Kinetic regulation of coated vesicle secretion

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The secretion of vesicles for intracellular transport often relies on the aggregation of specialized membrane-bound proteins into a coat able to curve cell membranes. The nucleation and growth of a protein coat is a kinetic process that competes with the energy-consuming turnover of coat components between the membrane and the cytosol. We propose a generic kinetic description of coat assembly and the formation of coated vesicles and discuss its implication to the dynamics of COP vesicles that traffic within the Golgi and with the endoplasmic reticulum. We show that stationary coats of fixed area emerge from the competition between coat growth and the recycling of coat components, in a fashion resembling the treadmilling of cytoskeletal filaments. We further show that the turnover of coat components allows for a highly sensitive switching mechanism between a quiescent and a vesicle-producing membrane, upon a slowing down of the exchange kinetics. We claim that the existence of this switching behavior, also triggered by factors, such as the presence of cargo and variation of the membrane mechanical tension, allows for efficient regulation of vesicle secretion. We propose a model, supported by different experimental observations, in which vesiculation of secretory membranes is impaired by the energy-consuming desorption of coat proteins, until the presence of cargo or other factors triggers a dynamical switch into a vesicle-producing state.

Transport vesicle | protein coat | COP vesicles | self-assembly | nonequilibrium phase transition

The plasma membrane and the membrane of cell compartments such as the endoplasmic reticulum (ER) and the Golgi continually produce vesicles for cargo transport. Vesicle formation generally involves specific proteins that aggregate into semirigid coats of dimensions in the 100-nm range, well visible by electron microscopy (1–3). The process of vesicle formation is now rather well established (4) and is sketched in Fig. 1. First, various cytosolic proteins assemble on the membrane to form a coat by building units, called monomers. The membrane-bound monomers then polymerize into a coat structure that locally bends the membrane and recruits cargo molecules. As the coat expands, the coated membrane invaginates until it forms a nearly spherical vesicle containing cargo (5, 6) that is eventually released from the membrane. The coat components soon disassemble and are ready to participate in the formation of a new vesicle.

The coats are classified in three major classes, COPII, COPI, and clathrin. Although they involve distinct proteins, the three types of coat share many common features, from their size and shape to the mechanism by which polymerization, cargo recruitment, and membrane deformation is achieved (4). Our approach is primarily aimed at studying the formation of COPI and COPII vesicles. However, the generality and robustness of its outcome suggest relevance for the more sophisticated clathrin coats as well. The assembly of COPs and clathrin and the fission of COP vesicles can now be reconstructed on purified liposomes with a restricted number of components (7–9). Those experiments point out the robustness of the coat-formation process. They also confirm that coat polymerization is spontaneous, only driven by weak short-range attractions between the monomers, whereas coat disassembly requires the presence of an energy source. More precisely, the assembly and disassembly of COP coat components follow the cycle of activation—inactivation of a GTPase protein, Sar1 for COPII and Arf1 for COPI (4). Once activated, the GTPases bind to the membrane and recruit individual coatamer complexes (the monomers) that later polymerize into coats (10). The inactivation of the GTPase, triggered by the hydrolysis of its bound GTP, leads to its unbinding from the membrane and to the monomer disassembly if the GTPase belongs to a monomer.

Strikingly, fluorescence recovery after photobleaching (FRAP) experiments suggest that the exchange kinetics of coat components is much faster than the rate of vesicle secretion (10). In other words, many futile monomers are released to the cytosol during the expansion of a coat. So, whereas new membrane-bound monomers polymerize at the coat periphery, others within the coat disassemble and are expelled to the cytosol. Paradoxically, the consumption of energy via GTP hydrolysis seems to work against coat growth and to prevent vesicle formation. This resembles microtubules dynamics and by analogy to the treadmilling of microtubules, it has been suggested that the competition between growth and unbinding may produce stable coats of fixed area (10–13).

In this article, we investigate theoretically the consequence of futile release of coat components on the distribution of size and shape of protein coats and, more practically, on the amount of secreted vesicles. In our model (Fig. 1), monomers are continuously “dropped” onto a membrane and proceed to aggregate into coats of growing size that curve the membrane. Monomers leave the membrane either individually after GTP hydrolysis or collectively as part of a completed vesicle. Intuitively, one expects GTPase inactivation to decrease the rate of vesicle formation by reducing the lifetime of membrane-bound monomers. However, our generic approach reveals that a deeper understanding of vesicle secretion requires a quantitative statistical model. Indeed, we report the existence of a discontinuous dynamical transition from a quiescent to a vesicle-producing membrane, upon variation of the rate of GTP hydrolysis. In other words, the apparently counterproductive energy consumption that favors the unbinding of coat components provides secretory membranes with a highly sensitive switch to regulate vesicle release, triggered, for instance, by a variation of the cargo concentration or the mechanical tension of the membrane.

Description of the Model
Our goal is to describe the collective behavior of a population of evolving membrane domains (coats) formed by the aggregation of identical units (monomers), which are themselves continuously recycled between the membrane and a reservoir (the cytosol). Our...
starting point is the course of events depicted in Fig. 1. We consider a patch of membrane much larger than the size of individual coats, which is subjected to a constant and homogeneous influx of monomers \( J_{on} \). The monomers have a finite lifetime on the membrane before being recycled to the cytosol, at a rate \( k_{off} \). While on the membrane, they diffuse and eventually aggregate into coated protein coats. Coats that manage to reach a critical size leave the membrane as coated vesicles.

**Monomers.** The formation of a new monomer on the membrane involves a succession of steps (GTPase binding on membrane and activation and the recruitment of coat proteins) that are not individually described in the present model. The rates associated with these processes enter a unique parameter, the mean number of monomers \( J_{on} \), formed on the membrane per units of time and area.

**Coat Growth.** Coat expansion proceeds by polymerization of monomers at the coat edge. Monomer–monomer binding is spontaneous and results from weak short-range interactions. The binding energy \( \gamma \) should be in the range of a few \( k_B T \) (\( k_B T \) is the energy available from thermal fluctuations, with \( k_B \) the Boltzmann constant and \( T \) the temperature in Kelvin), because the 10\( k_B T \) provided by GTP hydrolysis is sufficient to break the bonds. The polymerization is thus thermally reversible and solely driven by the minimization of the free energy of the coat.

