ATP-triggered stress granule formation via phase separation

Jean David Wurtz
Department of Bioengineering, Imperial College London, South Kensington Campus, London SW7 2AZ, U.K.

Chiu Fan Lee
Department of Bioengineering, Imperial College London, South Kensington Campus, London SW7 2AZ, U.K.
E-mail: c.lee@imperial.ac.uk

Abstract. Stress granules (SG) are droplets of proteins and RNA that form in the cell cytoplasm during stress conditions. We consider minimal models of stress granule formation based on the mechanism of phase separation regulated by ATP-driven chemical reactions. Motivated by experimental observations, we identify a minimal model of SG formation triggered by ATP depletion. Our analysis indicates that ATP is continuously hydrolysed to deter SG formation under normal conditions, and we provide specific predictions that can be tested experimentally.

Submitted to: New J. Phys.
1. Introduction

In order to function, a biological cell has to organise its interior contents into compartments that can dynamically evolve depending on the environment and moment in the cell cycle. For example, cytoplasmic stress granules (SG) form when the cell is under stress [1] and P granules localise to one side of the embryo of *C. elegans* prior to the first cell division [2] [3] [4]. Both SG and P granules belong to the class of ribonucleoprotein (RNP) granules. RNP granules consist of RNA and RNA-binding proteins and lack a surrounding membrane. Recent experiments provided strong evidence that the intriguing mechanism behind their formation and dynamical regulation, in the absence of a membrane, is based on the physical phenomenon of phase separation [5] [6]. Phase separation refers to the spontaneous partitioning of a system into compartments of distinct macroscopic properties, such as the condensation of water vapour into droplets when fog forms. Phase separation under equilibrium condition is a well-understood phenomenon [7], however the phase separation in the cytoplasm can be driven out of equilibrium by a number of effects that include activity of motor proteins shuttling along the cytoskeleton [8], and chemical reactions affecting the phase-separating behaviour of RNP granule constituents [9] [10] [11] [12] [13].

In this paper, we apply the theoretical formalism developed in [13] to investigate minimal models of SG regulation based on non-equilibrium, chemical reaction-controlled phase separation. SG are a particularly dynamic type of RNP granules as they form quickly, in the order of 10 min, when the cell is under stress (e.g., heat shock, chemical stress, osmotic shock, etc.), and also dissolve away rapidly when the stress is removed [14] [15]. The cell’s reaction to external stresses by forming SG is important for its survival [16] [1]. Although specific functions of SG remain unclear, they are thought to be involved in protecting messenger-RNA by recruiting them into SG away from harmful conditions [17] [18] [19]. In addition, SG malfunction is associated with several degenerative diseases such as amyotrophic lateral sclerosis and multisystem proteinopathy [20].

1.1. Experimental observations

Experimental studies have shown that SG assemble in response to multiple types of stress situations [21] [22] [23]. Several pathways for SG formation have been identified but the precise mechanisms of SG formation remain unclear. Interestingly, while various stress conditions causing SG assembly also cause a depletion of the cytoplasmic ATP concentration [21] [24] [25]. ATP depletion alone has been shown to trigger SG formation [23]. Here, we will focus exclusively on how changes in ATP levels can regulate SG formation. In other words, we will make the assumption that the ATP concentration directly triggers SG formation through ATP-dependent biochemical reactions. In addition to this central premise, we will use two other pieces of biological observations to guide our modelling: 1) During normal conditions, i.e. without imposed stress, the ATP concentration is at the normal level and SG are absent, or at least are so small that they are undetectable microscopically. 2) When external stress is imposed, ATP can fall by 50% [21] [25], and SG assemble, with sizes of the order of a micrometer [16]. Hence a relatively mild change in the ATP level can lead to a very large change in SG size. We will therefore impose the following two constraints on our modelling: 1) a drop in the ATP level leads to the growth of SG, and 2) the response is switch-like.
Figure 1. (a) Distinct schemes of ATP-driven chemical reactions that control cytoplasmic SG formation. Phase-separating form of the constituent molecules in SG is denoted by $P$ and the soluble form is denoted by $S$. In model A, ATP promotes conversion from $P$ to $S$, while it is the reverse in model B. In model C, ATP drives both conversions. (b) Phase diagram. At low $P$ concentration, molecules distribute homogeneously (‘□’ symbol). When the concentration of $P$ increases beyond the phase boundary denoted by the dashed line, $P$-rich droplets form and are surrounded by a $P$-poor phase (‘△’ symbol).

