On the fluorescence properties of chromophores near metallic nanostructures
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Chapter 3

Binding of 4-amino napthalamide derivatives on gold nanoparticles using thioctic acid as a bidentate disulfide linker*

Abstract

We synthesised a fluorescent 4-amino napthalamide derivative (TNI), which was functionalised with a bidentate disulfide, i.e. a cyclic disulfide within a five-membered ring. The photophysics of TNI are complicated by the presence of the disulfide group. We found that upon excitation, the investigated compound partially undergoes intramolecular photoinduced electron transfer (PET).

Noble metal nanoparticles affect the photophysical properties of nearby molecules and upon binding of TNI with gold nanoparticles (AuNPs) the fluorescence intensity is reduced to 23%. In this case, it was found that this ligand irreversibly adsorbs onto the gold surface. A competition experiment using both TNI and perylene monoimide based chromophore bearing a linear disulfide, confirmed that the two types of disulfides have different binding efficiencies with the gold surface. Functionalisation with TNI of silver nanoparticles, or alloy nanoparticles of different gold/silver compositions, has not been successful.

* Manuscript in preparation
Introduction

Metal nanoparticles (MNPs) strongly affect the optical properties of nearby chromophores. The local electromagnetic field is influenced by surface plasmon resonance of the free electrons present in the nanoparticles. Resonant coupling with a chromophore in close proximity may greatly enhance the absorption and fluorescence of the chromophore. In various theoretical studies, the effect of the enhanced field on the transition rates of the chromophore (rate of absorption, rate of radiative decay and rate of non-radiative decay) has been investigated. In these models, the chromophore is usually treated as a point dipole. A substantial increase of all the rates which is caused by the presence of a metal particle is typically predicted. When the absorption of the chromophore overlaps with the surface plasmon band of the MNP, a drastic increase of the absorption rate is found. Likewise, resonant coupling also enhances the radiative decay rate. Both factors depend on the relative orientation of the chromophore with respect to the MNP. The non-radiative rate of the system, however, is also affected because of energy transfer from the chromophore to the metal nanostructure. This can lead to strong quenching of the fluorescence. In the calculations on such systems performed by our collaborators, the description of the chromophore is based on a quantum chemical (QM) model, which is much more chemically sound than treating the chromophore as a point dipole. Their calculations confirmed that various factors, such as the distance of a chromophore towards the surface of the nanoparticle, its relative orientation, and the shape and size of the nanoparticle, are important variables for the enhancement of the rate factors. In particular, these factors result in either dramatic quenching or substantial enhancement of the fluorescence intensity, which is consistent with several experimental studies. It is invariably predicted that the fluorescence of chromophores is quenched when these are situated in very close proximity (< 5 nm) of colloidal gold nanospheres (AuNPs).

The effects that metal nanoparticles have on the fluorescent behaviour of nearby chromophores have been investigated experimentally. A way to study the quenching effect, and possibly find suitable parameters for enhancing fluorescence, is to connect the chromophores onto AuNPs in solution. Sulfur containing groups such as thiols or disulfides have a high affinity for gold surfaces. These groups are commonly used to form self-assembled monolayers on gold surfaces. Thiols (S - H) or disulfides (S - S) can readily chemisorb onto the surface, but there is still some uncertainty whether the thiol or disulfide bonds of the adsorbates are dissociated upon adsorption on the metal surface. Nuzzo et al. have performed XPS studies and stated that the S - S bond of disulfides is cleaved, whereas the S - H bond of thiols remain intact. Other studies using surface enhanced Raman
scattering (SERS) reveal that in the spectrum of a ligand with a disulfide or thiol group adsorbed to a gold surface, the vibrations caused by the $S - S$ or the $S - H$ bond are absent, indicating that the disulfide bond and the thiol bond are both cleaved upon adsorption on the surface.\textsuperscript{8} It is believed that the species resulting from adsorption on the surface is comprised of an alkyl thiolate ($RS^- - Au^+$) adsorbed on the gold surface.\textsuperscript{9}

Because of their propensity to bind to gold surfaces, it seemed appropriate to combine a chromophore with a disulfide group, after which the chromophore could be attached to gold nanoparticles. It was found, however, that thiolates do not bind so strongly onto the gold surface and tend to desorb from the gold surface under ambient conditions in solution (See Chapter 2). An equilibrium between bound and free ligands is found (association constant $K = 5 \times 10^5$ M$^{-1}$). Instead, we now opted for a cyclic disulfide so that upon binding onto AuNPs, this group is attached via two sulfur atoms (Figure 1). In this way, desorption from the gold surface is minimised, as this would require two gold-sulfur bonds to be broken. In our case, the disulfide stems from thiocic acid and is inside a five-membered ring. This disulfide function has already been successfully used to immobilise systems on the surfaces of a gold film or bridging of gold nanoparticles.\textsuperscript{10,11}

![Figure 1: Schematic representation of functionalisation of AuNPs with a cyclic disulfide.](image)

Like in Chapter 2, a chromophore has been equipped with a linker and the aforementioned specific disulfide function. It was also found that gold nanoparticles remain stable in certain water and organic solvent mixtures. Therefore, the incorporation of an ethylene glycol chain to increase the water-solubility is no longer a necessity, and for our purposes a long aliphatic chain will suffice. The chromophore under investigation in this Chapter is a 4-amino substituted naphthalimide derivative. This type of chromophore has a reported fluorescence quantum yield of $\leq 84\%$.\textsuperscript{12} The lowest excited state of 4-amino substituted naphthalimides gives rise to a broad absorption band between 385 and 450 nm.\textsuperscript{13} This band is not present in unsubstituted naphthalimides and is assigned to a transition with charge-transfer (CT) character because of the electron donating nature of the amino group.
Indeed, the crystal structure of N-butyl-4-(butylamino)-1,8-naphthalimide\textsuperscript{14} suggests some charge transfer character in the ground state: the C – N bond is shorter than a usual single bond, indicating a considerable double bond character and additionally, the bond angles around the amine N atom are close to 120\degree. The naphthalimide with the electron donating amino group is a “push-pull” chromophore with large internal charge transfer in the first electronic excited state. This type of chromophore is therefore also strongly solvatochromic. Naphthalimides with an amino group can be readily prepared and this results in bright yellow/ strongly green fluorescent solids. Lucifer Yellow is a commercial dye that is based on this structure and is, for example, used for staining of neurons.\textsuperscript{15} For our purposes, the chromophore has, like the perylene monoimide derivative discussed in Chapter 2, suitable properties: a rigid structure, photostability, and it exists as a single emitting species in solution.

