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CHEMICAL REACTIONS OF EXPLOSIVE
MOLECULES FOR DETECTION APPLICATIONS

BY

CHRISTOPHER A. LATENDRESSE

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
CHEMISTRY

UNIVERSITY OF RHODE ISLAND

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OF
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ABSTRACT

Explosive analytes and their decomposition products are of great interest to the scientific community, in large part due to events of international terrorism and warfare, but also as environmental pollutants. Chapter 1 of this dissertation shows for the first time that the hydroxide adducts of trinitrobenzene (TNB) and trinitrotoluene (TNT), TNB-OH^- and TNT-OH^- , are emissive while TNT^- is not. This has great impact on pre-existing sensors, which may be affected negatively by an increase in emission competing with the observation of a quench. Additionally, we described a competing reaction with the solvent, *N,N*-dimethylformamide (DMF), which is also capable of nucleophilic attack upon TNT and observable due to its overwhelming quantity.

Chapter 2 of this dissertation shows the similarities of TNT products formed by OH^- exposure and amine exposure, covering a wide variety of amines. ^1H NMR and rapid absorbance measurements showed formation of TNT^- or TNT adducts, the relative rates of which are observed to be strongly dependent on solvation. Intrinsic rate constants in methanol implied that all amine reactions were forming the same adduct (TNT-OCH_3^-). The credibility of amine adduct formation was further explored in Chapter 3 through computational approaches. High level *ab initio* calculations were performed to obtain models of reactants and products of the investigated reactions, and their relative energies and thermodynamic quantities were computed. The data showed a striking disparity in the calculated thermodynamics, with OH^- and OCH_3^- adduct formation being much more favorable than amine adduct formation. Charge

transfer (CT) complexes between TNT and the explored amines were converged and also found to be of higher energy than alkoxide adducts.

In Chapter 4, a sensing array based on highly fluorescent reporter molecules in DMF solution is described. Eight xanthene-based fluorophores were chosen based on their high quantum yields, and their interactions with twelve relevant explosive analytes were interpreted through absorbance and emission data. The resulting array showed promise in observing an identifying "fingerprint" response to each class of analyte, with the largest responses coming from the formation of TNB and TNT products. In addition, these products were observed to be involved in an electron transfer (ET) mechanism where they donated energy to cationic fluorophores, enhancing the fluorophores' emission.

The findings of this dissertation indicate the need for caution in TNT sensor development, as trace water provides OH^- and common solvents are capable of competing for TNT reactivity. Trace water should be monitored and minimized for appropriate sensor testing and subsequent use, and solution based sensors require an understanding of solvent competition. Amines are not capable of direct deprotonation of TNT, and do not compete significantly with OH^- or alkoxides in forming a sigma adduct with TNT. Rather, the formation of colorful complexes that has been attributed to a TNT- NR_3 adduct in the literature is due to the deprotonation of ambient water or solvent molecules, which in turn react with TNT. Finally, xanthene-based fluorophores may selectively interact with explosive analytes, with trinitroaromatic products capable of electron transfer to fluorescent reporters leading to emission enhancement.

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factory." To Mom, for imprinting me with the right balance of self-esteem and humility. All of your hard work and perseverance gave me the opportunity to be the first in our family to graduate from college, and now to defend a Ph.D. I wouldn't be anywhere close to where I am without you. To Hapme, for being a third parent and providing a grandmother's love. To Becca, Julie, and Emma, for constant motivation in words of support.

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PREFACE

The following research is presented in manuscript format according to the guidelines provided by the Graduate School of the University of Rhode Island. The dissertation is separated into four chapters.

The first chapter is entitled "Fluorescent Species Formed by the Reaction of Trinitroaromatics With N,N-Dimethylformamide and Hydroxide" and has been accepted and published ASAP by the *Journal of Physical Chemistry A*.

The second chapter is entitled "Comparing the Roles of Hydroxide and Amines in TNT Reactions: Clarifying Absorbance Spectra Assignments For Use in Detection Methods" and is in preparation for submission to the *Journal of the American Chemical Society*.

The third chapter is entitled "Relative Thermodynamics of TNT Reactions Commonly Used for Sensor Applications: Computational Modeling in Vacuo and in Solution" and is in preparation for submission to the *Journal of the American Chemical Society*.

The fourth chapter is entitled "A Fluorometric Sensing Array for the Detection of Military Explosive and IED Materials" and is in preparation for submission to *Analyst*.

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CHAPTER 1

Fluorescent Species Formed by the Reaction of Trinitroaromatics With *N,N*- Dimethylformamide and Hydroxide

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The following is submitted to the *Journal of Physical Chemistry A*, and is presented
here in manuscript format.

ABSTRACT

Trinitrobenzene (TNB) and trinitrotoluene (TNT) react in *N,N*-dimethylformamide (DMF) to form multiple species in solution. Despite structural similarities, electronic spectra show that the reactivity is different for TNB and TNT. In addition to reaction with the DMF solvent, residual water in nominally dry DMF generates sufficient hydroxide for reaction with TNB and TNT. Multiple sigma adducts are formed and observed to be fluorescent, which has not been previously reported. Both TNB and TNT show the capacity to form sigma adducts with hydroxide and DMF, while methyl hydrogens of TNT can be deprotonated by hydroxide.

KEYWORDS: trinitrobenzene (TNB); trinitrotoluene (TNT); Meisenheimer complex.

1. Introduction

Common reactions of nitroaromatic molecules are of interest to the scientific community, especially for the application of explosive detection. Many products of these reactions have absorbances in the visible range, which allow for a multitude of detection approaches including colorimetry^{1,2} and fluorescence methods.³⁻⁸ 2,4,6-Trinitrotoluene (TNT) and 1,3,5-trinitrobenzene (TNB) are perhaps the most studied of the nitroaromatic family, having the highest electron deficiency in their pi system from their three nitro groups; this leaves them the most susceptible to nucleophilic attack, thereby converting the original species with no visible absorbance features to a vibrantly colorful product (commonly referred to as a "Meisenheimer complex").

While TNT and TNB share this type of reactivity, TNT also has acidic hydrogens that can be abstracted to form the TNT anion (TNT^-).⁹⁻¹² Since both TNT products have absorbances in similar locations in the visible range,^{9,10} and basicity and nucleophilicity tend to go hand in hand, it is a precarious process to be able to differentiate TNT sigma adducts from TNT^- .

It is commonly believed that nitroaromatics and their nucleophilic substitution products are not fluorescent, and therefore additional mechanisms need to be imposed in order to address their sensing using a fluorescence method. Many sensors rely on the fluorescence quenching of a reporter molecule, where the emission of a selected fluorophore decreases as a function of interacting with an explosive analyte. This process may result from either dynamic or static quenching, but is most often observed as a combination of both mechanisms.¹³⁻¹⁸ Since quenching decreases the observed signal as a function of addition of analyte, these collective mechanisms are commonly referred to as "turn-off" mechanisms.

Another typical approach utilizes absorbance features of an analyte in a resonance energy transfer mechanism (RET). In this mechanism, the excited state of a donor molecule is deactivated by the transfer of energy across space to an acceptor molecule, promoting it to its excited state. Since the mechanism does not necessitate direct contact between donor and acceptor, a sensor developed using RET has the capacity to be more sensitive. If the acceptor is emissive, then the observation is ratiometric with a decrease of donor emission concurrent with an increase in acceptor emission; otherwise, the observation is just a quenching of the donor's emission. Because many explosive analytes do not have visible absorbance features or react

under the same conditions, this is also touted as a selectivity parameter to differentiate nitroaromatics. Many proposals in the literature^{3,6-8} use the visible absorbance features of nitroaromatic products to justify their use as a RET acceptor, but since these products lack emissive features the sensing remains a "turn-off" mechanism.

We describe herein the observation of fluorescence emission from TNT and TNB products formed in *N,N*-dimethylformamide (DMF), without addition of any strong base or nucleophile. The competing equilibria are complicated. TNT is observed to react with OH⁻ (present from the trace water in nominally dry DMF) forming the non-emissive TNT⁻ or an emissive sigma adduct with DMF or OH⁻. TNB also shows the ability to form emissive sigma adducts with either DMF or OH⁻. In addition, the emission of TNT adducts may give a long desired method to clarify their abundance from that of TNT⁻. This is the first description of these products as being emissive, with evidence of the formation of products through a reaction with DMF. This work also demonstrates the need to understand the role of even trace amounts of water in working TNT sensors.

2. Materials and Methods

2.1. Materials. TNB was purchased from Fisher Scientific wetted with ca. 40% water with 98.0% purity. Concentrations of TNB solution were thus calculated using a value of 60% TNB solid by mass. TNT was obtained from Drs. Jimmie Oxley and James Smith and was used without further purification. DMF (≥99.5%) was purchased from Fisher Scientific and used as received. H₂O (HPLC grade) was

purchased from Sigma and used as received. Aqueous 0.1 M NaOH and HCl solutions were purchased from Anachemia.

2.2. Absorbance and Fluorescence Measurements. Absorbance spectra were obtained between 300 and 800 nm on a Perkin-Elmer Lambda 900 UV/Vis/NIR spectrophotometer. Fluorescence spectra were obtained with a Horiba Fluorolog-3, Xe arc lamp excitation source, 3 nm excitation and emission slits for TNB, 5 nm excitation and emission slits for TNT, 0.1 sec integration time, and ambient temperature. TNB and TNT titration emission scans were excited at 446 nm, while additional samples were investigated using excitations every 10 nm from 350 to 600 nm. TNB and TNT excitation scans were monitored at an emission wavelength of 550 and 700 nm.

2.3. Peak Fitting Method. The commercially available software PeakFit© and SigmaPlot© were employed to fit emission spectra to constituent peaks. Initially, spectra were fit with an increasing number of Gaussian peaks with vibrationally related maxima, FWHM (full width at half maximum), and height until a consistent set of peaks were obtained for each individual spectrum in a given titration. After this, a global fit was undertaken upon all related spectra at the same time using the average initial parameters as a starting point. Converged fits were obtained without unrealistic or inconsistent parameters to support chemical mechanisms.

2.4. Computational Analysis. Spartan '10 computational software¹⁹ was used to propose the relative absorbance maxima for the isomers of [TNT-OH]⁻. Structures were optimized at the HF/6-311+G** level without imaginary vibrational frequencies.

TDDFT energies and absorbance spectra were calculated using the converged structures at the B3LYP/6-311+G** level.

3. Results and Discussion

3.1. UV/Vis Absorption

The addition of a few crystals of TNB or TNT into DMF rapidly generates colorful products visible to the naked eye. To monitor these products, a 7×10^{-5} M solution of TNB and a 1.1×10^{-4} M TNT solution were prepared in DMF and immediately observed using absorbance and fluorescence spectroscopy. The initial absorbance spectrum for the TNB solution (Figure 1a) shows maxima at 452 nm and 523 nm, reminiscent of other "Meisenheimer" type adducts reported in literature with a narrow feature around 420 nm and a broader set of features around 500 nm.^{9,10}

Figure 1. Initial and final absorbance spectra from trinitroaromatics titrated with water in DMF: (a) 7×10^{-5} M TNB in DMF; (b) TNB + 30 μ L H₂O in DMF; (c) 1.1×10^{-4} M TNT in DMF; (d) TNT + 30 μ L H₂O in DMF.

Only two possible products are expected to be formed by TNB, which are sigma adducts with DMF or OH^- (TNB-DMF and $[\text{TNB-OH}]^-$, respectively - see Scheme 1); the absorbance changes observed suggest an equilibrium between the DMF adduct and the hydroxide adduct with free TNB. Titration of water to the TNB solution was done in increments of 5 μL . The initial concentration of water in the TNB solutions was 6×10^{-4} M due to the availability as a wetted solid, whereas TNT was not pre-wetted. While the autoionization constant of water in DMF is not available, we estimate the initial $[\text{OH}^-]$ in the TNB solutions to be on the order of 10^{-10} M using K_w in pure water as an approximation. The initial $[\text{OH}^-]$ in the TNT solutions will be less than this, as the same amount of water is not carried in by solid addition. Titration of water gives evidence of a tight isosbestic point at 318 nm early in the titration and a second isosbestic point at 475 nm later in the titration (see Supplementary Information, Fig. S1). Addition of water decreases the initial absorbance features at higher wavelengths while increasing the new features at lower wavelengths; after 30 μL of water are added, new maxima are observed at 438 nm and 514 nm. This likely indicates the indirect conversion of the TNB-DMF adduct to the $[\text{TNB-OH}]^-$ adduct. The isosbestic point that is well defined at 318 nm represents the loss of UV features of TNB in solution with the rise of an absorbance peak from $[\text{TNB-OH}]^-$. The late forming isosbestic point at 475 nm shows a decrease in absorbance at higher wavelengths from the loss of TNB-DMF while features at lower wavelength increase from the generation of $[\text{TNB-OH}]^-$; the late isosbestic point formation may be resultant from a third species that completely disappears after the second addition of water - this may be a dinitrobenzene impurity in the TNB or a charge-transfer complex that goes below

limits of detection. TNB CT complexes have been reported in the literature, with maxima ranging from the UV to the violet end of the visible spectrum.²⁰

To help confirm our assignments, we monitored the absorbance of the TNB solution with the addition of 5 μL of 0.1 M NaOH/ H_2O and the addition of 5 μL of 0.1 M HCl/ H_2O . Figure 2a shows the absorbance increase from the addition of NaOH and the decrease from the addition of HCl.

Figure 2. (a) Absorbance spectra and (b) emission spectra resultant from 7×10^{-5} M TNB in DMF (—); + 5 μL of 0.1 M NaOH/ H_2O ($[\text{OH}^-]/[\text{TNB}] = 3.6$) (---); + 5 μL of 0.1 M HCl/ H_2O ($[\text{H}^+]/[\text{TNB}] = 3.6$) (---). Samples were excited at 446 nm. The addition of NaOH increases the concentration of $[\text{TNB-OH}]^-$ in solution, increasing the initial absorbance and emission spectra. The addition of HCl decreases

the concentration of $[\text{TNB-OH}]^-$, leaving TNB-DMF as the only observed absorbing and emitting species in the visible range. The insets show the lower intensity features closer to baseline.

The initial spectral shape is retained with the NaOH addition, supporting that the initial spectrum is at least in part resultant from the formation of $[\text{TNB-OH}]^-$. There is a small shift in the absorbance maximum, initially observed at 450 nm shifting to 440 nm. This indicates that there is another absorbing species involved. The addition of HCl helps to clarify this spectrum, as it removes OH^- from solution. Two very weak signals are retained after the acidification, as seen in the inset on Figure 2a. We attribute these features to be from the TNB-DMF adduct, since there is still an overwhelming amount of DMF available that is not protonated. These experiments support our assignments of the generation of $[\text{TNB-OH}]^-$ and TNB-DMF in our titration of water into TNB/DMF solution.

The addition of TNT to DMF shows a very different absorbance spectrum (Figure 1c), suggesting different products. Maxima for this initial spectrum are observed at 375 nm, 509 nm, 529 nm, and 645 nm. Since TNT has acidic methyl protons whereas TNB does not, it is not surprising that this absorbance spectrum has been previously attributed to TNT^- ;⁹⁻¹² in this particular system, hydroxide from ambient water is likely acting as the Brønsted-Lowry base. The addition of water leads to the observation of isosbestic points at 339 nm, 414 nm, and 451 nm (see Supplementary Information Fig. S2), suggesting the contribution of only two absorbing species. This can be explained through the loss of TNT^- and the increase in

[TNT-OH]⁻, while there is little evidence here to suggest a DMF adduct. If there is a DMF sigma adduct, it either has a low molar absorptivity or the concentration is much lower than that of the corresponding hydroxide adduct. In similar fashion to our TNB experiments, we added 5 μ L of 0.1 M NaOH/H₂O and 5 μ L of 0.1 M HCl/H₂O to our TNT solution to help confirm our spectral assignments. Figure 3a shows the absorbance growth of the TNT/DMF solution with the addition of NaOH, and the decrease of the absorbance spectrum with the addition of HCl. As expected, the addition of NaOH created more TNT⁻, increasing the absorbance but retaining nearly the same spectral shape throughout the visible range. The new feature at 443 nm comes from the generation of [TNT-OH]⁻, where the growth in the water titration occurred. Addition of HCl obliterated the initial absorbance spectrum; this is consistent with the initially observed product being TNT⁻, which is fully protonated as the solution is acidified. The inset on Figure 3a shows the baseline, where there is a very small absorbance component remaining. This may indicate a small amount of TNT-DMF that is not affected by HCl addition. Again, these experiments have helped to support our original assessment that OH⁻ from the water titration is responsible for the absorbance changes, forming both TNT⁻ and [TNT-OH]⁻ and possibly TNT-DMF. Overall, the absorbance spectra indicate sigma adduct formation for both TNB and TNT along with acid-base reactivity for TNT. The possible reaction products are summarized in Scheme 1.

Figure 3. (a) Absorbance spectra and (b) emission spectra resultant from 1.1×10^{-4} M TNT in DMF (—); + 5 μL of 0.1 M NaOH/ H_2O ($[\text{OH}^-]/[\text{TNT}] = 2.3$) (---); + 5 μL of 0.1 M HCl/ H_2O ($[\text{H}^+]/[\text{TNT}] = 2.3$) (---). Samples were excited at 446 nm. The addition of NaOH increases the concentration of $[\text{TNT-OH}]^-$ in solution, increasing the initial absorbance and emission spectra. The addition of HCl decreases the concentration of $[\text{TNT-OH}]^-$, leaving TNT-DMF as the only observed absorbing and emitting species in the visible range. The insets show the lower intensity features closer to baseline.

Scheme 1. Proposed reactions of TNB/TNT with DMF or OH⁻.

3.2. Emission & Excitation Spectra of TNB Products

Emission spectra were collected for both systems before and after each addition of water in the titration. In the case of TNB, the samples were excited at 446 nm in an effort to observe possible emissions from each species. Figure 4 shows the emission spectra for the initial addition of TNB into DMF and also after the addition of 30 μL H_2O . The initial spectrum has a maximum at 650 nm, which decreases as water is added (see Supplementary Information, Fig. S3), while a new maximum is observed at 586 nm, which increases with the addition of water. In correlation to the absorbance spectra, the initial emission spectrum represents major features of the TNB-DMF adduct, which then gives way to the $[\text{TNB-OH}]^-$ adduct. The previously discussed NaOH and HCl additions support this assignment. Figure 2b shows the emission spectra of the TNB/DMF samples with the addition of NaOH and HCl, excited at 446 nm. We observe increase in emission at 591 nm for TNB + NaOH, indicating this feature to be resultant of the formation of $[\text{TNB-OH}]^-$. This feature overwhelms the spectrum, such that the original feature at 637 nm can no longer be seen. The addition of HCl to the TNB/DMF solution causes a decrease in emission at higher energy, but an increase in emission beyond 600 nm. Combined with our assessment of the absorbance spectra, this indicates the loss of $[\text{TNB-OH}]^-$ concurrent with the increase in TNB-DMF. This is likely the result of the reversion of $[\text{TNB-OH}]^-$ back to free TNB, which then shifts the TNB-DMF formation equilibrium forward.