**Coat Structure.** Electron microscopy (1, 2) supports the assumption that the optimal area per monomer \( s_0 \approx 100 \text{ nm}^2 \) and the optimal radius of curvature of the coat \( R_0 \approx 50 \text{ nm} \) are homogeneous within the coat and remain constant during coat growth. We thus adopt a model in which the state of a coat is fully characterized by a single, slowly varying parameter: the number of polymerized monomers \( \ell \) it contains, taken as a continuous variable for commodity. All other internal degrees of freedom in the coat (protein density and coat shape) are considered to adjust to their optimal configuration faster than the typical rates of coat growth and GTP hydrolysis. Under these assumptions, a given coat of size \( \ell \) can be described as a spherical cap of constant curvature (defined as the dimensionless quantity \( c = \sqrt{s_0/(4\pi R_0^2)} \approx 1/20 \)). A full spherical coat (\( \ell c^2 = 1 \)) with these properties contains several hundred monomers.

**Monomer Release.** Contrary to the reversible monomer polymerization, monomer desorption is an energy-consuming process driven by GTP hydrolysis. It occurs at a rate \( k_{off} \), assumed constant here for simplicity [see supporting information (SI) Appendix for a discussion of this assumption]. This rate can be estimated from FRAP experiments (10, 12, 14, 15), \( k_{off} \approx 0.1 – 10 \text{ s}^{-1} \). Under the assumption of constant protein density in the coat, the dissociation of monomer from the coat is followed by a rapid rearrangement of the coat structure and by a slight shrinkage of the coat; monomer inactivation thus opposes coat expansion.

**Vesicle Release.** A mature coat containing a number \( \ell_c \) (\( \ell_c c^2 \approx 1 \)) of bound monomers forms a nearly closed sphere connected to the rest of the membrane by a thin neck. At this stage, the coat cannot grow further and is eventually released into the cytosol as a coated vesicle. The details of the scission mechanism vary between classes of coat, and may involve additional proteins (4). Here, we merely assume that once a domain reaches the critical size \( \ell = \ell_c \), it leaves the membrane as vesicles at a constant rate \( k_v \).

**Membrane Properties.** The curvature of the coat imposes a deformation to the membrane that is opposed by membrane tension \( \sigma \). Membrane tension \( \sigma \) thus favors coat depolymerization, and can have a sizable effect on coat growth if it is larger than \( \gamma / \ell \sigma \sqrt{\ell_c} \approx 10^{-2} \text{ J/m}^2 \). (A detailed model for the elastic properties of the protein-covered membrane is discussed in SI Appendix, section II.B.) Tensions of the Golgi, the ER, and the plasma membranes are typical in the range \( \sigma \approx 10^{-6} – 10^{-4} \text{ J/m}^2 \) (17), and may thus play a role in vesicle secretion. Hereafter, membrane tension will be expressed in natural units: \( \bar{\sigma} = \sigma s_0 \approx 10^{-2} - 1 \). The population of membrane coats is characterized by its size distribution \( n(\ell) \). The mean concentration of coats of size \( \ell \) (between \( \ell \) and \( \ell + d\ell \)) at a time \( \ell \) is \( n(\ell) = \ell^2 d\ell \), and the mean concentration of isolated monomers is \( n(0) \). Our purpose is thus to compute, \( n(\ell) \) and \( n_1 \) at steady state, for given values of the parameters \( \gamma \), \( \bar{\sigma} \), \( k_{off} \), \( J_{on} \), \( k_v \), and \( \ell_c \). More practically, we will compare the fluxes of coat components leaving the membrane as inactive monomers \( J_{off} \) and as part of a vesicle \( J_v \) (Fig. 1).

**Theoretical Framework**

**Monomer Fluxes and Conservation Relations.** In this section, we derive the kinetic equations for the evolution of the coat size distribution \( n(\ell) \). The monomer cycle can be divided into four steps, to which correspond four different fluxes, as shown in Fig. 1.

- \( J_{on}(\ell) \) is the influx of single monomers binding to the membrane, taken as an input in our model.
- \( J_p(\ell, t) \) is the flux of monomer-joining domains of size \( \ell \), a balance between polymerization and depolymerization for this domain size. Integrated over the entire population, it gives the total flux of monomers incorporated into domains \( J_p = \int_0^{\ell_c} d\ell J_p(\ell, t) \).
- \( J_{off}(\ell, t) \) is the flux of monomers expelled from domains of size \( \ell \) into the cytosol after GTP hydrolysis. Under the assumption of uniform release, it is given by \( J_{off}(\ell) = k_{off} n(\ell) \). Integrated over the entire population, it gives the total flux of individual inactive monomers leaving the membrane \( J_{off} = \int_0^{\ell_c} d\ell J_{off}(\ell, t) \).
- \( J_v(\ell) \) is the flux of mature coats released as vesicles. Introducing the rate of vesicle formation \( k_v \), we have \( J_v = k_v n(\ell_c) \). The total flux of monomers leaving the membrane as part of a vesicle is \( J_v(\ell) = j_v \ell_c \).

The evolution of the coat size distribution satisfies (see SI Appendix):

\[
\frac{\partial n(\ell)}{\partial t} = -\partial_\ell [J_p(\ell) - J_{off}(\ell)],
\]

and the polymerization current \( J_p \) reads:

\[
J_p(\ell) = -k_p n_1 (\partial_\ell n(\ell) + n(\ell) \partial_\ell \Delta E(\ell)).
\]
$j_p$ is proportional to the density of available monomers $n_1$, and to the rate of monomer binding onto coats $k_p$ (see below). It contains a diffusive term accounting for random polymerization and depolymerization induced by thermal noise and a convective term describing the drift of monomers toward domains of lower energy, driven by the “force” $-\partial_\ell \Delta E$. The free energy difference $\Delta E$ (in $k_B T$ units) between a coat of size $\ell$ and $\ell$ isolated monomers diffusing on the membrane is obtained treating the coat as a rigid spherical cap (18) (see also SI Appendix):

$$\Delta E(\ell) = \gamma \sqrt{\ell(1 - c^2 \ell)} + \sigma c^2 \ell^2 - \mu(n_1) \ell$$  \[3\]

where

$$\mu = \ln n_1 + \gamma \sqrt{1 - c^2 + \sigma c^2}$$  \[4\]

is a chemical potential including the entropy of the freely diffusing monomers, see SI Appendix.

The net current $j(\ell) = j_p(\ell) - j_{\text{off}}(\ell)$ accounts for polymerization and desorption. It can be written in terms of an effective energy $\tilde{E}(\ell) = \Delta E(\ell) + \frac{k_{\text{off}}}{2\sigma n_1} (\ell^2 - 1)$:

$$j = -k_p n_1 (\partial_\ell \tilde{E}) + n \partial_\ell \tilde{E},$$  \[5\]

with

$$\tilde{E}(\ell) = \gamma \sqrt{\ell(1 - c^2 \ell)} + \Sigma(n_1) \ell^2 - \mu(n_1) \ell + \text{const.,}$$  \[6\]

$$\Sigma(n_1) = \sigma c^2 + \frac{k_{\text{off}}}{2k_B n_1}.$$  \[7\]

This equation introduces an effective tension $\Sigma$ that illustrates the fact that desorption of inactive monomers and membrane tension formally play the same role in hindering coat maturation and vesicle secretion. Note that more generally, the counterterm binding or inactivation rates may depend on the coat size, in which case monomer desorption enters the effective energy as $j d\ell (k_{\text{off}}(\ell)/k_p(\ell))$.

