Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary information

Fluorescent assay for quantitative analysis of trimethylamine *N*-oxide

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Table S1 Comparison of several assay methods for determining TMAO in blood samples.

Methods	Sample preparation	LOD (μM)	LOQ (μM)	Response time for single sample (min)	Consumed time for 80 samples (min)	Determination of multiple samples concurrently	Costly isotope labeled internal standards	Uniquely trained personnel	Expensive equipment
LC-MS/MS [1]	Required	0.013	0.08	10	800	Difficult	Required	Required	Required
LC-MS/MS [2]	Required	0.02	0.05	5	400	Difficult	Required	Required	Required
UHPLC–MS/MS [3]	Required	0.09	0.26	6	480	Difficult	Required	Required	Required
LC-ToF-MRM [4]	Required	0.05	0.10	2.5	200	Difficult	Required	Required	Required
LC-ESI-MS/MS [5]	Required	0.067	0.13	10	800	Difficult	Required	Required	Required
NMR [6]	Not required	3.0	3.3	6	480	Difficult	Not required	Required	Required
This study	Required	0.62	2.05	40	40	Easy	Not required	Not required	Not required

UHPLC–MS/MS, Ultra-High Performance Liquid Chromatography–Tandem Mass Spectrometry; LC-ToF-MRM, Liquid Chromatography-Time of Flight Mass Spectrometry with Multiple Reaction Monitoring; LC-ESI-MS/MS, Liquid Chromatographic Electrospray Ionization Tandem Mass Spectrometry; NMR, Nuclear Magnetic Resonance.

References for Table S1

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2. Z. Wang, B. S. Levison, J. E. Hazen, L. Donahue, X. M. Li and S. L. Hazen, *Analytical biochemistry*, 2014, **455**, 35-40.
3. H. M. Awwad, J. Geisel and R. Obeid, *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, 2016, **1038**, 12-18.
4. L. M. Heaney, D. J. Jones, R. J. Mbasu, L. L. Ng and T. Suzuki, *Analytical and bioanalytical chemistry*, 2016, **408**, 797-804.
5. J. Liu, M. Zhao, J. Zhou, C. Liu, L. Zheng and Y. Yin, *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, 2016, **1035**, 42-48.
6. E. Garcia, J. Wolak-Dinsmore, Z. Wang, X. S. Li, D. W. Bennett, M. A. Connelly, J. D. Otvos, S. L. Hazen and E. J. Jeyarajah, *Clinical biochemistry*, 2017, **50**, 947-955.

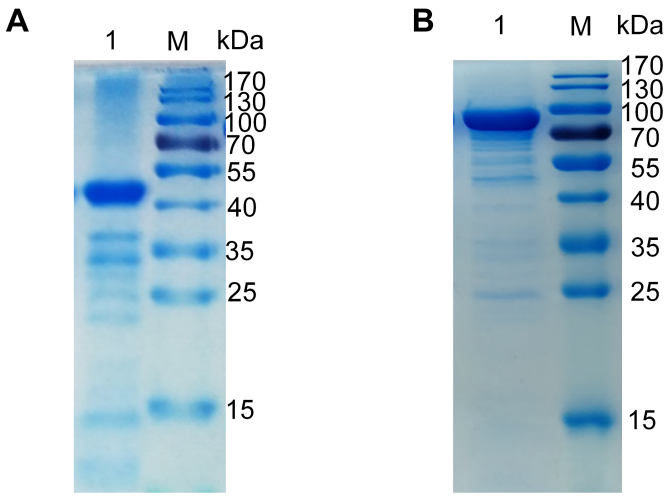

Figure S1 Preparation of the recombinant proteins. (A) SDS-PAGE of the prepared FADH. M, marker; 1, the obtained FADH. (B) SDS-PAGE of the prepared TDM. M, marker; 1, the obtained TDM.

