Supporting Information

Human NAD(P)H:quinone oxidoreductase type I (hNQO1) activation of quinone propionic acid trigger groups

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Enzyme assay

Enzyme assays were performed on the quinone propionic acid derivatives by monitoring the decrease in NADH absorbance at 340 nm using the conditions below.

Reagents in the well assay

- 0.007 % BSA solution in 0.1 M PB/0.1 M KCl pH 7.10
- Diluted quinone values vary depending on the quinone
- 100 μM NADH
- Enzyme amount – depends on the quinone

Procedure

The 0.007% BSA solution (1 μM) was prepared as follows: approximate 7 mg of BSA were added to a 100-mL volumetric flask that was then filled with 0.1 M PB/0.1 M KCl pH 7.1 to the calibration mark. The appropriate amount of quinone was introduced into a 10-mL volumetric flask and dissolved with 100 μL of ethanol followed by addition of BSA solution to the calibration mark. From the quinone stock solution, several diluted quinone solutions were generated by pipetting a pre-calculated volume of quinone stock solution into different 10-mL volumetric flasks and filling them with BSA solution to the calibration mark to obtain the desired quinone concentrations. Then, a 400 μM solution of NADH was prepared by addition of approximately 2.93 mg of NADH into a 10-mL volumetric flask and filled with BSA solution to the calibration mark. Enzyme solutions

were made by adding BSA solution to the frozen vial of enzyme and then transferring it into a bigger container that previously had BSA solution to reach the desired enzyme concentration. All solutions were kept at room temperature and in the dark prior to their use in the plate reader. A multipipette was used to deliver each solution into the corresponding wells. The quartz plate was placed inside the plate reader instrument and allowed to sit for 5 min before starting the experiment.

Instrument Parameters

- Wavelength = 340 nm
- Shaking time = 5 seconds
- Data collection = every 0.4 or 0.8 seconds (20 flashes) – each well 100 seconds
- Extinction coefficient = 4390 M^{-1} cm^{-1}
- Sample and Blank replicates = 4
- Control replicates = 3

Dispensing amounts

- 100 μL of the corresponding quinone solution
- 50 μL of enzyme solution
- 50 μL of NADH solution

} sample wells

- 100 μL of the corresponding quinone solution
- 50 μL of BSA solution
- 50 μL of NADH solution

} control wells

- 200 μL of BSA solution ⟶ Blank wells

Enzyme Time

The enzyme time assay was performed on several quinone derivatives to find the appropriate amount of enzyme needed to have an accurate measurement of each quinone point. Below are the specifications for the assay where the decrease in absorbance of NADH was measured at 340 nm.

Reagents in the well assay

- 0.007 % BSA solution in 0.1 M PB /0.1 M KCl pH 7.10
- 30 μM of the corresponding quinone
- 100 μM NADH
- Different enzyme amounts

Procedure

The 0.007% BSA solution (1 μM) was prepared as follows: approximately 7 mg of BSA were added to a 100-mL volumetric flask that was then filled with 0.1 M PB/0.1 M KCl pH 7.10 to the calibration mark. The appropriate amount of quinone was introduced into a 10-mL volumetric flask, and it was dissolved with 100 μL of ethanol, followed by addition of BSA solution to the calibration mark. From the quinone stock solution, one diluted quinone solution was generated by pipetting a pre-calculated volume of quinone stock solution into a 10-mL volumetric flask and filling it with BSA solution to the

calibration mark. Then, a 400 μM solution of NADH was prepared by addition of approximate 2.93 mg of NADH into a 10-mL volumetric flask that was then filled with BSA solution to the calibration mark. Enzyme solution was made by adding BSA solution to the frozen vial and then transferring it into a bigger container that previously had BSA solution to reach the desired enzyme concentration. All solutions were kept at room temperature and in the dark prior to their use in the plate reader. A multipipette was used to deliver each solution into the corresponding wells. The quartz plate was placed inside the plate reader instrument and allowed to sit for 5 min before starting the experiment.

Parameters

- Wavelength = 340 nm
- Shaking time = 5 seconds
- Data collection = every 0.4 seconds (20 flashes) – each well 100 seconds
- Extinction coefficient = 4390 M^{-1} cm^{-1}
- Sample and Blank replicates = 5
- Control replicates = 3

Dispensing amounts

- 100 μL of 60 µM quinone solution
- 20,25,30,35,40, or 50 μL of enzyme solution } sample wells
- 30,25,20,15,10, or 0 μL of BSA solution
- 50 μL of NADH solution
- 100 μL of 60 μM quinone solution
- 50 μL of BSA solution } control wells
- 50 μL of NADH solution
- 200 μL of BSA solution → Blank wells

Receptor	Score (kJ mol^{-1})	Match	Lipo	Ambig	Clash	Rot
1	-25.2	-19.7347	-12.0594	-6.8892	3.8815	4.2000
2	-24.7	-19.0412	-11.8116	-7.0926	3.6185	4.2000
3	-24.2	-18.5762	-12.2120	-6.9455	3.9675	4.2000
4	-23.6	-17.8762	-11.8138	-7.4545	3.9220	4.2000
5	-24.2	-19.7347	-11.2013	-6.7019	3.8636	4.2000
6	-23.7	-19.0412	-10.9535	-6.9053	3.6006	4.2000

Table S1. Associated energies for Q_{Br}-COOH when docked in hNQO1 active site. Above is the lowest score energy for each receptor and the components of the Böhm scoring function.

