Supplementary information: Structure, dynamics and phase behavior of short rod inclusions dissolved in a colloidal membrane

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Analyzing fluid-fluid interface fluctuations with fluorescence microscopy: We describe the algorithms used to analyze the fluctuations of the fluid-fluid interfaces – *fd wt* and *litmus*, *fd*-Y21M and *litmus*, and *litmus* outer edge – shown in Fig. 7. In all cases, the principal challenge is to locate the edge of a region of fluorescently labeled rods with subpixel precision. Our technique is schematically illustrated in Fig. S1. Our analyses use custom Python code that draws heavily on the *numpy* and *scipy* libraries. A portion of a raw fluorescence image of the membrane shown in Fig. 7b is shown in Fig. S1a; the analysis of the membrane in Fig. 7a is similar. First, we calculate the magnitude of the gradient of the raw image by applying Sobel operators (Fig. S1b). This gradient magnitude image is large at the edges of the bright fluorescent region in the original image. Second, we use Canny edge detection[29] to roughly locate the fluorescent edges in the raw image to within a pixel. We briefly summarize the Canny edge detection algorithm. The original image is smoothed with a Gaussian filter, and then the horizontal and vertical components of the gradient of the smoothed image are computed using Sobel operators. A set of candidate edge pixels is then determined by choosing pixels in the magnitude of the gradient of the smoothed image that exceed a threshold value. Since the set of candidate edge pixels is usually more than one pixel thick, a non-maximum edge suppression scheme then retains only candidate edge pixels that are local maxima along the direction of the gradient at that pixel. Occasionally, small clusters of spurious pixels remain. We remove the spurious pixels by eliminating any pixels that are not directly connected to at least one pixel whose gradient magnitude exceeds a second, higher threshold value. The result is a one-pixel-thick set of edge points (Fig. S1c).

Third, we refine the edge locations. For each of the two edges (*fd*-Y21M and *litmus*, and *litmus* outer edge) in Fig. S1c, we first fit cubic splines with a smoothing parameter to the Canny edge points (Fig. S1d). The smoothing parameter allows the spline curves to not pass exactly through the Canny edge points. Then, we proceed along each rough spline curve in arc length increments of one pixel width. At each location along a rough spline curve, we examine the intensity of the gradient magnitude image along a direction perpendicular to the rough spline curve, using linear interpolation to determine the gradient magnitude at any points that do not correspond to an exact pixel location. We fit Gaussians to the resulting cut through the gradient magnitude to determine the location of the maximum to subpixel precision (Fig. S1e). This results in a set of refined edge points (Fig. S1f).

Finally, we fit a new set of cubic splines with no smoothing to the refined edge points. This refined spline curve is constrained to pass through all of the refined edge points and facilitates the computation of the tangent angles to the edge. The averages of the squares of the Fourier components of these tangent angles lead to the fluctuation spectra shown in Fig. 7c. The fluctuation spectra are not sensitive to the exact values of the empirically-determined parameters used in this algorithm (namely, the Canny thresholds, Gaussian filter width, and cubic spline smoothing parameter).

