

Photochemical & Photobiological Sciences

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

Specific excitonic interactions in the aggregates of hyaluronic acid and cyanine dyes with different length of methine group

H. Tobata and T. Sagawa

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Absorption and circular dichroism (CD) spectra of three kinds of cyanine dyes with different length of methine group (3,3'-diethylthiadicarbocyanine iodide; DTDC, 3,3'-diethylthiacarbocyanine iodide; DTC, 3,3'-diethylthiacyanine iodide; DTTHC) in aqueous solution were compared with or without hyaluronic acid (HA) that has a helical structure. DTDC forms chiral H- and J-aggregates, while DTC and DTTHC are unable to form any aggregation. DTDC also forms H- and J- aggregates in the presence of sodium polyacrylate (PA) with random coil, however the PA-DTDC aggregates has no chirality. These results suggest that the chirality of HA-DTDC aggregates induced by the helical structure of HA. In 2.4 vol% and 10 vol% methanol, HA-DTDC aggregates showed the different patterns of the temperature dependence, while no aggregation was observed in 30 vol% methanol. Solubility of DTDC against the mixed solvent of water and methanol is generally improved by the increment of methanol, which prevents the aggregation of DTDC.

Introduction

Hyaluronic acid (HA) is distributed widely in connective tissues and present mainly in skin, cartilage and vitreous body. HA has played diverse biological roles, such as wound healing, infection prevention and lubrication by holding water in intracellular matrix and forming a jelly matrix interacted to proteins or other polysaccharides.¹ HA is alternate copolymer repeated disaccharide subunits of D-glucuronic acid and N-acetyl-D-glucosamine, and one of the materials that are widely used in the fields of cosmetics and foods. Several studies have shown that the secondary structure of HA is characterized by intermolecular hydrogen bonding involving water molecules in aqueous solution, it is confirmed the left-handed duplex helical structure by X-ray scattering.²⁻⁴ Recently, The solution conformation of HA is investigated using molecular dynamics (MD) simulation and high-field nuclear magnetic resonance (NMR).⁵⁻⁶ Almond *et al* suggested that HA conformation is close to a contracted left-handed 4-fold helix.⁵ Gargiulo *et al* reported that HA can adopt two different arrangements, which can be described by a three or four-folded left-handed helix, with a higher occurrence of the first one, by the combined use of NMR residual dipolar couplings and MD simulations.⁶

Cyanine dyes are used for photosensitizing, information

recording and cell staining with fluorescence. Although cyanine dye is an achiral molecule, it forms a chiral dimer on duplex DNA and α -helical polyamine.⁷⁻⁹ Akimkin *et al* reported that the noncovalent interaction with HA of four cyanine dyes having similar structures, 3,3', 9-trimethylthiacarbocyanine (Cyan 2), 3,3'-dimethylthiacarbocyanine (Cyan 45), 3,3'-diethylthiacarbocyanine (DTC), and 3,3'-diethyl-9-methylthiacarbocyanine (DMTC). Cyan2, Cyan 45 and DMTC were shown to form H-aggregates in the presence of HA and the dyes can be arranged in the series Cyan 2 > Cyan 45 > DMTC > DTC.¹⁰

In the previous study, we reported that DMTC forms H-aggregates on HA and the chirality of H-aggregates change from *R* to *S* as lowering the temperature.¹¹ In order to regulate the aggregation of cyanine dyes with different length of methine group (3,3'-diethylthiadicarbocyanine iodide; DTDC, 3,3'-diethylthiacarbocyanine iodide; DTC, 3,3'-diethylthiacyanine iodide; DTTHC), we investigated the specific spectroscopic behaviour of the cyanine dyes in the presence of HA or sodium polyacrylate (PA) in aqueous solution.

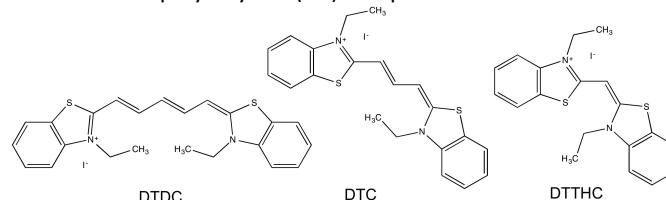


Figure 1. Chemical structure of the cyanine dyes with different length of methine group

