Supplementary Information

How membrane geometry regulates protein sorting
independently of mean curvature

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The supplementary information contains:

46 pages
8 figures
2 tables
Here we introduce to the geometry of curved lipid bilayers and explain in detail how we use a molecular field theory to calculate the physical properties of curved lipid bilayers, with a special focus on the importance of Gaussian curvature on protein discrimination between cylindrical and spherical membrane curvature.

Introduction to the geometry of curved lipid bilayers. Our brief discussion of the curvature concepts used throughout this work is best initiated by considering a simple planar curve in two dimensions. Every point on any two-dimensional surface can be characterized by a local curvature tensor, and for the simple case of a planar curve in two dimensions, the curvature tensor is a scalar with a magnitude equal to the inverse radius of a circle that locally follows the curve at the point of interest. Figure 6 contains a planar curve that we will use to highlight these key concepts. A tangent line, labeled T, is drawn through the point of interest, labeled P. We will introduce a coordinate system labeled as $x$ that is zero at P. The curve can be fully described by determining the function $h(x)$ that is a measure of the height of the curve above T. We will write the function $h(x)$ as a Taylor series expansion through $h(x_0) = 0$ as follows,

$$h(x) = \left( \frac{d^2 h}{dx^2} \right)_{x_0} \frac{x^2}{2} + ..., \quad (IV)$$

knowing that the first derivative is zero since the coordinate runs along the tangent to point P,

$$\left( \frac{dh}{dx} \right)_{x_0} = 0.$$

Now consider a circle of radius $r$ tangent to T and point P. We can relate the coordinate $x$ to the angle, $\theta$, measured with respect to the normal vector at P pointing toward the center of the circle, with the
following expression from trigonometry, $x = r \sin \theta$. For small angles, we can expand sine to second order and show that $\theta = \frac{x}{r}$. Using the circle to obtain another expression for $h(\theta)$, we get $h(\theta) = r(1 - \cos \theta)$. By expanding the cosine function to second order in the new expression for $h(\theta)$ and using the connection between the angle and $x$, we get the following for $h(x)$,

$$h(x) = \frac{1}{r} \frac{x^2}{2} \quad (V)$$

Comparing this expression to the Taylor series expansion for $h(x)$, we see that $\left( \frac{d^2 h}{dx^2} \right) = \frac{1}{r}$, and this provides us with an intuitive concept for the curvature at point P being equal to $1/r$, where $r$ is the radius of a circle that locally follows the curve at P.

A similar analysis applies to a two-dimensional surface $S$ in three dimensions. Now, at any point P on the surface we have a tangent plane instead of a tangent line that we had for the planar curve. Let $x_1$ and $x_2$ be coordinates in an orthogonal coordinate system on the tangent plane measured with respect to an origin at point P (this is analogous to the definition of $x$ in the planar curve system). The height of the surface above the tangent plane is then written as

$$h(x) = \frac{1}{2} c_{i,j} x_i x_j, \quad (VI)$$

where $c_{i,j}$ are the components of a 2x2 real symmetric tensor $c$, which has two real eigenvalues $r_1^{-1}$ and $r_2^{-1}$ and associated orthonormal eigenvectors in the tangent plane. $r_1$ and $r_2$ are called the principle radii of curvature of the surface at P and correspond, respectively, to the radii of circles in the directions of the eigenvectors. It can be shown that the principle radii of curvature correspond
to the maximum and minimum curvatures of all of the normal sections through P that can be generated
by rotating the axis of the coordinate system in the tangent plane around the point P.

There are two scalar invariants of the curvature tensor that can be used to completely characterize the
curvature at a point P on a two-dimensional surface $S$. They are the trace ($Tr$) of the tensor and the
determinant (det) of the tensor defined as follows,

$$ Trc = r_1^{-1} + r_2^{-1} = 2H \quad \text{and} \quad \det c = \frac{1}{r_1 r_2} = K, \quad (VII) $$

where $H$ is defined as the mean curvature and $K$ is defined as the Gaussian curvature. There are two
geometries of primary interest in this work, and those are the spherical surface and the cylindrical
surface. Both the sphere and the cylinder have constant values for their mean and Gaussian curvatures
over their entire surface. The mean curvature for a cylinder is $H_{cyl} = \frac{1}{2r_{cyl}}$, and the mean curvature of
a sphere is $H_{sph} = \frac{1}{r_{sph}}$. If one were interested in comparing a sphere and a cylinder with the same
mean curvature, then the radius of the cylinder would be half the radius of the sphere. The Gaussian
curvature of a cylinder is $K_{cyl} = 0$, and the Gaussian curvature of a sphere is $K_{sph} = \frac{1}{r_{sph}^2}$.

There are two common reasons for the dismissal of the importance of the Gaussian curvature in the
physics of curvature sensing, and these two reasons are the Gauss-Bonnet theorem and the relative
magnitude of the Gaussian curvature term in relation to the mean curvature.
Let us first focus on the Gauss-Bonnet theorem. This is a very important result in the field of differential geometry and mathematically can be written as follows\textsuperscript{39,40},

$$\int \int K dS = 4\pi (1 - g), \quad \text{(VIII)}$$

where the integral is of the Gaussian curvature over the surface $S$, and $g$ is an integer called the genus that is equal to the number of holes in the surface. $g$ is equal to 0 for spheres and $g$ is equal to 1 for the cylinders studied in this work. In other words, the integral over a surface is a topological invariant, so as long as holes in the surface are not created or destroyed during the bending process then the terms related to Gaussian curvature can be neglected. There are three major issues with dismissing the importance of the Gaussian curvature in protein curvature sensing using the Gauss-Bonnet theorem, even when the genus is the same, when the curvature is high enough. The first is the issue with only using the lowest order expansion of the free energy for all curvatures. The traditional Helfrich energy is expanded to second order in mean and first order in Gaussian curvature, and this expression is what is tied with the Gauss-Bonnet theorem to disregard the Gaussian curvature; however, when the largest of the two radii of curvature is lower than 150 nm for a given geometry, we find that the traditional Helfrich energy expanded to second order is insufficient to capture the energy of curvature. In other words, higher order curvature terms are needed for physiologically relevant smaller radii of curvature, and neglecting the Gaussian curvature due to the Gauss-Bonnet theorem is inappropriate.

The second issue can be explicitly shown in the volume of the outer leaflet as a function of curvature,

$$V_{\text{outer leaflet}} = A(0) \left[ I + HI^2 + \frac{1}{3} KI^3 \right]. \quad \text{(IX)}$$
where \( A(0) \) is the area of the midplane, and \( l \) is the height of outer leaflet above the bilayer midplane (the magnitude is on the order of 1 nm). The volume of the outer leaflet is shown below to be critical in the physics of curvature sensing. When the magnitude of \( K \) is small enough, the contribution of the Gaussian curvature to the outer leaflet volume is negligible, and this agrees with the conclusion drawn from the Gauss-Bonnet theorem coupled with the lowest order Helfrich expansion. So, as long as the curvature is high enough, equation (IX) clearly shows that the Gaussian curvature cannot be neglected due solely to the Gauss-Bonnet theorem. The third issue is seen in equation (VIII) that the theorem is utilized in bilayer mechanics when integrating over the entire closed surface of the membrane system. A theoretical description of the insertion of membrane binding domains into localized regions of a lipid membrane does not require an integration of the entire closed surface of the membrane.

