

Structured and Intrinsically Disordered Domains Within Amphiphysin1 Work Together to Sense and Drive Membrane Curvature.

Wade F. Zeno^a, Wilton T. Snead^a, Ajay S. Thatte^a, and Jeanne C. Stachowiak^{a,b}

^aDepartment of Biomedical Engineering, The University of Texas at Austin, Austin, TX 78712; ^bInstitute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX 78712

Correspondence and requests for materials should be addressed to J.C.S. (email: jcstach@austin.utexas.edu)

SUPPLEMENTARY INFORMATION

Supplementary Figures

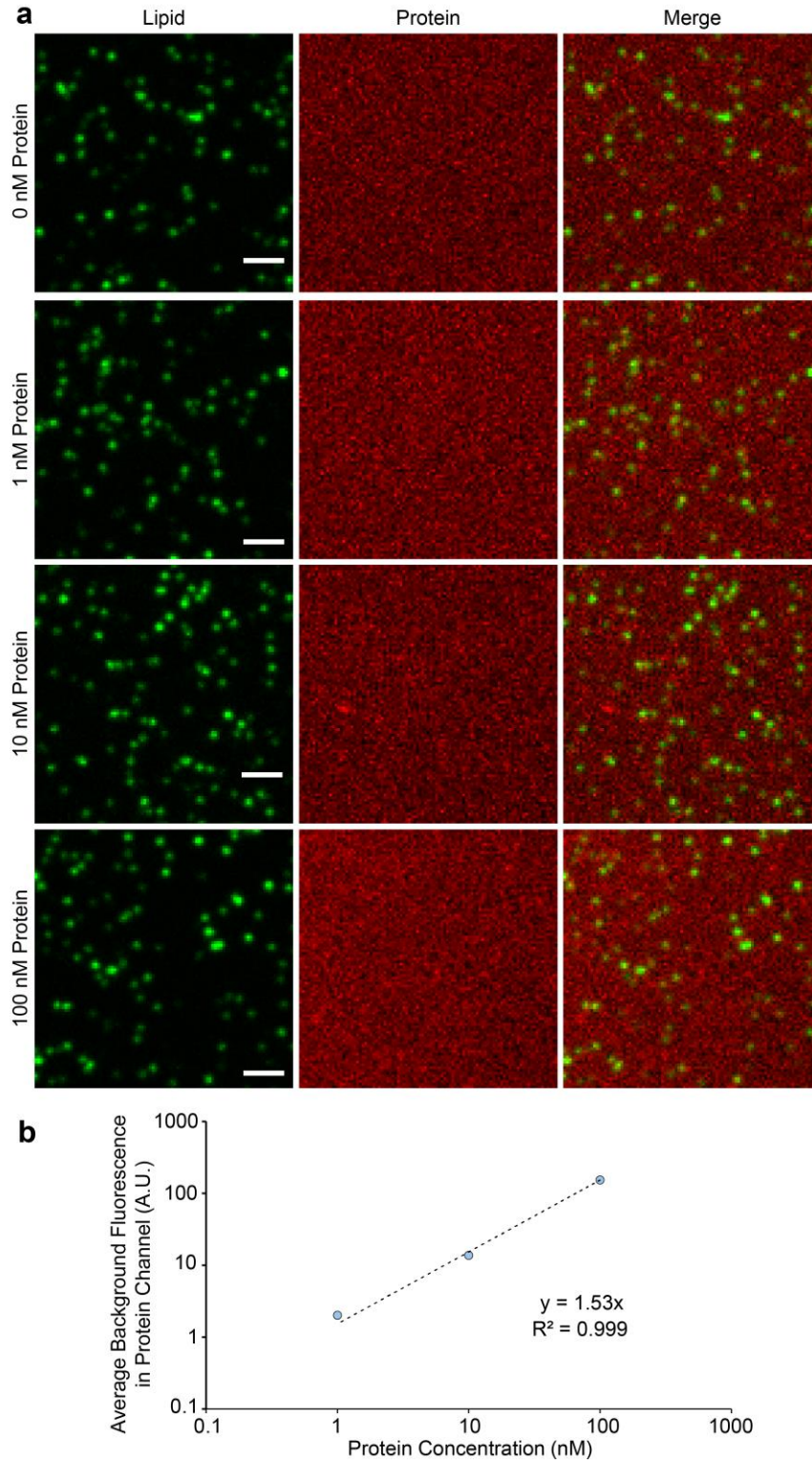


Figure S1: The C-terminal domain of Amphiphysin1 does not bind to the membrane directly. (a) Images of vesicles that were tethered and incubated with 0, 1, 10, or 100 nM AmphCTD. Fluorescence intensities in the protein channels were adjusted to achieve maximum contrast (i.e. to promote the best visualization of bound protein). **(b)** The average fluorescence background in the protein channel as a function of solution

concentration. The linear increase in intensity indicates an increase of fluorescent protein concentrations in solution. Scale bars in **a** represent 2 μm .

