Supplementary Information for:

Examining Reaction Specificity in PvcB, a Source of Diversity in Isonitrile-containing Natural Products

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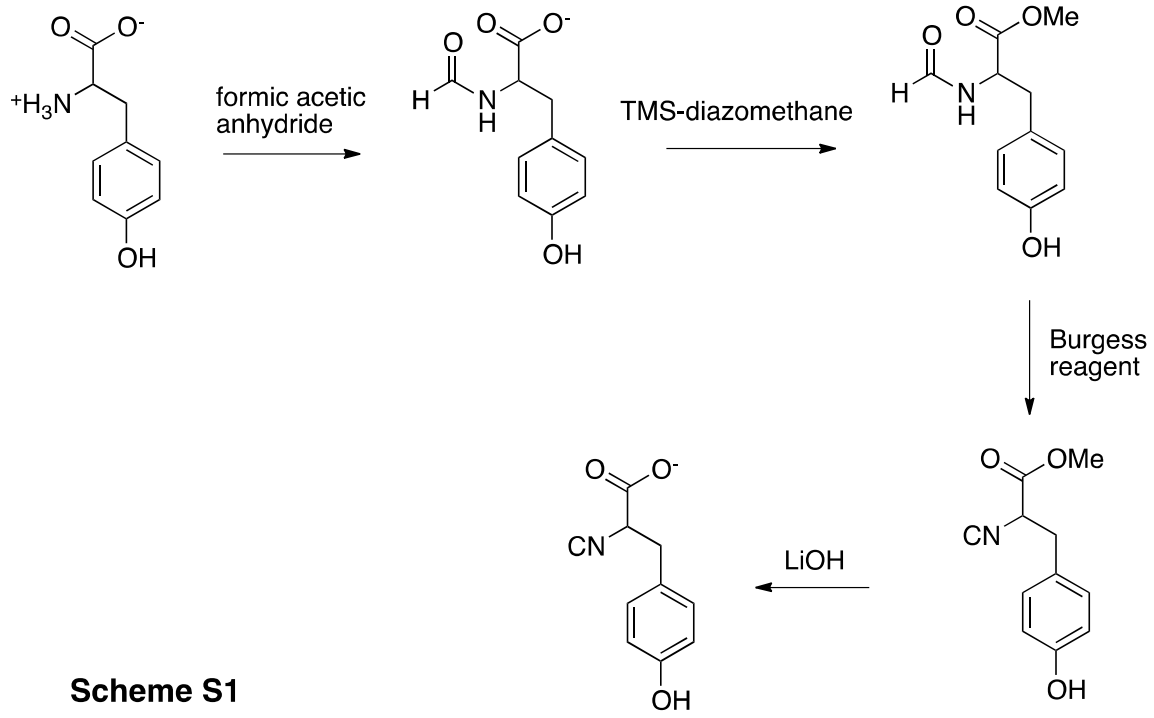
Synthesis of tyrosine isonitrile

The route for synthesis of tyrosine isonitrile is given in Scheme S1. ^{1}H NMR spectrum of the product is shown in Figure S1.

