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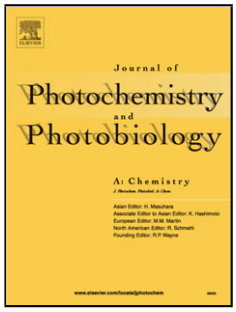

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Examination of tryptamine-squaraine complexes as both colorimetric and fluorometric stains in gel electrophoresis.

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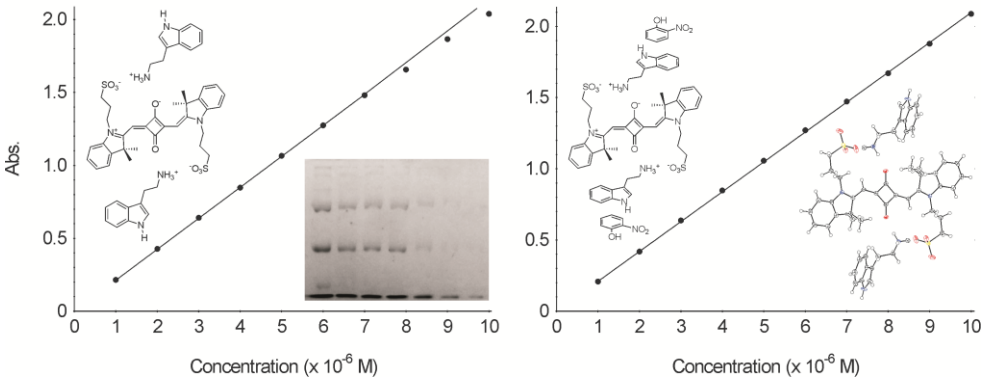
GRAPHICAL ABSTRACT



HIGHLIGHTS

- Use of bis-tryptaminium dual-sulfonate bis(indolenine)squaraine salt
- Ternary complexes indicate molecular interactions, observed in UV-vis
- No enhanced fluorescence (above that of other squaraines) in protein solutions
- X-ray crystal structures of two tryptamine proton-transfer salts are reported
- Electrophoresis gels stained with bis-tryptaminium squaraine fluoresce

ABSTRACT

A water-soluble dual pendent squaraine with tryptaminium cations has been mixed with mono-nitroaromatics in solution to create ternary complexes with the aim of creating indole-nitroaromatic charge-transfer pairs. Examination of the X-ray crystal structures of two tryptamine-nitroaromatic complexes, without the squaraine moiety, revealed that neither demonstrated charge-transfer pair or stacking associations between the indole and nitroaromatic rings. Examination of the fluorescence performance upon illumination with 300 nm light of each ternary squaraine complex, particularly in the presence of an equimolar amount of protein, showed that the levels across the series were no more than that observed for the base bis-tryptaminium squaraine, as was observed in a previous study. However, electrophoresis gels stained with the base bis-tryptaminium squaraine, whose X-ray crystal structure is also reported, were found to fluorescence; the only other squaraine dye of this type to do so in addition to the bis-piperidinium squaraine of previous studies.

Keywords: Squaraine dyes; X-ray crystal structure; Gel electrophoresis; Tryptaminium salts; Protein detection; Fluorescence

1. Introduction

Electrophoresis gel documentation systems (or gel docs), those commonly employed to fluorometrically image electrophoresis gels, are fitted with an ultraviolet (UV) light illuminator source emitting UV light at 310 ± 40 nm. For this reason, dyes such as ethidium bromide, which fluoresces a bright orange colour when excited with UV light, is equally commonly employed as a fluorescent tag in detecting nucleic acids in gel electrophoresis techniques [1], where the gels are imaged using a gel doc. In contrast,

bis(indolenine)squaraine dyes, studied as fluorescent protein-sensitive molecular probes, fluorescence in the near-infrared region of the visible spectrum and thus require excitation at the wavelength of maximum visible light absorbance (i.e. λ_{max}), which is normally > 600 nm [2-4]. It was therefore surprising to discover that the bis-piperidinium salt of a dual pendant sulfonate bis(indolenine)squaraine (**1**) (Fig. 1) fluoresced an intense pink color when a protein loaded gel, stained with this particular dye, was imaged in fluorescence mode (i.e. illumination with the UV light source) on a gel doc [5]. No other variant in that series of dual pendant sulfonate squaraine dyes, including dyes charge-balanced with alkali metals, could emulate this [6]. Compound **1** does not specifically absorb in the UV region so a synthetic strategy, and subsequent study, was devised that incorporated charge-transfer complexes with the squaraine dye in an attempt to increase the UV absorption of the squaraine-based compound [7]. This was done with the aim of promoting energy transfer from the charge-transfer component to the squaraine moiety to increase the squaraine fluorescence. The synthetic strategy adopted, similar to that illustrated in figure 2, involved the use of di-nitro aromatics, which are known to form charge-transfer complexes with the indole ring of the tryptamine cation. However, examination of each of these squaraine-based compounds against that of the squaraine-tryptaminium salt (**2**), both with and without the presence of protein, showed no improvement in fluorescence output upon illumination of UV light (at 300 nm). As a continuation to that study, here we report the use of four mono-nitro aromatics for potential incorporation with compound **2** to create compounds **3** – **6** (Fig. 2). The single crystal structures of **2** and two of the individual complexes employed (**7** and **8**), illustrated in figure 3, are reported, as are the results following use of two of the ternary complexes plus compound **2** as both colorimetric and fluorometric stains for gel electrophoresis.

2. Experimental

2.1. Synthesis

All chemicals were purchased from Sigma-Aldrich and were used as received without further purification. The water used in this study was purified using reverse osmosis techniques. Infrared spectra were recorded using a Perkin-Elmer Spectrum 100 FT-IR spectrometer. ^{1}H NMR data were recorded in DMSO-d_6 on a Bruker AVIIIHD 500 MHz FT-NMR spectrometer equipped with a SMART probe. Details of the synthesis of 2,3,3-trimethyl-1-(propan-3-sulphonyl)-indolenine, bis-piperidinium 2,4-bis-(3,3-dimethyl-(1-propan-3-sulphonate)-2-indolinylidenemethyl)cyclobutene-1,3-diolate (**1**) [5] and bis-(2-(1*H*-indol-3-yl)ethanaminium) 2,4-bis-(3,3-dimethyl-(1-propan-3-sulphonate)-2-indolinylidenemethyl)cyclobutene-1,3-diolate (**2**) [7] have been previously published, with the crystal structure of **1** (as a trihydrate) also previously reported [6].

