CHEMISTRY

Supercooled water reveals its secrets

Experiments provide evidence for two liquid phases in supercooled water droplets

By **Paola Gallo**[1] *and* **H. Eugene Stanley**[2]

When a substance remains liquid below its melting point, it is said to be in a metastable supercooled state. In the region where the substance can be supercooled, the crystal is still the stable state, but crystallization can be avoided if the cooling occurs fast enough. The supercooled phase diagram of water has received particular attention (*1*). The anomalous thermodynamic properties of water point to the possible existence of two different liquid phases—one with high density and the other with low density—that become identical at a liquid-liquid critical point in the supercooled phase (C′, see the figure). But whereas mild supercooling of water is moderately easy to achieve, the deeply supercooled region has been out of the reach of experiments. On page 1589 of this issue, Kim *et al.* (*2*) use an evaporative cooling technique to cool micrometer-sized water droplets to deeply supercooled temperatures and provide evidence for the postulated critical point.

It was 25 years ago that Poole *et al.* (*3*) proposed, on the basis of computer simulations, the existence of two coexisting forms of liquid water—a high-density liquid (HDL) and a low-density liquid (LDL)—with a well-defined coexistence line in the pressure-temperature plane terminating in a second liquid-liquid critical point in the supercooled state. The second-order nature of this liquid-liquid critical point has been demonstrated in simulations, and the border between HDL and LDL has been shown to be a coexistence line separating the two phases (*4*).

This hypothesis is consistent with the experimental fact that thermodynamic quantities such as the isothermal compressibility, the isobaric specific heat, and the coefficient of thermal expansion display anomalous increases upon cooling and that, at low temperature, two forms of amorphous solid water exist: one with high density and one with low density.

Several potentials for water show a liquid-liquid critical point and a Widom line above the critical point (*5*, *6*). To understand the meaning of the Widom line, we must first consider a fundamental property of liquids. Below the critical point, the two phases (gas and liquid, or HDL and LDL) are well separated, with a coexistence line that marks the sharp change from one phase to another (see the figure). Above the critical point, these phases merge into a single phase. If a substance is in this one-phase region and gets close enough to the critical point, bubbles of one phase start to form inside the other, giving rise to strong density fluctuations. Because of these fluctuations, in the one-phase region, thermodynamic quantities such as the isothermal compressibility (which is proportional to the mean square density fluctuations) display maxima that merge on a single line terminating at the critical point. This line is made by the maxima of the correlation length, a quantity that measures the maximum distance for which the fluctuations in two regions of the space of interest are correlated. The correlation length diverges at the critical point.

It is this line that is called the Widom line, named after the chemist Benjamin Widom, who emphasized that one cannot take data at the critical point, but only nearby. To recognize the critical point, one can detect the locus of maxima of correlation length by measuring the locus of maxima of a quantity, such as compressibility, and then extrapolate to the location of the critical point. A useful metaphor might be to imagine locating Mount Everest when its top is cloud-covered. If we are close enough and find a path that is the local maximum, for example, a ridge, and follow this path upward, we will move toward the summit.

In water, the Widom line exists in the one-phase region above the liquid-gas critical point (*7*) (see the figure), but no convincing experimental evidence had been reported to date for a Widom line in supercooled water. Xu *et al.* (*5*) have argued that if such evidence were found, it would point to the existence of the hypothesized liquid-liquid transition terminating in a liquid-liquid critical point. The Widom line is also connected to dynamics, because the diffusive behavior changes upon crossing the Widom line (*5*, *7*, *8*).

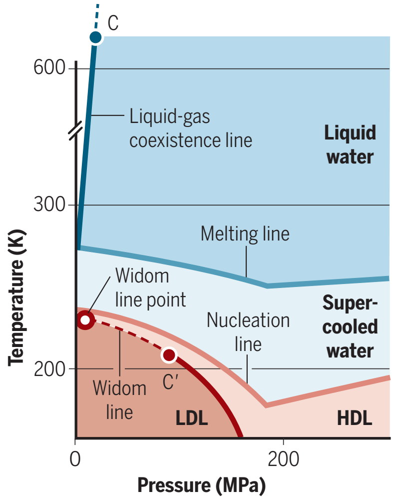
Extensive computer simulations for a wide range of models of liquid water largely confirm the picture from the initial studies. The nature of the two phases, the LDL and

When water goes supercooled

Scientists have long predicted the existence of two different liquid states of supercooled water. Kim *et al*. now provide experimental evidence for their existence.

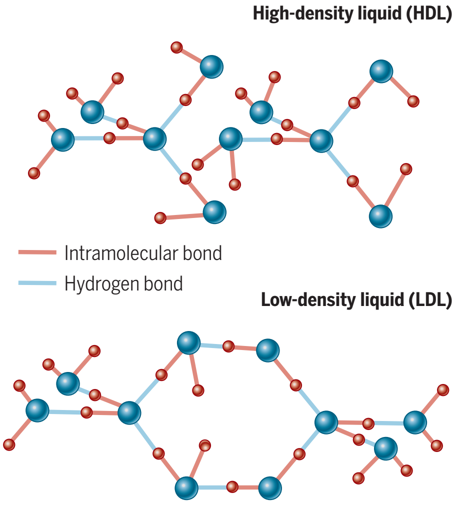
Experimental confirmation

Kim *et al.* provide experimental evidence for the Widom line and, hence, of a second-order critical point in the supercooled region of the water phase diagram.



Two forms of liquid

Clusters of water molecules approach each other in fundamentally different ways in the two forms of supercooled water.