Determination of the Extinction Coefficient of NADH

	Trial 1	Trial 2	Trial 3		
NADH (μM)	Abs. (a.u.)	Abs. (a.u.)	Abs. (a.u.)	Average (a.u)	St. Dev.
10	0.0304	0.0192	0.0112	0.0203	0.0096
30	0.1232	0.11105	0.1062	0.1135	0.0088
50	0.1866	0.2258	0.2128	0.2084	0.0200
80	0.3336	0.3412	0.3264	0.3337	0.0074
100	0.3873	0.4078	0.4058	0.4003	0.0113
120	0.4828	0.5258	0.5028	0.5038	0.0215
150	0.597	0.6348	0.598	0.6099	0.0215
200	0.84255	0.8534	0.8306	0.8422	0.0114
250	1.0858	1.10255	1.0726	1.0870	0.0150
300	1.3153	1.3058	1.276	1.2990	0.0205

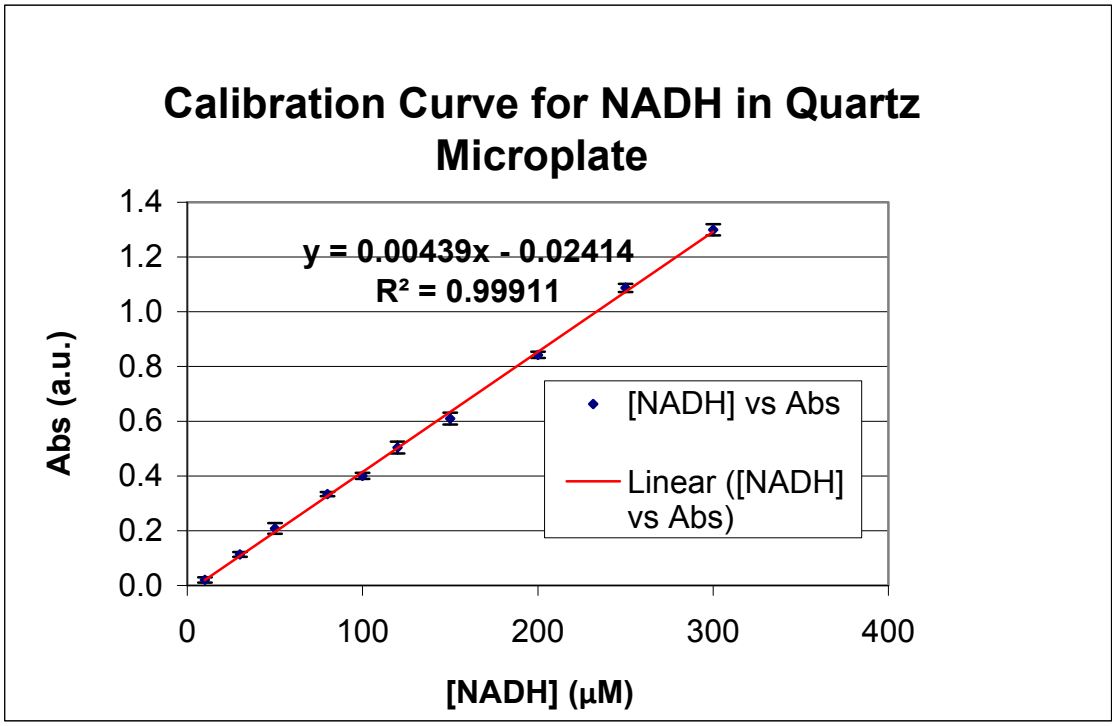


Figure S1. Determination of molar extinction coefficient for NADH in 0.1 M PB/0.1 M KCl pH 7.10 buffer. Error bars represent one standard deviation (n = 3).

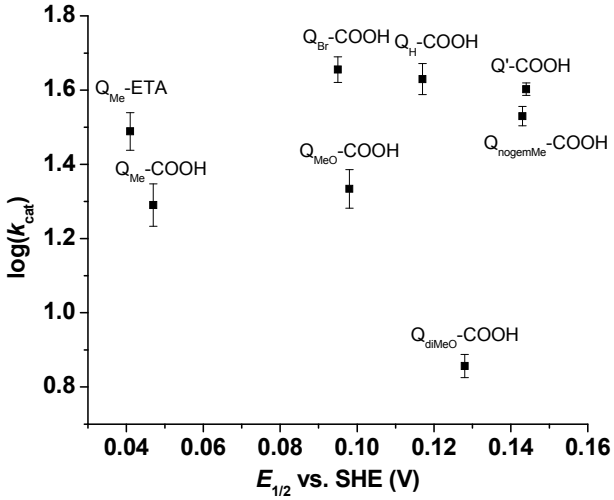


Figure S2. Relationship between log(k_{cat}) and $E_{1/2}$ of the QPAs. Error bars represent one standard deviation (n = 3).

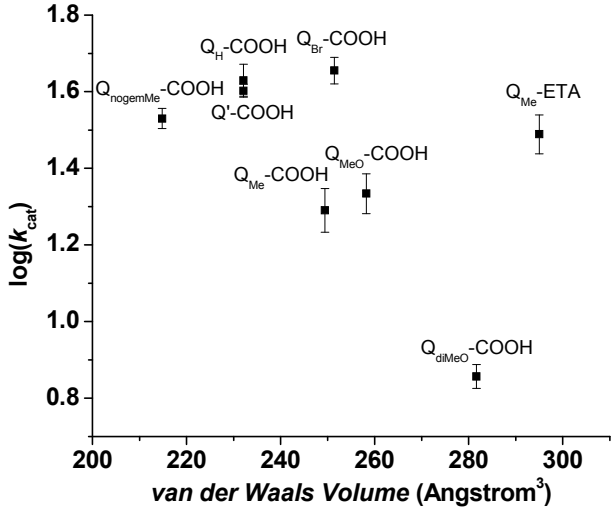


Figure S3. Relationship between log(k_{cat}) and van der Waals Volume of the QPAs. Error bars represent one standard deviation (n = 3).

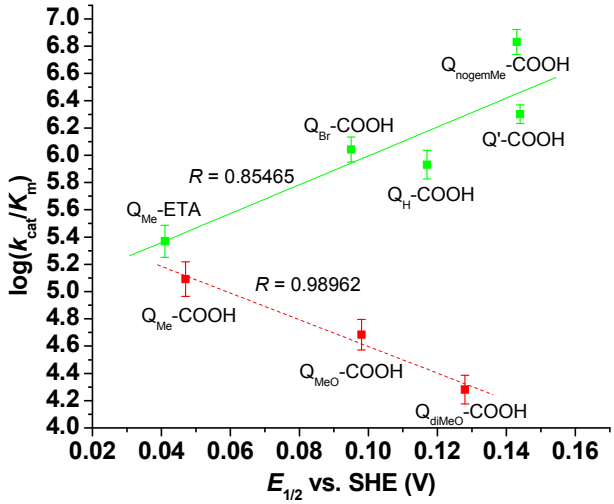


Figure S4. Relationship between log(k_{cat}/K_m) and $E_{1/2}$ of the QPAs. Error bars represent one standard deviation (n = 3).