We now set out to construct minimal models that are compatible with these salient experimental findings on SG regulation.

2. Minimal models

We first describe a set of minimal models of SG regulation based on the principle of phase separation controlled by ATP-driven chemical reactions. A similar reaction scheme has recently been employed to study centrosomes [10], and the associated physics has started to be explored in [10, 13]. We consider the cell cytoplasm as a ternary mixture of molecules: the phase-separating form of the SG constituent molecules ($P$), the soluble form of the same molecules ($S$), and the remaining molecules in the cytoplasm ($C$). Note that the constituents of SG consist of many distinct proteins and mRNA [14]. Therefore, $P$ and $S$ are meant to represent the average behaviour of the set of proteins and mRNA responsible for SG formation via phase separation [26]. The same applies to the component $C$, which represents the average behaviour of the cytoplasmic molecules not involved in SG formation. To control phase separation, we further assume that $P$ and $S$ can be inter-converted by chemical reactions that are potentially ATP-driven:

$$P \xrightleftharpoons[k_h]{k} S$$

Stress granule formation via phase separation

where \( k, h \) denote the forward and backward reactions. In the biological context these reactions can be protein post-transcriptional modifications. For example the phase behaviour (phase-separated or homogeneous) of intrinsically disordered proteins, a class of proteins that lack a well defined secondary structure, can be controlled via their phosphorylation/dephosphorylation \[27, 28\]. In our minimal description, we assume that there is no cooperativity in the chemical reactions, i.e. \( k, h \) do not depend on the concentrations of the molecular components. However, the rates can be influenced by the ATP concentration, denoted by \( \alpha \), in a linear manner. With these simplifications, we can categorise the distinct schemes into three models: A, B, and C (Fig. 1(a)). In model A, ATP promotes the conversion from the phase-separating state \( P \) to the soluble state \( S \). In model B, ATP promotes the reverse reaction, and in model C, both reactions are driven by ATP. Specifically, we have:

Model A:
\[
\begin{align*}
    k_A(\alpha) &= \alpha K_A, \\
    h_A(\alpha) &= H_A
\end{align*}
\]

Model B:
\[
\begin{align*}
    k_B(\alpha) &= K_B, \\
    h_B(\alpha) &= \alpha H_B
\end{align*}
\]

Model C:
\[
\begin{align*}
    k_C(\alpha) &= \alpha K_C, \\
    h_C(\alpha) &= \alpha H_C
\end{align*}
\]

with \( K_i, H_i \) being constants, where \( i = A, B, C \) refers to the model under consideration.

3. Steady-state of a multi-droplet system

Given the three minimal models depicted in Fig. 1(a), we now aim to determine which one of them is the most compatible with experimental observations. To do so, we will first elucidate the salient features of the mechanism of chemical reaction-driven phase separation by considering a single phase-separated droplet of radius \( R \) co-existing with the dilute medium.

Denoting the concentrations of \( P \) and \( S \) by the same symbols, we assume they obey the reaction-diffusion equations:

\[
\begin{align*}
    \partial_t P_{\text{in/out}} &= D \nabla^2 P_{\text{in/out}} - k_i(\alpha) P_{\text{in/out}} + h_i(\alpha) S_{\text{in/out}} \\
    \partial_t S_{\text{in/out}} &= D \nabla^2 S_{\text{in/out}} + k_i(\alpha) P_{\text{in/out}} - h_i(\alpha) S_{\text{in/out}}
\end{align*}
\]