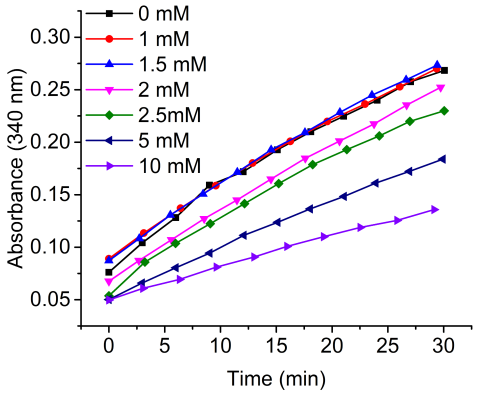

Figure S2 Imidazole inhibits the activity of FADH. The reaction mixtures were performed by using HEPES buffer (0.1 M, pH 8.0), 125 μM NAD^+, 3 mM FA, 0.0075 $mg \cdot ml^{-1}$ FADH and various concentrations of imidazole (mM) at 37 °C.

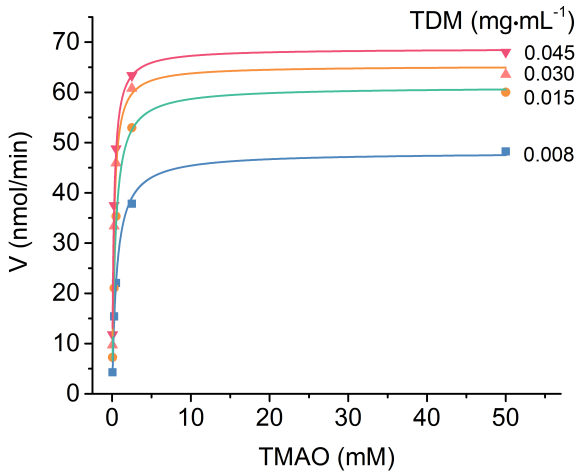

Figure S3 The effect of different amounts of TDM on activities of the TDM and FADH-catalyzed coupled reaction under variable TMAO concentrations.

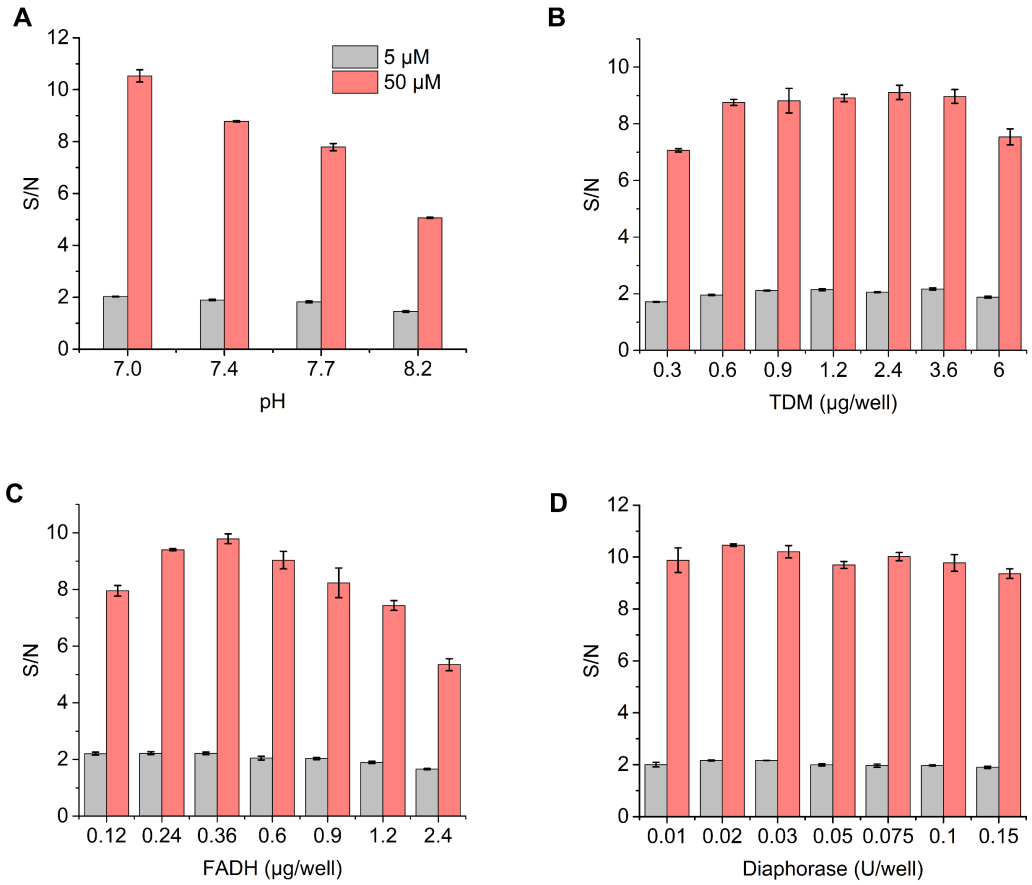

Figure S4 Effect of pH (A) and usage amounts of TDM (B), FADH (C) and diaphorase (D) on signal-noise (S/N) ratios of the assay. Values are the average ± SD (*n*=3).