2.1.1. Preparation of tryptamine-squaraine-nitroaromatic complexes

Bis-(2-(1*H*-indol-3-yl)ethanaminium) 2,4-bis-(3,3-dimethyl-(1-propan-3-sulphonate)-2-indolinylidenemethyl)cyclobutene-1,3-diolate hemihydrate (**2**) (2.90 mg, 3.0 μmol) was dissolved with twice molar amounts of 2-nitrophenol (0.83 mg, 6.0 μmol), 4-nitrophenol (0.83 mg, 6.0 μmol), 5-nitroquinoline (1.0 mg, 6.0 μmol) and 5-nitroisoquinoline (1.0 mg, 6.0 μmol), in water (100 mL) with sonication to produce 30 μM stock solutions of **3**, **4**, **5** and **6** respectively.

2.1.2. Synthesis of tryptaminium salts for X-ray analyses

Tryptamine (100 mg, 0.62 mmol) was reacted with an equimolar amount of either 2-nitrophenol (86 mg), 4-nitrophenol (86 mg), 5-nitroquinoline (108 mg), or 5-nitroisoquinoline (108 mg) by warming in ethanol (3 mL). Total evaporation of the ethanol

from each reaction yielded yellow crystals for **7** and **8**, a yellow-brown gel for **9** (that solidified after several days) and yellow-brown powder for **10**, each identified as organic salt complexes (for **7** and **8**) or a co-crystal adduct (for **9** and **10**) using infrared spectroscopy techniques. Compounds **7** and **8** yielded crystals from which specimens were cleaved for the X-ray analyses.

2.1.3. X-ray crystallographic analysis

Crystallographic data for **2** was collected at 100(1) K on a Rigaku Saturn724+ diffractometer using monochromatized Mo-Kα x-ray radiation (λ = 0.71075 Å) equipped with an Oxford Cryosystem low temperature device, and for **7** and **8** was collected at 200(1) K on an Oxford Diffraction Gemini-S CCD-detector diffractometer using monochromatized Mo-Kα X-ray radiation (λ = 0.71073 Å). All structures were solved by direct methods SHELX97 [8], and refined by full-matrix least-squares calculations. Crystal data for **2**: $C_{52}H_{60}N_6O_8S_2$, Mw = 961.18, monoclinic, $P2_1/n$, Z = 2, a = 14.3879(9), b = 5.8044(3), c = 29.186(2) Å, β = 99.645(7)°, D_{calcd} = 1.328 g cm^{-3}, T = 100(1) K, $F(000)$ = 1020, μ = 0.173 mm^{-1}, 13756 reflections were collected, 4230 unique (R_{int} = 0.0698), 2780 observed ($I > 2\sigma(I)$), 310 parameters, R_1 = 0.0528, wR_2 = 0.1161.Crystal data for **7**: $C_{16}H_{17}N_3O_3$, Mw = 299.33, monoclinic, $P2_1/c$, Z = 4, a = 14.334(3), b = 4.9651(8), c = 20.658(4) Å, β = 94.59(2)°, D_{calcd} = 1.357 g cm^{-3}, T = 200(1) K, $F(000)$ = 632, μ = 0.096 mm^{-1}, 5773 reflections were collected, 2878 unique (R_{int} = 0.0619), 1542 observed ($I > 2\sigma(I)$), 226 parameters, R_1 = 0.0754, wR_2 = 0.1277. Crystal data for **8**: $C_{16}H_{17}N_3O_3$, Mw = 299.33, triclinic, $P\bar{1}$, Z = 2, a = 8.4490(9), b = 9.7178(8), c = 9.7199(9) Å, α = 74.751(7), β = 74.168(8), γ = 84.662(7)°, D_{calcd} = 1.333 g cm^{-3}, T = 200(1) K, $F(000)$ = 316, μ = 0.094 mm^{-1}, 4949 reflections were collected, 2925 unique (R_{int} = 0.0209), 2307 observed ($I > 2\sigma(I)$), 211 parameters, R_1 = 0.0419, wR_2 = 0.0916. Crystallographic data (**2** (CCDC 1421976), **7** (CCDC

1054363) and **8** (CCDC 1054364)) have been deposited at the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK.

2.2. Materials

Bovine serum albumin (BSA) was purchased from Sigma-Aldrich and used as received without further purification. A stock solution of 100 μg/mL BSA was prepared by dissolving BSA (10 mg) in water (100 mL), for immediate use.

2.3. Spectroscopic measurements

Visible absorption spectra were recorded on a Shimadzu UV-1650 UV/visible spectrometer. Absorbance spectra for **7 – 10** were recorded between 200 – 800 nm in a 3 mL cell at a concentration of 70 μM whereas the spectra for **2** – **6** were recorded at a concentration of 10 μM. Visible absorption spectra of tryptamine, 5-nitroquinoline, 5-nitroisoquinoline, **9** and **10**, were recorded in aqueous solutions with 10% v/v dimethylsulfoxide, to ensure full solubility of the compound. For this reason the UV-visible spectrum in each of these cases is cut-off below 240 nm. All other spectra were recorded in water.

2.4. Fluorescence measurements

Solution fluorescence measurements were made on a Perkin-Elmer luminescence spectrometer LS50. Arbitrary fluorescence emission intensities were recorded between 330 – 800 nm (excitation: 300 nm) in a 3 mL fluorescence cell at a concentration of 1 μM. For the solutions without the presence of protein, 100 μL of squaraine stock solution was diluted to 3 mL in the fluorescence cell. Solutions containing protein had 2 mL of BSA stock solution plus 100 μL of squaraine stock solution diluted to 3 mL in the fluorescence cell.

2.5. Gel electrophoresis experiments

All electrophoresis gels were prepared according to previously described methodologies [5]. For each gel, lanes 2 - 8 were consecutively loaded with 20, 15, 12.5, 10, 7.5, 5, and 2.5 μL of unstained Precision Plus Protein standard from Bio-Rad. Staining with selected squaraines was undertaken using 200 mL aqueous solutions containing a dye concentration of 5 x 10^{-4} M. All squaraine staining solutions were mixed for 16 h and filtered before use. All gels were imaged, immediately following destaining, using a Bio-Rad Gel Doc EZ Imager system.

