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There's lots of things that you can do alone, but it takes two to tango.

Louis Armstrong

Contents

8.1	Introduction and Overview		211
8.2	Different Processes Induced by Particle–Membrane Interactions		212
	8.2.1	Basic Particle Properties: Size, Shape and Surface Chemistry	212
	8.2.2	Particle Adsorption	213
	8.2.3	Particle Incorporation	213
	8.2.4	Particle Translocation	214
	8.2.5	Particle Engulfment	214
8.3	B Basic Aspects of Particle Engulfment		214
	8.3.1	Curvature-Dominated versus Tension-Dominated Regimes	214
	8.3.2	Generic versus Specific Particle–Membrane Adhesion	215
8.4	Engulfment of Rigid Spherical Particles		216
	8.4.1	Adhesion Length	216
	8.4.2	Energy Landscapes and Engulfment Regimes	216
	8.4.3	Stability Relations	218
	8.4.4	Energy Landscapes and Curvature-Induced Forces	220
	8.4.5	Adhesion-Induced Segregation of Membrane Components	220
8.5	Engulfment of Complex Particles		221
	8.5.1	Nonspherical Particles	221
	8.5.2	Deformable Particles and Fluid Droplets	221
8.6	Engulfment of Multiple Particles		221
	8.6.1	Engulfment Patterns	221
	8.6.2	Particle Aggregation into Membrane Tubes	222
	8.6.3	Janus Particles and Membrane-Mediated Interactions	222
8.7	Summ	223	
List of Abbreviations			224
Glossary of Symbols			224
References			224

8.1 INTRODUCTION AND OVERVIEW

Membranes are present in every living cell, and represent an essential component of life as we know it. They separate the inside from the outside of cells, and they form many smaller membrane-bounded compartments within the cell. Because of their fluidity, biological membranes are highly dynamic and can remodel their architecture in response to external and internal signals. In this chapter, we will be concerned with those remodeling processes that are involved in the entry of nanometer-sized particles into cells. Indeed, nanoparticles are increasingly used for targeted delivery of drugs to biological cells (Panyam and Labhasetwar, 2003; Singh and Lillard, 2009), reaching difficult targets such as tumors (Paciotti et al., 2004; Cho et al., 2008) or crossing the blood-brain barrier (Kreuter, 2001; Lockman et al., 2002). Novel magnetic nanoparticles are being developed as contrast agents in magnetic resonance imaging (Lee et al., 2007; Sun et al., 2008), and gold nanoparticles are being used in X-ray imaging and photothermal therapies (Hainfeld et al., 2006; Huang et al., 2006).

Nanoparticles are also more and more common in industrial processes, which has raised some concerns about their cytotoxicity. The world production of nanoparticles is projected to increase 25-fold in the period between 2008 and 2020 (Lewinski et al., 2008), with nanoparticles being extensively used in cosmetics, food, paints, powders, and surface treatments (Schmid and Riediker, 2008). Both *in vitro* and *in vivo* studies have found that nanoparticles can be cytotoxic when applied in high doses, with toxicity levels depending on nanoparticle size, shape, and surface chemistry (Fischer and Chan, 2007; Pan et al., 2007; Lewinski et al., 2008). A further particularly important class of nanoparticles is provided by viruses. Viral capsids typically range in size from 20 to 300 nm, and enter cells *via* the same pathways as artificial nanoparticles (Mercer et al., 2010). Insights into the nanoparticle pathways may thus help in creating new tools against viral infection.

In order to enter a cell, a particle must first cross the cellular membrane. This process is termed *endocytosis*, and starts with the adhesion and engulfment of the particle by the membrane, followed by membrane fission (Mukherjee et al., 1997; Sahay et al., 2010). The particle then ends up inside the cytoplasm, fully enclosed by the membrane. Several distinct endocytic pathways used by cells to take up nanoparticles have been described, depending on the molecular machinery involved. The most thoroughly studied pathway is clathrin-mediated endocytosis, which is assisted by the formation of a strongly curved protein coat on the inner side of the membrane (Mukherjee et al., 1997).

As demonstrated throughout this book, giant unilamellar vesicles (GUVs) are very important model systems that can provide useful insight into the structure and dynamics of cellular membranes. In this context, engulfment of rigid particles by GUVs (Dietrich et al., 1997; Koltover et al., 1999; Fery et al., 2003; Meinel et al., 2014; Strobl et al., 2014; van der Wel et al., 2016), smaller liposomes (Le Bihan et al., 2009; Michel et al., 2014), as well as polymer vesicles (Jaskiewicz et al., 2012) has been observed to occur spontaneously in experiments. In this way, spontaneous engulfment by GUVs may be seen as a membrane-driven mimetic mechanism of endocytosis in cells. Other experiments have observed adsorption of nanoparticles onto the GUV surface (Dinsmore et al., 1998; Natsume et al., 2010; Li et al., 2016; van der Wel et al., 2016), as well as particleinduced disruption of the membrane structure (Luccardini et al., 2006; Laurencin et al., 2010; Li and Malmstadt, 2013; Wei et al., 2015). Membrane disruption in GUVs illustrates one aspect of the cytotoxic capabilities of nanoparticles.

In this chapter, we consider the interaction between rigid particles and membranes, with particular emphasis on particle adhesion and engulfment (see also Chapters 9 and 25 for theoretical and experimental reviews of the interactions of membranes and polymers, which may be considered as soft particles). In Section 8.2, we will describe the basic particle properties that determine particle-membrane interactions and introduce the different processes arising from these interactions. Subsequent sections will be devoted to particle adhesion and engulfment. In Section 8.3, we introduce some basic aspects of particle engulfment, followed by a detailed theoretical description of the engulfment of rigid spherical particles in Section 8.4. In Section 8.5, we review some theoretical work on engulfment of nonspherical and deformable particles. Finally, we will review existing work on simultaneous engulfment of many particles, particle aggregation and membrane-mediated particle-particle interactions in Section 8.6, followed by some closing remarks and an outlook in Section 8.7.

8.2 DIFFERENT PROCESSES INDUCED BY PARTICLE-MEMBRANE INTERACTIONS

8.2.1 BASIC PARTICLE PROPERTIES: SIZE, SHAPE AND SURFACE CHEMISTRY

From a theoretical point of view, we would like to identify the basic principles underlying particle-membrane interactions. A first problem arises from the wide variety of particles that are used in applications. Indeed, the term "nanoparticle," as found in the scientific literature, encompasses an astonishing variety of particles, made from different materials and with widely differing characteristics, such as fullerenes (Kroto et al., 1985) and quantum dots (Alivisatos, 1996) as well as silver (Rai et al., 2009), gold (Daniel and Astruc, 2004) and silica (Slowing et al., 2008) nanoparticles.

As a first approximation, we may identify three particle properties of particular relevance for particle–membrane interactions (Albanese et al., 2012):

- 1. Size: Nanoparticles used in applications cover a wide range of sizes, from below 1 nm, in the case of spherical fullerenes (Kroto et al., 1985), up to a few hundreds of nanometers, in the case of large metallic (Daniel and Astruc, 2004; Dykman and Khlebtsov, 2014) or silica nanoparticles (Slowing et al., 2008). In experiments with model GUVs, latex particles as large as several micrometers have been used (Dietrich et al., 1997). Naturally, particles that are smaller than the membrane thickness, $\ell_{\rm me} \approx 5$ nm, will interact with the membrane in very different ways than those that are of comparable size or much larger than the membrane thickness.
- 2. Shape: Commonly used particles are most often spherical but they can also have elongated quasi-one-dimensional shapes as in the case of carbon nanotubes (Odom et al., 1998) or gold nanorods (Jana et al., 2001) as well as flat disk-like shapes as in the case of graphene nanosheets. In all of these cases, it will be important whether one, two or all of the particle dimensions are larger or smaller than the membrane thickness, $\ell_{\rm mc}$.
- 3. Surface chemistry: Hydrophilicity and hydrophobicity, as well as surface charge, strongly influence particle– membrane interactions. In addition, nanoparticles may be functionalized by coating them with lipids, polymers or specific protein ligands that can bind to receptors on the membrane surface (Verma and Stellacci, 2010; Monopoli et al., 2012; Mahmoudi et al., 2014). It is also possible to produce "patchy" particles with a nonhomogeneous surface, consisting of patches with different coatings or functionalization (Pawar and Kretzschmar, 2010; Poon et al., 2010). A particularly important kind of patchy particles are "Janus" particles, consisting of two distinct hemispherical patches or faces (Lattuada and Hatton, 2011; Li et al., 2016).

Depending on these properties, particles may (i) adsorb onto the membrane, (ii) incorporate into the hydrophobic core of the bilayer, (iii) translocate through the membrane, or (iv) be engulfed by the membrane.

