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Interfacial electrostatic properties and ice nucleation efficiency on functionalized gold surfaces

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Surface properties play a critical role in determining the heterogeneous ice nucleation ability of a material, with important implications for atmospheric processes and climate models. However, understanding how these properties, such as surface chemistry, structure, and morphology, influence ice nucleation is experimentally challenging, as it depends on nanoscale variations that are difficult to control and characterize. To investigate these effects, drop-freezing experiments and scanning Kelvin probe microscopy (SKPM) were performed on atomically flat gold (Au) surfaces functionalized with self-assembled monolayers (SAMs) of alkanethiols ($\text{HS}-(\text{CH}_2)_n-\text{X}$) terminating in chemical groups of varying polarity ($-\text{NH}_2$, $-\text{CONH}_2$, $-\text{CH}_3$, $-\text{COOH}$, $-\text{OH}$). The results revealed significant differences in ice nucleation efficiency depending on the terminal functional group, independently of surface wettability. Surface dipoles, inferred from SKPM surface potential measurements, exhibited a strong correlation with ice nucleation efficiency among polar functional groups, regardless of the polarity's sign. These findings offer molecular-level insights into ice nucleation mechanisms and suggest the potential to predict the ice nucleation performance of natural and engineered surfaces based on surface chemical functionality.

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1 Introduction

The process of ice formation, specifically, when and how water freezes, remains poorly understood at the microscopic level, despite its central role in climate science,^{1–3} biology^{4,5} and various technological applications.^{6–8} Water freezes only when an ice nucleus, formed at temperatures below 0 °C, surpasses the nucleation free energy barrier by reaching a critical size, typically a few nanometres.^{9,10} In natural environments, most ice nucleation events are catalysed by foreign surfaces (e.g., mineral dust, pollen, bacteria) through heterogeneous nucleation, which reduces the free energy barrier. Therefore, to better understand ice formation processes on Earth, it is essential to investigate how ice nucleation is influenced by the surface properties of solids,¹¹ including their structural alignment with ice,^{12–15} surface polarity,^{16,17} exposed chemical groups^{18–20} or confinement effects.^{21–25}

Although heterogeneous ice nucleation is intrinsically stochastic, identifying the specific surface properties that govern nucleation activity remains a major experimental challenge. Recent studies have nevertheless demonstrated that freezing is frequently initiated at only a small number of highly active sites, rather than uniformly across the entire substrate.^{26–28} This finding suggests that substrate heterogeneities are likely to play a significant role in determining the observed freezing temperature (T_f), while stochasticity primarily contributes to the variability in freezing temperatures associated with a given site.

Identifying the physicochemical characteristics of these active sites is therefore crucial. Given that critical ice nuclei are nanometre-scale in size, these characteristics must be examined with nanoscale precision. A promising strategy for this involves fabricating surfaces with nanoscale control over their chemical and physical properties.³ In particular, the distribution of chemical species on a material's surface is known to influence both the structure¹⁴ and dynamics²⁹ of interfacial water, thereby affecting the formation of ice nuclei. On the other hand, it has been shown that surface energy alone is not a reliable predictor of nucleation efficiency, whereas the specific chemical nature of surface-bound functional groups can provide important insights.^{19,20} For example, hydroxyl ($-\text{OH}$) groups are well known to promote ice nucleation^{26,30,31}

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although their exact role at the microscopic level remains under active investigation.^{32,33}

Self-assembled monolayers (SAMs) offer a well-established technique for chemically modifying surfaces.³⁴ When prepared on atomically flat substrates such as silicon or gold (Au), SAMs yield surfaces with minimal topographical variation and high homogeneity in physicochemical properties at the nanoscale.³⁵ Such surfaces can be fabricated over areas of several square centimetres, enabling the observation of numerous ice nucleation events under consistent conditions. Alkanes (C_nH_{2n+2}), particularly alkoxy-silanes and halogenated derivatives, are commonly used in SAM formation to modulate surface hydrophobicity³⁶ and friction.³⁷ Alkane SAMs on silicon have also been applied to study the effect of alkyl chain length on ice nucleation behaviour²⁰ and halogen end groups.¹⁹ These studies suggest that changes in nucleation temperature may be associated with variations in the interfacial electrostatic properties of the surface, including contributions from molecular dipoles.

To build on this work, we investigate the ice nucleation behaviour of gold surfaces functionalized with SAMs composed of alkanethiols terminated with a single functional group type: $-NH_2$, $-CONH_2$, $-CH_3$, $-COOH$ or $-OH$. These groups span a range of polarities and are relevant in both organic chemistry and biology. Importantly, all alkanethiols had an identical chain length (C16), ensuring consistent morphology and terminal group density across all samples—parameters that are highly sensitive to both chain length and gold substrate structure.^{34,35} The resulting surfaces (NH_2 -SAM, $CONH_2$ -SAM, CH_3 -SAM, $COOH$ -SAM, and OH -SAM) were atomically smooth with a homogeneous distribution of a single chemical group on each surface.

We quantified the ice nucleation properties of these SAMs by performing drop-freezing experiments using pure water droplets. From the T_F and lag time data, we calculated ice nucleation rates and free energy barriers, allowing a direct quantitative comparison of the nucleation efficiency associated with each functional group. Using scanning Kelvin probe microscopy, we measured the surface potentials of each functionalized surface and compared these to the ice nucleation efficiencies. This allowed us to investigate possible relationships between terminal-group chemistry, surface potential, and ice nucleation efficiency.

2 Experimental

2.1 Materials

The substrates used to prepare the gold surfaces were undoped, (100)-oriented silicon wafers (Siegert Wafer). Epo-Tek 353 ND epoxy resin (Epoxy Technology) and standard microscope glass slides (Labbox) were employed to assemble the final samples. Ultrapure water with a resistivity of 18.2 M Ω cm was dispensed using a Milli-Q system and used throughout all experiments.

