

Novel Analysis Method for Condensate Wetting Identifies Charge-Dependent Tau-Membrane Interactions

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Condensed biomolecular phases are fundamental compartments in cells and play intricate roles for cellular organization. Although often referred to as “membraneless organelles,” evidence has emerged that condensates associate with membranes through wetting interactions, which are physiologically important but not well understood. Here we report a novel method that provides a detailed description of how condensates interact with surfaces. Combining technically advantageous flat membranes and reconstituted condensates, we developed an accurate method that automatically analyzes the three-dimensional geometry of single, μm -sized condensates acquired as multicondensate confocal image stacks using 96-well plates. We validated our computational routine by quantifying wetting of *in vitro* and *in silico* condensates for precisely controlled solution and supported lipid membrane biochemistries. Further, we applied our method to analyze the wetting of condensates formed from the Alzheimer disease-associated protein tau in its phosphorylated and nonphosphorylated forms. Our results demonstrate that both membrane charge and tau posttranslational modifications affect wetting and that our method allows building a systematic, quantitative wetting database, which catalogues conditions controlling wetting in multiple dimensions. We anticipate that upscaled in a high-throughput pipeline, our screening approach will efficiently support efforts to construct a comprehensive wetosome database, which will unravel the mechanisms underlying condensate-membrane interactions and, thus, decipher the molecular grammar governing wetting.

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I. INTRODUCTION

In the past decade, condensates have gained increasing focus as dynamic subcellular compartments with defined function [1]. Often called “membraneless organelles,” they are intracellular droplets enriched in distinct biomolecules, commonly a combination of proteins and nucleic acids [2]. Condensates form distinct subcellular microenvironments that serve to partition specific components and have the potential to drive specific reactions depending on their exact composition [3].

Aside from their diverging biochemical properties, the condensates’ physical characteristics allow them to interact with other intracellular structures in unique ways, for example by wetting membranes [4]. This condensate-membrane interaction depends on both membrane and condensate material properties, and can result in various morphologies and phenomena in cells including the formation of membrane sheets and cups, dividing condensates, membrane bending and vesiculation [4–7]. It has been shown that such complex shape changes directly impact conserved physiological processes including autophagosome formation, which is the key step of a recycling pathway called autophagy that is fundamentally important for many human diseases [8]. During autophagosome formation, molecular and physical condensate properties can determine how much of a condensate is engulfed within the developing autophagosome and eventually degraded [6].

Tau is a protein that binds, regulates and stabilizes microtubules, primarily in cells of the nervous system. Tau does not have a well defined structure, and is classified as an intrinsically disordered protein. Its amino acid sequence encodes an unstructured, mostly negatively charged N-terminal part,

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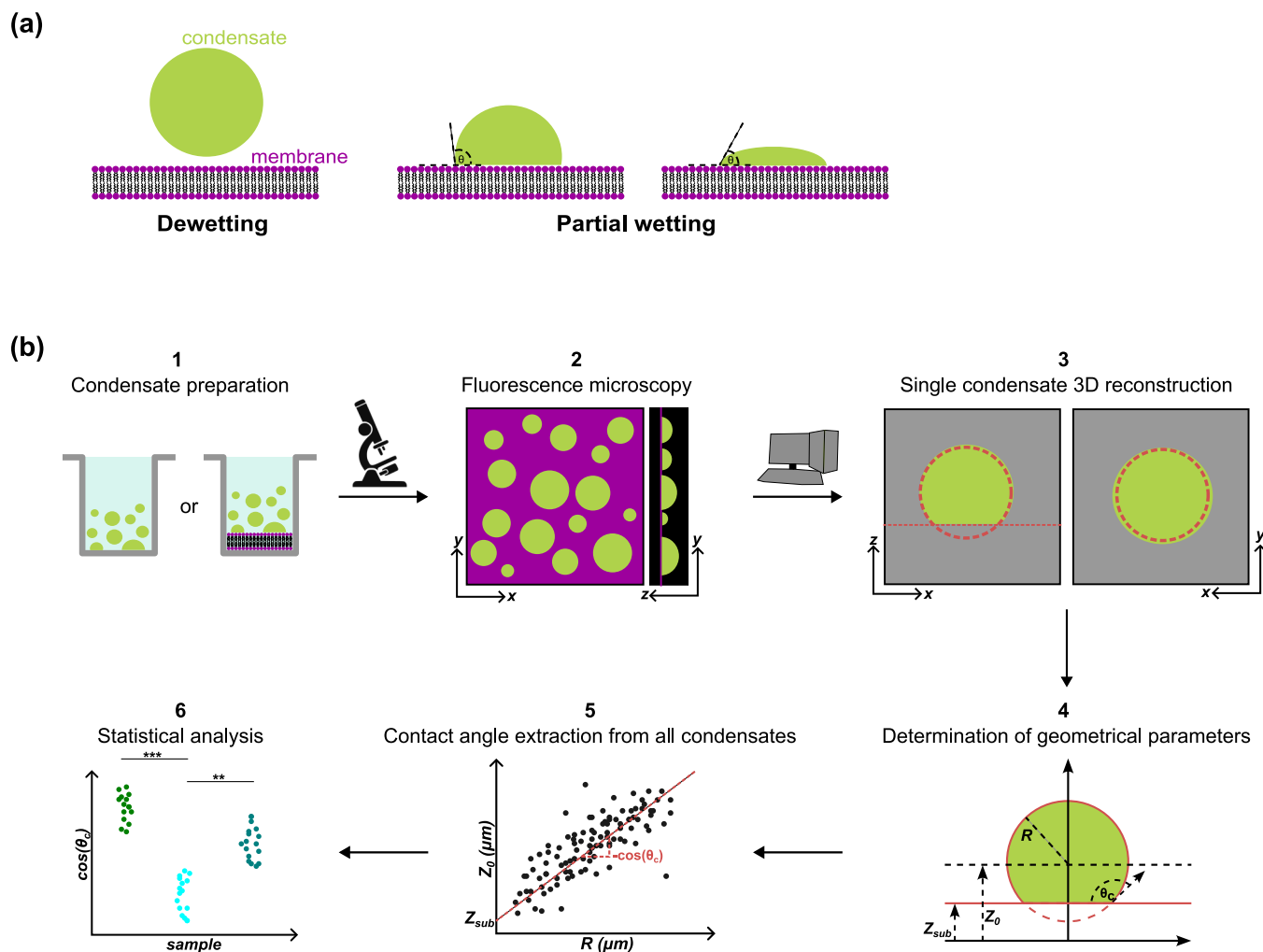


FIG. 1. Novel method to analyze condensate wetting. (a) Condensates wet surfaces in varying degrees: No surface contact, also called dewetting, corresponds to a contact angle $\theta_c = \pi$ between the surface (here, a membrane) and the condensate. Increasing condensate-substrate interactions lead to varying degrees of partial wetting, characterized by a decreasing contact angle θ_c . Partial wetting is defined by contact angles $0 < \theta_c < \pi$. (b) Overview of the experimental and analysis procedure. (1) In a microscopy dish, such as a 96-well plate, condensates settle on a surface of choice, e.g., glass or a membrane in the form of a supported lipid bilayer. (2) Using confocal microscopy, Z-stacks with multiple individual condensate images are recorded. (3) Our analysis reconstructs 3D volumes of each single condensate, shown here exemplarily as 2D projections for the xz and xy planes. (4) From these reconstructions, geometric parameters of individual condensates are extracted and (5) the average contact angle for the whole stack is calculated from linear regression of all condensates analyzed. (6) Statistical analysis for each stack is performed automatically for different conditions, in a statistical analysis program of choice.

positively charged proline-rich and microtubule binding domains in the central region, and a short negatively charged C-terminal tail [9]. Tau can form various assemblies, the most studied being β -structure-rich tau filaments that define different tauopathies [10]. Both physiological and pathogenic tau exhibit various phosphorylation states, which change the overall charge of the protein [11].