The molecular structure of the chromophore, T\textsubscript{NI} (thioctic acid-naphthalimide), is depicted in Scheme 1. It consists of the fluorescent probe, an aliphatic linker and the cyclic disulfide from thioctic acid connected via an amide bond. In addition, a reference compound BuNI is prepared (Scheme 1).

\begin{center}
\includegraphics[width=\textwidth]{Scheme1.png}
\end{center}

\textbf{Scheme 1:} Structures of BuNI and T\textsubscript{NI}.

Firstly, the photophysical behaviour of T\textsubscript{NI} is described and compared to that of BuNI. The differences caused by the presence of the disulfide ring are discussed. Secondly, the interactions of T\textsubscript{NI} with AuNPs in terms of the binding efficiency and the effects on the fluorescent properties are studied. Also the binding efficiency of T\textsubscript{NI} is compared to that of a perylene-monoimide dye coupled to a linear disulfide via a tetraethyleneglycolalkyl chain (PMIdS, See Figure 1 in Chapter 2) in a competition experiment.
Results and Discussion

Photophysics of naphthalimide chromophores

Absorption and emission spectra

The syntheses of TNI and BuNI are described in the Experimental Section. We studied the photophysical properties of the reference chromophore BuNI and the ligand TNI in solution, before carrying out fluorescence titrations concerning the interaction of TNI with gold nanoparticles. The chromophores have a broad absorption peak in the region 380 - 450 nm and an emission peak in the range of 480 - 520 nm; they are solvatochromic with a 20 nm bathochromic shift in absorption and a 40 nm shift in emission for BuNI from toluene to DMF (Figure 2). Compared to BuNI, both the absorption and emission of TNI are shifted towards longer wavelengths. The shift in emission is rather large in toluene (17 nm) compared to the shift in DMF (5 nm). Moreover, TNI is found to be less fluorescent by 23% than BuNI in toluene. These differences between the two chromophores have been studied in more detail and are discussed below.

Figure 2: Normalised absorption and emission spectra (λexc. ≈ 420 nm) of TNI (green) and BuNI (red) in toluene and DMF.

The absorption and emission spectra of BuNI and TNI were measured in various solvents of different polarities. The emission spectra of BuNI and TNI are shown in Figures 3 and 4; the samples were excited at their corresponding absorption maxima. They both display the solvatochromic behaviour typical of 4-amino substituted naphthalimides; the absorption maximum of BuNI shifts 47 nm from n-hexane (λmax. = 408 nm) to DMSO (λmax. = 445 nm) and its emission maximum shifts by almost 80 nm from n-hexane (λmax. = 453 nm) to DMSO (λmax. = 531 nm). The red shift of the spectra of TNI compared to BuNI is not limited to only toluene and DMF, but appears in all studied solvents.
The red shift is more pronounced in non-polar solvents than in polar solvents. The emission spectra of TNI are also broader than those of BuNI.

It can be imagined that in non-polar solvents, the amide group in TNI forms hydrogen bonds with the naphthalimide moiety, by folding of the flexible aliphatic chain. This may result in changes in the local environment and thereby the properties of the chromophore itself. In solution, more than one conformation may thus be present (conformational heterogeneity). The observed red shift in both the absorption and emission spectra of TNI, compared to BuNI, as well as the fact that the emission spectra of TNI are broader than that of BuNI, can be attributed to the presence of another emitting species in solution as a result of intramolecular hydrogen bonding.

Figure 3: Normalised emission spectra of BuNI in various solvents.

Figure 4: Normalised emission spectra of TNI in various solvents.
Table 1: Emission maxima of BuNI and TNI in various solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>BuNI $\lambda_{em.}$</th>
<th>TNI $\lambda_{em.}$</th>
<th>$\Delta \lambda$ (nm)</th>
<th>Solvent</th>
<th>BuNI $\lambda_{em.}$</th>
<th>TNI $\lambda_{em.}$</th>
<th>$\Delta \lambda$ (nm)</th>
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a $\lambda_{exc}$ is at the maximum absorption of the sample.
b Difference in emission maximum between BuNI and TNI.
c Vibronically structured band.

Fluorescence lifetime measurements

The fluorescence decay times of BuNI and TNI were measured using the time correlated single photon counting (TC-SPC) setup in both toluene and DMF, using $\lambda_{exc.} = 323$ nm or $\lambda_{exc.} = 420$ nm, and multiple detection wavelengths ranging from 500 nm to 540 nm. In both solvents the fluorescence decay of BuNI is monoexponential (7.9 ns in toluene; 9.8 ns in DMF) and is independent of the detection wavelength. Unlike BuNI, the fluorescence decay curves of TNI cannot be fitted monoexponentially, but they need to be fitted biexponentially (Figure 5). The long component $\tau_1$ is slightly shorter than the decay time of BuNI (6.8 ns in toluene; 9.2 ns in DMF). The short component $\tau_2$ is in the order of 1.5-2 ns (Table 2).

Figure 5: Fluorescence decay curve of TNI in toluene ($\lambda_{exc.} = 323$ nm, $\lambda_{det.} = 520$ nm). A: monoexponential fit; B: biexponential fit.
Table 2: Fluorescence lifetimes of BuNI and TNI in DMF and in toluene.

