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with Titrations Using Dimethyl Sulfoxide**

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JOURNAL
OF THE
AMERICAN
CHEMICAL
SOCIETY®

Reprinted from
Volume 118, Number 50, Pages 12614–12623

Predicting the Relative Stabilities of Multiparticle Hydrogen-Bonded Aggregates Based on the Number of Hydrogen Bonds and the Number of Particles and Measuring These Stabilities with Titrations Using Dimethyl Sulfoxide

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Received August 8, 1996[®]

Abstract: This paper describes an experimental method for determining the relative stabilities of hydrogen-bonded aggregates in terms of the mole fraction of dimethyl sulfoxide (DMSO) in chloroform solution, χ^{DMSO} , required to cause their dissociation. It also describes three indices— I_{Tm} , I_G , and $I_G/(N-1)$ —that estimate the relative stabilities of hydrogen-bonded aggregates. Each of these indices depend on two variables: HB and N . HB is the number of hydrogen bonds holding the aggregate together; N is the number of particles in the aggregate. The melting-point index, $I_{\text{Tm}} = \text{HB}/(N-1)$, corresponds conceptually to a “melting point” for the aggregate, that is, a temperature at which it would dissociate into separate particles. This index is the most useful of the three for “rule of thumb” estimation of relative stability if assembly occurs *cooperatively*. The free energy index, $I_G = 2.8\text{HB} - 16(N-1)$, corresponds to a free energy of assembly, ΔG , with units kcal/mol. The index $I_G/(N-1) = 2.8\text{HB}/(N-1) - 16$ corresponds conceptually to a free energy of association per particle, $\Delta G/(N-1)$. This third index is most useful if assembly occurs *noncooperatively*.

Introduction

We and others are interested in the design, synthesis and characterization of aggregates held together by non-covalent interactions (especially hydrogen bonds).^{1–12} Estimating the stabilities of these aggregates is a fundamental challenge for non-covalent synthesis, since they typically involve competing (and sometimes large) terms for the enthalpy and entropy of association. Qualitatively, the larger the number of interacting particles in the aggregate, the larger the area of molecular surface that is brought into contact and (in principle) the more favorable the enthalpy of formation; correspondingly, the larger the number of interacting particles, the more unfavorable the entropy of formation.

Theoretical and computer-based models for estimating enthalpies of interaction of organic groups are highly developed.¹³ Although the extension of these techniques to non-covalent

systems is at an earlier stage than those for covalent systems, the collective intuition of organic chemists about *enthalpies* is strong. The capability to estimate *entropies* is considerably weaker: characteristics of both the aggregating molecules and of the solvent contribute to the total entropy. The equations describing these contributions are too complicated for “back-of-the-envelope” use, and computer simulations are still too slow to estimate changes in entropies for association of large non-covalent molecules and aggregates.¹³

Although it is difficult to estimate free energies of formation of aggregates, the increasing importance of non-covalent aggregation to organic chemistry—especially to molecular recognition and to the association of biological molecules—requires methods to guide thinking and design and to rationalize experimental results. A series of studies—starting with reviews and analyses of experimental studies by Page and Jencks,^{14,15} extending to the detailed discussions of Williams,^{16–20} and including computational studies of Still,^{21,22} Jorgenson^{23,24} and Gavezzotti²⁵—represent the most helpful analyses to date.

[®] Abstract published in *Advance ACS Abstracts*, December 1, 1996.

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We have been studying a series of hydrogen-bonded aggregates based on a single, non-covalent motif: the cyclic $\text{CA}_3\cdot\text{M}_3$ rosette from derivatives of cyanuric acid (or barbituric acid) and melamine.⁸ We needed a rule of thumb to help us estimate the competing contributions of different patterns of covalent and non-covalent connectivity among large numbers (up to 10) of particles based on this hydrogen-bonded motif. More interacting particles implied a larger and more favorable enthalpy of aggregation from the networks of hydrogen bonds, and a larger and more unfavorable entropy of aggregation; this unfavorable entropy could be mitigated by linking appropriate particles covalently but at increased synthetic effort.

The impetus for this report is our empirical observation that the relative stabilities of the series of hydrogen-bonded aggregates seem to be reflected in the magnitude of the ratio $\text{HB}/(N-1)$, where HB is the number of hydrogen bonds and N is the number of particles. Conceptually, this ratio reflects the competing contributions of enthalpy and entropy: formation of larger numbers of hydrogen bonds would be reflected in more favorable enthalpy; aggregation of larger numbers of particles would be reflected in more unfavorable entropy. We observe that aggregates that have higher values of $\text{HB}/(N-1)$ are more "stable" than those with lower values.

The meaning of "stability" requires careful definition. The practical definition of a stable aggregate is one in which the components are largely in the form of the aggregate at a concentration of interest. The stability is *not* reflected simply in the magnitude of ΔG° for aggregation, since the effect of concentration (and therefore of entropy) on the extent of aggregation is not indicated by ΔG° (which assumes that both aggregates and components are at 1 M).

To make this point clear, consider the aggregation of N simple monomers, A , into the monocomponent aggregate A_N (eq 1). The most useful, intuitive measure of stability is the extent of aggregation, θ , (eq 2) at a total concentration of monomer (A_0 , eq 3) added to the solution; θ represents the completeness of conversion of monomer units into aggregate. Here, K is the equilibrium constant (eq 4). Rearranging eq 4 yields eq 5, and the concentration of free monomer, $[A]$, is given by the solution to eq 5.



$$\theta = \frac{N[A_N]}{[A_0]} = 1 - \frac{[A]}{[A_0]} \quad (2)$$

$$[A_0] = [A] + N[A_N] \quad (3)$$

$$K = \frac{[A_N]}{[A]^N} = \frac{[A_0] - [A]}{N[A]^N} \quad (4)$$

$$KN[A]^N + [A] - [A_0] = 0 \quad (5)$$

$$\theta_{N=2} = 1 + \left(\frac{1 - \sqrt{1 + 8K[A_0]}}{4K[A_0]} \right) \quad (6)$$

Figure 1 gives θ for $N = 2$ (eq 5 is solved analytically in this case and yields eq 6) and $N = 6$ (eq 5 is solved numerically). Compare, for instance, the two-particle and the six-particle

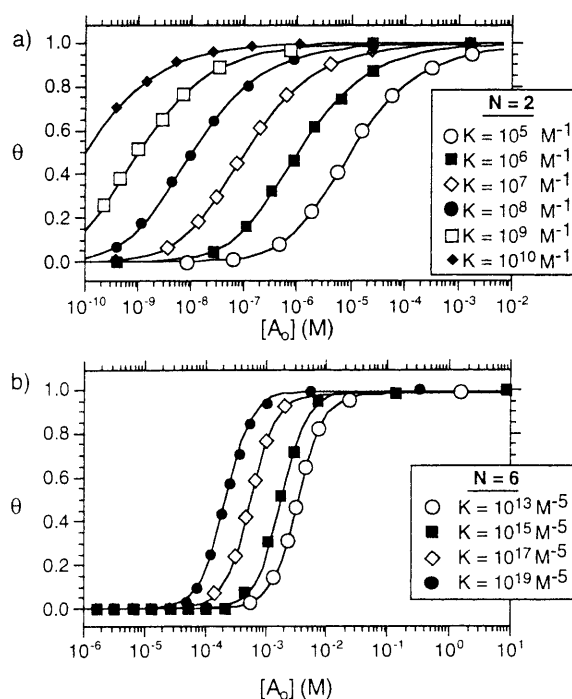


Figure 1. The dependence of the extent of formation of the aggregate, θ , on the total concentration of monomer, $[A_0]$, and the equilibrium constant (K_N) using eqs 5–6. (a) Two-particle aggregate; $N = 2$. (b) Six-particle aggregate; $N = 6$.

aggregates with $K_6 = 10^{15} \text{ M}^{-5}$ ($\Delta G \sim -21 \text{ kcal/mol}$) and $K_2 = 10^6 \text{ M}^{-1}$ ($\Delta G \sim -8 \text{ kcal/mol}$). At first, it might seem that the six-particle assembly is more "stable", since it has the greater value of K and the more negative value of ΔG . Figure 1 shows, however, that at $[A_0] = 100 \mu\text{M}$, the six-particle aggregate is almost fully dissociated ($\theta = 0.05$), whereas the two-particle aggregate is almost fully associated ($\theta \sim 0.95$). The key issue here is that the value of θ depends strongly on N ; K_N and ΔG° do not correlate in a simple way to θ .

