

Substrate and cofactor binding to nitrile reductase: a mass spectrometry based study

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Supporting information

Kinetic measurements:

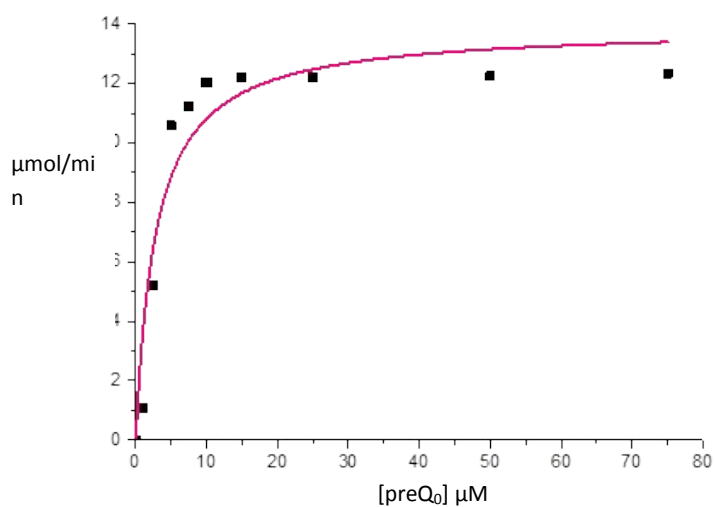


Figure S1. Michaelis-Menten curve

In order to determine the K_m for PreQ₀, the concentration of the cofactor was kept constant at 100 μM, while changing the substrate concentration from 1-75 μM. Error margins or standard deviation 5-8%.

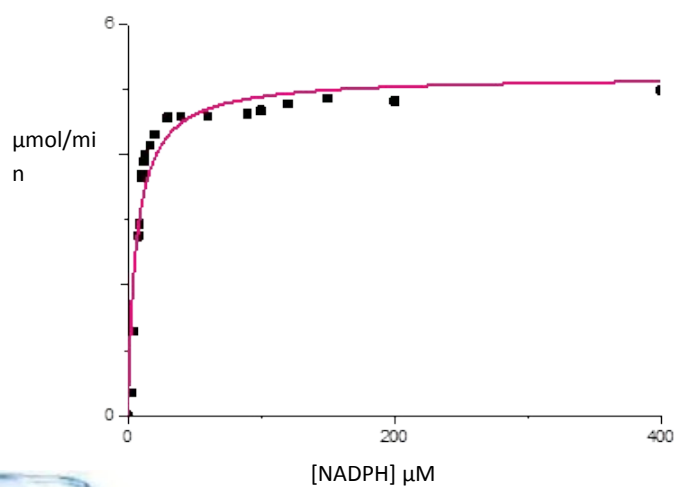


Figure S2. Michaelis-Menten curve

In order to determine the K_m for NADPH, the concentration of PreQ₀ was kept constant at 50 μM, while changing the cofactor concentration from 1-400 μM. Error margins Standard deviation 5-8%.

