

Helical Assembly of Flavin Mononucleotides on Carbon Nanotubes as Multimodal Near-IR Hg(II)-Selective Probes

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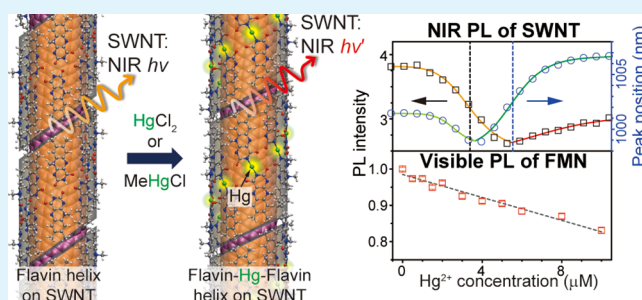
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Supporting Information

ABSTRACT: The development of novel methods to detect mercury is of paramount importance owing to the impact of this metal on human health and the environment. We observed that flavin mononucleotide (FMN) and its helical assembly with a single-walled carbon nanotube (SWNT) selectively bind Hg^{2+} arising from HgCl_2 and MeHgCl . Absorption spectroscopic studies show that FMN preferentially forms a 2:1 rather than a 1:1 complex with Hg^{2+} at high FMN concentrations. On the basis of the analogy to the thymine–Hg–thymine complex, it is proposed that the 2:1 complex between FMN and Hg^{2+} comprises a Hg-bridged pair of FMN groups, regardless of the presence of SWNT. Upon addition of as little as a few hundred nanomoles of Hg^{2+} , both FMN and FMN–SWNT exhibit absorption and photoluminescence (PL) changes. Moreover, FMN–SWNT displays simultaneous multiple sigmoidal changes in PL of SWNT tubes having different chiral vectors. Assessment of binding affinities using the Hill equation suggests that 2:1 and 1:1 complexes form between Hg^{2+} and FMN groups on the FMN–SWNT. Theoretical calculations indicate that optical changes of the FMN–SWNT originate from Hg-mediated conformational changes occurring on the helical array of FMN on the SWNT. High-resolution transmission electron microscopy revealed that the presence of Hg^{2+} in complexes with the FMN–SWNT enables visualization of helical periodic undulation of FMN groups along SWNT without the need for staining. Circular dichroism (CD) study revealed that FMN–SWNT whose CD signal mainly originates from FMN decreases dichroic bands upon the addition of Hg^{2+} owing to the formation of a centrosymmetric FMN–Hg–FMN triad on SWNT. The binding mode specificity and multimodal changes observed in response to Hg^{2+} ions suggest that systems based on FMN–SWNT can serve as *in vivo* NIR beacons for the detection of various mercury derivatives.

KEYWORDS: mercury(II) chloride, flavin mononucleotide, carbon nanotube, binding mode, organomercurial



INTRODUCTION

Mercury, one of the most prevalent heavy metals in the environment, has become a growing environmental and human health problem. Absorption of mercury into the body leads to damage of the central nervous and endocrine systems.^{1,2} Generally, toxic forms of mercury, including $\text{Hg}(0)$ and HgCl_2 , and organomercurials, such as methyl and phenyl derivatives, are released into the environment from a variety of anthropogenic and natural sources.³ Following its release and uptake in living systems, mercury bioaccumulates creating harmful levels in species at the top of the food chain. As a result, consumption of these species can result in ingestion and transmission of high levels of mercury to the brain and kidneys, leading to damage to the central nervous and endocrine systems.^{1,2}

Owing to health and environment issues, novel methods for detecting mercury are in great demand. Traditional methods to

detect Hg^{2+} in aqueous environments utilize a number of instrumental techniques including gas chromatography, atomic absorption spectroscopy, and cold vapor atomic fluorescence spectrometry.⁴ Many of these approaches require preliminary, sophisticated, multistep procedures for sample preparation. To avoid tedious sample preparation and enhance detection limits, more recently small molecules,^{3,5} biomacromolecules,⁶ and nanomaterials^{7–9} have been developed as sensors for monitoring both inorganic mercury and organomercurials. The most common systems of this type rely on “turn-on” and “turn-off” responses of photoluminescence (PL) chromophores upon binding with Hg. One of the common strategies is to utilize Hg^{2+} -chelating crown ethers or calixarene,

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containing amine or thiol groups tethered to chromophores, which undergo changes in their electronic structures upon mercury binding.^{3,5} Although many are reversible, several PL enhancement protocols are based on chemodosimeters, which undergo irreversible reactions with Hg^{2+} .^{10,11} Mercury sensors that operate by PL enhancement have advantages associated with sensitivity and multiple-color light emission that enables multiplexing. However, these methods often suffer from false positive signals caused by nonspecific binding with other metals. In this regard, ratiometric sensing utilizing both absorption and PL bands offers several additional advantages.¹²

Nanomaterials, which have large extinction coefficient and/or emission quantum yields, have also been utilized in Hg^{2+} detection strategies that rely on the surface plasmon phenomenon,^{8,13} on ^{on/off} PL, and PL anisotropy.¹⁹ These approaches often use various thiol-containing groups that bind to Hg^{2+} . Also, thymine (T) has been exploited extensively because of its ability to complex with Hg^{2+} to form the triad T– Hg^{2+} –T.⁸ Nanoparticles, tethered to T or polyT in single-stranded DNA, have been employed as detectors, in which Hg^{2+} induces aggregation to promote surface plasmon changes⁸ or PL modulation.¹⁸ Similar strategies using single-walled carbon nanotubes (SWNTs)^{9,20} have also been devised. In these systems, the SWNT plays a passive role in which it quenches PL of a dye tethered to single-stranded T-rich DNA. However, intrinsic near-IR (NIR) PL of various SWNTs with different chiral vectors $[(n, m)]$ corresponding to the degree of offset of the carbon rows upon roll formation has not been fully utilized in this regard. Importantly, tubes with different chiral vectors have different band gaps, which give rise to their potential use in multiplexing applications.

On the other hand, flavin mononucleotide (FMN, Figure 1a), a phosphate analogue of vitamin B₂, is a well-known

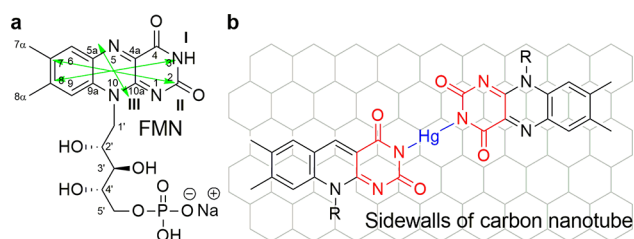


Figure 1. (a) Chemical structure of FMN. Roman characters and green arrows indicate transition dipoles I, II, III, and their directions, corresponding absorption bands at 447, 371, and 271 nm, respectively. (b) Proposed 2:1 mode of Hg^{2+} binding to FMN on the sidewalls of the SWNT. R denotes a D-ribityl phosphate group.

cofactor for numerous oxidoreductases.²¹ Specifically, upon sonochemical dispersion, FMN containing a chiral D-ribityl phosphate group has been shown to wrap carbon nanotubes in a helical self-assembly to produce FMN–SWNT, which is maintained by quadruple hydrogen bonding between opposing isoalloxazines, π – π interaction between isoalloxazine and SWNT sidewalls²² (Figure 1b), and anionic D-ribityl phosphate endowing colloidal dispersion.²³ Such helical wrapping differentiates SWNTs according to (n, m) chirality,^{24–27} handedness (i.e., left and right),^{28–30} and electrical type (semiconducting vs metallic tubes)^{25,31,32} in terms of binding affinity with co-surfactants. Those distinguished features discerned from other surfactants originate from the formation of a positive (P)-FMN helix on SWNT.^{28,29} In addition, FMN is extended to disperse two-dimensional materials including graphene^{33,34} and hex-

agonal boron nitride.³⁵ More recently, a flavin motif on SWNT has been utilized for thin-film transistors,³¹ composite materials,³⁶ photovoltaics,³⁷ and electrocatalysts.^{38,39} Moreover, flavin derivatives are known to be adsorbed onto a mercury electrode⁴⁰ and are possible to be utilized as Hg probes.

In the investigation described below, we developed a sensor system (FMN–SWNT), containing FMN pairs bonded in a helical motif to SWNTs, that selectively binds Hg^{2+} . Specifically, among nine metal ions, FMN–SWNT selectively detects Hg^{2+} by undergoing changes in the intensity and wavelength of near-IR (NIR) PL. Information about the sensing mechanism, gained using FMN as a model, showed that a 2:1 complex with Hg^{2+} is generated at high FMN concentrations. The proposal that Hg-bridged FMN pairs are formed on FMN–SWNT was assessed using Fourier transform infrared (FT-IR) spectroscopy and high-resolution transmission electron microscopy (HRTEM). Furthermore, Hg^{2+} titration of FMN–SWNT was found to promote multiple changes in the PL intensity and wavelength, which along with the Hill equation can be used to determine binding affinities. The origin of multiple modes of binding Hg^{2+} to FMN–SWNT was explored utilizing density functional theory (DFT) calculations. Finally, binding of methyl mercury chloride to FMN–SWNT was examined.

RESULTS AND DISCUSSION

In the initial phase of this study, we assessed the use of the FMN–SWNT complex for selective sensing of Hg^{2+} . FMN–SWNT was prepared by sonochemical debundling of powdery HiPco SWNT²³ having a median diameter (d_t) of ca. 1 nm (see Methods). Briefly, a mixture of FMN and HiPco SWNT in water was probe-sonicated followed by 80 000g ultracentrifugation to obtain a well-dispersed SWNT sample. The 80% supernatant, containing FMN–SWNT, was collected and used for metal ion detection studies. Addition of 0.5 equiv of chloride salts containing Fe^{3+} , Cr^{3+} , Mg^{2+} , Mn^{2+} , Pb^{2+} , Co^{2+} , Ni^{2+} , and Zn^{2+} to solutions of FMN–SWNT resulted in formation of black precipitates (Figure 2a), indicating that aggregation of the SWNT took place. Although appearing transparent, the solution resulting from Pb^{2+} addition contains an SWNT precipitate (see the zoom-in photograph in Figure S1). The formed mixtures were vigorously hand-shaken to disperse the precipitate and then subjected to absorption spectroscopic measurements. In contrast, addition of HgCl_2 to a solution of the FMN–SWNT does not result in the appearance of a black precipitate. Compared with the samples containing the FMN–SWNT and other metal ions, the one resulting from Hg^{2+} addition has absorption maxima, corresponding to the FMN chromophore at 267 and 446 nm⁴¹ (Figure 2b), that stem from the oxidized form of FMN²¹ and are greatly red-shifted by 5 and 3 nm, respectively (Figure 2c). Considering a sodium phosphate salt of FMN acting as a pH buffer,²⁶ this result indicates that Hg^{2+} ion binds with FMN groups that are present in FMN–SWNT.