Figure 4. Initial and final emission spectra from trinitroaromatics titrated with water in DMF: (a) 7×10^{-5} M TNB in DMF; (b) TNB + 30 μL H_2O in DMF; (c) 1.1×10^{-4} M TNT in DMF; (d) TNT + 30 μL H_2O in DMF. TNB spectra normalized at 650 nm, TNT spectra normalized at 591 nm.

In an effort to understand the exchange between the emissive species in the titration, a separate pair of samples were prepared with identical concentration of nitroaromatic and water as the final point in the titration. These samples were allowed to equilibrate for one hour before obtaining emission and excitation data. Both sets of emission spectra were fit to a modeled set of Gaussian peaks, based on initial parameters from selective excitation of individual products; the TNB and TNT samples show a unique excitation spectrum for emission at 550 nm and evidence of a separate species at 700 nm emission (see Figure 5).

Figure 5. Excitation spectra from trinitroaromatics with water in DMF: 7×10^{-5} M TNB + 30 μ L H₂O in DMF (a) 550 nm emission, (b) 700 nm emission; 1.1×10^{-4} M TNT + 30 μ L H₂O in DMF (c) 550 nm emission, (d) 700 nm emission.

Excitation maxima for the TNB solution are observed at 433 nm and 515 nm for 550 nm emission and 448 nm, 527 nm and 575 nm for 700 nm emission. This corresponds well to the absorbance features described previously, where $[\text{TNB-OH}]^-$ absorbance and excitation features are blue-shifted relative to TNB-DMF. The maximum observed for TNB-DMF at 527 nm may have slight overlap from a $[\text{TNB-OH}]^-$ emission contribution at 700 nm. Figure 6a shows the $[\text{TNB-OH}]^-$ emission spectrum resultant from 390 nm excitation of the TNB sample, indicating that three Gaussian peaks were required for a proper fit. As expected, the emission spectrum is similar in shape only to the lower energy features in the excitation spectrum; this indicates that all emission is occurring from the first electronic excited state.

Figure 6. Emission spectra of OH^- adducts fit with a three Gaussian spectrum for initial modeling parameters: (a) 7×10^{-5} M TNB + 30 μL water in DMF, 390 nm excitation; (b) 1.1×10^{-4} M TNT in DMF + 30 μL water, 370 nm excitation. Scatter points represent raw emission data, dashed lines represent constituent Gaussian peaks, and solid lines represent the sum of the Gaussian peaks for the overall modeled spectrum.

The TNB-DMF emission spectrum was observed using an excitation of 600 nm, as seen in Figure 7a modeled with two Gaussian peaks. These sets of peaks were used as initial parameters in the establishment of the overall model, fitting both species' spectra at the same time. An intensity ratio was held between peaks in the same spectrum, normalizing one intensity to the highest energy feature. Since emission generally occurs through transition from the ground vibrational state of the first electronic excited state to an excited vibrational excited state in the ground electronic

excited state, the energy spacing between peaks in the same spectrum should indicate a vibrational mode for the ground state of an emitting species. Due to this, a vibronic energy parameter was included in the equation, separating the peak maxima equally, for partial validation of data fitting. The complete set of parameters accounting for each emissive species in our model can be found in Table 1. The model's equation can be found in Supplementary Information.

Figure 7. Emission spectra of DMF adducts fit with a two Gaussian spectrum for initial modeling parameters: (a) 7×10^{-5} M TNB + 30 μ L water in DMF, 600 nm excitation; (b) 1.1×10^{-4} M TNT in DMF + 30 μ L water, 600 nm excitation. Scatter points represent raw emission data, dashed lines represent constituent Gaussian peaks, and solid lines represent the sum of the Gaussian peaks for the overall modeled spectrum.

Table 1. Fitting parameters for TNB/TNT emission product spectra.

	Intensity Ratio (a.u.)	Peak max (cm ⁻¹)	FWHM (cm ⁻¹)	Vibronic Energy (cm ⁻¹)
[TNB-OH]⁻	a ₁ 1*	λ ₁ 17496 ± 7	FWHM ₁ 640 ± 10	ν ₁ 990 ± 10
	a ₂ 0.89 ± 0.02	16510 ± 10	FWHM ₂ 850 ± 10	
	a ₃ 0.49 ± 0.01	15530 ± 20	FWHM ₃ 1220 ± 10	
TNB-DMF	a ₄ 1*	λ ₂ 15370 ± 30	FWHM ₄ 626 ± 3	ν ₂ 1000 ± 30
	a ₅ 0.76 ± 0.02	14370 ± 40	FWHM ₅ 900 ± 20	
[TNT-OH]⁻ C₁	a ₁ 1*	λ ₁ 17317 ± 6	FWHM ₁ 530 ± 30	ν ₁ 770 ± 30
	a ₂ 1.04 ± 0.03	16550 ± 30	FWHM ₂ 710 ± 40	
	a ₃ 0.48 ± 0.02	15780 ± 60	FWHM ₃ 1220 ± 40	
TNT-DMF	a ₄ 1*	λ ₂ 15390 ± 10	FWHM ₄ 440 ± 20	ν ₂ 630 ± 20
	a ₅ 2.8 ± 0.9	14760 ± 20	FWHM ₅ 1190 ± 60	
[TNT-OH]⁻ C₃	a ₆ 1*	λ ₃ 17930 ± 20	FWHM ₆ 499 ± 3	N/A

*Normalized in fitting equation

In the assigned [TNB-OH]⁻ spectrum, the energy difference between the two modeled peaks is 990 cm⁻¹, while the difference in the TNB-DMF spectrum is 1000 cm⁻¹. These energies may represent out of plane ring bending modes, formed by the nucleophilic attack. To verify that these modeling parameters are valid, the TNB fitting model was applied to emission data with varying excitation wavelengths. Figure 8a shows emission data excited from 410 to 450 nm with the TNB model overlaid on the raw data. The model clearly applies well to data collected across a range of excitation wavelengths that excite each species in varying quantities.

Figure 8. Emission spectra of trinitroaromatics with water in DMF as a function of excitation wavelength (410 to 450 nm excitation): (a) 7×10^{-5} M TNB + 30 μ L water in DMF; (b) 1.1×10^{-4} M TNT in DMF + 30 μ L water. Scatter points represent raw emission data, and lines represent the fit resultant from each established model.

Figure 9 shows emission spectra for the individual species fit to the initial, middle, and final point of the water titration. The two modeled species' spectra fit the overall spectrum very well throughout the titration, indicating the decrease of TNB-DMF relative to $[\text{TNB-OH}]^-$. The entire set of TNB titration spectra with the model fitting can be found in the Supplementary Information (Figure S4). Using these modeled fits, the area under each species' spectrum was calculated at each titration point.

Figure 9. Emission spectra from TNB titration with water in DMF, fit with a two species model for TNB- OH^- (— — —) & TNB-DMF (- - - -). (a) 7×10^{-5} M TNB in DMF; (b) + 15 μL H_2O ; (c) + 30 μL H_2O . Scatter points represent raw emission data, and lines represent the fit resultant from each established model.

Figure 10 shows the trend of each spectrum's integrated area as a function of the addition of water. The general trend supports our original assessment: $[\text{TNB-OH}^-]$ is observed to increase while TNB-DMF decreases with the addition of water.

Figure 10. Normalized modeled emission spectrum area as a function of increasing concentration of water.

3.3. Excitation and Emission Spectra of TNT Products

For TNT, the emission spectra of the titration do not show a great deal of shape change (see Supplementary Information S5). Figure 4 shows the normalized emission of the initial TNT solution and after the addition of 30 μ L water, excited at 446 nm. These two spectra appear to be superimposable, however the excitation data in Figure 5 refutes the existence of only one species. In similar fashion to the TNB data, two distinctly different excitation spectra are acquired using 550 nm emission and 700 nm emission. As previously mentioned, the methyl group on TNT gives additional

reactions that TNB cannot exhibit, so identification of emissive species requires caution. Since the emission intensity increases (Supplementary Information Fig. S5) while the absorbance of TNT^- is decreasing, TNT^- cannot be the emissive species. Also, the established TNT^- absorbance spectrum^{9,10} has absorbance at lower energy beyond 700 nm; this rules out TNT^- emitting at higher energy wavelengths. These emission spectra show similarity to the products that are formed from TNB, which does not have the ability to be deprotonated. In addition, Figure 5 shows that the excitation spectra are similar for $[\text{TNB-OH}]^-$ and the TNT product after adding 30 μL of water; with an observed emission at 550 nm, excitation maxima are observed at 424 nm and 526 nm. This supports that this excitation spectrum belongs to one of the possible $[\text{TNT-OH}]^-$ species. Features similar to TNB-DMF are seen for the excitation spectrum at 700 nm emission, with maxima around 460 nm and 590 nm, but are not as pronounced relative to the hydroxide sigma adduct features. Figure 3b shows the emission spectra from the aforementioned addition of NaOH and HCl to the TNT/DMF solution. The addition of NaOH causes a sizable increase in emission at 603 nm, too large of a change to be consistent with the more modest increase in the absorbance of TNT^- . Instead, this is consistent with the magnitude of the absorbance increase seen for $[\text{TNT-OH}]^-$. The addition of HCl to the TNT/DMF solution eliminates the $[\text{TNT-OH}]^-$ emission signal, again by removing hydroxide from the formation equilibrium. The inset on Figure 3b shows a component of the original emission that remains around 655 nm after the acidification, which is consistent with the analogous formation of a DMF adduct, TNT-DMF.

To further understand the TNT reactions, the emission data were also modeled using the same approach as described above. Figure 6b shows the emission spectrum resultant from 370 nm excitation, where only $[\text{TNT-OH}]^-$ is excited. The outcome was a three peak spectrum with slight differences from the $[\text{TNB-OH}]^-$ model, which was not able to reasonably fit all spectra in the titration by itself even when allowing for relaxation of initial parameters. TNT-DMF emission peaks were initially fit using a 600 nm excitation (see Figure 7b), and subsequently iterated with the $[\text{TNT-OH}]^-$ parameters to establish an overall model. It became obvious that there was an additional emitting feature for several reasons. First, the emission maximum appears to shift to higher energy as the excitation shifts to higher energy (see Figure 8b), which was not observed for TNB. Also, the initial fits for $[\text{TNT-OH}]^-$ and TNT-DMF were unable to account for all of the observed emission data collected between samples excited at varying wavelengths, even when the parameters were allowed to change dramatically. The addition of one extra Gaussian peak at a higher energy maximum than $[\text{TNT-OH}]^-$ was able to globally satisfy all data without drastically altering the initial $[\text{TNT-OH}]^-$ and TNT-DMF fits. The complete equation for the TNT model can be found in the Supplementary Information.

The existence of a third species seems to go against the observation of tight isosbestic points, which typically indicate only two absorbing species in solution. TNT^- and $[\text{TNT-OH}]^-$ are well established and clearly evident here. TNT-DMF has been observed through fluorescence, and supported through analogous chemistry to the formation of TNB-DMF. As a third contributing species, we cannot ascertain the magnitude of TNT-DMF's molar absorptivity nor its quantum yield. The addition of

another $[\text{TNT-OH}]^-$ species appears to cloud this even further; however, there are systems that may exhibit isosbestic points with three contributing species. Such a situation can arise in a system with absorbance contributions from a Lewis acid with two binding sites and one Lewis base, where the free acid and each form of the adduct absorb in the same range with different molar extinction coefficients.²¹ In our case, TNT acts as the Lewis acid, which has two binding sites (C_1 & C_3 - see numbering for TNT carbons in Scheme 1). While TNT does not absorb in the visible range, TNT^- does; the three products of the reaction of TNT with hydroxide (TNT^- , $[\text{TNT-OH}]^- \text{C}_1$, and $[\text{TNT-OH}]^- \text{C}_3$) should therefore still show isosbestic points if TNT-DMF is at or below absorbance detection limits. The question arises as to which form of $[\text{TNT-OH}]^-$ belongs to which modeled spectrum. While Fyfe *et. al.* previously described $[\text{TNT-OCH}_3]^- \text{C}_3$ as the only observable sigma adduct via ^1H NMR⁹, we cannot appropriately correlate this to our measurements without knowing how significantly the sterics affect methoxide attack versus hydroxide attack. We also do not know each species' molar absorptivity and quantum yield. If we estimate that both $[\text{TNT-OH}]^-$ species have molar absorptivities and quantum yields on the same order of magnitude, then the three peak emission spectrum that we initially fit plausibly belongs to $[\text{TNT-OH}]^- \text{C}_1$. Also, TDDFT UV-Vis calculations may support this assignment by projecting the C_3 adduct as having higher energy transitions (Supplementary Information Fig. S6). Absorbance computations at the B3LYP/6-311+G**//HF/6-311+G** level suggest that the HOMO/LUMO gap for the C_1 sigma adduct is of lower energy than the C_3 form. If each hydroxide sigma adduct has a similar Stokes

shift, this would assign the higher energy, single peak emission spectrum to $[\text{TNT-OH}]^- \text{C}_3$.

Figure 11 shows the initial emission spectrum of the TNT/DMF solution, the addition of 15 μL of water, and addition of 30 μL of water. All spectra in this titration fit well using our established model (full titration fitting can be found in Supplementary Information Fig. S7). The converged parameters for the TNT product emission spectra model can be found in Table 1. The vibronic energy for $[\text{TNT-OH}]^- \text{C}_1$ is 770 cm^{-1} and 630 cm^{-1} for TNT-DMF; these energies may represent ring bending modes in each respective species. The integrated area of each spectrum as a function of water addition is found in Figure 10. Unlike the trend for TNB, all emissive species are observed to grow as more water is added to the system. This could be an indication that TNT-DMF formation requires $[\text{TNT-OH}]^- \text{C}_3$ as an intermediate with a subsequent $\text{S}_{\text{N}}2$ step.

Figure 11. Emission spectra from TNT titration with water in DMF, fit to a three species model for TNT-OH⁻ C₁ (— — —), TNT-DMF (- - - -), and TNT-OH⁻ C₃ (— · — · —). (a) 1.1×10^{-4} M TNT in DMF; (b) + 15 μ L H₂O; (c) + 30 μ L H₂O.

4. Conclusions

In summary, we have shown that TNB has the capacity to form two sigma adducts in DMF, [TNB-OH]⁻ and TNB-DMF, as shown in Scheme 1. Absorbance data for TNB/DMF solutions supports the existence of two species that absorb in the

region expected for sigma adducts. Further investigation into excitation and emission data supports the two species. The development of a multi-Gaussian model (with parameters given in Table 1) clarifies the relative quantities of emission from each species, showing the two to be linked by equilibrium. The methyl group of TNT causes different reactivity than TNB. TNT has the ability to form four products in DMF (Scheme 1), leading to spectral differences and similarities which require careful analysis to discern. The major absorbing species, TNT^- , is shown to be non-emissive. Absorbance features consistent with sigma adducts of TNT are observed to grow as a function of water addition. The three possible sigma adducts, $[\text{TNT-OH}]^- \text{C}_1$, $[\text{TNT-OH}]^- \text{C}_3$, and TNT-DMF, all exhibit excitation and emission features that have distinct similarity to their TNB counterparts. A multi-Gaussian model was also established to differentiate the TNT sigma adducts' relative emission (Table 1), showing that all grow together as a function of added water. This is not observed for the TNB sigma adducts, and implies different mechanisms for DMF sigma adduct formation.

This is the first report of fluorescent emission from Meisenheimer adducts of TNB and TNT, with both nitroaromatics capable of nucleophilic attack by hydroxide and the less sterically hindered TNB capable of attack by DMF. All reactions show significant changes within minutes, which allow these reactions to be further explored in the context of sensor development. The emissions may be able to be enhanced by using a resonance energy transfer from a donor fluorophore with a long excited state lifetime to these adducts as acceptors; this could further amplify this form of a "turn-on" mechanism. Alternatively, a solid state sensor capable of capturing these nitroaromatics through stronger nucleophilic attack may be able to serve in a facile

turn-on approach. Future work should compare dinitrotoluene (DNT) emission characteristics to those described here. DNTs are common impurities in TNT, but since DNTs have a much higher vapor pressure than TNT they will each deliver similar amounts in the vapor phase to a sensor. Depending on the relative equilibrium constants for each adduct formation and their relative quantum yield, it may be found that DNTs are more observable in this capacity. In addition, the different reactivity between TNT and TNB means that caution must be used when using TNB as a surrogate for TNT in the development and testing of TNT sensors. Finally, this work shows that TNT sensors are expected to perform differently under wet or humid conditions than under dry conditions. Water can react with nitroaromatics even at trace levels.

ASSOCIATED CONTENT

Supporting Information.

UV/Vis Absorbance spectra, excitation and emission spectra of trinitroaromatics in DMF with water titration. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

TNB, trinitrobenzene; TNT, trinitrotoluene; DMF, *N,N*-dimethylformamide.

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Supplementary Information for:

Fluorescent Species Formed by the Reaction of Trinitroaromatics With *N,N*-Dimethylformamide and Hydroxide

by

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Figure S1: Absorbance spectra evolution from TNB titrated with water in DMF. Solution titrated directly after addition of TNB to DMF.

Figure S2: Absorbance spectra evolution from TNT titrated with water in DMF. Solution titrated directly after addition of TNT to DMF.

Figure S3: Emission spectra evolution from TNB titrated with water in DMF ($\lambda_{\text{ex}} = 446\text{nm}$). Solution titrated directly after addition of TNB to DMF.