We introduce three indices— I_{Tm} , I_G , and $I_G/(N-1)$ (eqs 7–9)—that reflect the compromise between enthalpy and entropy in determining the stabilities of self-assembling, non-covalent aggregates: the formation of hydrogen bonds favors assembly enthalpically; the association of multiple particles disfavors assembly entropically. This paper summarizes an analysis of the problem of estimating a *stability* in terms of these three indices.

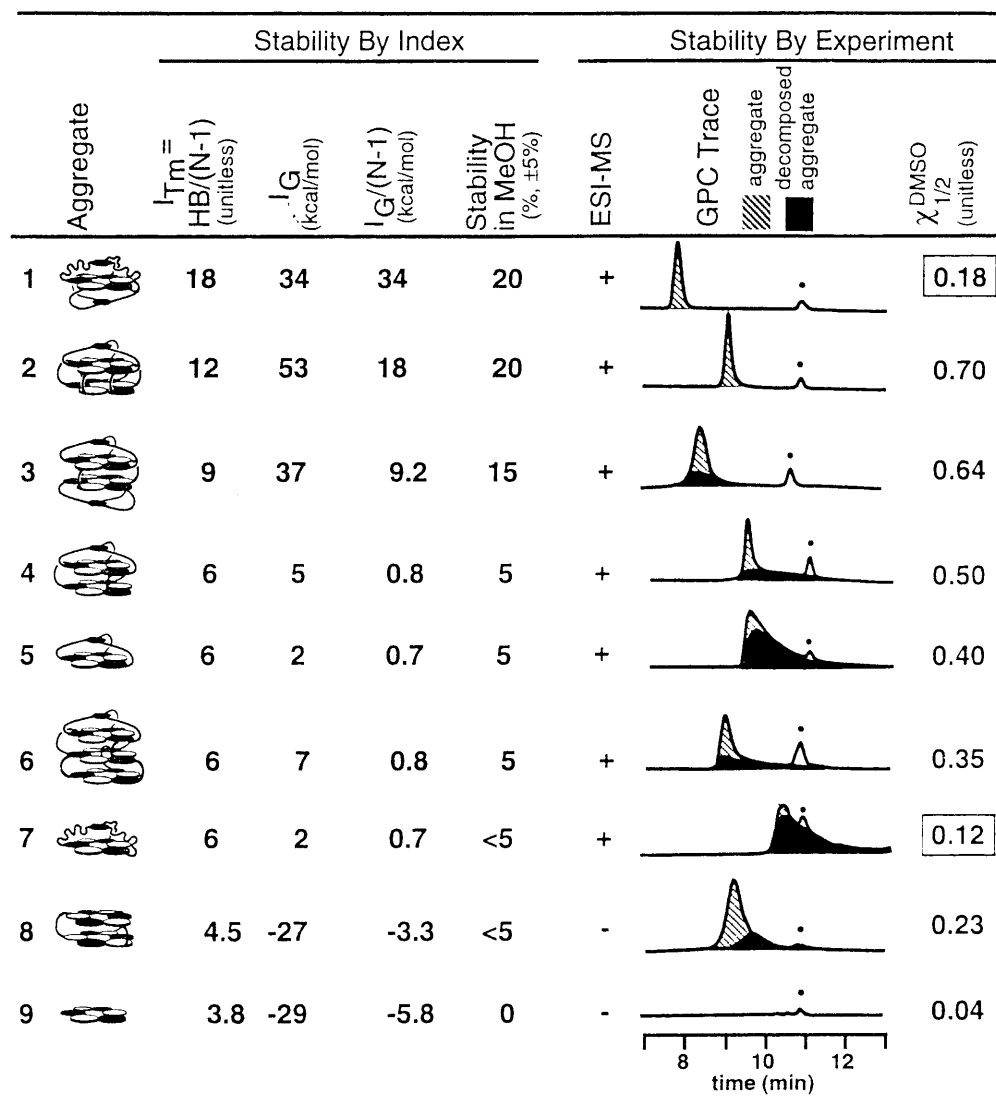
$$I_{\text{Tm}} = \frac{\text{HB}}{N-1} \quad (7)$$

$$I_G = 2.8(\text{HB}) - 16(N-1) \quad (8)$$

$$\frac{I_G}{N-1} = 2.8 \frac{\text{HB}}{N-1} - 16 \quad (9)$$

Our concepts of I_{Tm} , I_G , and $I_G/(N-1)$ are different. Briefly, I_{Tm} is proportional to a temperature at which $\Delta G = 0$. For assemblies that are highly cooperative, I_{Tm} is proportional to a "melting temperature", much like the melting temperature of double stranded DNA.²⁶ In this study, we measure "melting" in terms of a change in the state of aggregation as a function of

(26) Cantor has shown the melting temperature of double-stranded DNA is affected by the mole fractions of GC and AT base-pairs. That is, I_{Tm} is a function of the number of hydrogen bonds, HB, in the complex; with double stranded DNA, N is constant and equals two.

