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ARTICLE

Photo-driven Near-IR Fluorescence Switch: Synthesis and Spectroscopic Investigation of Squarine-Spiropyran Dyad

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With the objective of developing near-infrared fluorescence switch molecules for potential applications, synthesis of new dyad with two methyl 3-(3',3'-dimethyl-6-nitrospiro[chromene-2,2'-indolin]-1'-yl)propanoate (**6**), spiropyran (**SP**), units as the photochromic acceptors and a near-infrared fluorescence probe, (E)- 4-((1-(2-hydroxyethyl)-3,3-dimethyl-3H-indol-1-ium-2-yl)methylene)-2-((E)-(1-(2-hydroxyethyl)-3,3-dimethylindolin-2-ylidene)methyl)-3-oxocyclobut-1-en-1-olate (**SQ**), (**3**) as fluorescent donor are described. Two **SP** units are attached to the two indole nitrogen of squarine core with almost no conjugation with the **SQ** unit. The spectroscopic properties of the newly synthesized dyad, **SP-SQ-SP** (**7**), and corresponding model compounds (**SP, SQ**) have been studied in acetonitrile solution, poly(ethylene glycol) (PEG) polymer matrices and in nanoparticle form dispersed in aqueous medium. These dyes (**3** and **7**) exhibited absorption in the range 550-670 nm, with significant absorption coefficients ($10^{-5}M^{-1}$ cm^{-1}) in the ACN. The fluorescence emission spectra of these dyes cover very broad range from 630 to 750 nm and fluorescence quantum yields are of the order of 0.2 in ACN solution. External stimulations (ultraviolet light and visible light) generate reversible changes in the structure of **7**, resulting in changes in the absorption specta due to the presence of the two spiropyran units. The absorption spectrum of the **MC**, a ring open form of **SP**, in dyad **SP-SQ-SP** (**7**) has large spectral overlap with the fluorescence spectrum of the **SQ** unit. Thus, the fluorescence intensity of dyad **7** is modulated by reversible conversion among the two states of the photochromic spiropyran units and the fluorescence resonance energy transfer (FRET) between the **MC** form and the **SQ** unit. High-contrast "*on/off*" fluorescence switching is successfully achieved with remarkably fatigue resistance in solution, in polymer film and in nanoparticle form of dyad (**7**). The described results indicate that this system may represent an efficient fluorescent switch molecule in potentially rewritable high-density optical data or image storage utilizing near-infrared luminescence intensity readout schemes.

Introduction

The quest to develop smart material with properties that can be optically modulated has attracted immense attention in the field of material science because of high demand of such material in many scientific areas, including biotechnology and advanced optics.[1] Photo-responsive molecules, in particular photochromic molecules can be exploit to accomplish this objectives, as they can be transformed reversibly by light between two states with different spectroscopic identities.[2–5] This unique trait can then be judicially exercised to modulate or switch various functions at the molecular as well as supramolecular level by using light as a trigger. Photo-reversible fluorescence modulation or switching of fluorescence spectroscopic properties of the photo-responsive molecules are of much interest not only due to their wide applications in molecule-based logic systems, such as AND, OR, XOR, NAND, NOR,[6] but also for fluorescence spectroscopy as it plays an pivotal role in different molecular device design which includes fluorescence switch,[7] fluorescence sensor [8] and other photonic devices,[9] primarily because of its ease of use. For non-fatigue fluorescence modulation, fluorescence resonance energy transfer (FRET) is a desirable mechanism for fluorescence quenching because, unlike electron transfer, it does not directly create redox-active ions as photoproduct that could lead to photodamage or other unwanted processes.

Photochromic Spiropyran (**SP**) shows reversible unimolecular transformation between two states, spirocyclic (**SP**) and ring opened merocyanine (**MC**) forms, having different absorption spectra. Owing to this intriguing light-driven molecular transformation fluorophore-coupled spiropyran derivatives have gained substantial attention for designing smart materials with fluorescence "*on/off*" switching behaviours. Fluorescence emission from the fluorophore-

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coupled photochromic derivatives is generally observed when the photochromic compound is in the **SP** form, while emission is rigorously quenched when the photochromic compound is in the **MC** form due to fluorescence resonance energy transfer (FRET) from the excited state of the fluorophore to the **MC** form of the photochromic compound. Irradiation with visible light at the peak of the **MC** absorption results in transformation of the **MC** to the **SP** form of the compound and complete recovery of the fluorescence of fluorophore. By using this approach, a variety of spiropyran-derived fluorophore-coupled photochromic compounds have been designed and probed in molecular switching applications.[7-10] Recently, we have shown that Photoswitchable Oligo(p-phenylvenyl)s-Spiropyran **(SP-OPV-SP)** dyad takes part in fluorescence switching behaiour both in solution and as well as in solid state forming gel through self-assembly.[11]

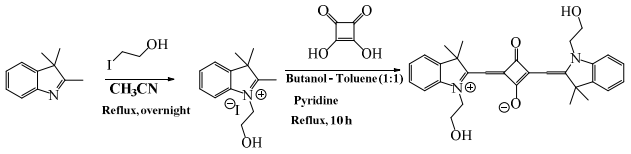
For effective functioning of a fluorescence-coupled spiropyran photoswitch, we have extend our quest with squarine dye which has strong absorption at 520-650nm and very high fluorescent quantum yield with emission at 600-750 nm range of electrometric spectrum in acetonitrile solvent. Hence, the red edge of absorption spectra of **MC** form of **SP** clearly overlaps to the blue edge of emission spectrum of **SQ**. As a consequence, the spiropyran–SQ system is proficient for incorporation into a FRET based photoreversible switching system. Here we report the synthesis and spectral studies of as prepared dyad in solution, in polymer matrices as well as in nanoparticle form of **SP-SQ-SP (7)**, (see Scheme 5). The ease of synthesis allows us to covalently attached two **SP** units to the both side of an **SQ** unit (**SP-SQ-SP**). Demonstrated results confirm that the fluorescence intensity of the **SQ** unit can be regulated by alternate application of ultraviolet light and visible light or thermal stimulation. Hence, this **SP-SQ-SP** dyad can potentially be used in molecular device design at the single molecular level in view of processing and communicating information. In order to compare the intermolecular communicating behaviour of the mixture solution of an **SQ** derivative (compound **3**, see Scheme 1) and a **SP** molecule (reference compound **6**; see Scheme 2) (in a molar ratio of 1:2) were also investigated.

Experimental

Materials and Instrumentation

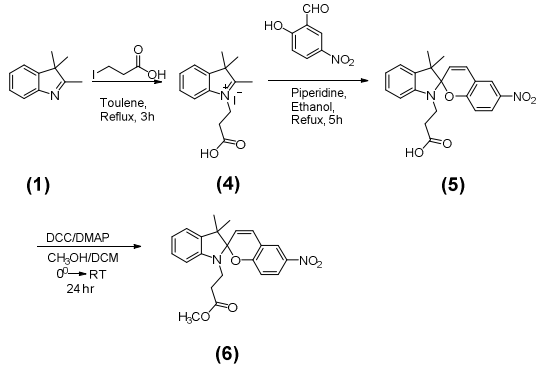
The solvents and the reagents were purified and dried by usual methods prior to use. 3, 4-Dihydroxycyclobut-3-ene-1,2-dione, Ethanol, 4-Bromophenylhydrazine hydrochloride, 3-Methyl-2-butanone and poly(ethylene glycol) were purchased from Sigma-Aldrich, USA. 2-iodoethanol, Acetic acid, NaOH, potassium carbonate, 2-Hydroxy-5-nitrobenzaldehyde and Acetonitrile were used as received from commercial suppliers.