As you can see by the definition of the Gaussian curvature, it is tempting to just ignore its contribution due to the Gaussian curvature having such a relatively small magnitude when compared to the mean curvature for all but very small radii of curvature. However, as we show below and in the main document (Fig. 3 and Supplementary Fig. 7) Gaussian curvature becomes an important metric to include in the physics of curvature sensing for radii of curvature below 150 nm, which is very relevant for curvatures of lipid bilayers in cells.

**Elucidating the importance of Gaussian curvature on protein discrimination between cylindrical and spherical membrane curvature using a molecular field theory.** In the next few sections we will describe the molecular theory that we use to determine the physical properties of curved lipid bilayers that are important for protein recruitment by cylindrical and spherically curved membrane
geometries. In particular, we will be calculating the lateral stress profiles across the membrane, the lipid area per molecule in both leaflets, the electrostatic potential, the distribution of hydrophobic interactions, and the protein density profiles for lipid membranes of cylindrical and spherical geometry. By doing so, we aim to elucidate how Gaussian curvature contributes to the thermodynamics of protein binding as described in the sections below.

Calculating the physical properties of curved lipid bilayers. The basic concept of the theory is to consider each possible conformation of the lipids and formulate a free energy in terms of the probability of each of those conformations. The minimal free energy provides us with an explicit expression for the probability of each molecular conformation in terms of the intermolecular and intramolecular interactions. We consider a fluid lipid bilayer of hydrophobic thickness equal to $2 \cdot l$, where $l$ is the monolayer, or leaflet, fatty-acid chain thickness. The bilayer studied in this work is composed of lipids that contain two mono-unsaturated tails of equal length (18 carbons) with a single $\text{cis}$-double bond between the 9th and 10th carbons to model the fatty-acid chains in DOPC. The conformations of the chains are described by Flory's Rotational Isomeric States model\textsuperscript{41-43} in which each CH$_2$ group is in one of three configurations: the lowest energy $\text{trans}$, or the $\text{gauche-plus}$ or $\text{gauche-minus}$, both of which are of an energy, 500 cal/mol, greater than that of the $\text{trans}$ configuration. The lipid bilayer is in contact with a salt solution of NaCl in water. The water is treated as dissociable from the neutral H$_2$O into H$_{\text{(aq)}}$ and OH$_{\text{(aq)}}$, each of the three with a volume of 0.03 nm$^3$. It is convenient to define a local volume fraction, $\phi_i(z)$, which is a function of position, $z$, and is related to the local number density, $\rho_i(z)$, by $\phi_i(z) = \rho_i(z) v_i$, where $v_i$ is the molecular volume of that chemical species.

We define the $z$-axis to be perpendicular to the interfaces with its origin at the midplane of the bilayer. We performed a second order free energy expansion about the area per molecule and the two principle
radii of curvature, and we found that the midplane of the bilayer is the surface of inextension (surface of constant area per molecule upon bending) in order to decouple the area from the pure curvature contributions to the free energy\textsuperscript{41,43,44}. The total area at distance $z$, $A(z, c)$, parallel to the midplane of the bilayer, is given by\textsuperscript{43,44}:

$$A(z, c) = A(0)[1 + (c_1 + c_2)z + c_1c_2z^2]$$

where $c_1$ and $c_2$ are the principal curvatures, $c_i = 1/r_i$, and $r_i$ is the radii of curvature of the midplane of the bilayer. For spherical curvature, $c_1 = c_2 = 1/r$, and for cylindrical curvature, $c_1 = 1/r$ and $c_2 = 0$.

As a result of this difference in principal curvatures, spherical and cylindrical membranes of the same radius will have different areas at a given $z$. We use $a(z, c) = A(z, c)/N$, where $N$ is the number of molecules in the exterior leaflet of the bilayer, to denote the average area available per molecule at a distance $z$ from the midplane of the bilayer. The intermolecular repulsions are modeled as excluded volume interactions and therefore we include them through packing constraints. Namely, we solve our molecular theory under the constraint that the total density is constant at each position $z$ (i.e. that it is incompressible)\textsuperscript{43-45}. The constant density constraint implies that the average volume fraction of the molecules in any layer must be equal to unity. That is,

$$\sum_{\delta} \langle \phi_{\delta}(z) \rangle + \phi_o(z) + \phi_{I+}(z) + \phi_{I-}(z) + \phi_{E+}(z) + \phi_{E-}(z) = 1$$

where $\delta = I, E$ and $I$ and $E$ stand for the interior and exterior leaflet of the bilayer, respectively. The subscript $(+)$ stands for the dissociated sodium cation and the subscript $(-)$ stands for the dissociated
chlorine anion. Throughout the rest of this section, we will use $x_{L,\delta}$ to denote the mole fraction of lipids in leaflet $\delta$.

The molecular theory uses a free energy functional that is constructed by explicitly writing each of the energetic/entropic contributions and then minimizing the free energy with respect to the free variables (for more details please see previous work with this model$^{43-45}$). We input the physical conformations of the chains and through free energy minimization we obtain the probability of each of those conformations as a function of the constraints imposed on the system. Through this method we are able to obtain the molecular level equilibrium physical parameters (such as local lateral stresses, area per molecule, lipid flip flop, and local molecular organization and architecture) that we need to elucidate the fundamental molecular driving forces for protein recruitment. The free energy of a membrane is given by

$$\frac{\beta W}{N_L} = \sum_\delta x_{L,\delta} \left[ \ln \left( \frac{x_{L,\delta} \sigma_\delta^2}{\alpha(0)} \right) - 1 \right] + \sum_\delta \sum_{\sigma_\delta} 2x_{L,\delta} \left[ P_{\sigma,\delta}(\alpha_\delta) \left( \beta \epsilon_\delta(\alpha_\delta) + \ln(P_{\sigma,\delta}(\alpha_\delta)) \right) \right]$$

$$+ \sum_\delta \sum_{\sigma_\delta} x_{L,\delta} \left[ \frac{\beta \epsilon_\delta(\sigma_\delta)}{2N_L} \int \langle \rho_{\delta,\sigma}(r) \rangle \psi(r) \right] \frac{\nabla^2}{2} \left( \nabla \psi(r) \right)^2 dr + \frac{\beta \chi_{L,L}}{2N_L} \int \langle \rho_{L,L}(r) \rangle \psi^2(r) dr$$

$$+ \frac{\beta \chi_{L,L}}{2N_L} \int \langle \rho_{L,L}(r) \rangle \psi^2(r) dr + \frac{1}{N_L} \int \rho_{L}(r) \left[ \ln(\rho_{L}(r)) - 1 - \beta \mu_{L} \right] dr$$

$$+ \frac{1}{N_L} \int \rho_{H}(r) \left[ \ln(\rho_{H}(r)) - 1 - \beta \mu_{H} \right] dr + \frac{1}{N_L} \int \rho_{OH}(r) \left[ \ln(\rho_{OH}(r)) - 1 \right] dr$$