To gain evidence for this proposal, Hg^{2+} and the other metal ions were added to solutions containing the same concentration of noncomplexed FMN. Similar observations made when using FMN–SWNT were made using FMN alone. For example, addition of chloride salts containing Pb^{2+} , Zn^{2+} , Fe^{3+} , and Cr^{3+} (0.7 mM) to 1.2 mM FMN led to formation of turbid mixtures (Figure S2a), suggesting FMN aggregation. Moreover, the solution created by adding HgCl_2 displays an

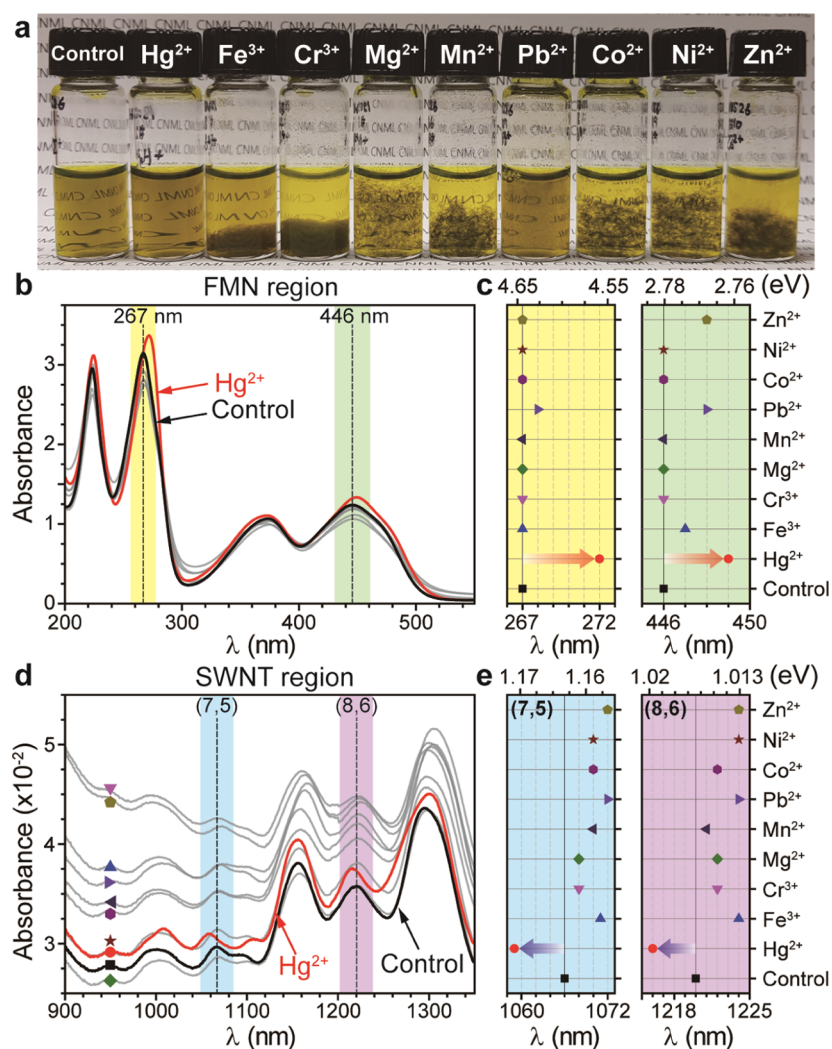


Figure 2. (a) Photograph of FMN-HiPco dispersions after the addition of 0.5 equiv of various metals in which FMN concentration was 1.2 mM. (b) Absorption spectra of the FMN region of FMN-HiPco using a cuvette with 1 mm path length. (c) Corresponding absorption maximum shifts of an FMN sample after the addition of 2:1 molar ratios of metal ions as initial wavelengths at 267 nm (left) and 446 nm (right). The absorption spectra were recorded by shaking the precipitated samples. Symbols denote metal ions. (d) NIR absorption spectra of SWNT regions. (e) Corresponding absorption maximum shifts of (7, 5) and (8, 6) chiral vector peaks at 1066 and 1220 nm, respectively.

absorption maximum at 449 nm (Figure S2b) that is 3 nm red-shifted from that of the corresponding band in FMN (Figure S2c). Solutions arising from addition of Fe^{3+} , Cr^{3+} , and Pb^{2+} exhibit absorption bands that are slightly red-shifted as a consequence of FMN aggregation caused by interactions of these multivalent metal ions with the FMN phosphate group.⁴² The absorption maxima of solutions created by addition of the other metal ions remain the same (ca. 446 nm) as that of FMN. The result suggests that Hg^{2+} selectively binds to the isoalloxazine ring of FMN.

Changes in the NIR absorption region of the FMN-SWNT caused by the presence of metal ions were also monitored. The HiPco nanotube undergoes electronic transitions (E_{ij} , where i denotes the transition order) in the NIR region stemming from E_{11} .⁴³ Because they correspond to unique (7, 5) and (8, 6) chiral vectors,²³ the respective maxima at 1066 and 1220 nm in the NIR spectrum were employed to determine the effects of metal ions (Figure 2d). Addition of various metal ions other than Hg^{2+} to solutions of FMN-SWNT (gray traces) promotes up to 6 nm bathochromic shifts in the 1066 and 1220 nm bands. As can be seen by viewing the spectra

recorded using various metal concentrations, shown in Figure S3a–i, red shifts occur over a wide metal concentration range. Because addition of these metal ions to solutions of the FMN-SWNT leads to precipitation, the observed red shifts are a consequence of formation of SWNT bundles. Notably, it was reported earlier that addition of metal-chloride salts induces SWNT aggregation⁴⁴ and results in well-known red shifts of SWNT absorption bands.⁴⁵ In contrast, addition of Hg^{2+} (red trace) causes 7 and 4 nm hypsochromic shifts of the respective 1066 and 1220 nm bands. This result indicates that Hg^{2+} ions bind to FMN-SWNT in a manner that is different from that of other metal ions.

The isoalloxazine group contains a ring that bears a structural resemblance to thymine, which has been demonstrated to selectively detect mercury ions by forming the triad T- Hg^{2+} -T (the red structure in Figure 1b).^{6,8,18,46} Thus, the selectivity of FMN must originate from the ability of the nitrogen bases in the thymine-like group to selectively capture Hg^{2+} over other metal ions in the form of an FMN- Hg^{2+} -FMN triad. To gain information to support the proposed binding mode of Hg^{2+} to FMN in FMN-SWNT, a titration

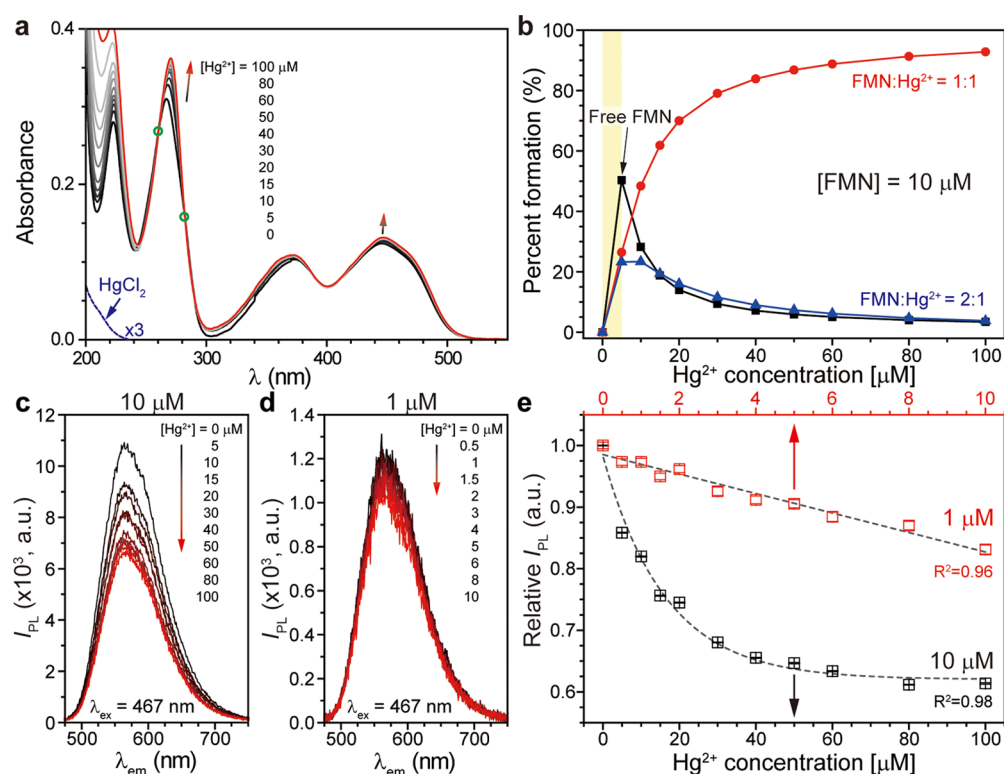


Figure 3. (a) Absorption spectroscopic changes upon the titration of HgCl_2 against 10 μM FMN solution. Green circles denote isosbestic points. The absorption spectrum of 50 μM HgCl_2 (blue) is also given. (b) Fitted plots of percentages of complex formation from 10 μM FMN with increasing Hg^{2+} concentrations. Corresponding PL spectroscopic changes upon titration of (c) 10 μM and (d) 1 μM FMN solutions. $\lambda_{\text{ex}} = 467$ nm. Excitation slit size: 0.05 nm. (e) Normalized I_{PL} vs Hg^{2+} concentration plot. Dotted lines are fitted curves.

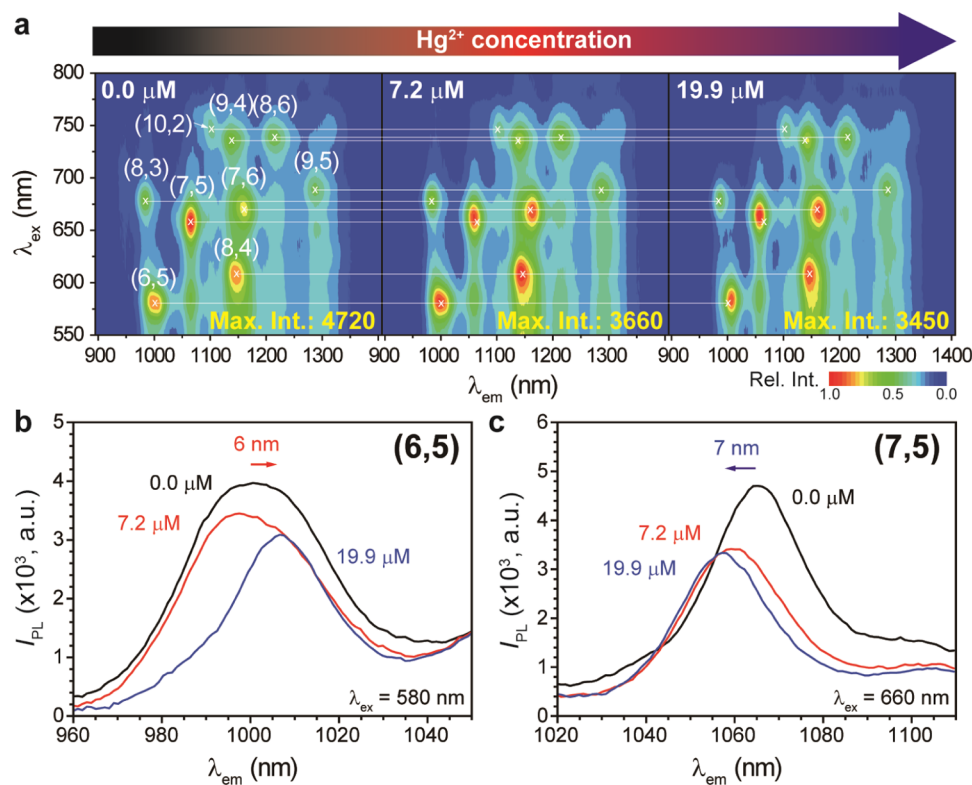


Figure 4. NIR PL changes of FMN–SWNT upon the addition of Hg^{2+} . Intensity-normalized PLE map changes of FMN–SWNT (a) before and after addition of 0, 7.2, and 19.9 μM Hg^{2+} . Note that maximum intensities are listed at the bottom and white lines marked by X are given to guide the initial position of each SWNT chiral vector. The corresponding PL emission spectroscopic changes of (b) FMN–SWNT (6, 5) and (c) FMN–SWNT (7, 5).

was carried out by adding HgCl_2 to an aqueous solution of the 10 μM model FMN while monitoring absorption spectroscopic changes (Figure 3a). The absorption bands of FMN experienced a gradual bathochromic shift as the Hg^{2+} concentration in the solution increases, with clear isosbestic points (denoted by circles) at 261 and 282 nm. The Hg^{2+} –FMN binding stoichiometries and association constants were estimated using the spectroscopic data (Figure 3b) and a nonlinear curve fitting analysis using commercially available Hypspec (HySS) software.⁴⁶ The results show that a 1:2 complex is formed between a Hg^{2+} ion and FMN through the intermediacy of a 1:1 complex and that the first and second association constants are 5.65 and 10.61 M^{-1} , respectively.