8.2.2 PARTICLE ADSORPTION

Particles that are both hydrophilic—so that they do not incorporate into the bilayer membrane—and small with a size comparable to the head group of the lipids—may adsorb onto the surface of the membrane (Lipowsky and Döbereiner, 1998; Lipowsky, 2013; Curtis et al., 2015; Różycki and Lipowsky, 2015). The latter process is illustrated by the molecular dynamics (MD) simulation snapshot in Figure 8.1a, which displays a hydrophilic particle of diameter 1 nm after adsorption onto a 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine membrane. Such "particles" may also be atomic ions or small molecules and solutes.

If a large number of such particles are present in the aqueous solution, they will adsorb onto the membrane surface with a certain coverage, Γ , proportional to the molar concentration, X, of particles in the solution. If the concentration of particles in the interior and exterior of the GUV are different, $X_{in} \neq X_{ex}$, the coverage of adsorbed particles will also differ between the interior and exterior surfaces of the membrane, $\Gamma_{in} \neq \Gamma_{ex}$. In this case, the asymmetric adsorption of particles onto the bilayer will generate a spontaneous curvature, m, in the membrane, with m as defined in Chapter 5, given by

$$m \approx \frac{k_{\rm B}T}{4\kappa} \ell_{\rm me} (\Gamma_{\rm ex} - \Gamma_{\rm in})$$
 (8.1)

This relation was first predicted theoretically by using a Langmuir adsorption model (Lipowsky and Döbereiner, 1998; Lipowsky, 2013) and later confirmed by MD simulations (Różycki and Lipowsky, 2015).

8.2.3 PARTICLE INCORPORATION

Hydrophobic particles such as spherical fullerenes, which have a size comparable to or smaller than the membrane thickness, ℓ_{me} , can be incorporated into the hydrophobic core of the bilayer membrane, as observed in many experiments (Hetzer et al., 1997; Gopalakrishnan et al., 2006; Bothun, 2008; Chen et al., 2010; Mai and Eisenberg, 2010; Rasch et al., 2010; Liu et al., 2013) and simulations (Ginzburg and Balijepalli, 2007; Wong-Ekkabut et al., 2008; D'Rozario et al., 2009; Curtis et al., 2015). A typical series of simulation snapshots is displayed in Figure 8.1b. Thus, very small hydrophobic nanoparticles behave just like hydrophobic molecules and can create "oily" pockets inside membranes, as observed in experiments (Hayward et al., 2006) and simulations (Greenall and Marques, 2012).

Some molecular simulations also found that hydrophilic particles that have a size comparable to the membrane thickness, $\ell_{\rm me} \approx 5$ nm, and exhibit an attractive interaction with the lipid headgroups may be incorporated into the membrane (Figure 8.1c) (Noguchi and Takasu, 2002; Ginzburg and Balijepalli, 2007; Smith et al., 2007; Guo et al., 2013; Yue et al., 2014; Curtis et al., 2015). However, this type of particle incorporation has not been observed experimentally and may be suppressed in real systems. Indeed, in order to completely surround the particle and maximize the contact with the hydrophilic head groups, the membrane must change its topology and form an interior hydrophilic "pocket," with a line of T-junction defects around the particle (Figure 8.1c). This defect line involves a line energy proportional to the particle diameter. It is likely that the coarse-grained, "soft" potentials used in the simulations underestimate the energetic cost of such T-junction defects, which are expected to be rather large for real lipid bilayers.



Figure 8.1 Different processes based on the interactions between particles and membranes. Time-series snapshots (cross sections) of molecular dynamics simulations of (a) adsorption, (b, c) incorporation, (d) translocation, and (e) engulfment of particles. The hydrophilic heads of the lipids are dark blue in (a–c), red in (d), and turquoise in (e); the hydrophobic tails of the lipids are turquoise in (a–c), yellow in (d), and black in (e). In (a), the particle is hydrophilic with radius $R_{pa} < \ell_{me}$ and adsorbs onto the membranes surface. In (b), the particle is hydrophobic with radius $R_{pa} < \ell_{me}$ and incorporates into the membrane core. In (c), the particle is hydrophilic with radius $R_{pa} \sim \ell_{me}$ and changes the topology of the bilayer. In (d), an elongated particle of a size comparable to ℓ_{me} and subject to an external force passes through the membrane. In (e), a hydrophilic particle of radius $R_{pa} \gg \ell_{me}$ is gradually engulfed but remains connected to the mother membrane by a small neck. ([a–c] Curtis, E.M. et al., *Nanoscale*, 7, 14505–14514, 2015. Reproduced by permission of The Royal Society of Chemistry; [d] Reprinted by permission from Macmillan Publishers Ltd. *Nat. Nanotechnol.*, Yang, K. and Ma, Y.-Q., 2010, copyright 2010; [e] Reprinted with permission from Smith, K.A. et al., *J. Chem. Phys.*, 127, 84703, 2007. Copyright 2007 by the American Institute of Physics.)

8.2.4 PARTICLE TRANSLOCATION

Lipid bilayers are permeable to small uncharged molecules such as H_2O , O_2 , and CO_2 (Lodish et al., 2000) (see also Chapter 20), which can therefore pass through the membrane without disrupting the bilayer structure. One may then ask whether such a translocation process is also possible for larger, artificial nanoparticles.

In order to permeate the membrane, particles with a completely hydrophilic surface will have to cross a large energy barrier provided by the hydrophobic core of the bilayer. On the other hand, if the particle surface is completely hydrophobic, the particle will prefer to insert into the hydrophobic core as described in Section 8.2.3. It was first proposed, based on simulation studies, that nanoparticles with weakly bound amphiphilic ligands (Ding et al., 2012) or with mixed hydrophilic–hydrophobic surface domains (Li et al., 2012) may be able to pass through lipid bilayers more easily. As far as we know, there is no experimental evidence for such a translocation process facilitated by heterogeneous surfaces. However, recent experiments indicate that lipid-covered hydrophobic nanoparticles with a diameter of about 6 nm can translocate through membranes (Guo et al., 2016).

Nanoparticles can be forced to penetrate through membranes by external forces that push (or pull on) the particles. Thus, a carbon nanotube that is attached to an atomic force microscope can be pushed through cell membranes (Chen et al., 2007). In simulations, the minimal forces required to make particles of different size, shape and surface chemistry pass through a lipid bilayer have been measured (Yang and Ma, 2010; Li et al., 2012). An example is shown in Figure 8.1d, which displays simulation snapshots of an ellipsoidal particle that is moved through a bilayer by applying an external force to the particle's center of mass.

8.2.5 PARTICLE ENGULFMENT

In all the cases described so far, the size of the particles was comparable to or smaller than the membrane thickness, ℓ_{me} . As soon as the particles become larger (in all dimensions) than a few times the membrane thickness, incorporation or translocation become energetically unfavorable and the membrane will instead spread onto the particle and engulf it (Figure 8.1e) (Lipowsky and Döbereiner, 1998; Smith et al., 2007; Roiter et al., 2008) if the interaction between the hydrophilic surface of the membrane and the particle is attractive. The engulfment process ends with the particle fully covered by the membrane but still connected to the mother membrane by a small neck, as in the rightmost snapshot of Figure 8.1e. This neck might then break via membrane fission, which implies that the particle has been effectively transported from one side of the membrane to the other. In the case of a closed vesicle, we will distinguish between endocytic engulfment, for which the particle originates from the exterior compartment of the vesicle, and exocytic engulfment, for which the particle originates from the interior compartment.

In the rest of this chapter, we will focus on the process of particle engulfment for three main reasons. First, engulfment applies to the largest range of particle sizes: Whereas adsorption, incorporation or translocation require particles of a size comparable or smaller than the membrane thickness, engulfment can occur for particles ranging in size from a few nanometers to several micrometers. Second, the study of particle engulfment by model membranes provides fundamental insights into the process of endocytosis, the main pathway of particle entry into cells. Finally, from a theoretical perspective, particle engulfment can be understood in the context of the curvature elasticity theory of membranes as introduced in Chapter 5, in which the molecular details of the membrane are coarse-grained into two basic elastic parameters: the bending rigidity κ and the spontaneous curvature *m*. The processes of adsorption, incorporation or translocation, on the other hand, depend on the rearrangement of just a few lipids and are therefore dependent on the molecular details of the membrane. As a consequence, they are typically studied using simulations.

8.3 BASIC ASPECTS OF PARTICLE ENGULFMENT

8.3.1 CURVATURE-DOMINATED VERSUS TENSION-DOMINATED REGIMES

As explained in Chapter 5, the equilibrium shapes of free vesicles are governed by the curvature elasticity of membranes, as long as the vesicles are large compared with the membrane thickness, that is, with diameters larger than about 50 nm. In typical experimental conditions, a vesicle membrane has a fixed total number of lipid molecules, and therefore a fixed rest area, A. In addition, experiments are typically carried out in aqueous solutions that contain osmotically active particles, such as salts or sugars. In such conditions, the volume, V, enclosed by the vesicle is also fixed (Seifert et al., 1991; Seifert, 1997). The equilibrium shape of such a free vesicle then depends only on its volume-to-area ratio or reduced volume

$$v \equiv \frac{V}{\frac{4\pi}{3} \left(\frac{A}{4\pi}\right)^{3/2}}$$
(8.2)

and on its membrane spontaneous curvature, *m*. For v = 1, the vesicle has a spherical shape and is typically "tense," not displaying strong shape fluctuations. For volumes 0 < v < 1, the vesicle will be "flaccid," with a nonspherical fluctuating shape. Note that we will often use the simple term "volume" as an abbreviation for reduced volume.

