

Liquid-Liquid Phase Separation Enables Highly Selective Viral Genome Packaging

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In many viruses, hundreds of proteins assemble an outer shell (capsid) around the viral nucleic acid to form an infectious virion. How the assembly process selects the viral genome amidst a vast excess of diverse cellular nucleic acids is poorly understood. It has recently been discovered that many viruses perform assembly and genome packaging within liquid-liquid phase separated biomolecular condensates inside the host cell. However, the role of condensates in genome packaging is poorly understood. Here, we construct equilibrium and dynamical rate equation models for condensate-coupled assembly and genome packaging. We show that when the viral genome and capsid proteins favorably partition into the condensate, assembly rates, yields, and packaging efficiencies can increase by orders of magnitude. Selectivity is further enhanced by the condensate when capsid proteins are translated during assembly and packaging. Our results suggest that viral condensates provide a mechanism to ensure robust and highly selective assembly of virions around viral genomes. More broadly, our results may apply to other types of selective co-assembly processes that occur within biomolecular condensates and suggest that liquid-liquid phase-separated condensates could be exploited for selective encapsulation of microscopic cargo in human-engineered systems.

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I. INTRODUCTION

Capsid assembly around the viral genome is an essential step in the lifecycle of many viruses. To form an infectious virion, the capsid proteins must selectively encapsulate the viral RNA amidst excess cellular RNA, and many do so with high specificity (e.g., 99% [1,2]). How viruses achieve such selective packaging remains a critical unsolved problem in virology [3,4]. More broadly, assembly around specific cargoes has also become a key design challenge for biotechnology, e.g., to form gene or drug delivery vectors or nanoreactors [5–7]. It has recently become clear that many viruses perform assembly and genome packaging within liquid-liquid phase separated biomolecular condensates [8–14]. In this article, we use theoretical modeling to show that coupling of assembly and genome packaging with condensates can lead to extremely selective packaging of the viral genome. These insights shed light on viral lifecycles and could help to identify targets for antiviral agents that interfere with viral condensates. Our models also show how to apply principles from virus assembly to human-engineered applications, by using liquid-liquid phase separation (LLPS) to drive specific cargo packaging.

Several mechanisms have been identified for selective genome packaging. Intense experimental and theoretical research has shown that for many viruses, the genome contains

one or more sequence-specific packaging signals that interact with packaging sites on capsid proteins [3,15–23]. Furthermore, the charge and structure of viral RNA molecules is optimal for encapsidation by their capsid proteins [2,24–27]. Other mechanisms include coordinated capsid protein translation and assembly [28–30], as well as subcellular localization of viral components [31–34]. Despite this evidence, how selective packaging depends on these or other as yet unidentified mechanisms remains poorly understood for most viruses. In this article, we investigate how coupling assembly and genome packaging to condensates can significantly promote the latter two mechanisms.

Biomolecular condensates are liquid- or gel-like droplets composed of proteins and (often) nucleic acids. They act as membraneless organelles that locally concentrate specific components to facilitate cellular processes, such as signaling [35,36], the cellular stress response [37–40], transcription [41–45], and cell division. Condensates can also control self-assembly within cells. Examples include the assembly of clathrin cages during endocytosis [46], formation of post-synaptic densities [47] and presynaptic vesicle release sites (active zones) [48,49] at neuronal synapses, assembly of actin within polypeptide coacervates [50], and modulating aggregation of α -synuclein [51]. Beyond normal cellular functions, many pathogens construct or exploit condensates within their host cells. For example, many viruses perform assembly and packaging of the viral nucleic acid within condensates known as virus factories, replication sites, Negri bodies, inclusion bodies, or viroplasm [8–13,52–69].

Condensate formation is driven by weak, multivalent interactions among the constituents leading to LLPS. Importantly, condensates are not simply regions of locally enhanced density of proteins and nucleic acid: instead, condensates are representatives of a dense phase, coexisting with a dilute

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background phase. Many condensates thus display the hallmarks of LLPS: they are droplets with well-defined interfaces, whose surface tension drives them to gradually coarsen into a single dense phase over time [70], unless coarsening is arrested by elasticity or other mechanisms [71,72]. Additionally, at equilibrium, condensates maintain a composition which is independent of the total concentration of their constituents. However, in nonequilibrium situations (such as during virus assembly), the condensate composition will change over time. Further, condensates can become gel-like or glassy over time [73–77]. Indeed, condensates are also regulated by nonequilibrium processes including internal chemical reactions [39,41–45,71,78–134]. Components that drive phase separation are called scaffolds, while components that preferentially partition into condensates but do not provide the primary phase separation driving force are called clients [135]. Scaffold phase separation is driven by a subtle interplay between various intermolecular forces, including hydrophobic, electrostatic, hydrogen bonding, and π - π and π -cation interactions [89]. The precise balance between these different interactions depends on the system; in rotavirus condensates, for example, electrostatic, hydrophobic, and cation- π interactions appear to be the most important [56].

In most viral condensates, favorable interactions drive preferential partitioning (i.e., increase the local concentration) of viral RNA, capsid proteins, and other components. The exact molecular origin of these interactions is still unknown for most viruses, but partitioning into condensates is generally driven by geometrical, chemical, and/or sequence-specific interactions [136]. For example, in rotavirus, whose viral condensates are one of the best-studied, the viral RNA polymerase VP1, a client protein which binds to the scaffold protein NSP5, recruits viral RNA by binding to an untranslated region on rotavirus transcripts [137]. In many negative-strand ssRNA viruses [57,60,69,138,139], coronaviruses [64,140,141], and retroviruses [142,143], the nucleocapsid protein is the scaffold and primary constituent of the condensate. However, in other viruses, such as norovirus [144], adenovirus [145], rotavirus [56,62,146] and reovirus [68], auxiliary viral proteins are the scaffolds.

While there have been comparatively few investigations of condensate-coupled assembly, *in vitro* experiments have demonstrated that viral proteins and/or nucleic acids form condensates (e.g., Refs. [57,64,140,147,148]), and that condensates can accelerate assembly of capsids [57]. On the theoretical side, recent works using chemical-kinetics-based rate equations [51,81,103,149–153] and molecular dynamics (MD) simulations [154] have suggested that preferential partitioning of subunits into condensates can significantly enhance assembly rates and robustness against parameter variations. However, models have yet to include genome packaging or other forms of co-assembly within a condensate.

Here, we advance beyond these previous studies by considering the assembly of capsids around nucleic acids within a condensate. We find that the condensate can significantly increase the assembly rates and yields of capsids filled with nucleic acids. Moreover, preferential partitioning (i.e., increased local concentration) of the viral RNA into the condensate can lead to highly selective packaging, and this effect is amplified if nonviral RNA is preferentially excluded from

the condensate. Under these conditions, we observe essentially 100% selectivity across a broad range of physiologically relevant conditions. Furthermore, motivated by the observation that some viruses include ribosomes in their condensates [155–160], we find that capsid protein translation during assembly can further increase yields and selective packaging when the condensate is present. Based on these observations, we suggest that viral condensates provide a potent mechanism for selective genome packaging. This mechanism could work in synergy with previously proposed mechanisms for selective packaging [3,161], such as packaging signals. More broadly, our model is sufficiently general that it could be used to design approaches that utilize LLPS to encapsulate specific cargoes in a variety of biological and nanomaterials systems.

II. MODEL

We consider a minimal model for the effect of condensates on selective packaging of RNA (Fig. 1). We focus on ssRNA viruses, and we model the case in which nonstructural proteins are scaffolds (e.g., the norovirus protein NS7, an RNA-dependent RNA polymerase [144]), while the RNA and assembly subunits are clients. We assume that subunits are sufficiently dilute that their effects on condensate stability can be neglected. However, our model can be readily extended to the case where the capsid protein is the scaffold (which we are considering in ongoing work), and to viruses with dsRNA (e.g., rotavirus) or DNA genomes.