Because FMN has a strong binding affinity to the sidewalls of SWNT with a binding energy as high as 2 eV by theoretical calculations,⁴⁷ the local concentration of FMN on the SWNT is much greater than that of noncomplexed FMN in the dispersion. To understand binding modes at possible higher FMN concentrations like on SWNT, we were able to estimate the extents of complex formation at Hg^{2+} concentrations of 100 and 1000 μM using HySS software (Figure S4a,b, respectively). The simulation indicates that the percentage of formation of the 1:2 complex between Hg^{2+} and FMN would be 62 and 86% when 0.5 equiv of a Hg^{2+} ion are added to 100 and 1000 μM solutions of FMN, respectively.

PL spectra of 10 μM FMN solutions were also recorded during titrations with Hg^{2+} (Figure 3c). PL of FMN has a maximum at 566 nm. Sequential addition of Hg^{2+} to a 10-fold equivalent excess over FMN results in a ca. 39% decrease in the PL intensity (I_{PL}). When 1 μM FMN is used (Figure 3d), addition of a 10-fold excess of Hg^{2+} leads to a ca. 17% decrease in the PL intensity. Also, addition of Hg^{2+} to a 1 μM solution of FMN causes a linear decrease in I_{PL} with increasing Hg^{2+} concentration (the red trace of Figure 3e), whereas addition of this metal ion to a 10 μM FMN solution results in an exponential decrease in the PL intensity. Moreover, the fact that addition of a 10-fold excess of Hg^{2+} , which should promote predominant formation of the 1:1 complex, does not completely quench the PL of FMN suggests that, along with chemical bonding with FMN, Hg^{2+} might be involved in modulation of the aggregation state of FMN.^{48,49} $\text{Hg}(\text{II})$ is known to bind with bases of DNA and RNA,⁵⁰ resulting in quenching of nucleotide PL owing to the heavy-atom effect.⁵¹ This is the primary reason for quenching of FMN PL. Moreover, a higher FMN concentration is known to facilitate FMN aggregates such as a dimer,⁴⁹ which alters the excitation profile of FMN, might be the reason for different I_{PL} decreases from different FMN concentrations.

The absorption spectroscopic measurements described above were made only on the (7, 5) and (8, 6) SWNT chiral vectors. To further pursue this issue, we utilized photoluminescence excitation (PLE) data to track precise E_{22} and E_{11} positions of other SWNT chiral vectors. For this purpose, a map consisting of emission spectra recorded using different excitation wavelengths (λ_{ex})⁴³ was obtained. The PLE maps obtained before and after addition of 0, 7.2, and 19.9 μM Hg^{2+} , concentrations that are well below 0.5 equiv relative to FMN, are shown in Figure 4a. Initially, we observed that I_{PL} of the SWNT decreases by nearly 30% upon addition of higher concentrations of Hg^{2+} and that the distribution of I_{PL} associated with different chiral vectors of the SWNT remains largely unchanged. A closer inspection of the maps reveals that peak shifts occur in SWNT with different chiral vectors. For

instance, addition of 7.2 μM Hg^{2+} , E_{11} of (6, 5) (Figure 4b), induces a blue shift of this band, whereas addition of 19.9 μM causes a red shift. Moreover, E_{11} of (7, 5) (Figure 4c) exhibits a continuous blue shift upon addition of higher concentrations of this metal ion. Finally, peaks associated with the other SWNT chiral vectors (Figure S6a–g in the SI) undergo lesser degrees of peak shift.

To determine the origin of spectral changes taking place during these titrations, a series of PLE maps were generated using Hg^{2+} concentration intervals as small as 0.6 μM . The changes occurring during the titrations, illustrated in Figure 5a

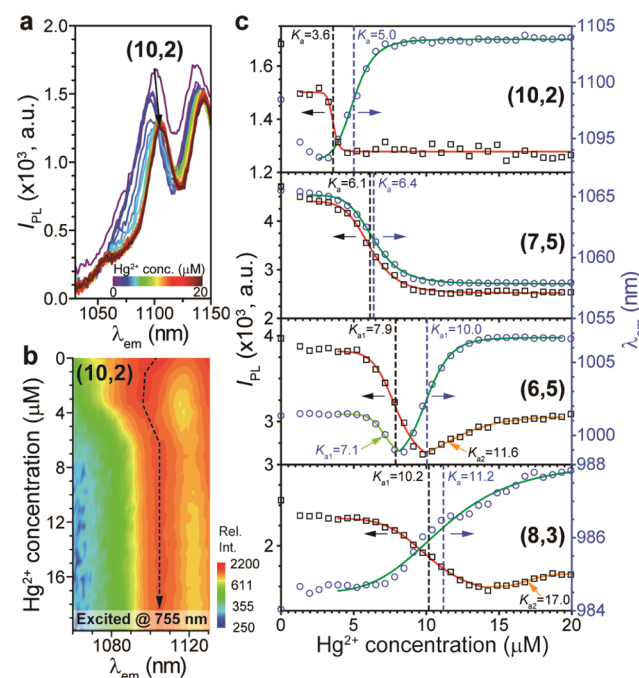


Figure 5. PL spectroscopic changes of FMN–SWNT upon titration with Hg^{2+} . (a) PL spectroscopic change of FMN–SWNT (10, 2) upon titration with Hg^{2+} . (b) Corresponding contour map of PL spectra of SWNT (10, 2). (c) Overlaid plot of I_{PL} and peak position of four SWNT chiral vectors upon titration [from the top to the bottom: (10, 2), (7, 5), (6, 5), and (8, 3)].

for the SWNT with a (10, 2) chirality vector, clearly show that both I_{PL} and peak position decrease as the concentration of Hg^{2+} increases. Inspection of the contour map of the PL spectrum (Figure 5b) demonstrates that the peak, after experiencing the initial hypsochromic shift, undergoes a bathochromic shift from 1093 to 1102 nm as the Hg^{2+} concentration nears 4 μM . Also, an overlay of I_{PL} and peak position plots (Figure 5c) shows that the I_{PL} and concurrent peak shift of the SWNT with the (10, 2) chiral vector display nearly parallel sigmoidal changes.

The binding affinity of Hg^{2+} to FMN was quantitatively assessed using the I_{PL} and peak position data and the Hill equation, $\theta = \left[\left(\frac{K_a}{[\text{titrant}]} \right)^\gamma + 1 \right]^{-1}$, where K_a is the mid-point of the sigmoidal transition, θ is the fraction based on the I_{PL} and peak shift caused by addition of Hg^{2+} to the FMN–SWNT, and γ is the Hill coefficient representing the cooperativity displayed by Hg^{2+} . The I_{PL} and peak position changes of the FMN complex of the SWNT having the (10, 2) chiral vector is well fitted employing a sigmoidal curve (solid lines in the top

plot in Figure 5c). The results of this analysis indicate that although K_a , derived using the I_{PL} plot, is $3.6 \mu\text{M}$, K_a arising from the peak shift data is $5.0 \mu\text{M}$. An extension of this analysis to other SWNT chiral vectors including (7, 5), (6, 5), and (8, 3) was conducted using the PL spectroscopic change shown in Figure S7. Analysis of the other plots, given in Figure 5c, shows that K_a values derived from I_{PL} and peak positions are slightly different and that the values exhibit complex behaviors. Several common trends arising from this analysis include the following: (i) independent of their chiral vectors, all tubes exhibit a decrease in I_{PL} with increasing Hg^{2+} concentrations even though the directions of their peak shifts vary [i.e., red shift: (10, 2), (6, 5), and (8, 3); blue shift: (7, 5)]; (ii) the K_a values derived from I_{PL} data are always smaller or are nearly equal to those derived from peak shift data; and (iii) the γ values are greater than 2 and are often larger than 20 (Table S1), indicating cooperative binding of Hg^{2+} to the FMN–SWNT. In some cases, as exemplified by the (6, 5) tube, plots of peak positions display double sigmoidal behavior, which is interpreted in terms of two binding constants, K_{a1} and K_{a2} . A comparison of their binding affinities with Hg^{2+} shows that the tube with the (10, 2) chiral vector has the smallest K_a (i.e., $3.6 \mu\text{M}$), whereas others have K_a values as large as $10.2 \mu\text{M}$ [e.g., for the (8, 3) tube] and display variations in γ . Table S2 contains a summary of the energies, E_{11} and E_{22} , for the initial, minimum, and maximum values of several SWNT tubes with different chiral vectors after Hg^{2+} addition. Most of the PL tubes exhibit minimum and maximum peak positions other than the initial position.

Binding of Hg^{2+} to FMN–SWNT was also explored using FT-IR spectroscopy (Figure 6a). For the first phase of this

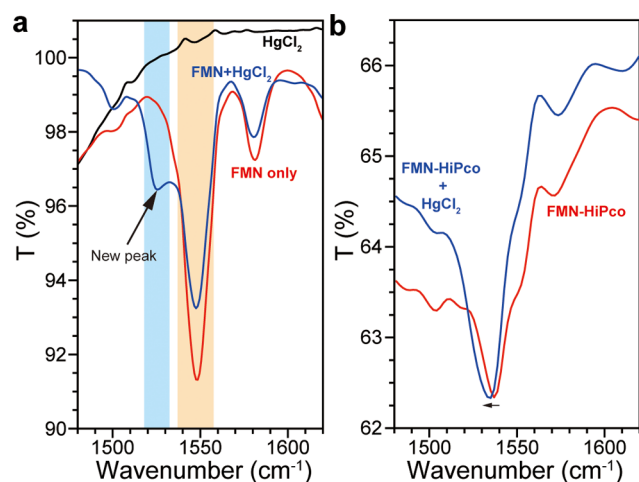


Figure 6. FT-IR spectra of (a) FMN in D_2O and (b) FMN–HiPco films in the presence and absence of 0.5 equiv of Hg^{2+} along with that of HgCl_2 in D_2O .

analysis, spectra were recorded using D_2O solutions of $10 \mu\text{M}$ FMN only and a 2:1 molar ratio of $10 \mu\text{M}$ FMN and HgCl_2 . FMN exhibits a strong FT-IR absorption band at 1548 cm^{-1} and a relatively weak band at 1581 cm^{-1} , which have been assigned to respective $\text{N}(5)=\text{C}(4a)$ and $\text{C}(10a)=\text{N}(1)$ stretching transitions using isotope-exchanged FT-IR experiments^{22,52} (see Figure 1a for the numbering). Upon addition of 0.5 equiv of Hg^{2+} , a new band arises at 1525 cm^{-1} . The results of FT-IR titration experiments and, specific measurements of the area ratios of the 1525 and 1548 cm^{-1} peaks,

indicate that the approximate ratio of free FMN to the FMN–Hg complex is 2:1 when the ratio of FMN to Hg^{2+} is 2:1, in agreement with the absorption spectroscopic measurements. Similarly, the FT-IR spectrum (Figure 6b) of a film of the FMN–SWNT, prepared by filtration of a 10 mL dispersion of FMN and HiPco to remove free FMN, contains bands at 1571 and 1537 cm^{-1} . In the presence of Hg^{2+} , the 1539 cm^{-1} band shifts to 1534 cm^{-1} , which is similar to the band shift occurring upon addition of HgCl_2 to FMN.