We can now distinguish two qualitatively different situations with respect to particle engulfment, depending on whether the vesicle has enough excess area to accommodate the particle or not. Suppose that we have a particle, not necessarily spherical, with volume V_{pa} and area A_{pa} . After completely engulfing the particle, the vesicle will enclose a volume $V \pm V_{pa}$, where the plus and minus signs apply to endocytic and exocytic engulfment respectively (Figure 8.2). The area of the remaining free membrane segment of the vesicle, not bound to the particle, will be $A - A_{pa}$. The new reduced volume of the vesicle after complete engulfment of the particle is then given by

$$v' \equiv \frac{V \pm V_{\text{pa}}}{\frac{4\pi}{3} \left(\frac{A - A_{\text{pa}}}{4\pi}\right)^{3/2}}$$
(8.3)

Because the new reduced volume v' always exceeds the original v, the vesicle becomes more spherical after a particle has



Figure 8.2 Endocytic (a) and exocytic (b) engulfment. In (a), the vesicle initially encloses a volume V (striped region) and engulfs a particle of volume V_{pa} originating from the exterior compartment. After engulfment, the effective volume enclosed by the mother vesicle includes the particle volume and is equal to $V + V_{pa}$ (striped region). In (b), the vesicle initially encloses a volume V (striped region), which includes the volume of the particle. After engulfment, the effective volume enclosed by the mother vesicle is $V - V_{pa}$ (striped region). In both (a) and (b), the area of the mother vesicle after engulfment is $A - A_{pa}$, where A is the area of the original vesicle and A_{pa} is the surface area of the particle.

been engulfed. We will now use the volume v' to distinguish a curvature-dominated from a tension-dominated regime. The curvature-dominated regime is defined by $v' \leq 1$, which implies that the vesicle has enough excess area to fully accommodate the particle. The curvature-dominated regime will be discussed in detail in Section 8.4 for the simplest case of a spherical particle. If, on the other hand, we find that v' > 1, the vesicle does not have enough excess area, and the vesicle membrane would need to stretch in order to accommodate the particle, leading to an increase in the mechanical tension of the membrane. This argument can naturally be extended to the engulfment of multiple particles: After complete engulfment of N particles, the new volume-to-area ratio v' of the vesicle is given by Eq. 8.3, with NV_{pa} and NA_{pa} instead of V_{pa} and A_{pa} .

As described in Chapter 5, the mechanical tension of the membrane can be related to the stretching of the membrane via $\Sigma = K_A \Delta A / A$, where K_A is the area compressibility modulus of the membrane and $\Delta A / A$ is the relative area stretching of the membrane with respect to the original (relaxed) vesicle membrane area. The energetics is then dominated by tension over bending if $\Sigma A_{\text{Da}} \gg \kappa$. In this tension-dominated regime, the equilibrium geometry of the system is dictated by a competition between particle-membrane adhesion and vesicle membrane tension, akin to the well-studied case of substrate wetting by droplets (Seifert and Lipowsky, 1990). Indeed, for vesicles that are initially close to spherical $v \approx 1$, it was found by Dietrich et al. (1997) that the experimentally observed engulfment geometries could be explained in close analogy to the Young-Dupré equation for wetting. Furthermore, when the membrane was stretched beyond ~ 4% by the particle engulfment, that is, for $\Delta A / A \ge 0.04$, the membrane was observed to rupture. The intermediate case in which the bending and stretching contributions to the total energy are comparable was studied using an approximate numerical method by Deserno and Gelbart (2002).

8.3.2 GENERIC VERSUS SPECIFIC PARTICLE-MEMBRANE ADHESION

As we have seen, in order to engulf a particle, the membrane needs to bend and, in the tension-dominated regime, it also needs to stretch. Both of these processes are energetically unfavorable, so what constitutes, then, the driving force for engulfment? This driving force is provided by attractive interactions between the surface of the particle and the surface of the membrane, leading to favorable adhesion between the two. Generally, we can speak of two qualitatively different types of particle–membrane interactions: generic surface interactions; and specific interactions mediated by ligands on the particle that attach to corresponding receptors or "stickers" present on the membrane, thus forming receptor–ligand pairs; see Chapter 17 for an overview of adhesion of GUVs to substrates.

A thorough description of generic interactions in membranes can be found in the review by Lipowsky (1995). These interactions are always present as a combination of short-ranged repulsion arising from hydration forces and longer-ranged electrostatic and van der Waals forces that may be attractive or repulsive depending on the particle-membrane separation. These generic interactions are renormalized by membrane fluctuations in the proximity of the rigid particle (Lipowsky and Leibler, 1986). Due to their complexity, a detailed description of these forces lies outside the scope of this chapter. Suffice to say that, when adding all the contributions together, a typical attractive potential between the surface of the particle and the membrane will show (i) repulsion for short distances, (ii) a minimum at a certain distance and (iii) attraction at longer distances. Several such phenomenological effective potentials have been used in theoretical research on particle engulfment, such as a Morse potential (Raatz et al., 2014) or a Lennard-Jones type of potential (Šarić and Cacciuto, 2012).

In practice, the typical range of such generic attractive interactions is on the order of just a few nanometers, usually even smaller than the membrane thickness. In order to study the overall shape of the adhering membrane, one may ignore the molecular details and focus on the adhesive strength $|W_{\rm gen}|$ of the membrane-surface interactions that corresponds to the adhesion (free) energy per area (Seifert and Lipowsky, 1990). This coarse-grained description of the membrane-surface interactions in terms of a single material parameter is in accordance with the separation of length scales that is used to describe the curvature elasticity of the membranes (see Chapter 5).

In addition to the generic interactions just discussed, biological membranes interact *via* membrane-anchored receptors and ligands (Lipowsky, 1996; Hu et al., 2013). Such specific interactions enable cellular membranes to selectively mediate binding of cells to other cells, an essential step in immune response or tissue development, as well as binding to small cargo that is to be internalized by the cell *via* endocytosis. In experiments, a commonly used pair of receptor–ligand molecules is provided by biotin and streptavidin, two molecules that can be easily used for the adhesion of particles and vesicles. The binding free energy, |U|, of such a receptor–ligand pair contains both enthalpic and entropic contributions. If the binding enthalpy of a single bond is |H|, its free energy can be estimated by $|U| = |H| - k_B T \ln(\rho_{\rm lig}/\rho_0)$, where $\rho_{\rm lig}$ is the density of ligands on the particle surface and ρ_0 that of receptors on the membrane before contact with the particle (Gao et al., 2005). The second term represents the loss of translational entropy by the receptor–ligand bond. If this binding free energy is large compared with k_BT , all ligands will be bound to a receptor and the total energetic contribution of the specific interactions can be incorporated into a single adhesive energy per unit area of particle–membrane contact $|W_{\rm spe}| \equiv |U| \rho_{\rm lig}$.

In order to obtain a simple description of particle adhesion, we can combine both generic and specific attraction into a single parameter: the adhesive strength $|W| \equiv |W_{\text{gen}}| + |W_{\text{spe}}|$. In this way, the whole complexity of the adhesive interactions can be encoded into a single parameter, which can also be measured experimentally (see Table 5.2 in Section 5.8 of Chapter 5). The adhesion energy of a particle in contact with a membrane segment of area \mathcal{A}_{bo} is then given simply by

$$\mathcal{E}_{ad} = -|W|\mathcal{A}_{bo} \tag{8.4}$$

Such an approach was first used in the study of vesicle adhesion to planar substrates (Seifert and Lipowsky, 1990). Values for |W| have been measured experimentally for many different systems, and can vary widely from 10^{-5} mJ/m² for 1,2-dioleoyl-*sn*-glycero-3phosphocholine/1,2-dioleoyl-*sn*-glycero-3-phospho-rac-(1-glycerol) (DOPC/DOPG, see Appendix 1 of the book for structure and data on these lipids) membrane and glass surfaces (Gruhn et al., 2007), known as ultraweak adhesion, to strong adhesion with 0.5 mJ/m² for 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC, see Appendix 1 of the book for structure and data on this lipid) and silica surfaces (Anderson et al., 2009) (see Table 5.2 in Chapter 5).