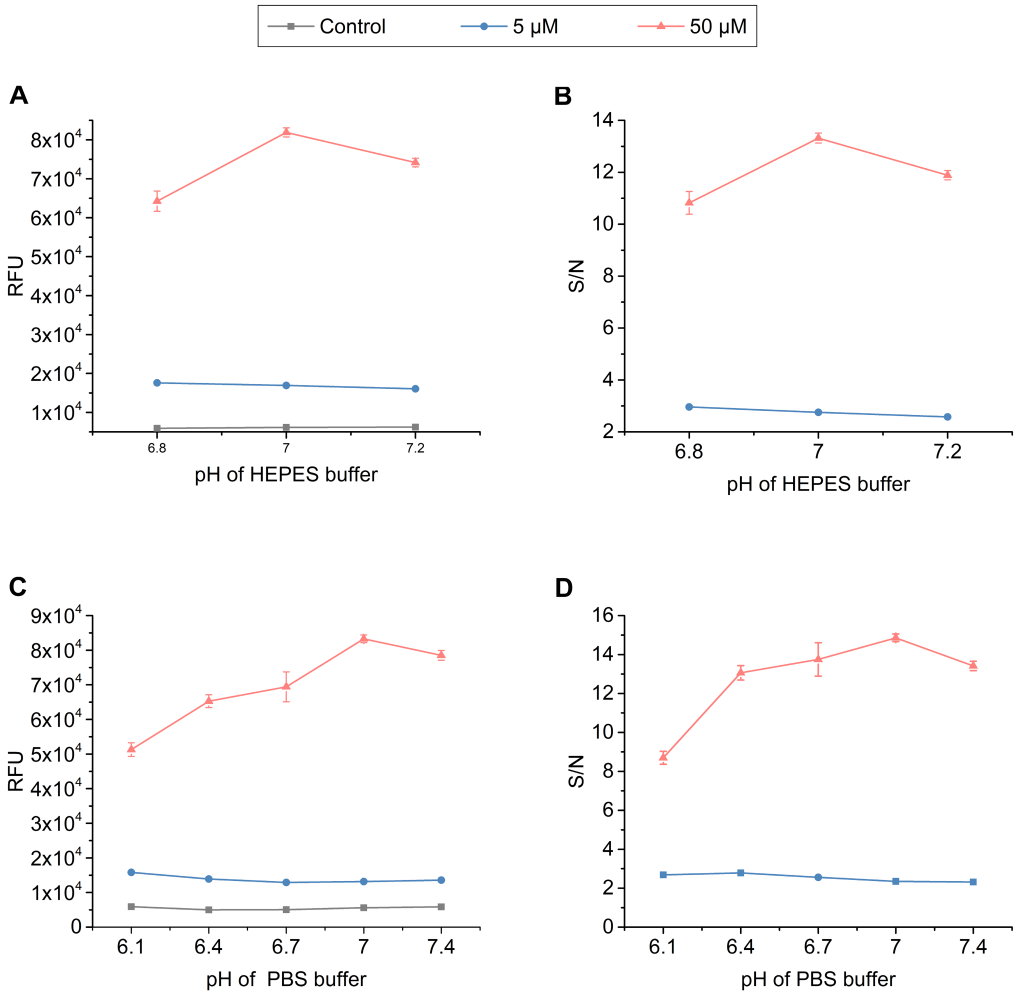

Figure S5 Effect of pH of HEPES buffer or PBS buffer on the fluorescence intensities (A or C) and signal-noise ratios (S/N) (B or D). Values are the average ± SD (*n*=3).

