Supporting Information

Mechanism and Equilibrium Thermodynamics of Hand J-aggregate Formation from Pseudo Isocyanine Chloride in Water

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S1 Datasets of the trimer spectra and the J-spectrum

Table S1. Digitalized versions of the trimer spectra and the J-aggregate spectrum established in

 the present work.

	$oldsymbol{arepsilon}_{trimer}$	\mathcal{E}_{trimer}	6
Λ	with $K_T = 200000 L^2 mol^{-2}$	with $K_T = 285000 L^2 mol^{-2}$	ک J-aggregate
nm		L mol ⁻¹ cm ⁻¹	
450	65642.5	65012.6	7121.0
451	68729.5	68037.0	7606.3
452	71660.4	70907.1	8163.3
453	74300.4	73496.4	8731.6
454	76816 7	75969.2	9236.9
455	79250 3	78347.4	9978.0
455	81//9 3	80512.0	10572.7
450	81449.3 92793 E	80312.0	112/1 2
457	03702.3	02773.9 94613 E	11241.5
438	80522.0	88420.0	12102.0
400	00038.4	00453.5	13102.9
401	90938.4	01077 2	13558.9
462	92428.3	91377.2	13965.0
463	93322.9	92376.8	14248.2
464	94385.8	93525.0	14224.8
465	95563.4	94782.4	14069.6
466	96820.4	96143.9	13678.3
467	98060.4	97526.2	13548.1
468	99385.9	99056.7	13007.7
469	101541.7	101341.7	12462.1
470	104246.2	104176.1	11704.6
471	107359.4	107429.9	11051.1
472	110644.7	110909.6	10571.2
473	115104.8	115461.7	9577.5
474	118691.2	119303.8	9377.8
475	122915.5	123723.0	8957.9
476	126653.9	127708.5	9079.1
477	130221 3	131538 3	8947 5
478	134109.2	135560.8	7451 4
478	135264 0	137155 8	8015.0
480	135636 /	137877 0	7544 1
480	126420.2	129802 4	7 J44.1
481	124202 5	127120 4	6502.1
482	134203.5	137130.4	6592.1
403	131404.5	134701.5	0004.5
484	120309.4	130137.1	8/58.4
485	120442.7	124689.3	11302.8
486	114217.5	118868.1	13919.7
487	106828.6	111935.5	16952.8
488	100380.3	105745.1	20036.9
489	93039.5	98759.5	22942.2
490	86404.2	92345.2	25979.7
491	79870.3	85991.2	29493.3
492	73491.1	79802.3	32355.6
493	67847.8	74258.1	34854.4
494	62127.9	68674.9	36702.3
495	57217.3	63829.8	37557.7
496	52550.1	59224.8	37601.2
497	48178.3	54941.9	36444.1
498	44089.4	50955.6	34816.9
499	40280.4	47271.5	32662.9
500	37061.8	44142.8	30439.3
501	33707.4	40967.2	28381.0
502	30284.0	37808.2	26700.8
503	27666 7	35379.8	25158.8
503	2/ 700 6	32737.8	240/13 1
504	24700.0	20527 0	227043.1
505	22193.1	30337.U 20404.0	23224.2
500	13/33.3	20494.9	22059.9
507	1///2.2	20821.4	22058.3
508	15332.7	24864.4	21991.4
509	135/4.5	23527.1	21842.5

