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Green AOT Reverse micelles as nanoreactors for Alkaline Phosphatase. The hydrogen bond "dances" between water and the enzyme, the reaction product, and the reverse micelles interface.

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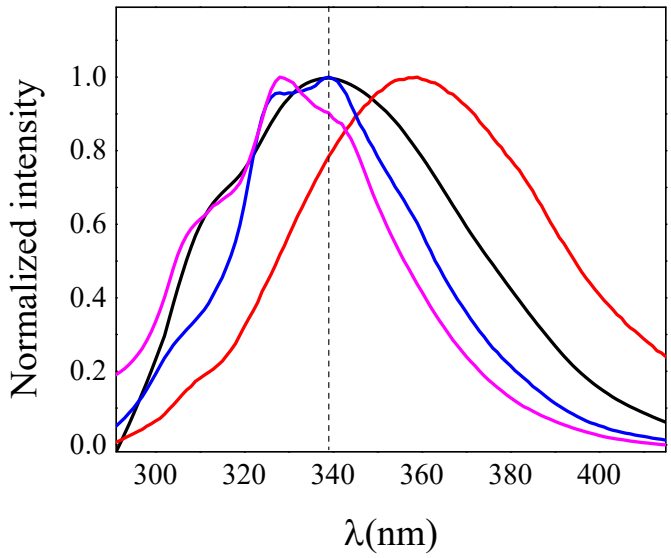

Figure S1. Emission spectra of AP in different media. pH=10 (black), 8M urea aqueous solution (red), ML/AOT/water RMs at W_0=10 and, pH=10 (blue), IPM/AOT/water RMs at W_0=10, pH=10 (pink). [AOT] = 0.1 M. [AP] = 1×10^{-7} M. [1-NP] = 5×10^{-5} M. λ_{exi}=280 nm.

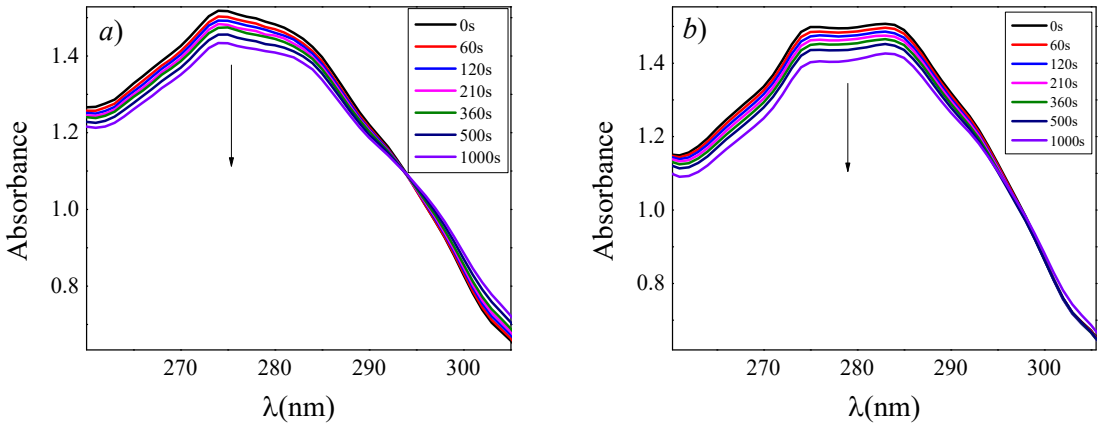

Figure S2. UV-vis spectra for 1-NP hydrolysis catalyzed by AP at different times in different RMs at W_0=10, pH=10, and T= 35°C. *a*) ML/AOT/water RMs. [1-NP] = 1.5×10^{-4} M; *b*) IPM/AOT/water RMs [1-NP] = 2×10^{-4} M. [AOT] = 0.1 M. [AP] = 1×10^{-7} M.

