Single Molecule Detection of Nitric Oxide Enabled by d(AT)_{15} DNA Adsorbed to Near Infrared Fluorescent Single-Walled Carbon Nanotubes

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1. **Deconvolution of SWNT photoluminescence spectra**

The fluorescence spectra were fitted using a sum of $N = 9$ Lorentzian lineshapes (8 nanotube peaks and 1 G-prime peak). The fluorescence intensity at any energy, $E$, is a sum over the contributions of all the species present in solution:

$$ I(E) = \sum_{i=1}^{N} \frac{C_i}{2\pi} \frac{\Gamma_i}{(E - E_{0,i})^2 + \Gamma_i^2/4} $$

The parameters to be estimated for the Lorentzian profile of the $i^{th}$ entity have been outlined below.

- $C_i$ – area under the peak
- $\Gamma_i$ – full width at half maximum (FWHM, meV)
- $E_{0,i}$ – peak center in terms of energy (meV)

Initial guesses for the peak areas were calculated from the control spectrum. The area under the $i^{th}$ peak was expressed as a fraction of the total area under the spectrum. This fraction was determined from the intensity of the investigated peak. The initial guesses for the FWHM of different nanotubes were obtained either from 2D excitation-emission profile similar to reported[1] or scaled according to their diameters[2] to ensure a good fit. The FWHM and peak center for the G-prime peak were kept constant (11 meV and 1258.72 meV respectively) and only its peak area was floated. In all, 31 parameters were used to fit a single fluorescence spectrum. Each $\Gamma_i$ ($E_{0,i}$) was constrained within a 10 meV (50 meV) window to maintain the physical validity of the fit. For responses such that the degree of quenching is over 50% the shifting response is set to zero due to the difficulty in distinguishing between actual shifting and relative intensity change of different species.
2. KMC simulating on single molecule adsorption and desorption on SWNT; stochastic analysis

KMC is implanted by using the algorithm reported by reference[3], and KMC simulated traces are generated considering $N = 10$, and 1000. $N = 10$ is chosen because there are maximum of 10 quenching steps observed experimentally, and the number is consistent with maximum number of exciton diffusion-limited segments [4-6] on the SWNT for which the average length is about 1-2 $\mu$m. $N = 1000$ is chosen to check the consistency of the MLE analysis method through comparing stochastic solution with deterministic solution. For each $N$ value, 10000 traces were generated for each sets of input rates, $(k_{a,j}, k_{d,j}) = (0.1, 0.00001), (0.01, 0.00001), (0.001, 0.00001), (0.0001, 0.00001)$. Units are in $[s^{-1}]$. For each $N$ value and set of input rates, the birth-and-death stochastic analysis method was then used to extract a value of $\hat{k}_{a,M}$ from every trace. This same analysis was performed several times while varying observation time from 600, 3000 to 30000 s. In addition, we examined how the mean and standard deviation of $\hat{k}_{a,M}$ vary when only a subset of the total number of traces is used (Traces = 10, 100, 1000, 10000). These values are summarized for each observation time and set of input rates, and each $N$.

3. Maximum likelihood estimator, $\mu$ and $\lambda$, based on birth-and-death Markov process

We would like to derive the MLE estimators, or $\hat{k}_{a,M}$ and $\hat{k}_{d,M}$ for the birth-and-death process represented in equation (5). Here, we use $\mu$ and $\lambda$ for now instead of $k_{a,j}$ and $k_{d,j}$ to keep the mathematical expression concise. For a birth-and-death process, the process parameter space $\tilde{\theta} = (\mu, \lambda)$ can be estimated through deriving the likelihood function, $L_{\tilde{\theta}}(\hat{\theta})$, and
computing \( \hat{\theta}_{max} \) that maximizes \( L(\hat{\theta}) \) by taking the first order derivative. And the process parameter \( \hat{\theta}_{max} \) is also named as maximum likelihood estimator (MLE), and in this case is a two dimensional vector.

To discuss this in detail, let \( X_t \) be the population size at time \( t \) of the birth-and-death process and the maximum number of population is \( N \). And the Markov process can be described

\[
P(X_{t+h} = j \mid X_t = i) = \begin{cases} 
(N - i)\lambda h + o(h) & (j = i + 1), \\
1 - (N - i)\lambda h - i\mu h + o(h) & (j = i), \\
i\mu h + o(h) & (j = i - 1), \\
o(h) & \text{(otherwise)}. 
\end{cases} \tag{1}
\]

We are considering the maximum likelihood estimation of the parameters \( \mu \) and \( \lambda \) assuming that the process has been observed continuously over some time interval. For a Markov jump process, the likelihood is

\[
L(\tilde{\theta}) = \prod_{i=1}^{n(t)} [ f(X_j \mid X_{j-1})\lambda(X_{j-1})] \exp \int_0^{\lambda(X_{j-1})DW} \tag{2}[7]
\]

where \( \theta \) is the parameter space, and \( n = n(t) \) is the number of jumps till time \( t \), and we assume that \( X_0 \), or the initial population size is non-random.