^{1}H NMR was recorded on 500 MHz (Bruker ARX500) and ^{13}C NMR spectra were recorded on Bruker 300 MHz spectrometer at room temperature in $CDCl_3$. The chemical shifts are reported in ppm (d) tetramethylsilane (TMS) as internal standard and coupling constant (J) are expressed in Hz. All Uv/vis spectra were recorded using Hitachi U-2910 spectrophotometer. All steady state fluorescence spectra were recorded at room temperature as well as low temperature by Fluorolog-3 spectrofluorimeter of Horiba Jobin Yvon, USA. Reactions were monitored by thin-layer chromatography (TLC) using 0.20-0.25 mm silica gel plates. Column chromatography was performed with silica gel (60-120 and 100-200 mesh). High resolution Mass spectra were recorded by electrospray ionization mass spectrometry (ESI-MS) on QSTAR XL hybrid ms/ ms system (Applied Bio systems/MDS sciex, foster city, USA). FT-IR spectra were recorded using a Thermo Nicolet Nexus-670 Fourier Transform Infrared Spectrometer with reference to KBr. Size of the nanoparticles was characterized using scanning electron microscope (Hitachi, S-3000N) and dynamic light scattering (DLS) measurement with zetasizer nano-ZS(Malvern Instrument, UK) using 173^0 scattering angle and laser of 633nm wavelength.



Scheme 1: Synthetic route of the Squarine **SQ (3)** dye.

1-(2-Hydroxyethyl)-2,3,3-Trimethyl-3H-Indolium Iodide (2):
A mixture of 2,3,3-trimethyl-3H-indole (**1**), (5.0 g, 31.4 mmol), and 2-iodoethanol (8.2 g, 47.7 m mol) was dissolved in 30-ml acetonitrile and the solution refluxed for 6 h. The reaction mixture was then cooled to room temperature and 20 ml of diethyl ether was added. The crude product precipitated and was collected by filtration and washed with 2–3 small portions of ether to give Compound **2** (ref:12) as brown crystals (8.3 g, 80%) 1H-NMR (500 MHz, d6-DMSO) δ 1.57 (6H, s, 2 × CH3), 2.85 (3H, s, CH3), 3.90 (2H, t, J 5.0 Hz, CH2), 4.62 (2H, t, J 5.0 Hz, CH2), 5.01 (1H, s, –OH), 7.64–7.62 (2H, m, ArH), 7.88–7.86 (1H, m, ArH), 7.99–7.89 (1H, m, ArH); 13C-NMR (125 MHz, d6-DMSO) δ 14.5, 21.9, 50.3, 54.2, 57.7, 115.5, 123.5, 128.7, 129.2, 141.0, 141.7.



Scheme2: Synthetic route of the Spiropyran (**SP**) **(6)**

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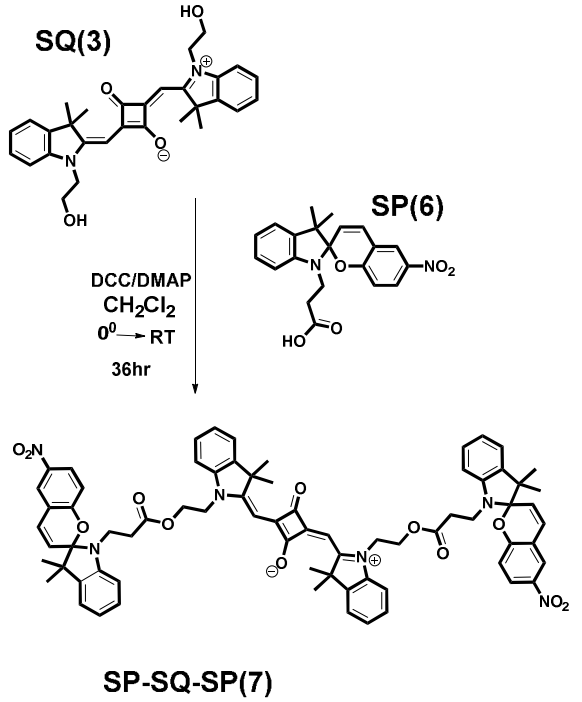
4-((1-(2-hydroxyethyl)-3,3-dimethyl-3H-indol-1-ium-2-yl)methylene)-2-((1-(2-hydroxyethyl)-3,3-dimethylindolin-2-ylidene)methyl)-3-oxocyclobut-1-en-1-olate, SQ (3):A mixture of 2 (2.8 g, 8.76 mmol) and 3,4-dihydroxycyclobut-3-ene-1,2-dione (0.5 g, 4.38 mmol) was taken in a 100 ml round bottom flask and it was dissolved in toluene (15 ml) after that added n-butanol and 1 or 1.5 ml of pyridine added, under inert condition reflux 18 hours. The reaction mixture was then cooled to room temperature and it was poured into water and extracted with dichloromethane. The organic layer was concentrated to give the corresponding purified by column chromatography [SiO_2: Hexane/CH_2Cl_2 (1:9 v/v) to afford 3 (ref:13) as thick blue solid (217 mg, ~70%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (t, J = 7.9 Hz, 4H), 7.17 (t, J = 7.3 Hz, 2H), 7.10 (d, J = 7.8 Hz, 2H), 5.89 (s, 2H), 4.36 (s, 2H), 4.02 (t, J = 6.1 Hz, 4H), 1.76 (t, J = 49.8 Hz, 16H). 13C-NMR (125 MHz, $CDCl_3$) δ: 195, 187,173.1, 172.8, 152, 145, 141.4, 141.2, 128, 125, 120.8, 120.1, 116.8, 111, 108.6, 94, 63.4, 59.8, 55.6, 51.1, 49.5, 26.8, 24.7. FT-IR (KBr) ν_{max} = 679, 774, 834, 935, 1053, 1095, 1165, 1269, 1354, 1450, 1486, 1577, 2922, 3362 cm^{-1}.



Scheme 3: Synthetic route of SP-SQ-SP (7) from SQ (3) and SPCOOH (5).

I-(β-carboxyethyl)-2,3,3-trimethylindolenine iodide (4): A mixture of 2,3,3-Trimethylindolenine (2.5 g, 15.7 mmol) and 3-iodopropanoic acid (3.14 g, 15.7 mmol) were dissolved in toluene (5 mL) and heated under nitrogen at 100 °C for 3 h. The resulting solution was evaporated; the remaining product dissolved in water (100 mL) and was washed with chloroform (50 mL) for 3 times. Evaporation of water gave product **4** (4.07 g, 72 %) as a red oil. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.53 (s, 6H), δ 2.85 (s, 3H), δ 2.96-3.0 (t, 2H), δ 4.63-4.67 (t, 2H), δ 7.61-7.64 (m, 2H), δ 7.82-7.84 (dd, 1H), δ7.97- 8.0(dd, 1H).

