Supporting Information for

Electronic Measurements of the Single-Molecule Catalysis

by cAMP-Dependent Protein Kinase A

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1. Protein Kinase A Mutagenesis

A mutation introduced into the gene encoding protein kinase A (PKA) placed a free thiol on the protein surface for bioconjugation to the SWCNT. This mutation, T32C, was synthesized using the DNA sequence for the core catalytic domain of wild-type PKA as a template for splice overlap extension PCR using the primers PKA-T32C-F (5'-GAAAAAATGGGAGCTTCTCAGAATAC-3') and PKA-T32C-R (5'-GTATTCTGAGAAGCTCCCATTTTTTC-3'). A second round of PCR was carried out using the primers Wild-Type PKA-F (5'-CATGCCATGGCAGCAACGCCGCCGCC-3') and Wild-Type PKA-R (5'-CCGCTCGAGCGGCACTCCTTGCCACA-3'). The subsequent DNA sequence encoding T32C PKA variant was then subcloned into the pET28 vector for DNA sequencing and protein overexpression.

2. Protein Kinase A Expression and Purification

The T32C PKA variant was overexpressed in *E. coli* using the following representative protocol adapted from previous reports.[1,2] After transformation of the pET28-T32C PKA plasmid into BL21 DE3 *E. coli* cells, agar plates supplemented with kanamycin (40 μg/mL) were incubated overnight at 37 °C. The following day, a single transformed colony was inoculated into LB media (5 mL) supplemented with kanamycin (40 μg/mL) and shaken at 37 °C overnight. The starter culture (5 mL) was used to inoculate LB media (1L) before incubation at 37 °C, and the culture was allowed to reach an OD_{600} of 0.8 before induction with 500 μM IPTG. Next, the culture was incubated overnight at 23 °C with shaking. The culture was then centrifuged at 6 krpm (4032 g) for 25 min at 4 °C, and the supernatant was decanted. The cell pellet was resuspended in 15 mL low salt lysis buffer (20 mM Tris, 10 mM NaCl, pH 7.2) before cell lysis by sonication. The resultant cell culture was centrifuged at 16 krpm (17203 g) for 45 min at 4 °C. Subsequently, the supernatant was decanted and filtered through a 0.45 μm pore size filter. The cell lysate was applied to a cation exchange column on a BioLogic DuoFlow FPLC. The purified T32C PKA fractions were concentrated by ultrafiltration with a 10 kDa cutoff microconcentrator and applied to a Superdex size-exclusion column. The T32C variant of PKA was eluted in PBS buffer (138 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2). Another protocol adapted from conversations with Dr. Michael Deal (in Professor Susan S. Taylor's laboratory at the University of California, San Diego) applied weak and then strong cation

exchange chromatography for purification.[1,2] As shown by the 15% SDS-PAGE (Figure S1), the resultant protein was >95% homogenous.

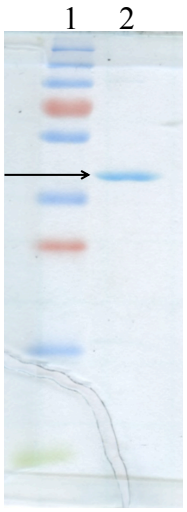

Figure S1: Expression and purification of T32C PKA variant. This 15% SDS-PAGE provides an illustrative example of the purity of the expressed PKA variant. The arrow indicates the expected MW for the migration of T32C PKA (41.8 kDa). Lane 1 is the PageRuler Plus Prestained Protein Ladder (Fermentas).

3. Protein Kinase A Activity Assay:

The PKA activity assay applied a previously reported protocol.[3] From the results, it can be concluded that wild-type PKA activity is demonstrated by a decrease in absorbance as compared to the absorbance of the reaction with no PKA present. Wild-type PKA activity is similar to T32C PKA activity, as shown in Figure S2.

