RESEARCH ARTICLE

Rose petal effect: A subtle combination of nano-scale roughness and chemical variability

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Abstract

Rose petals may involve high water contact angles together with drop adhesion which are antagonistic wetting properties. Petal surfaces have a cuticle which is generally considered a continuous, hydrophobic lipid coating. The peculiar properties of rose petals are not fully understood and have been associated with high surface roughness at different scales. Here, the chemical and structural features of natural upper and lower petal surfaces are analyzed by atomic force microscopy (AFM). Both rose petal surfaces are statistically equivalent and have very high roughness at all scales from 5 nm to 10 μm. At the nanoscale, surfaces are fractal-like with an extreme fractal dimension close to \(d_f = 2.5\). A major nanoscale variability is also observed which leads to large (nanoscale) wettability changes. To model the effect of roughness and chemical variability on wetting properties, a single wetting parameter is introduced. This approach enables to explain the Rose petal effect using a conceptually simple scheme. The described fundamental mechanisms leading to high contact angles together with drop adhesion can be applied to any natural and synthetic surface. Apart from introducing a new approach for characterizing a biological surface, these results can trigger new developments on nanoscale wetting and bio-inspired functional surfaces.

KEYWORDS

cuticle, fractal surface roughness, nanoscale wettability, plant surfaces, wetting parameter
Characterizing the multifunctional properties of biological surfaces such as plant leaves, is interesting not only under a Life sciences perspective, but also for the development of bio-inspired materials and Biomimetics.\[11\] Being the interface between aerial plant parts and the surrounding environment, plant surfaces play a major role for plant growth and survival.\[12\] The morphology and chemical composition of plant surfaces determine a wealth of fundamental processes; in particular, wettability, adhesion or repulsion of water drops and transport phenomena across the cuticle.\[3\] The outer surface of most aerial plant organs is protected by epidermal cells of different shape, function and composition.\[4\] The external cell wall of epidermal cells has a lipid-rich area named cuticle,\[5,6\] which is a composite material made of rather hydrophobic (lipids) and hydrophilic (polysaccharides) chemical constituents.\[7\] The cuticle has traditionally been considered a continuous lipid layer covering aerial plant parts, as stated in the first half of the XIX Century.\[8,9\] While the chemical heterogeneity of the cuticle has been highlighted in recent studies,\[7,10–13\] it is still generally regarded as a continuous, hydrophobic coating chiefly made of cutin and waxes embedded (intra-cuticular) or deposited (epi-cuticular) onto the surface.\[5,10\]

For Materials Science researchers, the properties of biological surfaces and chiefly plant organs from some species, are unexpected and fascinating in fundamental and technological terms.\[14–16\] being self-cleaning one of the most widespread applications.\[17,18\] Drop adhesion on (super) hydrophobic surfaces is another surprising and apparently antagonistic effect.\[19,20\] Foucussing on plants, special attention has been paid to two technologically important wetting effects observed for sacred lotus leaves\[17\] and rose petals.\[21\] High contact angle and low drop adhesion is called Lotus effect,\[17,22\] while high contact angle and high drop adhesion is known as Rose petal effect.\[21,23\] Both phenomena are generally attributed to (very) high surface roughness and its fine tuning at different structural scales.\[24–26\]

An important constraint for characterizing plant materials such as petals or leaves of many species, is their perishable nature and ease to dehydrate and lose structure shortly after detachment.\[27\] Hence, to date wettability studies aimed at explaining the Rose petal effect have only been performed with synthetic petal surface replicas.\[20,21\]

The behavior of liquids on solid surfaces is determined, on the one hand by the competition of the intermolecular forces of the atoms (or molecules) of the liquid and those of the solid—and thus by the chemical properties of the liquid and the solid—and on the other hand, by the specific properties of the interface, in particular by its roughness.\[28–30\] For the simplest case of a flat, homogeneous surface, wetting of a liquid on a surface depends only on three surface energies, that is, $\gamma_L$ (of the liquid), $\gamma_S$ (the solid) and $\gamma_{SL}$ (the solid-liquid interface); if $\gamma_S > \gamma_L + \gamma_{SL}$, the liquid covers the surface completely (total wetting) to minimize the total surface energy by covering the high energy solid surface. Otherwise, for $\gamma_S < \gamma_L + \gamma_{SL}$, equilibrium is reached by forming a (spherical) liquid drop, whose contact angle $\theta_0$ with the solid surface is given by the well-known Young Equation: $\cos(\theta_0) = (\gamma_S - \gamma_{SL})/\gamma_L$.\[28–31\] For flat surfaces, the maximum (equilibrium) contact angle is $120^\circ$–$130^\circ$ (for Teflon-like compounds\[19,30,32\]).