To support the observations of the Hg^{2+} -promoted shift to lower frequencies of the FT-IR band of FMN–SWNT, DFT calculations at the B3LYP level (see Methods) were carried out using the structurally simpler analogue lumiflavin (LF) instead of FMN. As can be seen by viewing simulated spectra of LF and LF–Hg–LF (Figure S8 of SI), generated using the calculations, a low-frequency shift of the $\text{C}=\text{N}$ stretching band takes place upon formation of the 2:1 complex, which is in qualitative agreement with the experimental FT-IR results.

DFT calculations were further utilized to gain additional information about the nature of Hg^{2+} binding to FMN in the FMN–SWNT. The geometry-optimized structures of LF–LF, LF–Hg–LF, and the LF–Hg–Cl pair are given in Figure 7a–c, which are the respective models of noncomplex and its 2:1 and 1:1 complexes with Hg^{2+} . In the optimized structure of LF–LF (Figure 7a), the LF rings are nearly coplanar and the $\text{N}(3)–\text{N}(3)$ distance ($3.92 \text{ \AA}$) is short owing to the presence of two $\text{C}=\text{O} \cdots \text{H}$ hydrogen-bonding interactions. In contrast, the LF rings in LF–Hg–LF (Figure 7b) are nearly perpendicularly oriented with respect to each other, and the $\text{N}(3)–\text{N}(3)$ distance is $4.1 \text{ \AA}$. The coplanar version of LF–Hg–LF has a similar electronic energy (see Figure S9 and Table S3 in the SI), which is the case on SWNT. Likewise, the structure of the LF–Hg–Cl pair (Figure 7c) is characterized by out-of-plane protrusion of the chlorides in a near-diamond-like four-membered ring with a Hg–Cl length of $2.6 \text{ \AA}$, suggesting the existence of a substantial interaction between Hg and Cl. The $\text{N}(3)–\text{N}(3)$ distance in the LF–Hg–Cl pair is $8.0 \text{ \AA}$, a value that is much longer than those in LF–LF and LF–Hg–LF. Using the geometry-optimized structures, three LF complexes of Hg^{2+} on the SWNT having an (8, 6) chiral vector (Figure 7d–f) were constructed using an 8_1 helix motif following a procedure described previously.²³ The results show that the $\text{N}(3)–\text{N}(3)$ distance among LF groups in LF–LF on the SWNT is slightly elongated to $4.2 \text{ \AA}$ owing to the sliding of the isoalloxazine rings and introduction of quadruple hydrogen bonding. However, the $\text{N}(3)–\text{N}(3)$ distance in LF–Hg–LF is unchanged when bound to the SWNT. Moreover, binding of chloride to LF–LF, forming the 1:1 complex LF–Hg–LF, results in a similar distance like that in the LF–Hg–Cl pair. These variations should promote large changes in optical properties because the excitonic nature of the SWNT is highly sensitive to the local dielectric constant.⁵³ Also, the conformational changes experienced by the LF pair are the likely reason for the PL variation observation for complex formation between Hg^{2+} and FMN–SWNT.

High-resolution transmission electron microscopy (HRTEM) images were acquired to assess Hg^{2+} binding to FMN–SWNT. HRTEM images of FMN–HiPco in the absence (a and c) and presence (b and d) of $36 \mu\text{M}$ Hg^{2+} are given in Figure 8a–d. Both the HRTEM images of FMN–SWNT (Figure 8a) and Hg^{2+} -added FMN–SWNT (Figure 8b) produced following 2% uranyl acetate staining exhibit individualized SWNTs decorated with FMN helices (insets,

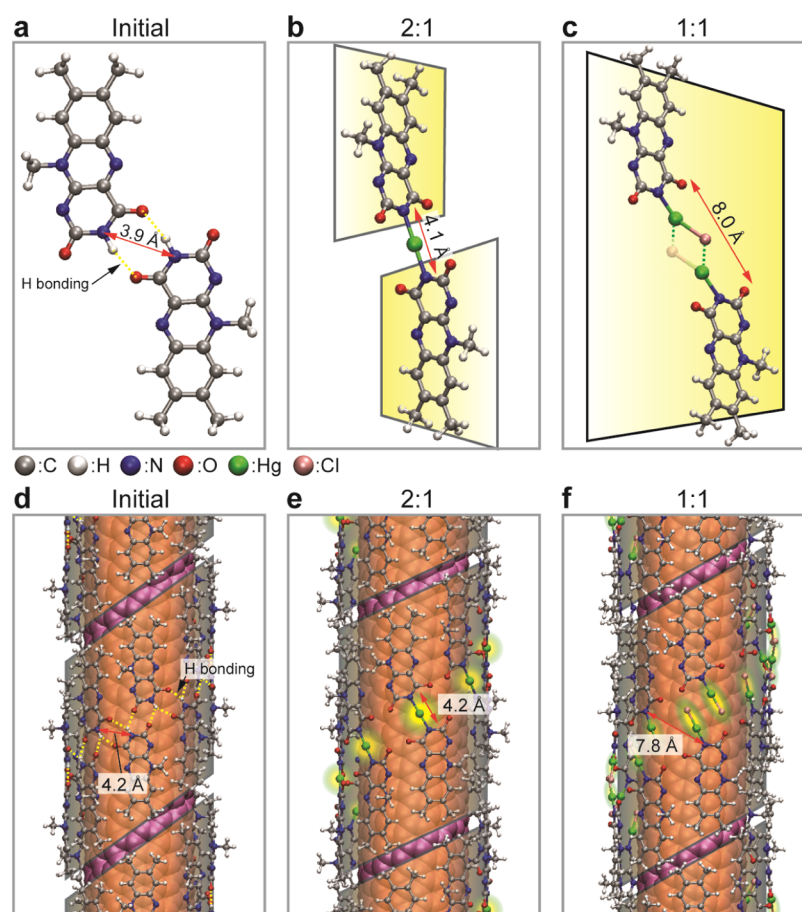


Figure 7. Geometry-optimized structures of (A) LF pair, (B) LF–Hg–LF, and (C) LF–Hg–Cl pair. (D–F) Corresponding structure of LF with SWNT (8, 6) constructed using an 8_1 helix where the unit length is ca. 2.5 nm.

and see additional images in Figure S10a–d). Upon a closer investigation of the insets, the spacing among helical wraps on SWNT is found to be ca. 5.0 nm (indicated by an arrow), which is about twice that of the unit length of an FMN 8_1 helix (i.e., 2.5 nm). This periodic undulation originates from the collapsed conformations of adjacent D-ribityl phosphate moieties.²³ For the unstained cases, although the image from FMN–SWNT (Figure 8c), generated in the absence of uranyl acetate staining, exhibits a vague contrast along SWNT, the image recorded on the complex of Hg^{2+} with FMN–SWNT (Figure 8d) displays vivid ca. 2.5 nm spaced undulations. Further changes of an FMN helix on SWNT when Hg^{2+} is present are hampered by the resolution limit (i.e., ± 0.4 nm). The results indicate that higher-atomic-number Hg serves as a staining agent for FMN–SWNT.

The question about why the changes in PL wavelength and I_{PL} occurring upon addition of Hg^{2+} indicate the existence of different K_a values is interesting. As determined using absorption-based mercury titrations, the predominance of complexes of FMN–SWNT having 2:1 and 1:1 binding modes with Hg^{2+} depends on the local FMN concentration. The preferential affinity between the isoalloxazine ring and SWNT sidewalls⁴⁷ promotes high concentration of FMN on SWNT as compared with that of free FMN in solution. That the high local concentration of FMN favors 2:1 binding suggests that titration using very small concentration intervals might reveal the existence of two binding modes. For this purpose, Hg^{2+} titration carried out by following PL from FMN–(10, 2) was

conducted using concentration intervals as small as $0.08 \mu\text{M}$. The results, shown in the overlays of I_{PL} and peak position (Figure S11a), reveal that an additional weak sigmoidal region exists prior to the large sigmoidal behavior occurring at $8.3 \mu\text{M}$. As a result, the K_a values found between I_{PL} and the first shift in peak position become nearly identical. The reason for the smaller increase associated with the first sigmoidal in the plot of peak wavelength seems to result from the similar longitudinal distance between the FMN pair and FMN–Hg–FMN. The results indicate that PL wavelength reflects the existence of both 2:1 and 1:1 binding modes. Moreover, on top of double sigmoidal transitions, initial I_{PL} and peak position changes of SWNT further increase detection level of Hg^{2+} . Figure S11b displays zoomed-in I_{PL} and peak position changes from the FMN–(10, 2) tube with addition of HgCl_2 with ~ 82 nM intervals, showing simultaneously decreased I_{PL} and increased peak wavelength. The initial change of FMN–SWNT prior to the sigmoidal transition was previously ascribed to the imperfect helical organization of FMN on SWNT located near defect sites such as ends of SWNTs.^{25,36} As compared with a stable FMN helix producing sigmoidal binding, less stability of imperfect FMN helix was advantageously utilized to promote changes in I_{PL} and peak position as small as few hundred nanomoles of Hg^{2+} .

The chemical resemblance of FMN to nucleotides further prompts us to investigate circular dichroism (CD) activity. Owing to the low signal, $\sim 33 \mu\text{M}$ FMN in the presence and absence of SWNT and Hg^{2+} was subjected to CD measure-

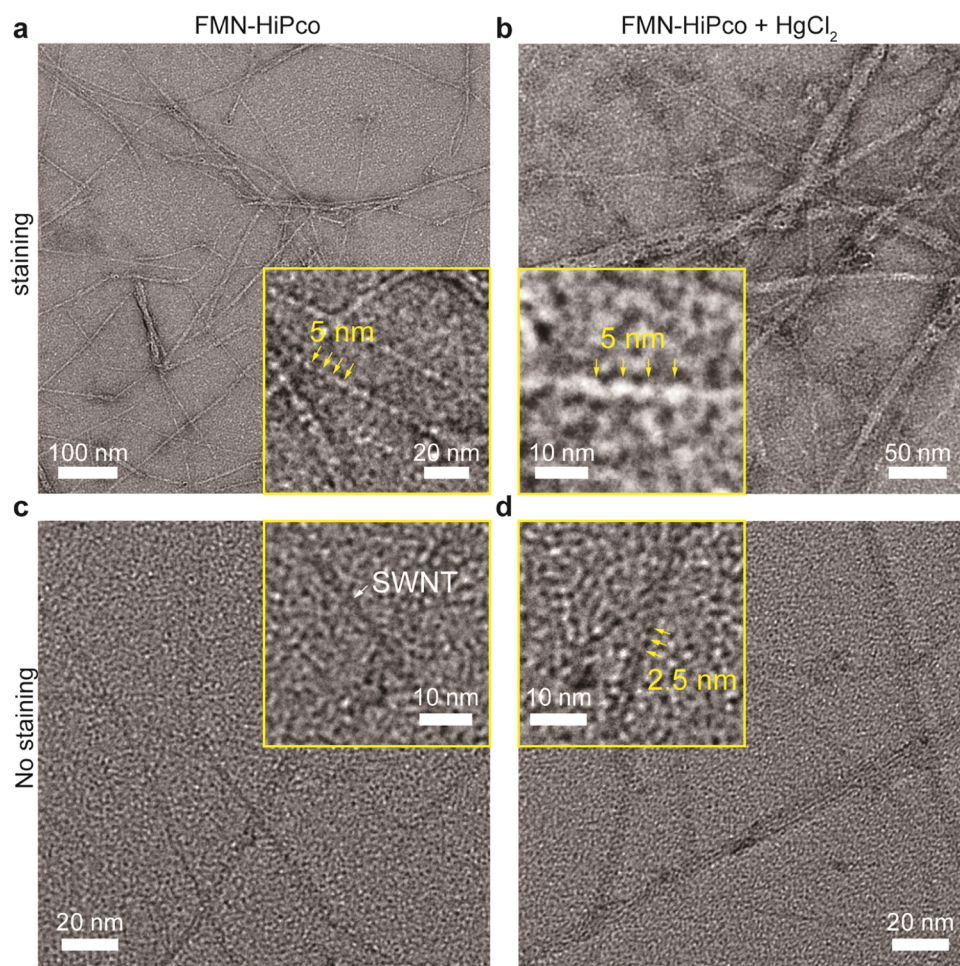


Figure 8. HRTEM images of FMN–SWNT in the presence (b, d) and absence (a, c) of HgCl_2 . Samples for (a) and (b) were stained by 2% uranyl acetate.