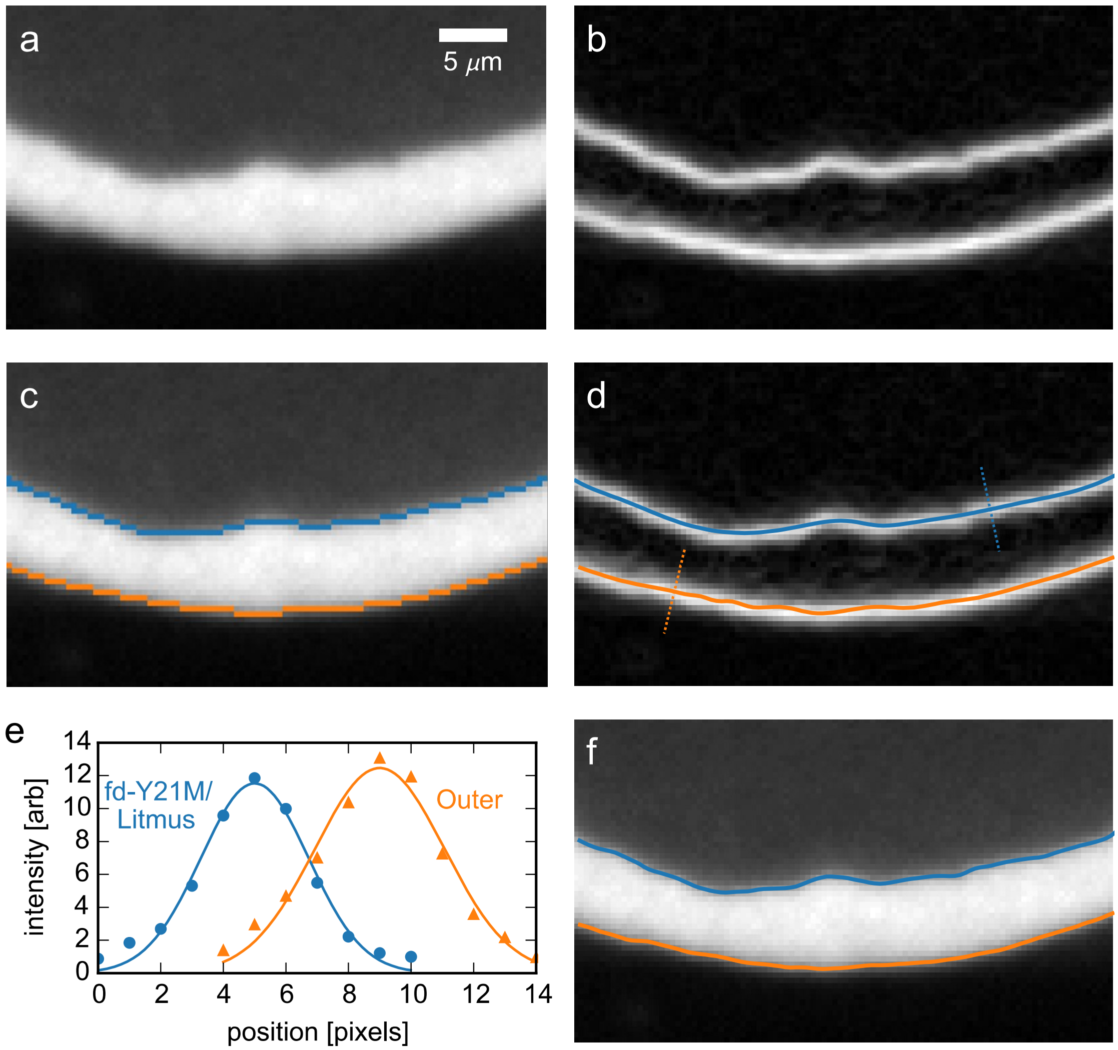

Figure S1: Algorithm used to locate fluorescent edges with subpixel precision. (a) Portion of a raw fluorescence image of a membrane containing both *fd*-Y21M and *fd-litmus* rods; the *litmus* rods are fluorescently labeled. (b) Gradient magnitude of the image in (a). (c) Rough edge points for *fd*-Y21M/*litmus* interface (blue) and *litmus* outer edge (orange) from Canny edge detection algorithm. The rough edge points are overlaid on the raw fluorescence image. (d) Rough cubic splines fit to rough edge points. The rough spline curves are overlaid on the gradient magnitude. (e) Gradient magnitude intensity along the dotted lines in (d). Solid points: gradient intensity; solid curves: best-fit Gaussians. Points corresponding to the *litmus* outer edge are horizontally offset for clarity. (f) Refined edge cubic spline curves overlaid on raw fluorescence image.