I-(β-carboxyethyl)-3',3'-dimethyl-6 -nitrospiro (indoline-2',2 [2H-1] benzopyran) (5): The product 4 (2.52 g, 7 mmol), 5-nitrosalicylaldehyde (1.16 g, 7 mmol), and piperidine (0.76 mL, 0.7 mmol) were dissolved in anhydrous ethanol (50 mL). The mixture was refluxed for 5 h. The resultant dark purple mixture was cooled in an ice bath and filtered, and the filter cake was washed with cold ethanol. The precipitate was recrystallized from ethanol and dried in vacuum to yield **5** (ref:14, 15) (1.71 g, 65 %). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.07 (s, 3H), δ 1.19 (s, 3H), δ 2.45-2.57 (t, 2H), δ 3.34-3.40 (t, 2H), δ 5.98-6.01 (d, 1H), δ 6.65-6.67 (d, 1H), δ 6.78-6.82 (t, 1H), δ 6.85-6.88 (d, 1H), δ 7.11-7.14 (t, 1H), δ 7.19-7.22 (d, 1H), δ7.98- 8.21 (dd, 1H), δ 8.21 (s, 1H). FT-IR (KBr) ν_{max} = 749, 803, 946, 1026, 1087, 1162, 1269, 1330, 1480, 1510, 1575, 1607, 1707, 2681, 2764, 2935, 2968 cm^{-1}.

Methyl 3-(3',3'-dimethyl-6nitrospiro[chromene-2,2'-indolin]-1'-yl) propanoate (6):
Compound DCC (97 mg, 0.47 mmol) was added to a solution of **4** (180 mg, 0.47 mmol), methanol (1 mL, 25 mmol) and DMAP (3.8 mg, 0.03 mmol) in dry CH_2Cl_2 (100 mL) and temperature was initially maintained at 0°C under Ar. Then, the mixture was allowed to warm up to ambient temperature over 12 h and stirred for a further 12 h. Solvent was evaporated and the residue dissolved in chloroform. It was then precipitated by the addition of methanol and filtered. The crude mixture was then purified by column chromatography [SiO2: Hexane/$CHCl_3$ (1:1 v/v) to afford **6** (ref:11,14,15) (130 mg, 70%). ^{1}H NMR (500 MHz, CDCl3): δ 1.07 (s, 3H), δ 1.19 (s, 3H), δ 2.45-2.57 (t, 2H), δ 3.34-3.40 (t, 2H), δ 3.7 (s, 3H), δ 5.98-6.01 (d, 1H), δ 6.65-6.67 (d, 1H), δ 6.78-6.82 (t, 1H), δ 6.85-6.88 (d, 1H), δ 7.11-7.14 (t, 1H), δ 7.19-7.22 (d, 1H), δ7.98- 8.21 (dd, 1H), δ 8.21 (s, 1H).

SP-SQ-SP (7): Compound DCC (29 mg, 0.14 m mol) was added to a solution of 5 (35 mg, 0.09 mmol), 3 (69.5 mg, 0.14 mmol) and DMAP (2 mg, 0.01 mmol) in dry CH_2Cl_2 (100 mL) and temperature was initially maintained at 0°C under Ar. Then, the mixture was allowed to warm up to ambient temperature over 12 h and stirred for a further 24 h. Solvent was evaporated and the residue dissolved in chloroform. It was then precipitated by the addition of methanol and filtered. The crude mixture was then purified by column chromatography [SiO_2: Hexane/$CHCl_3$ (1:1 v/v) to afford **7** (74 mg, 70%). HRMS (ES^+): calculated for $C_{72}H_{68}N_6O_{12}$ (M+): 1209.490; found, 1209.4943. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.02-7.96 (m, 4H), 7.35-7.32 (d, 2H), 7.25-7.21 (t, 3H), 7.05-7.03 (d, 4H), 6.98-6.96 (d, 2H), 6.90-6.84 (dd, 4H), 6.70-6.67 (d, 2H), 6.47-6.44 (d, 2H), 5.98(s, H), 5.8-5.76 (d, 2H), 4.45-4.41 (t, 4H), 4.31-4.08 (m, 5H), 3.52-3.3 (m, 6H), 2.6-2.4 (d, 2H), 1.96-0.86 (m, 24H).^{13}C NMR (101 MHz, $CDCl_3$) δ 171.4, 159.3, 146, 141, 135, 128.4, 127.8, 125.9, 124, 122.8, 122.4, 121.8, 119.8, 118.5, 115.5, 106.7, 52.9, 38.9, 33, 29.7, 29.5, 27, 25, 19.7. FT-IR (KBr) ν_{max} = 744, 806, 951, 1069, 1185, 1282, 1337, 1457, 1496, 1603, 1739, 2853, 2922, 2958, 3409 cm^{-1}.

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Preparation of SP-SQ-SP(7) incorporated PEG film

In order to incorporate **SP-SQ-SP(7)** into polymer matrices, we have taken 0.8 g of poly (ethylene glycol) and ~0.01 g of **SP-SQ-SP(7)** and dissolved them in 5 ml of dichloromethane. Few drops of as prepared solution was cast onto quartz plate and then drier in ambient condition over a period of 2 days. A thick light blue colour film was obtained (Fig. 2B, Inset).

Preparation of SP-SQ-SP nanoparticles

To prepare nanoparticles we adopted 'reprecipitation method' and it is described elsewhere.[16] In brief, first of all stock solution **SP-SQ-SP** (**7**) of milli Molar concentration were prepared in tetrahydrofuran (HPLC grade, Aldrich) and stored in the dark place at room temperature. Then few micro litters (30-50 µl) of stock solution of **SP-SQ-SP** (**7**) was rapidly injected into large excess of deionised water at room temperature and sonicated for few minutes. A very light blue colloid was obtained and used for characterization. The sizes of the as prepared nanoparticles are found to be ~ 60-100 nm (Fig. 1B, Inset), for more see (ESI, Fig. 8S).