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[1]*Dipartimento di Matematica e Fisica, Università Roma Tre, Via della Vasca Navale 84, 00146 Roma, Italy.* [2]*Physics Department and Center for Polymer Studies, Boston University, Boston, MA 02215, USA. Email: gallop@fis.uniroma3.it*

HDL, continues to be probed by sophisticated experiments and detailed simulations.

Kim *et al.* now present experimental data for a region of the supercooled-water phase diagram that was not accessible to experiments before. The authors cooled their droplets down to 227.7 K for water (H_2O) and 232.5 K for heavy water (D_2O). These two temperatures are below the temperatures of homogeneous nucleation for the respective liquids. By probing the droplets with femtosecond x-ray scattering, the authors measured both the structure and the maxima in isothermal compressibility and correlation length. Because a maximum in the correlation length is a feature of the Widom line, they unambiguously prove the existence of this line and, hence, of a second-order critical point. These results not only pave the way for the full exploration of the forbidden region of supercooled water, but are also important for fields such as cryopreservation, astrobiology, and the food industry where crystallization must be avoided.

The basis for the liquid-liquid transition in water lies in its intermolecular interactions, which are distinguished by two characteristic length scales of interaction. Two pentamers—that is, the tetrahedral structure formed by a water molecule and its four neighbors—can approach each other in two distinct fashions: a "handshake" configuration, where two corners of one pentamer approach two corners of another pentamer, and a "tango" configuration, where one pentamer is rotated 90° with respect to the other (see the figure). This reasoning suggests the possibility that a liquid-liquid phase transition might also occur in other liquids with local tetrahedral structure. This interesting idea is being explored in simulations and experiments on a number of liquids, including the common materials silicon and silicon dioxide (*9*–*12*) and colloids (*13*). ■

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PLANT BIOLOGY

Complex regulation of plant sex by peptides

How the pollen tube is regulated to deliver sperm cells to produce seeds is characterized

By **Martin Stegmann** *and* **Cyril Zipfel**

Most of our food—whether fruit or grain—is the direct result of flowering plant fertilization. As this also underlies the transmission of genetic information over generations, it is essential to understand the molecular events controlling plant reproduction. During fertilization, pollen (the male gametophyte, or reproductive cell) forms a pollen tube which is guided toward the ovule (the female gametophyte), where, upon bursting, it will deliver its sperm cells (*1*). All steps—from the perception of compatible pollens by the flower pistil (the female reproductive organ) to pollen tube growth, guidance and, ultimately, rupture—are controlled by a complex molecular dialogue involving numerous plant receptor kinases and endogenous signaling molecules, including secreted peptides (*2*). On pages 1596 and 1600 of this issue, Ge *et al.* (*3*) and Mecchia *et al.* (*4*), respectively, characterize signaling events between the male and female gametophytes, which offer exciting insights into the molecular mechanisms controlling plant fertilization and seed setting.

> ***"...these studies have far-reaching implications beyond plant reproduction."***

The *Catharanthus roseus* RLK1-like (CrRLK1L) receptor kinases (of which there are 17 in the model plant *Arabidopsis thaliana*) play critical roles in the interaction between the pollen and the ovule where the pollen will release sperm cells, thus allowing fertilization and subsequent seed development (*5*). The founding member of this receptor family, FERONIA (FER), is involved in numerous processes including fertilization, immunity, and growth, and endogenous RAPID ALKALINIZATION FACTOR (RALF) peptides are its ligands (*6*, *7*). RALF peptides (of which there are at least 36 in *A. thaliana*) may also be ligands for other CrRLK1L family members. The FER paralogs ANXUR1 (ANX1) and ANX2 are key regulators of pollen tube growth and integrity in *A. thaliana* (*8*, *9*). However, no ligand had been reported for them until now.

Based on gene expression profiles, Ge *et al.* and Mecchia *et al.* identified *RALF4* and *RALF19* as being preferentially expressed in mature pollen grains and tubes (*3*, *4*). Notably, simultaneous loss-of-function mutations of *RALF4* and *RALF19* led to male-specific fertilization defects associated with premature pollen tube bursting (*3*, *4*)—a phenotype also observed in *anx1* and *anx2* double mutants (*8*–*10*). Interestingly, genetic analyses indicated that ANX1 functions upstream of RALF4 and RALF19 (*4*). Consistently, Ge *et al.* showed that RALF4 and RALF19 directly bind to ANX1 and ANX2 ectodomains (the region of the receptors in the extracellular space) with high, nanomolar affinities (*3*), strongly implicating RALF4 and RALF19 as ligands for ANX1 and ANX2 in an autocrine (i.e., signals from the same cell type) manner to control pollen tube integrity so that it does not burst prematurely.

Surprisingly, Ge *et al.* identified two additional CrRLK1Ls, BUDDHA'S PAPER SEAL 1 (BUPS1) and BUPS2, that are preferentially expressed in mature pollen grains and tubes, and in which mutations similarly affected male-specific fertility and pollen tube integrity (*3*). BUPS1 and BUPS2 ectodomains can also bind RALF4 and RALF19 with affinities similar to those of ANX1 and ANX2 ectodomains. Furthermore, BUPS1 and BUPS2 could form complexes with ANX1 and ANX2 (*3*), suggesting that they form a receptor complex for RALF4 and RALF19 at the plasma membrane of pollen tubes (see the figure).

The ectodomains of CrRLK1Ls contain malectin-like domains, which show homology to the animal carbohydrate-binding protein malectin. Thus, CrRLK1Ls are proposed to be sensors of the carbohydrate-rich cell wall (*5*). Their role in fertilization is consistent with the requirement of ade-

The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, UK. Email: cyril.zipfel@tsl.ac.uk

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