<table>
<thead>
<tr>
<th></th>
<th>BuNI</th>
<th>TNI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\tau_1$ (ns)</td>
<td>$\tau_1$ (%) $^a$</td>
</tr>
<tr>
<td>Toluene</td>
<td>7.9</td>
<td>6.8 (64%)</td>
</tr>
<tr>
<td>DMF</td>
<td>9.8</td>
<td>9.2 (91%)</td>
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</table>

$\lambda_{\text{exc.}} = 323$ nm or 420 nm
$\lambda_{\text{det.}} = 490 - 530$ nm (toluene), 510 - 540 nm (DMF).

$^a$ Percentages are relative amplitudes

The relative amplitude of $\tau_2$ is independent of the concentration of the chromophore TNI and the short component must originate from an intramolecular process. Some possible explanations for the presence of a second time constant for TNI have been considered. The sulfur atoms in TNI might increase the probability of intersystem crossing to the triplet state (internal heavy-atom spin-orbital coupling$^{17}$). It is known that 1,8-naphthalimides undergo intersystem crossing,$^{18}$ and it may occur in 4-amino-naphthalimides as well. However, only a few accounts have been found of sulfide-enhanced intersystem crossing.$^{19}$ Simple enhancement of ISC does not explain biexponential decay, but heavy atom effect may be distance-dependent. Therefore, the presence of two conformers can explain biexponential decay: one conformer has no/little enhanced ISC, whereas the other displays increased ISC leading to a shorter decay time. The contribution and the rate constant of the short component $\tau_2$ are lower in DMF (~9% and 2.2 ns) than in toluene (~36% and 1.4 ns). If intersystem crossing would be the main reason for the second time constant, this difference can be explained: it is known for 1,8-naphthalimides that intersystem crossing is more efficient in non-polar solvents than in polar solvents.$^{20}$ The polarity of the solvent influences the relative energy of the electronic states involved in the intersystem crossing process. As a consequence, the mixing of the two states will be less important and the fluorescence quantum yield increases.

Alternatively, as the absorption maximum of thioctic acid is around 350 nm, energy transfer from the disulfide part to the naphthalimide part is possible when exciting at 323 nm. However, the biexponential decay is also observed with $\lambda_{\text{exc.}} = 420$ nm, which indicates that energy transfer cannot be the reason for the presence of $\tau_2$.

A more probable reason for the occurrence of $\tau_2$ in TNI as opposed to BuNI is intramolecular photoinduced electron transfer (PET). Naphthalimide derivatives are often used as electron acceptors because of the electron withdrawing imide group.$^{18}$ Although the amino group lowers the potential for 1-electron reduction of these molecules by 0.2 V,$^{21}$ still nearly quantitative intramolecular photoinduced electron transfer (PET) from tertiary amines attached to the naphthalimide through a
short aliphatic chain on the 4-position can take place in polar solvents, leading to strong quenching of
the fluorescence and complex emission kinetics. Likewise, studies involving the same
naphthalimide ring connected to p-methoxyaniline reveal that fast and nearly quantitative
intramolecular electron transfer takes place.

When the disulfide is constituted in a five-membered ring, it can be more easily oxidised than
open-chain analogues: it has been found that the oxidation potential is almost 0.3 V lower for thioctic
acid compared to linear disulfides because of the ring strain and a smaller CS-CS torsional angle in
the cyclic structure. The thioctic acid-derived disulfide can thus be seen as a possible electron
donating group within TNI and the naphthalimide unit as the electron accepting group.

To verify the possibility of electron transfer within the molecule, the redox potentials $E^\circ$ of the
two components of TNI (thioctic acid as donor and the reference chromophore BuNI as acceptor)
were measured in dichloromethane with cyclic voltammetry. The reduction potential $E_{\text{red}}^{\text{cyclic}}$ of the
chromophore BuNI is $-1.95$ V vs Fc, and is close to the reported value of 4-amino-1,8-naphthalimide
($E_{\text{red}}^{\text{cyclic}} = -1.46$ V vs SCE). Because of the electron donating amino-substituent, this value is more
negative than that of N-methyl naphthalimide ($E_{\text{red}}^{\text{cyclic}} = -1.31$ V vs SCE), or 3,5-di-tert-butyl-1,8-
naphthalimide derivatives ($E_{\text{red}}^{\text{cyclic}} = -1.41$ V vs SCE). In addition, BuNI also has a low oxidation
potential at $E_{\text{ox}}^{\text{cyclic}} = +0.90$ V vs Fc, due to the electron donating nature of the 4-amino group. Thioctic
acid has a lower oxidation potential (+0.74 V vs Fc) than BuNI. The $E_0$ energy of TNI (2.69 eV in
toluene and 2.60 eV in DMF) is close to the difference in the measured redox potentials ($E_{\text{red}}^{\text{cyclic}} - E_{\text{ox}}^{\text{cyclic}}$)
of the donor and acceptor. Electron transfer from the thioctic acid group to the naphthalimide is
feasible, and at short enough distances, intramolecular PET may take place in TNI (Figure 6). The
flexible aliphatic linker between the disulfide and the naphthalimide can fold by forming hydrogen
bonds, which renders a smaller distance between donor and acceptor than would be in an extended
form. We postulate that both conformations are present in solution (conformational heterogeneity)
and that intramolecular PET occurs more efficiently in the folded conformation than in the extended
conformation. This can explain the complex biexponential decay of the fluorescence.

* To convert the redox potential values reported versus Fc/Fc* to the saturated calomel electrode (SCE) scale, one should
add 0.38 V; therefore the reduction potential $E_{\text{red}}^{\text{cyclic}}$ of BuNI is $-1.59$ vs SCE. See: Pavlishchuk, V. V.; Addison, A. W.
Figure 6: Intramolecular PET from the disulfide part of TNI to the naphthalimide part.