Graduate School of Energy Science, Kyoto University, Yoshida-Honmachi, Sakyo-Ku, Kyoto 606-8501, Japan
E-mail address: tobata.haruko.86w@st.kyoto-u.ac.jp (H. Tobata), sagawa.takashi.6n@kyoto-u.ac.jp (T. Sagawa)

Results and Discussion

Absorption spectra of cyanine dyes

Absorption spectra of cyanine dyes ($10 \mu\text{mol L}^{-1}$) in phosphate buffer solution ($\text{Na}_2\text{HPO}_4 / \text{KH}_2\text{PO}_4$, $\text{pH} = 7.0 \pm 0.1$, $I = 0.01$) containing 2.4 vol% methanol were shown in Figure 2. It was reported that a cyanine dye of 1-carboxydecyl-4-[3-[3-methyl-3H-benzothiazol-2-ylidene]-propenyl]-quinolinium; TO-3 forms aggregate by lowering the temperature.¹² Similar to the case of TO-3, aggregation behaviour of DTDC was investigated by lowering the temperature. Indeed, the absorption at 650 nm, which corresponds to the DTDC monomer, was decreased and the absorption at 580 nm was increased. On the other hand, absorption spectra of DTTHC and DTC did not show the temperature dependence.

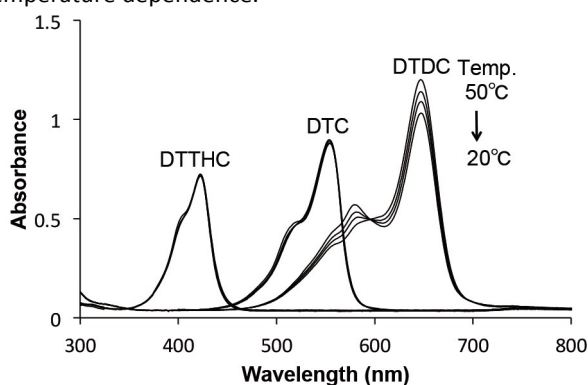


Figure 2. Absorption spectra of cyanine dyes ($10 \mu\text{mol L}^{-1}$) in 2.4 vol% methanol-phosphate buffer ($\text{pH} = 7.0 \pm 0.1$, $I = 0.01$). Temperature range is from 50 to 20°C at 10°C intervals.

Absorption of HA and PA- cyanine dyes aggregates

Absorption spectrum of DTDC ($10 \mu\text{mol L}^{-1}$) in the presence of HA ($20 \text{ unit-}\mu\text{mol L}^{-1}$) was shown in Figure 3. Adding of HA to DTDC led to the decrease of the absorbance at 650 nm, which corresponds to the DTDC monomer and appeared broad absorption band ranging from 500 nm to 850 nm. According to the Kasha's exciton theory, the absorption spectra of HA-DTDC indicates DTDC forms H- and J-aggregates in the presence of HA.¹³ Although DTDC are known to form H-aggregates in the minor groove of a DNA template,^{8,9} DTDC forms not only H-aggregates but also J-aggregates with HA in this study. *Sayama et al* reported that cyanine dyes on nanocrystalline TiO_2 form "J-like" aggregates with broad absorption spectra in the similar wavelength region from 450 nm to 780 nm.¹⁴ They indicated that broadening of the absorption ascribed to the J-like aggregates formation becomes wider with increasing the amount of the adsorbed dye onto the TiO_2 .¹⁵ Similar spectral changes of DTDC were observed in the presence of PA as shown in figure 3b. PA has a random coil structure in aqueous solution. Addition of PA to DTDC led to the decrease of the absorbance at 650 nm, which corresponds to the DTDC monomer and appeared broad absorption band ranging from 500 nm to 850 nm (Figure 3b, solid line).

