Title

Asymmetric crowders and membrane morphology at the nexus of intracellular trafficking and oncology.

Permalink

Authors

Parihar, Kshitiz

Ko, Seung-Hyun

Bradley, Ryan

et al.

Publication Date

2024-09-01

DOI

10.1016/j.mbm.2024.100071

Peer reviewed

HHS Public Access

Asymmetric crowders and membrane morphology at the nexus of intracellular trafficking and oncology

Kshitiz Parihar[a], **Seung-Hyun B. Ko**[b], **Ryan P. Bradley**[a], **Phillip Taylor**[a], **N. Ramakrishnan**[b], **Tobias Baumgart**[c], **Wei Guo**[d], **Valerie M. Weaver**[e], **Paul A. Janmey**[b,f], **Ravi Radhakrishnan**[a,b,*]

[a]Department of Chemical and Biomolecular Engineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA, USA

[b]Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA, USA

[c]Department of Chemistry, School of Arts & Sciences, University of Pennsylvania, Philadelphia, PA, USA

[d]Department of Biology, School of Arts & Sciences, University of Pennsylvania, Philadelphia, PA, USA

[e]Department of Surgery, Center for Bioengineering and Tissue Regeneration, University of California, San Francisco, San Francisco, CA, USA

[f]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Abstract

A definitive understanding of the interplay between protein binding/migration and membrane curvature evolution is emerging but needs further study. The mechanisms defining such phenomena are critical to intracellular transport and trafficking of proteins. Among trafficking modalities, exosomes have drawn attention in cancer research as these nano-sized naturally occurring vehicles are implicated in intercellular communication in the tumor microenvironment, suppressing anti-tumor immunity and preparing the metastatic niche for progression. A significant question in the field is how the release and composition of tumor exosomes are regulated. In

this perspective article, we explore how physical factors such as geometry and tissue mechanics regulate cell cortical tension to influence exosome production by co-opting the biophysics as well as the signaling dynamics of intracellular trafficking pathways and how these exosomes contribute to the suppression of anti-tumor immunity and promote metastasis. We describe a multiscale modeling approach whose impact goes beyond the fundamental investigation of specific cellular processes toward actual clinical translation. Exosomal mechanisms are critical to developing and approving liquid biopsy technologies, poised to transform future non-invasive, longitudinal profiling of evolving tumors and resistance to cancer therapies to bring us one step closer to the promise of personalized medicine.

Keywords

Multiscale modeling; Curvature sensing; Morphological transitions; Trafficking; Oncology

---

1. Introduction

Cells respond to mechanical stimuli (e.g., changes in substrate stiffness) by altering their mechanical state (e.g., cell stiffness, motility, adhesion, and contractility).[1] These phenotypic properties of cells are modulated by the organization of the cytoskeleton,[2] which plays a significant role in mechanosensing.[3–5] Dynamic reorganization of the cytoskeleton is a part of cellular homeostasis and often goes awry in cancer. Deregulated cytoskeletal assembly is implicated in tumor cell migration, invasion, and metastasis.[6–8]

Communication between a cell and its surroundings is potentiated by trafficking that involves membrane environments. Endocytosis causes the internalization of cargo at the plasma membrane and contributes to several cellular processes.[9,10] Endosomal cargo is recycled to the plasma membrane or slated for lysosomal or secreted extracellularly in the form of exosomes. Endocytic pathways are deregulated in cancer cells:[11] enhanced integrin recycling promotes invasive migration,[12,13] mechanisms that undermine lysosomal degradation of growth factor receptors (such as EGFR and MET) enhance proliferation,[14] altered E-cadherin trafficking induces epithelial-to-mesenchymal transition,[15,16] and differential expression of chemokine receptors are observed during metastasis.[17–19]

Among various trafficking vesicles in the extracellular microenvironment, exosomes are 100 nm vesicles generated through the endocytic pathway and secreted via exocytosis.[20,21] Exosomes are efficient potentiators of cell–cell communication, with the exosomal cargo actively mediating signaling between parent cells and recipient cells.[20] Tumor exosomes are implicated in cancer progression and metastasis, their roles in tumor cell invasion,[22] migration,[23,24] and immune interactions[25–27] are being appreciated with recent and ongoing studies.

From endocytosis at the plasma membrane to exosome generation at the endosomes, membrane curvature remodeling is an essential phenomenon in trafficking pathways. Among several mechanisms for membrane remodeling,[28] peripheral membrane proteins capable of generating curvature play a central role: these include epsin N-terminal homology (ENTH) domain 29, Bin-Amphiphysin-Rvs (BAR) domain 30, Endosomal

Sorting Complex Required for Transport (ESCRT) III protein complexes,[31] to name a few. Curvature generation by these proteins will depend on the membrane's mechanical properties (such as tension and bending rigidity). Moreover, forces from the cytoskeleton will influence membrane deformability.[32,33] Cytoskeleton-related tension will, therefore, affect the function of curvature-inducing proteins.

Altered ECM stiffness in tumorigenesis will influence cytoskeletal dynamics to define a new mechanosensitive mechanism in tumor cells for regulating trafficking pathways. Specifically, this mechanism can connect cortical tension and vesicle biogenesis, conferring an intriguing mechanosensitivity paradigm in tumor initiation and progression. Intracellular transport mechanisms (endocytosis, exocytosis, endosomal sorting, and exosome release) dynamically regulate and impact signaling by modulating receptor activity and protein localization.[34,35] Our outlook is to bridge molecular and cellular principles surrounding membrane curvature generation with rewiring of cellular signals. Here, we describe a general framework that integrates signaling with other cellular functions like trafficking, cell–cell and cell–matrix adhesion, and motility. We are guided by how a physical change in cell membrane configuration caused by external stimuli alters trafficking and signaling. We apply this framework to explore the link between trafficking, signaling in the tumor microenvironment, and cell fate.