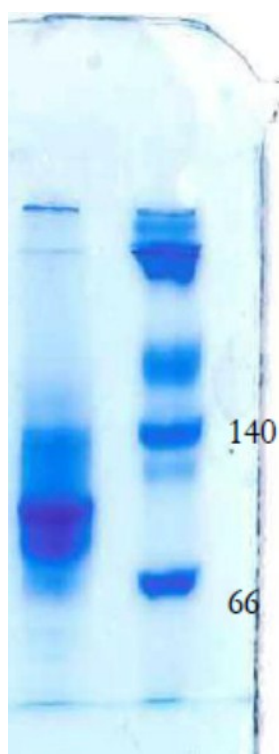


Figure S3. Native gel electrophoresis

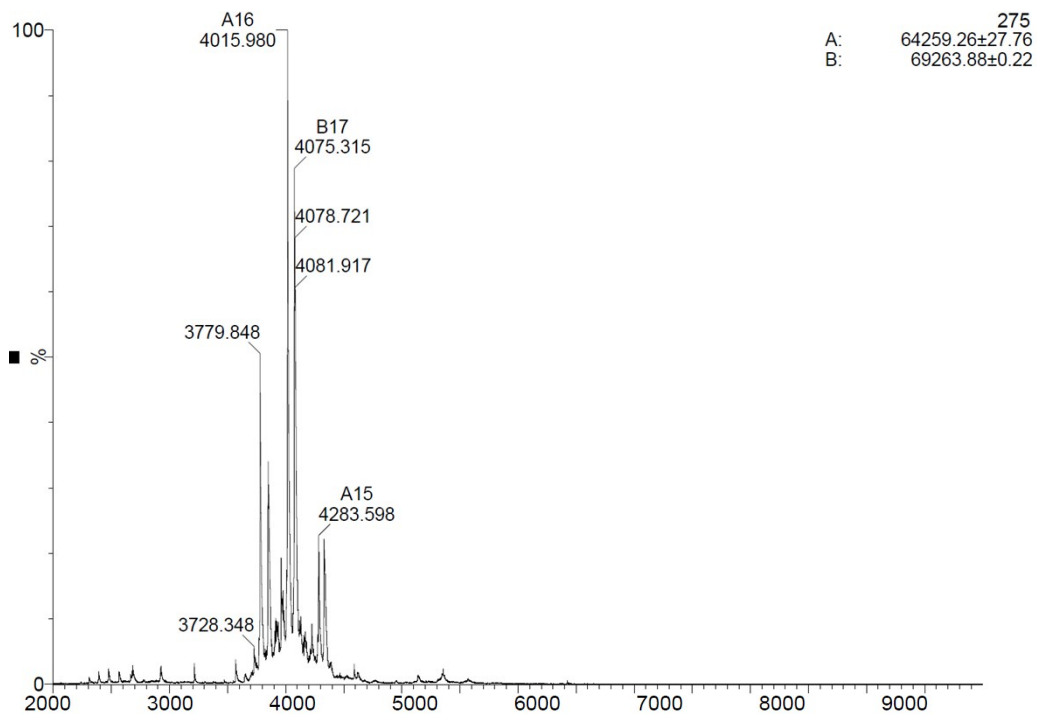


Figure S4. Mass spectra of free EcoNR, A16 multiple charge ion ($z=16^+$) of dimeric EcoNR- 2 tags, B17 multiple charge ion ($z =17^+$) of dimeric EcoNR-1 tag.