We extend rate equation models that we previously developed to describe assembly of empty capsids (containing no RNA) within condensates [153]. Our system consists of capsid subunits, cognate RNA, and noncognate RNA with total concentrations ρ_T , $\rho_{\text{cog},T}$, and $\rho_{\text{NC},T}$, respectively. Subunits can adsorb to and subsequently assemble around both cognate and noncognate RNA, forming RNA-filled capsids of size N subunits. These components are immersed in a multicomponent solution which consists of a minority phase (the viral condensate) coexisting with a majority phase (the “background”). We refer to the volumes of the condensate and background as V_c and V_{bg} , which are related to the total system volume by $V_{\text{tot}} = V_c + V_{bg}$. We present results in terms of the condensate size ratio (volume fraction), $V_r \equiv V_c/V_{bg}$. We showed in our previous work [153] that assembly yields are typically maximized when $V_r \ll 1$, and the same is true of the model considered here (see Fig. S1 [162]). Thus, unless otherwise noted, we set $V_r = 10^{-3}$. For simplicity, we consider a single compartment, but our model is readily extended to multiple compartments and doing so does not qualitatively change the results.

A. Rate equation model

1. Partition coefficients

Due to favorable interactions between viral components and condensate scaffold molecules, the compartment is enriched in the subunits and cognate RNA. This effect is quantified by partition coefficients K_c , K_{cog} , and K_{NC} for subunits (i.e., capsid proteins), cognate RNA, and noncognate RNA molecules, which set the equilibrium ratios of concentrations

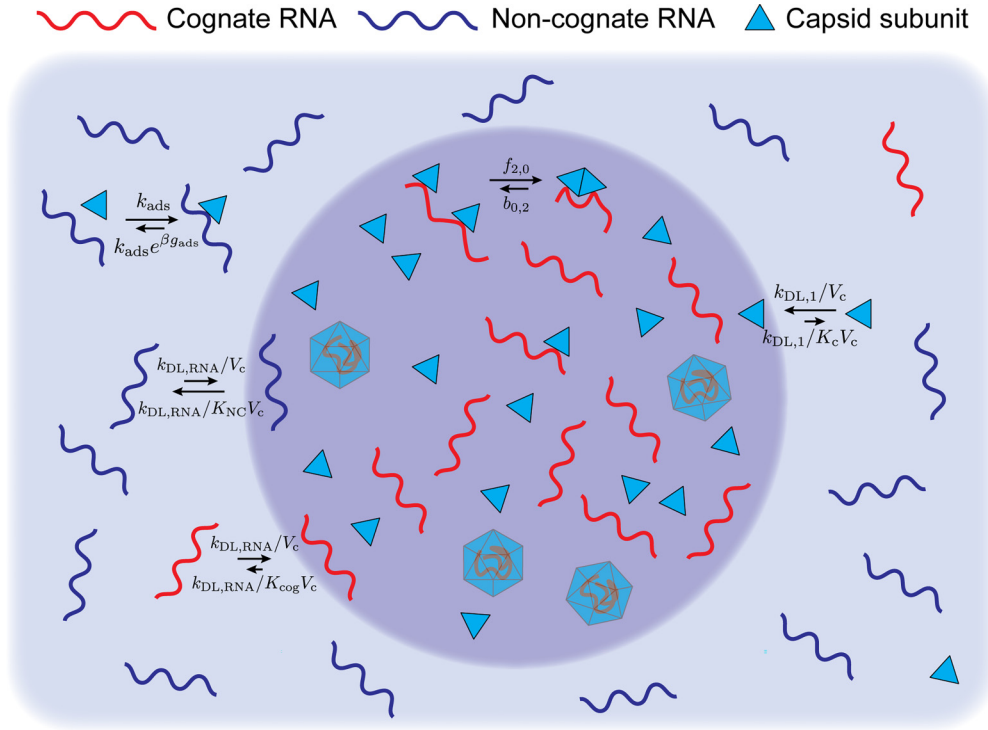


FIG. 1. Schematic of viral genome packaging within biomolecular condensates formed by liquid-liquid phase separation (LLPS). The viral condensate (light purple circle) is enriched in viral components by recruiting viral RNA (red) and capsid subunits (cyan) while disfavoring host RNA (blue). The condensate has volume V_c , while the surrounding background has volume $V_{bg} = V - V_c$. We define the volume fraction $V_r = V_c/V_{bg}$; unless otherwise noted, $V_r = 10^{-3}$. Subunits and RNA strands enter the condensate with diffusion-limited rate constants $k_{DL,1}/V_c$ and $k_{DL,RNA}/V_c$, respectively [Eqs. (A2b) and (A3c)], and exit with rate constants $k_{DL,1}/K_c V_c$ and $k_{DL,RNA}/K_v V_c$, for $v = \{\text{cog}, \text{NC}\}$. Subunits adsorb onto RNA with rate constant k_{ads} and binding free energy g_{ads} , such that the unbinding rate constant is $\sim k_{ads} e^{\beta g_{ads}}$. Adsorbed subunits can subsequently assemble; an RNA molecule with n adsorbed subunits and m assembled subunits undergoes dimerization (if $m = 0$) or association of a subunit to the intermediate (if $m \geq 2$) with rate $f_{n,m}$. Disassembly occurs with rate $b_{n,m}$.

in the condensate and background:

$$\begin{aligned} K_c &= \rho_1^c / \rho_1^{bg}, \\ K_{cog} &= \rho_{cog,(0,0)}^c / \rho_{cog,(0,0)}^{bg}, \\ K_{NC} &= \rho_{NC,(0,0)}^c / \rho_{NC,(0,0)}^{bg}. \end{aligned} \quad (1)$$

Here, ρ_1^α is the concentration of monomers in phase $\alpha = \{c, bg\}$ and $\rho_{v,(n,m)}^\alpha$ is the concentration of RNA of species $v = \{\text{cog}, \text{NC}\}$ in phase α with n adsorbed and m assembled subunits. We emphasize that in Eqs. (1) all concentrations are at their equilibrium values. The partition coefficient K_v is related to the change in solvation free-energy Δg_v for a molecule v that transitions from the background to the condensate as $K_v = e^{-\beta \Delta g_v}$ with $\beta = 1/k_B T$, where $k_B T$ is the thermal energy. For simplicity, we assume that solvation interactions are additive and thus partition coefficients are multiplicative; i.e., the partition coefficient for a cognate RNA with n adsorbed and m assembled subunits is given by

$$K_{cog}(n, m) = K_{cog} K_c^{(n+m)}. \quad (2)$$

2. Capsid assembly model

To describe the assembly kinetics, we adapt a rate equation formalism originally developed by Zlotnick [168–174] to describe the 2D polymerization of shells (capsids) in bulk solution. We have previously used versions of this model

to describe condensate-coupled assembly of empty capsids [153] and capsids assembling around nanoparticles in bulk solution [175]. The rate equations describe the time evolution of subunit and RNA concentrations:

$$\frac{d\rho_1^\alpha}{dt} = \mathcal{N}_1^\alpha + \mathcal{D}_1^\alpha + \mathcal{T}^\alpha, \quad (3a)$$

$$\frac{d\rho_{v,(n,m)}^\alpha}{dt} = \mathcal{N}_{v,(n,m)}^\alpha + \mathcal{M}_{v,(n,m)}^\alpha + \mathcal{D}_{v,(n,m)}^\alpha, \quad (3b)$$

where $v = \{\text{cog}, \text{NC}\}$ and $\alpha = \{c, bg\}$. The quantities $\mathcal{N}_1^\alpha$, $\mathcal{D}_1^\alpha$, and $\mathcal{T}^\alpha$ represent, respectively, the rate of change in free subunit concentration due to adsorption and desorption, the diffusive exchange of free subunits, and (when applicable) the rate of subunit translation from mRNA molecules. Similarly, the quantities $\mathcal{N}_{v,(n,m)}^\alpha$, $\mathcal{M}_{v,(n,m)}^\alpha$, and $\mathcal{D}_{v,(n,m)}^\alpha$ are the rates of change of RNA concentrations due to adsorption, assembly, and RNA diffusion, respectively, with n and m as the numbers of adsorbed and assembled subunits. Detailed expressions for these terms are given in Appendix A; here, we briefly describe them. We assume that subunits adsorb from solution (in background or condensate) onto the RNA with rate constant k_{ads} and free energy g_{ads} due to nonspecific electrostatic interactions between positive charges on capsid proteins and negative charges on the RNA backbone; the unbinding rate constant $k_{des} = \rho_{SS} k_{ads} e^{\beta g_{ads}}$, with the standard state concentration $\rho_{SS} = 1\text{M}$, follows from detailed balance.