Chart 1. Schematics of the Nine Aggregates Examined in This Work^a

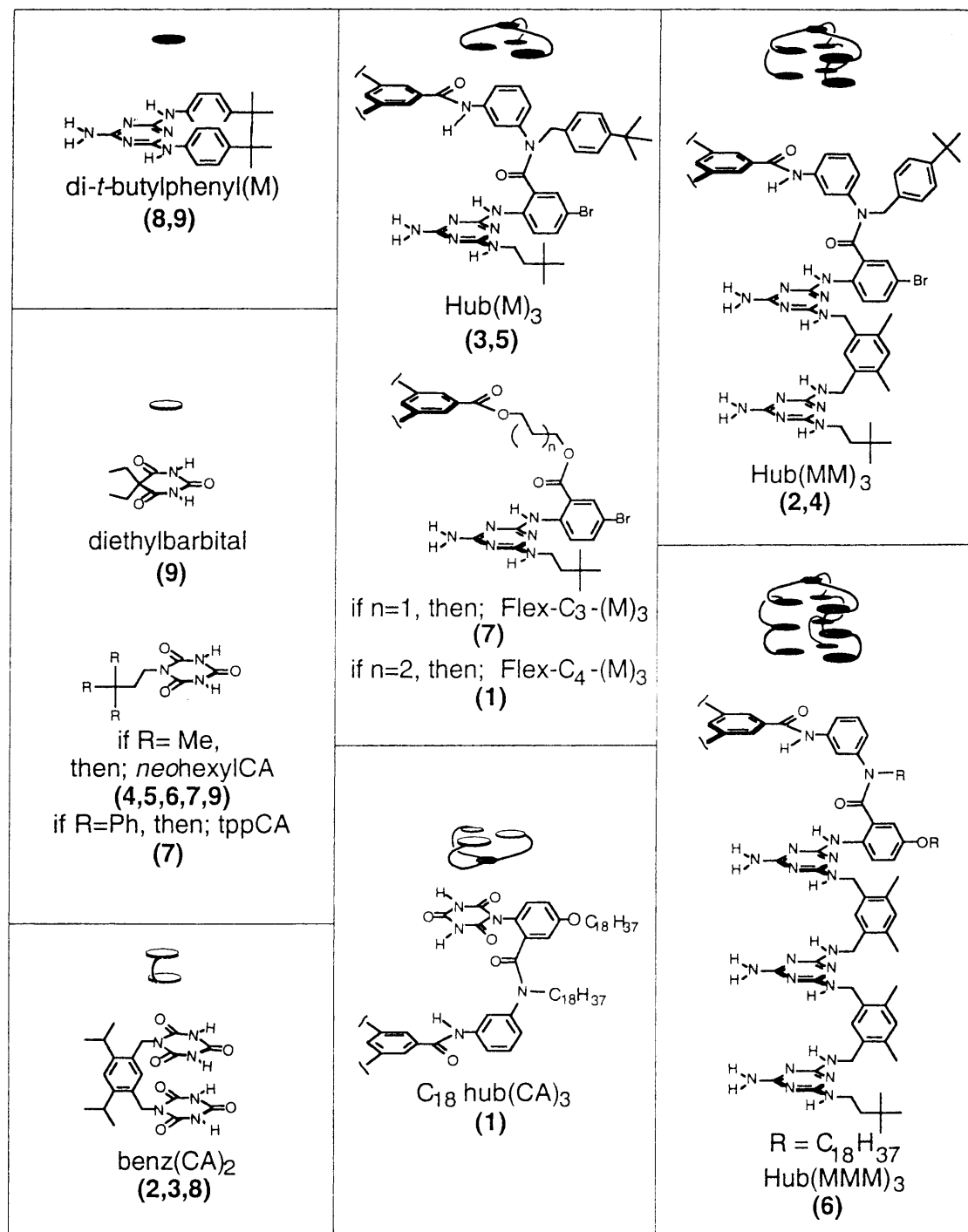
the concentration of a hydrogen-bonding solvent (such as DMSO); we do not measure melting as a function of temperature. We will clarify later the equivalence of solvent and temperature for measuring melting. I_{G} takes the form of the equation for free energy, and has units of kcal/mol. Finally we propose an index, $I_{\text{G}}/(N-1)$, that we feel may be cumbersome, but may be the most accurate of the three in its predictions of stability for noncooperative assembly: $I_{\text{G}}/(N-1)$ is a condensation of the first two indices (eq 9), and correlates roughly to the average free energy of association of each particle in the assembly. For stable, noncooperative assemblies, $I_{\text{G}}/(N-1)$ must be above some minimum value for aggregation to yield a stable species.

These three indices provide useful measures of stability; we believe that they will be helpful to the community studying molecular recognition. As both HB and N are known prior to the synthesis of the aggregate, we currently use both $\text{HB}/(N-1)$ and $I_{\text{G}}/(N-1)$ to guide our own choice of target molecules.

This paper introduces an experimental measure of stability: $\chi_{1/2}^{\text{DMSO}}$. This index is defined as the mole fraction of DMSO in chloroform solution at which half of the aggregate has decomposed into components (that is, at which $\theta = 0.5$). The value of $\chi_{1/2}^{\text{DMSO}}$ is a convenient experimental measure that correlates well both with our intuitive measure of stability (magnitude of θ) as well as with $\text{HB}/(N-1)$ (and, therefore, with both I_{Tm} and $I_{\text{G}}/(N-1)$).

Organization of this Paper. We begin by introducing the aggregates prepared to date and discussing the experimental methods used to determine their relative stabilities, including $\chi_{1/2}^{\text{DMSO}}$ (obtained by titrating solutions of aggregates with DMSO- d_6). We tabulate values of I_{Tm} , I_{G} and $I_{\text{G}}/(N-1)$ for the aggregates and interpret these indices. Finally, we discuss the assumptions we have made in relating ΔH and ΔS to HB and $N-1$.

Hydrogen-Bonded Aggregates and the Experimental Determination of Their Stabilities. Chart 1 shows the nine aggregates used in arriving at I_{Tm} , I_{G} and $I_{\text{G}}/(N-1)$. Chart 2 provides the molecular structures of all of these aggregates. The nine aggregates are all based on the lattice formed by alternating molecules of isocyanuric acid (CA, white disks) and melamine (M, dark disks). We refer to the motif common to all of these aggregates—six molecules of CA and M arranged in a circle—as a “rosette”. Each rosette is stabilized by 18 hydrogen bonds. These aggregates are shown in order of stability, with two notable exceptions: aggregates 1 and 7 are less stable than their positions in the chart reflect, mainly due to large changes in conformational entropy during assembly. The arms that comprise the M components in both these structures are composed almost entirely of single bonds (Chart 2), and are conformationally flexible; these arms are shown as wavy lines. We have discussed the techniques used to characterize these aggregates

Chart 2. Molecular Structures of the Components Comprising the Nine Aggregates Examined in This Work^a

elsewhere.^{8,27} We have inferred the stability of these aggregates using four semiquantitative and qualitative techniques: "titration" using hydrogen bonding solvent, gel permeation chromatography (GPC), and electrospray ionization mass spectrometry (ESI-MS). The results from these techniques are shown in Chart 1.

Dimethyl Sulfoxide Titrations. We obtained a significantly more accurate measure of stability by using ¹H NMR to establish the extent of aggregation, θ , as a function of the mole fraction of DMSO-*d*₆, χ^{DMSO} (Figure 2), in chloroform. Phase separation was not a problem using DMSO-*d*₆. Heating and sonication of the samples prior to data collection, and collection of data of the same sample for many days, helped to assure that the

aggregates were not trapped in a kinetically stable (but thermodynamically unstable) state. We used one of two methods to estimate the value of θ : monitoring the appearance and growth of a new peak corresponding to dissociated components with increasing χ^{DMSO} ; monitoring a gradual change in chemical shift from a position corresponding to aggregate to one corresponding to dissociated components. Generally, the first method is appropriate when the kinetics of assembly are slow on the NMR time scale, and the second is appropriate when the kinetics of assembly are fast. Both of these methods require that we assume a two-state model (fully associated aggregate going to fully dissociated components).

