The Free Energy Density of a Fluid and its Role in Solvation and Binding

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Abstract

The concept that a fluid has a position-dependent free energy density appears in the literature but has not been fully developed or accepted. We set this concept on an unambiguous theoretical footing via the following strategy. First, we set forth four desiderata that should be satisfied by any definition of the position-dependent free energy density, $f(R)$, in a system comprising only a fluid and a rigid solute: its volume integral, plus the fixed internal energy of the solute, should be the system free energy; it deviates from its bulk value, f_{bulk}, near a solute but should asymptotically approach f_{bulk} with increasing distance from the solute; it should go to zero where the solvent density goes to zero; and it should be well-defined in the most general case of a fluid made up of flexible molecules with an arbitrary interaction potential. Second, we use statistical thermodynamics to formulate a definition of the free energy density that satisfies these desiderata. Third, we show how any free energy density satisfying the desiderata may be used to analyze molecular processes in solution. In particular, because the spatial integral of $f(R)$ equals the free energy of the system, it can be used to compute free energy changes that result from the rearrangement of solutes, as well as the forces exerted on the solutes by the solvent. In particular, we discuss the thermodynamic analysis of water in protein binding sites to inform ligand design. Finally, we discuss related literature and address published concerns regarding the thermodynamic plausibility of a position-dependent free energy density. The theory presented here has applications in theoretical and computational chemistry and may be further generalizable beyond fluids, such as to solids and macromolecules.

1 INTRODUCTION

The molecules of living systems reside in an aqueous solvent, and most laboratory and industrial chemistry takes place in either an aqueous or organic solvent. In all of these settings, the solvent can strongly influence the thermodynamics of important processes, such as conformational changes of the solute (e.g., protein folding[1–4]), molecular binding events (such as protein-protein and protein-drug association[5–11]), and chemical reactions[12]. In order to understand how solvent affects chemistry and to support various applications, researchers have developed theories and associated computational tools to optimize the conformational sampling of solvent configurations in and around solute molecules in molecular simulations[13–24], and that spatially resolve a solute's solvation free energy (and sometimes its enthalpy and entropy) into contributions from locations around the solute. Approaches to the spatial mapping of solvation thermodynamics include inhomogeneous solvation theory (IST)[25–28], which underpins the WaterMap[29–31], STOW[32], Grid Inhomogeneous Solvation Theory (GIST)[33–39], and related[40,41] technologies; classical density functional theory (DFT)[42–45]; Grid-Cell Theory (GCT)[46]; the three-dimensional reference interaction site model (3D-RISM)[47–50], and others[51–55], as recently reviewed[56]. Such spatial decompositions have been of particular interest in studies of the physical chemistry of noncovalent binding[33,57–62] and as guides to host-guest chemistry and structure-based drug design[63–72]. For example, a ligand that displaces thermodynamically unfavorable water from a receptor site is expected bind the receptor more tightly than one that does not, other things being equal[29,35,73]. Alternatively, a modification to the ligand that stabilizes water within the site may enhance binding[9,74–79]. This body of work assumes that one can meaningfully discuss the local densities of free energy, enthalpy, and entropy, in a fluid, and that concepts such as "unfavorable", "disfavored", "unstable", "high energy", or "low entropy" water are well-founded in theory.

There are macroscopic precedents for the spatial localization of thermodynamic properties in a material at equilibrium. The surface tension of a liquid is a particularly clear-cut case. The total free energy of, for example, a large water droplet increases in proportion to its surface area, while the properties of water deep within the droplet are essentially those of the bulk (i.e., unperturbed) liquid. It is thus evident that water at and near the surface makes a different contribution to the system free energy than does bulk water away from the surface. Another example comes from macroscopic electrostatic theory, which is the foundation of widely used implicit solvent models[80–84]. Thus, classical electrostatics provides a well-defined, position-dependent, free energy density, $\epsilon E(\mathbf{R})/2$, associated with an electrical field $E(\mathbf{R})$ at location $\mathbf{R}$ in a medium with a uniform (i.e., not position-dependent) dielectric constant ϵ. This is a free energy density, rather than a potential energy density, because the dielectric response can include a contribution from orientational polarization[85], which is associated with a change in entropy and which makes the dielectric constant, and hence the field energy, temperature-dependent.

There are also microscopic perspectives that support the legitimacy of a spatial decomposition of solvation thermodynamics. In 1955, Stratonovich derived an expression for the entropy in a subvolume of a container of interacting particles, subject to the physically reasonable requirement that particle-particle correlations die off quickly enough with distance[86]. A few years later, Nettleton and Green built on this work to provide an expression for the entropy density in a subvolume of a fluid in terms of potentials of mean force[87]. These early efforts set the stage for the subsequent derivation of

IST, referenced above. More recently, the expectation that solvation thermodynamics is local to the solute was used by Gallicchio and coworkers to facilitate simulation studies of solvation[88] . Furthermore, Chen and workers documented excellent agreement between the numerical integral of the hydration enthalpy density near a solute, computed with GIST, with the result provided by a reliable reference method, while addition of an IST-based entropy approximation yielded hydration free energies close to those obtained by formal thermodynamic integration[89]. Classical density function theory (DFT) of fluids also offers various approaches to the formulation of a free energy density[43,90–92], and the three dimensional reference interaction site model (3D-RISM), an integral equation approach to solvation, can yield a position-dependent free energy density around a solute[47,50].

Nonetheless, the concept that the density of free energy, energy, or entropy, in the presence of an inhomogeneity can be regarded as a spatially varying, localizable, quantity is subject to controversy. (Here the inhomogeneity usually is a solute or interface, but it can be anything whose interaction with the fluid varies with position.) Thus, Ben-Naim[93–95] and Ben-Amotz[96,97] have argued that, because the chemical potential of the solvent is not a function of position, even in the presence of an inhomogeneity, and because the chemical potential is a measure of stability, it does not make sense to speak of a free energy density that varies with position. Similarly, it has been asked whether it makes sense to speak of "thermodynamically unfavorable" water at a given location in a system at equilibrium, given that water is in fact at that location: after all, if it is unfavorable there, shouldn't it go somewhere else?[98,99] In addition, the existence of distinct, competing, formulations of the solvation free energy density, such as IST, GCT, 3DRISM, and classical DFT, raises the question of how to compare their validity and usefulness, as recently highlighted by Persson[99].

Motivated by its practical value and the controversies it has generated, we offer a new analysis of this topic. First, in Section 2, we lay out a set of desiderata that a free energy, enthalpy, or entropy density should satisfy in order to be physically meaningful and useful. Section 3 summarizes definitions of the free energy density, energy density, and entropy density, that are expected to satisfy the present desiderata for fluids with arbitrary potential functions, made up of either rigid or flexible molecules. (We do not insist, however, that these are the only definitions which could satisfy the desiderata.) Section 4 connects the free energy density to solvation free energy and surface tension, and introduces a useful definition of the local chemical potential of a fluid. Section 5 discusses the concept of "thermodynamically unfavorable solvent", and resolves the apparent paradox that a system at equilibrium can nonetheless have regions where the fluid is thermodynamically unfavorable. Section 6 discusses the application of these concepts to noncovalent binding in solution, with a focus on applications to protein-ligand binding. Sections 7-9 provide derivations of the thermodynamic densities summarized in Section 3, offer insights into the various terms of the energy and entropy densities, and consider the relationship of the present theory to prior work. An extended Appendix supports the main text with derivations of the solvation free energy in terms of a local integral of the free energy density, a surface-energy analysis of dewetting of the gap between parallel plates in a liquid, a demonstration that our expression for the entropy density yields the correct result, i.e., the Sackur-Tetrode equation, in the special case of an ideal gas, and an approach to defining the energy density for an arbitrary potential function.

2 DESIDERATA FOR THE FREE ENERGY DENSITY OF A FLUID

Because multiple definitions of the free energy density of a fluid may be proposed, at least in principle, it is useful to lay out the desiderata that we think any definition of free energy density, *f(R)*, of a fluid as a function of location in the lab frame, *R*, should satisfy for it to be useful and physically coherent. In the following, we write only *f(R)*, but analogous desiderata apply to the potential energy and entropy densities, *u(R)* and *s(R).*

To be definite, we first specify the general nature of the system under discussion. We consider the Helmholtz free energy, F, of an equilibrium system of N solvent molecules at 1 atm pressure, temperature T, and volume V. There may also be a single inhomogeneity, such as a solute or an interface, which is rigid and fixed in the lab frame of reference. The value of N is large enough that the chemical potential of solvent, even in the presence of any inhomogeneity, closely approximates the value for bulk solvent $\mu_{bulk} = F_{bulk} / N$ at the same pressure as the system of interest, so that the number density of solvent far from the inhomogeneity closely approximates the bulk density of the fluid, ρ_{bulk}. The Helmholtz free energy of the system is $F = -RT \ln \int e^{-\beta U(\mathbf{r}^N)} d\mathbf{r}^N$, where $U(\mathbf{r}^N)$ is the potential energy as a function of the translation, rotational, and internal coordinates of the solvent molecules, $\mathbf{r}^N$.

With this general system in mind, we propose the following desiderata for the Helmholtz free energy density of the fluid in the absence or presence of an inhomogeneity:

D1. The Helmholtz free energy of the system should be given by the integral of the free energy density over the volume of the fluid, i.e., $F = \int f(\mathbf{R}) d\mathbf{R}$, where R is the coordinates of a point within the volume, plus the internal energy of the rigid solute. This should hold in both the absence and presence of an inhomogeneity. In the absence of an inhomogeneity, the free energy density should be independent of R and thus must equal $f_{bulk} = \mu_{bulk}\rho_{bulk}$. This is the most fundamental requirement of a free energy density.

D2. Near the inhomogeneity, if one is present, the free energy density can deviate from f_{bulk}, but it should asymptotically approach f_{bulk} with increasing distance from the inhomogeneity. This makes physical sense, enables useful applications, and avoids a trivial definition of the free energy density as the product of the chemical potential and the number density.

D3. The free energy density should go to zero where the density of the fluid goes to zero, so that the free energy density is clearly connected with the molecules of the fluid and so that one can reason about the thermodynamic consequences of displacing solvent from the neighborhood of a solute, as detailed in Section 6.2.

D4. The free energy density should be definable not only for a pairwise additive potential function acting among rigid molecules, but also for potential functions of any higher order, including ones without a known multibody expansion, and for flexible molecules, so that the free energy can be understood as a physical quantity rather than a mathematical construct that might apply only in artificial special cases.

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In summary, the free energy density of a fluid is a scalar field whose integral over the entire volume of the fluid (plus the internal energy of any rigid solutes or inhomogeneities present) equals the system free energy, and which is expected to trend asymptotically to the bulk value of the solvent with increasing distance from an inhomogeneity or solute. There may be systems for which no definition of the local free energy density can satisfy Desideratum D2, notably a fluid near its critical point, because this can exhibit such long-ranged correlations that the perturbation induced by an inhomogeneity cannot be localized[99] as required by Desideratum D2. However, we anticipate that the solvation of an inhomogeneity is local enough for most fluid systems of chemical and biological interest that Desideratum D2 is satisfied, as discussed in Section 3 and previously demonstrated in several examples[89,100]. Although this paper, for simplicity, focuses on rigid solutes and inhomogeneities, the present theory can readily be generalized to address conformational flexible solutes and inhomogeneities in fluids.

3   CONSTRUCTING A FREE ENERGY DENSITY

This section proposes a formulation of the free energy density, as well as the energy and entropy densities, of a fluid, that we believe satisfies the desiderata, at least in many cases of interest. The free energy density as a function of position in the lab frame of reference, $f(\mathbf{R})$, will be written in terms of the potential energy density $u(\mathbf{R})$ and the entropy density $s(\mathbf{R})$. In turn, the potential energy density, which will be termed the energy density, and the entropy density, are both written as multibody expansions which converge to the exact result at full order:

$$f(\mathbf{R}) = u(\mathbf{R}) - Ts(\mathbf{R})$$

$$u(\mathbf{R}) = u^{(1)}(\mathbf{R}) + \frac{1}{2}u^{(2)}(\mathbf{R}) + \frac{1}{3}u^{(3)}(\mathbf{R}) + \dots \qquad (1)$$

$$s(\mathbf{R}) = s^{(1)}(\mathbf{R}) + \frac{1}{2}s^{(2)}(\mathbf{R}) + \frac{1}{3}s^{(3)}(\mathbf{R}) + \dots$$

Here $u^{(n)}(\mathbf{R})$ is the thermodynamic mean of the sum of all n-body interactions engaged in by molecules at $\mathbf{R}$, including their interactions with any solute or inhomogeneity that may be present. For example, $u^{(2)}(\mathbf{R})$ is the mean pair interaction of molecules at $\mathbf{R}$ with all other molecules in the system. The factors of $\frac{1}{n}$ ensure that $U = \int u(\mathbf{R})d\mathbf{R}$ and that the n-body energy contribution from each n-tuple of molecules is distributed equally among its n molecules. Similarly, $s^{(1)}(\mathbf{R})$ is the one-body entropy of molecules at $\mathbf{R}$, and $s^{(n)}(\mathbf{R})$ for $n > 1$ accounts for the total mutual information of order n of the molecules at $\mathbf{R}$ with all other molecules in the system. The expressions in Eq (1) are developed in Section 7, which explicitly links them to the total free energy, energy and entropy and thus makes clear that they satisfy Desideratum D1.

