Processivity and collectivity of biomolecular motors extracting membrane nanotubes

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Membranes are fluid-lipid interfaces of remarkable biological functionality. They mediate transport processes at the cellular level, exhibiting soft mechanical properties and highly adaptive geometries. Vesicles, for instance, hold a spherical shape under most equilibrium conditions. But in the presence of spatiotemporal stimuli, membrane tubes can emerge over lengths of several micrometers with diameters in the nanometer scale [1,2]. This is the case of sensorial tentacles developed during phagocytosis [3] and intercellular protrusions, tether networks, and sensorial tentacles. Here we focus on the extraction of a single tube from a vesicle. Via a force balance coupled to binding kinetics, we analytically determine the phase diagram of tube formation as function of the motor processivity, the surface viscosity of the membrane \( \eta_m \), and the density of motors on the vesicle \( \rho \). Three tubulation mechanisms are identified: (i) tip pulling, due to the accumulation of motors at the leading edge of the membrane, (ii) viscous drag, emergent from the translation of motors along the tube, and (iii) hybrid extraction, such that tip pulling and viscous drag are equally important. For experimental values of \( \eta_m \) and \( \rho \), we find that the growth of bionanotubes tends to be driven by viscous forces, whereas artificial membranes are dominated by tip pulling.

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The force necessary to extract a membrane tube is typically five times larger than the pulling scale \( f_0 \approx 5 \text{ pN} \) associated with a single motor protein [9–15]. Overcoming this barrier requires at least two physical conditions: (i) a sufficiently high density of motors on the vesicle and (ii) a sustainable kinetics of motor binding to the substrate. Furthermore, the mechanical work done by each bound motor depends on its processivity. Kinesin, for example, can walk hundreds of steps before unbinding from the substrate. Nonclaret disjunctional (Ncd) proteins, on the other hand, detach just after a couple of steps. According to fluorescence imaging experiments [9], Ncd motors spread out along the tube, while kinesins cluster at the tip.

From the theoretical standpoint, the picture of tip clustering has been extensively studied in terms of stochastically interacting particles [10,11,13,16,17], deterministic dynamical systems [12], and mean field equations [14]. Nevertheless, the models proposed so far have not considered how the tubulation phenomenon depends on the processivity of the motors and on the surface viscosity \( \eta_m \) of the membrane. Both aspects are relevant, since nonprocessive motors execute key tasks at the cellular level and biological membranes may be significantly more viscous than artificial ones. Phosphatidylcholine (PC) membranes, for instance, have \( \eta_m \approx 10^{-10} - 10^{-9} \text{ Pa m s} \) [18,19], whereas values as high as \( \eta_m' \approx 10^{-4} \text{ Pa m s} \) are reported for red blood cells [20,21]. This fact, together with the lack of comparable differences in the bending rigidity \( k_c \approx 10^{-20} - 10^{-19} \) J [21–23] and in the stretching modulus \( k_A \approx 0.1–1 \text{ Nm} \) [22,23], raises questions about the role of \( \eta_m \) in motor-membrane interactions. In particular, what is the impact of \( \eta_m \) on the driving force behind tube formation? Can \( \eta_m \) affect the spatial distribution of motors along the tube? And what is the interplay between viscous and (non)processivity effects? The present Rapid Communication addresses these issues via a simple force balance coupled to the binding kinetics of motors.

**Model setup.** As shown in Fig. 1, consider a spherical vesicle of radius \( R \) immersed in an fluid of viscosity \( \eta_w \). The vesicle is coated with a density \( \rho \) of molecular motors, which bind and unbend to the substrate at constant rates \( k_b \) and \( k_u \), respectively. While bound, each motor takes unidirectional steps of length \( \ell \), exerting a force on the membrane. This induces the formation of a tube, which we define as a cylinder of length \( L \) capped by a hemisphere of radius \( r \approx \sqrt{k_c/(2\sigma)} \) [1,2].