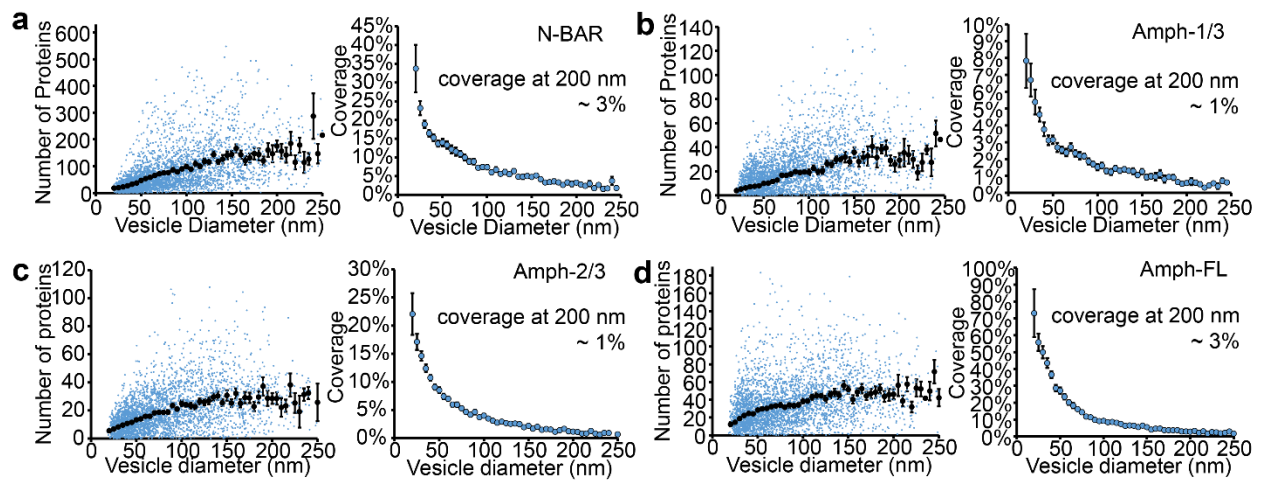


Figure S2: Raw data for protein binding to vesicles. Average number of membrane-bound proteins (left) and percent coverage of the membrane surface by proteins (right) for (a) N-BAR, (b) Amph-1/3, (c) Amph-2/3, and (d) Amph-FL. In the left panels, blue dots correspond to individual vesicles and black dots correspond to the moving average. Error bars correspond to the standard error of the mean within each bin. Coverage corresponds to the percentage of the vesicle surface area occupied by proteins. Coverage was calculated using the moving average data from the corresponding left panel. Coverage is equal to $(\text{number of proteins}) \times (\text{projected area of a single protein on the membrane surface}) / (\text{surface area of the spherical vesicle})$. A projected area of $23.5 \text{ nm}^2/\text{monomer}$ was used for N-BAR.¹ Projected areas of $25 \text{ nm}^2/\text{monomer}$, $50 \text{ nm}^2/\text{monomer}$, and $75 \text{ nm}^2/\text{monomer}$ were used for Amph-1/3, Amph-2/3, and Amph-FL, respectively, based on measurements and polymer scaling approximations performed previously.²

