Supporting Information

Site-specific measurement of water dynamics in the substrate pocket of ketosteroid isomerase using time-resolved vibrational spectroscopy

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Table S1. Mean vibrational frequencies of C183-nitrile in different solvent conditions.

Solvent condition	$\bar{\nu}_{CN}$ **(FWHM) (cm^{-1})**
In pH 5 buffer	2238.2 (7.1)
In pH 9 buffer	2226.3 (7.7)
In DMSO	2228.9 (5.7)
In KSI substrate pocket	2227.2 (7.3)

Figure S1. Kinetics of change in transient IR signal intensity of the nitrile in electronic ground state bleach (dark blue triangles and dark blue line) and the electronic excited state (dark red circles and dark red line). (A) C183 in pH 9 buffer; (B) C183 bound to KSI at pH 7.2. The decays in panel A fit to a double exponential equation with time constants of ~13 ps and ~455 ps for electronic ground state bleach and ~11 ps and ~420 ps for the electronic excited state. The decays in panel B fit to a single exponential equation with a time constant of ~80 ps, both for electronic ground state bleach and for the electronic excited state.

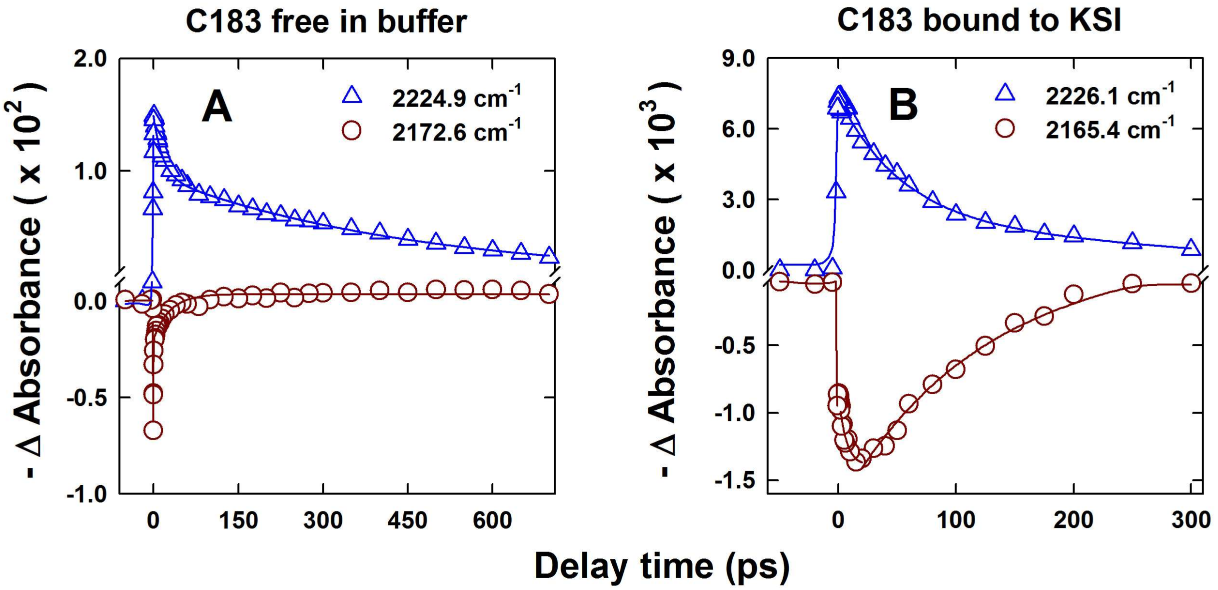

Figure S2. Changes in transient difference IR absorption spectra of the C183-nitrile when C183 is bound to different variants of KSI at pH 7.2. (A) C183 bounded to F86C/D40N mutant form of KSI. (B) C183 bounded to M116C/D40N mutant form of KSI. The inset in panel (A) and (B) is an expanded view of the evolution of the IR-absorption frequency of –CN in the electronic excited state at different times after the photo-excitation of C183. The temporal behavior clearly indicates the static vibrational frequencies in the electronic excited state, indicating the restricted dynamics of H-bonded water molecules at the active site of KSI.