The adsorbed subunits then undergo assembly into capsids of size N . An RNA molecule with n adsorbed subunits and m assembled subunits undergoes dimerization (if $m = 0$) or association of a subunit to an assembly intermediate (if $m \geq 1$) with rate $f_{n,m}$; disassembly occurs with rate $b_{n,m}$. Subunits and RNA molecules (with adsorbed and assembled subunits) can diffuse between the condensate and background; the associated rate constants are $k_{DL,1}$ and $k_{DL,RNA}$, respectively. For the results in Fig. 5, subunits are synthesized from cognate RNA with rate constant k_{tr} ; in all other main text figures, $k_{tr} = 0$. If $k_{tr} = 0$, then the rate equations obey mass conservation:

$$\sum_{\alpha=c,bg} \left[V_{\alpha} \rho_1^{\alpha} + V_{\alpha} \sum_{\nu=cog,NC} \sum_{n,m} \rho_{\nu,(n,m)}^{\alpha} (n+m) \right] = V \rho_T, \quad (4a)$$

$$\sum_{\alpha=c,bg} \left[V_{\alpha} \sum_{n,m} \rho_{\nu,(n,m)}^{\alpha} \right] = V \rho_{\nu,T}, \quad \nu = \{cog, NC\}, \quad (4b)$$

where α runs over condensate and background phases and ν runs over cognate and noncognate RNA.

B. Yields and selectivities

We report two measures of assembly. (1) We define the *total yield* x_{enc}^T as the fraction of RNA molecules in complete capsids, including cognate and noncognate in the condensate and the background. Noting that the concentration of RNA molecules in capsids is equal to the concentration of complete capsids ρ_N ,

$$x_{enc}^T = \rho_N / \rho_{RNA,T}, \quad (5)$$

where $\rho_{RNA,T} = \rho_{cog,T} + \rho_{NC,T}$ is the total RNA concentration. We analogously define the *cognate yield* x_{enc}^{cog} and *noncognate yield* x_{enc}^{NC} as

$$x_{enc}^{cog} = \rho_N^{cog} / \rho_{cog,T}, \quad (6)$$

$$x_{enc}^{NC} = \rho_N^{NC} / \rho_{NC,T},$$

with ρ_N^{cog} and ρ_N^{NC} the concentrations of capsids enclosing cognate and noncognate RNA, respectively. Yields of either species in the condensate and background are defined similarly (see Appendix B).

(2) We define the selectivity as $s = \rho_N^{cog} / \rho_N^{NC} = \rho_{cog,T} x_{enc}^{cog} / \rho_{NC,T} x_{enc}^{NC}$; we then define the normalized selectivity, $\bar{s}$, as

$$\bar{s} = \frac{2}{1 + x_{enc}^{NC} / x_{enc}^{cog}} - 1, \quad (7)$$

such that $-1 \leq \bar{s} \leq 1$. When $\bar{s} = 1$, only cognate RNA is encapsidated; when $\bar{s} = -1$, only non-cognate RNA is encapsidated; when $\bar{s} = 0$, cognate and noncognate RNAs are encapsidated to an equal extent relative to the total concentration of each species. That is, when $\bar{s} = 0$, $\rho_N^{cog} / \rho_N^{NC} = \rho_{cog,T} / \rho_{NC,T}$.

C. Equilibrium assembly theory

We also develop an equilibrium theory for our system for three reasons. First, the equilibrium theory identifies conditions under which assembly is thermodynamically favorable, and the maximum (infinite-time) values of yield and selectivity. Second, we can derive approximate expressions that provide easily understandable relationships for how yields

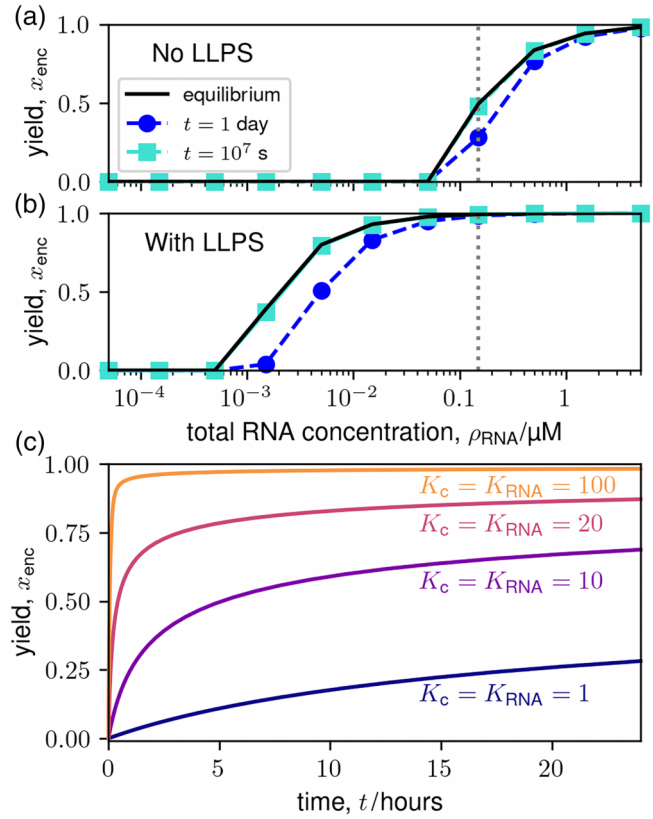


FIG. 2. LLPS increases the robustness and rate of genome packaging. This figure shows results for one RNA species (cognate). (a), (b) The yield (x_{enc}^T), defined as the fraction of RNA molecules in complete capsids [Eq. (5)] as a function of RNA concentration $\rho_{RNA,T}$ without (a) and with (b) LLPS for the subunit partition coefficient $K_c = 100$ and the RNA partition coefficient $K_{RNA} = 100$. The total subunit concentration is $\rho_T = N\rho_{RNA}$, so there are just enough subunits to encapsidate every RNA molecule. The dashed black lines are the equilibrium yield (Sec. S2B [162]), the blue lines with circular markers are results from the rate equation model [Eq. (A1)] after $t = 1$ day, and the turquoise lines with square markers are results from the rate equation model after $t = 10^7$ s. The dotted gray vertical lines mark the RNA concentration ($0.15 \mu M$, close to a typical physiological RNA concentration of $\approx 0.1 \mu M$ (BNID 109916 [176]) at which the results in (c) were obtained. (c) Yield as a function of time from the rate equation model for indicated values of the subunit and RNA partition coefficients.

and selectivities depend on the key control parameters. Third, although in the long-time limit the rate equation results (with $k_{tr} = 0$) should closely match the equilibrium predictions (see Fig. 2), at finite times slow nucleation or kinetic traps may cause the rate equation results to deviate significantly from equilibrium.

We calculate the equilibrium yields and selectivities using mass conservation, the law of mass action, and the definitions of the partition coefficients [Eqs. 1 and (2)]. In the Supplemental Material (Ref. [162]), Sec. S2 [162], we use these relationships to derive self-consistent equations for the equilibrium values of the yields [Eqs. (5), (6), and (B1)]. While these equations are complicated, we obtain simpler expressions for the equilibrium yield and selectivity in the

limit of excess RNA ($\rho_{\text{RNA},T} \gg \rho_T/N$):

$$x_{\text{enc}}^T \approx \begin{cases} \frac{\rho_T}{N\rho_{\text{RNA},T}}(\rho_{\text{CAC}}/\rho_T)^N, & \text{for } \rho_T \ll \rho_{\text{CAC}} \\ \frac{\rho_T}{N\rho_{\text{RNA},T}}(1 - \rho_{\text{CAC}}/\rho_T), & \text{for } \rho_T \gg \rho_{\text{CAC}} \end{cases} \quad (8)$$

and

$$s \approx \frac{\rho_{\text{cog},T}(1 + V_r K_{\text{NC}})(1 + V_r K_{\text{cog}} K_c^N)}{\rho_{\text{NC},T}(1 + V_r K_{\text{cog}})(1 + V_r K_{\text{NC}} K_c^N)}. \quad (9)$$

Here, ρ_{CAC} is the critical assembly concentration:

$$\rho_{\text{CAC}} \approx e^{\beta(G_{\text{ads}} + G_N/N)} \frac{1 + V_r K_c}{1 + V_r} \times \left(\sum_{v=\text{cog},\text{NC}} \frac{1 + V_r K_v K_c^N}{1 + V_r K_v} \rho_{v,T} \right)^{-\frac{1}{N}},$$

where the term $e^{\beta(G_{\text{ads}} + G_N/N)}$ (with G_N the free energy of an assembled capsid) reflects the subunit-RNA and subunit-subunit interactions, and the remaining terms account for translational entropy costs and interactions of RNA and subunits with the condensate.