2. Multiscale computational approach

We adopt a multiscale computational approach to enable the bridging between molecular mechanisms of curvature sensing, cellular localization, and curvature generation and the subsequent link to trafficking and signaling. Coarse-grained molecular dynamics simulations were performed in order to determine the molecular mechanisms of curvature generation and sensing. Established simulation protocols (shared at github.com/bradleyrp/automacs) make it possible to simulate protein/lipid systems at many concentrations systematically. The resulting MARTINI/GROMACS simulations scale linearly on high-performance computing platforms, making generating these simulation data efficiently possible. To predict the deformation fields for a particular protein-bilayer system, we simulated it using coarse-grained simulation methods. Specifically, we conducted the CGMD simulations of the protein domains embedded in mixed DOPC: DOPS bilayers with physiological concentrations of phosphoinositide lipids (1–4%)[36–38] using the MARTINI coarse-graining schemes and force fields.[39–41] Each protein is bound to a single PI(4,5)P2 molecule parameterized from atomistic simulations which remains bound to the amphipathic helix H0 near the known binding pocket.[29] Simulations lacking PI(4,5)P2 failed to show measurable curvature and in some cases the ENTH domains even detached from the bilayer. This result is consistent with experiments that show that ENTH domains require binding[42] and motivates our use of a single PI(4,5)P2 which is sufficient to retain ENTH at the bilayer in our model. The sensitivity of our results to the optimization parameters were tested extensively and the robustness of the results was confirmed in our prior work.[43]

We require a similarly systematic method for measuring the deformation field to complete the molecular portion of the simulation-curvature-morphology calculation. We adopt an atom-to-field mapping, which translates the structural details of the membrane at

the molecular level to phenomenological parameters at the mesoscale and describes a methodology that can investigate the membrane protein system at both these scales and transfer information between them. We have previously estimated curvature fields induced in protein-bilayer simulations for ENTH domains[44] and the protein Exo 70[45] by directly analyzing the statistics of deformations in the simulation trajectories. For our protein-bilayer system consisting of 1 million particles, a typical dynamics run for a length of 500 ns required 48 h of computing time on a 48-core (3 nodes, 16 processors each), therefore requiring 1920 CPU hours. In principle, the methods have linear scaling for 1000s of cores enabling exploration of large systems.[46]

Our studies also revealed that the induced curvature is of the same scale as natural undulations, making it challenging to convolve the two effects.[47] To overcome this challenge, we utilize an analysis method for a molecular description of protein-induced bilayer curvature where we quantify the protein-induced deformation at low surface coverages in a CGMD simulation using a continuum model based on the Canham-Helfrich Hamiltonian, which relates these deformation fields to height fluctuations in the bilayer in the presence of an intrinsic curvature field.[48,49] The atom to field mapping and the different ways to compare to experiments are discussed in two earlier works.[43,44] The main limitation is that single molecule studies, while most quantitative, may fail to capture the mapping because of cooperativity which is hard to decipher especially a priori. On the other hand, cellular experiments which include the cooperativity may not yield a quantitative estimate of curvature at the nanoscale.

At the micron scale, where a cell and its internal organelles display extraordinary complexity in their shape and organization, the physical properties of a biological membrane were explored using phenomenological continuum scale models. We have a generalized approach to studying membrane morphologies under high curvature such that they can be successfully applied to morphological transitions in cell membranes (such as vesiculation and tubulation).[44,49,50] Using the machinery of dynamically triangulated Monte Carlo or DTMC simulations,[50,51] we explored the roles of cooperative protein–protein and protein-membrane interactions clathrin-mediated endocytosis.[52] In our model, multiple epsins localized spatially and orientationally templated on the clathrin coat collectively play the role of a curvature-inducing coat.

We have also successfully extended these results to consider how thermal fluctuations influence the vesicle morphologies,[44,52,53] see Fig. 1 (left). We have similarly applied the anisotropic curvature model in the DTMC simulations to capture the collective role of Exo 70 domains found in the Exocyst complex proteins in reshaping membranous vesicles. When attached to the outer surface of giant unilamellar vesicles (GUVs), wildtype proteins induce inward-growing tubes. This phenomenon is predicted in our simulations by choosing a negative value for the anisotropic curvature field,[45] see Fig. 1 (middle); in particular, the equilibrium membrane conformation stabilized in the model shows striking similarity to that obtained from experiments. In experiments, it has been observed that the ESCRT proteins are localized to the neck region of the budded vesicle.[54] Using the anisotropic curvature model and localizing the protein curvature field to an annular region, we demonstrated a

novel phenomenon where a negatively curved vesicular bud is formed due to the constrictive action of either positively or negatively curving proteins, see Fig. 1 (right).

3. Results

3.1. Geometry through extracellular matrix (ECM) dimensionality tunes cytoskeletal tension to modulate cellular stress

Kai et al. investigated how engagement with a three-dimensional extracellular matrix influences cellular stress in cancer cells.[55] They observed that cells within a three-dimensional extracellular matrix decreased and redistributed the actin crosslinker filamin to reduce their cortical tension. They studied the effect of cortical actin tension on membrane morphology using mesoscale models described in section 2. Cortical actin regulates membrane protrusions through membrane pinning, influencing membrane topology.[56] Membrane topology controls the binding of curvature-generating proteins and cell signaling and the trafficking of cellular proteins, including their recycling and secretion.[57] Consistent with these observations, cells engaging the ECM in 3D increased recycling and secretion (SEC61 expression), suggesting cortical actin tension regulates secretory protein translocation into the ER lumen. DTMC simulations were conducted to explore the morphology of highly curved membranes.[44,49,50] Using this framework, Kai et al. quantified the surface deformations induced by membrane proteins as curvature fields and studied the emergent morphologies of the membrane using isotropic and anisotropic curvature models. They simulated membrane patches with different excess areas, which is the variable conjugate to the membrane tension and depends on cortical actin tension.[58] The model predicted that the protrusions in cells engaging the ECM in 2D (reflecting high cortical actin tension) would be shorter and more transient. By contrast, the membrane protrusions in cells engaging the ECM in 3D (reflecting lower cortical actin tension) would be longer and more stable. Consistent with these predictions, live cell microscopy showed that the cells in 2D formed dynamic, short membrane protrusions, whereas cells in 3D formed longer, more stable protrusions.