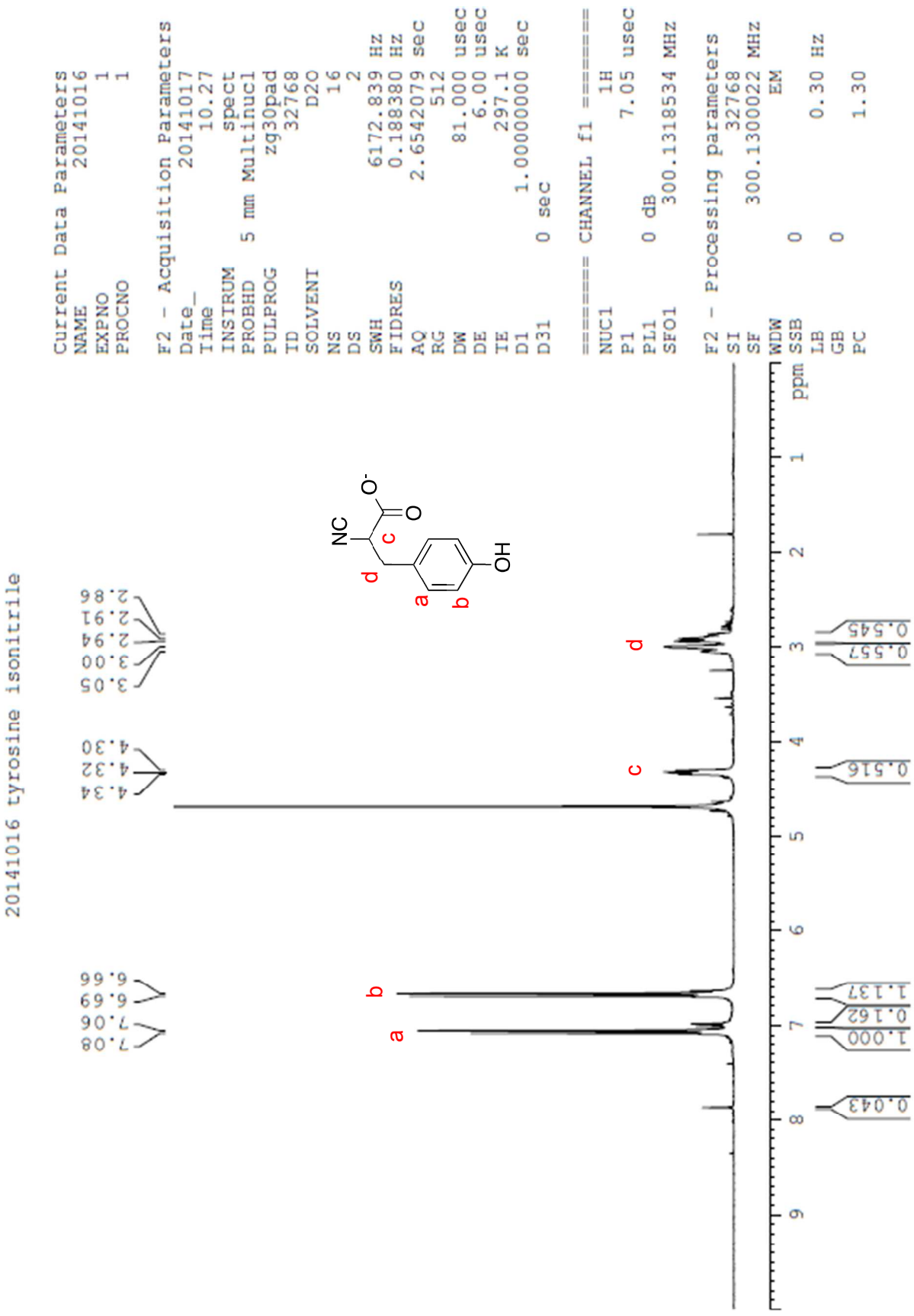


Scheme S1

Liquid chromatography-mass spectrometry

10 μl of the samples were injected into an integrated Thermo-Finnigan LC system equipped with a 150 x 4.6 mm C18 reverse phase column (Sigma-Aldrich). An LC method with a mobile phase of H_2O supplemented with 0.1% formic acid (Solvent A) and MeOH (Solvent B) was used: t = 0 min, 5%B; t = 5 min, 5%B; t = 30 min, 30%B; t = 35 min, 30%B; t = 45 min, 80%; t = 50 min, 80%B; t = 55 min, 5%B; t = 60 min, 5%B. HPLC separation of different components in the sample was followed by the mass analysis using a Finnigan TSQ7000 triple-quadrupole mass spectrometer equipped with an Atmospheric Pressure Chemical Ionization (APCI) source operating in positive ion mode.

