

Application of a Fluorescent Biosensor in Determining the Binding of 5-HT to Calmodulin

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Supplementary Materials

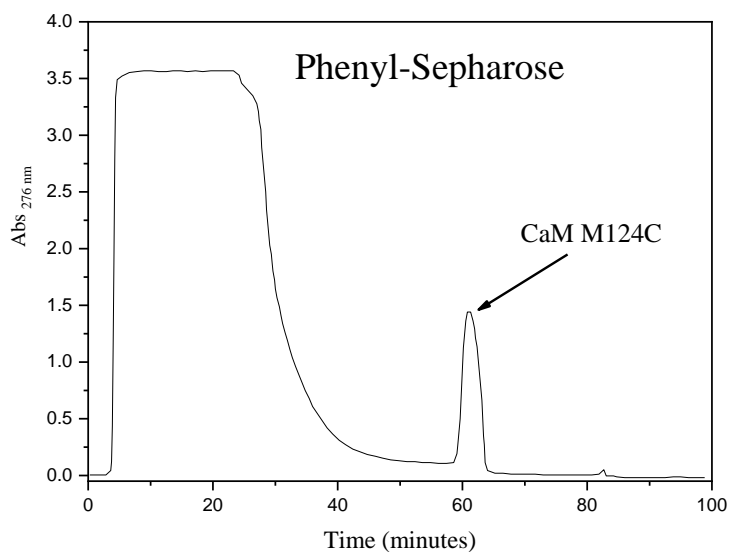


Figure S1. Chromatogram of the hydrophobic exchange purification of the CaM M124C protein, using the Phenyl-Sepharose CL-4B column. The mobile phase used at a flow rate of 1 ml / min was: 50 mM Tris-HCl pH 7.5, 0.5 mM DTT, 0.1 mM CaCl₂ and 500 mM NaCl. CaM M124C was eluted with the elution buffer which contains 50 mM Tris-HCl pH 7.5, 0.5 mM DTT, 1 mM EDTA and 150 mM NaCl; absorbance detection at λ_{276} . The fractions corresponding to the CaM M124C protein are eluted at 58-64 minutes.

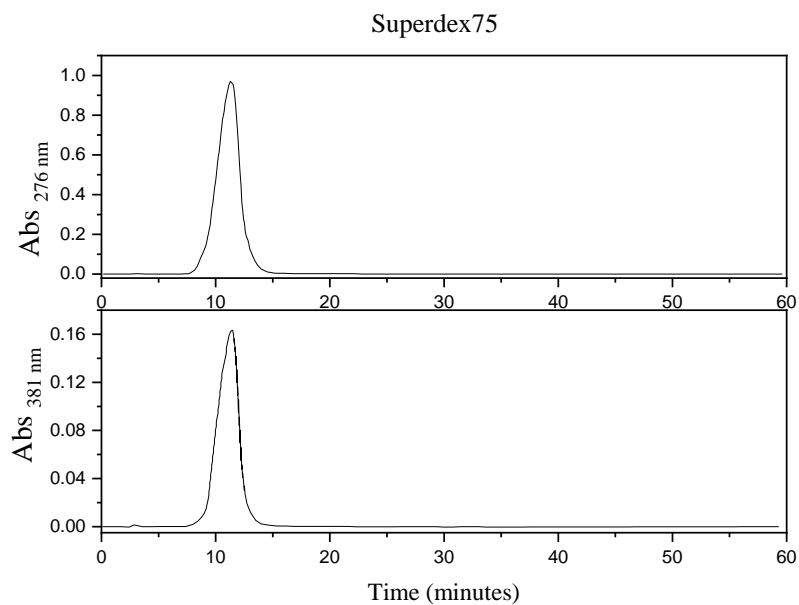


Figure S2. Chromatogram of the CaM M124C-*mBBR* protein, on a size exclusion column (superdex75). The mobile phase used was Tris-HCl pH 7.5, at a flow rate of 1 ml/min. At the top using an absorbance detection at λ_{276} and at the bottom an absorbance detection at λ_{330} .