Plasma membrane protrusions are cylindrically shaped. While the neck or annulus of the protrusion generates positive membrane curvature, the cytoplasmic length of the protrusion generates a negative curvature. Membrane curvature regulates the binding affinity of membrane curvature-associated proteins.[28] To explore this relationship, the DTMC model was used to predict protein recruitment. Kai et al. computed the free energy to recruit a protein to these three spatial regions (protrusion, annulus, and basal) as a function of cortical actin tension. The calculations show a more favorable recruitment of such proteins in 3D than in 2D. Given that Exo 70 is a negative curvature-inducing protein that is also a component of the exocyst complex targeting secretory vesicles to the plasma membrane, Kai et al. predicted that Exo70 could be regulated by cortical actin tension to facilitate secretory protein trafficking.[45] Consistent with model prediction, in the experiments of Kai et al., Exo70 showed better plasma membrane localization in 3D (low cortical tension) as revealed by a strong co-localization of the EGFP-Exo70 with membrane-tagged mCherry as compared to minimal membrane associations observed in 2D. The data suggest cortical

tension regulates secretory protein trafficking to the plasma membrane by controlling plasma membrane protrusion activity and topology.

The results implied that how a cell ligates its ECM (2D or 3D) profoundly affects ER function and protein secretion. The results implicated filamin-dependent cortical actin tension as a critical regulator of this ECM ligation-mediated ER homeostasis. Prolonged ER stress responses compromise cell viability, and impeding secretory protein trafficking can severely compromises ER function.[59] Cells with high cortical actin tension had significantly reduced cell viability compared to cells with low cortical actin tension. Accordingly, the viability of cells in 2D could be significantly extended if their myosin activity was inhibited. Also, knocking down the negative curvature-generating binding protein Exo 70, which has been implicated in protein secretion and membrane topology regulation, severely compromised the viability of the cells. The studies underscore the importance of geometrical context in organelle homeostasis and tissue behavior. Cells in 3D showed reduced endoplasmic reticulum stress response signaling, secreted and assembled a polarized endogenous basement membrane, and survived better under chemotherapeutic and radiation doses.

3.2. Regulation of trafficking vesicles by ECM stiffness

One of the main findings of the application described in section 3.1 is that trafficking vesicles potentiate the link between the dimensionality of ECM engagement and cell survival. Therefore, this section describes a causal mechanism that further illuminates this link.[60] Cells respond to mechanical stimuli by altering their mechanical properties, such as cell stiffness, motility, adhesion, and contractility.[1] The mechanical properties of cells are modulated by the organization of the cytoskeleton.[2] From endocytosis at the plasma membrane to biogenesis of exosomes as intraluminal vesicles in late endosomes, membrane curvature remodeling is an essential component of trafficking pathways. As discussed in section 3, curvature generation by these proteins will depend on the membrane's mechanical properties (such as tension and bending rigidity). Forces from the cytoskeleton also strongly influence membrane deformability by modulating the effective tension in the membrane through membrane-to-cortex attachments.[32,33] Cytoskeleton-related tension will, therefore, affect the curvature generating capabilities of the curvature-inducing proteins.

It has been postulated that cell–cell communication can be tuned through mechanisms of mechanosensitive biogenesis of trafficking vesicles.[20,59] Exosomes are nano-sized vesicles produced through the endocytic pathway and secreted via exocytosis, are efficient mediators of cell-to-cell communication,[20,21,61] and tumor-derived exosomes have been implicated in cancer progression,[61] tumor cell invasion,[22,23] metastasis,[24] and evasion of immune surveillance,[25,26,27] thereby linking to several cancer hallmarks. We thus wondered whether stiff ECM affects the membrane curvature generation needed in trafficking pathways through its effect on cortical tension.[62] Parihar et al.,[62] studied the dependence of cell mechanics of different cancer cells on ECM stiffness and composition to answer this question; this study reported extensive analysis on the physical behavior (namely, cell stiffness) spanning different cancer cell lines grown on different ECM stiffness and composition. The study utilized the biophysical modeling framework involving the DTMC-

based mesoscale membrane model to elucidate how ECM mediated changes in cortical tension of cells can influence production of vesicles.

While another study reported the experimental results for the ECM dimensionality corresponding to a particular cell type,[55] the mechanistic insights from the accompanying biophysical model (similar to the one mentioned above) about how the change in cytoskeleton tension due to ECM dimensionality effects membrane protrusion can be extended to other cell types.

In order to understand which proteins could be implicated in cell response to mechanosensing, the authors[62] analyzed a proteomics dataset which involved the study of 20 normal and cancer cell lines in seven different ECM substrates. They focused on the list of cytoskeletal-related proteins in modulating cell stiffness in response to ECM mechanics, including the cytoskeleton's major components. A neural network with these protein counts as input predicted with balanced accuracy (BACC) of 98% on a test dataset whether a cell paired with a given substrate was likely to belong to the low cortical stiffness or high cortical stiffness category.[62] This analysis reveals a highly diverse behavior among different cancer cell lines on varied ECM substrates, which can be captured through a low-dimensional fingerprint of 18 selected cytoskeleton-related proteins.

The study also conducted DTMC simulations to delineate how membrane deformations depend on cortical tension. Section 2 described the spatial localization of curvature-inducing proteins as a critical feature in the curvature generation mechanism. DTMC simulations have revealed a micellization-like mechanism for membrane tubulation, wherein tubes formed by self-assembling curvature-inducing proteins are analogous to micelle aggregates formed by self-assembling surfactant molecules.[48] To address how the curvature-generating capabilities of curvature-inducing proteins are affected by cortical tension, DTMC simulations were used to explore the morphological conformational space of planar to highly curved membranes.[44,48,50] The dependence of cortical tension is incorporated by simulating membrane patches subject to different excess areas, where increasing excess area corresponds to decreasing cortical tension.[58] Parihar et al. analyzed how the membrane morphology changes with growing excess area for a given total number of protein-induced curvature fields. The theory they developed predicted a Hill-type ultrasensitivity for the effect of cortical tension on membrane vesiculation,[62] and this behavior has since been verified experimentally.[63]