Finally, the monomer influx $J_{\text{on}}$ and the flux of secreted vesicle $J_v$ are accounted for via the boundary conditions (see SI Appendix):

$$j_p(1) = J_{\text{on}} - J_p,$$  \[8\]

$$j_p(\ell_v) = j_{\text{off}}(\ell_v) = j_v.$$  \[9\]

**Steady State.** At steady state, all fluxes are balanced and $\partial_\ell n = 0$. Eqs. 1–9 reduce to two conditions to be satisfied by $n_1$ and $n(\ell)$:

$$j(\ell) = j_v = \text{constant.}$$  \[10\]

$$J_{\text{on}} = J_{\text{off}} + J_v.$$  \[11\]

The former equation enforces that the size distribution is constant, and the latter that the flux of monomer binding to the membrane balances the flux of monomer leaving the membrane, either after inactivation or by vesiculation.

**Results.** In this section, we focus on the steady state of a membrane receiving a constant influx of monomer, each having a finite lifetime at the membrane. The full characterization of the coat population and of vesicle secretion follows two steps. First, the stationary distribution of coat size $n(\ell)$ is computed for a given concentration of free monomers $n_1$ with Eq. 10. Second, $n_1$ is self-consistently derived for a given monomer influx $J_{\text{on}}$ by imposing that the influx matches the total monomer outflow (Eq. 11). While the first step relies entirely on the properties of the free energy landscape $\tilde{E}(\ell)$, the second introduces collective effects emerging from the competitive growth of many domains, which ultimately give rise to the “secretory switch.”

**Low monomer concentration.** At low monomer concentration, the large energy barrier to vesiculation at $\ell = \ell_v$ prevents coats to mature into vesicles ($J_v \simeq 0$) (Fig. 2, states a and b). The coat size distribution resembles a distribution at thermal equilibrium: $n(\ell) \simeq e^{-E(\ell)}/\ell^\ell$ (19), and the membrane follows a classical scheme common to many self-assembling systems [e.g., surfactants in solution (19)]. The local energy minimum at $\ell^\ell$ appears above a critical concentration $n_1^{\text{cmc}}$, analogue to the “critical micellar concentration,” or “cmc” at which surfactants in solution start forming micellar.

[Fig. 2. Effective energy landscape $E(\ell)$ (left, in $k_B T$ units, from Eq. 6) and the corresponding coat size distribution $n(\ell)$ (right, in $n_1^{\text{cmc}}$ units, from Eqs. 5–10), for different values of the free monomer density $n_1$, $n_1/n_1^{\text{cmc}} = 0.986$ (state a - Top green curve), 1.039 (state b - Top red), 1.054 (state c - Middle) and 1.160 (state d - Bottom). The variation of $E(\ell)$ and $n(\ell)$ upon a slight increase of $n_1$ above the given value are shown in blue dashed lines. Other parameters are $\gamma = 5 k_B T$, $\sigma = 0$, $k_v = 0.13 s^{-1}$, $k_{\text{off}} = 0.12 s^{-1}$, and $\ell_v = 500$. With those values, $n_1^{\text{cmc}} = 0.009$.]
aggregates (see ref. 19 and SI Appendix). For \( n_1 < n_1^{\text{cmc}} \) (Fig. 2 state a), entropy dominates, and the effective energy increases monotonously with the coat size \( \ell \). Monomer aggregation is unfavorable, and the membrane contains mainly single monomers and few small transient domains formed by fluctuation. For \( n_1 > n_1^{\text{cmc}} \) (Fig. 2 state b), long-lived coats can nucleate, at a rate fixed by the nucleation barrier, and grow up to the optimal size \( \ell^* \). Maturation into coated vesicles (\( \ell = \ell^* \)) is prevented by monomer desorption and membrane tension.

**Larger monomer concentration.** The height of the energy barrier to vesiculation at \( \ell = \ell^* \) decreases with increasing monomer concentration. When it falls below the nucleation energy barrier (Fig. 2 state c), domains may mature into fully formed vesicles and vesicle secretion becomes increasingly probable. The optimal coat size \( \ell^* \) increases with \( n_1 \) and eventually exceeds the critical size for vesiculation \( \ell_v \) at high monomer concentration (Fig. 2 state d), under which conditions any nucleated domain matures into a fully formed vesicle.

The growth of individual coats is controlled by the amount of free, active monomers \( n_1 \). On the other hand, the pool of free monomers is depleted by their binding onto growing coats and is thus influenced by the coat population. As we shall see next, this feedback induces remarkable collective effects within the coat population, which presents a discontinuous transition between a state of arrested growth and a state of abundant vesiculation within a narrow range of kinetic parameters.

### Vesicle Secretion is Controlled by Collective Effects.

The solution of the coupled Eqs. 10 and 11 is graphically represented on Fig. 3 as the intersection of the monomer in-flux \( J_m \) and total outflux \( J_{\text{off}} + J_v \). It may fall in four different regimes (a–d), corresponding to the four distributions plotted in Fig. 2. In a wide range of parameters (see below), \( J_{\text{off}} \) displays the remarkable property of being nonmonotonous, with a sharp peak at a critical concentration of free monomers. This behavior dramatically influences the membrane’s ability to secrete vesicles. Indeed, a given monomer influx may correspond to three distinct dynamical states of the membrane. We will show below that regimes b and d represent respectively a quiescent membrane and a membrane secreting large amount of vesicles, whereas regime c is dynamically unstable. The secretory membrane thus constitutes a bistable dynamical system able to abruptly switch vesicle secretion on and off at prescribed monomer turnover rates.

Because all membrane-bound monomers are inactivated with the same rate \( k_{\text{off}} \), the total flux of monomer leaving the membrane after inactivation \( J_{\text{off}} \) is directly proportional to the total amount of monomer on the membrane. The peak of \( J_{\text{off}} \) in Fig. 3 stems from the complex relationship between the concentration of isolated monomer \( n_1 \) and the total amount of coat components on the membrane.