3. Results and discussion

3.1. Synthesis strategy

The background to the strategy adopted for this current study is detailed in the previous paper [7] and involves the incorporation of charge-transfer complexes with a squaraine dye with the aim of increasing the absorption of the overall complex at 300 nm. As mentioned in the previous paper, the use of tri-nitro aromatics was dismissed on health and safety grounds, even though they may display the biggest improvement in complex absorption at 300 nm. Results from the di-nitro aromatics demonstrated that formation of the desired complexes was achieved but no additional improvement in the squaraine fluorescence was achieved with excitation at 300 nm. With this in mind, two mono-nitro aromatics chosen for examination in this study were 2-nitrophenol (**2NP**) and 4-nitrophenol (**4NP**). There were many other possibilities but the one advantage of using these two mono-nitro aromatics was the fact that, at low concentration (i.e. 30 μM) both are soluble in water. Although the use of 10% dimethylsulfoxide (DMSO) in water was required in the last study to dissolve certain chemicals, water solubility was an important consideration in this study for the choice of

mono-nitro aromatics. However, as stated in the previous study, two choices pre-determined for this study were 5-nitroquinoline (**NQ**) and 5-nitroisoquinoline (**NIQ**). The intention of using nitro-substituted aromatics in this study is that they (being considered as acceptor rings) should form charge-transfer complexes with the tryptamine ring (a strong donor ring), as observed with the di-nitro aromatics in the last study [7], thus increasing the absorption of the complex across the blue – UV region of the visible spectrum.

3.2. X-ray crystallographic analysis

Fine needle crystals of **2** formed out of the DMSO-d_6 solution used to record the ^{1}H NMR spectrum, and were suitable for X-ray crystal structure elucidation. The structure of **2** is illustrated in figure 4 and shows a centrosymmetric squaraine molecule in the *trans* conformation, similar to the structure of **1** [6]. However, unlike **1**, and all of the all of the other previously reported crystal structures in this series bar the structure of the bis-quinolinium dye [5,6], the structure of **2** does not contain any water molecules. The main squaraine moiety of **2** is slightly twisted with a dihedral angle of 21.0(3)° between the plane of the squarate group and the plane of the indolenine ring. All previous dihedral angles observed in the crystal structures for this series of dyes are listed in Ref. [6]. The dihedral angle between the squarate group and the indole ring of the tryptaminium cation is 89.4(3)°. The indole N—H hydrogen bonds to an adjacent sulfonate oxygen atom O4 [2.925(4) Å; $-x - ½, y + ½, -z + ½$], whereas the three aminium hydrogen atoms sequentially hydrogen bond to the squarate oxygen O1 [2.746(3) Å], and the two remaining sulfonate oxygen atoms O2 [2.763(3) Å] and O3 [2.796(3) Å; $x, y + 1, z$]. For reference to those listed in Ref. [6], the three torsion angles, in the structure of **2**, that define the pendant sulfonate group are C13-N1-C14-C15 [78.4(3)°], N1-C14-C15-C16 [175.5(2)°] and C14-C15-C16-S1 [-84.3(3)°]. Furthermore, although the squaraine moieties in **2** are off-set stacked, the intermolecular

distances between the closest atoms are > 4 Å, thus no charge transfer associations are noted. Similarly, no such associations are observed for the indole ring in the tryptaminium cation, which (being a strong electron donor ring) could possibly charge transfer stack with the strong electron-accepting squarate group.

Figure 5 shows each of the associated molecular pairs for **7** and **8** with a common atom numbering scheme used for easy comparison of the cation (A) and the anion (B) species in each of the structures. Figures 5a and 5b show two confirmations of the tryptamine cation in the structure of **7** due to the indole ring rotating around a line running approximately through C3A, C9A and C6A. This is a very unique type of disorder for an indole molecule. The angle between the two six-membered rings is 37.7(4)°, and 41.8(4)° for the five-membered rings. Interestingly, the ethanaminium chain is not involved in the disorder. For both structures (**7** and **8**), the tryptaminium cations show no regular side-chain conformational features, as indicated by the defining torsion angles C2A –C3A-C31A-C32A[1] and C3A-C31A-C32A-N32A (tabulated values have been deposited as supplementary material). However, neither crystal structure demonstrates any cation-anion charge-transfer interactions, which is interesting because such interactions are observed in both the crystal structures of tryptamine with benzoic acid [9] and 4-chlorobenzoic acid [10], respectively, and nitrophenols are expected to be stronger acceptor rings than either benzoic acid or it's 4-chloro derivative. The crystal packing found in the structures of **7** and **8** is described in more detail in the Supplementary material.

[1] C21A-C3A-C31A-C32A and C22A-C3A-C31A-C32A for **7**.

3.3. Spectroscopic investigations

3.3.1. UV-visible spectroscopy

Figure 6 is a collection of solution absorbance spectra across the UV-visible range for compounds **2** – **6** and **7** – **10**. Figures 6a, 6c, 6e and 6g are the combined spectra for compounds **7** – **10**, respectively, and their constituent components. Synthesis of these compounds, through the addition of one component dissolved in ethanol to the other (dissolved in ethanol), yielded an observable increase in colour intensity for the combined mixture. This increase in colour intensity (upon mixture of solutions of the constituent molecules) is commonly observed in the preparation of charge-transfer complexes (if the appropriate molecules are present) although it is not the definitive sign for the construction of such complexes [11]. UV-visible examination of solutions of the resultant complexes corroborates an increase in colour intensity for compounds **7** (Fig. 6a) and **9** (Fig. 6e), but not for **8** (Fig. 6c) or **10** (Fig. 6g). None of these spectra, in corroboration with the crystal structures of **7** and **8**, indicate the presence of charge-transfer associations because no additional absorption, other than across the wavelengths absorbed by the individual components, is observed. This is interesting because the previously determined structure of indole-2-carboxylic acid with **NQ** arranges in charge-transfer stacks [12].

Figures 6b, 6d, 6f and 6h are the combined spectra for compounds **3** – **6**, respectively, and their constituent components including **2**. This latter spectral series all show increased absorption below 400 nm, in comparison with the spectra of their constituents. The two crystal structures of **7** and **8** and the spectra of **7** – **10** would each suggest that these increases in absorption are not due to charge-transfer associations. However, squaraine dyes consist of a donor-acceptor-donor structure in their rings and this structure has been the subject of much comment throughout the history of squaraines and it is possible that the nitro aromatics studied in this paper associate (in some form) with one of the two donor rings in the

squaraine moiety, in much the same way that Smith *et al*. utilize donor rings to associate with the central acceptor ring in the squaraine to form their squaraine rotaxanes [13].