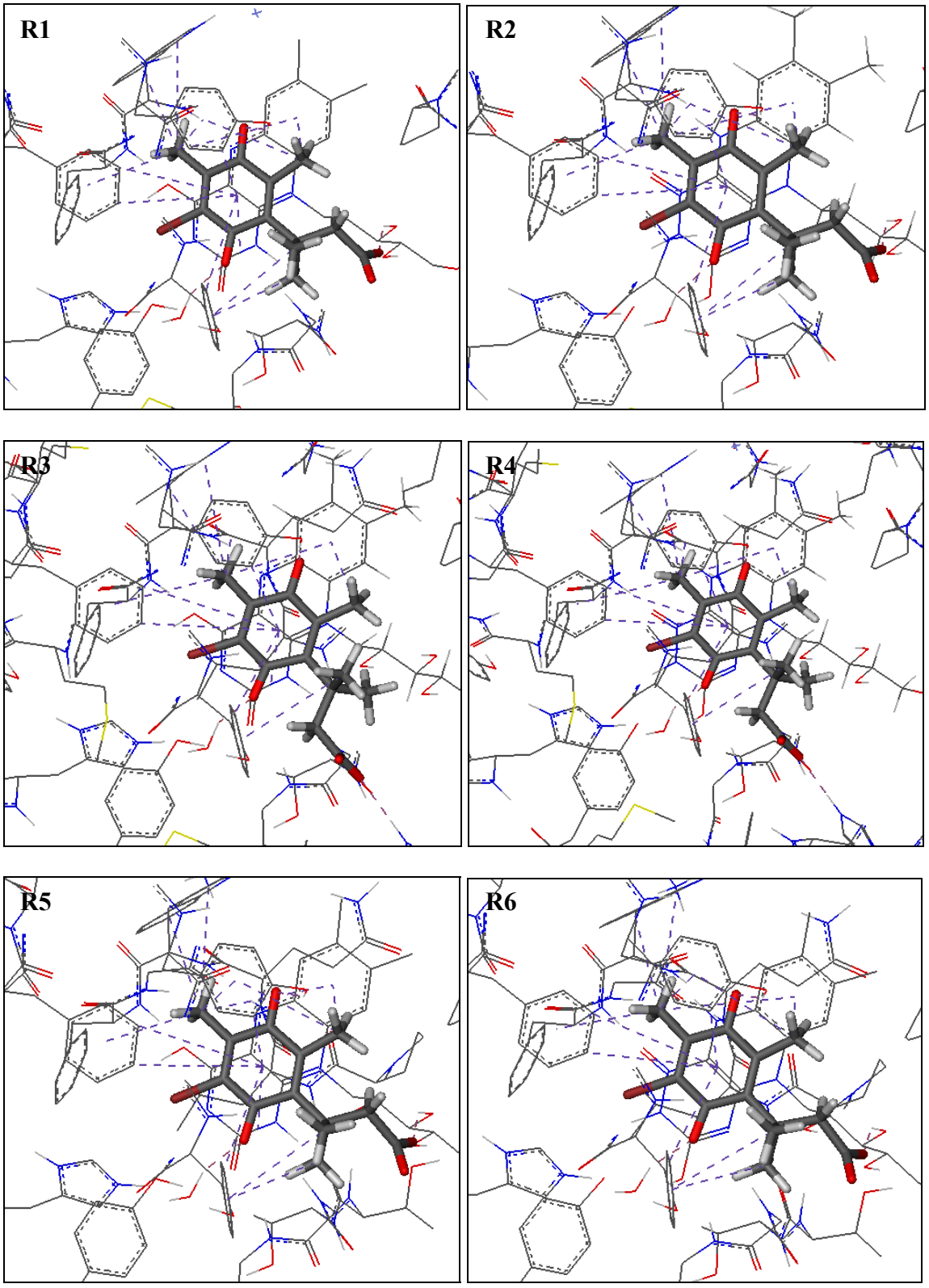
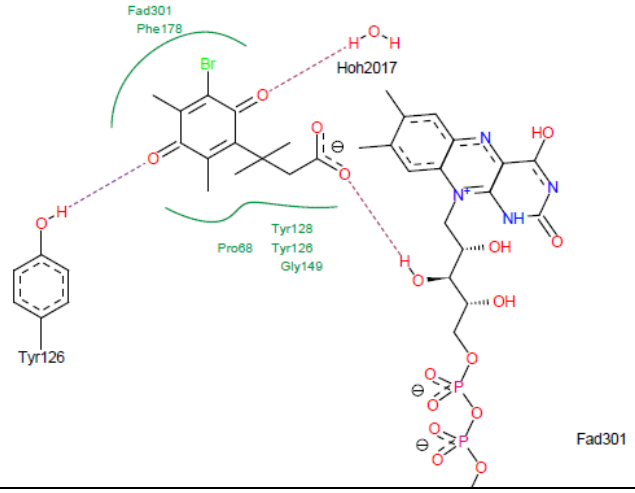
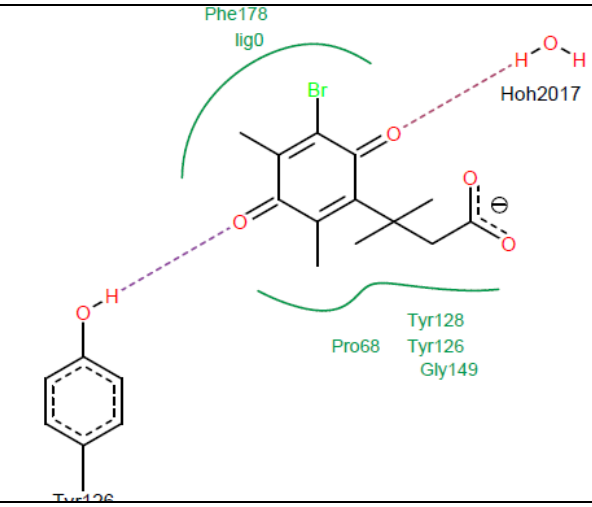


Figure S5. Lowest score frames of Q_{Br}-COOH in all the receptors.
Representation of Q_{Br}-COOH (stick display; color by atom type, carbon atoms colored grey); amino acids and FAD (lines display; color by atom type, carbon atoms colored grey). Dashed purple lines corresponded to hydrophobic interactions.

