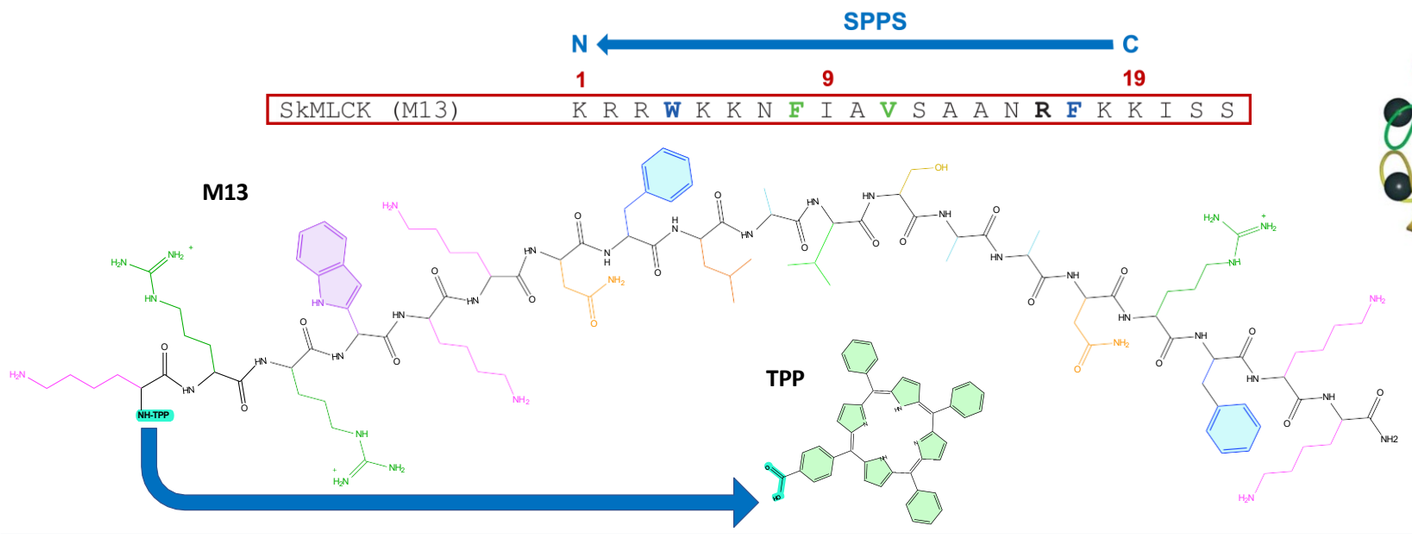
ABSTRACT

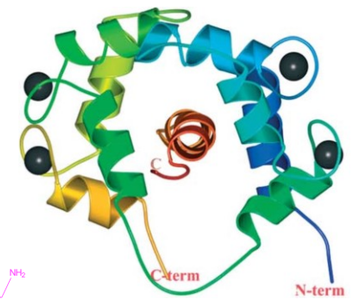
Calmodulin (CaM) is a protein involved in cellular signaling and undergoes specific conformational changes in the presence of Ca^{2+} ions and target peptides like M13.[1] In this study, some CaM mutants were labeled with two different nitroxide probes. Then, M13 was synthesized via SPPS and an aliquot was labeled with a porphyrin. Using EPR spectroscopy, CaM's conformational changes were observed both in buffer and in cell-mimetic environments, leading to evaluate the suitability of the selected probes, i.e., their resistance to reduction. Continuous Wave EPR reduction kinetics and pulsed EPR (DEER) experiments confirmed nitroxide probes' suitability and showed the major conformational changes of CaM in terms of interspin distances. Preliminary studies of nitroxide-labeled CaM's interaction with porphyrin-labeled M13 assessed triplet probe adequacy in perspective of distance measurements using dipolar EPR techniques coupled with photoexcitation. [2]