Corroboration that **3** – **6** in solution are ternary complexes comes in two forms. The first is that, similar to the water insoluble di-nitro aromatics in the previous paper [7], the water insoluble constituents in this paper, **NQ** and **NIQ**, are both dissolved (at 30 μM concentration) in a water solution containing **2**. The second is indicated in a study of λ_{max} for each ternary complex with increasing solution concentration, as opposed to the same for **2**. Figure 7a is a plot of λ_{max} verses solution concentration for a solution of **2** in water and it can be seen that **2** is linear to a concentration of 7 μM (or 7 x 10^{-6} M). Interestingly, **1** is also linear to 7 μM (refer Supplementary material). But, similar plots of **3** – **6** (Fig. 7b) all show linearity to 10 μM (or 1 x 10^{-5} M) and above. Plots of **2** with 3,5-dinitrobenzoic acid (**DNBA**), 3,5-dinitrosalicylic acid (**DNSA**), and 2,4-dinitrophenol (**DNP**) show that although **2** with **DNBA** is linear to 8 μM, the other two are linear to (and above) 10 μM (refer Supplementary material). There are a number of possible reasons why higher concentrations of both **1** and **2** reduce from linearity, and most of those reasons are due to the possible aggregational forms that **1** and **2** can take at higher concentrations, but incorporation of additional species that can interact with either the tryptamine ring or squaraine moiety in **2** must disrupt the formation of those aggregational forms thus allowing linearity to increased concentrations.

3.3.2. Fluorescence spectroscopy

Figure 8 is a collection of comparative solution fluorescence spectra for compounds **2** – **6** (figure 8a – 8e respectively) both with (upper) and without (lower) an equimolar amount of BSA, plus BSA itself (Fig. 8f), all irradiated using 300 nm light. All spectra were recorded at a concentration of 1 μM (or 1 x 10^{-6} M) for the squaraine and, as observed in the previous

paper for **2** with **DNBA** and **2** with **DNP** [7], none indicate any increase in fluorescence intensity over that of **2** by itself upon irradiation with 300 nm light. Although these ternary complexes have been interesting to study and the results thus far obtained have demonstrated the principle of ternary formation in solution, with a multitude of further possibilities; the primary aim of improving upon the fluorescence of **2** by creating a charge-transfer complex has not been achieved.

3.4 Gel electrophoresis

The purpose of the solution fluorescence studies thus far undertaken on the ternary squaraine complexes previously reported [7] and in this paper was to screen the fluorescence performance of any particular complex (upon excitation at 300 nm), to minimize the number of complexes that required full experimentation on gel electrophoresis. With the spectroscopic results (across all ternary complexes except for **2** with **DNSA**) indicating no difference between ternary complex and compound **2**, reliance fell upon the X-ray crystal structure results to determine which complexes should be further studied in the gels. Evaluation of all X-ray crystal structure results (both previously reported in Ref. [7] and in this paper) suggested that only two ternary complexes, those of **2** with **DNBA** and **2** with **DNSA** (both from Ref. [7]), were worth studying in electrophoresis gels because they were the only complexes where there was a definite indication of charge-transfer stacking interactions between the nitroaromatics and the tryptaminium moiety. As a 'control' to gels stained with **2** with **DNBA** and **2** with **DNSA**, gels were also stained with compound **2** by itself. The performances of these three stains were further compared against the performance of compound **1**. Limits to the solubility of compound **2** meant that staining solutions were half the concentration of those previously utilized (i.e. 1 x 10^{-3} M) [5,6]. The colorimetric performance of **2** with **DNBA, 2** with **DNSA** and compound **2** itself on gels containing

separated proteins was equal across all gels; judged on stain intensity and protein band resolution (Fig. 9). Gels stained with these three complexes were colorimetrically less intense than comparative gels stained with compound **1**. Staining at the reduced concentration (of 5 x 10^{-4} M) certainly resulted in reduced destain procedures, with only two or three washes within an hour being required to return gels with a clear background, but colorimetric results were not as good as those previously determined for **1** and other water-soluble dual pendent squaraine dyes used at 1 x 10^{-3} M [5,6]. In this respect, compound **1** currently remains the best performing colorimetric stain in this entire series of water-soluble dual pendent squaraine dyes.

When the stained gels from this study were examined fluorometrically (upon illumination at 300 nm) (Fig. 9), the three stains based on **2** fluoresced with equal intensity, with no indication of any quenching from **2** with **DNSA**. In certain respects, the fact that these three stains can be visualized fluorometrically (upon illumination at 300 nm) achieves the aim of this and the previous paper [7]. But, considering the equal fluorescence performance of **2** with **DNBA** and **2** with **DNSA** verses that of just compound **2** then it can be assumed that the addition of either **DNBA** or **DNSA** to compound **2** contributes nothing to the performance of **2** itself. It is possible that the tryptamine group in compound **2** interacts with available electron-accepting aromatics / heterocyclics within the proteins to form charge-transfer complexes. Possible candidates could arise from histidine, tyrosine and phenylalanine, although the aromatic / heterocyclic rings in these amino acids are not considered as strong electron-accepting ring systems (certainly not as strong as the nitroaromatics utilized in this and the previous study [7] that did not charge-transfer stack with the tyrptaminium molecule) but it is worth noting that in the X-ray crystal structure of tryptaminium benzoate [14] the two rings are charge-transfer stacked, indicating that such interactions may still be possible with weaker electron-accepting rings. However, this

approach cannot be used to explain the fluorescence behaviour of these stains in the gels because this type of charge-transfer association (with the protein) is not possible for compound **1**.

It is interesting that gels stained with **2** fluoresce because it is the only water-soluble dual pendent squaraine compound other than **1** to demonstrate this phenomenon. However, in the original paper that first reported the gel electrophoresis behaviour of compound **1** [5] it was stated that prior to that work there was only one previous report of the use of a squaraine dye as a gel stain [15]; this is now known to be incorrect, a second report utilizing a 1,3-symmetric core substituted squaraine (Fig. 10) had also been previously published [16]. Furthermore, the gel stained with this squaraine was imaged fluorometrically. Unfortunately, the dissimilarities between compounds **1**, and **2**, and the squaraine shown in figure 10 do not yet indicate a molecular design direction for the preparation of future squaraine dyes that can be used as fluorometric stains for gel electrophoresis.