This tension-dependent recruitment of curvature-inducing proteins combined with our model's prediction of curvature generation suggests an interesting mechanosensitive axis that could be exploited to hijack intracellular trafficking mechanisms by cancer cells to gain fitness advantage. For instance, stiff ECM can increase the secretion of exosomes by cancer cells and also influence the composition of the bioactive cargo carried by exosomes.[64] Specifically, exosomes produced by cancer cells on stiff ECM have been shown to increase proliferative signaling in the target cells,[63] promote migration of recipient cells,[65] and found to carry increased composition of specific adhesion receptors that mediate their organotropism for establishing pre-metastatic sites.[66]

To illustrate how essential signature proteins (Rab, SNX, EXOC and ATG) involved in regulating vesicle trafficking might be altered in the patients of cancer types, one can use the cBioPortal (http://cbioportal.org) to query for and analyze the mutations and copy number variations of the signature proteins in the extensive patient-derived cancer genomics data from The Cancer Genome Atlas (TCGA). Table 1 summarizes the results from this genomic analysis. We can see that a significant proportion of cancer patients across different cancer types showcase genotypic variations in the protein families critical in trafficking pathways, further highlighting the importance of the signaling (regulatory) portion of intra- and inter-cellular cargo trafficking machinery in tumorigenesis.

4. Conclusion

A correlation exists between cancer cell mutations, which confer a fitness advantage to cells with a gain of essential cellular functions like cell adhesion and motility, signaling, transcriptional regulation, and intracellular trafficking. Defective signaling and vesicular trafficking of growth factor receptors and their subsequent alteration of cell fate by influencing the downstream signaling pathways are the hallmarks of cancer cells.[14] The central goal of cancer biology is to find a mechanistic link between the above two factors, which has important implications for biomarker development and therapeutic efficacy. The main goal of oncology is to translate these findings into treatment regimens where optimization of therapeutic regimens can be customized to the genetic and physiological make-up of individualized patients or patient cohorts. Mechanistic studies that support the correlations with a causal link, such as those described here, can serve as a conduit for rational therapies.

Metastatic cancer is a major clinical challenge that accounts for over 500,000 deaths per year.[67–70] Delineating mechanisms would identify actionable biomarkers and novel therapeutic targets. Tumors develop within a cellular microenvironment characterized by altered extracellular matrix stiffness and composition, blood vessel architecture, and immune cell interactions. These microenvironment components are causal in tumor amplification and metastasis.[71–74] The immune cells that are initially recruited to the tumor are anti-tumorigenic; however, as malignancy proceeds, it shifts towards a pro-tumor phenotype. Also, the frequency of the tumor-infiltrating immune cells is reduced. The reprogramming of immune cells to a pro-tumor phenotype and immune cell depletion and exhaustion are traits associated with an aggressive metastatic tumor.[75–85]

A stiff extracellular matrix and cytoskeletal tension promote tumor cell growth and survival and stimulate cell invasion in culture.[86–93] Studies in vivo using genetically engineered mouse models demonstrated that stiff ECM promotes malignant transformation.[90,94–99] A definitive understanding of the interplay between protein binding/migration and membrane curvature evolution is emerging but still needs to be completed. The mechanisms underpinning such behavior are crucial in intracellular assembly and stability of organelles (which often sustain extreme curvatures), and in intracellular transport and sorting of proteins and cargo. Exosomes have drawn tremendous interest in cancer research as tumor-derived exosomes are implicated in modulating the tumor microenvironment, suppressing anti-tumor immunity, and preparing the metastatic niche for progression.[26,27,100–107]

We explored how tissue mechanics regulates cell cortical tension to influence exosome production and cargo composition by co-opting the biophysics and the signaling dynamics of intracellular trafficking pathways discussed in Section 3. How these exosomes contribute to suppressing anti-tumor immunity and promoting metastasis could be a subject of future investigations.

The impact of the described multiscale modeling approach goes beyond the fundamental investigation of specific cellular processes toward actual clinical trials. Exosomal mechanisms are critical to developing and approving liquid biopsy technologies, which are slated to be the future of non-invasive, longitudinal profiling of evolving tumors and resistance to therapies in cancer patients to bring us one step closer to the promise of personalized medicine.[108] Moreover, delineating the mechanobiological regulation of exosomes is critical to our understanding of the evolution of solid tumors under physical stress from ECM and the interaction of tumors with the immune system, making it central for therapy, including immunotherapy. Already, clinical studies have revealed that exosomal profiling can classify responders and non-responders to immunotherapy in the treatment of some solid cancers.[27]

Acknowledgments

References

1. Janmey PA, Fletcher DA, Reinhart-King CA. Stiffness sensing by cells. Physiol Rev. 2020;100(2):695–724. [PubMed: 31751165]

2. Fletcher DA, Mullins RD. Cell mechanics and the cytoskeleton. Nature. 2010;463(7280):485–492. [PubMed: 20110992]

3. Gupta M, Sarangi BR, Deschamps J, et al. Adaptive rheology and ordering of cell cytoskeleton govern matrix rigidity sensing. Nat Commun. 2015;6:7525. [PubMed: 26109233]

4. Solon J, Levental I, Sengupta K, Georges PC, Janmey PA. Fibroblast adaptation and stiffness matching to soft elastic substrates. Biophys J. 2007;93(12):4453–4461. [PubMed: 18045965]

5. Trichet L, Le Digabel J, Hawkins RJ, et al. Evidence of a large-scale mechanosensing mechanism for cellular adaptation to substrate stiffness. Proc Natl Acad Sci U S A. 2012;109(18):6933–6938. [PubMed: 22509005]

6. Yamaguchi H, Condeelis J. Regulation of the actin cytoskeleton in cancer cell migration and invasion. Biochim Biophys Acta. 2007;1773(5):642–652. [PubMed: 16926057]

7. Sun BO, Fang Y, Li Z, Chen Z, Xiang J. Role of cellular cytoskeleton in epithelial-mesenchymal transition process during cancer progression. Biomed Rep. 2015;3(5):603–610. [PubMed: 26405532]

8. Hall A The cytoskeleton and cancer. Cancer Metastasis Rev. 2009;28(1–2):5–14. [PubMed: 19153674]

9. Grant BD, Donaldson JG. Pathways and mechanisms of endocytic recycling. Nat Rev Mol Cell Biol. 2009;10(9):597–608. [PubMed: 19696797]

