Formulating rationally via statistical thermodynamics

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Abstract

Formulators do not naturally turn to statistical thermodynamics for experimental inspiration. However, with the newer, intuitive approach to statistical thermodynamics the formulator gains deep insights into the hitherto confusing effects of "cosolvents", "hydrotropes", "solubilizers" that affect properties such as solubility, gelation or conformational stability. The historical confusion has arisen from classical approaches which simply cannot disentangle causes and effects. The aim of this review is to demonstrate how a formulator can work with statistical thermodynamics towards a rational design of experiments and an unambiguous interpretation of the driving forces behind cosolvent effects.

1. Formulation via solvation control by additives (cosolvents)

This review deals with an important question in formulation science (Figure 1), namely to understand how

A. transition, solubility, stability, denaturation, sol-gel transition, aggregation, self-association, binding, dispersion,

can be controlled by adding

B. cosolvents, hydrotropes, micelles, surfactants, Hofmeister salts, chaotropes, kosmotropes, osmolytes, crowders, inert polymers, denaturants, stabilizers, gelling agents, excipients.

For convenience, let us use "transition" as a general term for A, and "cosolvents" for B throughout this review. The molecule that goes through transition is referred to as "solute".

How general and wide the scope of this question is can be felt by the diversity in the types of transition (list A, Figure 1(a)) and the wide-ranging solute size scales (Figure 1(b)). Moreover, the multiplicity of synonyms for cosolvents, covering different degrees of self-association (list B, Figure 1(c)), led to the long-standing misconceptions and confusions that the different class of cosolvents should obey different theories and explanations. The purpose of this review is to

persuade the reader that one universal theory can be used to explain all these transitions and cosolvents.

All the combinations above rely on the control of solvation by cosolvents. Hence, the first point of recourse for a formulator is the solubility prediction software, such as the Hansen solubility parameters (HSP) [1] or COSMO-RS [2], that can be used to quantify solvation phenomena in general. If they work perfectly there is no need for this review. However, systems like water and aqueous solutions pose notorious difficulties to such methods [3] which renders them unable to deal with the subtle ordering that drives the effects. In the absence of solubility theories, a formulator is forced to carry out

1. computer simulation, such as molecular dynamics,
2. measurements of physical properties.

For the reason to be discussed in detail in Section 7, option 1 is not yet mature enough completely to replace the current de-facto standards like HSP or COSMO-RS. Hence, here we focus on what can be known from the measurements of basic physical properties.

2. Why solvation is so confusing

Measuring physical properties to understand transition processes (option 2) has two uses: to validate the option 1, above, and to gain molecular insight itself. But a formulator is soon faced with a need to make decisions (Figure 1), namely to choose

(a) the suitable experimental approach(es) out of multitude of candidates that claim to give insight into solution-phase interactions,
(b) the appropriate theory or model – from several options – to quantify solution-phase interactions, and even
(c) the correct explanation from mutually-contradicting hypotheses that may or may not come with numbers or quantifiable models.

Conundrum in (a) is particularly serious when different experimental techniques proposed to measure the same phenomena give contradictory results. For example, the “osmotic pressure” dependence and hydrostatic pressure dependence of a biomolecular process can be in opposite directions and differs by a few orders of magnitude [4,5], even though they are claimed to probe the same thing: biomolecular hydration change. (This will be resolved in Section 4.)

The problem of (b) is that there are assumptions made in developing these models. One example is the partitioning model approach to preferential (competitive) solvation (Figure 2(b)), which has to introduce a boundary that divides the solvation shell from the bulk solution, without which the partition coefficient cannot be defined [6]. But where should such a boundary be? Instead of an answer there is an assumption. (See Section 4 for a statistical thermodynamic alternative). We shall examine the binding model (Figure 2(b)), which caused much confusion and controversy, in more detail in later sections.

Regarding (b), there is a tradition in solution chemistry that the study of "solution structure" (i.e., structure of bulk solvent-cosolvent mixture in our focus on two-component solutions) can somehow explain solvation of any solute. The "water structure" hypothesis and the hydrotrope self-association hypothesis belong to this category. According to the most articulate advocate of the water structure hypothesis [7], urea (as an example here) enhances the hydrophobic effect not through direct binding; the possibility was eliminated by the positive urea-hydrophobe interaction enthalpy. Hence, urea must act indirectly to the hydrophobe by breaking the structure of its hydration water [7]. The hydrotrope (i.e., a class of strongly self-associating cosolvents, Figure 1(c)) self-association hypothesis comes from the observation that a sigmoidal onset of solubilization is observed along the increasing hydrotrope concentration; and a loose analogy with critical micelle concentration has led to the proposal that hydrotrope self-association is the driving force (see [8–11] for review). The problem with these hypotheses is that they are dependent on questionable assumptions or unquantifiable premises. Statistical thermodynamics can judge their validity (see Section 6).

Therefore, the questions remain: which experiment(s) should be carried out? How should the experiment(s) be analysed to yield information on interactions taking place in solution? What are the driving forces for solubilization, aggregation, stabilization, and conformational changes?