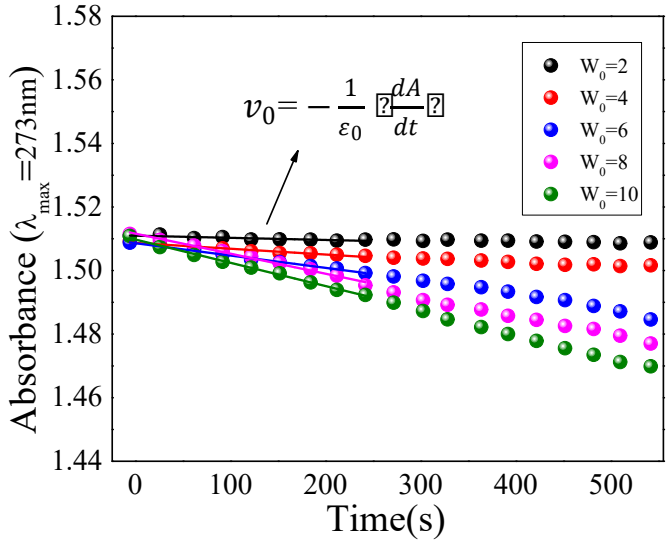

Figure S3. Representative absorbance profiles (λ_{max}=273nm) as a function of time for the 1-NP hydrolysis catalyzed by AP in RMs of ML/AOT/water at different W_0, pH=10, and T=35°C. Initial rate of reaction corresponding to the disappearance of the substrate (1-NP). [AOT] = 0.1 M. [AP] = 1×10^{-7} M. [1-NP] = 1.5×10^{-4} M.

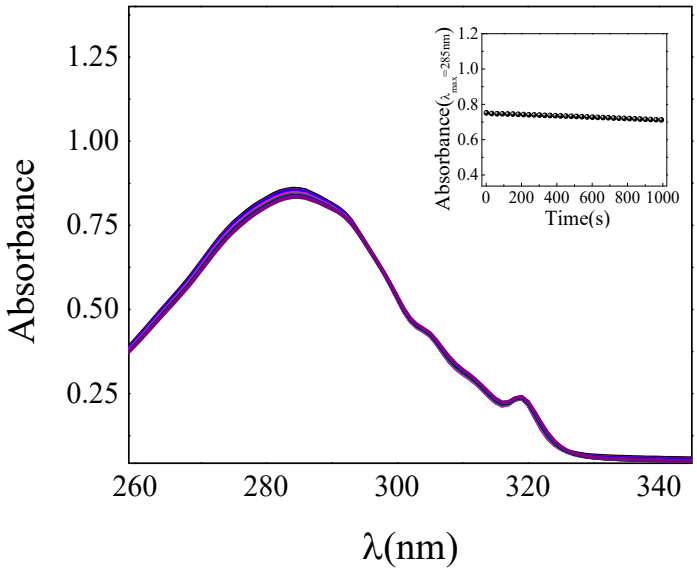

Figure S4. UV-vis spectra for the 1-NP hydrolysis catalyzed by AP in water (pH=7) at T=35 °C. Spectra were acquired every 30 seconds, for 1000 seconds. Insert: Absorbance values (λ_{max}=285 nm) of 1-NP in water at different times. [1-NP] = 1×10^{-4} M. [AP] = 1×10^{-7} M.

Experimental Section

Materials and methods

Methyl laurate (≥98% purity), isopropyl myristate (≥98% purity), sodium 1-naphthyl phosphate (>98% purity), and alkaline phosphatase (AP) from bovine intestinal mucosa (lyophilized powder 160 kDa) were purchased from Sigma-Aldrich and used as received. The surfactant AOT, from Sigma (>99% purity), was dried at reduced pressure before use. Milli-Q (Millipore) equipment was used to obtain ultrapure water.

The pH of the bulk water solution has been maintained at 10 using a 10 mM sodium carbonate/sodium hydrogen carbonate (Na_2CO_3-$NaHCO_3$) buffer solution, pH=10. This pH value was found to be optimal for this enzymatic reaction in homogeneous media. In the RM media, it is known that the pH cannot be measured inside the polar core of the aggregate.[1] In this sense, the value of the pH inside the polar core is referred to the homogeneous buffer solution.