TNB Model:

The overall equation governing our TNB model (intensity as a function of wavelength) is given by:

$$I_{em}(\lambda) = a_1 * \left[e^{-\frac{1}{2} \left(\frac{\lambda - \lambda_1}{FWHM_1} \right)^2} + a_2 * e^{-\frac{1}{2} \left(\frac{\lambda - (\lambda_1 - \nu_1)}{FWHM_2} \right)^2} + a_3 * e^{-\frac{1}{2} \left(\frac{\lambda - (\lambda_1 - 2\nu_1)}{FWHM_3} \right)^2} \right] + a_4 * \left[e^{-\frac{1}{2} \left(\frac{\lambda - \lambda_2}{FWHM_4} \right)^2} + a_5 * e^{-\frac{1}{2} \left(\frac{\lambda - (\lambda_2 - \nu_2)}{FWHM_5} \right)^2} \right]$$

where a_1 and a_4 are the intensities of the $[\text{TNB-OH}]^-$ and TNB-DMF spectra respectively, a_2 is the fixed ratio of intensity for the peaks that comprise the $[\text{TNB-OH}]^-$ spectrum, a_4 is the fixed ratio of intensity for the peaks that comprise the TNB-DMF spectrum, λ_1 represents the peak maximum for the highest energy feature of $[\text{TNB-OH}]^-$, λ_2 represents the peak maximum for the highest energy feature of TNB-DMF, $FWHM_i$ represents the bandwidth of each peak, and ν_i represents the vibrational energy gap in the ground electronic state.

Figure S4: Emission spectra evolution from TNB titrated with water in DMF ($\lambda_{ex} = 446\text{nm}$), fit using static model established through spectral peak fitting.

Figure S5: Emission spectra evolution from TNT titrated with water in DMF ($\lambda_{\text{ex}} = 446\text{nm}$). Solution titrated directly after addition of TNT to DMF.

TNT Model:

The overall equation governing our TNT model is given by:

$$I_{em}(\lambda) = a_1 * \left[e^{-\frac{1}{2} \left(\frac{\lambda - \lambda_1}{FWHM_1} \right)^2} + a_2 * e^{-\frac{1}{2} \left(\frac{\lambda - (\lambda_1 - \nu_1)}{FWHM_2} \right)^2} + a_3 * e^{-\frac{1}{2} \left(\frac{\lambda - (\lambda_1 - 2\nu_1)}{FWHM_3} \right)^2} \right] + a_4$$

$$* \left[e^{-\frac{1}{2} \left(\frac{\lambda - \lambda_2}{FWHM_4} \right)^2} + a_5 * e^{-\frac{1}{2} \left(\frac{\lambda - (\lambda_2 - \nu_2)}{FWHM_5} \right)^2} \right] + a_6 * \left[e^{-\frac{1}{2} \left(\frac{\lambda - \lambda_3}{FWHM_6} \right)^2} \right]$$

where a_1 , a_4 , and a_6 are the intensities of the $[\text{TNT-OH}]^-$ C_1 , TNT-DMF, and the $[\text{TNT-OH}]^-$ C_3 spectrum respectively. All other parameters are as described previously for the TNB model.

Figure S6: UV/Vis Computations performed at the B3LYP/6-311+G**//HF/6-311+G** level for the two hydroxide adducts of TNT, in vacuum and at 273.15K. Note that while the computed maxima are generally poor for this approach, the relative position of the maxima are typically representative of experimental observation.

Figure S7: Emission spectra evolution from TNT titrated with water in DMF ($\lambda_{\text{ex}} = 446\text{nm}$), fit using static model established through spectral peak fitting.

Comparing the Roles of Hydroxide and Amines in TNT Reactions: Clarifying Absorbance Spectra Assignments For Use in Detection Methods

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ABSTRACT

Many contemporary TNT sensors rely on the reaction of primary amines with TNT to form a species that absorbs in the visible range. The mechanism of this reaction has been attributed to charge transfer (CT) complexes, the deprotonation of TNT's methyl group forming TNT^- , or the nucleophilic attack upon TNT forming the zwitterionic sigma adduct TNT-NR_3 . All of these species are commonly used to justify similar absorbance spectra, which are especially important in the development of RET based sensors. Anionic base reactions with TNT are well established, but amine reactions with TNT have not been thoroughly investigated in the literature. We clarify herein the role of amines in TNT reactions in solution. Amines are found to be ineffective in directly deprotonating TNT. Amines also do not compete significantly in the formation of sigma complexes with alkoxide or hydroxide bases, which may be generated through acid/base reactions between amines and solvent molecules or ambient water. Since direct amine reactions are not observed as a primary rapid reaction with TNT, they are not expected to be an effective means of ultra-trace rapid sensing of TNT vapor. Additionally, solvent choice and the quantification of water content are crucial to the development and effective use of TNT sensors, in both solution and vapor phase.

INTRODUCTION

The detection of trinitrotoluene (TNT) has become an increasingly prevalent research topic over the past decade, due in part to the rise in international terrorism and the toxic nature of TNT in the environment. Many methods of detection have

been proposed in literature, including methods that rely on reactivity from TNT's electron deficient aromatic ring. Since these reactions generate species that absorb in the visible range, simple colorimetric sensors have been developed in the solution phase.¹⁻⁴ More complex and sensitive fluorometric sensors have been developed,⁵⁻⁸ with many using the colorful species in a resonance energy transfer (RET) sensing mechanism.⁹⁻¹² In such a sensor, a donor fluorophore is chosen based on significant overlap of its emission spectrum with the absorbance spectrum of the TNT product acceptor. In this fashion, a sensor can be tailored for a species that absorbs in a specific range.

Alkoxide reactions with TNT have been studied for a number of years, with two major competing reactions resulting. Scheme 1 shows the possible reactions between TNT and hydroxide or a generic Lewis base. The literature reports the ability of hydroxide or alkoxide to deprotonate the methyl group of TNT, forming TNT^- , or to attack the ring forming the sigma adduct TNT-OR^- .¹³⁻¹⁹ While alkoxides are believed to attack TNT's C_3 carbon, hydroxide is much less sterically hindered and is able to form the TNT-OH^- C_1 adduct, pushing TNT's methyl group out of plane. Since this study and others established C_1 as the most favorable nucleophilic attack site, this is commonly attributed as the site of attack by weaker nucleophiles, such as amines.^{3,4,9-12,20-29} However, given the scope of our study, we also indicate here the possibility of a Lewis base attack on C_3 as a function of decreased sterics at that site. While there are other proposals for TNT mechanisms, these are the possible initial elementary steps for any further reactions; therefore, these are the most crucial to understand in the context of a rapid TNT sensor. Due to this, further reactions such

Scheme 1. Proposed reactions of TNT with hydroxide and amine bases. TNT can be deprotonated at its methyl group forming TNT^- , can be attacked by a nucleophile at

the C₁ or C₃ carbon forming TNT-OH⁻ or TNT-B, or can have electron density donated to its electron deficient ring forming a CT complex, TNT:B.

as the "Janovsky complex"¹⁴ (TNT⁻ attacking TNT) will not be discussed in this study.

Given the multitude of TNT sensor proposals in literature that depend on TNT's interaction or reaction with amines, it is perhaps surprising that a thorough mechanistic study has not been undertaken previously. These types of sensors have been developed for both solution phase and vapor phase detection of TNT. Amine reactivity is proposed to work in similar fashion to anionic bases, where deprotonation to TNT⁻,²⁰⁻²³ the formation of a zwitterionic sigma complex (TNT-NR₃),^{3,9-12,24-27} and charge transfer (CT) complexes^{4,28,29} are all possible. Scheme 1 indicates these possibilities as the reactivity of a generic base (:B). A typical literature approach is to examine the absorbance spectrum of TNT in solution with an amine reactant before incorporating that reactant into a solid phase sensor, to project the reactivity of TNT vapor. The time domain of these studies are poorly documented; Fant et. al. are commonly referenced for the rapid reaction of TNT with amino acids and other amines, but their reactions were performed over a 24 hour period.³ The reactions are commonly done in solvents that may have competing reactions with TNT, as well as solvents which are not easily dried of trace water. Therefore it is possible that in this early stage of development, the reactivity may not be coming directly from the amines, but rather from alternative sources like OH⁻ generated through acid/base equilibria. Additionally, the observed absorbance spectra are commonly misattributed to the incorrect major absorbing species. There is definite confusion as to the actual product(s) being formed in solution, as numerous accounts of the same or similar

reactions lead to a different proposed product; for example, the same absorbance spectrum has been explained by a charge transfer complex,²⁸ TNT^- ,²¹ or a sigma adduct with an amine.²⁷ The overall goal of this study is to clarify the product formation of the reaction of TNT with amines, and address the relative rates of the TNT reactions with hydroxide against those of amines. In addition, we set out to clarify the timescale in which amine functionalized TNT sensors may or may not be applicable.

MATERIALS AND METHODS

TNT was obtained from Drs. Jimmie Oxley and James Smith and was used without further purification. NMR experiments were conducted in either d_8 -THF (99.5% D) or in d_4 -MeOD (99.8% D), both purchased from Cambridge Isotope Laboratories. DCl/D₂O (35% w/w, 99% D) solution and NaOD/D₂O (40% w/w, 99.5% D) solution were purchased from Sigma Aldrich. Additional D₂O (99.8% D) for dilution was purchased from Acros. TNT solutions were quantitatively prepared and ¹H and ¹³C spectra were acquired before and after the addition of base on a Bruker 300 MHz NMR. Time delay from the start of reaction to data acquisition was approximately five minutes per spectrum, due to solvent locking and shimming.

Solutions containing TNT for absorbance measurements were made quantitatively using either HPLC grade THF (99.9%, inhibitor-free) or HPLC grade MeOH (99.9%), both purchased from Sigma. TNT stock solutions were added to a cuvette and observed prior to addition of amine. While stirring, amine solutions were quantitatively added using a micropipette. To rapidly gather absorbance data, a setup

was constructed in house with a Peltier temperature controlled cuvette holder held at 20.00°C and a white light tungsten lamp directed by OceanOptics© fiber optics upon the sample. Our setup was capable of acquiring one spectrum about every 50 msec, limiting our temporal resolution to that value.

In order to address the kinetic evaluation in a reasonable fashion, a pseudo-first order kinetic approach was used. Experiments were prepared so that one reactant concentration would be 100x excess of the other, so that the loss of the excess reactant was negligible, simplifying the calculations. Data were fit to exponential trends using commercially available graphing software (Microsoft Excel, SigmaPlot).

RESULTS AND DISCUSSION

To elucidate the reaction between TNT and aliphatic or aromatic amines, our group utilized a similar approach to Fyfe et. al. by exploring relationships between ^1H NMR and absorbance spectra. In order to be consistent with what is known about hydroxide's reaction with TNT, we were able to perform our experiments first with hydroxide and subsequently with a group of selected amines of various basicity, nucleophilicity, and sterics. The aliphatic amines explored in this study included diethylamine (DEA), triethylamine (TEA), ethylamine (EA), propylamine (PA), and n-butylamine (BA). The aromatic amines explored in this study included 2,6-lutidine, collidine, pyridine, and aniline. Color changes were not observed with the addition of reasonable amounts of TNT and aromatic amines. Visible range absorbances were detected only when tens of milligrams of TNT were added to these as solvents; because of this, the further study of TNT reactions with aromatic amines was

discarded and only the aliphatic amines were used. In our attempt to investigate the formation of TNT products in differing environments, methanol and tetrahydrofuran (THF) were used as solvents to assess polar and slightly polar surroundings respectively.

Product identification through NMR:

^1H NMR was employed to determine the possible contributors to visible range absorbance. While we recognize the time discrepancy between our ability to acquire ^1H NMR data (on the order of 5 minutes) and our ability to monitor visible absorbance changes (on the order of tens of milliseconds), we were able to correlate our spectral data to faster data acquisitions from the literature.¹³ Since we anticipate ambient water to be present in even the most nominally dry solvents, our first investigation was the reaction of TNT with OH^- .

Hydroxide/methoxide as competing species:

In order to understand the relation of TNT/amine products in systems with any trace water, reactions of TNT and sodium deuterioxide in deuterium oxide solution ($\text{NaOD}/\text{D}_2\text{O}$) were carried out in deuterated THF and deuterated methanol. Figure 1 represents the reaction with increasing concentration of $\text{NaOD}/\text{D}_2\text{O}$ in d_8 -THF, while Figure 2 represents the same reaction in d_4 -MeOD.

Figure 1. ^1H NMR spectra for the reaction of 1.3×10^{-2} M TNT with increasing concentration of NaOD/ D_2O in d_8 -THF: (a) NaOD:TNT = 0; (b) NaOD:TNT = 0.14; (c) NaOD:TNT = 0.29; (d) NaOD:TNT = 0.58; (e) NaOD:TNT = 0.94; (f) NaOD:TNT = 1.30; (g) NaOD:TNT = 1.37; (h) subsequent addition of one drop of 30% $\text{DCl}/\text{D}_2\text{O}$.

Figure 1 shows the aromatic and methyl protons of TNT decreasing as a function of NaOD addition in d_8 -THF, as expected. TNT has two equivalent aromatic protons represented by the singlet at 8.98 ppm, as well as three equivalent methyl protons showing as a singlet at 2.62 ppm. Both peaks disappear as a function of the addition of NaOD, as a product grows in. The growing singlet peaks (8.31 ppm, 5.63 ppm) are observed to have a 1:1 integration ratio; this is consistent with the formation of TNT^- , while inconsistent with the formation of TNT-OH^- . The two aromatic protons on C_3 and C_5 of TNT^- give rise to the singlet at 8.31 ppm while the two equivalent vinyl-type protons on C_1 show as a singlet at 5.63 ppm. With no other

features apparent in this figure, TNT^- appears to be the exclusive product on this relative timescale of approximately one hour. The relative peak positions are consistent with the report of TNT^- formation in DMSO/MeOH using alkoxide bases from Fyfe et. al.¹³ Small shifts in unreacted TNT's methyl peaks are likely related to H/D exchange. Also, the final addition of excess DCl to the system would be expected to re-deuterate the methylene group on TNT^- , forming a CH_2D methyl group. The observation of three singlet peaks in the methyl region supports this, as does the restoration of the aromatic proton peaks to nearly their original chemical shift. Since the addition of excess DCl re-establishes an equilibrium state, a ^{13}C DEPT 135 spectrum was acquired with the final sample of the titration (Supplementary Information Fig. S2). The unreacted TNT peaks for the methyl carbons and the ring carbons (C_3 & C_5) with aromatic protons were apparent, given the hydrogens associated with them, while the other carbons did not show signals with a one hour acquisition time. After the deprotonation and subsequent deuteration, the DEPT 135 spectrum shows the aromatic carbons remaining at the same chemical shift; however, the methyl carbon peak is no longer visible. This is likely a result of two phenomena: the removal of a hydrogen from the methyl carbon lowers its relative enhancement factor, and the addition of a deuterium splits the signal to a triplet, further lowering the S/N ratio.

Figure 2. ^1H NMR spectra for the reaction of 1.5×10^{-2} M TNT with increasing concentration of NaOD/ D_2O in d_4 -MeOD: (a) TNT:NaOD = 0; (b) TNT:NaOD = 0.24; (c) TNT:NaOD = 0.49; (d) TNT:NaOD = 0.97; (e) TNT:NaOD = 1.95; (f) TNT:NaOD = 2.44; (g) addition of one drop of 30% DCl/ D_2O . "*" indicates an impurity in the sample.

In similar fashion, TNT^- features can also be observed as a primary product in d_4 -MeOD; however, TNT^- is not the only product in this system. TNT^- is evidenced by the growth of singlets at 8.30 ppm and 5.68 ppm, for the C_3/C_5 protons and the remaining C_1 protons respectively. In this more polar solvent, deuterium exchange can be observed with the methylene group on TNT^- , as the peak at 5.68 ppm diminishes and subsequently disappears as a function of time while the aromatic protons on TNT^- remain visible. This indicates the full deuteration to a CD_3 group. H/D exchange on TNT in basic deuterated media has been observed in the literature,³⁰ and is indicative of the formation of TNT^- being a reversible process.

The other species which appears to be growing in at a slower rate and as a function of increased NaOD concentration has a singlet at 8.41 ppm and another at 5.30 ppm. Their relative integration ratio is 3:2 which is consistent with the C₁ deuterioxide adduct formation, where the lower field feature represents the two aromatic protons (on C₃ and C₅) and the higher field feature represents the intact but shifted methyl group protons. This differs from Fyfe's observation of a 1:1 ratio for the C₃ adduct of methoxide, where two singlets were detected at 8.45 ppm and 6.18 ppm in 87.5% DMSO/12.5% MeOH. It is also important to note that the products only make up a small percentage of the total integration at equimolar TNT and NaOD concentration; this suggests that the equilibria lie much further towards reactants in MeOH than in THF. While there are unfamiliar features developed from the addition of excess DCl at the end of this titration, the implication of this product does not appear to impact our further absorbance investigation as we will discuss later in this paper. Also, TNT was exposed to DCl as a control to this experiment, which showed no change to the original TNT features. After the addition of DCl to the system, reaching a rapid equilibrium state, a ¹³C DEPT 135 experiment was undertaken. The data (Supplementary Information, Fig. S4) also support the full deuteration of TNT⁻, creating a CD₃ group; in this case, we observe the features to be negative whereas the features were not observable in *d*₈-THF. The CD₃ group is expected to show as a septet, which may be what we see as the negative features at 15 ppm with very low S/N. This may be additionally hindered by the removal of the pre-existing protons, which enhance that carbon's signal. No other features besides unreacted TNT are

apparent from the carbon spectrum, implying that TNT^- is the major early product of this reaction.

Reaction of TNT with amines:

With an understanding of hydroxide's role in TNT reactivity, we subsequently investigated the formation of products of TNT with various amines using ^1H NMR, as seen in Figure 3.

Figure 3. ^1H NMR spectra for the reaction of TNT with addition of excess bases in d_8 -THF: (a) unreacted TNT; (b) TNT + NaOD; (c) TNT + DEA; (d) TNT + TEA; (e) TNT + EA/ H_2O ; (f) TNT + PA; (g) TNT + BA. The data at higher chemical shift have been cut off due to similarity to unreacted TNT, while lower chemical shifts are overwhelmed by amine features.

In a qualitative assessment of reaction products, it is first apparent that TNT^- features exist in the reaction of TNT with EA or PA while similar features are not

apparent for TEA, DEA and BA. This observation does not correlate well with the expected basicity of each amine in water; however, it does correlate well to their expected water content. EA is a gas at ambient temperatures, and thus was only available as an aqueous solution. As a larger quantity of PA was necessary for product observation, a small water impurity may exist in this system as well. Since the addition of water directly generates hydroxide, it is difficult to clarify which species seen here are resultant from hydroxide or amine reactivity. Other features are seen in these spectra, which may relate to amine adducts, but are barely observable over the baseline noise and are a very small contributor even with excess amine – this suggests that the equilibrium constant for these reactions is low, with mainly unreacted TNT being observed at equilibrium.