where \( i = A, B, C \) and the subscripts “in” and “out” denote the concentration profiles inside and outside the drop, and \( D \) is the protein diffusion coefficient assumed to be equal for both molecules \( P \) and \( S \), both inside and outside the droplet. In addition to the dynamical equations, we need to supply the boundary conditions. To this end, we assume further that the system is close to equilibrium to the extent that local thermal equilibrium applies around the interfacial region, which means that

\[
\begin{align*}
    P_{\text{in}}(R_-) &= \hat{P}_{\text{in}} \\
    P_{\text{out}}(R_+) &= \hat{P}_{\text{out}} \left(1 + \frac{\ell_c}{R}\right) \\
    S_{\text{in}}(R_-) &= S_{\text{out}}(R_+),
\end{align*}
\]

where we have assumed spherical symmetry with the centre of the droplet being the origin of our coordinate system. In the equations above, \( R_\pm \equiv R \pm \epsilon \) with \( \epsilon \) being an infinitesimal constant. In other words, \( R_+ \) denotes the position just outside the droplet and \( R_- \) denotes the position just inside the droplet. \( \hat{P}_{\text{in/out}} \) are the equilibrium coexistence concentrations given by the phase boundary (see Fig. 1(b)) and Eq. (7) is
Chemical reactions induce concentration gradients inside and outside a phase-separated droplet. The reaction-diffusion equations (Eqs (5)) solved with the local thermal equilibrium conditions at the interface (Eqs (6)-(8)) predict concentration gradients of length scale $\xi$ (double arrow, Eq. (11)), and subsequent in-flux of $P$ (red arrow) and out-flux of $S$ (blue arrow) at the droplet’s interface.

The Gibbs-Thomson relation with $l_c$ being the capillary length. In addition, we assume that $S$ is inert to phase separation so that its concentration profile is continuous at the interface, hence Eq. (3). The boundary conditions above are further complemented by the no-flux boundary conditions:

$$0 = \partial_r P_{\text{in}}(r) \bigg|_{r=0} = \partial_r S_{\text{in}}(r) \bigg|_{r=0} = \partial_r P_{\text{in}}(r) \bigg|_{r=L} = \partial_r S_{\text{in}}(r) \bigg|_{r=L},$$

where $L$ is the system size and $r$ is the distance from the droplet’s centre.

Another crucial approximation we will employ is the quasi-static assumption as done in the Lifshitz-Slyozov theory for equilibrium phase separation [29], which assumes that the time scale at which concentration profiles reach a steady-state is much faster than the time scale at which a droplet radius changes by a non-negligible amount. With this separation of time scale, we only need to solve for the steady-state solutions of Eqs (5) in order to study the droplet’s dynamics in the system.

Setting the left hand sides of Eqs (5) to zero, the generic solutions for $P_{\text{in/out}}$, assuming spherical symmetry, are of the form [13]

$$P_{\text{in/out}}(r) = U_0 + \frac{R}{r} \left( U_1 e^{r/\xi} + U_{-1} e^{-r/\xi} \right)$$

where

$$\xi = \sqrt{\frac{D}{k_i + h_i}},$$

which corresponds to the length scale of the concentration gradients, and $U_n$ are independent of $r$ but model-parameter dependent. Specifically, $U_n$ depend on $k_i, h_i, P_{\text{in}}, P_{\text{out}}, l_c, L$ and $R$ as well as the overall combined concentration $\phi$ of both phase-separating and soluble forms of the molecules.

At the steady state, $U_n$ are determined by the boundary conditions and the molecule number conservation. Note that there are three constants to be determined.
in Eq. (10) because we started with two coupled second-order differential equations with the constraint that the total number of molecules $P$ and $S$ in the system is fixed. Eq. (10) indicates that chemical reactions affect the concentration profiles and introduce a new length scale ($\xi$) into the problem.

At thermal equilibrium ($k, h = 0$), a finite, phase-separating system in the nucleation and growth regime (which is the regime relevant to our biological context) can only be in two steady-states: either the system is well-mixed (i.e., no granules) or a single granule enriched in $P$ co-exists with the surrounding cytoplasm that is dilute in $P$ [7]. Furthermore, due to surface tension, there exists a critical radius $R_c$ below which droplets are no longer thermodynamically stable. The critical radius can be estimated as a trade off between the surface energy ($\propto R^2$) that penalises having two phases and the bulk free energy in the droplet ($\propto R^3$) that promotes droplet formation.