$$+ \frac{1}{N_L} \int \rho_{\omega}(r) \left[ \ln(\rho_{\omega}(r)) - 1 \right] dr + \frac{1}{N_L} \int \rho_{\omega}(r) \left[ \ln(\rho_{\omega}(r)) - 1 \right] dr + \frac{1}{N_L} \int \beta \pi(r) \left[ \sum_\delta \langle \phi_{L,\delta}(r) \rangle + \phi_{\omega}(r) + \phi_{H}(r) + \phi_{OH}(r) + \phi_{H}(r) + \phi_{\omega}(r) - 1 \right] dr$$
where $W$ is the constrained free energy of the system, $P_{\delta,t}(\alpha_\delta)$ is the probability of the fatty-acid tails in leaflet $\delta$, $N_L$ is the total number of lipids, $\alpha_\delta$ is the conformation of the fatty-acid chain of the lipid molecule, $\sigma_\delta$ is the conformation of the lipid headgroups, $\epsilon_i$ is the internal energy of the chain that arises from having gauche dihedral angles in a particular conformation, $V_{L,t}$ is the volume of a $CH_2$ unit that is equal to 0.027 nm$^3$, and $\beta = 1/k_BT$. The first term in the above equation is the contribution to the total free energy from the translation of the lipids, where $\lambda_\delta$ is the thermal de Broglie wavelength of the lipid molecules. The second term accounts for the conformational entropy and internal energy of the chains. The third term accounts for the conformational entropy of the headgroups. We modeled the PC headgroup of the lipids in a very similar way to previous publications using this theory$^{46}$, where instead of modeling the volume of the headgroup as two spheres we again used the Rotational Isomeric States model (RIS). The two glycerol carbons bonded to the phosphate group are fixed in space, and the remainder of the headgroup, comprised of two $CH_2$ groups, and a tri-methylated tertiary amine group, move according to the RIS model. The location of the phosphate group has a fixed charge of $-1e$, and the movable amine group has a fixed charge of $+1e$, where $e$ is the fundamental charge. The fourth term is the contribution of electrostatics$^{47}$, where $\psi(r)$ is the electrostatic potential, and $\epsilon$ is the dielectric coefficient, which is taken to be that of water (78.5 times the value of the dielectric value of vacuum). $\left\langle \rho_q(r) \right\rangle$ is the ensemble averaged number density of charges given by:

$$
\left\langle \rho_q(r) \right\rangle = \sum_{\delta} \left\langle \rho_{L,\delta,h,q}(r) \right\rangle e + \rho_+(r)e + \rho_-^*(r)e - \rho_-^*(r)e - \rho_{OH}(r)e,
$$
where \( \rho_{L,r,h,a}(r) \) is the local density of charges on the headgroups. The fifth, sixth, and seventh terms are the contributions of the hydrophobic interactions in the lipids system. \( \rho_{L,r,h,c}(r) \) is the local density of \( CH_2 \) and \( CH_3 \) groups in the headgroups of the lipids. \( \chi = -5k_B T \) is the strength of the hydrophobic interactions. The eighth and ninth terms in the free energy represent the translational free energy of the cations and anions of the salt as well as the bulk chemical potential of both species (the chemical potential is used to impose a bulk salt concentration of 0.1M). The tenth, eleventh, and twelfth terms represent the translational free energy of the dissociated \( H^+_{(aq)} \) and \( OH^-_{(aq)} \) species along with that of the water. The bulk pH in all of the calculations is set at 7 and the pKw is set at 14.0. The final term accounts for the repulsive steric interactions that are accounted for in an incompressibility constraint. \( \pi(r) \) is a Lagrange multiplier that is introduced to enforce the incompressibility constraint. The probability distributions for the headgroups and fatty-acid tails of the lipids (here written with the explicit curvature dependence) are
\begin{equation}
\begin{align*}
P_{\delta,t}(\alpha_{\delta}, e) &= \frac{1}{q_{\delta,t}(e)} \exp \left[ -\beta \epsilon_{t}(\alpha_{\delta}) - \int \beta \pi(z, e) v_{L,\delta,t}(z, \alpha_{\delta}) dz - \int \beta \chi v_{L,t} \int \left[ \langle \rho_{L,t,j}(z, e) \rangle + \langle \rho_{L,E,t}(z, e) \rangle \right] n_{t,\delta}(z, \alpha_{\delta}) dz \right] \\
P_{\delta,h}(\sigma_{\delta}, e) &= \frac{1}{q_{\delta,h}(e)} \exp \left[ -\int \beta \pi(z, e) v_{L,\delta,h}(z, \sigma_{\delta}) dz - \int \beta \psi(z, e) e n_{L,\delta,h}(z, \sigma_{\delta}) dz - \int \beta \chi v_{L,t} \int \langle \rho_{L,\delta,h}(z, e) \rangle n_{h,\delta}(z, \sigma_{\delta}) dz \right]
\end{align*}
\end{equation}

( XIV)

where \( q_{\delta,t}(e) \) and \( q_{\delta,h}(e) \) are the single molecule partition functions (normalization of the probability) defined as

\begin{equation}
\begin{align*}
q_{\delta,t}(e) &= \sum_{\alpha_{\delta}} \exp \left[ -\beta \epsilon_{t}(\alpha_{\delta}) - \int \beta \pi(z, e) v_{L,\delta,t}(z, \alpha_{\delta}) dz - \int \beta \chi v_{L,t} \int \left[ \langle \rho_{L,t,j}(z, e) \rangle + \langle \rho_{L,E,t}(z, e) \rangle \right] n_{t,\delta}(z, \alpha_{\delta}) dz \right] \\
q_{\delta,h}(e) &= \sum_{\sigma_{\delta}} \exp \left[ -\int \beta \pi(z, e) v_{L,\delta,h}(z, \sigma_{\delta}) dz - \int \beta \psi(z, e) e n_{L,\delta,h}(z, \sigma_{\delta}) dz - \int \beta \chi v_{L,t} \int \langle \rho_{L,\delta,h}(z, e) \rangle n_{h,\delta}(z, \sigma_{\delta}) dz \right]
\end{align*}
\end{equation}

( XV)

\( \beta \pi(z, e) \) and \( \beta \psi(z, e) \) are determined self-consistently for each curved state of the bilayer, and these field variables explicitly control the probability distributions of the lipid tails and headgroups.
In order to determine the lateral pressure profile, a system of equations is created through discretizing space along \( z \) into 0.2 nm layers. The probability of each conformation that is derived by the extrema of the free energy is used in the discretized incompressibility constraint and Poisson equation \(^{47}\) to set up a system of coupled nonlinear algebraic equations. These equations are solved using the KINSOL software package in the SUNDIALS suite. KINSOL specializes in solving systems of coupled nonlinear equations. Since the system is curved, the volumes of each of the discretized layers are not constant and therefore depend on the distance away from the midplane of the bilayer. The volume of layer \( i \), \( V(i) \), is given by the following expressions for spheres and cylinders:

\[
V(i) = \int_{(i-1)\times0.1\text{nm}}^{i\times0.1\text{nm}} A(z) \, dz
\]

\[
V_{\text{Cylinder}}(i) = A(0) \left[ z + \frac{z^2}{2R} \right]^{\times0.1\text{nm}}_{(i-1)\times0.1\text{nm}}
\]

\[
V_{\text{Sphere}}(i) = A(0) \left[ z + \frac{z^2}{R} + \frac{z^3}{3R^2} \right]^{\times0.1\text{nm}}_{(i-1)\times0.1\text{nm}}
\]