Real surfaces are generally neither flat nor homogenous, the correct description of wetting being then much more complicated; indeed wetting of random rough and heterogeneous surfaces is still an open field\[16,33–35\] and it is generally recognized that fundamental phenomena have to be studied at very small scales, correspondingly nanoscale wetting is receiving more and more attention.\[36,37\] The effect of roughness on a chemically homogeneous surface is described by the well-known Wenzel relation,\[28,38,39\] $\cos(\theta_{eff}) = r \cos(\theta_0)$, where $\theta_{eff}$ is the (macroscopic) contact angle of the rough surface, and $r = A_{tot}/A_0$ is the roughness parameter, with $A_{tot}$ the total area, and $A_0$ the projected area (see Equation 1 below). According to this relation, roughness increases the intrinsic properties of the material, that is, if the surface is hydrophilic, a rough surface is even more hydrophilic, while conversely, if the surface is hydrophobic, a rough surface is even more hydrophobic.\[28,38\] The effect of chemical heterogeneity on a flat surface is described by the Cassie equation,\[28,40\] $\cos(\theta_{eff}) = f_A \cos(\theta_0A) + f_B \cos(\theta_0B)$ where $f_A, f_B$ are the fractions of area and $\theta_0A, \theta_0B$ the (equilibrium) contact angles of the materials A and B which make up the heterogeneous surface. While the Cassie-Baxter relation as just discussed is applied to flat surfaces (of two or more materials), it can also be applied to rough surfaces composed of a single material.\[28,41\] Very high curvature of the surface morphology (“edges”) and chemical heterogeneity lead -on the nanoscale- to contact line pinning\[42,43\] and -meso/macroscopically- to advancing $\theta_{adv}$ and receding $\theta_{rec}$ contact angles that differ from the equilibrium contact angle (with $\theta_{rec} \leq \theta_0 \leq \theta_{adv}$)\[31,44\] and thus to contact angle hysteresis, which implies on the one hand friction processes (of the contact line) and on the other hand, that the concept of “reaching equilibrium” is (highly) non-trivial.\[19,45,46\] In this context, a superhydrophobic surface—such as the upper side of sacred Lotus (Nelumbo nucifera) leaves- is defined as a surface with very high (pseudo-equilibrium) contact angle ($\theta_0 > 150^\circ$, and thus larger than the maximum possible on a flat surface) and very low contact angle hysteresis (with $\Delta \theta_{hys} = \theta_{adv} - \theta_{rec} < 5\cdot10^\circ$).\[22,33,47,48\] while the
Rose petal effect implies a surface with very high (pseudo-equilibrium) contact angle and very high contact angle hysteresis ($\theta_0 > 120^\circ$, and $\Delta \theta_{hys} > 50^\circ$).\textsuperscript{[20,21,23,49]}

Aware of the existing wettability literature and new findings on the nature of plant surfaces as described above, our hypothesis is that the difference between the Rose petal and the Lotus Effects cannot be simply explained via surface roughness, but requires consideration of further aspects associated with surface chemical composition. We hence used atomic force microscopy (AFM) as tool to simultaneously measure the roughness, wetting properties and chemical variations of natural rose petal surfaces on a 5 nm to 50 $\mu$m scale. Existing studies only focused on the upper petal surface which has protruding epidermal cells known as papillae.\textsuperscript{[20,21,27,49]} Since we observed a similar wetting performance of the upper and lower petal surface, we carried out a detailed physico-chemical characterization of both petal sides to assess the potential effect of hierarchical surface structure, as taken for granted in most investigations.\textsuperscript{[20,21,23,49]}

## RESULTS

### 2.1 Rose petal surface structure

Figure 1 shows petal cross sections acquired by optical microscopy (OM) and transmission electron microscopy (TEM), after tissue staining with Toluidine Blue or heavy metals, respectively. Epidermal cells (cell walls are stained in blue color in OM micrographs) of different size and shape can be observed in the adaxial (in Figure 1, termed “upper” for simplicity from now on) and abaxial (in Figure 1, termed “lower” from now on) surface of the petal (Figure 1A-C). Hence, adaxial epidermal cells (ADEC) and abaxial epidermal cells (ABEC) are the interface between rose petals and the surrounding environment. Irregular papillae are present in the upper surface (Figure 1A,B) versus larger and more regular pavement cells occurring in the lower petal side (Figure 1A,B). When examining surface cross-sections by TEM at higher magnification (Figure 1C-G), it is possible to distinguish the epidermal cell
wall as an extracellular material which is considered an integral part of the epidermal cell.\cite{4} For TEM observation, samples were stained with various heavy metals and polysaccharide cell wall material (named as CW, for simplicity) has a dark coloration (Figure 1D-G).\cite{5} By contrast, the outermost part of the epidermal cell wall (i.e., the cuticle) which is lipid rich, has a whitish to grey appearance (Figure 1D-G).\cite{5,7} Both the upper and lower surface of petals have a cuticle as outermost extracellular material, but the lower side has a larger number of cuticular folds formed on the rather smooth pavement cell surface (Figure 1E), compared to the folds developed on the concave surface of papillae (Figure 1D). When observing cuticle cross-sections at very high magnification (Figure 1G), thin dark strands can be distinguished, indicating the presence of polysaccharides, as described in several cuticular ultrastructure studies.\cite{5,10,50,51} The structural pattern observed in both adaxial and abaxial cuticle cross-section of rose petals by TEM, can be assigned to Cuticular Structural Type 4: that is, all regions reticulate.\cite{5}