It is interesting to note here the possible appearance of two sigma adducts in the reaction of TNT with DEA, as Figure 3c shows two quartets in the spectrum for this reaction. The quartets may come from the inequivalence of the alkyl groups on DEA after covalently bonding to TNT, but may also come from two different attack sites. As the size of the nucleophile increases and sterics begin to play a larger role, it is conceivable that DEA can attack either the C₁ carbon or the C₃ carbon with similar energies. Computational analysis supports this proposal, as the relative energies of formation for the two sigma adducts shows the C₃ adduct to be slightly more favorable (*vide infra*).

Figure 4. ^1H NMR spectra for the reaction of TNT with addition of excess bases in MeOD: (a) unreacted TNT; (b) TNT + NaOD; (c) TNT + DEA; (d) TNT + TEA; (e) TNT + EA/H₂O; (f) TNT + PA; (g) TNT + BA. The data at higher chemical shift have been cut off due to similarity to unreacted TNT, while lower chemical shifts are overwhelmed by amine features.

With similar respect to the TNT/amine reactions in THF, Figure 4 shows the vinyl region of the ^1H NMR spectra for the same reactions in MeOD. The same major features appear here for the TNT^- anion at 8.3 ppm and 5.6 ppm, with the "wet" amines showing higher amounts. As seen in the NaOD titration in MeOD, there is obvious H/D exchange of the methylene hydrogens leading to the loss of the signal at 5.6 ppm relative to the aromatic protons at 8.3 ppm. The addition of amines to MeOD may also be generating methoxide through an acid/base equilibrium, which is also capable of deprotonating TNT. Since the TNT^- features are barely above the level of noise, it is also possible that a TNT-OCH_3^- adduct may be formed at an undetectable amount. This is clearly important, as it adds the possibility of a similar, but different

set of absorbance features in our further experiments; it also implicates the sensitivity of TNT reactions to competition with solvent molecules. The unreacted TNT signals (not shown) are the predominant features with approximately 500 times the intensity of the products. Clearly, the equilibria for these reactions lie much closer to reactants than products or the kinetics for these reactions are very slow.

Absorbance Characteristics/Kinetic Evaluation

Although absorbance spectroscopy is not a clear identification method for product structure, it can be a powerful tool when coupled with a method like NMR. Here, we set out to observe similarities in absorbance measurements to propose that there is no significant visible absorbance from TNT-amine adducts. In addition, we put to question whether amines are basic enough to deprotonate the methyl group on TNT. A simple experiment was conducted by dropping a few crystals ($\approx 10^{-3} - 10^{-4}$ M) of TNT into a few milliliters of dry TEA and observing the absorbance.

Surprisingly, no visible absorbance features were generated. This observation has dual consequence. First TEA is one of the most basic of the aliphatic amines, and although its steric hindrance is enough to rule out a sigma complex it would not be expected to limit its ability to deprotonate TNT's methyl group. Secondly TEA is one of the highest known solvents on Gutmann's Donor Number (DN)³¹ scale, as it has the capacity to donate electron density to an electron-poor species, forming a charge-transfer (CT) complex. Trinitrobenzene (TNB) has been shown to form CT complexes with aliphatic amines (including TEA) in cyclohexane, but their absorbance maxima are in the UV range.³² TNT is not fully planar like TNB, so the

formation of a CT complex may be comparably hindered by sterics. Since we know TNT's aromatic ring is electron-poor, and there are literature proposals of TNT forming CT complexes which absorb well into the visible range, this seems to stand in contrast to those proposals and indicate that they incorrectly identify the absorbing species.

To determine the rate constants for the concurrent formation of TNT^- and TNT-OR^- ($\text{R} = \text{H}, \text{CH}_3$), we derived an expression to fit absorbance data as a function of time. The full derivation can be found in the Supplementary Information. Briefly, we are dealing with the following competing equilibria under pseudo-first order conditions:

Under pseudo-first order conditions, the concentration of each product can be monitored as a function of time using a single exponential rise function:

$$[\text{Product}] = a(1 - e^{-kt})$$

where "a" and "k" are functions of intrinsic rate constants as well as initial concentrations of reactants. Substituting in absorbance for concentration using Beer's Law yields:

$$A_{Product} = \epsilon_{Product}ba(1 - e^{-kt})$$

Since water content clearly plays a role in the TNT^- equilibrium if OH^- is the reactant, its concentration must be accounted for in our experiments. We have previously described the equilibria linking water concentration to these species in DMF solution.³³

Figure 5. Initial five minute absorbance experiment observing the products of 100x excess NaOH with 4.2×10^{-5} M TNT in THF. (a) 5.21 sec; (b) 22.96 sec; (c) 95.13 sec; (d) 153.70 sec; (e) 214.10 sec; (f) 289.78 sec. Note the spectral shape change early on, consistent with the OH^- sigma adduct giving way to TNT^- as the major absorbing species.

Figure 6. Initial five minute absorbance experiment observing the products of 100x excess 2.2×10^{-3} M TNT with NaOH in THF. (a) 0.00 sec; (b) 0.38 sec; (c) 0.75 sec; (d) 5.33 sec; (e) 14.35 sec; (f) 61.57 sec; (g) 108.63 sec.

Having good evidence for the products formed by TNT and hydroxide, we conducted experiments in which we observed the visible absorbance spectrum for five minutes after the reactions were started. In Figure 5, we observe the reaction using excess NaOH in THF with the formation of two products: one which grows in immediately and the other which equilibrates much more slowly. However, the second product is clearly the major absorbing species even before equilibrium is established. The early literature confirms the species which give rise to these absorbance features; the early product here has the shape of an anionic adduct, TNT-OH^- , while the second spectrum belongs to TNT^- .^{13,14} This is consistent with our ^1H NMR assessment; due to sample preparation time, instrumental setup and data acquisition, TNT-OH^- had already disappeared and TNT^- was the only observed species. Figure 6 shows the reaction of 100x excess TNT with NaOH in THF.

Similar spectral shape to the excess NaOH experiments is seen here, with possible TNT-OH^- formation early and a slower formation of TNT^- . A control experiment was conducted to determine the effect of a possible scattering component of NaOH in THF on our kinetic fits. We observed a reasonably significant baseline increase with the addition of NaOH to THF, which dissipated within a few seconds. This lead us to omit some initial data when the absorbance signal was on a similar order of magnitude as the scattering.

Figure 7. Initial five minute absorbance experiment observing the products of 100x excess NaOH with 2.5×10^{-4} M TNT in MeOH. (a) 0.14 sec; (b) 0.79 sec; (c) 4.09 sec; (d) 9.02 sec; (e) 55.69 sec; (f) 172.51 sec; (g) 268.17 sec. Note the pseudo-isosbestic point established at 494 nm, once the TNT^- absorbance begins to decay. The inset shows absorbance data from 520 nm and 700 nm fit to an exponential rise function, where the observed rate constant is the exponential variable; in this case, the data at 520 nm required two exponential rise functions to fit properly.

The same experimental approach was also taken in MeOH. Figure 7 indicates a different observation as a function of time. TNT^- is the predominant absorbing

species early on, which then gives way to absorbances at lower wavelengths from TNT-OH^- . This is consistent with the order of appearance of these species in our NMR. While we are able to fit our entire five minute data set to a phenomenological set of exponential rise and decay functions (see Supplementary Information Figs. S11-13), we are not able to identify the secondary products nor do they appear to absorb strongly in the visible range. In addition, there is no analytic solution for the sequential equilibria that we have phenomenologically modeled. Due to this and the stronger importance of the initial processes on TNT detection, we continued by modeling the early absorbance evolution in MeOH.

The inset on Figure 7 shows a relatively close relationship between the observed rate constants at 520 nm and 700 nm, indicating the observation of the same species. However, it is interesting to note the late appearance of pseudo-isosbestic points, as seen in Figure 7 at 494 nm; these inflection points are apparent in each experiment but are not observed at the same location, which invalidates their assignment as a true isosbestic point. Instead, these points seem to shift to higher energy as the $[\text{TNT}]$ decreases. This may serve to imply a visible absorbance component of an additional species, or it may indicate that TNT^- and TNT-OH^- are not directly mechanistically linked. It may also result from lingering scattering component that does not fully dissipate. The scattering component from NaOH addition was smaller in MeOH than observed in THF, but still significant enough to warrant the omission of some initial data points.

Figure 8. Initial absorbance experiment observing the products of 100x excess TNT ($4.1 \times 10^{-2} \text{ M}$) with NaOH in MeOH. The spectrum increases then decreases, with no additional features appearing during the observation. The inset shows absorbance data from 520 nm and 700 nm fit to an exponential rise function, where the observed rate constant is the exponential variable.

Figure 8 shows the excess TNT experiment in MeOH, where a new dominant species is observed. These spectral features are consistent with TNT-OH^- formation, while there is still slight evidence to suggest a small TNT^- component given the curvature of the spectrum beyond 700 nm. Also, note that the relative absorbance of the feature at 450 nm and the feature at 520 nm changes slightly over the course of the observation; this implies that more than one species is being observed. The observed rate constants are virtually identical however, suggesting that we are observing TNT-OH^- formation at both wavelengths. This is also important because this indicates that TNT-OH^- does have an absorption component at 700 nm, although relatively small.

The early absorbance growths fit well to a set of exponential growths, as anticipated in our derivation and indicated on the insets to the absorbance evolution figures. The observed rate constants can be found collectively in Table 1.

	[TNT] (M)	[OH ⁻] (M)	k_{obs} A(520 nm)	k_{obs} A(700 nm)	Species Observed
THF	4.2E-05	4.2E-03	6.7E-03	7.6E-03	TNT ⁻
	3.2E-05	3.2E-05	6.1E-03	7.1E-03	
	2.2E-05	2.2E-03	7.0E-03	7.8E-03	
	2.2E-03	2.2E-05	3.9E-03	7.3E-03	TNT ⁻
	1.7E-03	1.7E-05	5.7E-03	7.0E-03	
	1.3E-03	1.3E-05	5.3E-03	8.0E-03	
MeOH	2.5E-04	2.5E-02	9.1E-01	7.3E-01	TNT ⁻
	1.9E-04	1.8E-02	8.3E-01	8.5E-01	
	1.5E-04	1.4E-02	9.0E-01	7.5E-01	
	4.1E-02	4.0E-04	1.2E+00	1.2E+00	TNT-OH ⁻
	2.9E-02	2.9E-04	2.1E+00	2.2E+00	
	2.2E-02	2.2E-04	1.7E+00	1.6E+00	

Table 1. Observed rate constants for pseudo-first order TNT + NaOH experiments in THF and MeOH. The observed rate constants were calculated by fitting absorbance data at 520 nm or 700 nm to a single exponential rise function.

Note that the observed rate constants are reproducible within the anticipated errors of the kinetic method. In THF, the major absorbing species is TNT⁻, which has absorbance at both observed wavelengths. In MeOH, the excess NaOH experiments show TNT⁻ as the major absorbing species, while the excess TNT experiments show TNT-OH⁻ as the major absorbing species. The observed rate constants for TNT⁻ are equivalent to $k_{-1}[H_2O]_o$; since the excess water concentration is relatively high for the replicate experiments and k_{obs} is not directly related to [TNT], we expect k_{obs} not to change dramatically with differing [TNT] or [OH⁻]. In the case of TNT-OH⁻, the observed rate constant is equal to k_{-2} and so reproducibility of this parameter is also

expected. These observations indicate that our kinetic model is appropriate for the reactions of interest.

The molar absorptivity of TNT^- (ϵ_{TNT^-}) was calculated in THF, under the assumption that all TNT was deprotonated by 100x excess OH^- ; this is shown to be appropriate given the TNT/OH^- ^1H NMR titration. If we assume that TNT-OH^- does not significantly absorb at 700 nm,^{13,14} this gives us the capacity to quantify the $[\text{TNT}^-]$ at any point of time and to calculate kinetic parameters by starting with TNT^- at 700 nm. Also, if we assume that the absorbance component from TNT-OH^- is negligible at equilibrium, we can determine ϵ_{TNT^-} at 520 nm as well. Since there is little absorbance evidence for TNT-OH^- in THF at later time, this is an acceptable assumption. Using the extrapolated absorbance at equilibrium and the initial $[\text{TNT}]$, the resulting calculations yielded 4000 L/mol and 11200 L/mol for ϵ_{TNT^-} at 700 nm and 520 nm respectively. Unfortunately, the same reaction in MeOH is shown to be more complicated by NMR, so the same approach may not be appropriate to calculate the molar absorptivity of TNT^- in MeOH. We estimate the molar absorptivities to be the same for both solvents, within reasonable approximation.

A similar approach could not be taken to calculate the molar absorptivity of TNT-OH^- ($\epsilon_{\text{TNT-OH}^-}$) at 520 nm, since there is no way of calculating $[\text{TNT-OH}^-]$ in any of our explored systems. Knowing that $\epsilon_{\text{TNT-OH}^-}$ should be a constant, we proceeded able to calculate the product of the molar absorptivity and the forward rate constant ($\epsilon_{\text{TNT-OH}^-}k_2$). If there is no significant observation of an amine sigma adduct, then each amine experiment should yield this same parameter within experimental error. Given that the excess TNT experiments in MeOH gave the

clearest observation of TNT-OH⁻ formation, we used the same conditions for the amine experiments. In addition, we were unable to satisfy the excess OH⁻ pseudo-first order condition using excess amine, since this would have required large volumes of amine.

	[TNT] (M)	[OH ⁻] (M)	[H ₂ O] (M)	k ₁ A(520 nm)	k ₁ A(700 nm)	k ₁ A(520 nm)	k ₁ A(700 nm)
THF	4.2E-05	4.2E-03	2.1E+00	1.7E+00	2.0E+00	3.1E-03	3.5E-03
	3.2E-05	3.2E-05	1.6E+00	1.8E+00	2.1E+00	3.8E-03	4.4E-03
	2.2E-05	2.2E-03	1.1E+00	3.1E+00	3.4E+00	6.4E-03	7.1E-03
	2.2E-03	2.2E-05	1.1E-01	3.6E-02	4.7E-02	3.5E-02	6.5E-02
	1.7E-03	1.7E-05	8.8E-02	5.3E-02	4.8E-02	6.5E-02	8.0E-02
	1.3E-03	1.3E-05	6.6E-02	4.7E-02	5.8E-02	8.0E-02	1.2E-01
MeOH	2.5E-04	2.5E-02	1.3E+01	4.4E+00	5.2E+00	7.1E-02	5.7E-02
	1.9E-04	1.8E-02	9.3E+00	8.4E+00	7.5E+00	8.9E-02	9.2E-02
	1.5E-04	1.4E-02	6.8E+00	7.1E+00	5.4E+00	1.3E-01	1.1E-01

Table 2. Intrinsic rate constants for the formation of TNT⁻ calculated using kinetic model and absorbance data at 520 nm and 700 nm.

Upon calculation of the intrinsic rate constant for the TNT⁻ reaction (in Table 2), we observe a major discrepancy between the excess NaOH and excess TNT experiments. The intrinsic rate constants should be the same for each condition, but they differ by at least an order of magnitude. We have shown above the relationship between the reverse rate constant and [H₂O], so this is expected to be accounted for in our proposed mechanism. However, the strong disparity between the dielectric constant of THF ($\epsilon = 7.5$) and of H₂O ($\epsilon = 80$) may be a key factor here. In the addition of excess NaOH, a relatively large amount of water is delivered to the otherwise non-polar solvent system. If water plays a role in solvating TNT or the

transition state in the formation of TNT^- , this trend may show up in our intrinsic rate constants. This is consistent with what we observe in the solvent change from THF to MeOH; the relative rate of formation of TNT^- vs. TNT-OH^- shifts in favor of TNT^- with higher dielectric. Also, in the excess NaOH experiments in THF the forward and reverse rate constants increase as the $[\text{H}_2\text{O}]$ decreases. This may be resultant from the effect of ambient water on our calculations. As the added water component from the NaOH solution decreases, the relative error in our $[\text{H}_2\text{O}]$ due to the hygroscopic nature of each solvent system increases. Since k_{-1} is calculated using $[\text{H}_2\text{O}]$, this would account for the observed increase in k_{-1} . It would also account for the observed increase in k_1 , as the calculation of k_1 depends on k_{-1} in our model. With these factors in mind, performing the amine experiments in MeOH with excess TNT and intentionally added water should minimize any of these effects on our analysis, as well as the scattering effect of NaOH in MeOH.

Amine reactions in MeOH:

Solutions of TNT ranging from 10^{-2} to 10^{-1} M were prepared, as well as diluted amine solutions in MeOH. Using the same techniques described above, the amine solutions were quantitatively added to the TNT solutions in a stirring cuvette while absorbance measurements were taken. Absorbance spectra evolved very rapidly and equilibrated within several seconds, as in the case of OH^- addition. Figure 9 shows the absorbance evolution of the reaction of excess TNT with EA, DEA, and TEA in MeOH.

Figure 9. Absorbance evolution for the reaction of 100x excess TNT with amines in MeOH. (a) 8.4×10^{-2} M TNT + EA, (b) 9.5×10^{-2} M TNT + DEA, (c) 9.0×10^{-2} M TNT + TEA.

Insets on Figure 9 indicate the observed rate constants for the reaction, which are equivalent to k_{-2} . The observed rate constants at 520 nm and 700 nm are similar for each individual experiment, as seen in the hydroxide reactions. They are not similar, however, for the reactions with different amines; the EA and DEA experiments agree within random error, but the TEA reaction shows a much larger observed rate constant. The shape of each set of spectra are strikingly similar, which is not expected for these three amines. TEA is too sterically hindered to form a sigma complex with

TNT, while EA and DEA are expected to form sigma complexes to different degrees. A normalized set of spectra from the initial equilibrium are found in Figure 10a. All spectra were normalized to their shared amine reaction absorbance maximum at 450 nm. The amine reaction spectra overlap well from about 420 to 460 nm, implying that only one species absorbs in this range, while the OH^- reaction spectrum shows slightly different shape. It is likely that TNT-OH^- is the major absorbing species formed in the OH^- reaction, and that the amines are deprotonating MeOH leading to the formation of TNT-OCH_3^- . This would account for the similar spectral shape, since both sigma adducts are expected to have similar MO energies. Towards lower energy, the spectra diverge but retain similar spectral shape consistent with the established spectrum of TNT^- . Difference spectra of the amine reaction spectra subtracted from the OH^- reaction spectra are found in Figure 10b, which further establish the similar spectral shape of TNT^- formation. Different amounts of TNT^- are expected at equilibrium, due to the different strengths of the amine bases deprotonating water or methanol and slightly different amounts of water. Due to the similar spectral shapes in Figure 10, this supports the assessment of only two significantly absorbing species in the visible range (TNT^- and TNT-OCH_3^-), which cannot include an amine sigma adduct.