Preparation of micellar systems

The methyl laurate/AOT/buffer and isopropyl myristate/AOT/buffer RMs were prepared by mass and volumetric dilution. To obtain optically clear solutions, they were placed in an ultrasonic bath and the water amount was added using a calibrated microsyringe.

Reaction in water and RMs

In a homogeneous medium, in a thermostated cell at 35°± 0.1°C that contained the AP in buffer pH = 10, the enzymatic reaction was initiated by adding different μL of the sodium 1-naphthyl phosphate stock solution to that 2 ml of buffer solution have the desired concentrations of substrate and AP. The concentrations of sodium 1-naphthyl phosphate and AP in the reaction media were around $2x10^{-5}$ M - $3x10^{-4}$ M and $1x10^{-7}$ M, respectively.

For the experiments performed in RMs, a solution was prepared containing 1-naphthyl phosphate in RMs of AOT dissolved in methyl laurate or isopropyl myristate, as appropriate, and another RM solution with AP at the same W_0. The enzymatic reaction was initiated by mixing the two AOT RM systems by magnetic stirring in a cell thermostated at 35°.

Kinetics procedure and Michaelis-Menten model

The enzymatic hydrolysis was followed by measuring the decrease in the maximum absorption band of the sodium 1-naphthyl phosphate substrate in each system, λ_{H_2O}= 285 nm (ε=4381 M^{-1} cm^{-1}), λ_{ML}=273 nm (ε=2764 M^{-1} cm^{-1}), λ_{IPM}=273 nm (ε=3474 M^{-1} cm^{-1}). Kinetic measurements were carried out in a quartz cell with a volume of 3ml and 1cm path length and

spectra were recorded using a UV-vis spectrophotometer (HP / Agilent 8453), with a thermostatic cell at 35±0.1 °C.

The substrate and AP are hydrophilic compounds that do not partition with the external nonpolar solvent. In this way, they are completely incorporated into the aqueous pool in RM of ML/AOT/buffer and IPM/AOT/buffer, respectively. Therefore, the hydrolysis catalyzed by AP follows the Michaelis-Menten model [2,3], represented in the Equation S1:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} E-S \xrightarrow{k_{cat}} E + P \tag{S1}$$

Where *E*, *S*, *E-S*, and *P* represent the enzyme, substrate, enzyme-substrate complex, and product, respectively. Applying the steady-state approximation to *E-S*, the rate law given in Equation S2 is obtained:

$$\frac{v_0}{[E]} = \frac{k_{cat}[S]}{K_M + [S]} \tag{S2}$$

Where v_0 is the initial reaction rate (M s^{-1}), [*E*] and [*S*] are the analytical enzyme and substrate concentration, respectively, k_{cat} is the catalytic rate constant, and K_M (M) is the Michaelis constant defined by Equation S3:

$$K_M = \frac{k_{-1} + k_{cat}}{k_1} \tag{S3}$$

The maximum absorbance (λ_{H_2O}= 285 nm, $\lambda_{ML\ or\ IPM}$= 273 nm) as a function of time was recorded and the initial rate (v_0) was obtained from the slope of [1-naphthyl phosphate] vs time profiles considering the initial stage of the reaction (Figure S4). Then, the values of v_0 plotted as a function of substrate concentration. Then, a fit was made according to equation S2 to obtain the values of k_{cat} and K_M. The hydrolysis of 1-naphthyl phosphate and consequently the formation of 1-naphtholate depended linearly on the reaction time during the first 30 minutes of reaction. The pooled standard deviation of the kinetic data, by using different samples, was less than 5%.

Emission spectroscopy experiments

The stability of the enzyme was evaluated by emission experiments. Emission spectra were recorded using a Horiba Fluoromax 4 spectrofluorometer with a thermostatic cell at 35±0.1 Emission spectra were measured with a volume of 3ml and 1cm path length, from 2 ml of AP

solution in homogeneous medium (buffer) and confined medium (RMs), respectively. The enzyme was dissolved in 8M urea solution for complete denaturing. The excitation wavelength was set at 280 nm, and the fluorescence emission spectra were scanned from 290 to 410 nm.

References

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