Because both intermolecular interactions and intermolecular correlations die away with distance in a fluid (away from its critical point), these local thermodynamic quantities approach their

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bulk values with increasing distance from a heterogeneity, as required for Desideratum D2. Indeed, even the longest-ranged perturbations in the free energy density of a liquid, those induced by a solute with a non-zero net electrical charge, will normally die off as r^4, where r is the distance from the solute. In this case, classical electrostatics and dielectric theory is expected to give an increasingly accurate estimate of the energy density of the field with increasing range as $\epsilon E^2(\mathbf{R})$, where ϵ is the dielectric constant of the liquid and $E(\mathbf{R})$ is the magnitude of the electrical field generated by the solute.

Because the field strength dies off as r^{-2}, the energy density dies off as r^{-4}, implying only a local perturbation of the liquid by a charged solute. It has also been shown that the contribution of orientational correlations to the entropy of liquid water derives almost entirely from correlations among water molecules in each other's first hydration shells[101].

The thermodynamic densities proposed in Eq (1) go to zero where the fluid density is zero, as required by Desideratum D3. To satisfy Desideratum D4, Section 7.2 defines an entropy density that accounts for conformational flexibility of the molecules of the fluid, and Sections 7.3 and **13.4** show that the energy density may be defined even if one does not have an explicit multibody expression for the potential function.

The energy and entropy densities in Eq (1) may be estimated using a molecular simulation of solvent molecules with or without a solute molecule fixed in the lab frame, much as done currently with the GIST method[33]. For a pairwise additive potential function, such as the AMBER[102], CHARMM[103], OPLS[104], or OpenFF[105] force fields, the energy density expansion terminates after the pairwise term and is reasonably straightforward to evaluate. However, estimating mutual information requires extensive sampling (i.e., long simulations), and it may be difficult to generate well converged results even for the pairwise term in the entropy expansion[34,34,101,106,107].

We have so far focused on the Helmholtz free energy density, as this is a simple sum of the potential energy density and the entropy density. However, when considering a process (such as adding a solute to solvent) carried out at constant pressure, one is generally more interested in the change in the Gibbs free energy, because this equals the reversible work associated with the constant-pressure process. The difference between the Helmholtz free energy and the Gibbs free energy, is PV. For many processes of interest, the change in PV is trivially small, so it is often neglected. Nonetheless, it is of interest to consider a density formulation of this term. We propose that a natural definition of the "PV density" $\delta_{PV}(\mathbf{R})$ is the product of the number density of each molecular species, s, present in the system as a function of position, $\rho_s(\mathbf{R})$, and the partial molar volume of the species v_s:

$$\delta_{PV}(\mathbf{R}) = \sum_s \rho_s(\mathbf{R})v_s \tag{2}$$

In the systems considered here, the molecular species would be the solvent and, if present, the solute. Note that this definition will in general place a small amount of Gibbs free energy density in the solvent-excluded region of a solute, so the Gibbs free energy density will not fully satisfy Desideratum D3.

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4 SOLVATION THERMODYNAMICS, SURFACE TENSION, AND A DEFINITION OF THE LOCAL CHEMICAL POTENTIAL

The free energy of solvation, ΔF_{solv}, is the change in free energy of the system between an initial state comprising the solute molecule at a fixed location in vacuum and a container with N solvent molecules located far enough from the solute that their interactions are negligible, and a final state with the solute at a fixed location within the container of solvent, with both systems at the same pressure, so that the free energy density of the fluid far from the solute is the same as the bulk or pure value for both systems[i]. Desideratum 1 implies that the Helmholtz solvation free energy for this process can be written as the difference between the integrals of the solvent free energy density with and without the solute, i.e., in the final and initial states of the solvation process, where both integrals are taken over the entire volume of the respective systems and hence encompass the same number of solvent molecules. (Note that, due to the constant pressure condition, the second volume is larger than the first by ΔV, the partial molar volume of the solute.) That is,

$$\Delta F_{solv} = F - F_{bulk}$$
$$F = \int f(\mathbf{R}) d\mathbf{R} \qquad (3)$$
$$F_{bulk} = V f_{bulk} = N \mu_{bulk}$$

where $f(\mathbf{R})$ is the free energy density of the liquid in the presence of the solute, F and F_{bulk} are the free energies of the final (solute in solvent) and initial (bulk solvent) states, respectively, and V in line 3 is that of the pure solvent, while the volume of the solvent/solute system is V+V_S, where V_S is the partial molar volume of the solute in the solvent. Desideratum 2 implies that the change in the Helmholtz free energy upon solvation can be approximated via an integral over only the region of space near the solute, where the free energy density and number density as a function of position differ significantly from bulk[108]:

$$\Delta F_{solv} \approx \int_{near} f(\mathbf{R}) d\mathbf{R} - N_{near} \mu_{bulk} \qquad (4)$$

where N_{near} is the mean number of water molecules in the "near" region. This result is derived in Section 13.1. For a free energy density satisfying Desideratum D2, the approximation in Eq (4) will asymptotically approach the exact result with increasing size of the "near" region. See Section 13.1 for a derivation of this result.

Note that the reversible work of inserting the solute at constant pressure is not the change in the Helmholtz free energy at constant pressure considered above but by the change in Gibbs free energy. To obtain this, one must add $\Delta(PV) = P\Delta V$. This PV contribution, which represents the work

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[i] If the pressure is not held constant, the proposed method will still accurately describe the Helmholtz free energy densities of the initial and final systems, but their difference – i.e., the solvation free energy density will not be fully localized near the solute, because the solvent density far from the solute will have changed on going from the initial (pure solvent) to the final (solvent+solute) state.

done by the system on its surroundings and is negligibly small for many processes of interest, is considered at the end of Section 3.

We furthermore define the solvation free energy density as a function of position, $\phi(\mathbf{R})$:

$$\phi(\mathbf{R}) = f(\mathbf{R}) - \rho(\mathbf{R})\mu_{bulk} \tag{5}$$

where $\rho(\mathbf{R})$ is the number density in the presence of the solute. Note that $\phi(\mathbf{R})$ is expected to asymptotically approach zero with increasing distance from the solute, so that

$$\Delta F_{solv} = \int \phi(\mathbf{R})d\mathbf{R} \approx \int_{near} \phi(\mathbf{R})d\mathbf{R} \ . \tag{6}$$

The parallelism of the two terms on the right hand side of Eq (5) lead naturally to the definition of a local chemical potential as

$$\mu(\mathbf{R}) = \frac{f(\mathbf{R})}{\rho(\mathbf{R})} \tag{7}$$

so that

$$\phi(\mathbf{R}) = \left[\mu(\mathbf{R}) - \mu_{bulk}\right]\rho(\mathbf{R}) \tag{8}$$

Closely related expressions have appeared previously in applications of IST[42,109] and the classical DFT of liquids[90,91]. Analogous molar quantities for the potential energy and entropy may also be defined as $u(\mathbf{R})/\rho(\mathbf{R})$ and $s(\mathbf{R})/\rho(\mathbf{R})$, respectively.

Note that $\mu(\mathbf{R})$ is not the work of inserting a solvent molecule at $\mathbf{R}$, except in the limit where $\mathbf{R}$ is far enough from the solute that $\mu(\mathbf{R})$ goes to μ_{bulk}. Note, too, that $\mu(\mathbf{R})$ is not the excess chemical potential $\mu_{ex}(\mathbf{R})$. The latter is given by[110,111] $\mu_{ex}(\mathbf{R}) = \mu_{bulk} - RT \ln \rho(\mathbf{R})$, and integrating $\rho(\mathbf{R})\mu_{ex}(\mathbf{R})$ does not equal yield the system free energy or a solvation free energy, so it does not satisfy or connect in any clear way with the Desiderata in Section 2, particularly D1, which requires that the volume integral of the free energy density yield the free energy. In fact, as previously pointed out[42], the excess chemical potential is the same as the free energy density associated with the first order entropy term of IST.
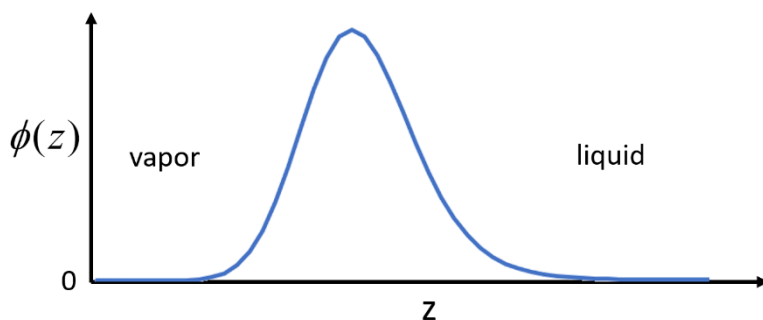
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Figure 1. Qualitative depiction of the solvation free energy density as a function of position along the vector (z coordinate) normal to a locally flat vapor-liquid interface.

Like the liquid near a solute, the liquid at a liquid-vapor interface will in general have a perturbed free energy density ($f(\mathbf{R}) > f_{bulk}$) and hence a nonzero solvation free energy density ($\phi(\mathbf{R}) > 0$). For a sufficiently expansive system, such as a macroscopic water drop, $\phi(\mathbf{R})$ varies along only the surface normal, z. For a system at equilibrium, the local chemical potential $\mu(z)$ asymptotically approaches the same bulk value μ_{bulk} deep in the liquid ($z \gg 0$) and deep in the vapor phase ($z \ll 0$), so $\phi(z) \to 0$ deep in each phase, per Eq (8). Thus, a graph of $\phi(z)$ has the general form shown in Figure 1. Because the surface tension of the liquid, γ, is the increment in the system free energy per unit surface area, it is apparent that the surface tension is given by the line integral of $\phi(z)$ through its peak:

$$\gamma = \int_{z_g}^{z_l} \phi(z) dz \tag{9}$$

where the limits of integration, (z_g, z_l), must fully contain the peak of $\phi(z)$, and γ approaches its exact value as the integration domain expands into each phase. Note that this approach to the surface tension, which appears to be novel (though prefigured by Lazaridis[27]) does not require one to choose a potentially arbitrary definition of the location of the surface along the z axis. We have thus derived the well accepted concept of the surface tension from the less familiar concept of a free energy density which varies with position in a system at equilibrium.

To the extent that the interface is rough on a size scale less than or equal to the distance over which molecules of the liquid are correlated, the definition of the surface tension becomes ambiguous, because the local surface normals can intersect within the perturbed region just below the surface, making it unclear which part of the surface, or surface element, should be assigned the free energy density of the nearby liquid. Similarly, if the distance separating two interfaces (e.g., two nonpolar plates) is larger than the correlation length of the liquid, then one may readily define the surface tension at each interface, but if the distance becomes smaller than the correlation length, the effective surface tension at each interface will change and may become difficult to define.

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5 THERMODYNAMICALLY UNFAVORABLE SOLVENT, SOLVENT STABILITY, AND DEWETTING

In order to discuss this topic rigorously, we start by defining terms. *Thermodynamically unfavorable solvent* is defined as solvent whose local chemical potential (Eq (7)) is greater than the fluid's bulk chemical potential (i.e., $\mu(\mathbf{R}) > \mu_{bulk}$), due to interactions with a nearby solute or inhomogeneity. Conversely, *thermodynamically favorable solvent* is said to exist where $\mu(\mathbf{R}) < \mu_{bulk}$. We define thermodynamic favorability in terms of the local chemical potential, *μ(R)*, rather than the free energy density, *f(R)*, for two reasons. First, this definition avoids classifying a bulk vapor as being either favorable or unfavorable relative to a liquid phase with which it is at equilibrium: although the local chemical potentials of these two phases are the same, their free energy densities differ, due to the large difference between their respective number densities. Second, the thermodynamically favorability defined in terms of *μ(R)* has a specific physical meaning: thermodynamically favorable and unfavorable solvent regions make, respectively, favorable and unfavorable contribution to the solvation free energy of the solute or inhomogeneity, per Equations (6) and (8).

Note, however, that the integrals of $\phi(\mathbf{R})$ or $f(\mathbf{R})$ over a subregion of the system do not yield the free energy change of removing the solvent from the subregion. They are merely the contributions of the subregion to the overall solvation free energy, ΔF_{solv}, or free energy, F, respectively, of the current state of the system, in their integral formulations (Equations (6) and (3), respectively). Accordingly, we recommend not using the phrase "unstable solvent" for thermodynamically unfavorable solvent. Indeed, if the solvent is present at equilibrium, it is, manifestly, stable. Also, to avoid confusion between potential energy and free energy, we recommend reserving the expression "high energy water"[57,58,77,112] for water in regions where the potential energy per molecule, $u(\mathbf{R}) / \rho(\mathbf{R})$ is greater than its bulk value.

