Electrochemical and Structural Coupling of the Naphthoquinone Amino Acid

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SUPPLEMENTARY INFORMATION

General Methods. All reactions for the synthesis of ^{15}N-labled naphthoquinone amino acid, Naq, were as before[1] with the use of ^{15}N-(diphenylmethylene)-glycine tert-butyl ester, which was prepared as described.[2] ^{15}N-glycine was purchased from Cambridge Isotope Laboratories, Inc. and Fmoc-^{15}N-Alanine was purchased from Sigma-Aldrich. HPLC grade solvents were purchased from Fisher Scientific. Peptide synthesis was carried out either a Pioneer Peptide Synthesis System or CEM Liberty Microwave Synthesiser using standard Fmoc chemistry (protected natural amino acids from Novabiochem) with HATU/HOAt (Genscript Corporation) as the coupling reagents (4-5.0 mol equivalents of activator/Fmoc-amino acid) on PAL-PEG-PS resin (Applied Biosciences). After cleavage and deprotection of the natural amino acids (Reagent R -- 90:5:3:2 TFA/Thioanisole/Ethane Dithiol/Anisole) under argon, the resin was filtered and washed with 10 mL TFA (3x). The TFA was removed *in vacuo*, and the crude peptide precipitated with cold methyl tert-butyl ether. HPLC purification of all peptides, before and after activation, was performed on a C18 preparative column using a gradient of water and acetonitrile, both with 0.1% TFA, for elution. The identities of all peptides, sequences in Supplementary Table 1, were confirmed with MALDI-TOF-MS, using either α-cyano-4-hydroxycinnamic acid or sinapic acid (Sigma-Aldrich) as the matrix. MALDI-TOF-MS determinations were performed on a PerSeptive Biosystem Voyager-DE RP. Kjedahl analysis was performed by Galbraith Laboratories, Inc. UV/*vis* spectra were acquired on an Agilent 8453 UV-Visible Spectrophotmeter or a Varian Cary-50 Spectrophotometer. A generic buffer, hereafter referred to as Common Buffer, composed of 20 mM cacodylate, 100 mM KCl, pH 6.9 was used for peptide stock solution, CD spectroscopy, CD titrations, NMR spectroscopy, and Kjedahl analysis.

Supplementary Table 1. Peptide Sequences

Peptide	**Sequence**•
P2Naq(ox)	Ac-DKDGDGYISAAEAQAQ-NH_2
P2Naq(red)	″″
RCNaq	Ac-AEAQAE-NH_2
P2NaqExt	Ac-DKDGDGYISAAEAQAQAAAAEAAAAEAAAAE-NH_2
PolyNaqABC	EAAAAEAAAAEAQAAEAQAAEAQAAEAAAAEAAAAE-NH_2
PolyNaqAC	EAAAAEAAAAEAQAAEA A AAEAQAAEAAAAEAAAAE-NH_2
PolyNaqB	EAAAAEAAAAEA A AAEAQAAEA A AAEAAAAEAAAAE-NH_2

