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Table of Contents

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EDITORIAL

Nanobubbles are not a Superficial Matter

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1. Introduction

*I*t has become almost a cliché now in the study of ecosystems to say that boundaries—estuaries, hedgerows, forest margins—are the locations of greatest richness and diversity. The same is true in physical chemistry: where different phases or substances meet, new things are possible. Until recently that message has tended to be neglected. Of course, it is impossible to ignore the fact that, even for interfaces modelled as step-like discontinuities—where a bulk-like liquid changes to a bulk-like solid, say—merely the breaking of symmetry in the normal direction creates surface-specific phenomena such as interfacial tension. And the excess free energy at an interface makes it a locus for further heterogeneity: gases absorb, perhaps forming monolayers and wetting films. Surfaces may sequester impurities from a fluid phase. They are where corrosion or catalysis or dissolution takes place. In short, surfaces and interfaces seem to act as agents of their own complication. One must wonder whether, had the early pioneers of surface science been confronted with the full complexity and heterogeneity of interfaces now laid bare by microscopic probes, rather than being at liberty to approximate them as smooth, uniform slabs, they might not have abandoned their program in despair at its intractability. Wolfgang Pauli famously attested to the challenging nature of the problem when he asserted that surfaces are the work of the devil.

The more closely we look, the greater this complication at interfaces appears to be. Liquids at solid surfaces abandon their bulk-like character. They may take on a layered structure, a consequence of purely geometric packing effects but modified by the short-range attractive component of intermolecular forces.[1,2] This ordering may acquire a lateral component too: the liquid starts to look solid-like, and there is good reason to believe that properties such as viscosity may be changed by orders of magnitude by confinement between solid surfaces.[3,4] On the other hand, incompatibility between a liquid and an adjacent solid—water against a hydrophobic surface, say—can lead to a depletion in the density of the fluid phase at the interface.[5–13] These deviations from bulk properties can exacerbate the chemical inhomogeneities of interfaces, altering for example the dielectric or solvation characteristics there.[14] Furthermore, ions may be depleted or enriched at the solid–water or air–water interfaces relative to the bulk,[15–21] an effect that involves subtle balances of enthalpic and entropic factors. Even so apparently simple a question as whether the air-water interface is acidic or basic is still fiercely contested.[22–29]

All this is of far more than just academic interest. Interfaces, and in particular the solvation processes associated with them, have long been recognized as central to chemical sciences with strong applied relevance, such as electrochemistry, catalysis and corrosion. But there has been no systematic, unified effort to understand these phenomena. Research has tended to proceed in a somewhat piecemeal and semi-empirical manner that fails to acknowledge the full extent of heterogeneity and the subtle influences that interfaces may have on the structure of a solvent and, in consequence, its solvating behaviour. With the advent of sophisticated techniques for probing and modelling these complex systems at the nanoscale and molecular scale, it seems likely that solvation science will emerge as a major frontier bridging diverse disciplines such as electrochemistry, biochemistry and green chemistry.

This perspective is perhaps most relevant of all to molecular and cell biology. The explosion of interest in the solvation of proteins[30–36]—and as a consequence, the recognition of how intimately and exquisitely the structure and dynamics of proteins and their aqueous environment seem to be linked—attests to the overdue acknowledgement that biomolecular interactions cannot be fully understood without a far more sophisticated picture of their interfacial behaviour. This applies equally to cell membranes, nucleic acids and other biological structures. There is an urgent need to understand how biologically relevant behaviour is mediated and influenced by the heterogeneities and dynamic restructuring of these interfaces over a wide range of length scales.

2. The Problem with Nanobubbles

*I*t was with these considerations in mind that a workshop on "Nanobubbles at Biological Interfaces" was convened in May 2011 by the Washington-based biotechnology company Revalesio, who suspect that nanobubbles might lie behind some of their recent findings. Given that the basic phenomenology of nanobubbles, even down to the issue of their existence, remains unclear and in many ways controversial,[37,38] it might seem premature to be asking what they imply for biological systems. However, recent studies of biological interfaces have made that question impossible to avoid.

In brief, gas-filled nanoscale bubbles have been identified on a variety of solid surfaces,[39–45] and are attributed to the heterogeneous nucleation of dissolved gas (Figure 1). These nanobubbles appear to be stable for very long times, of the order of days to months. Yet as Seddon et al. explain in this issue,[46] such bubbles should not exist at all, according to the classical view of the air–water interface, since their small radius of curvature implies a high Laplace pressure inside the bubble that should drive gas diffusion across the interface and cause the bubbles to dissolve almost instantly. If the structures seen
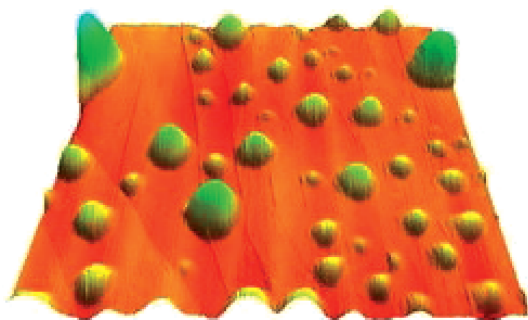
Nanobubbles