The solvents and reagents used in the preparation of the samples included absolute ethanol (EtOH, HPLC grade, Scharlab), dichloromethane (DCM, 99.8%), sulfuric acid (H_2SO_4 , 98%), and hydrogen peroxide (H_2O_2 , 30%) (all from Labkem). The alkanethiol compounds used for surface functionalization

were: 16-mercaptohexadecanoic acid (C_{16} -COOH, 90%); 16-mercaptohexadecanamide (C_{16} -CONH₂, 97%); 1-hexadecanethiol (C_{16} -CH₃, 95%) (all from Sigma-Aldrich); 16-hydroxy-1-hexadecanethiol (C_{16} -OH, 95%) (Dojindo Laboratories) and 16-amino-1-hexadecanethiol hydrochloride (C_{16} -NH₂, 96%) (Tebubio).

2.2 Preparation of gold surfaces

Gold layers with a thickness of 200 nm were deposited onto (100)-oriented silicon wafers *via* electron beam (e-beam) evaporation to produce smooth Au surfaces. In parallel, standard microscope glass slides were cut into smaller pieces: 1 cm $\times$ 1 cm for surface characterization and 2 cm $\times$ 1.25 cm for ice nucleation experiments. Glass substrates were cleaned using a piranha solution ($H_2SO_4:H_2O_2$, 3:1 v/v) for 40 min, followed by sequential rinsing with ultrapure water, absolute ethanol, and dichloromethane. The substrates were then dried under a nitrogen (N_2) stream. For sample assembly, the two-part Epo-Tek 353 ND epoxy resin (comprising an elastomer and a curing agent) was mixed carefully to avoid air bubble formation and then sonicated for 5–10 min to ensure homogeneity. A few drops of the prepared resin were deposited onto the cleaned glass pieces, which were then brought into contact with the Au-coated side of the silicon wafers. This created a layered structure of glass/epoxy/Au/Si. The assembled stack was allowed to rest at room temperature for 1 h to permit epoxy expansion, followed by curing at 80 °C for 16 h. After curing, the epoxy-hardened system was carefully separated from the silicon wafer using a scalpel, resulting in glass/epoxy/Au substrates; hereafter referred to as Au surfaces (see the schematic representation of the procedure in Fig. S1a, SI).

2.3 Functionalization of gold surfaces by the self-assembled monolayer technique

Five separate 10^{-2} M solutions of alkanethiol compounds (C_{16} -NH₂, C_{16} -CONH₂, C_{16} -CH₃, C_{16} -COOH, and C_{16} -OH) were prepared in absolute ethanol using 20 mL glass vials. The compounds were weighed or measured to ensure 0.2 mmol per solution: 62 mg of C_{16} -NH₂, 58 mg of C_{16} -CONH₂, 62 μ L of C_{16} -CH₃, 58 mg of C_{16} -COOH, and 55 mg of C_{16} -OH.

Au surfaces were vertically immersed in the respective thiol solutions and incubated at room temperature under gentle agitation for 24 h. After incubation, the surfaces were removed from the solutions, rinsed thoroughly with absolute ethanol to eliminate any unbound molecules, and dried under a nitrogen stream. The functionalized surfaces were stored in a dry environment to prevent contamination and degradation (see a scheme of the procedure in Fig. S1b, SI).

This process yielded five distinct SAMs: NH_2 -SAM, $CONH_2$ -SAM, CH_3 -SAM, $COOH$ -SAM, and OH -SAM, each terminating in a specific chemical group ($-NH_2$, $-CONH_2$, $-CH_3$, $-COOH$, and $-OH$, respectively), enabling systematic comparison across a range of surface polarities. The untreated Au surface is included as a reference substrate prior to functionalization. It is not intended to represent an inert or ice-nucleation-free blank, and the principal comparisons throughout this work are made between the different chemically functionalized SAMs. Indeed, a direct comparison between bare Au and

SAM-functionalized Au surfaces is not straightforward, since functionalization modifies not only the surface chemistry but also the surface structure, both of which can influence the organization of interfacial water and, consequently, the ice nucleation process. For completeness, the ice nucleation activity of untreated Au is reported in the Results section as a reference.

2.4 Surface characterization

Surface wettability was assessed by water contact angle (WCA) measurements using a Drop Shape Analyzer DSA 100. A 2 μL droplet of ultrapure water was dispensed at a rate of 267 $\mu\text{L s}^{-1}$, and the contact angle was determined using the Young–Laplace fitting method. For each surface, three contact angle measurements were performed at different locations and averaged. The reported contact angle is expressed as the mean value of 3, 5 or 9 surfaces, depending on the surface functional group. Chemical composition was analysed *via* X-ray photoelectron spectroscopy (XPS) using a SPECS Phoibos 150 hemispherical energy analyser with a monochromatic Al K α X-ray source (1486.6 eV, 300 W). Survey scans were acquired with a pass energy of 1 eV, while high-resolution scans (0.5 eV) were performed over five cycles for regions of interest (C1s, O1s, Au4f, S2p, and N1s).

Topography of Au and SAM samples was characterized using atomic force microscopy (AFM) in tapping mode. Surface potential was characterized using scanning Kelvin probe microscopy (SKPM) in two-pass mode. Measurements were conducted on a Cypher SPM (Oxford Instruments Asylum Research) using Pt/Ir₅-coated tips with nominal resonance frequencies of ~ 330 kHz and spring constants of ~ 42 N m $^{-1}$. The contact potential difference (CPD) between the tip and the surface was measured a few nm from the surface, and the voltage needed to compensate the CPD was applied to the tip – as the sample surface was grounded. Surface potential was extracted from CPD measurements referenced against a clean Au surface scanned under identical conditions. All SKPM analyses were performed on 5 $\mu\text{m} \times 5 \mu\text{m}$ image areas.