As a result, in the early stage of phase separation when the mixture is homogeneous, droplets larger than $R_c$ need to be nucleated either by the stochastic fluctuations of the concentrations in the case of homogeneous nucleation, or by the help of a third party such as impurities or other proteins or RNA acting as an aggregation site in the case of heterogeneous nucleation. Once multiple droplets are nucleated, a multi-droplet system is always unstable and coarsen by Ostwald ripening [29] – the mechanism by which large droplets grow while small droplets dissolve – and/or coalescence of droplets upon encountering via diffusion [30]. Since the diffusion of protein complexes in the cytoplasm is strongly suppressed [31], we will ignore droplet diffusion completely here and focus on Ostwald ripening. The phenomenon of Ostwald ripening is caused by 1) the Gibbs-Thomson effect that dictates that the solute concentration ($P$) outside a large droplet is smaller than that outside a small droplet (Eq. (7)); and 2) the dynamics of the solute in the dilute medium is well described by diffusion (Eq. (5a) with $k = h = 0$). These two effects combined lead to diffusive fluxes of solute from small droplets to large droplets and thus eventually a single droplet survives in a finite system [29].

Surprisingly, Ostwald ripening can be suppressed when non-equilibrium chemical reactions are present ($k, h > 0$) [32][10][13]. The physical reason lies in the new diffusive fluxes set up by the chemical reactions. For instance, $P$ is highly concentrated inside the droplets, and gets converted to $S$ continuously in the interior. At the steady-state, the depletion of $P$ inside the droplet has to be balanced by the influx of $P$ from the outside medium. Intuitively, we expect that the depletion of $P$ inside a droplet to scale like the droplet volume ($\propto R^3$), and the influx of $P$ through its droplet boundary to scale like the surface area ($\propto R^2$). Taken together, we can see that as $R$ increases, the depletion rate of a droplet will eventually surpass the influx of molecules from the medium and thus the growth will stop at a steady radius. As a result, multiple droplets of around the same size can co-exists in the system. We note that the above argument, although qualitatively correct, ignore pre-factors that also depend on the droplet radius $R$, which means that the quantitative calculation is more involved. Indeed, the influx of $P$ actually scales like $R$ instead of $R^2$ [13].

An important consequence of having a stable system of multiple droplets is that the overall concentrations of $P$ and $S$ in the whole system, denoted by $\bar{P}$ and $\bar{S}$ respectively, are controlled solely by the chemical reaction rates, as we now demonstrate. In a one droplet system,

$$\bar{P}V = \int_{0 \leq r < R} d^3r P_{\text{in}}(r) + \int_{R \leq r \leq L} d^3r P_{\text{out}}(r)$$  \hspace{1cm} (12)
where $V = 4\pi L^3/3$ is the system’s volume. Now, by integrating over the steady-state solution to Eq. (5a), we obtain
\[ 0 = \int_{0 \leq r < R} d^3r \left[ D \nabla^2 P_{\text{in}}(r) - k_i(\alpha)P_{\text{in}}(r) + h_i(\alpha)S_{\text{in}}(r) \right] \]
\[ + \int_{R \leq r \leq L} d^3r \left[ D \nabla^2 P_{\text{out}}(r) - k_i(\alpha)P_{\text{out}}(r) + h_i(\alpha)S_{\text{out}}(r) \right] \]
(13)
(14)
Since in the steady-state, the gradients of $P_{\text{in}}$ and $P_{\text{out}}$ at the interface have to be identical, the diffusion terms in the squared brackets above cancel each other. The same conclusion applies to $\bar{S}$. Therefore, we have
\[ 0 = V \left[ -k_i(\alpha)\bar{P} + h_i(\alpha)\bar{S} \right] \, , \]
(15)
Denoting the overall concentration of SG constituent molecules (whether phase-separating or soluble) by $\phi = \bar{P} + \bar{S}$, we have
\[ \bar{P} = \frac{h_i(\alpha)}{k_i(\alpha) + h_i(\alpha)} \phi \, , \quad \bar{S} = \frac{k_i(\alpha)}{k_i(\alpha) + h_i(\alpha)} \phi \, . \]
(16)
In particular, the total concentration of phase-separating molecules $\bar{P}$ depends on the chemical reaction rates, the ATP concentration $\alpha$ as well as the model under consideration (A, B or C).