3. Experiments without a statistical thermodynamic foundation is a recipe for confusion

Here, we briefly illustrate that any attempt to understand the cosolvent effect, despite its long history [12–14], is prone to confusion when approached without the rigour of statistical thermodynamics. (For a fuller account, see Ref [15]). Let us consider a transition of a solute (referred to as species u) as listed in Figure 1(a). Let K be the accompanying equilibrium

constant. According to the classical canon [4,16], how K changes with water activity a_1 (or the activity of principal solvent in general) can be expressed as a *competition*

$$\left(\frac{\partial \ln K}{\partial \ln a_1}\right)_{T,P,c_u\to 0} = \Delta N_{u1} - \frac{c_1}{c_2}\Delta N_{u2} \quad (1)$$

between ΔN_{u1} (the change in number of bound water to the solute u) and ΔN_{u2} (of cosolvent molecules) [4,16–18]. (Note that c_1 and c_2 represent the bulk water and cosolvent concentrations, respectively.) Eq. (1) was derived assuming (i) the existence of solvent binding sites on solutes and (ii) solvation as competitive stoichiometric binding of solvent and cosolvent.

There is lack of clarity in Eq. (1) as to (i) what, where, and how many are the solvent binding sites and (ii) how to account for the solvent-cosolvent size disparity. But the most serious problem is its inability to deal with sugars, polyols, and "kosmotropic" salts, that are strongly and preferentially excluded from biomolecular surfaces [4,16–18]. Since they are not bound to biomolecules, the number of bound cosolvents is zero. This renders Eq. (1), when used alongside with $\Delta N_{u2} = 0$, a powerful tool to evaluate hydration changes, by modulating water activity with "inert" or strongly excluded cosolvents [18,19]. Subsequent controversy, fuelled by grossly overestimated hydration via this method, has even questioned

(a) whether ΔN_{u1} and ΔN_{u2} have any real physical meanings [5,19,20], and

(b) whether two interaction parameters can in principle be determined from a single relationship (Eq. (1)) [5,19,20].

Only with rigorous statistical thermodynamics can such a controversy resolved [21,22].

3. Clarifying what we want from experiments via statistical thermodynamics

Clarity comes from rigorous statistical thermodynamics. By "rigorous" we do not mean "pages of impenetrable derivations". Rather, we mean nothing other than the use of its basic principles without any models or assumptions. By "clarity" we mean with regards to

i. the definition of solute-solvent and solute-cosolvent interactions (Figures 3 and 4), and

ii. how i. can be determined from experiments (Figure 5).

which the classical canon could not attain.

For a complete understanding of (i.), ΔN_{u1} and ΔN_{u2} must clearly be defined (Figure 3) [21,23]. By statistical thermodynamics a given conformation of a solute (or when there is no need to consider conformational transition), N_{u1} and N_{u2} are defined as excess numbers, i.e., the difference between the number of solvents (or cosolvents) in the vicinity of the solute and in the bulk solution of the same volume (Figure 3(a)) [21,23]. The boundary must be taken large enough, and after a certain size N_{u1} and N_{u2} ceases to depend on the volume encompassed by the boundary. ΔN_{u1} and ΔN_{u2} have thus been given a clear meaning as the change of excess number between the two conformational states (Figure 3(b)).

Having resolved i., we can apply the insight from it to tackle ii. When divided by the bulk phase concentrations, ΔN_{u1} and ΔN_{u2} become ΔG_{u1} and ΔG_{u2} (Figures 3 and 4) and are called the Kirkwood-Buff integrals (KBI). Introduced originally in 1951 [24], they have been applied to study the structure of solution mixtures via thermodynamic measurements [25–27], small angle scattering [28,29] and simulations [30,31]. KBI are the spatial integration of the increment of radial distribution function (RDF) from its bulk value (Figure 4). Besides its close relationship to scattering and simulation, the adoption of RDF is beneficial as it makes an arbitrary shell-bulk boundary redundant (see Figure 2(b)). Moreover, it encompasses both strong and specific binding (see Figure 2(b)); such binding corresponds to a very high first peak of RDF.

Consequently, using KBIs, Eq. (1) can be rewritten as

$$\left(\frac{\partial \ln K}{\partial \ln a_1}\right)_{T,P,c_u \to 0} = c_1(\Delta G_{u1} - \Delta G_{u2}) \tag{2}$$

which is simpler in form, hence easier for interpretation.

Thus, statistical thermodynamics has clarified what must be obtained from the experimental data – namely, the KBIs, the KBIs, which are the universal measure of solution structure and interaction strength [21–23].

4. Guiding experimental design by statistical thermodynamics

Statistical thermodynamics has identified the KBIs as the key descriptors of preferential solvation, thereby achieving the objectives of Section 3. Here, we show how KBIs can be determined from experimental data.