2.5 Water freezing experiments

Droplet freezing techniques are widely employed to investigate immersion-mode freezing, wherein water droplets supercooled and their freezing events tracked over time and temperature.^{38–40} With this purpose, our experimental setup consists of a temperature-controlled stage (Linkam LTS120) housed in a sealed chamber and monitored by a high-resolution camera to record freezing events and the corresponding freezing temperatures (T_f) following well-established protocols.⁴¹ Ultrapure water droplets (1 μL) were dispensed onto the SAM-functionalized Au surfaces using an electronic micropipette (Easy-W variable volume motorized digital micropipette, 0.5–10 μL , Labbox) and manually arranged in approximately regular arrays (typically $\sim 7 \times 5$ droplets per experimental run), with inter-droplet spacing greater than the droplet diameter to prevent contact-mode freezing between neighbouring droplets. The same micropipette and droplet volume were used throughout all experiments to ensure

consistent droplet volumes. The spacing and freezing behaviour were verified *via* high-speed optical microscopy, allowing visualization of ice-front propagation within and between droplets.⁴² Cooling was applied *via* a programmed temperature ramp, and continuous nitrogen gas flow into the chamber suppressed condensation. No visible evaporation or condensation was observed throughout the experiments, as confirmed by optical microscope images. Once freezing was complete, samples were thawed by increasing the temperature, and surfaces were cleaned before reuse. Each SAM surface underwent up to four freezing–thawing cycles. For each chemical group, multiple surfaces were tested, resulting in over 200 freezing temperature data points per SAM variant and cooling rate. The temperature uncertainty for all measurements was ± 0.2 °C.

3 Results and discussion

3.1 Effect of functional group on freezing temperatures

The effect of different terminal functional groups on ice nucleation was investigated by monitoring the freezing behaviour of ultrapure water droplets deposited on the SAM-functionalized surfaces using drop-freezing experiments described above. AFM topography images revealed a surface roughness of approximately 0.3 nm, indicating an almost atomically flat surface (see Fig. S2 in the SI). This flatness makes the sample well-suited for investigating chemical effects on freezing by minimizing the influence of topographical effects. In addition, prior to the freezing assays, X-ray Photoelectron Spectroscopy (XPS) was employed to confirm the presence of the intended terminal chemical groups on the SAMs. Each SAM type is illustrated schematically in Fig. 1a and accompanied by representative XPS spectra highlighting the relevant regions of interest (Fig. 1b). The characteristic peaks corresponding to each functional group were clearly identified, confirming successful surface functionalization *via* the SAM protocol (the complete spectra for each SAM are provided in the SI, Fig. S3–S8 and Table S1). After verifying the chemical composition, the wettability of the surfaces was assessed using water contact angle (WCA) measurements (Fig. 1c). The average contact angles (θ) for the different SAMs ranged from hydrophobic to highly hydrophilic: $110^\circ \pm 4^\circ$ (CH₃-SAM), $37^\circ \pm 7^\circ$ (COOH-SAM), $35^\circ \pm 12^\circ$ (NH₂-SAM), $27^\circ \pm 5^\circ$ (CONH₂-SAM), and $24^\circ \pm 5^\circ$ (OH-SAM). The non-functionalized Au surface exhibited an average contact angle of $82^\circ \pm 8^\circ$. These results are consistent with previously reported values for alkanethiol-based SAMs and bare gold surfaces.^{43,44}

Fig. 2a shows a series of representative images of water droplets on OH-SAM and CH₃-SAM surfaces during a drop-freezing experiment. It is clearly observed that freezing events are triggered at higher temperatures on the OH-SAM surface. Fig. 2b presents the fraction of frozen droplets, $f_{\text{ice}}(T)$, as a function of temperature during a cooling ramp at 1 °C min $^{-1}$. The results reveal clear differences in freezing behaviour depending on the terminal functional group of the SAM. Non-functionalized gold surfaces (*i.e.*, without SAMs) are included as a reference. A summary of the freezing characteristics for