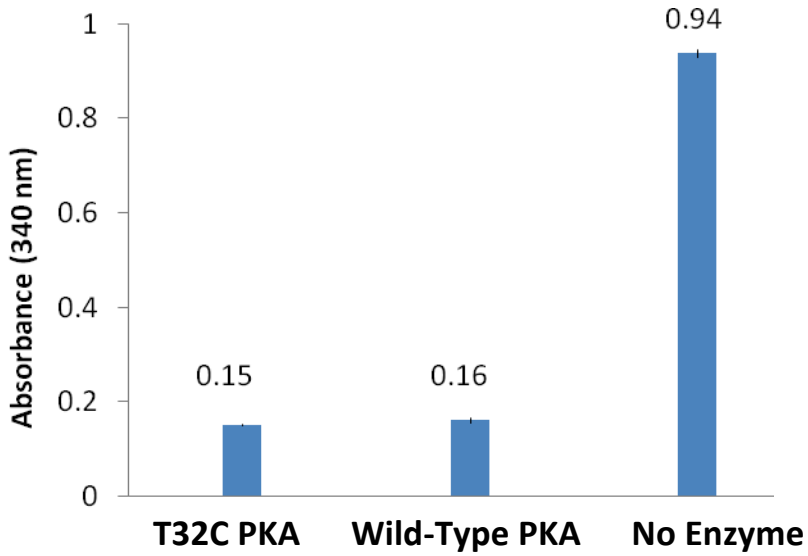

Figure S2: A PKA activity assay with the T32C and wild-type PKA variants. A negative control condition with no enzyme present under identical condition showed essentially no activity. Error bars represent standard deviation (n = 3).

4. SWNT Synthesis and Device Fabrication

SWNTs were grown by chemical vapor deposition (CVD) directly onto 4" Si wafer using standard techniques.[4] In our implementation, a 0.1% dilution of a saturated solution of FeMo catalyst nanoparticles in ethanol was spin-coated onto a wafer surface.[5,6] The deposited catalyst clusters were oxidized at 700 C (air, 600 s), then at 940 C (520 sccm H_2 in 3000 sccm, 300 s), and then exposed to carbon feedstock (1000 sccm CH_4, 520 sccm H_2, and 3000 sccm Ar, 180 s).

After CVD, SWNT-based electronic devices were built. Ti electrodes with source-drain separations of 2 to 3 μm were patterned on the randomly-grown SWNTs using optical lithography with an undercut bilayer resist (S1808 over LOR-A1, MicroChem). The device was electrically probed and characterized. To ensure that the device comprises only one SWNT, to determine the diameter of the SWNT, and to discern that the SWNT is free of particulates, the device was imaged by non-contact atomic force microscopy (AFM, NT-MDT).

After initial characterization, the device underwent a passivation step to insulate the majority of the surface including the source and drain electrodes from the protein conjugation and measurement solutions. The device was coated with an electron beam resist (A3 PMMA, MicroChem), and then patterned using electron beam lithography to expose an active SWNT window of 0.5 – 1.0 μm in length.

5. Protein Conjugation

A bi-functional linker molecule, pyrene maleimide, was used to functionalize the SWNT of the device. The pyrene functionality, through pi-pi stacking, adheres strongly to sidewall of SWNT while the maleimide group allows for the formation of a stable thioether bond with the free thiol of a cysteine sidechain in the protein.[7,8] The device was submerged in a saturated solution *N*-(1-pyrenyl)maleimide (Sigma-Aldrich) in ethanol for 45 minutes without agitation. Following this, the device was rinsed with 0.1% Tween-20 (Acros Organics) in ethanol to remove excess *N*-(1-pyrenyl)maleimide and then rinsed with de-ionized water.