Figure 1. Nanobubbles on a hydrophobic surface as revealed by an AFM. Image courtesy of Xuehua Zhang, University of Melbourne.

in microscopy truly do correspond to bubbles filled with bulk-like gas—and this is still not universally accepted[47]—then evidently something is amiss with this reasoning. Explanations based on thermodynamics,[48,49] kinetics,[50,51] or both,[52] have been put forward, but there is still no consensus theory of nanobubble stability. They represent an awkward but conspicuous instance of "surface misbehaviour".[53]

Since physiological fluids carry significant amounts of dissolved gases such as oxygen and carbon dioxide, the hypothesis that nanobubbles in water are created and sustained by the nucleation of gases from the solvated phase at hydrophobic surfaces would seem to imply that they should be common in biological systems. Most proteins have an appreciable surface area that is hydrophobic, and moreover such regions might be especially liable to nucleate and stabilize bubbles if they are concave. In addition, supersaturation of gas is not a requirement for nanobubble formation, and there seems to be a temperature threshold for bubble nucleation in aerated water (at least on hydrophobized silicon) that is slightly below physiological temperature, with optimal nucleation more or less at this temperature.[54,55]

Their alleged physical stability means that, as Zimmerman et al. recently put it, "nanobubbles, once formed, are highly persistent".[56] If they do appear in a system, they are hard to remove. And some recent findings suggest that "impure" aqueous solutions are likely to contain abundant nanobubbles: Ohgaki et al. claim that "almost no gas samples are dissolved

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homogeneously in the aqueous solution and the vast majority is present in the form of nanobubbles".[57] If this is true (and as yet the "if" remains), it would be more of a puzzle if nanobubbles were *not* present inside living cells.

3. Do Nanobubbles Matter in Biology?

*I*f they are, such bubbles might be expected to affect not only the self-assembly and aggregation of proteins but their functional interactions—that is to say, the interactome that characterizes the network of gene interdependencies.[58] Nanobubbles were first identified in studies of the long-range hydrophobic interaction: an attractive force between hydrophobic surfaces with an inordinately long reach of up to 300 nm or so, well beyond what could plausibly be explained by van der Waals forces.[59,60] It was suggested by Parker et al. that this attraction might be caused by nanobubbles bridging the surfaces, which are then pulled together by the menisci.[61] This now seems indeed a likely origin of the long-range attraction,[53] in which case nanobubbles on proteins could act to cause aggregation even when the macromolecules are apparently well separated.

Cavitation-induced attraction via a so-called dewetting transition has been postulated over shorter (several nm) distances as a mechanism for the attraction of large hydrophobes[62] and, in consequence, as a contribution to the folding of polypeptides into their native state. This effect has been seen in the aggregation of simple hydrophobic surfaces and particles,[63,64] the collapse of hydrophobic polymers[65] and the aggregation of protein subunits.[66] The formation of gas-like dry voids between hydrophobic surfaces in these cases has so far been discussed as a form of capillary evaporation that draws on the intrinsic density fluctuations in the liquid state.[67] But the presence of dissolved gas ought to exacerbate such an effect by nucleating the voids, and the diminution of the long-range hydrophobic attraction in degassed solutions[53,68] adds support to that idea. Although its general relevance to protein folding and self-assembly remains under debate,[69–71] this burgeoning topic of research already establishes a conceptual link to the possible influence of nanobubbles.

Whether or not dewetting transitions assist protein folding and aggregation, there seems to be strong reason to suspect that they play a part in the gating of protein pores. Some ion channels contain narrow hydrophobic constrictions in their interior passage, where small changes in conformation can trigger a switch from a filled (wet) state to an empty (dry) state. Without water filling the channel, ion motion through the pore may be blocked by the free energy penalty of stripping away the ion's hydration sphere.[72] Such drying-induced gating has been proposed, for example, for potassium channels[73,74] and mechanosensitive ion channels.[75,76] A nanobubble that nucleates in or migrates to the pore neck might similarly gate the pore. Such a mechanism has been proposed as an explanation of the anaesthetic action of rare gases such as xenon.[72]

Nanobubbles are expected to sequester impurities at their surfaces, which might stabilize the bubble by hindering gas

diffusion.[52] If so, it seems almost inevitable that nanobubbles in biological media will acquire a heterogeneous surface mediated by surfactant-like molecules such as lipids. Other explanations for the puzzling stability of nanobubbles invoke the possibility that they are sealed by unusually strong hydrogen bonds that again reduce gas diffusion.[57] This is a notion that appeals to old ideas about hydrophobic particles creating a highly ordered, ice-like hydration shell,[77,78] or high-charge-density ions acting as "structure-makers" in aqueous solution,[79] which now look increasingly hard to sustain.[80–82] If, however, there is indeed any change in the hydrogen-bonding around nanobubbles, they might be expected to affect the hydration of nearby macromolecules by competing for solvation water.