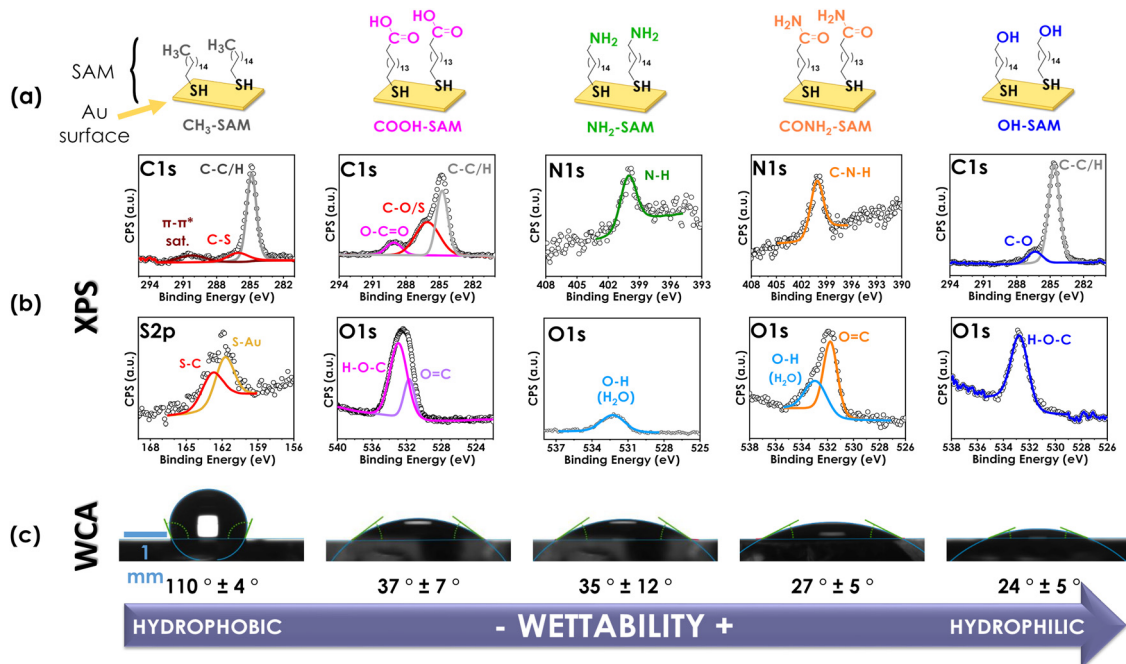


Fig. 1 Characterization of SAM-functionalized surfaces. (a) Schematic representations of the five self-assembled monolayers (SAMs), each terminated with a distinct functional group. (b) X-ray photoelectron spectroscopy (XPS) spectra for each SAM type, highlighting the characteristic peaks within the regions of interest. The presence of these peaks confirms successful functionalization of the gold surfaces via the SAM protocol. (c) Water contact angle (WCA) measurements for each SAM type, indicating surface wettability. Values represent the mean contact angle (CA) obtained from 3–5 samples per SAM type, with standard deviation as the error. A representative image of an ultrapure water droplet corresponding to the average CA is shown for each case.

each surface—namely, the temperature at which the first droplet froze, the temperature corresponding to $f_{ice} = 0.5$ (i.e., 50% of droplets frozen), and the total freezing temperature range—is provided in Table 1. We define T_F as the mean freezing temperature, calculated from all recorded freezing events per surface, as illustrated in Fig. 2c. These results are based on more than 200 individual droplet measurements per surface type, with standard deviations shown as error bars. To assess the reproducibility of the freezing trends, drop-freezing experiments were repeated at three different cooling rates: 1 °C min^{−1}, 5 °C min^{−1}, and 10 °C min^{−1}. The observed freezing behaviour remained consistent across rates. Regardless of the cooling rate, the trend in mean freezing temperature follows the order: $T_F(-OH) > T_F(-NH_2) > T_F(-CONH_2) > T_F(-COOH) > T_F(Au) > T_F(-CH_3)$.

In the experimental setup used—where water droplets rest on a surface— T_F is influenced by interfacial factors such as the contact area between the droplet and the surface. While all droplets in this study had a fixed volume of 1 μL, the contact area varied due to differences in surface wettability among the functionalized samples.

As shown in Fig. 1c, the contact angle varied significantly depending on the hydrophobicity of the terminal chemical groups, resulting in notable differences in droplet-surface interactions and, consequently, in ice nucleation behaviour. To further analyse the ice nucleation behaviour, we derived the cumulative freezing spectrum for each surface. This method is widely employed in atmospheric ice nucleation research^{40,45} as

it assumes that surface-related heterogeneity in nucleation efficiency is more influential than time-dependent (stochastic) effects.^{38,46} Under this framework, it is assumed that each SAM surface contains a distribution of active sites where ice nucleation can be triggered at different temperatures. Given an initial number of droplets N_0 , and the number of frozen droplets $N_F(T)$ at temperature T , the cumulative number of active sites or $N_{sites}(T)$ can be expressed as follows:

$$N_{sites}(T) = \frac{1}{A}[\ln N_0 - \ln N_L(T)] = -\frac{1}{A} \ln[1 - f_{ice}(T)] \quad (1)$$

where $N_L(T) = N_0 - N_F(T)$ is the number of unfrozen droplets at temperature T , and A is a normalization factor (e.g., per unit mass, volume, or surface area). In our case, A corresponds to the droplet-surface contact area. Following standard nomenclature in the literature, we denote the normalized value as $n_s(T)$.⁴⁰ The uncertainty associated with $n_s(T)$ was estimated by error propagation (Fig. 3), considering the experimental uncertainty in the droplet-surface contact area from the contact angle measurements.

The ice nucleation activity of bare Au is also included in Fig. 3 as a reference for comparison with the SAM-functionalized surfaces. The differences between bare Au and the SAMs support the interpretation that the observed ice nucleation activity is associated with the newly formed functionalized surfaces rather than with the underlying Au substrate. The following analysis therefore focuses on the differences among the SAM-functionalized surfaces. It is interesting to