PKA was then conjugated to the SWNT. A solution of PKA (52μM) in phosphate buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 2 mM $MgCl_2$, 2 mM ATP, 100 μM TCEP, pH 7.4) was prepared. The $MgCl_2$ and ATP are used to sterically block bonding to C199 and C343 located near the active site of the protein.[9] The device was soaked in the protein kinase solution for 60 min without agitation at room temperature. The device was then rinsed with phosphate buffer and soaked in another solution (100 mM NaCl, 20 mM Na_2HPO_4, pH 2.5) for 1 min with gentle shaking to remove non-specifically adsorbed protein from the surface of device. Following this, the device was rinsed and stored in a buffered aqueous solution (100 mM MOPs, 9 mM $MgCl_2$, 100 μM TCEP, pH 7.2)

To confirm protein conjugation before the electrical measurement, the device was imaged by in-liquid atomic force microscopy (NT-MDT) in the buffered aqueous solution.

6. Electrical Measurement Techniques

All measurements were performed with the active portion of the device submerged in buffered aqueous solution (pH 7.2). The potential of the electrolyte was controlled using Pt counter and reference electrodes, and held at -0.3 V vs. Pt using a Keithley 2400 sourcemeter. The source-drain bias was held at 100 mV. A Keithley 428 preamplifer operating at 10^8 V/A

gain and with a 40 μs rise time was used to measure the source-drain current of the device. Data was collected for at least 300 s for each measurement condition.

7. Control Experiments

Control measurements were conducted using a device from the same chip that had a successful protein attachment. The control and functionalized devices simultaneously underwent the protein attachment protocol, but the control device failed to yield an attachment. This device was then electrically measured in the buffered aqueous solution (pH 7.2), buffered aqueous solution (2 mM ATP), buffered aqueous solution (100 μM Kemptide), and buffered aqueous solution (2 mM ATP, 100 μM Kemptide). As shown in Figure S3, no processive fluctuations from the baseline were observed.