Conclusions

A water-soluble dual pendent squaraine with tryptaminium cations has been mixed with mono-nitroaromatics in solution to create ternary complexes with the aim of creating indole-nitroaromatic charge-transfer pairs. Examination of the X-ray crystal structures of two tryptamine-nitroaromatic complexes, without the squaraine moiety, revealed that neither demonstrated charge-transfer pair or stacking associations between the indole and nitroaromatic rings. The absence of charge-transfer associations was evident in the UV-visible absorption spectra of each complex, although intermolecular associations (of unspecified form) were evident in the spectra of the mixtures incorporating the squaraine. Examination of the fluorescence performance upon illumination with 300 nm light of each ternary squaraine complex, particularly in the presence of an equimolar amount of protein,

showed that although there was a ten-fold increase in the fluorescence of the squaraine moiety (at ~ 650 nm) the levels across the series were no more than that observed for the base bis-tryptaminium squaraine. However, electrophoretic gels stained with the base bis-tryptaminium squaraine were found to fluorescence; the only other squaraine dye of this type to do so in addition to the bis-piperidinium squaraine of previous studies.

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Appendix A. Supplementary data

The comparative infrared spectra of tryptamine and each of 2-nitrophenol, 4-nitrophenol, 5-nitroquinoline, and 5-nitroisoquinoline with **7** – **10**, respectively; the sequential visible absorption spectra of **1 – 6** plus the three ternary compounds from the previous paper [7] (ranging from 1 μM – 10 μM in steps of 1 μM); the comparative fluorescence spectra of **2** – **6** both with and without an equimolar amount of BSA (excitation: 300 nm and 624 nm); the ^{1}H NMR data for **2**; descriptions of packing arrangements for **7** and **8**; tables of the conformational data on the tryptaminium cations / tryptamine molecule in the X-ray crystal structures of compounds **7** and **8**; and tables of the hydrogen-bond geometry for

the X-ray crystal structures of **7** and **8** have all been deposited as Supplementary data, which can be found, in the online version, at http://

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Figure captions

Figure 1 Chemical diagram for compound **1**.

Figure 2 Schematic showing the synthetic route for compound **2** and complexes **3** – **6**.

Figure 3 Chemical diagrams of complexes **7** and **8**, as indicated by their X-ray crystal structures, and as suspected for **9** and **10**.

Figure 4 ORTEP and atom naming scheme for **2** (a = -*x*, -*y* + 1, -*z*) (displacement ellipsoids are drawn at 50% probability levels) (atom C19, C20 and C24 are not named for image clarity).

Figure 5 ORTEP and atom naming scheme for a,b) **7** and c) **8** (displacement ellipsoids are drawn at 50% probability levels).

Figure 6 Comparative UV-visible absorption spectra for a) **7** (———), **2NP** (– – –) and tryptamine (------) (conc. 70 μM); b) **3** (———), **2NP** (– – –) and **2** (------) (conc. 10 μM); c) **8** (———), **4NP** (– – –) and tryptamine (------) (conc. 70 μM); d) **4** (———), **4NP** (– – –) and **2** (------) (conc. 10 μM); e) **9** (———), **NQ** (– – –) and tryptamine (------) (conc. 70 μM); f) **5** (———), **NQ** (– – –) and **2** (------) (conc. 10 μM); e) **10** (———), **NIQ** (– – –) and tryptamine (------) (conc. 70 μM); and f) **6** (———), **NIQ** (– – –) and **2** (------) (conc. 10 μM).

Figure 7 Comparative plots of absorbance λ_{max} verses concentration for a) **2** and b) **3** ranging from 1 μM – 10 μM in steps of 1 μM.

Figure 8 Comparative fluorescence spectra (irradiated at 300 nm) for a) **2** with BSA (upper) and **2** (lower) (conc. 1 μM); b) **3** with BSA (upper) and **3** (lower) (conc. 1 μM); c) **4** with BSA (upper) and **4** (lower) (conc. 1 μM); d) **5** with BSA (upper) and **5** (lower) (conc. 1 μM); e) **6** with BSA (upper) and **6** (lower) (conc. 1 μM); and f) BSA (conc. ~67 μg/mL).

Figure 9 Electrophoresis gels loaded with 20, 15, 12.5, 10, 7.5, 5, and 2.5 μL of unstained Precision Plus Protein standard (Lanes 2 - 8), stained with a 5 x 10^{-4} M aqueous solution of **2** and imaged using a Bio-Rad Gel Doc EZ Imager in (left) colorimetric and (right) fluorescent image mode.

Figure 10 The 1,3-symmetric core substituted squaraine from reference [16].