For a dilute solute in a binary mixture, the two KBIs (ΔG_{u1} and ΔG_{u2}) are responsible for solute-solvent and solute-cosolvent mixture. Linear algebra tells us that in order to determine two independent KBIs, two independent relationships (equations) are necessary, which led the resolution of the controversy (point (b) of section 2) [21]. For example, the change of volume accompanying the transition ΔV_u, which is thermodynamically equivalent to the pressure-dependence of K, is

$$\Delta V_u = -RT\left(\frac{\partial \ln K}{\partial P}\right)_{T,x_2,c_u\to 0} = -c_1 V_1 \Delta G_{u1} - c_2 V_2 \Delta G_{u2} \quad (3)$$

can be solved together with Eq. (2) as a pair of simultaneous equations (Figure 6).

An insight into experimental design comes from a realization that Eqs. (2) and (3) are a pair of simultaneous equations. This gives a resolution to the mysterious discrepancy between "osmotic pressure" dependence (Eq. (2)) and hydrostatic pressure dependence (Eq. (3)) of a transition [4,5]. While the "osmotic pressure" dependence can be expressed from Eq. (2) in terms of the KBIs as [22]

$$-RT\left(\frac{\partial \ln K}{\partial \Pi}\right)_{T,P,c_u\to 0} = \Delta G_{u1}^0 - \Delta G_{u2}^0 \quad (4)$$

the hydrostatic pressure dependence comes from Eq. (3) as [22]

$$-RT\left(\frac{\partial \ln K}{\partial P}\right)_{T,x_2,c_u\to 0} = -\Delta G_{u1}^0 \quad (5)$$

where the superscript 0 denote the $c_2 \to 0$ limit at which these two pressure dependencies were discussed. The conclusion is that the two experiments play a complementary role for the determination of the two KBIs and that hydration ΔG_{u1} should be estimated via hydrostatic pressure [21,22].

So far, we have focused on a dilute solute in the presence of solvent and cosolvent for the sake of simplicity. Nonetheless, the theory presented above can readily be extended to multiple components [11,22,32]. Although the following may sound like a small print, it may be of fundamental importance for mesoscale particles. Let us now consider a dilute solute in n component solutions, which involve n solute-solvent KBIs, for which n independent experimental measurements are required to determine them all, by changing n thermodynamic variables [11,22,32]. According to the Gibbs phase rule, n component system in a single phase has $n+1$ independent thermodynamic variables, hence all n KBIs can be determined at a given temperature (which corresponds to one thermodynamic variable) [11,22,32]. Yet, when

u is no longer considered to be the part of solution, the system is considered to be biphasic, thereby reducing the number of independent KBIs by one [11,22,32].

Thus, statistical thermodynamics clarifies the needs and problems of measurements. Firstly, how to complement different experimental techniques to quantify all the necessary interactions. Secondly, how many experiments are necessary in principle, which is essential to know for the efficiency of experimental design. As a practical note, it is often straightforward to make estimates of bulk thermodynamic parameters (such as density, volume and activity) or to identify parameters that make minor contribution, thereby decreasing the need for experimental work. The present author has used this technique many times when analysing valuable historical data that lacked the ideal range of experimental information [3,33–37].

5. Judging the validity of classical hypotheses by statistical thermodynamics

Statistical thermodynamics can judge the validity of classical hypotheses that have been invoked for a long time. As examples, let us examine the validity of the water structure (Section 2), solvent binding, "osmolyte exclusion = zero binding" hypotheses (Section 3), as well as the textbook canon of preferential solvation (Section 3).

Water structure hypothesis. If this hypothesis were true, then $\left(\frac{\partial \ln K}{\partial \ln a_1}\right)_{T,P,c_u \to 0}$ in Eq. (2) would be dominated by ΔG_{u1} while ΔG_{u2} is negligibly small. This is contrary to the majority of transitions, such as protein stability [38], allosteric transition [22], aggregation and gelation [15,39], hydrotrope solubilization of small solutes [8,9,34], where ΔG_{u2} is much larger than ΔG_{u1}. For example,

- Protein stability [38]: ΔG_{u2} for ribonuclease thermal denaturation is 2643 $cm^3\ mol^{-1}$ in the presence of dilute guanidine hydrochloride, and is −2617 $cm^3\ mol^{-1}$ in the presence of trehalose, both much larger than $\Delta G_{u1} = -16$ $cm^3\ mol^{-1}$.
- Gel stability [39]: ΔG_{u2} for the melting of agarose gel is −2530 $cm^3\ mol^{-1}$ in the presence of dilute sucrose, much larger than $\Delta G_{u1} = -16$ $cm^3\ mol^{-1}$.
- Solubilization [34]: ΔG_{u2} for model drugs in the presence of hydrotropes are much larger than ΔG_{u2}, especially in the hydrotrope concentration range where solubility increase takes place.

This is underscored further by the widespread observation that protein transitions are so far more sensitive to the "osmotic pressure" than to the hydrostatic pressure [40,41]. This, according to Eqs. (4) and (5), signifies $|\Delta G_{u1}^0 - \Delta G_{u2}^0| \gg |-\Delta G_{u1}^0|$, namely $|\Delta G_{u2}^0| \gg |\Delta G_{u1}^0|$, which underscores our previous conclusion on the dominance of ΔG_{u2} over ΔG_{u1} which is contradictory to the water structure hypothesis.

