

# Curvature of double-membrane organelles generated by changes in membrane size and composition

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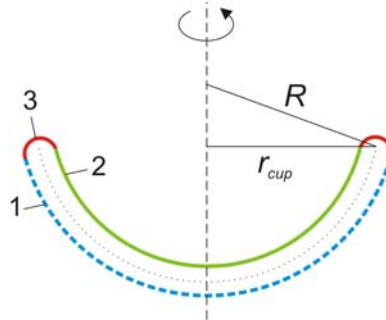
## Text S1

### Continuous deformation of a flat double-membrane sheet into a closed vesicle via cup-shaped intermediates

In the limit of  $r_{sheet} \gg r_{rim}$ , the bending energy of a sheet with non-zero homogeneous preferred curvature of the membrane ( $m_1 \neq 0$ ,  $m_2 \neq 0$ ,  $m_3 \neq 0$ ) is

$$E_{sheet} \approx \pi^2 \kappa \frac{r_{sheet}}{r_{rim}} (1 - 2m_3 r_{rim})^2 + 2\pi \kappa r_{sheet}^2 (m_1^2 + m_2^2) \quad (S1)$$

For the special case of nonhomogeneous preferred curvature of the membrane whereby  $m_1 = m_2 = 0$  and  $m_3 \neq 0$ , this expression is reduced to the first term only.



**Figure S1: Geometrical parameters of a cup-shaped intermediate.** The radius of the cup  $r_{cup}$  and the curvature radius  $R$  are shown. The lower (dashed blue), upper (solid green) and the rim (solid red) segments are indicated with 1, 2 and 3, respectively.

In the limit of  $R \gg r_{rim}$ , the bending energy of a vesicle or an organelle with asymmetric preferred curvature of the inner and outer membranes, i.e.,  $m_1 \neq m_2$  is

$$E_{ves} \approx 8\pi \kappa \left[ 1 + (1 - 2Rm_{12})^2 + 2R^2 m_1 m_2 \right] \quad (S2)$$

where the asymmetry parameter  $m_{12} = (m_1 - m_2)/2$ ; see also Fig. S1. For membranes with molecules symmetrically bound on both sides of the double membrane shape  $m_{12} = 0$ .

The total bending energy of a cup-shaped organelle is

$$E = E_1 + E_2 + E_3, \quad (S3)$$

where  $E_1$  and  $E_2$  arise from the bending of the two, initially flat surfaces (corresponding to  $E_{ves}$  for the closed organelle) and  $E_3$  is the contribution arising from the strongly bent rim (corresponding to  $E_{sheet}$  for the flat sheet conformation). For the limit of  $R \gg r_{rim}$ , the sum  $E_1 + E_2$  for the cup-shaped organelle with the total surface area

$A \approx 2\pi r_{sheet}^2$  is given by [1]:

$$E_1 + E_2 \approx 4\pi\kappa r_{sheet}^2 \left[ (M_1 - m_{12})^2 + m_{12}^2 + m_1 m_2 \right] \quad (S4)$$

Here, we consider a cup-shaped organelle with molecules asymmetrically bound on both sides of the shape, i.e.,  $m_{12} \neq 0$ .

The rim energy  $E_3$  can be approximately estimated considering the initial sheet and introducing the effective rim tension  $\lambda_{eff}$ :

$$\lambda_{eff} = \frac{E_{sheet}}{2\pi r_{sheet}} = \frac{\pi\kappa}{2r_{rim}} (1 - 2m_3 r_{rim})^2 \quad (S5).$$

Then for  $r_{cup} \gg r_{rim}$ , a cup-shaped intermediate with length of the rim  $2\pi r_{cup}$  will be characterized by the rim energy:

$$E_3 \approx 2\pi\lambda_{eff} r_{cup} \quad (S6)$$

Taking into account that  $r_{cup} = r_{sheet} \sqrt{1 - (r_{sheet} M_1 / 2)^2}$ , we obtain

$$E_3 \approx \pi^2 \kappa \frac{r_{sheet}}{r_{rim}} (1 - 2m_3 r_{rim})^2 \sqrt{1 - (r_{sheet} M_1 / 2)^2} \quad (S7).$$