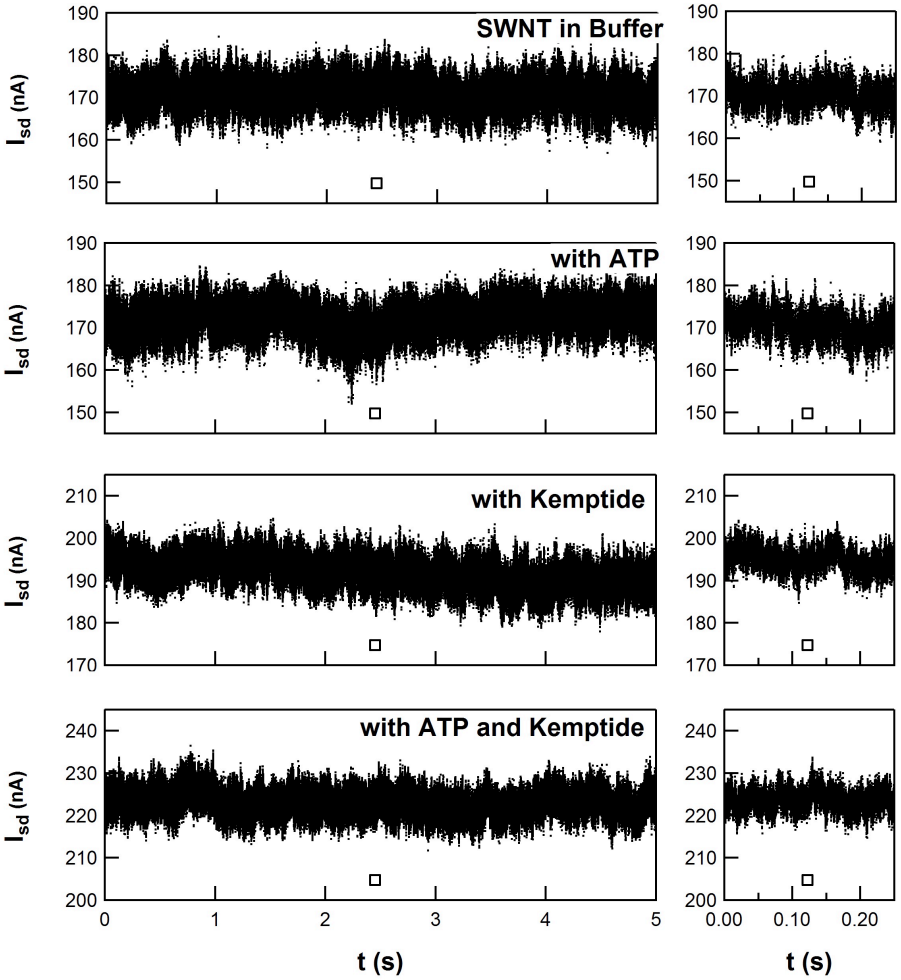

Figure S3: Control measurements of a pyrene-maleimide coated SWNT device, with no attached PKA. In the presence of ATP and/or Kemptide, *I(t)* through the device shows no fluctuations except for the characteristic pink noise found in all SWNT electronic devices.

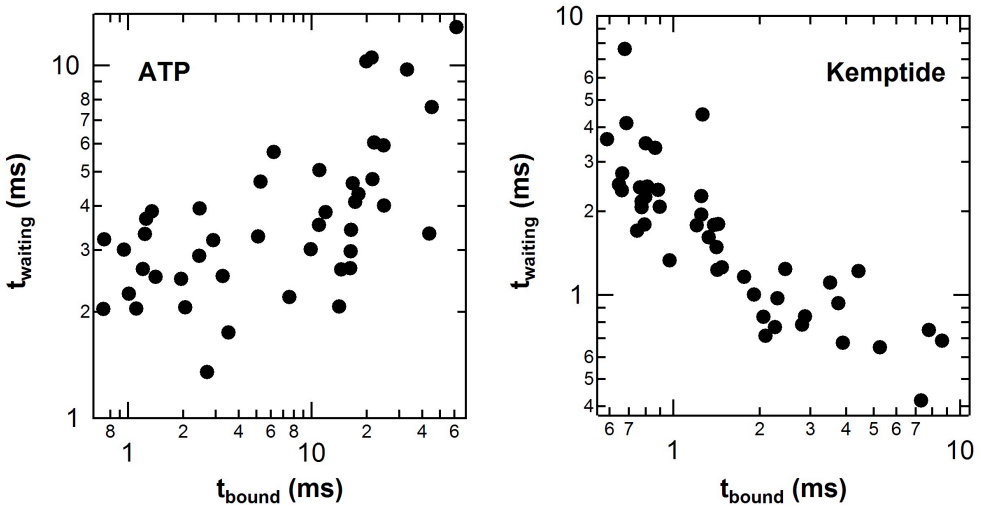

Figure S4: Scatter plot of $<t_{bound}>$ and $<t_{waiting}>$ using 1-second averages for ATP or Kemptide. For ATP, $<t_{bound}>$ and $<t_{waiting}>$ are weakly correlated with r = 0.71. This positive correlation suggests a shared mechanism such as such as a conformational change distant from the ATP binding site, which may modify the energy barrier between the bound and unbound states. For Kemptide, $<t_{bound}>$ and $<t_{waiting}>$ are anti-correlated with r = -0.84. An anti-correlated behavior suggests that as PKA slowly undergoes viscoelastic motions the binding affinity for Kemptide can either increase or decrease.

