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Cryo-transmission electron microscopy reveals mesoscopic H- and J-aggregates of near infrared cyanine dyes

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ABSTRACT

Cryogenic transmission electron microscopy (cryo-TEM) studies were performed to probe the morphological structure of aggregates formed by the two near-infrared dyes IR-140 and Indocyanine Green (ICG) in water. Both dyes formed sheet-like monolayered J-aggregates of mesoscopic size. This structure is consistent with a stacking geometry known for other cyanine dye J-aggregates. H-aggregates, which dominate in freshly prepared aqueous ICG solutions, proved to be linear aggregates of mesoscopic size that are characterized by a broad size distribution. Besides small oligomer particles up to tens of nanometers long thread-like aggregates were detected.

1. Introduction

Dyes that are active in the near-infrared (NIR) region between 650 and 900 nm have attracted ongoing attention in the past years because they provide the basis for the expanding field of non-invasive optical imaging techniques with diverse applications in pharmacology, molecular and cellular biology, diagnostics and material science [1–3]. Cyanine dyes represent an important class of NIR dyes that are readily available. Among them is the only clinically approved NIR fluorescent dye to date, the well-known 2-[7-[1,3-dihydro-1,1-dimethyl-3-(4-sulfobutyl)-2H-benzo[e]-indole-2-ylidene]-hepta-1,3,5-trienyl]-1,1-dimethyl-3-(4-sulfobutyl)-1H-benzo[e]indolium hydroxide inner salt sodium salt also called Indocyanine Green (ICG) or Cardiogreen. ICG dissolved in dimethylsulfoxide is also used as a NIR laser dye under the name IR-125 [4].

Despite the broad exploitation of the ICG's photophysical properties in different fields of research and application [5–9] effects of structural organization of the dye on these properties were only insufficiently characterized. It is known for a long time [5,10], that ICG just as many other cyanine dyes [11–14] undergoes molecular interaction in aqueous solvent already at low concentration, leading to the formation of dimers, oligomers or higher molecular aggregates. The coupling of the chromophors transition dipole moments upon aggregation results in a delocalisation of electronically excited states and is generally connected with substantial changes in the optical properties. Depending on the organization pattern of monomers within the aggregates, bathochromic (J-band) or hypsochromic shifts (H-band) of the aggregates absorption band with respect to the monomer band are observed. Aqueous ICG solutions show both types of aggregate spectra as a function of the particular conditions of preparation.

In a series of studies Penzkofer and co-workers investigated the J-aggregation of aqueous ICG solutions by using absorption spectroscopy [15,16], Mie scattering [17], and conventional transmission electron microscopy (TEM) of air-dried samples [18]. These authors came to the conclusion, that the J-aggregates are submicrometer sized loosely packed porous particles with crystalline regions.

In recent years we investigated the microstructure of several types of cyanine dye H- and J-aggregates on the nanometer-to-micrometer scale by using cryogenic transmission electron microscopy (cryo-TEM) [19–24]. This technique provided highly resolved direct images of the aggregates in their native environment that are free of the drying artifacts due to the specific sample preparation technique. Motivated by the success of these studies we decided to investigate the morphology of the ICG J-aggregates once more, now applying the cryo-TEM technique. The results are reported here.

For comparison we investigated a further NIR dye: 5-chloro-2-[2-(3-[2-[5-chloro-3-(4-sulfobutyl)-3H-benzothiazol-2-ylidene]-ethylidene]-2-diphenylamino-cyclopent-1-enyl)-

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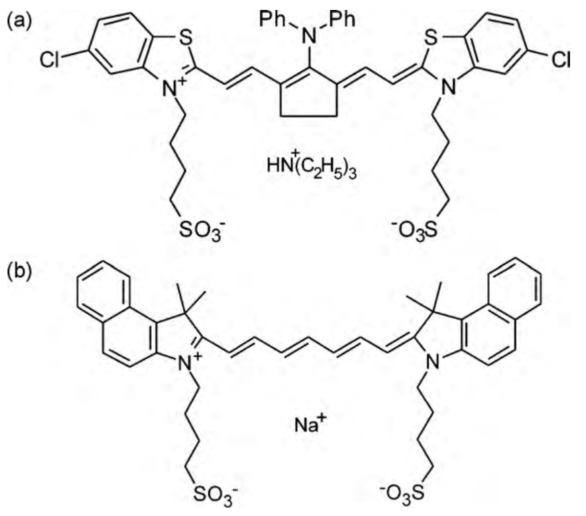


Scheme 1. Structural formula of IR-140 (a) and ICG (b) dyes.

vinyl]-3-(4-sulfobutyl)-benzothiazolium hydroxide inner salt triethylammonium salt, also known as IR-140. Instead of the water-insoluble and non-aggregating diethyl derivative [25], however, we used the sulfobutyl-substituted derivative. The exchange of the respective side-chains increases the solubility of the dye, which is then able to form J-aggregates in water as well [26]. In both cases extended sheet-like morphologies were found, which resemble structures observed earlier for other cyanine dyes. Unexpectedly, in the case of ICG we detected a second population of small particles that coexist with the sheet-like aggregates and appeared to represent H-aggregates.