For convenience we introduce the dimensionless reduced energy

$$\bar{E} = E / 4\pi\kappa - r_{sheet}^2 (m_1 + m_2)^2 / 4 \quad (S8)$$

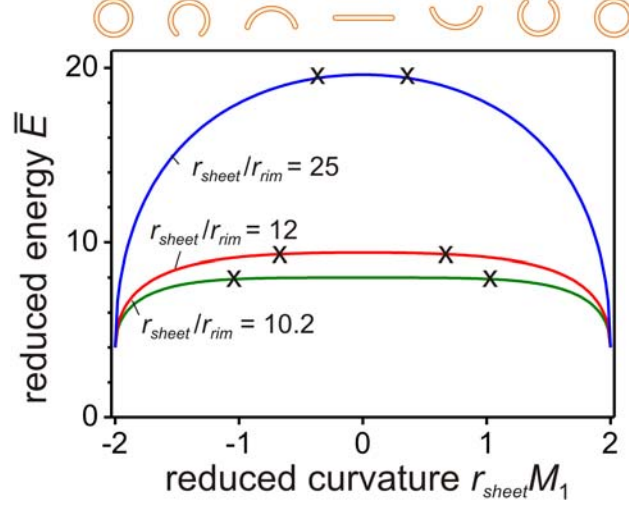
The second term in this expression is constant for various curvatures  $M_1$  of the cup-shaped vesicle. From expressions (S3), (S4) and (S7) we obtain

$$\bar{E} \approx r_{sheet}^2 (M_1 - m_{12})^2 + \pi \frac{r_{sheet}}{4r_{rim}} (1 - 2m_3 r_{rim})^2 \sqrt{1 - (r_{sheet} M_1 / 2)^2} \quad (S9)$$

which depends mainly on the preferred curvature  $m_{12}$  and the curvature  $M_1$  of the cup-shaped organelle.

Results for the reduced total energy of double-membrane sheets with vanishing asymmetry parameter ( $m_{12} = 0$ ) are plotted in Fig. 3 in the manuscript and in Fig. S2. Slow growth may kinetically trap the sheet close to the critical size flattening the energy landscape, see Fig. S2. At the critical size, the energy landscape exhibits a large, flat plateau around the sheet state and even very pronounced shape changes corresponding to the curvature range  $-1 < r_{sheet} M_1 < 1$  do not change the total energy significantly (crosses in Fig. S2 correspond to a decrease of the bending energy by 1%). This suggests that the sheet may be kinetically trapped slowing down the sheet closure. On the other hand, fast growing sheets will pass the critical size and can become large compared to this size. In this state, relatively small morphological changes will be associated with a significant decrease in the bending energy and closure will be fast.

Results for the reduced total energy of double-membrane sheets with different values of the asymmetry parameter  $m_{12}$  are plotted in Fig. S3. Nonzero asymmetry parameter breaks the symmetry of the energy profile; compare with Fig. 3 in the main text.