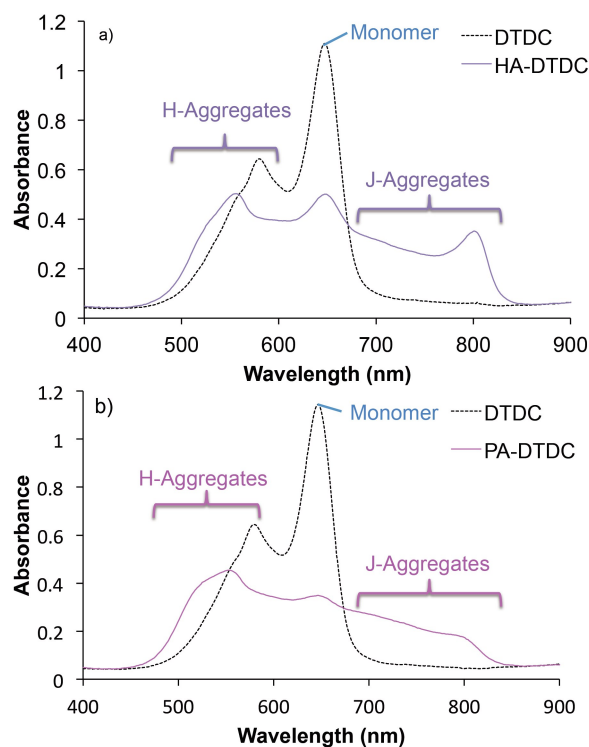


Figure 3. a) Absorption spectra of DTDC and HA-DTDC and b) Absorption spectra of DTDC and PA-DTDC in 2.4 vol% methanol-phosphate buffer ($\text{pH} = 7.0 \pm 0.1$, $I = 0.01$) at 20°C . Dot line is DTDC ($10 \mu\text{mol L}^{-1}$), solid line is HA or PA-DTDC aggregates ($20 \text{ unit-}\mu\text{mol L}^{-1}$, $10 \mu\text{mol L}^{-1}$, respectively).

It is confirmed that DTDC forms H- and J- aggregates in the presence of PA. In this context, broad absorption spectra observed from 500 nm to 850 nm both in the cases of HA-DTDC and PA-DTDC might be originated from broad assemblies of H- and J-aggregates.

Akimkin et al reported that Cyan2, Cyan45 and DMTC formed H-aggregates in the presence of HA, however DTC with HA caused only the decrease of the dye-absorbance without the appearance of new bands.¹⁰ In this study, DTC also showed a decrease in the absorbance at 555 nm without the appearance of new bands. This result indicates that DTC interacts with HA, although DTC doesn't form H- or J-aggregates with HA. On the other hand, DTTHC did not show any spectral changes with HA (Support information).

CD spectra of HA and PA-DTDC aggregates

In the CD spectrum of HA-DTDC ($20 \text{ unit-}\mu\text{mol L}^{-1}$, $10 \mu\text{mol L}^{-1}$), split-Cotton effects were observed at around 500 nm-600 nm, 650 nm-750 nm and 750 nm-850 nm, although a very weak CD intensity (almost zero) of DTDC ($10 \mu\text{mol L}^{-1}$) was observed (Figure 4a). The CD pattern centred at 550 nm is assigned to H-aggregates of DTDC, which bring *R*-chiral formation. Furthermore, the CD patterns centred at 700 nm and 800 nm indicate that J-aggregates of DTDC forms *S* or *R* chiral formation.

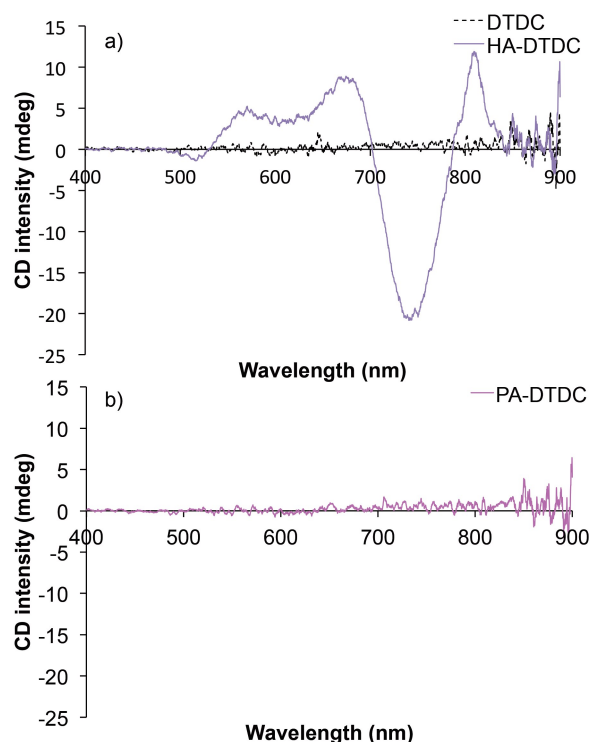


Figure 4. a) CD spectra of DTDC and HA-DTDC and b) CD spectra of PA-DTDC in 2.4 vol% methanol -phosphate buffer (pH = 7.0 \pm 0.1, l = 0.01) at 20°C. Dot line is DTDC (10 $\mu\text{mol L}^{-1}$), solid line is HA or PA-DTDC aggregates (20 unit- $\mu\text{mol L}^{-1}$, 10 $\mu\text{mol L}^{-1}$, respectively).