The lower contribution of $\tau_2$ in DMF is somewhat counterintuitive if it is caused by intramolecular PET, since a more polar solvent would be more capable of stabilising the radical cation and anion arising from the PET. Probably, in this system, electron transfer can only take place when the donor and acceptor are in close proximity. In non-polar solvents, as stated before, the folded conformation is more abundant than in polar solvents, leading to a net higher efficiency of intramolecular PET and therefore a higher contribution of $\tau_2$ in toluene than in DMF.

Adsorption on gold nanoparticles

Titration experiments

The complex photophysics of the chromophore TNI is not likely to affect the experiments with gold nanoparticles (AuNPs), since these experiments are performed in polar solvent mixtures (pure DMF or DMF : H$_2$O 8 : 2 v/v). When TNI is mixed with AuNPs, it can bind to the surface with its disulfide moiety. Unlike PMIdS (See Chapter 2), the disulfide is part of a cyclic five-membered ring and when it adsorbs onto the gold surface, the chromophore will bind to the nanoparticle with two gold-sulfur bonds. Even though we concluded that the gold-sulfur bond is not so strong in dilute samples, it is less likely to desorb from the surface, since for that two bonds need to be broken.

The experiments of functionalising AuNPs with TNI were carried out with excess-citrate stabilised gold nanoparticles of ~12 nm (xCS-AuNPs; $N_{\text{sites}} \approx 3000$) in a mixture of DMF : H$_2$O 8 : 2 v/v and with tetra(n-octyl)ammonium bromide stabilised gold nanoparticles of ~4.5 nm (TOAB-AuNPs; $N_{\text{sites}} \approx 380$) in pure DMF. The number of binding sites of the particle $N_{\text{sites}}$ is estimated from the particle diameter and the surface that a thiol-groups requires on an Au(111) surface.$^{27}$ For simplicity, we assume that the cyclic disulfide linker takes an equal amount of space on the metal surface as two thiol-groups. The chromophore concentration was kept at approximately 1 µM in all experiments. The fluorescence spectra were all obtained in a right angle configuration (See Experimental Section). Two types of experiments have been done to investigate the fluorescent
behaviour of TNI upon adsorption on AuNPs. First, in a titration experiment, a solution of TNI in DMF was prepared inside a cuvette to which small amounts of a solution of TOAB-AuNPs of ~4.5 nm were added (10 μL per step; \( c_{\text{particle}} = 7.4 \times 10^{-7} \) M). After each addition the fluorescence intensity was measured. In the second type of experiment—a so-called series experiment—a series of solutions was prepared with equal concentration of TNI, while varying the concentration of AuNPs. The graphs below show the results of the two different experiments (Figure 7: titration experiment; Figure 8: series experiment) done with TOAB-AuNPs.

**Figure 7**: Absorption (A) and emission spectra (B; \( \lambda_{\text{exc.}} \approx 458 \) nm) of TNI (1.1 \( \times 10^{-6} \) M, corresponding to 2.2 μM of ligating sulfur atoms) in DMF (2.9 mL) with addition of TOAB-AuNPs. In this experiment, a total of 90 μL of a concentrated solution of AuNPs (maximum concentration in sample: 2.0 \( \times 10^{-8} \) M, corresponding to 7.5 \( \times 10^{-6} \) M binding sites) was added to the solution. The emission spectra shown in Panel B are not corrected for the change in concentration of TNI by addition of AuNPs.

**Figure 8**: Emission spectra (\( \lambda_{\text{exc.}} \approx 458 \) nm) from “series experiments” of TNI in DMF with TOAB-AuNPs. The concentration of TNI was held constant at 1.1 μM, while varying the concentration of AuNPs (maximum concentration: 4.0 \( \times 10^{-8} \) M). The peaks marked with * are due to Rayleigh scattering.

The relative fluorescence intensity \( \frac{I}{I_0} \) is determined by the emission integral, after correction for the ‘inner filter effect’ at both the excitation pathway and the emission pathway. In the titration
experiments, the $I / I_0$ is also corrected for the change in concentration of the chromophore; since the concentrations that are used are very low, a linear relationship between the concentration and the fluorescence intensity can be assumed. Upon addition of AuNPs, the relative fluorescence intensity of TNI decreases linearly with the amount of AuNPs added until it reaches a plateau. The decrease in intensity is caused by quenching of the fluorescence due to binding of the chromophore onto the AuNPs. Because of the low concentration of AuNPs it is unlikely that they will affect the fluorescent properties of the chromophores without binding to them. As a control experiment, also an experiment was done with BuNI and xCS-AuNPs; here no quenching of fluorescence was observed upon addition of AuNPs (data not shown).

The point where $I / I_0$ stops decreasing (saturation point) occurs when the number of binding sites of the total AuNPs in solution is equal to the number of chromophores present (Figure 9). At this point all the chromophores in solution are bound to the surface of the AuNPs. This is true for both kinds of AuNPs. After the saturation point, $I / I_0$ does not decrease further upon adding AuNPs, indicating that no additional changes occur in the solutions. The relative fluorescence intensity of the TNI-AuNPs species is ~23%, which is higher than the predictions on the basis of quantum chemical calculations which had suggested nearly quantitative quenching of the fluorescence. Unfortunately, due to technical difficulties, it was not possible to perform time resolved fluorescence lifetime measurements on these systems; the fluorescence quantum yield of these bound species could thus not be quantified. The remaining fluorescence may also in part be caused by impurities in TNI that do not bind with the AuNPs.

![Figure 9](image)

**Figure 9:** Normalised integrated fluorescence intensity ($I / I_0$), corrected for inner filter effects, as a function of binding site concentration ($N_{sites}$ 5nm AuNPs: ~380; $N_{sites}$ 12nm AuNPs: ~3000).
Comparable studies on the self assembly of oligo(π-phenylene vinylene) (OPV) connected to thioctic acid shows similar results. In these studies, a solution of TOAB-AuNPs of ~4.1 nm in toluene is titrated to a solution of OPV in toluene and the ensuing OPV fluorescence is monitored. A quenching of the fluorescence is observed as the gold particles are added. The saturation point, where the fluorescence intensity does not decrease further, corresponds to $1.3 \times 10^2$ OPV molecules per particle. This number is in good agreement with complete coverage of the Au surface by thiolates. Finally, the fluorescence of these systems does not drop to zero as a fluorescence quantum yield of 0.078 has been found for OPV-Au adducts. As both calculations and lifetime measurements imply that the remaining fluorescence should be considerably lower than the measured values, the authors argue that the rest emission originates largely from OPV molecules not bound to the Au particle.