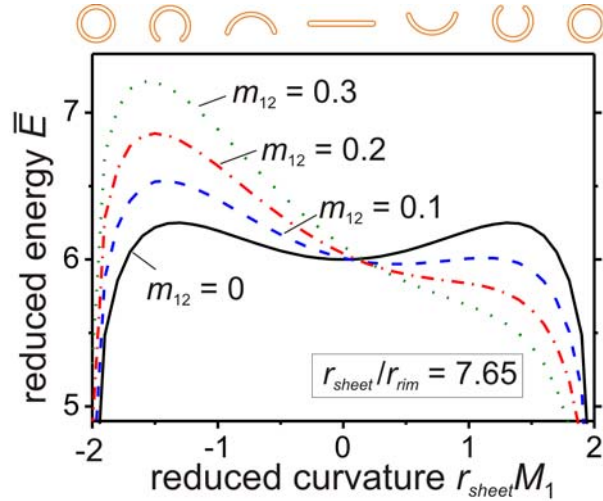


**Figure S2: Reduced energy  $\bar{E}$  of double-membrane organelles as a function of the reduced curvature  $r_{sheet}M_1$  for  $m_{12} = 0$  and  $m_3 = 0$ .** At and above the critical size,  $r_{sheet}/r_{rim} = 10.2$ , no barrier exists anymore, the closed organelle is the shape of minimal energy and bending of the flat sheet is energetically favorable. The points where the energy is decreased by 1 % of the energy of the initial sheet are marked with a cross (x). Small organelles close to the critical size can deform strongly without considerable change in the bending energy, while large organelles reduce their bending energy already at comparably small deformations. Thus, a large sheet has a high probability to close within a short time. Small sheets, even if larger than their critical size, will close after a considerable lag-time.

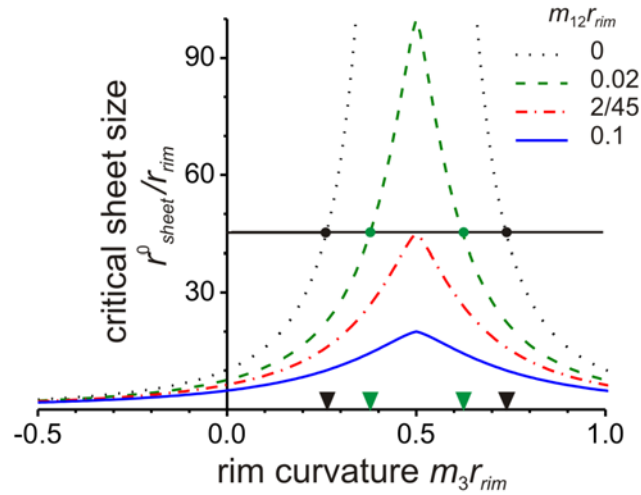
Having obtained an expression for the energy of the double-membrane shapes, we can now proceed to determine the reduced critical sheet size  $r_{sheet}^0/r_{rim}$  as in reference [1] which leads to:

$$\frac{r_{sheet}^0}{r_{rim}} = \frac{32}{\pi} \left( |1 - 2m_3 r_{rim}|^{4/3} + |16m_{12} r_{rim} / \pi|^{2/3} \right)^{-3/2} \quad (S10)$$

This result shows that the reduced critical sheet size depends only on the reduced rim curvature  $m_3 r_{rim}$  and the curvature asymmetry  $m_{12} r_{rim}$ . The expression (S10) for the critical sheet size is plotted in Fig. 5 for the symmetric case  $m_{12} = 0$  and in Fig. S3 for  $m_{12} \neq 0$ . For double-membrane spherical organelles with the reduced size  $r_{sheet}^0/r_{rim} \cong 45$  as considered in the main text, the reduced rim curvatures of symmetric sheets ( $m_{12} = 0$ ), and of sheets with asymmetry parameter  $m_{12} r_{rim} = 0.02$  are indicated in the figure caption. Inspection of Fig. S3 also reveals that sheets with this size and preferred curvature  $m_{12} r_{rim} > 2/45$  will never be stable irrespective of the value of the sheet rim curvature.



**Figure S3: Reduced bending energy of double-membrane shapes,  $\bar{E}$ , as a function of the reduced curvature  $r_{sheet}M_1$  calculated for different values of the effective preferred curvature  $m_{12}$ .** Asymmetrical distribution of molecules on both sides of the shape changes the effective preferred curvature  $m_{12}$  and favors a certain direction of bending. The reduced energy is plotted for different values of  $m_{12}$ , effective size  $r_{sheet}/r_{rim} = 7.65$  and rim preferred curvature  $m_3 = 0$ . For  $m_{12} = 0$  (curve) as presented in Fig. 3 in the main text, the probabilities for upward or downward curving are equal. Nonzero  $m_{12}$  breaks the symmetry of the energy profile.