Tube growth evolves slower than the speed \( v_0 \) of a free molecular motor. Typically, \( L \approx 0.15v_0 \) [9,14], suggesting that the population of bound motors experiences a velocity drop along the axial length \( L \). A convenient way to approach this effect consists in decomposing the tube into two parts: stem and tip [12,13], as illustrated in Fig. 2. The tip is formed by \( n_b \) bound motors that walk at the same speed \( \ell \) of the tube, pulling the membrane with a force \( f_b \). The remaining \( N_b \) bound motors have a velocity surplus \( v_0 - L \) that defines the stem. Their viscous drag force on the membrane is \( F_b \).

**Stem force.** In the course of their walk on the substrate, stem motors impart momentum to the membrane tube. The corresponding force can be expressed as \( F_b \equiv N_b F_c \), where \( F_c \equiv \xi (v_0 - L) \) denotes the viscous drag of a single motor on the membrane. The coefficient \( \xi \) is nontrivial, because it involves the following: (i) the size \( \lambda \) of the protein domain moving in the membrane and (ii) the local radius of curvature \( r \) of the membrane relative to the Saffman-Delbrück length \( \ell_b \equiv \eta_m' / \eta_w \) (the scale below which \( \eta_m' \) dominates the viscous dissipation [24]). According to recent experiments [25] and theoretical analyses [24,26], the axial drag coefficient in a
cylindrical membrane of radius \( r \ll \ell_0 \) is given by [24]
\[
\zeta = \frac{4\pi \eta_m'}{\ln (r/\lambda) + \frac{1}{2}}.
\]
(1)

In the context of this Rapid Communication, we take \( r = \sqrt{\kappa_c/(2\sigma)} \) and identify the length \( \lambda \) with the tail of the molecular motor. The resulting stem force,
\[
F_b = N_b \frac{4\pi \eta_m'(v_0 - \bar{L})}{\ln (r/\lambda) + \frac{1}{2}},
\]
(2)
is to be compared with its counterpart \( f_b \) at the tip.

**Tip force.** Towards the hemispherical cap of the tube, the membrane exhibits a curvature change around which \( n_b \) bound motors have their velocities reduced to \( L \). Such a drop depends on the force-velocity properties of the molecular motors. In its simplest form, the force \( f_1 \) due to a single motor is given by \( f_1 = f_0(1 - \mu/v_0) \), where \( f_0 \) denotes the stall force and \( v_0 \) the motor velocity under load [27]. On the basis of this individual contribution, we write the force \( f_b \equiv n_b f_1(\bar{L}) \) on the tip of the tube as
\[
f_b = n_b f_0 \left( 1 - \frac{\bar{L}}{v_0} \right).
\]
(3)

**Multimotor force versus membrane force.** The multimotor drive \( F_b = F_b + f_b \) is opposed by the membrane force \( F_m \). But how does \( F_m \) depend on the mechanical and geometrical properties of the vesiculotubular structure? We assume \( F_m = F_c + F_A \), such that \( F_c = 2\pi \sqrt{2\sigma \kappa_c} \) is the barrier for tube formation [1,2] and \( F_A \) the stretching force. The latter arises from the energy \( \mathcal{E}_A = \kappa_A (A - A_0)^2/(2A_0) \), where \( A_0 \) and \( A \) are the areas of the unstretched and stretched membranes, respectively [1]. For tube extraction from a spherical vesicle, \( A_0 = 4\pi r^2 \), \( A - A_0 \approx 2\pi r L \), and \( \mathcal{E}_A = \frac{\kappa_A}{2} \pi^2 \rho L^2 \).

Thus, \( F_A \equiv \partial \mathcal{E}_A/\partial L = \kappa_A \pi (r/R)^2 L \), leading to
\[
F_m = 2\pi \sqrt{2\sigma \kappa_c} + \kappa_A \pi \left( \frac{r}{R} \right)^2 L.
\]
(4)

Balancing \( F_b \) and \( F_m \), one readily finds
\[
\frac{v_0 - \bar{L}}{v_0} = \frac{2\pi \sqrt{2\sigma \kappa_c} + \kappa_A \pi \left( \frac{r}{R} \right)^2 L}{N_b \xi v_0 + n_b f_0}.
\]
(5)

Equation (5) governs the evolution of the tube length \( L \) and the current \( J_b \equiv (v_0 - \bar{L}) N_b/L \) of bound motors towards the tip [12]. Both are coupled to the binding kinetics.