**No vesicle secretion regimes a and b.** If no coat can form (low monomer concentration: state a), the monomers outflux is dominated by the desorption of free monomer: \( J_{\text{off}} \approx k_{\text{off}} n_1 \). If coats can form, but do not mature into vesicles (state b), the outflux is dominated by the desorption of monomers belonging to coats of size \( \ell^* \): \( J_{\text{off}} \approx k_{\text{off}} \ell^* n_1(\ell^*) \). In this regime, monomers reaching the membrane tend to join a coat and the density of free monomer is almost insensitive to the fluxes: \( n_1 \approx n(\ell^*)^{1/\gamma} \) with \( \gamma \gg 1 \), see SI Appendix. A small increase of \( n_1 \) requires a pronounced increase of the total amount of coat material on the membrane, which explains the sharp rise of the total monomer out-flux \( J_{\text{off}} \) with \( n_1 \) in Fig. 3. In this regime, the optimal coat size is also insensitive to the monomer fluxes, and is obtained from the minimization of the effective energy \( \hat{E} \) (Eq. 6)

\[
\ell^* \propto \left( \frac{\gamma}{\Sigma(n_1^{\text{cmc}})} \right)^{2/3}.
\]  

**The unstable regime c.** For intermediate monomer density, metastable coated pits have a high probability to grow into fully formed vesicle owing to the small barrier to vesiculation (Fig. 2 state c). The rate of vesicle formation increases with \( n_1 \), so the total amount of membrane-bound material actually decreases with increasing concentration of free monomer. This is shown by the dashed blue line in Fig. 2 state c, and explains the decrease of \( J_{\text{off}} \) in Fig. 3. This situation cannot be maintained at steady state, and spontaneously evolves toward either state b or d.

**Steady Vesicle Secretion Regime D.** If the monomer concentration on the membrane is large, the effective coat energy exhibits a nucleation barrier but no intermediate minimum (Fig. 2 state d). After nucleation, a coat grows at nearly constant velocity until it reaches the critical size \( \ell_v \), where it remains trapped for a time \( 1/k_b \) before being released as a vesicle. In this regime, both \( J_m \) and \( J_{\text{off}} \) increase with \( n_1 \), Fig. 3. The bistability exhibited by the coats dynamics relies on the existence of an unstable steady state and holds as long as there exist a (meta)stable coat of intermediate size. This feature is conserved even if the effective energy contains higher-order terms, to be expected if the ratio of monomer dissociation to binding rates \( (k_{\text{off}}/k_{\text{on}}) \) increases with the coat size (see SI Appendix). Furthermore, the switch exists if there is a metastable state within the accessible size range: \( \ell^*/\ell_v \approx \gamma c^2 \) < 1. From Eq. 12, this condition amounts to \( \Sigma > \gamma c^2 (\approx 10^{-4}) \). The effective tension \( \Sigma \) (Eq. 7) accounts both for the membrane mechanical tension \( (\sigma c^2 \approx 10^{-2} \text{ to } 10^{-3}) \) and the ratio \( (k_{\text{off}}/k_{\text{on}}) \). The binding rate is assumed to be limited by monomer diffusion and is expected to be of the order of the inverse monomer diffusion time over its own size \( (k_b \approx D/\ell_0 \approx 10^5 \text{ s}^{-1}, \text{with } D \approx \mu m^2/s \text{ the membrane diffusion coefficient}) \). With a dissociation rate \( k_{\text{off}} \approx 1 \text{ s}^{-1} \), and a monomer density \( n_1 \approx 1 \text{ cm}^{-2} \) (or \( k_b \approx 10^5 \text{ s}^{-1} \)), we find that secretory membranes are well into the bistable regime \( (\Sigma \approx 10^{-2} \gg \gamma c^2) \) and should exhibit the secretory switch discussed below.

**Discussion**

The growth of coated pits and the secretion of coated vesicles result from a kinetic balance between the polymerization and the inactivation of coat components. It is thus to be expected that coat maturation can only proceed if the coatomers turnover at the membrane is sufficiently slow (10, 11, 13). Our model goes beyond this intuitive analysis, and shows that secretory membranes are able to abruptly switch between a quiescent and a
and low thresholds). Between these two rates, the secretory membrane works in an all-or-nothing fashion and can switch between quiescent and vesicle-producing states. We also show how some apparently unrelated observations on COP vesicles naturally fit into our global picture of coated-vesicle secretion.

The Secretary Switch. Consider a secretory membrane receiving a fixed amount of coatamer per unit time. As coatamer accumulates and aggregates, the membrane eventually reaches a steady state in which the flux of coatamer desorption from the membrane $k_{\text{off}}$. The transition to vesiculation is discontinuous, and is characterized by an hysteretic cycle (arrows), with high and low turnover thresholds. The parameters are the same as those used for Figs. 2 and 3.

**Fig. 4.** Number of secreted vesicle per $\mu$m$^2$ s ($v = j_x \times t_{\text{m}}$) as a function of the rate of coatamer desorption from the membrane $k_{\text{off}}$. The transition to vesiculation is discontinuous, and is characterized by an hysteretic cycle (arrows), with high and low turnover thresholds. The parameters are the same as those used for Figs. 2 and 3.

Regulation of Vesicle Secretion by Cargo. The adsorption flux $J_{\text{on}}$ and the desorption rate $k_{\text{off}}$ of coatamer at the membrane tightly control vesicle secretion. Recent fluorescence experiments on COPs coat suggest that these rates vary with the amount of cargo present at the membrane. For COPII, the presence of extra cargo leads to significant increase of the amount of coat components at the Golgi membrane, which could reflect either the increase of $J_{\text{on}}$ or the decrease of $k_{\text{off}}$ (12). For COPI, FRAP experiments show that the coatamer exchange rate between the ER membrane and the cytosol is doubled in the absence of cargo. This has been attributed to the increase of $k_{\text{off}}$ with decreasing cargo density (15).

Our model predicts that vesicles can only be secreted if the recycling rate $k_{\text{off}}$ is below a critical value (Fig. 4). By increasing the lifetime of the coatamers at the membrane, the presence of cargo is thus expected to promote vesicle secretion, and a minimal amount of cargo at the membrane might actually be required for transport vesicle to be secreted. The two, high and low, recycling thresholds of Fig. 4 would then correspond to two critical cargo densities (low and high, respectively).