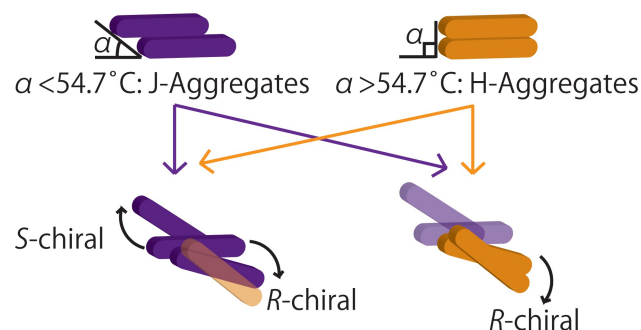


Figure 5. Schematic illustrations of *S*- and *R*-chiral formation of DTDC aggregates in the presence of HA.

Figure 5 indicates schematic representation of *S*- and *R*-chiral formation of DTDC aggregates in the presence of HA with various angles (α) and distances, though precise assignment of the *S*-chiral and *R*-chiral formation is not clear.

On the other hand, the CD intensity of PA-DTDC (20 unit- $\mu\text{mol L}^{-1}$, 10 $\mu\text{mol L}^{-1}$) was almost zero (Figure 4, b). These results suggest that the PA-DTDC aggregates have no chirality and the chirality of HA-DTDC aggregates is induced by the helical structure of HA.

Temperature dependence of the absorption of HA-DTDC aggregates with different concentration of methanol

Figure 6 shows absorption spectra of HA-DTDC (20 unit- $\mu\text{mol L}^{-1}$, 10 $\mu\text{mol L}^{-1}$) in phosphate buffer solution containing 2.4 or 10 vol% methanol in the temperature range from 50 to 20°C at 10°C intervals. In phosphate buffer solution containing 2.4 vol% methanol, the absorption spectrum of the HA-DTDC has three absorption peaks at 550 nm, 650 nm and 800 nm. As lowering the temperature, the absorbance at 650 nm, which corresponds to the DTDC monomer, decreased and the absorbance at 800 nm increased slightly. On the other hand, in phosphate buffer solution containing 10 vol% methanol, the absorption spectrum of HA-DTDC at 50°C has two peaks at 550 nm and 650 nm similar to DTDC without HA. As lowering the temperature, the absorbance at 650 nm, which corresponds to the DTDC monomer, significantly decreased and the absorbance at 550 nm and 800 nm increased. In phosphate buffer solution containing 30 vol% methanol, the absorption spectrum of HA-DTDC at 50°C has two peaks at 550 nm and 650 nm and there is no appearance of new absorption at around 800 nm as lowering the temperature (Support information).

Stadler *et al* reported that the cyanine dyes within the minor groove of DNA template and showed the temperature dependence of absorbance spectra of cyanine dye aggregates with DNA in 20 vol% methanol-10mmolL⁻¹ phosphate buffer solution.

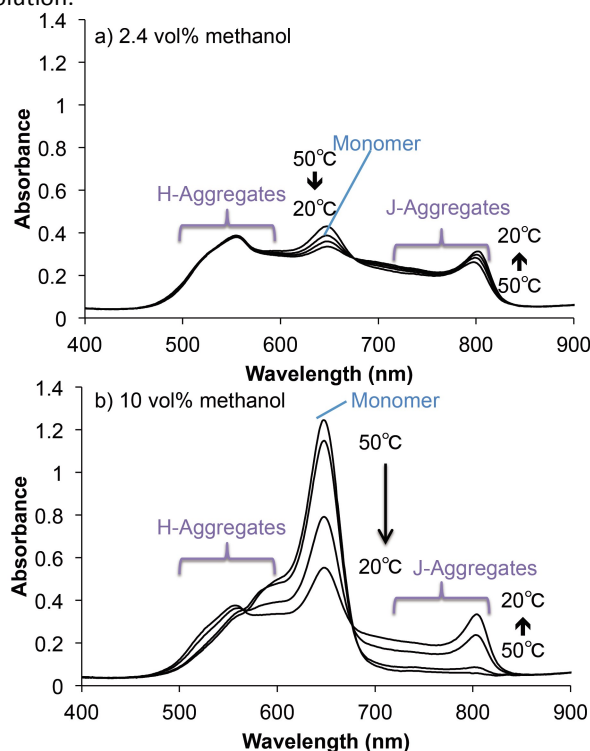


Figure 6. Absorption spectra of HA-DTDC (20 unit- $\mu\text{mol L}^{-1}$, 10 $\mu\text{mol L}^{-1}$, respectively) in a) 2.4 vol% or b) 10 vol% methanol-phosphate buffer (pH = 7.0 \pm 0.1, l = 0.01). Temperature range is 20 to 50°C at 10°C intervals.

