Supporting Information

1- Fundamentals of the Modulation Excitation Spectroscopy with phase sensitive detection (MES-PSD)

In the time-resolved spectra, the static signal belonging to the cyclic dimeric form of ketoprofen at ~1740 cm^{-1} is more intense than the periodic signal belonging to the interaction of the profen with the protein (see Figure 3A within the manuscript). In this context, the spectator species such as the dimeric ones and the active reversible intermediate species such as the acyl enzyme are not clearly distinguishable. The periodic perturbation of the system in the steady state (in our case the CALB enzyme under a flow of carbon tetrachloride) by an external parameter, such as concentration (a stream of R/S-ketoprofen 0.16 M in CCl_4) modifies only the formation of the reversible species whose concentration oscillates in a quasi steady-state at the same frequency as the stimulation [32]. At difference with the spectator species, the signal of the reversible species shows a phase delay φ with respect to the phase of the stimulation. The amplitude and the phase delay of the response are dependent on the stimulation-frequency. The signal after reaching the quasi steady-state can be averaged into one period improving the signal to noise ratio considerably. The so called phase sensitive detection (PSD) or demodulation is a further enhancement of the averaged signal that is achieved by a mathematical treatment given by the equation (1):

$$A(\phi_k^{\mathrm{PSD}}) = \frac{2}{T}\int_0^T A(t)\cdot \sin(k\cdot\omega\cdot t + \phi_k^{\mathrm{PSD}})\mathrm{d}t \qquad (1)$$

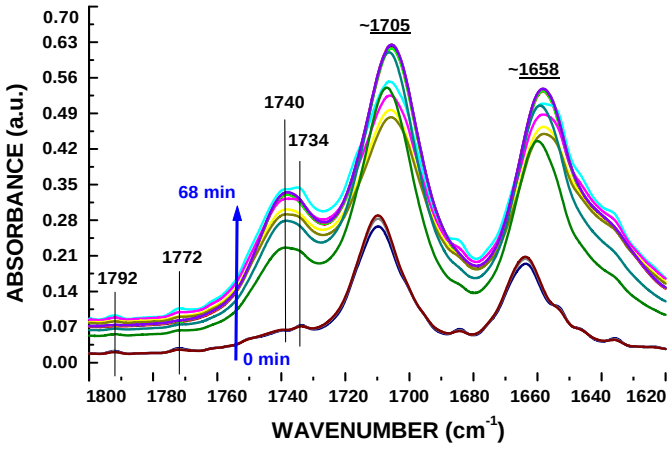
where, T is the length of one period, ω is the stimulation frequency, k is the demodulation index, ϕ_k^{PSD} is the demodulation phase angle for $k\omega$ demodulation, and A(t) and A(ϕ_k^{PSD}) are the active species response in time and phase-domain, respectively.

The equation (1) applied to the averaged signal converts a time-domain response to the phase-domain response. The figures 6A and 6B (within the manuscript) show the time-resolved and phase-resolved ATR-FTIR spectra after demodulation obtained in the MES experiments of ketoprofen interacting with CALB.

2- Speciation of R/S-ketoprofen in CCl_4: blank experiment in the ATR-FTIR *in situ* cell

The spectra obtained in the ATR-FTIR experiments with pure ketoprofen dissolved in carbon tetrachloride at various concentrations i.e. 0.02 M and 0.16 M without the presence of the enzyme are presented in the Figures S1 and S2.