Considering that newly synthesized cargo is brought to the membrane at a (slow) steady rate and is removed by vesiculation, membrane-bound cargo accumulates in the no-secretion regime, thereby decreasing $k_{\text{off}}$ and moving the system toward the secretion regime. Above the high cargo-density threshold, the coat machinery abruptly escapes the stationary-pits regime and switches to vesicle production (Fig. 4). This results in a decrease of membrane-bound cargo, which increases $k_{\text{off}}$ and moves the system toward the quiescent state. Below the low cargo-density threshold, vesicle secretion is switched off, letting the cargo accumulate until the high-density threshold is reached and vesicle production is resumed, starting a new cycle. Under constant cargo influx, the system should thus periodically switch between quiescent phases and phases of vesicle secretion, following the hysteretic loop of Fig. 4. If cargo synthesis is irregular, the membrane waits for sufficient accumulation of cargo between transient residences in the secreting state, where the accumulated cargo is released. The vesicle flux over time would then appear as an irregular pulsed signal. Recoding the total vesicle outflux over an extended patch of secretory membrane over time should thus be of high interest. Oscillatory or pulsed vesicle secretion would be a signature of the secretory switch uncovered by our theoretical analysis (within our framework, steady secretion would indicate that cargo synthesis is sufficiently fast to compensate the secreted cargo).

This accumulator mechanism would provide functional efficiency to the secretory membrane, because it would prevent the futile delivery of empty vesicles. Strikingly, analysis of COP vesicles in mutant cells where arf1 is unable to hydrolyse GTP has revealed a much lower cargo content than in normal cells (20). This observation supports our prediction. Indeed, in the absence of GTP hydrolysis ($k_{\text{off}} = 0$), the switch is gone, and vesicle secretion remains "on" regardless of the available amount of cargo.

The existence of an hysteretic cycle can also positively impact on the rate of cargo delivery. At steady state, the flux of cargo delivered by a secretory membrane that would not possess an unstable regime would automatically adjust to the flux of synthesized cargo. Here, and while the system is traveling along the secretion branch of the hysteretic cycle, the flux of secreted cargo is mainly controlled by the kinetics of coat formation and can potentially be much larger than the rate of cargo synthesis.

Effects of the Membrane Tension: Regulation of Vesicle Formation and Coat Flattening. Our calculation has shown that membrane tension plays essentially the same role as coatamer recycling in opposing vesicle secretion (Eq. 6). Vesiculation is only possible below a high tension threshold, and the oscillatory behavior...
described above may also result from variations of tension. Secretion removes membrane area from the organelle and may increase its tension, whereas the fusion of incoming vesicles (21) or other regulatory mechanisms (18), dynamically relieves tension. Vesicle secretion would thus occur only when enough membrane area has been accumulated to relax the tension. Such a mechanism suggests a coordination of purely mechanical origin between absorption and release of vesicles and could prevent the uncontrolled shrinking of secretory compartments. Remarkably, the Golgi strikingly crumbles in cells where the GTP hydrolysis in COPI-coat is rendered inoperant (20).

In Eq. 3, the effect of membrane tension was computed assuming that the coat rigidity κ (unit of energy) was sufficiently strong to impose the domain curvature, which remained constant regardless of the coat size and the membrane properties. This simplification ceases to be valid under high membrane tension (σ ≈ κ/R₀², where R₀ is the radius of curvature of a tensionless coat). Higher tensions result in a flattening of the coat (see SI Appendix, section II.B). Beyond a tension threshold σ > κ/(2R₀) (≈ 10⁻⁴ J/m² for the coat rigidity κ = 100k_B T), the formation of a closed sphere becomes impossible, and protein aggregates should grow as flat patches. High membrane tension would thus favor the formation of flat coatomer aggregates whose size is limited by coatamer recycling (22). Such “flat lattice” are indeed observed for clathrin coats at the basal membrane of adhered cells (23), where adhesive proteins are expected to generate high membrane tensions.

Concluding Remarks. The model presented here is the simplest implementation of a kinetic model of coated vesicle formation where coat growth competes with the inactivation of coat components. The secretary switch revealed by our work is very robust and relies solely on the (nonequilibrium) thermodynamics of coat formation. Beyond the qualitative agreement with experimental findings discussed in the previous section, we hope that future experiments can further test some of our predictions, which include: (i) the existence of metastable domains of intermediate size, (ii) the role of membrane tension in preventing the formation of curved protein coat and eventually its involvement in the formation of flat lattices, and (iii) the oscillary or pulsed secretion of vesicles in time.

In this study, we have used the crudest possible description of the coat structure, thus avoiding having to deal with structural details of specific protein coats. Further experimental observation, e.g., on biomimetic systems, could motivate the building of models focusing on the dynamics of a single coat. Supplementary degrees of freedom for the coat shape could be considered, allowing for the competitive growth of structures of various morphologies [tubules (6), spherical caps, and flat lattices (23)], observed in living cells and biomimetic systems.

In the same spirit, the heterogeneties of the coat structures may be included in the model. The coupling between the GTP hydrolysis rate and the curvature (COPI) or the degree of polymerization (COPII) revealed in recent experiments (14, 24), suggest that monomers inactivation may be enhanced at the coat center and limited at the boundary. One could then imagine the formation of layers of active monomers preventing coat disassembly (14, 24). Such properties suggest a strong analogy with microtubules (11), and it is tempting to imagine exotic growth dynamics with shrinking cascades such as those observed for microtubules (25).

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Supporting Information for “Kinetic regulation of coated vesicle secretion”

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I. KINETIC EQUATIONS

We provide here some details on the derivation of the kinetic equations (1-6) of the article.

A. Fluxes and conservation relations in a discrete model

The time evolution of the concentration \( n_\ell(t) \) of coats made of \( \ell \) linked monomers is first derived assuming \( \ell \) is a discrete quantity. A master equation is written, that includes monomer binding to (rate \( \ell k_1 \)) and unbinding from (rate \( \ell k_0 \)) \( \ell \)-sized coats. This equation also includes the exchange of monomer with the cytosol, via the release of individual monomer from coats at rate \( \ell k_0 \) (assumed constant here for simplicity). The boundary conditions at \( \ell = 1 \) and \( \ell = \ell_v \) must be receive special treatment, as monomers (\( \ell = 1 \)) are (i) adsorbed on the membrane at a rate \( \ell_j \), (ii) lost when they join any polymerizing domain, and (iii) produced by domain depolymerization. Note that two monomers are produced (lost) by the formation (break-up) of a dimer. Furthermore, vesicles are produced from domains reaching the critical size \( \ell_v \) at a rate \( \ell_v \). Finally, no domain smaller than \( \ell = 1 \) or larger than \( \ell = \ell_v \) may form. Including all these effects:

\[
1 < \ell < \ell_v \quad \partial_t n_\ell = -n_\ell n_\ell k_1 + n_\ell n_{\ell-1} k_{\ell-1} - n_\ell k'_\ell + n_{\ell+1} k'_{\ell+1} + k_0 (n_{\ell+1}(\ell + 1) - n_\ell), \tag{1}
\]

\[
\ell = 1 \quad \partial_t n_1 = \ell_j + k_0 (2n_2 - n_1) - n_1^2 k_1 + n_2 k'_2 + \sum_{\ell' = 2}^{\ell_v} (n_{\ell'} k'_{\ell'} - n_1 n_{\ell' - 1} k_{\ell' - 1}), \tag{2}
\]

\[
\ell = \ell_v \quad \partial_t n_{\ell_v} = n_1 n_{\ell_v - 1} k_{\ell_v - 1} - n_{\ell_v} k'_{\ell_v} - k_0 n_{\ell_v} \ell_v - k_v n_{\ell_v}. \tag{3}
\]

