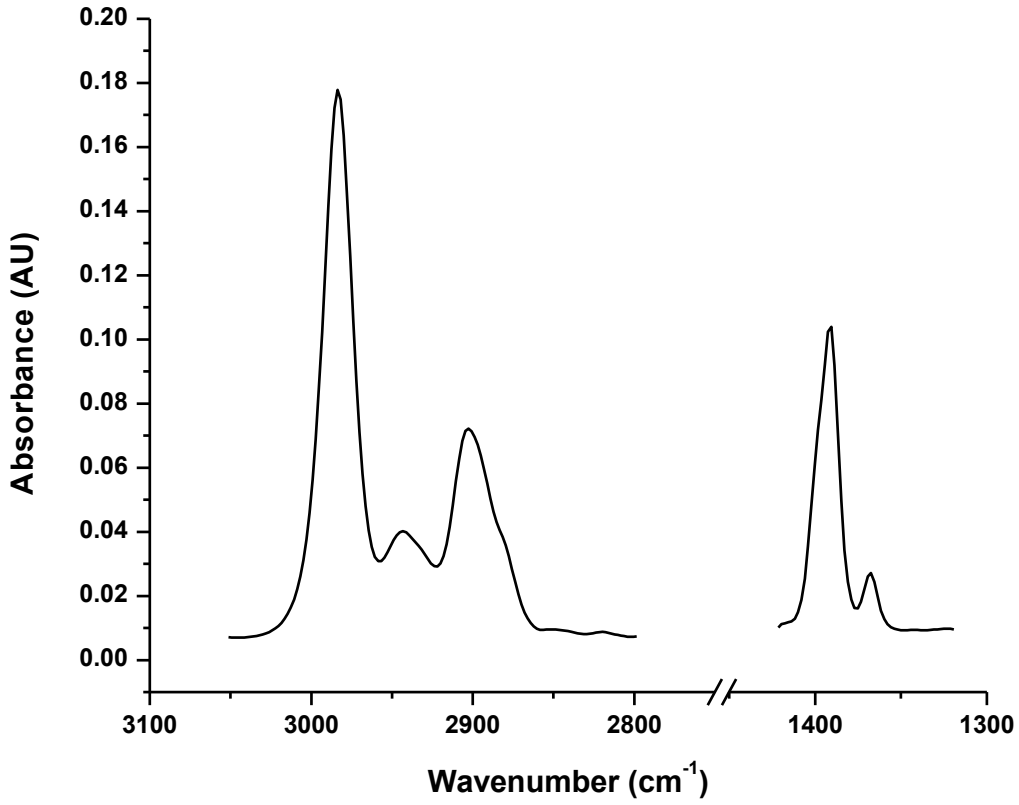
Supplementary Material

Experimental Section

Measurements were conducted on beamline 01B1-01 (MidIR) at the Canadian Light Source. The endstation is composed of a Bruker IFS 66v/S interferometer coupled to a Hyperion 2000 IR microscope (Bruker Optics, Billerica, MA, USA). Light was focused and collected by 36x Schwarzschild condenser and objectives and detected by a liquid nitrogen cooled narrowband MCT detector with a 100 μm sensing element. Data was collected at a spectral resolution of 4 cm^{-1}. Single channel traces were obtained using the fast Fourier transform algorithm, no zero-filling, after applying a Blackman-Harris 3-Term apodization function. The confocal apertures were set at 15x15 μm^2, providing a light spot at the sample close to the diffraction limit. Spectra were collected using 1024 scans per spectrum with a 40 kHz acquisition rate and repeated at 4 min intervals.
Data analysis was performed using OPUS version 6.5 (Bruker Optics, Billerica, MA, USA) and data plotting was performed using Origin version 8.0 (OriginLab, Northampton, MA, USA). The second derivative of absorption spectra was calculated by performing a coupled 13-points Savitzky-Golay smoothing. Fourier Self-Deconvolution was performed using a Gaussian line shape, a band deconvolution factor of 2 and a noise reduction factor of 0.5, as defined in OPUS.
The molar extinction coefficient for ethanol at 1983 cm^{-1} was measured using a solution of 1.7 M Ethanol (95%) in 9/1 v/v D_2O/glycerol.
Wild-type C. reinhardtii cultures were grown heterotrophically in full nutrient TAP (tris-acetate-phosphate) medium, [1] under low-light conditions. Prior to measurement, cells were suspended in D_2O to reduce spectral obstruction by H_2O, with glycerol added to prevent cellular movement, to give a final composition of 1/8/1 glycerol/D_2O/H_2O. Cells were then placed between two calcium fluoride IR optical windows separated by a 15 μm spacer, enclosed in a sample holder for liquids held at 20 °C and transferred to the microscope stage for immediate measurement. Cells are measured a with two-dimensional cell density of the order of 100-500 cells/mm^2 and without significant exposure to photosynthetically active visible radiation (400-700 nm). Under such conditions, the cell culture becomes quickly anaerobic.
D_2O (Sigma-Aldrich, St. Louis, MO, USA) in use throughout the experiment has 99% isotopic purity. 95% Ethanol (Sigma-Aldrich, St. Louis, MO, USA) was used as a reference. Other chemicals (Sigma-Aldrich, St. Louis, MO, USA) were of the highest available purity.