510	12435.2	22763.5	21580.8
511	11304.3	22049.8	21532.1
512	10880 /	21085.0	21202 7
512	10003.4	21985.9	21295.7
513	10038.5	21584.5	21406.9
F14	11005 0	22700.9	20704 4
514	11065.6	22/99.0	20764.4
515	11742.5	23699.3	20558.1
54.6	12502 7	21702.1	20266.2
516	12592.7	24780.4	20266.2
517	14128 4	26430.6	19806 7
517	14120.4	20430.0	156660.7
518	15576.4	27985.6	19538.0
519	17169 9	29648 7	19265 9
515	17105.5	25040.7	15205.5
520	19114.5	31549.4	19037.5
521	20959 3	33318.8	18819.6
522	2000010	0001010	1001510
522	22734.1	34962.2	18710.8
523	24126.0	36215 3	18704 2
525	24120.0	50215.5	10704.2
524	25634.0	37490.9	18776.2
525	26696.8	38314.4	19175 2
525	20050.0	30314.4	1017.5.2
526	27369.5	38/30.3	19930.7
527	27850.4	38912 5	20897 9
527	27030.4	50512.5	20057.5
528	27756.8	38548.3	22103.2
520	28474.0	29797 5	22817 7
525	28474.0	56767.5	22017.7
530	27902.8	37893.0	23802.2
521	26049 0	26621 /	216176
531	20948.0	30031.4	24047.0
532	27274.1	36401.4	24650.8
E22	256496	24510.2	24504 5
533	20048.0	34510.2	24594.5
534	26041.7	34274.0	22944.0
535	24204.2	22464.2	21721.0
535	24204.3	32161.2	21/21.8
536	23430 5	30925.9	19745 5
500	2010010	20022010	17050.0
537	22684.3	29698.6	17850.2
538	22620.6	29059 5	15708 6
500	2152010	25005.5	10000
539	21594.4	27623.5	14219.9
540	21261.2	26777 7	12703 9
540	21201.2	20777.7	12705.5
541	20834.6	25873.8	11449.8
542	20744 3	25261 7	10353.6
542	20744.5	25201.7	10555.0
543	20285.1	24378.2	9527.6
544	10077 2	22502 7	8802 0
544	19922.5	23393.7	8892.0
545	19832.5	23061.1	8344.3
EAG	10060.2	2100E 4	9110 E
540	19009.5	21995.4	8119.5
547	19384.0	21854.0	7599.7
F 4 9	19402.0	20722.2	7611.2
548	18493.9	20/33.2	7611.3
549	17600.5	19639.2	7763.7
550			6620.4
550			0020.4
551			6341.2
553			6251.2
552			0251.3
553			6318.1
554			65.40.7
554			6548.7
555			6993 4
			7010 4
556			7610.4
557			8430.6
			0.170.1
558			9476.1
559			10801.1
			12404.0
560			12404.9
561			14456.3
FC3			17000 5
502			1/009.5
563			20213.0
564			24202.0
504			24202.9
565			29345 3
505			20040.0
566			36016.0
567			45223.2
560			522012
568			58466.4
569			78635.8
530			110000 1
570			110229.1
571			158877 4
571			130022.4
572			225502.1
572			246404 4
5/3			246404.4
574			170129.9
E75			106646.0
5/5			106646.2
576			67996.3
573			46424.4
577			46131.1
578			33476.4
570			24704.2
5/9			24781.2
580			17610.9
			_, 010.5

S2 Details on sample composition above the threshold curve

The percentage of dimers as a function of the total PIC concentration shown in **Figure S1** includes a broad maximum, which coincides with the concentration regime applied by the present experiments. The broad maximum offers an explanation for the invariance of the relative dimer concentration.



Figure S1. The dependence of the relative dimer concentration on the total concentration, calculated via eqs. 9 and 12.

In **Figure S2** the evolution of the relative concentrations of monomers, dimers and trimers with total concentration and temperature is plotted.



Figure S2. A) Relative concentrations of monomers (black), dimers (red) and trimers (blue) plotted versus the total concentration [PIC] resulting from decomposition of UV-Vis spectra according to eq. 8 at $T = 25^{\circ}$ C. B) Temperature dependence of the relative concentrations of monomers (black), dimers (red) and trimers (blue) resulting from decomposition of UV-Vis spectra according to eq. 8 at *[PIC]* = 4 mM.

S3 Determination of the J-aggregate spectrum

Figure S3 shows all J-spectra independently established with three different samples at four temperatures (*[PIC]* = 7 mM), two temperatures (*[PIC]* = 6.5 mM) and five temperatures (*[PIC]* = 6 mM). All twelve spectra were used to form the averaged spectrum in the regime of $\lambda < 550$ nm shown in **Figure 9**. **Figure S4** shows the data used for determining the J-spectrum for $\lambda > 550$ nm together with the whole J-aggregate spectrum. **Figure S5** shows the result of subtracting the J-aggregate contribution from experimental spectra and renormalizing the excess spectra with [PIC]_{m,o}.



Figure S3. Established J-spectra of all investigated samples which are in the aggregated regime. Experimental spectra were corrected according to the oligomer/monomer contribution and the resulting excess spectra were normalized with the concentration of J-aggregates, which were taken from the aggregation threshold as the difference of the total concentration and the concentration of "non-aggregated" PIC [*PIC*]_{*m,o*}.