Figure 10. (a) Absorbance overlay of TNT reactions with hydroxide and amine bases at equilibrium established within 10 seconds. Spectra have been normalized to $A(450 \text{ nm})$. The spectra overlay very well up to around 460 nm, where TNT^- begins to absorb, indicating the formation of the same adduct species for each reaction. (b) Difference spectra calculated using normalized data from (a). Since the addition of hydroxide is observed to generate the most TNT^- , the amine reaction spectra were subtracted from it. The residual spectra show the same shape, consistent with the only other significantly absorbing product, TNT^- .

Amine	K_{eq} (MeOH)	[TNT] (M)	[NR ₃] (M)	[H ₂ O] (M)	$\varepsilon(\text{TNT-OCH}_3)_2$ A(520 nm)	$\varepsilon(\text{TNT-OCH}_3)_2$ A(700 nm)	k_2 A(520 nm)	k_2 A(700 nm)
DEA		6.9E-02	6.9E-04	1.2E-03			7.0E-01	7.3E-01
		7.9E-02	7.9E-04	1.2E-03			9.2E-01	9.1E-01
		9.5E-02	9.5E-04	1.2E-03			7.3E-01	7.0E-01
							7.8E-01	7.8E-01
TEA	9.0E-07	1.1E-01	1.1E-03	1.2E-03	7.6E+04	7.6E+03	1.7E+00	2.0E+00
		9.8E-02	9.8E-04	1.2E-03	5.4E+04	5.6E+03	1.8E+00	2.0E+00
		9.0E-02	9.0E-04	1.2E-03	4.4E+04	5.4E+03	1.3E+00	1.7E+00
			avg		5.8E+04	6.2E+03	1.6E+00	1.6E+00
EA	2.0E-06	5.9E-02	5.9E-04	1.9E-03	1.0E+05	1.3E+04	5.4E-01	5.2E-01
		7.7E-02	7.7E-04	2.1E-03	7.5E+04	8.3E+03	3.8E-01	3.6E-01
		8.4E-02	8.4E-04	2.2E-03	1.2E+05	1.3E+04	6.8E-01	7.0E-01
			avg		9.8E+04	1.2E+04	5.4E-01	5.3E-01
PA	8.6E-06	7.6E-02	7.5E-04	1.1E-03	4.1E+04	4.8E+03	6.0E-01	5.8E-01
		6.5E-02	6.4E-04	1.1E-03	3.7E+04	4.7E+03	5.5E-01	5.4E-01
		8.0E-02	7.9E-04	1.1E-03	5.0E+04	5.7E+03	5.3E-01	5.3E-01
			avg		4.3E+04	5.0E+03	5.6E-01	5.5E-01
BA	5.8E-06	9.6E-02	9.5E-04	1.2E-03	7.3E+04	7.3E+03	5.3E-01	5.1E-01
		1.0E-01	1.0E-03	1.2E-03	5.0E+04	4.6E+03	4.5E-01	4.3E-01
		1.0E-01	1.0E-03	1.2E-03	5.7E+04	5.3E+03	4.5E-01	4.3E-01
			avg		5.7E+03	6.0E+04	4.8E-01	4.6E-01

Table 3. Calculated rate parameters for reactions of excess TNT + amines in MeOH.

Table 3 summarizes the calculated rate parameters for the reactions of excess TNT with amines in MeOH. As mentioned above, the reverse rate constants for TEA differs substantially from those of the less sterically hindered amines. This could be explained through the early formation of a charge-transfer complex, although we saw no evidence in the visible range to support the formation of such a species. Foster reported the formation of CT complexes of trinitrobenzene (TNB) with aliphatic amines in cyclohexane, where the CT bands for EA, DEA, and TEA all had absorbance maxima in the UV. TNT has a different conformation than TNB, with the methyl group forcing the nitro groups out of plane with the ring; this not only decreases the electron withdrawing capacity of the nitro groups, but adds a steric component that TNB does not have. This increases the relative electron density of the ring and alters the relative MO energies. Due to these two factors, the formation of a CT complex would be hindered and may not form as readily and may have absorbance maxima at higher energies. Therefore, it is possible that CT species are being formed with TNT but out of our observational range, as well as overlapping with the TNT absorbance in the UV. This is consistent with our observation that the reversibility of the TEA reaction is fastest, as it has the most favorable CT complex formed earlier to compete with.

The forward rate constant product ($\epsilon_{TNT-OH}k_2$) was calculated for the amine reactions under the assumption that OCH_3^- is the major attacking nucleophile, using the acid/base equilibrium constants for the amine deprotonation of MeOH.³⁴ These calculated values agree very well between TEA, PA, and BA, indicating that the same forward reaction is occurring. The EA reaction has a $\epsilon_{TNT-OH}k_2$ value that is about

twice as large. This may be related to the water content in this reaction relative to the other amine reactions. Since EA is delivered from an aqueous solution, the water which accompanies it may cloud this calculation. Not only does the water shift the overall dielectric higher to a more polar environment, but the extra water also carries extra hydroxide generated by the deprotonation of water by EA. In light of this, the observed reaction of EA may be two sigma attack reactions occurring at the same time with similar kinetics, or a poor assessment of the actual amount of OCH_3^- formed prior to reaction with TNT. However this may be, the consistency of the $\epsilon_{\text{TNT-OH}}-k_2$ parameter through the PA, BA, and TEA reactions clearly indicates that the amines are not directly involved in the reactions that generate the visible range absorbing species in the initially observed reactions. It is plausible that direct amine attack is much slower, or perhaps requires a substitution reaction where it replaces an anionic nucleophile. Therefore, their use for ultra-trace vapor detection of TNT is not appropriate.

CONCLUSIONS

To conclude, we have shown through related ^1H NMR and visible absorbance experiments that amines do not play a direct role in the initial formation of decomposition products of TNT. Amines are not basic enough to directly deprotonate the methyl group of TNT, nor are they strong enough nucleophiles to compete with the small quantity of anionic species that they form through acid/base reactions in solution. The observation of visible range absorbances, which are commonly used in sensor development, instead are resultant from TNT^- or TNT-OR^- depending on the

solvent identity and dielectric. This finding may indicate the need for reassessment of existing sensors for better mechanistic clarification, as well as caution regarding the choice of solvent in similar systems. Also, this study shows the need to assess trace amounts of water in solution or vapor during the development and employment of sensors that rely on these reactions.

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Supplementary Information for:

**Comparing the Roles of Hydroxide and Amines in TNT Reactions: Clarifying
Absorbance Spectra Assignments For Use in Detection Methods**

by

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Euler*

Figure S1. ¹³C NMR spectrum of TNT in *d*₈-THF prior to addition of base.

Figure S2. ^{13}C DEPT 135 spectrum of TNT + NaOD in d_8 -THF with quick addition of excess DCl.

Figure S3. ^{13}C NMR spectrum of TNT in MeOD before addition of base.

Figure S4. ^{13}C DEPT 135 spectrum of TNT + NaOD after the addition of excess DCl in d_4 -MeOD. The inset is a zoomed view of the 14 ppm to 17 ppm region, where negative features can be observed consistent with the deprotonation and re-deuteration of TNT.

Kinetic Model Derivation:

For the competing equilibrium reactions between TNT and hydroxide (or methoxide):

We cannot assume one equilibrium is much faster than the other, because we observe both on a similar timescale. Therefore, they must be solved simultaneously.

The mass balance and charge balance conditions require:

$$[TNT]_o = [TNT] + [TNT^-] + [TNTOH^-] \quad [3]$$

$$[OH^-]_o + [H_2O]_o = [OH^-] + [H_2O] + [TNTOH^-] \quad [4]$$

$$[OH^-]_o = [OH^-] + [TNT^-] + [TNTOH^-] \quad [5]$$

The latter two equations can be combined to show:

$$[H_2O] = [TNT^-] + [H_2O]_o \quad [6]$$

The differential equations are:

$$\frac{d[TNT^-]}{dt} = k_1[TNT][OH^-] - k_{-1}[TNT^-][H_2O]$$

$$\frac{d[TNTOH^-]}{dt} = k_2[TNT][OH^-] - k_{-2}[TNTOH^-]$$

Derivation can proceed under either pseudo-first order condition, as long as water concentration remains in excess over TNT^- concentration.

Case 1: $[\text{OH}^-]_o \gg [\text{TNT}]_o, [\text{H}_2\text{O}]_o \gg [\text{TNT}^-]$,

Letting $k'_1 = k_1[\text{OH}^-]_o$, $k'_2 = k_2[\text{OH}^-]_o$, and $k'_{-1} = k_{-1}[\text{H}_2\text{O}]_o$, the differential equations become:

$$\begin{aligned}\frac{d[\text{TNT}^-]}{dt} &= k'_1[\text{TNT}] - k'_{-1}[\text{TNT}^-] \\ \frac{d[\text{TNTOH}^-]}{dt} &= k'_2[\text{TNT}] - k_{-2}[\text{TNTOH}^-]\end{aligned}$$

From charge balance, $[\text{OH}^-]_o = [\text{OH}^-] + [\text{TNT}^-] + [\text{TNTOH}^-]$ and since $[\text{OH}^-]_o \approx [\text{OH}^-]$ it follows that $[\text{TNTOH}^-] \approx -[\text{TNT}^-]$ and from the TNT mass balance $[\text{TNT}]_o = [\text{TNT}] + [\text{TNT}^-] + [\text{TNTOH}^-]$ or $[\text{TNT}]_o = [\text{TNT}] + [\text{TNT}^-] - [\text{TNT}^-]$ or $[\text{TNT}]_o = [\text{TNT}]$. Then the differential equations become:

$$\begin{aligned}\frac{d[\text{TNT}^-]}{dt} &= k'_1[\text{TNT}]_o - k'_{-1}[\text{TNT}^-] \\ \frac{d[\text{TNTOH}^-]}{dt} &= k'_2[\text{TNT}]_o - k_{-2}[\text{TNTOH}^-]\end{aligned}$$

which readily solve to give:

$$\begin{aligned}[\text{TNT}^-] &= \frac{k'_1[\text{TNT}]_o}{k'_{-1}}(1 - e^{-k'_{-1}t}) \\ [\text{TNTOH}^-] &= \frac{k'_2[\text{TNT}]_o}{k_{-2}}(1 - e^{-k_{-2}t})\end{aligned}$$

Case 2: $[TNT]_o \gg [OH^-]_o, [H_2O]_o \gg [TNT^-]$,

Letting $k_1'' = k_1[TNT]_o$, $k_2'' = k_2[TNT]_o$, and $k_{-1}' = k_{-1}[H_2O]_o$, the differential equations become:

$$\begin{aligned}\frac{d[TNT^-]}{dt} &= k_1''[OH^-] - k_{-1}'[TNT^-] \\ \frac{d[TNTOH^-]}{dt} &= k_2''[OH^-] - k_{-2}[TNTOH^-]\end{aligned}$$

From equation [5], $[OH^-] = [OH^-]_o - [TNT^-] - [TNTOH^-]$ and from equation [3], $[TNTOH^-] = [TNT]_o - [TNT^-] - [TNT]$. Substituting for $[TNTOH^-]$ gives $[OH^-] = [OH^-]_o - [TNT^-] - ([TNT]_o - [TNT^-] - [TNT]) = [OH^-]_o - [TNT]_o + [TNT]$. Since $[TNT]_o \sim [TNT]$, $[OH^-] = [OH^-]_o$. Then the differential equations become:

$$\begin{aligned}\frac{d[TNT^-]}{dt} &= k_1''[OH^-]_o - k_{-1}'[TNT^-] \\ \frac{d[TNTOH^-]}{dt} &= k_2''[OH^-]_o - k_{-2}[TNTOH^-]\end{aligned}$$

These solve to give:

$$\begin{aligned}[TNT^-] &= \frac{k_1''[OH^-]_o}{k_{-1}'}(1 - e^{-k_{-1}'t}) \\ [TNTOH^-] &= \frac{k_2''[OH^-]_o}{k_{-2}}(1 - e^{-k_{-2}t})\end{aligned}$$

1TNT + 100NaOH in THF

Figure S5. Absorbance evolution for the reaction of 1TNT + 100NaOH in THF, where $[\text{TNT}] = 4.2 \times 10^{-5} \text{ M}$ and $[\text{OH}^-] = 4.2 \times 10^{-3} \text{ M}$. The top figure shows absorbance growth in the visible range comprised of two discernible products, TNT-OH⁻ and TNT⁻. The bottom figure shows the absorbance data at 520 nm and at 700 nm as a function of time. The data have been fit to exponential rise functions, with the observed rate constants indicated.

Figure S6. Absorbance evolution for the reaction of 1TNT + 100NaOH in THF, where $[\text{TNT}] = 3.2 \times 10^{-5} \text{ M}$ and $[\text{OH}^-] = 3.2 \times 10^{-3} \text{ M}$. The top figure shows absorbance growth in the visible range comprised of two discernible products, TNT- OH^- and TNT $^-$. The bottom figure shows the absorbance data at 520 nm and at 700 nm as a function of time. The data have been fit to exponential rise functions, with the observed rate constants indicated.

Figure S7. Absorbance evolution for the reaction of 1TNT + 100NaOH in THF, where $[TNT] = 2.2 \times 10^{-5} \text{ M}$ and $[OH^-] = 2.2 \times 10^{-3} \text{ M}$. The top figure shows absorbance growth in the visible range comprised of two discernible products, TNT-OH⁻ and TNT⁻. The bottom figure shows the absorbance data at 520 nm and at 700 nm as a function of time. The data have been fit to exponential rise functions, with the observed rate constants indicated.

100TNT + 1NaOH in THF

Figure S8. Absorbance evolution for the reaction of 100TNT + 1NaOH in THF, where $[\text{TNT}] = 2.2 \times 10^{-3} \text{ M}$ and $[\text{OH}^-] = 2.2 \times 10^{-5} \text{ M}$. The top figure shows absorbance growth in the visible range comprised of two discernible products, TNT-OH^- and TNT^- . The bottom figure shows the absorbance data at 520 nm and at 700 nm as a function of time. The data have been fit to exponential rise functions, with the observed rate constants indicated.

Figure S9. Absorbance evolution for the reaction of 100TNT + 1NaOH in THF, where $[TNT] = 1.7 \times 10^{-3} \text{ M}$ and $[OH^-] = 1.7 \times 10^{-5} \text{ M}$. The top figure shows absorbance growth in the visible range comprised of two discernible products, TNT- OH^- and TNT $^-$. The bottom figure shows the absorbance data at 520 nm and at 700 nm as a function of time. The data have been fit to exponential rise functions, with the observed rate constants indicated.

Figure S10. Absorbance evolution for the reaction of 100TNT + 1NaOH in THF, where $[\text{TNT}] = 1.3 \times 10^{-3} \text{ M}$ and $[\text{OH}^-] = 1.3 \times 10^{-5} \text{ M}$. The top figure shows absorbance growth in the visible range comprised of two discernible products, TNT-OH⁻ and TNT⁻. The bottom figure shows the absorbance data at 520 nm and at 700 nm as a function of time. The data have been fit to exponential rise functions, with the observed rate constants indicated.

1TNT + 100NaOH in MeOH

Figure S11. Absorbance evolution for the reaction of 100TNT + 1NaOH in THF, where $[\text{TNT}] = 2.5 \times 10^{-4} \text{ M}$ and $[\text{OH}^-] = 2.5 \times 10^{-2} \text{ M}$. The top figure shows absorbance growth in the visible range comprised of two discernible products, TNT- OH^- and TNT $^-$. The top figure inset shows the phenomenological fit of the absorbance data at 520 nm and 700 nm over the first five minutes of reaction to a set of exponential rise and decay functions. The bottom figure shows the initial absorbance data at 520 nm and at 700 nm as a function of time. The data have been fit to exponential rise functions, with the observed rate constants indicated.

Figure S12. Absorbance evolution for the reaction of 100TNT + 1NaOH in THF, where $[TNT] = 1.9 \times 10^{-4}$ M and $[OH^-] = 1.8 \times 10^{-2}$ M. The top figure shows absorbance growth in the visible range comprised of two discernible products, TNT-OH⁻ and TNT⁻. The top figure inset shows the phenomenological fit of the absorbance data at 520 nm and 700 nm over the first five minutes of reaction to a set of exponential rise and decay functions. The bottom figure shows the initial absorbance data at 520 nm and at 700 nm as a function of time. The data have been fit to exponential rise functions, with the observed rate constants indicated.

Figure S13. Absorbance evolution for the reaction of 100TNT + 1NaOH in THF, where $[TNT] = 1.5 \times 10^{-4}$ M and $[OH^-] = 1.4 \times 10^{-2}$ M. The top figure shows absorbance growth in the visible range comprised of two discernible products, TNT-OH⁻ and TNT⁻. The top figure inset shows the phenomenological fit of the absorbance data at 520 nm and 700 nm over the first five minutes of reaction to a set of exponential rise and decay functions. The bottom figure shows the initial absorbance data at 520 nm and at 700 nm as a function of time. The data have been fit to exponential rise functions, with the observed rate constants indicated.

100TNT + 1NaOH in MeOH

Figure S14. Absorbance evolution for the reaction of 100TNT + 1NaOH in MeOH, where $[\text{TNT}] = 4.1 \times 10^{-2} \text{ M}$, $[\text{OH}^-] = 4.0 \times 10^{-4} \text{ M}$. The top figure shows absorbance growth in the visible range comprised of two discernible products, TNT-OH^- and TNT^- . The top figure inset shows the absorbance data at 520 nm and 700 nm over the first five minutes of reaction. The bottom figure shows the initial absorbance data at 520 nm and at 700 nm as a function of time. The data have been fit to exponential rise functions, with the observed rate constants indicated.

Figure S15. Absorbance evolution for the reaction of 100TNT + 1NaOH in MeOH, where $[TNT] = 2.9 \times 10^{-2}$ M, $[OH^-] = 2.9 \times 10^{-4}$ M. The top figure shows absorbance growth in the visible range comprised of two discernible products, TNT-OH⁻ and TNT⁻. The top figure inset shows the absorbance data at 520 nm and 700 nm over the first five minutes of reaction. The bottom figure shows the initial absorbance data at 520 nm and at 700 nm as a function of time. The data have been fit to exponential rise functions, with the observed rate constants indicated.

Figure S16. Absorbance evolution for the reaction of 100TNT + 1NaOH in MeOH, where $[TNT] = 2.2 \times 10^{-2}$ M, $[OH^-] = 2.2 \times 10^{-4}$ M. The top figure shows absorbance growth in the visible range comprised of two discernible products, TNT-OH⁻ and TNT⁻. The top figure inset shows the absorbance data at 520 nm and 700 nm over the first five minutes of reaction. The bottom figure shows the initial absorbance data at 520 nm and at 700 nm as a function of time. The data have been fit to exponential rise functions, with the observed rate constants indicated.