Fig.1

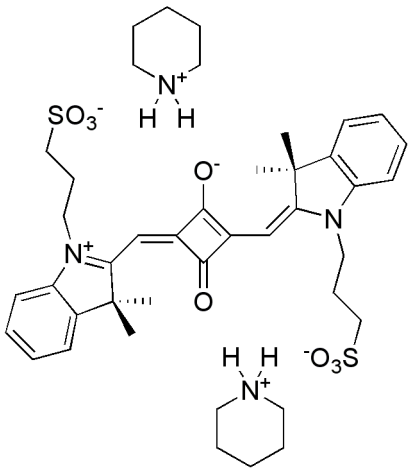

Fig.2

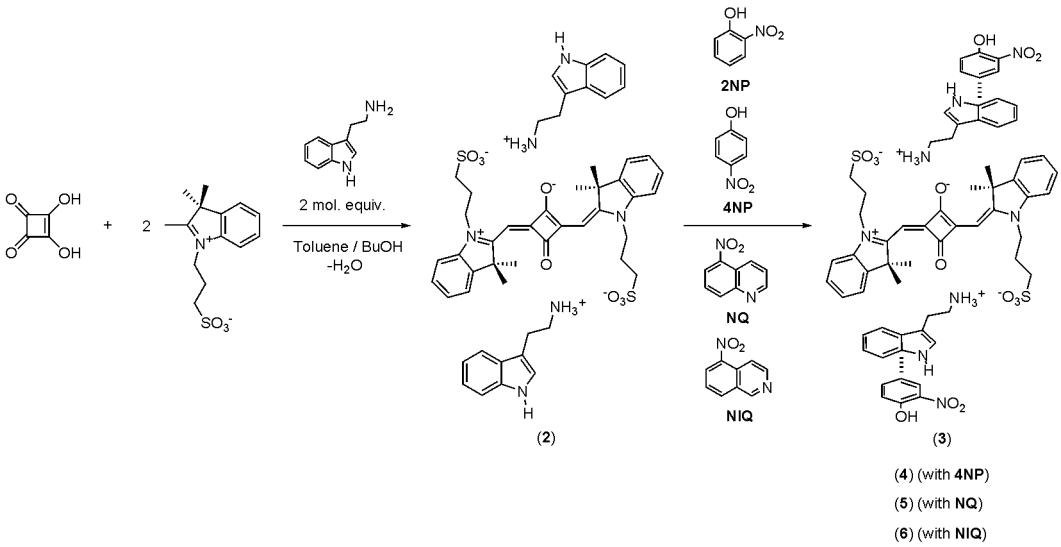

Fig.3

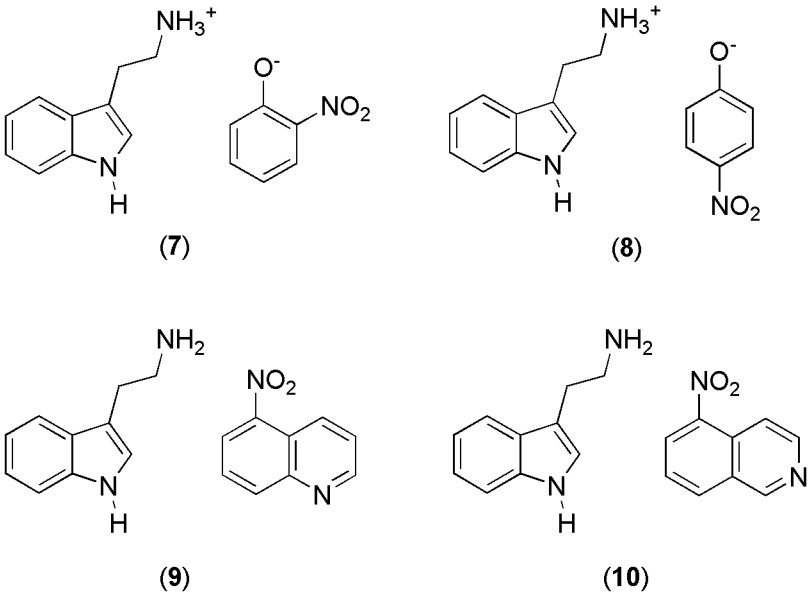