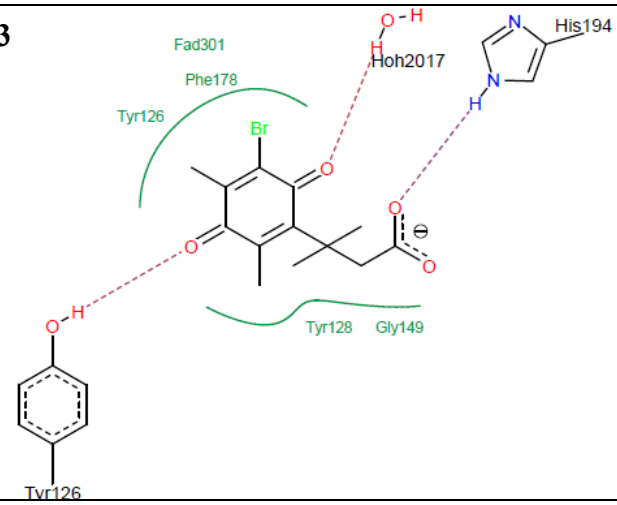
Receptor 1



Receptor 2



Receptor 3



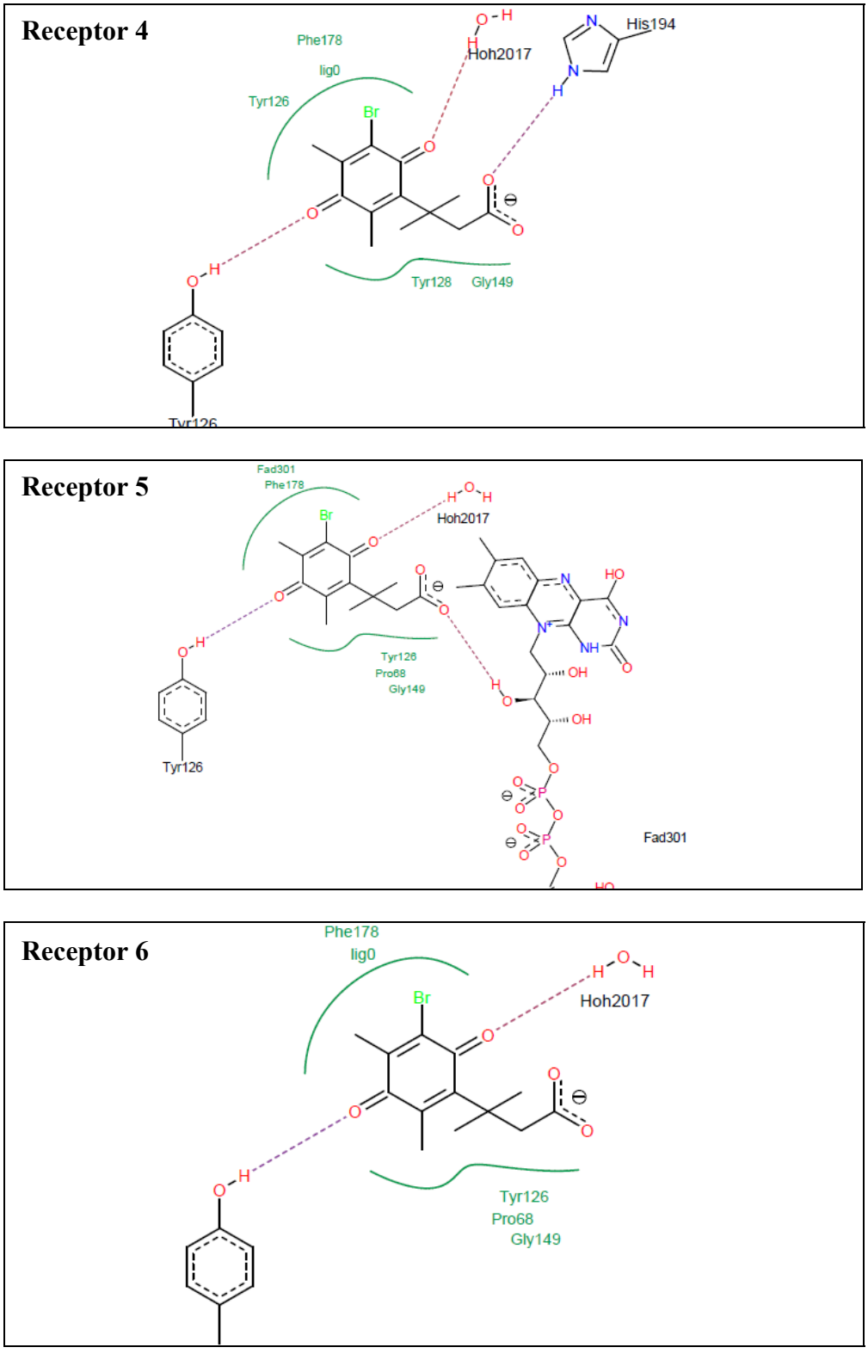


Figure S6. Poseview frames of Q_{Br}-COOH in all the receptors.
Representation of a 2D view of the docked pose of Q_{Br}-COOH (line display; color by atom type, carbon atoms colored black); amino acids and FAD labeled in green or structure line display; color by atom type, carbon atoms colored black. Hydrophobic interactions are displayed as green contact curves with only the names of the interacting residues attached to these lines. Dashed lines correspond to hydrogen bond interactions.