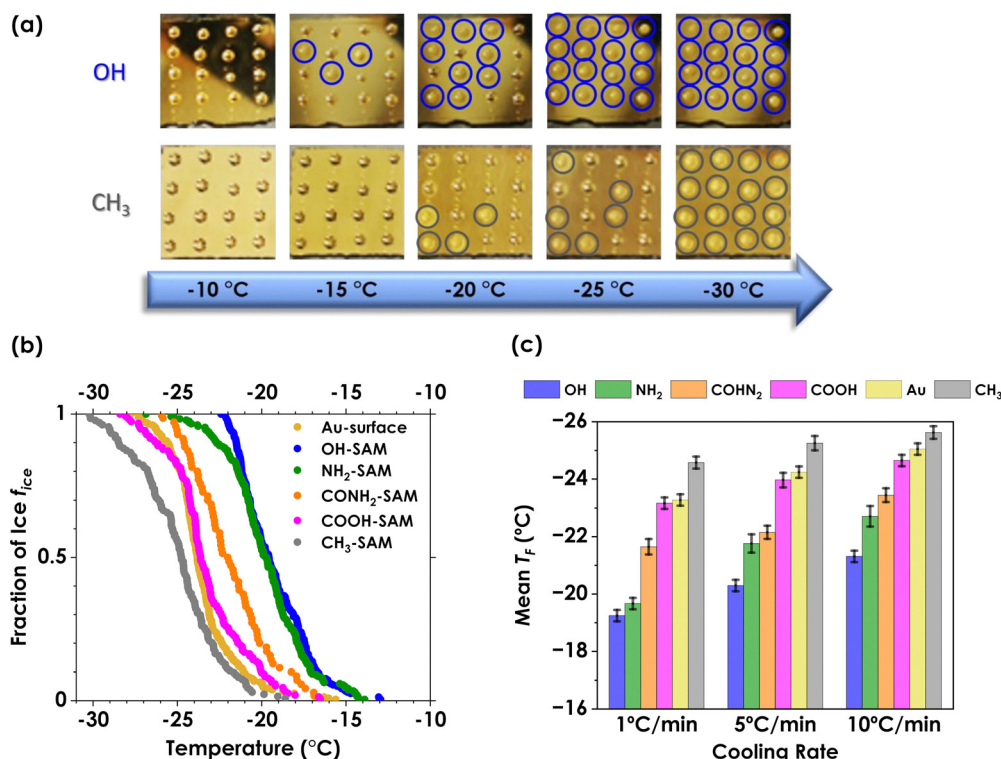


Fig. 2 Heterogeneous ice nucleation as a function of surface type, investigated via drop-freezing experiments. (a) Representative views of a region of the sample surface showing images of ultrapure water droplets on OH-SAM (top row) and CH₃-SAM (bottom row) surfaces during a drop-freezing experiment. Coloured circles indicate the droplets frozen at each temperature. (b) Fraction of frozen droplets, $f_{ice}(T)$, as a function of temperature for all surface types, including non-functionalized Au as a reference. (c) Mean freezing temperature (T_F) for each surface, averaged over approximately 200 droplets per surface type. Error bars represent the standard deviation, and results are shown for three different cooling rates.

Table 1 Ice-nucleation data obtained from drop-freezing experiments shown in Fig. 2b, including the mean contact angle of each sample

Surface type	Total number of droplets	$T(1^{st} \text{ droplet})$ (°C)	$T(f_{ice} = 50\%)$ (°C)	$T(f_{ice} = 100\%)$ (°C)	ΔT (°C)	Mean T_F (°C)	Mean contact angle (°)
Au (ref.)	234	−15.6	−23.8	−27.8	12.2	−23.3 ± 0.2	82 ± 8
−OH	235	−13.0	−19.6	−22.4	9.4	−19.2 ± 0.2	24 ± 5
−NH ₂	236	−13.9	−19.7	−26.9	13.0	−19.7 ± 0.2	35 ± 12
−CONH ₂	70	−16.8	−22.0	−25.9	9.1	−21.6 ± 0.3	27 ± 5
−COOH	154	−16.5	−23.6	−28.4	11.9	−23.2 ± 0.2	37 ± 7
−CH ₃	134	−18.6	−24.8	−30.2	11.6	−24.6 ± 0.2	110 ± 4

note that the cumulative freezing spectra shown in Fig. 3 reveals two distinct groups based on their onset temperatures. The OH[−] and NH₂[−] terminated SAMs activate ice nucleation around −13 °C, COOH and CONH₂ at approximately −17 °C, and CH₃ at around −19 °C. The first group (OH and NH₂) not only initiates freezing at higher temperatures, but also exhibits nearly an order of magnitude greater density of ice-nucleating sites compared to the other terminations. Surprisingly, although the CH₃-terminated SAM begins freezing at a significantly lower temperature, the density of ice-nucleating sites increases rapidly once nucleation starts, eventually reaching levels similar to those of the COOH- and CONH₂-terminated SAMs at around −20 °C. Different kinds of sites, depending on the chemical group of the SAM is suggested from all these results. For the COOH, CONH₂ and CH₃ groups, the presence of a different chemical group clearly induces a

freezing onset earlier on COOH, CONH₂ than on CH₃. Nevertheless, for temperatures below −20 °C, the density of ice nucleation sites is approximately equal for the three samples, suggesting that within this range, ice nucleation sites are likely related to structural ordering within the SAM layer itself, an effect independent of surface chemistry. In contrast, the earlier-activating site population is consistent with additional interfacial interactions involving the terminal functional groups.

An additional measure of the ice nucleation efficiency for each SAM surface can be obtained by calculating the heterogeneous ice nucleation rate per unit surface area at selected temperatures. According to classical nucleation theory (CNT), the heterogeneous nucleation rate coefficient $J_{het}(T)$, defined as the number of nucleation events per unit volume per unit time, depends on the Gibbs free-energy barrier for critical ice embryo

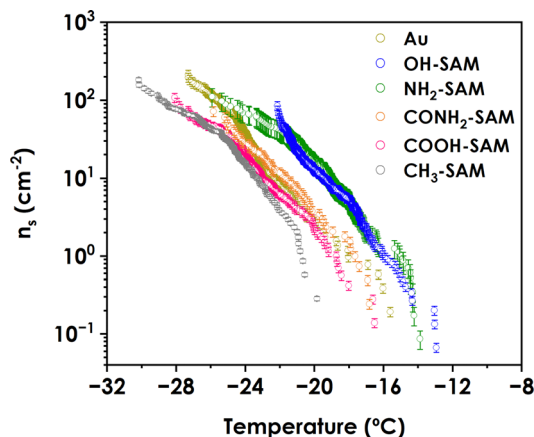


Fig. 3 Cumulative freezing spectra for each surface, showing the number of ice nucleation sites per unit contact area, $n_s(T)$. Values were calculated from the data in Fig. 2b using eqn (1). The uncertainty in $n_s(T)$ was determined by error propagation from the experimental uncertainties associated with the measured contact angle and droplet footprint. This representation provides a surface-normalized measure of ice nucleation activity across different functionalized SAMs and the gold surface.

formation and follows an Arrhenius-type expression:

$$J_{\text{het}}(T) = A_{\text{het}} \exp\left(-\frac{\Delta G^*(T)}{k_B T}\right) \quad (2)$$

where A_{het} is a pre-exponential factor, $\Delta G^*(T)$ is the free-energy barrier for ice nucleation, and k_B is the Boltzmann constant.