As a stable multi-droplet system can be viewed as a system with many copies of the one-droplet system, the above conclusion also holds when multiple droplets co-exist. We will now employ the formalism discussed to study the behaviour of the three minimal models introduced.

4. Model selection

4.1. Model B

We will start with model B. In this model, ATP drives the $S$ to $P$ conversion (i.e., $k_B = K_B$ and $h_B = \alpha H_B$). As such, a reduction in ATP will naturally suppress this conversion and thus lead to a decrease in $P$ and restrain phase separation. Therefore, depleting ATP cannot promote SG formation. As a result, we can eliminate this model since it contradicts our first biological constraint (Sec. 1.1).

4.2. Model C

In model C, ATP drives both conversions (i.e., $k_C = \alpha K_C$ and $h_C = \alpha H_C$). As a result, the overall concentrations $\bar{P}$ and $\bar{S}$ are independent of $\alpha$ (Eq. (16)). In fact, there are two qualitatively distinct regimes depending on the relative magnitude of $K_C/H_C$ and the parameter
\[ \lambda \equiv \frac{2(\phi - \bar{P}_{\text{out}})}{\bar{P}_{\text{in}}} \, . \]
(17)
4.2.1. $K_C/H_C \gg \lambda$ regime (Fig. 3(a)). This regime corresponds to the situation where the gradient length scale $\xi$ is much larger than the droplets [13], so droplets can be assumed to be homogeneous in concentrations ($P_{\text{in}}(r) = \bar{P}_{\text{in}}$). However, concentration gradients in the cytoplasm can be significant and by increasing $\alpha$, the
subsequent decrease in \( \xi \) leads to steep gradients in the cytoplasm (Fig. 2). Since the concentration of \( P \) is fixed at the interface, the gradients result in a higher cytoplasmic concentration of \( P \) away from the drop. As the total number of \( P \) is constant in this model, the droplet must shrink to compensate. Indeed, using the quantitative method developed in [13], we know that droplets tend to shrink as \( \alpha \) is increased. In Fig. 3(a) we show the range of stable droplet radii in a multi-droplet system as \( \alpha \) varies. Note that the droplet number density is variable and depends on the nucleation process and potentially the coarsening kinetics.

Similar to the equilibrium case \( (k, h = 0) \), there exists a critical radius \( R_c \) (red irregular dashed line) below which droplets dissolve (downward arrows). Systems with droplets larger than \( R_c \) are unstable and coarsen via Ostwald ripening leading to an increase of the droplet size (upward arrows). Above a critical radius (black slanted dashed line) there is a region where multi-droplet systems are stable (grey area) and this region is bounded by a maximal radius \( R_u \) (red continuous line) such that droplets larger than \( R_u \) shrink (downward arrows). Specifically, \( R_u \) has the following scaling form [13]:

\[
R_u \propto \alpha^{-1/2}.
\]

Namely, a fall of \( \alpha \) increases the size of stable SG, thus satisfying our first constraint discussed in Sect. 1.1. However, this ATP-controlled growth is sub-linear. For instance, to decrease the maximal SG radius \( R_u \) by two-fold, a four-fold decrease in ATP concentration is required. Therefore, depletion of ATP according to the scaling
relation in Eq. (18) alone cannot account for the switch-like behaviour, which is our second biological constraint.