10. Doherty GJ, McMahon HT. Mediation, modulation, and consequences of membrane-cytoskeleton interactions. Annu Rev Biophys. 2008;37:65–95. [PubMed: 18573073]

11. Mellman I, Yarden Y. Endocytosis and cancer. Cold Spring Harb Perspect Biol. 2013;5(12):a016949. [PubMed: 24296170]

12. Caswell P, Norman J. Endocytic transport of integrins during cell migration and invasion. Trends Cell Biol. 2008;18(6):257–263. [PubMed: 18456497]

13. Muller PA, Caswell PT, Doyle B, et al. Mutant p53 drives invasion by promoting integrin recycling. Cell. 2009;139(7):1327–1341. [PubMed: 20064378]

14. Mosesson Y, Mills GB, Yarden Y. Derailed endocytosis: an emerging feature of cancer. Nat Rev Cancer. 2008;8(11):835–850. [PubMed: 18948996]

15. Paredes J, Figueiredo J, Albergaria A, et al. Epithelial E- and P-cadherins: role and clinical significance in cancer. Biochim Biophys Acta. 2012;1826(2):297–311. [PubMed: 22613680]

16. Corallino S, Malabarba MG, Zobel M, Di Fiore PP, Scita G. Epithelial-to-Mesenchymal Plasticity harnesses endocytic circuitries. Front Oncol. 2015;5:45. [PubMed: 25767773]

17. Chow MT, Luster AD. Chemokines in cancer. Cancer Immunol Res. 2014;2(12):1125–1131. [PubMed: 25480554]

18. Marchese A Endocytic trafficking of chemokine receptors. Curr Opin Cell Biol. 2014;27:72–77. [PubMed: 24680433]

19. Zlotnik A, Burkhardt AM, Homey B. Homeostatic chemokine receptors and organ-specific metastasis. Nat Rev Immunol. 2011;11(9):597–606. [PubMed: 21866172]

20. Gurung S, Perocheau D, Touramanidou L, Baruteau J. The exosome journey: from biogenesis to uptake and intracellular signalling. Cell Commun Signal. 2021;19(1):47. [PubMed: 33892745]

21. Edgar JR. Q&A: what are exosomes, exactly? BMC Biol. 2016;14:46. [PubMed: 27296830]

22. Hoshino D, Kirkbride KC, Costello K, et al. Exosome secretion is enhanced by invadopodia and drives invasive behavior. Cell Rep. 2013;5(5):1159–1168. [PubMed: 24290760]

23. Sung BH, Ketova T, Hoshino D, Zijlstra A, Weaver AM. Directional cell movement through tissues is controlled by exosome secretion. Nat Commun. 2015;6:7164. [PubMed: 25968605]

24. Peinado H, Zhang H, Matei IR, et al. Pre-metastatic niches: organ-specific homes for metastases. Nat Rev Cancer. 2017;17(5):302–317. [PubMed: 28303905]

25. Shinohara H, Kuranaga Y, Kumazaki M, et al. Regulated polarization of tumor-associated macrophages by miR-145 via colorectal cancer-derived extracellular vesicles. J Immunol. 2017;199(4):1505–1515. [PubMed: 28696255]

26. Kurywchak P, Tavormina J, Kalluri R. The emerging roles of exosomes in the modulation of immune responses in cancer. Genome Med. 2018;10(1):23. [PubMed: 29580275]

27. Chen G, Huang AC, Zhang W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nature. 2018;560(7718):382–386. [PubMed: 30089911]

28. McMahon HT, Boucrot E. Membrane curvature at a glance. J Cell Sci. 2015;128(6):1065–1070. [PubMed: 25774051]

29. Ford MG, Mills IG, Peter BJ, et al. Curvature of clathrin-coated pits driven by epsin. Nature. 2002;419(6905):361–366. [PubMed: 12353027]

30. Dawson JC, Legg JA, Machesky LM. Bar domain proteins: a role in tubulation, scission and actin assembly in clathrin-mediated endocytosis. Trends Cell Biol. 2006;16(10):493–498. [PubMed: 16949824]

31. Henne WM, Stenmark H, Emr SD. Molecular mechanisms of the membrane sculpting ESCRT pathway. Cold Spring Harb Perspect Biol. 2013;5(9).

32. Diz-Muñoz A, Fletcher DA, Weiner OD. Use the force: membrane tension as an organizer of cell shape and motility. Trends Cell Biol. 2013;23(2):47–53. [PubMed: 23122885]

33. Sens P, Plastino J. Membrane tension and cytoskeleton organization in cell motility. J Phys Condens Matter. 2015;27(27):273103. [PubMed: 26061624]

34. Kutateladze TG. Translation of the phosphoinositide code by PI effectors. Nat Chem Biol. 2010;6(7):507–513. [PubMed: 20559318]

35. Bissell MJ, Hines WC. Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. Nat Med. 2011;17(3):320–329. [PubMed: 21383745]

36. Slochower DR, Huwe PJ, Bradley R, Radhakrishnan R, Janmey PA. Quantum and all-atom molecular dynamics simulations of protonation and divalent ion binding to phosphatidylinositol 4,5-bisphosphate (PIP2). J Phys Chem B. 2013;117(28):8322–8329. [PubMed: 23786273]

37. Slochower DR, Wang YH, Radhakrishnan R, Janmey PA. Physical chemistry and membrane properties of two phosphatidylinositol bisphosphate isomers. Phys Chem Chem Phys. 2015;17(19):12608–12615. [PubMed: 25901568]

38. Slochower DR, Wang YH, Tourdot RW, Radhakrishnan R, Janmey PA. Counterion-mediated pattern formation in membranes containing anionic lipids. Adv Colloid Interface Sci. 2014;208:177–188. [PubMed: 24556233]

39. López CA, Rzepiela AJ, de Vries AH, Dijkhuizen L, Hünenberger PH, Marrink SJ. Martini coarse-grained force field: extension to carbohydrates. J Chem Theor Comput. 2009;5(12):3195–3210.

40. Marrink SJ, Risselada HJ, Yefimov S, Tieleman DP, De Vries AH. The MARTINI force field: coarse grained model for biomolecular simulations. J Phys Chem B. 2007;111(27):7812–7824. [PubMed: 17569554]

41. Monticelli L, Kandasamy SK, Periole X, Larson RG, Tieleman DP, Marrink SJ. The MARTINI coarse-grained force field: extension to proteins. J Chem Theor Comput. 2008;4(5):819–834.