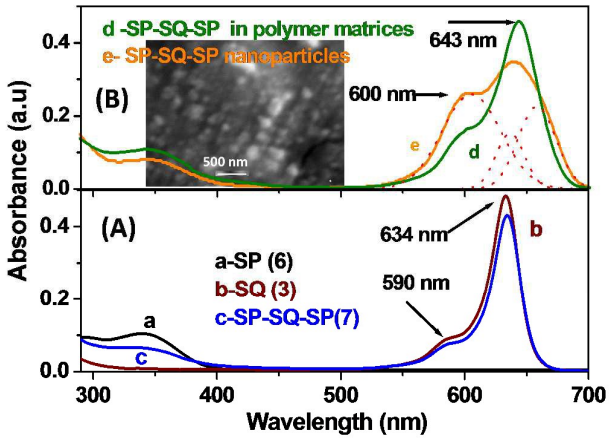


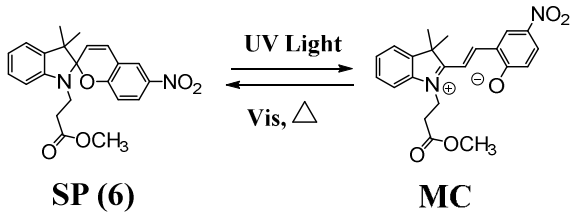
Figure1. (A) Absorption spectra of **SP(6)**, **SQ(3)** and **SP-SQ-SP (7)** in ACN solution (~10^{-6} M) at room temperature, (B) absorption spectra of **SP-SQ-SP (7)** in PEG matrices and it nanoparticles dispersed in water. Inset shows the SEM micrograph of nanoparticles

Results and Discussion

The synthesis of SP-functionalized dyad SP-SQ-SP (**7**) is shown in Scheme 3. Compounds **SQ** (**3**) and **SP** (**5**) were prepared according to the sequence shown in Scheme 1 and Scheme 2. Reaction of acid-alcohol coupling between **3** and **5** in the presence of DCC, DMAP led to **SP-SQ-SP** dyad **(7)** in 50% yield.

Absorption Spectra:

As prepared reference spiropyran compound **SP** (**6**) shows the typical reversible interconversion between spirocyclic **(SP)** and ring opened merocyanine **(MC)** states (Scheme 4) upon irradiation of ultraviolet (330 nm) and visible light (580nm), and it is identified by absorption spectra of respective conformer (ESI, Fig. 1S & 2S)[17]. Since, **SQ(3)** is known to form H-aggregates[18,19] we have performed concentration dependent studies of absorption spectra of **SQ(3)** as well as dyad **SP-SQ-SP(7)** in the range of 10^{-7} to 10^{-5} M concentration and found no substantial change in absorptions spectra (ESI, Fig. 3S) in the studied concentration range. Hence, all solution phase experiments are performed with 5-6 micro molar concentration of the dyad molecule.



Scheme 4: Two switching states of references SP (6) compound.

Figure 1A shows the absorption spectra of **SP(6)**, **SQ(3)** and **SP-SQ-SP(7)** dyad in ACN solution and Figure 1B shows the absorption spectra of **SP-SQ-SP (7)** in polymer matrices and its nanoparticle dispersed in water. As it is observed in solution, the absorption bands of **SP-SQ-SP (7)** are the exact superposition of absorption bands of photochromic **SP (6)** unit and fluorescence probe **SQ (3)** unit**.** This clearly indicates that in ground state, there is no interaction between **SP (6)** and **SQ(3)** moieties. Thus, **SP-SQ-SP (7)** turns out to be an ideal dyad molecule. In ACN solution, dyad **7** shows a strong absorption peak at 634 and a shoulder band at 590 nm corresponding to **SQ (3)** absorption bands and a relatively weak absorption peak at 330 nm corresponding to the absorption of **SP (6)**. In polymer matrices, no change in absorption spectra pertaining to **SQ (3)** moiety of dyad **7** but a systematic red shift of 10 nm is observed for the absorption bands corresponding to **SQ (3)** moiety along with the broadening of the spectral shape. In polymer matrices the ratio of the intensities of 643 nm band to 600 nm band decreases than that of in ACN solution. This result indicates that in polymer matrices **SP-SQ-SP (7)** get bit aggregated. Not surprisingly, the absorption band correspond to **SQ(3)** moiety of **SP-SQ-SP(7)** nanoparticles is found to be very broad and apparently composed of two almost equally intense band at 600 and 643 nm. However, non-linear least square fitting analysis to the absorption spectra yields three Gaussian peaks. An additional new peak at 660 nm is resolved along with two distinct bands at 600, 643nm. The appearance of this new band indicates that existence of J-type aggregates in the formation of **SP-SQ-SP** nanoparticles. No changes in absorption band correspond to **SP (6)** absorption was notices neither in PEG polymer matrices nor in nanoparticle form of **SP-SQ-SP**.

After the ACN solution of dyad **7** was irradiated at room temperature (27^0C) with ultraviolet light at 330 nm (150W Xenon lamp) for 30 seconds, the light blue colour ACN solution of dyad **7** turned to dark blue colour solution (Fig. 2A). The colourless **SP** solution turns blue upon 330 nm photo-irradiation due to transformation of **SP** to **MC** form.[10] Hence, this colour change of ACN solution of dyad **7** confirms the transformation of **SP** to **MC** in dyad **SP-SQ-SP (7)**. However,

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the absorption spectra of UV (330nm) light irradiated solution shows a very weak new band at round 555 nm (ESI, Fig. 4S). The characteristic absorption band of the **MC** form of **SP** which shows absorption maximum at 555 nm and it is superimposed with the absorption bands of **SQ** but the main absorption bands of the **SQ** unit remain unchanged (Figure 4S, Curve b). In comparison to the absorption spectrum of **SP (6)** upon ultraviolet light irradiation (ESI, Fig. 1S), it is confirmed that the formation of the corresponding **MC** form of **SP** in dyad **7** under the same conditions is occurred. This photogenerated isomer reverts to original one by thermal isomerisation within 30 seconds at room temperature in solution phase making it difficult to collect absorption spectra of **MC-SQ-MC** form with high fidelity. However, upon decreasing the solution temperature to 7^0 C, the rate of forward (i.e. **SP** to **MC**) transformation induced by 330 nm photo-irradiation does not change but the rate of backward reaction decrease dramatically and the absorption spectra of photo-irradiated **SP-SQ-SP** clearly shows new band at 555nm (Figure 2A curve b), a band which correspond to absorption spectra of **MC**. At this temperature, the **MC** form of **SP** is very stable and irradiation of 555 nm light for 20 minutes reverts the **MC** form to **SP**.

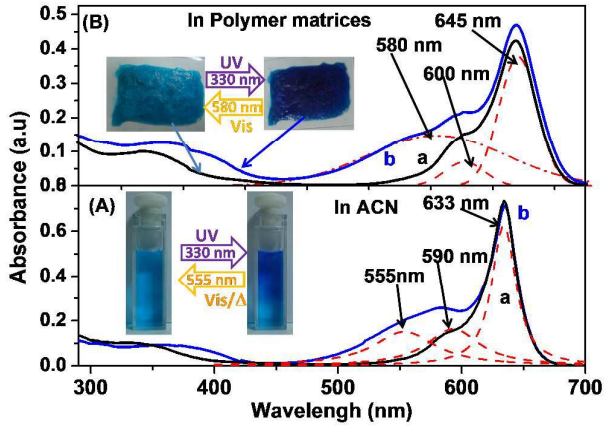

Figure 2. Absorption spectra of **SP-SQ-SP** (**7**), (A) in ACN solution (~10^{-6} M) at 280 K and (B) in PEG polymer matrices onto quartz plate at room temperature, 300K. (a) Black curve, before irradiation of UV light, (b) Blue curve, after irradiation of UV light (330 nm) for 30 sec in ACN solution of dyad **7** and polymer film respectively. Insets are photographic image of reversible colour change of **SP-SQ-SP** in solution and in polymer matrices at 300K.