8.4 ENGULFMENT OF RIGID SPHERICAL PARTICLES

8.4.1 ADHESION LENGTH

As mentioned in Section 8.3, in the curvature-dominated regime, spontaneous engulfment is the result of an interplay between membrane bending and particle adhesion: the gain in adhesion energy will be opposed by the energetic cost of bending the membrane around the particle. Such an energy balance was first considered by Lipowsky and Döbereiner (1998) for the case of a large vesicle (with zero spontaneous curvature) engulfing a small spherical particle. If the particle has radius R_{pa} , the completely engulfed particle will gain the adhesion energy $-|W| 4\pi R_{pa}^2$, which increases with particle size. On the other hand, it follows from the spontaneous curvature model (see Chapter 5) that the membrane segment bound to the particle has the bending energy $8\pi\kappa$ that is proportional to the bending rigidity κ and independent of the particle size. Ignoring the engulfment-induced changes in the bending energy of the unbound membrane segment, which should be negligible if the particle is much smaller than the vesicle, we find that complete engulfment is energetically favorable if $|W| 4\pi R_{\rm pa}^2 > 8\pi\kappa$ or, equivalently,

$$R_{\rm pa} > \sqrt{2\kappa / |W|} \equiv R_W. \tag{8.5}$$

Therefore, engulfment becomes energetically favorable for sufficiently large particles. The length scale R_{W} is an important material parameter

of the system that we will call the *adhesion length*. Values of the adhesion length for different combinations of membrane composition and adhesive material are displayed in Table 5.2 of Chapter 5.

A limitation of the approach leading to Eq. 8.5 is that it simply compares the energy of the free and completely engulfed states but does not inform us about the stability of each state, that is, about the energy landscape of the engulfment process. Indeed, the transition from the free to the completely engulfed state may in principle occur (i) discontinuously, reflecting an energy barrier between the two states, or (ii) continuously, *via* partially engulfed states in which the membrane is bound to the particle but does not cover the particle completely. In order to distinguish these two cases, one has to (numerically) calculate the energy of the system along an appropriate reaction coordinate for the engulfment process, as will be done in Section 8.4.2.

8.4.2 ENERGY LANDSCAPES AND ENGULFMENT REGIMES

We will focus in this subsection on the engulfment of particles by vesicles with an axisymmetric geometry, as depicted in Figure 8.3. A possible reaction coordinate for this engulfment geometry is the *wrapping angle* ϕ , as defined in the figure. The wrapping angle can vary from $\phi = 0$, representing a free particle, to $\phi = \pi$, corresponding to a completely engulfed particle. For values $0 < \phi < \pi$ the particle is partially engulfed.

The restriction to axisymmetric geometries is due to the fact that, for each value of the wrapping angle ϕ , we need to find the shape of the unbound membrane segment that minimizes the bending energy of this segment while still satisfying the constraints on the total vesicle area A and enclosed volume V. A numerical solution of the corresponding Euler-Lagrange equation is only feasible for axisymmetric geometries, using a shooting method as described by Seifert et al. (1991) and Agudo-Canalejo and Lipowsky (2016). Numerical solutions therefore only allow us to describe engulfment *at the poles* of axisymmetric vesicles, although we will overcome this limitation using analytical considerations in Sections 8.4.3, 8.4.4 and 8.6.1. The numerical minimization of the membrane bending energy for non-axisymmetric geometries



Figure 8.3 Vesicle membrane in contact with a spherical nanoparticle (gray) of radius R_{pa} . The vesicle shape is axially symmetric with respect to the vertical dashed line. The wrapping angle, ϕ , denotes the position of the contact line, which partitions the membrane into a bound (red) and an unbound (blue) segment. The wrapping angle varies from $\phi = 0$ for the onset of adhesion up to $\phi = \pi$ for the completely engulfed state. The bound and unbound membrane segment have the areas A_{bo} and $A_{un} = A - A_{bo}$, respectively.

requires the discretization or triangulation of these membranes, the shape of which can then be determined by energy gradient methods such as Monte Carlo simulations (see also Chapter 6).

Using the shooting method, we can obtain the energy of the unbound segment as a function of the wrapping angle, $\mathcal{E}_{un}(\phi)$, which comes exclusively from bending. The energy of the segment bound to the particle, on the other hand, can be calculated analytically and includes both bending and adhesion contributions that have the form

$$\mathcal{E}_{\rm bo} = [-2\pi |W| R_{\rm pa}^2 + 4\pi\kappa \left(1 \pm mR_{\rm pa}\right)^2](1 - \cos\phi) \qquad (8.6)$$

where the plus and minus signs apply to endocytic and exocytic engulfment, respectively. Notice that we have also included the possibility of a nonzero spontaneous curvature. For the case of zero spontaneous curvature m = 0, we recover the condition of energetically favorable engulfment as given by Eq. 8.5 by imposing that $\mathcal{E}_{bo} < 0$ in Eq. 8.6. The total energy, $\mathcal{E}(\phi)$, of the vesicle-particle system is then provided by the sum of the contributions from the bound and unbound segments, with $\mathcal{E}(\phi) = \mathcal{E}_{un}(\phi) + \mathcal{E}_{bo}(\phi)$.

We will now explore the energy landscapes for different sets of parameters. There are six parameters in the system: three material parameters, namely the bending rigidity of the membrane, κ ; its spontaneous curvature, m; and the adhesive strength, |W|; and three geometric parameters, given by the membrane area, A; the enclosed volume, V; and the particle radius $R_{\rm pa}$. By choosing the adhesive length, R_{W} , as a basic length scale and the bending rigidity as the basic energy scale, we are left with only four free parameters: the particle radius and the vesicle size, $R_{\rm ve} \equiv \sqrt{A/4\pi}$, in units of R_{W} , the spontaneous curvature in units of R_{W}^{-1} , and the reduced volume of the vesicle, v, as defined in Eq. 8.2.

For particles that are several times smaller than the vesicle, that is, for $R_{\rm pa} \lesssim 0.1 R_{\rm ve}$, there are four qualitatively different types of energy landscapes or *engulfment regimes*, as shown in Figure 8.4:

the free regime, \mathcal{F}_{st} , see (a); the partially engulfed regime, \mathcal{P}_{st} , see (b); the completely engulfed regime C_{st} , see (c); and the bistable regime \mathcal{B}_{st} , see (d–f). Which engulfment regime is present depends on the precise values of the four free parameters. In Figure 8.5, we show the engulfment regimes as a function of particle size and vesicle size, for an oblate vesicle with reduced volume v = 0.98 and three different values of the membrane spontaneous curvature, in the case of endocytic engulfment.

Particles that are much smaller than the adhesive length, R_{W} , as given by Eq. 8.5 are always found in the free regime, \mathcal{F}_{st} , in which the free state is stable and the completely engulfed state is unstable. Particles that are much larger than the adhesive length, on the other hand, are found in the completely engulfed regime, $C_{\rm sr}$, in which the free state is unstable but the completely engulfed state is stable. The most interesting behavior occurs for particles of intermediate size, on the order of the adhesion length: They may be either in a partially engulfed regime, $\mathcal{P}_{\rm st}{-\!\!\!-\!\!}$ in which both the free and completely engulfed states are unstable and a partially engulfed state is stable—or in a bistable regime \mathcal{B}_{st} —in which both the free and completely engulfed states are stable and separated by an energy barrier. These four different engulfment regimes are separated from each other by the two lines $L_{\rm fr}$ and $L_{\rm ce}$ (Figure 8.5), which mark the stability limits of the free and completely engulfed state, respectively.

In the \mathcal{P}_{st} regime, the contact area between the membrane and the particle changes in a continuous manner as we vary the particle radius, from a vanishingly small value at the instability line L_{fr} up to the total area of the particle at the instability line, L_{ce} . The system behavior is markedly different in the bistable regime, \mathcal{B}_{st} , in which the contact area changes abruptly or discontinuously as the particle size is varied. When, by increasing the particle size, the system crosses the instability line, L_{ce} , the free state remains stable and coexists with a metastable completely engulfed state until we cross the transition line, L_* , at which the free state remains metastable until the system crosses the line



Figure 8.4 Free energy landscapes $\Delta \varepsilon(\phi) = \varepsilon(\phi) - \varepsilon(0)$. (a) For the free regime, \mathcal{F}_{st} , the landscape is uphill, with a minimum at $\phi = 0$, which corresponds to the free state, and a maximum at $\phi = \pi$, which defines the completely engulfed state; (b) For the partial engulfment regime, \mathcal{P}_{st} , the landscape has maxima both at $\phi = 0$ and at $\phi = \pi$ and a minimum at an intermediate ϕ -value corresponding to a partially engulfed state; (c) For the complete engulfment regime, \mathcal{C}_{st} , the landscape is downhill, with a minimum at $\phi = \pi$ and a maximum at $\phi = 0$; (d–f) Three landscapes within the bistable regime, \mathcal{B}_{st} , with two local minima at $\phi = 0$ and $\phi = \pi$ that are separated by a free energy barrier, implying that both the free and completely engulfed state, respectively. Panel (e) corresponds to the transition line, L* (see also Figure 8.5), at which both free and completely engulfed states have the same free energy.