To better reflect our experimental conditions, in which ice nucleation occurs at the substrate–water interface, we adopted a model in which ice nuclei grow as spherical caps with a defined contact angle and a constant interfacial energy.⁴⁷ Within this framework, the temperature-dependent heterogeneous nucleation rate per unit surface area is given by:

$$J_{\text{het}}(T) = \frac{A_{\text{het}}}{S} \exp\left(-\frac{\Gamma}{T(T_m - T)^2}\right) \quad (3)$$

where $T_m = 273.15$ K is the melting temperature, S is the contact area between the water droplet and the solid substrate, and Γ is a scaling factor related to the ice nucleation free-energy barrier (details are provided in the supplementary material).

To extract $J_{\text{het}}(T)$ from the experimental data, the freezing temperature distribution was divided into temperature bins of width ΔT_i , centered at temperature T_i , each containing n_i freezing events. The nucleation rate for each bin was calculated using the following expression, where r is the cooling rate.

$$J_{\text{het}}(T) = \frac{r n_i}{S \Delta T_i \left(\frac{n_i}{2} + \sum_{j>i} n_j\right)} \quad (4)$$

To compare the ice nucleation efficiency across different surfaces, the nucleation free-energy barrier was inferred from the slope of a linear fit of $\ln J_{\text{het}}(T)$ versus $1/[T(T_m - T)^2]$. The derived Γ values serve as comparative indicators of the nucleation barrier for each SAM. This theoretical approach has been successfully applied in previous studies.^{19,21,48,49} It is important

to note that eqn (4) correctly describes $J_{\text{het}}(T)$ as an intrinsic property of a SAM exposing a single population of active sites with comparable ice-nucleating efficiency for a given chemical termination, provided that ice nucleation in our experiments is governed by a single population of nucleation sites.⁴⁶ On the other hand, ice nucleation can also be driven by a combination of active-site populations operating at different temperatures, for instance due to structural defects or chemical composition within the SAMs. In such cases, the $J_{\text{het}}(T)$ value calculated here should be regarded as an effective property of the studied SAMs that may reflect contributions from multiple populations of nucleation sites and may depend on factors such as defect density, local chemical heterogeneity, and interfacial water structure. To determine which scenario applies to our SAMs, we followed the FROST framework.⁵⁰ According to FROST, two parameters obtained from droplet-freezing experiments at different cooling rates, λ and ω , can be compared to distinguish between a single- and a multi-component system. The parameter λ represents the temperature-correction factor that aligns the $f_{\text{ice}}(T)$ curves obtained at different cooling rates, while ω corresponds to the slope of the linear fit of $\ln(R/S)$, where R is the freezing rate and S is the droplet-substrate contact area. If λ and ω are similar within experimental uncertainty, the system can be considered single-component; conversely, $\omega < \lambda$ indicates a multi-component population of active sites [50]. In our experiments, λ and ω exhibited comparable values for Au, CH₃-SAM, and COOH-SAM, indicating single-component behaviour. Ambiguous results were obtained for NH₂-SAM, whereas OH[−] and CONH₂-SAMs clearly showed two-component behaviour, as suggested by the differences between λ and ω (see Table S2 for values).

Fig. 4a shows that, for all tested substrates, the natural logarithm of the heterogeneous nucleation rate, $\ln J_{\text{het}}(T)$, exhibits a linear inverse relationship with the term $1/[T(T_m - T)^2]$, as predicted by CNT. Error bars for $\ln J_{\text{het}}(T)$ were calculated using a binomial distribution, following the approach described in [48]. The resulting Γ values for each functionalized surface are summarized in Fig. 4b and reveal a trend consistent with that observed for T_F : $\Gamma(\text{OH}) < \Gamma(\text{NH}_2) < \Gamma(\text{CONH}_2) < \Gamma(\text{COOH}) < \Gamma(\text{CH}_3)$. This ordering indicates that the chemical nature of the terminal functional group exerts a significant influence on the ice-nucleation free-energy barrier (see Table S3).

Interestingly, although COOH-SAM and NH₂-SAM both display similarly low contact angles—indicating a high degree of hydrophilicity—they differ markedly in their Γ values. This observation suggests that factors beyond wettability, such as molecular structure and intermolecular alignment, play key roles in determining ice-nucleation efficiency. Conversely, the CONH₂-SAM, which exhibits a lower contact angle than NH₂-SAM, shows a higher Γ value, further reinforcing the idea that wettability alone does not fully account for nucleation behaviour.

Previous experimental and theoretical studies have investigated the freezing behaviour of water droplets on monolayers of alkenes functionalized with alcohol or acid groups.^{30,32} These studies reported that monolayers of fatty acids promote ice nucleation at higher temperatures than monolayers of alcohols with the same carbon chain length. Our findings are in good

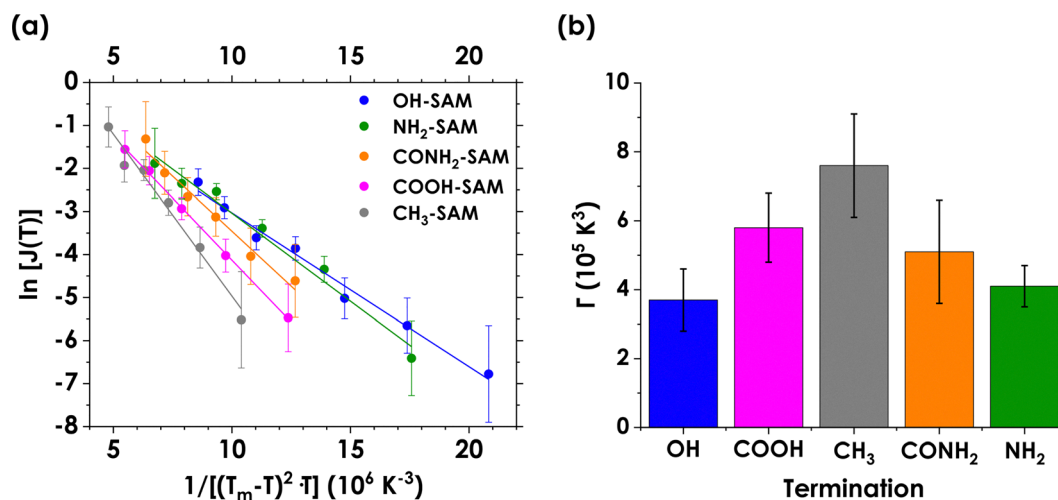


Fig. 4 (a) Heterogeneous ice nucleation rate, $J_{\text{het}}(T)$, derived from droplet-freezing experiments. The data are plotted as $\ln[J_{\text{het}}(T)]/1/[T(T_m - T)^2]$. The slope of the linear fit for each surface corresponds to the scaling factor Γ , which is related to the ice nucleation free-energy barrier. (b) Comparison of Γ values obtained from the linear fits in panel (a), highlighting differences in the ice nucleation energy barrier among surface functionalization.