Despite these definitions, the concept that water (for example) may be thermodynamically disfavored in one region relative to another, in a system at equilibrium, may seem facially incorrect. After all, as previously remarked[98,99], if the water is thermodynamically unfavorable there, why doesn't it move someplace more favored? The resolution to this seeming paradox is that the realizable physical process of vacating water from such a region would generate a vapor-filled bubble, leading to a net increase in the free energy of the system because of the high free energy density of water at a water-vapor interface (Figure 2a). Note that water at a vapor interface is likely to be even more thermodynamically unfavorable than the water at the surface of a hydrophobic solute, because a hydrophobic solute provides stabilizing dispersion interactions, whereas the near-vacuum inside a bubble provides virtually no stabilizing interactions. Further insight into this issue can be gained by considering a model system comprising two parallel, hydrophobic plates in water (Figure 5). As detailed in Section 13.2, thermodynamically unfavorable water will be present in the space between the plates unless they are close enough together that the free energy cost of forming a new cylindrical vapor-water interface around the newly vacated (i.e., dewetted) region between the plates is low enough[113]. Because the area of the new vapor-water interface is proportional to the distance between the plates, dewetting occurs only when the distance between the plates falls below a threshold, which can be determined from the parameters of the problem. This example highlights the distinction between

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thermodynamic unfavorability, as defined here, and stability, the latter being defined based on physically realizable and clearly specified initial and final states of the system. Section 6.2 develops these concepts further in the context of ligand-protein binding.
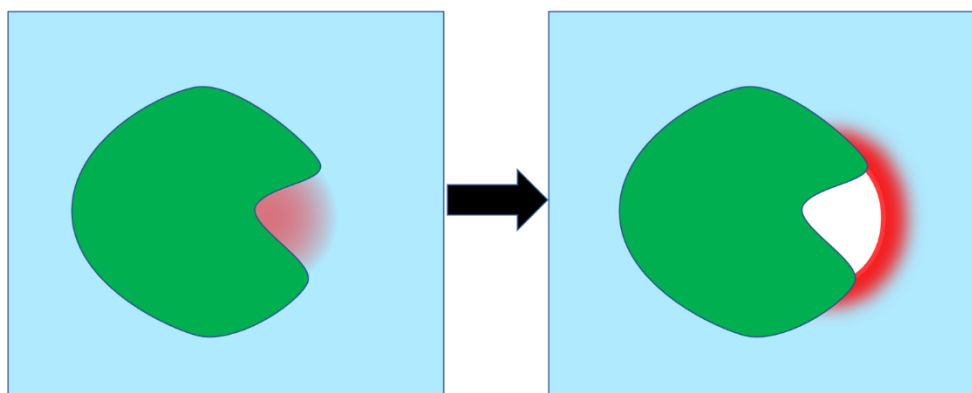
Figure 2. Hypothetical dewetting of a protein binding site. Left: hydrophobic surface of binding site contains thermodynamically unfavorable water (pale red). Right: if the binding site water relocates to the bulk solvent, the binding site then is occupied by a bubble whose interface with the solvent induces highly unfavorable water (deep red). Therefore, the system with the binding site filled with mildly unfavorable water (left) is more stable overall than the system with the empty binding site (right).

Note that unfavorable water can generate forces on solutes and inhomogeneities and can do work if the solutes or inhomogeneities are movable. For example, the binding of two hydrophobic molecules in solution is driven by the "release" of unfavorable water from their surfaces into bulk upon binding, leading to a fall in the overall free energy of the system. A non-spherical water drop in zero gravity provides another example: here, the high local chemical potential of water at the surface, manifested as surface tension, drives the drop to become spherical. The change of shape reduces the number of unfavorable surface waters, releasing them instead into the bulk at the interior of the drop and, again, lowering the overall system free energy. Similarly, the free energy falls when two spherical water drops merge to form a single spherical drop, because the single drop has a smaller surface area and hence less thermodynamically unfavorable water. More generally, the fact that water is at equilibrium does not imply that there is no stored free energy in the water at the surface of a hydrophobic solute, much as an equilibrium system comprising a spring compressed in a clamp has energy stored in the spring. Note, too, that the mechanical energy density is much higher in the spring than in the clamp, so again we have a system at equilibrium with varying energy densities in its different parts.

It has been argued that the presence of unfavorable water at the surfaces of two hydrophobic solutes in water cannot explain their tendency to associate with each other, because the chemical potential of water near the solute is the same as the chemical potential of water far from the solute[93,97,98]. However, the chemical potential is not a suitable quantity to assess a position-dependent free energy density. The chemical potential of water is the change in system free energy upon adding one unrestrained water molecule to the system[114]. Even if one computes the work of initially adding a water molecule at a fixed position near a solute, one must then add in the work of releasing it to wander the whole system like all the other water molecules. The same goes for a calculation where the water
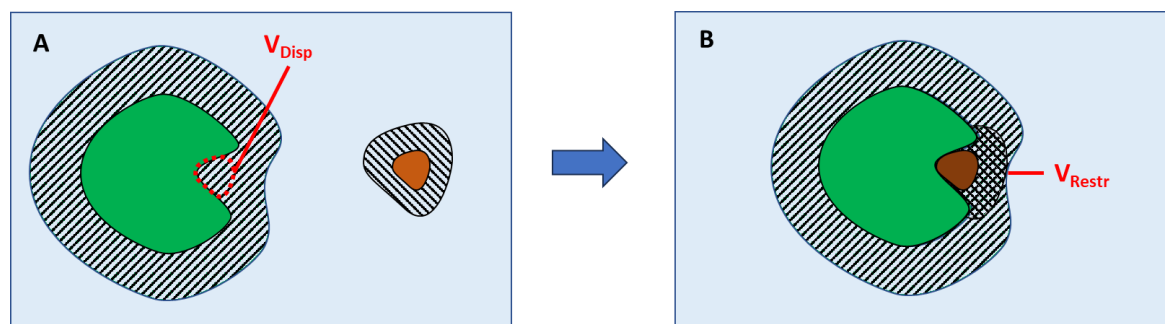

Figure 3. Displacement and restructuring of binding site water upon ligand binding. A: protein (green) surrounded by thermodynamically perturbed (favorably or unfavorably) water (upward hatching), and unbound ligand (orange) surrounded by thermodynamically perturbed water (downward hatching). B) Protein-ligand complex where ligand has displaced water from region V_{disp}, demarcated by red dashed boundary in panel A, and water in region V_{restr} (cross-hatched) has been restructured so that its thermodynamic properties differ from both those of the corresponding regions around the free ligand and protein.

molecule is initially added far from the solute. If one obtains different results from these two calculations, then one must be computing something other than the chemical potential. Thus, the chemical potential is incapable of reporting on the local properties of the solvent in a system at equilibrium. In contrast, the free energy density and local chemical potential developed in the present work are localizable via their construction in terms of local molecular interactions and correlations (Eq (1)), and the spatial integral of the free energy density gives the free energy of the system and therefore can be used to compute free energy changes that result from the rearrangement of solutes, as well as the forces exerted on the solutes by the solvent.

6   CONTRIBUTIONS OF SOLVATION TO BINDING THERMODYNAMICS

6.1   BINDING AND THE SOLVENT FREE ENERGY DENSITY

The theory presented above provides a framework to analyze the role of solvation free energy in the association of two molecules in solution, such as a protein and a small molecule ligand. Once again, it will be essential to consider the initial and final states of the system. In order to focus on the contribution of the solvent to the binding free energy, we consider the association of a rigid receptor and a rigid ligand (Figure 3A) to form a rigid complex (Figure 3B), where the free and bound species are all considered to be rotationally and translationally fixed. However, these ideas are readily generalized to models with full solute degrees of freedom. With these simplifications, the binding free energy may

be written as

$$\Delta F_{bind} = \Delta U_{PL} + \Delta\Delta F_{solv}$$

$$\Delta\Delta F_{solv} = \int \phi_{PL}(\mathbf{R})d\mathbf{r} - \int \phi_{P\&L}(\mathbf{R})d\mathbf{r} \tag{10}$$

where ΔU_{PL} is the interaction potential energy between the protein and ligand in the bound state, $\Delta\Delta F_{solv}$ is the change in the solvation free energy of the two solutes upon binding, $\phi_{PL}(\mathbf{R})$ is the solvation free energy density around the protein-ligand complex, and $\phi_{P\&L}(\mathbf{R})$ is the solvation free energy density fields around the free protein (P) and ligand (L) far apart in solution. The integrals are over the entire volume of the system and the coordinates of the protein are considered to remain unchanged on binding. (Note that, maintaining the pressure at 1 atm means that there will, in general, be a small difference in the volume of the system before and after binding, because the sum of the partial molar volumes of the protein and ligand is not identical to the partial molar volume of their bound complex.) Because the solvation free energy densities are expressed as volume integrals, partitioning the system into subvolumes allows partitioning the change in solvation free energy on binding into various contributions, and any such partitioning is valid as long as it encompasses the entire volume of the system. One can also construct efficient approximations by focusing only on regions near enough to the solutes where $\phi_{PL}(\mathbf{R}) - \phi_{P\&L}(\mathbf{R})$ deviates significantly from zero.

6.2 THE INITIAL STATE DISPLACEMENT APPROXIMATION

In prior work, we[35,115,116] and others[31,73,117] have used what we will term the initial state displacement (ISD) approximation to estimate the solvent contribution to the protein-ligand binding free energy. This approximation is based upon the supposition that the main contributions to ΔF_{solv} come from the displacement of water from the binding site by the ligand and from the desolvation of the ligand on binding. The region from which water will be displaced, V_{disp}, is demarcated by a dashed red line in Figure 3A and is covered by the ligand (orange) in Figure 3B. Thus, we have:

$$\Delta F_{solv} \approx \Delta F_{disp} + \Delta F_{lig,solv}$$

$$\Delta F_{disp} = \int_{V_{disp}} \phi_{PL}(\mathbf{R})d\mathbf{R} - \int_{V_{disp}} \phi_{P\&L}(\mathbf{R})d\mathbf{R}$$

$$= -\int_{V_{disp}} \phi_{P\&L}(\mathbf{R})d\mathbf{R} \tag{11}$$

$$\Delta F_{lig,solv} = -\int_{V_{lig}} \phi_{P\&L}(\mathbf{R})d\mathbf{R}$$

where V_{lig} is the region near the uncomplexed ligand where the solvation free energy density is significantly different from zero. The second equality stems from the fact there is, by definition, no water in the displacement volume in the bound state (PL), so the solvation free energy density is zero there, in accordance with Desideratum 3. The final equality is provided mainly for completeness, because applications of the ISD approximation typically handle the change in solvation of the ligand by some auxiliary method, rather than by integrating its solvation free energy density -- although this approach could, in principle, be used. Note that ΔF_{disp} does not correspond to the actual physical process of vacating of water from the binding pocket, but is simply the integral of $-\phi_{P\&L}(\mathbf{R})$ within the

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displacement volume. In contrast, physically vacating the water from this region would lead to the thermodynamically costly formation of a vacuum/liquid interface, as discussed in Section 5. Note, too, that the entire displacement approximation is predicated on the applicability of Desideratum 3, since this requires that solvent is present everywhere that the free energy density is nonzero and that the free energy density goes to zero wherever solvent has been displaced.

The ISD approximation leads to the design concept that one may boost ligand affinity by identifying unfavorable regions of solvation free energy in the binding site of the free protein and ensuring that such regions are non-existent in the bound state. Multiple studies have used this approximation to inform rational lead compound modification, where the initial state is the protein complexed with an unmodified ligand and the final state is the protein complexed with a modified ligand. In addition, we[116] and Uehara[117] have used this approximation in protein-ligand docking calculations by mapping out regions in the binding site of the unbound protein that contain thermodynamically unfavorable water and accounting for ligand occupancy of these regions with a modified scoring function. This solvent-centric perspective must, of course, be integrated with other determinants of affinity, such as direct ligand-protein interactions, ligand desolvation, and configurational entropy.

The free energy density of binding site water may also be useful in assessing the druggability of a binding pocket, because the presence of unfavorable water there increases the potential for development of a ligand that gains affinity by displacing it. This capability may be particularly relevant for tasks such as identifying protein-protein interfaces susceptible to disruption by small-molecule ligands – a classically difficult problem – and to determining whether a shallow allosteric site is amenable to targeting with a high-affinity ligand. Molecular dynamics simulations are often used to discover hitherto unknown—i.e., cryptic -- binding pockets in proteins, and one might expect that two cryptic sites that appear with equal probability should be equally suitable for targeting with a small molecule drug. However, if one site contains water that is more thermodynamically unfavorable than the other, this may make it easier to target with a high-affinity ligand, due to the free energy benefit of displacing its high free energy water to bulk.