Tau forms condensates both *in vitro* and in cells [12,13]. Tau condensation can be induced in various conditions, including addition of the crowding agent polyethylene glycol (PEG) and co-phase separation with negative biopolymers such as RNA or heparin [14,15]. These condensates have been shown to nucleate microtubules [16], and are also a possible starting point for tau filament formation.

Changes in lipid metabolism commonly accompany neurological diseases, including tauopathies such as frontotemporal

dementia and Alzheimer's disease [17]. The cellular impact of these alterations is currently under debate. Modified lipid compositions and membrane properties, however, not only affect signaling cascades but also can redistribute membrane-associated proteins [18]. This could affect tau localization in neurons, both as condensates and as filaments. Tau-membrane interactions are therefore of interest as potential contributors to tau pathogenicity.

Condensate-membrane wetting has been observed in living cells, and in *in vitro* studies combining condensates reconstituted from purified proteins with model membranes. In these experimental systems, wetting can be quantified using the contact angle that arises at the three-phase contact line between the condensate, the membrane and the surrounding solution [Fig. 1(a)]. Analysis of sessile droplet contact angles is established for a range of droplet materials and for

sizes close to mm-scale [19–22]. Using such large proteinaceous condensates for systematic screens requires prohibitive amounts of purified protein. Quantifying contact angles of μm -sized condensates that are randomly oriented at the surface of free-standing membrane vesicles is a tedious process as it typically requires manual determination of the geometric parameters of both the condensate and the membrane on the basis of individual contacts [5]. This approach requires careful determination of the axis of symmetry of the condensate, identification of the condensate boundary along a cross section, followed by a nonlinear fitting of a circular arc to this boundary. As an alternative, wetting of μm -sized condensates on supported lipid membranes has been quantified [23] but using a setup and analysis routine that do not fit large-scale studies.

Here, we aimed to speed-up and automate the analysis of μm -sized condensates on supported lipid membranes, to enable comparable analyses of various protein condensates and experimental conditions in an upscalable setup suitable for high-throughput screening. Our approach [Fig. 1(b)] is based on confocal microscopy of multiple condensates sedimented on technically advantageous flat membranes and three-dimensional (3D) reconstruction of single condensates, followed by a volumetric moment analysis that completely sidesteps individual condensate fitting procedures and instead allows for the contact angle to be extracted as a global property of the sample. In particular, this allows for contact angle determination even in samples where the condensates are barely above image resolution (a few pixels in diameter) as long as there are many condensates of varying sizes in the sample. Moreover, it is not necessary to measure the vertical position of the substrate, which is indirectly estimated from the global fit. A comparison between the estimated and directly measured substrate position can be used as an additional quality check on the analysis.

The paper is organized as follows. In Sec. II, we first introduce our experimental methods, as well as our novel method for the automatic determination of wetting contact angles (Sec. III). The method is then first tested using polymeric condensates on a glass substrate (Sec. III A). Next, using computer-generated condensates of defined size and known contact angles, we validate our method by characterizing in detail its systematic errors and confirming that our automated pipeline reliably captures condensate size and contact angles (Sec. III B). Finally, in Sec. III C we analyze the wetting of tau on supported lipid bilayers with diverging membrane charges. We find that tau wetting depends on membrane charge, in line with previous results [15], and further that wetting of tau condensates changes when we employ a protein kinase A (PKA)-phosphorylated form of the protein. These results indicate that tau modifications change the intracellular wetting of tau condensates on membranes, which could play a role in pro-pathological interactions of tau with the plasma membrane and with organelles.

II. MATERIALS AND METHODS

A. Preparation of supported lipid bilayers

Supported lipid bilayers (SLBs) were prepared on cleaned surfaces using small unilamellar

vesicles (SUVs) composed of 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC, Avanti Research), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP, Avanti Research), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS, Avanti Research) and a minor fraction of a fluorescently labeled lipid DiI-C18 (Sigma-Aldrich). The desired lipid compositions in chloroform (Sigma, 366927) were desiccated under vacuum and resuspended in phosphate buffered saline (PBS) to a final lipid concentration of 2 mM. SUVs were prepared by 20 freeze-thaw-vortex cycles (freeze in liquid N_2 , thaw, vortex 15 s). SUVs were diluted 1:15 in 10 mM HEPES pH 7.4, 150 mM NaCl [24].

In parallel, a 96-well glass-bottom plate (IBL, P96-1.5H-N) was plasma cleaned for 20 min at 100% power in a plasma cleaner (Zepto BLS, Diener). Immediately after cleaning, 50 μl diluted SUVs were added per well, and incubated for SLB formation 30 min at 30 °C. Each well was washed five times with the desired assay buffer, always keeping residual buffer in the well so that SLBs formed do not dry. SLB integrity was checked by confirming membrane fluidity using fluorescence recovery after photobleaching.

B. PEG-phosphate condensate preparation

PEG-phosphate condensates were prepared by mixing 230 mg/ml PEG 8 kDa, 200 mM NaCl, in 100 mM KPO_4 buffer pH 7. To stain condensates, we added 0.5 mg/ml BSA/BSA-Atto488 (50:1 ratio). Samples were briefly vortexed, 50 μl of the condensate-containing solution was placed in one well of 96-well plates and used for microscopy. In some experiments we varied acidity (achieved by different pH KPO_4 buffers) and salinity (by increasing NaCl molarities). In these cases, conditions used are given directly in the corresponding figure.