Osmolyte exclusion = zero binding hypothesis. The dominance of ΔG_{u2} over ΔG_{u1} is a universal observation, applicable to strongly-excluded osmolytes, which is contradictory to the statement that the osmolyte binding is zero, namely, $\Delta N_{u2} = \Delta G_{u2} = 0$ [18,19]. Indeed,

- Protein stability [38]: ΔG_{u2} for ribonuclease thermal denaturation is −2617 cm^3 mol^{-1} in the presence of trehalose.
- Gel melting [39]: ΔG_{u2} for the melting of agarose gel is −2530 cm^3 mol^{-1} in the presence of dilute sucrose.

There are many other counterexamples to this hypothesis.

Solvent binding hypothesis. Contrary to this hypothesis, the excess number of cosolvents to a solute (or a conformational state) may not be positive. G_{u2} takes a negative value when the local number of cosolvents are lower around the solute than in the bulk (Figure 7(a)). That cosolvent depletion [42] or exclusion (i.e., crowding [43]) plays a major role to chemical processes was beyond the reach of the solvent binding perspective [21,22]. (Note that there is always a large, negative contribution for macromolecules to G_{u1} and G_{u2} arising the excluded volume effect. Indeed, ΔG_{u2} of ribonuclease in aqueous urea and trehalose solutions both take negative values; the less negative ΔG_{u2} in urea comes from urea accumulation around the protein [44]).

Preferential solvation paradigm. ΔG_{u1} and ΔG_{u2} can be calculated independently, contrary to the expectation of the classical hypothesis. In addition, it is ΔG_{u2} that contributes dominantly to many transitions. Hence, we focus on the distribution of cosolvents based on the following approximation for Eq. (3):

$$-\left(\frac{\partial \ln K}{\partial \ln a_1}\right)_{T,P,c_u \to 0} \simeq c_1 \Delta G_{u2} \tag{6}$$

There is no longer any need to consider the "preferential" solvation, namely the difference $\Delta G_{u2} - \Delta G_{u1}$, except for a few cases (see below).

Cosolvent accumulation and exclusion as the true driving force. As the simultaneous equations (Eqs. (2) and (3)) have established the negligibility of ΔG_{u1}, we can focus on ΔG_{u2} as the driving fore (Figure 6(b)). When cosolvents are excluded from the solutes, aggregation of the solute pair reduces the solute surface from which cosolvent are excluded, hence $\Delta G_{u2} > 0$. This, according to Eq. (6), leads to the enhancement of aggregation. When cosolvents tend to be accumulated around the solutes, the aggregation of the solute pair makes them less exposed to cosolvents, thereby reducing the number of cosolvents around them, leading to $\Delta G_{u2} < 0$. This, according to Eq. (6), leads to the suppression of aggregation [15].

Thus, we have shown that most of the classical hypotheses cannot be supported by simple experimental observations. We have replaced them with the KBI-based interpretation.

6. Minor role of bulk solution structure revealed by statistical thermodynamics

As discussed in Section 2, bulk solution structure has long been expected to provide an explanation for solvation and solvation-induced equilibrium shifts in the presence of cosolvents. Now, we test an alternative hypothesis: hydrotrope self-association is responsible for solubilization (see Section 2).

To evaluate this hypothesis, it is important to bear in mind that solubilization is measured along the molarity of hydrotrope concentration. Hence, it is useful to express the following molar solubilization gradient (l.h.s.) in terms of KBIs

$$\left(\frac{\partial \ln s_u}{\partial c_2}\right) = \frac{G_{u2} - G_{u1}}{1 + c_2(G_{22} - G_{21})} \tag{7}$$

where G_{22} and G_{21} are the hydrotrope-hydrotrope and hydrotrope-water KBIs [8,9].

Hydrotrope self-association, according to Eq. (7), is in the denominator. The larger G_{22} the more the denominator. Therefore, the hydrotrope self-association reduces the per-molar solubilization efficiency, contrary to this hypothesis [8,9]. Moreover, the driving force of solubilization is still the positive $G_{u2} - G_{u1}$. (Note, in this case, that it is convenient to consider the KBI difference, because both G_{u1} and G_{u2} contain a large negative, yet cancelling, contributions from solute's excluded volume [8,9].

For example, let us compare the cases of solubilization of p-aminobenzoic acid with urea and nicotinamide as hydrotropes [9,34]. Solubilization by urea driven by positive G_{u2}, peaking at around 760 cm^3 mol^{-1}. In addition, the low self-association of urea ($G_{22} \simeq -40$ cm^3 mol^{-1}) does not contribute to the solubilization inefficiency, because the denominator of Eq. (7) hardly deviate from 1. With nicotinamide, G_{u2} is much higher, peaking around 1450 cm^3 mol^{-1}. However, a larger G_{22} around 1200 cm^3 mol^{-1}, which can make the denominator of Eq. (7) reach 2 when c_2 is around 1 M, which means that nicotinamide self-association halves the solubilization efficiency [9,34]. Further examples can be found in Refs [9] and [34].

Thus, we have shown that the hydrotrope self-association hypothesis is incorrect based on our quantitative calculations on KBIs, which the readers are encouraged to go through interactively through the web-based apps [34].