6.3 THERMODYNAMIC CONTRIBUTIONS OF WATER RESTRUCTURING AROUND THE COMPLEX.

The ISD approximation assumes that the difference between $\phi_{PL}(\mathbf{R})$ and $\phi_{P\&L}(\mathbf{R})$ is negligible near the protein except insofar as the ligand displaces solvent. This is partly justified by the assumption of protein rigidity and the locality of solvent perturbations (Desideratum D2), which, in combination, imply that the solvation free energy density of solvent far from the binding site is likely to be minimally changed
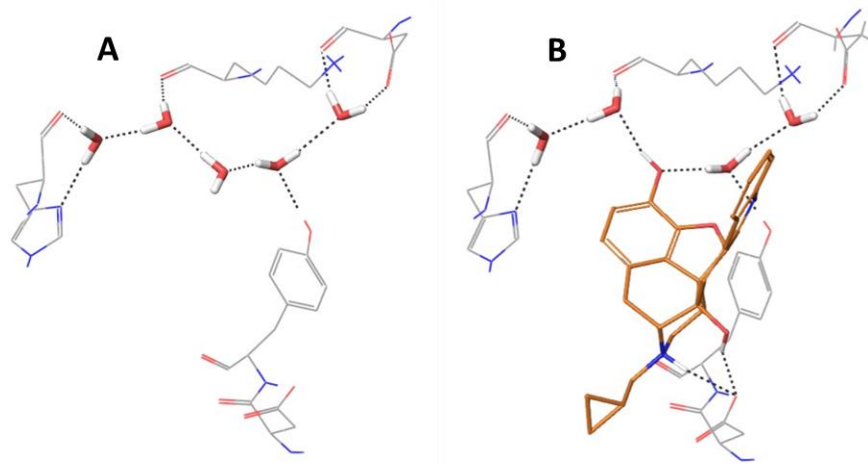

Figure 4. A ligand integrates with an existing water network in a protein binding site. A) Water network modeled into the binding site of the human delta opioid receptor (PDBID 4N6H) by explicit solvent MD simulations and analysis with SSTMAP. B) The hydroxyl group of the crystallographic ligand in 4N6H displaces one water from the network but maintains the rest of the network by making hydrogen bonds which mimic those made by the displaced water. See text for citations.

when a ligand binds. However, although use of this approximation has led to some apparent successes, it is becoming clear that the thermodynamics of restructuring water around the protein on binding should not be neglected. Restructuring, as opposed to simple displacement, occurs where the water is perturbed by both the ligand and protein in the final state. One such scenario is illustrated in Figure 3B, where the cross-hatched region labeled V_{restr} contains water that is strongly perturbed by both the protein and the ligand in the bound state, and thus has been restructured by binding. The change in solvation free energy from this region can be written as:

$$\Delta F_{restr} = \int_{V_{restr},PL} \phi_{PL}(\mathbf{r})d\mathbf{r} - \int_{V_{restr},PL} \phi_{P+L}(\mathbf{r})d\mathbf{r} \tag{12}$$

Another scenario occurs when the final state includes a water-filled region which did not exist in the initial state, such as when the initial state has a large ligand in the binding site and the final state has a smaller bound ligand [22]. In both scenarios, it is essential to consider the free energy density of the final-state water in question, because this can impact ligand affinity. Water trapped in a hydrophobic cavity between the protein and ligand will have a high free energy density and will therefore make an unfavorable contribution to the overall system free energy, opposing ligand binding. However, if the final-state water makes good hydrogen bonds with the protein and ligand, its free energy density will be low, so it can make a favorable contribution to the system free energy, favoring ligand binding.

This reorganization of binding site water has been shown to be important in a number of studies, where a common theme is the design of ligands that form thermodynamically favorable interactions with retained water molecules or water networks in the binding site[76,77,118,119]. For example, in Figure 4, a ligand hydroxyl group displaces one water from a stable water network in human delta opioid

receptor[120] (modeled by molecular dynamics and analyzed with SSTMAP[37]), but maintains the network's stability by substituting its own hydrogen bonds for those made by the displaced water[121].

7 DERIVATION OF A GENERAL FREE ENERGY DENSITY

This section provides the theoretical underpinnings of the density definitions in Section 3, using the mutual information expansion for the entropy, including the case of flexible fluid molecules, and demonstrating the form of the energy density for interaction potentials that are not pairwise additive.

7.1 SYSTEM DEFINITION

We consider an equilibrium system made up of N>>1 indistinguishable molecules in a fluid state at pressure P ~1 atm and temperature T, and enclosed in a volume V. The system may also include a rigid solute molecule immersed in the fluid (or another inhomogeneity, such as a free surface) that is fixed in the lab coordinate frame. Solvent molecule i is considered to have three translational coordinates $\mathbf{R_i}$. Unless it is monatomic, it also has orientational and internal coordinates $\mathbf{q_i}$. Thus, the full coordinate set of molecule i is $\mathbf{r}_i = (\mathbf{R}_i, \mathbf{q}_i)$ and the full set of system coordinates is $\mathbf{r}^N = (\mathbf{R}_1, \mathbf{q}_1, \ldots, \mathbf{R}_N, \mathbf{q}_N)$. In the classical approximation of statistical thermodynamics, the Helmholtz free energy is given by

$$F = -k_B T \ln\left(\frac{Z}{N!}\right)$$
$$Z = \int e^{-\beta U(\mathbf{r}^N)} d\mathbf{r}^N \qquad (13)$$

where $U(\mathbf{r}^N)$ is the potential energy of the system as a function of the molecular coordinates and $\beta = 1/k_B T$ with k_B the Boltzmann constant. We have omitted momenta and a factor involving Planck's constant, as these will cancel in the calculation of any properties of interest, such as the binding free energy of two molecules in solution[122]. The factor of N! also will not contribute, but is temporarily included in order to clarify its appearance in the expression for the entropy. A more complete treatment derivation, which includes momenta, is provided in Section 13.3.

We consider the Helmholtz free energy, rather than the Gibbs free energy, because it is simpler, captures all of the important physics, and is close to the Gibbs energy for processes of interest here. For applications, where the Gibbs free energy is more important, one may simply add PV to the Helmholtz free energy. Alternatively, one may treat P as an additional contribution to the free energy density, but this would violate Desideratum in, for example, the case of a dewetted cavity, and it is not clear what insight this would provide.

The ensemble averaged potential energy, U, is given by

$$U = \int p(\mathbf{r}^N) U(\mathbf{r}^N) d\mathbf{r}^N \qquad (14)$$

where $p(\mathbf{r}^N)$ is the probability density over the fluid's coordinates:

$$p(\mathbf{r}^N) = \frac{e^{-\beta U(\mathbf{r}^N)}}{Z} \tag{15}$$

The entropy is given by[86]

$$S = -k_B \int p(\mathbf{r}^N) \ln p(\mathbf{r}^N) d\mathbf{r}^N - k_B \ln N! \tag{16}$$

The following check confirms that this expression gives the expected relationship between S, U, and F:

$$
\begin{aligned}
S &= -k_B \int p(\mathbf{r}^N) \ln p(\mathbf{r}^N) d\mathbf{r}^N - k_B \ln N! \\
&= -k_B \int p(\mathbf{r}^N) \ln\left[N! p(\mathbf{r}^N) \right] d\mathbf{r}^N \\
&= -k_B \int p(\mathbf{r}^N) \ln\left[\frac{N! e^{-\beta U(\mathbf{r}^N)}}{Z} \right] d\mathbf{r}^N \\
&= -k_B \int p(\mathbf{r}^N) \left[-\beta U(\mathbf{r}^N) - \ln\left(\frac{Z}{N!} \right) \right] d\mathbf{r}^N \\
&= \frac{U - F}{T}
\end{aligned}
$$

(17)

where the second line follows from $\int p(\mathbf{r}^N) d\mathbf{r}^N = 1$, the third line follows from Eq (15), and the fourth line follows from Eq (13) and Eq (14). Because the $k_B \ln N!$ contribution to S is constant over a change in the probability density, such as that induced by addition of a solute inhomogeneity to the system, it does not contribute to the entropy of solvation or any other change that does not change N. We therefore omit it in what follows.

7.2 ENTROPY DENSITY OF A FLUID VIA THE MUTUAL INFORMATION EXPANSION

The mutual information expansion (MIE)[123–125], a series expansion of the entropy of a multivariate probability density function in 1-body, 2-body, 3-body, and higher order, contributions, allows the entropy density as a function of position to be written in a form analogous to that of the energy density, as written in Eq (1). We demonstrate this here by deriving the first and second order terms, starting with the expression for the system entropy given in Eq (16), but omitting the $N!$ term as explained above:

$$S = -k_B \int p(\mathbf{r}^N) \ln p(\mathbf{r}^N) d\mathbf{r}^N \tag{18}$$

For simplicity, we consider any solute or inhomogeneity present to be rigid, but this derivation generalizes to the flexible case. To construct a second order approximation of the entropy density, we

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truncate the MIE after the 2-body term and thus obtain the sum of all single-molecule marginal entropies, S_i, and the sum of all molecule-molecule mutual information terms, M_{ij}:

$$
\begin{aligned}
S &\approx S^{(1)} + S^{(2)} \\
S^{(1)} &= \sum_{i=1}^{N} S_i \\
S^{(2)} &\equiv -\sum_{i=1}^{N}\sum_{j>i}^{N} M_{ij} \\
M_{ij} &= S_i + S_j - S_{ij} \\
S_i = S_j &= -k_B \int p_i(\mathbf{R},\mathbf{q}) \ln p_i(\mathbf{R},\mathbf{q}) d\mathbf{R}d\mathbf{q} \\
S_{ij} &= -k_B \int p_{ij}(\mathbf{R},\mathbf{q},\mathbf{R}',\mathbf{q}') \ln p_{ij}(\mathbf{R},\mathbf{q},\mathbf{R}',\mathbf{q}')\, d\mathbf{R}d\mathbf{q}\, d\mathbf{R}'d\mathbf{q}'
\end{aligned}
\tag{19}
$$

Here S_{ij} is the joint marginal entropy of molecules i and j, $p_i(\mathbf{R},\mathbf{q})$ and $p_{ij}(\mathbf{R},\mathbf{q},\mathbf{R}',\mathbf{q}')$ are the single-molecule and two-particle marginal probability distribution functions, and $S_i = S_j$ because of the indistinguishability of the molecules. Following Lazaridis[27,28], we use the product rule of probability to treat the translational coordinates $\mathbf{R}$ of each molecule separately from its orientational and internal coordinates $\mathbf{q}$:

$$
\begin{aligned}
p_i(\mathbf{R},\mathbf{q}) &= p_{q|R,i}(\mathbf{q}\,|\,\mathbf{R})\, p_{R,i}(\mathbf{R}) \\
p_j(\mathbf{R},\mathbf{q}) &= p_{q|R,j}(\mathbf{q}\,|\,\mathbf{R})\, p_{R,j}(\mathbf{R}) \\
p_{ij}(\mathbf{R},\mathbf{q},\mathbf{R}',\mathbf{q}') &= p_{q|R,ij}(\mathbf{q},\mathbf{q}'\,|\,\mathbf{R},\mathbf{R}')\, p_{R,ij}(\mathbf{R},\mathbf{R}')
\end{aligned}
\tag{20}
$$

where $p_{q|R,i}$ is the probability distribution of the orientational and internal coordinates of molecule i conditioned on its location, and $p_{R,i}(\mathbf{R}) = \int p_i(\mathbf{R},\mathbf{q})d\mathbf{q}$ is the marginal probability distribution of molecule i over its location, i.e., its probability density function. Substituting into the expression for S_i in Equation (19) and using the fact that molecules i and j are identical and therefore have identical distribution functions, we obtain

$$
\begin{aligned}
S_i = S_j &= -k_B \int p_{q|R,i}(\mathbf{q}\,|\,\mathbf{R}) p_{R,i}(\mathbf{R}) \ln\!\left[p_{q|R,i}(\mathbf{q}\,|\,\mathbf{R}) p_{R,i}(\mathbf{R}) \right] d\mathbf{R}d\mathbf{q} \\
&= -k_B \int p_{q|R,i}(\mathbf{q}\,|\,\mathbf{R}) p_{R,i}(\mathbf{R}) \ln\!\left[p_{R,i}(\mathbf{R}) \right] d\mathbf{R}d\mathbf{q} - k_B \int p_{q|R,i}(\mathbf{q}\,|\,\mathbf{R}) p_{R,i}(\mathbf{R}) \ln\!\left[p_{q|R,i}(\mathbf{q}\,|\,\mathbf{R}) \right] d\mathbf{R}d\mathbf{q} \\
&= -k_B \int p_{R,i}(\mathbf{R}) \ln\!\left[p_{R,i}(\mathbf{R}) \right] d\mathbf{R} + \int p_{R,i}(\mathbf{R}) S_i^q(\mathbf{R}) d\mathbf{R} \\
S_i^q(\mathbf{R}) &= -k_B \int p_{q|R,i}(\mathbf{q}\,|\,\mathbf{R}) \ln\!\left[p_{q|R,i}(\mathbf{q}\,|\,\mathbf{R}) \right] d\mathbf{q}
\end{aligned}
\tag{21}
$$