Fig.4

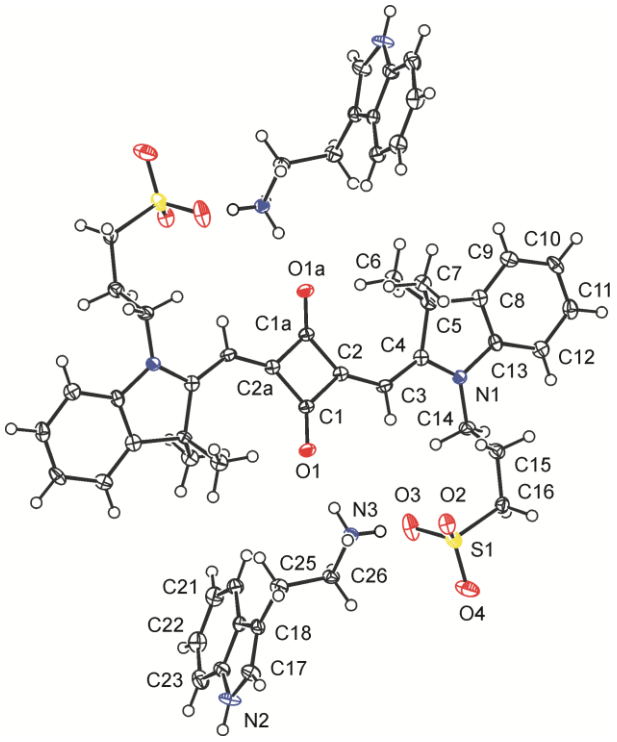

Fig.5

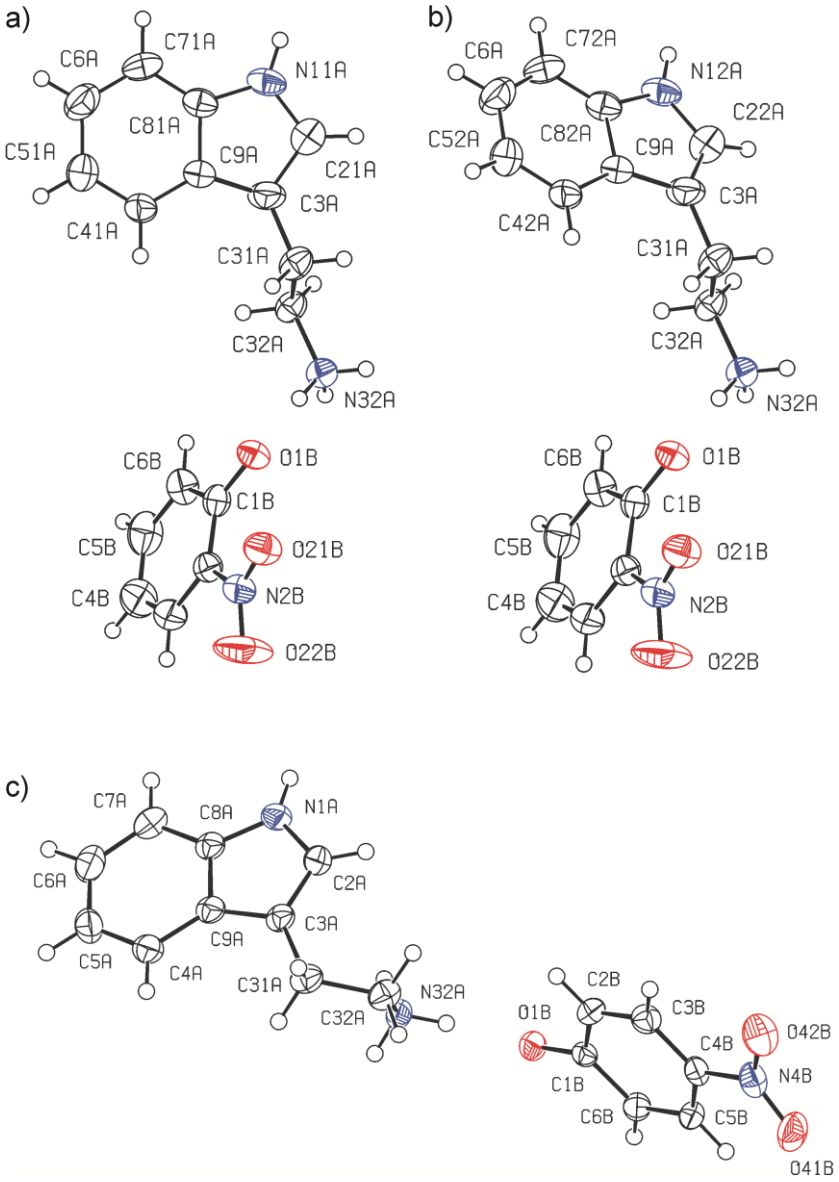

Fig.6

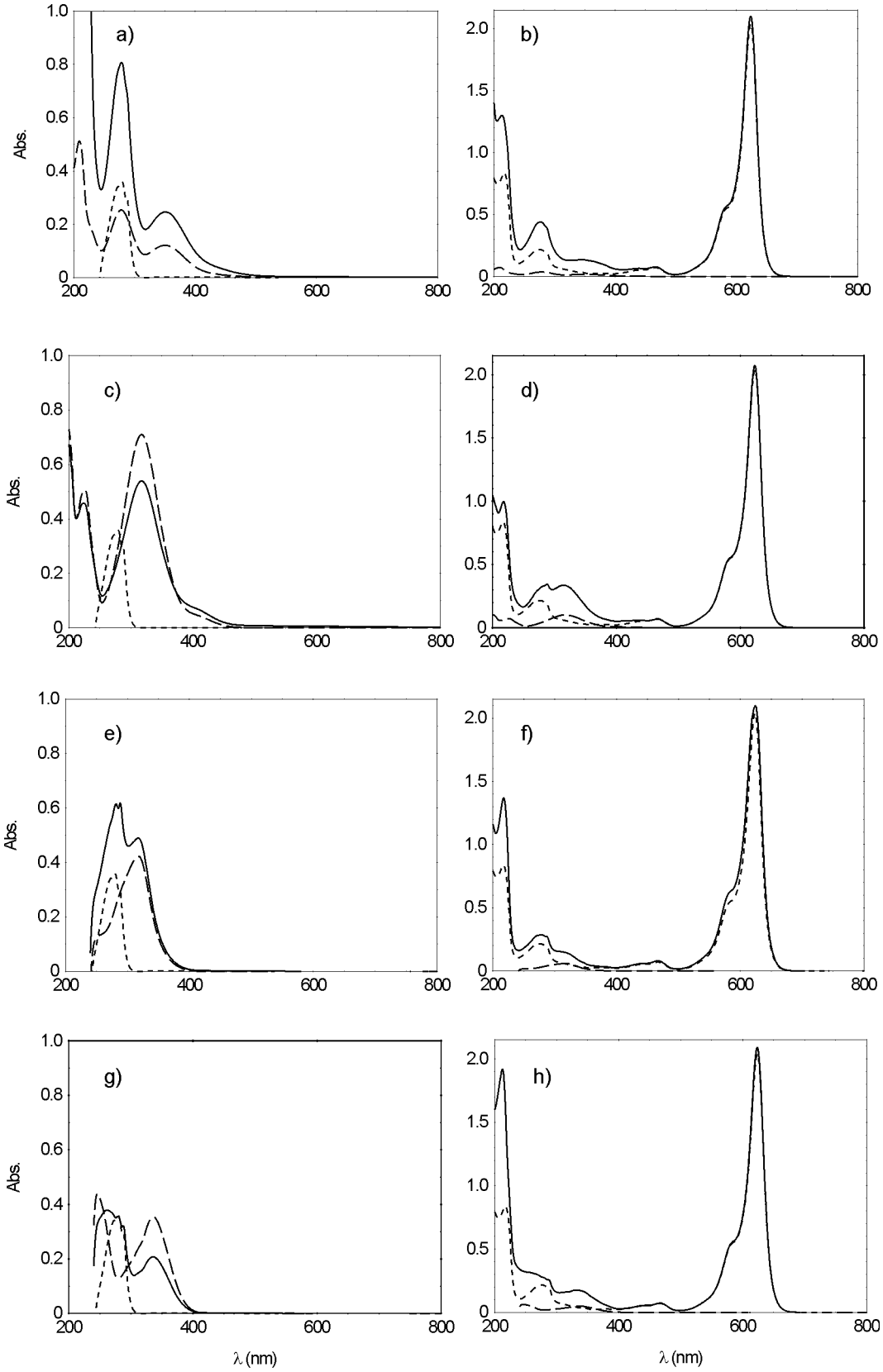

Fig.7