They claimed that property of the cyanine dyes and hydrophobic effects affect predominantly for the dimerization and/or the aggregation of the cyanine dyes with DNA than the effect of the donor-acceptor interaction. HA-DTDC showed the temperature dependence as shown in Figure 6. These results support that the formation of HA-DTDC aggregates also depends on the dispersion ability of the cyanine dyes.

In the previous study, we reported that DMTC forms H-aggregates on HA and the chirality of H-aggregates change their form from *R* to *S* as lowering the temperature.¹¹ Meanwhile, DTDC is able to aggregate in the presence of HA at 50°C in 2.4 vol% methanol. Higher concentration of methanol suppressed the aggregation of DTDC, and no aggregation was observed in 30 vol% methanol (Support information).

Solubility of DTDC against the mixed solvent of water and methanol is generally improved by the increment of methanol, which prevents the aggregation of DTDC. While, the helicity of HA is enhanced by the addition of organic solvent into the aqueous solution.¹⁶ Therefore, 2.4 vol% methanol is appropriate concentration in terms of HA-DTDC aggregation with high chirality.

Experimental

Materials

HA powder made from chicken comb, the molecular weight range was 530,000 to 1330,000, was provided by Kewpie Corporation. PA, the polymerization degree was 22,000 to 70,000, was purchased from Wako Pure Chemical Industries, Ltd. Cyanine dyes with different spacer were purchased from Sigma-Aldrich corporate.

Preparations

0.1 unit-mmol L⁻¹ of HA and PA were dissolved in phosphate buffer solution (Na₂ HPO₄/KH₂ PO₄, pH = 7.0 ± 0.1, I = 0.01), respectively. Cyanine dyes (DTTHC 5.0 mg, DTC 5.4 mg, DTDC 5.6 mg) was dissolved in 25 mL of methanol (0.42 mmol L⁻¹), respectively. Those solutions stored at 4°C in a refrigerator. Sample solution for the measurements of UV-visible or CD spectra was prepared that 2 mL of 0.1 unit-mmol L⁻¹ HA solution or 0.1 unit-mmol L⁻¹ PA solution, 0.24 mL of 0.42 mmol L⁻¹ cyanine dye solution and phosphate buffer solution were mixed to total volume of 10 mL.

UV-visible spectroscopy and CD spectroscopy

UV-visible absorption spectra were measured using Ultraspec 4000 UV/Visible Spectrophotometer (Pharmacia Biotech) and CD spectra were measured using J-1100 CD spectrometer (JASCO, Japan). Those spectra measured using a quartz cell with 10 mm optical path length.

Conclusions

DTDC formed chiral H- and J-aggregates in the presence of HA, while DTC and DTTHC did not show any aggregation with or without HA. DTDC also forms H- and J- aggregates in the presence of PA with random coil, however the PA-DTDC

aggregates has no chirality. These results suggest that the chirality of HA-DTDC aggregates originated from the helical structure of HA.

Increment of methanol suppressed the aggregation of DTDC, and no aggregation was observed in 30 vol% methanol. This result indicates that solubility of DTDC against the mixed solvent of water and methanol is generally improved by the increment of methanol, which prevents the aggregation of DTDC.

The aggregation behavior of cyanine dyes is able to be regulated by changing the length of the methine group and the addition of HA effectively amplifies both of the H- and the J-aggregation in the different absorption regions with different *R*-chiral formation for H-aggregate and /or *S* or *R* chiral formation for J-aggregate. Therefore, the chirality of the HA-DTDC aggregates originated from the helical structure of HA.

It was also found that the solubility is able to be optimized for the thermos-responsible and reversible colour tuning aggregation behaviour of the dye remarkably by adjusting the ratios of methanol and water.

Consequently, conformationally modulated and amplified aggregation of cyanine dyes having three different molecular lengths in the combination with hyaluronic acid was systematically investigated and found the optimized interacting distance between the dyes and the macromolecules.