where we have used the fact that, for a given value of $\mathbf{R}$, $p_{q|R,i}(\mathbf{q}\,|\,\mathbf{R})$ is a normalized probability density function, i.e., $\int p_{q|R,i}(\mathbf{q}\,|\,\mathbf{R})d\mathbf{q}=1$. The third line of Eq (21) expresses the single-body entropy as the sum of a translational part and an orientational/conformational part, where the orientational/conformational entropy of molecule i as a function of location, $S_i^q(\mathbf{R})$, is weighted by the local probability density $p_{R,i}(\mathbf{R})$. Similarly, substituting the last line of Eq (20) into the expression for S_{ij} in Eq (19), we obtain:

$$
\begin{aligned}
S_{ij} &= -k_B \int p_{q|R,ij}(\mathbf{q},\mathbf{q}'\,|\,\mathbf{R},\mathbf{R}')\,p_{R,ij}(\mathbf{R},\mathbf{R}')\ln\left[p_{q|R,ij}(\mathbf{q},\mathbf{q}'\,|\,\mathbf{R},\mathbf{R}')\,p_{R,ij}(\mathbf{R},\mathbf{R}') \right] d\mathbf{R}d\mathbf{q}d\mathbf{R}'d\mathbf{q}' \\
&= -k_B \int p_{R,ij}(\mathbf{R},\mathbf{R}')\ln\left[p_{R,ij}(\mathbf{R},\mathbf{R}') \right] d\mathbf{R}d\mathbf{R}' + \int p_{R,ij}(\mathbf{R},\mathbf{R}')S_{ij}^q(\mathbf{R},\mathbf{R}')d\mathbf{R}d\mathbf{R}' \\
S_{ij}^q(\mathbf{R},\mathbf{R}') &= -k_B \int p_{q|R,ij}(\mathbf{q},\mathbf{q}'\,|\,\mathbf{R},\mathbf{R}')\ln\left[p_{q|R,ij}(\mathbf{q}_i,\mathbf{q}'\,|\,\mathbf{R},\mathbf{R}') \right] d\mathbf{q}d\mathbf{q}'
\end{aligned}
$$
(22)

The notation here is analogous to that in Eq (21).

Because the solvent molecules are mutually indistinguishable, we may introduce one symbol for the one-body and two-body probability density functions, $p_i(\mathbf{R},\mathbf{q}) = p_j(\mathbf{R},\mathbf{q}) = p^{(1)}(\mathbf{R},\mathbf{q})$ and $p_{ij}(\mathbf{R},\mathbf{q},\mathbf{R}',\mathbf{q}') = p_{kl}(\mathbf{R},\mathbf{q},\mathbf{R}',\mathbf{q}') = p^{(2)}(\mathbf{R},\mathbf{q},\mathbf{R}',\mathbf{q}')$ for all i,j,k,l, and each molecule accordingly makes an identical contribution to the one-body and two-body number densities of the fluid, $\rho(\mathbf{R})$ and $\rho(\mathbf{R},\mathbf{R}')$, and every pair of molecules ij has the same the two-body mutual information M_{ij}. Therefore, using the fact that there are N molecules $\dfrac{N(N-1)}{2}$ molecule pairs[126], we obtain

$$
\begin{aligned}
\rho(\mathbf{R}) &= N p_R^{(1)}(\mathbf{R}) = N\int p^{(1)}(\mathbf{R},\mathbf{q})d\mathbf{q} \\
\rho(\mathbf{R},\mathbf{R}') &= \frac{N(N-1)}{2}p_R^{(2)}(\mathbf{R},\mathbf{R}') = \frac{N(N-1)}{2}\int p^{(2)}(\mathbf{R},\mathbf{q},\mathbf{R}',\mathbf{q}')d\mathbf{q}d\mathbf{q}'
\end{aligned}
$$
(23)

It also follows that

$$
\begin{aligned}
S^{(1)} &= N S_i \\
S^{(2)} &= -\frac{N(N-1)}{2}M_{ij}
\end{aligned}
$$
(24)

For any i,j. Equations (23) and (24) allow us to write the first order approximation of the entropy, $S^{(1)}$, as follows:

$$S^{(1)} = NS_i = -k_B \int \rho(\mathbf{R}) \ln\left[\frac{\rho(\mathbf{R})}{N}\right] d\mathbf{R} + \int \rho(\mathbf{R}) S^q(\mathbf{R}) d\mathbf{R}$$

$$S^q(\mathbf{R}) = -k_B \int p_{q|R}^{(1)}(\mathbf{q} \mid \mathbf{R}) \ln\left[p_{q|R}^{(1)}(\mathbf{q} \mid \mathbf{R}) \right] d\mathbf{q} \tag{25}$$

Examination of the first line of Eq (25) shows that $S^{(1)}$ can be written as the spatial integral of the first-order entropy density, $s^{(1)}(\mathbf{R})$:

$$S^{(1)} = \int s^{(1)}(\mathbf{R}) d\mathbf{R}$$

$$s^{(1)}(\mathbf{R}) = \rho(\mathbf{R}) S^q(\mathbf{R}) - k_B \rho(\mathbf{R}) \ln\left[\frac{\rho(\mathbf{R})}{N}\right] \tag{26}$$

This is our first-order estimate of the entropy density of the fluid.

The second-order contribution (see Eq (19)) is obtained analogously. We first write

$$S_{ij} = -k_B \int \frac{2\rho(\mathbf{R},\mathbf{R}')}{N(N-1)} \ln\left[\frac{2\rho(\mathbf{R},\mathbf{R}')}{N(N-1)}\right] d\mathbf{R} d\mathbf{R}' + \int \frac{2\rho(\mathbf{R},\mathbf{R}')}{N(N-1)} S_{ij}^q(\mathbf{R},\mathbf{R}') d\mathbf{R} d\mathbf{R}'$$

$$S_{ij}^q(\mathbf{R},\mathbf{R}') = -k_B \int p_{q|R}^{(2)}(\mathbf{q},\mathbf{q}' \mid \mathbf{R},\mathbf{R}') \ln\left[p_{q|R}^{(2)}(\mathbf{q}_i,\mathbf{q}' \mid \mathbf{R},\mathbf{R}') \right] d\mathbf{q} d\mathbf{q}' \tag{27}$$

Now, because there are N(N-1)/2 pairs, we have:

$$S^{(2)} = -\frac{N(N-1)}{2} M_{ij} = -\frac{N(N-1)}{2}\left(2S_i - S_{ij}\right)$$

$$= -N(N-1)S_i + \frac{N(N-1)}{2} S_{ij}$$

$$= N(N-1)\left(k_B \int p(\mathbf{R}) \ln\left[\frac{\rho(\mathbf{R})}{N}\right] d\mathbf{R} - \int p(\mathbf{R}) S^q(\mathbf{R}) d\mathbf{R} \right)$$

$$-\frac{N(N-1)}{2} k_B \int p^{(2)}(\mathbf{R},\mathbf{R}') \ln\left[p^{(2)}(\mathbf{R},\mathbf{R}') \right] d\mathbf{R} d\mathbf{R}' + \frac{N(N-1)}{2} \int p^{(2)}(\mathbf{R},\mathbf{R}') S^q(\mathbf{R},\mathbf{R}') d\mathbf{R} d\mathbf{R}'$$

$$= (N-1)\left(k_B \int \rho(\mathbf{R}) \ln\left[\frac{\rho(\mathbf{R})}{N}\right] d\mathbf{R} - \int \rho(\mathbf{R}) S^q(\mathbf{R}) d\mathbf{R} \right)$$

$$-k_B \int \rho(\mathbf{R},\mathbf{R}') \ln\left[\frac{2\rho(\mathbf{R},\mathbf{R}')}{N(N-1)}\right] d\mathbf{R} d\mathbf{R}' + \int \rho(\mathbf{R},\mathbf{R}') S^q(\mathbf{R},\mathbf{R}') d\mathbf{R} d\mathbf{R}'$$

$$\tag{28}$$

where

$$S^q(\mathbf{R},\mathbf{R}') = -k_B \int p_{q|R}^{(2)}(\mathbf{q},\mathbf{q}' \,|\, \mathbf{R},\mathbf{R}') \ln\left[p_{q|R}^{(2)}(\mathbf{q},\mathbf{q}' \,|\, \mathbf{R},\mathbf{R}') \right] d\mathbf{q}\, d\mathbf{q}' \tag{29}$$

Note that the one-body and two-body number densities need to evaluate these expressions, $\rho(\mathbf{R})$ and $\rho(\mathbf{R},\mathbf{R}')$, may be estimated from molecular simulations[33,34]. When the molecules of the fluid are uncorrelated, as in the special case of an ideal gas, the mutual information M_{ij} should be zero for all pairs of molecules ij, making $S^{(2)} = 0$. This may be confirmed by substituting $p^{(2)}(\mathbf{R},\mathbf{R}') = p^{(1)}(\mathbf{R}) p^{(1)}(\mathbf{R}')$, where $p^{(1)}(\mathbf{R}) = p^{(1)}(\mathbf{R}')$ and $S^q(\mathbf{R},\mathbf{R}') = S^q(\mathbf{R}) + S^q(\mathbf{R}')$

and $S^{(q)}(\mathbf{R}) = S^{(q)}(\mathbf{R}')$ into Eq (28):

$$
\begin{aligned}
S^{(2)} = {} & N(N-1)\left(k_B \int p^{(1)}(\mathbf{R}) \ln\left[p^{(1)}(\mathbf{R}) \right] d\mathbf{R} - \int p^{(1)}(\mathbf{R}) S^q(\mathbf{R}) d\mathbf{R} \right) \\
& - \frac{N(N-1)}{2} k_B \int p^{(1)}(\mathbf{R}) p^{(1)}(\mathbf{R}') \ln\left[p^{(1)}(\mathbf{R}) p^{(1)}(\mathbf{R}') \right] d\mathbf{R}\, d\mathbf{R}' \\
& + \frac{N(N-1)}{2} \int p^{(1)}(\mathbf{R}) p^{(1)}(\mathbf{R}') \left[S^q(\mathbf{R}) + S^{(q)}(\mathbf{R}') \right] d\mathbf{R}\, d\mathbf{R}' \\
= {} & 0
\end{aligned}
\tag{30}
$$

Referring to Eqs (1) and (28), we can now write the second order contribution to the entropy density, $s^{(2)}(\mathbf{R})$, as:

$$
\begin{aligned}
\frac{1}{2} s^{(2)}(\mathbf{R}) = {} & (N-1)\left(k_B \rho(\mathbf{R}) \ln\left[\frac{\rho(\mathbf{R})}{N} \right] + \rho(\mathbf{R}) S^q(\mathbf{R}) \right) \\
& - k_B \int \rho(\mathbf{R},\mathbf{R}') \ln \frac{\rho(\mathbf{R},\mathbf{R}')}{N(N-1)} d\mathbf{R}' + \int \rho(\mathbf{R},\mathbf{R}') S^q(\mathbf{R},\mathbf{R}') d\mathbf{R}'
\end{aligned}
\tag{31}
$$

Thus, we have derived first- and second-order estimates of the entropy density, whose integrals over the system volume give the corresponding first- and second-order estimates of the system entropy, as required for Desideratum D1. Section 13.3 confirms that this entropy expansion approach avoids the need for correction terms like those introduced by Baranyai and Evans[127], which also appear in IST[27].

Note that the MIE can be used to derive higher order approximations of the entropy density in order to account for 3-body correlations, 4-body correlations, etc. It has been argued, based on numerical experiments, that the MIE can converge well as a function of the order of truncation (e.g. 2-body) for systems without long-ranged correlations, but that this can become problematic for highly correlated systems[128]. We do not expect long-ranged correlations in non-critical fluids, and there is evidence that accounting for water-water (i.e. 2-body) correlations suffices to capture the entropy of pure water to within about 20% error[101], so we anticipate that truncating the MIE at second order will be enough to capture much relevant physics for aqueous systems. (See also related work on nonaqeuous fluids[129].) Nonetheless, it is of interest that, by expressing the entropy density in terms of probability density functions, instead of correlation functions as done in IST, the present derivation opens the possibility of replacing the MIE with alternative entropy expansions, such as the maximum information spanning tree (MIST)[130], which might have advantageous convergence properties in some settings.