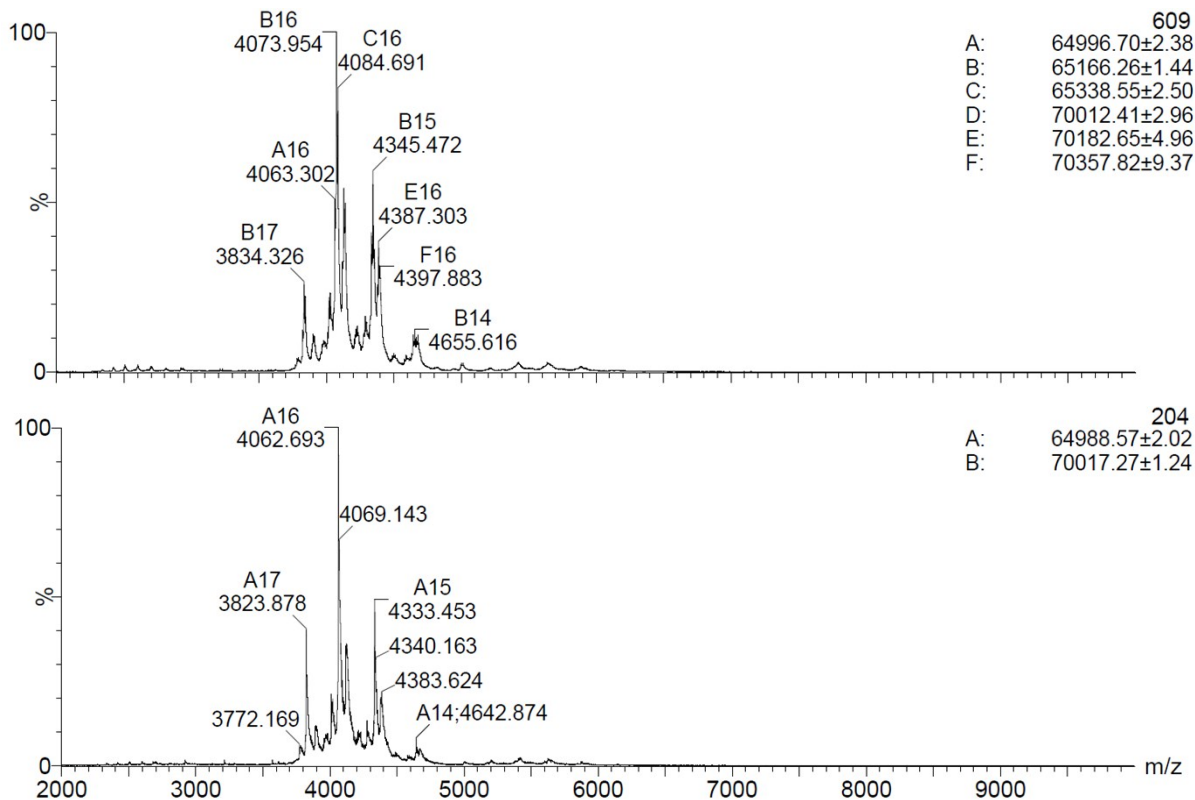


Figure S5. A) *EcoNR* treated with one equivalent of PreQ₀ and NADPH each per monomer B) The mass peak $m/z=4063$ is correlated to the mass of the dimeric *EcoNR* +NADPH when an excess of cofactor was used.

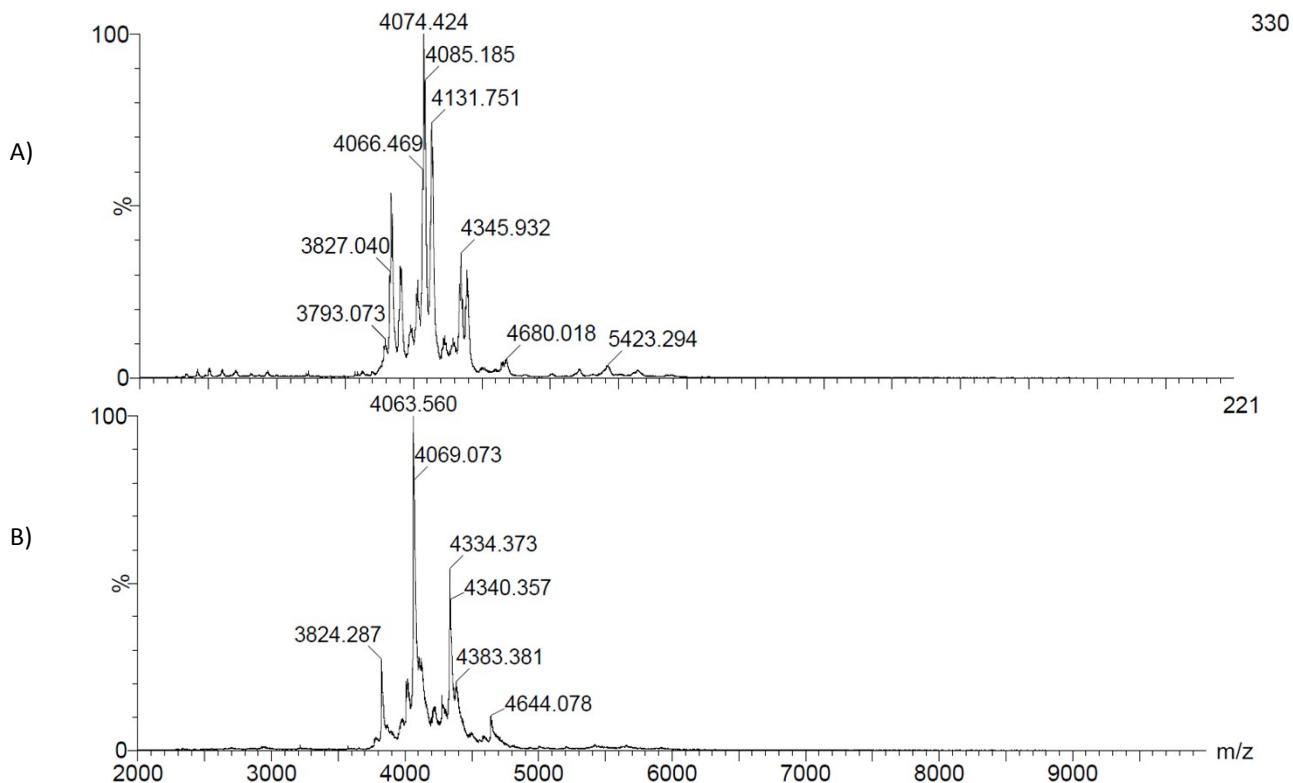


Figure S6. A) *EcoNR* treated with one equivalent of NADPH and PreQ₀ each per monomer B) The mass peak $m/z=4063$ is correlated to the mass of the dimeric *EcoNR* +NADPH when an excess of cofactor was used.

