Supporting Information

FAD Roles in Glucose Catalytic Oxidation Studied by Multiphase Flow of Extractive Electrospray Ionization (MF-EESI) Mass Spectrometry

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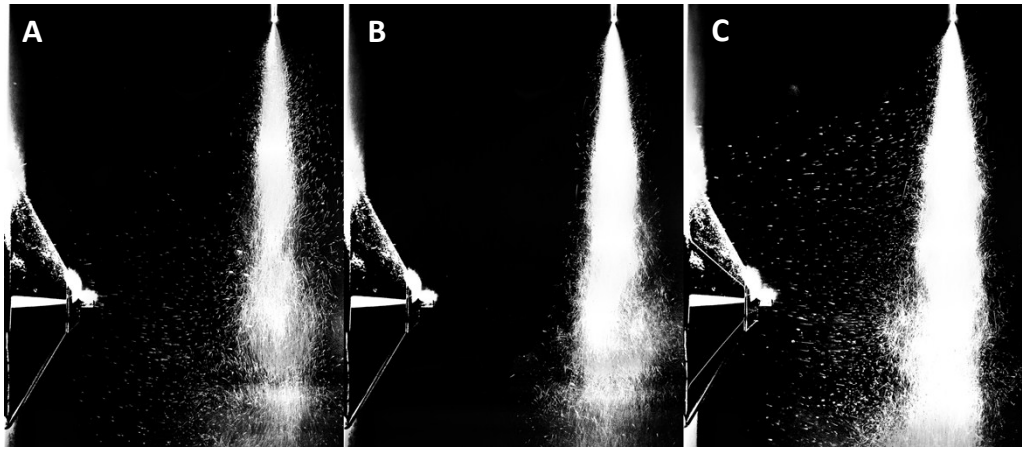


Figure S1. Actual pictures of introduction of ions from different sprays into the MS inlet. (A) Extracted sample. (B) 50 μL·min^{-1} methanol, (C) extracted sample with 50 μL·min^{-1} methanol.

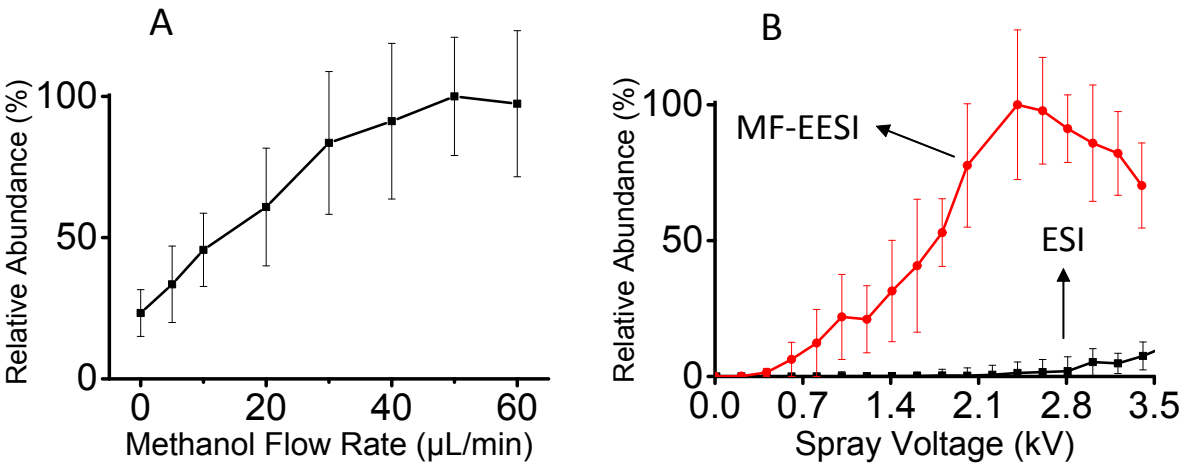


Figure S2. Optimization of MF-EESI. (A) Relative abundance of the ion of gluconic acid at *m/z* 195.0 as a function of the methanol flow rate. (B) Comparison of signals (gluconic acid at *m/z* 195.0) obtained by MF-ESI and ESI at different spray voltage.

