

Supplementary Information

Vibrational Analysis of Acetylcholine Binding to the M₂ Receptor

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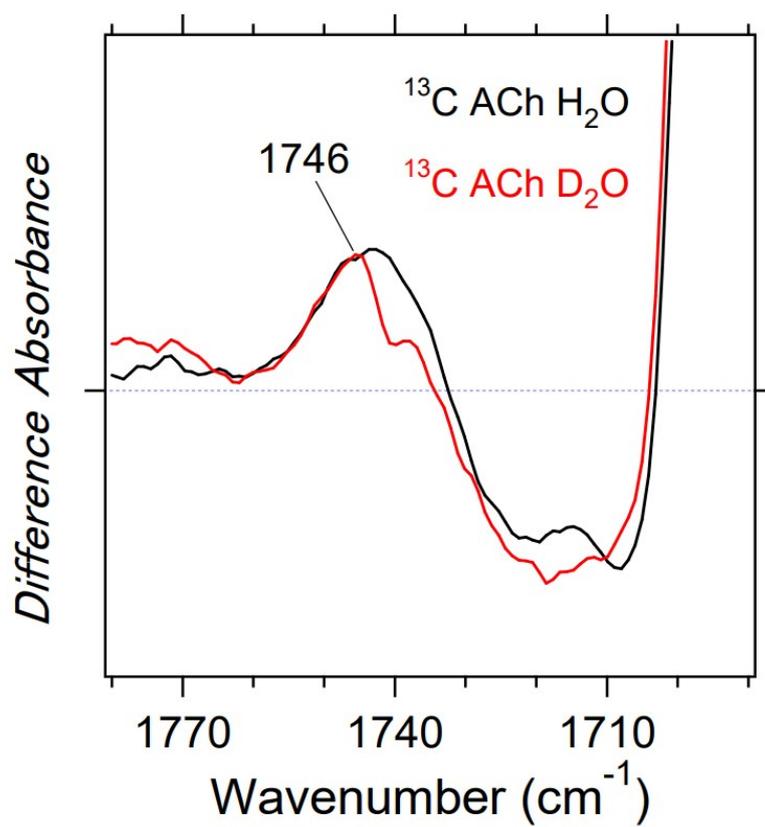


Figure S1. Difference ATR-FTIR spectra upon binding of 2- ^{13}C -labeled acetylcholine to M_2R . Black and red lines are the spectra in H_2O and D_2O , respectively. This figure is expanded from Figure 3b.