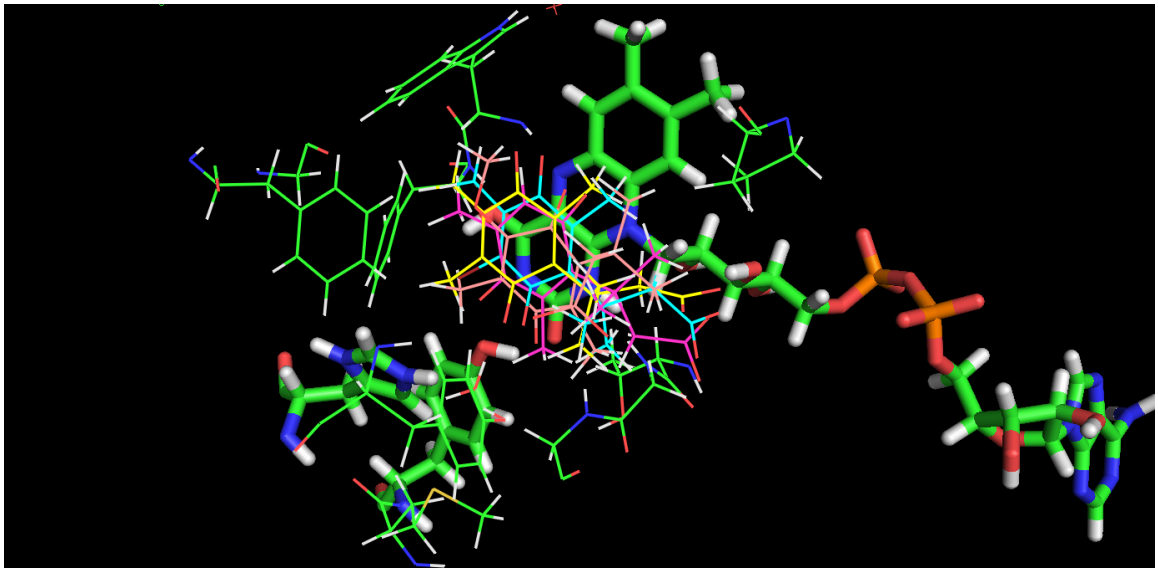


Figure S7. Superimposed images of trimethyl-locked quinones in the active site of hNQO1.
Representation of Q_{Br}-COOH (sky blue), Q_{H}-COOH (magenta), Q_{Me}-COOH (yellow), Q_{MeO}-COOH (light pink); amino acids (lines display; color by atom type, carbon atoms colored green); Tyr 155, His 161 and FAD (stick display; color by atom type, carbon atoms colored green).

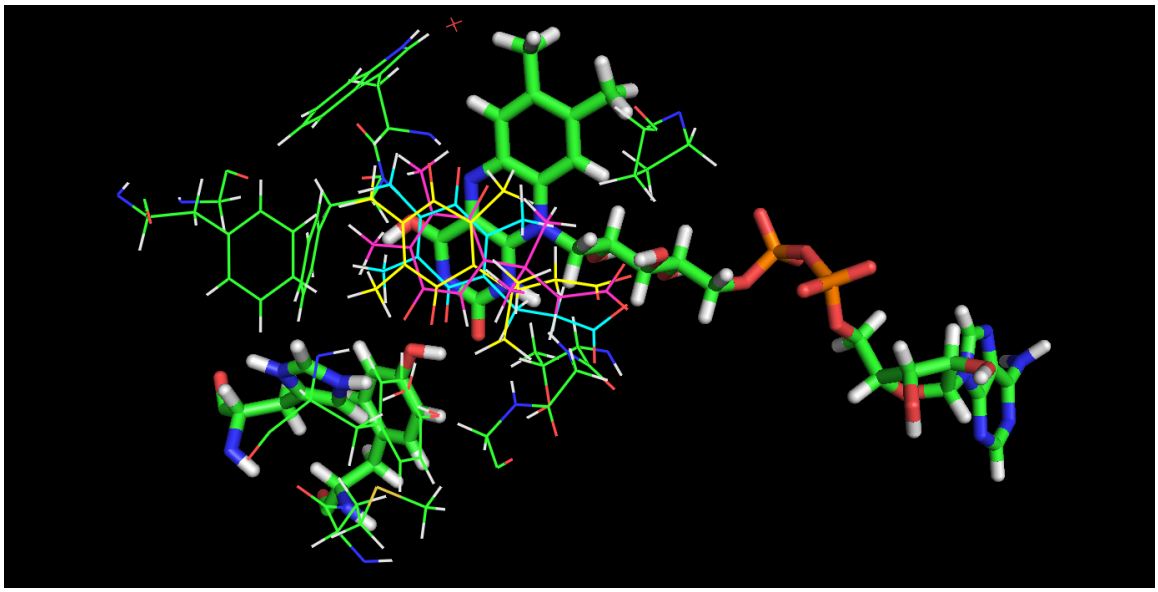


Figure S8. Superimposed images of quinones, with and without the trimethyl-lock motif, in the active site of hNQO1.
Representation of $Q_{nogemMe}$-COOH (sky blue), Q'-COOH (magenta), Q_{Me}-COOH (yellow); amino acids (lines display; color by atom type, carbon atoms colored green); Tyr 155, His 161 and FAD (stick display; color by atom type, carbon atoms colored green).