**Competition experiments**

To see the differences in binding efficiencies of the type of disulfide, a titration experiment with a mixture of TNI and PMIds in DMF was performed. The TOAB-AuNPs were used in this experiment. The solution contains more TNI than PMIds (ratio ~5 : 1) because the absorption coefficient at $\lambda_{\text{exc}}$ of TNI is nearly a factor three lower than that of PMIds and also because TNI has a substantially lower fluorescence quantum yield. The fluorescence of TNI can be distinguished in the emission spectra of the mixtures as a shoulder underneath the emission band of PMIds (Figure 10). As expected, the total fluorescence intensity decreases when AuNPs are added to the solution. The fluorescence intensity of TNI and PMIds can be reconstructed from the spectra by fitting the corresponding spectra of pure TNI and PMIds in DMF (See insets in Figure 10). In this way it is possible to calculate the relative fluorescence intensity of each chromophore separately (Figure 11). The emission peak of TNI decreases faster than that of PMIds, which is a clear indication that TNI binds more strongly to the AuNPs than the disulfide group in PMIds.

When the concentration of binding sites of the AuNPs is lower than the concentration of ligating sulfur atoms ($c_{\text{binding sites}} < c_{\text{sulfur atoms}}$), the relative fluorescence intensity of TNI decreases linearly, similar to the binding efficiency of pure TNI. In contrast, $I / I_0$ of PMIds does not decrease to the same extent as that of pure PMIds. We therefore conclude that a smaller amount of PMIds binds to the AuNPs because of competition with TNI which binds irreversibly. At the point $c_{\text{binding sites}} = c_{\text{sulfur atoms}}$, mostly TNI would thus be adsorbed on the gold surface. Likewise, only a slight decrease of $I / I_0$ of TNI is observed, whilst after the equivalence point the curve of PMIds has a similar shape as that of pure PMIds, i.e. gradually decreasing even after excess of AuNPs, which is a clear confirmation of the weak binding that was found between PMIds and AuNPs.
Chapter 3

Figure 10: Titration experiment (λ_{exc.} ≈ 458 nm) of a mixture of T_{NI} (9.7 × 10^{-7} M) and PMIdS (1.8 × 10^{-7} M; corresponding to total concentration of ligating sulfur atoms of 2.9 µM) in DMF (2.9 mL). Total added TOAB-AuNP stock solution: 60 µL (maximum concentration in sample: 1.5 × 10^{-8} M). Inset A: reconstruction of the fluorescence spectrum of the initial mixture from that of pure T_{NI} and PMIdS in DMF. Inset B: reconstruction of the final solution.

Figure 11: Relative fluorescence intensity of T_{NI} (green diamonds) and PMIdS (blue diamonds), corrected for inner filter effects, upon addition of TOAB-AuNPs to the mixture as a function of binding site concentration (N_{sites} AuNPs: ~380). Gray diamonds are from the titration experiments with pure T_{NI} and pure PMIdS, respectively.

Fluorescence recovery

In an additional experiment, a solution of TOAB-AuNPs in toluene has been added to a solution of T_{NI} in toluene. Here, it was found that the functionalised AuNPs were not stable in solution and after one day precipitation had occurred. The fluorescence intensity of the solution, after precipitation of the AuNPs, increased to about 90% of that of the original solution (Figure 12). This evidently
indicates that quenching of the fluorescence is solely due to binding of the chromophore onto the gold surface; after precipitation of the AuNPs, the chromophore is liberated and goes back into solution. The red-shift of the emission peak is caused by the addition of tetra-n-octylammonium bromide (TOAB), which is present as stabiliser in high concentrations in the AuNP stock-solution.

Figure 12: Emission spectrum ($\lambda_{exc} \approx 458$ nm) of a sample of TNI (4.4 × 10^{-6} M) in toluene, without AuNPs (blue) and with an excess of TOAB-AuNPs (200 µL; 4.7 × 10^{-8} M) added (green). Solid line: immediately after addition; dashed line: after precipitation of the AuNPs overnight.

Silver and alloy nanoparticles

The absorption band of the chromophore TNI does not have the ideal overlap with the surface plasmon band of gold (~520 nm), and from quantum chemical calculations no situations leading to fluorescence enhancement caused by AuNPs have been found.29 However, there is an excellent overlap between the absorption of TNI and the surface Plasmon band of silver nanoparticles (~400 nm). In addition, the large Stokes shift in naphthalimide derivatives results in almost no spectral overlap and minimal effects on the emission efficiency of TNI are expected. From quantum chemical calculations,29 approximately a 100-fold amplification of the absorbance for TNI is predicted in the presence of a silver metal nanoparticle that can effectively lead to a 4-fold enhancement in the relative brightness of the chromophore.

Similar titration experiments as described above have been done with TNI and silver nanoparticles or alloy nanoparticles. Alloy nanoparticles could be prepared by co-reduction of equimolar mixtures of HAuCl$_4$ and AgNO$_3$ under the same conditions as used when preparing normal xCS-AuNPs.30 This method, however, leads to a larger size distribution than for the xCS-AuNPs. The affinity of disulfides for silver surfaces is less than that for gold surfaces and in these experiments no evidence of binding of TNI on silver nanoparticles has been observed. By mixing TNI with alloy nanoparticles, followed by centrifugation of these samples, it was possible to prepare
nanoparticles functionalised with the chromophore. Unfortunately, no time resolved fluorescence lifetime measurements could be carried out and these samples have not been examined further. More studies are needed to be able to quantify the extent of fluorescence enhancement or quenching by alloy nanoparticles.