Note that equation (2) is equivalent to

\[
L(\tilde{\theta}) = \prod_{i=0}^{n(t)-1} [ f(X_{j+1} \mid X_j)\lambda(X_j)] \exp \int_0^{\lambda(X_j)}dW \tag{3}
\]

If we consider the birth-and-death process described by equation (1), then we have
\[ \tau(X_i) = (N - X_i) \lambda + X_i \mu \]

\[
f(X_i + d | X_i) = \begin{cases} 
(N - X_i) \lambda / \tau(X_i) & (d = 1), \\
i \mu / \tau(X_i) & (d = -1), \\
0 & \text{(otherwise)}.
\end{cases}
\] (4)

Substituting in equation (3),

\[
L_i(\theta) = \prod_{j=0}^{n(t)-1} [g(X_{i+1} | X_i)] \exp \int_0^{X_i} \tau(X_i) \, du
\]

\[
= \lambda^n \mu^D \prod_{j=0}^{n(t)-1} [h(X_{i+1} | X_i)] \exp \int_0^{X_i} \tau(X_i) \, du
\]

\[
= \lambda^n \mu^D \exp \int_0^{X_i} [h(X_{i+1} | X_i)] \prod_{i=0}^{n(t)-1} [h(X_{i+1} | X_i)]
\]

\[
= \lambda^n \mu^D \exp - \int_0^{X_i} \frac{(N - X_i) \lambda + X_i \mu}{\tau(X_i)} \prod_{i=0}^{n(t)-1} [h(X_{i+1} | X_i)]
\] (5)

where

\[
g(X_i + d | X_i) = \begin{cases} 
(N - X_i) \lambda & (d = 1), \\
i \mu & (d = -1), \\
0 & \text{(otherwise)}.
\end{cases}
\]

\[
h(X_i + d | X_i) = \begin{cases} 
(N - X_i) & (d = 1), \\
i & (d = -1), \\
0 & \text{(otherwise)}.
\end{cases}
\]

and \(B_i\) and \(D_i\) being the number of birth and death in the time interval \([0, t]\), and \(B_i + D_i = n(t)\). \(S_t\) is defined as \(\int_0^t X_u \, du\), the total time lived by the population in the time interval \([0, t]\).
The maximum likelihood estimators (MLE) of \((\mu, \lambda)\) are obtained by maximizing \(L(\theta)\)

\[
\frac{\partial \ln(L(\theta))}{\partial \lambda} = \frac{B_t}{\lambda} + S_t - N_t = 0
\]

\[
\frac{\partial \ln(L(\theta))}{\partial \mu} = \frac{D_t}{\mu} - S_t = 0
\]

So the MLE are \(\hat{\lambda}_{MLE} = \frac{B_t}{N_t - S_t}, \hat{\mu}_{MLE} = \frac{D_t}{S_t}\).

4. **Unbiased (consistent) MLE estimator for multiple traces**

Although central limit theorem states that sufficiently large number of independent random variables will be approximately normally distributed, a consistent estimator for multiple traces is not simply a mean of estimator of each traces, especially if the sample size is limited. In this case, a consistent MLE estimator for multiple traces is required. In fact, we can derive that the \(\hat{k}_a\) for multiple traces can be expressed by the following formula,

\[
\hat{k}_a = \frac{\sum_j D_{t,j}}{\sum_j S_{t,j}} = \frac{\sum_j (S_{t,j} \hat{k}_{a,M,j})}{\sum_j S_{t,j}} = w_j \hat{k}_{a,M,j}
\]

where \(j\) indicates the \(j^{th}\) population, and \(D_{t,j}\) and \(S_{t,j}\) are the number of deaths, the total time lived by the population in the time interval \([0, t]\) for the \(j^{th}\) population, and we define,

\[
w_j = \frac{S_{t,j}}{\sum_j S_{t,j}}
\]
Therefore, \( \hat{k}_a \) is just a \( S_t \)-weighted average of \( \hat{k}_a \) obtained from a single population (trace).