We applied the first method based on the appearance and disappearance of isolated peaks to all aggregates *except* for aggregate **9**; these remaining eight aggregates gave peaks that

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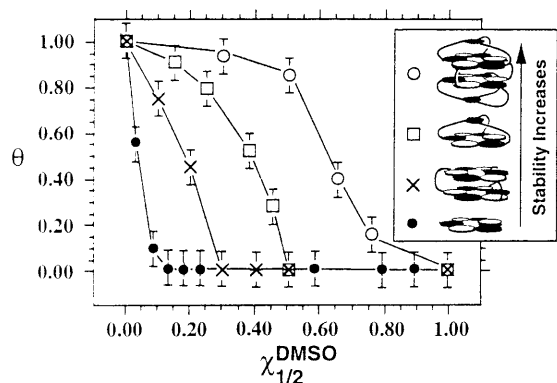


Figure 2. We used ^1H NMR spectroscopy to measure the extent of aggregation, θ , as a function of the mole fraction of $\text{DMSO-}d_6$, $\chi_{1/2}^{\text{DMSO}}$; four representative plots are shown. We estimate the values of θ using the areas of peaks corresponding to the associated aggregate or dissociated components for all aggregates except aggregate 9. For this aggregate, we use changes in the value of δ , the shift (downfield from TMS, measured in ppm) of lines corresponding to the methylene protons of diethylbarbital as a function of $\chi_{1/2}^{\text{DMSO}}$. The value of $\chi_{1/2}^{\text{DMSO}}$ in this case is defined as the value of $\chi_{1/2}^{\text{DMSO}}$ where the shift of the protons is half that separating the diethylbarbital in aggregate and in solution in the same solvent (that is where $\Delta\delta = (1/2)\Delta\delta_{\text{max}}$; $\theta = 0.5$). The error in the values of θ is less than 15%, as reflected in the error bars. Lines connecting the data do not reflect a mathematical function, but serve as guides to the eye.

were characteristic of either the fully associated aggregate (in chloroform) or the fully dissociated components (in DMSO). We used the integrated areas of these characteristic peaks ($A_{\text{associated}}$ and $A_{\text{dissociated}}$) to estimate the value of θ (eq 10). Two examples of such characteristic peaks are the hydrogen bonded hydrogens of CA (which appeared as two singlets at $\delta_{\text{NH}} = 14\text{--}16$ ppm downfield from TMS in the associated form, and as a singlet at $\delta_{\text{NH}} \sim 10.4$ ppm in the dissociated form) and the benzylic hydrogens of $\text{Hub}(\text{M})_3$ (which are diastereomeric and appear as two pairs of doublets at $\delta_{\text{CH}_2} = 4.2$ ppm and $\delta_{\text{CH}_2} = 5.8$ ppm in the associated form, and as a singlet at $\delta_{\text{CH}_2} = 5.0$ ppm in the dissociated form).²⁸ These peaks are sufficiently isolated and their splitting pattern sufficiently characteristic that we identified them readily and were able to obtain accurate integrations: other regions of the spectrum—especially the aromatic region between 6 and 10 ppm—are crowded with many overlapping peaks. For these aggregates, we define the mole fraction of $\text{DMSO-}d_6$ that causes half the aggregate to dissociate (that is, where $A_{\text{associated}} = A_{\text{dissociated}}$; $\theta = 0.5$) as $\chi_{1/2}^{\text{DMSO}}$.

$$\theta_{\text{associated}} = \frac{A_{\text{associated}}}{A_{\text{associated}} + A_{\text{dissociated}}} \quad (10)$$

Aggregate 9, which we suspect is the least stable among the aggregates we examined, showed no isolated, characteristic peaks that corresponded to the associated aggregate that were distinguishable from peaks of the dissociated aggregate. For this aggregate we applied the second method of determining θ as a function of $\chi_{1/2}^{\text{DMSO}}$. We monitored δ , the shift (downfield from TMS, measured in ppm) of lines corresponding to the methylene groups $-\text{CH}_2-$ of diethylbarbital. For each mole fraction of DMSO ($\chi_{1/2}^{\text{DMSO}}$; defined as the moles of DMSO divided by the sum of the moles of DMSO and chloroform),

(28) Use of either set of peaks gave the same results. It may be noteworthy, however, that for aggregates comprised of $\text{Hub}(\text{M})_3$, there appeared a new peak at approximately 11.5 ppm that seemed to grow with increasing mole fraction of methanol, then disappeared completely at 100% DMSO. It is possible that this peak represents a partly dissociated aggregate and that a three-step model more accurately describes the dissociation of certain of the aggregates.

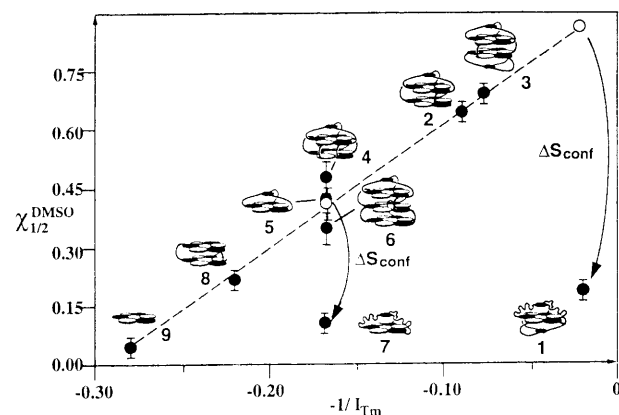


Figure 3. The experimental values of $\chi_{1/2}^{\text{DMSO}}$ are plotted against $-1/I_{\text{Tm}}$ (Figure 4 derives the forms of this plot). The slope gives the value of $TC_s/D_{\text{HB}} = 3.45$ (unitless). The open circles indicate the position of the flexible aggregates that is predicted using the stability indices, and the closed circles are the measured values. The error bars represent a 5% error (in aggregates 1–3, 7–9) or a 10% error (aggregates 4–6) in the value of $\chi_{1/2}^{\text{DMSO}}$.

we determined the shifts of these protons in two solutions: one solution contained only diethylbarbital ($\delta_{\text{CH}_2}^{\text{D}}$); the other solution contained diethylbarbital and the appropriate number of equivalents of the melamine component ($\delta_{\text{CH}_2}^{\text{exp}}$). If the shift of the $-\text{CH}_2-$ groups of diethylbarbital in the aggregate is $\delta_{\text{CH}_2}^{\text{A}}$, the mole fraction of the aggregate in the mixture ($\theta_{\text{A}}^{\text{DMSO}}$) is then given by eq 11. We define $\chi_{1/2}^{\text{DMSO}}$ as the value of $\chi_{1/2}^{\text{DMSO}}$ at which $\Delta\delta = (1/2)\Delta\delta_{\text{max,CH}_2\text{Cl}}$; $\theta = 0.5$.

$$\theta_{\text{A}}^{\text{DMSO}} = \frac{\delta_{\text{CH}_2}^{\text{exp}} - \delta_{\text{CH}_2}^{\text{D}}}{\delta_{\text{CH}_2}^{\text{A}} - \delta_{\text{CH}_2}^{\text{D}}} = \frac{\Delta\delta}{\Delta\delta_{\text{max,CH}_2\text{DMSO}}} \quad (11)$$

There is undoubtedly a great deal of information in the precise shape of the dissociation curves, especially regarding cooperativity of assembly and disassembly. We restrict this work to trends in relative stability and do not explicitly address mechanisms of assembly; that is, we do not analyze the shapes of these curves in this work. We extract values of $\chi_{1/2}^{\text{DMSO}}$ for four aggregates that span a wide range of stabilities (Chart 1). The value of $\chi_{1/2}^{\text{DMSO}}$ correlates with $-1/I_{\text{Tm}}$ (we derive this relationship later). Since $I_G/(N-1)$ correlates with I_{Tm} , the value of $\chi_{1/2}^{\text{DMSO}}$ also correlates with $I_G/(N-1)$.

