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Nanoreporter of an Enzymatic Suicide Inactivation Pathway

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Abstract

Enzymatic suicide inactivation, a route of permanent enzyme inhibition, is the mechanism of action for a wide array of pharmaceuticals. Here, we developed the first nanosensor that selectively reports the suicide inactivation pathway of an enzyme. The sensor is based on modulation of the near-infrared fluorescence of an enzyme-bound carbon nanotube. The nanosensor responded selectively to substrate-mediated suicide inactivation of the tyrosinase enzyme via bathochromic shifting of the nanotube emission wavelength. Mechanistic investigations revealed that singlet oxygen generated by the suicide inactivation pathway induced the response. We used the nanosensor to quantify the degree of enzymatic inactivation by measuring response rates to known small molecule tyrosinase modulators. This work resulted in a new capability of interrogating a specific route of enzymatic death. Potential applications include drug screening and hit-validation for compounds that elicit or inhibit enzymatic inactivation, and single-molecule measurements to assess population heterogeneity in enzyme activity.

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Supporting Information

Please see attached file “Supporting Information” which contains additional spectroscopic and imaging data, discussion, and materials and methods.

Keywords

High throughput assay; Drug screening; Drug development; enzymology; Reactive oxygen species

Introduction

Enzymatic inactivation processes involve the reversible or irreversible abrogation of enzyme function, often mediated by enzyme-substrate interactions^{1–5}. Constitutively activated or dysregulated enzymes are often a cause of a wide array of human diseases^{6–12}. The measurement of these interactions and resulting enzymatic inactivation is of critical value for a variety of applications ranging from industrial processes to drug development^{13–17}. In particular, detecting and characterizing pathways of enzymatic death and permanent inactivation plays an increasingly crucial role in drug development^{16, 18, 19}.

Current methodologies to study enzyme inactivation, such as mass spectrometry, calorimetry, or radioactive labeling are relatively slow, require extensive synthesis and purification steps, may not be selective to the inactivation pathway itself, and cannot achieve single-molecule level resolution of enzyme-substrate interactions or dynamic measurements²⁰. Thus, new methods to selectively and transiently report single-molecule and population-level enzyme inactivation will improve basic and applied investigations of these processes²¹.

Nanomaterial-based biosensors have been employed in chemical biology and biochemistry studies due to their versatility in reporting on a wide array of substrates and chemical reactions^{22–25}. Specifically, nanoscale materials have increasingly overlapped with studies of enzymes, creating a sub-field of “nanoenzymology.” Recent studies highlight a growing interest in using nanomaterials to add unique capabilities to conventional methods aimed at characterizing enzyme-substrate interactions^{26–28}.

One class of nanomaterial, single walled carbon nanotubes (CNTs) have unique electronic/optical properties that are useful in developing quantitative sensors²⁹. They emit near-infrared photoluminescence which is sensitive to the local environment³⁰, and the emission

is uniquely photostable, enabling quantitative and long-term monitoring of small-molecules, proteins, and nucleic acids *in vitro* and *in vivo* ^{31–34}.

Tyrosinase is an enzyme involved in the production of melanin within tissues of mammalian, plant, and fungal species ³⁵. Tyrosinase can undergo permanent suicide inactivation as a result of specific substrate binding. This phenomenon occurs when a phenolic substrate forms a covalent bond with the enzyme's copper(II) center, causing its ejection from the binding pocket ^{36, 37}. Suicide inactivation is reported to promote the generation of reactive oxygen species (ROS) that enhance the inactivation kinetics. ^{1–4}. For this investigation, mushroom tyrosinase was chosen as a model enzyme for its well-characterized chemical pathways, inhibitors, and structure as well as its suicide inactivation through substrate binding.

In this work, we report the optical transduction of the suicide inactivation pathway of tyrosinase based on modulation of the photoluminescence of a carbon nanotube. The carbon nanotubes exhibit a distinct bathochromic (red) shift upon initiation of the suicide pathway that was also measured at the single-nanosensor level. We found that the optical response of the sensor is a result of the generation of reactive oxygen species, specifically singlet oxygen (¹O₂), on the nanotube during the enzymatic reaction. Notably, inhibition of ¹O₂ generation, and concomitantly, the sensor response, increased melanin production via the diphenolase (non-inactivation) pathway of the enzyme. Libraries of known suicide inducers and tyrosinase inhibitors were interrogated, resulting in the quantification of suicide pathway inhibition. This work presents a technology to investigate a specific enzymatic inactivation mechanism and to assess compounds that induce or inhibit specific enzymatic inactivation pathways.