7. A meeting point between experiments and simulations is provided by statistical thermodynamics

There are reasons as to why the ever-accelerating CPU speed and some potentially game-changing innovations in free-energy calculations [45] still have not made solubility prediction a relic of the past. Simulations of the liquid state still have to rely on classical molecular dynamics requiring force field parameterization, which is still a matter of active research even for the most common cosolvents (such as urea) for aqueous solutions [46,47].

What makes force field parameterization difficult is that not only the basic physical properties of a solution (such as density, activity, compressibility, enthalpy, or heat capacity) should be reproduced over a wide range of temperature and pressure, but also the simulated solution structure must be accurate. Hence, the accurate reproduction of KBIs has been adopted as the guiding principle of parameterization [46], as well as the important benchmark [47].

Hence, the calculation of KBIs from experimental data is useful not only in rationalizing how cosolvents work but also as a benchmark for molecular dynamics simulations that must be carried out to elucidate the cosolvent action in more microscopic detail.

8. Conclusions with a pointer to practical manuals

Our goal was to understand and explain why certain cosolvents (additives) affect solubility, conformational stability, aggregation, or gelation. To answer this question quantitatively and with clarity, we need statistical thermodynamics (i) to define interactions between component species via the Kirkwood-Buff integrals (KBIs) that have a definite microscopic meaning, (ii) to guide experimental data acquisition and analysis by efficiently choosing a set of experiments that yields KBIs, and (iii) to judge the classical hypotheses on cosolvent action by quantitatively validating each hypothesis via KBIs.

The present article was written with an emphasis, i.e., to persuade the reader that statistical thermodynamics should underpin all three steps as summarised above. For a more hands-on guide to start calculating KBIs for particular applications, I would like to point the readers to the following recent reviews of ours that are more focused in scope:

- the use of headspace analysis to quantify flavour and fragrance stabilization [35];
- solubilization by cosolvents, and hydrotropes [11,34];
- biomolecular stability, food gel formation and gelatinization via calorimetry [15,48];
- green solvents and supercritical extraction [33];
- chromatographic determination of KBIs [37]

Some of the articles are accompanied by interactive web-based apps (see https://www.stevenabbott.co.uk/practical-solubility/kb.php which includes a general tutorial) to assist the readers to analyse their own experimental data. In addition, the review by Abbott in the present issue contains references to many useful interactive tools to make models and theories useful and accessible for the practitioners of formulation.

Not only can statistical thermodynamics guide us through experimental design, analysis and interpretation but also helps us keep confusion at bay. Statistical thermodynamics is not confined inside the ivory tower. It is a branch of practical science.

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Figures

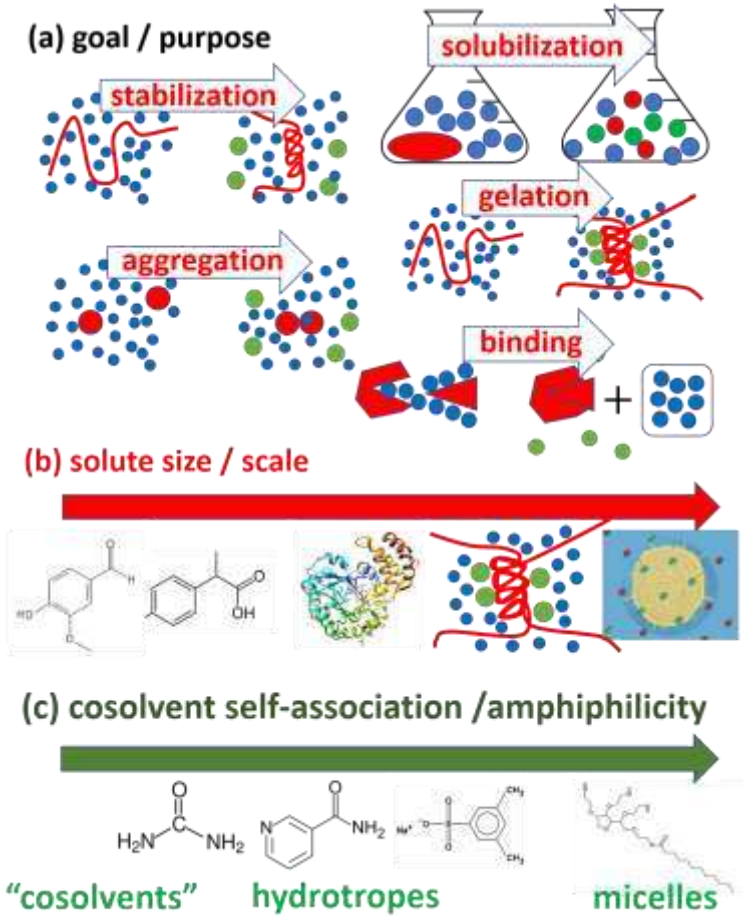

Figure 1. The scope of this review. (a) Our goal is a universal understanding of how cosolvent (green) addition modulates solubilization, aggregation, binding, conformational stabilization and gelation of solutes (red). These phenomena involve (b) solute sizes all the way from small hydrophobic solutes via macromolecules and their assemblies towards granules in the presence of (c) cosolvents with varying amphiphilicity and self-aggregation tendencies.