C. Recombinant purification of tau

Human full-length wild-type tau (2N4R, hTau40) was expressed in *Escherichia coli* BL21 Star (DE3) (Invitrogen) and purified as previously described [15]. Protein expression was induced with 0.5 mM IPTG at $\text{OD}_{600} = 0.6$ for ~ 2 h at 37 °C. For wild-type tau, cells were harvested, resuspended in lysis buffer (20 mM MES, 1 mM EGTA, 0.2 mM MgCl_2 , 1 mM PMSF, 2 mM DTT, protease inhibitors (Pierce Protease Inhibitor Mini Tablets, EDTA-free)) and lysed using a French press. Samples were supplemented with 500 mM NaCl and boiled at 95 °C for 20 min. Cell debris was removed by centrifugation and the supernatant was dialyzed against low salt buffer (20 mM MES, 50 mM NaCl, 1 mM MgCl_2 , 1 mM EGTA, 2 mM DTT, 0.1 mM PMSF, pH 6.8), filtered (0.22 μm membrane filter), run through a cation exchange column (Hi-Trap SP HP, 5 ml, GE Healthcare), and eluted with a high salt buffer (20 mM MES, 1 M NaCl, 1 mM MgCl_2 , 1 mM EGTA, 2 mM DTT, 0.1 mM PMSF, pH 6.8). Fractions containing tau were pooled, concentrated using spin column concentrators (Protein concentrators; 10–30 kDa MWCO, Pierce), and run through a size exclusion column (Superose 6 10/300, GE Healthcare). Fractions containing purified monomeric tau were concentrated as before, the buffer exchanged to PBS, 1 mM DTT, pH 7.4, final tau concentration was determined

using BCA assay (BCA kit, Pierce) and proteins were stored at -80°C .

D. *In vitro* phosphorylation of tau

Tau proteins were incubated in phosphorylation buffer (25 mM HEPES, 100 mM NaCl, 5 mM MgCl_2 , 2 mM EGTA, 1 mM DTT, protease inhibitors (ROCHE, Complete)) with recombinant PKA kinase (New England Biolabs, P6000; 5 mg/ml tau, 0.5 mg/ml PKA) and 1 mM ATP overnight at 30°C . To denature and remove the kinase from the sample, NaCl was added to a final concentration of 500 mM and the protein solution boiled for 10 min at 95°C , and centrifuged at 100 000 g for 30 min. The phosphorylated tau in the supernatant was dialyzed against phosphate buffered saline (PBS) containing 1 mM DTT. It has been shown that PKA phosphorylates up to five tau amino acids (S214/S324/S356/S409/S416) [11].

E. Fluorescent labeling of tau

Tau was fluorescently labeled using amine-reactive DyLight488-NHS ester (Thermo Scientific, 46403) following the manufacturer's instructions. The dye was dissolved in DMSO to a final concentration of $10\text{ }\mu\text{g}/\mu\text{l}$, then added in a 5-fold molar excess to the protein in PBS with 1 mM DTT for 2 h at room temperature at 250 rpm shaking. Excess dye was removed by dialysis (Pur-A-Lyzer Mini Dialysis tubes, Sigma-Aldrich; MWCO 12–14 kDa for full-length Tau) against PBS, pH 7.4, 1 mM DTT overnight at 4°C . The labeling degree (amount of dye / molecule protein) was determined by measuring the final protein concentration and correlating it to the maximum absorbance of the attached dye according to manufacturer instructions.

F. Reconstitution of tau condensates

SLBs were washed five times with the assay buffer (25 mM HEPES pH 7.4, 10 mM NaCl, 1 mM DTT), supplemented with a final concentration of 5% PEG 8 kDa and incubated for 1 h. Condensates formed in wells upon addition of tau at a final concentration of 0.1 mg/ml and settled at the well bottom during a 1 h incubation prior to imaging.

G. Image acquisition

Fluorescence images were acquired on an Olympus FV1000 microscope using a UPlanSApo 60x/1.2 water immersion objective. GFP was excited using a 488 nm laser. Red dyes including Rhodamine B and DiI were excited with a 559 nm laser. Three-dimensional confocal stacks were recorded with 512×512 pixel resolution and $0.5\text{ }\mu\text{m}$ z-spacing. The Olympus.oib image files were directly used for automated image analysis.

H. Statistical analysis

Biological samples were analyzed in GraphPad Prism 10. Samples were tested for normality using Shapiro-Wilk test. As the samples were not distributed normally, significance was tested using nonparametric Kruskal-Wallis test with Dunn's *post hoc* test for multiple comparisons.

I. Automated image analysis

1. Condensate segmentation

To measure the contact angle between the phase-separated protein condensates and the substrate on which the condensate sediments, we first segment and reconstruct the individual condensates from a 3D confocal microscopy stack with multiple condensates. Using MATLAB's image processing toolbox, we first apply a Gaussian filter to the grayscale 3D image to filter out noise. Next, we binarize the grayscale image using automatic thresholding (Otsu's method), fill in possible holes inside the binarized condensates, and further smooth out the binarization by morphologically opening it with a disk of radius 1 pixel. Finally, we segment individual condensates using the connected components method. Having segmented individual condensates, we can proceed to analyze their shape to measure the contact angle.

2. Moments of a spherical cap and their relation to the contact angle

In Fig. 1(b4) we show the key geometric parameters for an axisymmetric spherical cap. Here, θ_c is the contact angle, Z_0 and R are the vertical position of the center and the radius of the spherical cap, respectively, and Z_{sub} is the vertical position of the substrate. Note that, through simple geometry, these four quantities bear the relation

$$Z_0 = Z_{\text{sub}} - R\cos(\theta_c). \quad (1)$$

This relation will be used later to determine the contact angle as an aggregate parameter from a global fit of all the condensates in the sample. First, however, we show how to measure R , $\cos(\theta_c)$, and Z_0 for each individual condensate from an analysis of the moments of the intensity distribution of the binarized condensate and how they compare with those of an idealized spherical cap.

Consider a continuous intensity distribution $I(\mathbf{r})$ where $\mathbf{r} = (X, Y, Z)$. The relevant moments for our purpose are the zeroth order moment or volume, $V = \int I(\mathbf{r})dV$, the first moments or coordinates of the center of mass, $U_c = (1/V) \int UI(\mathbf{r})dV$ with $U = X, Y, Z$, and the second ($n = 2$) and third ($n = 3$) order moments $S_U^{(n)} = (1/V) \int (U - U_c)^n I(\mathbf{r})dV$. Now consider an idealized spherical cap, with "intensity" $I(\mathbf{r}) = 1$ inside and $I(\mathbf{r}) = 0$ outside. Due to axisymmetry, we necessarily have that $S_X^{(3)} = S_Y^{(3)} = 0$. The nontrivial moments can be calculated by direct integration as

$$V = \frac{\pi}{3}R^3(2 + c^3 - 3c), \quad (2)$$

$$Z_c = Z_0 + \frac{3}{4}R\frac{(1 + c)^2}{2 + c}, \quad (3)$$

$$S_X^{(2)} = S_Y^{(2)} = \frac{R^2}{20}\frac{(1 - c)(3c^2 + 9c + 8)}{2 + c}, \quad (4)$$

$$S_Z^{(2)} = \frac{R^2}{80}\frac{(1 - c)^2(3c^2 + 18c + 19)}{(2 + c)^2}, \quad (5)$$

$$S_Z^{(3)} = \frac{R^3}{160}\frac{(1 - c)^3(1 + c)^2(7 + c)}{(2 + c)^3}, \quad (6)$$

where for conciseness we have defined $c \equiv \cos(\theta_c)$. From these, we note that there are two dimensionless ratios which are in one-to-one correspondence with the contact angle:

$$\frac{S_Z^{(2)}}{S_X^{(2)}} = \frac{1}{4} \frac{(1-c)(3c^2 + 18c + 19)}{(2+c)(3c^2 + 9c + 8)} \quad (\text{Method 1} \equiv M_1), \quad (7)$$

$$\frac{(S_Z^{(3)})^2}{(S_Z^{(2)})^3} = \frac{20(1+c)^4(7+c)^2}{(3c^2 + 18c + 19)^3} \quad (\text{Method 2} \equiv M_2). \quad (8)$$