Conclusions and outlook

The photophysics of $T_{NI}$ have been studied and compared to a reference chromophore $BuNI$. The complex photophysics of $T_{NI}$, among which biexponential fluorescence decay, are most probably caused by intramolecular PET from the cyclic disulfide to the naphthalimide group. Electron transfer is a likely process based on the redox potentials of the naphthalimide group and the disulfide. Further studies, such as nanosecond time-resolved transient absorption measurements, are needed to observe the naphthalimide radical anion. However, due to technical difficulties, it has not yet been possible to perform these measurements.

The fluorescence of $T_{NI}$ is quenched upon binding onto the surface of the AuNPs. Similar to the experiments with $PMIdS$ (Chapter 2), there is no dependence on the size of AuNPs. In the experiments, it was found that the chromophore does not desorb from the gold surface, which was the case for $PMIdS$. Furthermore, the difference in binding efficiency of the disulfide is confirmed when AuNPs are added to a mixture of both chromophores $T_{NI}$ and $PMIdS$. In this experiment both chromophores act independently from each other and bind to the gold surface with efficiencies identical to that of pure $T_{NI}$ and of pure $PMIdS$. This shows that stronger multidentate ligands are better suited for the construction of hybrid organic-inorganic nano-objects, when robustness is a requirement, such as in medical diagnostics or biological imaging. Furthermore, longer spacers, extending beyond the reach of small ligands, would be helpful in further studying the energy transfer from organic dyes to metal nanoparticles, as well as the inclusion of noble metal particles of different sizes and composition.
Experimental Details

UV/VIS-fluorescence

The UV/Vis absorption spectra were recorded on a double beam Varian Cary 3E spectrophotometer, spectral range 190 to 900 nm, with bandwidths down to 0.2 nm. The spectra were recorded in rectangular 10 mm quartz cuvettes. The fluorescence excitation and emission spectra were recorded on a Spex Fluorolog 3 spectrometer, equipped with double grating monochromators in the excitation and emission channels. The excitation light source was a 450W Xe lamp and the detector a Peltier cooled R636-10 (Hamamatsu) photomultiplier tube. The fluorescence spectra were corrected for the wavelength response of the detection system. Fluorescence decay times were measured using time correlated single photon counting (TC-SPC) on a set-up that has been described elsewhere.31 Two excitation wavelengths were used: either $\lambda_{\text{exc.}} = 323$ nm from a frequency-doubled cavity-dumped DCM dye layer, or $\lambda_{\text{exc.}} = 420$ nm from a frequency-doubled Coherent Chameleon titanium-sapphire laser of which the repetition rate was reduced to 4 MHz by an APE Pulse-Select pulse picker.

For characterisation of the gold nanoparticles in various solvents and their interaction with TNI and BuNI, UV/Vis absorption spectrometric measurements were performed using an optical fibre-based system (OceanOptics, USA) incorporating a USB4000-VIS-NIR CCD spectrometer and a LS-1 tungsten-halogen light source equipped with a BG34 colour correction filter. Fluorescence measurements were carried out on another optical fibre-based system (OceanOptics, USA) using a QE65000 thermo-electrically cooled, back-thinned CCD spectrometer and a stabilised light-emitting diode (LS-450, 455 nm centre wavelength) as an excitation source. Emission spectra from this spectrometer were not corrected for the wavelength dependence of spectrometer detection efficiency. The efficiency curve is expected to be rather flat in the region of BuNI and TNI emission. Moreover, virtually no changes in the shapes of the emission spectra are found during the titration measurements, making the measurement of relative integrated intensities as needed for fluorescence titrations reliable. In the calculations of the relative fluorescence intensity $I / I_0$, the inner filter effects, such as re-absorption, are considered.32

Synthesis of AuNPs

The synthesis of 4.5 nm TOAB-stabilised gold nanoparticles (TOAB-AuNPs) and the 13 nm citrate-stabilised gold nanoparticles (xCS-AuNP) is described in Chapter 2.
Synthesis of naphthalimide chromophores

The reference compound BuNI and TNI have both been synthesised from 4-bromo-1,8-naphthalanhydride. The synthesis of BuNI is depicted in Scheme 2. The synthesis of BuNI also yielded a side product Me₂N-NI in relatively high amounts, from a reaction of BrNI with dimethylamine that is present as impurity in the solvent DMF. The synthesis of TNI is shown in Scheme 3. The first attempt to prepare the mono-substituted intermediate C12NI from 1,12-dodecyldiamine resulted predominantly in the dialkylated product, as has also been reported for other reactions with symmetric diamines. The second attempt of the reaction was performed using molten dodecyldiamine as ‘solvent’, but this did not increase the yield significantly. The final step was an amide coupling of thioctic acid with C12NI to yield TNI. The purity of TNI was checked with normal-phase HPLC with absorption and fluorescence detection (eluens: heptane/acetone, see further). Assuming that all chromophores detected with the HPLC have the same molecular absorption coefficient, from that analysis the purity was estimated at ~90%.

Scheme 2: Synthesis of BuNI.

