**Figure 3–figure supplement 1**

**Coexpression of the M2 receptor and a non-binding sensor.** (**A**) CHO cells were cotransfected with the plasmids coding for eGFP-M2 and mCh-M2(D103A)-FCM. The cells were treated with FlAsh, and those expressing both receptors were identified by the presence of both eGFP and mCherry. (**B**, **C**) The emission was recorded in absence of ligand (**B**) and in the presence of NMS (1 **M) (**C**), and the spectra were unmixed according to Equation 11 to obtain the contribution of each fluorophore. The circles are the measured spectrum, and the solid lines are the best fits of Equation 11. The dashed lines depict the constituent spectra as follows: eGFP, blue; FlAsH, green; mCherry, red. The FRET efficiency between FlAsH and mCherry was computed from the unmixed contributions according to Equation 12.