(XVI)

remembering that \( A(z, c) \) is a function of mean and Gaussian curvature. The mathematical expression for the lateral pressure profile is determined by taking the appropriate derivative of the free energy equation. The lateral pressure profile, \( \beta \Pi(z, c) \), is related to the surface tension, \( \gamma \), through the following expression
The equilibrium value for the surface tension is zero for the planar bilayer and this sets the area per molecule of the surface of inextension for all of the theoretical calculations presented in this work. The lateral pressure profile generated from equation (XVII) is plotted in Supplementary Figure 7a. It is important to note that, as seen in equation (XVII), the translational contributions of the lipids are not included in the lateral pressure profiles presented by the theory. The term including the translation of the lipids does not make any contribution to the comparison plots in Supplementary Figure 7b, since the areal density at the midplane of the lipids is a constant in all of the calculations. When comparing our lateral pressure profiles with those obtained in similar systems by molecular simulation, it is useful to remember that the translational contribution of the lipids will increase the magnitude of our lateral pressure profile. The exact distribution of the translational contribution to the lateral pressure profile is unfortunately unattainable with the current construction of the molecular theory; however, even without the translation of the lipids explicitly in the lateral pressure profile, the comparison of the theory with simulation is very favorable.
Any curved lipid bilayer will display an asymmetric transbilayer pressure profile as the lipids in the exterior leaflet relax, due to the expansion above the midplane of the bilayer \((z = 0\) in the expression for the area above\), and the lipids in the interior leaflet become more frustrated, due to the compression below the midplane. A chemical potential gradient between the bilayer leaflets is immediately established upon bending, where lipids from the interior leaflet will flip to the outer leaflet to reduce the imposed stresses and chemical potential gradient. In reference\(^{44}\) we used the molecular theory to show that the amount of lipid flip-flop between the leaflets has considerable influence on the physical properties on a curved bilayer. We introduced a term called the relaxation ratio, 
\[
\eta = \frac{2 \left( \frac{\partial x_F}{\partial C} \right)}{l \left( \frac{\partial C}{\partial C} \right)},
\]
where \(C\) is the sum of the principle curvatures. For the results in this manuscript, we used a relaxation ratio of 0.4 because it yielded a bending modulus for DOPC of \(20k_BT\), which matched the experimental result for the bending modulus of DOPC in reference\(^{51}\). The fraction of lipids in the exterior leaflet for spherical and cylindrical curvatures, \(x_E\), are listed in Supplementary information Table 3.

**Theoretical calculations of tN-Ras, Syt and Anx recruitment to spherically and cylindrically curved lipid bilayers using the potential-distribution theorem.** We first calculate the theoretically predicted protein recruitment for the simplest case, tN-Ras. When a bulk solution of proteins with lipid chain anchors are in equilibrium with a proportion of proteins bound to a curved lipid bilayer, then thermodynamics requires that the chemical potential of the chain anchor, \(\mu_A\), must be equal in the bulk and bilayer phases, 
\[
\mu_A^{\text{bulk}} = \mu_A(c).
\]

The chemical potential of the chain anchors can be determined by the potential-distribution theorem\(^{45,52}\). Valid usage of the potential-distribution theorem for Syt and Anx’s MBDs require a dilute concentration of the proteins. Previous experimental work verifies that the absolute binding
concentrations of these MBDs are quite dilute in relevant membrane systems\textsuperscript{53,54}. For our molecular theory (please see reference\textsuperscript{45} for more specific details) the areal density of the proteins at a given membrane curvature, $c$, normalized to the areal density of the proteins in a planar bilayer, $c = 0$, is written as

$$\frac{\rho_d(c)}{\rho_d(c = 0)} = \frac{q_{\text{linker}}(c) \prod_{k=1}^{2} q_{k,E}(c)}{q_{\text{linker}}(c = 0) \prod_{k=1}^{2} q_{k,E}(c = 0)}$$

( XVIII)

where $q_{k,E}(c)$ is the single molecule partition function (normalization of the probability) for $k$ being equal to 1 for the palmitoyl chain and 2 for the farnesyl chain, and $\rho_d(c)$ is the exterior aerial density of the proteins as a function of curvature. By examining equations (XV) given above for the single molecule partition function, it can be seen that the normalized protein areal density depends explicitly on the three major contributions to the lateral pressure profiles, $\beta \pi(z,c)$, $\beta \psi(z,c)$, and $\langle \rho_{L,E}(z,c) \rangle$, which are calculated for a given temperature, composition, and curvature of the bilayer.

Performing these calculations allows us to extract the equilibrium binding densities for tN-Ras on both vesicles and tubes. Comparing the theoretically calculated tN-Ras densities versus curvature with the experimental data revealed excellent agreement for both tubes and vesicles (Fig. 2). Furthermore, we compared the theoretically calculated lateral pressure profiles along the bilayer normal for planar (dark line) and curved (pale line, 50 nm diameter) membranes (Supplementary Fig. 7a). The curvature dependent relief in the relative lateral pressure of the outer monolayer, $\Delta P$, is calculated as the total area between the curves (shaded area in Supplementary Fig. 7a) and found to
be significantly larger for 50 nm spheres than tubes (Supplementary Fig. 7a). The exterior lipid area is calculated by using equation (V) with \( z = 1.3 \) nm (Supplementary Fig. 7a). Equation (X) provides us with the absolute area as a function of the distance from the midplane, so in order to get the area per lipid for the exterior leaflet, we had to divide by the number of lipids in the exterior leaflet obtained by using the values of the fraction of lipids in the exterior leaflet provided in Supplementary information Table 3.

The recruitment of Syt and Anx can also be calculated from the molecular theory, again using the potential-distribution theorem\(^45,52\), if we can estimate the hydrophobic distribution, shape, charge distribution, and total volume of the membrane binding domains (MBDs) of the proteins, by the following expression:

\[
\frac{\rho_\lambda(c)}{\rho_\lambda(c = 0)} = \exp\left[\frac{\int_{z_{\text{min}}}^{z_{\text{max}}} \beta \pi(z,c) v_p(z) dz - \int \beta \psi(z,c) n_{p,q}(z) dz}{-\beta \chi_{L, t} \int [\langle \rho_{L, \delta, h}(z,c) \rangle] n_{p,c}(z) dz}
\right]
\]

\[
= \exp\left[\frac{\int_{z_{\text{min}}}^{z_{\text{max}}} \beta \pi(z,c = 0) v_p(z) dz - \int \beta \psi(z,c = 0) n_{p,q}(z) dz}{-\beta \chi_{L, t} \int [\langle \rho_{L, \delta, h}(z,c = 0) \rangle] n_{p,c}(z) dz}
\right]
\]

( XIX )

where \( v_p(z) dz \) is the volume contribution of the insertion motif at position \( z \) in the bilayer. \( n_{p,c}(z) dz \) is the number of \( CH_2 \) and \( CH_3 \) groups in the protein located at position \( z \), and \( n_{p,q}(z) dz \) is the number of charged units in the protein located at position \( z \). The integration over \( v_p(z) dz \) from \( z_{\text{min}} \) to \( z_{\text{max}} \) yields the total volume of the MBD. From this expression, it is evident that the size, shape, hydrophobicity, charge distributions, and penetration depth of the insertion domains are critical parameters for determining the relative recruitment. Equation (XIX) differs from equation (XVIII)
because the MBD of tN-Ras consists of two fatty-acid chains that have $10^8$ conformations that need to be averaged over to obtain the equilibrium binding thermodynamics. Equation (XIX) is used for the MBD of Syt and Anx because these domains require considerably less conformations to be considered for a given membrane curvature and MBD penetration depth.