5. Validation of the birth-and-death MLE as a consistent estimator

In order to validate the consistency of the MLE estimator, we ran KMC simulation at \( N = 1000 \), and applied the same stochastic analysis to the simulated traces as these traces simulated at \( N = 10 \) (discussed in the main paper). The results are summarized in Table S2. Because as \( N \) increases, the time trace approaches to an analytical solution of equation (2) in the main text which can be described by an exponential decay. As expected, \( \hat{k}_{a,M} \) approaches a deterministic output, and even for a single trace, the error \( \hat{k}_{a,M} \) is less than 4% off the true value. In addition, the distribution converges to a delta function (Figure S8a), resulting in a significantly reduced standard deviation. Simulation results show that the lower limit of the standard deviation is only 3% of \( k_{a,i} \) (Table S2) at \( N = 1000 \), which is only 10% of the lower bound of the standard deviation at \( N = 10 \).

6. Contribution of slightly larger standard deviation at low \( k_{a,i} \)

Close examination of the distribution of \( \hat{k}_{a,M} \) from \( k_{a,i} \) indicates that the slightly larger standard deviation comes from many zero-transition traces (Figure 7a-i), and the MLE estimation method breaks down when there is no transition occurs. The solution to this issue is to increase observation time. It is worth noting that for small \( k_{a,i} \), increasing the observation time is rather effective in reducing the standard deviation to its lower bound. This is probably because at high \( k_{a,i} \) including 0.1 and 0.01 s\(^{-1}\), majority of the traces have shown completely quenching within 600 s, and prolonging observation time would not affect either \( D_i \) or \( S_i \) in equation (5) (main text), and later observation becomes ineffective. In contrast, increasing observation time can decrease the standard deviation for small \( k_{a,i} \) (0.0001 s\(^{-1}\)),

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because at low $k_{-j}^\prime$, only a few transitions occur at the first 600 s. Therefore, prolonging the observation diminishes any deviation that is due to insufficient transitions. However, because NO is rather diffusive, prolonging the experiments also means losing either spatial or temporal resolution, therefore is not recommended in this particular case. In fact, at an $k_{-j}^\prime$ as low as 0.0001 s$^{-1}$, notice that even 10 traces consistently yield a $\hat{k}_{a,M}$ with less than 5% error, and therefore this method is very accurate and effective. In general, this simulation-check approach also provides guidance in the experimental design, and provides a more fundamental understanding on experimental data.

7. Molecular model on AT$_{15}$-SWNT structure

Computations were performed using commercial software package, HyperChem (HyperCube, FL). d(AT)$_{15}$ oligonucleotides was obtained from the nucleic acid database, and was drawn in the vicinity to the SWNT. After geometry optimization was performed on the d(AT)$_{15}$ DNA, energy minimization using Amber force field was conducted on the AT$_{15}$-SWNT complex. The simulation was run for 1600 psec until the conformation reaches equilibrium.
Table S1 Concentration of analytes listed in the high-throughput screening assay.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration (mM)</th>
<th>Analyte</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-α-Estradiol</td>
<td>0.10</td>
<td>L-Ascorbic acid</td>
<td>0.50</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>0.48</td>
<td>L-Cittrulline</td>
<td>0.11</td>
</tr>
<tr>
<td>Acetylcholine chloride</td>
<td>0.54</td>
<td>L-Histidine</td>
<td>0.10</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>0.51</td>
<td>L-Thryoxine</td>
<td>0.10</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.51</td>
<td>Melatonin</td>
<td>0.49</td>
</tr>
<tr>
<td>ATP</td>
<td>0.11</td>
<td>NADH</td>
<td>0.51</td>
</tr>
<tr>
<td>cAMP</td>
<td>0.10</td>
<td>Nitric oxide</td>
<td>0.06</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.10</td>
<td>Quinine</td>
<td>0.01</td>
</tr>
<tr>
<td>Cytidine</td>
<td>0.48</td>
<td>Riboflavin</td>
<td>0.10</td>
</tr>
<tr>
<td>D-Aspartic acid</td>
<td>0.02</td>
<td>Salicylic acid</td>
<td>0.49</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>10.80</td>
<td>Serotonin</td>
<td>0.11</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>5.00</td>
<td>Sodium azide</td>
<td>0.51</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>10.90</td>
<td>Sodium pyruvate</td>
<td>0.50</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>10.30</td>
<td>Sucrose</td>
<td>0.10</td>
</tr>
<tr>
<td>Dopamine</td>
<td>0.49</td>
<td>Thymidine</td>
<td>0.52</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.50</td>
<td>Tryptophan</td>
<td>0.25</td>
</tr>
<tr>
<td>Guanosine</td>
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<td>Tyramine</td>
<td>0.49</td>
</tr>
<tr>
<td>Histamine</td>
<td>0.51</td>
<td>Urea</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Table 2 Effect of observation time and number of traces on $\hat{k}_{a,M}$ estimation. KMC simulated traces are generated using $N = 1000$. 1000 traces were generated for each sets of input rates, $(k_{a,i}, k_{a,j}) = (0.1, 0.00001), (0.01, 0.00001), (0.001, 0.00001), (0.0001, 0.00001)$. Units are in $[s^{-1}]$. For each pair of input rates, the birth-and-death stochastic analysis method was then used to extract an estimated value of $\hat{k}_{a,M}$ from every trace. This same analysis was performed several times while varying observation time from 600, 3000 to 30000 s. In addition, we examined how the $S_r$-weighted average and standard deviation of $\hat{k}_{a,M}$ vary when only a subset of the total number of traces is used (number of traces = 1, 10, 100, 1000). These values are summarized for each observation time and set of input rates.
Figure S1  Typical AFM (tapping mode) images of AT_{15}-SWNT deposited on the oxygen plasma
pre-wetted silicon dioxide surface. (a) Phase image of the same sample as in Figure 1. (b) Height (left) and phase (right) image of another scanning position on the same sample with scan size of 2 µm. (c) Height (left) and phase (right) image of the SWNT indicated by the red arrow in b) with scan size of 0.5 µm.