Typical parameter ranges

In typical experiments, the condensate volume will be small compared to the background, $V_r \ll 1$. For example, rotavirus viroplasms vary from 0.1 μm –5 μm in diameter [62], which gives $V_r \approx 10^{-7}$ – 10^{-2} for a typical mammalian cell radius of 10 μm . The typical total concentration of mRNA molecules in mammalian cells is on the order of 0.1 μM (BNID 109916 [176]), while viral RNA concentrations can vary from ≈ 10 nM to a few μM [177]. These quantities can be varied over much larger ranges with *in vitro* experiments.

For convenience, we define all model parameters and give their values in Table I of the Appendix.

III. RESULTS

A. LLPS promotes robust encapsidation

We first investigate how LLPS affects assembly when there is only one species of RNA, and subunits and the RNA preferentially partition into the condensate, according to the partition coefficients $K_c = \rho_1^c/\rho_1^{\text{bg}}$ and $K_{\text{RNA}} = \rho_{\text{RNA}}^c/\rho_{\text{RNA}}^{\text{bg}}$ (see the Model section). We consider a range of RNA concentrations, and for simplicity, a constant stoichiometric ratio of subunits to RNA, $\rho_T = N\rho_{\text{RNA},T}$, so there are just enough subunits to encapsulate all RNA molecules. Both the equilibrium and rate equation results show that the condensate increases the range of concentrations leading to productive assembly by orders of magnitude. Figure 2(a) shows the yield (the fraction of RNA molecules in complete capsids, x_{enc}^T) as a function of RNA concentration without the effect of LLPS (i.e., no partitioning, $K_c = 1$, $K_{\text{RNA}} = 1$), while Fig. 2(b) shows yields with strong partitioning ($K_c = 100$, $K_{\text{RNA}} = 100$). The equilibrium theory predicts that above a critical concentration ρ_{CAC} the yield monotonically increases with RNA concentration until reaching nearly 100%. While the rate equation yields are somewhat lower at the finite time of 1 day, they match the equilibrium results by long times (10^7 s). LLPS increases the range of concentrations at which assembly occurs by two

orders of magnitude, by increasing the local concentration (i.e., within the condensate) by a factor of $K_c = K_{\text{RNA}} = 100$, which lowers ρ_{CAC} by a factor of ≈ 100 [see Eq. (10)].

Additionally, the rate equation predicts that the condensate vastly increases assembly rates. Figure 2(c) shows yields as a function of time, demonstrating that both the assembly rate and the final yield dramatically and monotonically increase with K_c and K_{RNA} . These kinetics are shown for an RNA concentration of $\rho_{\text{RNA},T} = 0.15$ μM [indicated by the gray dotted lines in Figs. 2(a) and 2(b)], which is roughly consistent with typical RNA concentrations in mammalian cells (BNID 109916 [176,178]). Thus, partitioning subunits and RNA into condensates enables rapid, high-yield encapsidation over a wide range of concentrations.

Note that the monotonic increase in yields at finite time with concentration contrasts with previous studies on empty capsid assembly, which find that the yield decreases at high concentrations due to a kinetic trap in which overly rapid capsid nucleation starves the system of free subunits before assembly can complete. This trap does not occur for our results at the stoichiometric ratio $\rho_T = N\rho_{\text{RNA},T}$ because the amount of nucleation is limited by the number of RNA molecules, as shown previously for assembly of capsids around nanoparticles [175]. However, at sufficiently high concentrations, empty capsid assembly and malformed assemblies [24,175,179,180], which we have neglected in this study, would deplete finite-time yields.

B. LLPS provides a mechanism for selective viral genome packaging

We now consider a system with two RNA species: cognate (viral) and noncognate (host). Consistent with the early stages of viral infections, where viral RNA is scarce compared to host RNA, we focus on conditions where the fraction of total RNA that is cognate is relatively small, $f_{\text{cog}} = \rho_{\text{cog},T}/(\rho_{\text{cog},T} + \rho_{\text{NC},T}) = 0.1$. In the main text results, we consider a stoichiometric ratio at which there are just enough subunits to encapsulate all the cognate RNA, $\rho_T = Nf_{\text{cog}}\rho_{\text{RNA},T}$, but we present results in Supplemental Material (Ref. [162]), Sec. S1 (Figs. S4–S6 [162]) for other values of f_{cog} and ρ_T , which we discuss later in this section.

Cognate RNA molecules favorably partition into the condensate (with partition coefficient K_{cog}) due to favorable interactions with condensate components, while noncognate RNA tends to be excluded from the condensate (with partition coefficient K_{NC}). Our model is agnostic to the molecular details of how this partitioning occurs. The partitioning mechanism is currently unknown for norovirus, whose ssRNA genome and nonstructural protein condensate most closely reflect our model [144]. However, in rotavirus condensates (which have dsRNA genomes), the client protein VP1 binds viral RNA to drive its partitioning [137]. We will extend our model to account for such dsRNA genomes in future work. Host RNA could be excluded by less specific effects, e.g. an entropic penalty [124,181]. While either selective incorporation of cognate RNA into condensates or exclusion of non-cognate RNA from condensates alone can promote selective partitioning, we find that selectivity is most robust when both mechanisms operate. Note, however, that binding

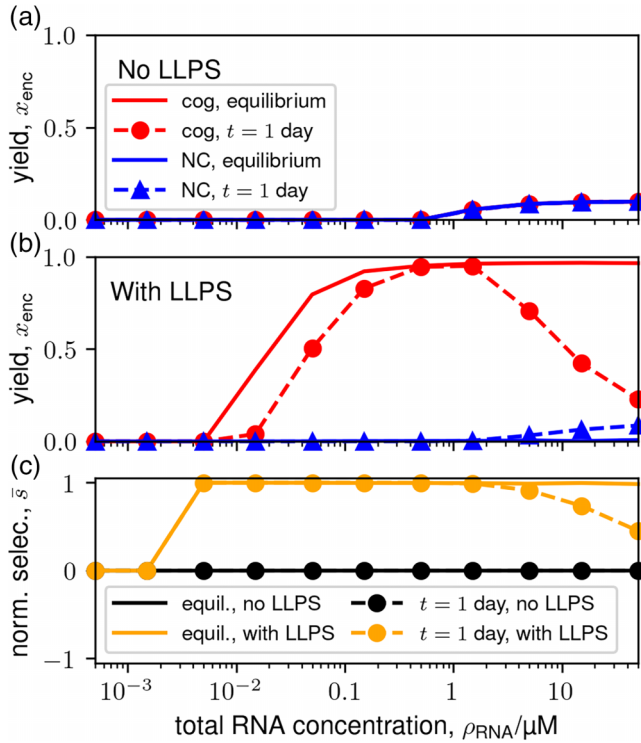


FIG. 3. LLPS drives selective packaging of cognate RNA over a wide range of concentrations. (a), (b) Yields of cognate (cog, red, circles) and noncognate (NC, blue, triangles) RNA versus total subunit concentration, without (a) and with (b) LLPS. Solid lines denote equilibrium results (Sec. S2B [162]), while dashed lines with markers correspond to rate equation results [Eq. (A1)] at $t = 1$ day. (c) Normalized selectivity [Eq. (7)] with (orange) and without (black) LLPS. Solid lines denote equilibrium results, while dashed lines with markers correspond to rate equation results at $t = 1$ day. For (a) and for black lines in (c), $K_c = K_{\text{cog}} = K_{\text{NC}} = 1$, while for (b) and for orange lines in (c), $K_c = K_{\text{cog}} = 100$ and $K_{\text{NC}} = 10^{-2}$. For these results, $f_{\text{cog}} = \rho_{\text{RNA,cog}} / (\rho_{\text{RNA,cog}} + \rho_{\text{RNA,NC}}) = 1/10$ and $\rho_T = N \rho_{\text{RNA,cog}}$, so there are just enough subunits to encapsidate every cognate RNA strand.

of subunits to either species of RNA increases its partition coefficient [see Eq. (2)].