Figure 8.5 Different engulfment regimes for the endocytosis of a nanoparticle at the pole (see text) of an oblate vesicle with reduced volume v = 0.98. The three panels (a–c) correspond to the three values $m = -0.05 / R_W$, 0, +0.05 / R_W of the spontaneous curvature. The different regimes are defined by the two instability lines, $L_{\rm fr}$ and $L_{\rm ce}$, for the free and the completely engulfed state. The asymptotes of the two instability lines for large $R_{\rm ve}$ are indicated by vertical dotted lines. The bistable regimes, $\mathcal{B}_{\rm st}$, contain the transition lines, L_* (dashed), at which the free and completely engulfed states coexist. For panel (c), the two instability lines $L_{\rm fr}$ and $L_{\rm ce}$ intersect. Close to the intersection point, the system is "multicritical" and reacts sensitively to small changes in both particle and vesicle size. The shaded area (gray) close to the x-axes indicates the size regime in which the vesicle does not have enough room to accommodate the completely engulfed particle, leading to a tension-dominated regime. (Reprinted from Agudo-Canalejo, J. and Lipowsky, R., ACS Nano, 9, 3704–3720, 2015a.)

 $L_{\rm fr}$, at which the free state becomes unstable and a particle in contact with the membrane would suddenly become completely engulfed. If we now reduce the particle size, the completely engulfed state would remain (meta)stable until we crossed the $L_{\rm cc}$. The system therefore exhibits a marked hysteresis in this region.

As shown in Figure 8.5 for the case of endocytic engulfment, the intermediate size regime consists of a bistable regime for negative or zero values of the spontaneous curvature $m \le 0$. For positive values of the spontaneous curvature (Figure 8.5c), we find again a bistable regime for small vesicles but a partially engulfed regime for large vesicles. In the latter case, the two instability lines cross each other. Around the intersection point, the system is "multicritical" in the sense that small changes in the particle and vesicle size will lead to large modifications of the system behavior.

As a particular example, let us consider GUVs made from DOPC/DOPG, and glass particles with a monolayer-coated surface (Gruhn et al., 2007). From Table 5.2 in Chapter 5, we see that the adhesion length corresponding to this system is $R_{W} \simeq 500$ nm. Particles with radii sufficiently smaller than R_{W} will remain free, whereas particles sufficiently larger than R_{W} should be completely engulfed. On the other hand, particles with radii in the vicinity of 500 nm are expected to explore the intermediate regimes of the engulfment diagrams in Figure 8.5. For GUVs with reduced volume v = 0.98, positive spontaneous curvature $m = +0.05 / R_{W} \approx 1 / (10 \,\mu\text{m})$, and a size around $R_{\rm ve} = 30 R_W \approx 15 \,\mu{\rm m}$, we expect particles with radii between 510 and 540 nm to be partially engulfed (Figure 8.5c). Conversely, if we consider particles with a radius of about 510 nm, they should become partially engulfed by vesicles with sizes larger than 15µm, but remain free in the presence of smaller vesicles. Furthermore, vesicles with sizes below 6µm will not lead to partial engulfment of intermediate-sized particles because they will display a bistable regime instead of the partially engulfed regime. We emphasize again, however, that due to the requirement of axisymmetry in numerical calculations, Figure 8.5 only describes the engulfment of a particle at one of the weakly curved poles of the oblate vesicle. This limitation is overcome below, where we provide analytical equations that can describe engulfment at any arbitrary point on the vesicle surface.

8.4.3 STABILITY RELATIONS

As mentioned above, the boundaries between the four different engulfment regimes are given by two instability lines, corresponding to the stability limits of the free and completely engulfed states. Interestingly, exact analytical expressions exist (Agudo-Canalejo and Lipowsky, 2015a) for the two instability lines, which are found to depend strongly on the local geometry of the membrane in contact with the particle.

The free, nonadhering state of the particle is unstable and the membrane starts to spread over the particle for particle radii

$$R_{\rm pa} > \left[R_{\rm W}^{-1} \mp M_{\rm ms} \right]^{-1} \equiv R_{\rm fr} \tag{8.7}$$

where $M_{\rm ms}$ is the mean curvature of the membrane before contact with the particle (Figure 8.6a), and the minus and plus signs apply to endocytic and exocytic engulfment respectively. The corresponding instability line $L_{\rm fr}$ is given by the equality $R_{\rm pa} = R_{\rm fr}$.

In the completely engulfed state, the particle is still connected to the original mother membrane by a closed neck. This state becomes unstable and the closed neck starts to open up if the particle is too small, with

$$R_{\rm pa} < \left[R_W^{-1} \pm M'_{\rm ms} \mp 2m \right]^{-1} \equiv R_{\rm ce} \tag{8.8}$$

where now $M'_{\rm ms}$ is the mean curvature of the mother membrane at the position of the neck (Figure 8.6b), and the upper and lower signs apply again to endocytic and exocytic



Figure 8.6 Definition of the mean curvatures of membrane segments relevant to the stability of free and completely engulfed particles. In (a), the mean curvature of the membrane before contact with the free particle is M_{ms} . In (b), the completely engulfed particle is still connected to the mother membrane by a narrow neck. The mean curvature of the mother membrane at the position of the neck is M'_{ms} .

engulfment respectively. The associated instability line L_{ce} is given by the equality $R_{pa} = R_{ce}$. Equation 8.8 can be viewed as a generalization of the closed neck condition for the budding of vesicles (see Chapter 5) to the case of "budding" around an adhesive particle (Agudo-Canalejo and Lipowsky, 2016), and has also been successfully applied to the interaction between ESCRT proteins and membranes (Agudo-Canalejo and Lipowsky, 2018).

Given that the two instability lines govern the engulfment behavior, the two relationships as provided by Eqs. 8.7 and 8.8 allow for a complete understanding of the engulfment process, without the need to perform numerical calculations. In particular, for the case of a planar membrane without spontaneous curvature, we have $M_{\rm ms} = M'_{\rm ms} = m = 0$, and we find that the free state becomes unstable and the completely engulfed state becomes stable when $R_{\rm pa} > R_{\rm W}$, therefore recovering the previously known result in Eq. 8.5. Moreover, by combining Eqs. 8.7 and 8.8, we find that they intersect whenever $m = (M_{\rm ms} + M'_{\rm ms})/2$. For convexly curved vesicles, such as the oblate vesicle with v = 0.98 in Figure 8.5, the mean curvatures $M_{\rm ms}$ and $M'_{\rm ms}$ are positive. This explains why the two instability lines do not intersect for negative or zero spontaneous curvature (Figure 8.5a and b) but do cross for positive spontaneous curvatures, as in Figure 8.5c. For the endocytic case, we find that $R_{\rm fr} < R_{\rm cc}$ if $m > (M_{\rm ms} + M'_{\rm ms})/2$, in which case particles of intermediate size are in the partially engulfed regime and the engulfment transition is continuous; whereas $R_{\rm fr} > R_{\rm cc}$ if $m < (M_{\rm ms} + M'_{\rm ms})/2$, in which case, particles of intermediate size are in the bistable regime and the engulfment transition is discontinuous. The opposite is true for exocytic engulfment, with the transition being discontinuous if $m > (M_{\rm ms} + M'_{\rm ms})/2$ and continuous otherwise.

In the particular case of a convex vesicle (so that $M_{\rm rns}$ and $M'_{\rm rns}$ are positive) with no spontaneous curvature, such a stability analysis predicts that endocytic engulfment is always discontinuous, whereas exocytic engulfment is always continuous. This was confirmed *via* numerical calculations in (Bahrami et al., 2016). Likewise, the predictions of the stability analysis in Eqs. 8.7 and 8.8 are also corroborated by the numerical results in (Góźdź, 2007; Cao et al., 2011). Furthermore, Eqs. 8.7 and 8.8 can be used to extract the material parameters of the system, such as the spontaneous curvature *m* and the adhesion length R_{W} , from experimental or computational studies of particle engulfment (see Box 8.1).