Results and Discussion

We assembled a supramolecular complex consisting of a carbon nanotube (CNT) wrapped by single-stranded DNA containing a PEG linker (PEG₁₂-GT₁₅-Amine) (Figure 1A). The ssDNA-nanotube complex exhibited colloidal stability typical of DNA-CNT complexes³⁸. The tyrosinase enzyme was then chemically conjugated via 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) coupling to the 3'-terminal amine group on the PEG linker portion of the DNA-CNT (as we have done previously ³⁹). The solution was dialyzed against 10% PBS for 48 hours to remove free enzyme and reagents. We conducted liquid-phase atomic force microscopy (AFM) to observe the tyrosinase-conjugated DNA-nanotube (TYR-CNT) complexes bound to a mica substrate, which showed regions of raised elevation at positions situated along the length of the carbon nanotube (Figure 1B). Compared to DNA-CNTs, the height profiles of TYR-CNTs showed several 2–4 nm elevated regions (Figure 1C). On average, the height of TYR-CNTs was twice the height of DNA-CNTs suggesting that multiple enzymes were immobilized on the DNA-nanotube complexes, as height variations due to DNA on the nanotube surface are normally ~0.5 nm ⁴⁰ (Figure S1). Dynamic light scattering (DLS) measurements also showed a larger size of TYR-CNT as compared to DNA-CNT (Figure S2 and Supplementary Text).

The tyrosinase-nanotube complexes were challenged with known tyrosinase substrates to assess the optical response of the nanotube to an enzyme-catalyzed reaction. As the complexes were exposed to increasing concentrations of the tyrosinase substrate dopamine, the emission exhibited red-shifting and attenuation (Figure 1D–E). The emission center wavelength exhibited monotonic bathochromic behavior across all chiralities (Figure 1F). This response was compared to another known tyrosinase substrate, L-tyrosine (Figure 1G). The magnitude of the response (total wavelength shift) of the complex was more than an order of magnitude greater for dopamine than for L-tyrosine. We explored the response of the TYR-CNT complex to other tyrosinase substrates. A library of twelve phenolic substrates at concentrations ranging from 0.01 to 1 mM was then introduced to the complex (Figure 1H). Pronounced differences in the magnitude of shifting of the nanotube emission wavelength were apparent, depending on the substrate.

We compared the enzyme activity to the rate of optical response of the TYR-CNT complex. We found an inverse correlation between melanin production rate (as a benchmark for tyrosinase activity), and the TYR-CNT optical response rate, as a function of enzyme substrate (Figure 1I). For instance, compared to dopamine, L-tyrosine induced a substantially lower rate of change of the optical response of the TYR-CNT complex (0.0281 nm/min), though it induced a higher rate of melanin production (0.25 OD/min). Conversely, pyrogallol induced a higher response rate of the TYR-CNT complex (0.75 nm/min), but its activity, according to its melanin production, was 0.09 OD/min.

We hypothesized that the differences in the optical response of the tyrosinase-nanotube complexes among substrates of tyrosinase may be due to differences in substrate-dependent inactivation routes of the enzyme. For instance, dopamine, a diphenolic substrate, undergoes a single tyrosinase-catalyzed reaction to a quinone. During this reaction, there is a substantial probability that the enzyme undergoes suicide inactivation⁴¹. Conversely, L-tyrosine, a monophenolic substrate, is much less likely to induce the suicide inactivation pathway in tyrosinase⁴². In addition, the response of the complexes to tyramine, a direct metabolic precursor to dopamine, is significantly slower than the response to dopamine. Of the non-monophenolic substrates, those which directly induce suicide inactivation of tyrosinase, such as pyrogallol and pyrocatechol⁴³, caused large optical responses of the enzyme-nanotube complex. Conversely, other non-phenolic substrates such as 4-nitrocatechol and gallic acid, which are weaker suicide substrates of tyrosinase (k_{cat} values of 0.9 s^{-1} and 28.2 s^{-1} , respectively)⁴⁴ did not cause large optical responses. By comparison, L-dopa has a k_{cat} value of 102.6 s^{-1} ⁴⁴. Previous studies posited that the presence of electron withdrawing groups at the C-4 position of catechols greatly reduces potency of a tyrosinase suicide inhibitor⁴⁴. Therefore, although isoproterenol has not been investigated as a suicide substrate of tyrosinase, the presence of an electron withdrawing group at its C-4 position would explain its low activity in this scenario. These findings suggest that the magnitude of the TYR-CNT wavelength shift correlates to its efficiency as a suicide substrate. The results indicate that the optical response of the TYR-CNT complex is selective for substrates of tyrosinase that induce the suicide inactivation pathway, suggesting that the complex acts as a sensor for suicide inactivation of the enzyme. Moreover, when the optical response of the nanosensor was fitted to a Michaelis–Menten kinetics model, the values for each substrate exhibited a striking correlation with their catalytic constants (K_{Cat})