100TNT + 1DEA in MeOH

Figure S17. Absorbance evolution for the reaction of 100TNT + 1DEA in MeOH. (a) $[\text{TNT}] = 9.5 \times 10^{-2} \text{ M}$, $[\text{DEA}] = 9.5 \times 10^{-4} \text{ M}$; (b) $[\text{TNT}] = 7.9 \times 10^{-2} \text{ M}$, $[\text{DEA}] = 7.9 \times 10^{-4} \text{ M}$; (c) $[\text{TNT}] = 6.9 \times 10^{-2} \text{ M}$, $[\text{DEA}] = 6.9 \times 10^{-4} \text{ M}$. Each shows absorbance growth in the visible range comprised of two discernible products, TNT-OCH_3^- and TNT^- . The figure insets show the absorbance data at 520 nm and 700 nm over the first several seconds of reaction to the first established equilibrium. The data have been fit to exponential rise functions, with the observed rate constants indicated.

100TNT + 1TEA in MeOH

Figure S18. Absorbance evolution for the reaction of 100TNT + 1TEA in MeOH. (a) $[TNT] = 1.1 \times 10^{-1} \text{ M}$, $[TEA] = 1.1 \times 10^{-3} \text{ M}$; (b) $[TNT] = 9.8 \times 10^{-2} \text{ M}$, $[TEA] = 9.8 \times 10^{-4} \text{ M}$; (c) $[TNT] = 9.0 \times 10^{-2} \text{ M}$, $[TEA] = 9.0 \times 10^{-4} \text{ M}$. Each shows absorbance growth in the visible range comprised of two discernible products, $TNT-OCH_3^-$ and TNT^- . The figure insets show the absorbance data at 520 nm and 700 nm over the first several seconds of reaction to the first established equilibrium. The data have been fit to exponential rise functions, with the observed rate constants indicated.

100TNT + 1EA in MeOH

Figure S19. Absorbance evolution for the reaction of 100TNT + 1EA in MeOH. (a) $[TNT] = 8.4 \times 10^{-2}$ M, $[EA] = 8.4 \times 10^{-4}$ M; (b) $[TNT] = 7.7 \times 10^{-2}$ M, $[EA] = 7.7 \times 10^{-4}$ M; (c) $[TNT] = 5.9 \times 10^{-2}$ M, $[EA] = 5.9 \times 10^{-4}$ M. Each shows absorbance growth in the visible range comprised of two discernible products, $TNT-OCH_3^-$ and TNT^- . The figure insets show the absorbance data at 520 nm and 700 nm over the first several seconds of reaction to the first established equilibrium. The data have been fit to exponential rise functions, with the observed rate constants indicated.

100TNT + 1PA in MeOH

Figure S20. Absorbance evolution for the reaction of 100TNT + 1PA in MeOH. (a) $[TNT] = 8.0 \times 10^{-2} \text{ M}$, $[PA] = 7.9 \times 10^{-4} \text{ M}$; (b) $[TNT] = 7.6 \times 10^{-2} \text{ M}$, $[PA] = 7.5 \times 10^{-4} \text{ M}$; (c) $[TNT] = 6.5 \times 10^{-2} \text{ M}$, $[PA] = 6.4 \times 10^{-4} \text{ M}$. Each shows absorbance growth in the visible range comprised of two discernible products, $TNT-OCH_3^-$ and TNT^- . The figure insets show the absorbance data at 520 nm and 700 nm over the first several seconds of reaction to the first established equilibrium. The data have been fit to exponential rise functions, with the observed rate constants indicated.

100TNT + 1BA in MeOH

Figure S21. Absorbance evolution for the reaction of 100TNT + 1BA in MeOH. (a) $[TNT] = 1.0 \times 10^{-1} \text{ M}$, $[BA] = 1.0 \times 10^{-3} \text{ M}$; (b) $[TNT] = 1.0 \times 10^{-1} \text{ M}$, $[BA] = 1.0 \times 10^{-3} \text{ M}$; (c) $[TNT] = 9.6 \times 10^{-2} \text{ M}$, $[BA] = 9.5 \times 10^{-4} \text{ M}$. Each shows absorbance growth in the visible range comprised of two discernible products, $TNT-OCH_3^-$ and TNT^- . The figure insets show the absorbance data at 520 nm and 700 nm over the first several seconds of reaction to the first established equilibrium. The data have been fit to exponential rise functions, with the observed rate constants indicated.

Relative Thermodynamics of TNT Reactions Commonly Used for Sensor Applications: Computational Modeling in Vacuo and in Solution

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ABSTRACT

Computational analysis was performed at the B3LYP/6-311+G**//HF/6-311+G** level to determine the relative thermodynamics of reactions involving trinitrotoluene (TNT). The products of the modeled TNT reactions are commonly invoked for the use in colorimetric and fluorometric detection. The deprotonation of the methyl group of TNT was explored through the formation of free ions and ion pairs, and the sigma attack of hydroxide and various aliphatic amines at two plausible sites on TNT were also modeled. Additionally, charge transfer (CT) complexes were investigated. The SM8 implicit solvation model was applied to converged geometries, which suggests a strong solvation effect upon product formation. Thermodynamic analysis suggests a significant disparity between the favorability of hydroxide attack versus amine attack, indicating that TNT-OH⁻ or TNT-OCH₃⁻ formation is most favorable at any modeled dielectric.

INTRODUCTION

Trinitrotoluene (TNT) is a commonly used military grade explosive, due to its relatively high explosive output and its low shock sensitivity. TNT is also a complicated reactive substrate: TNT's methyl group can be deprotonated in basic solution forming TNT⁻, and TNT can undergo nucleophilic attack forming a sigma complex commonly referred to as a "Meisenheimer" complex.¹⁻⁴ Both reactions are promoted by the TNT's electron poor ring, which is able to distribute more electron density through each of the aforementioned reactions.

These transient products have strong absorbances in the visible range. Due to this, these reactions are commonly invoked for the design of TNT sensors, both in solution and in solid/vapor phase. Colorimetric indication is commonly used for sensing TNT as an environmental toxin in the solution phase,⁵⁻⁸ whereas more sensitive fluorometric methods have been developed to detect trace quantities of TNT vapor.⁹⁻¹³ The competing reactions of TNT forming TNT^- or a sigma complex, TNT-B, serve to complicate the design of these sensors, especially those that rely on resonance energy transfer (RET).^{11,12,14,15} RET-based TNT sensors typically employ a strong fluorophore, whose emission is quenched as it donates energy from its excited state to the excited state of the TNT product acceptor. The choice of donor fluorophore is inherently dependent on the absorbance features and subsequent molecular orbital energies of the TNT product, which is not always identified properly in contemporary literature. In some cases, these absorbance features have even been attributed to the formation of a charge-transfer (CT) complex.^{10,16-18} Further, the rate of formation of these products is also an important feature, as environmental analysis has a much lower demand for rapid detection than the detection of landmines or improvised explosive devices (IEDs). Our group and others have investigated the relative kinetics of the formation of each of these TNT products,¹⁹⁻²¹ which has proven to be intimately tied to solvation effects. In many cases, one product will act only as a transient species, yielding the dominance of the visible absorbance range to the second product rapidly. With a change in solvation, these roles can be observed to reverse, causing the other product to appear as the transient species. This indicates the importance of considering the effect of solvation on TNT sensor development.

Finally, the relative thermodynamics of each competing reaction are also solvent dependent, and secondary reactions are observed to occur removing TNT^- or TNT-B from detection.

All of the discussion up to this point is made more convoluted by the proposed reaction of aliphatic amines with TNT to form TNT^- ^{13,18,22,23} or zwitterionic sigma adducts.^{6,11,12,14,15,24,25} With lower basicity, we question herein the ability of aliphatic amines to deprotonate TNT significantly, as well as their ability to form a thermodynamically stable sigma complex relative to that of alkoxide bases. For many of these reasons, experimental approaches have proven to be limited in their ability to assess the actual chemistry that is being observed. Several groups have computationally addressed similar systems/reactions previously. However, some restricted their structural optimization computations to molecular mechanics forcefields,²⁶ semi-empirical methods,²⁷ and DFT methods.^{28,29} No computational studies have been performed that compare alkoxide and amine reactivity through TNT^- formation, sigma adduct formation, and CT complex formation using the same method, let alone a high level *ab initio* method and solvation modeling. By computationally modeling the reactants and products of these proposed reactions, we are able to calculate the relative thermodynamics of each reaction and observe a significant trend in solvation. Our results may also shed some light on the role of water in functional solid/vapor sensors, as the amines used in these sensors may only be deprotonating water to form hydroxide, which then gives rise to its related TNT products.

COMPUTATIONAL METHODS

In order to gain further insight into the proposed reactions, computational modeling was employed using the commercially available Spartan '10 software.³⁰ Reactants and products of TNT reactions with OH^- , CH_3O^- , NH_3 , ethylamine (EA), diethylamine (DEA), and triethylamine (TEA) were modeled; the generic set of reactions of TNT with base (B) can be found in Scheme 1. The amines were chosen to cover a range of basicity and sterics. EA is a good nucleophile and is an appropriate stand-in for the primary amines that are commonly used in the aforementioned sensors. DEA is more basic than EA but has greater steric hindrance. TEA is the best electron donor out of the modeled amines, but is so sterically hindered that it cannot attack the TNT ring. The deprotonation reaction was modeled using TNT^- with a subsequent conjugate acid as free molecules/ions or as an ion pair. Sigma complexes formed through nucleophilic attack at either the C_1 or the C_3 carbon of TNT were modeled ($\text{TNT-OH}^- \text{C}_1$, $\text{TNT-OH}^- \text{C}_3$, $\text{TNT-NH}_3 \text{C}_1$, etc. - see numbering convention on TNT in Scheme 1). Also, CT complexes between free TNT and the aliphatic amines were converged. All species were geometrically optimized initially using the Hartree-Fock (HF) method with the 6-311+G** basis set. Outputs with reasonable geometries and lacking imaginary frequencies were used in a single point B3LYP functional energy calculation with the 6-31G* basis set. ^1H NMR estimations were also computed for each species at the same level of theory, while molecular orbital energies were obtained using the B3LYP functional with the higher basis set, 6-311+G**. In addition, the SM8 solvation model³¹ was applied to propose relative thermodynamics in a wide range of dielectrics, to assist in our description of observed

Scheme 1. Proposed reactions of TNT with hydroxide and amine bases. TNT can be deprotonated at its methyl group forming TNT^- , can be attacked by a nucleophile at the C_1 or C_3 carbon forming TNT-OR^- (where $\text{R} = \text{H}$ or CH_3) or TNT-B , or can have electron density donated to its electron deficient ring forming a CT complex, TNT:B .

TNT

TNT⁻

TNT-OH⁻ C₁

TNT-OH⁻ C₃

TNT-OCH₃⁻ C₁

TNT-OCH₃⁻ C₃

[NH₄⁺][TNT⁻]

TNT-NH₃ C₁

TNT-NH₃ C₃

$[\text{H}^+-\text{EA}][\text{TNT}^-]$

TNT-EA C_1

TNT-EA C_3

$[\text{H}^+-\text{DEA}][\text{TNT}^-]$

TNT-DEA C_1

TNT-DEA C_3

$[\text{H}^+-\text{TEA}][\text{TNT}^-]$

Figure 1. Converged equilibrium geometries of TNT and possible acid/base and sigma attack products. Note that we were able to converge a sigma adduct for all bases except for TEA, which is expected to be too sterically hindered to attack the ring covalently. CT complexes were omitted from this figure due to the number of converged structures.

solvation dependency. In these solvation computations, the converged structures from the HF outputs were used to perform single point energy calculations at the B3LYP/6-31G* level; this approach was chosen since it was used as a fitting method for the development of the SM8 model. To cover a wide range of dielectric constants, the following solvent models were used: vacuum ($\epsilon = 0$), hexane ($\epsilon = 2.02$), tetrahydrofuran ($\epsilon = 7.52$), 1-propanol ($\epsilon = 20.1$), methanol ($\epsilon = 33$), formic acid ($\epsilon = 58$), water ($\epsilon = 80$), and formamide ($\epsilon = 109$). All computations were performed using an input temperature of 298.15 K and a pressure of 1 atm.

DISCUSSION

STRUCTURAL ASSESSMENT

The set of converged structures for the acid/base and sigma attack reactions can be found in Figure 1. Although we investigated the formation of a multitude of CT complexes, they are omitted here for simplicity. We have also omitted the converged structure of the isolated protonated amines, which were used in the deprotonation reaction calculations to form free ions. The ion pairs are shown, and converged so that the positive hydrogens on the alkylammonium cation interact directly with the oxygens from the nitro group, where the greatest electron density from the TNT anion is located. This distorts the methylene group much further out of the plane of the ring than calculated for the isolated TNT anion. As expected, we were able to converge sigma adducts for all of the amines with the exception of TEA. These optimizations pushed TEA away from the ring, and stabilized in an electrostatic interaction or CT complex type orientation.

Table 1 shows some related structural parameters from each species' optimized geometry. As expected, the nucleophiles show a general trend of increasing bond length as their nucleophilic strength decreases and their sterics increase. The largest bond angle distortions from that of TNT are seen for the TNT-OH⁻ and TNT-OCH₃⁻

Species	Bond Lengths (Å)	Bond Angles (°)		Dihedral Angles (°)		
	Nu – C	C ₂ -C ₁ -C ₆	C ₂ -C ₃ -C ₄	C ₁ -C ₂ -C ₃ -C ₄	C ₇ -C ₁ -C ₂ -C ₃	Nu-C ₁ -C ₂ -C ₃
TNT	-	114	118.09	1.26	174.56	-
TNT ⁻	-	110.38	120.91	4.90	162.29	-
TNT-OH ⁻ C ₁	1.408	106.59	-	3.67	112.09	126.92
TNT-OCH ₃ ⁻ C ₁	1.418	106.37	-	1.46	112.75	121.44
TNT-NH ₃ C ₁	1.541	107.88	-	4.74	115.67	126.68
TNT-EA C ₁	1.540	107.81	-	4.49	115.01	127.84
TNT-DEA C ₁	1.597	106.77	-	1.34	122.49	118.93
TNT-OH ⁻ C ₃	1.422	-	108.46	20.29	177.53	100.79
TNT-OCH ₃ ⁻ C ₃	1.420	-	107.64	29.28	159.57	90.78
TNT-NH ₃ C ₃	1.561	-	110.48	12.68	169.12	109.28
TNT-EA C ₃	1.554	-	110.15	13.37	168.87	110.81
TNT-DEA C ₃	1.584	-	109.06	24.13	164.56	97.53

Table 1. Structural parameters from TNT products optimized at the HF/6-311+G** level. Note that "Nu" refers to the bonding site of the nucleophile.

adducts, stretching the ring in plane. These adducts also distort the calculated dihedral angles to the greatest degree of all of the nucleophiles, bending the ring out of plane. The distortions may play a large contributing factor to the destabilization of the frontier MOs, whose density surfaces are localized on the ring. Since the amine adducts do not distort the ring as much as OH⁻ or OCH₃⁻, their MOs have closer relative energies to those of TNT. The amine adducts show a definite trend of increasing sterics resulting in increased distortion. TNT⁻ formation distorts the bond

angles and dihedrals the least, which is expected due to the enhanced resonance stabilizing the ring. The C₁ adducts appear to distort the ring less than their C₃ counterparts, which may explain their slightly greater MO stability. In addition, the C₁ adducts push the electron donating methyl group out of plane of the ring, replacing a portion of its electron donation with that of the nucleophile. Although the C₃ adducts are not displacing an electron donating group, their nucleophiles are significantly more orthogonal to the ring, which negatively affects their ability to donate electron density. This may be another stabilizing advantage of the C₁ adduct over the C₃ adduct.

	Species	Computational (Vacuum)	THF	MeOH
TNT	Aromatic H's	8.320 (1)	8.97 (1)	8.96 (1)
	Methyl H's	2.107 (1)	2.62 (1)	2.64 (1)
TNT ⁻	Aromatic H's	7.752, 7.733 (1)	8.30 (1)	8.29 (1)
	Methyl H's	4.854, 4.829 (1)	5.64 (1)	5.68 (1)
TNT-OH ⁻	Aromatic H's	7.597, 7.482 (1)	NOT OBSERVED	8.41 (1)
	Methyl H's	1.461 (1)	NOT OBSERVED	5.28 (1)

Table 2. ¹H NMR Computations compared with experimental results for TNT and alkoxide reactions. Peak multiplicity is indicated in parentheses.

Since the TNT/hydroxide reactions show much greater thermodynamic favorability and more significant experimental signal, NMR computations were done using those products. Table 2 shows the comparison of the computations (in vacuum) to the experimentally observed features in tetrahydrofuran (THF) and methanol. Note that some features are computed to be reasonably close but not identical, indicating the limitation of slight asymmetry of the calculated aromatic hydrogens. Although the values differ substantially based on the lack of solvation in the calculations, the

differences are in a reasonable range to account for solvent shifts. As we have shown previously,¹⁹ there are only small chemical shifts effects when changing from the non-polar THF to the more polar methanol system. The calculated chemical shifts, in comparison, are found at lower field than their experimental counterparts as we would expect. The magnitude of the solvation shifts (from calculated to experimental) are almost all approximately the same, at about 0.5 to 0.9 ppm shifted downfield. The one significant outlier here is the computation for the methyl protons in TNT-OH⁻; while the change of the adjacent carbon from sp² to sp³ hybridization would be expected to lower the chemical shift, the inclusion of the hydroxyl group in close proximity would be expected to raise the chemical shift. With additional reorientation of the nitro groups, it is not implausible to observe a shift higher than 5 ppm, as we have in methanol.

Given the literature discussion of charge transfer (CT) complexes in which TNT acts as an acceptor, we investigated several converged CT structures. In addition, since TEA has the largest Gutmann donor number (DN)^{32,33} of the amines in our investigation, we added solid TNT to dry TEA. No color change was observed, with no apparent absorbance features in the visible range. In a comparison of the computed TNT:TEA CT complexes, the relative energies of the formation reactions were much lower than the TNT⁻ formation reactions; as the dielectric increases, the reactions have energies on the same order of magnitude, but the relationship between their relative molar absorptivities remains unknown. This suggests that the formation of any CT complex will not play an observable role in the overall equilibrium of these systems at low concentration. Politzer et. al.²⁸ have reported computations of similar

TNT-amine CT structures, with calculations well into the UV range; this seems to correspond well with literature reports of TNB CT complexes with EA, DEA, and TEA with absorbance maxima between 280 and 420 nm.³⁴ This is consistent with the lack of CT bands in our previously reported absorbance spectra.