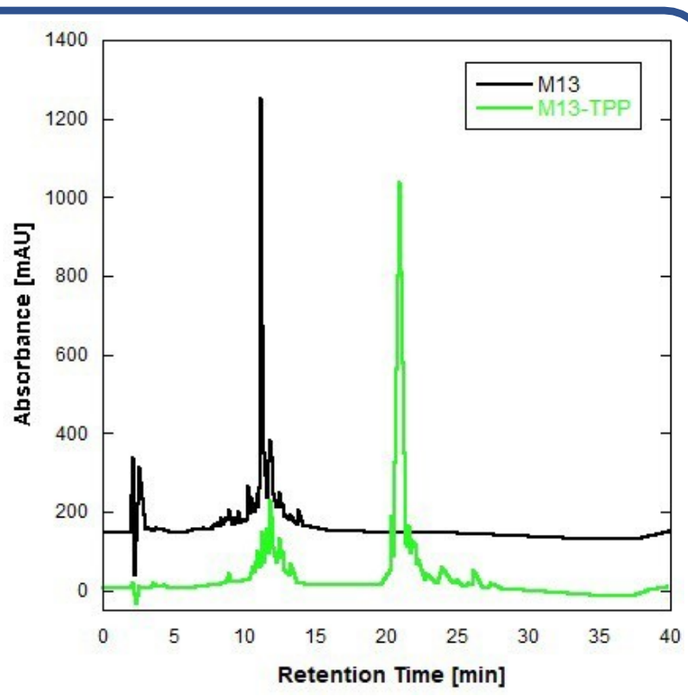


M13 pep'de synthesis

CaM's target peptide, M13, has a sequence rich in hydrophobic amino acids, allowing good interaction with CaM, which literally wraps the peptide creating a hydrophobic tunnel around it.[3,4] M13 (residues 1-19 of the peptide) was obtained through solid-phase peptide synthesis (SPPS), with a 63% final yield and a 92% purity. Porphyrin TPP was added to the N-terminal unprotected amine as TPP-COOH, by a standard coupling reaction.