However, we cannot yet rule out this model because of another intriguing feature of this type of non-equilibrium phase-separating systems. When \( \alpha \) is greater than a critical value \( \alpha_c \), even though the overall concentrations \( \bar{P} \) and \( \bar{S} \) remain constant, one can still eliminate droplets completely by quenching the stable radii below the nucleus radius \( R_c \). An estimate of an upper bound of \( R_c \) can be given by the smallest granule observed, which we take to be of the order 100 nm [16]. As we demonstrate in Appendix A.1, the scaling law (Eq. (18)) remains valid until \( R_u \simeq R_c \) so we can use it to estimate the maximal SG size that would form upon varying \( \alpha \) by a factor of two in the vicinity of \( \alpha_c \). As a conservative estimate, if we assume that the tip of the phase boundary where \( \alpha = \alpha_c \) (Fig. 3) corresponds to the ATP concentration in normal conditions, and that the corresponding droplet size is \( R_c \), then a reduction of 50% of \( \alpha \) can only lead to a maximal SG radius of around 100 \( \times 2^{1/2} \approx 140 \) nm according to the upper bound law \( R_u \). This radius is too small compared to experimental observations (Sec. 1.1) and we thus rule out model C in this regime.

### 4.2.2. \( K_C/H_C \ll \lambda \) regime (Fig. 3(b))

This regime corresponds to the case where gradients can be significant inside droplets (\( \xi \ll R \)) [13]. Here, \( \alpha \) also controls the droplet stability but droplets have unbounded radii and cannot be dissolved irrespectively of \( \alpha \). Since one cannot control droplet assembly and dissolution based on the magnitude of \( \alpha \) we can eliminate model C in this regime as well.

In summary, we have shown that model C does not provide the switch-like response compatible with our second biological constraint (Sec. 1.1). We can therefore rule out this particular model.

### 4.3. Model A

In model A only the forward reaction rate \( k \) is amplified by an increase of \( \alpha \) and the backward rate is constant (i.e., \( k_A = \alpha K_A \) and \( h_A = H_A \)). Again, there are two distinct regimes, depending on the magnitude of \( H_A \) relative to the parameter

\[
\kappa \equiv \frac{D}{l_c^2} \left( \frac{\phi - \bar{P}_{\text{out}}}{P_{\text{out}}} \right)^2. \tag{19}
\]

#### 4.3.1. \( H_A \ll \kappa \) regime (Fig. 4(a))

When \( \alpha \) is larger than the value \( \alpha^* = \lambda H_A/K_A \), droplets are always much smaller than \( \xi \) so the mechanism that control droplet size is similar to the one discussed in model C [13]: increasing \( \alpha \) increases cytoplasmic gradients that enrich the cytoplasm in \( P \), leading to droplet shrinkage. However a major difference in model A is that the overall \( P \) concentration (\( \bar{P} \)) is not fixed any more but decreases as \( \alpha \) increases (Eq. (16)). Compared to model C we therefore expect that the droplet radius decreases more drastically as \( \alpha \) increases. This is indeed the case and the maximal radius of a stable droplet is [13]:

\[
R_u \propto \sqrt{\frac{\bar{P} - \bar{P}_{\text{out}}}{\alpha}} \tag{20}
\]

\[
= \sqrt{\frac{1}{\alpha} \left( \frac{H_A \phi}{\alpha K_A + H_A} - \bar{P}_{\text{out}} \right)}. \tag{21}
\]
Stress granule formation via phase separation

Figure 4. Stability diagram of model A. Two regimes can be distinguished depending on the magnitude of $h_0$ with respect to the parameter $\kappa$ (Eq. 19). (a) $H_A \ll \kappa$: droplets can exist below a critical ATP concentration $\alpha_c$ (black vertical dashed line). Droplets of radius smaller than the nucleus radius $R_c$ (discontinuous red curve), or larger than the maximal radius $R_u$ (continuous red curve) are unstable and dissolve (downward arrows). Droplets larger than $R_c$ but smaller than a critical radius (black dashed curve) are unstable and coarsen via Ostwald ripening, leading to an increase of the average droplet radius (upward arrows). There exists another critical ATP concentration $\alpha^*$ below which droplets radius are not maximally bounded. (b) $K_A/H_A \ll \lambda$: droplets can exist only below a critical ATP concentration $\alpha_c$ and are always unstable and coarsen via Ostwald ripening (upward arrows). Parameters: $\phi = 0.2 \, \mu M$, $\hat{P}_{\text{out}} = 0.04 \, \mu M$, $\hat{P}_{\text{in}} = 40 \, \mu M$, $l_c = 1 \, \text{nm}$, $D = 1 \, \mu m^2s^{-1}$, $K_A = 5 \times 10^{-3} \, \text{mM}^{-1}s^{-1}$. (a): $H_A = 5 \times 10^{-3} \, \text{s}^{-1}$. These parameters are meant to be generic in order to elucidate the system’s behaviour.