Synthesis of N-n-butyl-4-bromo-1,8-naphthalimide (BrNI)

A flask containing a mixture of 4-bromo-1,8-naphthalanhydride (1.49 g, 5 mmol), and n-butylamine (2.6 mL, 20 mmol, 4 equiv.) in ethanol (40 mL) was heated in an 80 °C oil bath. The mixture was refluxed with stirring overnight. The resultant brown mixture was cooled to -10 °C in an acetone/ice bath and the suspension was filtered. The residue was washed with ice-cold ethanol and collected (1.75 g, 98%). The product was analysed with ¹H NMR.
\[ ^1H \text{NMR (400MHz, CDCl}_3) : \delta 8.58 (dd, 1H, J = 8 \text{ Hz}, 1 \text{ Hz}), 8.56 (dd, 1H, J = 8 \text{ Hz}, 1 \text{ Hz}), 8.41 (d, 1H, J = 8 \text{ Hz}), 8.03 (d, 1H, J = 8 \text{ Hz}), 7.83 (t, 1H, J = 8 \text{ Hz}), 4.17 (t, 2H, J = 7 \text{ Hz}), 1.70 (\text{quin.}, 2H, J = 7 \text{ Hz}), 1.40 (\text{sext.}, 2H, J = 7 \text{ Hz}), 0.96 (t, 3H, J = 7 \text{ Hz}). \]

Synthesis of \(N\)-butyl-4-(\(n\)-butylamino)-1,8-naphthalimide (BuNI)

In a round-bottom flask \(\text{BrNI} (153 \text{ mg}, 0.46 \text{ mmol})\) was dissolved in DMF (20 mL). To this solution excess \(n\)-butylamine (1 mL, 10 mmol) and DIPEA (2 mL, 12 mmol) were added and the mixture was stirred overnight in an oil bath of 130 °C. The solvent was removed by rotary evaporation. Purification by column chromatography over silica (eluent: CH\(_2\)Cl\(_2\)) yielded two products; firstly eluting was a side product \(N\)-butyl-4-(\(N\),\(N\)-dimethylamino)-1,8-naphthalimide (Me\(_2\)N-NI) (25.6 mg, 0.086 mmol, 18%), followed by the product BuNI (51.4 mg, 0.16 mmol, 34%). The purity of the two compounds is >99% based on analysis on the HPLC, using a normal phase column and a mixture of heptane and acetone (10% acetone) as eluens. Both compounds have been analysed by \(^1H\) NMR and mass spectroscopy.

\(\text{Me}_2\text{N-NI}: ^1\text{H NMR (400 MHz, CD}_2\text{Cl}_2) : \delta 8.51 (dd, 1H, J = 8 \text{ Hz}, 1 \text{ Hz}), 8.44 (dd, 1H, J = 8 \text{ Hz}, 1 \text{ Hz}), 8.65 (d, 2H, J = 8 \text{ Hz}), 7.63 (t, 1H, J = 8 \text{ Hz}), 7.11 (d, 1H, J = 8 \text{ Hz}), 4.11 (t, 2H, J = 7 \text{ Hz}), 3.09 (s, 6H), 1.67 (\text{quin.}, 2H, J = 7 \text{ Hz}), 1.41 (\text{sext.}, 2H, J = 7 \text{ Hz}), 0.96 (t, 3H, J = 7 \text{ Hz}). ^{13}\text{C NMR (100 MHz, CD}_2\text{Cl}_2) \delta 164.95, 164.39, 157.47, 132.77, 131.72, 131.14, 130.75, 125.86, 125.34, 123.82, 115.59, 113.76, 45.14, 40.36, 30.81, 30.25, 20.96, 14.23. \text{HR-MS (FAB+) m/z} = 297.1603 (M-H\(^+\)); (calcd. C\(_{18}\)H\(_{20}\)N\(_2\)O\(_2\) = 296.1603).

\(\text{BuNI}: ^1\text{H NMR (400 MHz, CD}_2\text{Cl}_2) : \delta 8.48 (d, 1H, J = 8 \text{ Hz}), 8.36 (d, 1H, J = 8 \text{ Hz}), 8.10 (d, 1H, J = 8 \text{ Hz}), 7.57 (t, 1H, J = 8 \text{ Hz}), 6.69 (d, 1H, J = 8 \text{ Hz}), 5.39 (t, 1H, NH\(_2\)), 4.09 (t, 2H, J = 7 \text{ Hz}), 3.39 (t, 2H, J = 7 \text{ Hz}), 1.78 (\text{quin.}, 2H, J = 7 \text{ Hz}), 1.65 (\text{quin.}, 2H, J = 7 \text{ Hz}), 1.52 (\text{sext.}, 2H, J = 7 \text{ Hz}), 1.40 (\text{sext.}, 2H, J = 7 \text{ Hz}), 1.00 (t, 3H, J = 7 \text{ Hz}), 0.95 (t, 3H, J = 7 \text{ Hz}). \text{HR-MS (FAB+) m/z} = 325.1915 (M-H\(^+\)); (calcd. C\(_{20}\)H\(_{25}\)N\(_2\)O\(_2\) = 325.1916). \text{UV/VIS (toluene): } \lambda_{\text{max}} = 421 \text{ nm}, \varepsilon_{\text{max}} (\text{M}^{-1} \text{ cm}^{-1}) = 1.13 \times 10^4. \text{ Fluorescence (toluene, } \lambda_{\text{exc.}} = 420 \text{ nm): } \lambda_{\text{max}} = 481 \text{ nm.}

\(4-(12\text{-aminododecylamino})\)-\(N\)-\(n\)-butyl-1,8-naphthalimide (C\(_{12}\)NI)

\text{First attempt:} A mixture containing \(\text{BrNI} (300 \text{ mg}, 0.9 \text{ mmol})\) and 1,12-dodecyldiamine (500 mg, 2.5 mmol, 2.5 equiv.), tris(\(\sigma\)-dibenzylideneacetone)dipalladium(0) (18 mg, 0.02 mmol, 2 mol % Pd), and BINAP (25 mg, 0.04 mmol, 2 equiv. compared to Pd) in nitrogen purged toluene (10 mL) was refluxed in a Schlenk flask for 3h. The solution was allowed to cool to room temperature. The crude oil was purified further by flash chromatography on silica gel (gradient, CH\(_2\)Cl\(_2\) to CH\(_2\)Cl\(_2\) : MeOH 8 : 1 v/v). Firstly the dialkylation product \(\text{C}_{12}\text{-NI}_2\) was obtained as a side product (213 mg, 30%) and its...
structure was confirmed with mass spectroscopy; this was followed by the desired product $\text{C12NI}$ (55 mg, 12%). The product has been analysed with $^1\text{H}$ NMR and mass spectroscopy.