2. Material and methods

IR-140 and ICG were supplied by FEW Chemicals (Wolfen, Germany) and Fluka, respectively, and were used as received. The structural formulas are shown in Scheme 1. The molar extinction coefficients in methanol were found to be $\varepsilon = 9.4 \times 10^4$ L/(mol cm) (IR-140) and 2.25×10^5 L/(mol cm) (ICG). Due to their high water-solubility solutions were prepared by direct dissolving the dyes in Millipore water under stirring.

Absorption spectra were measured at room temperature (21 °C) with a Lambda 9 spectrophotometer (Perkin-Elmer) using quartz cells of different optical path lengths.

The samples for cryo-TEM were prepared at room temperature by placing a droplet (5 μL) of the solution on a hydrophilized (60 s Plasma treatment at 8 W using a BALTEC MED 020 device) perforated carbon filmed Quantifoil (Jena, Germany) grid. The excess fluid was blotted off to create an ultrathin layer (typical thickness of 100 nm) of the solution spanning the holes of the carbon film. The grids were immediately vitrified in liquid ethane at its freezing point (−184 °C) using a standard plunging device. Ultra-fast cooling is necessary for an artifact-free thermal fixation (vitrification) of the aqueous solution avoiding crystallization of the solvent or rearrangement of the assemblies. The vitrified samples were transferred under liquid nitrogen into a Philips CM12 transmission electron microscope using the Gatan cryoholder and stage (Model 626). Microscopy was carried out at −175 °C sample temperature using the microscopes low dose protocol at a primary magnification of 58,300×. The defocus was chosen in all cases to be 1.5 μm.

For staining electron microscopy, aliquots of 5 μL dye solution were absorbed for 1 min to glow-discharged carbon-coated collodium films on 400-mesh copper grids. After blotting and staining with 1% phosphotungstic acid (PTA) the grids were air-dried. TEM micrographs were taken at a primary magnification of 58,300× and 28,000× using a defocus of 0.8 and 1.4 μm, respectively.

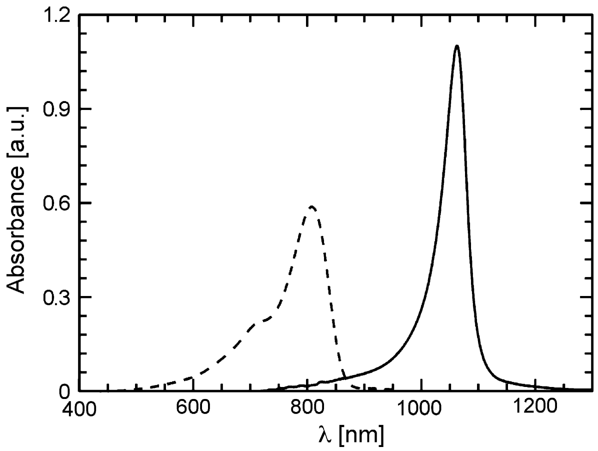


Fig. 1. Absorption spectra of 5.8×10^{-5} M IR-140 solution in MeOH (broken line) and 1.28×10^{-3} M IR-140 solution in water (solid line).

3. Results

3.1. IR-140

The absorption spectrum of dye IR-140 dissolved in MeOH (broken line in Fig. 1) showed a broad band located at 807 nm due to the 0-0 transition of the monomer and an additional vibronic sub-band at 707 nm [27]. When water was used as solvent, dramatic spectral changes occurred. A significantly narrowed single band that is red-shifted with respect to the monomer band appeared (solid line in Fig. 1) due to the formation of J-aggregates by self-assembly of the dye molecules in the polar environment [12]. The J-band is slightly asymmetric, possessing an absorption tail toward shorter wavelengths.

The observed change of spectral pattern is typical for many cyanine and merocyanine dyes [28–39] and can be explained theoretically by the model of molecular excitons [40]. By influencing the intermolecular interactions of the chromophors, their packing has a strong effect on the collective optical properties of the molecules within the aggregate [41]. Thus, changes in the absorption spectra of J-aggregates occurring over a period of weeks could be traced back to changes in the structural organization of aggregates [20–23]. On the other hand, also monomers, dimers and higher aggregates coexisting with the J-aggregates produce spectral contributions, which might lead to a complex overall absorption spectrum. In the case of IR-140, however, the situation seems to be quite simple. The absorption spectrum is dominated by a single and intense J-band centred at 1060 nm. The minute absorbance in the 500–900 nm wavelength range in water points to nearly complete self-assembly of all dye monomers (at the selected dye concentration of $\sim 1.3 \times 10^{-3}$ M) into J-aggregates. The immediate appearance of the J-band upon dissolution of the dye in water supports this finding and underlines the surprisingly strong preference of IR-140 to form J-aggregates.