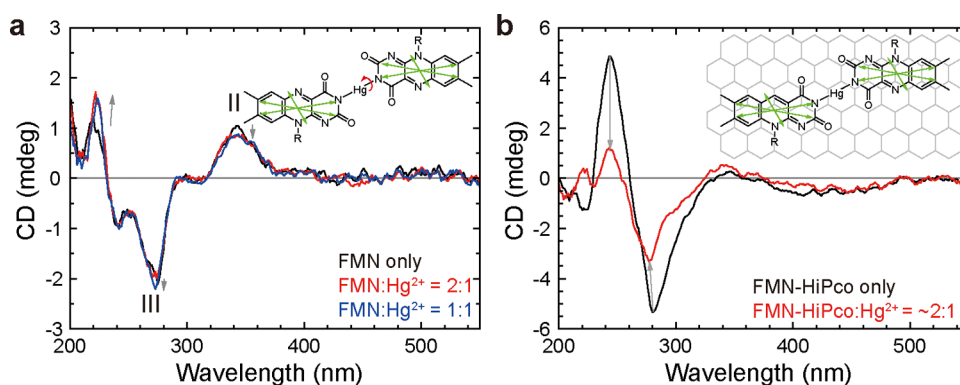


Figure 9. (a) CD spectra of FMN (black) and FMN in the presence of 0.5 equiv (red) and 1 equiv (blue) addition of Hg^{2+} . The inset illustrates transition dipoles of FMN (green arrows) from FMN–Hg–FMN, which has rotational freedom of the Hg axis. (b) CD spectra of FMN–SWNT (black) and FMN–SWNT with nearly 0.5 equiv addition of Hg^{2+} (red). The inset depicts FMN–Hg–FMN on the SWNT surface.

ments. Since D-ribyl phosphate sodium salt is a chiral side chain, it is expected to induce chiral changes of isoalloxazine chromophores upon binding. As depicted by green arrows in Figure 1a, the isoalloxazine group has absorption bands at 271, 371, and 447 nm originating from π – π^* transitions with transition dipoles III, II, and I, respectively.^{41,54} With this in mind, the CD spectrum of the pristine FMN (Figure 9a) displays negative dichroic bands at 241, 273, and 343 nm and positive bands at 223 and 343 nm, in good agreement with

riboflavin analogues.^{55,56} Bands at 273 and 343 nm are derived from transition dipoles III and II, respectively. It is noteworthy that the dichroic band from transition III is the strongest among three transitions, and transition I is nearly absent presumably owing to the vicinity effect of a chiral side chain.⁵⁶ Upon progressive addition of HgCl_2 as 0.5 and 1 equiv to FMN, dichroic bands at 241 and 273 nm are slightly increased and 340 nm band is weakened in both cases. According to exciton theory,⁵⁷ a transition dipole is strengthened or

weakened according to the relative orientation of a chromophore. As mentioned in theoretical calculations, the energetic barrier for the rotation of FMN along the Hg axis is not expensive. Therefore, this result seems to be related with the association of D-ribityl phosphate groups of an FMN–Hg–FMN triad (see the inset of Figure 9a) as seen in 5 nm undulation of TEM measurements, leading to increased transition dipole III and partial canceling of transition dipole II with opposing isoalloxazine dipoles. On the other hand, CD signals of FMN–SWNT dispersion (Figure 9b), which were obtained from a dialysis to eliminate free FMN to produce $\sim 33 \mu\text{M}$ FMN concentration, mainly originate from FMN, displaying positive dichroic bands at 243 and 343 nm and a negative one at 280 nm, whereas SWNT does not contribute CD signals since the racemic mixture of SWNT displays silent CD activity,⁵⁸ which is the case of FMN–SWNT dispersion used in this protocol.²⁹ Interestingly, albeit the similar FMN concentration, the CD signals of ~ 280 nm are about 2–3 times stronger than those of the pristine FMN, whereas ellipticity derived from 343 nm is suppressed. This is rationalized that unlike the case of FMN and the FMN–Hg–FMN triad, which have rotational freedom along the Hg axis, a quadruply hydrogen-bonded FMN...FMN dimer has partially aligned and restricted structure on sidewalls of SWNT, leading to suppression of the 343 nm band owing to the cancellation of transition dipole II. A previous study²⁸ suggested that the energy-minimized hydrogen-bonded FMN helix on SWNT can adapt herringbone-type FMN dimer structure owing to van der Waals interaction, suggesting that transition dipoles II and III might not be completely eliminated. Upon nearly 0.5 equiv addition of a Hg^{2+} ion, overall CD intensity decreases by half while maintaining its spectrum. This result signifies that the centrosymmetric FMN–Hg–FMN triad on SWNT (the inset of Figure 9b) results in the strengthening of the cancellation of transition dipoles II and III as compared with the initial hydrogen-bonded FMN helix on SWNT owing to a covalent bond. CD experiments are well coordinated with the suggested helical structures of FMN–SWNT before and after Hg addition.

In the final phase of this investigation, we investigated binding of FMN–SWNT to methyl mercury chloride (MeHgCl).³ Figure 10a displays representative PL spectra of FMN–SWNT (6, 5) obtained using increasing MeHgCl concentrations. It can be seen by viewing the spectra that the PL band for this tube displays an initial increase and later decrease in wavelength. Note that, unlike for HgCl_2 addition, I_{PL} initially increases upon MeHgCl addition. The I_{PL} behaviors observed during the initial stages of HgCl_2 and MeHgCl titrations suggest that the mercury substituent is responsible for initial I_{PL} behavior of SWNT other than sigmoidal bindings. Analysis of the PL spectra (Figure 10b) shows that, after addition of $65 \mu\text{M}$ MeHgCl , the I_{PL} and PL position changes match those observed using HgCl_2 titration (Figure 5c). In the case of MeHgCl , the K_a value based on sigmoidal transitions in I_{PL} and peak positions is ca. 26 times larger than that of HgCl_2 . This finding demonstrates that the use of FMN–SWNT can be extended to the detection of organomercurials.

CONCLUSIONS

In the effort described above, we explored the selective binding of Hg^{2+} in inorganic mercury and organomercurial compounds to FMN in helical assemblies on single-walled carbon

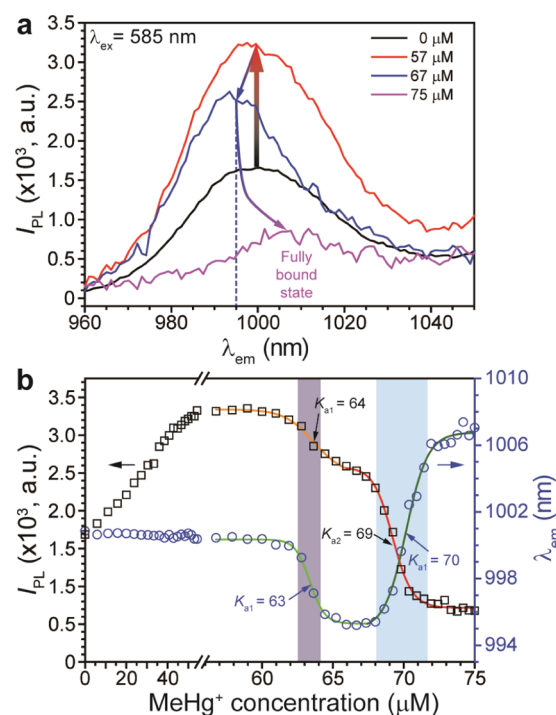


Figure 10. (a) PL spectroscopic changes of FMN–SWNT upon titration with MeHgCl . (b) Simultaneous I_{PL} and PL positions of the FMN-(6, 5) tube upon titration with MeHgCl .

nanotubes. The results, obtained using FMN as a model, demonstrate the existence of a novel scheme for binding Hg^{2+} to an opposing pair of FMN groups. The Hg^{2+} binding events are associated with bathochromic shifts in FMN absorption maxima. The changes show that multiple binding modes exist in which a 2:1 complex between FMN and Hg^{2+} is dominant at high FMN concentrations. Moreover, the binding behavior is more distinct and specific between Hg^{2+} and FMN–SWNT, in which opposing FMN groups constitute helical wrappings around the tubes. Binding of FMN–SWNT with HgCl_2 induces changes in absorption and PL spectra. Conformational changes of the FMN helix on the SWNT promoted by binding Hg^{2+} lead to a sigmoidal decrease of I_{PL} and changes of peak positions. Especially significant, although I_{PL} of FMN–SWNT displays a sigmoidal decrease upon forming a 2:1 complex between FMN and Hg^{2+} , changes in the PL position occur upon forming both 2:1 and 1:1 complexes. The different behavior in the PL intensity and position of the SWNT is proposed to be a consequence of the sequential longitudinal elongation of the FMN pair along SWNT. Furthermore, an imperfect FMN helix on SWNT increases sensitivity up to few hundred nanomoles of Hg^{2+} . Unlike other multivalent metal species, the FMN–SWNT serves as a stable, sensitive, and selective Hg^{2+} probe. NIR emission capability of FMN–SWNT over 1000 nm for detection of Hg^{2+} is especially merited for biological applications as compared with small molecular NIR chromophores whose emissions are typically below 800 nm.⁵⁹ Further enhancement of the detection limit for Hg^{2+} below the nanomolar regime is expected to occur upon the development of FMN-based SWNT thin-film transistors.^{31,60,61} This study also demonstrates the viability of a new strategy to sense mercury ions using a response in the near-IR region, which is potentially useful in biological applications. Finally, the specificity for binding Hg^{2+} to FMN

assemblies on SWNT could provide a corridor to the controlled chemical modification of SWNT sidewalls.

■ EXPERIMENTAL SECTION

Materials and Instrumentation. Flavin mononucleotide (sodium salt form, >95% purity) was purchased from TCI. All metal chlorides, of reagent grade, and either anhydrous (i.e., HgCl_2 , FeCl_3 , PbCl_2 , MgCl_2 , MnCl_2 , and ZnCl_2) or hexahydrates ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$), were purchased from Sigma-Aldrich, Alfa Aesar, Junsei, and Samchun. SWNTs, prepared using a high-pressure CO process (HiPco, raw grade, batch #: R1-831, with d_t distribution 1.00 ± 0.35 nm), were purchased from NanoIntegrus. UV–vis–near-IR (NIR) absorption spectra were recorded on a JASCO V-770 with a cuvette having a 10 mm beam path, unless otherwise noted. FT-IR measurements were conducted with a resolution of 4 or 8 cm^{-1} using a JASCO FT/IR-4700 with either an attenuated total reflection accessory or a CaF_2 flow cell. Transmission electron microscopy (TEM) images were recorded using a JEM-3011 HR and a JEM-1400 (JEOL, Japan). Sample staining was performed using 2% uranyl acetate. The pH of solution was acquired by a SevenMulti pH meter (Mettler Toledo), which, prior to measurement, was calibrated with three known pH references (i.e., pH = 4.01, 7.00, and 10.01).

FMN–SWNT Dispersion and Film. A mixture of 1 mg (0.08 mmol in carbon atom equivalency) of HiPco SWNT and 16 mg (0.03 mmol) of FMN in 4 mL of H_2O was initially bath-sonicated for 1 h and probe-sonicated for 5 h at a 300 W intensity. The resulting solution was centrifuged at 80 000g for 2 h using a swing-bucket rotor (SW 41 Ti, Beckman Coulter). The upper 80% of the supernatant was collected as a clear dark solution. Prior to optical measurements, the upper supernatant was diluted seven times with deionized (DI) water, resulting in ca 1.20 mM FMN. For FT-IR measurements, the upper supernatant (10 mL) was vacuum-filtrated using a 1 in. wide 0.45 μM Teflon filter.