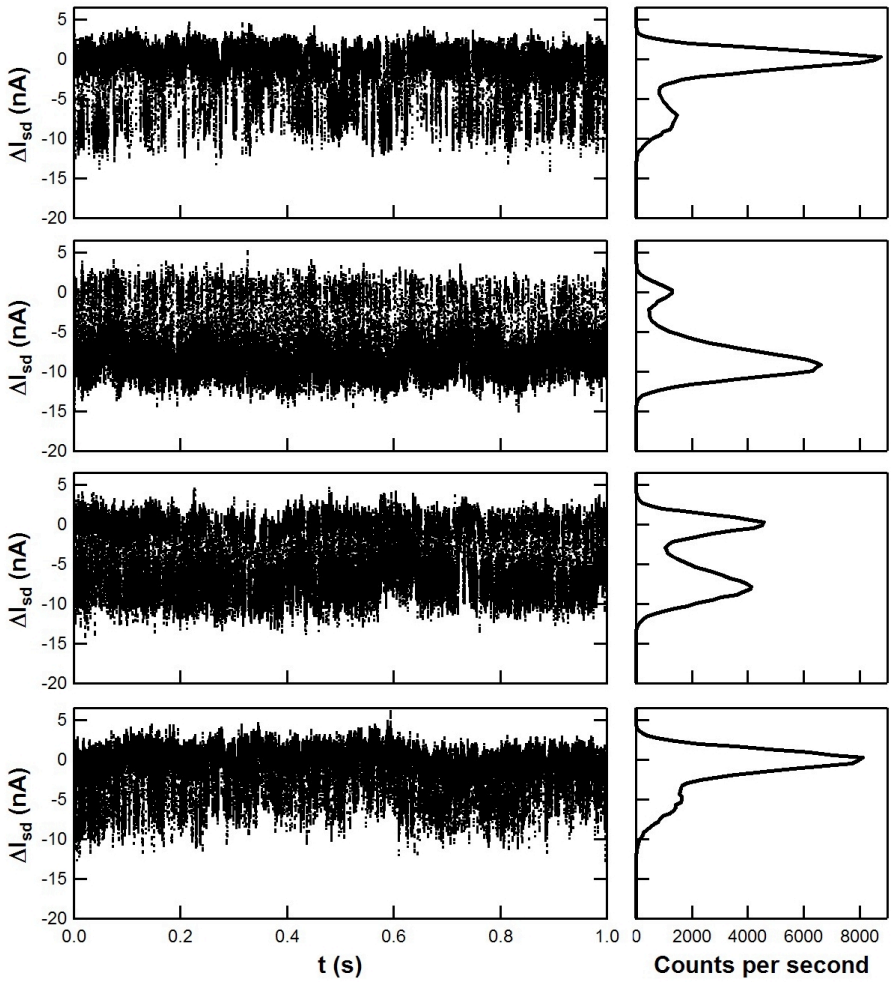

Figure S5: Conductance fluctuations of a single PKA device due to Kemptide binding and release. Over a ten minute measurement, $<t_{bound}>$ and $<t_{waiting}>$ varied dynamically such that $<t_{bound}>$ could be either larger or smaller than $<t_{waiting}>$. For one-second windows, the conductance histograms typify these dynamic, relative variations of $<t_{bound}>$ and $<t_{waiting}>$.