At room temperature, photo-irradiation at 330 nm (150 Xenon Lamp) light for just 30 seconds, the polymer film of dyad **7** undergoes to change the colour of the film and it turned into dark blue from light blue (Figure 2B, inset). Consequently, the absorption spectra of this film showed a prominent peak at 580 nm corresponding to the **MC** form of dyad **7** (Fig. 2B, curve b). It is important to mention here that unlike in solution the photogenerated isomer (**MC** form) of dyad **7** in film is very stable at room temperature. It reverts thermally very slowly to **SP** form and takes more than 24 hours to complete the conversion. However, upon 580 nm photo irradiation (150 W, xenon lamp) the rate of conversion from **MC** to **SP** enhanced and it takes 20 minutes to restore the original state. This cycle of photoinduced reversible change of dyad **7** was repeated several times without degrading the molecule both in solution and in polymer film.

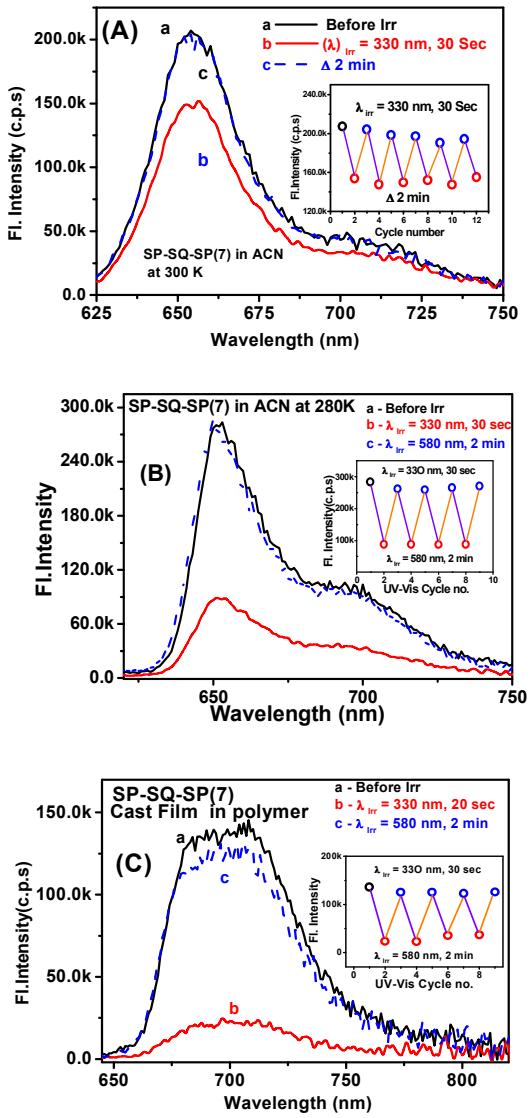

Figure 3. Fluorescence switching behaviour of SP-SQ-SP upon alternate application of UV and Vis/Δ in ACN solution at room temperature and at 7^0 C as well as in PEG matrices at room temperature. (A) in ACN at 300K, (B) in ACN at 280 K and (C) in PEG polymer matrices at 300K. Insets show the reversible modulation of the fluorescence intensity of the SQ moiety at the emission peak for the respective medium.

Fluorescence Spectra:

Although there was no noticeable change in concentration dependent absorption spectra of **SQ(3)** or dyad **SP-SQ-SP(7)** in ACN solution, a slight change in concentration dependent fluorescence spectra of **SQ** and dyad **SP-SQ-SP(7)** are observed in ACN solution. Upon increasing concentration of **SQ** or dyad **SP-SQ-SP** from 10^{-7} M to 10^{-6} M in ACN solution, a bathochromic shift of fluorescence emission maxima was observed and no further shift of emission peak was observed

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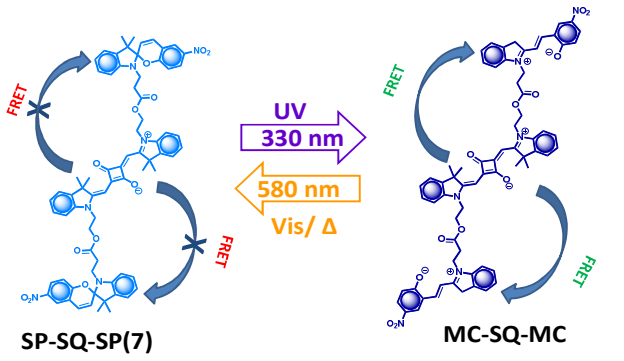
till few hundred of micro molar concentration of the **SQ** or **SP-SQ-SP** (ESI, Fig. 5S). Hence, all fluorescence spectroscopic studies are performed in 6-7 micromolar concentration of the compounds. It is also important to mention that no self quenching of fluorescence was observed for these compounds till few hundreds of micro molar solution. Excitation wavelength dependent studies of dyad **7** shows good linearity in the studied concentration range. However, very small fluorescence Stokes shift of dyad **7** limits the excitation wavelength to the blue side of the absorption spectra. Figure 3 shows the fluorescence spectra of the ACN solution (6 µM) of dyad **7** under different experimental conditions with an excitation wavelength of above 600 nm. Before exposing to ultraviolet light, dyad **7** showed a broad emission band in the range of 625-750 nm with the maximum around 656 nm (Figure 3A curve a,). This fluorescence spectrum is broadly similar to that of fluorescence emission spectra of reference **SQ (3)** in terms of fluorescence emission spectral structure. The fluorescence quantum yield of dyad **7** is found to be higher than that of SQ[20] and it is estimated to be 0.7(ESI, Fig. 6S). However, upon irradiation with ultraviolet light (330 nm, peak position of **SP**) for 30 seconds at room temperature, the intensity of the fluorescence band around 656 nm decreased and could be managed to estimate only 28% reduction to that of the initial solution (Figure 3A curve b,) and no new fluorescence peak was detected. It is important to note that in ACN solution the **MC** form of **SP** (**6**) shows fluorescence emission peaking at 650 nm (ESI, Fig. 1S). Upon irradiation with ultraviolet light, the **SP** moieties of dyad **7** transform into the **MC** conformer, resulting in the formation of **MC–SQ–MC** (Scheme 5) which quenches the excited state of the **SQ** unit through fluorescence resonance energy transfer. Energy transfer from the excited **SQ** state to **MC** produces the excited **MC** state, which should show a new emission band at around 650 nm. However, in the present case we do observe such a new fluorescence band relating to the fluorescence of the **MC** moiety of **MC–SQ–MC**, perhaps due to strong overlap of florescence of MC to that the red edge florescence of **SQ**. Since **SQ** has very high fluorescence quantum yield with respect to fluorescence yield of **MC**, the fluorescence emission of **MC** hides under the envelop of the fluorescence of dyad **SP-SQ-SP** even in reduced fluorescence condition. Moreover, **MC-SQ-MC** form of **SP-SQ-SP** thermally reverts very fast to dyad **SP-SQ-SP** and hinders to monitor efficient conversion of dyad **SP-SQ-SP**. However, **MC-SQ-MC** comes back to **SP-SQ-SP** form within 30 sec after photo-irradiation and fluorescence intensity is restored to its initial value without distortion of the fluorescence band shape (Figure 3A, curve c) and this cycle of fluorescence *"on/off"* states are repeated by application of 330 nm light followed by thermal conversion for several cycles without degrading the compound (Fig. 3A, Inset). This result confirms that light and thermal-driven transformation between two states of dyad **7** is fatigue resistant in the solution phase at room temperature. However, to control the rate of thermal conversion of **MC-SQ-MC** to **SP-SQ-SP** we have performed fluorescence switching experiment at 7^0 C in ACN Solution. As shown in Figure 3B, there is no substantial change in the shape of fluorescence spectra of **SP-SQ-SP(7)** in this low temperature but bit increase in florescence intensity. Upon irradiation with ultraviolet light (330 nm, peak position of SP) for 30 seconds, the intensity of the fluorescence band around 656 nm decreased to 70% of that of initial solution (Figure 3B, curve b) and it remains constant with time. It clearly reveals that a substantial amount of conversion occurs from **SP-SQ-SP** to its ring opening conformer **MC-SQ-MC** and **MC-SQ-MC** is quite stable at 7^0 C in ACN solution. However, after irradiation with 555 nm (absorption peak position of **MC** form of **SP**) light for 20 minutes, **MC–SQ–MC** completely changed to the **SP–SQ–SP** form and the fluorescence intensity was restored to its initial value without distortion of the fluorescence band shape (Figure 3B, curve c). This fluorescence *"on/off"* states are repeated by alternate application of 330 and 555 nm light for several cycles without degrading the compound (Figure 3B). This result confirms the light-driven transformation between two states of dyad **7** is also fatigue resistant in the solution phase.