Nanobubbles should thus induce partitioning of solutes—a fact already exploited in the use of surfactant-stabilized microbubbles (aphrons[83]) for extraction techniques. The proteins lactoferrin and lactoperoxidase may be separated from sweet whey in this manner, for example.[84] This suggests that, if nanobubbles can be reliably produced in large numbers, they might have valuable biotechnological applications. On the other hand, such sequestering in biological systems might be harmful if it happens in an uncontrolled way.

Solute adsorption onto and enrichment at the interfaces of microbubbles and aphrons can be electrostatically driven.[84,85] Given the tendency of ions to show either enhanced or reduced concentrations at the air–water interface,[15–21] it seems likely that nanobubbles will acquire a surface charge, which will then influence the energetics of their potential interactions with biological surfaces, just as is the case for nanoparticles.[86–89] The presence of nanobubbles at surfaces may also have hydrodynamic implications, especially if there is focused gas flow out of the bubble apex.[51]

These considerations raise two questions. First, if nanobubbles are already present, perhaps even common, in biological systems, have these systems adapted to exploit them to advantage, or at least to avoid possible deleterious effects? Second, regardless of whether or not there is a "natural biology of nanobubbles", might we introduce them by design for biomedical and technological purposes? Some indication that the latter might be possible was provided by Wagner et al., who reported that the generation of nanobubbles around gold nanoparticles using laser pulses could selectively both identify and ablate cancer cells xenografted into zebrafish.[90] Here the nanobubbles appear to exert their effect by "brute force", releasing energy during growth and collapse that mechanically disrupts the cells. And Rapoport et al. have used co-polymer-stabilized micro- and nanobubbles as vectors for the anticancer drug doxorubicin, injecting them into tumours where the bubbles were burst using ultrasound.[91] Delivery of drugs across the blood-brain barrier has been enhanced by the disruptive effect of ultrasound-induced microbubbles.[92,93]

These are tentative first steps which draw hardly at all on the emerging understanding of the nature and properties of nanobubbles, but which perhaps establish the possibility of achieving much more by rational design. As well as using nanobubbles for controlled transport and release of drugs, one might imagine for example applications in the delivery and storage of gases—as Pan and Yang report in this issue, oxygen nanobubbles stabilized on porous mineral particles might act as gas delivery vehicles for addressing the environmental problems of eutrophication and resulting anoxia in natural waters.[94]

4. How Ubiquitous are they?

Now that the existence of nanobubbles at surfaces seems rather well attested (if not universally accepted), it seems timely to confront the still more controversial question of whether they may exist in bulk solution. There is not yet any direct and convincing evidence for this, partly because—in contrast to the case for surfaces—there is no experimental technique that seems well suited to their unambiguous detection. However, there are highly suggestive hints. For example, Jin et al. have reported depletion forces between two surfaces apparently induced by the exclusion of bulk, *charged* nanobubbles from the intervening gap.[95] And Ohgaki et al. have presented freeze-fracture images of bulk liquids that apparently retain an imprint of nanobubbles containing nitrogen, methane and argon.[57] They estimated that there are around 1.9×10^{16} nanobubbles per dm^3 of water.

Some of the mechanisms proposed to explain how nanobubbles are formed and sustained rely on the active participation of a solid phase, for example in playing the role of sequestering an adsorbed layer of gas molecules to feed a dynamic nanobubble state and counterbalance dissolution.[50,51] But purely thermodynamic arguments for nanobubble stability cannot invoke the influence of the dispersion forces of a surface, since these have far too small a range to stabilize a bubble whose apex is several nanometres from the wall. One proposed mechanism by which bulk nanobubbles might be rendered dynamically stable invokes a mutual shielding against the diffusive outflow of gas if such bubbles are sufficiently close together.[96]

The notion that nanobubbles might complicate the bulk phase too, making it an inhomogeneous colloidal state, has implications for several current areas of research in cell biology, such as the effect of molecular crowding,[97–99] the formation of macromolecular aggregates[100,101] and anomalous rates of molecular diffusion in the cytoplasm.[102,103]

It is too early to make any definitive claims about how nanobubbles might affect biology at the molecular and mesoscale, either by accident or design. But as the following papers hint, there is a compelling case to start examining that issue. This may turn out to be another phenomenon that challenges the picture of the cell's fluid phase as a simply a passive solvent in which the active agents are solvated, forcing us to consider it instead as a truly complex and participatory fluid.

Nanobubbles

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