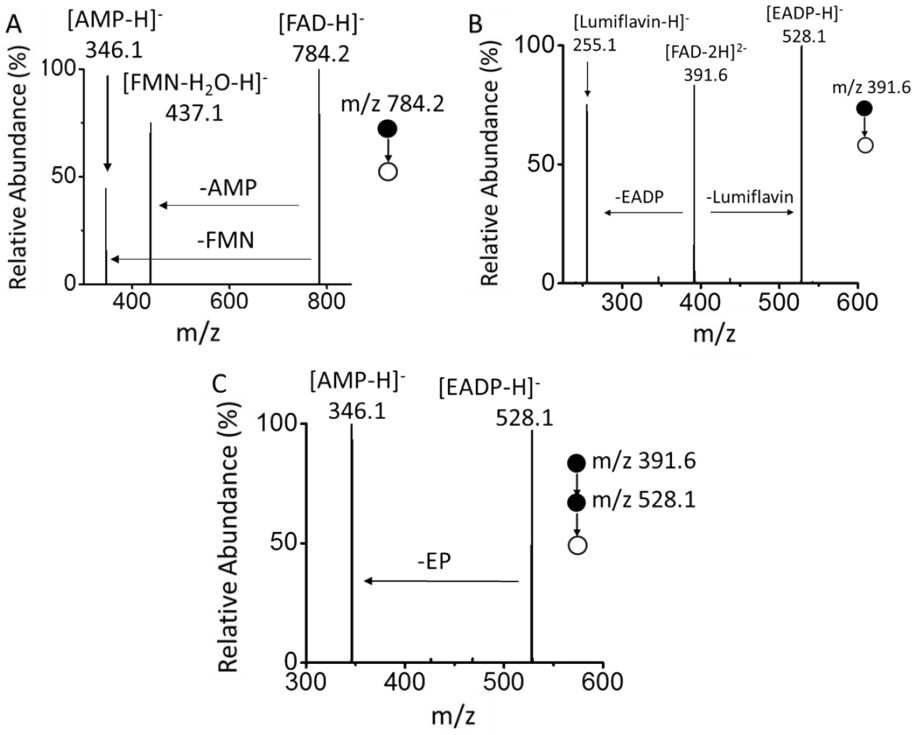
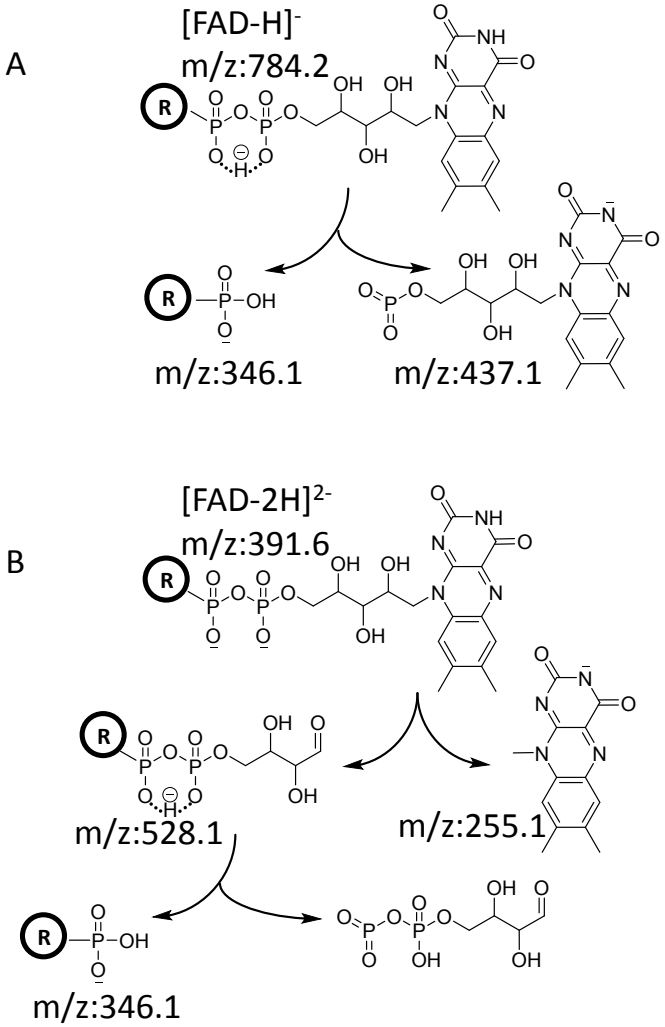


Figure S3. Structural confirmation of the FAD-Glucose I complex from the mixture of FAD and glucose by CID. (A) MS^2 CID of the ion at m/z 784.2. (B) MS^2 CID of the ion at m/z 391.6. (C) MS^3 CID of m/z 528.1.