Sequentially, similar types of experiments were performed with dyad **7** within PEG polymer matrices at room temperature. As shown in Figure 3C, the fluorescence emission spectrum of dyad **7** became broad and the peak position was red shifted by 10 nm compared to that in ACN solution, peaking at 670 nm (Figure 3B curve a,). This change of emission spectra of **SP-SQ-SP** in the polymer matrices is perhaps due to some aggregation effect of **SQ** moiety. However, after irradiation with 330 nm light for just 30 seconds at room temperature, the fluorescence intensity of the **SQ** moiety of dyad **7** was quenched by 85% of its initial value (Figure 3B, curve b) and it did not substantially change with time. This result not only confirms the efficient **FRET** between the **SQ** and **MC** moieties in polymer matrices but also confirms the hindrance of thermal conversion of **MC-SQ-MC** to **SP-SQ-SP** at room temperature. To check the photo-reversibility, the polymer film was irradiated with 580 nm light for 2 minutes and the fluorescence of the **SQ** moiety came back almost to 90% of its initial value (Figure 3C, curve c). This cycle was repeated several times and no remarkable degradation of the compound was observed (Figure 3C, Inset).



Scheme 5. Reversible fluorescence switching cycle of dyad **7** under different external stimuli: ultraviolet light (330 nm) and visible light (580 nm).

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The as prepared water dispersed nanoparticles of dyad **7** shows good fluorescence with emission peak at 675 nm. At room temperature, upon UV irradiation (330 nm) for 30 sec, the florescence intensity of the nanoparticles reduced to 50 % of its initial value (ESI, Fig. 7S) and after two minutes it reverts to its initial value. However, irradiation by visible light enhances the reversion process and complete reversion is observed by irradiation of 580 nm light for 30 sec. This cycle is repeated several times and no change of initial fluorescence intensity is observed. It is worthy to note that at room temperature in nanoparticle form, the rate of back reaction i.e. **MC-SQ-MC** form to **SP-SQ-SP (7)** form is slowed by two times than that in ACN solution. Like in solution phase, on lowering the temperature to 7^0C, efficiency of thermal reversion tends to be zero and visible light induced reversion becomes predominantly active.

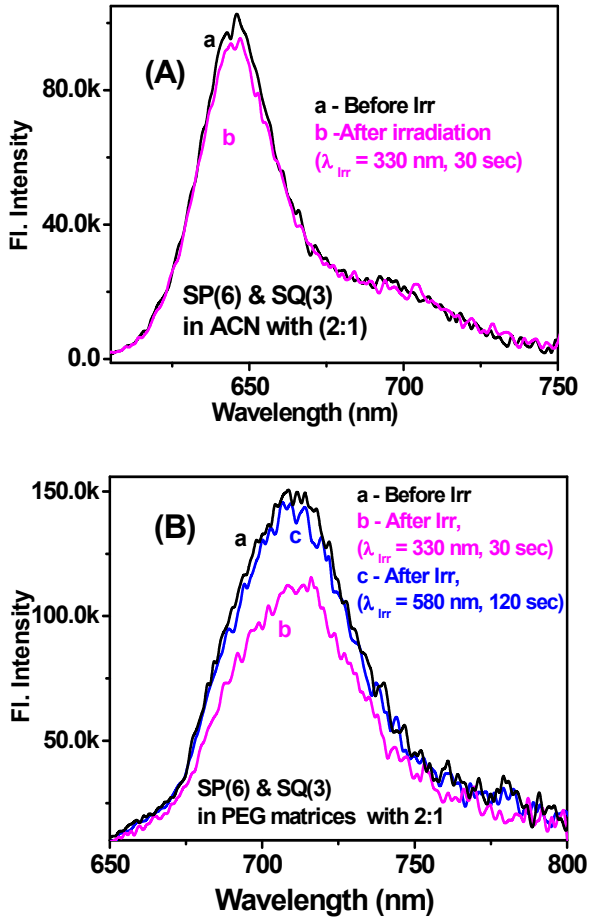

Figure 4: Fluorescence switching behaviour of the mixture of SP(6)and SQ(3) in 2:1 ratio upon alternate application of UV and Vis/Δ in ACN solution and in PEG polymer matrices at room temperature. (A) in ACN at 300K, (B) in PEG polymer matrices at 300K.

For comparative studies, similar set of experiments were performed for the mixtures of reference compounds **SP(6)** and **SQ** (**3**) (in a molar ratio of 2:1) both in ACN solution and in PEG polymer matrices at room temperature (300K) in order to compare the difference between intra and inter molecular effect in fluorescence switching behaviour (Figure 4). Before ultraviolet light irradiation, the solution showed the exactly similar fluorescence emission spectra to that of dyad **7** (Figure 4A, curve a). After irradiation of the mixture solution at 330 nm for 5 min, less than 5% reduction of the fluorescence intensity was observed (Figure 4A, curve b) and it returns to its original value upon irradiation of 550 nm light for 2 minutes. However, after irradiation of PEG polymer film incorporated with 2:1 mixture of **SP (6)** and **SQ (3)** at 330 nm for 30 seconds reduction of the fluorescence intensity was observed only about 21 % to its initial value (Figure 4B, curve b) and it returns to its original value upon irradiation of 580 nm light for 2 minutes, (Figure 4B, curve c) similar to what is described above for dyad **7**. Although the extent of fluorescence reduction is quite low, but these unavoidable reduction of fluorescence intensity is due to the quenching of the excited state of the **SQ** unit by the corresponding MC form generated from the SP upon ultraviolet light irradiation both in solution and in polymer matrices. As compared to dyad **7**, the fluorescence intensity reduction for the mixed solution of **3** and **6** is significantly less. According to the Förster theory,[21] the energy transfer efficiency is strongly dependent on the donor-acceptor distance. The donor and acceptor units in dyad **7** are much closer to each other than those in the case of the mixed solution of **3** and **6** (intermolecular) and it strongly depends on rate of diffusion of the molecules. However, the efficiency of FRET is bit enhanced in polymer matrices but it is quite low than for dyad **7** in same polymer matrices. Thus, it is quite clear that the intermolecular energy transfer is not as effective as that for the intramolecular cases.