Figure S1


20141016 tyrosine isonitrile
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PROCNO 1
F2 - Acquisition Parameters
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Time 10.27
INSTRUM spect
PROBHD 5 mm Multinucl
PULPROG zg30pad
TD 32768
SOLVENT D2O
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DS 2
SWH 6172.839 Hz
FIDRES 0.188380 Hz
AQ 2.6542079 sec
RG 512
DW 81.000 usec
DE 6.00 usec
TE 297.1 K
D1 1.00000000 sec
D31 0 sec
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P1 7.05 usec
PL1 0 dB
SFO1 300.1318534 MHz
F2 - Processing parameters
SI 32768
SF 300.1300022 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.30
7.08
7.06
6.69
6.66
4.34
4.32
4.30
3.05
3.00
2.94
2.91
2.86
1.000
0.162
1.137
0.043
0.516
0.557
0.545
a
b
c
d
NC
O
O
OH
ppm

SVD Analysis of PaPvcB product decomposition

Tyrosine isonitrile (0.2 mM), α-ketoglutarate (2 mM), ascorbate (50 μM), and $(NH_4)_2Fe(SO_4)_2$ (10 μM) were combined in 50 mM HEPES, pH 7.8, 150 mM NaCl in a total volume of 1.0 mL. Reaction was initiated by the addition of 4 μM PaPvcB. Spectra were collected every 0.1 min for 30 min.

Spectroscopic data were filtered so that only those collected 30 s apart were imported into KinTek Explorer v. 4.0 [1], where singular value decomposition was performed. The amplitudes of the first five eigenvectors derived from SVD are plotted in Figure S2. Based on visual inspection of these data, all but the first two eigenvectors were ignored for subsequent data analysis, and it was determined that 2 species were sufficient to account for the spectra.

The time evolution of the SVD spectral components and amplitudes were fitted to a model in which one species was converted irreversibly to another in a first order process.

$$P_1 \xrightarrow{k_1} P_2$$

In this model P_1 is the true product of the PaPvcB reaction, and P_2 is the species to which it converts nonenzymatically. The value of k_1 was determined to be 0.12 ± 0.05 min^{-1}. The time evolution of the first two eigenvectors were fitted to the model (Figure S3). Spectra reconstructed from the SVD spectra, the two eigenvectors, and the calculated value of k_1 are compared with the experimental data in Figure S4.

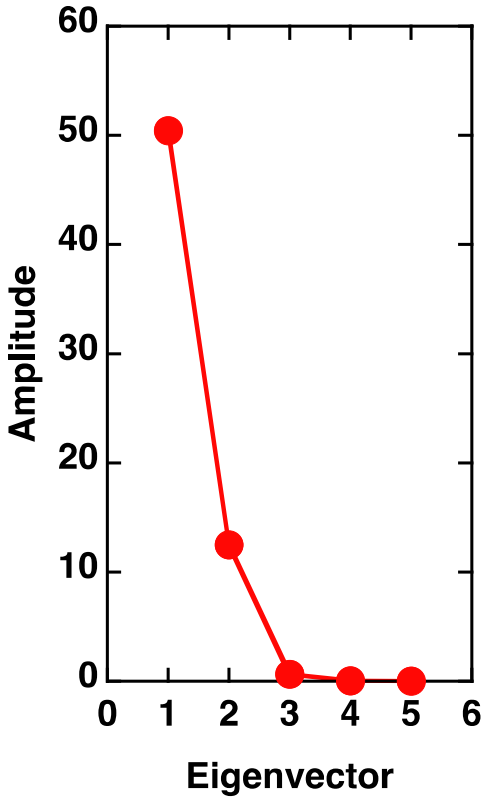

Figure S2: Amplitudes of the first 5 eigenvectors derived from singular value decomposition of spectra obtained during decomposition of the PaPvcB reaction product.