A



B

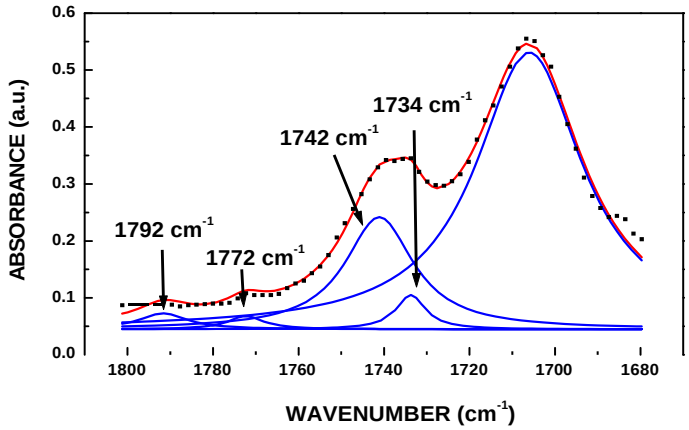
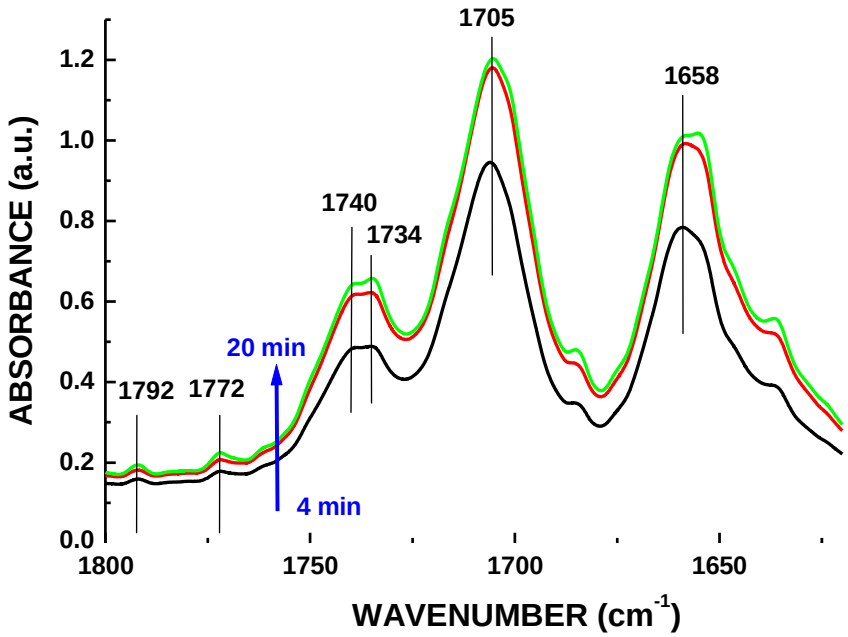


FIGURE S1. (A) Evolution of the infrared spectra of ketoprofen 0.02 M dissolved in CCl_4 at room temperature in an ATR cell. (B) Deconvoluted spectra of ketoprofen 0.02 M taken at 52 min.

A



B

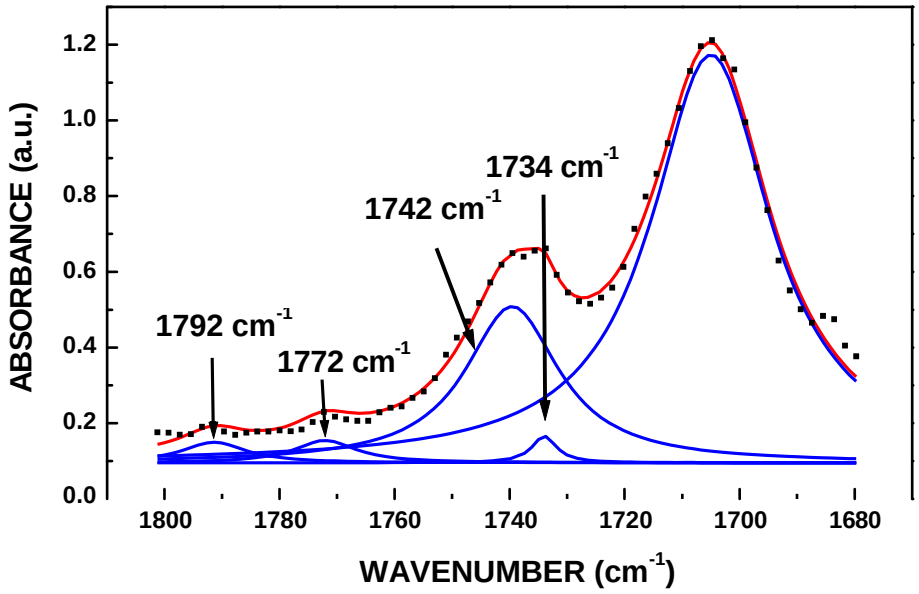


FIGURE S2. (A) Evolution of the infrared spectra of ketoprofen 0.16 M dissolved in CCl_4 at room temperature in an ATR cell. (B) Deconvoluted spectra of ketoprofen 0.16 M taken at 20 min.

The signals at 1705 cm^{-1} and 1658 cm^{-1} belong to the stretching vibration of the C=O group as discussed in the main body of this article. The profen shows also three distinctive signals at 1792 cm^{-1}, 1772 cm^{-1} and 1742 cm^{-1} ascribed to the stretching vibration of the carbonyl group of ketoprofen in its monomeric form, linear and cyclic dimeric forms, respectively [39].