**Figure S4: Dependence of the critical size  $r_{sheet}^0$  of the sheet as a function of the rim curvature  $m_3$  for different values of the curvature asymmetry  $m_{12}$ .** All quantities are given in units if the rim curvature radius  $r_{rim}$ . The regions below and above a curve correspond to conditions for stable sheets and double-membrane spherical organelles respectively. For  $r_{sheet}^0/r_{rim} \cong 45$  (black solid line) corresponding to the autophagosome size with  $r_{rim} = 20$  nm in Fig. 2C, the rim curvature  $m_3 \cong 1/(76$  nm) or  $1/(28$  nm) for the case of symmetric sheets, i.e.,  $m_{12} = 0$ , see black arrowheads, while for a sheet with asymmetry parameter  $m_{12}r_{rim} = 0.02$   $m_3 \cong 1/(54$  nm) or  $1/(32$  nm), see green arrowheads.

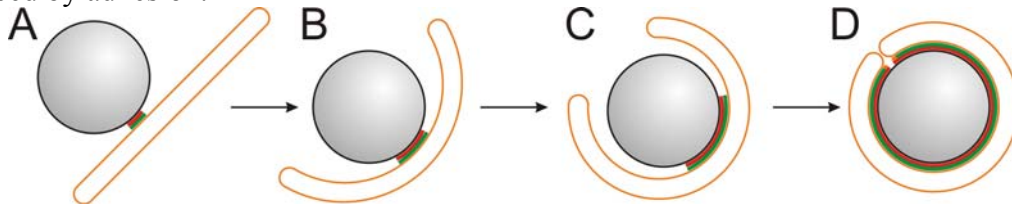
## Text S2

### Enclosing of substrates with specific interaction with the membrane

The basis of specific autophagy is the interaction between a particular ligand with a certain receptor. This interaction is attractive and reduces the free energy of the whole system. The total energy of this system is described by:

$$E = E_1 + E_2 + E_3 + E_{ad} \quad (11)$$

With the adhesion energy  $E_{ad} = WA_{ad}$ . The adhesion energy per unit area,  $W$ , is negative and  $A_{ad}$  is the adhesion area. A receptor can bind its ligand provided they are sufficiently close to each other. If a flat membrane sheet and a specific substrate approach each other, the adhesion area  $A_{ad}$  for initial binding is very small and thus will not contribute significantly to the total energy of the sheet  $E$ , see Fig. S5 which illustrates a case of weak adhesion. The more the sheet bends, the closer the curvature of the substrate and the membrane are and the larger the area of adhesion will be. Consequently, the effect of adhesion energy will be the stronger the closer the reduced curvature  $r_{sheet}M$  to the substrate curvature is, see Fig. S5. Accordingly, the energy barrier can be significantly reduced by adhesion.



**Figure S5: Weak adhesion and bending of a double-membrane sheet around a substrate.** The surface of the substrate (grey) is covered with receptors and the surface of double membrane with ligands. The regions where ligands (green) and receptors (red) are sufficiently close together to interact are indicated. (A) The flat membrane sheet adhering to the curved substrate has a very small area of interaction. (B, C) As the sheet bends the curvatures of the substrate and the membrane approach each other and the adhesion area increases. (D) The substrate is fully covered by the membrane and the sheet closes.

In the main text, we showed that a reduction of the critical size will generally lead to a smaller size of the final double-membrane organelle. Thus, specific interactions of any kind between the membrane and another particle or organelle will theoretically decrease the size of the double-membrane organelle. In reality, the organelle size will be determined by the size of the particle to a large extent.

## References

1. Lipowsky R (1992) Budding of membranes induced by intramembrane domains. *Journal De Physique II* 2: 1825-1840.