

Contribution of dynamic and static quenchers for the study of protein conformation in ionic liquids by steady-state fluorescence spectroscopy

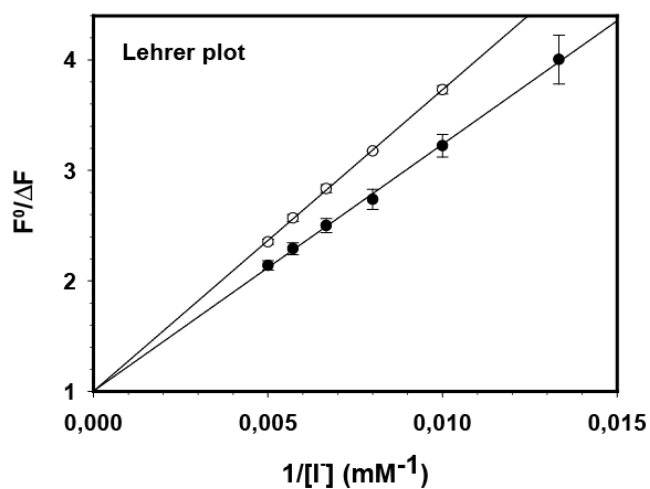
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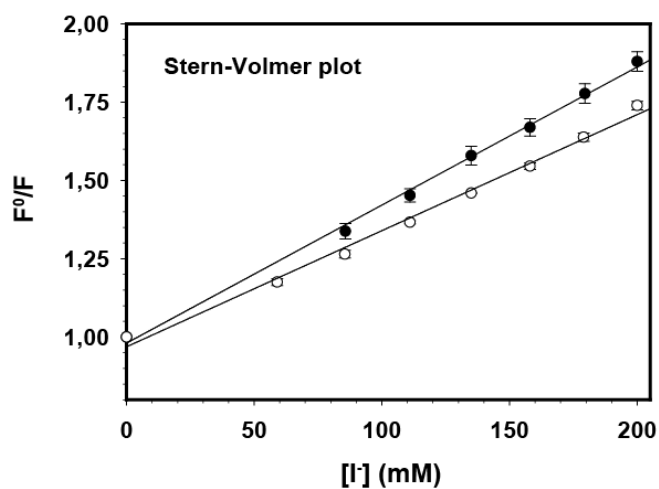
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Supporting Information

1. Lehrer representation of the quenching of the FDH fluorescence by iodide in PBS buffer.



- Excitation 280 nm: $223.2/[I^-] + 1.004$; $R^2 = 0.998$
- Excitation 295 nm: $272.8/[I^-] + 1.002$; $R^2 = 0.999$



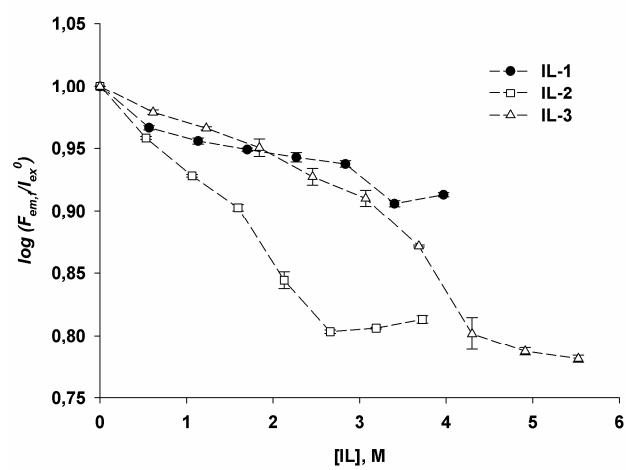
- Excitation 280 nm: $0.98 + 0.00441 [I^-]$; $R^2 = 0.997$
- Excitation 295 nm: $0.97 + 0.0037 [I^-]$; $R^2 = 0.993$

Excitation wavelength	Lehrer Plot		Stern-Volmer Plot
	α (M^{-1})	K_{SV}^{Iodide} (M^{-1})	K_{SV}^{Iodide} (M^{-1})
280 nm	0.99	4.48	4.28
295 nm	0.99	3.67	3.49