One can define three partial (lower case) and total (upper case) monomer currents,

\[
j_p(\ell) = n_1 n_{\ell-1} k_{\ell-1} - n_\ell k'_\ell \quad J_p = \sum_{\ell = 2}^{\ell_v} j_p(\ell), \tag{4}
\]

\[
j_{\text{off}}(\ell) = k_0 n_\ell \ell \quad J_{\text{off}} = \sum_{\ell = 1}^{\ell_v} j_{\text{off}}(\ell), \tag{5}
\]

\[
j_v = k_v n_{\ell_v} \quad J_v = j_v \ell_v, \tag{6}
\]

which are respectively the net current (balance of binding and unbinding) of single monomers toward the \( \ell \)-coats, the current of monomers expelled from the \( \ell \)-coats after GTP hydrolysis, and the current of released vesicles.

Using Eqs.(4-6), Eqs.(1-3) may then be rewritten:

\[
\partial_t n_\ell = (j_p(\ell) - j_{\text{off}}(\ell)) - (j_p(\ell + 1) - j_{\text{off}}(\ell + 1)), \tag{7}
\]

\[
J_p(1) = \ell_j - J_p \quad (8)
\]

\[
J_p(\ell_v + 1) - j_{\text{off}}(\ell_v + 1) = j_v. \tag{9}
\]

where one recognizes the discrete version of Eqs.(1,8,9) of the article. The total amount of coat material on the membrane evolves with time according to:

\[
\partial_t \sum_{\ell = 1}^{\ell_v} n_\ell \ell = \ell_j - J_{\text{off}} - J_v. \tag{10}
\]

In the next section, we explain how the polymerization current \( j_p \) is related to coat energy.
B. Expression of the polymerization current

Monomer binding to and unbinding from a coat are thermal processes, which rates satisfy detailed balance:

$$k_{\ell+1}e^{-E_{\ell+1}} = k_{\ell}e^{-E_{\ell}}$$

where $E_{\ell}$ is the free energy of an $\ell$-sized monomer aggregate. Under this constrain, the polymerization current depends on a single kinetic coefficient, for instance, the binding rate. It can be written as,

$$j_p(\ell) = k_{\ell} \left( n_1n_{\ell} - n_{\ell+1}e^{E_{\ell+1} - E_{\ell}} \right),$$

$$= k_{\ell}n_1 \left( n_{\ell} - n_{\ell+1}e^{\Delta E_{\ell+1} - \Delta E_{\ell}} \right),$$

where we have introduce the free energy difference between an $\ell$-sized aggregate and $\ell$ free monomer:

$$\Delta E_{\ell} = E_{\ell} - (E_1 + \ln n_1)\ell,$$

$E_1 + \ln n_1$ is the chemical potential of the free monomers on the membrane.

C. Continuous limit

For convenience, we use the continuous limit where the number of monomer per coat $\ell$ is taken as a continuous variable. The coat concentration, the currents and the energy are transformed into continuous functions: $n_{\ell} \rightarrow n(\ell)$. We then expand all quantities as $A_{\ell\pm 1} = A(\ell) \pm \partial_\ell A(\ell) + ...$, up to the first dominant order.

The continuous limit of Eqs.(7-9) hence read,

$$\partial_\ell n(\ell) = -\partial_\ell j_p(\ell) + \partial_\ell j_{off}(\ell),$$

$$j_p(1) = J_{on} - J_p,$$

$$j_p(\ell_v) - j_{off}(\ell_v) = j_v,$$

with unchanged expressions for the currents of monomer unbinding and vesicle release (Eqs.(5-6))

$$j_{off}(\ell) = k_{off}\ell n(\ell),$$

$$j_v = k_v n(\ell_v).$$

The expression of the polymerization current is obtained by expanding Eq.(12) and assuming a constant fusion rate $k_{\ell} = k_p$,

$$j_p(\ell) = -k_p n_1 (\partial_\ell n(\ell) + n(\ell)\partial_\ell \Delta E(\ell)) \quad \text{with} \quad \Delta E(\ell) = E(\ell) - (E_1 + \ln n_1)\ell.$$

In the continuous limit, the expressions of the global fluxes Eqs.(4-6) become,

$$J_p = \int_1^{\ell_v} j_p(\ell)d\ell \quad ; \quad J_{off} = \int_1^{\ell_v} j_{off}(\ell)d\ell \quad ; \quad J_v = j_v \ell_v.$$ (20)

Eqs.(14-20) are the ones reported in the text. Finally, we define the net growth current $j(\ell)$ as the concentration of coat that grow from the size $\ell - 1$ to the size $\ell$ per time unit. It results from the competition between polymerization (binding/unbinding at the coat edge) and release of inactivated monomers:

$$j(\ell) = j_p(\ell) - j_{off}(\ell).$$ (21)

The kinetic equation may then be written as a classical Fokker-Planck equation:

$$\partial_\ell n(\ell) = -\partial_\ell j(\ell), \quad \text{with} \quad j(\ell) = -k_p n_1 (\partial_\ell n(\ell) + n(\ell)\partial_\ell \hat{E}(\ell))$$ (22)

formally equivalent to a diffusion process in an effective energy landscape, given by:

$$\hat{E}(\ell) = \Delta E(\ell) + \int_1^\ell d\ell' \frac{\kappa_{off}}{k_p n_1}.$$ (23)

In the text, the off-rate and the rate of polymerization are assumed to be constant (see Section II.C), in which case monomer inactivation enters the energy as part of an effective surface tension (quadratic term in $\ell$, Eqs.6-7 of the main text).
D. Steady state