•In these sequences Q is the one letter character for Naq

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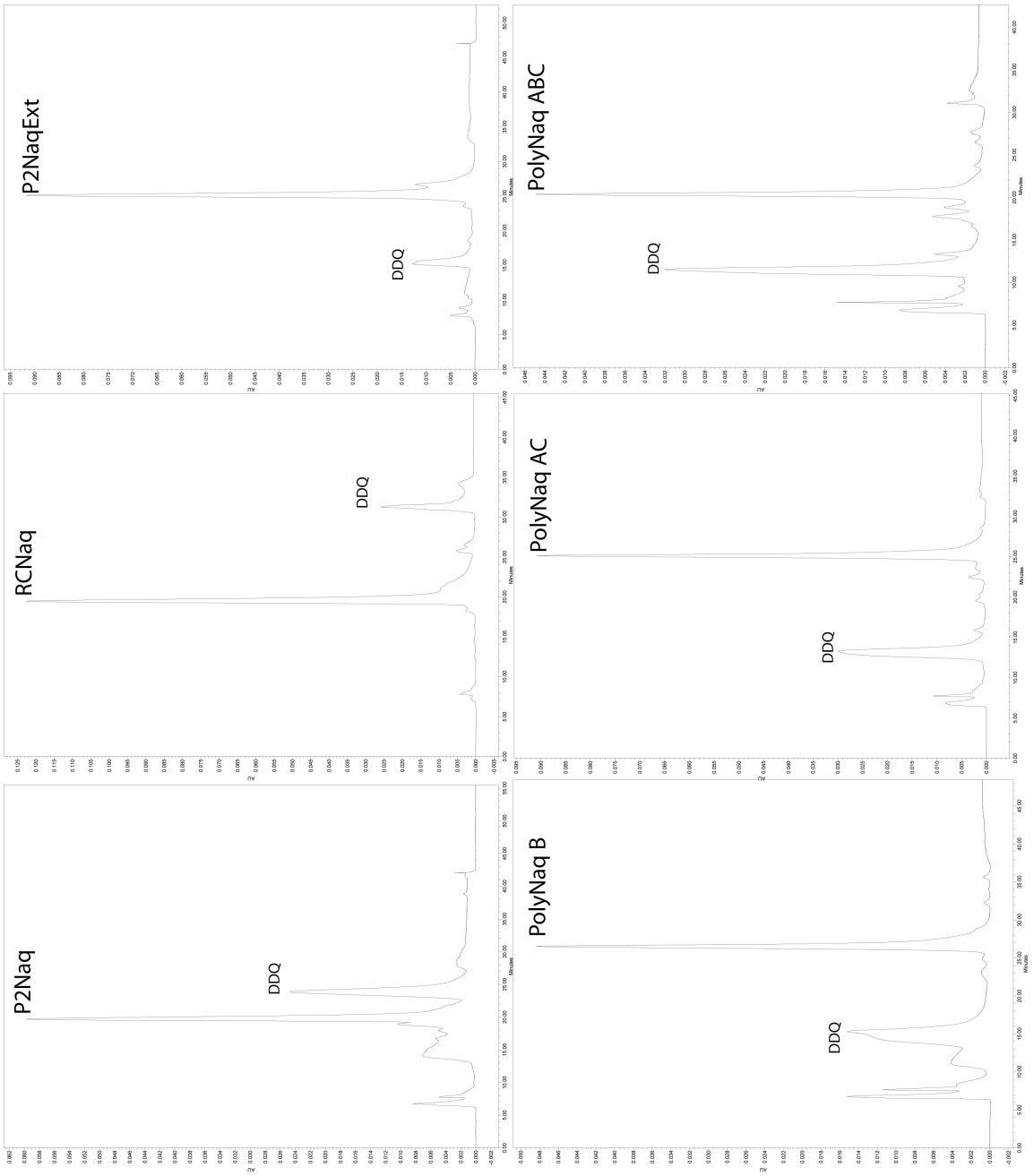
Naq(OMe/OMe) Deprotection. P2Naq(OMe/OMe) formed hard, transparent millimeter scale aggregates in water post-purification that only dissolved in 8 M GdnHCl or TFA. In a modification of the previously described procedure,[1] initial samples of P2Naq(OMe/OMe) were deprotected by use of cerium ammonium nitrate (CAN) in slight molar excess (>2 fold) in acidic (pH < 2 by TFA) aqueous GdnHCl solutions (peptide concentration was determined using a Naq(OMe/OMe) + Tyr ε_{301} 5065) at room temperature due to reduced solubility of GdnHCl on ice. However, because slightly higher selectivity (significantly curtailed oxidation of the tyrosine residue in P2Naq and P2NaqExt) and yields were observed using dichlorodicyanoquinone (DDQ), all peptides were ultimately deprotected using the following procedure: Naq(OMe/OMe) containing peptide was dissolved in 200 µL TFA and placed on ice. The concentration of the peptide in this solution was determined quickly by absorbance and exactly 1 equivalent of DDQ in MeCN (stock ~ 9 mg mL^{-1}) was added while shaking. The solution becomes dark upon DDQ addition and slowly lightens over the course of at least 30 minutes on ice. Dilution of the reaction with ddH_2O is followed immediately by HPLC purification, chromatographs in Supplementary Figure 1, and peptide identity confirmed by MALDI-TOF-MS, Supplementary Table 2. After deprotection, purification, and lyopholization, P2Naq, RCNaq, and P2NaqExt were dissolved in the Common Buffer, concentrations were determined by measured absorbance, and the solutions aliquoted and flash frozen.

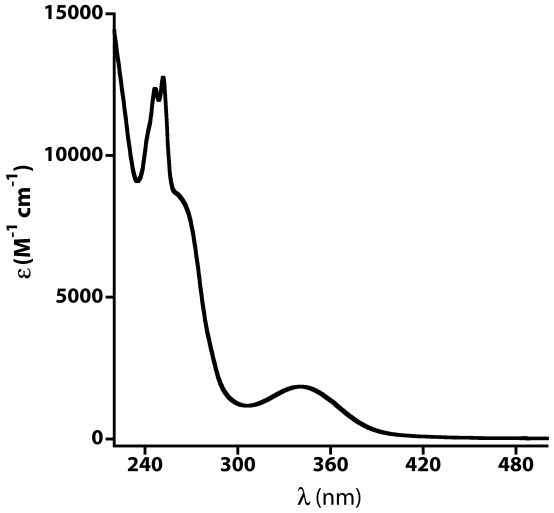


Supplementary Figure 1. HPLC chromatographs of the DDQ-based activation reactions of the indicated peptides.

