SUPPORTING INFORMATION

Multiplexed Electrochemistry of DNA-bound Metalloproteins

Catrina G. Pheeney, Anna R. Arnold, Michael A. Grodick, and Jacqueline K. Barton*

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125, USA

Email jkbarton@caltech.edu

Primer Sequences

Mutation	Primer
Y205H	5'- TGT GAA **CAC** AAA GAG AAA GTT GAC ATC TGA GGA TCC GGC TGC TAA C -3' (Forward)
	5'- GTT AGC AGC CGG ATC CTC AGA TGT CAA CTT TCT CTT T**GT** **G**TT CAC A -3' (Reverse)
E200K	5'- GCC CCG CTG TGG CTC TTG TAT TAT T**AA** **A**GA TCT TTG TGA ATA C -3' (Forward)
	5'- GTA TTC ACA AAG ATC **TTT** AAT AAT ACA AGA GCC ACA GCG GGG C -3' (Reverse)
K208E	5'- GTG AAT ACA AAG AG**G** **AA**G TTG ACA TCT GAG GAT CCG GCT GCT AAC -3' (Forward)
	5'- GTT AGC AGC CGG ATC CTC AGA TGT CAA C**TT** **C**CT CTT TGT ATT CAC -3' (Reverse)

Wild Type and Mutant EndoIII Purification Protocol

A stock of BL21star-(DE3)pLysS containing a pET11-ubiquitin-His_6-*nth* construct was used to inoculate a 100 mL culture of LB containing 100 µg/mL of ampicillin and 35 µg/mL of chloramphenicol. This culture was grown overnight at 37 °C with shaking. Then, 1 mL of the overnight culture was used to inoculate each of four 1L cultures of LB containing the same amount of ampicillin and chloramphenicol as the overnight culture. The 1L cultures were shaken at 37 °C until the OD_{600} reached ~0.6-0.8. Enough isopropyl β-D-1-thiogalactopyranoside (IPTG) was then added to bring the total concentration of IPTG to 300 µM. The cultures were subsequently shaken at 150 rpm for ~3.5 hours at 30° C. The cells were collected by centrifugation at 5,500 rpm for 15 minutes, flash-frozen, and stored at -80 ºC. All subsequent steps were carried out at 4 °C or on ice. On the day of the purification the pellet was resuspended in 250 mL of Buffer A (20 mM sodium phosphate, pH 7.4, 250 mM NaCl, 5 mM DTT, 5% glycerol, DNase (Roche), RNase (Roche), and EDTA-free protease inhibitor cocktail tablets (Roche)). The resuspended cells were lysed via microfluidization. The cell lysate was clarified by centrifugation at 17,000 *g* for 30 minutes. Enough NaCl was added to the resulting supernatant to bring the NaCl concentration to 500 mM. The supernatant was then loaded onto a Histrap HP column (GE Healthcare) that had been equilibrated with buffer B (20 mM sodium phosphate, pH 7.4, 500 mM NaCl, 1 mM DTT). After washing with buffer B, EndoIII was eluted using a gradient from 0-100% buffer C (20 mM sodium phosphate, pH 7.4, 500 mM NaCl, 500 mM imidazole, 1 mM DTT) over 20 column volumes. A yellow band that eluted at ~100 mM imidazole was collected. This imidazole-containing buffer was then immediately exchanged into buffer D (20 mM sodium phosphate, pH 7.4, 500 mM NaCl, 0.5 mM EDTA, and 20 % glycerol) using a HiPrep 26/10 desalting column (GE Healthcare). The protein solution, now in buffer D, was concentrated down to ~5 mL using 10,000 MWCO Amicon Ultra-15 centrifugation filter units (Millipore) and was loaded onto a HiLoad Superdex 16/600 75 pg size exclusion column (GE Healthcare) that had been equilibrated with the protein storage buffer (20 mM sodium phosphate, pH 7.4, 100 mM NaCl, 0.5 mM EDTA, 20 % glycerol). A clean peak that eluted after approximately 50-55 mL of buffer had passed through the column was collected and concentrated to achieve a final concentration of

~ 100 μM as quantified using an ε_{410} of 17,000 $M^{-1}cm^{-1}$ (30**)**. The protein was then aliquoted, flash-frozen in liquid nitrogen and stored at -80 °C. The approximate yield was 6 mg/L. The purity of the protein was determined to be >95% as analyzed by SDS-PAGE (data not shown).

$$Y = \frac{(y_n + m_n T) + (y_d + m_d T)\,exp\left(\frac{\Delta H_m}{R}\left(\frac{1}{T_m} - \frac{1}{T}\right)\right)}{1 + exp\left(\frac{\Delta H_m}{R}\left(\frac{1}{T_m} - \frac{1}{T}\right)\right)}$$

Equation S1. Circular dichroism (CD) thermal denaturation data was fit using a non-linear least-squares regression to a simple two-state unfolding model (32), where Y is the fractional ellipticity, T is the temperature in Celsius, ΔH_m is the enthalpy at the unfolding transition, T_m is the melting temperature (°C), R is the ideal gas constant, while m_n and y_n describe the pre-transition (native protein) slope and y-intercept and m_d and y_d describe the equivalent values post-transition (denatured protein). Reported errors in T_m values are derived from this fitting.