Box 8.1 Extracting material parameters from experimental or computational studies of particle engulfment

- As seen in Figure 8.4, the critical particle sizes R_{fr} and R_{ce} of the system are typically on the order of the adhesion length R_W. As shown in Table 5.2 of Chapter 5, values of the adhesion length can range from as little as 13 nm, for strong adhesion between DMPC and silica, to as much as 3 μm, for the ultraweak adhesion between DOPC/DOPG and glass. This implies that, for ultraweak adhesion, the different engulfment regimes should be accessible to optical microscopy experiments, whereas for strong adhesion the different engulfment states could be distinguished by cryoelectron or super-resolution microscopy. In the case of computer simulations, the strong adhesion regime should be accessible *via* coarse-grained MD simulations, whereas for ultraweak for ultraweak adhesion one could use Monte Carlo simulations.
- Suppose that, in experiments or simulations, the partially engulfed regime, P_{st} , is observed for the endocytic case. This implies that, for this system, $m > (M_{ms} + M'_{ms})/2$, and engulfment proceeds continuously with increasing particle size in the endocytic case. We could then probe the system with particles of varying size, and record the two critical particle sizes, R_{fr} and R_{ce} , below and above which particles are free and completely engulfed, respectively. Simultaneously, one could easily measure from the experimental images or the simulation snapshots the corresponding values of M_{ms} and M'_{ms} . Using Eqs. 8.7 and 8.8, we can directly determine the values of the adhesion length R_W and the spontaneous curvature m as

$$R_{W} = R_{\rm fr} \left(1 + R_{\rm fr} M_{\rm ms} \right)^{-1} \tag{8.9}$$

$$m = (R_{\rm fr}^{-1} - R_{\rm ce}^{-1} + M_{\rm ms} + M_{\rm ms}') / 2$$
(8.10)

• Suppose that, on the other hand, the partially engulfed regime, \mathcal{P}_{st} , is not observed for the endocytic case, implying that, for this system, $m < (M_{ms} + M'_{ms})/2$, and engulfment proceeds discontinuously with increasing particle size in the endocytic case. In this situation, only R_{fr} can be recorded, and therefore we would have access to the adhesion length R_W using Eq. 8.9 but not to the spontaneous curvature. However, we can now use the fact that, when $m < (M_{ms} + M'_{ms})/2$, engulfment proceeds continuously with increasing particle size in the exocytic case. Therefore, by repeating the same type of experiment or simulation, for the same particle–vesicle system, but this time for exocytic engulfment, we can now record the corresponding values of both R_{fr} and R_{ce} . The values of the adhesion length R_W and the spontaneous curvature m are then obtained as

$$R_{W} = R_{\rm fr} \left(1 - R_{\rm fr} M_{\rm ms} \right)^{-1}$$
(8.11)

$$m = (R_{\rm ce}^{-1} - R_{\rm fr}^{-1} + M_{\rm ms} + M_{\rm ms}')/2$$
(8.12)

8.4.4 ENERGY LANDSCAPES AND CURVATURE-INDUCED FORCES

The approach based on the stability of free and completely engulfed states presented in the previous section and Box 8.1 is very useful because it allows us to predict the fate of a particle coming into contact with a membrane, that is, whether the particle will remain free, be partially or completely engulfed or whether it will show bistability between the free and completely engulfed states. Unfortunately, the stability analysis does not provide us with any information about the height of the energy barriers for bistable regimes (Figure 8.4d–f) or about the binding energy and degree of engulfment for partially engulfed particles (Figure 8.4b).

Going beyond stability analysis, it is possible to develop an analytical approximation for the full energy landscapes experienced by particles coming into contact with vesicles, if we consider the limit of small particles with $R_{\rm pa} \ll R_{\rm ve}$. This analytical theory was developed in (Agudo-Canalejo and Lipowsky, 2017), through a systematic expansion of the free energy of the system to leading order in the particle-to-vesicle size ratio $R_{\rm pa}/R_{\rm ve}$. The free energy was found to behave as

$$\Delta \mathcal{E}(\phi) = 4\pi\kappa R_{\text{pa}} [2(R_{\text{pa}}^{-1} - R_W^{-1} \pm m)(1 - \cos\phi)$$
$$\pm (M_{\text{ms}} - m)\sin^2 \phi]$$
$$+ O(\kappa R_{\text{pa}}^2 / R_{\text{vc}}^2)$$
(8.13)

where the plus and minus signs correspond to endocytic and exocytic engulfment, respectively. We note that Eq. 8.13 depends on the local membrane curvature, $M_{\rm ms}$, and therefore can be used to describe particle engulfment at any location on the surface of a vesicle, even non-axisymmetric locations.

Equation 8.13 reproduces the four kinds of energy landscapes displayed in Figure 8.4, that is, the four different engulfment regimes. Furthermore, it gives us access to the height of the energy barriers in bistable regimes and to the binding energy of partially engulfed particles. More precisely, according to Agudo-Canalejo and Lipowsky (2017), we find that the typical magnitude of barrier heights and binding energies is given by $4\pi\kappa R_{\rm pa} \mid M_{\rm ms} - m \mid$. Two important features should be noticed: First, the binding strength of partially engulfed particles as well as the size of the energy barriers in bistable regimes both increase with increasing particle size. Second, for a given particle size, the binding energy of a partially engulfed particle depends on the local curvature, $M_{\rm ms}$.

More specifically, the binding of partially engulfed particles is more favorable for membrane segments with lower mean curvature in the case of endocytic engulfment, and for segments of higher mean curvature in the case of exocytic engulfment. This dependence of the binding energy of partially engulfed particles on the local mean curvature leads to curvature-induced forces that pull partially engulfed particles toward regions of lower or higher membrane curvature, in the case of endocytic or exocytic engulfment, respectively (Agudo-Canalejo and Lipowsky, 2017). Because particles with a chemically uniform surface as described here are only partially engulfed if their size and adhesiveness is in the right range (Figure 8.5), these curvature-induced forces can be most conveniently explored in experiments with partially adhesive Janus-like particles, which will be described in Section 8.6.3.

Finally, it is worth pointing out that the description of particle engulfment in the present section and Sections 8.4.2 and 8.4.3 is only strictly valid for particles that are many times smaller than the vesicle, with $R_{pa} \leq 0.1 R_{ve}$. On the other hand, if a particle is much larger than the vesicle, with $R_{pa} \gg R_{ve}$, we will recover the adhesion behavior of vesicles to planar substrates described in Chapter 5. Particles of a size comparable to the vesicle size thus represent an intermediate regime between these two limits. For particles that are smaller than the vesicle but still relatively large, with $R_{pa} \gtrsim 0.1 R_{ve}$, deviations from the ideal engulfment behavior described above lead to *satellite minima*, partially engulfed states with wrapping angles close to $\phi \approx 0$ or $\phi \approx \pi$ that are metastable and can coexist with free and completely engulfed states (Agudo-Canalejo and Lipowsky, 2015a, 2017).

8.4.5 ADHESION-INDUCED SEGREGATION OF MEMBRANE COMPONENTS

Biological membranes are always composed of a mixture of lipids and proteins (van Meer et al., 2008), and this compositional complexity can be mimicked in GUVs with multicomponent membranes. If a particle comes into contact with such a membrane, it is expected to preferentially attract and/or repel certain membrane components (Lipowsky et al., 2013) leading to adhesion-induced segregation of the membrane components. For example, if a charged particle comes into contact with a membrane that contains lipids of the opposite charge, these lipids are likely to be enriched in the membrane segment that is bound to the particle.

If the particle-bound segment has a different lipid composition, it will also have different elastic properties compared with the unbound segment. Thus, we will now consider the case in which the bound segment has bending rigidity, κ_{bo} , and spontaneous curvature, m_{bo} , whereas the unbound segment has a different bending rigidity, κ , and spontaneous curvature, m. The energy of such a segregated membrane is equal to the energy of a uniform membrane with bending rigidity, κ , and spontaneous curvature, m, provided we replace the adhesive strength, |W|, by the effective adhesive strength

$$W_{\rm eff} = |W| + \frac{2\kappa}{R_{\rm pa}^2} \left(1 \pm mR_{\rm pa}\right)^2 - \frac{2\kappa_{\rm bo}}{R_{\rm pa}^2} \left(1 \pm m_{\rm bo}R_{\rm pa}\right)^2 \tag{8.14}$$

as follows from the difference between the elastic parameters of the unbound and bound segments. As before, the plus and minus signs apply to endocytic and exocytic engulfment, respectively.

It turns out that the stability relations for the new system are now again given by Eqs. 8.7 and 8.8 provided we replace the adhesive length, R_W , by $\sqrt{2\kappa} / W_{\text{eff}}$. It is important to note that this substitution introduces a nonlinear dependence on the particle size. For sufficiently negative values of the spontaneous curvature of the bound segment, one now finds regions of the parameter space in which only intermediate-sized particles can be completely engulfed (Agudo-Canalejo and Lipowsky, 2015a). The latter parameter dependence for complete

engulfment explains the nonmonotonic size dependence of the uptake of gold nanoparticles by HeLa cells as observed experimentally (Chithrani and Chan, 2007).

We note that, in general, there will also be an energetic contribution proportional to the length of the contact line that separates the particle-bound and the unbound segments of the membrane. The associated line tension may be positive or negative. Positive line tensions will favor bistable regimes, because they provide an energy barrier for the engulfment process. Negative line tensions, on the other hand, will favor partially engulfed states, as well as completely engulfed states with nonaxisymmetric 'tight-lipped' membrane necks, as found for the engulfment of nanodroplets (Satarifard et al., 2018).

8.5 ENGULFMENT OF COMPLEX PARTICLES

8.5.1 NONSPHERICAL PARTICLES

Although the most commonly used nanoparticles in experiments and applications are spherical, it is also possible to produce nonspherical particles. Such particles may for example be ellipsoidal, cubic, or rod-like. In principle, the engulfment of nonspherical particles should not be conceptually different from that of spherical particles because it can still be understood as a competition between particle-membrane adhesion and membrane bending. In practice, however, the computations become more difficult. First of all, the interaction of nonspherical particles with membranes typically leads to non-axisymmetric configurations. Therefore, an exact solution to the problem of finding the minimum energy shapes of the vesicle as was done in Section 8.4.2 is not feasible, and approximate techniques such as energy minimization of discretized membranes (Bahrami, 2013; Dasgupta et al., 2013, 2014; Yi et al., 2014) or MD simulations (Vácha et al., 2011; Huang et al., 2013) have to be employed. These techniques are much more computationally expensive. Second, whereas the engulfment process of spherical particles could be described by a single reaction coordinate (Figures 8.3 and 8.4), the engulfment of nonspherical particles requires at least two such coordinates in order to describe the orientation of the particle with respect to the membrane.