Supplementary Table 2. MALDI-TOF Results of DDQ-Activated Peptides

Peptide	Observed Mass (Amu)	Expected Mass (Amu)
P2Naq	1780.2	1778.7 ($M+H^+$)
RCNaq (^{15}N-labeled at AQA, from Common Buffer)	801.7	799.25 ($M+K^+$)
P2NaqExt (^{15}N-labeled at AQA)	3025.2	3023.3 ($M+H^+$)
PolyNaqABC	3514.3	3508.5 ($M+H^+$)
PolyNaqAC	3356.9	3352.5 ($M+H^+$)
PolyNaqB	3199.4	3196.4 ($M+H^+$)

Determination of Molar Absorptivity of P2Naq. Kjedahl total nitrogen of solutions of oxidized P2Naq (deprotected as described above, dissolved in the Common Buffer) with known absorbance spectra were determined and the molar absorptivity spectrum calculated, Supplementary Figure 2. The molar absorptivity at 252 nm is 12700 M^{-1} cm^{-1}, and at 340 nm is 1840 M^{-1} cm^{-1}. The spectrum contains a small contribution from the tyrosine at 252 nm (316 M^{-1} cm^{-1}).[3] For reduced protein samples, a spectrum of the protein solution prior to reduction was used for protein concentration determination.



Supplementary Figure 2. Molar absorptivity of oxidized P2Naq in the Common Buffer as determined by Kjedahl total nitrogen analysis.

Reduction of Naq. The borane (particularly water stable ammonium borane) and borohydride reagents commonly used for the reduction of quinones interfere with lanthanide binding due to the formation of insoluble lanthanide borate salts. In addition, common redox mediator dyes used for maintenance of redox poises are generally not spectroscopically silent and tend to interfere with spectroscopic characterization. Enzymatic systems capable of low potential reduction, such as xanthine oxidase, proved to interfere either spectroscopically or by substrate binding to lanthanide. A relatively spectroscopically silent, non-interfering reducing system was realized by using colloidal platinum nanoparticles with a polyvinylpyrrolidinone (PVP) protective polymer in conjunction with hydrogen gas. The PVP polymer serves

to prevent platinum nanoparticle aggregation. A small amount of benzyl viologen indicator was added to ensure the system remained reduced, which is readily observed by a blue-purple color of the benzyl viologen radical (H_2 $E_{m, pH\ 6.9}$ -414 mV; benzyl viologen E_m -311 mV; expected Naq $E_{m, pH\ 6.9}$ ≈27 mV). Platinum nanoparticles with a 40 kD PVP protective polymer (40:1 monomer/platinum ratio) were prepared using standard conditions,[4] and dialyzed extensively against pure water to remove any free platinum that may have interfered with lanthanide binding. Platinum concentration in the colloidal platinum stock was calculated from the concentration of platinum in the preparation reaction. While there has been a previous report where a natural hydrogenase was used for the same purpose, [5] as far as we are aware, this is the first report of using easily prepared platinum nanoparticles for the reduction of a protein and maintenance of a low potential redox poise.

CD Spectroscopy. Circular dichroism spectra were acquired on an Aviv 410 CD Spectrophotometer. Automated titrations were performed with a Hamilton Company automated diluter controlled by a macro written in house for the Aviv CD Spectrophotometer. CD spectra of all peptides were taken at 25 °C in the Common Buffer (20 mM cacodylate, 100 mM KCl, pH 6.9). There was no observed concentration dependence of the CD spectra of the peptides at various concentrations, determined spectroscopically prior to data collection, Supplementary Figure 3. All CD samples containing $LaCl_3$ were at saturating lanthanide concentrations (either 2 mM, oxidized samples, or 10 mM, reduced samples). Reported spectra are averages of the mean residue molar ellipticity across all concentrations tested. Reduced protein samples were prepared in pear shaped round bottoms containing peptide solutions and benzyl viologen (100 μM). Hydrogen gas was flowed over the solution for at least 30 minutes with stirring before addition of colloidal platinum (10 μM platinum). Solutions would turn blue-purple upon benzyl viologen reduction, at which point the solution would be transferred via cannula into a CD cuvette with a positive pressure of hydrogen gas maintained during data collection.