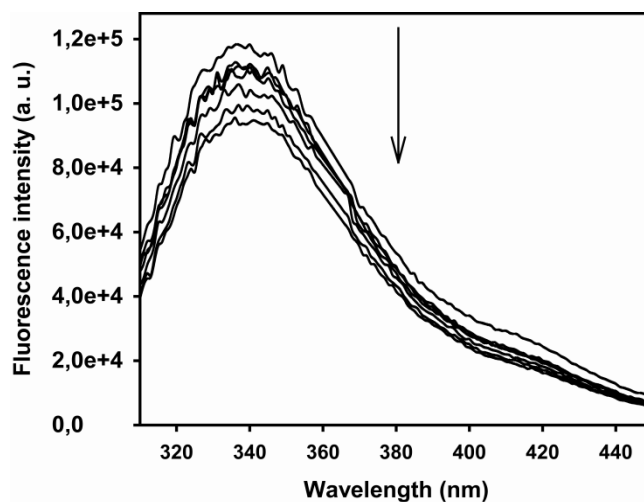
2. Structure of the FDH from *Candida boidinii*. Structure of the chains A (black) and D (white) of the formate dehydrogenase from *Candida boidinii* forming the dimeric form (pdb entry 2fss) represented in the ribbon form. Fluorescent residues are depicted as stick and sphere. Only residues of the chain A are shown for clarity. Tryptophanyl residues are shown in pink. Tyrosinyl residues are in green.