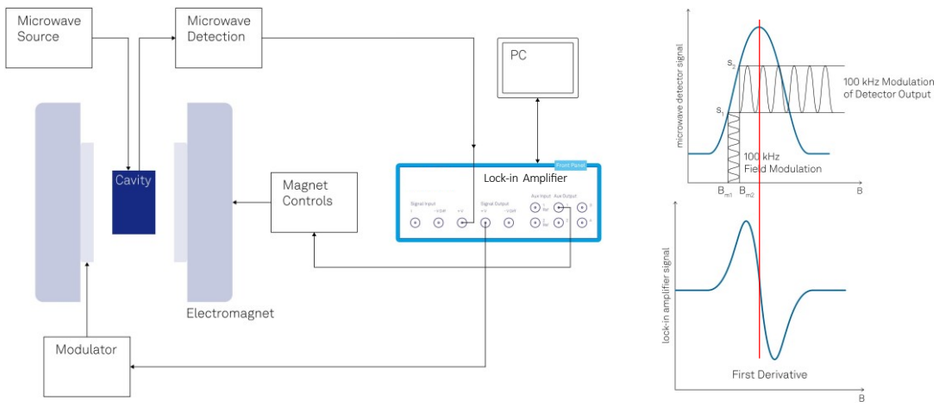




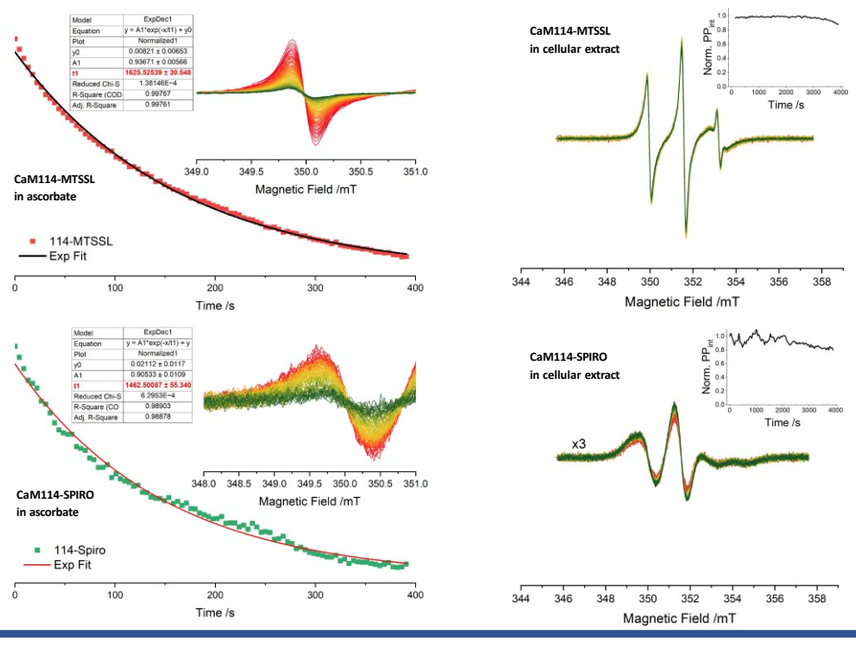


Con'nuous Wave EPR

Experimental setup. Block diagram of an EPR spectrometer.[5] The signal reflected from the resonant cavity is modulated at a specific frequency and then amplified by the lock-in amplifier. A derivative signal of the absorption spectrum, is obtained.

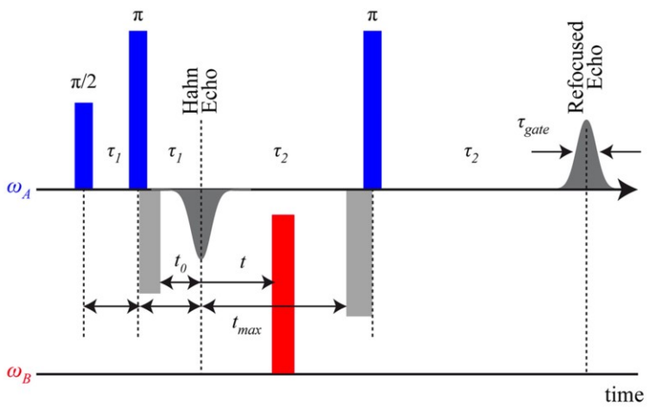


Experimental results. Kinetic measurements for the two different spin labels, MTSSL and SPIRO. In the figures below the results for the E114C mutant of CaM are reported, aquired in ascorbate and in cellular extract envoironments.

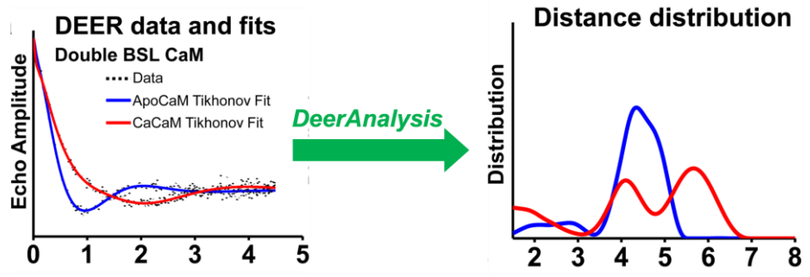


Double electron-Electron Resonance (DEER)