The approach we follow is then to first directly measure all relevant moments of the segmented condensates from the experiment, via the discrete definitions of the moments, i.e., $V = \sum_i I_i v$, $U_c = (1/V) \sum_i U_i I_i v$, and $S_U^{(n)} = (1/V) \sum_i (U_i - U_c)^n I_i v$, where the index i runs over all voxels in the segmented condensate, which have volume $v = \delta x \delta y \delta z$ where δx , δy , δz represent the lateral voxel size along each dimension. Next, we use either Eq. (7) (Method 1 or M_1 for short) or Eq. (8) (Method 2 or M_2 for short) to obtain a value of $c = \cos(\theta_c)$, i.e., the contact angle.

While both Methods M_1 and M_2 should give the same result for an idealized spherical cap measured with infinite resolution, the finite resolution of the experimental images, as well as experimental and imaging artifacts, can result in different estimates of $\cos(\theta_c)$ from each Method (as explored in Sec. III B). The two methods have different strengths and weaknesses. In particular, a notable feature of M_2 is that it depends only on the ratio of Z moments, and is thus robust to rescaling of the Z axis by an arbitrary constant factor. This implies that M_2 can automatically self-correct for optical artifacts that stretch or compress the Z axis, and similarly for gravitational flattening of large condensates, which to a good approximation can be modeled as a Z -rescaling that turns spherical caps into flattened ellipsoidal caps [25–27]. However, as we will see in Sec. III B, the fact that M_2 depends only on Z moments comes with an associated downside: at high wetting, the condensates only span across very few Z slices, so that the Z moments and the resulting estimate for $\cos(\theta_c)$ can become inaccurate. However, M_1 is well behaved for both low and high wetting, but is not robust to Z axis distortion (in particular, M_1 will underestimate or overestimate the extent of wetting if Z is stretched or compressed, respectively).

Finally, via Eqs. (2) and (3), we obtain the radius R and geometric center Z_0 of the condensate as

$$R = \left(\frac{3V}{\pi} \frac{1}{2 + c^3 - 3c} \right)^{1/3}, \quad (9)$$

$$Z_0 = Z_c - \frac{3}{4} R \frac{(1+c)^2}{2+c}. \quad (10)$$

In this way, all relevant geometric parameters of a segmented condensate can be measured. To obtain an aggregate measure of $\cos(\theta_c)$ that leverages all condensates within a given sample, and is therefore more robust, we use Eq. (1) to obtain a global fit of R vs Z_0 for all condensates in the sample, where the slope corresponds to $-\cos(\theta_c)$ and the intercept to the substrate position Z_{sub} [Fig. 1(b5)]. This allows for an additional consistency check across all condensates in the sample (they should fall on a straight line) and against an independent, direct experimental measurement of Z_{sub} , when

such a measurement is possible (which is the case when the supported lipid bilayer is fluorescently labeled).

As a final note, it is worth mentioning that our approach has several in-built methods to detect deviations of the segmented condensate shapes from an idealized spherical cap, which can serve as a quality of fit indicator. First of all, there are quantities that can signal deviations from axisymmetry. One such quantity is the deviation $S_X^{(2)} - S_Y^{(2)}$ between the measured X and Y second order moments, while another pair of such quantities is the third order moments $S_X^{(3)}$ and $S_Y^{(3)}$. These quantities should be zero for an axisymmetric condensate, and nonzero otherwise. Next, even in axisymmetric cases, one can construct quantities that signal deviations from a spherical cap, using the geometric relations in Eqs. (2), (4), (5), and (6). Indeed, these four equations correspond to an overdetermined nonlinear system for the two unknowns R and c . For an ideal spherical cap, a single (R, c) pair would satisfy all four equations, but this is not the case in general due to discretization effects, optical artifacts, or true deviations from a spherical cap shape in experiment. One can form six pairs of equations from which six (R, c) pairs can be correspondingly extracted. The extent to which the six values of R and c differ from each other, e.g., as measured by their standard deviation, can thus serve as a measure of deviation from an ideal spherical cap shape.

III. RESULTS AND DISCUSSION

Condensate wetting on surfaces depends on several variables, the major ones including condensate composition, surface properties, as well as salt conditions and pH of the buffer. Wetting can be quantified using the contact angle that arises at the three-phase contact line between the condensate, the surface and the surrounding solution. It allows to categorize the wetting into different stages: contact angles between 0° and 90° (or equivalently $\cos(\theta_c)$ between 1 and 0) correspond to high wettability, while contact angles between 90° and 180° (or equivalently $\cos(\theta_c)$ between 0 and -1) indicate low wettability. A contact angle of 180° ($\cos(\theta_c) = -1$) means that condensates do not wet a surface under these experimental conditions, Fig. 1(a). Our aim was to establish an experimental approach that allows systematic measurements of condensate wetting using standard wet-lab methods and a newly developed, robust automated image analysis routine that allows a fast, quantitative contact angle readout, Fig. 1(b).

To allow a direct adaptation of our procedure to any microscopy-suitable dish from self-assembled sample chambers to high-throughput systems, we set up our experimental procedure in 96-well glass bottom imaging plates combining technically advantageous flat supports and confocal microscopy, Figs. 1(b1) and 1(b2). Condensed phases are dense compared to dilute phases and settle on surfaces of choice, such as glass surfaces or SLBs. We choose 96-well plates for several reasons: (i) The volumes needed are as low as $50 \mu\text{l}$, which saves precious (protein) samples. (ii) Plates can be readily adapted for automated sample preparation in high-throughput screening facilities and even lower volumes with 384 or 1536-well formats. (iii) Automated imaging allows systematic screening of several buffer conditions, surfaces, membrane and condensate compositions of interest. (iv)

