librational modes) and thus changing how the sub-surface vibration couples with the surroundings. Common methods of optimizing the water-photolysis reaction conditions involve altering both the underlying semiconductor (through doping) and the electrolyte composition. This new vibrational mode could help to better understand the process on the molecular level, rather than relying on combinatorial methods and exploring an ever expanding parameter space.

The observed vibrational mode is rather unique and truly interfacial in that it is localized in the sub-surface and couples to both bulk environments that it is in contact with: the hard semiconductor and the soft aqueous layer. While Fano resonances have been observed before for vibrations of molecules that are bound to a surface and coupled to the electronic states of a substrate, this is the first observation of a vibrational-mode coupling to the quasi-continuum of vibrational or librational modes of a solvent. The identification of such a distinct interfacial sub-surface vibration is quite remarkable.

Not only is it remarkable, it could also be of great importance because monitoring chemical processes at interfaces is generally difficult. The interfacial region represents a very small fraction of any macroscopic sample, which makes singling out the interfacial molecules from the bulk experimentally challenging. Nonlinear optical experiments in the form of sum frequency generation spectroscopy can specifically probe surface vibrational modes while rejecting the large background of bulk vibrations due to the break of inversion symmetry at the surface[8]. However, such surface-specific experiments impose further restrictions on the vibrational modes and the kind of samples that can be studied. In contrast, the vibrational mode discovered by Cuk and co-workers is inherently interfacial: critically it exists only at the interface with no bulk equivalent. Accordingly, the vibration and associated dynamics can be followed with time-resolved reflection spectroscopy without having to turn to nonlinear optical experiments.

Because the oxyl-radical vibration couples to both the semiconductor electronic states responsible for driving the photocatalysis and the interfacial water librational modes directly involved in the water-splitting reaction, the new vibrational mode offers a window into the mechanism of and driving force for water photolysis. Cuk and co-workers observe that the vibration occurs within picoseconds of UV excitation and decays on the nanosecond time scale (subject to the reaction conditions). More generally, the unique vibrational mode could prove to be a new way of tracking the different steps of catalytic water-splitting: the exciton formation leading to the creation of the oxyl radical, followed by its coupling to the water librational dynamics inherently involved in the water-splitting reaction, and finally the reaction of the oxyl radical to form an O–O bond that ultimately leads to gaseous O_2 evolution. Although there are still plenty of challenges facing water photolysis for sating global energy consumption — such as long term stability of the cell components, regeneration, and supressing recombination — scientists now have a new tool to monitor the reaction at the molecular level providing detailed mechanistic insight into this important process. ❐

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MEMBRANELESS ORGANELLES

Phasing in and out

The low-complexity-protein, liquid phases of membraneless organelles have now been established to selectively partition biomolecules. The specialized microenvironment that they provide differs chemically from the surrounding medium and enables specific nucleic-acid remodelling reactions.

James Shorter

Eukaryotic cells contain a series of membrane-encapsulated organelles, which provide an interdependent network of discrete microenvironments with diverse physicochemical properties. The interior of each of these organelles is precisely regulated and tailored to facilitate a series of specific biochemical reactions. In addition, cells harbour various membraneless organelles, including nucleoli and Cajal bodies, as well as diverse ribonucleoprotein particle granules, such as stress granules and processing bodies[1]. These membraneless compartments resemble liquid droplets in a separate phase to the surrounding medium, and exhibit dynamic behaviour, such as rapid biogenesis and disassembly, in response to environmental cues[1].

A flurry of recent work suggests that membraneless organelles originate via liquid–liquid phase separation events driven by low-complexity domains of resident proteins[1,2]. The interior of membraneless organelles comprises a liquid-protein phase maintained by weak, multivalent interactions[1,2]. These protein liquids likely provide solvent conditions that are chemically different to the surrounding aqueous cytoplasm or nucleoplasm. However, it has been unclear whether these protein-liquid phases confer any physicochemical advantages for the biochemical reactions that might occur inside the membraneless organelle or whether they are merely inert scaffolds that enable spatiotemporal organization. Now, writing in *Nature Chemistry*, a team of Timothy Nott, Timothy Craggs and Andrew Baldwin have established that in addition to providing spatiotemporal organization, these proteinaceous liquids create a physicochemical microenvironment that selectively partitions biomolecules and simultaneously promotes specific nucleic-acid remodelling events, such as destabilization of double-stranded structures and stabilization of hairpin structures[3].