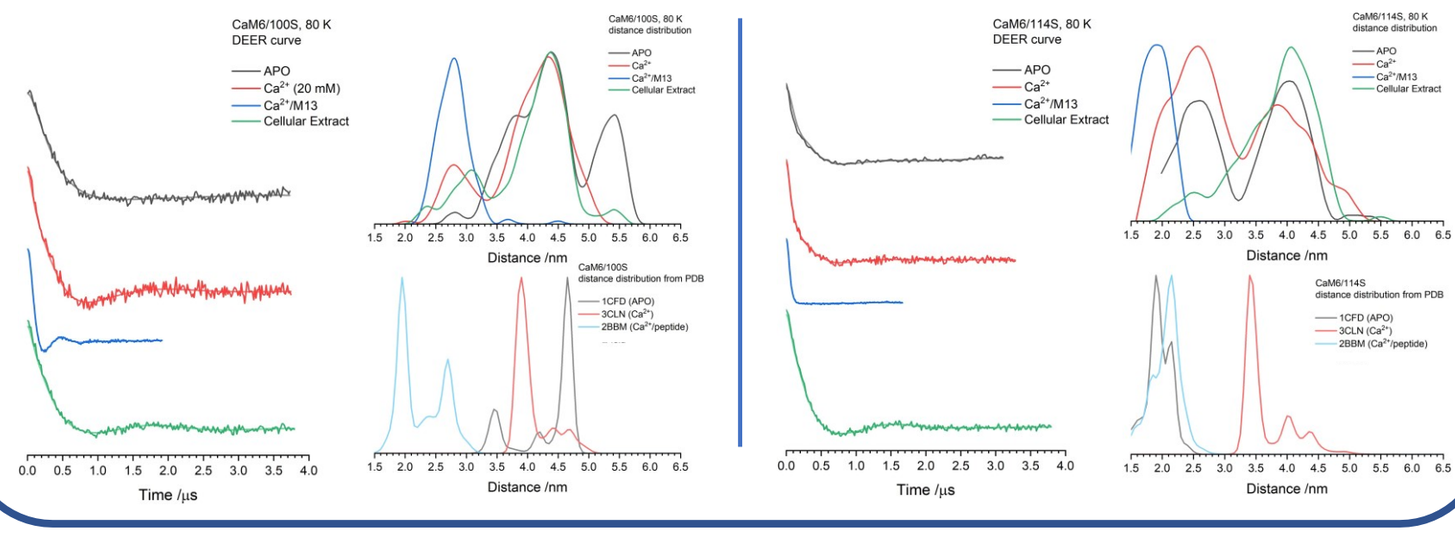
Experimental setup. DEER was used to obtain interspin distances in the protein complex. A four pulse sequence is employed, at two specific microwave frequencies, ω_A and ω_B.[6] The DEER signal is an oscillating and decaying function in time, whose modulations are given by the dipolar interaction between the interacting spins, i.e., depends on their mutual distance. The DEER traces in function of time are then converted into a distribution of distances.



$$\omega_{AB}(\theta, r) = -\frac{\mu_0 g_A g_B \mu_B^2}{4\pi\hbar}\frac{1}{r_{AB}^3}(3cos^2\theta - 1)$$

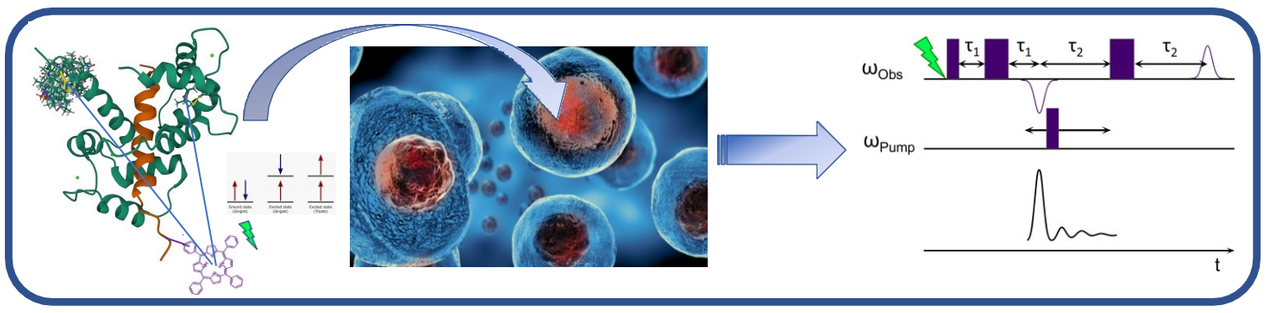


Experimental results. The DEER measurements provided structural distance costraints for the double mutants E6CI100C and E6CE114C of CaM, labeled with SPIRO. The results confirmed that CaM is at equilibrium between different conformations (closed/*apo*, open/*holo* in the presence of Ca^{2+}, compact in the presence of Ca^{2+} and M13). The results in cellular extract were comparable to the ones in buffer solution.



Future perspec'ves: light-induced pulsed EPR measurements in cell

The next steps include bringing EPR measurements directly into the cell, as well as the possibility of exploring the protein-ligand interaction by means of pulsed EPR measurements coupled to photoexcitation that could provide distance measurements between the protein and the porphyrin-labeled peptide.



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