42. Ford MG, Pearse BM, Higgins MK, et al. Simultaneous binding of PtdIns(4,5)P2 and clathrin by AP180 in the nucleation of clathrin lattices on membranes. Science. 2001;291(5506):1051–1055. [PubMed: 11161218]

43. Bradley RP, Radhakrishnan R. Curvature-undulation coupling as a basis for curvature sensing and generation in bilayer membranes. Proc Natl Acad Sci U S A. 2016;113(35):E5117–E5124. [PubMed: 27531962]

44. Tourdot RW, Bradley RP, Ramakrishnan N, Radhakrishnan R. Multiscale computational models in physical systems biology of intracellular trafficking. IET Syst Biol. 2014;8(5):198–213. [PubMed: 25257021]

45. Zhao Y, Liu J, Yang C, et al. Exo 70 generates membrane curvature for morphogenesis and cell migration. Dev Cell. 2013;26(3):266–278. [PubMed: 23948253]

46. Perilla JR, Goh BC, Cassidy CK, et al. Molecular dynamics simulations of large macromolecular complexes. Curr Opin Struct Biol. 2015;31:64–74. [PubMed: 25845770]

47. Hsieh WT, Hsu CJ, Capraro BR, et al. Curvature sorting of peripheral proteins on solid-supported wavy membranes. Langmuir. 2012;28(35):12838–12843. [PubMed: 22881196]

48. Tourdot RW, Ramakrishnan N, Baumgart T, Radhakrishnan R. Application of a free-energy-landscape approach to study tension-dependent bilayer tubulation mediated by curvature-inducing proteins. Phys Rev E. 2015;92(4).

49. Tourdot RW, Ramakrishnan N, Radhakrishnan R. Defining the free-energy landscape of curvature-inducing proteins on membrane bilayers. Phys Rev E - Stat Nonlinear Soft Matter Phys. 2014;90(2):022717.

50. Ramakrishnan N, Sunil Kumar PB, Radhakrishnan R. Mesoscale computational studies of membrane bilayer remodeling by curvature-inducing proteins. Phys Rep. 2014;543(1):1–60. [PubMed: 25484487]

51. Ramakrishnan N, Tourdot RW, Radhakrishnan R. Thermodynamic free energy methods to investigate shape transitions in bilayer membranes. Int J Adv Eng Sci Appl Math. 2016;8(2):88–100. [PubMed: 27616867]

52. Agrawal NJ, Nukpezah J, Radhakrishnan R. Minimal mesoscale model for protein-mediated vesiculation in clathrin-dependent endocytosis. PLoS Comput Biol. 2010;6(9):e1000926. [PubMed: 20838575]

53. Liu J, Tourdot R, Ramanan V, Agrawal NJ, Radhakrishanan R. Mesoscale simulations of curvature-inducing protein partitioning on lipid bilayer membranes in the presence of mean curvature fields. Mol Phys. 2012;110(11–12):1127–1137. [PubMed: 26500377]

54. Hurley JH, Hanson PI. Membrane budding and scission by the ESCRT machinery: it's all in the neck. Nature. 2010;11(8):556–566.

55. Kai F, Ou G, Tourdot RW, et al. ECM dimensionality tunes actin tension to modulate endoplasmic reticulum function and spheroid phenotypes of mammary epithelial cells. EMBO J. 2022;41(17):e109205. [PubMed: 35880301]

56. Bisaria A, Hayer A, Garbett D, Cohen D, Meyer T. Membrane-proximal F-actin restricts local membrane protrusions and directs cell migration. Science. 2020; 368(6496):1205–1210. [PubMed: 32527825]

57. Jarsch IK, Daste F, Gallop JL. Membrane curvature in cell biology: an integration of molecular mechanisms. J Cell Biol. 2016;214(4):375–387. [PubMed: 27528656]

58. Ramakrishnan N, Sreeja KK, Roychoudhury A, et al. Excess area dependent scaling behavior of nano-sized membrane tethers. Phys Biol. 2018;15(2):026002. [PubMed: 29116056]

59. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science. 2011;334(6059):1081–1086. [PubMed: 22116877]

60. Hynes RO. The extracellular matrix: not just pretty fibrils. Science. 2009;326(5957):1216–1219. [PubMed: 19965464]

61. Whiteside TL. Tumor-derived exosomes and their role in cancer progression. Adv Clin Chem. 2016;74(74):103–141. [PubMed: 27117662]

62. Parihar K, Nukpezah J, Iwamoto DV, Janmey PA, Radhakrishnan R. Data driven and biophysical insights into the regulation of trafficking vesicles by extracellular matrix stiffness. iScience. 2022;25(8):104721. [PubMed: 35865140]

63. Wu B, Liu DA, Guan L, et al. Stiff matrix induces exosome secretion to promote tumour growth. Nat Cell Biol. 2023;25(3):415–424. [PubMed: 36797475]

64. Parihar K, Liu DA, Hassan G, et al. Mechanical cues synergize with EVs in tumor progression. Nat Biomed Eng. 2024. in press.

65. Patwardhan S, Mahadik P, Shetty O, Sen S. ECM stiffness-tuned exosomes drive breast cancer motility through thrombospondin-1. Biomaterials. 2021;279:121185. [PubMed: 34808560]

66. Sneider A, Liu Y, Starich B, et al. Small extracellular vesicles promote stiffness-mediated metastasis. Cancer Res Commun. 2024.

67. Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol. 2006;24(14):2137–2150. [PubMed: 16682732]

68. Luchini C, Capelli P, Scarpa A. Pancreatic ductal adenocarcinoma and its variants. Surg Pathol Clin. 2016;9(4):547–560. [PubMed: 27926359]

69. Wang JD, Jin K, Chen XY, Lv JQ, Ji KW. Clinicopathological significance of SMAD4 loss in pancreatic ductal adenocarcinomas: a systematic review and meta-analysis. Oncotarget. 2017;8(10):16704–16711. [PubMed: 28053288]

70. Weigelt B, Peterse JL, van 't Veer LJ. Breast cancer metastasis: markers and models. Nat Rev Cancer. 2005;5(8):591–602. [PubMed: 16056258]

71. Awaji M, Singh RK. Cancer-associated fibroblasts' functional heterogeneity in pancreatic ductal adenocarcinoma. Cancers (Basel). 2019;11(3).