Acknowledgements

We are grateful to Prof. Hiroataka Ihara and Prof. Makoto Takafuji of Kumamoto University for helpful suggestions and discussions. We thank Kewpie Corporation for providing the HA powder. We also appreciate JASCO for kind assistance to measure the CD spectra.

Notes and references

1. I. Hargittai and M. Hargittai, Molecular structure of hyaluronan: an introduction, *Struct. Chem.*, 2008, **19**, 697-717
2. ICM. Dea, R. Moorhouse, D. A. Rees, S. Arnott, J. M. Guss and E. A. Balazs, Hyaluronic Acid: A Novel, Double Helical Molecule, *Science*, 1973, **179**, 560-562
3. J. K. Sheehan and E. D. T. Atkins, X-ray fibre diffraction study of conformational changes in hyaluronate induced in the presence of sodium, potassium and calcium cations, *Int. J. Biol. Macromol.*, 1983, **5**, 215-221
4. S. Arnott, A. K. Mitra and S. Raghunathan, Hyaluronic Acid Double Helix, *J. Mol. Biol.* 1983, **169**, 861-872
5. A. Almond, P. L. Deangelis and C. D. Blundell, Hyaluronan: the local solution conformation determined by NMR and computer modeling is close to a contracted left-handed 4-fold helix, *J. Mol. Biol.* 2006, **358**, 1256-1269
6. V. Gargiulo, M. A. Morando, A. Silipo, A. Nurisso, S. Pérez, A. Imberty, F. J. Cañada, M. Parrilli, J. Jiménez-Barbero and C. D. Castro, Insights on the conformational properties of hyaluronic acid by using NMR residual dipolar couplings and MD simulations, *Glycobiology*, 2010, **20**, 1208-1216

- 7 H. Ihara, M. Shibata and C. Hirayama, Molecular recognition using cyanine- α -helical poly (L-lysine) complexes in methanol, *Chem. Lett.*, 1992, 1731-1734
- 8 J. L. Seifert, R. E. Connor, S. A. Kushon, M. Wang and B. A. Armitage, Spontaneous assembly of helical cyanine dye aggregates on DNA nanotemplates, *J. Am. Chem. Soc.*, 1999, **121**, 2987-2995.
- 9 A. L. Stadler, B. R. Renikuntla, D. Yaron, A. S. Fang and B. A. Armitage, Substituent Effects on the assembly of helical cyanine dye aggregates in the minor groove of a DNA template, *Langmuir*, 2011 **27**, 1472-1479.
- 10 T. M. Akimkin, A. S. Tatikolov, I. G. Panova, and S. M. Yarmoluk, Spectral study of the noncovalent interaction of thiacyanocyanine dyes with hyaluronic acid, *High Energy Chemistry*, 2011, **45**, 515-520
- 11 T. Sagawa, H. Tobata and H. Ihara, Exciton interactions in cyanine dye-hyaluronic acid (HA) complex: reversible and biphasic molecular switching of chromophores induced by random coil-to-double-helix phase transition of HA, *Chem. Commun. (Cambridge, U. K.)*, 2004, 2090-2091
- 12 J. Ghasemi, A. Niazi, G. Westman, M. Kubista, Thermodynamic characterization of the dimerization equilibrium of an asymmetric dye by spectral titration and chemometric analysis, *Talanta*, 2004, **62**, 835-841
- 13 M. Kasha, H. R. Rawls and M. Ashraf El-Bayoumi, The exciton model in molecular spectroscopy, *Pure Appl. Chem.*, 1965, **11**, 371-392
- 14 K. Sayama, S. Tsukagoshi, T. Mori, K. Hara, Y. Ohga, A. Shinpou, Y. Abe, S. Suga and H. Arakawa, Efficient sensitization of nanocrystalline TiO₂ films with cyanine and merocyanine organic dyes, *Solar Energy Materials & Solar Cells*, 2003, **80**, 47-71
- 15 K. Sayama, S. Tsukagoshi, K. Hara, Y. Ohga, A. Shinpou, Y. Abe, S. Suga and H. Arakawa, Photoelectrochemical Properties of J Aggregates of Benzothiazole Merocyanine Dyes on a Nanostructured TiO₂ Film, *J. Phys. Chem. B*, 2002, **106**, 1363-1371
- 16 P. W. Staskus and W. C. Johnson, Jr, Conformational transition of hyaluronic acid in aqueous-organic solvent monitored by vacuum ultraviolet circular dichroism, *Biochemistry*, 1988, **27**, 1522-1527