Aggregates 1 and 7 deviate significantly from the pattern of stabilities predicted using the stability indices (Figure 3). Both of these aggregates share a common feature: the arms comprising the melamine component are flexible, and the changes in conformational entropy, ΔS_{conf} , on assembly may be significant. A significant change in the value of ΔS_{conf} disfavors assembly and decreases the mole fraction of DMSO required to dissociate the aggregate.²⁹

Gel Permeation Chromatography. Chart 1 shows the form of the GPC traces of these aggregates. GPC is a size exclusion technique. Small aggregates—with size defined as the hydrodynamic drag, which is usually estimated as mass raised to an exponent between 1/2 and 1, often 2/3—partition into the matrix to a greater extent than large ones, and take longer to emerge from the column. This technique is normally used to yield information on molecular weight; we infer stability from the shape of the peak. That is, stable aggregates elute from the

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column as sharp peaks; less stable aggregates that undergo slow dissociation on the column produce peaks that tail.³⁰

Electrospray Ionization Mass Spectrometry (ESI-MS). Aggregates that are stable to electrospray ionization (indicated with a "+" in Chart 1) gave peaks corresponding to the aggregate. We assume that other aggregates did not show any peak corresponding to the aggregate (indicated with a "-"), because they were either unstable to the ionization or fragmented rapidly during analysis.

Conceptual Bases For I_{Tm} , I_G and $I_G/(N-1)$. In this section, we discuss the concepts on which I_{Tm} , I_G and $I_G/(N-1)$ rest. We include a limited number of mathematical expressions, but most of the arguments are qualitative. The discussion is divided in three parts: I_{Tm} , I_G and $I_G/(N-1)$. The three indices are consistent with experimental trends of stability. The values for these indices for nine different aggregates are shown in Chart 1.

Stability is Proportional to $I_{Tm} = HB/(N-1)$. The stability of double stranded DNA is described universally in terms of a melting process.²⁶ The melting temperature, T_m , is the temperature (K) at which the double-stranded DNA joined by hydrogen bonds is half dissociated ($\theta = 0.5$) into the two component particles of single-stranded DNA (eqs 12 and 13).²⁶ This melting is cooperative for DNA.

$$\Delta G = \Delta H - T\Delta S = 0 \quad (12)$$

$$T_m = \frac{\Delta H}{\Delta S} \quad (13)$$

Substituting HB for ΔH and $(N-1)$ for ΔS , we arrive at eq 7, which relates I_{Tm} to readily available parameters. We discuss the validity of these substitutions later. Because I_{Tm} assumes that the dissociation of our hydrogen-bonded complexes can be treated as a fully cooperative phase-transition-like process, it will be incorrect to the extent that there are no intermediate, partially formed aggregates of comparable stability involved.

The ease at which I_{Tm} can be computed, and the consistency in its results, both make this index very useful in qualitative discussions of stabilities of proposed new aggregates.

The most important assumption in using these indices—especially in using $(N-1)$ as a surrogate for the entropy of assembly—is that the changes in conformational entropy, ΔS_{conf} , are comparable among the aggregates being compared. Two aggregates in this work, 1 and 7, are less stable than predicted using any of the stability indices. The failure of the stability indices in these two cases reflects a breakdown of the hypothesis that conformational entropy could be ignored.

Relationship between I_{Tm} and $\chi_{1/2}^{DMSO}$. The value of I_{Tm} is obtained by setting $\Delta G = 0$ and solving for T (eqs 12–13). Figure 4 illustrates that there are two limiting ways, in principle, that ΔG can be made to equal zero *experimentally*: the favorable enthalpy ΔH can be decreased until $\Delta H = T\Delta S$ (left side), or the unfavorable entropy $T\Delta S$ can be increased until $\Delta H = T\Delta S$ (right side). Experimentally, the ΔH can be decreased by

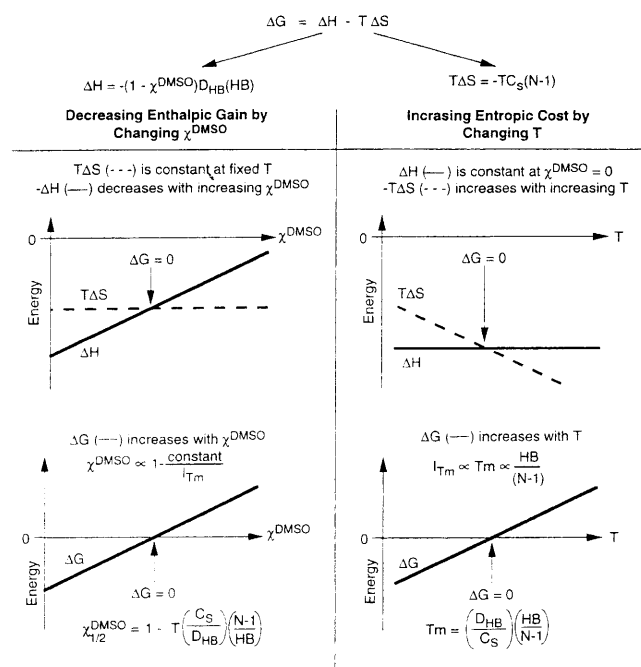


Figure 4. Relationship between I_{Tm} and the experimental values χ^{DMSO} and T , expressed in terms of ΔG , ΔH and ΔS (see text for details).

decreasing the net enthalpy of each hydrogen bond by adding a solvent that competes with the components of the aggregate for hydrogen bonds. Experimentally, the $T\Delta S$ can be increased by increasing the temperature. We chose the former strategy and titrated nine different aggregates in chloroform solution with DMSO.

Stability Proportional to $I_G = D_{HB}(HB) - TC_S(N-1)$. Unlike I_{Tm} , I_G does not assume a value for ΔG : the value of I_G for the assembly of N particles into one is proportional to ΔG_N° and has units of kcal/mol. We convert HB and $(N-1)$ into energy terms by introducing two constants: D_{HB} (the enthalpy of a single hydrogen bond in the aggregate from the separate components in $CHCl_3$ solution, in kcal/mol), and C_S (an entropy term with units $\text{kcal mol}^{-1} \text{ degree}^{-1} \text{ particle}^{-1}$). We estimate values for D_{HB} and C_S later in the text to arrive at a numerical expression for I_G (eqs 14–16) for small organic aggregates at room temperature.

$$\Delta G = \Delta H - T\Delta S \quad (14)$$

$$\Delta H \propto D_{HB}(HB) = 2.8(HB) \quad (15)$$

$$T\Delta S \propto TC_S(N-1) = 16(N-1) \quad (16)$$

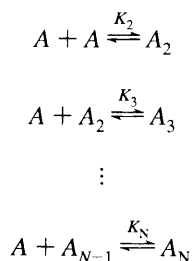
The success of I_G in predicting stabilities depends on the accuracy of the values used for D_{HB} and C_S . I_{Tm} is independent of these values.³¹ We have calculated values of I_G for all of the aggregates listed in Chart 1. As we illustrated earlier, the value of I_G (or of ΔG_N°) for self assembly is not the most useful measure of stability; that is, I_G is not related simply to θ . Instead, we propose that a version of I_G , namely $I_G/(N-1)$, is more useful, especially for noncooperative assembly. We discuss this parameter next.

Stability Proportional to $I_G/(N-1) = D_{HB}HB/(N-1) - TC_S$. This stability index is a condensation of the previous two, and perhaps conceptually the most meaningful for noncooperative

(30) The precise shape of the peak in the GPC trace is a complex function of the rate of disassembly (relative to the time of the GPC experiment), of the mechanism of disassembly, and of the solubility of the components (the CA components—especially those containing two and three CA groups—have low solubility in chloroform). For insoluble CA components, such as those in aggregates 1–3 and 8, the shape of the GPC traces implies a greater stability than determined from titrations using DMSO—experiments that we feel are more accurate than any of the other methods discussed. Specifically, aggregates 1 and 8 give reasonably sharp GPC traces but are dissociated by low mole fractions of DMSO.