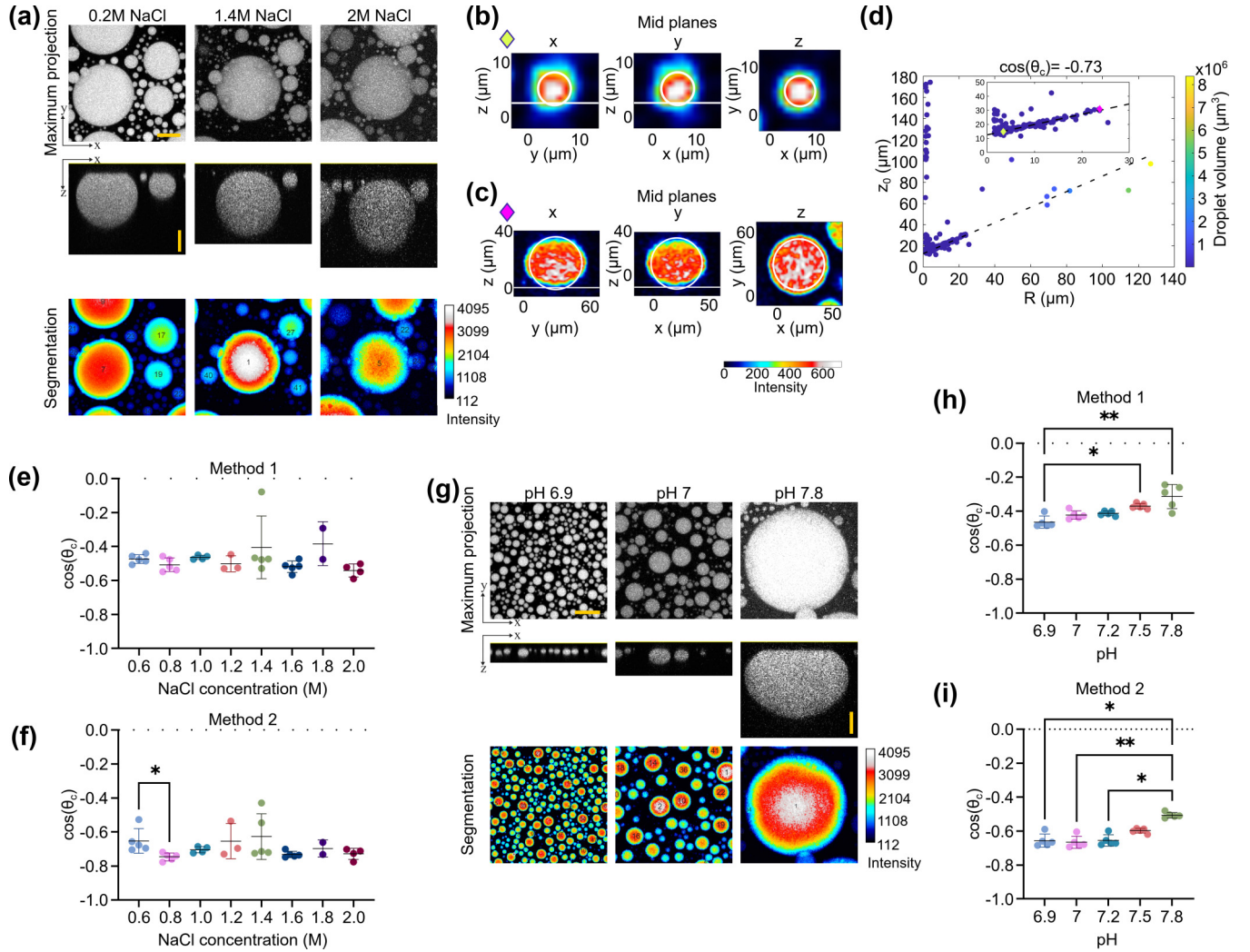


FIG. 2. Automated contact angle analysis of polymeric condensates wetting glass. (a) PEG-rich condensates form in the presence of phosphate buffer, and settle to the glass bottom of a 96-well plate. Fluorescently Alexa 488-tagged BSA partitions into the condensates and is used here as condensate stain for confocal microscopy. Top, maximum intensity projections of PEG-rich condensates in 100 mM phosphate buffer with indicated NaCl concentration. Middle, representative xz sections of the image stack. Bottom, segmentation of the condensates by the analysis code, color-coded by intensity using royal LUT. (b) Small and (c) large example condensates (1.8 M NaCl). Caps and positions of the substrate were fitted using M_2 . (d) Global linear fit (1.8 M NaCl) using M_2 . The inset shows enlargement of point cloud between 0 and 30 μm condensate radius. Condensates shown in panels (b), (c) are indicated with the correspondingly colored diamond. (e), (f) Contact angles for different salinities as calculated with M_1 (e) or M_2 (f). Every dot represents the mean of one image with multiple condensates, mean $\pm$ SD. (g) Top, maximum intensity projections of PEG-rich condensates in 100 mM phosphate buffer of the indicated pH. Middle, representative xz section of the image stack. Bottom, segmentation of the condensates. (h), (i) Contact angles for different pH, analyzed using M_1 in panel (h) or M_2 in panel (i). Every dot represents the mean of one image with multiple condensates, mean $\pm$ SD. Kruskal-Wallis with Dunn's *post hoc* test, * $p < 0.05$, ** $p < 0.01$. Scale bars 50 μm .

The resulting imaging files can be directly analyzed by our MATLAB code. Currently, the code is optimized for Olympus file formats, but this can be easily adjusted as we implemented Bio-Formats in the code [28]. Based on the confocal imaging stacks, we segment all individual condensates and directly measure the moments of their intensity distribution, allowing for a reconstruction of the geometric parameters of condensates without an explicit fit. A self-consistency equation [Eq. (1)] can then be used to obtain an aggregate value of $\cos(\theta_c)$ (global fit) from all condensates present in the sample via a simple linear regression.

A. Analysis of polymeric condensates wetting glass

To test our analysis strategy with experimental data, we used a simple and established condensate-forming system [29,30]. In the presence of phosphate buffer, PEG forms condensates into which the fluorescently tagged BSA protein partitions, allowing to stain the condensates for confocal microscopy [Fig. 2(a)] [31]. We chose to explore two easily adjustable experimental parameters, which we hypothesized to change wetting behaviors of the condensates on glass surfaces: both a change in salinity (achieved by varying NaCl concentration) and buffer pH can be expected to modify

electrostatic interactions between condensates and the surface, which should in turn lead to different wetting outcomes and contact angles.

The first observation upon changing salinity was a huge size heterogeneity with radii ranging from 1 μm to larger than 50 μm . We find that both methods M_1 and M_2 robustly fit both small and large condensates to a spherical cap, as shown exemplarily for condensates from the 1800 mM NaCl sample using M_2 in Figs. 2(b) and 2(c). Figure 2(d) shows the corresponding global fit using M_2 for all condensates extracted from a single image with multiple condensates, confirming that all condensate sizes show consistent, size-independent contact angles. For very small radii ($<5 \mu\text{m}$), we observed some artifact data points that did not significantly influence the global fit. Artifacts can arise due to a variety of factors including uneven dye distribution in condensates, larger condensates that settle in very close proximity to smaller condensates leading to overlapping individual 3D reconstructions or surface pinning effects. The latter effect we primarily observed in samples with very large condensates. Because huge condensates are rarely observed in samples reconstituted using purified cellular components, we are confident that with a larger number of wetting events per image stack, the observed noise can be largely ignored in physiologically relevant samples. Based on several analyzed image stacks per condition, we conclude that salt does not influence the contact angle of PEG condensates (see Figs. 2(e) and 2(f) for the analysis using M_1 and M_2 , respectively).

