Supplementary Materials 1. Methods for surfactant critical micelle / micellar concentration (CMC) determination

The surfactant CMC determination methods were collected from ISI data based from 1962-2010 and were classified as: 1) spectroscopic measurements using a fluorescence probe, [1-24], an absorbance dye [2, 25-37], and other probes; [25, 38-42] 2) electrochemical measurement using electrophoresis or capillary electrophoresis, [43-60] conductimetric; [22, 32, 61-72] 3) surface tension measurements, [29, 40, 67, 73-78] and contact angle measurement; [79] 4) optical measurements using light scattering, [71, 80-84] optical fibers [85-91], and refractometric; [92] 5) other methods such as ITC, [93-96] chromatography, [97] ultrasonic velocity [98], and others; [14, 99-114]. Computational modeling and predictions were also used for surfactants CMC studies. [115-148]

Supplementary Materials 2. Absorbance data workup for samples prepared in 96 well plates and then transferred to the odd and even wells of a 384 well-plate

Signals were highly reproducible between independent measurements for a given instrumental setting and microplate type, but were not uniform and showed positional dependence across the entire plate, causing a systematic error. We noticed that the signals were uniform within any group-of-12 wells at either even or odd positions of a 24-well-row in Greiner 384-well plates using the M5 plate reader, but were significantly different between these groups, attributed to the systematic error. Accordingly, an empirical protocol was developed in which the samples for repetitive measurement were prepared by serial dilution of surfactant using a 96-well plate contained references (1 mM Triton X-100 or buffer), and then direct transfer into a 384-well microplate for measurement. The 384-well microplate contained references for both the odd and even group-of-12 and measured signals were normalized by taking the ratios of the sample signals to the reference signals to adjust for systematic errors. The protocol enabled us to get highly reproducible signal over the whole plate if the same reference was used throughout the measurement. Different instrumental settings may give a different signal pattern and a similar strategy can be applied accordingly.

Supplementary Materials 3. Geometric path of refracted light

Rather than a simple lens-like effect as proposed by Cottingham,[149] the absorbance signals observed in a plate reader with a vertical detecting light beam, striking the curved liquid surface off the well center,

is a result of a two-step refraction. The light first refracts into the liquid and changes direction toward the edge of the well, a fraction may hit the wall of the well; the rest that passes through the liquid undergoes a second refraction and comes into air again with an additional direction change, and may partly be out of the range of the detector. The refractions also split the light with a fraction reflected backward. All of these contribute to the loss of detectable light and result in the absorbance-like signal which is dependent on liquid surface curvature and the width of the detecting light beam. In an instrument with a detecting light beam width comparable to the dimensions of the plate well, as is the case in the M5 plate reader, continuous liquid surface curvature change causes a gradual lost of light reaching the detector, resulting in a gradual absorbance change, which can be quantitatively calibrated to the liquid surface curvature. The quantitative relationship between absorbance value and liquid surface curvature can be deduced from Figure 1C based on the above analysis, although it is dependent on plate reader, microplate type, and instrumental settings.

Because of gravity and geometric restraints, R is not uniform across the liquid surface, but is approaching a uniform value in thinner cylindrical wells. n1 and n2 are constant; offset and h2 are instrumental constants; h1 is insensitive to liquid curvature change when offset is ~0.7 of well radius and can be considered as constant for the same liquid volume. The relationship between shift and R is quantitatively established by satisfying the following equations:

$\sin(\alpha) = \text{offset}/R;$

$\sin(\alpha)/\sin(\beta) = n2/n1;$

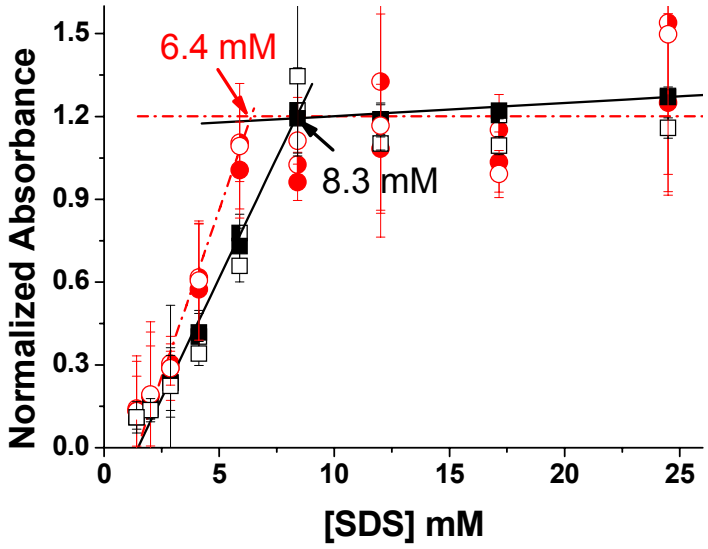
$\gamma = \alpha - \beta;\ \sin(\gamma)/\sin(\theta) = n1/n2;$

$d1 = h1*\tan(\gamma);\ d2 = h2*\tan(\theta);$

$\text{shift} = d1 + d2.$

The projection of a detecting light beam on the detector plane can be obtained by integrating the calculated light spots. The calculation predicts a certain degree of defocusing of the light beam and is independent of offset with constant R. Light also splits upon refraction with a fraction reflecting backwards. This contributes to additional detectable light loss which is also dependent on R and offset and is defined by Fresnel Equations (detailed reference at http://en.wikipedia.org/wiki/Fresnel equations). If the ratio of light spots falling within the detector range was set as $\eta1$, the ratio of light that is not reflected backward during the two refractions as $\eta2$, $\eta3$ respectively, the absorbance value contributed by the curved liquid surface is equal to $\log(1/\eta1*\eta2*\eta3)$.

Supplementary Materials 4. Determination of the CMC value from the inflection points of the surfactant concentration vs. absorbance curves, and the effect of on-site dilution in the 384-well microplate on the CMC determination.

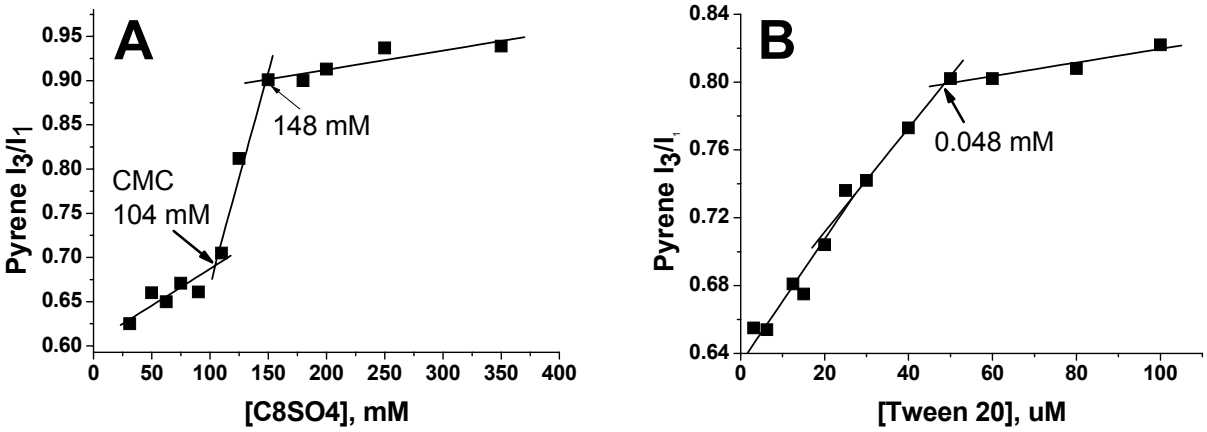


The figure shows the concentration-absorbance dependent curve of SDS with different measuring conditions. The inflection point in the curve was obtained from the intersection between straight lines obtained by fitting the data points on either side of the inflexion point. Samples dissolved in water were serially diluted in the 384-well microplate (circles); or serially diluted in the 96-well plate and then directly transferred into the 384-well microplate (squares). The open, half filled and filled symbols correspond to 384-well microplate measurements after the sample was incubated for 15, 60, and 150 minutes, respectively. The larger noise and longer stabilization time for samples prepared by 384-well on-plate dilution were due to the bubbles introduced during the dilution. The lower CMC value may be due to incomplete delivery of the small sample volume during the dilution; using longer and sharper tips may improve the measurement. Pre-dilution in 96 well plates used a larger sample volume and was therefore subject to less relative sample loss in the tips.