Furthermore, all the studies described here are qualitative in nature and the quantitative estimations were not performed. During the collection of fluorescence spectra irradiation was not carried out as well as the time gap after photo-irradiation and before collecting the spectral signature of photoproducts whether it fluorescence or absorption has not been considered. Hence, thermal reversion to the **SP** form could occur before and during the collection of the fluorescence spectra, especially in solution where conversion of **MC** to **SP** form is very fast. Finally, since **MC** and **SQ** absorb at 550 nm and irradiation at this wavelength creates only photostationary equilibrium between two forms (**SP** and **MC**) of spiropyran units, rather than complete conversion of the system to one form. The studied fluorescence modulation in solution at room temperature as well as low temperature, polymer matrices and in nanoparticle form observed for dyad **SP–SQ–SP (7**) can be rationalized with the switching cycles starting and ending with **SP–SQ–SP (7)** as shown in Scheme 5. There should exist several states of dyad **SP–SQ–SP (7**) due to the partial conversion between **SP** and **MC** or vice versa on exposure to ultraviolet or visible light. Since changes in the fluorescence intensity are the sole parameter to account for the conversion due to irradiation with UV/Vis light or thermal, we consider two ultimate states (Scheme 5) to clarify the mechanism. Upon irradiation with ultraviolet light, the SP

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moieties in dyad **7** are transformed to **MC** moieties, forming **MC–SQ–MC**, which quenches the excited state of the **SQ** moiety and the fluorescence intensity of the **SQ** moiety is reduced. On the other hand, upon irradiation of the solution of **MC–SQ–MC** with visible light or thermal exposure, complete conversion from **MC–SQ–MC** to **SP–SQ–SP(7)** occurs and the fluorescence intensity returns to its initial value. Thus, alternate application of ultraviolet light, visible light, and thermal exposure regulates explicitly the fluorescence intensity of the **SQ** moiety. This external stimulation-dependent fluorescence spectra of dyad **7** can be exploited to design integrated logic circuits on the molecular level.

Conclusion

We have successfully synthesized a new dyad **SP-SQ-SP(7)** and have examined their photophysical properties devoted to understanding their light driven fluorescence switching behaviour in solution, in PEG polymer film and in its nanoparticle form dispersed in water. The presence of **SP (6)** units controls the fluorescence switching '*on/off*' states of the **SQ(3)** moiety of dyad **7** by way of reversible interconversion among the two different states of the photochromic spiropyran moiety and fluorescence resonance energy transfer (**FRET**) between the **MC** and **SQ** moieties. At room temperature in solution phase **MC** form of SP in **SP-SQ-SP(7)** is found to be less stable resulting very fast thermal reversion of **MC** form to **SP** which could be advantageous in developing temperature sensor. Near-infrared fluorescence on/off states of nanoparticle dispersed in aqueous media may have potential applications in fluorescence imaging. Overall, these results may have implication for the invention of efficient near-infrared fluorescent probes for potential applications towards molecular device design with multiple inputs and a single output.