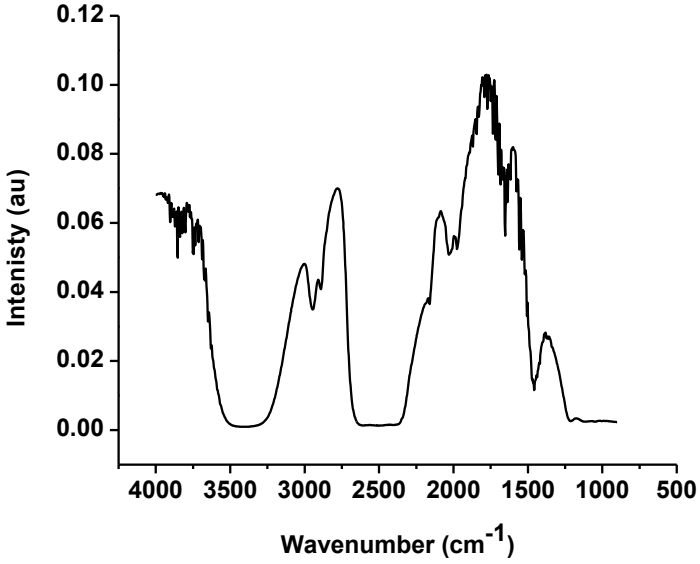
1. E. H. Harris, *The Chlamydomonas Sourcebook; Second Edition; Introduction to Chlamydomonas and its Laboratory Use; Volume 1*, 2009.

Ethanol Spectrum in D_2O/glycerol



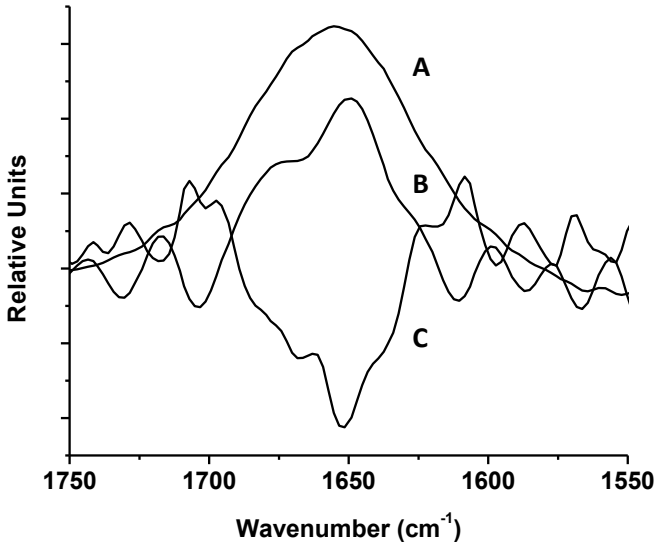
Supplementary Material. Figure S1. FTIR spectrum of 1.7 M Ethanol in 9/1 (v/v) D_2O/glycerol.

Single Channel Trace of Solvent



Supplementary Material. Figure S2. Single channel trace of a 15 μm thick sample of 1/8/1 glycerol/D_2O/H_2O, showing light throughput across the mid-infrared spectral region.

Second Derivative and Deconvolution of Peak V



Supplementary Material. Figure S3. Decomposition of Peak V into components. (A) Absorbance difference spectrum. (B) Results of Fourier Self-Deconvolution. (C) Second derivative.