⁴⁵. We named the nanosensor-derived term the “inactivation constant” (K_{Inac}) (Figure 1J). The new value represents the maximum chemical conversions of each substrate specifically towards the inactivation pathway. We surmise that the correlation of K_{Inac} with K_{Cat} occurs because the affinities of the substrates to the enzyme drive the rates of both reactions.

We assessed the emission response at the single-sensor level via spectral imaging. The TYR-CNT complexes were immobilized on cover glass and visualized using near-infrared hyperspectral microscopy ⁴⁶ to obtain spectra from individual regions of interest (ROIs) in the imaging field (Figure 2A). Upon introduction of dopamine to the sample, we observed a distinct shift in emission wavelength and attenuation of emission intensity of individual ROIs (Figure 2B–C). Resolution of single chiralities across the imaging field showed that nearly all ROIs contained single TYR-CNTs. The distinct response of individual, surface-immobilized TYR-CNT complexes to dopamine indicates that the sensor response does not occur due to aggregation or nanotube-nanotube bundling, a known cause of photoluminescence red-shifting ⁴⁷.

We then investigated the mechanism of the nanosensor emission response to suicide inactivation of tyrosinase. We considered that nanotube emission is known to exhibit red shifts in response to changes in the local environment via: nanotube aggregation/bundling, increasing local electrostatic charge ⁴⁸, and changes in the local dielectric environment towards a more polar environment on or near the nanotube interface⁴⁹. We investigated the following potential mechanisms that could result in the above phenomena: TYR-CNT complex aggregation/precipitation, enzyme structural changes that redistribute the electrostatic environment of the nanotube or increase exposure to water, binding of copper (released from the enzyme) to the DNA or nanotube ^{40,55}, melanin (product) binding to the nanotube surface, the intermediate product (quinone) binding to the nanotube, and exposure to ROS produced during the reaction. We ruled out most of these potential causes of the response (Figure 3A). Further investigations of exposure to water (Figure S3A), aggregation (Figure S3B), enzyme structural changes (Figure S3C–D), binding of copper (Figure S4A–B), melanin (product) (Figure S4D) and quinone exposure are discussed in the SI. By introducing copper salt (Cu^{+2}) and 1 mM hydrogen peroxide (H_2O_2), however, we observed a 16 nm red-shift - a similar magnitude as the TYR-CNT response to dopamine (10 nm) (Figure 3A). This result suggests ROS as a potential mediator of the TYR-CNT response. To confirm the generation of ROS during the enzymatic reaction with dopamine, we used a general ROS detection reagent (2',7'-dichlorodihydrofluorescein diacetate [H_2DCF]). Upon ROS generation by copper Fenton chemistry, the fluorescence intensity of the reagent brightened over 8-fold. (Figure 3B).

We then investigated the effect of generation of ROS on the TYR-CNT optical response. The most common subtypes of ROS species are hydroxyl radical ($\cdot\text{OH}$), superoxide anion ($\text{O}^{\cdot-}_2$), and singlet oxygen ($^1\text{O}_2$). To investigate the hypothesis that ROS caused the TYR-CNT response, we generated several ROS species in the presence of the DNA-CNT complexes via the copper Fenton reaction⁵⁰ (Figure 3C). We also made use of mannitol to quench $\cdot\text{OH}$ ⁵¹, (2,2,6,6-Tetramethylpiperidin-1-yl) oxyl or (2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl)TEMPO(to trap $\text{O}^{\cdot-}_2$ ⁵² and 1,4-diazobicyclo(2,2,2)-octane)DABCO(to quench $^1\text{O}_2$ ⁵³ (Figure 3C). To determine which ROS species were involved in the signal

transduction via enzymatic reaction, we repeated the ROS fluorescence measurement of the tyrosinase-dopamine reaction in the presence of ROS scavenging agents (Figure 3D). In the presence of TYR-CNT and 1 mM dopamine, the fluorescence intensity increased 4-fold, denoting ROS generation by the enzymatic reaction.