7.3 ENERGY DENSITY OF A FLUID WITH A MULTIBODY POTENTIAL ENERGY FUNCTION

The IST has been set forth for a two-body potential. Here, we demonstrate the generalization to higher order multibody potentials, where a "body" is one molecule of fluid, and the potential energy of a given configuration of the system is:

$$U(\mathbf{r}^N) = \sum_{i=1}^{N}\phi^{(1)}(\mathbf{R}_i,\mathbf{q}_i) + \frac{1}{2}\sum_{i=1}^{N}\sum_{j\neq i}^{N}\phi^{(2)}(\mathbf{R}_i,\mathbf{q}_i,\mathbf{R}_j,\mathbf{q}_j) + \frac{1}{3}\sum_{i=1}^{N}\sum_{j\neq i}^{N}\sum_{\substack{k\neq i\\k\neq j}}^{N}\phi^{(3)}(\mathbf{R}_i,\mathbf{q}_i,\mathbf{R}_j,\mathbf{q}_j,\mathbf{R}_k,\mathbf{q}_k) + \ldots$$

(32)

Here, the order of each ϕ defining the potential function is given by its superscript, and interactions with any solute or inhomogeneity are included implicitly (see Appendix). Note that any arbitrary potential, including one that accounts explicitly for electronic polarizability via, e.g., the widely used Silberstein/Applequist model[131–133] can be fully described by such a multibody expansion, and that this problem can be solved even when the potential function is not explicitly provided in terms of a multibody expansion (see Section **13.4**). We again limit attention, for simplicity, to the case of a rigid solute or inhomogeneity, while noting that this derivation generalizes to the flexible case. We define the energy associated with molecule i for a given system configuration $\mathbf{r}^N$ as

$$U_i(\mathbf{r}^N) = \phi^{(1)}(\mathbf{R}_i,\mathbf{q}_i) + \frac{1}{2}\sum_{j\neq i}^{N}\phi^{(2)}(\mathbf{R}_i,\mathbf{q}_i,\mathbf{R}_j,\mathbf{q}_j) + \frac{1}{3}\sum_{j\neq i}^{N}\sum_{\substack{k\neq i\\k\neq j}}^{N}\phi^{(3)}(\mathbf{R}_i,\mathbf{q}_i,\mathbf{R}_j,\mathbf{q}_j,\mathbf{R}_k,\mathbf{q}_k) + \ldots \quad (33)$$

Note that the total energy is given by the sum of these molecular energies:

$$U(\mathbf{r}^N) = \sum_{i=1}^{N}U_i(\mathbf{r}^N)$$

(34)

The ensemble-averaged contribution of molecule i to the desired energy density at $\mathbf{R}$ is given by:

$$u_i(\mathbf{R}) = \left\langle \delta(\mathbf{R}_i - \mathbf{R})U_i(\mathbf{r}^N)\right\rangle = \int p(\mathbf{r}^N)\delta(\mathbf{R}_i - \mathbf{R})U_i(\mathbf{r}^N)d\mathbf{r}^N$$
$$= u_i^{(1)}(\mathbf{R}) + \frac{1}{2}u_i^{(2)}(\mathbf{R}) + \frac{1}{3}u_i^{(3)}(\mathbf{R}) + \ldots$$

(35)

where

$$u_i^{(1)}(\mathbf{R}) = \int p(\mathbf{r}^N)\delta(\mathbf{R}_i - \mathbf{R})\phi^{(1)}(\mathbf{R}_i,\mathbf{q}_i)d\mathbf{r}^N$$

$$u_i^{(2)}(\mathbf{R}) = \int p(\mathbf{r}^N)\delta(\mathbf{R}_i - \mathbf{R})\left[\sum_{j\neq i}^{N}\phi^{(2)}(\mathbf{R}_i,\mathbf{q}_i,\mathbf{R}_j,\mathbf{q}_j)\right]d\mathbf{r}^N$$

$$u_i^{(3)}(\mathbf{R}) = \int p(\mathbf{r}^N)\delta(\mathbf{R}_i - \mathbf{R})\left[\sum_{j\neq i}^{N}\sum_{\substack{k\neq i\\k\neq j}}^{N}\phi^{(3)}(\mathbf{R}_i,\mathbf{q}_i,\mathbf{R}_j,\mathbf{q}_j,\mathbf{R}_k,\mathbf{q}_k)\right]d\mathbf{r}^N$$

(36)

…

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The ensemble average energy of molecule i is given by the spatial integral of its energy density:

$$\left\langle U_i(\mathbf{r}^N) \right\rangle = \int u_i(\mathbf{R}) d\mathbf{R} \tag{37}$$

Finally, because all N molecules are indistinguishable, the total energy density at $\mathbf{R}$ is simply

$$u(\mathbf{R}) = \sum_{i=1}^{N} u_i(\mathbf{R}) = N u_i(\mathbf{R})$$

$$= u^{(1)}(\mathbf{R}) + \frac{1}{2} u^{(2)}(\mathbf{R}) + \frac{1}{3} u^{(3)}(\mathbf{R}) + \ldots \tag{38}$$

for any i, where

$$u^{(1)}(\mathbf{R}) = N \int p(\mathbf{r}^N) \delta(\mathbf{R}_i - \mathbf{R}) \phi^{(1)}(\mathbf{R}_i, \mathbf{q}_i) d\mathbf{r}^N$$

$$u^{(2)}(\mathbf{R}) = N \int p(\mathbf{r}^N) \delta(\mathbf{R}_i - \mathbf{R}) \left[\sum_{j \neq i}^{N} \phi^{(2)}(\mathbf{R}_i, \mathbf{q}_i, \mathbf{R}_j, \mathbf{q}_j) \right] d\mathbf{r}^N$$

$$u^{(3)}(\mathbf{R}) = N \int p(\mathbf{r}^N) \delta(\mathbf{R}_i - \mathbf{R}) \left[\sum_{j \neq i}^{N} \sum_{\substack{k \neq i \\ k \neq j}}^{N} \phi^{(3)}(\mathbf{R}_i, \mathbf{q}_i, \mathbf{R}_j, \mathbf{q}_j, \mathbf{R}_k, \mathbf{q}_k) \right] d\mathbf{r}^N \tag{39}$$

$$\ldots$$

The ensemble average of the total energy is given by the spatial integral of the energy density, as required by Desideratum D1:

$$U = \int u(\mathbf{R}) d\mathbf{R} \tag{40}$$

8 PHYSICAL INTERPRETATION OF THE ENERGY AND ENTROPY EXPANSION TERMS

The one-body potential energy term defined above comprises the mean internal (i.e., conformation-dependent) energy and the mean solvent-solute interaction energy (if any) of the fluid molecules as a function of their location in the system. The two-body energy term then accounts for the mean pairwise interaction of the fluid molecules at a given location with all other molecules in the system, the three-body term accounts for the mean three-body interactions of the molecules at a given location, and so on at higher orders. For simulations with a pairwise additive potential function[104,105,134–136], all terms above pairwise are identically zero, whereas a multibody potential function, such as a polarizable force field[133,137–141], will have nonzero higher order terms.

The one-body entropy term has a translational component that goes as the log of the local number density and that is thus closely related to the excess chemical potential[42], with higher density giving lower entropy; and an orientation/conformation component that falls as the molecules at a given location are more orientationally and conformationally restricted by any solute or inhomogeneity that may be present. The two-body translational term reflects the degree to which the presence or absence of a molecule at a given location correlates with the presence or absence of molecules elsewhere[34],

while the two body orientation/correlation component falls as the molecules at a given location become more orientationally and conformationally correlated with molecules elsewhere.

If no solute or other inhomogeneity is present, then all terms in the energy and entropy expansions are independent of position, reporting simply on properties of the pure fluid. In the presence of a solute or other inhomogeneity, the terms in the expansion tend to deviate from their pure fluid values near the inhomogeneity, while going asymptotically to their pure fluid values with increasing distance from it.

A solute that interacts strongly and directionally with solvent molecules (e.g., a sodium ion in water) will tend to generate a strongly favorable first order energy term nearby, along with a strongly unfavorable (low entropy) first order entropy term. However, the higher order entropy terms will tend to be small in magnitude, because molecules with sharply reduced positional, orientational, and conformational fluctuations cannot have large mutual informations with other molecules: in effect, there is little motion to be correlated in this setting. By contrast, a solute that interacts weakly with solvent molecules (e.g., a methane molecule in water), will yield only a small interaction energy with the solvent, and the first order entropy terms may not be particularly small, since the method does not strongly restrict nearby solvent molecules. However, because solvent molecules near the solute remain mobile, second and higher order entropy terms can become large, falling with increased correlation among solvent molecules near the solute. Accordingly, we expect much of the drop in water entropy near a hydrophobic solute, such as methane, to result from increased water-water correlations, rather than from restriction of water mobility in the reference frame of the solute. More broadly, a drop in the one-body entropy (due to restriction) is likely to anticorrelate with a drop in entropy due to higher order entropy terms (i.e., due to correlations), as indeed observed in the case of aqueous solvation[89].

9   RELATED CONTRIBUTIONS AND CONCEPTS

The definition of a free energy density presented here is closely related to inhomogeneous solvation theory (IST) and is perhaps best regarded as an elaboration of IST that explicitly handles systems with arbitrarily complex potential functions and flexible molecules. However, we reserve a detailed exploration of the relationship between IST and the present approach for future work. This generalization is theoretically significant because it confirms the existence of a rigorous and general definition of the free energy density of a fluid that can satisfy all of our Desiderata (Section 2). It thus demonstrates that the thermodynamic densities are general, in the sense that they are not limited to systems complying with the approximations normally associated with IST. In addition, the present derivation of the entropy density (Section 7.2), which is based on the MIE [123–125], avoids bookkeeping problems that can arise in the traditional correlation function-based derivation of the entropy density [127]. The MIE derivation also may be more transparent to researchers not versed in liquid state theory, and it should be easier to generalize it to more complex and heterogeneous systems, since the MIE does not require an assumption of particle indistinguishability. Note that connections between IST and MIE are well documented in the literature [40,53,106,124]. It is also worth mentioning that the energy and entropy densities derived here lead directly to tools for analyzing the outputs of molecular simulations, along the lines of WaterMap[73] and GIST[33].

The classical density function theory (DFT) of liquids is well founded in liquid state theory[126,142] and, as reviewed elsewhere, may be used to formulate definitions of the free energy density by either a

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perturbation[92] or a density expansion[90,91] approach[43]. Application of DFT typically avoids the need to run a molecular simulation, instead providing useful approximations through semi-analytic calculations. The perturbation approach is regarded as better suited to strongly heterogeneous systems[43] and appears to lead to definitions of the free energy that satisfies Desiderata D1, D2, and D4, but that may not go to zero where the solvent density is zero[92] and so may not satisfy Desideratum D3 (Section 2). Deeper insights into the relationship between a perturbative DFT approach and IST are provided by a prior study[42]. The density expansion approach yields a free energy density that includes a factor of the local particle density and thus is expected to satisfy Desideratum D3, but we are not aware of a formulations in the literature that yields the exact free energy as the volume integral of the free energy density for an arbitrary potential function. Overall, DFT appears to offer valuable and theoretically sound pathway to useful definitions of the free energy density, and progress has been made in applying DFT to heterogeneous systems of biological and biomedical interest[143,144].

Like DFT, the three-dimensional reference interaction site approach (3D-RISM)[48,49,145,146] provides estimates of the properties of liquids in heterogeneous systems without the need to run molecular simulations. It also is based on rigorous derivation that appears amenable to providing a definition of the free energy density that satisfies all four desiderata, along with numerical approximations that can be applied in a practical setting and that have been compared with IST results for water in protein binding sites[50].

Grid Cell Theory[46] (GCT) provides an approximation of the free energy density when applied to a molecular simulation, and has given encouraging agreement with solvation free energies obtained from free energy methods with explicit solvent molecular simulations. It can therefore be a valuable practical tool to analyze solvation. However, GCT does not approach an exact result for a given liquid system in any limit, such as that of infinite simulation sampling or infinitesimal cell volume, so it does not seem to address one of the questions posed in this work, namely, whether there exists a rigorous definition of the free energy that satisfies the present desiderata.

In summary, there are several other promising approaches to defining the free energy density of a fluid, and more study is needed to fully understand how they relate to each other, both theoretically and in terms of numerical accuracy and computational performance.

It is also natural to ask how the chemical potential, μ, and the excess chemical potential, $\mu_{ex}(\mathbf{R})$, of a fluid relate to the free energy density developed in this work. As discussed in Sections 4 and 5, the chemical potential is not a position-dependent quantity, but is instead a property of a molecular species—here of the solvent—in the system. Thus, although one could use it to formally define a free energy density that would satisfy Desideratum D1, i.e., $\mu\rho(\mathbf{R})$, this definition would not satisfy Desideratum D2. The excess chemical potential may appear to a more promising candidate for an alternative definition of the free energy density, because it varies with position in the context of an inhomogeneity[110,111]. In addition, it is given by the Widom particle insertion process[110], which may seem a logical way to probe the stability of water as a function of position. Indeed, the relationship between the excess chemical potential and IST has been previously explored[42]. However, the excess chemical potential is related to the chemical potential as $\mu_{ex}(\mathbf{R}) = \mu - RT \ln \rho(\mathbf{R})$ [110,111], so it is apparent that the integral of $\mu_{ex}(\mathbf{R})$ equals neither the free energy of the system nor the solvation free energy of an

inhomogeneity, so it does not satisfy Desideratum D1. More generally, we are not aware of any rigorous mapping from the number density of a liquid at location **R** to its free energy density at **R**.