Syt docks to the bilayer by inserting two C2 domains, C2A and C2B, at a 1.0 nm penetration depth (Supplementary Fig. 1c). The membrane-docked structures were produced using PDB structure files for C2A (PDB ID: 1BYN) and C2B (PDB ID: 1K5W), respectively. The positions of all the atoms correspond to successive Euler angle rotations, which has previously been described in detail. The positions of the atoms as a function of distance perpendicular to the $xy$-plane of the bilayer headgroups are then exactly determined. The volumes of the individual units are calculated by using the approximate van der Waals radii of 0.16 nm for carbon, 0.14 nm for oxygen, 0.145 nm for nitrogen, 0.173 nm for sulfur, and 0.05 nm for hydrogen. The locations of $\text{CH}_2$ and $\text{CH}_3$ groups contributed to $n_{p,c}(z)$ and the locations of the charged amino acids contributed to $n_{p,q}(z)$. The theoretical results for Syt recruitment are in excellent agreement with experiments, as visualized in Figure 2. We performed the calculations using different penetration depths of the C2 domain (Supplementary Fig. 7d). Based on a $\chi^2$ goodness-of-fit test, the best agreement between theory and the experimental density results, both on vesicles and tubes, is observed for a penetration depth of 1.0 nm (Vesicles $\chi^2$: 0.9 nm = 13.6, 1.0 nm = 10.0, 1.1 nm = 12.3 and Tube $\chi^2$: 0.9 nm = 1.1, 1.0 nm = 0.3, 1.1 nm = 2.8).

We started calculating Syt with an insertion depth of 1 nm below the glycerol carbons in the lipid headgroups based on the estimate in reference. We then increased and decreased the penetration depth for both spheres and cylinders and found that the literature estimate agreed best with the experimental data (Supplementary Fig. 7d).

The Anx bilayer insertion domain is comprised of amphiphilic alpha helices. We model these domains as a given number of rigid cylinders with the R groups extending radially. Each helix is
given a radius of 0.5 nm. Given the conditions of the experiments, we know that the helices insert shallowly into the bilayer with an orientation that is parallel to the lipid headgroups. We model the insertion of 4 helices, each with a length, \( L \), of 2.3 nm. This length corresponds to an average of 15 residues per helix, as has been approximated in a previous study. The total volume, \( V \), of insertion at a given distance \( z \) to the plane of the membrane for one helix is obtained from the expression:

\[
V = 2L \int_{z_{\text{min}}}^{z_{\text{max}}} \sqrt{r_{\text{helix}}^2 - z^2} \, dz
\]

where \( r_{\text{helix}} \) is the 0.35 nm approximated radius of the helix. We used the helical wheel in Figure 5 of ref to determine the location of the hydrophobic and charged groups. We did not have reliable literature estimates for the penetration depth for Anx, but there is evidence that the helices inserted shallowly for our experimental conditions. Again, we performed the calculations using different penetration depths of the Anx helix (Supplementary Fig. 7e). Based on a \( \chi^2 \) goodness-of-fit test, the best agreement between theory and the experimental density results is observed to be 0.1 for vesicles, whereas the comparison for tubes indicated very shallow insertion (Vesicles \( \chi^2 \): 0.1 nm = 1.07, 0.2 nm = 1.98, 0.3 nm = 7.22, 0.4 nm = 17.76 and Tube \( \chi^2 \): 0.1 nm = 1.19, 0.2 nm = 1.55, 0.3 nm = 3.61).

In order to accurately fit the experiments, we obtained a penetration depth into the hydrophobic phase of the lipid leaflet of 0.1 nm for spherical bilayers, while in cylindrical bilayers the protein fully penetrated the headgroup region, but did not insert into the hydrophobic region, both scenarios being well within the previously approximated insertion range. Consistent with the tN-Ras results, we found that the molecular theory predicts that there is very little curvature sensing when amino acids are not penetrating into the hydrophobic region of the bilayer.
Using the physical properties obtained from the mean field theory to provide insight into the molecular mechanisms that contribute to Gaussian curvature effects. As described in the main text, the major difference between spherical and cylindrically curved membranes is the presence or absence of Gaussian curvature, respectively. Here we wish to use the theoretical calculation to elucidate how the difference in Gaussian curvature between the two systems will affect lateral pressure and area per lipid.

Equation (XVI) describes the volume of the discretized membrane layers that essentially consist of a mean and Gaussian curvature component, originating from equation (IX). It is evident that comparing equation (XVI) for the spherical and cylindrical geometries, in a scenario where \( r_{\text{sphere}} = 2 \cdot r_{\text{cylinder}} \), leaves the Gaussian term \((z^3/3R^2)\) in the spherical geometry as the only difference between the two systems. The volumes of each layer are used to self-consistently solve for the lateral pressure profile in the calculation. Consequently, plotting the lateral pressure profile difference of a cylinder and sphere of twice the diameter will highlight the contribution of the Gaussian term in the calculation. The difference in the lateral pressure for a sphere of radius \( r \) and a cylinder of radius \( r/2 \), with \( r = 100 \) nm and \( r = 40 \) nm, is shown in Supplementary Figure 7b. The plot demonstrates that the influence of Gaussian curvature is distributed relatively evenly throughout the thickness of the bilayer, and that the higher the curvature of the bilayer, the greater is the effect of Gaussian curvature on the lateral pressure profile. In Figure 3b we plotted the ratio of the difference in the curved versus planar integrated lateral pressure for the whole exterior leaflet, \( \Delta P \), between a sphere of radius \( r \) and a cylinder of radius \( r/2 \). The \( \Delta P \) ratio displays a ~10\% increase for \( r = 75 \) nm and up to about 50\% for \( r = 20 \) nm. Since the change in \( \Delta P \) is believed to be important for curvature sensing membrane-binding proteins, this suggests that Gaussian curvature has a major role in protein recruitment.

To examine the contribution of Gaussian curvature to the area of exterior leaflet lipid headgroups, we plotted the area of exterior headgroups for a sphere of radius \( r \) divided by the area of the exterior
headgroups of a cylinder of radius \( r/2 \) (Fig. 3b). Equation (X) is used to calculate the exterior area with \( z = 1.3 \) nm. As seen in equation (X) the difference between \( A(z = 1.3 \text{ nm}) \) for cylinders of size \( r/2 \) compared to spheres of size \( r \) is the presence of the Gaussian curvature term. The resulting plot depicted in Figure 3b, demonstrates that the Gaussian curvature has almost no measurable effect on the area of the lipid headgroups in the exterior membrane leaflet, since the ratio between the lipid area of spheres and cylinders with radius \( r \) and \( r/2 \), respectively, never deviates more than 0.5\% from 1. This very small difference between the area per headgroup in spheres and cylinders might be a reason for the apparent lack of interest in the effects of Gaussian curvature in lipid bilayer physics up to this point.

Separating the interaction contributions to MBD curvature sensing. The relative density of MBDs bound to curved lipid bilayers can be written within the molecular theory as a product of four terms. To see this, first look at the full equation for the relative areal density of Syt and Anx as a function of curvature,

\[
\frac{\rho_A(c)}{\rho_A(c = 0)} = \exp \left[ -\int_{z_{\text{min}}}^{z_{\text{max}}} \beta \pi(z,c) \nu_p(z) dz - \int \beta \psi(z,c) n_{p,q}^\text{e}(z) dz \right. \\
\left. -\beta \chi_v \int \left\{ \rho_{L,h}^\text{e}(z,c) \right\} n_{p,e}^\text{e}(z) dz - \beta \chi_r \right]
\]

(XXI)

These four terms are the major contributors to the overall lateral pressure profile for the equilibrium bilayer and they are 1) the repulsive excluded volume contribution contained in the \( \beta \pi(z,c) \) function,
2) the electrostatic contribution contained in the $\beta \psi(z,c)e$ function, 3) the hydrophobic contribution contained in the $\langle \rho_{L,E,t}(z,c) \rangle$ function, and (4) the internal energy contribution defined by $\varepsilon(\alpha)$. 