These findings suggest that $^1\text{O}_2$ is the major ROS species generated as part of the tyrosinase inactivation. This evidence supports a previous study showing that the enzymatic inactivation pathway involves $^1\text{O}_2$ rather than $\cdot\text{OH}$ ⁵⁴. To confirm the effect of chemical trapping/quenching on TYR-CNT optical response in the tyrosinase inactivation assay, we analyzed sensor emission (Figure 3E). While mannitol and TEMPO succeeded in quenching the assay fluorescence intensity by 50%, DABCO succeeded in fully quenching the fluorescence. While the addition of mannitol and TEMPO resulted in a negligible effect on the optical response (less than 1 nm shift), DABCO succeeded in quenching the sensor optical response by over 70%. These findings led us to conclude that the primary cause of the nanosensor response was singlet oxygen. These findings led us to conclude that the primary cause of the nanosensor response was singlet oxygen. The mechanism of the singlet oxygen-based modulation of the nanotube emission was hypothesized by Weisman et al. ⁵⁵, who suggested that the reaction of singlet oxygen with DNA base pairs, specifically guanine, cause covalent conjugation of DNA to the surface of the nanotube. The DNA sequence thus affects the degree of the nanotube wavelength shifting response (also shown by Heller et al. ⁵⁶).

We further interrogated the effect of singlet oxygen on general nanotube wavelength emission by incubating the DNA-nanotube complex with 0.1mM Cu^{+2} and elevated levels of H_2O_2 in the presence of DABCO (Figure 3F). The results showed that the optical response correlated with an increase in concentration of H_2O_2 , and the addition of DABCO quenched the response at every H_2O_2 concentration.

We further tested the correlation between suicide substrates and generation of ROS species. We used ROS detection reagent to monitor the generation of ROS species when either dopamine or l-tyrosine were introduced to tyrosinase. In the presence of dopamine, the fluorescence intensity of the reagent brightened over 1.6-fold higher compares to l-tyrosine (Figure S3E). These findings suggest that the activity of suicide substrate is correlated with the ability to generate ROS species. Weaker suicide substrates will generate less ROS species and hence will divert the enzyme activity towards diphenolase pathway.

We also investigated ascorbic acid, which is both known to reduce dopaquinone back into dopamine ³⁵, inhibiting melanin production^{57, 58}, and also function as a general ROS scavenger ⁵⁹. We introduced ascorbic acid following an initial treatment with dopamine, which arrested dopamine-induced shifting of the emission wavelength of the TYR-CNT but did not cause the emission wavelength to reverse course (Figure 3G, S5A). We also incubated the DNA-CNT with copper and hydrogen peroxide to generate singlet oxygen species in the presence of ascorbic acid (Figure S5B). The results showed significant inhibition of the wavelength shift with higher concentrations of ascorbic acid (complete inhibition achieved at the concentration of 100 mM of ascorbic acid). Similar effects were seen when TYR-CNT was incubated with dopamine and several different concentrations of

ascorbic acid (Figure S5C). We further tested the inhibitory effect of ascorbic acid on the melanin production (Figure S5D). The results showed significant inhibition of melanin production at concentrations higher than 10 mM of ascorbic acid. On comparing the two inhibitory effects of ascorbic acid, we found a stronger effect of ascorbic acid on suppressing the generation of singlet oxygen species as opposed to melanin production. These findings suggest that ascorbic acid acts primarily to prevent suicide inactivation of tyrosinase which allows for increased tyrosinase activity for melanin production (via the diphenolase pathway).

To determine whether DABCO abrogated the TYR-CNT optical response through singlet oxygen quenching or direct enzyme inactivation, we measured melanin formation (Figure 3H).