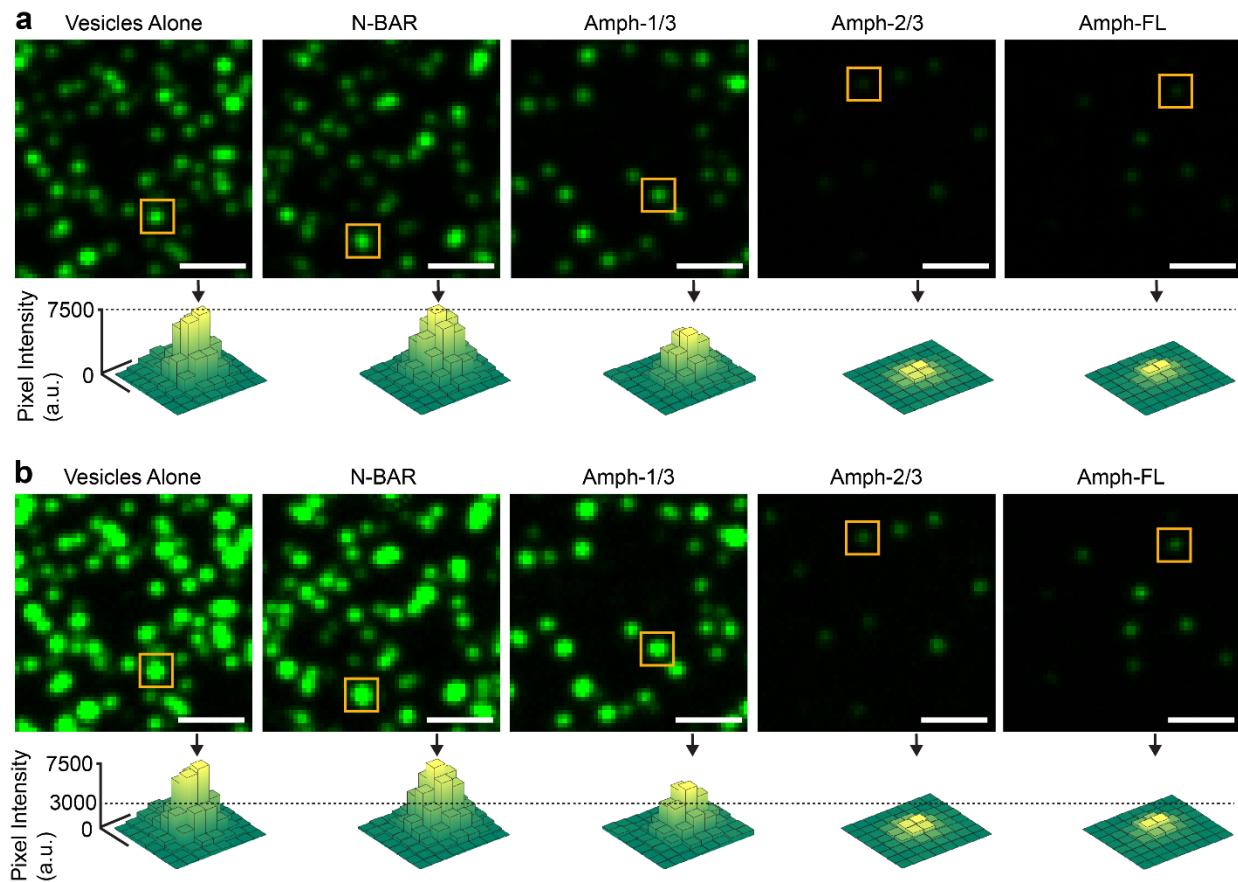


Figure S3: Fluorescence images of tethered vesicles with constant intensity settings. Images from Figure 2a with the pixel intensity scales held constant throughout each picture. Pixel intensity scales were set from (a) 0 – 7500 (a.u.) and (b) 0 – 3000 (a.u.). Scale bars represent 2 μm .

Supplementary References

1. F. Campelo, H. McMahon and M. Kozlov, *Biophysical Journal*, 2008, **95**, 2325-2339.
2. W. F. Zeno, U. Baul, W. T. Snead, A. C. DeGroot, L. Wang, E. M. Lafer, D. Thirumalai and J. C. Stachowiak, *Nature communications*, 2018, **9**, 4152.