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assembly. Consider the formation of the homoaggregate A_N from N particles of A as a series of simple equilibria.



If each of these steps is considered thermodynamically identical, then $K_2 = K_3 \dots = K_N$ (that is, the assembly is fully noncooperative), and K_N and ΔG_N° are given by eqs 17–18, where K_i and ΔG_i° are the binding constant and free energy for adding one particle to a partially formed aggregate.

$$K = K_2 K_3 \dots K_N = K_i^N \quad (17)$$

$$\Delta G_N^\circ = \Delta G_2^\circ + \Delta G_3^\circ + \dots \Delta G_N^\circ = (N-1)\Delta G_1^\circ \quad (18)$$

Thus one limiting way of comparing stabilities of aggregates having different numbers of particles might be to calculate the average free energy for each on dissociating one particle of aggregate (or a subaggregate) into two particles (that is, ΔG_i°). We have already shown that the total free energy of assembly ΔG_N° is proportional to the value of I_G . Thus eq 18 yields eq 19.

$$\Delta G_i^\circ = \frac{\Delta G_N^\circ}{N-1} \propto \frac{I_G}{N-1} = D_{HB} \left(\frac{HB}{N-1} \right) - TC_S \quad (19)$$

$I_G/(N-1)$ thus represents the average free energy of assembly per particle, and is equal to the enthalpy of assembly per particle minus a constant that is equal to the entropy of assembly per particle. For noncooperative assemblies, the complexation of each particle to a partially formed assembly must occur with a favorable free energy (that is, a favorable $I_G/(N-1)$). An example may be the formation of extended, hydrogen-bonded tapes in organic solvent. High values of $I_G/(N-1)$ therefore correspond to stable aggregates, and low values correspond to unstable aggregates.

HB Approximates ΔH ; $N-1$ Approximates ΔS . The final section of this paper reports the assumptions that we use to justify our approximations that $HB \propto \Delta H$ and that $(N-1) \propto \Delta S$, and estimate values for both proportionality constants.

Enthalpy (ΔH) is Proportional to HB. Four types of interactions contribute to the value of ΔH on assembly of N particles (eq 20): the enthalpy of van der Waals interactions (ΔH_{vdW}),³² of charge-charge interactions (ΔH_{CC}),^{33,34} of bond strain (ΔH_{BS}),^{35,36} and of hydrogen bonds (ΔH_{HB}).^{37–40}

$$\Delta H = \Delta H_{vdW} + \Delta H_{CC} + \Delta H_{BS} + \Delta H_{HB} \quad (20)$$

We expect that the total change in vdW interactions on assembly is small ($\Delta H_{vdW} \approx 0$): the strength of the vdW interactions among the particles in the assembly and with solvent are likely to be similar in magnitude. There are no charged or ionizable groups in any of the complexes we consider, and therefore $\Delta H_{CC} \approx 0$.

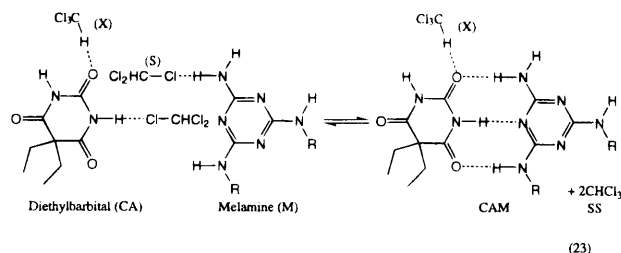
The magnitude of bond strain is difficult to estimate and probably differs among aggregates,^{36,41} but the aggregates that have similar structures should have similar values of ΔH_{BS} for assembly. We have previously synthesized four-particle aggregates that varied in the expected strain: the aggregate with the greatest strain was the least stable.³⁵ Since we are most interested in comparing the members within a family of related aggregates (that is, we are interested in the difference between values of ΔH , $\Delta \Delta H$), contributions to enthalpy that are *constant* for all members of the family of aggregates are unimportant to us; we therefore ignore the contribution of ΔH_{BS} ($\Delta \Delta H_{BS} \approx 0$) to ΔH .

We hypothesize that, within a series of aggregates of related structure, the relative enthalpy of formation is dominated by formation of hydrogen bonds (eq 21).

$$\Delta H \approx \Delta H_{HB} \quad (21)$$

If the assembly comprises particles that are connected by structurally similar hydrogen bonds, the value of ΔH_{HB} is a product of the *number of hydrogen bonds* (HB) and the *average net enthalpy of formation of each hydrogen bond* (D_{HB}) in the solvent of interest (eq 22). An example of calculating D_{HB} is given by eqs 23 and 24.

$$\Delta H_{HB} = -D_{HB}(HB) \quad (22)$$



$$\Delta H_{HB} = (\Delta H_{CAM} + \Delta H_{SS}) - (\Delta H_{CAS} + \Delta H_{MS}) = 2D_{HB} \quad (24)$$

HB Is Proportional to the Total Enthalpy of Association.^{42,43} The generality of eq 10 may be limited by at least three factors: (i) *The value of D_{HB} is dependent on solvent.* The value of D_{HB} depends on solvent (S) (eqs 13 and 14) (ΔH_{SS}). Table 1 suggests the range of D_{HB} in two systems similar to those examined here. Because the value of D_{HB}

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(42) In systems other than our own, VDW and CC interactions may not be negligible. If the energies of VDW and ES are either (i) proportional to the number of hydrogen bonds or (ii) similar in magnitude among the aggregates, then differences in HB are still proportional to the relative energies of assembly.

(43) In self-assembling systems based on charge-charge interactions or metal-ligand chelates, the analysis would be parallel to that reported here, with the number of charge-charge interactions or metal-ligand interactions replacing HB.

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(36) Bond strain occurs when the conformation adopted on assembly requires that bond lengths, bond angles, and torsional angles deviate from values in the uncomplexed particles. We believe that differences in BS are related to the degree of planarity (DP) of the rosette; we have addressed this issue in Chin, D.; Gordon, D. M.; Whitesides, G. M. *J. Am. Chem. Soc.* **1994**, 116, 12033.

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Table 1. Values of D_{HB} (kcal/mol) for Interactions between Adenine and Uracil and between Two Molecules of *N*-Butylacetamide^a

solvent	D_{HB}	
	2 <i>N</i> -butylacetamide ³⁵⁻⁴⁰	adenine + uracil ³⁵⁻⁴⁰
gas phase		7.2
CCl ₄	3.8	3.8
benzene	3.1	
CHCl ₃	2.6	3.1
dioxane	0.2	
water	0.0	1.4

^a HB = 2, $N = 2$ in both cases.

depends on solvent, we restrict our attention to comparisons of stabilities in a common solvent, and among aggregates in a common family. (ii) D_{HB} differs for different types of hydrogen bonds. Hydrogen bonds within a single assembly can differ in strength. We use a single *average* value. Again, this approximation limits comparisons to similar families of aggregates. (iii) *The value of HB is affected by spontaneous formation of alternate/undesired aggregates.* Our treatment assumes that only two states are involved: aggregate and dissociated, non-interacting components. In fact, the components can probably form non-specific hydrogen bonds with themselves and with each other.²⁸ Although the importance of these interactions is unknown, the broadening observed for solutions of the melamine components, and the low solubility of the barbiturate in chloroform both suggest that they may be significant. The arguments based on I_{Tm} are still valid if the propensity to aggregate non-specifically remains *constant* for the different molecules being compared.