It is precisely the rotation of elongated particles during engulfment that has been more thoroughly studied in theoretical and simulation work. These elongated particles tend to first attach to the membrane via their weakly curved surface segments but are then completely engulfed at their strongly curved tips. This engulfment-mediated rotation was first described by Bahrami (2013) and can be understood in terms of bending considerations. Initially, the membrane prefers to bind to the weakly curved side because it can gain the same amount of adhesion energy with a smaller cost in bending (Figure 8.7a). Close to complete engulfment, on the other hand, an elongated particle lying on its flat side would require that the membrane has already spread around both of its strongly curved tips, whereas if it stands against the membrane on its tip, the membrane can avoid wrapping around one of the strongly curved tips (Figure 8.7b). This conformational transition from "lying down to standing up" has also been observed in MD simulations (Huang et al., 2013).



Figure 8.7 Engulfment of a prolate ellipsoidal particle, from energy minimization of a triangulated vesicle. In (a), the particle area fraction bound to the membrane is small with $A_{bo}/A_{pa} = 0.35$, and the particle preferentially adheres *via* its weakly curved surface segment. In (b), the particle area fraction bound to the membrane is large with $A_{bo}/A_{pa} = 0.67$ and the particle has reoriented with its strongly curved tip pointing toward the vesicle interior. (Bahrami, A.H., *Soft Matter*, 9, 8642, 2013. Reproduced by permission of The Royal Society of Chemistry.)

8.5.2 DEFORMABLE PARTICLES AND FLUID DROPLETS

So far, we have considered particles that are rigid, that is, that do not change their shape as a result of their interaction with the membrane. A natural way to add complexity to the system would be to consider deformable particles. A simple example of a deformable particle that could be engulfed by a GUV would be a smaller lipid vesicle, which would have a bending rigidity comparable to that of the GUV, or a small polymer vesicle, whose bending rigidity would be on the order of 10 times that of the GUV (see Chapter 26). Another example of a deformable particle that may be engulfed by a GUV could be a fluid droplet, such as those arising in GUVs containing aqueous two-phase systems (see Chapter 29). Deformable particles may also be achieved by grafting a polymer brush onto rigid particles.

The particular case corresponding to the engulfment of a smaller fluid vesicle by a larger fluid membrane was considered in (Yi et al., 2011). In this case, the adhesion energy needed for complete engulfment increases as one decreases the bending rigidity of the smaller vesicle. This suppression of completely engulfed states is a consequence of a preference for partially engulfed states. Indeed, small vesicles that are less rigid than the engulfing membrane will tend to spread onto the larger membrane without deforming it.

A similar prominence of partially engulfed states is found for the engulfment of fluid droplets, as was studied in (Kusumaatmaja and Lipowsky, 2011; Satarifard et al., 2018). Small droplets again have a tendency to spread onto the membrane. In analogy to the engulfment of rigid particles, however, it is found that for large droplets, the partially engulfed state can become unstable and undergo a discontinuous transition to a completely engulfed state.

8.6 ENGULFMENT OF MULTIPLE PARTICLES

8.6.1 ENGULFMENT PATTERNS

We have until now described the engulfment of single particles by vesicles. It is, however, of practical relevance and theoretical interest to consider the case of simultaneous engulfment of many particles by a single vesicle. In Section 8.4, we described how the engulfment behavior of a single spherical particle is highly dependent on the local curvature of the engulfing vesicle, as described by the (in)stability conditions for the free and completely engulfed particle states in Eqs. 8.7 and 8.8. When we consider a vesicle with a complex, nonspherical shape, these instability conditions vary continuously as we move along the membrane surface. In particular, we can then take advantage of the local nature of these conditions to study the engulfment of many particles at non-axisymmetric locations of the vesicle shape.

Depending on the local curvature of the vesicle at the point of contact with the particle, this particle will either remain free, be partially or completely engulfed, or exhibit bistability between the free and the completely engulfed states. These four engulfment regimes can therefore coexist on the surface of a single vesicle, forming different engulfment patterns when a vesicle is exposed to many particles (Figure 8.8).

For a vesicle membrane with a laterally uniform composition, up to three different engulfment regimes can be simultaneously present on a single vesicle, if the particles are much smaller than the vesicle, which implies that the vesicle-particle system can form 10 possible engulfment patterns (Agudo-Canalejo and Lipowsky, 2015b). These patterns depend strongly on the vesicle shape, as defined by its reduced volume, on the spontaneous curvature, as well as on the particle size and adhesiveness. Therefore, small variations of any of these four parameters lead to morphological transitions between different engulfment patterns.

Completely engulfed particles in bistable, \mathcal{B}_{st} , or completely engulfed, C_{st} , segments are connected to the mother vesicle by a very narrow neck, and therefore create only a local deformation of the membrane that costs no energy. As a consequence, these particles cannot "feel" their surrounding membrane curvature gradient and will be subject to diffusive motion, unaware of each other. However, if these completely engulfed particles diffuse from the \mathcal{B}_{st} or C_{st} segments into \mathcal{F}_{st} or \mathcal{P}_{st} segments, they will completely or partially detach from the vesicle membrane, respectively. Once in a \mathcal{P}_{st}



increasing particle size and/or adhesiveness

Figure 8.8 Different engulfment patterns of nanoparticles (black) on a prolate vesicle. The spontaneous curvature of the vesicle membrane is positive in (a) and negative in (b). The patterns involve coexistence of the four different engulfment regimes along separate segments of the same vesicle: Free segments \mathcal{F}_{st} with no engulfment (red) and bistable segments \mathcal{B}_{st} with activated engulfment and release (yellowish orange), as well as segments decorated by partially engulfed, \mathcal{P}_{st} (blue), and completely engulfed, \mathcal{C}_{st} (green), particles. A change in particle size or adhesiveness leads to continuous morphological transitions between these patterns. (Reprinted from Agudo-Canalejo, J. and Lipowsky, R., Nano Lett., 15, 7168–7173, 2015b.)

segment, partially engulfed particles can "feel" the surrounding curvature gradient and will be subject to curvature-mediated forces, see Section 8.4.4, as well as membrane-mediated particle–particle interactions that may lead to aggregation of particles. The latter processes will be discussed in the following two subsections.

8.6.2 PARTICLE AGGREGATION INTO MEMBRANE TUBES

Aggregation of partially engulfed particles into membrane tubes has been observed in two independent simulation studies using triangulated membranes and multiple spherical particles (Bahrami et al., 2012; Šarić and Cacciuto, 2012). As was clarified later (Bahrami et al., 2014; Raatz et al., 2014), this cooperative engulfment process turns out to be energetically favorable, compared with the individual engulfment of each particle, when the range of the interaction potential is sufficiently large. For potential ranges on the order of a few percent of the particle radius, these tubular structures are found to be very stable against thermal fluctuations. For a typical adhesion potential range of 1 nm, tubular aggregates should be favorable for particles with radii on the order of tens of nanometers.

8.6.3 JANUS PARTICLES AND MEMBRANE-MEDIATED INTERACTIONS

So far, we have considered particles that have a chemically uniform surface, that is, particles with a single value of the adhesive strength, |W|. As discussed in Sections 8.4 and 8.6.1, such particles are found in partially engulfed states only for a narrow region of the parameter space that includes the particle size and adhesive strength, as well as the vesicle shape and size, and the membrane spontaneous curvature and bending rigidity. Particles that are too small or too large will instead be found in free or completely engulfed states. As a consequence, it is a challenging task to explore the membrane-mediated interactions that arise between partially engulfed particles with a chemically uniform surface.

A better option to study such membrane-mediated interactions is provided by Janus or "patchy" particles, the surface of which contains both a strongly adhesive and a non-adhesive patch. If the adhesive strength, |W|, of the sticky patch is sufficiently large, with $|W|R_{pa}^2 \gg \kappa$, the membrane will always spread over this patch until it reaches the boundary between the adhesive and the nonadhesive patches, where it becomes "pinned." This pinning is caused by the abrupt change of the contact curvature as one crosses the boundary (Lipowsky et al., 2005). As a consequence, Janus or patchy particles will always be found in a partially engulfed state, independent of the size and shape of the vesicle or the membrane spontaneous curvature, and provide perfect candidates for the study of membrane-mediated particle–particle interactions.

Partially engulfed particles deform the surrounding membrane (Figure 8.3). If two identical Janus particles are sitting on the same vesicle, their deformation fields will overlap, and the shape as well as the bending energy of the membrane will depend on the distance between the two particles. This distance-dependence of the bending energy necessarily translates into a membrane-mediated force between the two particles, as they will tend to move in the direction that minimizes the bending energy of the membrane.