Figure S4. A) Normalized J-peak of experimental spectra below the aggregation threshold after subtraction of the contribution of oligomers/monomers. B) Averaged normalized J-peak of all spectra shown in A). C) The merged J-spectrum. The part below $\lambda = 550$ nm is found in Figure 9 and the part above $\lambda = 550$ nm is seen in B).



Figure S5. A) Experimental spectra used for verifying the validity of the J-aggregate spectrum. These spectra were not used in the process of determining the J-aggregate spectrum given in **Figure S4.** B) The resulting excess spectra after subtraction of the contribution of J-aggregates from experimental spectra. Additionally, the modelled oligomer spectrum is plotted for comparison.

S4 Determination of the molar mass M_J of J-aggregates

Figure S6 shows the result of calculating the molar mass M_J of J-aggregates with and without taking the scattering contribution of oligomers/monomers into account. The insignificant deviation between both approaches below T < 20 °C justifies neglection of the scattering contribution from oligomeric/monomeric PIC in eq 19a.



Figure S6. Molar mass M_J of J-aggregates. Black spheres refer to the data points from Figure 11, which were calculated by neglecting the contribution of oligomeric/monomeric PIC. Red spheres were established by taking the scattering contribution of oligomeric/monomeric PIC into account. Hollow black spheres represent the ratio of M_J with and without implementing the oligomer/monomer correction.

The result of the calculation of M_J using the aggregation threshold is shown in **Figure S7**. The three different datasets are established by taking either the threshold curve itself or the upper/lower error margin of it to calculate the concentration of J-aggregates.



Figure S7. Determination of the molar mass M_J of J-aggregates. Data were established by means of eq 19c with the concentration exclusively of J-aggregates. Black data points were calculated based on the concentration received from the aggregation threshold shown in Figure 1 at [PIC] = 7mM. Blue data points refer to the lower error margin and red data points refer to the upper error margin of the aggregation threshold shown in the inlet. The horizontal dashed line represents the value $M_J = 2.55 \cdot 10^6$ g/mol determined by Herzog et al.¹ in 0.01 M NaCl solution.

S5 Bimodality of field-time correlation functions and resulting SLS curves

Figure S8 shows three examples of bimodal correlation functions which were recorded at the onset of aggregation.



Figure S8. Field-time correlation functions $g_1(\tau)$ at three temperatures close to the threshold value of the formation of J-aggregates. The color index goes from brown to green and represent the scattering angle from 30° to 86° in 8° increments.

In **Figure S9** the scattering curves of the monomer/oligomer mixture and of J-aggregates at the onset of aggregation are plotted.



Figure S9. Evaluation of the scattering contribution of J-aggregates at the onset of aggregation. A) Scattering curves of J-aggregates and of B) oligomers/monomers with $\Delta R_i(q) = \Delta R(q) \cdot b_i(q)$. The color index goes from brown to green and represents different temperatures from 21.3 °C to 20.5 °C. C) Results of applying eq 2 on the scattering curves of J-aggregates shown in A) with a dummy concentration of 1 g/L. The increase in M_w with decreasing T indicates an accumulation of J-aggregates. The radius of gyration is constant and amounts to R_g = 188±2 nm.

<u>S6 Simulation of SLS curves based on $M_{m,o}$ and M_J </u>

The evolution of b_i with time shall be used to approximate the concentration of both species. It is shown in **Figure 11** that determination of the J-aggregate concentration c_J depends on the particular choice of the threshold line and thus is charged with an increasing uncertainty while approaching the respective line. One route to circumvent this problem is to approximate the concentrations at the onset of aggregation with help of b_i . These concentrations are then used to predict scattering curves and compare the corresponding R_g and M_w parameters with the experimental values shown in **Figure 10**.

The weighting factors b_i , the molar masses M_i and their mass concentrations c_i are related as follows:

$$\frac{c_{m,o}}{c_I} = \frac{b_{m,o}}{b_I} \cdot \frac{M_J}{M_{m,o}}$$
(S1)

In order to determine concentrations via b_i , it is in general necessary to know the molar mass of the entities or, if only R_h is known, to know the relationship between R_h and M_w .² The molar mass of monomers/oligomers is extracted from the monomer/oligomer phase, shown in the inlet in **Figure 10**, and amounts to 850 g/mol. The molar mass of J-aggregates was set to $M_J = 1.48 \times 10^6$ g/mol corresponding to the best estimate based on **Figure 11**.