In order to characterize the J-aggregates we investigated a 3.9×10^{-3} M IR-140 solution by cryo-TEM and additionally by conventional TEM using the negative staining preparation technique. A representative cryo-TEM image is shown in Fig. 2a. Although the image contrast is rather low, objects slightly darker than the background having a typical dimension of 100 nm × 500 nm can be discerned near the border of holes in the supporting carbon film. These objects are images of thin sheet-like structures. Their particular distribution is an effect of segregation caused by the blotting

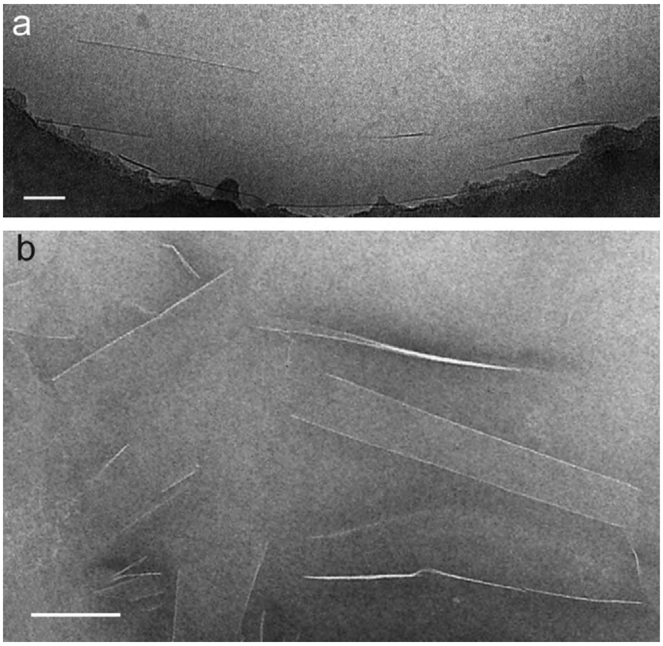
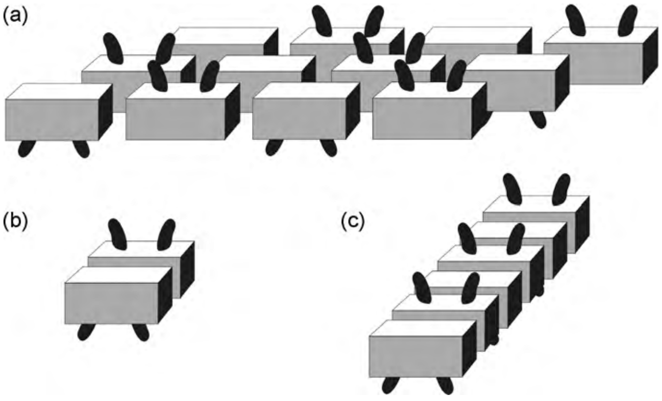


Fig. 2. Cryo-TEM (a) and PTA-stained TEM (b) micrographs of a 3.9×10^{-3} M aqueous IR-140 solution revealing sheet-like J-aggregates. Scale bars: 50 nm (a) and 100 nm (b).



Scheme 2. Schematic representation of a brickwork-like monolayered J-aggregate (a), a sandwich-like dimer (b), and a face-to-face stacked linear H-aggregate (c).

step during the sample preparation. The sheets are often accompanied by dark lines, which typically emerge from folds of the sheets and represent direct images of their cross-section [42]. An effective thickness of 2.9 ± 0.5 nm was estimated from the micrographs pointing to a monolayered arrangement of molecules, whose long axes lie parallel to the layer plane (cf. Scheme 2 in the discussion showing different imaginable molecular arrangements) [20,24]. In a former study [20] of the structurally and spectroscopically similar sheet-like J-aggregates of 5,5′,6,6′-tetrachloro-1,1′-diethyl-3,3′-di-(4-sulfobutyl)-benzimidacarbocyanine (TDBC), the respective thickness has also been measured by small-angle neutron scattering. Here a slightly smaller value of ~2 nm has been determined. Comparing both values one has to consider, however, that the present value is a quite rough estimate from averaging only a few cross-section profiles and potentially affected by Fresnel fringe effects [24]. On the other hand, the analysis of several TEM micrographs gave no indication for an assembly of the dye into bilayers or multilayers.

The sheet-like morphology of J-aggregates could also be confirmed by negative staining TEM. A representative micrograph is shown in Fig. 2b. Here, the overall morphology remained unchanged, while the layers and folds appeared bright due to the inverted contrast. Again, a vertical stacking of dye layers was not observed.