Estimating the Value of D_{HB} . The enthalpy of association has been measured by both calorimetry and by van't Hoff analysis for a large number of hydrogen-bonding pairs (see, for example, Table 1). In chloroform, the average value for D_{HB} for a hydrogen bond between an amide or imide proton and a pyridine or carbonyl group is approximately 2.8 ± 0.3 kcal/mol.^{33,34,44-49}

Entropy (ΔS) is Proportional to $(N-1)$. We partition the total change in entropy on aggregation into translational entropy (ΔS_{trans}), rotational entropy (ΔS_{rot}), conformational entropy (ΔS_{conf}) and entropy of the solvent (ΔS_{sol}) (eq 25). Each term can be evaluated separately using expressions taken from statistical thermodynamics (eqs 26 and 27).⁵⁰

$$\Delta S = \Delta S_{trans} + \Delta S_{rot} + \Delta S_{conf} + \Delta S_{sol} \quad (25)$$

$$\Delta S_{trans} = (N-1) \left(\frac{5}{2} R + R \ln \frac{V}{N} (2\pi k T \bar{M}_P)^{3/2} \right) - R \ln \frac{V}{N} \left(2\pi k T \left(\frac{M_{ass}}{M_P} \right)^{3/2} \right) = C_{trans} (N-1) - \delta_{trans} \approx C_{trans} (N-1) \quad (26)$$

$$\Delta S_{rot} = (N-1) R \ln \left(\frac{8\pi^2 e k T}{h^2} \right) (\bar{I}_P) - R \ln \left(\frac{8\pi^2 e k T}{h^2} \right) \left(\frac{I_{ass}}{\bar{I}_P} \right) = C_{rot} (N-1) - \delta_{rot} \approx C_{rot} (N-1) \quad (27)$$

The terms in eqs 26 and 27 are the fundamental constants R , k , e , π and h , and the variables concentration (N/V), the average mass of the particles that comprise the assembly (\bar{M}_P), the mass of the assembly (M_{ass}), the average moment of inertia of the particles that comprise the assembly (\bar{I}_P), and the moment of inertia of the assembly (I_{ass}). In eq 26, the value of δ_{trans} depends on the ratio M_{ass}/\bar{M}_P . We will show later that for the aggregates in this study, the value of δ_{trans} is less than 20% of the value of C_{trans} ; we therefore ignore it. Similarly, for ΔS_{rot} , the value of the small correction δ_{rot} is less than 20% of the value of C_{rot} ; we therefore ignore it as well. Both ΔS_{trans} and ΔS_{rot} are then directly proportional to $(N-1)$.

Estimating the contribution from conformational entropy (ΔS_{conf}) is difficult: we believe that comparing the entropy of assembly of different aggregates within a family is possible only if the contributions from conformational entropy are expected to be similar (for systems in which the flexibility of the melamine components are similar).⁵¹ In this work, we provide two examples (aggregates **1** and **7**) where ΔS_{conf} may be significant, and for which the aggregates are less stable than predicted using the stability indices. For example, hub(M)₃:3CA (hub) (aggregate **5**) and flex(M)₃:3CA (flex) (aggregate **7**) are both assemblies of four particles that form a monorosette. Although the value of $I_{Tm} = 6$ for each aggregate, the aggregate based on hub (which is conformationally rigid) is more stable (has a higher value of $\chi_{1/2}^{DMSO}$) than the aggregate based on flex (which is conformationally flexible) (Figure 4).³⁵

We ignore the entropy of the solvent (ΔS_{sol}) in the final analysis for several reasons: (i) *The value of ΔS_{sol} may be negligible.* When adenine and uracil associate, 2–4 molecules of solvent are released into the bulk solvent. The change in translational entropy for this process varies inversely with the concentration of the molecules (N/V ; eq 9). The concentration of molecules of solvent in the liquid phase is, in general, large (the concentration of CHCl₃ in pure liquid at 25 °C is 8 M), and the change in translational entropy for release of 2–4 particles into bulk solvent is correspondingly low. (ii) ΔS_{sol} is proportional to $(N-1)$. If the number of molecules of solvent associated with each particle is *constant*, then the total change in translational and rotational entropy for release of solvent into the bulk is proportional to $(N-1)$ (eq 28), and is accounted for (if only crudely) by our model.

$$\Delta S_{sol} \approx -C_{sol} (N-1) \quad (28)$$

(iii) ΔS_{sol} is constant. If all the individual particles within a family of aggregates have a similar number of associated molecules of solvent, then the *difference* in ΔS_{sol} among the different members in the family ($\Delta \Delta S_{sol}$) may be negligible.

While the entropy of solvent is ignored for associations in *organic* solvent, formation of multiparticle assemblies in *aqueous* solution is often driven by the changes in the entropy of the solvent.^{31,52} Thus our discussions are limited to systems in which entropy disfavors aggregation. Combining eqs 25–28 yields eq 29. As an approximation, we conclude that for an aggregate of small particles, *the entropy of association is*

(51) If the change in conformational entropy is either (i) proportional to the number of particles, $(N-1)$ or (ii) of similar magnitude in the aggregates, then differences in $(N-1)$ will still be predictive of relative entropic cost of assembly.

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proportional to $(N-1)$.⁵³ It is noteworthy that ΔS is proportional to $(N-1)$ and not to N . The physical origin of the choice of $(N-1)$ rather than N is that $N-1$ characterizes the *net change* in the total degrees of freedom upon assembly. Of course, for large values of N , $(N-1)$ and N have similar values.

$$\Delta S = C_{\text{trans}}(N-1) + C_{\text{rot}}(N-1) + \Delta S_{\text{conf}} + C_{\text{sol}}(N-1) \approx C_S(N-1) \quad (29)$$

Estimating the Value of C_S . The simplest approach, and the one we take in this paper, is to assume that eqs 26 and 27 are valid in the condensed phase, and assign reasonable (or typical) values to the variables in these equations.^{54–56} In this zeroth level approximation, and for the reasons discussed earlier, we ignore any changes in both conformational entropy and the entropy of the solvent. The value of TC_S is then given by eq 30, where the expressions for C_{trans} and C_{rot} are taken from eqs 26 and 27, respectively, at 25 °C.

$$TC_S = TC_{\text{trans}} + TC_{\text{rot}} = 9 + 7 = 16 \text{ kcal/mol} \quad (30)$$

The average mass of particles in the aggregates is dominated by the mass of CA (approximately 300–350 g/mol). In eq 26, the value of ΔS_{trans} is proportional to $TC_{\text{trans}}(N-1) - T\delta_{\text{trans}}$. The value of $T\delta_{\text{trans}}$ depends on the ratio of the mass of the aggregate to the average mass of the particle. The mass of the assembly varies from 1700–3200. Using standard conditions for the remaining variables ($T = 300 \text{ K}$, $N/V = 1 \text{ M}$), the value of $T\delta_{\text{trans}}$ is then $0.8 \ln(M_{\text{ass}}/M_p)$, which is equal to approximately 1–2 kcal/mol for the aggregates 1–9. This value for $T\delta_{\text{trans}}$ is small relative to $(N-1)TC_{\text{trans}}$, and we therefore ignore it. The value of TC_{trans} is approximately 9 kcal/mol. For similar reasons, we ignore the small value of $T\delta_{\text{rot}}$. The value of TC_{rot} is approximately 7 kcal/mol. Both $T\delta_{\text{trans}}$ and $T\delta_{\text{rot}}$ decrease the entropic cost of assembly by a small amount; by ignoring them, we may therefore be overestimating ΔS_{tot} . From these estimates, we conclude that the value for C_S is approximately 16 kcal/mol in our systems. The numerical expression for I_G is given by eq 2. The units for I_G are kcal/mol, and I_G is proportional to the free energy (ΔG) of assembly. Since we have ignored the values of δ_{trans} and δ_{rot} , the absolute value of I_G does *not* determine the spontaneity of reaction: a positive value of I_G does not imply that assembly occurs spontaneously; a negative value of I_G does not imply that assembly does not occur. If the value for C_S is accurate, however, the relative values of I_G (ΔI_G) should correlate with $\Delta \Delta G$ of assembly.

