The strange world of short-range potentials

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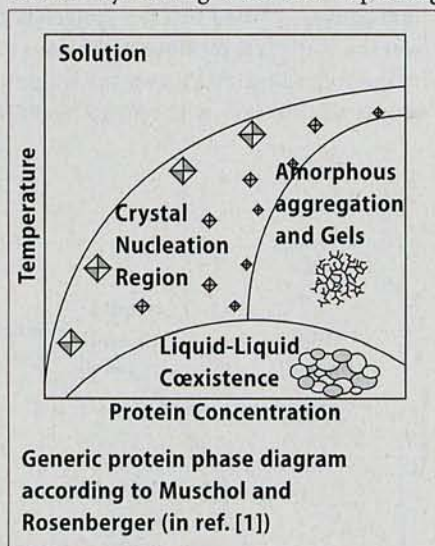
hot topic Protein Crystallization

*T*he English poet Rupert Brooke wrote: "And sure, the reverent eye must see a purpose in Liquidity". Since we all owe our existence to the occurrence of the liquid state, we must agree with this sentence. But is this little pocket we call liquid an unavoidable feature of the phase diagram? Or can a thermodynamic system display just two stable phases, namely a solid and a single fluid phase? Apparently this is possible, provided that the range of the attractive part of the interaction potential is much shorter than the particle hard core diameter.

For such a potential, the critical point "sinks" within the fluid-crystal coexistence region, and the gas-liquid transition becomes metastable with respect to crystallization. This model can hardly find a physical realization in simple fluids, where the range of the potential is always comparable to the molecular size, but is verified experimentally in colloidal dispersions. Light scattering measurements show that both the phase diagram and the osmotic compressibility of a protein solution prone to crystallisation conform to those expected for very short-range attractive potentials.

Indeed, a phase separation into two fluid-like phases is often seen to come ahead of crystallisation for protein solutions

brought into the crystal nucleation region, while above the metastable critical point large fluctuations of concentration can considerably speed up the crystal growth kinetics. Moreover, for strong supersaturation, protein crystallization is often hindered by the formation of long-live metastable amorphous aggregates, another typical signature of very short-range potentials (see *figure*). However, the microscopic origin of such a short range potential is still unclear. Proteins are often crystallized by adding salts and exploiting

Generic protein phase diagram according to Muschol and Rosenberger (in ref. [1])

what is called the "salting-out" effect. But the amount of salt needed to trigger crystal nucleation greatly exceeds what is required for simply screening the electrostatic interactions due to protein surface charges. Moreover, the salting-out effect is strongly salt-specific. A well-defined scale of efficiency for different electrolytes in precipitating proteins was established more than a century ago. This scale, known as the "Hofmeister series", also controls many other thermodynamic properties of aqueous electrolyte solutions, like the surface tension or the critical micellar concentration of solubilized surfactants. Neutron scattering measurements by Leberman and Soper suggest that salts induce strong changes in the solvent structure like those induced by a pressure jump, but so far no microscopic quantitative model is available for these effects. Since obtaining protein crystals is a fundamental step in order to derive protein structure and to elucidate their biochemical role, further progress along these lines could shed additional light on that beautiful condensed matter system we call life.

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Further reading
A collection of recent papers relating to protein phase behaviour and crystallization can be found in *Journal of Crystal Growth* 196 (1998) • Protein crystallization methods are discussed in Crystallisation of Nucleic Acids and Proteins: A Practical Approach edited by A. Ducruix and R. Giegè (Oxford University Press, Oxford, 1992) • For a review of the Hofmeister series see K. D. Collins and M. W. Washabaugh *Quarterly Review of Biophysics* 18 323 (1985)

Counting the molecules

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hot topic Theories of Freezing

*I*t seems easy to distinguish fluids from solids, but their microscopic similarity is the main difficulty in the study of freezing. Theoretically, liquids are characterized by their density and short-ranged correlation structure. However, these aspects do not show a clear contrast in relation to crystals. The qualitative difference comes from the long-ranged molecular order, which produces rigidity and other macroscopic characteristics of crystals— but it is difficult to include its effects in the relative thermodynamic stability of fluids and solids. Hard spheres are the simplest representation of freezing and

exposes its subtleties: the model is purely entropic (allowed configurations have no potential energy) so that it freezes when the entropy in the "ordered" crystal becomes larger than in the "disordered" fluid, contrary to the usual view of entropy as a measure of "disorder". How do we calculate this entropy?

The answer comes from the density functional techniques developed for inhomogeneous fluids in external potentials. Crystalline order implies that the effects of boundaries extend over the whole system, while in fluids they disappear over microscopic distances. Thus, crystals may be de-

scribed as self-maintained inhomogeneous fluids, with density distributions consisting of narrow peaks arranged in regular lattices, fixed by distant boundaries. The development of density functional approximations for hard spheres during the 1980s, was rewarded with accurate predictions for their freezing, but only within a variational use of the formalism, with the crystal density distribution restricted to normalized peaks, describing the positions of each molecule. However, the full minimization of the free energy gave much poorer results, with wrong unit cell occupation. The approximations where good enough to calculate the entropy of inhomogeneous density distributions, but not to see the molecular discreteness.

This problem has been solved by recent advances within fundamental measure theory, a geometrical approach based directly on the molecular shape, rather than on the usual excluded volume (which for