Determining Metal Bindings. FMN–HiPco dispersions and FMN solutions were subjected to metal ion binding determinations. Aliquots (50 μL) of nine metal-chloride solutions (10 mM) were quickly injected into 2 mL of FMN–HiPco dispersions within cuvettes with a 1 mm beam path whose FMN concentration was 1.2 mM. The metal ion concentrations varied as 0.24, 0.48, and 0.70 mM, in which the molar ratios of FMN/metal ions decreased from 4.9 to 1.7, were subjected to optical measurements. Furthermore, a similar screening experiment was conducted using noncomplexed FMN at the same concentration.

Binding Mode Measurements with HgCl_2 and MeHgCl . Various concentrations of FMN in aqueous solutions were used as the host solutions. Aqueous HgCl_2 (200 μM), used as the guest solution, was added in 0.05, 0.10, and 0.20 mL increments to 2 mL of host solutions for absorption and PL intensity measurements. The binding mode of FMN with Hg^{2+} was determined using commercially available Hypspec software. The software was also utilized to simulate binding modes that exist at 100 and 1000 μM . For measurements of organomercurial binding, aliquots from a 0.1 mM stock solution of MeHgCl were added to FMN solutions to generate final concentration intervals as small as 0.4 μM .

PLE Measurements and Titration. PLE measurements were conducted using a Spex Nanolog 3-211 spectrofluorometer equipped with a photodetector in the UV–vis range, a single-channel InGaAs detector for the NIR range, and an iHR320 spectrometer with 150 lines/mm grating. Titrations were conducted by carrying out PLE measurements on solutions formed by adding Hg^{2+} in concentrations in intervals of 800 nM to 3 mL solutions of FMN–SWNT. All titrations were conducted at 293 K, and both the excitation and emission light intensities were corrected against instrumental variations using sensitivity correction factors. For measurement of FMN PL, PL spectra were recorded with various acquisition times (i.e., 0.1 s for 1 and 10 μM ; 1 s for 100 nM). The spectra were well fitted with the Asym2Sig function from the Origin program, and an error bar was obtained from background noise. For the measurement

of SWNT PL, the ranges of the excitation wavelength and emission wavelength were 550–800 nm in 1 or 5 nm steps and 900–1350 nm in 1 nm steps, respectively. The pH values of the solution before and after the titration were 7.2 and 7.0, respectively. The recorded spectra were adjusted by a dilution factor, and each SWNT chirality was deconvoluted with a Lorentzian function to determine the I_{PL} and peak position. The I_{PL} trajectory was fitted using the Hill equation implemented in the Origin program.

Determining Relative K_a Using the Hill Equation. A PLE map array was used to generate concentration-dependent traces of I_{PL} using either the PL positions of SWNT chiral vectors^{23,25} or manual peak picking. K_a and γ of the mercury species were obtained using the Hill function implemented in the Origin program.

DFT Calculation. DFT calculations of LF derivatives are carried out using the Gaussian 09 package.⁶² Geometry optimization and energy minimization were performed using an effective core potential using Def2SVP from B3LYP with a 6-31G(d,p) basis set to incorporate orbitals of a Hg atom.⁶³ Atomistic structure LF-(8, 6) was constructed according to the literature. (8, 6) nanotubes (ca. 2.5 nm in unit length) terminated with hydrogen atoms were wrapped with an LF helix with an 8_1 helix (45° rotation followed by translation of 1/8 times by the unit length of LF derivatives).²³ Visual Molecular Dynamics (VMD) software was used for molecular visualization.⁶⁴ Calculated IR frequencies were broadened by 4 cm^{-1} .

CD Measurements. FMN–SWNT dispersion was dialyzed against DI water to remove free FMN and adjust FMN concentration to ~ 30 μM . CD spectra were recorded by a JASCO J-815. Prior to measurement, the sample chamber was purged with N_2 at room temperature. CD spectra whose maximum absorbances were adjusted below 2.0 by dilution were recorded with 100 nm/min scanning speed and eight times accumulation with a 10 mm quartz cuvette.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.8b18781.

Photograph of FMN–HiPco dispersions after addition of Hg^{2+} and Pb^{2+} (Figure S1); photograph of FMN solution after the addition of various metal ions (Figure S2); absorption spectrum change of FMN–HiPco upon the addition of various metal ions (Figure S3); titration simulation results of Hg^{2+} ions against higher FMN concentrations (Figure S4); PL spectrum change of 10 μM FMN without HgCl_2 addition (Figure S5); representative PL emission of FMN–SWNT in the absence and presence of Hg^{2+} ions (Figure S6); PL spectrum change of FMN–SWNT according to SWNT chiralities during Hg^{2+} titration (Figure S7); simulated vibrational spectra using LF and LF–Hg–LF (Figure S8); chemical structure of LF–Hg–LF whose relative angle is 0° (Figure S9); additional HRTEM images of FMN–SWNT in the presence/absence of Hg^{2+} ions and uranyl acetate staining (Figure S10); overlaid I_{PL} and peak position via fine titration of Hg^{2+} ions against the FMN-(10, 2) tube (Figure S11); K_a and γ of the I_{PL} and peak position trajectories on Hg titration (Table S1); PLE-derived E_{11} and E_{22} positions of FMN–SWNT with and without Hg^{2+} ions according to chiral SWNTs (Table S2); calculated electronic energy of an LF dimer according to binding mode with Hg^{2+} ions (Table S3) (PDF)

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Notes

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Helical Assembly of Flavin Mononucleotides on Carbon Nanotubes as Multimodal Near IR Hg(II) Selective Probe

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Figure S1. Zoomed-in photograph of FMN-HiPco dispersions after addition of Hg^{2+} and Pb^{2+} . Unlike Hg^{2+} added sample, SWNT bundle were observed with the naked eye for Pb^{2+} added sample.

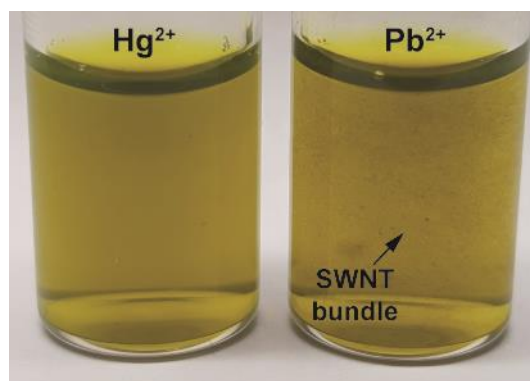


Figure S2. (a) Photograph of vials containing 1.2 mM FMN solution after the addition of various metal ions with 0.7 mM. (b) The absorption spectra of FMN after the addition of various metal ions. (c) Absorption maximum shift of FMN peak at 267 and 446 nm after the addition of metal ions with the molar ratio of 2:1.

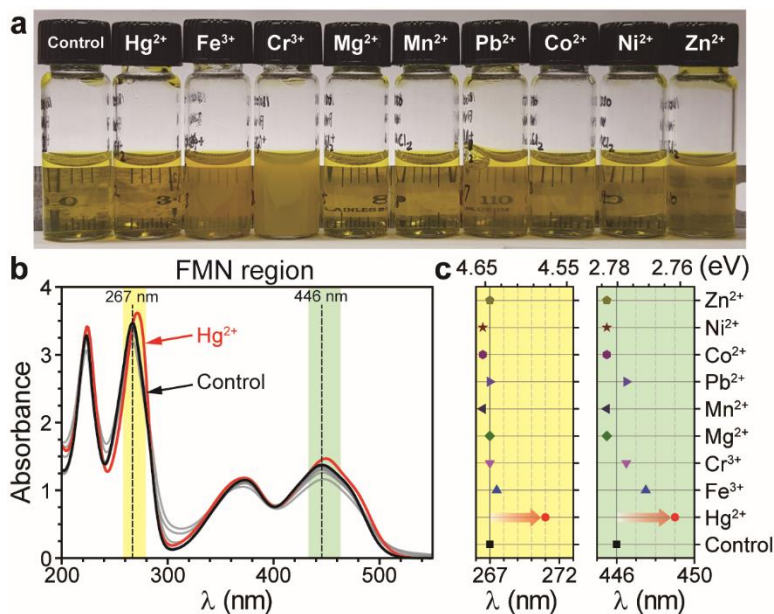


Figure S3. (a-i) Absorption spectrum change of FMN-HiPco upon the addition of various metal ions.

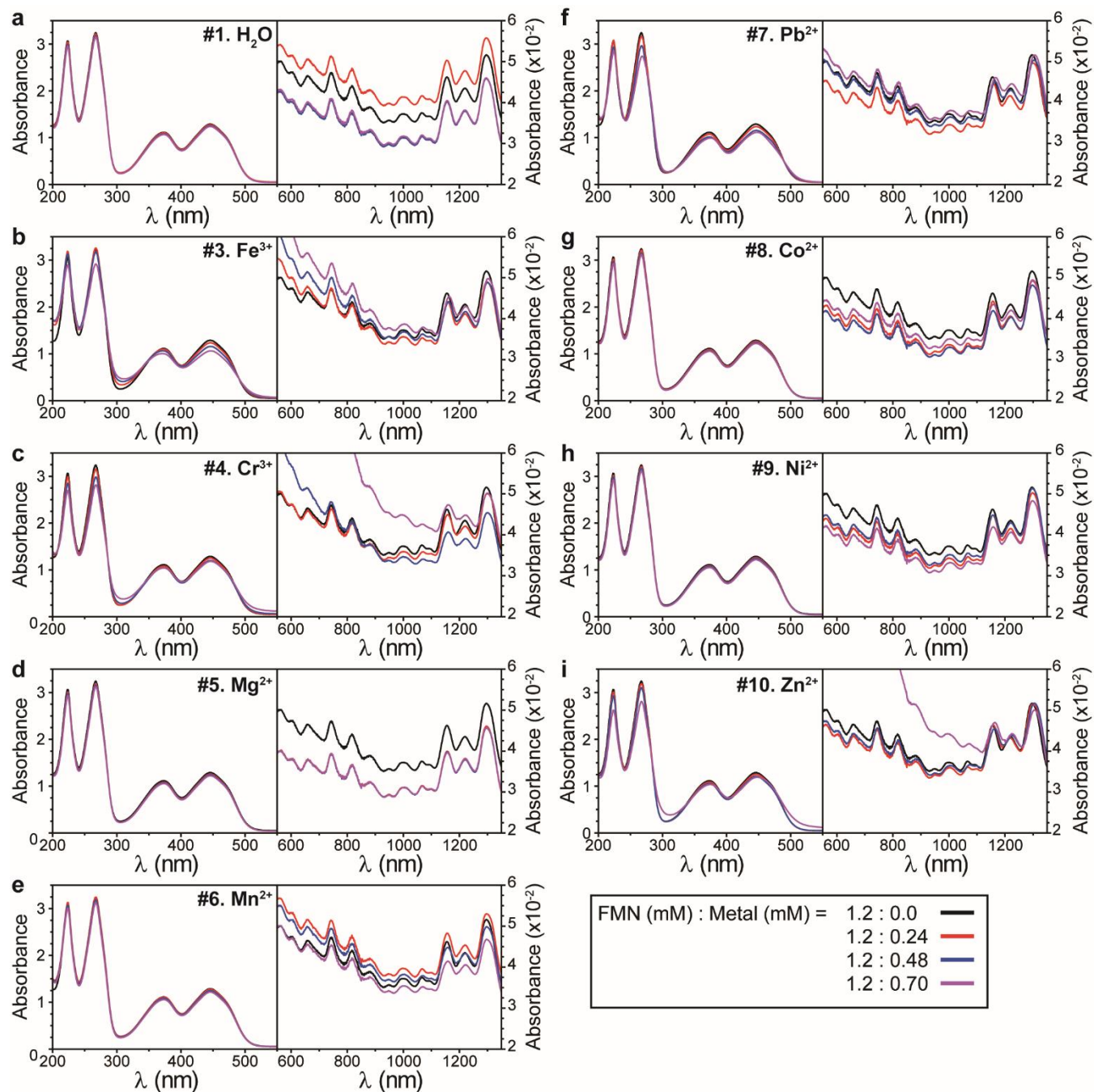


Figure S4. Titration simulation results of Hg^{2+} ion against (a) 100 μM and (b) 1000 μM FMN solutions, respectively.