Figures 3(b) and 3(c) show that these conditions with strong partitioning ($K_c = K_{\text{cog}} = 100$ and $K_{\text{NC}} = 10^{-2}$) drive essentially perfectly selective packaging of cognate RNA over a wide range of subunit concentrations. In particular, partitioning lowers the critical assembly concentration for cognate RNA by roughly two orders of magnitude, and above this threshold drives an equilibrium $x_{\text{enc}}^{\text{cog}}$ approaching 100%, with no assembly around noncognate RNA ($\bar{s} \approx 1$). In Fig. S2 [162], we show how the cognate yield varies with time for several different values of the partition coefficients. As in the case of a single species of RNA [Fig. 2(c)], partitioning increases both the assembly rate and the final ($t = 1$ day) yield. For a wide range of $\rho_{\text{RNA,T}}$, the rate equation results nearly reach equilibrium by 1 day. However, at high RNA concentrations $\rho_{\text{RNA,T}} \gtrsim 1$ μM (an order of magnitude above typical physiological concentrations), the cognate yield drops markedly and noncognate yield increases slightly. This difference between finite time and equilibrium reflects a kinetic trap: At such high

concentrations, the nucleation rate in the background becomes comparable to the rates of subunit diffusion into and assembly within the condensate (see Fig. S3 and Sec. S3 [162] for timescale estimates). Consequently, subunits become stuck in partially or fully assembled capsids containing noncognate RNA.

Figure 3(a) shows the control example with no LLPS effects ($K_c = K_{\text{cog}} = K_{\text{NC}} = 1$). Here, there is no distinction between cognate and noncognate RNA, and thus no selectivity [$\bar{s} = 0$, black lines in Fig. 3(c)]—capsids assemble around RNA species in proportion to their concentrations. The overall yield (fraction of each RNA species enclosed, x_{enc}^T) is limited to $f_{\text{cog}} = 0.1$ by the subunit stoichiometric ratio ($\rho_T / N \rho_{\text{RNA,T}} = f_{\text{cog}}$); higher stoichiometric ratios of subunits lead to higher yields (Fig. S4 [162]).

1. Varying stoichiometric ratio and fraction cognate

The results thus far consider a limiting subunit stoichiometric ratio $\rho_T = N \rho_{\text{cog,T}}$ and one value of $f_{\text{cog}} = 0.1$. Figure S4 [162] shows results for a higher subunit stoichiometric ratio $\rho_T = N \rho_{\text{RNA,T}}$, sufficient to encapsidate *all* RNA molecules. LLPS still leads to selective encapsidation in this case, although over a significantly narrower range of concentrations compared to Fig. 3(b). Figure S5 [162] shows that, for fixed $\rho_{\text{NC,T}}$, the yields decrease with decreasing f_{cog} , consistent with the simplified equilibrium expression Eq. (8) due to the increasing chemical potential cost to remove a cognate RNA from solution as its concentration decreases. Importantly though, selectivity remains high throughout the range over which cognate RNA is encapsidated. Moreover, high yields can be achieved even at low f_{cog} by increasing the total RNA concentration $\rho_{\text{RNA,T}}$ and thus the cognate concentration (Fig. S6 [162]).

2. Varying partition coefficients

Figure 4 shows how selectivity depends on the partition coefficients for the typical noncognate RNA concentration $\rho_{\text{RNA,T}} = 0.1$ μM . Figure 4(a) shows varying subunit partition coefficient K_c , demonstrating that selectivity remains extremely high even for low K_c under strong partitioning/exclusion of cognate/noncognate RNA. For $K_c \gtrsim 2$, capsids assemble almost exclusively around cognate RNA ($\bar{s} \approx 1$). Note that the approximate equilibrium selectivity [Eq. (9)], which depends only on the ratio of total cognate and noncognate concentrations and not on the concentrations themselves, closely matches the exact equilibrium result and the rate equation results at $t = 1$ day. Thus, Eq. (9) serves as a simple and accurate predictor of how much partitioning is required to achieve a desired selectivity. Figure 4(b) shows Eq. (9) as a function of K_{cog} and K_{NC} for $K_c = 100$, demonstrating that when subunit partitioning is strong, only modest partitioning of cognate into, and/or noncognate out of, the condensate is required to achieve high selectivity. Figures 4(c) and 4(d) show how the selectivity depends on K_{cog} and K_{NC} for weak subunit partitioning $K_c = 3$ and noncognate [Fig. 4(c)] or cognate [Fig. 4(d)] RNA partitioning. Selectivities close to one are attained for $K_{\text{cog}} \gtrsim 10$ [Fig. 4(c)] and $K_{\text{NC}} \lesssim 0.1$ [Fig. 4(d)]. Taken together, these results show that LLPS enables

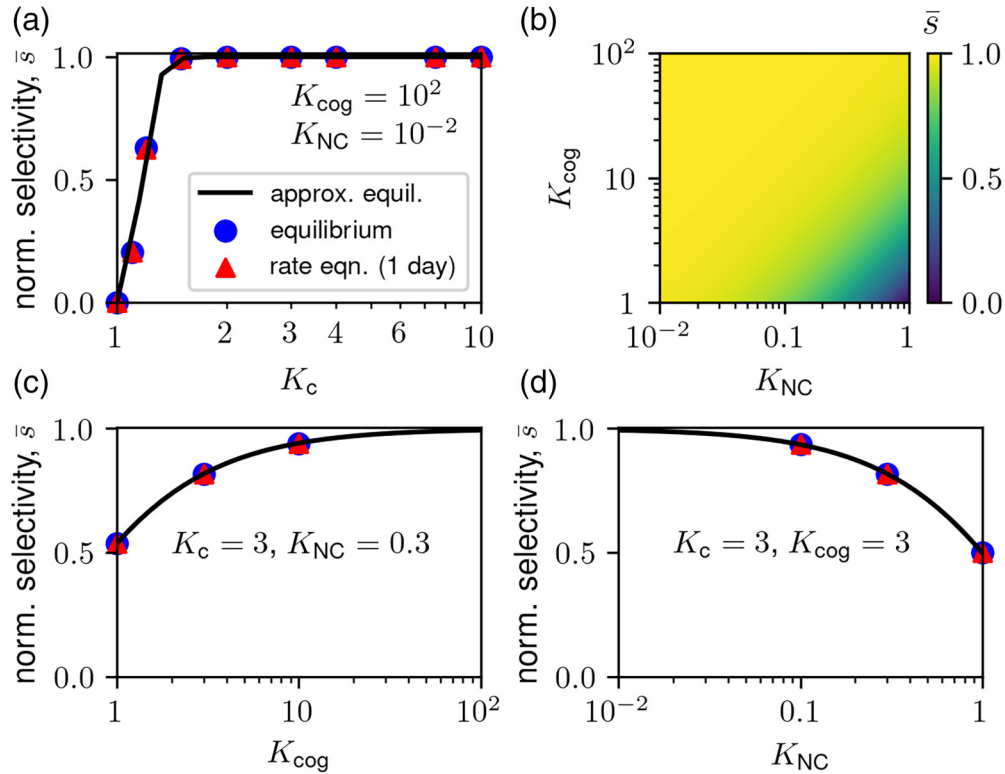


FIG. 4. LLPS promotes selective viral genome packaging over a wide range of partition coefficients. (a) Normalized selectivity [$\bar{s}$, Eq. (7)] as a function of K_c . Selectivity is shown for dynamics at $t = 1$ day (red triangles), exact equilibrium (blue circles), and the approximate equilibrium result [Eq. (9)] for $\rho_{\text{NC},T} = 0.1 \mu\text{M}$, $f_{\text{cog}} = 0.1$, $K_{\text{cog}} = 10^2$, and $K_{\text{NC}} = 10^{-2}$. (b) Approximate equilibrium selectivity [Eq. (9)] as a function of K_{cog} and K_{NC} for $\rho_{\text{NC},T} = 0.1 \mu\text{M}$, $f_{\text{cog}} = 0.1$, and $K_c = 10^2$. (c) Selectivity as a function of K_{cog} for $\rho_{\text{NC},T} = 0.1 \mu\text{M}$, $f_{\text{cog}} = 0.1$, $K_c = 3$, and $K_{\text{NC}} = 0.3$. (d) Selectivity as a function of K_{NC} for $\rho_{\text{NC},T} = 0.1 \mu\text{M}$, $f_{\text{cog}} = 0.1$, $K_c = 3$, and $K_{\text{cog}} = 3$. For all rate equation and exact equilibrium results in this figure, $\rho_T = N\rho_{\text{cog},T}$. Note that the approximate equilibrium result, Eq. (9), is independent of ρ_T .

highly selective packaging of cognate RNA at physiologically realistic concentrations and partition coefficients.