Figure 2 shows scanning electron microscopy (SEM) and AFM topography images of the upper and lower side of rose petals. On the upper surface (Figure 2A,C) papillae (epidermal cells)—arranged in a more or less organized pattern—are clearly seen, having a typical spacing of approximately 20 μm and a height of up to 25 μm, as determined from the AFM data (gold-colored insets of Figures 2A,B). On a smaller scale (1-2 μm), characteristic cuticular folds are found. The lower side (Figures 2B,D) has a different, more uniform topography, forming a mosaic pattern of small-scale folds (1-2 μm). This mosaic pattern, again with a typical lateral scale of 10–20 μm, is generated by local height variations of cuticular folds. On a large scale, due to the occurrence of papillae, roughness is much higher on the upper petal surface than on the lower one (about 25 μm vs. 3 μm, peak to peak). Clearly, the structures observed in these AFM and SEM images are in good agreement with the images of petal cross-sections (Figure 1), in particular, the papillae of the upper surface and the cuticular folds of the lower surface of rose petals are clearly resolved.

When the resolution is increased (smaller length scale), the morphological differences between the upper and lower petal side become less and less evident.
2.2 Power spectral density of surface roughness and local orientations

Many surfaces in nature are fractal,[52] which implies having roughness over a large range of length scales with a statistically well-defined distribution. To analyze the morphology of the rose petal surfaces in a statistically well-defined and detailed way, the power spectral density of surface roughness (PSD, see Supporting Information for more details) was calculated from the AFM images.[53,54] Essentially the measured surface data $z(x,y)$ is developed as Fourier series $z(X,Y) = (\Sigma a_{kx,ky} e^{i(kxX+kyY)}/N^{1/2})$, the PSD of surface roughness is then the (modulus of the squared) amplitude $a^2_{kx,ky}$, averaged over all angles. For self-similar surfaces, the PSD curves $\log[a^2_{kx,ky}]$ versus $\log[k]$ are linear and their slope is determined by the Hurst Exponent and the fractal dimension. As observed in Figure 3A, the PSD of the upper surface shows increased roughness at small $k$ values (large wavelength) due to the contribution of papillae. In other regards, the PSD curves of the upper and lower petal surfaces are essentially equivalent and linear. Having equivalent PSD in the range between 5 nm to 10 μm is the mathematical proof of the statement: “at a small scale, the upper and lower surfaces are alike”. Moreover, from the linearity and slope of the PSD-curves, we conclude that the upper and lower petal surfaces are fractal with a fractal dimension $d_f \approx 2.45$ (in the range between 5 nm to 10 μm).

As discussed in detail below, not only surface roughness but also the local slope $\nabla z(x,y)$ is important for wetting. To access these slopes, Nanogoniometry[55] is applied to the AFM images, in order to determine the angles of surface orientation that occur in an AFM topography image. This technique essentially calculates the surface orientation of each image point by computing the local normal vector of the surface $z(x,y)$, from which $n^2$ normal vectors are obtained (with n: number of data points per line). The corresponding statistical distribution of these surface normals is shown in Figure 3B,C. Interestingly, these normals are not distributed uniformly: angles up to a maximum of about 50° are measured. Moreover, by far the most common slope observed in AFM images—for both, the upper and lower rose petal surface— is precisely close to this maximum value of 50° (Figure 3B,C).

2.3 Nanoscale variations of surface wettability

AFM offers two powerful advantages over other techniques: on the one hand, it directly measures the topography $z(x,y)$ and, on the other hand, it can simultaneously provide maps $c(x,y)$ related to the chemistry of the
FIGURE 4 Structural and chemical heterogeneity of the upper surface of rose petals in relation to wettability. A, Topographic image of a papilla on the upper surface of a rose petal (12.5 μm lateral side and 3.6 μm height). B, Chemical map of a papilla. C, Chemical map corresponding to the small area shown in (B). D, Composed three-dimensional image combining the morphology (A) and the chemistry (B), represented by the color of the surface; blue: more hydrophilic, towards red: more hydrophobic. E, Profiles A and B (shown in (A)-(C)) show the characteristic features of papillae. The vertical height of these profiles corresponds to the surface morphology, and the color again to nanoscale wetting. The inset corresponds to profiles a and b of the enlarged region shown in (C). F, Images showing (macroscopic) contact angles $\theta$ of water drops deposited onto the upper side of rose petals. The “outer” images show the (pseudo-) equilibrium contact angle $\theta_0$ with the rose petal surface having different orientations upwards-sideways-downwards, while the “inner” figures show advancing/receding contact angles ($\theta_{\text{adv}}/\theta_{\text{rec}}$) with the rose petal measured horizontally. More precisely, for the “outer” images: The image with the drop oriented “upwards” corresponds to $\theta_0$ when the upper rose petal surface is oriented upwards (“normal orientation”), the drop oriented “downwards” corresponds to $\theta_0$ when the upper rose petal surface is turned upside down and the drop is “hanging” from the surface, and finally the drop oriented “sideways” shows how the drop adheres to the upper surface of the rose petal when oriented perpendicular to gravitational force.

In our experiments, we associate chemical information with the interaction generated by the formation of liquid necks.[57] Chemical maps thus describe local hydrophilicity/hydrophobicity (see Supporting Information). Topography and chemical maps were measured on the upper (Figure 4) and lower side (Figure 5) of rose petals (see also Figures S1 and S2).