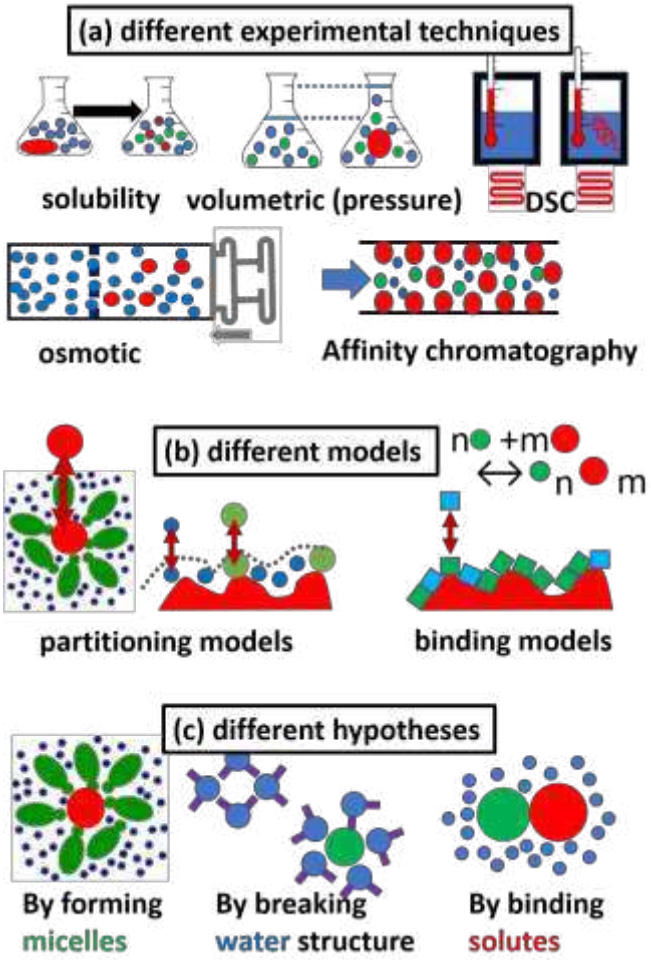

Figure 2. Options and choices are available, but often without clarity. (a) Different experimental techniques available to quantify cosolvent effects. (b) Different models proposed to analyse some of the experiments in (a) to yield solvation changes based on simplified assumptions on solvation. (c) Different hypotheses to rely on how cosolvent works.

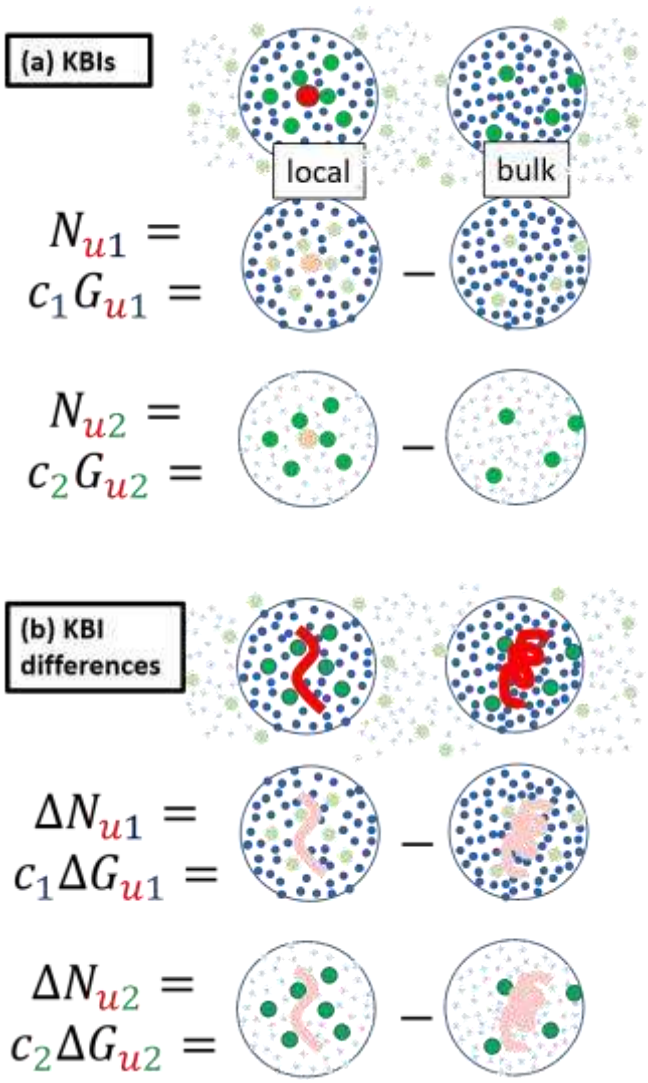

Figure 3. The Kirkwood-Buff integrals (KBIs) are a universal measure of solvation. (a) KBIs between solute and water (G_{u1}) and between solute and cosolvent (G_{u2}) are defined through the increment of water (or cosolvent) molecule from the bulk by the presence of a solute. (b) The difference in solute-water (ΔG_{u1}) and solute-cosolvent (ΔG_{u2}) KBIs between denatured and native states of a protein. KBI differences can be used to quantify the role of solvents not only on protein stability as in here but also biomolecular gelation, aggregation and binding.