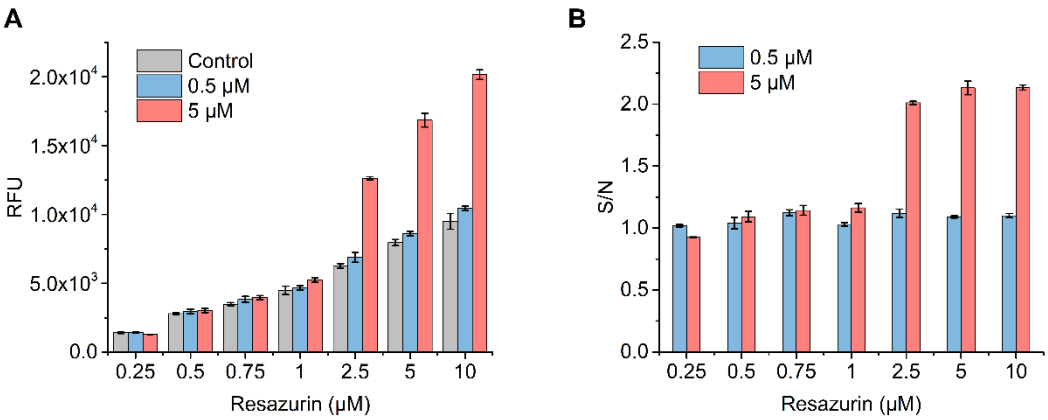

Figure S6 Resazurin levels were optimized to improve fluorescent readouts (A) and signal-noise ratios (B) in lower concentration ranges of TMAO. Values are the average ± SD (*n*=3).

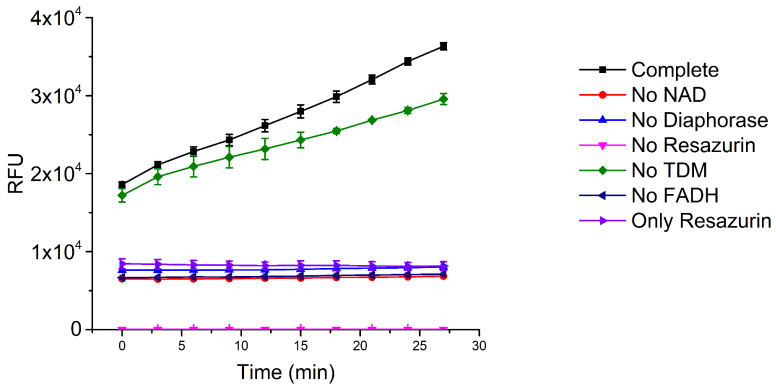

Figure S7 The effect of each component in the assay mixture on the production of background signals were determined in the absence of TMAO. As shown, the coexistence of FADH, NAD, resazurin and diaphorase contributes the most background signals. Time-dependent monitoring of fluorescence measures the assay mixtures for many times, and this mode may aggravate the generation of background signals. Thus, single end-point measurement is highly recommended.

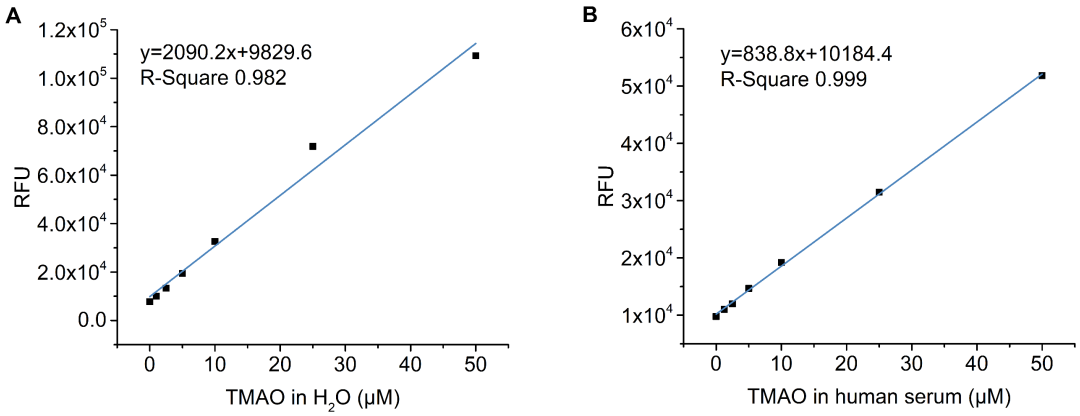

Figure S8 The calibration curve of fluorescent intensity for quantifying TMAO in water (A) or human serum (B) were determined.