Supplementary Materials 5. Measurement of CMC using pyrene as a fluorescent probe.[24]

The method used followed closely that described by Kalyanasundaram and Thomas. Fluorescence measurements were carried out on a Perkin Elmer LS55 Fluorescence Spectrometer with excitation wavelength of 334 nm and the emission spectrum was recorded from 350 to 450 nm; the

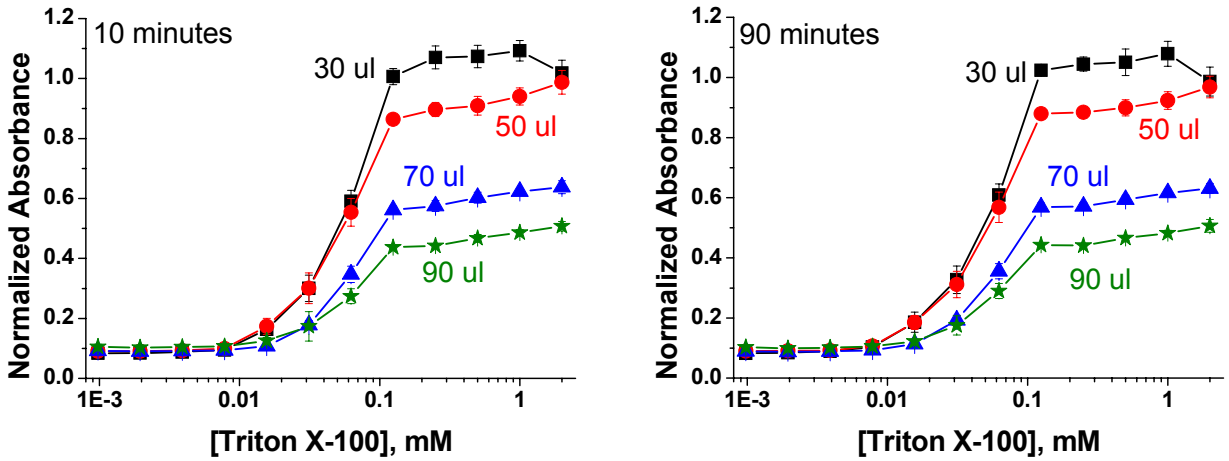
excitation/emission slits were set as 3/3 mm (the lowest allowed setting is 2.5/2.5 mm). Before each measurement, 1.5 µl of 0.2 mM pyrene dissolved in ethanol was added into 1 ml surfactant sample, mixed, and the fluorescence spectrum recorded immediately. The fluorescence intensities of the peaks at ~372 nm (I_1) and ~383 (I_3) were extracted from the spectra, and the I_3/I_1 value vs. surfactant concentration was used for CMC determination. Two inflection points were observed for most surfactants (Figure A), except Tween 20 which showed only one obvious inflection point at higher surfactant concentration (Figure B). The lower inflection point was selected as the CMC, as reported. The samples for fluorescence measurement were recovered and the same samples were subjected to absorbance measurements for comparison of the two methods, so that the samples in both measurements contained 0.3µM pyrene and ~30 mM ethanol.



Supplementary Materials 6. Comparison with literature values of CMC

The literature reported CMC values for the same surfactant displayed a wide-range because different experimental conditions were used. Our measured CMC values fall in the reported ranges. CMC dependence on different buffer and measurement conditions are well documented, [149-179] and detailed analysis of the variations will be the subject of further study.

Supplementary Materials 7. The CMC values are independent of sample volume used in the measurements.



The figures show the absorbance-concentration dependent curve of Triton X-100 solution dissolved in 50 mM phosphate buffer, pH 7.0. The sample volumes used in a 384-well microplate were 30, 50, 70, 90 µl per well. For comparison of the effect of sample volume on dynamic range, a reference of buffer without surfactant was used. The volume of samples used in the measurement had an effect on the dynamic range of the measured signals, but did not affect the inflection point in the curves, or the measured CMC value. Signals remained unchanged for > 90 minutes.

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