C. Subunit translation further enhances packaging selectivity

The concentration of viral proteins is not constant in infected cells, since subunits are synthesized from (positive sense) viral RNA by ribosomes. Some viruses incorporate ribosomes within viroplasm [155–160], suggesting the possibility of protein translation within condensates. Therefore, we now consider the consequences of subunit translation for condensate-mediated selective packaging. We assume that subunit translation occurs at a rate proportional to local cognate RNA concentration (see the Model section). However, since the actual rate will depend on ribosome concentration along with other factors, we treat the translation rate constant k_{tr} as a variable parameter.

Figures 5(a) and 5(b) show how the yield and selectivity vary with the total RNA concentration for a range of translation rates. Figure 5(a) shows that increasing translation rates makes assembly significantly more robust, increasing the range of total RNA concentrations that result in high cognate yields by up to 100-fold. Figure 5(b) shows that the effect of translation on selectivity is more nuanced at these parameters. Translation enables high selectivity at low RNA concentrations ($\rho_{\text{RNA},T} \lesssim 0.01 \mu\text{M}$, where no assembly occurs

with $k_{\text{tr}} = 0$), and modestly increases the selectivity at very high RNA concentrations ($\rho_{\text{RNA},T} \gtrsim 1 \mu\text{M}$). The latter effect arises because subunits are preferentially being synthesized inside the condensate, circumventing the monomer starvation kinetic trap due to subunits binding to noncognate RNA in the background. However, since selectivity is already 100% over the range $\rho_{\text{RNA},T} \in (0.01, 1) \mu\text{M}$ at these parameters, translation has little effect in this range except for slightly decreasing selectivity at extremely high rates ($k_{\text{tr}} = 10^{-3} \text{s}^{-1}$). This effect arises because high translation rates lead to high subunit stoichiometric ratios (in both the condensate and background), causing capsids to assemble around noncognate and cognate RNA.

IV. DISCUSSION

Our results show that coupling self-assembly to biomolecular condensates is a powerful strategy for making viral genome packaging robust, rapid, and selective. Despite the significant excess noncognate RNA present in an infected cell, preferential partitioning of subunits and cognate RNA into the condensate leads to high yields of capsids containing viral genomes and high selectivity—low yields of capsids with noncognate RNA. For physically relevant ranges of partition coefficients, we observe essentially perfect selectivity over wide ranges of RNA and capsid protein concentrations. Although selective packaging does not require exclusion of host

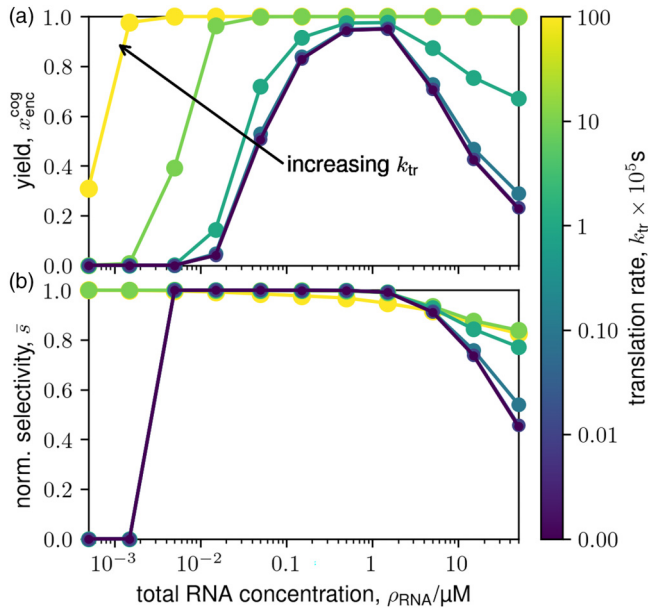


FIG. 5. Translation of capsid proteins in the condensate increases selective packaging of the viral genome. (a) Fraction of cognate RNA in complete capsids (x_{enc}^{cog}) as a function of total RNA concentration for indicated capsid protein translation rates. (b) Normalized selectivity $\bar{s}$ for cognate RNA as a function of total RNA concentration for indicated translation rates. Other parameters are $K_c = K_{cog} = 10^2$, $K_{NC} = 10^{-2}$, $f_{cog} = 0.1$, and $V_t = 10^{-3}$. For $k_{tr} = 0$, we set $\rho_T = N\rho_{cog,T}$, so there are just enough subunits to encapsidate all cognate RNA strands.

genetic material from the condensate, selectivity is optimized when viral condensates both enrich themselves with viral structural components and exclude host genetic material. We also find that partitioning of subunits and cognate RNA into the condensate can increase assembly rates and robustness (the range of concentrations leading to high yields) by orders of magnitude, consistent with previous studies of empty capsid assembly [153,154].

Motivated by the observation of ribosomes in or near some viral condensates, our model predicts that protein translation can enhance selective packaging. While previous work has shown that assembly can be improved by addition of capsid proteins during assembly [28,182,183], a key difference in our system that enhances selectivity is that translation is proportional to the local viral RNA concentration, which is higher in the condensate.

A. Comparison with packaging signals and other mechanisms for selective packaging

While several mechanisms have been proposed for selective viral packaging [3], most work has focused on packaging signals, which are specific RNA sequences that interact with sites on capsid proteins. Here, we show that an alternative mechanism, co-recruitment of capsid proteins and viral RNA molecules into a condensate, is also a powerful mechanism to drive selectivity. While, for simplicity, we did not consider packaging signals in this model, it seems likely that selectivity could be further enhanced by combining packaging signals

with condensate-coupled assembly. Thus, a natural next step will be to incorporate packaging signals into our model.

Indeed, a number of viruses that employ condensates during genome packaging are known to have packaging signals, such as HIV [142,143,184–187], rotavirus [188], and reovirus [189]. It is also worth noting that the viral condensate scaffolds must have a mechanism to distinguish viral from host RNA, which likely involves sequence-specific interactions; for example, rotavirus RNA is recruited by the viral RNA binding protein VP1. Alternatively, favorable capsid protein-condensate interactions among capsid proteins adsorbed to viral RNA could facilitate partitioning of viral RNA (as observed for ebolavirus [69,190]). Finally, noncognate RNA, which does not have such preferential interactions with viral proteins, could be preferentially excluded from the condensate by generic entropic forces [124,181].

B. Extending to alternative coarse-grained models

Our rate equation model builds on previous work by Zlotnick [168–174] and ourselves [153,175], and is closely related to the Becker-Döring equations [191], but many other coarse-grained frameworks for modeling self-assembly exist, including approaches based on Smoluchowski coagulation [192], Hohenberg-Halperin dynamics [193] (applied to capsid assembly by Zandi and van der Schoot [194,195]), Cahn-Hilliard dynamics [103,196], and other chemical kinetics models [103,152,197–200]. These approaches could also be extended to model LLPS-mediated selective packaging. Although model predictions may differ quantitatively (e.g., the precise increases in rate and yield with partition coefficients), we expect that they will all give similar qualitative results to our model as long as cognate RNA and capsid subunits tend to partition into the condensate and noncognate RNA tends to be excluded. Many of these coarse-grained approaches are limited by selecting a low-dimensional order parameter (e.g., assembly size) to describe the assembly kinetics and by neglecting the possibility of malformed intermediates. These assumptions can be justified if assemblies attain local equilibrium at each size, but in practice this is often not the case [182]. Approaches that do not make this assumption, such as particle-based molecular dynamics simulations, could be used to test coarse-grained model predictions.