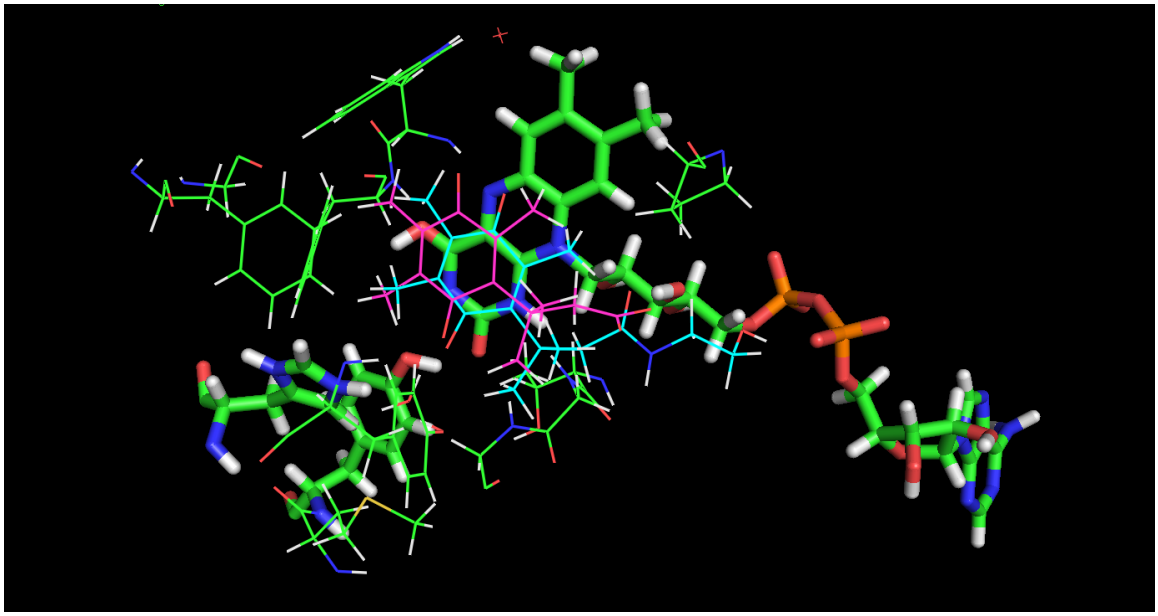


Figure S9. Superimposed images of charged quinone versus neutral quinone in the active site of hNQO1.
Representation of Q_{Me}-ETA (sky blue), Q_{Me}-COOH (magenta); amino acids (lines display; color by atom type, carbon atoms colored green); Tyr 155, His 161 and FAD (stick display; color by atom type, carbon atoms colored green).

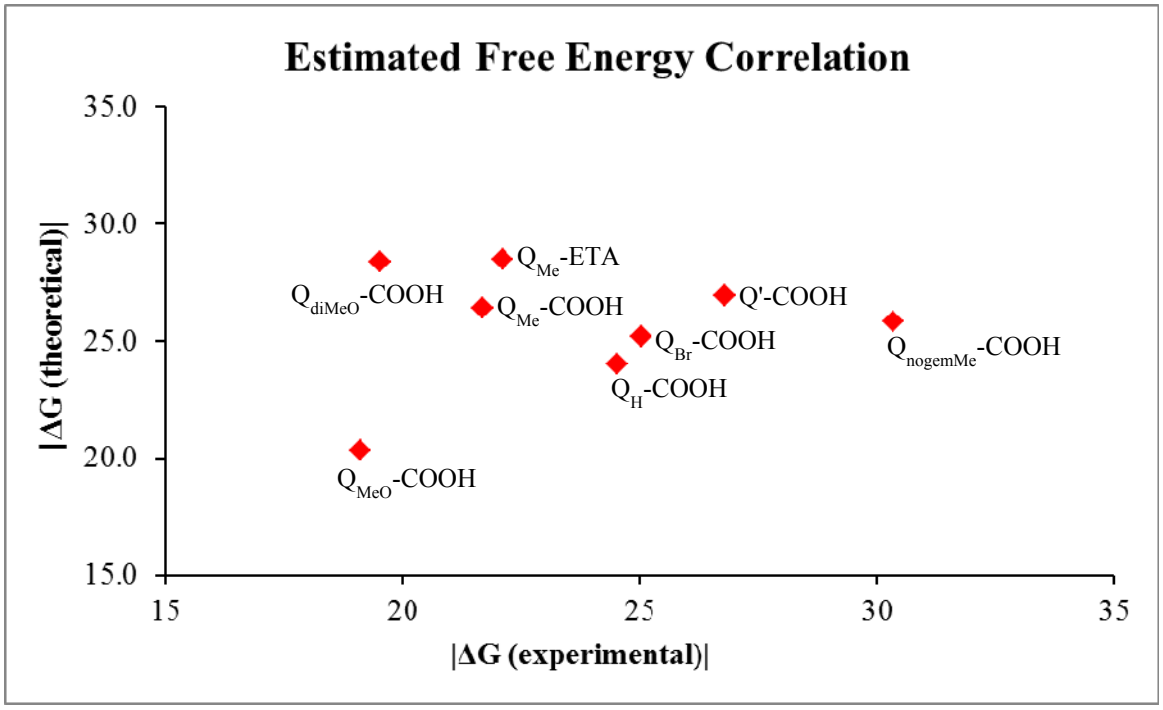


Figure S10. Relationship between estimated theoretical and experimental free energies of binding.

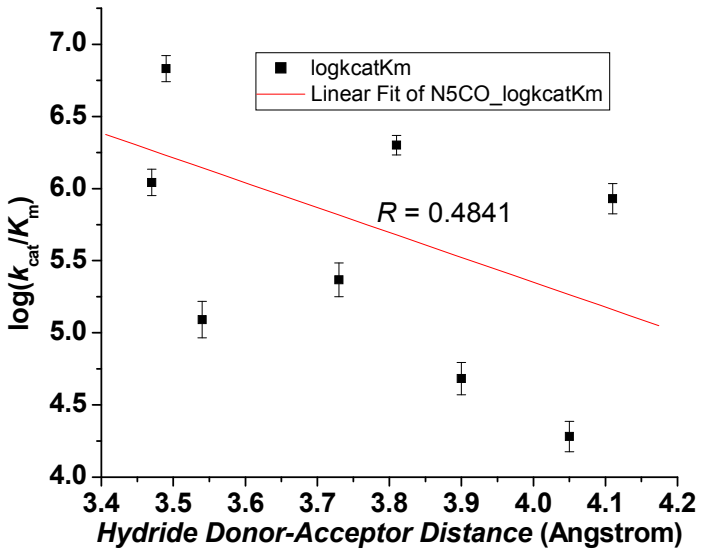


Figure S11. Relationship between quinone propionic acid specificity and FAD N_5-quinone acceptor site (C_B) distance. $R = 0.4841$ for the best linear-least-squares fit (red line). Error bars represent one standard deviation ($n = 3$).