10 CONCLUSION

We have provided a rigorous definition of the free energy density of a fluid, elucidated its meaning and interpretation, and brought forward approximations often used when analyzing the role of solvent in binding thermodynamics. Although we have developed one specific formulation for the free energy density (as well as the potential energy and entropy densities), other formulations may also satisfy the desiderata for a free energy density laid out in Section 2. If so, these should, similarly, provide a solvation free energy density that allows the solvation free energy of a solute to be estimated from a local integral and whose integral along a surface normal yields the surface tension (Section 4), and they should also support drug discovery applications based on analysis of the thermodynamic properties of binding-site water (Section 6). However, they will provide somewhat different spatial distributions of the free energy density and solvation free energy density. There is room for further research on such formulations and the definition of a metric that could be used to determine whether, or in what settings, one formulation should be preferred over another.

The present work also sets the free energy density in the context of related literature. In particular, we have shown that that the concept of a position-varying free energy density in a fluid at equilibrium makes physical sense and indeed is consistent with and explanatory of familiar concepts such as the surface tension of a liquid (Section 4). We also note that neither the chemical potential nor the excess chemical potential provide free energy densities that satisfy the desiderata: the chemical potential is not a position-dependent quantity, and although the excess chemical potential is position-dependent, the product $\mu_{ex}(\mathbf{R})\rho(\mathbf{R})$ does not integrate to the system free energy. Instead, we provide a definition of the local chemical potential which does have this property, and which can be used to thermodynamically characterize a fluid in a manner that is clearly linked to the overall free energy of the system. We hope that the concepts and derivations introduced here will be found useful in future theoretical and computational analyses of the role of solvent in molecular processes.

11 ACKNOWLEDGEMENTS

12 AUTHOR DECLARATIONS

MKG has an equity interest in and is a cofounder and scientific advisor of VeraChem LLC. TK is founder and scientific advisor of Deep Waters NYC, LLC; has an equity interest in and is a scientific advisor for

13 APPENDIX

13.1 SOLVATION FREE ENERGY AS A LOCAL INTEGRAL OF THE FREE ENERGY DENSITY NEAR THE SOLUTE

Referring to Section 4, the free energy of a solute-solvent system may be written in terms of the free energy density of the solvent in the presence of the solute. Here, we divide the system into a strongly perturbed region "near" the solute and a weakly perturbed region "far" from the solute:

$$F = \int f(\mathbf{R})d\mathbf{r} = \int_{near} f(\mathbf{R})d\mathbf{r} + \int_{far} f(\mathbf{R})d\mathbf{r} \approx \int_{near} f(\mathbf{R})d\mathbf{R} + V_{far}f_{bulk}$$

$$V_{far} = N_{far} / \rho_{bulk}$$

$$f_{bulk} = \mu_{bulk}\rho_{bulk}$$

$$V_{far}f_{bulk} = N_{far}\mu_{bulk} = (N - N_{near})\mu_{bulk}$$

$$F \approx \int_{near} f(\mathbf{R})d\mathbf{R} + (N - N_{near})\mu_{bulk} \tag{41}$$

$$N_{near} = \int_{near} \rho(\mathbf{R})d\mathbf{R}$$

$$F_{bulk} = N\mu_{bulk}$$

Hence: $\Delta F_{solv} = F - F_{bulk} \approx \int_{near} f(\mathbf{R})d\mathbf{R} - N_{near}\mu_{bulk}$

Here N_{near} and N_{far} are the mean number of solvent molecules in the near and far regions, V_{far} is the volume of the far region, and we have used that, far from the solute, the free energy density $f(\mathbf{R}) \to f_{bulk}$ and the single-molecule number density $\rho(\mathbf{R}) \to \rho_{bulk}$.
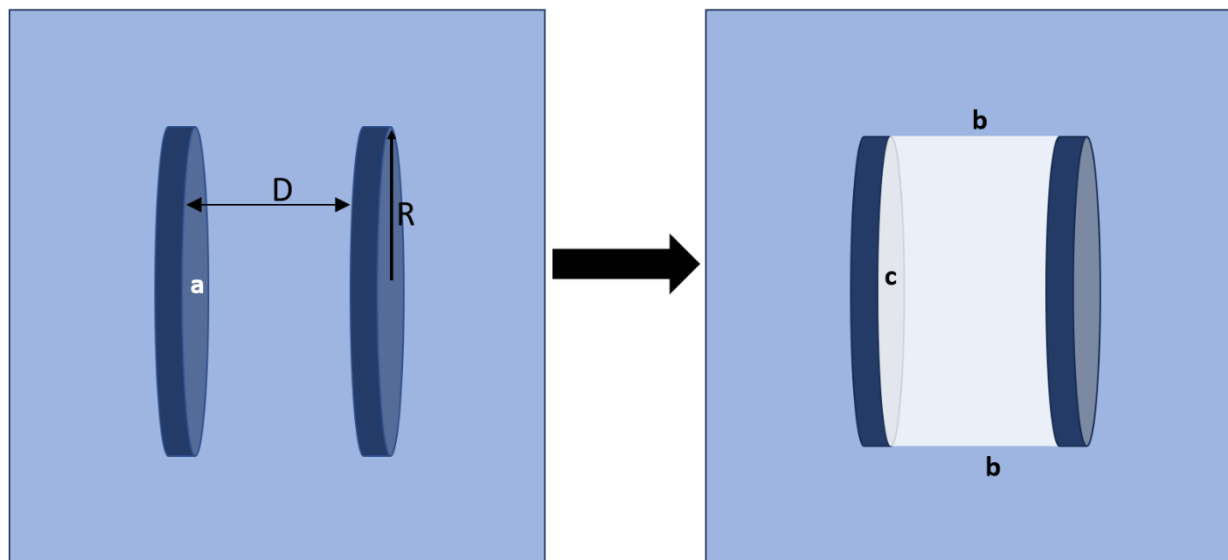
Figure 5. Dewetting of the cylindrical space between two circular plates immersed in a liquid. D: distance between plates. R: radius of plates. a: liquid-surface interface. b: liquid-vapor interface. c: solute-vapor interface.

13.2 SURFACE-ENERGY ANALYSIS OF DEWETTING BETWEEN PARALLEL PLATES IN A LIQUID

In Section 5, we used a system with two parallel, nonpolar plates immersed in water as a model to help explain when and how thermodynamically unfavorable water may stably exist in a system at equilibrium. Here, we support the model with a detailed analysis. In analogy with the hypothetical dewetting of a receptor binding site to allow unfavorable water to escape to bulk, we consider the free energy change upon dewetting the cylindrical region between two solid circular plates of radius R, separated by distance D, and immersed in water (Figure 5). For a system at 1 atm pressure, the pressure-volume contribution to the free energy of dewetting is negligible as long as R is not very large, so we omit it. Following prior work[113,147], we develop this in macroscopic terms, using the liquid-vapor, liquid-solute, and solute-vapor surface tensions, respectively γ_{lv}, γ_{ls}, and γ_{sv}. Focusing only on the inner plate surfaces (**a** in Figure 5), since the outer surfaces will not be dewetted, we write the initial free energy of the system with water between the plates as:

$$F_1 = 2\gamma_{ls}A = 2\pi R^2 \gamma_{ls} \tag{42}$$

because the surface of each plate has total surface area $A = \pi R^2$. The free energy following dewetting of the space between the plates, again neglecting the outer surfaces as well as the small pressure-volume work, is:

$$F_2 = 2\pi R^2 \gamma_{sv} + 2\pi RD\gamma_{lv} \tag{43}$$

where the first term gives the energy of the solute-vapor interfaces (**c** in Figure 5), and the second term gives the free energy of the liquid-vapor interface at the boundary of the cylindrical dewetted region (**b** in Figure 5). The change in free energy thus is:

$$\Delta F = F_2 - F_1 = 2\pi R^2 (\gamma_{sv} - \gamma_{ls}) + 2\pi RD\gamma_{lv} \tag{44}$$

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For hydrophobic plates – analogous to a hydrophobic receptor binding site – the first term in Eq (44) is negative due to the unfavorable surface tension of water at the hydrophobic surface ($\gamma_{ls} > 0$). This term thus favors expulsion of water to dewet the space between the plates, opening a vapor-filled bubble. However, this thermodynamic driving force is opposed by the final term in Eq (44), which is proportional to the liquid-vapor surface tension, which is expected to be even higher than the liquid-solute surface tension, because the vapor affords no favorable interactions with the liquid. In net, the space between the plates will contain water so long as $\Delta F > 0$, even though this water is thermodynamically unfavorable due to its unfavorable liquid-solute surface energy. Dewetting occurs when the plates are close enough that the unfavorable surface energy of a new vapor-liquid boundary (**b** in Figure 5) is not high enough to oppose the favorable drop in free energy that results from drying of the inner surfaces of the plates.

This system highlights the importance of accounting for the free energy penalty of forming a new liquid-vapor interface when consider whether thermodynamically unfavorable water will exit a region of space, as discussed in Section 5.

13.3 CONSISTENCY OF THE MIE APPROACH WITH THE SACKUR-TETRODE EQUATION FOR THE ENTROPY OF AN IDEAL GAS

Inhomogeneous Solvation Theory (IST)[27,28] builds on earlier work of Wallace[26], who expanded the entropy of a homogeneous liquid with N molecules in terms of n-molecule correlation functions of the form $g_N^{(n)}(\mathbf{r}_1, \ldots, \mathbf{r}_n)$. Baranyai and Evans[127] subsequently showed that Wallace's expression contained a flaw, which could be corrected by adding a correction term at each level of the expansion. In contrast, the mutual information expansion (MIE) approach used here gives the correct entropy for an ideal gas, i.e., the Sackur-Tetrode equation[114] written as Eq 7 of Baranyai and Evans, without requiring any correction terms. To demonstrate this, we begin by restating the problem in terms that include momentum, since the Sackur-Tetrode equation includes the momentum contribution to the entropy[148].

We consider an equilibrium system made up of N>>1 indistinguishable molecules, each made up of n atoms, in a fluid state at pressure P ~1 atm and temperature T, and enclosed in a container of volume V. The system may also include a rigid solute molecule immersed in the fluid (or another inhomogeneity, such as a free surface) that is fixed in the lab coordinate frame. The full sets of spatial and momentum coordinates, respectively, are denoted $\mathbf{r}^N$ and $\mathbf{p}^N$. In the classical approximation of statistical thermodynamics, the Helmholtz free energy is given by

$$F = -k_B T \ln\left(\frac{Q}{N!}\right)$$

$$Q = \frac{1}{h^{3nN}} \int e^{-\beta H(\mathbf{p}^N, \mathbf{r}^N)} d\mathbf{r}^N d\mathbf{p}^N$$

$$H = \sum_{i=1}^{N} \sum_{j=1}^{n} \frac{p_{ij}^2}{2m_{ij}} + U(\mathbf{r}^N) \qquad (45)$$

$$Q = \left(\frac{2\pi \mathcal{M} k_B T}{h^2}\right)^{3nN/2} \int e^{-\beta U(\mathbf{r}^N)} d\mathbf{r}^N$$

$$\mathcal{M} \equiv \left(\prod_{j=1}^{n} m_{ij}\right)^{\frac{1}{n}}$$

Here $\mathbf{p}^N$ and $\mathbf{r}^N$ are the vectors of momentum and position for the n atoms of molecule N, p_{ij} and m_{ij} are, respectively, the momentum and mass of atom j of molecule i, and H is the Hamiltonian which give the energy as the sum of the kinetic and potential energies. Thus, the free energy may be written as:

$$F = -k_B T \ln\left[\frac{1}{N!}\left(\frac{2\pi \mathcal{M} kT}{h^2}\right)^{3nN/2} \int e^{-\beta U(\mathbf{r}^N)} d\mathbf{r}^N\right]$$

$$= -k_B T \ln\left(\frac{1}{N!}\right) - k_B T \ln\left(\frac{2\pi \mathcal{M} kT}{h^2}\right)^{3nN/2} - k_B T \ln \int e^{-\beta U(\mathbf{r}^N)} d\mathbf{r}^N \qquad (46)$$

The entropy is the partial derivative of the free energy with respect to temperature:

$$S = -\frac{\partial}{\partial T}\left[-k_B T \ln\left(\frac{1}{N!}\right) - kT \ln\left(\frac{2\pi \mathcal{M} k_B T}{h^2}\right)^{3nN/2} - k_B T \ln \int e^{-\beta U(\mathbf{r}^N)} d\mathbf{r}^N\right]$$

$$= \left[k_B \ln\left(\frac{1}{N!}\right)\right] + nNk\left[\ln\left(\frac{2\pi \mathcal{M} kT}{h^2}\right)^{3/2} + \frac{3}{2}\right] + \left[k_B \ln \int e^{-\beta U(\mathbf{r}^N)} d\mathbf{r}^N + \frac{\langle U \rangle}{T}\right] \qquad (47)$$

$$= \frac{F - \langle K \rangle - \langle U \rangle}{T}$$

The last line confirms our expectation that $F = E - TS$, where the mean system energy, E, is the sum of the mean kinetic energy $\langle K \rangle = \frac{3nNk_B T}{2}$ and the mean potential energy $\langle U \rangle$. Further intuition can be gained by recognizing that the next-to-last line breaks down the entropy into the three bracketed parts:

$$S_{indist} = k_B \ln\left(\frac{1}{N!}\right)$$

$$S_{momentum} = nNk_B \ln\left(\frac{2\pi \mathcal{M} k_B T}{h^2}\right)^{3/2} + \frac{3nNk_B}{2} \qquad (48)$$

$$S_{space} = k_B \ln \int e^{-\beta U(\mathbf{r}^N)} d\mathbf{r}^N + \frac{\langle U \rangle}{T}$$

The first, part, S_{indist}, results from the indistinguishability of particles. The second, $S_{momentum}$, results from the momentum degrees of freedom, and can be interpreted as $TS_{momentum} = F_{momentum} - <K>$, where $F_{momentum}$ is the free energy contribution from the momentum degrees of freedom. The third, S_{space}, result from the spatial degrees of freedom and can be interpreted as $TS_{space} = F_{space} - <U>$, where F_{space} is the free energy contribution from the spatial degrees of freedom (before accounting for indistinguishability).