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The team focused on nuage granules (also called chromatoid bodies), which are membraneless organelles that host components of an RNA interference pathway that safeguards spermatocytes against deleterious transposable elements[4]. A primary component of nuage granules is Ddx4, a DEAD-box RNA helicase[4]. The disordered, N-terminal low-complexity domain of Ddx4, termed Ddx4^{N1}, undergoes a liquid-phase transition that drives assembly of nuage granules[4]. Ddx4^{N1} is enriched in glycine (18.4%), serine (13.9%), arginine (9.8%) and asparagine (8.2%) residues[3], and harbours di-RG and tri-RG motifs[5]. Baldwin and co-workers demonstrated that in the test tube, pure Ddx4^{N1} liquid droplets provide a microenvironment akin to an organic solvent, which sequesters and concentrates short single-stranded RNAs and DNAs (Fig. 1)[3]. Similar behaviour was also observed in synthetic liquid droplets formed by disordered portions of three other RNA-binding proteins: EWSR1, Ddx3x and EIF4H (ref. 3).

Short, regulatory RNAs, such as microRNA, short interfering RNA and Piwi-interacting RNA (piRNA), adopt hairpin conformations containing double-stranded (stem) and single-stranded (loop) regions[3]. Remarkably, RNA hairpins and natural piRNAs were partitioned to the interior of Ddx4^{N1} liquid droplets more effectively than unstructured RNAs of similar length[3] (Fig. 1). Furthermore, the secondary structure of hairpin RNAs was stabilized inside the Ddx4^{N1} liquid phase, indicating that the crowded droplet interior favours compact oligonucleotide structures[3]. A key function of nuage granules *in vivo* — the concentration of specific short RNA hairpins — was therefore reconstituted *in vitro* using pure Ddx4^{N1} liquid droplets[3]. In contrast, rigid double-stranded DNA, RNA, and hybrid RNA–DNA duplexes of 20 bp or longer were largely excluded from Ddx4^{N1} liquid droplets[3] (Fig. 1). In a surprising result, the team also showed that double-stranded nucleic acids were converted to single-stranded forms upon entering the Ddx4^{N1} droplet[3] (Fig. 1). This unwinding and separation of double-stranded DNA and RNA is typically catalysed by specific ATP-dependent helicases; however, Ddx4^{N1} lacks the DEAD-box helicase domain, which couples ATP hydrolysis to RNA helicase activity. This finding establishes that the interior of membraneless organelles can function as a passive helicase. The specialized microenvironment is also likely to be more conducive for various nucleic acid remodelling, cleavage, and polymerization reactions that are catalysed by enzymes.

In another important experiment, Baldwin and co-workers showed that the partitioning of proteins into Ddx4^{N1} liquid droplets depends on the identity and properties of the protein[3] (Fig. 1). Fluorescently tagged versions of proteins that are known to be present in nuage granules — such as full-length Ddx4 and PiwiL1 — were readily absorbed; whereas the small heat-shock proteins Hsp16.5 and αB-crystallin, which are not known to reside in such membraneless organelles, were excluded[3]. Protein absorption was not readily predicted by molecular weight or isoelectric point, but could be predicted by amino acid composition[3]. Proteins rich in arginine and tyrosine were readily absorbed. Crucially, proteins that selectively partitioned into the Ddx4^{N1} liquid droplets could also import and unwind double-stranded DNA that would otherwise be excluded[3]. This finding illustrates that low-complexity liquid phases can function as biomolecular filters that alter the structural properties of retained nucleic acids.