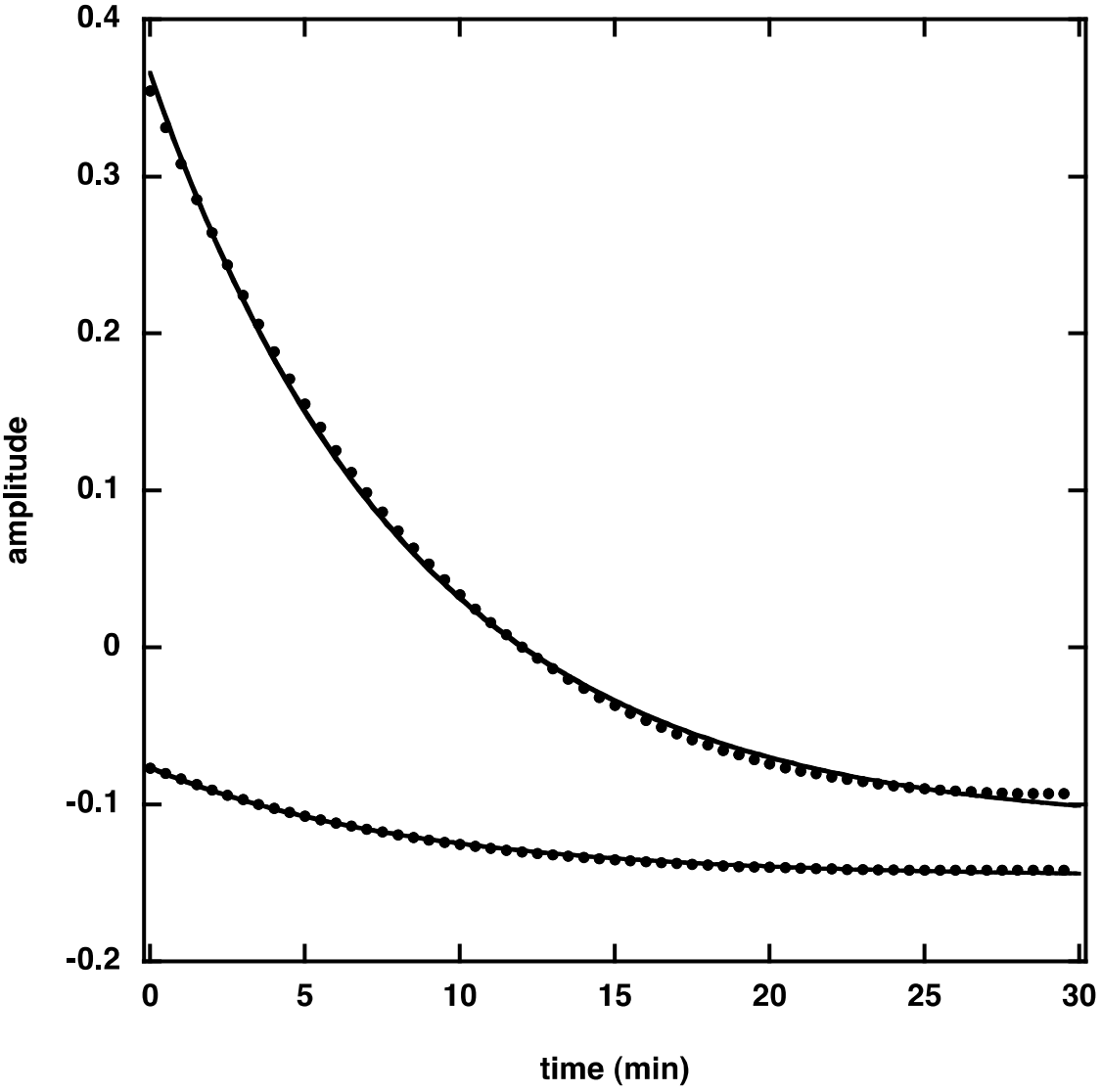

Figure S3: Time dependence of the first two eigenvectors derived from SVD analysis. Experimental values shown as circles (•) and fitted curves shown as solid lines.