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Notes and references

1 M. Bossi, V. Belov, S. Polyakova, S.W. Hell, *Angew. Chem*., 2006, **118**, 7623; *Angew. Chem. Int. Ed*., 2006, 45, 7462.
2 M. Irie, S. Kobatake, M. Hirochi, *Science* 2001, **291**, 1769.
3 J. AndrLasson, Y. Terazono, B. Albinsson, T. A. Moore, A. L.Moore, D. Gust, *Angew. Chem.,* 2005, **117**, 7763 *Angew.Chem. Int. Ed.,* 2005, 44, 7591.
4 G. K. Such, R. A. Evans, T. P. Davis, *Macromolecules* 2006, **39**, 9562.
5 S. Just, A. Aemissegger, P. Guntert, O. Zerbe, D. Hilvert, *Angew.Chem*. 2006, **118**, 6445; *Angew. Chem. Int. Ed.,* 2006, **45**, 6297.
6 (a) A. P. de Silva, S. Uchiyama, *Nat. Nanotechnol*. 2007, **2**, 399; (b) A. Credi, *Angew. Chem.,* 2007, **119**, 5568; *Angew. Chem. Int. Ed.,* 2007, **46**, 5472; (c) X. Chen, Y. Wang, Q. Liu, Z. Zhang, C. Fan, L. He, *Angew. Chem*., 2006, **118**, 1791; *Angew. Chem. Int. Ed.*, 2006, **45**, 1759; (d) D. H. Qu, Q. C. Wang, H. Tian, *Angew. Chem.,* 2005, **117**, 5430; *Angew. Chem. Int. Ed*., 2005, **44**, 5296; (e) H. Miyaji, H. K. Kim, E. K. Sim, C. K. Lee, W. S. Cho, J. L. Sessler, C. H. Lee, *J. Am. Chem. Soc*., 2005, **127**, 12510; (f) U. Pischel, *Angew. Chem*., 2007, **119**, 4100; *Angew. Chem. Int. Ed*., 2007, **46**, 4026; (g) E. Katz, V. Privman, *Chem. Soc. Rev*., 2010, **39**, 1835; (h) S. Angelos, Y. W. Yang, N. M. Khashab, J. F. Stoddart, J. I. Zink, *J. Am. Chem. Soc.*, 2009, **131**, 11344.
7 A. P. de Silva, D. B. Fox, T. S. Moody and S. M. Weir, *Trends Biotech.*, 2001, **19**, 29.
8 (a) E. A. Lemke and C. Schultz, *Nat. Chem. Biol*., 2011, **7,** 480;(b) K. P. Carter, A. M. Young and A. E. Palmer, *Chem. Rev.,* 2014, **114,** 4564.
9 (a) Y. Li, T. Liu, H. Liu, M.-Z. Tian and Y. Li, *Acc. Chem. Res*., 2014, **47,** 1186; (b) A. P. De Silva, H. Q. N. Gunaratne,T. Gunnlaugsson, A. J. M. Huxley, C. P. Mccoy, J. T. Rademacher and T. E. Rice, *Adv. Supramol. Chem*., 1997, **4**, 1.
10 (a) F.M. Raymo , S. Giorgani, *J. Am. Chem. Soc.,* 2002, **124**, 2004; (b) F. M. Raymo and S. Giorgani, *Org. Lett.,* 2001, **3**, 1833; (c) F. M. Raymo , S. Giorgani, *Org. Lett.,* 2001, **3**, 3475.(d) E. Deniz, S. Sortino , F. M. Raymo, *J. Phys. Chem*. *Lett.,* 2010, **1**, 1690.
11 D. Siva, B. Ramakrishna, Y. Venkatesh, P. R. Bangal, *RSC Adv*., 2015, **5**, 56855.
12 (a) M. D. H. Bhuiyan , G. J. Gainsford , Y. Kutuvantavida , J. W. Quilty , A. J. Kay , G. V. M. Williams & M. R. Waterland *Mole.r Cryst. and Liq. Crys.*, 2011, **548**, 272; (b) S. Kuster, T. Geiger, *Dyes and Pigments*, 2012, **95**, 657; (c) D. B. Liu, W. W. Chen, K. Sun, K. Deng, W. Zhang, Z. Wang, X. Y. Jiang, *Angew. Chemie Int. Ed.*, 2011, **50**, 4103.
13 (a) S. Sreejith, P. Carol, P. Chithra, A. Ajayaghosh, *J. Mater. Chem.,* 2008, **18**, 264; (b) A. jayaghosh, *Acc. Chem. Res.,* 2005, **38**, 449; (c) K. Funabiki, H. Mase, Y. Saito, A. Otsuka, A. Hibino, N. Tanaka, H. Miura, Y. Himori,T. Yoshida, Y. Kubota, M. Matsui, *Org. Lett.,* 2012, **14,** 5.
14 (a) R. Klajn, *Chem. Soc. Rev*., 2014, **43**, 148; (b) A. Nayak, H. Liu, G. Belfort, *Angew. Chem. Int. Ed*., 2006, **45**, 4094; (c) S. Wu, Y. Luo, F. Zeng, J. Chen, Y. Chen, Z. Tong, *Angew. Chem. Int. Ed*., 2007, **46**, 7015; (d T. Satoh, K. Sumaru, T. Takagi, K. Takai, T. Kanamori, *Phys. Chem. Chem. Phys.,* 2011, **13**, 7322; (e) J. Whelan, D. Abdullah, J. Wojtyka, E. Buncel, *J. Mat. Chem*., 2010, **20**, 5727; (f) L. Chen, J. Wu, C. Schmuckb, H. Tian, *Chem. Comm*., 2014, **50**, 6443; (g) J. Chen, F. Zeng, S. Wu, Q. Chen, Z. Tong, *Chem. Eur. J*. 2008, **14**, 4851; (h) S. R. Keum, S. M. Ahn, S. J. Roh, S. Y. Ma, *Dyes and Pig.,* 2010, **86**, 74; (i) X. Li, Y. Wang, T. Matsuura, J. Meng, *HETEROCYCLES*, 1999, **51**, 11; (j) J. Zhang, M. Riskin, R. T. Vered, H. Tian, I. Willner, *Chem. Eur. J*. 2011, **17**, 11237; (k) K. Namba, S. Suzuki, *Bull. of the chem. soc. of jap*., 1975, **48**, 1323.
15 (a) G. Berkovic, V. Krongauz and V. Weiss, *Chem. Rev.,* 2000, **100**, 1741; (b) S. Kawata and Y. Kawata, *Chem. Rev.,* 2000, **100**, 1777; (c) G. E. Collins, L. S. Choi, K. J. Ewing, V. Michelet, C. M. Bowen and J. D. Winkler, *Chem. Comm*., 1999, 321; (d) M. Inouye, K. Akamatsu , H. Nakazumi*, J. Am. Chem. Soc.,* 1997, **119**, 9160; (e) A. S. Dvornikov, J. Malkin , P. M. Rentzepis, *J. Phys. Chem*., 1994, **98**, 6746; (f) J. T. C. Wojtyk, P. M. Kazmaier and E. Buncel, *Chem. Commun.,* 1998, 1703; (g) J. T. C. Wojtyk, P. M. Kazmaier and E. Buncel, *Chem. Mat*., 2001, **13**, 2547; (h) D. B. Liu, W. W. Chen, K. Sun, K. Deng, W. Zhang, Z. Wang and X. Y. Jiang, *Angew. Chem., Int. Ed*., 2011, **50**, 4103; (i) F. M. Raymo, S. Giordani, *J. Am. Chem. Soc*., 2001, **123**, 4651.
16 (a) H. Kasai, H. S. Nalwa, H. Oikawa, S. Okada, H. Matsuda, N. Minami, A. Kakuta, K. Ono, A. Mukoh , H. Nakanishi, *Jpn.*

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DOI: 10.1039/C5RA20252C

J. Appl. Phys. Part 2, 1992, 31, L1132; (b) A. K. Perepogu, P.R. Bangal, *J. Chem. Sci.,* 2008, **120**, 485.
17 A. Ajayaghosh and S. J. George, *J. Am. Chem. Soc.,* 2001, **123**, 5148.
18 (a) A. Moliton, R. C. Hiorns, *Polym. Int.,* 2004, **53**, 1397; (b) H. Chen, K.Y. Law, J. Perlstein, D. G. Whitten, *J. Am. Chem. Soc.*, 1995, **117**, 7257; (c) K. Liang, K.Y. Law, D. G. Whitten, *J. Phys. Chem.* ,1994, **98**, 13379.
19 (a) K. G. Thomas, K. J. Thomas, V. Madhavan, S. Das, D. Liu, P. V. Kamat, M. V. George, *J. Phys. Chem*., 1996, **100,** 17310; (b) K. T. Arun, B. Epe, D. Ramaiah, *J. Phys. Chem. B,* 2002, **106**, 11622.
20 20 S. F. Völker, S. Uemura, M. Limpinsel, M. Mingebach,C. Deibel, V. Dyakonov, C. Lambert, *Macromol. Chem. Phys*., 2010, **211**, 1098.
21 L. Giordano, T. M. Jovin, M. Irie and E. A. Jares-Erijman, *J. Am. Chem. Soc.*, 2002, **124**, 7481.

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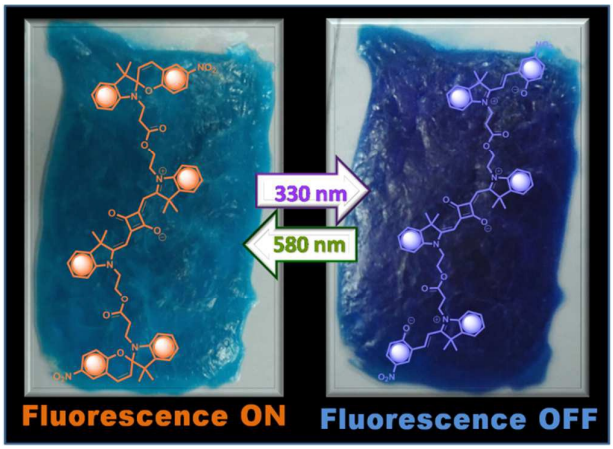

Novel near-infrared fluorescent dyad molecule was synthesized by coupling squarine derivatives (SQ) and Spiropyran (SP) and characterized in solution, in polymer matrices and in its nanoparticle form. Light driven reversible transformations between ring close and ring open isomers of SP modulate the near-infrared fluorescence of SQ leading to fluorescence "ON/OFF" switching behaviours.