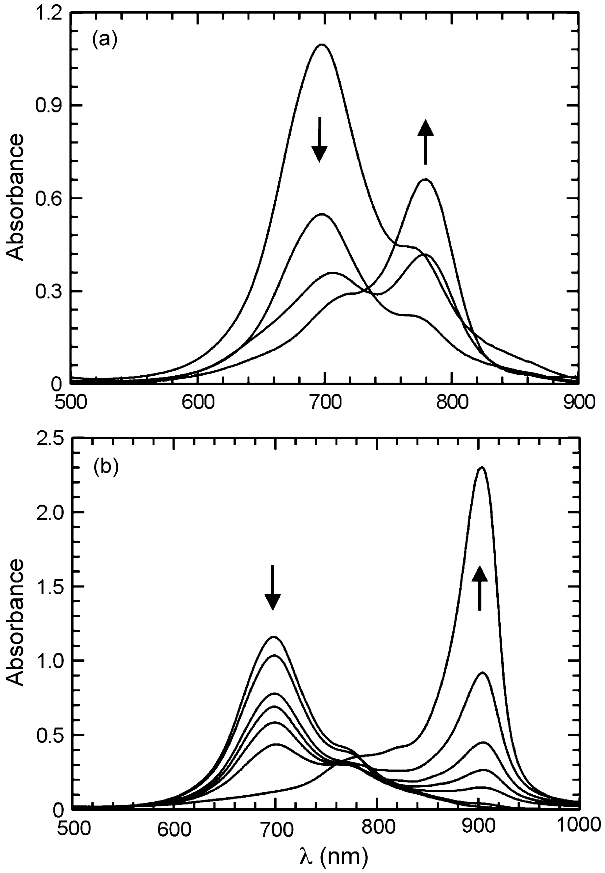


Fig. 3. (a) Change of absorption spectra of an 1-day-old 7×10^{-3} M aqueous ICG solution upon dilution with water. Arrows indicate the effect of decreasing dye concentration. Dye concentration (in M): 1×10^{-3}, 5×10^{-4}, 5×10^{-5}, 5×10^{-6}. The dashed curve represents the normalized absorption spectrum of a 1.28×10^{-4} M ICG solution in MeOH (molar extinction coefficient: $\varepsilon = 2.25 \times 10^5$ L/(mol cm)). (b) Change of absorption spectra of a 7×10^{-3} M aqueous ICG solution during storage at room temperature. Arrows indicate the effect of increasing storage time (in days): 1, 6, 8, 11, 14, 20, 47.

3.2. ICG

The absorbance of aqueous ICG solutions is known to depend strongly on the dye concentration [5,10]. Fig. 3a shows a family of absorption curves measured after stepwise dilution of a freshly prepared 7.0×10^{-3} M stock down to a concentration of 5.0×10^{-6} M. For comparison, the normalized absorption spectrum of a solution in MeOH is added to the figure. This methanolic solution showed again the typical monomer spectrum of the cyanine dye, consisting of a broad band located at 784 nm due to the 0-0 transition and a vibronic sub-band at 720 nm. Compared with that, all aqueous solutions showed spectra with two bands of different relative intensities located at around 697 and 779 nm, respectively. The appearance of the hypsochromically shifted band at 697 nm is caused by dye–dye interactions and reveals the presence of dimers or higher H-type aggregates [10]. The second band at 779 nm can be ascribed to monomers, that coexist with the H-aggregates in aqueous solutions. The general trend observed upon increasing dilution is the decrease of the portion of H-aggregates. One should note, however, that even at the lowest dye concentration of 5.0×10^{-6} M the absorbance at around 720 nm still exceeds

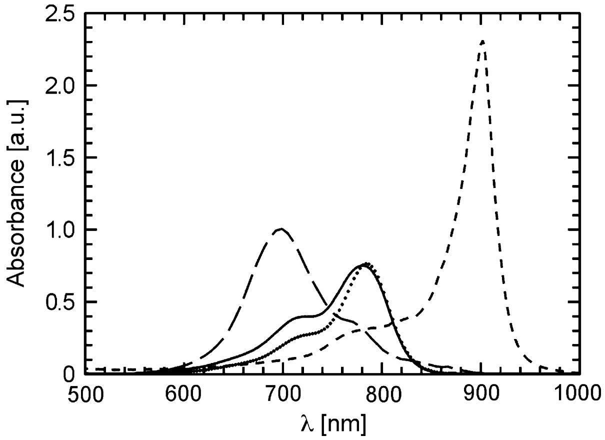


Fig. 4. Absorption spectra of a 7×10^{-3} M aqueous ICG solution 1 day (long dashes) and 3 months (short dashes) after preparation, respectively. The solid curve represents the absorption spectrum of an aqueous 6.4×10^{-4} M ICG solution containing 45 wt% of MeOH, while the dotted curve represents the monomer spectrum (measured in 100% MeOH) normalized to equal maximum absorbance.

that of pure monomers (dashed curve) by about 10%, i.e. a small population of H-aggregates is obviously still remaining.

At dye concentrations exceeding ~1.3×10^{-3} M the spectral behavior is more complex. A red-shifted band located at around 900 nm slowly evolved to became increasingly strong and narrow within several weeks of storage at room temperature [5,15]. Fig. 3b shows the measured family of absorption curves for a 7.0×10^{-3} M aqueous solution as a function of storage time. The reason for the spectral change is a slow structural transformation of the H-aggregates into J-aggregates. The process of transformation can be accelerated by a thermal treatment [15]. Just as in the case of coexisting H-aggregates and monomers at low dye concentration, one has to assume a dynamic equilibrium to exist between the different species. Thus, after several weeks a stable spectrum was obtained (see Fig. 3b), which showed a remarkable short-wavelength absorption tail, that is characterized by a clear shoulder at the position of the monomer band (780 nm) and still noticeable absorption at the position of the H-band; i.e. monomers, H- and J-aggregates obviously coexist. While the equilibrium between the different species can in principle be described by phenomenological models [15], a molecular model of the structural transition does not exist. This is due to the difficulties in obtaining quantitative structural data of the involved species.