In classical thermodynamics, S_{indist} and $S_{momentum}$ are independent of the potential function, $U(\mathbf{r}^N)$, and we have analytic expressions for them. As a consequence, they are commonly ignored in liquid state theory and molecular simulations, and our main interest is in estimating S_{space}, which does depend on the potential function, and this is what we have done in the main text, where in fact we have implicitly used S for S_{space}.

However, we want to confirm that, in the case of an ideal, monatomic, gas, our expression for the entropy reduces to that of Baranyai and Evans's Eq 7, which does include these contributions and is identical with the Sackur-Tetrode equation. For an ideal monatomic gas, each atom has a fixed potential energy U_{atom}, so $\langle U \rangle = NU_{atom}$, and $\int e^{-\beta U(\mathbf{r}^N)} d\mathbf{r}^N = \int e^{-\beta NU_{atom}} d\mathbf{r}^N = e^{-\beta NU_{atom}} V^N$. In addition, $n = 1$, and $\mathcal{M}$, the geometric mean of the atomic masses in a molecule goes simply to the mass, m, of the single atom; i.e., $\mathcal{M} \to m$. Therefore, the spatial component of the entropy for an ideal gas becomes:

$$\begin{aligned} S_{ig,space} &= k_B \ln \int e^{-\beta NU_{atom}} d\mathbf{r}^N + \frac{NU_{atom}}{T} \\ &= k_B \ln\left(e^{-\beta NU_{atom}} V^N\right) + \frac{NU_{atom}}{T} \qquad (49) \\ &= Nk_B \ln V \end{aligned}$$

The indistinguishability and momentum components do not change on going to the ideal gas limit. Thus, we obtain the following expression for the entropy of the ideal gas, where the second line uses Stirling's approximation that $\ln N \backsimeq N \ln N - N$ for large N.

$$S_{ig} = k_B \ln\left(\frac{1}{N!}\right) + Nk \ln\left(\frac{2\pi m k_B T}{h^2}\right)^{3/2} + \frac{3Nk}{2} + Nk_B \ln V$$

$$\approx -Nk_B \ln N + Nk_B + Nk_B \ln\left(\frac{2\pi m k_B T}{h^2}\right)^{3/2} + \frac{3Nk}{2} + Nk_B \ln V$$

$$= Nk_B \ln\left(\frac{2\pi m k_B T}{h^2}\right)^{3/2} + \frac{5Nk_B}{2} + Nk \ln \frac{V}{N}$$

$$= Nk_B \ln\left[\left(\frac{2\pi m k_B T}{h^2}\right)^{3/2} \frac{V}{N}\right] + \frac{5Nk_B}{2}$$

(50)

The final line is recognizable as the Sackur-Tetrode equation, and is equivalent to Baranyai and Evans's Eq 7, where ρ, the number density, is given by N/V, and the de Broglie wavelength, Λ, is given by

$$\Lambda \equiv \frac{h}{\sqrt{2\pi m k_B T}}$$

(51)

Recalling that the main text applies the MIE to the spatial component of the entropy, S_{space} (though it uses the symbol S for simplicity), we now demonstrate that, for an ideal, monatomic, gas, the second order MIE gives $S_{space} = S_{ig,space} = Nk_B \ln V$, in accordance with Eq (49). In Eq (25), we have $\rho(\mathbf{R}) = N/V = Np(\mathbf{R})$ due to the ideality of the gas, and we omit the term in S^q because a monatomic gas has no orientational or internal degrees of freedom, so the first order estimate of the entropy (omitting the "space" subscript) is

$$S_{ig}^{(1)} = -k_B \int \rho(\mathbf{R}) \ln \frac{\rho(\mathbf{R})}{N} d\mathbf{R} = -k_B \int \frac{N}{V} \ln \frac{1}{V} d\mathbf{R} = Nk_B \ln V$$

(52)

Comparison with Eq (49) shows that this first order MIE approximation provides the correct spatial component of the entropy of an ideal gas. The mutual information of two uncorrelated variables is zero, and the atoms of an ideal gas are not correlated with each other, so we expect all of the higher order MIE terms to be zero. We now confirm this for the second order term (Eq (28), using the fact that the pair density $\rho(\mathbf{R},\mathbf{R}') = \frac{N(N-1)}{2V^2} = \frac{N(N-1)}{2} p^2(\mathbf{R})$:

$$S^{(2)} = -\frac{N(N-1)}{2} M_{ij}$$

$$= -(N-1)\left(-k_B \int \frac{N}{V} \ln\left[\frac{1}{V}\right] d\mathbf{R}\right) + \left(-k_B \int \frac{N(N-1)}{2V^2} \ln \frac{1}{V^2} d\mathbf{R} d\mathbf{R}'\right)$$

$$= -N(N-1)k_B \ln V + k_B \frac{N(N-1)}{2}(2\ln V) = 0$$

(53)

Thus, the second order MIE approach yields the correct spatial component for the entropy of the ideal gas. Because the lack of correlation among gas molecules means that all mutual information terms are zero, the same result is expected to hold to all orders of the expansion.

13.4 ENERGY DENSITY FOR AN ARBITRARY POTENTIAL ENERGY FUNCTION

This section shows a way of formulating the potential energy density, $u(\mathbf{R})$, when an explicit multibody expansion of the potential energy is unavailable, such as when one uses a quantum chemical method to compute the energy of the system. This is done not so much to support practical applications as to allay possible concerns that the energy density is well-defined only if one knows such a multibody expansion. To accomplish this goal, we only need a suitable method of assigning an energy $U_i(\mathbf{r}^N)$ to each solvent molecule i for a given configuration of the system, $\mathbf{r}^N$, as done in Eq (33) via the multibody expansion, so that it can be assigned in turn to a location in the lab frame, as done in Eq (35). In what follows, we first state the approach and then demonstrate that it yields the correct result when applied to a sample potential with a known multibody expansion.

In brief, the energy associated with a solvent molecule in a given configuration, $U_i(\mathbf{r}^N)$, is defined as the average change of the system's energy upon adding molecule i to the system, potentially in the presence of the rigidly fixed solute molecule, where the average is taken over all $N!$ possible orders of adding the N solvent molecules to construct the configuration $\mathbf{r}^N$. More specifically, the total energy of the system, $U(\mathbf{r}^N)$, is defined relative to a reference state with all molecules infinitely separated and in some arbitrarily selected molecular conformation. The energy of the system, $U(\mathbf{r}^N)$, is the sum of the energies of bringing each molecule, i, from infinity to its location, orientation, and conformation, as defined by $\mathbf{r}^N$. The energy contribution of a given molecule depends on the order in which the molecules are brought back from infinity to reconstruct the system, and we index the $N!$ possible orderings, or reconstructions, by $l = (1, \ldots, N!)$. For a conservative force field, the sum of the contributions for a given reconstruction l is always the same, equaling the total energy of the system in the configuration of interest, i.e., $U(\mathbf{r}^N)$. Thus, if the contribution of molecule i to the overall energy for a given reconstruction of the system is $U_{il}(\mathbf{r}^N)$, then

$$U(\mathbf{r}^N) = \sum_{i=1}^{N} U_{il}(\mathbf{r}^N) \quad (1)$$

for all orderings l. We finally define the configuration-dependent but reconstruction-independent energy of molecule i, as the average of $U_{il}(\mathbf{r}^N)$ over all possible reconstructions:

$$U_i(\mathbf{r}^N) = \frac{1}{N!}\sum_{l=1}^{N!} U_{il}(\mathbf{r}^N) \quad (2)$$

Note that the total system energy equals the sum of these molecular energies:

$$U(\mathbf{r}^N) = \sum_{i=1}^{N} U_i(\mathbf{r}^N) \qquad (3)$$

From a practical standpoint, recomputing energies, as required here, for the vast number of molecular permutations would be computationally intractable. However, the energy of placing one molecule in the system depends strongly only on the locations of other nearby molecules, so it might be possible to factorize the calculation into a set of local reconstructions, treating long-ranged interactions approximately, such as by a mean-field approximation.

We now show that this construction approach provides the desired outcome when there is a defined multibody potential. For simplicity, we first analyze a single configuration of 4 molecules and no solute interacting via a 3-body potential, and focusing on how the energy of molecule $i=1$ is assigned by the permutation averaging process. Because there is only one configuration, we omit $(\mathbf{r}^N)$ from the notation. The multibody potential comprises ϕ_1, which is the internal energy of molecule 1; the pairwise interaction energies of molecule 1 with molecules 2, 3, and 4, ϕ_{12}, ϕ_{13}, ϕ_{14}, respectively; and the triplet interaction energies ϕ_{123}, ϕ_{124}, ϕ_{134}. As shown in Table 1, U_1, the average over permutations of the energy change on adding molecule 1, comprises ϕ_1, half of its 2-body interactions, and a third of its 3-body interactions. Thus, the permutation-averaged method yields the desired result for a defined multibody potential (Section 7.3).

Looking beyond this simple system, one may see that the permutation-averaged approach generalizes. In a system of any size, the energy change on introducing molecule 1 includes a contribution of ϕ_1 for every permutation, so the full one-body term is always included in the average taken to obtain U_1. However, for half of the permutations, molecule 2 is added after molecule 1, so the mean contribution of the corresponding 2-body term is $\frac{1}{2}\phi_{12}$. Similarly, molecule 1 is added after molecules 2 and 3 in one third of the permutations, so the corresponding 3-body contribution is $\frac{1}{3}\phi_{123}$. Clearly, this pattern leads to the desired energy assignments for multibody potentials of any order. This consistency supports the reasonableness of using permutation-averaged energy assignments in the more general case where no multibody potential has been defined.

Table 1.

Permutation (l)	Order of reconstruction for permutation l	Energy change upon adding molecule 1 in permutation l (U_{1l})
1	1234	ϕ_1
2	1243	ϕ_1

34

3	1324	ϕ_1
4	1342	ϕ_1
5	1423	ϕ_1
6	1432	ϕ_1
7	2134	$\phi_1 + \phi_{12}$
8	2143	$\phi_1 + \phi_{12}$
9	2314	$\phi_1 + \phi_{12} + \phi_{13} + \phi_{123}$
10	2341	$\phi_1 + \phi_{12} + \phi_{13} + \phi_{14} + \phi_{123} + \phi_{124} + \phi_{134}$
11	2413	$\phi_1 + \phi_{12} + \phi_{14} + \phi_{124}$
12	2431	$\phi_1 + \phi_{12} + \phi_{13} + \phi_{14} + \phi_{123} + \phi_{124} + \phi_{134}$
13	3124	$\phi_1 + \phi_{13}$
14	3142	$\phi_1 + \phi_{13}$
15	3214	$\phi_1 + \phi_{13} + \phi_{12} + \phi_{123}$
16	3241	$\phi_1 + \phi_{12} + \phi_{13} + \phi_{14} + \phi_{123} + \phi_{124} + \phi_{134}$
17	3412	$\phi_1 + \phi_{13} + \phi_{14} + \phi_{134}$
18	3421	$\phi_1 + \phi_{12} + \phi_{13} + \phi_{14} + \phi_{123} + \phi_{124} + \phi_{134}$
19	4123	$\phi_1 + \phi_{14}$
20	4132	$\phi_1 + \phi_{14}$
21	4213	$\phi_1 + \phi_{14} + \phi_{12} + \phi_{124}$
22	4231	$\phi_1 + \phi_{12} + \phi_{13} + \phi_{14} + \phi_{123} + \phi_{124} + \phi_{134}$
23	4312	$\phi_1 + \phi_{14} + \phi_{13} + \phi_{134}$
24	4321	$\phi_1 + \phi_{12} + \phi_{13} + \phi_{14} + \phi_{123} + \phi_{124} + \phi_{134}$
Average (U_1)		$\phi_1 + \dfrac{1}{2}\left(\phi_{12} + \phi_{13} + \phi_{14}\right) + \dfrac{1}{3}\left(\phi_{123} + \phi_{124} + \phi_{134}\right)$

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