We emphasize that the numerical estimation of C_S is approximate. There is some evidence that in condensed phases (in solution), the calculated value for C_{trans} using eq 26 is twice as large as the experimental value, and that we have therefore significantly overestimated the value of ΔS_{tot} .^{29,57–60} In this case, the value for TC_S would be approximately 11 kcal/mol. The major sources of uncertainty in the value of C_S are probably the values of ΔS_{trans} in solution (eq 14 is accurate in the gas

phase, but not in the condensed phase), and ΔS_{conf} . We find that the experimental value for the ratio of $D_{\text{HB}}/TC_S = 3.45 \pm 0.5$ (from the slope of the line in Figure 3); although this value is consistent with a value of $D_{\text{HB}} = 3 \text{ kcal/mol}$, and $TC_S = 11 \text{ kcal/mol}$, we do not evaluate these parameters independently in this work.

Conclusions

We have shown that the values of ΔH and ΔS for assembly of hydrogen-bonded aggregates can be approximated by D_{HB} (HB), and $C_S(N-1)$, respectively. We have also shown that the stability index $I_{\text{Tm}} = \text{HB}/(N-1)$ can be related to $\Delta H/\Delta S$ theoretically, and to $\chi_{1/2}^{\text{DMSO}}$ experimentally. I_{Tm} may be the most useful of the three for fully cooperative assemblies. We have shown theoretically that the stability index I_G can be related to the value of ΔG of assembly. Finally, we have shown that $I_G/(N-1)$ is theoretically related to $\Delta G/(N-1)$ theoretically: this value is a free energy of assembly per particle. This last index requires evaluation of parameters— D_{HB} and C_S —whose values depend on the system and are uncertain in some cases. $I_G/(N-1)$ is therefore difficult to use, but may be the most predictive of relative stability among a group of related aggregates that assemble noncooperatively (for example, hydrogen-bonded tapes). We have shown that for our aggregates, $I_G/(N-1)$ correlates with the experimental measure of stability, $\chi_{1/2}^{\text{DMSO}}$.

We believe that these indices I_{Tm} and $I_G/(N-1)$ represent two limiting models for self assembly of particles in solution. I_{Tm} is related conceptually to a melting point, and is most useful for fully cooperative processes. $I_G/(N-1)$ is conceptually related to a free energy per particle, and is most useful for fully noncooperative processes. Interestingly, $I_G/(N-1)$ is related linearly to I_{Tm} . Both will be useful as “rules of thumb” in the design and evaluation of hydrogen-bonded aggregates. In all systems where the assumptions of the derivations of $\Delta H = D_{\text{HB}}$ (HB) and $\Delta S = C_S(N-1)$ are met, this approach to evaluating stability prior to time-consuming synthesis may be useful to those interested in constructing large, multiparticle arrays in organic solvent.

A substantial assumption underlying the use of these stability indices is that the changes in the value of ΔS_{conf} are insignificant (or at least the same) among the aggregates being compared. If the value of ΔS_{conf} for one aggregate is significant (or higher than for the other aggregates), then the stability (as estimated using $\chi_{1/2}^{\text{DMSO}}$) is lower than that predicted by the stability indices. We describe two examples (aggregates 1 and 7) of such cases in this work.

The arguments presented here can be extended easily to assemblies held together by non-covalent interactions other than hydrogen bonds. For example, in Lehn's assemblies of molecules held together by Cu^{2+} -bipyridyl interactions,^{61,62} OM, the number of the organometallic interactions, would replace HB in both I_{Tm} and I_G . In the assemblies of Hunter,⁶³ the components are held together by porphyrin-nitrogen interactions, and again, OM would replace HB, in principle. The values of D_{HB} and C_S , the constants multiplying HB and $(N-1)$, would have different values from those in this work; the values of these constants may be evaluated using a combination of the equations contained in this work, and experimental enthalpies of various organometallic interactions. In both cases, a weak

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chelating agent would replace DMSO, and an equivalent experimental parameter would replace $\chi_{1/2}^{\text{DMSO}}$.

Experimental Section

General. The synthesis and characterization of the aggregates studied here have been described^{27,64–68} and reviewed.⁸ Solvents used for NMR spectroscopy were obtained from Cambridge Isotope Laboratories and were used as received. To limit the amount of water,⁶⁹ newly-opened bottles of deuterated solvent were used for each experiment; compounds were dried thoroughly under low pressure; NMR tubes were heated in an oven.

NMR Spectroscopy. All spectra were recorded on a Bruker AM-400 Spectrometer. We determined the 90° PW for each sample (typically between 5 and 15 s). Recycler delays of 2 s were used; increasing the recycler delay did not affect the integration of the peaks of interest. Other parameters include: SW (sweep width) = 20 ppm;

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(69) Trace water was not possible to avoid even in fresh bottles of deuterated solvent, and was present in proportion to the mole fraction of DMSO. Because the ratio of DMSO to water was approximately 20000:1, and because the DMSO, as a hydrogen bond acceptor, is comparable to water, the contribution of this trace water to dissociation of the aggregate was ignored.

SI = TD = 32 K; and NS (number of scans) = 64 or 32, depending on the quality of the spectra. All NMR samples were sonicated and heated to reflux in an attempt to reach equilibrium. Spectra recorded after one week were identical to those recorded initially. The total concentration of aggregate was 5 mM in all cases.

Aggregates. Below we detail observations made during these titration experiments. We group the aggregates by characteristic. (i) *Remains homogeneous throughout the course of experiments:* aggregates **1**, **3**, **5**, **7**, and **9**. (ii) *Precipitates somewhat from solutions containing both DMSO and CDCl₃, but is soluble in both pure solvents:* **2** and **8**.⁷⁰ (iii) *Loose gel formation from solutions containing both DMSO and CDCl₃, but is soluble in both pure solvents:* **4** and **6**.⁷¹

Acknowledgment. This work was supported by the NIH Grants GM 30367 and CHE 91-22331. The NMR facilities at Harvard were supported by NIH grant 1-S10-RR04870-01 and NSF Grant CHE88-14019. E.E.S. (1995) and M.M.(1994) gratefully acknowledge Eli Lilly for predoctoral fellowships.

JA9627681

(70) Certain CA derivatives—especially those containing two or three CA groups—precipitate slightly from solution when not part of a stable assembly; this precipitation may thermodynamically *lessen* the likelihood of formation of stable aggregates, and the value of the mole fraction of DMSO required for dissociating the aggregate may be slightly underestimated.

(71) These gels could be brought completely into solution by diluting the sample from 5 mM to 2.5 mM. Quantitatively, this increases the translational and rotational entropic cost of assembly by less than 10% in most cases, but this increase does depend on the value of N. The spectra collected for aggregate **5** were indistinguishable at 2.5 mM and 5 mM over the full range of χ^{DMSO} .