To allow a precise correlation of morphological and chemical data, from these two raw images, a combined three-dimensional image is generated, where its height $z(x,y)$ corresponds to the topography and the color of each data point represents the chemical interaction $c(h(x,y))$ and thus the nanoscale wetting properties (Figures 4D and 5D). We found not only a very high roughness (typically “as high as large”), but also very large variations of chemical composition, with different regions separated by only few nanometers (Figures S1 and S2). These large variations of local wetting—observed on the upper and lower side of rose petals—are incompatible with the assumption that aerial plant surfaces are covered with a continuous lipid layer (the cuticle and epicuticular waxes). Instead, we propose that the cuticle locally exposes hydrophilic cell wall polysaccharides (likely cellulose), giving rise to an extreme wetting variability.

In this context, we recall that the plant cuticle is considered a composite membrane made of hydrophobic (lipids) and hydrophilic (cell wall polysaccharides) chemical constituents.[7,10] Hence, the hydrophobic and hydrophilic areas detected by AFM can be unequivocally

![Figure 4](image-url)
Figure 5 Structural and chemical heterogeneity of the lower surface of rose petals in relation to wettability. Essentially, this image is equivalent to the previous Figure 4, but the data corresponds to “the other side” of the rose petal. A, Topographic image of pavement cell of the lower surface (10 μm lateral side and 3 μm height) and (B) corresponding chemical map, (C) chemical map corresponding to the small area shown in (A) and (B). D, Image combining the morphology (A) and the chemistry (B). E, Colored profiles A and B (shown in (A) and (B)). As in Figure 4, the vertical height corresponds to the surface morphology, and the color to nanoscale wetting, blue: more hydrophilic, towards red: more hydrophobic. F, Images showing (macroscopic) contact angles θ of water drops deposited onto the lower side of rose petals. The “outer” images show the (pseudo-) equilibrium contact angle θ₀ with the rose petal surface having different orientations upwards-sideways-downwards, while the “inner” figures show advancing/receding contact angles (θₐdv / θₐrec). More precisely, for the “outer” images: the image with the drop oriented “upwards” corresponds to the contact angle when the lower rose petal surface is oriented upwards, the drop oriented “downwards” corresponds to the contact angle when the lower rose petal surface in its “normal orientation” and the drop is “hanging” from the surface, and finally the drop oriented “sideways” shows how the drop adheres to the lower surface of the rose petal when oriented perpendicular to gravitational force.

associated with lipid zones (rather apolar and hydrophobic compounds) and polysaccharides (prone to hydrogen-bonding and non-dispersive interactions), respectively.

To correlate macro- with nano-scale wetting properties, the (equilibrium) contact angle (θ₀) of upper and lower rose petal surfaces with different liquids was measured. For water, advancing θₐdv and receding θₐrec contact angles as well as contact angle hysteresis, are also shown (Table 1, and Figures 4F and 5F). In agreement with the data found in the literature, [20,21] we determined very high contact angles and very high contact angle hysteresis, which is the fingerprint of the rose petal effect. What we find quite remarkable is that, particularly for water, the equilibrium contact angles θ₀ on the upper and lower side are essentially the same (within our experimental error), although the total surface roughness is almost 10 times larger on the upper side: 25 μm (upper side) versus 3 μm (lower side). In addition, the (pseudo-) equilibrium contact angle is much closer to the advancing contact angle than to the receding one; specially for the case of the lower side, both are essentially the same, within our experimental error. Drops of the three different liquids had very strong adhesion to both petal surfaces, even when turning the petals sideways or upside down (see Figures 4F and 5F).
which are both based on biology—are two important and
determine macroscopic wetting, [34–37] and we believe that
is far from clear how nanoscale chemistry and structure
still an open issue. In particular, for the case of wetting, it
ing of the structure-function relation of these interfaces is
most extracellular material and interface between organs
and the surrounding environment. A precise understanding
of the structure-function relation of these interfaces is
still an open issue. In particular, for the case of wetting, it
is far from clear how nanoscale chemistry and structure
determine macroscopic wetting,[34–37] and we believe that
nature is still ahead of “first principles calculations”. That
is, if surfaces with well-defined and robust wetting properties
have to be designed, it is still more effective to “look” at
what Nature offers, rather than relying on theoretical model-
ing. In this context, the Lotus and Rose petal effects—
which are both based on biology—are two important and
well-studied examples.

With respect to the results of the present work, we would
first like to note that we have proven for the first time,
the occurrence of hydrophilic and hydrophobic nanoscale
areas in a plant (and probably in a living biological) sur-
face. Second, the micro- and nano-structure of the upper
petal surface provided a similar wettability effect as the
one associated with the sole occurrence of (micro- and
nano-) cuticular folds in the comparatively smoother
surface of the pavement cells protecting the lower petal
surface. It is remarkable that both petal sides had a sim-
ilar fractal-like morphology, in spite of the very differ-
ent (absolute) total roughness (25 versus 3 μm). As dis-
cussed in more detail below, the fractal-like structure of
the petal surface generates a large amount of excess surface
area on the nanoscale, which determines the energetics of
liquid-air and solid-liquid interfaces at the (macroscopic)
boundary between the drop and the rose petal surface.
Third, our results showing the smart combination of chem-
ical and structural roughness observed in both rose petal
surfaces, provide evidence for the fundamental contribu-
tion of chemical composition to the so-called Rose petal
effect. Finally, to describe the observed combination of
topographic roughness and chemical variability in a simple
scheme, we define a single wetting parameter that allows
to extend the Wenzel relation to the nanoscale, in order to
model nanoscale wettability as determined by local mor-
phology and chemistry (more precisely: nanoscale contact
angle).