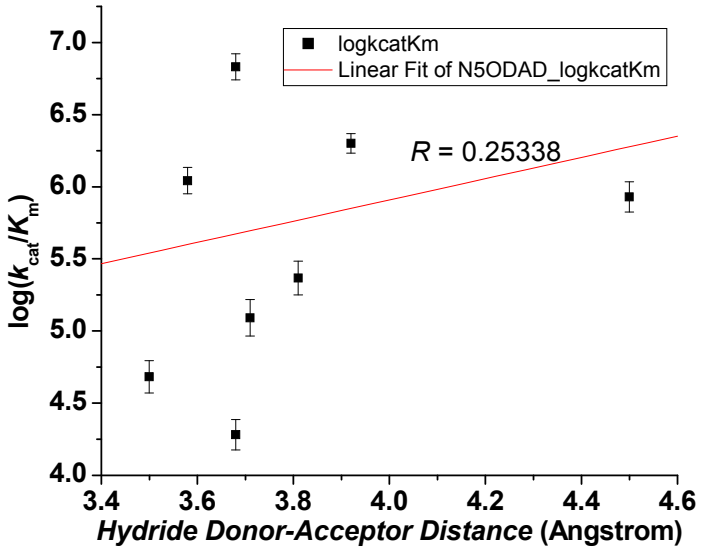


Figure S12. Relationship between quinone propionic acid specificity and FAD N_5-quinone acceptor site (O_C) distance. $R = 0.25338$ for the best linear-least-squares fit (red line). Error bars represent one standard deviation ($n = 3$).

Preparation of N-(2-hydroxyethyl)-3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanamide (Q_{Me}-ETA). The quinone ethanolamine derivative was prepared by adding Q_{Me}-NHS [1] (150 mg, 0.4328 mmol) into a 25-mL round bottom flask and letting the solid dry under high vacuum for 15 min. Then argon was purged through the flask to have it under inert atmosphere; the flow was stopped for two minutes and 4.5 mL of dichloromethane was added. The flow of argon was reestablished, and the reaction mixture was cooled to 0 °C. Then triethylamine (310 µL, 2.2241 mmol) was added dropwise, followed by ethanolamine (40 µL, 0.6647 mmol), using glass syringes. The argon flow was stopped, and then the round bottom flask was sealed from the atmosphere. The mixture was stirred for 4 hours. Progress of the reaction was followed by thin-layer chromatography (3:1:2 dichloromethane/methanol/hexanes) until no Q_{Me}-NHS remained. After the reaction was complete, the contents of the round bottom were diluted with 50 mL of dichloromethane, and the resulting mixture was then washed with 5 % of sodium bicarbonate (2 X 100 mL). The organic layer was dried over sodium sulfate, and after filtration, the solvent was evaporated to yield 87.8 mg of a yellow solid (75 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 6H), 1.95-1.97 (d, 6H), 2.14 (s, 3H), 2.85 (s, 2H), 3.32-3.33 (q, 2H), 3.65 (t, 2H), 5.82 (s, 1H).

^{13}C NMR (400 MHz, $CDCl_3$): δ 12.13, 12.68, 14.15, 28.98, 38.34, 42.20, 49.14, 62.48, 137.95, 138.09, 143.45, 153.12, 173.06, 187.54, 191.35.

HRMS (ESI, positive mode) *m/z* $[M+H]^+$, calcd = 294.1706 (calcd for $C_{16}H_{24}NO_4$), obsd = 294.1709; 0.9 ppm error.

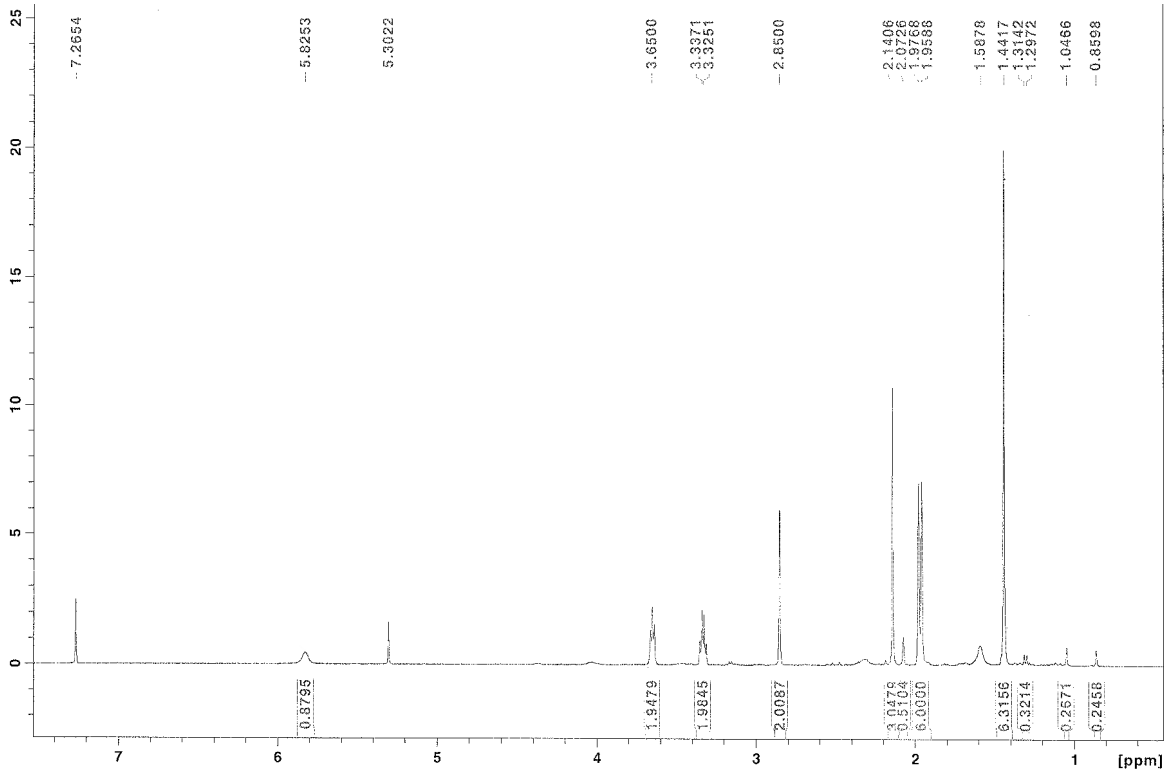


Figure S13. ^{1}H NMR for Q_{Me}-ETA ($CDCl_3$, 400 MHz)