To test the influence of pH on wetting of the PEG condensates, we used KPO buffers in the range of pH 6.9 to pH 7.8. Interestingly, small pH changes led to pronounced changes in the condensate size distribution, as shown in Fig. 2(g) and Fig. S1 in the Supplemental Material [32]. The more basic the pH, the larger condensates appeared, with sizes up to 100 μm for pH 7.8. We observed no change in wetting between pH 6.9 and 7.2 and a small, nonsignificant increase at pH 7.5 (see Figs. 2(h) and 2(i) for the analysis using M_1 and M_2 , respectively). At pH 7.8, we observed a significant increase in wetting from a $\cos(\theta_c)$ of -0.65 to -0.5 . Since this correlates also with the strong size increase, we ascribe this change in wetting not to the acidity itself, but to gravity effects on these super large condensates. Comparison of the results obtained using M_1 and M_2 for both NaCl concentration [Figs. 2(e) and 2(f)] and pH [Figs. 2(h) and 2(i)] variation shows that both methods deliver the same trends, although M_1 systematically gives slightly larger values of $\cos(\theta_c)$ than M_2 .

B. Method validation using computer-generated data

To verify the accuracy and precision of M_1 [Eq. (7)] and M_2 [Eq. (8)] and to compare their performance quantitatively, we generated wetting condensates computationally, which allowed us to systematically control condensate volume distribution and contact angles. To mimic experimental conditions closely, we also included random noise that is present in all experimental data. We used these computer-generated data to assess systematic errors of our contact angle analysis routine. Of particular interest were inaccuracies that creep into the contact angle data due to the coarse resolution of experimental data along the Z axis.

First, we generated binarized condensates all with the same contact angle θ_c and volumes sampled from a Gaussian distribution $P(V)$ with mean $\langle V \rangle$ and standard deviation σ_V , and placed them at random locations on a virtual substrate within a 3D box (Fig. 3(a) and Figs. S2(a) and S2(b) in the Supplemental Material [32]), ensuring that they do not overlap with each other. To mimic the experimental conditions, we added noise by randomly flipping voxels between 0 and 1, with probability p .

Second, we then applied the same analysis pipeline as for the experimental data [see Figs. 3(b)–3(d)], and compared the $\cos(\theta_c)$ obtained from global fit of the whole sample to the true value that we input in the construction of the computer-generated condensates, for both M_1 and M_2 . The results as a function of the input contact angle $\cos(\theta_c)$ and the Z -plane spacing δz are shown in Fig. 3(e). In particular, Fig. 3(e) shows the global fit results for both M_1 [Figs. 3(e)(i) and 3(e)(ii)] and M_2 [Figs. 3(e)(iii) and 3(e)(iv)]; with the accuracy as measured by the difference between the globally fitted $\cos(\theta_c)$ and the true value shown in Fig. 3(e)(i, iii), and the precision as measured by the standard error SE of the slope

$$SE = \sqrt{\frac{\sum_i (Z_{0i} - \hat{Z}_{0i})^2}{(n-2) \sum_i (R_i - \langle R \rangle)^2}},$$

where $\hat{Z}_{0i}$ represents the prediction of the fit and $\langle R \rangle$ is the average across all data points, shown in Figs. 3(e)(ii) and 3(e)(iv).

The results clearly show that the global fit [Fig. 3(e)] does an excellent job at capturing the true contact angle, particularly for M_1 across all contact angles and for M_2 at low wetting. For strong wetting ($\cos(\theta_c) > 0$) and large Z -plane spacing $\delta z > 1 \mu\text{m}$, however, the results show that M_2 produces strong systematic deviations from the true value, suggesting that M_2 cannot be trusted in this regime. Indeed, because M_2 is based on the comparison of intensity moments (second and third) only along the Z direction, a severe degradation of its performance is expected for strongly wetting condensates that span only a few pixels across the Z direction. Our results also indicate that the global fit method is accurate even at low condensate numbers in the sample, as low as 50, see Fig. S2(c) in the Supplemental Material [32].

C. Tau condensation on charged membranes

To test our analysis on physiologically relevant condensates and surfaces, we reconstituted condensates using the tau protein. Tau is a condensate-forming, primarily positively charged intrinsically disordered and microtubule binding protein, which has been linked to the development of frontotemporal dementia and Alzheimer's disease pathology [13]. In cells, tau and its condensates have been observed in direct proximity to strongly negatively charged microtubules [16,33] and, consistent with this, tau condensates have been shown to wet glass surfaces, which are also negatively charged [15]. Interestingly, tau has been reported to interact with membranes *in vitro* [34] and in cells [35]. Here, we take advantage of the adjustable charge of supported lipid bilayers providing us with a flat membrane model to systematically test the wettability of tau condensates.