$v_p(z)dz$ is the volume contribution of the insertion motif at position $z$ in the bilayer. $n_{p,e}(z)dz$ is the number of CH$_2$ and CH$_3$ groups in the protein located at position $z$, and $n_{p,q}(z)dz$ is the number of charged units weighted by the valency of the charges in the protein located at position $z$. The integration over $v_p(z)dz$ from $z_{min}$ to $z_{max}$ yields the total volume of the MBD. The total volume (both inside the hydrophobic tail and headgroup regions of the bilayer) are 6.1 nm$^3$ for Syt in both spheres and tubes, 1.5 nm$^3$ for Anx inside of a sphere, 1.3 nm$^3$ for Anx inside of a tube, and 1.7 nm$^3$ for tN-Ras in both spheres and tubes. From this expression, it is evident that the size, shape, hydrophobic, internal energy and charge distributions are the contributing parameters for determining the relative recruitment.

In order to highlight the relative contributions of the four interactions, it is useful to write the above expression in the following form
\[
\frac{\rho_d(c)}{\rho_d(c = 0)} = \left[ \text{Excluded Volume} \right] \left[ \text{Electrostatic} \right] \left[ \text{Hydrophobic} \right] \left[ \text{Internal Energy} \right]
\]

\[
\left[ \text{Excluded Volume} \right] = \frac{\exp \left[ - \int_{z_{\text{min}}}^{z_{\text{max}}} \beta \pi(z,c) v_p(z,\alpha) dz \right]}{\exp \left[ - \int_{z_{\text{min}}}^{z_{\text{max}}} \beta \pi(z,c = 0) v_p(z,\alpha) dz \right]}
\]

\[
\left[ \text{Electrostatic} \right] = \frac{\exp \left[ - \int \beta \psi(z,c) \text{en}_{p,q}(z,\alpha) dz \right]}{\exp \left[ - \int \beta \psi(z,c = 0) \text{en}_{p,q}(z,\alpha) dz \right]}
\]

\[
\left[ \text{Hydrophobic} \right] = \frac{\exp \left[ - \beta \chi_{L,t} \int \left( \rho_{L,\beta,\delta}(z,c) \right) n_{p,c}(z,\alpha) dz \right]}{\exp \left[ - \beta \chi_{L,t} \int \left( \rho_{L,\beta,\delta}(z,c = 0) \right) n_{p,c}(z,\alpha) dz \right]}
\]

\[
\left[ \text{Internal Energy} \right] = \frac{\exp \left[ - \beta \varepsilon(\alpha) \right]}{\exp \left[ - \beta \varepsilon(\alpha) \right]}
\]

(XXII)

where the terms in brackets are the integrated energetic contributions relative to the planar bilayer.

For tN-Ras, the relative contributions do not factorize as nicely as the other two MBDs, so we had to approximate the relative contributions by taking the ensemble average of the excluded volume, hydrophobic, and electrostatic contributions for each chain over all of the conformations and multiplying them together. The internal energy contribution was back calculated to make sure that the product of the four terms matched the molecular field calculation.

The excluded volume term increases with decreasing \( r \) (a reduction of a repulsive contribution) for each MBD, because the net effect of curving the bilayer is to increase the volume accessible in the outer leaflet of the bilayer to allow the MBD to insert (Fig. 3c and Supplementary Fig. 7f).
hydrophobic term decreases with decreasing $r$ (a reduction of an attractive contribution) for each MBD, due to the decreasing local density of the hydrophobic fatty-acid tails in the outer leaflet as the curvature increases. The electrostatic contribution is a negligible contributor to the relative density curvature sensing due to the low and relatively symmetric areal charge density near both leaflet headgroups for every geometry studied. The pure component zwitterionic PC headgroups used for all of the theoretical results in this work do not create a sizable enough electrostatic potential asymmetry to be a factor in curvature sensing. The experimental system did contain a low concentration of anionic PS lipid headgroups, however, several theoretical calculations that included the PS headgroups demonstrated that this low and symmetric overall charge distribution on the lipid bilayer did not significantly affect MBD curvature recruitment. The internal energy does not show any variation with curvature; though bending the bilayer did change the probability of each of the MBDs configurations, bending did not noticeably change the relative probability of the configurations in relation to each other. In order for the internal energy to have a significant contribution to curvature sensing, the relative importance of the configurations to the ensemble average needs to change, and that did not happen with the MBDs studied in this work.

Given the relative importance of the excluded volume and hydrophobic interactions to geometry discrimination, we introduce a new variable in Figure 3 that quantifies the relative density of a MBD for a given shape and radius, normalized to that same shape at a radius of 200 nm (diameter of 400 nm), calculated by only considering the excluded volume and hydrophobic interactions, $\Delta I_{\text{VolHyd}}$. Supplementary Figure 7g takes this new variable for vesicles and divides it by the corresponding tube variable at half the radius, again to elucidate the precise effect of Gaussian curvature. This new variable then provides a novel way of elucidating the importance of geometry discrimination for a given MBD. Supplementary Figure 7g does this for the three MBDs calculated by the molecular theory at a vesicle diameter of 40 nm to connect the results to Figure 2g and compare the theoretical
prediction to experimental results. We extend the elucidative power of this new variable by mapping it onto the volume filling representation of Syt’s C2A binding domain in Figure 3d. The molecular theory requires a discretization in the direction parallel to the bilayer normal, so an integration of the color mapping over the volume of the C2A domain will provide its contribution to Supplementary Figure 7g. The color map shows how the specific residues of the C2A domain contribute to the geometry discrimination as a function of the spatial distribution of specific chemical groups.

Verifying that α-syn did not show binding to tubes above the background. We tried to perform a side-by-side comparison of the binding of α-syn to vesicles and tubes. Since we were not able to detect any binding of α-syn to tubes using our normal settings we increased the laser intensity and number of accumulated frames, which introduced an amount of cross excitation of the membrane dye into the α-syn channel, before addition of protein (Supplementary Fig. 4e and f). However we did not, even for greatly increased acquisition settings, record any binding to tubes above the cross excitation (Supplementary Fig. 4g and h). Our normal imaging conditions, which was used throughout this work, was set to minimize the cross excitation of the DiD membrane label into the protein channel (Supplementary Fig. 4i and j). The observation that α-syn is not recruited to tubes under our experimental conditions (10 mole% PS, 1 mM Ca\(^{2+}\), 95 mM NaCl) supports that recruitment of α-syn to membrane tubes is less potent than to vesicles\(^{39}\) and require conditions that are favoring electrostatic interactions\(^{60,61}\), i.e. highly negatively charged membranes (>20 mole% PS).

The binding of all MBDs to vesicles and tubes are studied under identical experimental conditions (protein concentration, lipid composition, ionic strength, pH, incubation time etc.) to facilitate a direct comparison. Here we designed the systems to include 10 % negatively charged lipids to ensure that electrostatics is present, but do not dominate the membrane interaction and thus potentially mask the membrane curvature dependent contributions to MBD recruitment. Therefore we did not address the
membrane geometry selectivity at higher membrane charges (~50%), for which α-syn has previously been shown to bind to tubes\textsuperscript{62}. Therefore it is likely that a higher membrane charge, facilitating electrostatic binding of α-syn, would result in a different membrane geometry discrimination. However, we take the inability of α-syn to bind to tubes under these specific experimental conditions to mean that the most conservative estimate of the discrimination efficiency for α-syn is ~ 12 fold, since $R_{\text{ves}} = 12.2 \pm 2.3$ for α-syn (Supplementary Table 1).