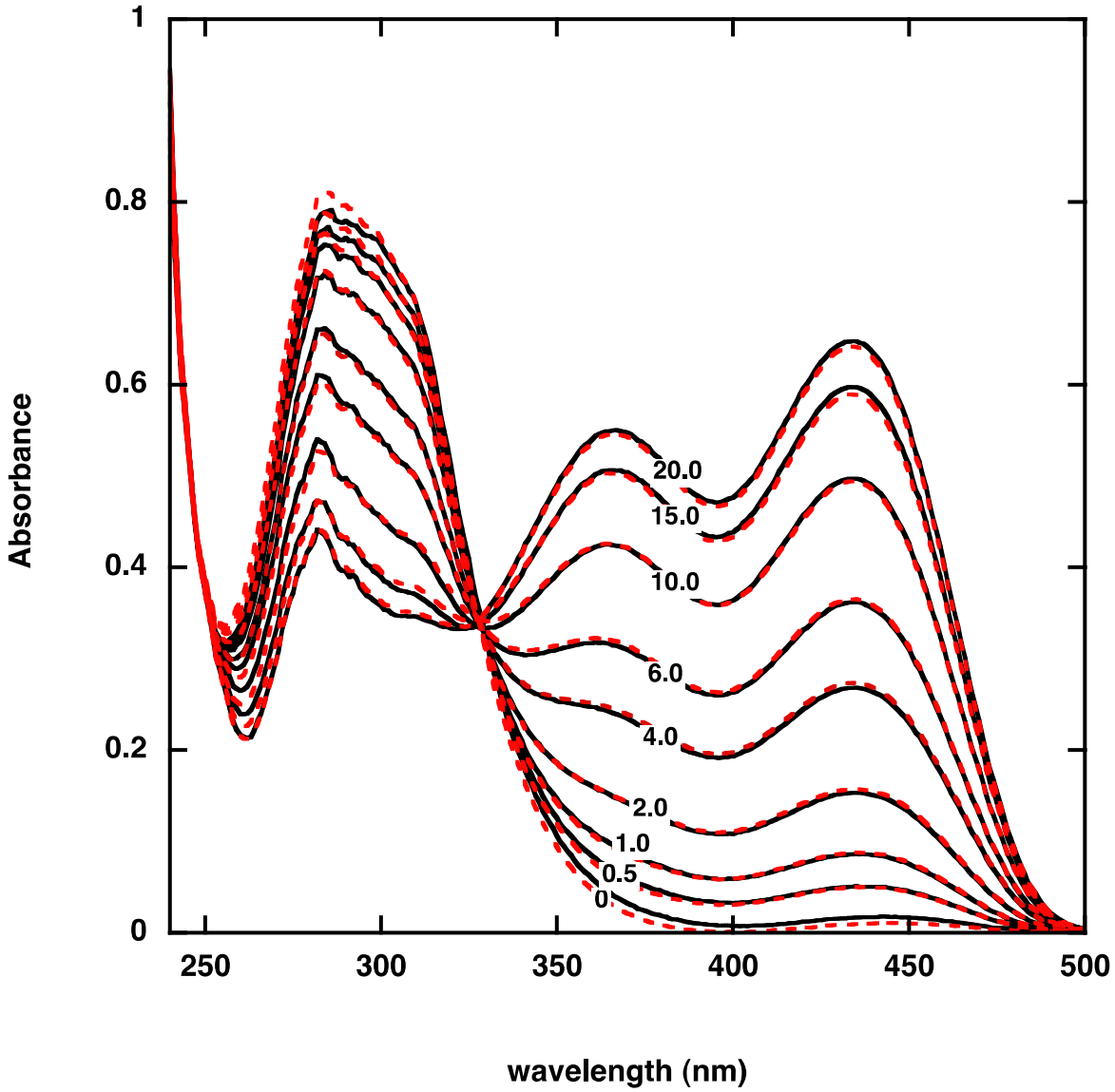

Figure S4: Comparison between selected experimental spectra and reconstructed spectra from SVD analysis of the PaPvcB reaction. Experimental conditions are described in the manuscript; black solid lines are experimental, red dashed lines are reconstructed. Spectra were acquired at the times indicated on the figure.

References

[1] Johnson, K. A., Simpson, Z. B., and Blom, T. (2009) Global Kinetic Explorer: A new computer program for dynamic simulation and fitting of kinetic data, *Analytical Biochemistry 387*, 20-29.