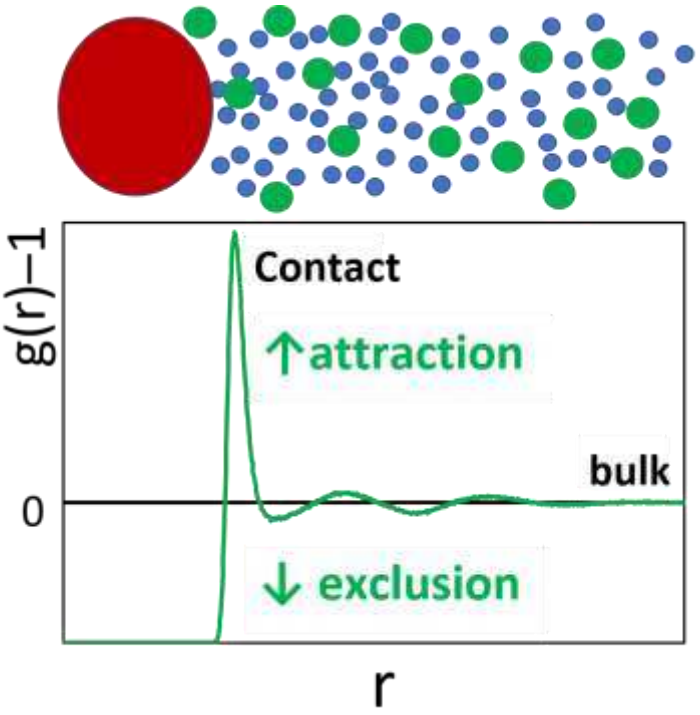

Figure 4. KBIs have microscopic interpretation, which has been illustrated using solute-cosolvent KBI as an example. A KBI is an integration of the increment of radial distribution function from its bulk value (namely, 1), through which thermodynamic or macroscopic measurements listed in Figure 1 will be unified with the structural data attainable by light, X-ray and neutron scattering.

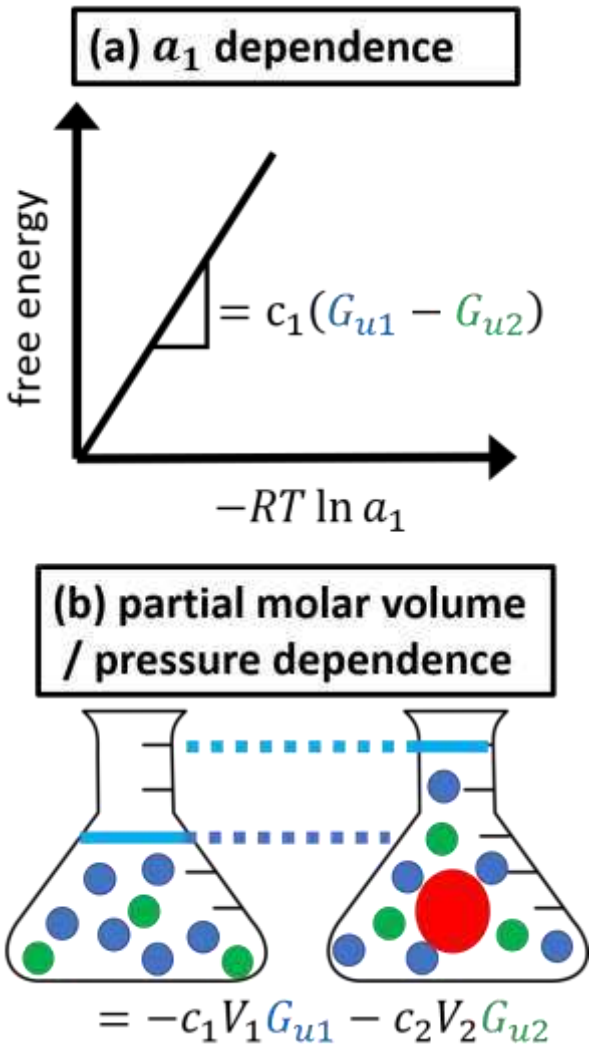

Figure 5. KBIs can be determined from experimental data alone. For example, calculation of the two KBIs (solute-solvent and solute-cosolvent) requires two independent experiments, such as (a) water activity dependence of the solvation free energy and (b) partial molar volume, or the hydrostatic pressure dependence of the solvation free energy.