At steady state, the condition $\partial_t n(\ell) = 0$, implies that the growth current is independent of the coat size (Eq.(22))

$$j(\ell) = j_v = \text{const.}$$

This condition implies that the steady coat size distribution reads,

$$n(\ell) = e^{-\tilde{E}(\ell)} - \int_1^\ell d\ell \left( \frac{j_v}{k_p n_1} e^{\tilde{E}(\ell') - \tilde{E}(\ell)} \right),$$

where we have used $n_1 = n(1)$. Then, using Eq.(18) one can obtain the final expressions of the steady coat distribution and vesicle flux:

$$n(\ell) = e^{-\tilde{E}(\ell)} \frac{e^{\tilde{E}(\ell_c)} + \int_1^{\ell_c} d\ell' \frac{k_p}{k_p n_1} e^{\tilde{E}(\ell')}}{e^{\tilde{E}(\ell_c)} + \int_1^\ell d\ell' \frac{k_p}{k_p n_1} e^{\tilde{E}(\ell')}}, \quad j_v = \frac{k_p}{e^{\tilde{E}(\ell_c)} + \int_1^\ell d\ell' \frac{k_p}{k_p n_1} e^{\tilde{E}(\ell')}}.$$

which have been used to obtain the distribution of the Fig.2 of the text. The steady-state expression Eq.(26) still depend upon the concentration of single monomers $n_1$. The latter is fixed by imposing the conservation of the total amount of coat components $(\partial_t \int d\ell n(\ell) = 0)$, equivalent to (Eq.(10)):

$$J_{on} = J_{off} + j_v.$$  

This equation has to be solved numerically, e.g in terms of the monomer density $n_1$ that satisfies a given monomer influx $J_{on}$. Its graphical solution is presented Fig.3 of the paper and discussed in detail in the text.

II. ELASTIC MODEL FOR THE PROTEIN COAT

The purpose of this section is to derive the expression of the coat free energy that enters the dynamic equation. The coat is modeled as a rigid sheet of non-zero spontaneous curvature bound to a tension-bearing membrane of zero spontaneous curvature. In our model, the monomer density ($s_0^{-1}$) and the coat radius of curvature ($R$), and its preferred radius of curvature $R_0$ are assumed to be homogeneous within the coat. The dimensionless coat curvature and spontaneous curvature used in the main text is defined as $c = \sqrt{s_0/4\pi R}$ and $c_0 = \sqrt{s_0/4\pi R_0^{-1}}$. The coat forms a spherical cap of area $S$ that contains $\ell = S/s_0$ monomers.

A. Coat free energy

The energy of an $\ell$-sized, membrane-bound coat of curvature $c$ reads:

$$E(\ell, c) = -\varepsilon \ell + \gamma \sqrt{\ell(1 - c^2 \ell)} + \tilde{\sigma} c^2 \ell^2 + 8\pi \kappa (c - c_0)^2 \ell + 8\pi \kappa_m c^2 \ell.$$

where:

- The first term is the bulk free energy of the monomers within the coat. $-\varepsilon$ is the energy per monomer.
- The second term accounts for the loss of binding energy of the monomers at the coat periphery. It is proportional to the number of monomer at the boundary ($\sim \sqrt{\ell(1 - c^2 \ell)}$ for the spherical cap) times the binding energy loss per peripheral monomer $\gamma$.
- The third term accounts for the work done against membrane tension when bending of a patch of membrane. It is proportional to the excess surface area ($\Delta S = S_0 c^2 / s_0 = s_0 c^2 \ell^2$ for a spherical cap) times the tension $\sigma$ (with $\tilde{\sigma} = \sigma s_0$).
- The last two terms are bending energies for the protein coat and the membrane. They are proportional to their bending rigidities ($\kappa$ and $\kappa_m$, respectively), to the square of the deviation from the spontaneous curvatures ($c_0$ and zero, respectively), and to the coat area.
B. Coat equilibrium curvature

Assuming that the relaxation of internal degrees of freedom within the domain is faster than domain growth, the domain curvature relaxes to its optimal value \( c^* \), that minimize the domain energy for a given domain size: \( \partial E(\ell, c) / \partial c |_{c^*} = 0 \), and the coat free energy is a function of the monomer number only: \( E(\ell) = E(\ell, c^*(\ell)) \). The optimal curvature is given by:

\[
e^* \left( 1 + \frac{\kappa_m}{\kappa} + \frac{\bar{\sigma} \ell}{8\pi k} - \frac{\gamma \sqrt{\ell}}{16\pi k \sqrt{1-c^{*2}\ell}} \right) = c_0
\]  

(29)

The coat bending rigidity favors the curvature \( c^* = c_0 \). The line energy also favors a curved shape that reduces the coat perimeter, but is negligible for 100nm-sized domains (\( \ell \ll (16\pi k / \gamma)^2 < 10^5 \)), except for nearly spherical domains (\( c^2\ell \approx 1 \)). The membrane rigidity favors flat domains, and tends to reduce the coat curvature by a factor (\( 1 + \kappa_m / \kappa \)). Its effect should be small as the protein coat is expected to be much more rigid than the membrane (\( \kappa \gg \kappa_m \)). Furthermore, it is independent of the domain size and can be readily included in the model by modifying the preferred curvature of the coated membrane. The membrane tension also favors a flat structure (\( c^* = 0 \)), and its influence increases with the domain size. The equilibrium curvature results in principle from a balance of these antagonistic effects and may depend upon the coat size \( \ell \).

1. High coat rigidity limit

In the article, we have assume that the coat curvature remains constant during coat growth. This is valid if the membrane tension is not too large: \( \bar{\sigma} \ll 8\pi k / \ell \). In this case, the membrane curvature is the preferred curvature of the coat \( c^* = c_0 \) (since \( \kappa \gg \kappa_m \)) and the coat energy reduces to:

\[
E(\ell) = \gamma \sqrt{\ell(1-c_0^2\ell)} - \epsilon \ell + \bar{\sigma} c_0^2 \ell^2 + 8\pi k c_0^2 \ell.
\]  

(30)

As shown by Eqs.(12,19) the polymerization kinetic is controlled by the free energy difference (13) between coat and free monomers. According to (28) and (13), it can be written

\[
\Delta E(\ell) = \gamma \sqrt{\ell(1-c_0^2\ell)} - \epsilon \ell + \bar{\sigma} c_0^2 \ell^2.
\]  

(31)

All the contributions linear in \( \ell \) have been regrouped in the term \( \mu \ell \) where,

\[
\mu = \left( E(1) + \ln n_1 \right) - \left( - \epsilon + 8\pi k c_0^2 \right),
\]  

(32)

is chemical potential difference between free and assembled monomers. Assuming that the energy expression (28) keeps its validity for \( \ell = 1 \), the chemical potential reduces to,

\[
\mu = \ln n_1 + \gamma \sqrt{1-c_0^2} + \bar{\sigma} c_0^2.
\]  

(33)

One recovers the equation (3,4) of the article where we have simply denoted \( c \equiv c_0 \). It should be noted that the terms in \( \epsilon \) compensate in (32) and have no influence on the polymerization kinetic. Thus, within this formalism, the monomer-monomer interaction energy is solely parametrized by \( \gamma \) in the kinetic equations.

The energy difference \( \Delta E \) of Eq.(31) shows an inflexion point allowing for the occurrence of a local minimum at intermediate coat size provided \( \bar{\sigma} > \gamma c_0 \).