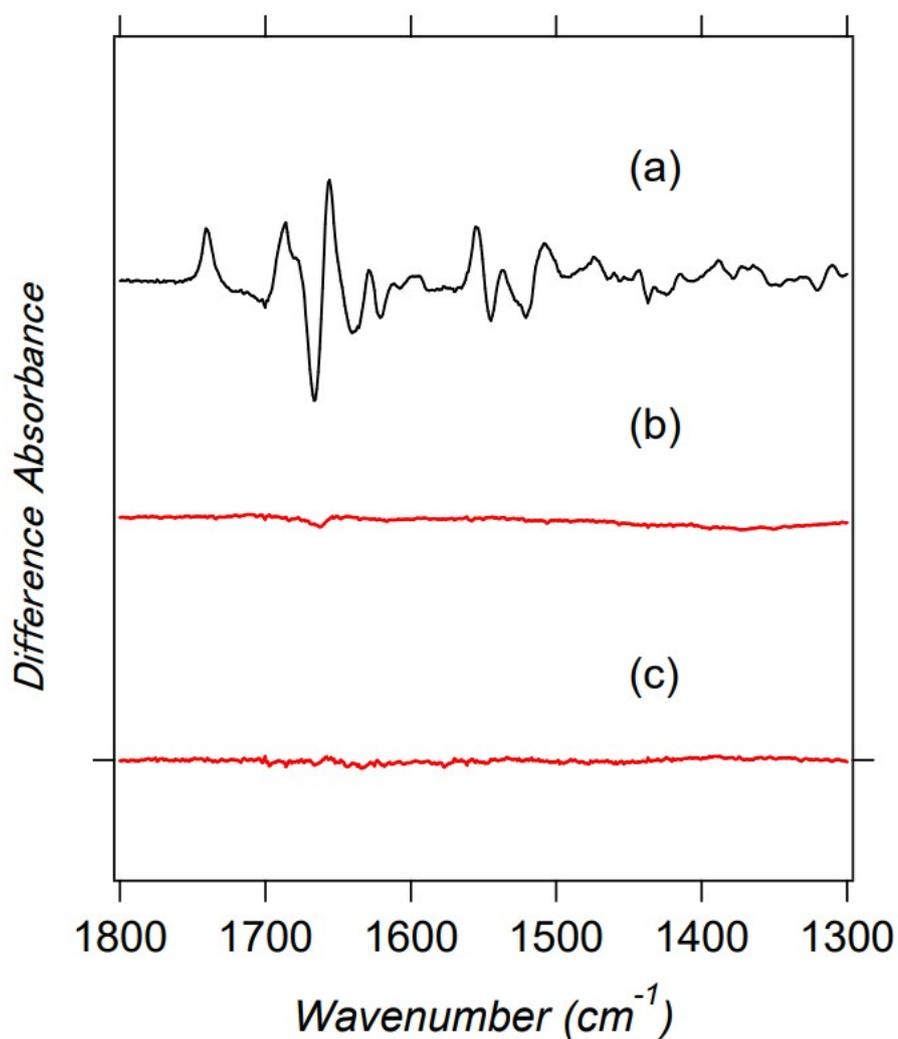


Figure S2. (a) Difference ATR-FTIR spectrum upon binding of acetylcholine (1 mM) to M₂R, where concentration of NaCl is 140 mM. (b) Difference ATR-FTIR spectrum of M₂R between NaCl and KCl (140 mM) in the absence of acetylcholine. (c) Difference ATR-FTIR spectrum of M₂R between NaCl and KCl (140 mM) in the presence of acetylcholine (1 mM).

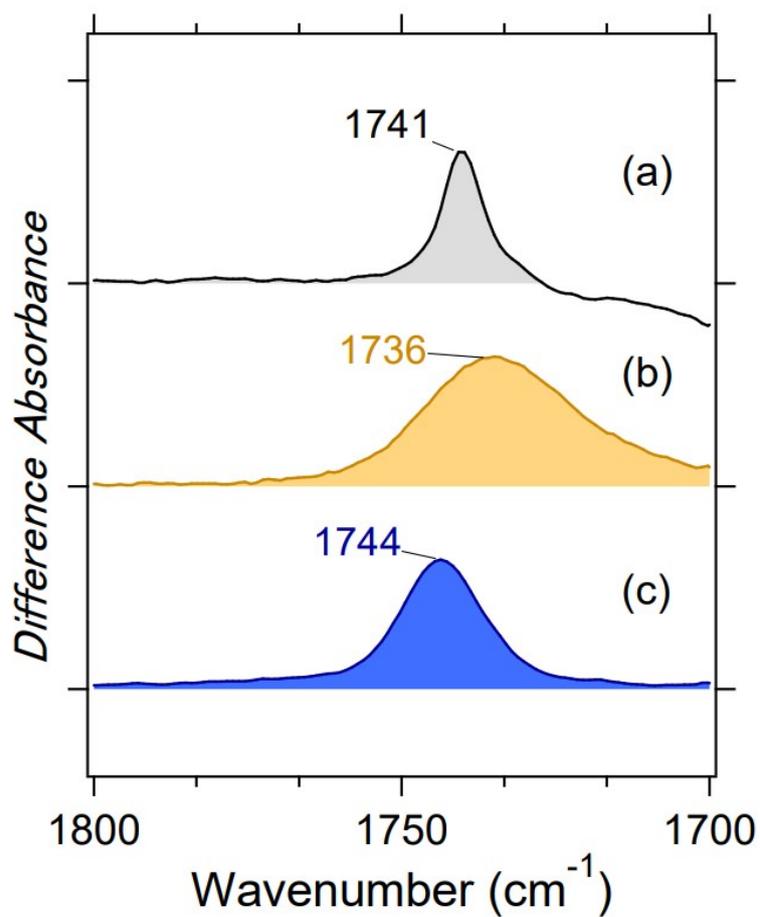


Figure S3. Spectral comparison of the C=O stretch of ACh in M₂R (a), in aqueous solution (b), and in dimethylsulfoxide (DMSO) (c), where peak absorbances are normalized. (a) and (b) are reproduced from Figure 5b. In the gas phase, the C=O stretch of ACh is reported at 1751, 1778, and 1794 cm⁻¹,²² suggesting that the C=O stretches with and without hydrogen bond appear at 1750-1730 cm⁻¹ and 1795-1775 cm⁻¹, respectively.