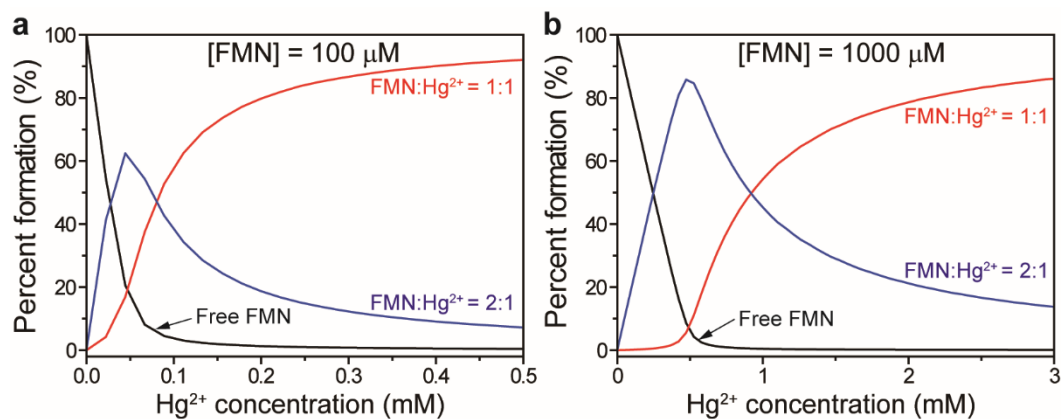


Figure S5. PL spectrum change of 10 μM FMN without HgCl_2 addition. $\lambda_{\text{ex}} = 467 \text{ nm}$. Excitation slit size: 0.05 nm.

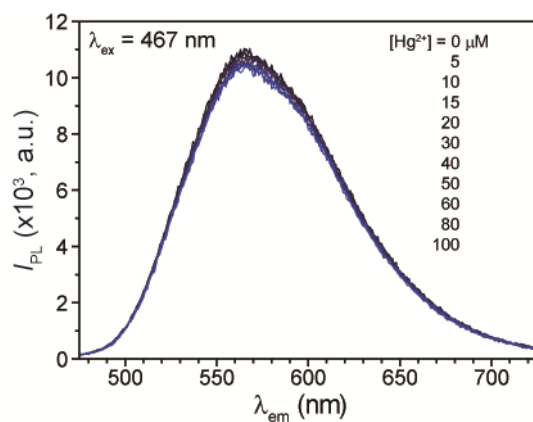


Figure S6. Representative PL emission of FMN-SWNT in absence and presence of Hg^{2+} ion. (a) (8,3), (b) (8,4), (c) (10,2), (d) (7,6), (e) (9,4), (f) (8,6), and (g) (9,5) tubes.

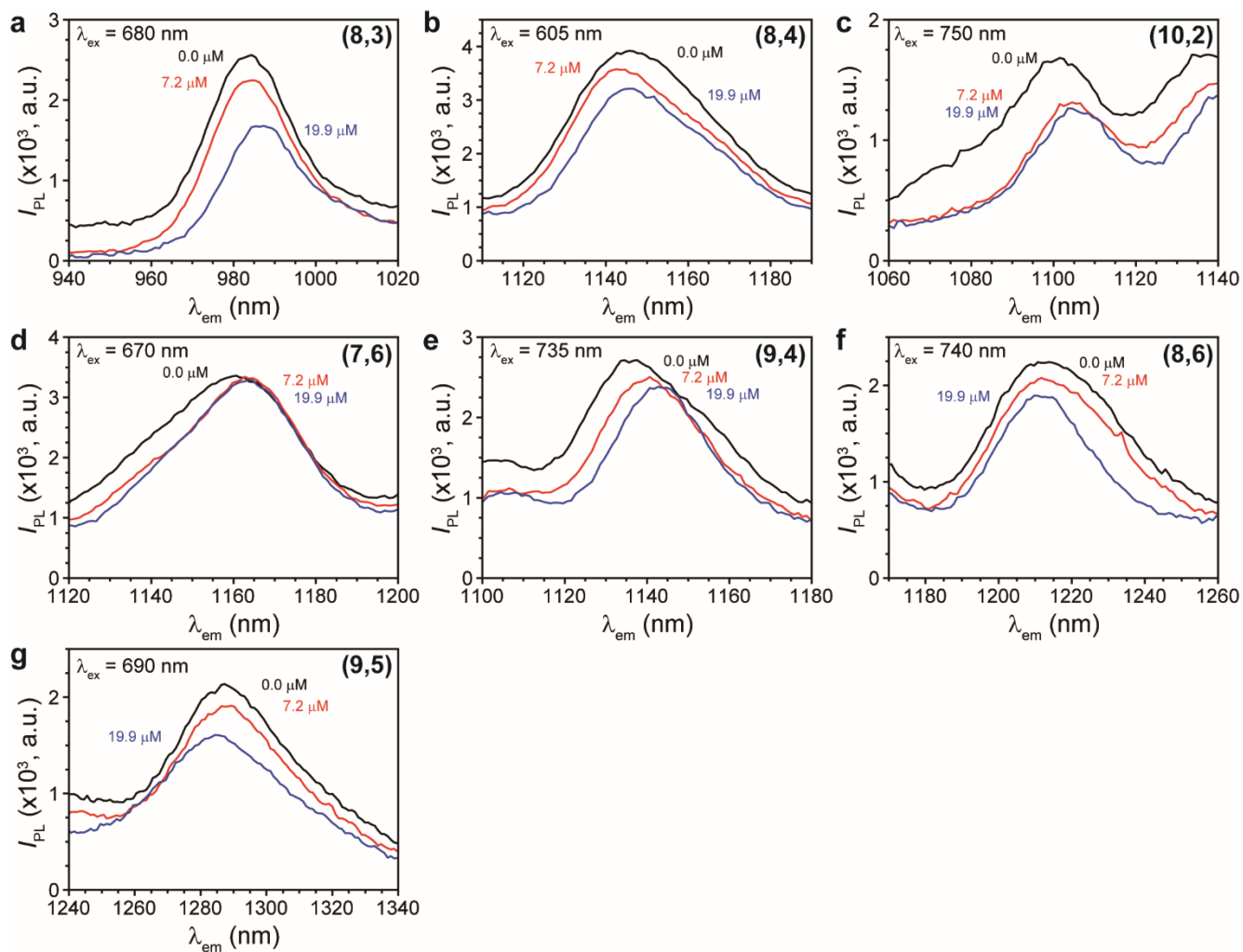


Figure S7. The PL spectrum change of FMN-SWNT according to SWNT chiralities during Hg^{2+} titration. (a) (6,5), (b) (8,3), (c) (7,5), (d) (8,4), (e) (7,6), (f) (9,4), (g) (8,6), and (h) (9,5).

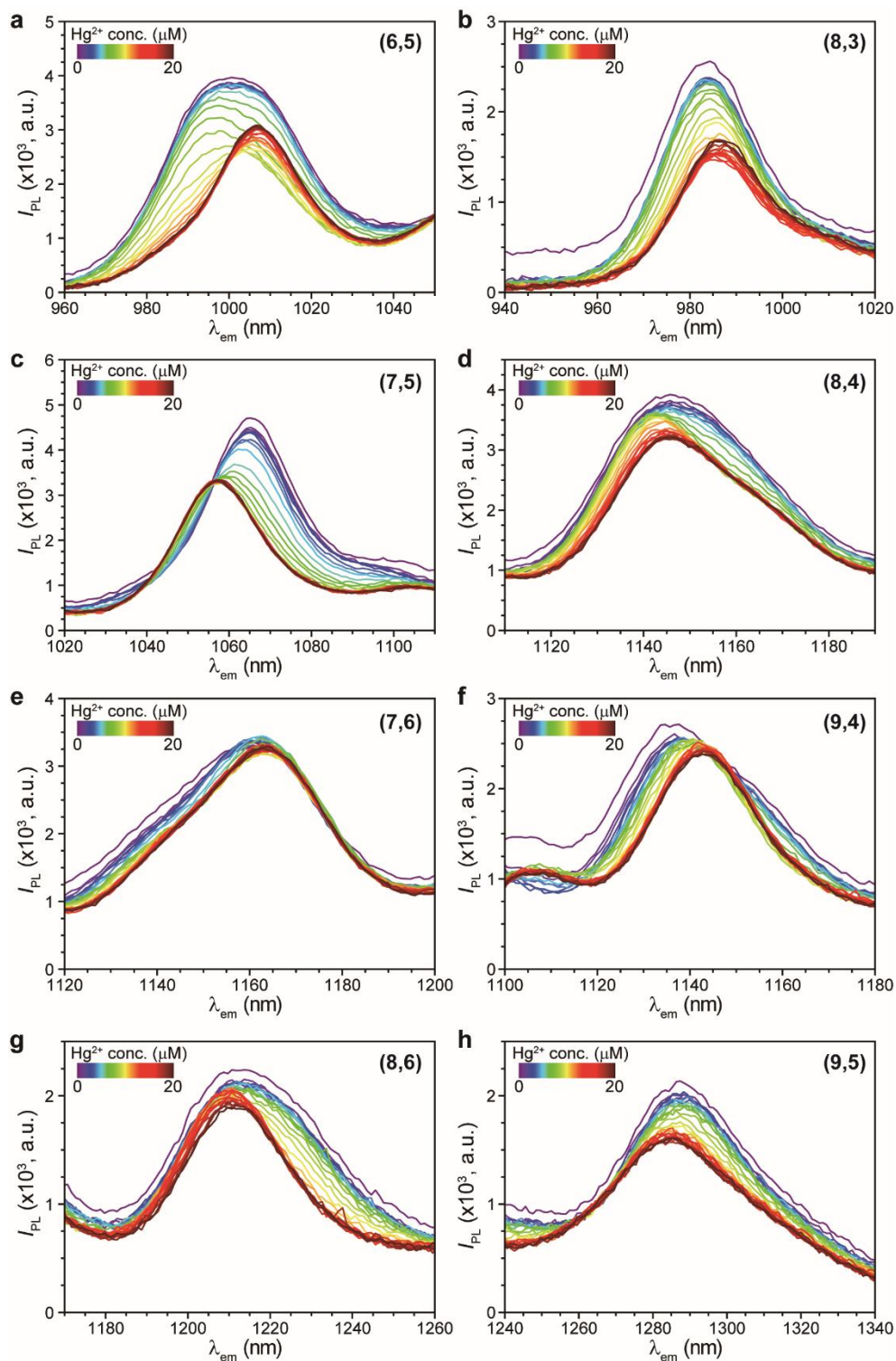


Figure S8. Simulated vibrational spectra using LF (black) and LF-Hg-LF (red).

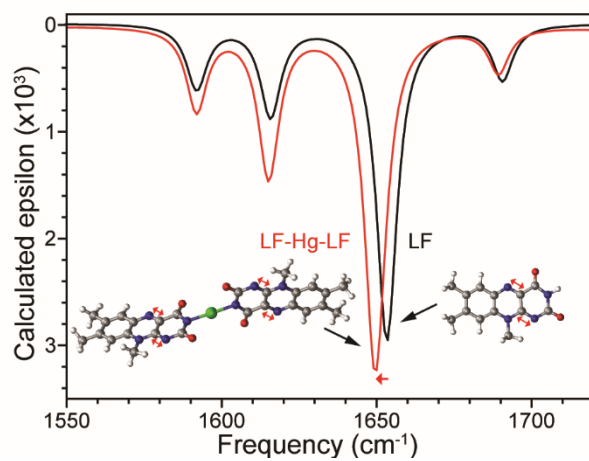


Figure S9. Chemical structure of LF-Hg-LF whose relative angle is 0°.