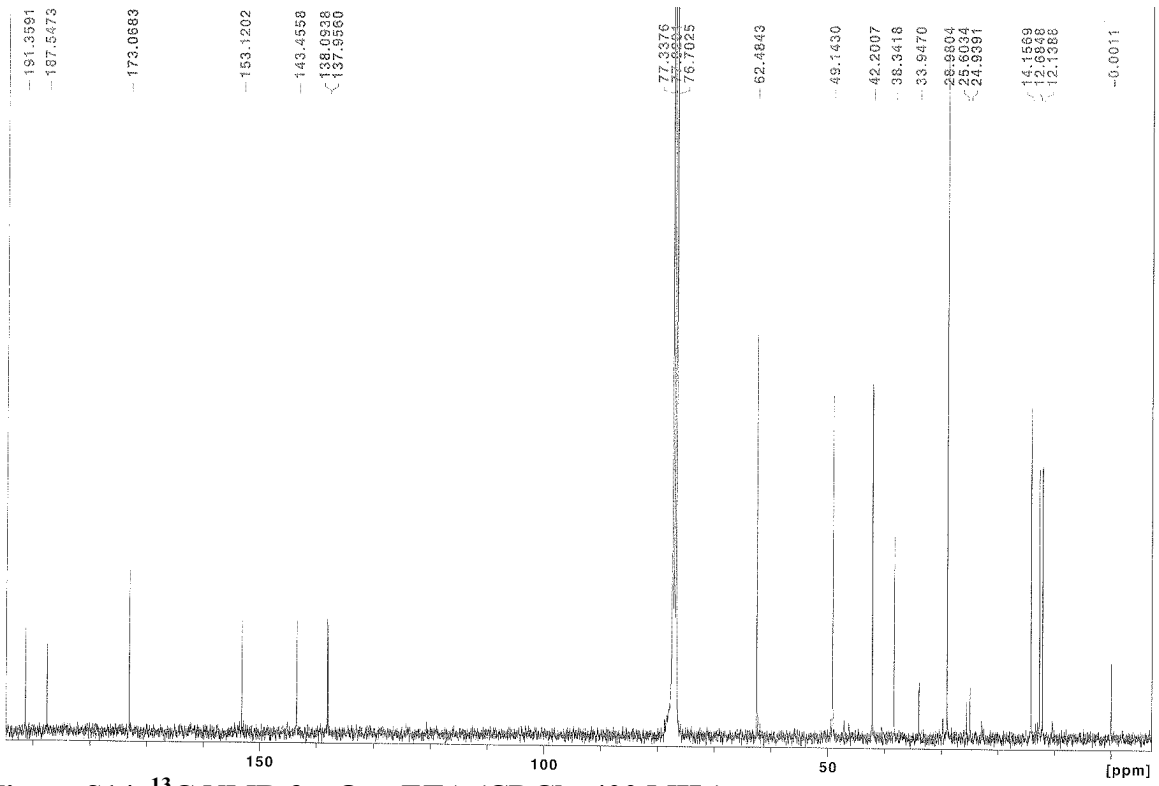


Figure S14. ^{13}C NMR for Q_{Me}-ETA ($CDCl_3$, 400 MHz)

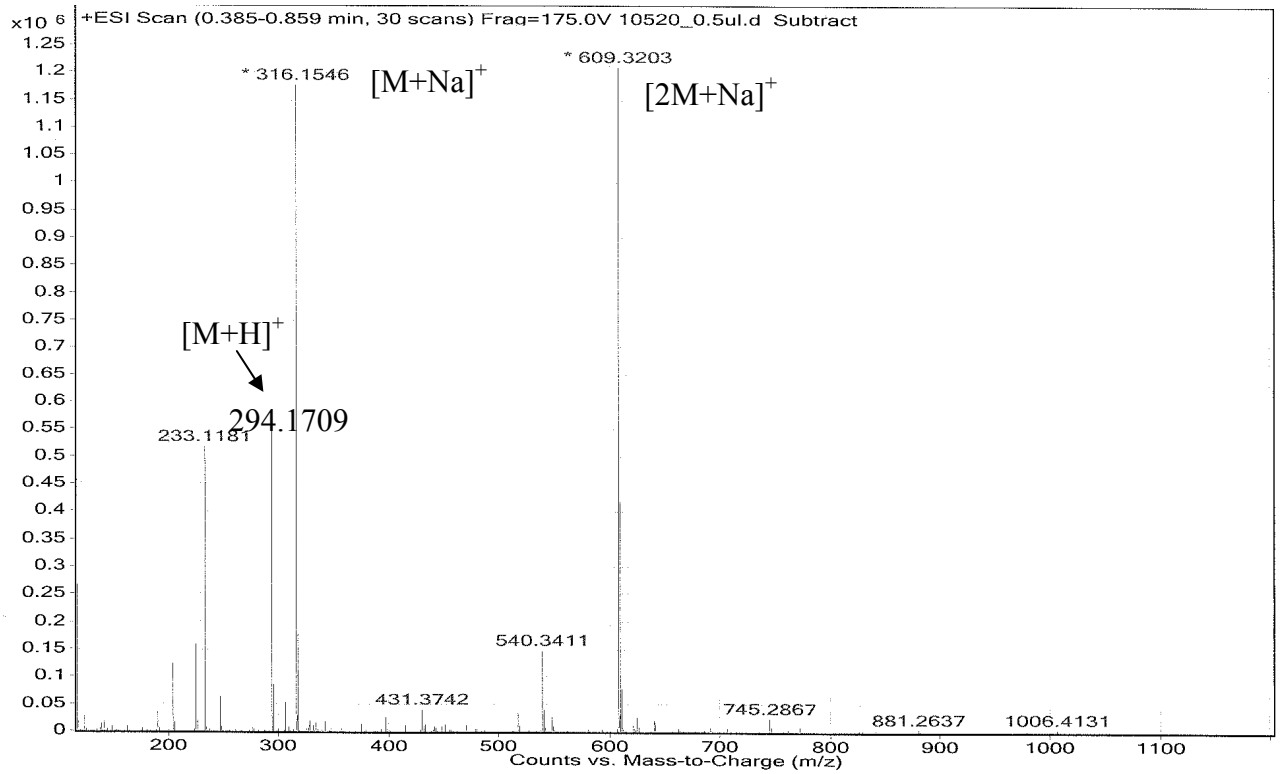


Figure S15. High resolution mass spectrum (positive ion, electrospray ionization) for Q_{Me}-ETA

References

1. Carrier, N. H. (2011) Redox-active liposome delivery agents with highly controllable stimuli-responsive behavior, Ph.D. Dissertation, Department of Chemistry, Louisiana State University, Baton Rouge, LA.