Figure S2 Height (a) and phase (b) AFM images (tapping mode) of AT_{15}-SWNT deposited on APTES pre-treated silicon dioxide surface. White dots indicated by red arrows were insoluble APTES from the sample.
**Figure S3** Setup for high throughput screening assay and data analysis. Polymer and DNA oligonucleotides of various sequences were used to suspend SWNTs and screened against a custom-designed analyte tray with 36 biologically relevant common molecules. SWNT PL was measured using a home-built near-infrared fluorescence microscope, excited at 785 nm. Fluorescence peak center and intensity of each nanotube species were obtained through spectral deconvolution.
**Figure S4** Contour plot of fluorescence intensity versus excitation and emission wavelengths for AT$_{15}$-SWNT and nanotube assignment.
Figure S5 Comparison of selectivity of AT\textsubscript{15}-SWNT containing and without free DNA. Bar charts represent the percentage of quenching (1-I/I\textsubscript{0}) of the (7,5) nanotube species when wrapped in: AT\textsubscript{15}-SWNT containing free DNA (a), AT\textsubscript{15}-SWNT without free DNA(b) and AAT\textsubscript{10}-SWNT (c) upon exposure to the 36 analytes. Red boxes highlight the responses of the polymer-SWNT complexes to dopamine, riboflavin and NO.
Figure S6  Absorption spectra of AT₁₅, GT₁₅, AAT₁₀ and PVA suspended SNWT upon 1 hour exposure to NADH, L-ascorbic acid, dopamine, and riboflavin. Concentration of SWNT is maintained at 2 mg/l in the buffer. Noise at 380 nm results from fast scanning which is the only solution in order to synchronize all measurements after 1 hour exposing the SWNT solution to the analytes. Absorption of dopamine, riboflavin and NADH at low wavelength cause the sloping base line at 400-500 nm (riboflavin and NADH) and 400 - 800 nm (dopamine).
Figure S7 AT$_{15}$-SWNT response to hydroxyl radical. Fluorescence intensity (I/I$_0$, intensity/initial intensity) of (7,5) species of AT$_{15}$-SWNT measured 10 minutes and 12 hours after addition of H$_2$O$_2$/FeSO$_4$ (Concentration of each reagent is indicated in the x-axis). SWNT is at 2 mg/l in PBS (50 mM).
Figure S8 Comparison of response time of AT$_{15}$-SWNT to NO (red circle) and peroxynitrite (black square). Fitted using a first-order kinetics (exponential decay) results in a sensor response time of $t_{1/2} = 1.1$ sec for NO and 28 sec for peroxynitrite.
Figure S9 Fluorescence intensity comparison among SWNT (2 mg/l) suspended in 8 DNA sequences. Laser (785nm) power is 150 mW at the sample.
Figure S10  Effect of observation time and number of traces on estimation for KMC traces ($N = 10$). (a) An example to study the effect of observation time and number of traces on the $\hat{k}_{a,M}$ estimation. Histogram of $\hat{k}_{a,M}$ estimated from 10 (a-i, a-iv), 100 (a-ii, a-v), 1000 (a-iii, a-vi) KMC traces using 600 s (top) and 3000 s (bottom) as observation time. Input values are $k_{a,i} = 0.01 \text{ s}^{-1}$, $k_{d,i} = 0.00001 \text{ s}^{-1}$, and $N = 10$. (b) The $\hat{k}_{a,M}$ and standard deviation of the distribution as a function of $k_{a,i}$ of the KMC in a log$_{10}$-log$_{10}$ scale, and fitted with a linear trend with slope indicated in the figure (square, $\hat{k}_{a,M}$ of multiple traces; error bar, standard deviation). Each panel represents the $\hat{k}_{a,M}$ obtained with a different observation time (b-i, 600 s; b-ii, 3000 s).