72. Dougan SK. The pancreatic cancer microenvironment. Cancer J. 2017;23(6):321–325. [PubMed: 29189327]

73. Kai F, Drain AP, Weaver VM. The extracellular matrix modulates the metastatic journey. Dev Cell. 2019;49(3):332–346. [PubMed: 31063753]

74. Thomas D, Radhakrishnan P. Tumor-stromal crosstalk in pancreatic cancer and tissue fibrosis. Mol Cancer. 2019;18(1):14. [PubMed: 30665410]

75. Allard B, Aspeslagh S, Garaud S, et al. Immuno-oncology-101: overview of major concepts and translational perspectives. Semin Cancer Biol. 2018;52(Pt 2):1–11.

76. Binnewies M, Roberts EW, Kersten K, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. Nat Med. 2018;24(5):541–550. [PubMed: 29686425]

77. Burugu S, Asleh-Aburaya K, Nielsen TO. Immune infiltrates in the breast cancer microenvironment: detection, characterization and clinical implication. Breast Cancer. 2017;24(1):3–15. [PubMed: 27138387]

78. Cotechini T, Medler TR, Coussens LM. Myeloid cells as targets for therapy in solid tumors. Cancer J. 2015;21(4):343–350. [PubMed: 26222088]

79. Flores-Borja F, Irshad S, Gordon P, et al. Crosstalk between innate lymphoid cells and other immune cells in the tumor microenvironment. J Immunol Res. 2016;2016:7803091. [PubMed: 27882334]

80. Law AM, Lim E, Ormandy CJ, Gallego-Ortega D. The innate and adaptive infiltrating immune systems as targets for breast cancer immunotherapy. Endocr Relat Cancer. 2017;24(4):R123–R144. [PubMed: 28193698]

81. Padoan A, Plebani M, Basso D. Inflammation and pancreatic cancer: focus on metabolism, cytokines, and immunity. Int J Mol Sci. 2019;20(3).

82. Quigley DA, Kristensen V. Predicting prognosis and therapeutic response from interactions between lymphocytes and tumor cells. Mol Oncol. 2015;9(10): 2054–2062. [PubMed: 26607741]

83. Ruffell B, Coussens LM. Macrophages and therapeutic resistance in cancer. Cancer Cell. 2015;27(4):462–472. [PubMed: 25858805]

84. Vitale I, Manic G, Coussens LM, Kroemer G, Galluzzi L. Macrophages and metabolism in the tumor microenvironment. Cell Metab. 2019;30(1):36–50. [PubMed: 31269428]

85. Broz ML, Krummel MF. The emerging understanding of myeloid cells as partners and targets in tumor rejection. Cancer Immunol Res. 2015;3(4):313–319. [PubMed: 25847968]

86. Dupont S, Morsut L, Aragona M, et al. Role of YAP/TAZ in mechanotransduction. Nature. 2011;474(7350):179–183. [PubMed: 21654799]

87. Guilluy C, Swaminathan V, Garcia-Mata R, Timothy O'Brien E, Superfine R, Burridge K. The Rho GEFs LARG and GEF-H1 regulate the mechanical response to force on integrins. Nat Cell Biol. 2011;13(6):722–727. [PubMed: 21572419]

88. Ishihara S, Inman DR, Li WJ, Ponik SM, Keely P. Mechano-signal transduction in mesenchymal stem cells induces prosaposin secretion to drive the proliferation of breast cancer cells. Cancer Res. 2017;77(22):6179–6189. [PubMed: 28972074]

89. Laklai H, Miroshnikova YA, Pickup MW, et al. Genotype tunes pancreatic ductal adenocarcinoma tissue tension to induce matricellular fibrosis and tumor progression. Nat Med. 2016;22(5):497–505. [PubMed: 27089513]

90. Levental KR, Yu H, Kass L, et al. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell. 2009;139(5):891–906. [PubMed: 19931152]

91. Miroshnikova YA, Rozenberg GI, Cassereau L, et al. alpha5beta1-Integrin promotes tension-dependent mammary epithelial cell invasion by engaging the fibronectin synergy site. Mol Biol Cell. 2017;28(22):2958–2977. [PubMed: 28877984]

92. Mouw JK, Yui Y, Damiano L, et al. Tissue mechanics modulate microRNA-dependent PTEN expression to regulate malignant progression. Nat Med. 2014;20(4):360–367. [PubMed: 24633304]

93. Paszek MJ, DuFort CC, Rossier O, et al. The cancer glycocalyx mechanically primes integrin-mediated growth and survival. Nature. 2014;511(7509):319–325. [PubMed: 25030168]

94. Egeblad M, Rasch MG, Weaver VM. Dynamic interplay between the collagen scaffold and tumor evolution. Curr Opin Cell Biol. 2010;22(5):697–706. [PubMed: 20822891]

95. Feig C, Gopinathan A, Neesse A, Chan DS, Cook N, Tuveson DA. The pancreas cancer microenvironment. Clin Cancer Res. 2012;18(16):4266–4276. [PubMed: 22896693]

96. Hewitt RE, Powe DG, Ian Carter G, Turner DR. Desmoplasia and its relevance to colorectal tumour invasion. Int J Cancer. 1993;53(1):62–69. [PubMed: 7677932]

97. Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. Cold Spring Harb Perspect Biol. 2011;3(12).