Calculating the bud scheme in Supplementary Figure 8. We obtained the mean and Gaussian curvatures for three progressing stages of a budding membrane for Supplementary Figure 8. The program COMSOL Multiphysics (v. 4.3b) allowed us to mathematically map the surface geometry of the buds and project an overlay of color, with wavelength proportional to the value of the curvature of interest. All figures were constructed as 3-D simulations in the SolidWorks CAD program, with specified dimensions, and then imported into COMSOL for surface analysis. Mean and Gaussian curvature values were determined in nm\textsuperscript{-1} and nm\textsuperscript{-2}, respectively, and the results of this analysis are in Supplementary Figure 8a, b. With the known mean and Gaussian curvatures, we were able to use the molecular theory described above to determine the relative binding of tN-Ras to each point on the surface of the budding membranes shown in Supplementary Figure 8c.
Supplementary Fig. 1 | Mass spectra and scheme of peptide No. 2 (tN-Ras-Alexa488), structures of the Syt C2AB domains and scheme of the four membrane binding domains investigated.

a, Mass spectra obtained for tN-Ras-Alexa488 (tN-Ras). b, Scheme of the minimal anchoring motif of N-Ras (tN-Ras). c, Structure of the cytoplasmic C2A and C2B domains [Protein Data Bank accession numbers: 1BYN and 1UOW. The labeling site is indicated by the arrow and Ca^{2+} binding sites by grey spheres. The box in the top indicates a lipid membrane.
Supplementary Fig. 2 | Correlating measured tube diameter with integrated tube intensity for size determination of diffraction-limited tubes.

a, Micrograph of tubes adsorbed on the SLB illustrating the typical number of tubes in a single field of view. Scale bar is 10 μm. Insert of z-slice micrograph of tube and SLB confirms no significant
deformation of the tube upon adhesion to the SLB. Scale bar is 1 μm. b, Micrograph of DiD-labeled tubes below (bottom) and above (top) the diffraction limit of the microscope (left) and the intensity profile along the red line in the micrograph (right). The scale bar is 1 μm. c, Intensity profile of the tube in b. A double Gaussian function is fitted to extract the integrated intensity, I(DiD), with the tube diameter, d(tube), measured as the peak-to-peak distance. d, Integrated intensity versus tube diameter for seven tubes above the diffraction limit displaying a clear linear correlation. The slope S of the linear fit is subsequently used to convert the integrated DiD intensity of tubes below the diffraction limit to absolute tube diameter in nanometer. e, Histogram of integrated DiD intensities for 627 tubes below the diffraction limit, measured under identical conditions. The intensity values are spanning more than an order of magnitude (200,000 – 6,000,000 A.U.). f, A histogram of the intensities in e converted to size in nm using the slope S found in d, resulting in diameters spanning 30 nm to 430 nm. g, Histogram of the percentage error on the tube diameter for 627 tubes. Each tube size was calculated as the average of three independent measurements on the same tube, with the error found as the standard error of the mean. The error on the size measurement is below 10 % for approximately 75 % of the tubes. h, Percentage error for individual tubes plotted against tube diameter reveal that only for the smallest tube diameters does the error exceed 20 %. i, Histogram of the percentage error on the quantified tN-Ras densities. All integrated intensities used for density calculations are averages of three independent measurements on each tube, with the error reported as the SEM. These individual errors on the intensities are propagated to a final density error, the majority of which lies between 0% and 20%.
Supplementary Fig. 3 | Independent method verifies the linearity between integrated tube intensity and tube diameter.

a, Micrographs of DiDC18 (DiD) labeled tubes of two different diameters pulled from GUVs. The diameter is determined by controlling the aspiration pressure in the micropipette holding the GUV. Scale bars are 5 μm. b, Intensity line profiles, taken as an average of 50 pixels, across the two tubes in a. The integrated DiD intensity is quantified by fitting of Gaussian functions. c, Integrated intensity versus diameter for five tubes of different diameters. The integrated intensity is presented as the average of three independent measurements on the same tube and the error bars are SEM. The good agreement between the linear fit and the data confirms that the integrated intensity scales linearly with tube diameter.
Supplementary Fig. 4 | Comparison between the curvature-dependent recruitment of the membrane-binding domains and the negative control Streptavidin and a quantitative
comparison of protein ability to discriminate between spherical and cylindrical membrane geometries.

a, The normalized densities versus tube diameter plotted for each individual MBD together with the negative control, Streptavidin (Strep). tN-Ras and Syt display significant curvature-dependent recruitment as compared to Strep. Anx shows similar binding as Strep and thus no curvature-dependent recruitment. The binding of α-Syn to tubes was at the level of the background. b, Control experiment to exclude that the recruitment of tN-Ras by membrane curvature is an artifact of the quantification method. Tubes and vesicles containing two premixed membrane dyes were prepared, DiO-C18 (DiO) and DiD, (DOPC:DOPS:DOPE-Biotin:DiD:DiO 89.4:10:0.2:0.2:0.2) revealing no curvature dependence of the dye distribution for tubes (left) or vesicles (right). This serves as a control verifying that the assay and quantification method does not bias the density determination. α-syn did not show any detectable binding to tubes above the background. Micrographs and line scans of the same tube in the DiD channel (c and d) and in the α-syn channel (e and f) for greatly increased acquisition settings as compared to the settings used in the rest of the work, reveal a minor cross-excitation into the α-syn channel before addition of α-syn. Micrographs and line scans after addition of α-syn (g and h) reveal no binding to tubes above the cross excitation (Field of view slightly shifted after addition of protein). (i and j) Typical crosstalk of the DiD membrane label into the protein channel for our normal acquisition settings used throughout this work.
Supplementary Fig. 5 | Membrane tubes are in lateral diffusive contact with the SLB.

**a**, Micrographs of a DiD labeled tube on a SLB before bleaching (left), after bleaching (middle) and after 400 s of recovery (right) of the DiD channel. Insert shows micrographs of a DiD labeled vesicle with encapsulated Alexa-488 (lumen) sedimented on a SLB, before bleaching (left), after bleaching (middle) and 30 min after bleaching (right) of the DiD channel. Whereas the tube displays full recovery within 400 s, the vesicle did not display any recovery within 30 min. **b**, Quantification of fluorescence recovery on tubes. The average intensity as a function of time on the tube (grey) and on
the bilayer next to the tube (blue) within the ROIs depicted in a. The intensity traces are corrected for overall bleaching and normalized to the start intensity. c, Two mechanisms can lead to fluorescent recovery of membranes after bleaching; 1) lateral diffusion of lipids from the SLB (right) or 2) lipid flipping from the outer monolayer of the SLB to the outer monolayer of the adsorbed membrane (left). The sedimented vesicle can only recover through lipid flipping and thus illustrates the rate of recovery by this mechanism. The fact that the tube displayed full recovery before the vesicle had even started regaining any intensity verify that the tube is in lateral diffusive contact with the SLB and recover through lateral diffusion of lipids.
Supplementary Fig. 6 | Graphical representation of curved membranes and relative equilibrium MBD density over a curvature landscape comprising a range of spherical and cylindrical curvatures.
\textbf{a}, Graphical representation of key curvature concepts for a planar curve in two dimensions. T is the tangent line to the curve at point P. x is a coordinate system that has an origin at point P and runs along T. \( h(x) \) is a measure of the curve’s height above the tangent line T. R is the radius of a circle that locally follows the curve at P. The curvature at point P is 1/R. \textbf{b}, The fraction of lipids in the exterior leaflet for various spherical and cylindrical curvatures. \textbf{c}, Experimentally determined MBD density ratio values for a landscape of vesicle and tube diameters. Blue values represents increased density on vesicles, red represents increased density on tubes. The black dashed line is a guide to the eye representing the vesicle/tube diameters where there is no net preference for one of the geometries. \textbf{d}, Theoretically calculated tN-Ras density versus diameter of different curvature geometries vesicle (green), tube (blue) and saddle point (black).
Supplementary Fig. 7 | Membrane properties regulating curvature geometry discrimination.