**Figure S11** Effect of observation time and number of traces on estimation for KMC traces ($N = 1000$). (a) An example to study the effect of observation time and number of traces on the $\hat{k}_{a,M}$ estimation. Histogram of $\hat{k}_{a,M}$ estimated from 10 (a-i, a-iv), 100 (a-ii, a-v), 1000 (a-iii, a-vi) KMC traces using 600 s (top) and 3000 s (bottom) as observation time. Input values are $k_{a,d} = 0.01 \text{ s}^{-1}$, $k_{d,a} = 0.00001 \text{ s}^{-1}$, and $N = 1000$. (b) The $\hat{k}_{a,M}$ and standard deviation of the distribution as a function of $k_{a,d}$ of the KMC in a log$_{10}$-log$_{10}$ scale, and fitted with a linear trend with slope indicated in the figure (square, $\hat{k}_{a,M}$ of multiple traces; error bar, standard deviation). Each panel represents the $\hat{k}_{a,M}$ value obtained with a different observation time (b-i, 600 s; b-ii, 3000 s).
**Figure S12** Comparison between the effectiveness of applying the Chi-squared error-minimizing step-finding algorithm on the control data and the data where NO is added. (a) Time-trace of a diffraction limited spot (2x2 pixel) in a control experiment where no analytes are added (red) and fitted trace (Chi-squared error-minimizing step-finding algorithm, black). Over-fitting on noise is observed. (b) Time-trace after applying noise-reduction algorithm on the time-trace in (a) (red), and fitted steps on the noise-reduced trace (black). Over-fitting on noise is observed. (c) Time-trace of a diffraction limited spot (2x2 pixel) in an experiment where 19.4 μM NO is added (red) and fitted trace (Chi-squared error-minimizing step-finding algorithm, black) without applying the noise-reduction method. It appears that the intensity fluctuation is more obvious in the control dataset compared to the dataset of which the array is exposed to NO even for the same starting SWNT intensity. We are still investigating the source of this fluctuation.
**Figure S13a.** Fluorescence time traces of the 50 brightest diffraction limited spots (2x2 pixels) in a control experiment in which no NO is added. The trace images are displayed in a descending order of the starting fluorescence intensity. Traces of number 1,2,3,4,5,6,7,8,10,11,13,14,15,16,17,18,22,23,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,46,47,48,49,50 are identified as zero-transitions.
Trace No.: 1
Trace No.: 2
Trace No.: 3
Trace No.: 4
Trace No.: 5
Trace No.: 6
Trace No.: 7
Trace No.: 8
Trace No.: 9
Trace No.: 10
Trace No.: 11
Trace No.: 12
Trace No.: 13
Trace No.: 14
Trace No.: 15
Trace No.: 16
Trace No.: 17
Trace No.: 18
Trace No.: 19
Trace No.: 20
**Figure S13b.** Fluorescence time traces of the 50 brightest diffraction limited spots (2x2 pixels) upon exposure to NO solution (0.16 µM, 1x PBS) at $t = 0$ s. The trace images are displayed in a descending order of the starting fluorescence intensity. Traces of number 4,5,7,10,11,12,17,18,21,25,27,29,30,32,33,34,35,36,37,41,42,43,46,47,48,50 are identified as zero-transitions.
Figure S13c. Fluorescence time traces of the 50 brightest diffraction limited spots (2x2 pixels) upon exposure to NO solution (0.78 µM, 1x PBS) at t = 0 s. The trace images are displayed in a descending order of the starting fluorescence intensity. Traces of number of 17, 25, 26, 27, 31, 42, 45, 48, 50 are identified as zero-transitions.
**Figure S13d.** Fluorescence time traces of the 50 brightest diffraction limited spots (2x2 pixels) upon exposure to NO solution (3.9 µM, 1x PBS) at t = 0 s. The trace images are displayed in a descending order of the starting fluorescence intensity. Each trace contains at least one transition.
**Figure S13e.** Fluorescence time traces of the 50 brightest diffraction limited spots (2x2 pixels) upon exposure to NO solution (19.4 µM, 1x PBS) at t = 0 s. The trace images are displayed in a descending order of the starting fluorescence intensity. Each trace contains at least one transition.
Supplementary References