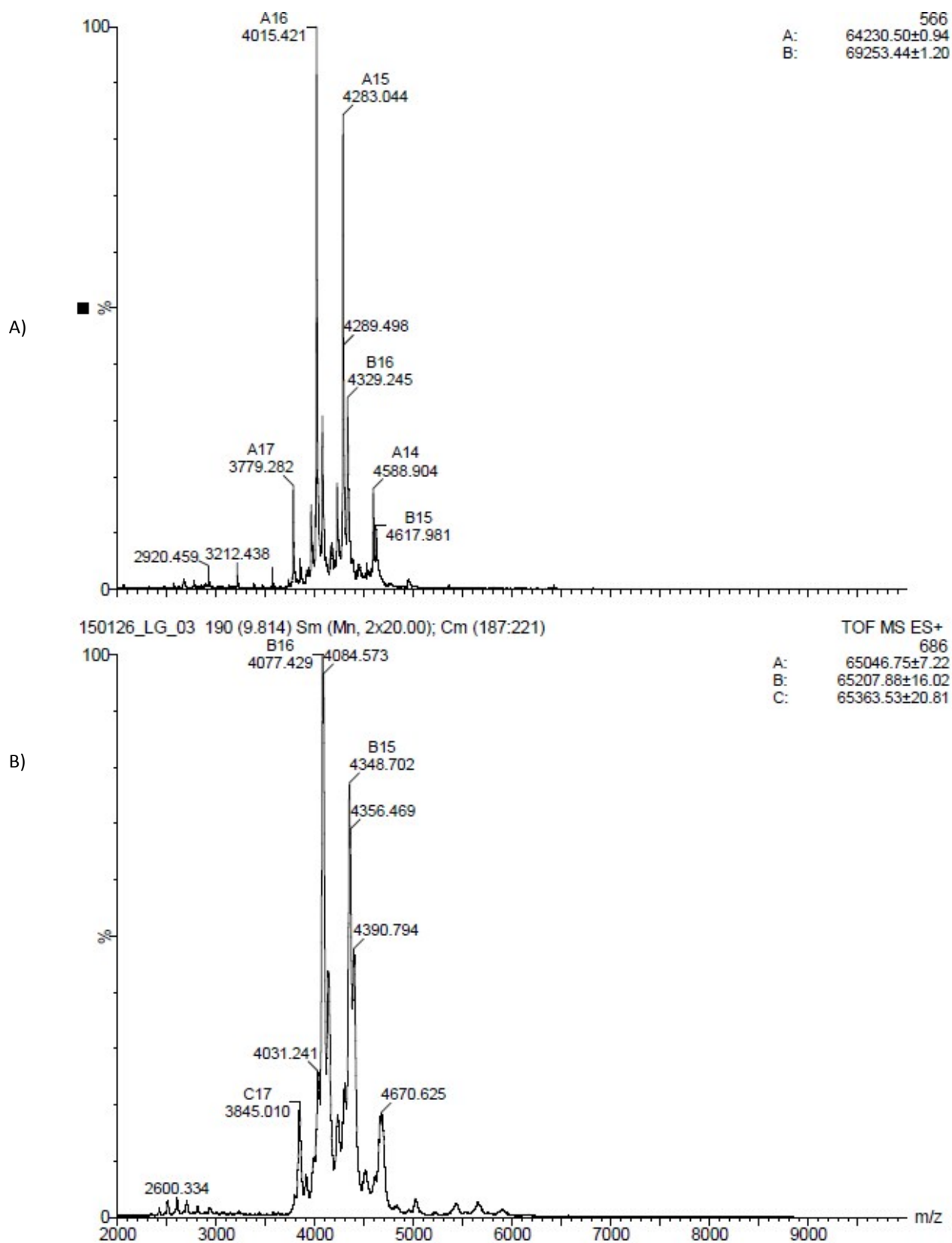


Figure S7. A) *EcoNR* with AMP and B) followed by the equimolar addition of PreQ₀ and NADPH.

NMR spectrum was recorded on a Bruker Avance spectrometer at 400 MHz (^1H) at room temperature.

^1H NMR in DMSO-d_6