a, Top panel depicts theoretically calculated lateral pressure profiles along the bilayer normal for the hydrophobic segment of either tubes (left, blue) or vesicles (right, green). The relative lateral pressure profile is depicted for either planar (dark line) or curved (pale line, 50 nm diameter) membranes. The curvature dependent relief in the relative lateral pressure of the outer monolayer, $\Delta P$, is calculated as the total area between the curves (shaded area). The bottom panel quantifies the theoretically calculated increase in the exterior area per lipid when curving a flat membrane to a 50 nm diameter tube (blue) or a 50 nm diameter vesicle (green) (left) and the theoretical calculated total relief in lateral pressure of the outer monolayer, $\Delta P$, when curving a flat membrane to a 50 nm diameter tube (blue) or a 50 nm diameter vesicle (green) (right). b, Theoretically calculated distribution of the Gaussian curvature mediated change in the lateral pressure for a low curvature (red) and a high curvature (black) scenario as a function of bilayer height. c, Density ratio for vesicle radius $r$ and tube radius $r/2$ plotted against vesicle diameter for both the experimental data and the theoretical calculations for the tN-Ras (orange), Syt (red) and Anx (magenta). d, Comparison of experimental and theoretically calculated Syt densities for various insertion depths as a function of tube (red) and vesicle (blue) diameter. e, Comparison of experimental and theoretically calculated Anx densities for various insertion depths as a function of tube (red) and vesicle (blue) diameter. f, Theoretically calculated interaction energy terms for Syt for either vesicles (left) or tubes (right); excluded volume (red circles), hydrophobic interactions (blue squares), electrostatic interaction (black triangles) and internal degrees of freedom (green triangles) plotted as a function of diameter. g, Theoretically calculated excluded volume and hydrophobic interaction energies was multiplied ($I_{\text{Vol} \times \text{Hyd}}$) and plotted as ratios for a vesicle of radius 40 nm and a tube of radius 20 nm against the experimentally determined discrimination efficiency values from Figure 2g.
Supplementary Fig. 8 | Quantitative theoretical prediction of N-Ras localization due to the complex geometrical transformations associated with vesicle budding, or caveolae formation.

**a,b**, The calculated distribution of **a** Gaussian and **b** mean curvatures in units of nm$^{-2}$ and nm$^{-1}$ respectively, for the surfaces used to generate the N-Ras binding densities in **c**. **c**, Quantitative
theoretical prediction of N-Ras localization due to the complex geometrical transformations associated with vesicle budding, or caveolae formation. Left to right: progressive deformation of a planar membrane to the ‘inverse omega’ shape commonly adopted by budding vesicles and caveolae. Color scale shows the density of N-Ras relative to a planar membrane. The model for the spherical and cylindrical parts of the membrane surface has been validated experimentally, while the model for saddle points has not. Local variations in the mean and Gaussian curvature of these complex geometries give rise to the depicted equilibrium protein density patterns.
### Supplementary information Table 1 | Summary of results for individual experiments

| Protein   | Assay     | Npts | Sorting Ratio (Ave±SD) |
|-----------|-----------|------|------------------------|
| **Streptavidin** | Tubes - Ex1 | 51   | 1.00                   |
|           | Tubes - Ex2 | 46   | 1.96                   |
|           | Tubes - Ex3 | 52   | 0.78                   |
|           | Tubes - Ex4 | 68   | 0.91                   |
|           | Tubes - Ex5 | 70   | 0.87                   |
|           | Ave±SEM:    |      | 1.10±0.24              |
|           | Vesicles - Ex1 | 1477 | 1.04                   |
|           | Vesicles - Ex2 | 1175 | 1.00                   |
|           | Vesicles - Ex3 | 1438 | 0.99                   |
|           | Ave±SEM:    |      | 1.01±0.01              |
| **tN-Ras** | Tubes - Ex1 | 58   | 1.92                   |
|           | Tubes - Ex2 | 60   | 2.51                   |
|           | Tubes - Ex3 | 130  | 2.31                   |
|           | Tubes - Ex4 | 162  | 2.67                   |
|           | Ave±SEM:    |      | 2.35±0.16              |
|           | Vesicles - Ex1 | 1648 | 3.54                   |
|           | Vesicles - Ex2 | 2066 | 4.05                   |
|           | Vesicles - Ex3 | 2207 | 7.34                   |
|           | Vesicles - Ex4 | 852  | 6.88                   |
|           | Ave±SEM:    |      | 5.45±0.97              |
| **Synaptotagmin** | Tubes - Ex1 | 45   | 6.02                   |
|           | Tubes - Ex2 | 43   | 6.78                   |
|           | Tubes - Ex3 | 80   | 2.63                   |
|           | Tubes - Ex4 | 99   | 2.21                   |
|           | Tubes - Ex5 | 103  | 5.87                   |
|           | Tubes - Ex6 | 94   | 8.01                   |
|           | Ave±SEM:    |      | 5.25±0.95              |
|           | Vesicles - Ex1 | 1037 | 43.04                  |
|           | Vesicles - Ex2 | 413  | 57.84                  |
|           | Vesicles - Ex3 | 220  | 29.00                  |
|           | Ave±SEM:    |      | 43.29±8.32             |
| **AnnexinB12** | Tubes - Ex1 | 38   | 1.00                   |
|           | Tubes - Ex2 | 13   | 1.12                   |
|           | Tubes - Ex3 | 63   | 0.88                   |
|           | Ave±SEM:    |      | 1.00±0.07              |
|           | Vesicles - Ex1 | 495  | 52.42                  |
|           | Vesicles - Ex2 | 387  | 33.27                  |
|           | Vesicles - Ex3 | 657  | 35.93                  |
|           | Ave±SEM:    |      | 40.54±5.99             |
| **α-Syn** | Vesicles - Ex1 | 2574 | 8.39                   |
|           | Vesicles - Ex2 | 1953 | 16.42                  |
|           | Vesicles - Ex3 | 688  | 11.66                  |
|           | Ave±SEM:    |      | 12.16±2.33             |
* p < 0.05, ** p < 0.01, *** p < 0.001, (NS) Not significant

| System comparison          | p value |
|----------------------------|---------|
| Tube Strep vs Tube tN-Ras  | **      | 0.0031  |
| Tube Strep vs Tube Syt     | **      | 0.0037  |
| Tube Strep vs Tube Anx     | NS      | 0.74    |
| Ves Strep vs Ves tN-Ras    | *       | 0.012   |
| Ves Strep vs Ves Syt       | **      | 0.0071  |
| Ves Strep vs Ves Anx       | **      | 0.0027  |
| Ves Strep vs Ves Amph      | ***     | 0.0007  |
| Tube tN-Ras vs Ves tN-Ras  | *       | 0.02    |
| Tube Syt vs Ves Syt        | ***     | 0.00026 |
| Tube Anx vs Ves Anx        | **      | 0.0027  |

Supplementary information Table 2 | Significance testing

A summary of the t-test evaluating 1) the significance of MBD sorting as compared to the negative control Strep and 2) the significance of the sorting difference between tubes and vesicles

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