agreement with these results. However, in those studies, the observed difference in freezing efficiency was attributed to a larger structural mismatch between the ice lattice and the acid monolayer compared to the alcohol monolayer. In our case, the monolayers differ in molecular architecture. Due to the geometry imposed by the gold–thiol bonding,³⁴ the structural mismatch is expected to be minimal. Therefore, the observed effect in our experiments is likely attributable to the chemical functional group itself rather than to structural mismatch.

3.2 Ice nucleation mechanisms

To gain further insight into the mechanisms underlying the observed differences in nucleation efficiency, we investigated the surface potential of the various functionalized substrates using Kelvin probe force microscopy (KPFM) under ambient conditions (22 °C and 40–60% relative humidity). KPFM measures the contact potential difference (CPD) between the sample surface and the AFM probe, providing information about surface dipole characteristics. Fig. 5 shows histograms of CPD values measured for each SAM-functionalized surface. The influence of the SAM on the surface potential, and its relationship to the molecular dipole moment, can be estimated by referencing the CPD of the bare gold substrate,^{20,51} as follows:

$$\Delta\text{CPD}_{\text{SAM-Au}} = \text{CPD}_{\text{SAM}} - \text{CPD}_{\text{Au}} = \frac{N\mu}{\epsilon_{\text{r,SAM}}\epsilon_0} \quad (5)$$

where N is the molecular surface density, μ is the dipole moment component normal to the surface, and $\epsilon_{\text{r,SAM}}$ and ϵ_0 are the relative permittivity of the SAM and vacuum, respectively.

Given the uniform preparation protocol and the use of alkanethiols with identical C16 chain length, major differences in overall monolayer organization are not expected. Nevertheless, subtle variations in molecular orientation, local packing density, hydration, ionization state, or defect density between different terminal groups cannot be excluded. Previous studies have shown that for CH₃-SAMs on silicon, the chain length

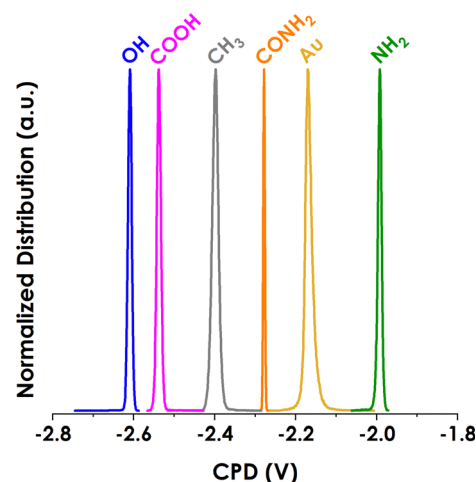


Fig. 5 Histograms of contact potential differences obtained from $5 \times 5 \mu\text{m}$ images in Kelvin probe microscopy for each SAM.

significantly influences nucleation behaviour *via* surface potential modulation.^{20,52} In particular, changes in surface potential are more strongly governed by chain length than by the dipole moment of the terminal group, rendering the latter a minor factor.⁵¹ Our CH₃-SAMs, with a 16-carbon chain length, exhibited a surface potential lower than that of bare Au, consistent with literature findings.⁵² In contrast, all SAMs in this study share identical chain lengths, thereby minimizing structural differences associated with chain length and allowing us to focus on the influence of terminal-group chemistry on the measured surface potential differences. However, upon exposure to moisture, either during the final rinsing step or from ambient humidity, surface potentials may be further altered by the adsorption of water molecules^{14,31,53,54} or by the formation of ionic species.^{55,56} This effect is particularly relevant for COOH and OH groups, which can yield negatively

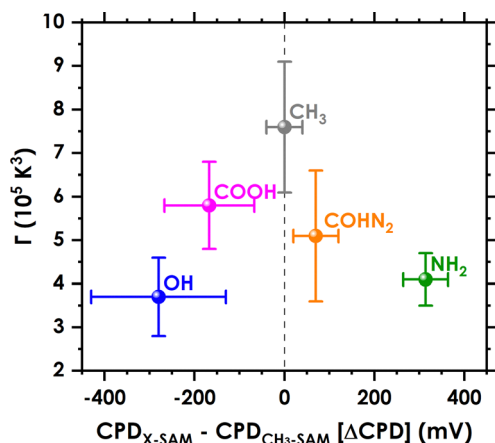


Fig. 6 Correlation between the surface potential of alkanethiol SAMs terminated with various polar functional groups (relative to non-polar CH₃ termination) and their corresponding ice-nucleation efficiency. Surface potential differences (Δ CPD) are plotted against the scaling factor Γ , which represents the ice nucleation free energy barrier. Lower Γ values correspond to higher nucleation efficiencies. The observed trend reveals a correlation between the measured interfacial electrostatic properties of the SAMs and their ice nucleation behaviour.

charged O[−] species, and for NH₂ and CONH₂ groups, which can lead to the formation of positively charged NH₃⁺ species. These ionic contributions can induce strong negative or positive shifts in the measured surface potential.^{55,56} As a result, the assumption of constant N in eqn (5) is strictly valid only for the CH₃-SAM, which does not significantly ionize. For the other SAMs, the surface dipole consists of contributions from both neutral and ionic species, whose densities are influenced by environmental exposure and cannot be precisely controlled. Nevertheless, a consistent trend is observed across all samples, offering valuable insight into how surface potential may influence ice-nucleation efficiency.