To characterize the morphology of aggregates two 7.0×10^{-3} M ICG samples were prepared. One was vitrified within 1 day of preparation, the other after 3 months of storage. The absorption spectra, which are shown in Fig. 4, are dominated either by a strong H-band or a strong J-band (indicated by long or short dashes, respectively). For comparison a third sample was prepared, which mainly contained dye monomers. For that we added 45 wt% of MeOH to an aqueous 6.4×10^{-4} M ICG solution. The corresponding absorption spectrum is also added to Fig. 4 (solid line). Comparison with the normalized spectrum of monomers (ICG solution in 100% MeOH, dotted line in Fig. 4) points, indeed, to a very low portion of remaining H-aggregates against an overwhelming content of non-aggregated dye.

Representative cryo-TEM micrographs of the different solutions are presented in Fig. 5. The micrographs in Fig. 5a and b were obtained for the solution showing the dominant J-band. Two different morphologies are visible. Sheet-like aggregates which resemble by size and shape those already found for IR-140 (Fig. 5a and b) and very small particles appearing as dark spots of 2.1 ± 0.5 nm size (Fig. 5b). Folds of single sheets are also visible. The sheet's effective thickness estimated from the folds cross-section is of the same order of magnitude as in the case of IR-140 J-aggregates. A value of 3.0 ± 0.5 nm was determined. The micrograph shown in Fig. 5c was taken from the solution with the H-band dominated absorption spectrum. Again, small particles were found, but in addition thread-like assemblies of variable length with a diameter of 2.5 ± 0.5 nm (marked by arrows). They obviously represent flexible cylindrical aggregates and therefore can be easily discriminated from the folds of crumpled monolayers. Fig. 5d was obtained from the 45% methanol containing solution with a high content of monomers and a low portion of H-aggregates. Again, the small particles were detected.

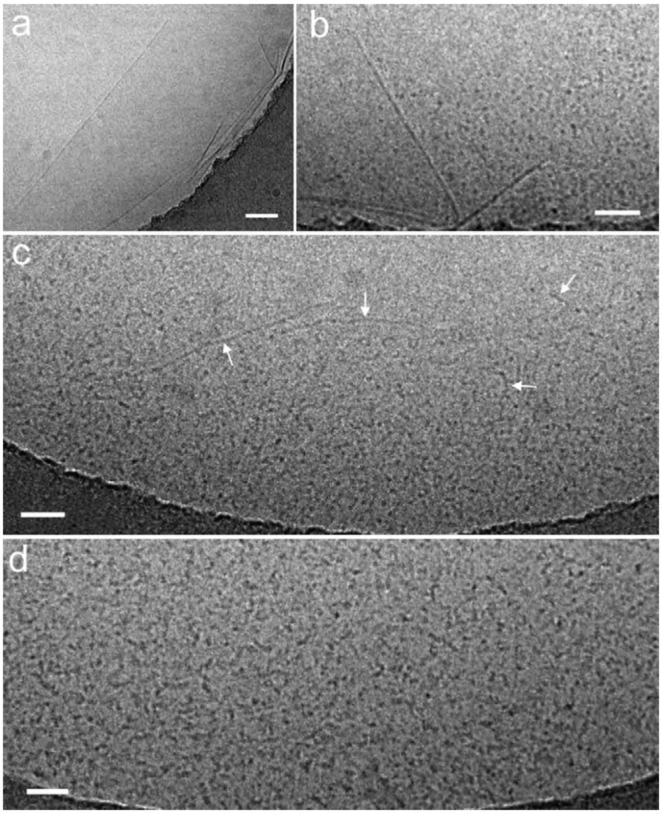


Fig. 5. Cryo-TEM micrographs of aqueous ICG solutions revealing J- and H-aggregates. (a and b) Within the 7×10^{-3} M ICG solution exhibiting a dominant J-band after 3 month of storage sheet-like J-aggregates and small H-aggregates coexist. (c) The freshly prepared 7×10^{-3} M ICG solution showing a strong H-band reveals H-aggregates of different sizes. The arrows mark thread-like particles. (d) The aqueous 6.4×10^{-4} M ICG solution containing 45 wt% of MeOH reveals H-aggregates. See Fig. 4 for the respective absorption spectra. Scale bars: 50 nm (a) and 30 nm (b–d).

The two different aggregate morphologies have also been detected by negative staining TEM. A representative micrograph shown in Fig. 6 reveals large sheet-like aggregates and flakes composed of granular material. The assembly of small particles into larger flakes is most likely an artifact due to the drying of samples for negative staining TEM. In the cryo-TEM preparation this effect was not observed.