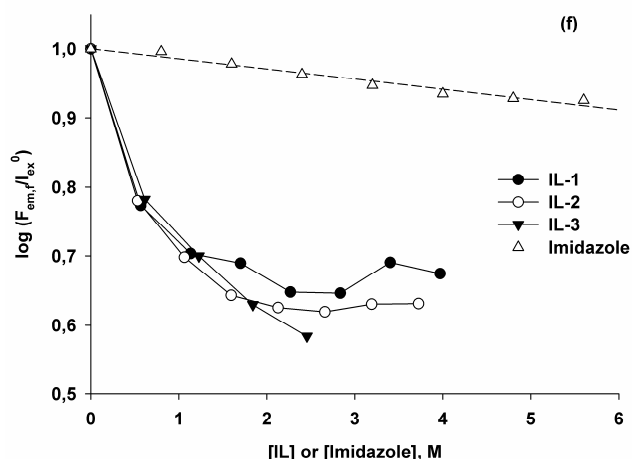
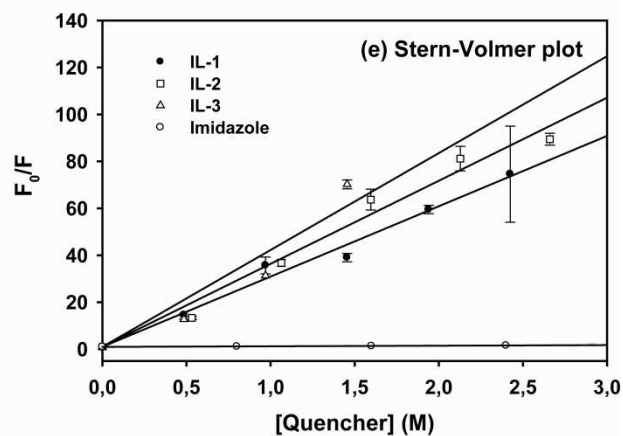
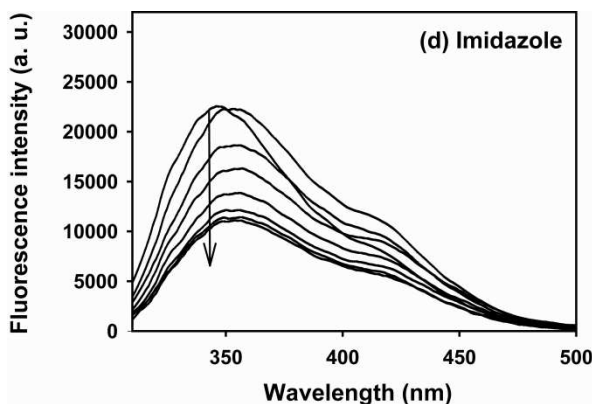
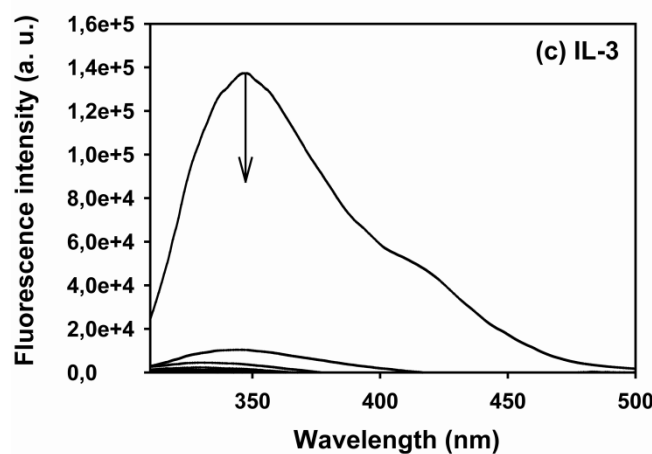
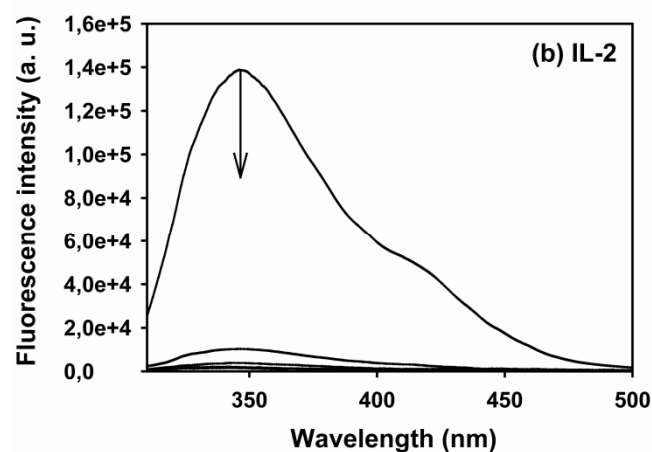
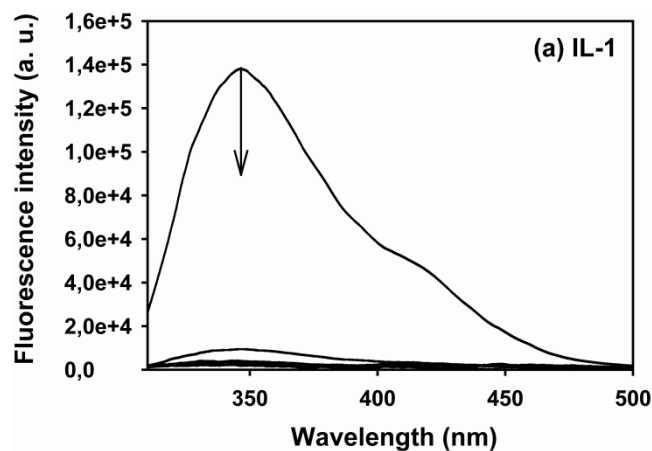
3. Emitted light intensity of the FDH at increasing IL concentration analyzed by equation (3)