The PSD curves of the upper and lower rose petal sides
are equivalent; and -most importantly- these curves are, to
a very good approximation, linear. In the range between
5 nm to 10 μm, petal surfaces are therefore fractal-like; with
a very high fractal dimension \( d_f \approx 2.45 \). Theoretical argu-
ments suggest that the fractal dimension of natural sur-
faces should be limited to \( d_f \leq 2.3 \), when fractal behav-
ior occurs over many orders of magnitude.[59] In our case,
the surfaces are “flat” below our experimentally accessi-
ble range (≈ 5 nm) and are again “flat” for scales larger
than 50–100 μm. On rose petals, Nature appears to “con-
centrate” roughness on the scale between 5 nm to 25 μm
to create a surface with accurately designed wetting and
adhesion properties.

In addition to surface roughness, also local slope \( \nabla z(x,y) \) is fundamental for wetting since it determines, among
other factors, the total area:

\[
A_{tot} = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \sqrt{1 + (\nabla z(x,y))^2} \, dx \, dy
\]  

(1)

through the term \( 1 + (\nabla z(x,y))^2)^{1/2} \). As discussed above
(see also Figures 4 and 5), the angles are not distributed
uniformly, the most common slope observed in the AFM
images are (very) large angles up to 50° (Figure 3B,C).

### Table 1

| Liquid       | Surface | Contact angles (°) | Equilibrium [θ₀] | Advancing [θ_adv] | Receding [θ_rec] | Hysteresis [Δθ_hys] |
|--------------|---------|--------------------|------------------|-------------------|-----------------|---------------------|
| Water        | Upper   | 131 ± 6            | 140 ± 4          | 85 ± 5            | 54 ± 6          |
|              | Lower   | 130 ± 4            | 132 ± 4          | 71 ± 5            | 61 ± 6          |
| Glycerol     | Upper   | 125 ± 5            |                  |                   |                 |
|              | Lower   | 122 ± 4            |                  |                   |                 |
| Diiodomethane| Upper   | 69 ± 5             |                  |                   |                 |
|              | Lower   | 61 ± 9             |                  |                   |                 |

For water, advancing (θ_adv), receding (θ_rec) contact angles and contact angle hysteresis (Δθ_hys) are provided. Data are means ± standard deviations.
While this result may appear surprising at first glance, it is consistent with the experimental results obtained from the topographic data: a profile through a surface with \( d_f = 2.5 \) will result in a curve such that each Fourier term induces the same slope because the decrease of the Fourier component \( a_k = c / k \) (\( c \): some appropriate constant) of the measured surface data \( z(x,y) \) (recall: \( z(x,y) = \sum a_{kX,kY} e^{i(kXx+kYy)} / N^{1/2} \), see SI) is compensated by a \( k \) from the derivative, which results in Fourier components \( (a_k k) \) of the slope \( \nabla z(x,y) \) that are essentially constant. This is precisely what we observe experimentally.

As discussed above, wetting properties are determined by both, roughness and chemical composition.\(^{[28]}\) The effect of roughness on a chemically homogeneous surface is described by the Wenzel relation,\(^{[28,38]}\) \( \cos(\theta_{eff}) = r \cos(\theta_0) \), where \( r \) is the roughness parameter \( r = A_{tot}/A_0 \), while the effect of chemical heterogeneity on a flat surface is described by the Cassie relation \( \cos(\theta_{eff}) = f_A \cos(\theta_A) + f_B \cos(\theta_B) \) where \( f_A, f_B \) and \( \theta_A, \theta_B \) are the fraction of area and the contact angle of the materials A and B which make up the surface.\(^{[40]}\) The Cassie–Baxter relation, as just discussed, is applied to flat surfaces. Interestingly, it can also be applied to rough surfaces composed of a single material.\(^{[28,41]}\) As shown in Figure 6, we may assume that this rough, chemically homogenous surface is effectively flattened as the valleys of the surface morphology are filled either with the liquid (if the surface is hydrophilic, \( \cos(\theta) \geq 0 \), Wenzel regime, upper right part of Figure 6, blue line), or with air (if the surface is hydrophobic \( \cos(\theta) < 0 \), Cassie-Baxter regime, lower left part of Figure 6, red line), leaving only a fraction \( f_s = f_A \) of surface material “uncovered”. For a chemically homogeneous but rough surface, this approach predicts a behavior \( \cos(\theta_{eff}) \) versus \( \cos(\theta) \) as sketched in Figure 6.\(^{[28,45]}\)