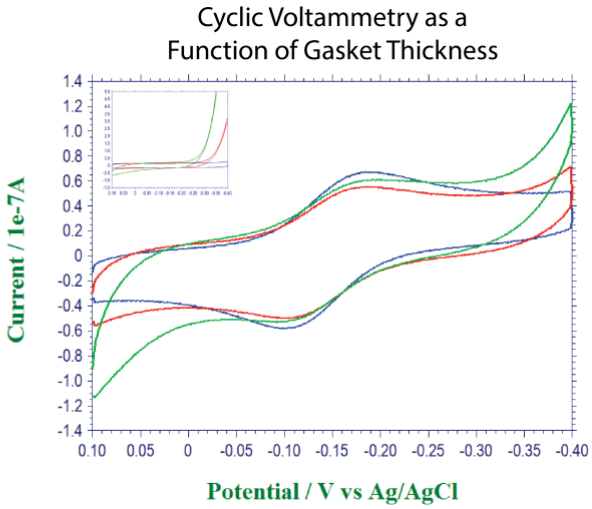


Figure S1. Cyclic voltammetry (scan rate = 100 mV/s) of EndoIII (30 μM) incubated on closely packed well-matched duplex DNA monolayers acquired in phosphate buffer (20 mM sodium phosphate, 100 mM NaCl, 0.5 mM EDTA, 20 % glycerol, pH 7.4) are presented. Three different thicknesses for the Buna N gasket, utilized during the multiplexed chip assembly, were tested: 0.064″ (green), 0.032″ (red), and 0.020″ (red). With decreasing gasket thickness the signal generated from EndoIII is shown to increase while the background contributions decrease. *Inset*: Background signals acquired prior to the addition of EndoIII in spermidine buffer (5 mM phosphate, 50 mM NaCl, 40 mM $MgCl_2$, 5 mM spermidine, pH 7.0).

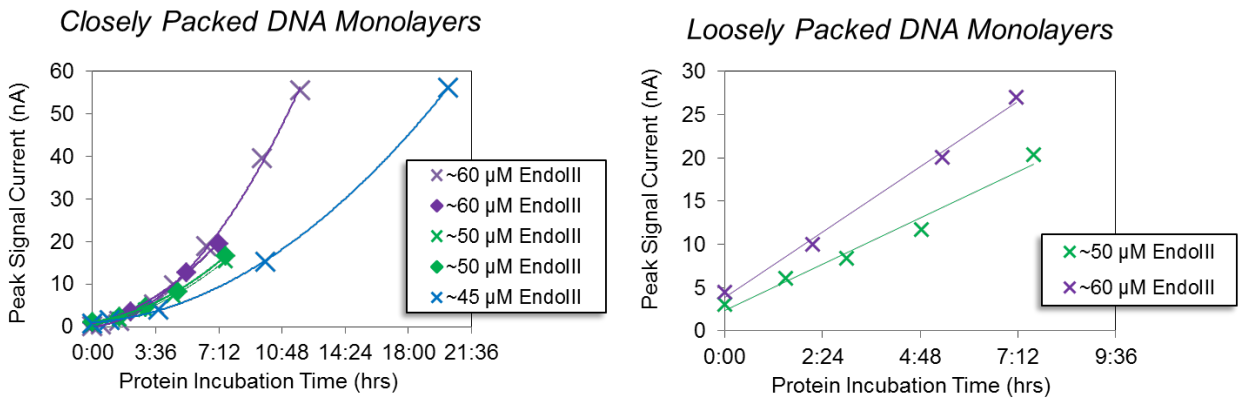
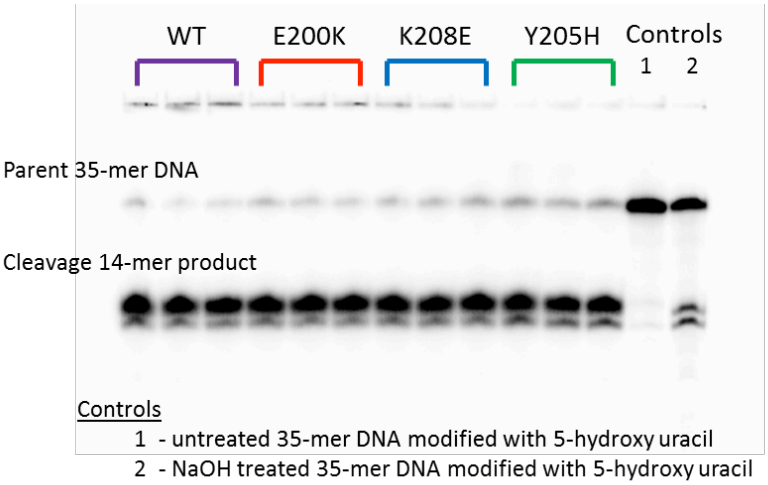