So, how does the Ddx4^{N1} liquid phase promote this specific biochemistry? Ddx4^{N1} liquid phases are maintained by weak, multivalent electrostatic and cation–π interactions that involve predominantly arginine and phenylalanine residues[4]. Thus, single-stranded nucleic acids are absorbed by transiently engaging arginine side chains, whereas double-stranded nucleic acids are destabilized and unwound via similar interactions with arginine side chains in the liquid phase[3]. In contrast, compact nucleic acid hairpins would cause a minimal disruption of the liquid phase and become stabilized via favourable interactions[3]. Finally, proteins that partitioned into the Ddx4^{N1} liquids were rich in arginine and tyrosine residues. These amino acids are compatible with the weak, multivalent electrostatic and cation–π interactions that maintain the liquid[3]. The Ddx4^{N1} liquids therefore provide a unique biochemical milieu with properties that are chemically distinct from those of bulk aqueous solvent (Fig. 1).

Many proteins harbour low-complexity domains that can drive liquid–liquid phase separation events[1–3,6]. However, these domains can have very different amino acid compositions. For example, prion-like domains are a type of low-complexity domain enriched in glycine and uncharged polar amino acids, such as glutamine, asparagine and tyrosine, but have a much lower proportion of arginine residues compared with the Ddx4 N-terminal low-complexity domain[6]. Prion-like domains drive liquid droplet formation by FUS and hnRNPA1 (ref. 6), but due to the distinct amino acid composition of the prion-like domains, the interior of these structures are likely to provide a different microenvironment to that of Ddx4^{N1} liquids. By extension, cells may form diverse membraneless organelles using low-complexity domains with highly distinctive amino acid compositions, which in turn could engender liquid phases with biochemical properties tailored to favour particular functional reactions or interactions between specific partners.

Finally, the formation of liquid phases by prion-like domains and perhaps

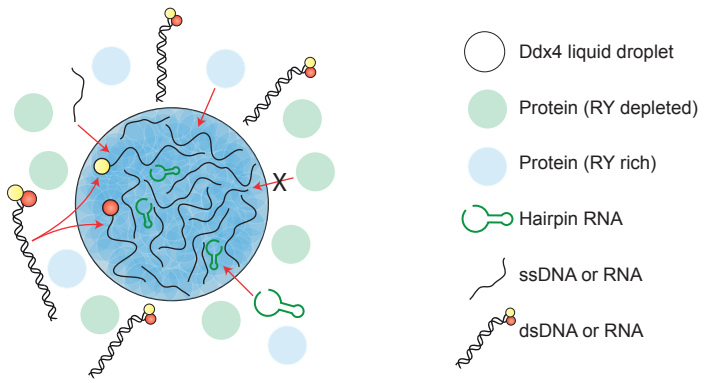

Figure 1 | Ddx4^{N1} liquid droplets selectively partition biomolecules and can alter nucleic acid structure. Ddx4^{N1} liquid droplets form a specialized microcompartment that selectively partitions biomolecules and dictates specific aspects of nucleic acid biochemistry. DNA or RNA double helices are melted, single-stranded DNA or RNA is readily absorbed, hairpin structures formed by regulatory single-stranded nucleic acids are absorbed and stabilized, and proteins are absorbed (if RY rich) or excluded (if RY depleted) according to their sequence. The Ddx4^{N1} liquid droplet is therefore a stringent sorting device with an interior that imposes biochemical rules distinct from the surrounding aqueous solution. Figure adapted from ref. 3, NPG.

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other low-complexity domains increases the risk that these domains may access self-templating, pathological fibrils that underpin fatal neurodegenerative diseases such as amyotrophic lateral sclerosis[2,6,7]. Indeed, single missense mutations in prion-like domains can accelerate the transition from liquid states to pathological fibrils and are an established cause of neurodegenerative disease[2,6,7]. Baldwin and co-workers establish that the C-terminal portion of EWSR1 (residues 451–656), which harbours two tri-RGG motifs, a zinc-finger domain and a tri-RG motif[5], and is RG-rich like the Ddx4^{N1} low-complexity domain, readily forms liquid droplets[3]. Intriguingly, several mutations in this C-terminal region of EWSR1 (which does not contain the N-terminal prion-like domain) have been connected to amyotrophic lateral sclerosis[8]. Thus, it will be of great interest to determine whether these mutations accelerate the maturation of EWSR1 liquids into pathological fibrils[8]. ❐

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