C. Testing in experiments

Because our models are general and do not depend on specific molecular details, their predictions can apply to diverse experimental systems in which assembly subunits and a cargo preferentially partition into phase-separated condensates. As noted in the Introduction, there are cellular systems in which multiple components preferentially partition into condensates to promote co-assembly, such as clathrin cages and cargo during endocytosis [85,201–205]. Selective partitioning and exclusion of different molecules is a common feature among biomolecular condensates [78,124,135,181,206,207], and thus likely represents a general strategy for promoting desired reactions while simultaneously preventing undesirable ones. Because selectivity depends on K_c as $s \propto K_c^N$ [see Eq. (9)], this strategy is particularly advantageous for high-order reactions (such as viral genome packaging).

The biological systems that most directly motivate this work are viral condensates in which capsids assemble around the genome (e.g., Refs. [56,68,144]). Our theoretical predictions could be tested in these systems if there is a way to manipulate partition coefficients of viral RNAs and capsid proteins (perhaps via phosphorylation [208]) or by controlling other variables such as RNA concentration. Our predictions of how the subunit translation rate affects selectivity could potentially be tested by introducing ribosomes and varying their concentration.

We note that many dsDNA viruses first assemble empty capsids, and subsequently package their genomes using molecular motors [209]. In this case, condensates could still enhance empty capsid assembly (as predicted by our previous work [153,210]). Condensates could improve selectivity for the DNA as well, but typically the packaging motor is highly selective for viral DNA [209].

However, it would be more straightforward to directly test our predictions with *in vitro* experiments on phase-separated condensates, which enable more direct control over concentrations and partition coefficients. For example, the capsid proteins and RNA from several viral systems have been shown to form condensates *in vitro* [56,57,67,68]. Measuring packaging selectivity (which can be achieved using established protocols [1,4]) as a function of RNA and subunit concentrations would enable testing our prediction that condensates enable selective packaging over a wide range of concentrations. More broadly, DNA origami and protein design have recently been used to engineer subunits that assemble into capsids [211–219]. In the DNA origami system, the capsids were further engineered to assemble around cargoes of DNA or functionalized nanoparticles. Our predictions could be tested by engineering such subunits to favorably partition into the condensate (e.g., through DNA handles or other types of interactions), and two species of cargoes that have been functionalized to have different partition coefficients for the condensate. Recent work by Kelley *et al.* [181] has shown that nanoparticles can be driven to partition into protein condensates when their surfaces are functionalized with biotin or oligonucleotides [181]. The nanoparticle partition coefficients can be controlled by changing the number (biotin) or length (oligonucleotides) of surface molecules. Such nanoparticles would be particularly attractive candidates for cargo molecules, since they could be used to test our predictions for how selectivity depends on cargo partition coefficients.

D. Outlook

Given that subunit translation can enhance selective packaging, it would be interesting to extend our model to consider the timing of translation, since previous computational studies suggest that continuously modulating the concentration of available subunits can optimize self-assembly [28,220]. Further, to simplify a large parameter space, we have assumed a fixed RNA concentration in this work, but the model can be extended to account for RNA transcription. We have also assumed that the condensate properties are independent of the local concentration of viral components. However, studies of ebolavirus [69] and SARS-CoV-2 [221] have shown that the material properties of condensates and even condensate formation itself can be sensitive to the concentrations of, e.g.,

nucleic acids. Future work should assess the interplay between condensates' phase behavior and the local concentration of viral components. Finally, it will be important to test model assumptions, such as the influence of excluded volume [154] and RNA structure [24,222–225], using particle-based molecular dynamics simulations.

Such studies will advance our understanding of condensate-mediated assembly as a fundamental mechanism for selective co-assembly in biology. They also have practical implications for biomedicine and nanomaterials. Understanding LLPS could lead to new antiviral therapy approaches that block assembly and/or selective genome packaging, and could guide the development of condensates as a powerful approach for nanoscale packaging in human-engineered systems.

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DATA AVAILABILITY

The data that support the findings of this article are openly available [226].

APPENDIX A: RATE EQUATIONS

To model the kinetics of LLPS-coupled genome packaging, we use the following rate equations:

$$\frac{d\rho_1^\alpha}{dt} = \mathcal{N}_1^\alpha + \mathcal{D}_1^\alpha + \mathcal{T}^\alpha, \quad (\text{A1a})$$

$$\frac{d\rho_{v,(n,m)}^\alpha}{dt} = \mathcal{N}_{v,(n,m)}^\alpha + \mathcal{M}_{v,(n,m)}^\alpha + \mathcal{D}_{v,(n,m)}^\alpha, \quad (\text{A1b})$$

where $v = \{\text{cog}, \text{NC}\}$ and $\alpha = \{\text{c}, \text{bg}\}$. The quantities $\mathcal{N}_1^\alpha$, $\mathcal{D}_1^\alpha$, and $\mathcal{T}^\alpha$ represent, respectively, the rate of change in free subunit concentration due to adsorption and desorption, the diffusive exchange of free subunits, and (when applicable) the rate of subunit translation from mRNA molecules:

$$\begin{aligned} \mathcal{N}_1^\alpha = & -k_{\text{ads}}\rho_1^\alpha \sum_{m=0}^N \sum_{n=0}^{N-m} (N - (n+m)) \sum_{v=\text{cog}, \text{NC}} \rho_{v,(n,m)}^\alpha \\ & + k_{\text{des}} \sum_{m=0}^N \sum_{n=0}^{N-m} n \sum_{v=\text{cog}, \text{NC}} \rho_{v,(n,m)}^\alpha, \end{aligned} \quad (\text{A2a})$$

$$\mathcal{D}_1^\alpha = \begin{cases} \frac{1}{V_c} k_{\text{DL},1} (\rho_1^{\text{bg}} - \rho_1^{\text{c}}/K_c), & \alpha = \text{c} \\ -V_r \mathcal{D}_1^{\text{c}}, & \alpha = \text{bg}, \end{cases} \quad (\text{A2b})$$

$$\mathcal{T}^\alpha = k_{\text{tr}} \sum_{m=0}^{N-1} \sum_{n=0}^{N-m} \rho_{\text{cog},(n,m)}^\alpha. \quad (\text{A2c})$$

TABLE I. Simulation parameter values and descriptions. Parameters without explicitly listed units (in parentheses) are dimensionless.

Parameter	Value(s)	Description
ρ_T (μM)	10^{-3} – 100	total capsid subunit concentration
$\rho_{\text{cog},T}$ (μM)	5×10^{-5} – 5	total cognate RNA concentration
$\rho_{\text{NC},T}$ (μM)	4.5×10^{-4} – 45	total noncognate RNA concentration
V_{tot} (μm^3)	4190	total volume
V_r	10^{-3}	condensate size ratio
V_c (μm^3)	4	condensate volume
V_{bg} (μm^3)	4186	background volume
K_c	1–100	subunit partition coefficient
K_{cog}	1–100	cognate RNA partition coefficient
K_{NC}	0.01–1	noncognate RNA partition coefficient
g_{nuc} ($k_B T$)	–5	subunit binding free energy in nucleation phase
g_{elong} ($k_B T$)	–10	subunit binding free energy in elongation phase
g_N ($k_B T$)	–20	binding free energy of final subunit
g_{ads} ($k_B T$)	–3.5	adsorption free energy
G_N ($k_B T$)	–200	free energy of assembled capsid
k_{ads} ($\mu\text{M}^{-1}\text{s}^{-1}$)	10^6	adsorption rate constant
N	20	number of subunits in complete capsid
n_{nuc}	3	critical nucleus size
f_{cog}	0.1	ratio of cognate RNA to total RNA
D_1 ($\mu\text{m}^2/\text{s}$)	10	subunit diffusion constant
D_{RNA} ($\mu\text{m}^2/\text{s}$)	1	RNA diffusion constant
f_0 (s^{-1})	10^4	assembly rate constant

Here, k_{ads} is the rate constant for adsorption of subunits to RNA, and g_{ads} is the adsorption free energy; the desorption rate constant $k_{\text{des}} = \rho_{\text{SS}} k_{\text{ads}} e^{\beta g_{\text{ads}}}$, with the standard state concentration $\rho_{\text{SS}} = 1\text{M}$, follows from detailed balance. The sums over n run over the number of adsorbed (but unassembled) subunits; the sums over m run over the number of assembled subunits, and the prime symbol indicates that the sum excludes $m = 1$ (there are no single assembled subunits); and the sums over v run over {cog, NC}. For simplicity, we assume that a maximum of N subunits can adsorb to a single RNA molecule. Thus, the sums over n have an upper limit of $N - m$. The diffusion-limited rate constant $k_{\text{DL},1} = 4\pi R_c D_1$ sets the speed at which subunits enter the condensate. The condensate radius is given by $R_c = (V_c/(4\pi/3))^{1/3}$, and we

set $D_1 = 10 \mu\text{m}^2/\text{s}$ (a typical value for proteins in the cytoplasm BNID 112087 [176]). Finally, k_{tr} is the translation rate constant. We consider the effects of translation in Sec. III C; in other cases, we set $k_{\text{tr}} = 0$. Note that when we do include translation, we assume that RNA with adsorbed and partially assembled subunits is still accessible for translation. We have tested this assumption by using alternative models in which either (1) only RNA which has no adsorbed subunits or (2) all RNA except those which are fully encapsidated are accessible for translation, and we find very similar behavior for all three models (see Fig. S7 [162]).