Scheme S1. The fragmentation mechanism of (A) $[FAD\text{-}H]^-$ at *m/z* 784.2 and (B) $[FAD\text{-}2H]^{2-}$ at *m/z* 391.6 generated by MS^n CID.

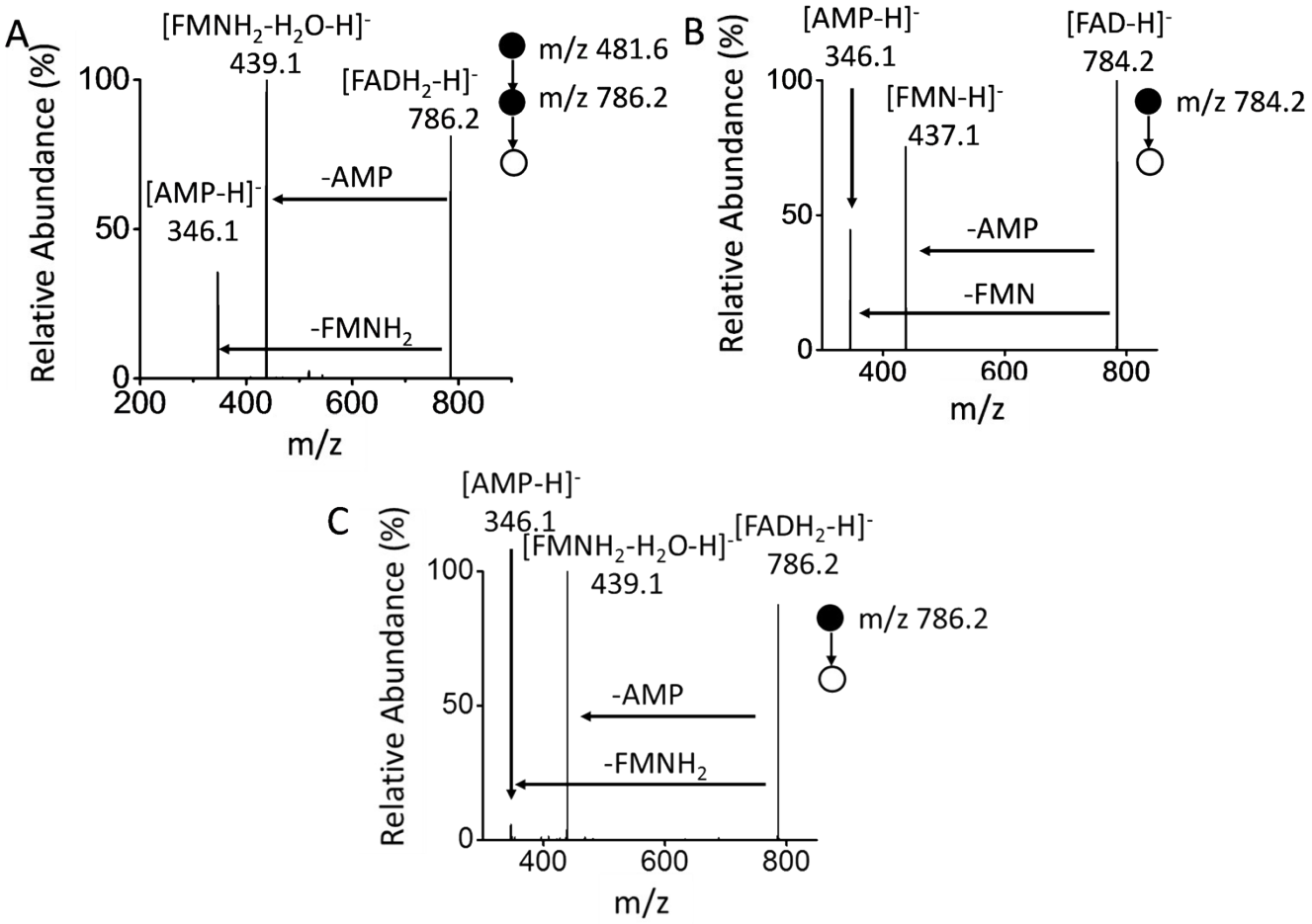
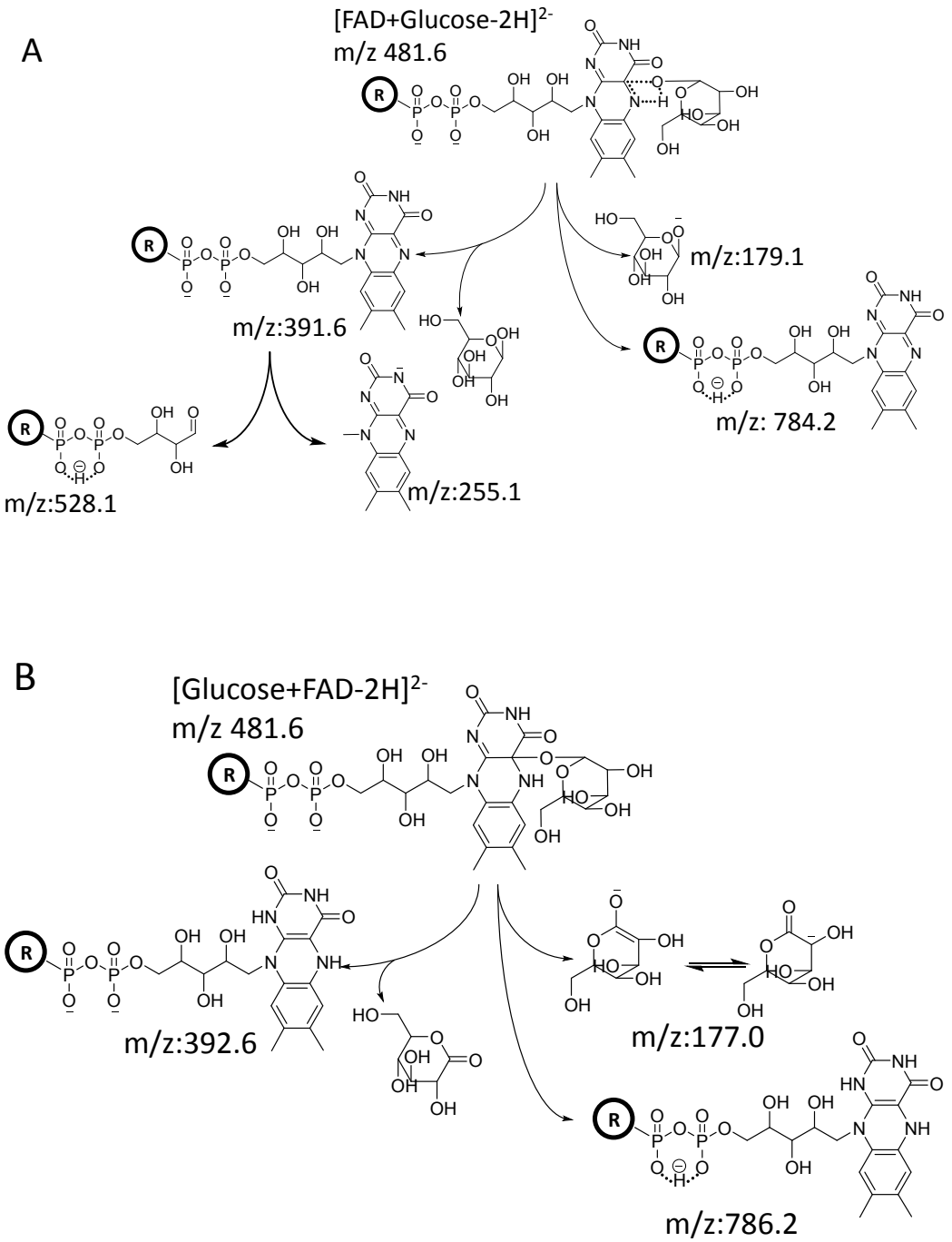


Figure S4. Structure confirmation by CID for the FAD-Glucose II complex from the mixture of FAD and glucose in the presence of GOx. (A) MS^3 CID of *m/z* 481.6; (B) MS^2 CID of the ion at *m/z* 784.2; (C) MS^2 CID of the ion at *m/z* 786.2.



Scheme S2. Schematic diagram for fragmentation mechanism of *m/z* 481.6 generated by MS^n CID in the reaction system (A) without GOx and (B) with GOx.