Eq S1 is used to calculate the concentrations in g/l of both modes for the aggregation process within the T-regime where the bimodality (shown in **Figure 12**) is unambiguously accessible.

With these concentrations, scattering curves of mixtures of monomeric/oligomeric PIC and Jaggregates were simulated according to the following approach. In case of a multimodal system, the excess Rayleigh ratio ΔR of the sample is approximated as the sum of excess Rayleigh ratios of all species according to

$$\Delta R(q) = \sum_{i} K_i \cdot c_i \cdot M_i \cdot P_i(q \cdot R_{g,i})$$
(S2)

In eq S3 K_i is the optical constant defined in eq 4, c_i is the concentration in g/l determined with eq S1, M_i is the molar mass, $R_{g,i}$ is the radius of gyration and $P_i(q)$ is the form factor of species *i*. In the specific case of the formation of J-aggregates, the system is approximated with distinguishable species. Since both species are based on PIC molecules, the refractive index increment dn/dc, and thus *K*, the same value is assumed for both species. The first species is monomeric/oligomeric PIC with a molar mass of $M_{m,o} = 850$ g/mol, derived from the inlet in **Figure 10**. The radius of gyration of this species is too low to be detectable, which results in a form factor of 1 throughout the whole *q*-regime under consideration. The second species is the J-aggregate with a molar mass of $M_J = 1.48 \times 10^6$ g/mol and a radius of gyration of $R_g = 188$ nm, with R_g stemming from the evaluation of ΔR_J shown in **Figure S9**. A form factor of a rod was assumed for the J-aggregate with the length of the rod *L* determined by means of $L = R_g \sqrt{12}$.³ Thus, eq S2 transforms to following expression:

$$\Delta R(q) = K \cdot (c_{m,o} \cdot M_{m,o} + (c - c_{m,o}) \cdot M_j \cdot P_{rod}(q \cdot R_g))$$
(S3)

A comparison of the resulting scattering curves from eq S3 and the corresponding data from the experiment is found in **Figure S10**. Extraction of M_w and R_g from the scattering curves simulated with eq S3 were carried out with the same approach applied to the scattering data from the experiment (See experimental Part for details). Results from eq S3 are compared with the experimental results in **Figure S11**.



Figure S10. Scattering curves from experiment (A)) and from simulation (B)). The temperature regime corresponds to the data shown in Figure 12. Brown data points were recorded at $T = 21.4^{\circ}C^{\circ}$ and the green data points were recorded at $T = 20.5^{\circ}C^{\circ}$.

Evaluation of the simulated scattering curves yields values for M_w and R_g comparable to the values obtained from the experiment, with simulated M_w being slightly larger. The simulated values follow the temperature dependent trend of the experimental data, which suggests a systematic shift as the origin for the larger M_w from simulation. A reason for this deviation could be originated in the uncertainties of $M_{m,o}$ and M_J . Another source of the deviation may be the neglect of the second osmotic virial coefficient A_2 by scattering curves simulated with eq S3. Due to the inherent positive charge of PIC, A_2 is most likely positive, and if known would yield lower values for M_w . In the light of these uncertainties, the small discrepancies between measured and calculated values in **Figure S11** are not considered to be significant and the extent of agreement confirms the applicability of the concept of chain growth on the description of the scattering data.



Figure S11. Temperature dependent evolution of the weight averaged molecular mass M_w and the radius of gyration R_g from a light scattering experiment (black) with [PIC] = 7 mM. The red spheres represent values for M_w and R_g obtained from the evaluation of simulated scattering curves with mass concentrations extracted from eq S1.

S7 Aggregation experiment triggered by a change in solvent

Figure S12 shows the result of an aggregation experiment recorded with light scattering where the aggregation was triggered by changing the solvent condition from aqueous to 0.01M NaCl solution. For this purpose, 7 mL of a 0.0114M NaCl solution was held at 4°C inside the device. 1 mL of an 8mM PIC solution, which was previously held at 30°C, was added and then mixed for approximately 30 seconds before starting the measurement.



Figure S12. Evolution of the mass weighted molecular weight M_w , the radius of gyration R_g and the hydrodynamic radius R_h for a sample with *[PIC]* = 1 mM in 0.01M NaCl solution at T = 4 °C. The aggregation was triggered by changing the solvent from aqueous solution to 0.01M NaCl solution.

REFERENCES

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- 3 A. Guinier, *Small-angle scattering of X-rays*, 1955.