2. Membrane flattening under high tension

Under high membrane tension (\( \bar{\sigma} \sim 8\pi k / \ell \)), Eq.(29) shows that the optimal domain curvature deviates from the preferred coat proteins curvature:

\[
c^*(\ell) = \frac{c_0}{1 + \frac{\bar{\sigma} \ell}{8\pi k}}.
\]  

(34)
We can see that the coat curvature decreases as the coat grows. The coat shape parameter \( \beta(\ell) = \ell \sigma^2 \) reaches a maximum \( \beta^* = 2\pi \kappa c^0 / \sigma \) for a given size \( \ell^* = 8\pi \kappa / \sigma \). A closed sphere corresponds to \( \beta = 1 \). The formation of a coated vesicle requires a tension low enough so that \( \beta^* \geq 1 \). In the opposite case, the coat initially grows as a curved protein domains, but increasingly flattens for \( \ell > \ell^* \), and eventually forms a large, flat domain. Coated vesicle formation thus imposes an upper bound to the membrane tension, given by

\[
\sigma < \frac{\kappa}{2R^2} \tag{35}
\]

C. Remark: Influence of the coat size on the binding and inactivation rates.

The rate of monomer binding onto coats and the rate of coatomer inactivation may both in principle depend on the size of the coat. The monomer binding rate \( k_p \) might be sensitive to the length of the coat edge, and could become slower as the pit matures into a spherical coat. The coatomer inactivation rate \( k_{\text{off}} \) of COPI coat is known to be exponentially sensitive to membrane curvature (Ref.[14] of the text). If the coat curvature increases with the domain size (i.e. because of collective effects between the coat proteins), \( k_{\text{off}} \) would effectively increase with the size of the coat. Both these effects would lead to an increase of the ratio \( (k_{\text{off}}/k_p) \) with the size \( \ell \). According to Eq.(23), higher order terms given by \( \int d\ell (k_{\text{off}}(\ell)/n_1 k_p(\ell)) \) would appear in the effective energy. We show in the main text that if the ratio \( k_{\text{off}}/k_p \) is constant, the switch exists when this ratio is larger than a critical value. If it is size dependent, additional parameters (e.g. a lengthscale) will come into play, and the condition for the existence of the switch will involve a model-dependent combination of all parameters. The existence of the switch stems from the fact that coats can nucleate, but not vesiculate. Higher order terms in the effective energy disfavor vesiculation more than nucleation, and will thus not abolish the switch.

III. SCALING RESULTS FOR PROTEIN SELF-AGGREGATION

In this section, we report some scaling result pertaining to protein self-aggregation in the complex energy landscape defined by Eqs.(23,31):

\[
\tilde{E}(\ell) = \gamma \sqrt{\ell(1-c_0^2\ell)} - \mu \ell + \Sigma \ell^2 + \text{const.} \quad \Sigma = \sigma c_0^2 + \frac{k_{\text{off}}}{2k_p n_1} \tag{36}
\]

A. Low concentration regime - No vesicle secretion

If no vesicle is secreted, the protein coat size distribution resembles the distribution at thermal equilibrium \( n(\ell) \sim e^{-\tilde{E}(\ell)} \). Domains can form if there exist a local minimum to the effective energy. An inflexion point \( (\partial_\ell \tilde{E} = 0 \text{ and } \partial^2_\ell \tilde{E} = 0) \) appears in the energy \( \tilde{E} \) Eq.(36) at a size \( \ell_n \) and for a chemical potential \( \mu_n \) (see Fig.2a,b of the text), defined by:

\[
\mu_n = \frac{3}{2} \gamma^{2/3} \Sigma^{1/3} \quad \ell_n = \frac{\gamma^{2/3}}{4\Sigma^{2/3}} \tag{37}
\]

For \( \mu > \mu_n \), metastable coats can nucleate (with a nucleation size \( \sim \ell_n \)), and grow up to an optimal size, defined by \( \partial_\ell \tilde{E} = 0 \) (see the peak in the size distribution, Fig.2b), which depends upon the chemical potential. The critical concentration \( n_1^{\text{mc}} \) defines the monomer concentration at which most monomers are in coats, analogue to the critical micellar concentration (cmc) in surfactant systems. At the cmc, the scaling of Eq.(37) still holds, so that \( \ell^* \sim (\gamma/\Sigma)^{2/3} \).

The total amount of coat component on the membrane is fixed by adsorption and desorption Eq.(27). In the absence of vesicle secretion, and assuming a two-state model where coat proteins are either isolated, or part of a coat with optimal size \( \ell^* \), the conservation relation reads

\[
\frac{J_{\text{on}}}{k_{\text{off}}} = n_1 + \ell^* n_\ell. \tag{38}
\]
For \( \mu < \mu_{\text{cmc}} \), coats are scarce (\( n_1 > \ell^* n_T \)) and the monomer concentration increases linearly with the in-flux: \( J_{\text{on}} / k_{\text{off}} \sim n_1 \). Beyond the cmc, coats dominate and \( J_{\text{on}} / k_{\text{off}} \sim \ell^* n_T \). In this regime, the equilibrium size distribution \( n(\ell) \sim e^{-\tilde{E}(\ell)} \) tells us that \( n_1 \sim e^\mu \sim n(\ell^*)^{1/\ell^*} \sim (J_{\text{on}} / k_{\text{off}})^{1/\ell^*} \). Since we expect the mean number of protein per coat to be large (\( \ell^* \gg 1 \)), both the density of monomer and the chemical potential are constant: \( \mu_{\text{cmc}} \sim \mu_n \) and \( \ell^* \sim \ell_n \). The coat size and the concentration of monomer are only weakly dependent of the in-flux, and any additional monomer reaching the membrane tends to join a coat.

### B. High concentration regime - Steady vesicle secretion

If the concentration of monomer on the membrane is large, the effective coat energy exhibits a nucleation barrier but no intermediate minimum (see Fig.2d of the text). After nucleation, a coat grows at nearly constant velocity, \( v = -k_p n_1 \partial_\ell \tilde{E} \simeq cste \), until reaching the critical size \( \ell_v \) where it remains trapped for a time \( 1/k_v \) before being released as a vesicle. The distribution of coat size is thus nearly flat at intermediate size \( n(\ell < \ell_v) = j_v / v \), Fig.2d) and shows a peak at the critical size, \( n(\ell_v) = j_v / k_v \). Conservation imposes that the current of released vesicles matches the current of nucleated coats, which is fixed by the nucleation energy barrier and increases with the monomer concentration: \( j_v \simeq k_p n_1 \exp(-\gamma^2/4\mu) \).