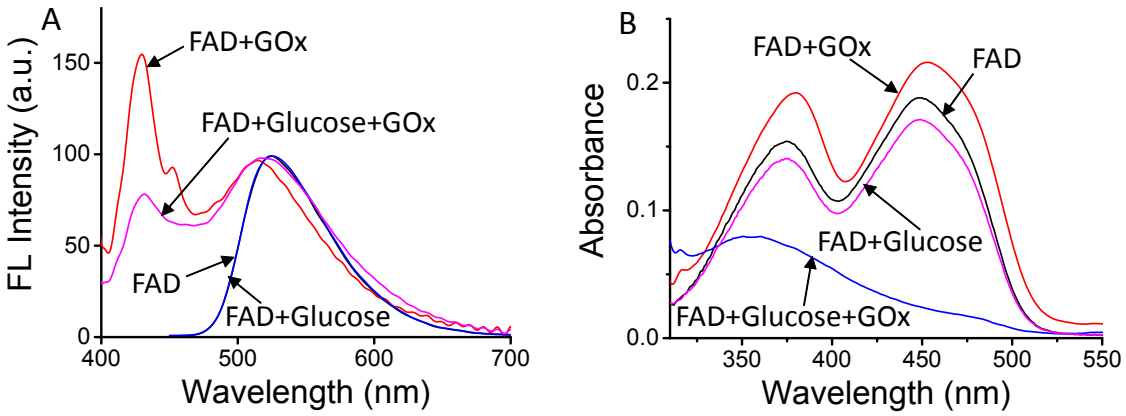


Figure S5. FL (A) and UV-Vis (B) spectra of FAD, the mixture of FAD and glucose, the mixture of FAD and GOx, and the mixture of FAD, Glucose and GOx. There are no noteworthy signals for glucose in both spectra.