Figure S2. Signal accumulation of EndoIII as a function of time at various concentrations on both closely and loosely packed dsDNA monolayers formed in the presence and absence of 100 mM $MgCl_2$ during assembly, respectively. The peak signal current was quantified based on the reductive signal of EndoIII in the cyclic voltammaogram (scan rate = 100 mV/s) acquired in phosphate buffer (20 mM sodium phosphate, 100 mM NaCl, 0.5 mM EDTA, 20 % glycerol, pH 7.4).



Mutant	% Activity Compared to WT
E200K	98.0 ± 1.5
K208E	95.8 ± 1.8
Y205H	91.6 ± 2.1

Figure S3. Enzymatic assay for EndoIII glycosylase activity. Glycosylase activity was determined for each mutant (Y205H, K208E, and E200K) compared to wild type EndoIII based on previously established methods (22). For this assay, protein samples (1 μM) were incubated for 15 min at 37 ºC with 5′-^{32}P-radiolabeled 35-mer duplex DNA (100 nM) modified with 5-hydroxy uracil, a substrate for EndoIII, in 10 mM Tris HCl, 1 mM EDTA, 50 mM NaCl, pH 7.6. Reactions were then quenched by adding of 1 M NaOH to a final concentration of 100 mM NaOH, dried, and electrophoresed through 20% denaturing PAGE at 90 W for 1.5 hours. Glycosylase activity of EndoIII results in the appearance of the 14-mer cleavage product in the denaturing gel. The glycosylase activity was determined as the fraction of 14-mer product observed relative to the total quantity of DNA and the percent activity compared to WT (purple) was then calculated for Y205H (green), K208E (blue), and E200K (red). Two control lanes containing the 35-mer duplexed DNA, yet lacking the enzymatic protein, were either untreated or treated with the 1 M NaOH.

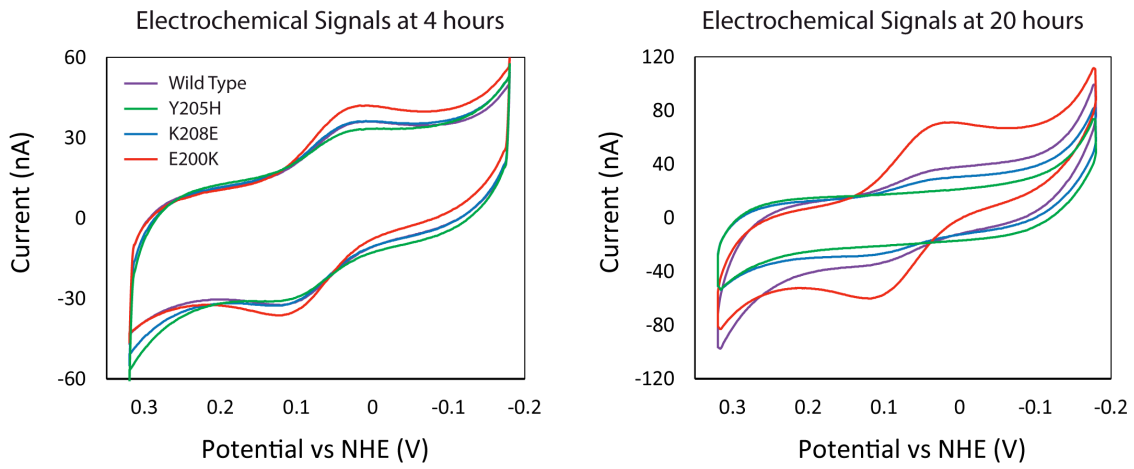


Figure S4. Electrochemical stability of EndoIII mutants. (*Left*) Protein concentrations were normalized for electrochemistry coupling to yield approximately equivalent signal sizes at early time points (~ 4 hours). Cyclic voltammetry (scan rate =100 mV/s) acquired in phosphate buffer (20 mM sodium phosphate, 100 mM NaCl, 0.5 mM EDTA, 20 % glycerol, pH 7.4) for wild type (purple), Y205H (green), K208E (blue), and E200K (red) EndoIII are presented. (*Right*) After extended incubation (~20 hours) on the multiplexed chip, the electrochemical signal from the electrostatic EndoIII mutants and wild type protein diminished based on their CVs. The degree of signal loss directly correlates with the stability and DNA CT proficiency of the proteins, with the remaining signal size decreasing in the following order: E200K (red), wild type (purple), K208E (blue), and finally Y205H (green) which had no discernible signal remaining.