The results showed that DABCO failed to inhibit melanin formation but instead resulted in enhanced production. We further compared the melanin formation and optical response rates of the sensor when challenged with dopamine, L-tyrosine, dopamine in the presence of DABCO, ascorbic acid, and EDTA (Figure 3I). While L-tyrosine induced a low sensor response rate and high rate of melanin production, and dopamine induced the opposite, the increasing DABCO concentrations in the presence of the dopamine substrate rescued the L-tyrosine-like response. Inhibitors of tyrosinase activity that do not induce suicide inactivation, ascorbic acid and EDTA, attenuated both the sensor response and melanin formation (Additional discussion and data in the Supplementary Information).

We therefore propose, based on the above data, that the sensor response is caused by the generation of singlet oxygen species via the suicide inactivation pathway, and that scavenging singlet oxygen diverted enzymatic activity to melanin production (Schematic in Figure 3J). By scavenging singlet oxygen, the suicide inactivation pathway of the enzyme was attenuated, and the diphenolase pathway, which leads to melanin formation, was enhanced (Additional discussion in Supplementary Text).

We interrogated a library of known tyrosinase inhibitors using the nanosensor. These TYR-CNTs were first pre-incubated with increasing concentrations of each inhibitor before challenging with 1mM dopamine. The inhibition effect was calculated as the degree of induced TYR-CNT signal attenuation from increasing concentrations of inhibitor; this result was used to construct dose-response curves. The results of this screen produced several findings. For example, kojic acid (KA), a known tyrosinase inhibitor, exhibited dose-dependent attenuation of the dopamine-mediated optical response rate (Figure 4A). This data was used to plot a response curve for kojic acid (Figure 4B–C) that was fit using a variable slope model. Analysis resulted in an IC_{50} value of 0.5 mM for KA inhibition. This value falls within the range of literature IC_{50} values, approximately 0.01 mM to 0.3 mM⁶¹. We repeated the interrogation of additional inhibitors of tyrosinase (Figure 4C).

Because inhibitors of tyrosinase in the library are known to act through different chemical mechanisms in the active site, but the conventional output assay involves melanin production, we surmise that some of the suicide nanosensor results would not necessarily give the same answers as reported IC_{50} results. We propose that the reporting of suicide

inactivation using this sensor would a modified IC₅₀-like value to describe the degree of suicide inactivation as a separate value from general inhibition of enzyme activity (Further discussion in SI).

Conclusions

We engineered a nanotube-based optical sensor that quantifies enzyme-substrate interactions in the context of the suicide inactivation pathway of tyrosinase. The sensor, composed of the tyrosinase enzyme covalently linked to DNA bound to a carbon nanotube, exhibited consistent bathochromic shifts in response to suicide substrates of tyrosinase. On deeper investigation, we found that these responses were due to the generation of the ROS species singlet oxygen produced by reactions of the suicide substrate by tyrosinase. The inhibition of ROS production prevented the suicide inactivation of the enzyme and promoted product formation. We measured suicide inactivation on the single-sensor level and interrogated a library of small molecule tyrosinase inhibitors and measured the sensor response attenuation to find IC₅₀ values of suicide inactivation. This work develops, to our knowledge, the first optical biosensor that selectively interrogates a suicide inactivation pathway of an enzyme. Applications of this technology include the screening of enzyme-drug interactions, single-molecule measurements to resolve variants within heterogeneous populations, and unbiased testing of novel reagents that may modulate enzyme activity through tuning or introducing suicide inactivation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflicts of Interest Statement

D.A.H. and P.V.J. are cofounders with equity interest in LipidSense, Inc. D.A.H. is a cofounder and officer with equity interest in Goldilocks Therapeutics Inc. and Nirova Biosense, Inc., as well as a member of the scientific advisory board of Concarlo Holdings, LLC and Nanorobotics, Inc. M.L. is a member of the scientific advisory board of Epi One, Inc.

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Figure 1. Characterization of TYR-CNT complexes and optical response to known tyrosinase substrates.

(A) Schematic representation of the TYR-CNT. (B) Fluid AFM image of the TYR-CNT and DNA-CNT (inset). (C) Histogram of the variations in height of the TYR-CNT and DNA-CNT complexes from the AFM image. (D) Near-infrared fluorescence spectra of TYR-CNT incubated with different concentrations of dopamine at time = 55 minutes. Inset: normalized emission band; peak center wavelength denoted by triangles. (E) Kinetics of the TYR-CNT emission wavelength response at several dopamine concentrations. (F) Response of different CNT chiralities (nanotube species) to 1 mM dopamine, after 60 minutes, $n = 3$, mean $\pm$

SEM. (G) Response of the TYR-CNT complexes upon introducing 1 mM of dopamine (black) or L-tyrosine (red) (added at time = 10 minutes). (H) Heat map of the emission response rate of TYR-CNT complexes to twelve phenolic substrates. (I) Melanin production and response rates of 1mM phenolic substrates after 60 minutes. $n = 3$, mean $\pm$ SEM; **** $p < 0.0001$, * $p = 0.035$, “ns” denotes not significant, unpaired t test. (J) Catalytic constants (K_{Cat}) of tyrosinase substrates, obtained from the literature, plotted with inactivation constants (K_{Inac}) calculated from nanosensor response rates.