4. Discussion

Both investigated NIR cyanine dyes form J-aggregates upon self-assembly in water, which are characterized by a single and narrow absorption band with a tail extending toward shorter wavelengths. The absorption spectra resemble that of other J-aggregating dyes, for example TDBC [31]. Hence, it is not surprising that the supramolecular architecture revealed by cryo-TEM of all the different J-aggregates is quite similar. The detected sheet-like aggregate morphology is consistent with the brickwork packing

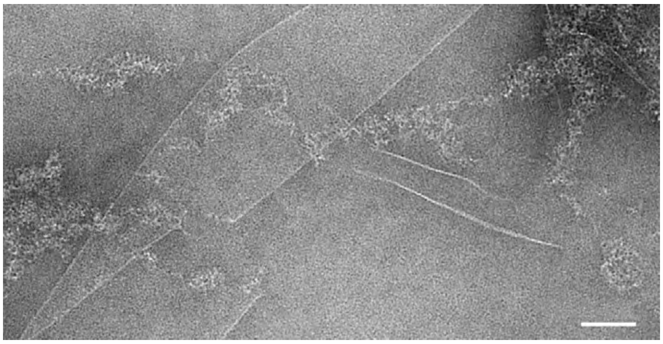


Fig. 6. The PTA-stained TEM micrograph of a 7 × 10^{-3} M aqueous ICG solution characterized by equally strong H- and J-bands 14 days after sample preparation reveals sheet-like J-aggregates and flakes of granular material. See Fig. 3b for the absorption spectrum. Scale bar: 50 nm.

model advanced by Kuhn and co-workers [43]. These authors modelled the J-aggregates by monomolecular layers of molecules, whose long axes lie parallel to the layer plane, as presented schematically in Scheme 2a. The chromophore is represented as a rigid "box" to which the two sulfobutyl groups are attached. For symmetry reasons the dye molecules are presumably arranged within the layers with their sulfobutyl groups uniformly pointing to both sides of the chromophore layer plane. To produce the red-shifted J-band, adjacent chromophores have to be arranged with large lateral displacements along the long molecular axis. It is true that the reported cryo-TEM results cannot yield data on the organization of molecules within the layer, but the results are, at least on supramolecular scale, consistent with the simple structure model.

A surprising finding is the appearance of a second population of mesoscopic dye aggregates coexisting with the J-aggregates in the case of ICG. The particularly wide and structured short-wavelength tail in the absorption spectrum indicates contributions from monomers and H-aggregates. Because dye monomers are too small to be resolved by cryo-TEM, the second species may be assigned to the H-aggregates. This interpretation is consistent with the observation, that a second species of different morphology has not been found for IR-140 and TDBC, which both do not form H-aggregates at all, and whose absorption spectra after aggregation indicate at best coexisting monomers. The ICG solution containing 45% of MeOH and whose absorption spectrum only comprises monomers and H-aggregates, on the other hand, shows the second species as well.

Recently Würthner and co-workers synthesized a class of merocyanine dyes, which self-aggregate to form mesoscopic H-aggregates in unpolar solvents. The supramolecular structure of some of these aggregates has been well characterized by several microscopic techniques [44–46]. The H-aggregates of cyanine dyes formed in water, however, have only scarcely been directly detected by structural investigations. To our knowledge there are only three studies demonstrating the formation of mesoscopic H-aggregates [24,47,48]. It is a widely held opinion that H-aggregates consist of only a few molecules. The dimer composed of two parallel aligned molecules (Scheme 2b) is the simplest model of an H-aggregate, but also untilted (Scheme 2c) or less tilted stacks of more than two molecules are feasible models of H-aggregates [49]. The estimated size of about 2 nm for the smallest particles points to oligomers consisting of few molecules or short molecular stacks, but just as in the case of J-aggregates cryo-TEM cannot yield data on the aggregates internal organization. At high dye concentration (Fig. 5b) only a few larger and threadlike aggregates are found. This behavior differs qualitatively from that of many other quasi-one dimensional dye aggregates, which show a high tendency toward linear growth [20], for example, the 1,1′-diethyl-2,2′-cyanine chloride (pseudoisocyanine chloride) that forms quasi-endless linear J-aggregates immediately after the beginning of aggregation [50].

In conclusion, we demonstrated that the two NIR dyes IR-140 and ICG form sheet-like monolayered J-aggregates of mesoscopic size upon self-assembly in aqueous solution. The two-dimensional organization of chromophors into soft molecular layers, has proved once more to be a general building principle of many cyanine dye J-aggregates. The early structure model of ICG J-aggregates supposing sub-micrometer sized loosely packed porous particles, however, cannot be confirmed. The H-aggregates dominating in freshly prepared aqueous ICG solutions are resolved by electron microscopy, as well. They turn out to be linear aggregates of broad size distribution that ranges from small oligomer particles up to tens of nanometers long thread-like aggregates.