**Binding kinetics.** The membrane tube hosts a total of \( N = 2\pi r L_0 \) motors, of which \( N_b = N_0 + n_b \) bound and \( N_a = N - N_b \) unbound to the substrate. We model the binding kinetics over the stem and tip regions by
\[
\frac{dN_b}{dt} = I - k_a N_b - J_b,
\]
(6)
\[
\frac{dn_b}{dt} = J_b - k_a n_b,
\]
(7)
where \( I \) denotes the influx of motors to the filament. This flux involves geometric constraints and excluded volume effects on binding. We assume \( I = k_0 N_a(1 - \phi_b) \), where \( N_a \) is the number of attachable motors and \( \phi_b \) the occupation of the filament. Letting \( N_0 \) denote the number of tracks accessible to a motor (see Fig. 3 and Ref. [13]), we estimate the number of stepping sites as \( N_0 L/\ell \) and \( \phi_b \approx (N_b + n_b)/(N_0 L/\ell) \).

Analogous arguments hold for \( N_a \). Considering that unbound motors tend to be uniformly distributed on the membrane, only those on the lower surface of the tube can bind to a track. The corresponding fraction of attachable motors is \( \phi_a \).
so that \( N_b = \phi_b N_a \). As shown in Fig. 3, \( \phi_b \approx (2\lambda)/(2\pi r) \) since a cross section of the tube hosts two unbound motors that neighbor the filament. In terms of such estimates, \( I = k_b \lambda (2\pi r \rho - N_b - n_b) (1 - N_b / N_a \ell) \).

Dimensionless equations. Introducing \( \tau \equiv t k_a \) and \( X \equiv L / \ell \), Eqs. (5)–(7) can be written in dimensionless form as

\[
\frac{dX}{d\tau} = P \left( 1 - \frac{E + SX}{N_b D + n_b} \right),
\]

\[
\frac{dN_b}{d\tau} = B \phi_a (MX - N_b - n_b) \left( 1 - \frac{N_b + n_b}{N_a X} \right)
- \frac{P N_b}{X} \left( \frac{E + SX}{N_b D + n_b} \right),
\]

\[
\frac{d n_b}{d \tau} = \frac{P N_b}{X} \left( \frac{E + SX}{N_b D + n_b} \right) - n_b,
\]

where

\[
P \equiv v_0 / (k_a \ell), \quad E \equiv 2 \pi \sqrt{2 \sigma \kappa_\ell / f_0}, \quad S \equiv \kappa_A \pi (r/R)^2 \ell / f_0, \quad D \equiv \zeta_0 v_0 / f_0, \quad B \equiv k_b / k_a, \quad M \equiv 2 \pi r \ell P. \quad \text{These dimensionless parameters comprise one indicator of collectivity (number } M \text{ of motors in a tube element of radius } r \text{ and length } \ell), \text{ two kinetic ratios (representing binding } B \text{ and processivity } P), \text{ and three force ratios (extraction barrier } E, \text{ viscous drag } D, \text{ and membrane stretching } S). \text{ In them, the dimensionless current } j_b \equiv j_b / k_a \eta \text{ is given by } j_b = P (N_b / X) (E + SX) / (N_b D + n_b).

Collectivity and processivity. Focus on motor collectivity and processivity suggests the study of Eqs. (8)–(10) as a function of \( M \) and \( P \). The former is experimentally controllable via the density \( \rho \) of motors on the vesicle. The latter involves stepping and unbinding properties, in such a way that \( P = 1 \) for an ideal nonprocessive motor and \( P \gg 1 \) for processive motors. Of particular interest is the question of how \( M \) and \( P \) are reflected in the driving force of tube extraction. To address this issue, we consider the ratio \( F_b / f_0 = D N_b / n_b \), where \( D = 4 \pi \eta_m v_0 f_0^{-1} / [\ln (r / \lambda) + 1 / 2] \). Depending on the surface viscosity \( \eta_m \) of the membrane, three tubulation mechanisms can emerge: (i) tip pulling, where \( f_b > F_b \), (ii) viscous drag, for which \( F_b > f_b \), and (iii) hybrid extraction, such that \( F_b = f_b \). Since hybrid extraction is a key limiting case, we shall determine its steady state solution \((\overline{X}, \overline{N_b}, \overline{n_b})\) and its locus on the \( \eta_m \times \rho \) plane.