RELATIVE THERMODYNAMICS

Thermodynamic quantities were calculated only in vacuum, since the SM8 solvation model assumes no change to the vibrational modes which the thermodynamic calculations directly depend on; however, these calculations were computed under the same level of theory as the solvation calculations. Enthalpy changes for each modeled reaction can be estimated given a system at constant temperature and volume, whereby $\Delta H_{\text{rxn}} \approx \Delta E_{\text{rxn}} \approx E_{\text{products}} - E_{\text{reactants}}$. Entropy values were also computed, in order to further assess the negligibility of the $T\Delta S$ term in the Gibb's free energy equation in vacuum. Given the assumption that this term is negligible, ΔG_{rxn} can be estimated by ΔE_{rxn} .

Table 3 shows the thermodynamic parameter ΔE_{rxn} , as well as ΔG_{rxn} , ΔH_{rxn} , and ΔS_{rxn} , which are calculated using vibrational mode analysis. Several points are immediately apparent from the data set. ΔS_{rxn} contributions from most of the reactions are significant, breaking down the approximation that $\Delta G_{\text{rxn}} \approx \Delta E_{\text{rxn}}$. As expected, the lowest values of ΔS_{rxn} are from the acid/base reactions, where proton transfer does not dramatically affect the overall entropy of the system. Here, the only calculated positive ΔS_{rxn} comes from hydroxide deprotonating TNT; this is an important distinction, as this is a driving force towards spontaneity and

thermodynamic favorability. The amine deprotonation reactions do not have this in their favor, but EA, DEA, and TEA have very similar ΔS_{rxn} values. All of the sigma adduct forming reactions have negative entropies, as they promote higher order in the system, with the trend of least negative to most negative as $\text{OH}^- > \text{NH}_3, > \text{CH}_3\text{O}^- > \text{EA} > \text{DEA}$.

<u>Reaction</u>	<u>ΔE_{rxn}</u> <u>(kJ/mol)</u>	<u>ΔG_{rxn}</u> <u>(kJ/mol)</u>	<u>ΔH_{rxn}</u> <u>(kJ/mol)</u>	<u>ΔS_{rxn}</u> <u>(J/molK)</u>
$\text{TNT} + \text{OH}^- \rightarrow \text{TNT}^- + \text{H}_2\text{O}$	-411.3	-412.4	-409.8	8.7
$\text{TNT} + \text{OH}^- \rightarrow \text{TNT-OH}^- \text{ C}_1$	-487.1	-435.6	-480.1	-149.2
$\text{TNT} + \text{OH}^- \rightarrow \text{TNT-OH}^- \text{ C}_3$	-460.7	-408.7	-453.1	-149.0
$\text{TNT} + \text{CH}_3\text{O}^- \rightarrow \text{TNT}^- + \text{CH}_3\text{OH}$	-293.8	-289.9	-286.1	12.8
$\text{TNT} + \text{CH}_3\text{O}^- \rightarrow \text{TNT-OCH}_3^- \text{ C}_1$	-348.1	-281.6	-335.3	-180.2
$\text{TNT} + \text{CH}_3\text{O}^- \rightarrow \text{TNT-OCH}_3^- \text{ C}_3$	-351.5	-283.4	-337.8	-182.4
$\text{TNT} + \text{NH}_3 \rightarrow \text{TNT}^- + \text{NH}_4^+$	487.3	499.3	495.1	-14.2
$\text{TNT} + \text{NH}_3 \rightarrow [\text{TNT}^-][\text{NH}_4^+]$	65.7	113.5	69.8	-146.5
$\text{TNT} + \text{NH}_3 \rightarrow \text{TNT-NH}_3 \text{ C}_1$	16.8	77.8	28.0	-167.0
$\text{TNT} + \text{NH}_3 \rightarrow \text{TNT-NH}_3 \text{ C}_3$	29.9	90.5	41.0	-165.8
$\text{TNT} + \text{EA} \rightarrow \text{TNT}^- + \text{H}^+ \text{-EA}$	431.7	440.4	438.8	-5.5
$\text{TNT} + \text{EA} \rightarrow [\text{TNT}^-][\text{H}^+ \text{-EA}]$	42.2	106.9	47.4	-199.5
$\text{TNT} + \text{EA} \rightarrow \text{TNT-EA} \text{ C}_1$	-6.1	68.0	4.4	-213.3
$\text{TNT} + \text{EA} \rightarrow \text{TNT-EA} \text{ C}_3$	6.9	81.4	17.7	-213.8
$\text{TNT} + \text{DEA} \rightarrow \text{TNT}^- + \text{H}^+ \text{-DEA}$	391.0	399.8	398.0	-5.8
$\text{TNT} + \text{DEA} \rightarrow [\text{TNT}^-][\text{H}^+ \text{-DEA}]$	23.5	94.0	29.1	-217.7
$\text{TNT} + \text{DEA} \rightarrow \text{TNT-DEA} \text{ C}_1$	36.9	115.4	47.4	-227.8
$\text{TNT} + \text{DEA} \rightarrow \text{TNT-DEA} \text{ C}_3$	30.1	108.4	40.2	-229.0
$\text{TNT} + \text{TEA} \rightarrow \text{TNT}^- + \text{H}^+ \text{-TEA}$	371.8	381.8	380.0	-6.2
$\text{TNT} + \text{TEA} \rightarrow [\text{TNT}^-][\text{H}^+ \text{-TEA}]$	56.1	128.6	63.8	-217.2

Table 3. Thermodynamic parameters calculated for each explored reaction between TNT and bases at the B3LYP/6-31G**//HF/6-311+G** level.

Similar trends continue to ΔH_{rxn} and ΔG_{rxn} . The hydroxide reactions have reasonably similar spontaneity to each other, with the formation of the sigma complex having the edge in vacuum. Besides the alkoxide reactions, all others are calculated to be significantly non-spontaneous. The deprotonation reactions forming free ions are less favorable than their sigma adduct forming counterparts; for the amines, the disparity is especially noticeable. This is partly expected, given the lack of a solvation parameter in these calculations to support an isolated charge. The capability of forming an ion pair improves the favorability substantially, and while NH_3 and EA still have a more favorable ΔG_{rxn} for sigma adduct formation, the larger DEA has a more favorable deprotonation reaction. The basicity of the amines in vacuum, as calculated by the ion pair formation ΔG_{rxn} , trends as $\text{DEA} > \text{EA} > \text{NH}_3 > \text{TEA}$.

Since most of the modeled reactions had a sizeable entropy component, the approximation of $\Delta G_{\text{rxn}} \approx \Delta E_{\text{rxn}}$ is not appropriate. However, the dramatic differences between the calculated values of ΔG_{rxn} for each hydroxide reaction and the most favorable amine reaction, the formation of TNT-EA, are significant. It is unlikely that the inclusion of a solvation model would bridge this gap; therefore, these computations indicate that amine reactions would not be expected to be observed with even trace amounts of water in solution, as hydroxide reactions with TNT would be predominant. This would especially be true in a methanoic solution, as a substantial amount of CH_3O^- would be produced by the methanol/amine acid/base equilibrium.

Figure 2. ΔE_{rxn} plotted as a function of dielectric constant for TNT reactions with bases. The plot indicates the solvation dependence of TNT reactions with hydroxide and selected amines. Energy calculations performed at B3LYP/6-31G*//HF/6-311+G** level of computation using the SM8 solvation model. Note that some points from the free ion computations at lower dielectric are off scale and are thus omitted from this figure. Also, note that the ΔE_{rxn} data for the methoxide adducts overlap such that the data points cannot be discerned.

Figure 2 shows the relative energy change of each explored TNT reaction as a function of solvent dielectric. The hydroxide and methoxide reactions appear to be relatively independent of solvation modeling, as the energy spacing between the computed species remains similar for any dielectric. The C_1 TNT- OH^- adduct is predicted to be the more thermodynamically favorable product, which is what we observe in our absorbance spectra in more polar solvents at long time periods. The TNT- OCH_3^- adduct isomers show very similar thermodynamics at all modeled

dielectrics, as the larger nucleophile is sterically hindered at the C₁ attack site. This also emphasizes the kinetic hindrance to the adduct formation, as TNT⁻ is typically observed prior to the adduct's appearance.

NH₃ and EA products follow a similar trend, where the adduct reactions are of lower relative energy than the ion pair or the free ions. By no surprise, the free ion formations are calculated to be the least thermodynamically favorable, especially towards low dielectrics which cannot stabilize the charges through intermolecular forces. In addition, the change in relative energy of the NH₃ and EA adduct formations is about an order of magnitude less than the hydroxide adducts in methanol ($\epsilon = 33$). In vacuum, the computation indicates a non-favorable reaction for the ammonia adduct, and a barely favorable adduct formation with EA. Since the hydroxide reactions are calculated to become even more thermodynamically favorable as the dielectric decreases, the relative energy gap between the hydroxide and amine adduct reactions increases substantially; this does not project well for the solid-vapor applications of TNT and primary amine reactions for TNT detection.

The size difference of DEA relative to EA changes the relative favorability of action as a Brønsted-Lowry base versus a nucleophile. The calculations predict the ion pair of [H⁺-DEA][TNT⁻] to be the most favorable reaction, with the free ions still more favorable than either adducts at dielectrics higher than that of 1-propanol ($\epsilon = 20.1$). It is interesting to note that the C₃ carbon is expected to be the more thermodynamically favorable attack site for the larger DEA nucleophile, but not so much so that we would not expect evidence of both adducts at equilibrium. This is consistent with our ¹H NMR observation for this reaction, where there is evidence of

two sigma adducts formed by DEA attack on TNT. Since TNT-TEA adducts were unable to converge, due in large part to the sterics of that attack, the only comparison to be made is that of TEA's capacity as a Brønsted-Lowry base. The TEA deprotonation reactions are not expected to be thermodynamically favorable at either of the solvent dielectrics which we observed experimentally, which correlates to our observation that TNT crystals dissolved in ultra-dry TEA did not exhibit any color change representing TNT deprotonation. Again, since the hydroxide reactions are computed to be much more favorable than either those of DEA or TEA, it would be expected that the products of these amine reactions would not be of appreciable concentration at equilibrium.

TD-DFT ANALYSIS

Figure 3 shows the computed relative energy of the frontier molecular orbitals of TNT and the discussed products of alkoxide and amine reactions. TNT is very stable, with its occupied MOs at the lowest energy relative to those of the other species. It also has the largest HOMO/LUMO gap, which is consistent with the experimental lack of visible absorbance maxima. The anionic species, TNT^- , $\text{TNT-OH}^- \text{C}_1$, $\text{TNT-OH}^- \text{C}_3$, $\text{TNT-OCH}_3^- \text{C}_1$, and $\text{TNT-OCH}_3^- \text{C}_3$ all have very similar MO energies. TNT^- is shown here to have the smallest HOMO/LUMO gap, which is consistent with the spectral assignments made previously in absorbance measurements. Of the two hydroxide adducts, the C_1 adduct has a lower HOMO/LUMO gap, and is expected to have an absorbance maximum at higher wavelength than the C_3 adduct; this relationship can be seen in context in

Figure 3. Computed MO diagram of explored TNT products with bases at the B3LYP/6-311+G**//HF/6-311+G** level. Note that electrons have been added to signify the HOMO for each species.

Figure 4a. While the absolute maxima from this DFT approach do not correlate well with experimental observation, the relative maxima are expected to. The amine adducts show a size-dependent trend of relative MO energies, where increased sterics are likely destabilizing the MOs. This is true for both the C₁ and the C₃ adducts. It is interesting that there is little difference in the relative MO energies of each C₁/C₃ adduct pair, but there is significant difference between the energies of the amine adducts versus the alkoxide adducts. This corresponds to the structural analysis, where the amines do not distort TNT's central ring as significantly as OH⁻ or CH₃O⁻;

since this is where the HOMO surfaces are localized, further distortion raises their relative energies.

Figure 4. Absorbance spectra computed at the B3LYP/6-311+G**//HF/6-311+G** level, normalized to individual species' computed maximum. (a) TNT-OH⁻ C₁ (——), TNT-OH⁻ C₃ (---); (b) TNT-OCH₃⁻ C₁ (——), TNT-OCH₃⁻ C₃ (---); (c) TNT-EA C₁ (——), TNT-EA C₃ (---); (d) TNT-DEA C₁ (——), TNT-DEA C₃ (---). Note that while the computed maxima are generally poor for

this approach, the relative position of the maxima are typically representative of experimental observation.

CONCLUSIONS

High level *ab initio* computations were performed to assess the relative thermodynamics of TNT product formation, from reaction with OH^- , CH_3O^- , and aliphatic amines. The deprotonation of the methyl group of TNT forming TNT^- , and the nucleophilic attack upon TNT forming TNT-B, are calculated in vacuum to be very favorable and spontaneous for OH^- and CH_3O^- while non-spontaneous for amines. The application of the SM8 solvation model decreases the favorability of OH^- and CH_3O^- reactions while improving the amine reactions as the solvent dielectric increases, but not enough to justify a significant amine reaction contribution at equilibrium. OH^- and CH_3O^- adducts are calculated to have the greatest degree of ring distortion out of the investigated adducts, resulting in destabilized MOs relative to TNT, but a lower HOMO/LUMO gap yielding visible range absorbances. This study indicates the significance of solvation effects on TNT reactions, as well as the role of OH^- and ambient water in the formation of these products. The use of methanol as a solvent system for TNT/amine reactions is likely to lead to CH_3O^- based products and subsequent visible absorbance features instead of amine based products. Therefore, solvent choice is crucial for further investigation of this and similar systems. Based on these calculations, amines employed in existing TNT sensors are not directly responsible for the sensing mechanism. Since OH^- plays a much more significant

thermodynamic role, especially in the gas phase, water content should be monitored and controlled for an accurate assessment of sensor effectiveness.

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A Fluorometric Sensing Array for the Detection of Military Explosive and IED Materials

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Abstract

A fluorometric sensing array was developed to detect and discern common military explosives as well as materials used in improvised explosive devices (IEDs). Xanthene-based fluorophores were chosen as reporter molecules and were exposed to explosive analytes in DMF solution. Unique responses were observed for each analyte, with the strongest observed responses resultant from trinitrobenzene (TNB) or trinitrotoluene (TNT) addition. Evidence for substantial electron transfer (ET) between TNB and TNT products and fluorophores was observed. Computational support indicates that anionic trinitroaromatic products are likely donating electrons to cationic fluorophores, leading to an observed enhancement of their emission.

1. Introduction

With the rise in domestic and international terroristic events in the past decade, the scientific community has been pushed by the need to develop rapid and sensitive means of detection of explosive analytes. Explosive sensors have been developed based on a number of methods of detection, including colorimetry,^{1,2} fluorescence,³⁻⁹ mass spectrometry,¹⁰⁻¹² electrochemical,¹³⁻¹⁵ and Surface Enhanced Raman Scattering (SERS),¹⁶⁻¹⁸ among others.

Sensitivity and selectivity remain at the heart of an effective sensor, and while many sensors are able to claim one, most cannot achieve both. Fluorescence sensing benefits from the inherently high sensitivity of contemporary fluorescence instrumentation, which can achieve attomolar detection for molecules with high quantum yields in solution.¹⁹ Additionally, detection limits may be improved by

another order of magnitude using metal enhanced fluorescence.^{20,21} Selectivity, although difficult to achieve using a single interactive sensor, can be induced by the use of a sensor array where multiple sensors can be exposed to the same analyte stream and their responses observed together. This “fingerprint” can be read as a function of a specific analyte; whereas one of the sensors may not be able to discern one analyte from another, it is unlikely that two analytes will interact with the multitude of sensors in the exact same fashion.

Perhaps surprisingly, the rhodamine family has not been well explored in the field of explosive detection. Meaney & coworkers investigated the fluorescence quenching of common fluorophores including Rhodamine 6G against nitrobenzene, 4-nitrotoluene and 2,6-dinitrotoluene, but did not explore more contemporarily relevant analytes.⁹ Rhodamines have notoriety as being very strong fluorophores, and were therefore selected to test as fluorescence reporters against explosive analytes. Rhodamines also have the advantage of possessing visible range excitations and emissions that are not subject to interference by absorbance bands of explosive analytes. The rhodamines investigated in this study were Rhodamine 560 Chloride (Rh560), Rhodamine 6G (Rh6G), Sulforhodamine B (SRhB), Rhodamine 640 Perchlorate (Rh640), Sulforhodamine 640 (SRh640), Rhodamine 700 (Rh700) and Rhodamine 800 (Rh800). Fluorescein 548 (Fl548) was also included, as another representative xanthene derivative with a high quantum yield. The structures of the selected fluorophores can be found in Supplementary Information Fig. S1. The analytes exposed to the aforementioned fluorophores were selected based on current military relevance: trinitrobenzene (TNB), trinitrotoluene (TNT),

cyclotrimethylenetrinitramine (RDX), and cyclotetramethylenetetranitramine (HMX) are common military grade secondary explosives; pentaerythritoltetranitrate (PETN) and triacetone triperoxide (TATP) have been used in improvised explosive devices (IEDs) and large scale terrorist events over the past two decades due to their relatively simple synthesis and high output;^{10,11,22} and 2,3-dinitrotoluene (2,3-DNT), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), 3,4-dinitrotoluene (3,4-DNT), 2-nitrotoluene (2-NT) and 4-nitrotoluene (4-NT) are common impurities in crude and refined TNT due to incomplete nitration.²³ Due to the much higher vapor pressures and saturated vapor densities of DNTs and NTs relative to TNT, these species may serve as useful analytes for the implication of TNT sensing. The structures of the aforementioned explosive analytes can be found in Supplementary Information Fig. S2.

2. Experimental

Chemicals and materials

DMF ($\geq 99.5\%$) was purchased from Fisher Scientific and used as received. Rh560, Fl548, Rh640, SRh640, Rh700, and Rh800 were purchased from Exciton (Dayton, Ohio) and used as received. Rh6G (99%) was purchased from Acros and used as received. SRhB was purchased from Lambda Physik and used as received. TNT, TATP, PETN, HMX, and RDX were obtained from Drs. Jimmie Oxley and James Smith and were used without further purification. TNB was purchased from Fisher Scientific wetted with ca. 40% water with 98.0% purity; 2,3-DNT (99%), 2,6-DNT (98%), and 3,4-DNT (99%) were purchased from Aldrich; 2,4-DNT (95%) was

purchased from Alfa Aesar; and 2-NT (99%) and 4-NT (99%) were purchased from Acros. All were used as received.