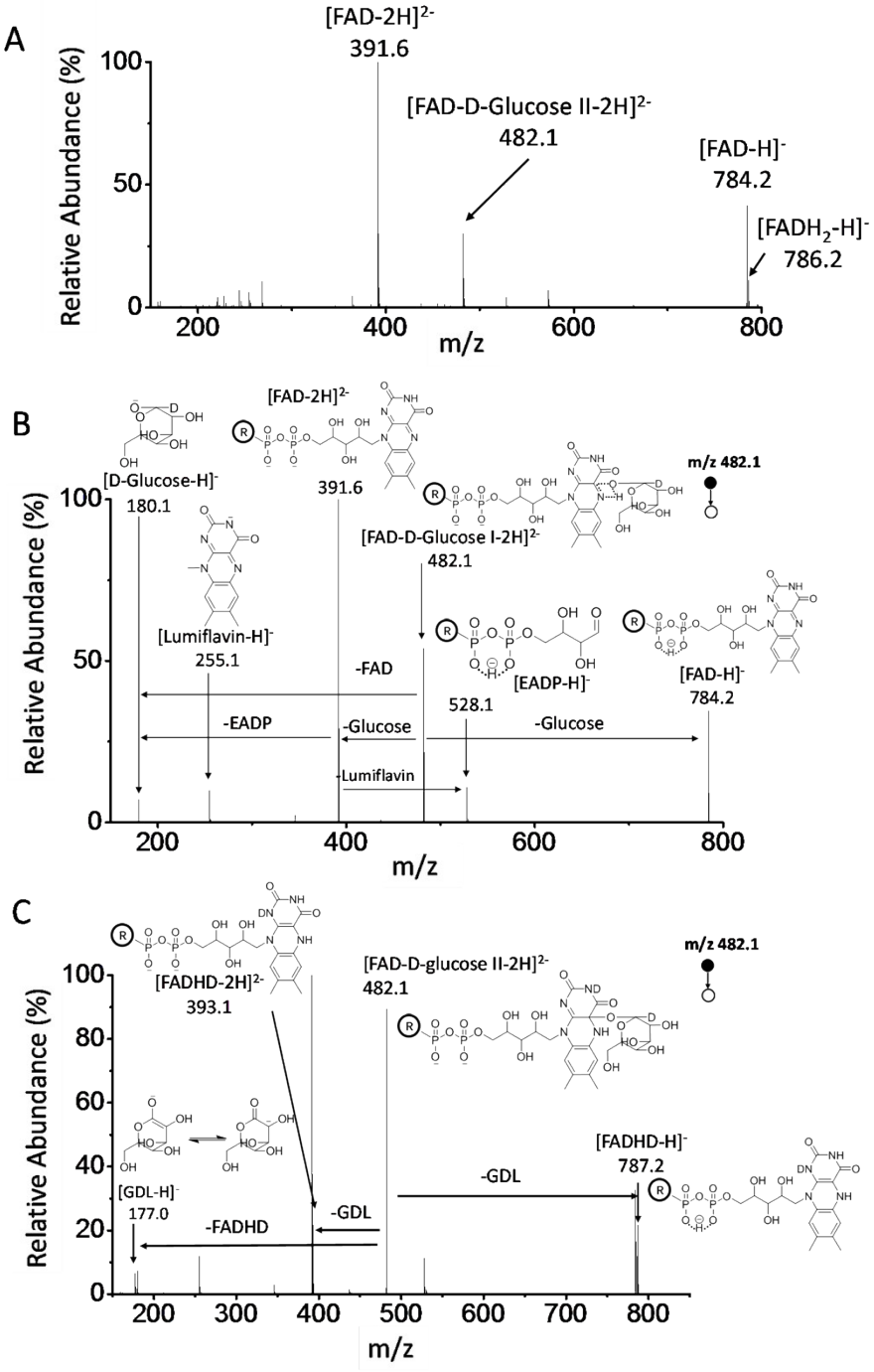


Figure S6. The isotope labeling experiment using deuterated reagent of D-glucose-1-d for the catalytic reaction. (A) Mass spectrum of the ternary mixture of D-glucose-1-d, FAD and GOx detected by MF-EESI MS. MS^2 CID of the two complexes of FAD and D-glucose-1-d at m/z 482.1 for (B) FAD-D-Glucose I obtained from the mixture of FAD and D-glucose-1-d and (C) FAD-D-Glucose II obtained from the mixture of FAD, D-glucose-1-d and GOx.

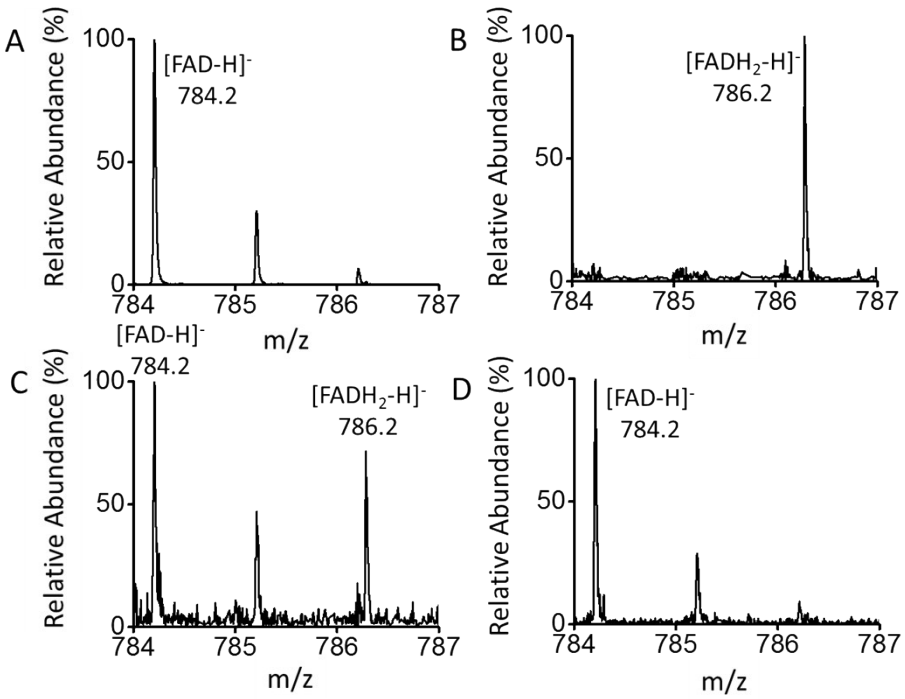


Figure S7. Mass spectra confirmation for cyclic conversion of FAD. (A) the mixture of FAD and glucose, (B) addition of GOx into the system, (C) introduction of O_2 into the catalytic oxidation system, and (D) after introduction of O_2.