Although the application of the Cassie-Baxter relation to a rough, homogenous surface as just discussed is quite successful, in our opinion it unfortunately does not apply to natural surfaces which may be chemically heterogeneous and rough. Moreover, it is based on the roughness parameter \( r = A_{tot}/A_0 \) (see above), which is an average quantity. Here, we present a scheme to describe and characterize the nanoscale wetting behavior of locally rough and heterogeneous surfaces. The true surface area (Equation 1) is calculated by integrating over the term \( (1+(\nabla z(x,y))^2)^{1/2} = (1+tan^2(\theta_{topo}(x,y)))^{1/2} = 1/\cos(\theta_{topo}(x,y)) \), where \( tan(\theta_{topo}(x,y)) \) is the local slope, and \( \theta_{topo}(x,y) \) is the angle of the plane tangent to the (global) surface, which is also the (total) deviation of local surface normal \( n(x,y) \) from the overall surface normal \( n_0 = (0,0,1) \), which is precisely the data calculated by nanogoniometry.
and shown in Figure 3B,C. We thus propose to define the local roughness parameter \( r(x,y) \), as well as a nanoscale effective contact angle \( \theta_{\text{neff}}(x,y) \) and a local effective wetting parameter \( w(x,y) \) as:

\[
r(x,y) = 1 / \cos(\theta_{\text{topo}}(x,y))
\] (2a)

and

\[
w(x,y) = \cos(\theta_{\text{neff}}(x,y)) = \cos(\theta_{\text{chem}}(x,y)) / \cos(\theta_{\text{topo}}(x,y))
\] (2b)

if \(-1 < \cos(\theta_{\text{chem}}(x,y)) / \cos(\theta_{\text{topo}}(x,y)) < 1\), and \( \pm 1 \) if this term exceeds these values.

The wetting parameter \( \cos(\theta_{\text{neff}}(x,y)) \) describes the wetting behavior of a particular surface location on the nanoscale, taking into account both local roughness (local tilting of the surface patch) and local chemical properties. Moreover, we may interpret that the two fundamental parameters controlling wetting, that is, chemistry (through \( \cos(\theta_{\text{chem}}(x,y)) \) and morphology (through \( \cos(\theta_{\text{topo}}(x,y)) \)), are measured “in the same units” (actually without units because angles are dimensionless). The \( \cos(\theta_{\text{eff}}) \) versus \( \cos(\theta_{l}) \) relation shown in Figure 6A, defines a macroscopic contact angle \( \theta_{\text{eff}} \) using average quantities. We may interpret the effective nanoscale contact angle \( \theta_{\text{neff}}(x,y) \) as the parameter allowing to extend the Wenzel relation down to the nanoscale. Essentially, we here propose that nanoscale wetting behavior is described by a \( \cos(\theta_{\text{neff}}(x,y)) \) versus \( \cos(\theta_{\text{chem}}(x,y)) \) relation. The effective nanoscale angle \( \theta_{\text{neff}}(x,y) \) describes how the contact line of a drop will “locally touch” the surface at that position. For \( \cos(\theta_{\text{neff}}(x,y)) = -1 \), the contact line will “leave” the surface (anti-) parallel (\( \theta_{\text{neff}} = 180° \)) to the overall surface plane in a “totally hydrophobic” configuration, while for \( \cos(\theta_{\text{neff}}(x,y)) = +1 \), the contact line “leaves” the surface parallel (\( \theta_{\text{neff}} = 0° \)) to this plane in a “totally hydrophilic” configuration, with the liquid “sticking” to the surface.

In Figure 6, the local wetting parameter calculated from the data shown in Figure 3 is shown. The experimental chemical map \( ch(x,y) \) and the calculated wetting parameter \( w(x,y) \) are similar, and regions which are recognized as “wetting” in the chemical map \( ch(x,y) \) also correspond to “wetting” in the wetting parameter images. However, we find that the contrast between the wetting and the nonwetting regions are even more extreme in the calculated wetting image \( w(x,y) = \cos(\theta_{\text{neff}}(x,y)) \) as compared to the experimental “chemical” image \( ch(x,y) \), due to the additional contribution of surface roughness.

A final remark regarding the high adhesion of drops onto rose petals: the high chemical variability of these surfaces implies that points (more precisely: curves on the surfaces separating regions of different materials) exist having a (very) high contact angle hysteresis. The (nanoscale) contact line is then pinned at these curves. We propose that, as the energetics of the rose petal is varied (“gedankenexperiment”: turn a petal with a drop upside down), the air-liquid interface near the contact line will be deformed and will change while being still locally pinned to this line. We suggest that as the total potential energy of the drop varies, potential energy is converted into surface energy \( E_{\text{surf}} = \gamma_L \delta A \), where \( \delta A \) is the surface area variation due to the deformation of the liquid interface at pinned contact lines and \( \gamma_L \) is the surface free energy of the liquid. Nanoscale contact angle hysteresis therefore allows the liquid-air interface to act as a “spring” where surface energy can be stored, as long as the contact line is deformed in a continuous way; enabling the drop to (macroscopically) adhere to the surface while varying its internal energy. If the contact line varies discontinuously and “jumps” to a new surface location, this will lead to contact angle hysteresis as well as energy dissipation/friction of the drop motion on the surface.

Our results showing the occurrence of nanoscale hydrophilic and hydrophobic areas in petal surfaces provide evidence that the cuticle is a composite material made of lipids (polyester cutin and waxes) and cell wall polysaccharides. In the case of rose petals, hydrophilic carbohydrate polymers extend from the epidermal cell wall to the outermost cuticle surface and thus to the petal-air interface, as also supported by our TEM observations. Thereby, the nanoscale chemical heterogeneity of the surface has a major impact on nano- and macroscale surface wettability, as shown here for rose petals. The ultra-structure of the petal cuticles analyzed is entirely reticulate and had a limited presence of epicuticular waxes.