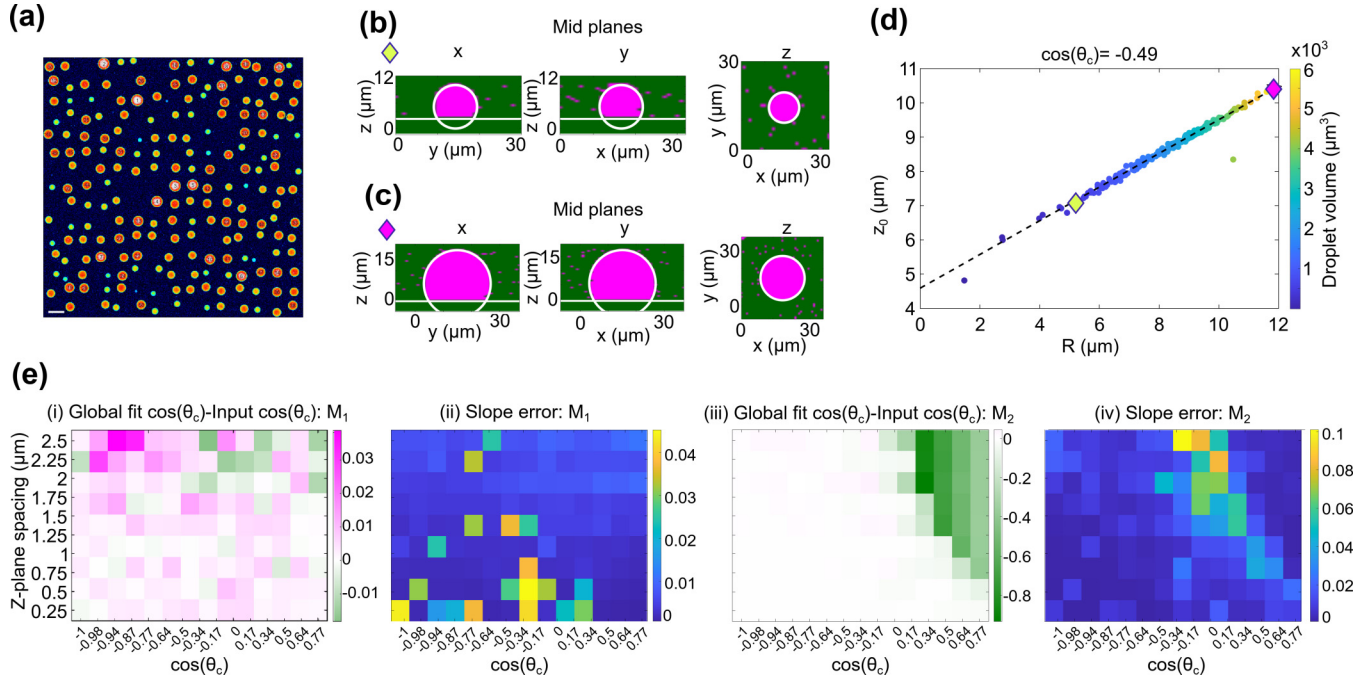


FIG. 3. Method validation using *in silico* condensates. (a) Cumulative intensity projection of a set of 200 computer-generated condensates using an input contact angle of $\theta_c = 120^\circ$ ($\cos(\theta_c) = -0.5$). Scale bar, 35 μm . Representative condensates (magenta) and estimated spherical caps extracted from a small (b) and a large (c) condensate. Caps and positions of the substrate (white lines) were extracted using M_2 . The pink spots outside the condensates originate from the random noise added to the computer-generated dataset. (d) Measurement of $\cos(\theta_c)$ using the global fit method, using data shown in panel (a) and including spherical caps displayed in panels (b), (c) as indicated. Values of R and Z_0 were extracted using M_2 . The color gradient of the data points indicates condensate volume. The output of the global fit is $\cos(\theta_c) = -0.49$. (e) Color maps showing (i) the difference between the estimated and input $\cos(\theta_c)$ values and (ii) corresponding slope error for the global fit, using M_1 , as a function of the input $\cos(\theta_c)$ and the Z -plane spacing δz . (iii) and (iv) show the same analysis for M_2 . Note the stark difference in the limit values of the color scale between (i) and (iii). In all panels, the pixel width in the xy direction is set to $\delta x = \delta y = 1 \mu\text{m}$. In panel (e), 200 condensates were generated with a Gaussian volume-distribution with $\langle V \rangle = 2000 \text{ pixel}^3$ and $\sigma_V = 1400 \text{ pixel}^3$, and random noise was added with $p = 0.03$. Note that the data in panel (e) was generated with equally spaced values of θ_c , and consequently the values of $\cos(\theta_c)$ on the x axis are not equally spaced.

We purified tau from *E. coli* cells, which do not perform any posttranslational modifications on proteins, as previously described [15]. As tau is typically present in cells with posttranslational modifications, we also phosphorylated tau *in vitro* using PKA [11]. To modulate membrane charge, we supplemented zwitterionic POPC bilayers with different percentages of negatively charged POPS lipids or positively charged DOTAP lipids, see Fig. 4(a). Tau condensate wetting on positively charged membranes is shown in Fig. 4(b), while wetting on neutral to negatively charged membranes is shown in Fig. 4(c). Visual inspection indicated that tau condensates wet highly positively and negatively charged membranes with a lower contact angle than charge-neutral membranes, because condensates formed under similar conditions occupied a larger membrane fraction. Despite the fact that phosphorylation can be expected to reduce the positive tau charge (the net charge of unphosphorylated tau at pH 7.4 is +3) by introducing negative charges, we noticed a similar trend for PKA-Tau. Contact angle measurements confirmed these observations; see Fig. 4(f). Consistently, both methods M_1 and M_2 quantify a lower $\cos(\theta_c)$ (higher contact angle, i.e., less wetting) for PKA-Tau compared to the nonphosphorylated tau.

Our analysis method automatically extracts the substrate position Z_{sub} using the global fit. Fluorescent labeling of the

SLBs allows us to independently determine the position of the membrane by fluorescence microscopy. To ensure that our analysis pipeline correctly extrapolates the substrate location, we compared the theoretical and experimental values finding that they show a strong linear relation with unit slope, as expected, although the theoretical values show a small, albeit systematic, overestimation of the substrate height relative to the experimental values, see Fig. 4(d). This independent confirmation gives further confidence on our automated pipeline working appropriately. We note that, in the experiments, the origin of the Z coordinate corresponds simply to the bottommost Z slice, and is arbitrary, typically several μm below the substrate. The values of Z_{sub} that we estimate and measure are thus of the order of several μm .

In Fig. 4(e) representative condensates for uncharged, +15 and +25 charged membranes are shown. Condensates showing little wetting on uncharged and +15 membranes were fitted using M_2 , while the condensate on +25 charged membranes was characterized by high wettability and fitted using M_1 . In combination, these methods reliably fit both weakly and strongly wetting condensates. The different performance of the two methods becomes clear when looking at the analysis of the dataset using both methods, see Fig. 4(f). Comparison of the two methods shows that, as in the case of