Steady state and phase diagram. Setting \( dX / d\tau = dN_b / d\tau = dn_b / d\tau = 0 \) in Eqs. (8)–(10) and invoking the condition \( F_b = f_b \), one analytically finds

\[
\overline{X} = \frac{P}{D}, \quad \overline{N_b} = \frac{E}{2D} + \frac{PS}{2D^2}, \quad \overline{n_b} = \frac{E}{2} + \frac{PS}{2D}.
\]

This leads to a current \( \overline{F_b} = \overline{n_b} \sim P / D \) that linearly increases with processivity and monotonically decreases with the membrane viscosity. The corresponding density of motors, though, has a more involved dependence on \( P \) and \( D \). It follows from Eq. (9) evaluated at (11),

\[
\overline{M} = \frac{(1 - \overline{\phi_b}) / N_a \overline{\phi_b}}{1 - \overline{\phi_b}},
\]

with \( \overline{\phi_b} \equiv (\overline{N_b} + \overline{n_b}) / (N_a \overline{X}) \). Here, the tube length \( \overline{X} \) and the number of stepping tracks \( \overline{N_b} \) limit the population of bound motors \( N_b + n_b \), so that Eq. (12) is physically relevant only if \( \overline{\phi_b} < 1 \). This constraint is violated at membrane viscosities (e.g., \( \eta_m \approx 10^{-9} \text{ Pa m s} \) for which \( \overline{F_b} = \overline{f_b} \) requires an excessive number of bound motors. Discarding \( \overline{\phi_b} \geq 1 \) from the graphical representation of Eq. (12), we plot in Fig. 4 the hybrid extraction density as function of the membrane viscosity, for motors at low (\( P = 2 \)) and at high (\( P = 100 \)) processivities.

Discussion. What are the biophysical implications of Fig. 4? On the one hand, we note that the experiments of Refs. [9–15] remarkably fall into the viscosity range \( \eta_m \approx 10^{-10–10} \text{ Pa m s} \) that is within the domain of the tip force, because of the artificiality of PC membranes rather than the processivity of the motors. In contrast, biological membranes are more viscous (\( \eta_m \approx 10^{-8} \text{ Pa m s} \) [20,21]) and hence susceptible to the drag exerted by stem motors. For instance, tubulation in a neuron (\( \eta_m \approx 10^{-8} \text{ Pa m s} \) [20,21]) by kinesins (\( P \approx 100 \text{ at, say, } \rho = 500 \text{ nm}^{-2} \)) falls into the stem regime. But as the processivity is decreased from \( P = 100 \) to \( P = 2 \), the hybrid extraction line is shifted to higher densities so that \( \rho \gtrsim 10^3 \text{ nm}^{-2} \). Physically, this degree of motor collectivity seems indeed required to compensate large membrane viscosities.

Summary and outlook. On the basis of forces (2)–(4) and binding kinetics (6) and (7), we sketched the phase diagram of membrane tubulation by molecular motors. Our results of Fig. 4 indicate that artificial (PC) membranes inexorably fall into the tip regime, whereas biological membranes can be dragged by stem motors. Such a contrast suggests that the surface viscosity \( \eta_m \) likely affects the spatial distribution of...
bound motors. In particular, since the current \( J_b \sim 1/\eta'_m \), we expect that the accumulation of processive motors at the tip of nanotubes becomes less pronounced for increasing \( \eta'_m \). Fluorescence imaging experiments could tackle this issue, along the lines of Ref. [9].

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