Fig. 5 presents the CPD values measured for the functionalized SAMs. Using the CH₃-SAM as a reference, the differences in Δ CPD between various functionalized SAM surfaces and CH₃-SAM can be defined as: $\Delta\text{CPD}_{\text{SAMX-CH}_3} = \text{CPD}_{\text{SAMX}} - \text{CPD}_{\text{CH}_3}$. Here, SAMX refers to surfaces functionalized with polar terminal groups (COOH, OH, NH₂, and CONH₂) capable of forming ionic species. As expected, COOH- and OH-SAMs show lower CPD values than CH₃-SAM, while NH₂- and CONH₂-SAMs show higher values. Translated to our Δ CPD definition, the observed trend goes from positive to more negative values, as follows: $\Delta\text{CPD}_{\text{NH}_2\text{-CH}_3} > \Delta\text{CPD}_{\text{CONH}_2\text{-CH}_3} > \Delta\text{CPD}_{\text{CH}_3\text{-CH}_3} \equiv 0 > \Delta\text{CPD}_{\text{COOH-CH}_3} > \Delta\text{CPD}_{\text{OH-CH}_3}$. This trend was consistently reproduced across all samples, suggesting that, despite possible variations in ionic-species density, the observed surface potential differences are strongly associated with the chemical nature of the terminal group, although contributions from hydration, ionization, and local structural variations cannot be completely excluded.

When comparing this Δ CPD trend with the Γ values (Fig. 6), a clear inverse relationship emerges: as the magnitude of Δ CPD increases, either positively or negatively, the corresponding Γ

value decreases, indicating a lower free-energy barrier for ice nucleation. At first glance, this observation might appear contradictory to prior studies on CH₃-SAM on silicon, where increased surface polarity was associated with suppressed ice nucleation. Those studies proposed that strong surface dipoles could enhance the alignment of interfacial water molecules, reducing entropy and thereby increasing the nucleation free-energy barrier.^{18,56} In such cases, the uniform orientation of water dipoles near charged surfaces was thought to disrupt favourable ice-like structures, thus inhibiting nucleation. However, other studies have demonstrated that surface dipoles can also promote ice nucleation if they facilitate the orientation of interfacial water molecules in a manner consistent with the ice lattice.^{17,18,57} This dual role of dipoles suggests that the effect of surface polarity is context-dependent, and not a simple predictor of nucleation behaviour.

Our findings indicate that SAMs exhibiting larger positive or negative CPD shifts relative to CH₃-SAMs also exhibit enhanced ice nucleation activity. While this observation suggests that interfacial electrostatic properties are closely linked to ice nucleation efficiency, the present experiments do not allow us to establish a unique causal relationship or exclude contributions from factors such as local heterogeneity, defect density, molecular organization, or adsorbed water structure.

This indicates that hydrophobicity alone is not a reliable indicator of ice-nucleation efficiency in SAMs on gold. Instead, the nature and orientation of dipoles or ionic species at the interface play a more critical role. Ultimately, the differences observed between our results and those of earlier studies can likely be attributed to variations in interfacial water structure, driven by specific hydrogen-bonding interactions between water molecules and the surface functional groups. Recent studies have demonstrated that the molecular-level interfacial water structure is a strong predictor of ice-nucleation efficiency,⁵⁸ further reinforcing the importance of surface chemistry in dictating nucleation behaviour.

4 Conclusions

In summary, we have investigated heterogeneous ice nucleation on gold surfaces functionalized with self-assembled monolayers (SAMs) of alkane thiols featuring different terminal chemical groups. The samples were prepared with very low roughness and functionalized with molecules of equal length, ensuring identical alkyl-chain lengths and minimizing structural differences between the tested surfaces. This approach allowed us to assess the ice-nucleating ability of surfaces with nanometer-level control over their physical and chemical properties, a critical factor in understanding ice nucleation mechanisms. The surfaces were functionalized with five distinct chemical groups of significant relevance in atmospheric chemistry and biology. We observed substantial differences in ice nucleation efficiency as a function of the functional group. These differences were quantified using established models from classical nucleation theory. Notably, our results demonstrate that the hydrophobicity of the SAMs cannot be used as a reliable predictor of a surface's ice nucleation ability. To further investigate

these differences, we measured the surface potential of the functionalized surfaces under ambient conditions. Interestingly, we found a clear correlation between the measured surface potential of the SAMs and their ice nucleation efficiency. These results suggest that the interfacial electrostatic properties associated with different terminal functional groups are closely linked to ice nucleation behaviour, likely through their influence on the structure and dynamics of interfacial water. However, within the limits of the present study, we cannot exclude additional contributions from local heterogeneity, defect density, ionization state, or specific populations of highly active nucleation sites. These insights are vital for understanding ice nucleation in the context of organic chemistry and for designing surfaces capable of controlling ice nucleation processes.

Author contributions

Julia Canet: investigation, formal analysis, data curation, visualization, writing – review and editing. Raquel Gimeno-Muñoz: methodology, investigation, formal analysis, data curation, visualization, writing – original draft, review and editing. Laura Rodríguez: investigation, conceptualization, methodology, formal analysis, data curation, visualization, writing – original draft, review and editing. Mark Holden: validation, writing – review and editing. Albert Verdager: funding acquisition, conceptualization, formal analysis, data curation, visualization, writing – original draft, review and editing, supervision, validation.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI), see DOI: <https://doi.org/10.1039/d6cp01787h>. Data for fraction of frozen droplets are available at <https://doi.org/10.5281/zenodo.17376843>.

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