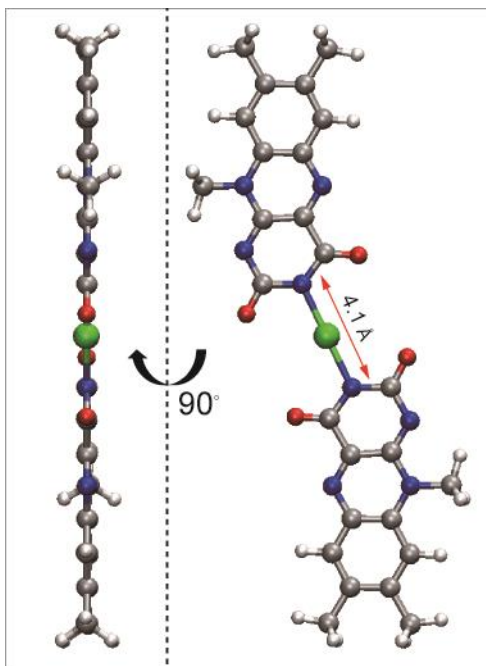


Figure S10. Additional HRTEM images of FMN-SWNT in presence/absence of Hg^{2+} ion and uranyl acetate staining.

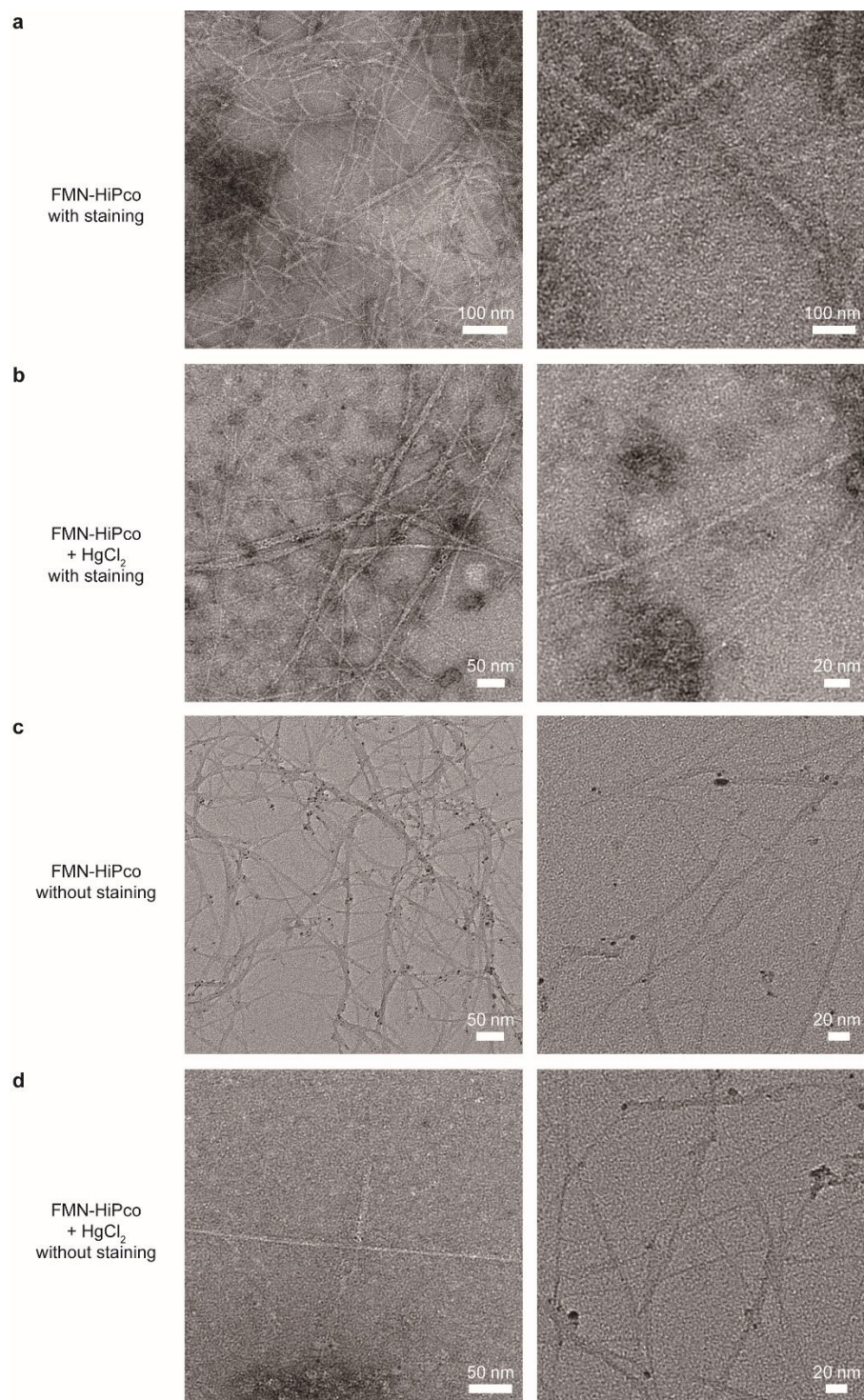


Figure S11. (a) The overlaid I_{PL} and peak position *via* fine titration of Hg^{2+} ion against FMN-(10,2) tube and (b) the corresponding zoomed-in plot up to 1000 nM titration of shaded area of (a).

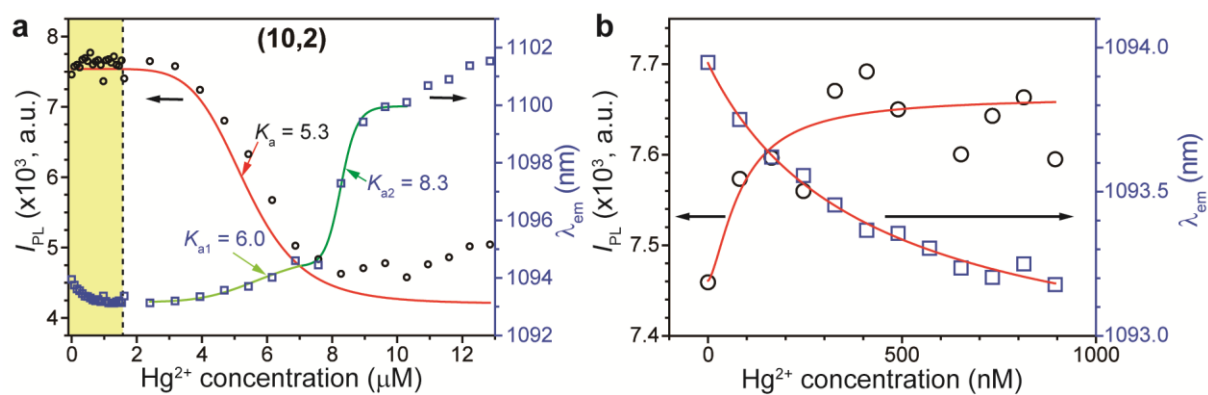


Table S1. K_a and γ of the I_{PL} and peak position trajectories on Hg titration at Figure 5C along with standard deviation (STD). On average, STD of K_a is less than 5%.

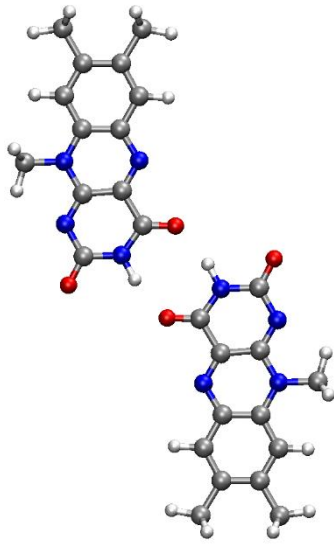
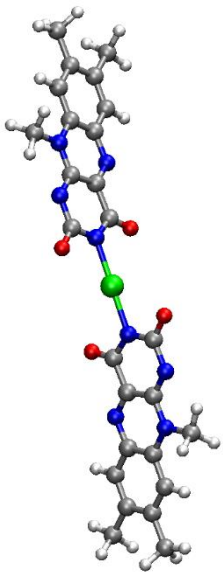
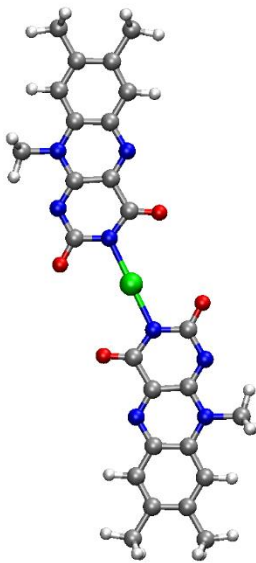
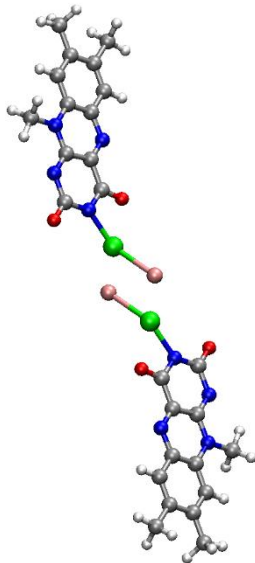
(n,m)		I_{PL}			Peak position		
		K_a [μ M]	STD of K_a	γ	K_a [μ M]	STD of K_a	γ
(6,5)	K_{a1}	7.89	0.13	9.70	7.13	0.11	14.57
	K_{a2}	11.59	0.54	6.96	10.04	0.07	12.16
(8,3)	K_{a1}	10.18	0.16	6.50	11.17	0.33	4.37
	K_{a2}	17.04	0.32	24.53	-	-	-
(7,5)		6.13	0.05	6.60	6.38	0.04	6.68
(10,2)		3.58	0.09	18.69	5.01	0.05	7.14

Table S2. PLE-derived E_{11} and E_{22} positions of FMN-SWNT with and without Hg^{2+} ion according to chiral SWNTs. d_t is based on C-C distance with 0.142 nm.

Assignment			Initial PL energy [eV]		PL energy after Hg^{2+} ion addition [eV]			
					Minimum		Maximum	
(n,m)	d_t [nm]	Family [2n+m]	E_{11}	E_{22}	E_{11}	E_{22}	E_{11}	E_{22}
(6,5)	0.75	17	1.238	2.134	1.230	2.126	1.241	2.120
(8,3)	0.77	19	1.260	1.826	1.257	1.829	1.260*	1.826*
(7,5)	0.82	19	1.165	1.884	1.165*	1.884*	1.173	1.865
(8,4)	0.83	20	1.083	2.043	1.081	2.036	1.085	2.036
(10,2)	0.87	22	1.126	1.651	1.120	1.660	1.130	1.652
(7,6)	0.88	20	1.069	1.851	1.066	1.854	1.068	1.852
(9,4)	0.92	22	1.092	1.687	1.085	1.685	1.092*	1.687*
(8,6)	0.96	22	1.022	1.673	1.022	1.682	1.024	1.680
(9,5)	1.01	23	0.962	1.800	0.962*	1.800*	0.963	1.797

* Asterisk indicated that initial PL energy was either minimum or maximum energies during the titration.

Table S3. Calculated electronic energy of LF dimer according to binding mode with Hg²⁺ ion.

Entry	Initial	2:1-twisted configuration	2:1-flat configuration	1:1
Geometry - optimized structure				
Minimized energy (atomic unit)	3779.6	4169.0	4170.8	5234.9