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References

[1] J. Fabian, H. Nakazumi, M. Matsuoka, Near-infrared adsorbing dyes, Chem. Rev. 92 (1992) 1197–1226.
[2] S. Daehne, U. Resch-Genger, O.S. Wolfbeis (Eds.), Near-infrared Dyes for High Technology Applications, NATO ASI Series, 3. High Technology, 52, Kluwer Academic, Dordrecht, 1998.
[3] J.O. Escobedo, O. Rusin, S. Lim, R.M. Strongin, NIR dyes for bioimaging applications, Curr. Opin. Chem. Biol. 14 (2009) 1–7.
[4] J.P. Webb, F.G. Webster, B.E. Plourde, Sixteen new IR laser dyes, IEEE J. Quant. Electron. 11 (1975) 114–119.
[5] M.L.J. Landsman, G. Kwant, G.A. Mook, W.G. Zijlstra, Light-absorbing properties, stability, and spectral stabilization of Indocyanine green, J. Appl. Physiol. 40 (1976) 575–583.
[6] K. Takechi, P.K. Sudeep, P.V. Kamat, Harvesting infrared photons with tricarbocyanine dye clusters, J. Phys. Chem. B 110 (2006) 16169–16173.
[7] R. Weigand, H. Crespo, R. Sastre, Study of the broad-band saturable absorption of Indocyanine green J-aggregates in polymeric films using 10-fs laser pulses, Appl. Phys. B 82 (2006) 303–308.
[8] M.Y. Berezin, H. Lee, W. Akers, S. Achilefu, Near infrared dyes as lifetime solvatiochromic probes for micropolarity measurements of biological systems, Biophys. J. 93 (2007) 2892–2899.
[9] T. Goswami, S.K.K. Kumar, A. Dutta, D. Goswami, Probing the ultrafast solution dynamics of a cyanine dye in an organic solvent interfaced with water, J. Phys. Chem. B 113 (2009) 16332–16336.
[10] R. Philip, A. Penzkofer, W. Bäumler, R.M. Szeimies, C. Abels, Absorption and fluorescence spectroscopic investigation of Indocyanine green, J. Photochem. Photobiol. A: Chem. 96 (1996) 137–148.
[11] E.E. Jelly, Spectral absorption and fluorescence of dyes in the molecular state, Nature 138 (1936) 1009–1010.
[12] G. Scheibe, Über die Veränderlichkeit der Absorptionsspektren in Lösung und die van der Waalsschen Kräfte als ihre Ursache, Angew. Chem. 50 (1937) 51.
[13] D. Möbius, Scheibe aggregates, Adv. Mater. 7 (1995) 437–444.
[14] T. Kobayashi, J-Aggregates, World Scientific, Singapore, 1996.
[15] F. Rotermund, R. Weigand, A. Penzkofer, J-aggregation and disaggregation of Indocyanine green in water, Chem. Phys. 220 (1997) 385–392.
[16] R. Weigand, F. Rotermund, A. Penzkofer, Aggregation dependent absorption reduction of Indocyanine green, J. Phys. Chem. A 101 (1997) 7729–7734.
[17] R. Weigand, F. Rotermund, A. Penzkofer, Degree of aggregation of Indocyanine green in aqueous solutions determined by Mie scattering, Chem. Phys. 220 (1997) 373–384.
[18] J. Zweck, A. Penzkofer, Microstructure of Indocyanine green J-aggregates in aqueous solution, Chem. Phys. 269 (2001) 399–409.
[19] H. von Berlepsch, C. Böttcher, L. Dähne, Structure of J-aggregates of pseudoisocyanine dye in aqueous solution, J. Phys. Chem. B 104 (2000) 8792–8799.
[20] H. von Berlepsch, C. Böttcher, A. Ouart, C. Burger, S. Dähne, S. Kirstein, Supramolecular structures of J-aggregates of carbocyanine dyes in solution, J. Phys. Chem. B 104 (2000) 5255–5262.
[21] H. von Berlepsch, M. Regenbrecht, S. Dähne, S. Kirstein, C. Böttcher, Surfactant-induced separation of stacked J-aggregates. Cryo-transmission electron microscopy studies reveal bilayer ribbons, Langmuir 18 (2002) 2901–2907.
[22] H. von Berlepsch, S. Kirstein, C. Böttcher, Supramolecular structure of J-aggregates of a sulfonate substituted amphiphilic carbocyanine dye in solution: methanol-induced ribbon-to-tubule transformation, J. Phys. Chem. B 108 (2004) 18725–18733.
[23] H. von Berlepsch, S. Kirstein, R. Hania, A. Pugžlys, C. Böttcher, Modification of the nanoscale structure of the J-aggregate of a sulfonate-substituted amphiphilic carbocyanine dye through incorporation of surface-active additives, J. Phys. Chem. B 111 (2007) 1701–1711.