Future studies should hence examine the occurrence of hydrophilic and hydrophobic areas in the surface of different plant and biological materials, because this will have many currently unexpected functional implications such as variable wetting, permeability to water and solutes or interactions with surface deposited matter (e.g., microorganisms or contaminants).

4 | CONCLUSION

In this study, we have analyzed in detail down to the nanoscale, the morphology and chemical properties of the upper and lower surface of rose petals. An extreme roughness on the 5 nm to 25 μm scale was measured. Although the total roughness is very different (25 versus 3 μm for the upper and lower petal surfaces, respectively), the PSDs of surface roughness of both petal sides are very similar and fractal with \( d_f \approx 2.45 \). For water, the contact angle
on both surfaces is within the same range, which proves that rather than total roughness, other parameters—and in particular, the fractal dimension—are relevant for their wetting behavior. In addition, we found an extreme chemical heterogeneity of the surfaces, with very hydrophilic and very hydrophobic patches being close together. Both, extreme roughness and chemical variability are the basis for the Rose petal effect. From our data, we conclude that a liquid drop will strongly adhere to nanoscale hydrophilic patches, and detach from all the other hydrophobic parts of the surface, since the combined effect of roughness and chemical (nanoscale) wetting properties will induce a high effective nanoscale contact angle (\( \cos(\theta_{\text{neff}}(x,y)) \)). This explains the surprising and in principle antagonistic properties associated with the Rose petal effect, that is, high contact angle and high drop adhesion.

We are convinced that, although this work is focused on rose petals, the fundamental mechanisms by which Nature is able to generate high contact angle and drop adhesion will apply to other surfaces with similar characteristics. In addition, the application of this fundamental mechanism will trigger the development of new functional surfaces by “learning from Nature”.

5 | EXPERIMENTAL SECTION

5.1 | Material preparation

The upper (adaxial) and lower (abaxial) surface of petals of a commercial orange rose (floribunda hybrid rose var. Lois lane) with Rose petal effect, were analyzed, either intact or after a mild process of fixation, dehydration and critical point drying to avoid papillae collapse. For AFM and SEM analyses, fixed, critical point-dried tissues were used due to the soft nature of petals that dehydrated and lost structures shortly after detachment. Fresh petals were collected, cut with a scalpel in \( \sim 4 \) mm\(^2\) pieces and were immediately immersed in phosphate buffer (7.2 pH) containing glutaraldehyde (2.5%) and paraformaldehyde (4%) (both from EMS) for 3 hours at 4°C. They were then rinsed in ice-cold phosphate buffer, pH 7.2, 4 times within a period of 6 hours and kept at 4°C for 12 hours. Tissues were post-fixed in a 1:1 aqueous solution of osmium tetroxide (2%, TAAB Laboratories, United Kingdom) and potassium ferrocyanide (3%, Sigma-Aldrich) for 1.5 hours. They were subsequently washed with distilled water (x3), dehydrated in a series of acetone (30, 50, 70, 80, 90, 95 and 100%); distilled water (\( \delta_t = 47.9 \) of MJ\(^{1/2}\) m\(^{-3/2}\)) \[60\]. Solutions were changed twice (10 minutes each) until ethanol (70%) in which samples were kept for 12 hours at 4°C. Then, petal tissues were further dehydrated in ethanol (x4 times, 10 minutes each in 80, 90, 95 and 100%) and subjected to critical point drying (Leica EM CPD300, Leica Microsystems, Germany).

5.2 | SEM

The upper and lower surfaces of immediately detached petals, were observed with a variable pressure SEM (Hitachi S-3000N, Hitachi High-Tech, Japan) at low vacuum, after gold (Au) coating (sputter Quorum Q150T-S, Quorum Technologies, United Kingdom). Papillae loss integrity during analysis, but it was possible to take some images of the surfaces by rapidly examining different petal areas. Additionally, adaxial and abaxial surfaces of fixed, critical point-dried (as described above), Au-coated (sputter Quorum Q150R-S) petal sections were examined with a high-pressure SEM (JEOL JSM6400-40 kV, Jeol Ltd., Japan).