Apparatus and Procedure

Fluorophore solutions in DMF were created and diluted to a maximum visible range absorbance of 0.1 abs. units. Solutions of Rh700 and Rh800 were acidified with trifluoroacetic acid (TFA) to prevent photobleaching prior to use. Absorbance data (PerkinElmer Lambda900 UV/Vis/NIR spectrophotometer) and emission data (Horiba Fluorolog-3, Xe arc lamp excitation) were obtained before and after the addition of a few crystals of solid explosive, or the addition of a small drop in the case of 2-NT_(l). Excitation and emission slits were used between 2 and 5 nm, but did not vary between initial and final spectra acquisitions.

Computational Analysis

Spartan '10 computational software²⁴ was used to propose the relative absorbance maxima for the isomers of [TNT-OH]⁻. Structures were optimized at the HF/6-311+G** level without imaginary vibrational frequencies. TDDFT energies and absorbance spectra were calculated using the converged structures at the B3LYP/6-311+G** level.

3. Results and discussion

Solutions of fluorophores were prepared in DMF, with absorbance and emission spectra acquired before and after the addition of explosive analyte. Figure 1

shows the absorbances characterizing the initial fluorophore solutions, spanning the visible range. In similar fashion, Figure 2 shows the normalized emission spectra from each initial fluorophore solution, spanning from 530 nm to 800 nm. Pairing absorbance and emission measurements together allows for determination of possible interactions between fluorophore and analyte, as both ground state and excited state profiles are examined.

Fig. 1 Absorbance overlay of selected fluorophores in DMF, normalized to visible absorbance maximum. Listed in order of decreasing energy maximum, the fluorophores are Rh560 (———), FI548 (- - - -), Rh6G (- - - -), SRhB (- - - -), Rh640 (- - - -), SRh640 (- - - -), Rh700 (- - - -), Rh800 (- - - -).

Fig. 2 Emission overlay of selected fluorophores in DMF, normalized to emission maximum. Listed in order of decreasing energy maximum, the fluorophores are Rh560 (———), FI548 (- - - -), Rh6G (- - - -), SRhB (- - - -), Rh640 (- - - -), SRh640 (- - - -), Rh700 (- - - -), Rh800 (- - - -).

Figure 3 shows the output of the sensing array, as indicated by emission difference spectra. Note that the ordinate axis for TNB and TNT interactions are set to 100% change in emission. All others are zoomed to 10% for clarity, with a select few zoomed to 40% marked by " $\times \frac{1}{4}$." The trinitroaromatics, TNB and TNT, both show very strong observed quenching. This is in large part due to the products which they form in DMF, reacting with hydroxide generated by trace amounts of water. Others have previously described the absorbance properties²⁵⁻²⁸ and recently we have described the emission properties of these products;²⁹ both of these factors complicate the observations made here as added variables. TNB and TNT are capable of nucleophilic attack by hydroxide and DMF; in addition, TNT is capable of deprotonation by hydroxide. The resulting strong absorbance features can be seen in Supplementary Information Figs. S3-S6. Consistently, these products are diminished in the observations with Rh700 and Rh800, as these solutions were slightly acidified to inhibit photobleaching during analysis. Since the product formation is reliant on hydroxide in solution, acidification removes hydroxide and shifts the equilibria back towards free TNT or TNB. The formation of these products serves to complicate the interactions with the fluorophores in solution, due to their inner filter effect and possible ground state interactions. Both of these factors will be discussed in greater detail later in the paper.

PETN, RDX, and HMX are all nitroaliphatics, which show a mixture of quenches and enhancements through interactions with the sensing fluorophores. There is no observed change in any fluorophore's absorbance upon these exposures (see Figs. S3-S6), implying that emission changes are only resultant from excited state

Fig. 3 Emission difference spectra for the exposure of explosive analytes to selected fluorophores in DMF solution, normalized to the initial emission maximum. Blue spectra indicate the observed quenching of emission, red spectra indicate the observed enhancement of emission, and black spectra indicate minimal change in emission. Full absorbance and emission spectra can be found in Supplementary Information Figs. S3-S6.

interactions. Emission decreases are likely resultant from dynamic mechanisms. Increases in emission may result from interactions that either decrease the lifetime of the excited state, or perhaps increase the rigidity of the fluorochrome leading to more favorable emission kinetics. Rh640 most strongly shows this capacity here with both RDX and HMX. TATP, the only explored peroxide based explosive, also shows this capacity with Rh640 and Rh6G. Since TATP is known to decompose to H_2O_2 and acetone in the presence of acid,^{30,31} it might be proposed that oxidation of Rh700 and Rh800 might occur by H_2O_2 due to the small amount of TFA in solution. Absorbance measurements do not show this, as there is no change to support such a ground state reaction. Instead, TATP or its decomposition products appear to quench Rh700 through a collisional mechanism.

The DNT isomers that were explored show a range of emission quenching and enhancement, but with fingerprint patterns that differentiate them from the other analytes. DNTs have a similar capacity to TNT to be deprotonated to a varying degree, and the ability to form a sigma complex with hydroxide from ambient water in solution. In some of our additions, absorbance features arise to support this assessment. For example, Fig. S5 shows an increase in absorbance for the addition of 2,4-DNT to SRh640 centered around 650 nm; this absorbance spectrum has been shown previously to be resultant from the deprotonation of 2,4-DNT.³² Likewise, Fig. S4 shows an increase in absorbance for the addition of 3,4-DNT to FI548 between 400 and 500 nm; this would be consistent with the formation of a hydroxide adduct. While these absorbance features contribute to an inner filtering effect, they are minimal compared to those of the TNB or TNT products, which will be described

later. In addition, there are observable emission changes for DNT additions that either do not show these absorbance growths or are not fully resultant from an inner filter effect. 3,4-DNT causes a larger decrease in the emission of FI548 than its product's absorbance growth (Fig. S4). Another example can be found in Fig. S6, where 2,4-DNT causes a dynamic quench to the emission of Rh700, while the absorbance spectrum is unchanged. The NT isomers also show differentiating patterns from each other, and from other analytes. Colorful products similar to those formed by hydroxide reacting with TNT or DNTs are not observed with NTs, and so all observed absorbance spectra remained unchanged upon addition of 2-NT or 4-NT. Dynamic quenching was observed from the addition of 2-NT and 4-NT to FI548 (Fig. S4), as well as 2-NT added to Rh640 or SRh640.

One issue with the trinitroaromatic products involves the degree of inner filter effect (IFE) caused by their absorbance. While we observe large quenches in emission, this may not be related to excited state interactions, but rather simple absorbance of emitted photons by other components in solution. To account for this, IFE corrections were done using the following equation:¹⁹

$$F_{corr} = F_{obs} \times 10^{\left(\frac{A_{ex}+A_{em}}{2}\right)}$$

where F_{corr} is the corrected emission intensity, F_{obs} is the observed emission intensity, A_{ex} is the absorbance at the excitation wavelength, and A_{em} is the absorbance at the emission maximum. Although the DNTs showed some slight evidence of product formation, which were consistent with sigma adduct formation or deprotonation, their absorbance changes were minute and did not have a dramatic impact on the IFE

correction calculations. The trinitroaromatic products had massive absorbances, which definitely required corrections; a comparison of observed vs. corrected emission changes can be found in Table 1.

	Rh560	Rh6G	FI548	SRhB	Rh640	SRh640	Rh700	Rh800
TNT obs.	-75	-68	-97	-54	-44	-64	-1	-4
TNT corr.	9	23	-98	4	104	-4	1	-6
TNB obs.	-5	-12	-44	-5	-44	-9	-2	2
TNB corr.	5	24	-29	0	47	-2	2	2

Table 1. Observed percent emission changes compared to inner filter effect corrected changes for the fluorophores with addition of TNB or TNT.

Once the maximum emissions were corrected using the above equation, the % change was recalculated. We estimate the relative error in the corrected emission change to be between 5-10%. The IFE corrected changes stand in stark contrast to the observed changes, indicating a hidden component of increased emission in the process. For example, the addition of TNT to Rh640 shows an observed quench of 44%. However, given the amount of TNT products that absorb in the visible range, IFE corrections should be applied. If no true collisional or static quenching occur, these corrections should bring the change back to 0%. Instead, the corrected change is calculated to be 104%, indicating a competing interaction that enhances the emission of the fluorophore. A similar effect can be seen for other rhodamine/trinitroaromatic pairings in Table 1, in which the corrected % changes are all more positive than the observed. The exceptions to this are the additions of TNT to FI548 as well as the additions to Rh700 and Rh800, which do not change significantly within the estimated

error. FI548 especially shows significant quenching with TNT and TNB, which does not appear to be competing with an enhancing phenomenon.

Although we have previously described the emission of TNB and TNT products in DMF,²⁹ there is little shape change in the emission spectra to justify their direct additive role in the calculated increase in emission. TNB reacts with hydroxide from trace water to form the sigma complex TNB-OH^- , which shows a narrow absorbance feature around 450 nm and a broader absorbance feature around 520 nm. Additional absorbance bands are expected from the TNB-DMF sigma adduct around 650 nm, which we see in varying amounts. TNT is capable of similar chemistry, by forming two possible TNT-OH^- adduct isomers, as well as a DMF adduct; both have been shown to be emissive. TNT is also capable of being deprotonated by OH^- , forming TNT^- , but this species is not emissive.

One plausible conclusion is that the increase results from resonance energy transfer (RET). This would cause the excited states of a trinitroaromatic product to donate energy to the fluorophore acceptor, populating their excited states leading to greater emission. Additionally, it appears that the addition of TNT leads to a greater enhancement than the TNB counterparts. While we cannot address RET efficiencies here without knowing the relative amount of each species and lifetimes, it is interesting to note the consistently higher response to TNT. To further investigate the possibility of RET between trinitroaromatic products and the fluorophores, the absorbance spectra of the fluorophore acceptors is compared to the emission spectrum of TNB product donors in DMF solution in Figure 4.

Fig. 4 Normalized absorbance overlay of fluorophores in DMF, compared with the normalized emission of TNB products. Listed in order of decreasing energy maximum, the fluorophores are **(a)** Rh560 (—), **(b)** FI548 (- - -), **(c)** Rh6G (- - -), **(d)** SRhB (- - -), **(e)** Rh640 (- - -), **(f)** SRh640 (····), **(g)** Rh700 (- - -), **(h)** Rh800 (- - -). The emission spectrum **(i)**, given by the thicker line width, is a composite of the emission of TNB-OH⁻ and TNB-DMF formed in the addition of TNB to DMF solution.

The emission spectrum is comprised of two components, TNB-OH⁻ and TNB-DMF, which emit at a maximum around 580 nm and 650 nm respectively.²⁹ The emission spectrum overlaps best with the absorbance spectra of Rh700 and Rh800, which do not exhibit enhancement when exposed to TNB in DMF. Also, the similar absorbance spectra of Rh640 and SRh640 but different response to TNB do not make sense in the context of RET shown here. In a RET mechanism, it would be expected that both Rh640 and SRh640 would show enhancements, which are not observed. In addition, the concentrations of each species in solution are on the order of 10⁻³ M at best, which would make the average distance between molecules greater than 100 Å. Given the strong distance dependence in a RET mechanism, this distance is higher than the typical Förster distance exhibited by similar molecules.¹⁹ The poor correlation of the spectral bands of the fluorophores and TNB products and the large intramolecular distances suggest that RET is not the enhancement mechanism.

Other possibilities for an enhanced fluorescence are charge transfer (CT) or electron transfer (ET) mechanisms. A CT complex may be formed if the HOMOs of the TNB or TNT products are close enough in energy to the LUMOs of the sensing fluorophores, for a small amount of overlap to occur. If either of the TNB or TNT products have HOMOs of high energy relative to the LUMOs of the fluorophores, ET may justify the enhanced excited state population and subsequent enhanced emission of the fluorophores. To help in our assignment of the mechanism of enhanced emission, molecular orbital energies were calculated using Spartan '10 computational software. Figure 5 shows the calculated relative energies of the HOMO and LUMO for TNB, TNB-OH⁻, TNT, TNT-OH⁻, TNT⁻, and the sensing fluorophores.

Fig. 5 HOMO/LUMO diagram for energy comparison between sensing fluorophores, trinitroaromatics, and trinitroaromatic products. Relative energies were computed at the B3LYP/6-311+G**//HF/6-311+G** level in vacuum at 298.15 K and 1 atm. Arrows are shown to designate the HOMO.

It is clear that there is a dramatic difference between the frontier orbital energies of the free trinitroaromatics, with the transient anionic products (TNT⁻ and the OH⁻ adducts)

being of much higher energy. The trinitroaromatic products have HOMOs so high in energy that they are very close to the energy of the LUMOs of some of the modeled fluorophores (eg. FI548, SRh640). This gives plausibility to the formation of a CT complex where the products may donate to the fluorophore. The HOMOs of TNB and TNT are reasonably close in energy to the LUMOs of several of the fluorophores, which indicates the possibility of a trinitroaromatic:fluorophore CT complex formation. However, the energy of this transition would be greater than the HOMO/LUMO gap of all of the fluorophores, suggesting that the CT band would not be in the visible range but rather in the UV. Since the formation of any CT complex would lead to a spectral shift, yet no shift is observed, no significant CT complex formation is occurring.

Photo-induced electron transfer (PET) is unlikely to play a role in our observations in the context of these calculations. Typically, PET causes a quench in emission if electron density is transferred to or away from a fluorophore, as this can lead to structural and lifetime changes.¹⁹ Emission enhancements can be justified through disruption of PET mechanisms, but since our initial solution is comprised of only the fluorophore in DMF this also seems unlikely. The only way that this might be conceived is if DMF is initially quenching the fluorophores, and that the displacement of DMF by explosive molecules in solvation leads to an observed enhancement. Given the relatively low quantity of analyte added relative to the overwhelming amount of DMF solvent, the small addition cannot account for the magnitude of enhancement observed.

In the context of a ground state ET mechanism, TNB and TNT have the lowest calculated HOMO energy of all of the modeled species. This feature as well as their electron deficient character makes it unlikely that the free trinitroaromatics would donate electrons to the fluorophores. The anionic products have a much higher calculated HOMO energy however, making them more likely electron donors. FI548 and SRh640 have LUMOs that are slightly higher in energy than the HOMOs of TNT⁻ and the OH⁻ adducts, making them poor candidates as electron acceptors. The calculated LUMO of SRhB is lower but still close in energy to the HOMO of the anionic products (within 0.3 eV); this is likely an insufficient energy gap to cause significant ET at ambient temperatures. Rh6G and Rh640 have LUMOs of very similar energy (-5.39 and -5.13 eV respectively), which are about 2.4 eV lower than the HOMO of TNB-OH⁻. Since these two fluorophores show the greatest enhancement with TNB and TNT addition in solution and their relative LUMO energies to TNB-OH⁻ are strikingly similar, electron transfer appears to be the most probable mechanism. The difference in energy is substantial enough to occur without significant reversibility. Rh560, Rh700, and Rh800 have LUMOs of lower energy than Rh6G and Rh640. Rh560 shows a calculated enhancement that is within the estimated error of our approach, and with a LUMO that is 3 eV lower than the HOMO of TNB-OH⁻ the magnitude of the enhancement might be expected to be higher. The correlation of the relative enhancements is beyond the scope of our calculations here, however Rh560 is likely to follow the trend of enhancement shown by Rh6G and Rh640. Rh700 and Rh800 have a difference of about 2.8 eV, but an electron transfer is hindered by the lack of OH⁻ adduct formation in the acidified solution. In these

cases, only slight changes in emission are observed after IFE calculations. It is also interesting to note that the three enhanced fluorophores (Rh560, Rh6G, Rh640) happen to all be cationic, whereas the remaining fluorophores (excluding Rh700 and Rh800 for aforementioned reasons) are either neutral in charge or anionic. It is easy to conceive the possible formation of an ion pair between the cationic fluorophores and the anionic trinitroaromatic products. This driving force would bring the species in close proximity to allow ET to occur, without the limitations of diffusion and collision in solution.

4. Conclusions

In summary, we have investigated a sensing array based on highly fluorescent molecules in DMF solution that shows promise for the detection of explosive analytes. In addition to typical collisional or static quenching, inner filter effect corrections indicate an ET component, which increases the emission of at least two of the fluorescent reporters (Rh6G and Rh640). The findings of ET may have an impact on pre-existing RET based sensors, as the emission of the fluorophores in this study was observed to enhance. The capability of trinitroaromatic products to be involved in ET may influence existing sensors that rely on TNB or TNT as a RET disruptor, leading to a competitive ET mechanism that would decrease the effectiveness of the sensor by diminishing an observed quench in emission. Further studies will be undertaken to expand the set of fluorescent reporter molecules and quantify the interactions with the most significant emission change, as well as experiments to give additional clarification of the ET with trinitroaromatic products.

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Supplementary Information for:
A Fluorometric Sensing Array for the Detection of Military Explosive and IED Materials

by

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Figure S1: Structures of fluorescent reporter molecules that comprise the sensing array.

Rhodamine 560

Rhodamine 6G

Fluorescein 548

Sulforhodamine B

Rhodamine 640

Sulforhodamine 640

Rhodamine 700

Rhodamine 800

Figure S2: Structures of the explosive analytes exposed to the sensing array.

TNB

TNT

RDX

HMX

PETN

TATP

2,3-DNT

2,4-DNT

2,6-DNT

3,4-DNT

2-NT

4-NT

Figure S3: Normalized absorbance and emission overlays from Rh560 and Rh6G. Data have been normalized to the maximum from the initial spectrum. Left vertical axis represents absorbance and right vertical axis represents emission. (—) Initial fluorophore absorbance spectrum, (- - -) + analyte, (- - -) initial fluorophore emission spectrum, (- · - ·) + analyte.

Figure S4: Normalized absorbance and emission overlays from FI548 and SRhB. Data have been normalized to the maximum from the initial spectrum. Left vertical axis represents absorbance and right vertical axis represents emission. (—) Initial fluorophore absorbance spectrum, (- - -) + analyte, (- - -) initial fluorophore emission spectrum, (- · -) + analyte.

Figure S5: Normalized absorbance and emission overlays from Rh640 and SRh640. Data have been normalized to the maximum from the initial spectrum. Left vertical axis represents absorbance and right vertical axis represents emission. (—) Initial fluorophore absorbance spectrum, (---) + analyte, (---) initial fluorophore emission spectrum, (---) + analyte.

Figure S6: Normalized absorbance and emission overlays from Rh700 and Rh800. Data have been normalized to the maximum from the initial spectrum. Left vertical axis represents absorbance and right vertical axis represents emission. (—) Initial fluorophore absorbance spectrum, (- - -) + analyte, (—) initial fluorophore emission spectrum, (- - -) + analyte.