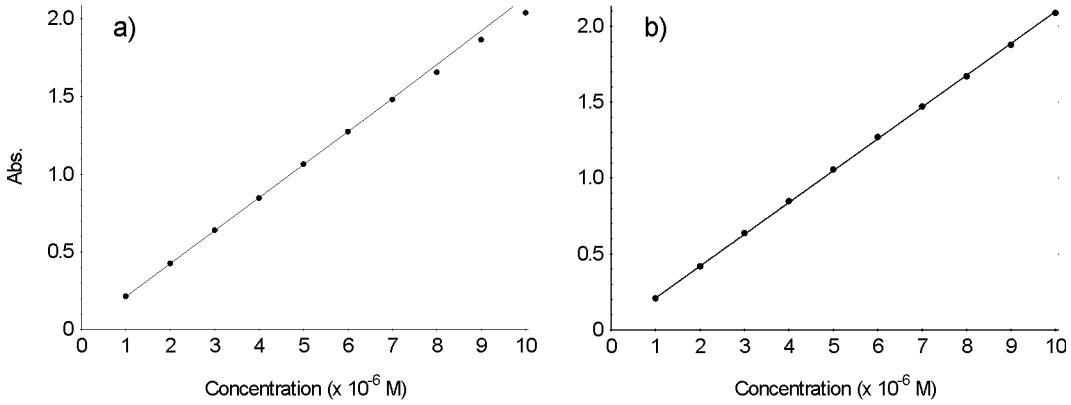

Fig.8

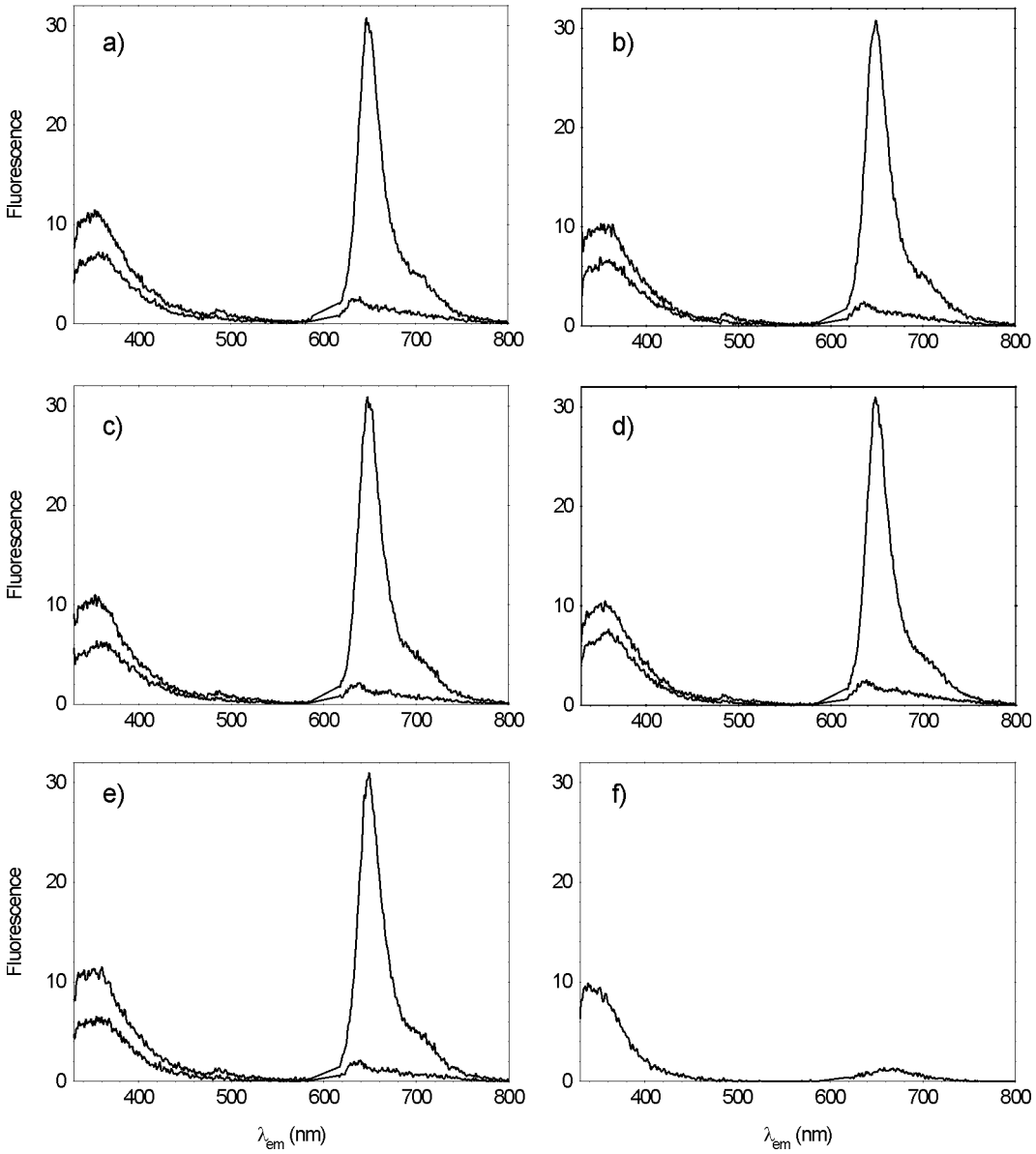

Fig.9

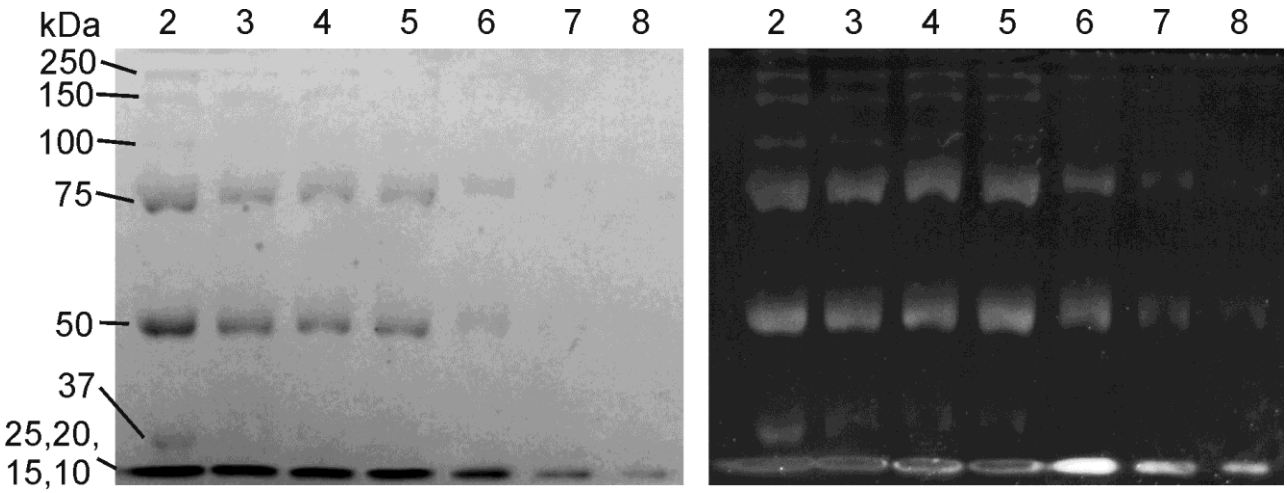

Fig.10