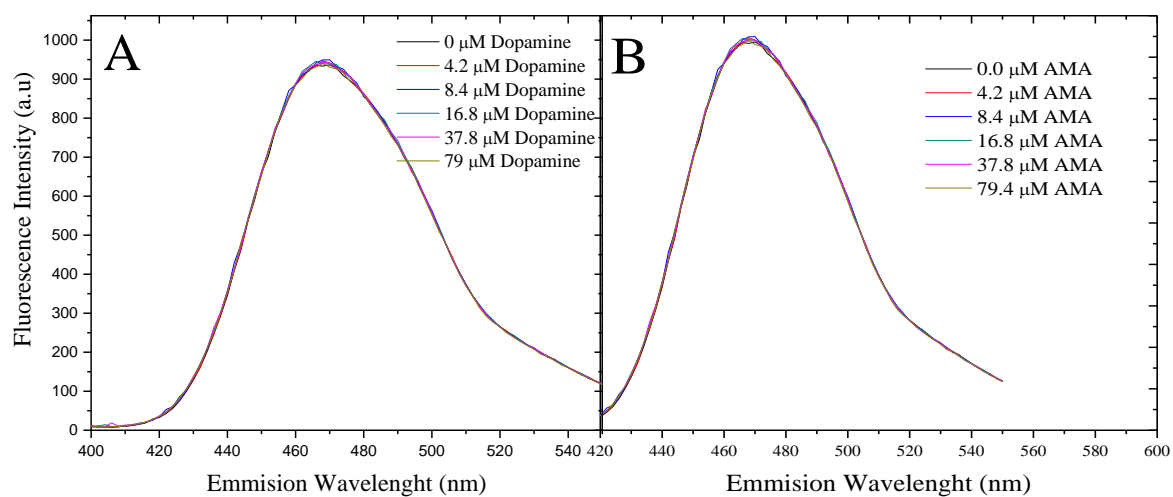


Figure S3. Fluorescence spectra of *h*CaM M124C-*mBBR* adding known concentrations of DOP and AMA. Figure 1s. Chromatogram of the hydrophobic exchange purification of the CaM M124C protein, using the Phenyl-Sepharose CL-4B column. The mobile phase used at a flow rate of 1 ml / min was: 50 mM Tris-HCl pH 7.5, 0.5 mM DTT, 0.1 mM CaCl₂ and 500 mM NaCl. CaM M124C was eluted with the elution buffer which contains 50 mM Tris-HCl pH 7.5, 0.5 mM DTT, 1 mM EDTA and 150 mM NaCl; absorbance detection at 1276. The fractions corresponding to the CaM M124C protein are eluted at 58-64 minutes.

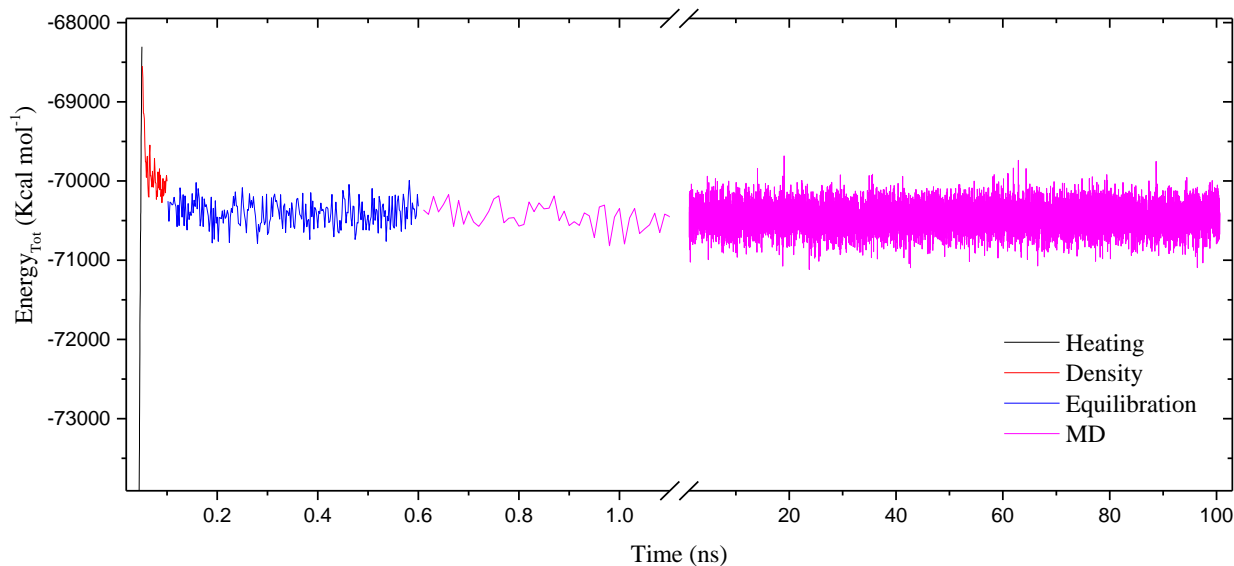


Figure S4. Progress of the molecular dynamics simulation of the CaM-CPZ complex. Total energy of the system vs time. The steps of the simulation comprise 50 ps of heating, 50 ps of equilibration at constant volume, 500 ps of equilibrium and 100 ns of molecular dynamics simulation.