Figure 2. Single nanosensor measurements.

(A) Near-infrared fluorescence image of TYR-CNT adsorbed to a glass surface. Scale bar = 100 μm . (B) Representative fluorescence spectra from a single complex upon introducing dopamine. (C) Emission wavelength of TYR-CNT complexes upon incubation with 1mM dopamine for 20 minutes; $n = 7$, mean $\pm$ SEM; **** $P < 0.0001$, 0 min and 5 min ** $P = 0.0015$, 0 min and 10 min ** $P = 0.0013$, unpaired t test.

Figure 3. Mechanism of sensor response.

(A) Emission wavelength of DNA-CNT and TYR-CNT complexes in different conditions after 60 minutes; denTYR = Heat-denatured tyrosinase, [Cu²⁺] = 0.1mM; [Melanin] = [DH-BQ] = [H₂O₂] = 1mM; n = 3, mean ± SEM. (B) ROS detection reagent normalized fluorescence intensity in different conditions. [ROS detection reagent] = 10μM, [Dopamine] = 1mM; n = 3, mean ± SEM; ****P < 0.0001, ***P = 0.0004, unpaired t test. (C) Schematic of Copper Fenton reaction with respective quenchers (figure adapted from Carrier et al. 2018⁶⁰). (F) ROS detection reagent fluorescence during incubation with TYR-CNT and 1mM

dopamine together with ROS scavengers for 60 minutes. [Mannitol] = [TEMPO] = [DABCO] = 10 mM; n = 3, mean $\pm$ SEM; ****P < 0.0001, ***P = 0.0004, unpaired t test (D) Emission wavelength response of TYR-CNT when incubated with 1 mM dopamine and 10 mM ROS scavengers for 60 minutes; n = 3, mean $\pm$ SEM; ****P < 0.0001; unpaired t test (C) Emission wavelength of DNA-CNT in different conditions after 60 minutes; n = 3, mean $\pm$ SEM. (G) Response of sensor emission wavelength to ascorbic acid during exposure to dopamine. Arrow denotes the time of introduction of ascorbic acid at 10 minutes after exposure to dopamine. (H) Absorbance measurements of melanin formation in different conditions. [EDTA] = 50 mM; [Ascorbic Acid] = 100 mM; n = 3, mean $\pm$ SEM; control and EDTA, **P = 0.0036; control and Ascorbic Acid, **P = 0.0013; control and DABCO, *P = 0.0272; unpaired t test. (I) Absorbance measurements of melanin formation and optical response rate in different conditions. Absorbance values were normalized to the control; Control = TYR-CNT + 1 mM dopamine. L-tyrosine group contained TYR-CNT + 1 mM L-tyrosine; Other groups are composed of control and DABCO/EDTA/Ascorbic Acid; n = 3, mean $\pm$ SEM. (J) Schematic illustration of the hypothesized TYR-CNT mechanism. Upon interrogation of TYR-CNT with substrates of the diphenolase pathway, no response is observed because the substrate causes no generation of singlet oxygen species. Upon interrogation with a substrate that induces suicide inactivation of the enzyme, singlet oxygen species are generated and react with the DNA-nanotube complex which cause to a red shift in the emission wavelength of the nanotube. ROS scavenger can inhibit the suicide inactivation pathway and as a result enhance the diphenolase pathway, resulting with higher production of melanin.

Figure 4. Measurement of abrogation of suicide inactivation on interrogation via a tyrosinase inhibitor library.

(A) Emission wavelength of TYR-CNT pre-incubated with kojic acid prior to challenging with dopamine. (B) Comparison of two methods of assessing enzyme inhibition: sensor response rate and absorbance of melanin. (C) Processed data from (B) plotted against Kojic acid concentration along with similar analyses for other inhibitors of tyrosinase.