5.3 | Observation of petal surface cross-sections

For OM and TEM examination, petals were cut into 4 mm\(^2\) pieces and fixed in glutaraldehyde (2.5%) and paraformaldehyde (4%) (both from EMS) for 3 hours at 4°C. They were then rinsed in ice-cold phosphate buffer, pH 7.2, 4 times within a period of 6 hours and kept at 4°C for 12 hours. Tissues were post-fixed in a 1:1 aqueous solution of osmium tetroxide (2%, TAAB Laboratories, United Kingdom) and potassium ferrocyanide (3%, Sigma-Aldrich) for 1.5 hours. They were subsequently washed with distilled water (x3), dehydrated in a series of acetone (30, 50, 70, 80, 90, 95 and 100%; x2, 15 minutes each concentration) and gradually embedded in acetone-Spur’s resin (TAAB Laboratories) mixtures (3:1 for 2 hours, 1:1 for 2 hours, 1:3 for 3 hours (v:v)) and finally pure resin (samples were always kept at approximately 25°C). After 12 hours, petal samples were placed in blocks, which were filled with pure resin, before incubation at 70°C for 3 days until complete resin polymerization. Ultra-thin sections (obtained with a Leica Ultracut E, Leica Microsystems) were consequently cut, mounted on nickel grids and post-stained with Reynolds lead citrate (EMS) for 5 minutes prior to sample analysis. Petal sections were observed with a Jeol 1010 TEM (Jeol Ltd., at 80 kV) equipped with a CCD Megaview camera. For analyzing the general structure of rose petals by OM, semi-thin cross-sections were cut, mounted in microscope slides and stained with toluidine blue, before observation with an epifluorescence microscope (Axioplan-2, Zeiss, Germany).

5.4 | Contact angle measurements

Equilibrium contact angles of drops of deionized water, glycerol (99% purity, Sigma-Aldrich, Germany) and
diiodomethane (99% purity, Sigma-Aldrich) were measured at room temperature (∼20 °C) with a Drop Shape Analysis System (DSA 100, Krüss, Germany). Using a dosing system holding a 1 mL syringe equipped with a 0.5 mm diameter needle, drops of approximately 2 μL of each liquid were deposited on to the adaxial and abaxial surface of immediately excised petals, collected from roses (at least six roses, 12 petals and 36 drops per liquid and surface [i.e., n = 36]) kept in water at 6°C. Side view images of drops were taken and contact angles were automatically calculated using the tangent equation to fit the captured drop shape with the calculated one. Water, glycerol and diiodomethane were used for assessing rose petal surface wetting by drops of liquids with different degrees of dispersive and non-dispersive components. Such liquids have the following total surface tension (γ) and surface tension components (γ LW, Lifshitz–van der Waals; and Acid-base positive (γ+) and negative (γ−) components): γ = 72.80, γ LW = 21.80 and γ+ = γ− = 25.50 mJ m−2 for water, γ = 63.70, γ LW = 33.63, γ+ = 8.41 and γ− = 31.16 mJ m−2 for glycerol and γ = γ LW = 50.80, γ+ = 0.56 and γ− = 0 mJ m−2 for apolar diiodomethane.[61]

Advancing (θadv) and receding (θrec) contact angles (n = 20) were determined as described by Priest et al (2009),[24] with some modifications following a similar experimental setting to the one described for equilibrium contact angle measurements. For determining θadv on upper and lower petal surfaces, droplets were formed at the end of a syringe needle and placed on to the surfaces. Droplets were found to experience a major adhesion for the surfaces (measurements were carried out with the needle remaining attached to the drops) and did not slide when moving the samples in the horizontal plane. Hence, drop volumes were gradually increased to 9–10 μL water, when motion of the contact line was observed to occur and maximum θ values were recorded. Receding contact angles were recorded after reducing drop volumes to approximately 1–2 μL water (the drop still attached to the needle). The water contact angle hysteresis Δθhys of the upper and lower side of rose petals was determined as the different between θadv and θrec.

5.5 | AFM data acquisition and processing

Data were acquired using Dynamic atomic force microscopy (DAFM) on a Nanotec Electronica AFM system (Madrid, Spain) with a phase-locked loop board (PLL, bandwidth ∼2 kHz), which maintained the cantilever at resonance. Images were acquired using the amplitude as signal for the feedback channel (Amplitude modulation dynamic mode; AM-DAFM) at (relatively) large oscillation amplitude (a fre = 25 nm), in the attractive regime. Silicon (Si) tips (ν ≈ 70 kHz) with a nominal force constant of 3 N m−1 were used. The nominal radius of the tip apex of these probes is specified as 15 nm by the manufacturer. The frequency shift δν(X,Y) = ν(X,Y) - ν0 (ν0: free resonance frequency) of the tip-sample resonance frequency was determined using the PLL system of our electronics, and δν(X,Y) was acquired as additional channel of information related to the chemical composition of the sample (see Supporting Information). WSeM software[63] was used for image processing. Typically, a plane filter was applied to topography images; no filter is applied to the frequency images. For the calculation of Nanogoniometry histograms H(θx,θy) (Figure 3, see Supplementary data), and for the calculation of the wetting parameter w(x,y) (Figure 6), specific algorithms were programmed in Mathematical code to compute the corresponding data directly from WSeM raw-data image files.

ACKNOWLEDGMENTS

Research has been supported by Ministerio de Ciencia e Innovación (MICINN, Spain and the European Union) through the projects, “Nano and Meso Scales: Modelling, Structure and Characterization” (PID2019-104272RB-C52 and C55), Fundación Séneca 20985/PI/18 and Omya International AG. Lisa Almonte and Carlos Pimentel acknowledge financial support by MICINN (Spain) and the European Union; Lisa Almonte is being supported by a postdoctoral project contract (ENE2016-79282-C5-4-R and PID2019-104272RB-C52) and Carlos Pimentel by a Juan de la Cierva - Formación postdoctoral contract (FJC2018-035820-I, MICINN, Spain). Enrique Rodríguez-Cañas is a recipient of a PTA contract (PTA2018-015394-I; MICINN Spain).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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