Supporting Information:

Methods:

The laser source for the vibrational sum-frequency generation experiments is a regenerative Ti:Sapphire amplifier (Coherent) producing 800 nm pulses at a 1 kHz repetition rate with a pulse duration of 35 fs and a pulse energy of 3.5 mJ. Approximately one third of the laser output is used to pump a home-built optical parametric amplifier and a difference-frequency mixing stage. This nonlinear frequency conversion produces broadband mid-IR pulses (tuneable from 2-10 μm, with a bandwidth of 600 cm\(^{-1}\) (FWHM), and a pulse energy of 10-20 μJ). The IR pulses have a sufficiently large bandwidth to measure the complete SFG spectrum of the OH stretch vibrations of H\(_2\)O. Another part of the 800 nm pulse is sent through an etalon to narrow down its bandwidth to \(\sim 15\) cm\(^{-1}\). The resulting narrow-band 800 nm pulse (VIS) and the broadband IR pulse are directed to the sample surface at angles of \(\sim 50^\circ\) and \(\sim 55^\circ\), respectively, to generate light at the sum frequency. The VIS and IR beams are focused in spatial and temporal overlap on the sample surface with 200 mm and 100 mm focal length lenses, respectively. The SFG light generated at the surface is sent to a monochromator and detected with an Electron-Multiplied Charge Coupled Device (EMCCD, Andor Technologies). The conventional VSFG spectra are background subtracted (blocked IR) and normalized to a reference SFG spectrum measured from z-cut quartz. The acquisition time of a typical VSFG measurement is 600s.

We use heterodyne-detected VSFG to determine the imaginary and real parts of \(\chi(2)\). In HD-VSFG the SFG electric field generated from the sample is combined with that of a local oscillator (LO) at the same frequency. The LO sum-frequency (LO-SFG) light is generated by first focusing the IR and VIS beams on a metal surface to generate a strong non-resonant \(\chi(2)\) SFG signal. This signal is delayed with respect to the IR and VIS beams by passing it through a silica plate (~ 1 mm). The LO-SFG and the IR and VIS beams are refocused on the sample surface using a spherical mirror. The IR and VIS beam generate the SFG signal of the sample. Subsequently, the LO-SFG and sample SFG beams are collimated, sent into a monochromator and detected with a CCD. The detected interference pattern contains cross terms from which we extract the real and imaginary \(\chi(2)\) part using Fourier transformation. To obtain the Im \(\chi(2)\) of the sample, we compare the signal with the HD-VSFG signal of a reference sample for which the phase of the SFG light is known. For this purpose we used a z-cut quartz crystal that was oriented in such a way that the bulk electric dipole contribution of quartz was maximized. For
HD-SFG measurements it is crucial that the HD-VSFG signal from the reference (z-cut quartz crystal) is generated at the same height as the sample. A mismatch in height would result in a phase shift of the recorded spectra and thus in the extracted real and imaginary $\chi(2)$. We control the height of the reference quartz crystal and the sample by monitoring the position of the VSFG signal on the CCD camera. This enables us to define the SFG signal on the camera with a precision of 1 pixel size (16 x 16 μm). Together with our setup geometry this leads to a phase uncertainty of $\sim\pi/10$ (~20 degrees). The phase uncertainty does not affect our experimental results nor their interpretation, as the possible flipping of vibrations as a function of pH would involve much larger phase changes $\sim\pi$ (180 degrees). The typical acquisition time of a HD-SFG spectrum is 120 s. All SFG measurements were performed in H$_2$O (Milipore) or D$_2$O (Cambridge Laboratories) and the pH was adjusted using hydrochloric acid or sodium hydroxide. The pH (Mettler Toledo FE20) of the samples was checked before and after each measurement. Class II hydrophobins were provided by VTT research and purified as described in Ref. 25.
Supporting Figure 1: Three-dimensional structure of T. reesei hydrophobin HFBII. Hydrophobins fold into compact structures with a β-barrel core and a distinguishable hydrophobic patch (colored in black).
Supplementary Figure 2: Comparison of the HD-VSFG magnitude \((\text{Imaginary } \chi(2))^2 + (\text{Real } \chi(2))^2\) and conventional VSFG spectrum of a HFBII film at the air-water interface. Spectra were obtained at different pH values as indicated in the figure.