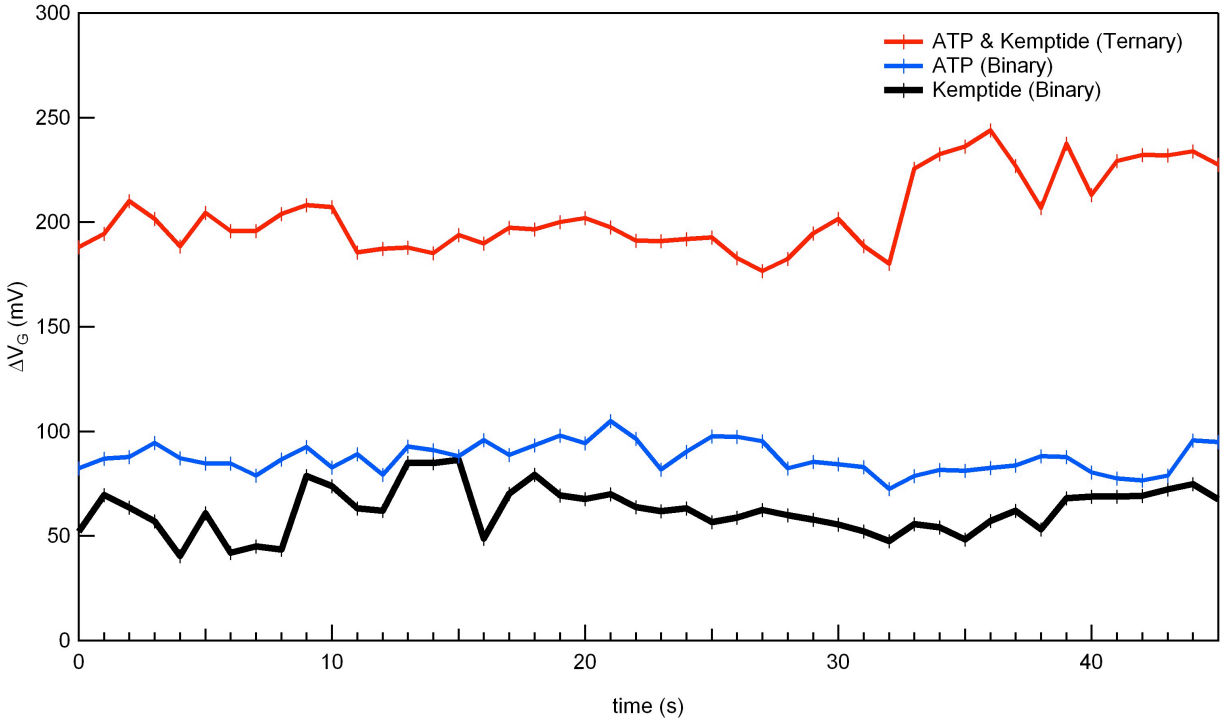

Figure S6: Time-dependence of the effective gating of a single PKA device. The transconductance of SWNT device provides a metric for measuring the device response to changes in the protein conformation. From the apoenzyme to binary complex formed by Kemptide (black), the ΔV_G is +65 ± 11 mV. From apoenzyme to binary complex formed by ATP (blue), the ΔV_G is +87 ± 7 mV From apoenzyme to the ternary complex, the ΔV_G is 221 ± 20 mV.

References

(1) Madhusudan; Akamine, P.; Xuong, N. H.; Taylor, S. S. *Nat. Struct. Biol.* **2002**, *9*, 273.

(2) Akamine, P.; Madhusudan; Wu, J.; Xuong, N. H.; Ten Eyck, L. F.; Taylor, S. S. *J. Mol. Biol.* **2003**, *327*, 159.

(3) Technikova-Dobrova, Z.; Sardanelli, A. M.; Papa, S. *FEBS Lett.* **1991**, *292*, 69.

(4) Goldsmith, B. R.; Coroneus, J. G.; Khalap, V. R.; Kane, A. A.; Weiss, G. A.; Collins, P. G. *Science* **2007**, *315*, 77.

(5) Müller, A.; Das, S. K.; Kögerler, P.; Bögge, H.; Schmidtmann, M.; Trautwein, A. X.; Schünemann, V.; Krickemeyer, E.; Preetz, W. *Angew. Chem. Int. Ed.* **2000**, *39*, 3413.

(6) An, L.; Owens, J. M.; McNeil, L. E.; Liu, J. *J. Amer. Chem. Soc.* **2002**, *124*, 13688.

(7) Chen, R. J.; Zhang, Y.; Wang, D.; Dai, H. *J. Amer. Chem. Soc.* **2001**, *123*, 3838.

(8) Hermanson, G. T. *Bioconjugate Techniques*; 2nd ed.; Academic Press, Inc.: Chicago, 2008.

(9) Nelson, N. C.; Taylor, S. S. *J. Biol. Chem.* **1983**, *258*, 10981.