For two identical partially engulfed cylindrical particles that both lie on the same side of the membrane parallel to their symmetry axis, it has been known for a long time that the membrane-mediated interaction between the two particles is repulsive (Weikl, 2003; Müller et al., 2005). For spherical particles, the behavior is more subtle. Particles whose adhesive patch corresponds to less than half of the particle, that is, which are partially engulfed up to wrapping angles $\phi < \pi / 2$ will experience repulsive membrane-mediated interactions (Weikl et al., 1998; Reynwar and Deserno, 2011). Particles whose adhesive patch is larger than half the particle, corresponding to partial engulfment with wrapping angle $\phi > \pi / 2$ will experience longrange repulsion, mid-range attraction and short-range repulsion (Reynwar and Deserno, 2011).

8.7 SUMMARY AND OUTLOOK

In this chapter, we described some recent theoretical insights on particle–membrane interactions. For engulfment of rigid spherical particles, in Section 8.4, we showed how the engulfment process is governed by the stability of the free and completely engulfed states of the particle, as described by Eqs. 8.7 and 8.8. These two analytical conditions provide a concise but detailed description of the engulfment behavior, by defining the boundaries between the four relevant engulfment regimes (Figure 8.5). This type of stability analysis can then be used to describe complex engulfment patterns when a single vesicle is exposed to many small particles, as was shown in Section 8.6.1.

A limitation of the stability analysis is that we cannot access the height of the energy barriers present in a bistable, \mathcal{B}_{st} , regime nor the binding energy or equilibrium wrapping angle of partially engulfed states in a partially engulfed, \mathcal{P}_{st} , regime. Obtaining these latter quantities typically requires numerical computations such as those described in Section 8.4.2. However, in the limit in which the particles are much smaller than the vesicle, we can obtain these quantities analytically, see Section 8.4.4. Most importantly, we find that partially engulfed particles can "feel" the local membrane curvature and experience curvature-mediated forces toward regions of lower or higher membrane curvature, depending on whether the particles originate from the exterior (endocytic engulfment) or interior (exocytic engulfment) of the vesicle.

For more complex particles, such as nonspherical and deformable particles, a unifying theoretical framework such as the one we have for rigid spherical particles is still lacking. It is attractive to hypothesize that the stability relations for the free and completely engulfed states in Eqs. 8.7 and 8.8 will continue to hold for nonspherical particles, provided that we substitute the particle radius, R_{pa} , by the inverse local mean curvature of the particle at the point of contact with the membrane, $1/M_{\rm pa}$, which varies along the surface of the particle. This hypothesis is plausible because the two instability lines depend only on the local curvature of the vesicle membrane at the point of contact with the particle. The stability relation of the free particle state would then imply that the minimal adhesive strength required for binding to the membrane at a weakly curved region of the particle is lower than at a strongly curved region. The same would be true for the minimal adhesive strength required for a stable closed neck in a completely engulfed state, that is, closed necks are more stable for weakly curved segments of

the particle surface. However, whereas for spherical particles the numerical results showed that these two instability lines determine the system behavior, with a partially engulfed state being stable only if the free and completely engulfed states are unstable (Figures 8.4 and 8.5), the same cannot be said for nonspherical particles. In the latter case, the energy landscapes are multidimensional and can display coexistence of several partially engulfed states, or of partially engulfed states.

All theoretical results described in this chapter are accessible to experiment. In particular, the theoretical predictions about engulfment patterns of vesicles with nonspherical shapes, as described in Section 8.6.1, could be studied by exposing lipid vesicles to fluorescently labeled particles. Vesicle membrane segments corresponding to the free, \mathcal{F}_{st} , or bistable, \mathcal{B}_{st} , regimes should appear dark, whereas completely engulfed, C_{st} , segments that belong to a partially engulfed, \mathcal{P}_{st} , regime, curvature-mediated interactions will lead to more complex behavior. Such experimental studies of engulfment patterns would also provide a direct test of the stability relations in Eqs. 8.7 and 8.8. Furthermore, using partially adhesive Janus-like particles in contact with deflated vesicles of nonspherical shape, it should be possible to experimentally study the curvature-induced forces described in Section 8.4.4.

It will also be interesting to directly probe the bistable regime, \mathcal{B}_{st} , in which both the free and completely engulfed particle states are (meta)stable, using experiments with optical tweezers. Indeed, we expect that, in a bistable regime, a particle in contact with the membrane will not be spontaneously engulfed. However, if the particle is pushed against the membrane by the tweezers, it should become completely engulfed, and it should remain engulfed even if the force exerted by the tweezers is switched off. In order to be released, the particle will have to be pulled out of the vesicle using the tweezers once again. The minimal forces required to push and pull particles into and out of the engulfing membrane are governed by the height of the energy barrier for engulfment in the bistable regime and by the energy difference between the free and completely engulfed states (Figure 8.4d–f).

Other experimental challenges that will prove interesting, and which will require an expansion of the theoretical ideas described in this chapter, involve the interaction of particles with more complex vesicles, such as (i) vesicles with phase-separated membranes and (ii) vesicles with nanotubes, both described in Chapter 5. In the case of phase-separated vesicles, the particles should be preferentially engulfed by liquid-disordered domains rather than by liquid-ordered domains, because liquid-disordered membranes have a lower bending rigidity, unless this effect is overcompensated by a stronger adhesion of the particles to the liquid-ordered domains. Furthermore, the positive line tension of the domain boundaries should favor particle engulfment, because the engulfment process will tend to reduce the length of the contact line between domains. In the case of vesicles with nanotubes, the nanotubes should act as an area reservoir that can be used to engulf particles if these are sufficiently adhesive in the same way that neutrophils can extract area from membrane reservoirs during phagocytosis of many large particles (Herant et al., 2005). Whereas spherical vesicles without nanotubes cannot engulf particles without having their membrane stretched and ruptured, see Section 8.3.1, spherical vesicles with nanotubes should be able to engulf a large number of particles.

LIST OF ABBREVIATIONS

MD molecular dynami	ic
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- DOPC 1,2-dioleoyl-sn-glycero-3-phosphocholine
- DOPG 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol)
- DMPC 1,2-dimyristoyl-sn-glycero-3-phosphocholine

GLOSSARY OF SYMBOLS

- A surface area of vesicle
- A_{pa} surface area of particle
- $\dot{A_{bo}}$ area of membrane segment bound to the particle
- A_{un} area of the unbound vesicle membrane segment
- ΔA increase in membrane area due to stretching
- \mathcal{B}_{st} bistable regime of engulfment
- $C_{\rm st}$ completely engulfed regime
- \mathcal{E}_{ad} adhesion energy
- Ebo energy of the particle-bound vesicle membrane segment
- \mathcal{E}_{un} energy of the unbound vesicle membrane segment
- $\mathcal{F}_{\mathsf{st}}$ free regime of engulfment
- Γ coverage by adsorbate
- H binding enthalpy of a receptor-ligand pair
- κ membrane bending rigidity
- κ_{bo} bending rigidity of the bound membrane segment
- *K*_A area compressibility modulus
- k_B Boltzmann's constant
- l_{me} membrane thickness
- L_{fr} stability limit of the free particle state
- L_{ce} stability limit of the completely engulfed particle state
- *L*^{*} transition line in the bistable regime
- m membrane spontaneous curvature
- *m*_{bo} spontaneous curvature of the bound membrane segment
- *M*_{ms} mean curvature of the vesicle membrane at the point of contact with the particle
- *M*[']_{ms} mean curvature of the vesicle membrane at the position of the narrow neck adjacent to a completely engulfed particle
- M_{pa} local mean curvature of particle surface
- $\mathcal{P}_{\mathrm{st}}$ partially engulfed regime
- ϕ wrapping angle
- R_{pa} radius of spherical particle
- R_{ve}^{r} effective radius of vesicle defined by $R_{ve} \equiv \sqrt{A/4\pi}$
- $R_{\rm W}$ adhesion length defined by $R_{\rm W} \equiv \sqrt{2\kappa / |W|}$
- *R*_{fr} critical particle radius above which the free state is unstable
- *R*_{ce} critical particle radius below which the completely engulfed state is unstable
- ho_{lig} density of ligands on the particle surface
- ρ₀ density of receptors on the membrane
- Σ mechanical tension of the membrane
- T temperature
- U binding free energy of a receptor-ligand pair
- V volume of vesicle
- V_{pa} volume of particle
- reduced volume (volume-to-area ratio) of the vesicle

- v' effective reduced volume of vesicle after particle engulfment
- $W_{\rm gen}$ adhesion (free) energy per area contributed by generic interactions
- $W_{\rm spe}$ adhesion (free) energy per area contributed by specific interactions
- W total adhesion (free) energy per area
- W_{eff} effective adhesion energy per area, see Eq. 8.14.
- *X* molar concentration of adsorbates in solution

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Particle-membrane interactions

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226

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