[24] H.V. Berlepsch, E. Brandenburg, B. Koksch, C. Böttcher, Peptide adsorption to cyanine dye aggregates revealed by cryo-transmission electron microscopy, Langmuir doi:10.1021/la100944d.
[25] J. Monhanty, D.K. Palit, J.P. Mittal, Photophysical properties of two infrared laser dyes-IR-144 and IR-140: a picosecond laser flash photolysis study, Proc. Ind. Natl. Sci. Acad., Part A 66 (2000) 303–315.
[26] H. Mustroph, private communication.
[27] H. Mustroph, K. Reiner, J. Mistol, S. Ernst, D. Keil, L. Hennig, Relationship between the molecular structure of cyanine dyes and the vibrational fine structure of their electronic absorption spectra, ChemPhysChem 10 (2009) 835–840.
[28] A.H. Herz, Dye-dye interactions of cyanines in solution and at AgBr surfaces, Photogr. Sci. Eng. 18 (1974) 323–335.
[29] H. Hada, C. Honda, H. Tanemura, Spectroscopic study on the J-aggregate of cyanine dyes. I. Spectral changes of UV bands concerned with J-aggregate formation, Photogr. Sci. Eng. 21 (1977) 83–91.
[30] A. Pawlik, A. Ouart, S. Kirstein, H.-W. Abraham, S. Daehne, Synthesis and UV/Vis spectra of J-aggregating 5,5′,6,6′-tetrachlorobenzimidacarbocyanine dyes for artificial light-harvesting systems and for asymmetrical generation of supramolecular helices, Eur. J. Org. Chem. (2003) 3065–3080.
[31] S. Makio, N. Kanamura, J. Tanaka, The J-aggregate 5,5′,6,6′-tetrachloro-1,1′-diethyl-3,3′-bis(4-sulfobutyl)-benzimidazolocarbocyanine sodium salt in aqueous solution, Bull. Chem. Soc. Jpn. 53 (1980) 3120–3124.
[32] I.A. Struganova, H. Lim, S.A. Morgan, The influence of inorganic salts and bases on the formation of the J-band in the absorption and fluorescence spectra of the diluted aqueous solutions of TDBC, J. Phys. Chem. B 106 (2002) 11047–11050.
[33] H. Yao, M. Omizo, N. Kitamura, Mesoscopic string structures of thiacyanine J aggregates in solution, Chem. Commun. (2000) 739–740.
[34] H. Yao, T. Isohashi, K. Kimura, Electrolyte-induced mesoscopic aggregation of thiacarbocyanine dye in aqueous solution: counterion size specificity, J. Phys. Chem. B 111 (2007) 7176–7183.
[35] T.D. Slavnova, A.K. Chibisov, H. Görner, Kinetics of salt-induced J-aggregation of cyanine dyes, J. Phys. Chem. A 109 (2005) 4758–4765.
[36] A. Gil, D. Möbius, I. Sández, A. Suárez, Different J-aggregates of an amphiphilic cyanine dye in monolayers at the air–water interface, Langmuir 19 (2003) 6430–6435.
[37] N. Vranken, M. Van der Auweraer, F.C. De Schryver, H. Lavoie, P. Bélanger, C. Salesse, Influence of molecular structure on the aggregation properties of thiacarbocyanine dyes adsorbed to Langmuir films at the air–water interface, Langmuir 16 (2000) 9518–9526.
[38] N. Kato, K. Saito, Y. Uesu, Structural phase transition of merocyanine J-aggregate induced by ion-recombination in the aqueous sub-phase, Chem. Phys. Lett. 326 (2000) 395–399.
[39] K. Tani, C. Ito, Y. Hanawa, M. Uchida, K. Otaguro, H. Horiuchi, H. Hiratsuka, Photophysical property and photostability of J-aggregate thin films of thiacyanine dyes prepared by the spin-coating method, J. Phys. Chem. B 112 (2008) 836–844.
[40] A.S. Davydov, Theory of Molecular Excitons, Plenum Press, New York, 1971.
[41] V. Czikkely, H.D. Försterling, H. Kuhn, Extended dipole model for aggregates of dye molecules, Chem. Phys. Lett. 6 (1970) 207–210.
[42] D. Danino, Y. Talmon, R. Zana, Cryo-TEM of thread-like micelles: on-the-grid microstructural transformations induced during specimen preparation, Colloid Surf. A: Physicochem. Eng. Aspects 169 (2000) 67–73.
[43] V. Czikkely, H.D. Försterling, H. Kuhn, Light absorption and structure of aggregates of dye molecules, Chem. Phys. Lett. 6 (1970) 11–14.
[44] S. Yao, U. Beginn, T. Gress, M. Lysetska, F. Würthner, Supramolecular polymerization and gel formation of bis(merocyanine) dyes driven by dipolar aggregation, J. Am. Chem. Soc. 126 (2004) 8336–8348.
[45] A. Lohr, M. Lysetska, F. Würthner, Supramolecular stereomutation in kinetic and thermodynamic self-assembly of helical merocyanine dye nanorods, Angew. Chem. Int. Ed. 44 (2005) 5071–5074.
[46] A. Lohr, T. Gress, M. Deppisch, M. Knoll, F. Würthner, Synthesis of merocyanine dye nanorods: the importance of solvent, kinetic and thermodynamic control, and steric effects on self-assembly, Synthesis No 19 (2007) 3073–3082.
[47] E.S Emerson, M.A. Conlin, A.E. Rosenoff, K.S. Norland, H. Rodriguez, D. Chin, G.R. Bird, The geometrical structure and absorption spectrum of a cyanine dye aggregate, J. Phys. Chem. 71 (1967) 2396–2403.
[48] H. Yao, K. Domoto, T. Isohashi, K. Kimura, In situ detection of birefringent mesoscopic H and J aggregates of thiacarbocyanine dye in solution, Langmuir 21 (2005) 1067–1073.
[49] M. Kasha, H.R. Rawls, M.A. El-Bayoumi, The exciton model in molecular spectroscopy, Pure Appl. Chem. 11 (1965) 371–392.
[50] H. von Berlepsch, C. Böttcher, Network superstructure of pseudoisocyanine J-aggregates in aqueous sodium chloride solution revealed by cryo-transmission electron microscopy, J. Phys. Chem. B 106 (2002) 3146–3150.