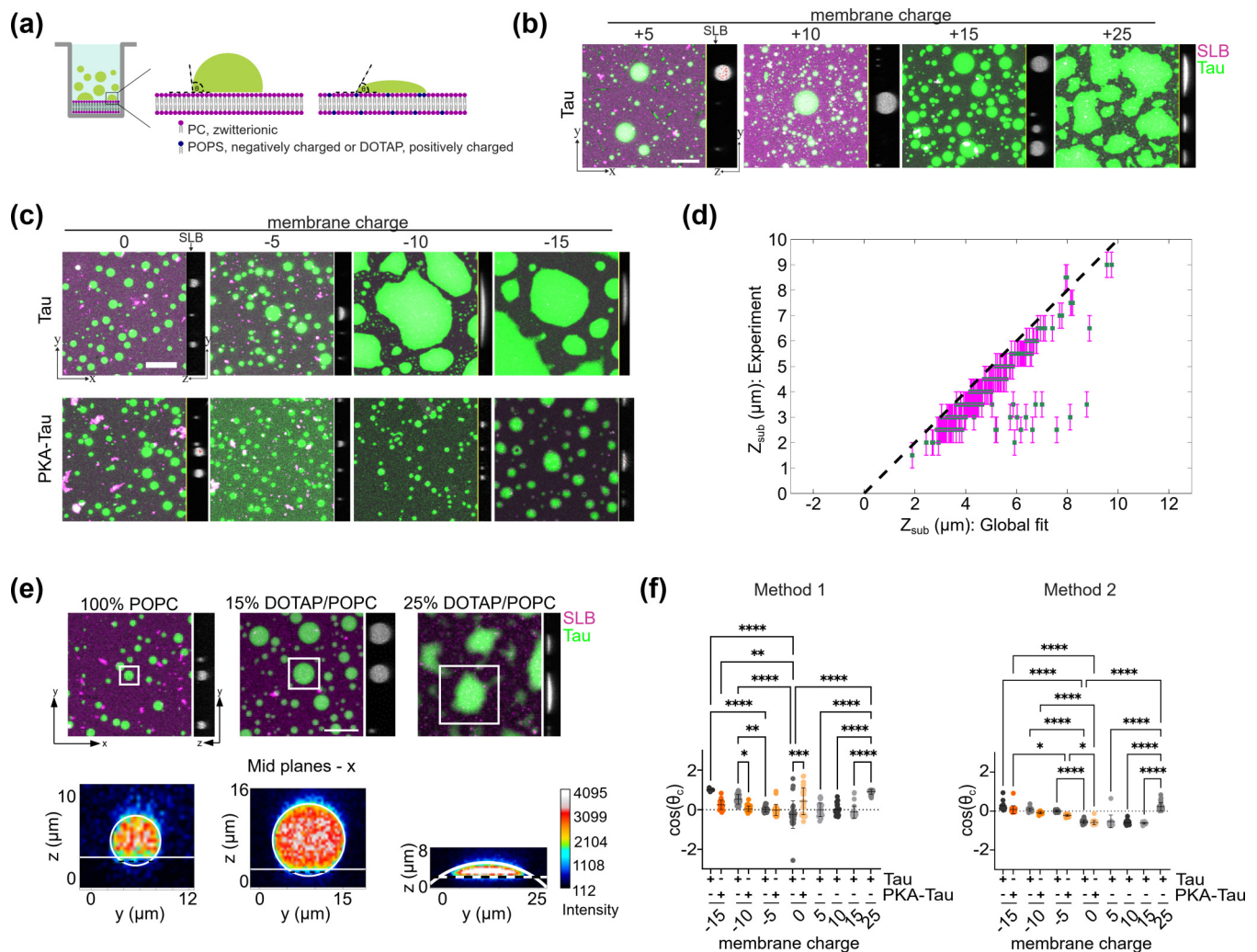


FIG. 4. Wetting of tau condensates depends on membrane charge and tau phosphorylation state. (a) Experimental setup: Condensates reconstituted from purified tau protein (green) sedimented on supported lipid bilayers (magenta) consisting of zwitterionic POPS supplemented with varying percentages of positively charged DOTAP or negatively charged POPS and a minor fraction of the fluorescent lipid DiI-C18. (b) Tau condensates on SLBs containing the indicated DOTAP fraction. Representative confocal images. (c) Tau and phosphorylated tau (PKA-Tau) condensates on SLBs with the indicated amount of negatively charged POPS. Experiments in panels (b), (c) were performed simultaneously and hence were also analyzed in parallel. (d) SLB position is consistent between experiment (using fluorescent lipids) and condensate analysis (global fit). The dashed line represents the ideal case without any deviation between experimental and theoretical values. (e) Spherical caps extracted from different wetting stages. Top, contrast-adjusted images shown in panel (c) with single condensate highlighted. Bottom, enlarged single condensates with estimated cap and substrate location (white lines). Condensates with SLBs containing 100% PC and 15% DOTAP were estimated using M_2 , the condensate on 25% DOTAP SLB was estimated using M_1 . (f) Result summary using M_1 (left) and M_2 (right). Each dot represents the mean of one image with multiple condensates as shown in panels (b), (c), $n > 5$. Kruskal-Wallis with Dunn's *post hoc* test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Scale bars 20 μm .

polymeric condensates on glass substrates, both methods deliver the same trends, although M_1 systematically gives slightly larger values of $\cos(\theta_c)$ than M_2 . Additionally, in line with the discussion of the strengths and weaknesses of both methods in Sec. III 2, M_1 shows a bigger spread of the data in low wetting conditions, while M_2 conversely shows greater spread in stronger wetting situations (cf. also Fig. S3 in the Supplemental Material [32]).

Reassuringly, with both methods we see significant differences both between different membrane charges for tau, and between tau and PKA-Tau. With increasing membrane charge both into positive and negative directions, we get an increase in $\cos(\theta_c)$ from -0.55 (M_2) to $\cos(\theta_c) = 1.00$

(membrane charge -15) or 0.90 (membrane charge $+25$) (M_1). The $\cos(\theta_c)$ values for PKA-Tau were significantly lower than those for tau and did not deviate strongly from a $\cos(\theta_c)$ of around 0, corresponding to a contact angle of 90° and hence intermediate wetting. This is in line with the visual results presented in Figs. 4(b) and 4(c). Phosphorylation of tau by PKA leads to both smaller, more homogeneously sized condensates, as well as clearly decreased wetting, especially on charged membranes. While it is surprising that tau wetting does not differentiate between positive and negative charge, it is feasible that wetting on negatively charged membranes is decreased as a result of the added negative charges on the protein which, depending on the condensate

organisation, potentially leads to repulsive electrostatic forces between membrane and condensate.

IV. DISCUSSION

We have developed and validated a novel method to analyze condensate wetting on flat substrates, which allows automated segmentation of confocal image stacks with multiple objects. In comparison with other methods in the literature that extract contact angles from wetting condensates [19–21,23], our method has several advantages. Because the method is based purely on volumetric moments rather than on a fitting of the condensate surface, it does not require identification of the condensate surface nor knowledge of the substrate location and it is less computationally expensive as it does not depend on additional optimization steps. Together with the self-consistency equation (1), which allows for a global fit to all condensates in each sample, our method can robustly extract contact angles and substrate location even for conditions in which condensates are only a few pixels in diameter, in a fully automated way.

One critical experimental limitation is the need for the condensate to be labeled fluorescently. Depending on the composition of the condensate, this can be done by including a minor fraction of fluorescently tagged proteins, or by partitioning of fluorescent dyes such as FITC and rhodamine derivatives into the condensates. We tested our method with three very different samples: a polymer-based condensate on a glass surface, a physiologically relevant protein condensate on a supported lipid bilayer, and a computer-generated dataset of wetting condensates. In all cases, our method reliably recognized the condensates and calculated the contact angle resulting from their wetting geometry.

We observed that posttranslational modifications of tau significantly change its wetting behavior. The most obvious explanation for this result is that phosphorylation adds negative charges to the mainly positively charged tau protein,

particularly on serine and threonine residues in the positively charged microtubule binding region and its flanking regions. However, as wetting of the tau condensates was also high on strongly positively charged membranes, there may be other additional contributions. Intracondensate organization has been shown to influence wetting characteristics [36]. As the intracondensate organization of tau condensates is largely unknown, it would be interesting to investigate its modulation by posttranslational modifications.

Due to its compatibility with a wide range of reaction volumes, multiwell formats and imaging platforms, our analysis approach is suited for implementation in high-throughput screening formats. Thus, it provides the basis for a systematic and quantitative mapping of wetting behavior by independently tuning condensate, solution and membrane physicochemical parameters across a broad parameter space. The resulting multidimensional “wetosome” dataset would catalog the conditions under which wetting emerges and present a quantitative framework to assess the generality of condensate wetting phenomena. Combined with data-driven analysis, this resource would allow the extraction of predictive relationships and physical constraints governing condensate wetting, thereby contributing to a quantitative description of the molecular grammar underlying wetting phenomena in cells.

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DATA AVAILABILITY

The data and code that support the findings of this article are openly available [37].

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