$\text{C12NI}$: $^1\text{H}$ NMR (400 MHz, CD$_3$OD): $\delta$ 8.50 (m, 2H), 8.32 (d, 1H, $J = 8$ Hz), 7.62 (t, 1H, $J = 8$ Hz), 6.74 (d, 1H, $J = 8$ Hz), 4.12 (t, 3H, $J = 7$ Hz), 3.42 (t, 2H, $J = 7$ Hz), 2.76 (t, 2H, $J = 7$ Hz), 1.68 (m, 2H), 1.64 (m, 2H), 1.43 (m, 4H), 1.22 (m, 16H), 1.00 (t, 3H, $J = 7$ Hz). $^{13}\text{C}$ NMR (100 MHz, CD$_2$Cl$_2$) $\delta$ 165.02, 164.42, 150.12, 134.63, 131.28, 130.35, 126.43, 125.11, 123.87, 120.76, 110.70, 104.72, 44.32, 42.17, 40.27, 32.49, 30.3-29.7, 27.68, 27.37, 20.98, 14.25. HR-MS (FAB+) $m/z$ = 452.3279 (M-H$^+$); (calcd. C$_{28}$H$_{42}$N$_3$O$_2$ = 452.3277). $\text{C12-NI}_2$: HR-MS (FAB+) $m/z$ = 703.4224 (M-H$^+$); (calcd. C$_{44}$H$_{54}$N$_4$O$_4$ = 703.4223).

\[ \text{BrNI} \xrightarrow{a} \text{C12NI} \ 12\% \quad + \quad \text{C12-NI}_2 \quad 30\% \]

\[ \text{C12NI} + \text{OH} \xrightarrow{b} \text{TNI} \]

a) 1,12-dodecyldiamine, 130 °C  
b) BOP, DIPEA, DMF, 18%

Scheme 3: Synthesis of T NI.

**Second attempt**: In a flask 1,12-dodecyldiamine (7 g, 35 mmol) was heated to melting under a nitrogen atmosphere. A solution of BrNI (200 mg, 0.60 mmol) in toluene (2 mL) was slowly added dropwise, the mixture was further heated to 130 °C and the mixture was left stirring for 2 days. The reaction mixture was poured into water and the product mixture was extracted four times into CH$_2$Cl$_2$. The organic layers were combined and filtrated over celite. Finally, the mixture was purified via column chromatography over silica eluting with a step-wise gradient starting from CH$_2$Cl$_2$ to CH$_2$Cl$_2$ : Acetone : Acetone : MeOH (5 : 1 v/v/v) to obtain the product (40 mg, 0.088 mmol, 15%).

$4$-(12-[5-(dithiolan-3-yl)pentanoylamino]dodecylamino)-N-$n$-butyl-1,8-naphthalimide ($\text{TNI}$)

A solution of 5-dithiolan-3-ylpentanoic acid (88.4 mg, 0.43 mmol) and BOP (398 mg, 0.90 mmol, 2 equiv.) in DMF (5 mL) was stirred for 2 h at room temperature. A solution of DIPEA (0.85 mL) and
**C12NI** (370 mg, 0.82 mmol, 2 equiv.) in anhydrous DMF (2 mL) was then added to the reaction mixture. The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the product was purified by column chromatography on silica gel, eluting with CH₂Cl₂ : MeOH (8 : 1). The obtained fraction was recrystallised in acetone to yield the product (50 mg, 18%). It was analysed with ¹H NMR and mass spectroscopy. The purity is ~90% based on analysis on the HPLC using a mixture of heptane and acetone (50% acetone) as eluens. The impurities detected with the HPLC all have similar absorption spectra as the product.

¹H NMR (400 MHz, CD₃OD) δ 8.53 (m, 2H), 8.35 (d, 1H, J = 8 Hz), 7.64 (t, 1H, J = 8 Hz), 6.78 (d, 1H, J = 8 Hz), 4.12 (t, 2H, J = 8 Hz), 3.45 (t, 2H, J = 7 Hz), 3.14 (m, 2H), 2.42 (sept., 1H, J = 7 Hz), 2.17 (t, 2H, J = 7 Hz), 1.79 (m, 4H), 1.64 (m, 6H), 1.28 (m, 24H), 0.99 (t, 3H, J = 7 Hz). HR-MS (FAB+) m/z = 640.3613 (M-H⁺); (calcd. C₃₆H₅₄N₃O₃S₂ = 640.3607). UV/VIS (toluene): λmax. = 423 nm, εmax. (M⁻¹ cm⁻¹) = 7.1 × 10³. Fluorescence (toluene, λexc. = 420 nm): λmax. = 499 nm.

**HPLC**

The HPLC system was equipped with two pumps (LC-20AT and LC-10AT vp; Shimadzu), a sample injector with a 100 µL loop, a solvent degasser (DGU-14A), a photodiode array (SPD-M10A vp) and a fluorescence detector (RF-10A XL). For analytical HPLC measurements, a Reprosil 100Si column (5 µm, 100 Å, 150 × 4.6 mm I.D.; Dr. Maisch GmbH) was used. The chromatographic mobile phase was a binary gradient (solvents heptane and acetone). A mobile phase condition of 10% acetone was used for the analysis of BuNI and Me₂N-NI, and 50% acetone for the analysis of TNI. The flow-rate of the mobile phase was 1 mL/min. The detection excitation and emission wavelengths were 420 and 500 nm, respectively.

**Cyclic Voltammetry**

Cyclic voltammograms were obtained of ~1 mM solutions in dichloromethane of the compounds of interest with tetrabutyl ammonium hexafluorophosphate as supporting electrolyte. The measurements were done in gas-tight, single-compartment, three electrode cells equipped with platinum working, coiled platinum wire auxiliary and silver wire pseudoreference electrodes. The cell was connected to a computer controlled Potentiostat Autolab PGSTAT 10 (Eco Chemie).
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References and notes: