J-aggregation of 5, 10, 15, 20-tetraphenyl-21H, 23H-porphinetetrasulfonic acid in a molecular crowding environment simulated using dextran

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Abstract

In a molecular crowding environment, different thermodynamics is often observed in a dilute solution. One such example is the promotion of the formation of amyloids, which are causal agents of Alzheimer’s disease. Although a considerable number of molecular crowding studies have been reported, its effect remains unclear. In this study, we investigated a J-aggregation of a porphyrin derivative, 5, 10, 15, 20-tetraphenyl-21H,23H-porphinetetrasulfonic acid (TPPS), in a molecular crowding environment simulated by dextran (Dex) in $\text{HClO}_4$, HCl, and NaCl solutions. The changes in the number of monomers in the J-aggregate ($n$) with the concentration of Dex ($C_{\text{Dex}}$) depended on the type of solution. No change in $n$ was observed in the NaCl solution, which indicated that the Dex solution did not affect the J-aggregation because of the ionic strength effect. In the HCl solution, the aggregation behavior changed with the pH. Further, at a low pH, the electrostatic interactions promoted J-aggregation by the volume exclusion of Dex, while the aggregation was suppressed at a high pH owing to steric hindrance. A different aggregation mechanism, involving the hydrogen bonding between NH in the center of the TPPS macrocyclic frame and the SO$_3$H and ClO$_4^-$ functional groups, was responsible for the J-aggregation in the HClO$_4$ solution. Moreover, the $n$ value increased owing to the volume exclusion effect. We expect that this study will be useful for further elucidation of the molecular crowding effect.

Keywords Molecular crowding · J-aggregation · Dextran

Introduction

Molecular crowding environments, which are omnipresent in biological cells, have attracted the attention of researchers working in the biochemical field. This is because the thermodynamic and kinetic reactions in such systems are different from those in dilute solutions [1–8]. A molecular crowding environment consists of many biological molecules, such as DNAs, RNAs, proteins, carbohydrates, and lipids. When inert molecules in a cell exclude reactant molecules, the volume occupied by the reactant molecules changes, which influences their reactivity in the cell. Liang et al. reported that the mixed macromolecule crowding agents bovine serum albumin (BSA) and Ficoll inhibited the amyloid formation of lysozyme [9]. Zinchenko et al. demonstrated that a DNA molecule and chromatin fibril were compressed into a globular form by concentrated BSA [10]. Therefore, determining the effect of molecular crowding on the reactivity in the cell is essential for elucidating complex biological reactions.

To simplify the molecular crowding system, reagents that mimic molecular crowding, such as polyethylene glycol (PEG), dextran (Dex), and Ficoll, have been used. Using these reagents, various molecular crowding effects such as the excluded volume effect [11–13], osmotic pressure [14, 15], and structural changes [16, 17] have been proposed. The molecular crowding environment limits the occupied volume of the reactant molecules, which increases their effective concentration. Minton proposed a relation between the activity of the reactant molecules and the excluded volume [18, 19]. Highly concentrated macromolecules (i.e., molecular crowding reagents) lower the osmotic pressure of the solution. This indicates that...
water activity decreases with the increasing concentration of the molecular crowding reagents. Sugimoto et al. revealed that DNA G-quadruplex formation was stabilized due to a change in the osmotic pressure, although destabilization of the DNA duplex was observed [17]. They summarized changes in hydration number due to molecular crowding in DNA–protein interactions [6]. The conformation of molecules such as proteins is influenced by contact between the molecules and the molecular crowding reagents, which in turn changes their enzymatic activity. Cheung et al. reported that the structure of phosphoglycerate kinase was stabilized at an optimized concentration of Ficoll and that it was approximately 15 times more active than in the dilute solution [20]. Although many researchers have contributed to molecular crowding studies, some questions remain unanswered.

Structural changes in the molecular crowding environment make it difficult to elucidate the molecular crowding effect because the thermodynamic and kinetic expressions for these structural changes are difficult to decipher [21]. Therefore, it is important to use rigid molecules that do not undergo any structural changes. In our previous study, the complexation reaction of 8-hydroxyquinoline-5-sulfonic acid with metal ions was promoted by the addition of a highly concentrated PEG solution due to the increase in reactant activity and the decrease in water activity [22]. We revealed that the acid dissociation behavior of 8-hydroxyquinoline-5-sulfonic acid in the PEG solution changed due to the contribution of the water activity [23]. These previous reports indicated that the use of a rigid molecule is useful for understanding the biochemical reactions in the molecular crowding environment.

Amyloid fibrils, formed inside or outside the cells, cause Alzheimer’s, Parkinson’s, and prion diseases [24–27]. These amyloid fibrils are generated when the unfolded proteins with β-sheet structures accumulate. In general, the β-sheet aggregation of proteins occurs through the hydrogen bonding between the amino acids [28]. Though the promotion and suppression of the amyloid fibril formation were reported in the molecular crowding environment such as volume exclusion and structural change [9, 29–31], the structural change of proteins may cause misunderstandings.

In analytical chemistry, the molecular crowding effect has recently received a lot of attentions. The increase in the complexation constant in the molecular crowding environment leads to the high sensitivity in a sensing system. For example, Actis et al. demonstrated a 1000-fold increase in the molecular count of proteins and sixfold increase in the peak current for DNA by the molecular crowding in the nanopore sensing system [32]. Sasaki et al. reported a 5.5-fold increase in the sensitivity of DNA padlock rolling circle amplification in the molecular crowding environment [33]. Although the molecular crowding effect is useful for the increase in the sensitivity of the sensing system, the detailed mechanism remains unclear.

In the present study, we investigated the J-aggregation behavior of 5, 10, 15, 20-tetraphenyl-21H,23H-porphine-tetrasulfonic acid (TPPS) in a molecular crowding environment. TPPS is one of the most popular porphyrin derivatives known to form J-aggregates [34–37]. Since TPPS is also a rigid molecule with a planar π-conjugate framework, it can be used as a model molecule for aggregation in molecular crowding. Dex was selected as the molecular crowding reagent over PEG as PEG changed the acid dissociation constant of molecules [23], whereas Dex did not. The J-aggregation of TPPS depends on the pH and ionic strength [38]. Thus, the dependence of the J-aggregation behavior on the Dex concentration was investigated in HClO₄, HCl, and NaCl solutions.

**Experimental section**

Dex, with an average molecular weight of 40,000, was used as the molecular crowding reagent. TPPS (Fig. S1) was purchased from Funakoshi Co. Ltd. (Tokyo, Japan). Sodium chloride, hydrogen chloride, and Dex were purchased from Fujifilm Wako Pure Chemicals Co. Ltd. (Osaka, Japan). Perchloric acid (HClO₄) was purchased from Kanto Chemical Co. Inc. (Tokyo, Japan).

The absorption spectra of TPPS at various concentrations (C_{TPPS} = 4, 5, 6, 7, 8, and 9 μM) were measured using a JASCO V-730 instrument and a cell with a path length of 0.1 cm. The prepared TPPS sample was allowed to stand overnight to stably form the J-aggregate. To simulate the molecular crowding environment, dextran solutions of 7.5, 10, 12.5, and 15w/% were prepared. The molar absorption coefficients of TPPS at 435 nm (ε₄₃₅) in the 0, 7.5, 10, 12.5, and 15w/% dextran solutions (C_{Dex}) were determined to be 4.80 × 10^5, 4.61 × 10^5, 4.20 × 10^5, 4.19 × 10^5, and 4.10 × 10^5 L mol⁻¹ cm⁻¹, respectively. The pH value and ionic strength affected the J-aggregation of TPPS. Thus, the absorption spectra of TPPS at various concentrations of NaCl (C_{NaCl}), HCl (C_{HCl}), and HClO₄ (C_{HClO₄}) were acquired. The following are the concentrations used: C_{NaCl} = 0.100, 0.150, and 0.200 M; C_{HCl} = 0.100, 0.130, and 0.200 M; C_{HClO₄} = 0.580, 0.116, and 0.232 M. Although the pH changed slightly owing to the addition of Dex, the difference was within ±0.05.

**Results and discussion**

**J-aggregation of TPPS in the dilute solution**

The absorption spectra of the TPPS solutions without Dex were measured to observe the J-aggregation. Figure 1 shows...
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...the absorption spectra of TPPS in the NaCl, HCl, and HClO$_4$ solutions, four characteristic peaks at 435, 492, 645, and 707 nm were observed. In particular, two sharp and intense peaks were obtained at 492 and 707 nm at high $C_{TPPS}$, which is attributed to the formation of J-aggregate [39]. On the other hand, the peaks obtained at 435 and 645 nm corresponded to the Soret and Q bands, respectively [39], resulting from the dianion of TPPS that is protonated at the nitrogen centers of the macrocycle at pH less than 4 [40]. Although the formation of J-aggregate was observed for all the solutions, the change in the absorbance varied with increasing $C_{TPPS}$. This indicated that the formation of J-aggregate in the aqueous solution is influenced by the pH and ionic strength.

Figure 2 shows the dependence of the absorption spectrum of the 4 μM TPPS solution on $C_{NaCl}$, $C_{HCl}$, and $C_{HClO_4}$. It was observed that as the concentrations, $C_{NaCl}$, $C_{HCl}$, and $C_{HClO_4}$ increased, the absorbances at 435 and 645 nm decreased, while the absorbances at 492 and 707 nm increased. This indicated that the TPPS monomer aggregates in all the solutions. Moreover, the isosbestic point at 662 nm suggested that the formation of J-aggregate for TPPS is the stoichiometric reaction [38]. In Fig. 2, it is shown that the J-aggregation behavior in $C_{NaCl}$, $C_{HCl}$, and $C_{HClO_4}$ is different; hence, it depends on the chemical species present in the solution. J-aggregation of TPPS occurred via π-interactions and due to the electrostatic interactions between the central nitrogen in the macrocyclic frame of TPPS and the deprotonated sulfonic acid group. Thus, the ionic strength and pH play important roles in J-aggregation because the decrease in pH may cause the protonation of the sulfonic acid group and the ionic strength influences the electrostatic interactions.

Mataga proposed a method for determining the number of monomers ($n$) in a polymer [40]. When only the monomer...
and polymer are present in the solution, the equilibrium constant, \( K \), for the equilibrium between the monomer and polymer is expressed by the following equation:

\[
K = \frac{x}{nC_M^{n-1}(1-x)^x} \tag{1}
\]

where \( x \) is the molar fraction of the polymer against the total quantity of monomer and polymer, and \( C_M \) is the concentration of the monomer. The molar extinction coefficients for the monomer and polymer are denoted as \( \varepsilon^M \) and \( \varepsilon^P \), respectively; the apparent \( \varepsilon \) (\( \varepsilon^{app} \)) at the monomer peak can be written as

\[
\varepsilon^{app} = \frac{1}{n}x\varepsilon^P + (1-x)\varepsilon^M \tag{2}
\]

From Eqs. (1) and (2), we obtain,

\[
\log \left( C_M \left( 1 - \frac{\varepsilon^{app}}{\varepsilon^M} \right) \right) = \log(AK) + n \log \left( C_M \frac{\varepsilon^{app}}{\varepsilon^M} - \frac{\varepsilon^P}{n^M} \right) \tag{3}
\]

\[
A = \frac{1}{n^p \left( n - \frac{\varepsilon^P}{\varepsilon^M} \right)^{n-1}}. \tag{4}
\]

Since \( \frac{\varepsilon^P}{n^M} \) is negligible as compared to \( \frac{\varepsilon^{app}}{\varepsilon^M} \), the following equation is obtained,

\[
\log \left( C_M \left( 1 - \frac{\varepsilon^{app}}{\varepsilon^M} \right) \right) = \log(AK) + n \log \left( C_M \frac{\varepsilon^{app}}{\varepsilon^M} \right) \tag{5}
\]

Equation (5) indicates that the \( n \) and \( K \) values can be determined by plotting graphs between \( \log \left( C_M \left( 1 - \frac{\varepsilon^{app}}{\varepsilon^M} \right) \right) \) and \( \log \left( C_M \frac{\varepsilon^{app}}{\varepsilon^M} \right) \).

Figure 3 shows the graphical relationship between \( \log \left( C_M \left( 1 - \frac{\varepsilon^{app}}{\varepsilon^M} \right) \right) \) and \( \log \left( C_M \frac{\varepsilon^{app}}{\varepsilon^M} \right) \) in the NaCl, HCl, and HClO4 solutions. It should be noted that the \( \varepsilon_{135} \) value described in the experimental section was used as the \( \varepsilon^M \) value. Linear relationships were obtained for all the solutions. These graphs were analyzed using Eq. (5), and the \( n \) and \( K \) values were obtained as follows: \( n = 4.3 \) and \( K = 4.6 \times 10^{18} \text{ M}^{-1} \) for NaCl, \( n = 4.9 \) and \( K = 1.9 \times 10^{21} \text{ M}^{-20} \) for HCl, and \( n = 8.8 \) and \( K = 9.4 \times 10^{41} \text{ M}^{-40} \) for HClO4. Kobayashi et al. reported that the \( n \) value of TPPS J-aggregates was 11 using these parameters: \( C_{\text{TPPS}} = 1.88-8.98 \text{ µM} \) and \( C_{\text{HClO4}} = 0.092 \text{ M} \) [42]. The experimental \( n \) value was almost the same as the reference value. Notably, the \( n \) value depends on the concentration range of \( C_{\text{TPPS}} \) and the solution composition [42].

Figure S2 shows the dependence of the relation between \( \log \left( C_M \left( 1 - \frac{\varepsilon^{app}}{\varepsilon^M} \right) \right) \) and \( \log \left( C_M \frac{\varepsilon^{app}}{\varepsilon^M} \right) \) on \( C_{\text{NaCl}}, C_{\text{HCl}}, \) and \( C_{\text{HClO4}} \). The \( n \) and \( K \) values obtained in this study are summarized in Tables 1, 2, 3. In the NaCl solution, the largest \( n \) value (\( = 5.1 \)) was obtained at \( C_{\text{NaCl}} = 0.150 \text{ M}. \) Perry et al. investigated the effect of salt concentration on the coacervation of vinyl polyelectrolytes and reported that the critical concentration of the phase separation was \( C_{\text{NaCl}} = 100-200 \text{ mM} \) [43]. The phase separation was
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suppressed at concentration values higher than this critical concentration. Leon et al. demonstrated that hydrophobic interactions were dominant for phenylalanine aggregation at high salt concentrations (> 250 mM), while π-interactions and electrostatic interactions such as charge-charge interactions played an important role in the aggregation at low salt concentrations (≤ 250 mM) [44]. These previous reports indicated that polymer aggregation occurs at a critical concentration of the solution, which is proposed to be \( C_{\text{NaCl}} = 150 \) mM for the J-aggregation of TPPS in the present study.

On the other hand, \( n \) decreased with increasing \( C_{\text{HCl}} \) and \( C_{\text{HClO}_4} \). However, the absorbance at 492 nm increased with increasing \( C_{\text{HClO}_4} \) and \( C_{\text{HCl}} \), as shown in Fig. 2. These results indicated that the total concentration of J-aggregate increased, although increasing the pH caused a decrease in the \( n \) value. The J-aggregate is formed via π-interactions and electrostatic interactions between the sulfonic group and nitrogen at the cationic center in TPPS. The \( pK_a \) values of the central nitrogen and benzenesulfonic acid were reported to be 4.9 [43] and –0.8 [45], respectively. Therefore, the sulfonic acid groups were partially protonated at \( C_{\text{HCl}} = 0.100–0.200 \) and \( C_{\text{HClO}_4} = 0.100–0.200 \) M while the central nitrogen was completely protonated. Since the protonation of the sulfonic acid group makes the J-aggregation by electrostatic interactions difficult, the \( n \) values decrease with increasing \( C_{\text{HClO}_4} \) and \( C_{\text{HCl}} \). The increase in the total concentration of J-aggregate in Fig. 2 indicated that many such J-aggregates with short lengths were formed instead of longer J-aggregates. Hence, we demonstrated J-aggregation of TPPS in NaCl, HCl, and HClO₄ solutions.

### J-aggregation behavior of TPPS in the molecular crowding environment

The J-aggregation behavior of TPPS was investigated in a molecular crowding environment. Figure S3 shows the dependence of the absorption spectra of 4 μM TPPS in NaCl, HCl, and HClO₄ solutions on the value of \( C_{\text{Dex}} \). The monomer absorbance (435 nm) decreased with increasing \( C_{\text{Dex}} \), while the absorbance of the J-aggregate (492 nm) increased, although the increasing order did not necessarily coincide with that of \( C_{\text{Dex}} \). One of the reasons for this decrease in the monomer absorbance could have been the decrease in \( \varepsilon_{435} \) with \( \varepsilon_{435} = 4.80 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1} \) at \( C_{\text{Dex}} = 0\% \) and \( \varepsilon_{435} = 4.10 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1} \) at \( C_{\text{Dex}} = 15\% \). Steer et al. revealed that the \( \varepsilon \) ratio of monomer to dimer for thiophosgene was 3.12 at 280 nm [46]. Belay showed that the \( \varepsilon \) value for caffeic acid dimer was 1.95 times larger than that for monomer in aqueous solution, while in acetonitrile, 0.40 times reduction was observed [47]. These results indicated that \( \varepsilon \) for polymer depends on the number of monomers and the solvent. Therefore, it was difficult to derive the changes in the concentrations of the monomer and J-aggregate from the spectral changes in Figure S3 because the change in \( C_{\text{Dex}} \) influences both the solvent properties and \( n \) value.

Figure S4 shows the absorbance spectra normalized at 491 nm in the NaCl, HCl, and HClO₄ solutions. Interestingly, the peak width for the HClO₄ solution increased with the addition of Dex, whereas no change in the peak width was observed for the NaCl solution. According to the exciton theory, energy gap between S0 and S1 changes based on the tilt angle of the J-aggregate [48]. Therefore, peak broadening of the J-aggregate indicated that many

### Table 2
Summary of the \( n \) and \( K \) values for the J-aggregation of TPPS in HCl solution

| \( C_{\text{HCl}} / M \) | \( C_{\text{Dex}} / \text{w/v\%} \) | \( n \) | \( \log K \) |
|----------------------|-----------------|------|-------|
| 0.100                | 0               | 4.9  | 21    |
| 7.5                  | 5.6             | 25   |
| 10                   | 5.9             | 26   |
| 12.5                 | 6.3             | 27   |
| 15                   | 6.6             | 31   |
| 0.130                | 0               | 4.5  | 20    |
| 7.5                  | 4.9             | 22   |
| 10                   | 4.4             | 19   |
| 12.5                 | 3.7             | 15   |
| 15                   | 3.3             | 13   |
| 0.200                | 0               | 3.3  | 13    |
| 7.5                  | 3.1             | 12   |
| 10                   | 2.7             | 9.8  |
| 12.5                 | 2.3             | 7.7  |
| 15                   | 2.9             | 11   |

### Table 3
Summary of the \( n \) and \( K \) values for the J-aggregation of TPPS in HClO₄ solution

| \( C_{\text{HClO}_4} / M \) | \( C_{\text{Dex}} / \text{w/v\%} \) | \( n \) | \( \log K \) |
|---------------------------|-----------------|------|-------|
| 0.116                     | 0               | 8.8  | 42    |
| 7.5                       | 5.9             | 27   |
| 10                        | 7.2             | 33   |
| 12.5                      | 8.6             | 41   |
| 15                        | 8.9             | 43   |
| 0.232                     | 0               | 6.9  | 33    |
| 7.5                       | 4.1             | 18   |
| 10                        | 5.0             | 22   |
| 12.5                      | 5.2             | 23   |
| 15                        | 5.4             | 24   |
| 0.580                     | 0               | 6.1  | 28    |
| 7.5                       | 4.8             | 21   |
| 10                        | 6.5             | 30   |
| 12.5                      | 6.6             | 31   |
| 15                        | 8.0             | 39   |

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J-aggregate molecules with various tilted angles were present in the HClO₄ solution, which is induced by Dex. In contrast, no change in the peak width in the NaCl solution indicated that monodisperse J-aggregates with the same tilted angle were formed.

Figures S5-S7 show relationships between 
\[ \log \left( C_M \left( 1 - \frac{C_M}{C_D} \right) \right) \]
and 
\[ \log \left( C_M \left( \frac{C_M}{C_D} \right) \right) \]
for NaCl, HCl, and HClO₄ solutions including Dex. All the relationships represent good linearity (correlation coefficient, \( r > 0.97 \)). These linear relationships were shifted to the upper left expect for the case of \( C_{NaCl} = 0.150 \) M. This indicated that the \( c^{app} \) values become smaller at high \( C_{NaCl} \), \( C_{HCl} \), and \( C_{HClO₄} \). Since the \( c_{435}^{app} \) value in each Dex solution was used as \( c^M \), this shift suggested that the concentration of TPPS monomer decreases with increasing \( C_{NaCl} \), \( C_{HCl} \), and \( C_{HClO₄} \). Therefore, the decreased amount of TPPS monomer should cause an increase in the \( n \) value and J-aggregate concentration. The relationships between the \( n \) and \( K \) values were analyzed based on Eq. (5), as shown in Figures S5-S7, and the obtained \( n \) and \( K \) values were added to Tables 1, 2, 3.

The \( n \) and \( K \) values clearly changed by the addition of Dex. The dissolution of solutes such as Dex at high concentrations may cause a change in the polarity of the solution, which may change the J-aggregation behavior. Thus, the polarity of the Dex solution was investigated using dansyl acid, which is often used as a solvatochromic dye [49]. When dansyl acid is added to the hydrophobic solution, the fluorescence wavelength shifts to a shorter wavelength. Figure S8 shows the fluorescence spectrum of dansyl acid in the Dex solution. An extremely small wavelength shift was observed: from 511.5 nm at \( C_{Dex} = 0\text{w/v}\% \) to 509.8 nm at \( C_{Dex} = 15\text{w/v}\% \); this indicated that the dissolution of Dex did not change the polarity of the solution. Therefore, we concluded that the changes in the \( n \) and \( K \) values resulted from other factors, such as the molecular crowding effect.

Figure 4 shows the relationship between \( C_{Dex} \) and \( n \) in the NaCl, HCl, and HClO₄ solutions. The change in the value of \( n \) seemed discontinuous, at \( C_{Dex} = 0-7.5\text{w/v}\% \). The addition of Dex may cause a significant change in the solvent properties. The difference in the fluorescence wavelength of dansyl acid between \( C_{Dex} = 0 \) and \( 7.5\text{w/v}\% \) (1.1 nm) was larger than that between \( C_{Dex} = 7.5 \) and \( 15\text{w/v}\% \) (0.6 nm). Therefore, the comparison of the \( n \) value in the dilute and Dex solutions was difficult. Hence, the dependence of \( n \) on \( C_{Dex} \) was evaluated. In the NaCl solution, the \( n \) values were almost constant as shown in Fig. 4A: \( n = 3.8-4.3 \) at \( C_{NaCl} = 0.100 \) M, \( n = 5.1-5.7 \) at \( C_{NaCl} = 0.150 \) M, and \( n = 3.3-4.2 \) at \( C_{NaCl} = 0.200 \) M. This indicates that Dex does not affect the \( n \) values of the J-aggregate in the NaCl solution, as well as the ionic strength of the solution. The decrease in the intermolecular distance due to volume exclusion may not work well here because NaCl weakens electrostatic interactions.

The \( n \) values at \( C_{HCl} = 0.100 \) M in Fig. 4B increased as \( C_{Dex} \) increased, while a decrease in the value of \( n \) for 0.130 and 0.200 M HCl solutions was observed. This difference in the \( n \) behavior with the varying values of \( C_{HCl} \) results from pH variation. As stated above, the pKₐ of benzenesulfonic acid was reported to be 0.8 [45]. Since the pH values at \( C_{HCl} = 0.100, 0.130, \) and 0.200 M are 1.00, 0.89, and 0.70, respectively, the sulfonic acid groups on TPPS structure protonate at high \( C_{HCl} \), leading to the suppression of the J-aggregation. In the case of the HCl solution, volume exclusion and steric hindrance effects may affect the \( n \) value.
In this study, Cl$^-$ does not affect J-aggregation, as shown in Fig. 4A. Therefore, J-aggregation via the counteranion intermolecular distance due to the volume exclusion of Dex should not occur in the HCl solution. The decrease in the molecular distance of TPPS molecules only overlap each other. When the number of TPPS monomer in the J-aggregate increases, the J-aggregation can no longer exist in the restricted space formed by Dex. Therefore, an interplay of opposite effects influences the J-aggregation mechanism in the HClO$_4$ solution. Luca et al. revealed that the J-aggregate of TPPS was formed due to the interactions between NH in the center of TPPS and the counteranion, and between SO$_3$H and the counteranion [50].

Interestingly, the $n$ values increased with increasing C$_{Dex}$ for all C$_{HClO_4}$. As discussed above, a decrease in the pH causes protonation of sulfonic acid, which suppresses J-aggregation. In other words, there should be a different aggregation mechanism in the HClO$_4$ solution. The increase in the intermolecular distance due to the volume exclusion of Dex strengthens the interaction between NH, SO$_3$H, and ClO$_4^-$, which leads to an increase in the $n$ value. Moreover, the results obtained in Fig. S4 support the J-aggregation of TPPS through the counteranion. When the counteranion is placed between the monomers in the J-aggregate, the flexibility of the resultant J-aggregate increases, forming several types of J-aggregates with various tilted angles. Thus, the J-aggregation behavior of TPPS in a molecularly crowded environment was demonstrated.

## Conclusion

In the present study, we demonstrated that the J-aggregation behavior of TPPS in the molecular crowding environment simulated by Dex changed with the pH and ionic strength of the solution. Dex did not affect J-aggregation in the NaCl solution. The $n$ value and polydispersity of the J-aggregate with different tilted angles in the NaCl solution did not change as C$_{Dex}$ increased. The $n$ value in the HClO$_4$ solution increased by the addition of Dex because the interactions among NH, SO$_3$H, and ClO$_4^-$ are effective owing to volume exclusion. In the HCl solution, the $n$ behavior changed with varying pH values. At low pH, J-aggregation via electrostatic interactions was promoted by volume exclusion, while high pH causes the protonation of sulfonic acid groups on TPPS, leading to the suppression of J-aggregation.

### Supplementary Information

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