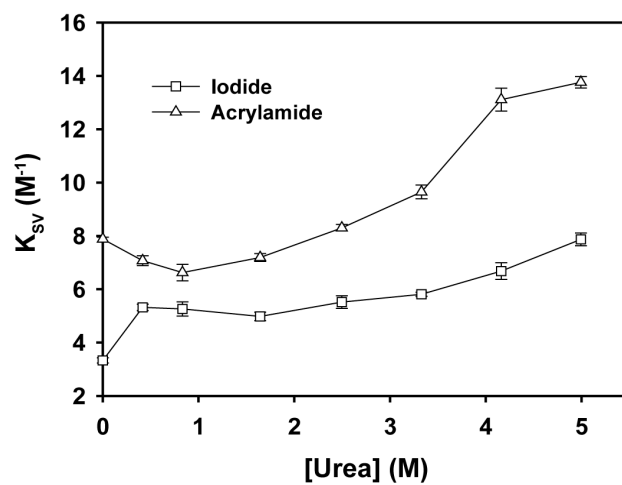
4. Quenching of the FDH fluorescence by imidazole. FDH fluorescence spectrum is recorded in the presence of 0, 118, 171, 222, 270, 316 and 359 mM of imidazole. Maximum fluorescence intensities at 335 nm are used for the K_{SV} determination. All the experiments are performed in triplicate and medium fluorescence is subtracted. As the quenching is very low, data are not well fitted.



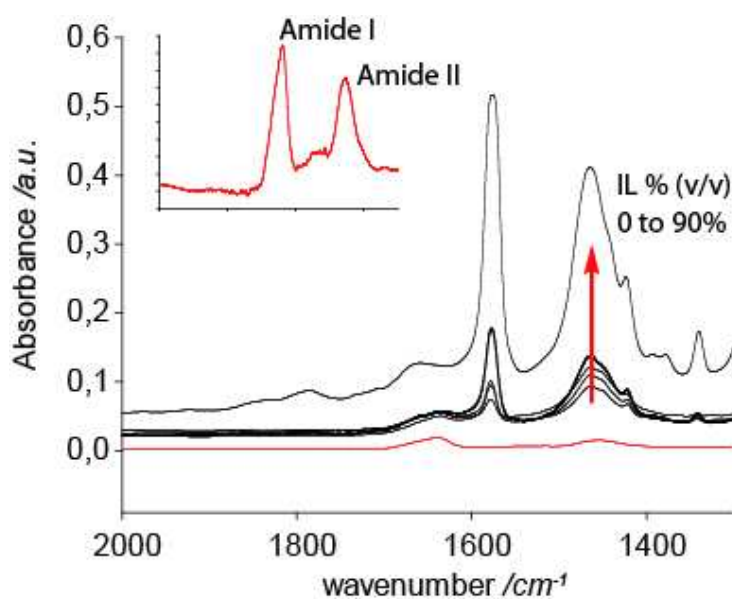
5. Quenching of a Trp solution by the ILs or imidazole. In the case of the tryptophan quenching by **IL-1-3**, the concentration of the solution of tryptophan is fixed at 50 μM to have a fluorescence intensity approximately in the same range than the FDH (Figure 3). In the case of tryptophan quenching by imidazole, its concentration is 10 μM . All the experiments are performed in triplicate and the fluorescence of the medium is subtracted. Spectra are recorded in the presence of (a) 0 - 3.969 M of **IL-1** by 0.567 M concentration steps, (b) 0 - 3.724 M of **IL-2** by 0.532 M concentration steps, (c) 0 - 4.298 M of **IL-3** by 0.614 M concentration steps and (d) 0 - 5.6 M of imidazole by 0.8 M concentration steps. (f) Emitted light intensity of the tryptophan solution at increasing IL concentration is analyzed by eq. (3).



6. Urea denaturation. Evolution of the K_{SV} after quenching of the FDH fluorescence by acrylamide or iodide in presence of different concentration of urea. Iodide (\square), Acrylamide (\triangle).



7. FTIR spectra of FDH in the absence (red) and in the presence of 10-90 % (v/v) [MMIm][Me₂PO₄] (IL-1) at ~10 mg ml⁻¹. No clear signal corresponding to the FDH could be observed in this IL. The solvent subtraction is not efficient. *Insert:* FTIR spectra of the FDH in PBS.



8. CD spectra of FDH in PBS (red) and in the presence of 10 % (v/v) [MMIm][Me₂PO₄] (IL-1) (up) and detector signal obtained for buffer (dotted) and of 10-30 % (v/v) [MMIm][Me₂PO₄] (IL-1) in buffer (down). Similar spectra are obtained with IL-2 and IL-3.