98. Lu P, Weaver VM, Werb Z. The extracellular matrix: a dynamic niche in cancer progression. J Cell Biol. 2012;196(4):395–406. [PubMed: 22351925]

99. Rubashkin MG, Cassereau L, Bainer R, et al. Force engages vinculin and promotes tumor progression by enhancing PI3K activation of phosphatidylinositol (3,4,5)-triphosphate. Cancer Res. 2014;74(17):4597–4611. [PubMed: 25183785]

100. Kalluri R The biology and function of exosomes in cancer. J Clin Invest. 2016;126(4):1208–1215. [PubMed: 27035812]

101. Bae S, Brumbaugh J, Bonavida B. Exosomes derived from cancerous and non-cancerous cells regulate the anti-tumor response in the tumor microenvironment. Genes Cancer. 2018;9(3–4):87–100. [PubMed: 30108680]

102. Nabet BY, Qiu Y, Shabason JE, et al. Exosome RNA unshielding couples stromal activation to pattern recognition receptor signaling in cancer. Cell. 2017;170(2):352–366 e13. [PubMed: 28709002]

103. Gao L, Wang L, Dai T, Jin K, et al. Tumor-derived exosomes antagonize innate antiviral immunity. Nat Immunol. 2018;19(3):233–245. [PubMed: 29358709]

104. Hoshino A, Costa-Silva B, Shen TL, et al. Tumour exosome integrins determine organotropic metastasis. Nature. 2015;527(7578):329–335. [PubMed: 26524530]

105. Becker A, Thakur BK, Weiss JM, Kim HS, Peinado H, Lyden D. Extracellular vesicles in cancer: cell-to-cell mediators of metastasis. Cancer Cell. 2016;30(6):836–848. [PubMed: 27960084]

106. Whiteside TL. Exosomes and tumor-mediated immune suppression. J Clin Invest. 2016;126(4):1216–1223. [PubMed: 26927673]

107. Xu R, Rai A, Chen M, Suwakulsiri W, Greening DW, Simpson RJ. Extracellular vesicles in cancer - implications for future improvements in cancer care. Nat Rev Clin Oncol. 2018;15(10):617–638. [PubMed: 29795272]

108. Ignatiadis M, Sledge GW, Jeffrey SS. Liquid biopsy enters the clinic — implementation issues and future challenges. Nat Rev Clin Oncol. 2021;18(5):297–312. [PubMed: 33473219]
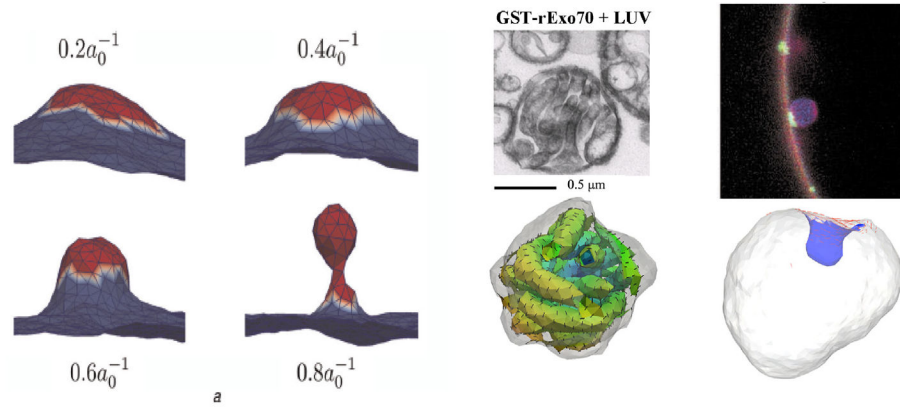
Fig. 1.
(**Left**) membrane deformation profiles under the influence of imposed curvature of the epsin shell model for three different coat areas; here $\kappa = 20\,k_BT$. For the largest coat area, the membrane shape is reminiscent of a clathrin-coated vesicle. Here, the vesicle morphologies subject to thermal fluctuations and entropic effects. (**Middle**) experimental observation of Exo 70 on GUV and (**Right**) results from the anisotropic curvature model. (**Right**) Fluorescence image shows the membrane in red and the location of ESCRT proteins in green; simulation results showing the novel phenomenon of budding by constriction – negative curved bud generated by negatively curved proteins localized to an annular region; the protein field does not localize the area of the bud.

Table 1

Genomic analysis performed using cBioPortal on patient-derived cancer genomic data from TGCA PanCancer Atlas. *Mut%* and *CNA%* correspond to percentage of samples with one or more mutations and copy number alterations respectively. Samples profiled is the number of patient samples having the genomic data needed for that particular analysis. While *max* is the maximum value of *Mut%* (*CNA%*) for a protein family, 4% refers to the proteins having *Mut%* (*CNA%*) greater than 4%. Rab: Rab GTPases, SNX: sorting nexins, EXOC: exocyst complex components, and ATG: autophagy-related proteins.

Tissue Type	Samples Profiled		Rab		SNX		EXOC		ATG	
			max	4%	max	4%	max	4%	max	4%
Skin	440	Mut%	5	Rab27B	5.7	SNX29, 13, 19, 31, 9	5	EXOC1, 7, 4, 6B	10	ATG2A, 2B, ULK1, 2
	367	CNA%	3.8		2.7		4.6	EXOC3	3	
Prostate	494	Mut%	0.6		0.8		0.6		0.8	
	489	CNA%	4.3	Rab2A, 3C	8.6	SNX14, 31, 3, 16	2.2		8.6	ATG5
Ovary	523	Mut%	0.6		1		1		0.8	
	572	CNA%	10.8	Rab8A, 19, 3D, 6A, 30, 25, 2A, 4A, 38, 3A, 13, 22A, 39B, 44	12.4	SNX31, 27	9.1	EXOC3, 4, 2, 8	7.9	ATG4D, 9B, 16L2, 3
Lung	566	Mut%	1.2		2.5		3		3.7	
	511	CNA%	9.4	Rab 13, 25, 22A, 29	10	SNX27, 6, 16, 31	12.9	EXOC3	2	
Colon	534	Mut%	2.6		4.9	SNX13	3.7		6.2	ATGA2A, 2B, 9B
	592	CNA%	7.6	Rab22A	7.6	SNX21	0.8		1.5	
Breast	1066	Mut%	0.7		0.7		1		1	
	1070	CNA%	9.2	Rab4A, 29, 13, 25, 2A, 22A, 37, 26, 34	11.1	SNX31, 27, 16	9.4	EXOC8	4	ATG16L2
Brain	397	Mut%	0.5		1.3		1.3		2	
	575	CNA%	1.4		1.7		5.2	EXOC1	1.6	