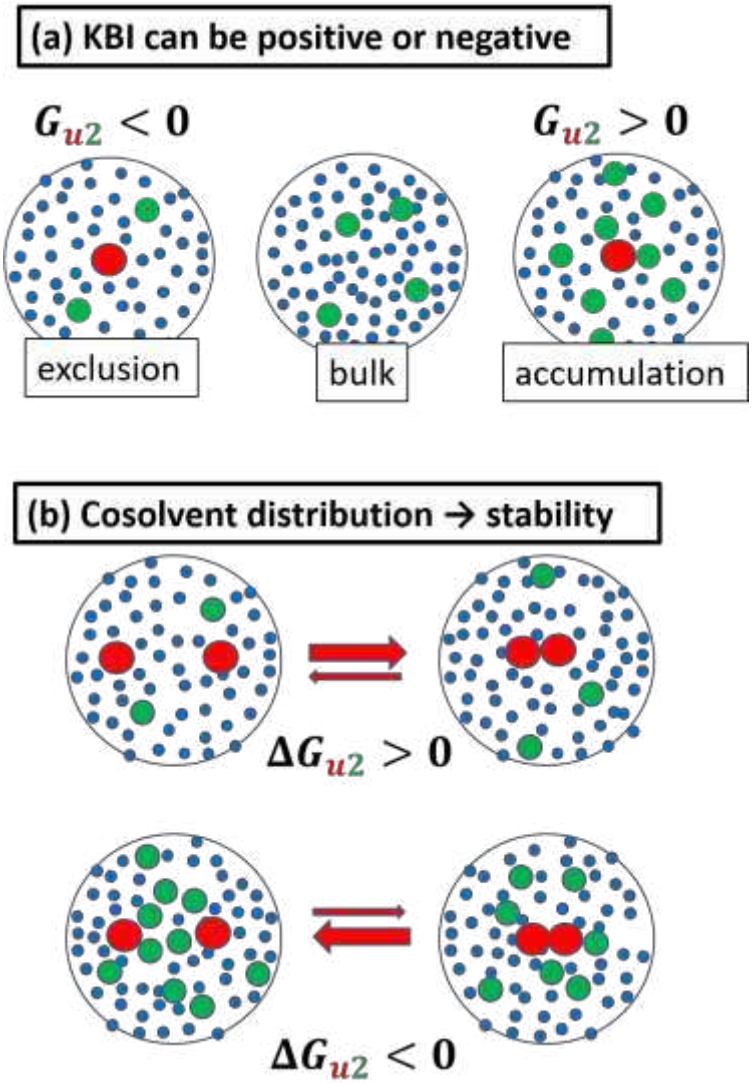

Figure 6. Distribution of cosolvents as the driving force. (a) G_{u2} can either be positive or negative, depending on the difference between local (solute's vicinity) and bulk concentrations of cosolvents. (b) ΔG_{u2} governs the transition, in this case aggregation. When cosolvents are excluded from the solutes, aggregation makes the cosolvent less excluded, hence $\Delta G_{u2} > 0$, leading to the enhancement of aggregation. When cosolvents are accumulated around the solutes, aggregation makes them less exposed to cosolvents and are less attractive, hence $\Delta G_{u2} < 0$, leading to the suppression of aggregation.

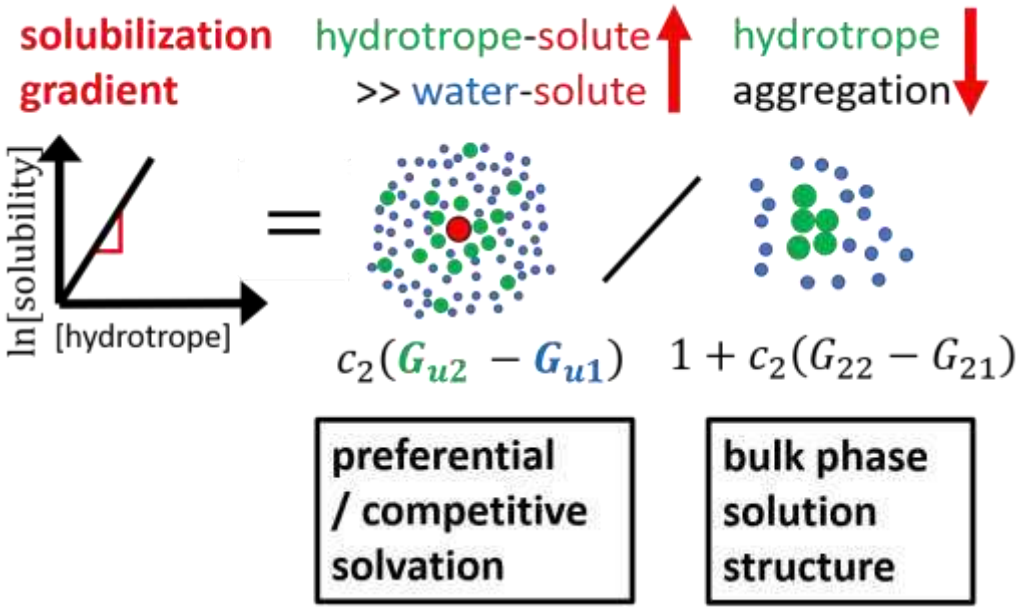

Figure 7. Two contribution to the cosolvent effect when viewed per molar clarified for the first time by KBIs. The major contribution is the preferential solute-cosolvent interaction, whereas the minor contribution is the per-molar inefficiency arising from the self-association of the cosolvent.