The quantities $\mathcal{N}_{v,(n,m)}^\alpha$, $\mathcal{M}_{v,(n,m)}^\alpha$, and $\mathcal{D}_{v,(n,m)}^\alpha$ are the rates of change of RNA concentrations due to adsorption, assembly, and RNA diffusion, respectively:

$$\mathcal{N}_{v,(n,m)}^\alpha = k_{\text{ads}} \rho_1^\alpha \rho_{v,(n-1,m)}^\alpha - k_{\text{ads}} \rho_1^\alpha \rho_{v,(n,m)}^\alpha - k_{\text{des}} \rho_{v,(n,m)}^\alpha + k_{\text{des}} \rho_{v,(n+1,m)}^\alpha, \quad (\text{A3a})$$

$$\mathcal{M}_{v,(n,m)}^\alpha = \begin{cases} b_{n-2,2} \rho_{v,(n-2,2)}^\alpha - f_{n+2,0} \rho_{v,(n+2,0)}^\alpha, & m = 0 \\ f_{n+2,0} \rho_{v,(n+2,0)}^\alpha - (f_{n,m} + b_{n,m}) \rho_{v,(n,m)}^\alpha + b_{n-1,m+1} \rho_{v,(n-1,m+1)}^\alpha, & m = 2 \\ f_{n+1,m-1} \rho_{v,(n+1,m-1)}^\alpha - (f_{n,m} + b_{n,m}) \rho_{v,(n,m)}^\alpha + b_{n-1,m+1} \rho_{v,(n-1,m+1)}^\alpha, & m > 2 \\ f_{n+1,N-1} \rho_{v,(n+1,N-1)}^\alpha - b_{n,N} \rho_{v,(n,N)}^\alpha, & m = N, \end{cases} \quad (\text{A3b})$$

$$\mathcal{D}_{v,(n,m)}^\alpha = \begin{cases} \frac{1}{V_c} k_{\text{DL,RNA}} (\rho_{v,(n,m)}^\alpha - \rho_{v,(n,m)}^{\text{bg}}) / (K_v K_c^{(n+m)}), & \alpha = \text{c} \\ -V_r \mathcal{D}_{v,(n,m)}^\alpha, & \alpha = \text{bg}. \end{cases} \quad (\text{A3c})$$

The RNA diffusion-limited rate constant is $k_{\text{DL,RNA}} = 4\pi R_c D_{\text{RNA}}$. We assume for simplicity that $D_{\text{RNA}} = 1 \mu\text{m}^2/\text{s}$, independent of how many subunits are adsorbed; the lower value of D_{RNA} compared to D_1 reflects the larger size of RNA compared to capsid proteins.

Molecular dynamics simulations have shown that capsid assembly on nucleic acids and other polymers involves a complex dynamics including cooperative polymer-subunit motions [24,179,227,228]. Importantly though, subunit association rates depend primarily on the concentration of

adsorbed but unassembled subunits, with a weak dependence on polymer length [227]. Therefore, for the simple model in this work we follow Dykeman *et al.* [229] and assume that intermediates can only associate with subunits at adjacent sites on the polymer. The forward assembly rates are then given by

$$f_{n,m} = \begin{cases} f_0 n / (N - m), & 0 < m < N \\ f_0 n (n - 1) / (2N), & m = 0, n > 0 \\ 0, & \text{otherwise.} \end{cases} \quad (\text{A4a})$$

Here the term $n / (N - m)$ reflects the probability that an adsorbed subunit is adjacent to the assembly intermediate; the additional factor $(n - 1) / 2$ in the second line of Eq. (A4a) accounts for the fact that dimerization can happen anywhere along the RNA molecule. The term f_0 is the association rate constant given the presence of an adjacent subunit. We set $f_0 = 10^4 \text{s}^{-1}$ (see Sec. S4 [162] and Ref. [229] for an explanation of this estimate).

The dissociation rate constants then follow from detailed balance as

$$b_{n,m} = \begin{cases} f_0 e^{\beta g_m}, & m > 0 \\ 0, & \text{otherwise.} \end{cases} \quad (\text{A4b})$$

1. Assembly model

Following Refs. [168–174], the assembly reaction is split into nucleation and elongation (or growth) phases to reflect the fact that smaller intermediates have fewer attractive contacts per subunit and are thus less stable compared to larger intermediates. Therefore, below a critical nucleus size n_{nuc} , the excess free energy of subunit binding is unfavorable at productive reaction conditions, whereas association in the elongation phase is favorable, driving forward-biased assembly. We set $n_{\text{nuc}} = 3$, $N = 20$, $g_{\text{nuc}} = -5k_B T$, $g_{\text{elong}} = 2g_{\text{nuc}}$, and $g_N = 4g_{\text{nuc}}$. The especially favorable value of g_N reflects the fact that in most capsid structures, the final subunit makes more contacts than previous steps in the elongation phase. We assume that g_{ads} and the subunit-subunit affinities are the same in the condensate and background.

This model assumes a single simplified assembly pathway, with a single intermediate per RNA molecule. While the approach can be extended to allow for additional assembly pathways [230], this can be done more efficiently using stochastic simulations [229,231–233]. Moreover, the spatial structure of assembly can be accounted for using particle-based MD simulations (e.g., Refs. [24,179,210,223,224,227,234–239]) or the reaction-diffusion framework NERDDS [240]. Accounting for the effects of RNA on assembly pathways and intermediate structures will be most straightforward to accomplish using MD simulations (e.g., Refs. [24,179,180,223–225,241,242]). We have also assumed that association of subunits in solution to intermediates occurs at negligible rates in comparison to RNA-bound subunits. Furthermore, we neglect the possibility of empty capsids since these will be present at extremely low populations in comparison to RNA-filled capsids for the parameters we study: the relative equilibrium concentrations of empty and filled capsids is $\frac{\rho_N^{\text{(filled)}}}{\rho_N^{\text{(empty)}}} = \rho_{\text{RNA},f} \exp(\beta N g_{\text{ads}}) \ll 1$, with $\rho_{\text{RNA},f}$ the free RNA concentration [175,243]. Consistent with this prediction, most viruses that use LLPS do not assemble empty virions.

APPENDIX B: YIELDS IN CONDENSATE AND BACKGROUND

Condensate cognate/noncognate and background cognate/noncognate yields are defined as

$$\begin{aligned} x_{\text{enc}}^{v,c} &= \frac{\rho_N^{v,c}}{\rho_{v,T}} \frac{V_r}{1 + V_r}, \\ x_{\text{enc}}^{v,bg} &= \frac{\rho_N^{v,bg}}{\rho_{v,T}} \frac{1}{1 + V_r}, \end{aligned} \quad (\text{B1})$$

where $\rho_N^{v,\alpha}$ is the concentration of capsids enclosing RNA of species $v = \{\text{cog}, \text{NC}\}$ in phase $\alpha = \{\text{c}, \text{bg}\}$. Figure S3 [162] shows $x_{\text{enc}}^{v,c}$ and $x_{\text{enc}}^{v,bg}$ versus time for moderate and high RNA concentrations. At moderate RNA concentrations, there is no assembly in the background at any time; at high RNA concentrations, there is a small yield in the background at short times, but these capsids quickly diffuse into the condensate.

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