The droplet shrinkage as $\alpha$ increases is more pronounced than in model C due to the additional $\alpha$ in the denominator of $\hat{P}$ (Eq. (16)). When $\alpha$ is greater than a critical value $\alpha_c$ (vertical dashed line in Fig. 4(a)), then $P \leq \hat{P}_{\text{out}}$ and all droplets dissolve ($R_u = 0$), which corresponds to the situation where the system falls outside the equilibrium phase-separating region (Fig. 4(a), ‘□’ symbol). By equating $R_u$ to zero we find the expression of $\alpha_c$:

$$\alpha_c = \left( \frac{\phi}{\hat{P}_{\text{out}}} - 1 \right) \frac{H_A}{K_A}. \tag{22}$$

As a result, in model A droplet dissolution can be achieved by depleting $P$ to the extent that the system crosses the equilibrium phase boundary. This suggests a stronger response than in model C which we quantify in Appendix A.2. In particular we show that the ratio $R_a/R_c$ for $\alpha \lesssim \alpha_c$ does not have to be of order 1 as in model C but is a function of the system parameters $\hat{P}_{\text{out}}, \hat{P}_{\text{in}}, \phi, D, l_c$ and $H_A$. Therefore in the $H_A \ll \kappa$ regime of model A, droplets can be formed in a switch-like manner by a two-fold decrease of $\alpha$, satisfying both our biological constraints (Sec. 1.1). Interestingly, for $\alpha < \alpha^*$ the size of stable droplets is not bounded ($R_u \to \infty$), which provides a strong experimental prediction that we will discuss in Sec. 5.1.

4.3.2. $H_A \gg \kappa$ regime (Fig. 4(b)). This regime corresponds to the situation where the chemical reactions are of rates fast enough that the gradient length scale $\xi$ is smaller
than the nucleus radius $R_c$. Although there exists a critical ATP concentration $\alpha_c$ beyond which all droplets dissolve (vertical dashed line), a multi-droplet system is always unstable and coarsen via Ostwald ripening (upward arrows). This is not a desirable feature for the formation of stable cytoplasmic organelles and we therefore discard model A in this regime.

In summary, we conclude that among the three minimal models introduced, model A in the $H_A \ll \kappa$ regime is the best suited to describe the physics of ATP-triggered SG formation.

5. Summary & Discussion

Starting from experimental observations of SG formation in the cell cytoplasm, we have formulated three minimal models based on chemical reaction-controlled phase separation to account for the appearance of SG upon ATP depletion. Applying the formalism developed in [13], we compared the models based on their qualitative features to salient experimental observations.

We eliminated model B because it does not predict SG growth when ATP concentration falls. Model C was discarded because although SG grow during ATP depletion, the response is not switch-like. Finally, we found that model A, where ATP drives only the $P \to S$ conversion, can satisfy both biological constraints. However, we have ruled out the $H_A \gg \kappa$ regime in model A because droplets cannot be stable as they always coarsen via Ostwald ripening.

We are thus left with a unique scenario, i.e., model A with $H_A \ll \kappa$ and we will now use estimates of physiological parameters to elucidate a particular scenario of our model (Fig. 5). In normal conditions the ATP concentration $\alpha$ is at its basal value (blue arrow) leading to a low concentration of molecule $P$. As a result, the system is outside the phase-separating region (‘□’ in the insert figure) and no droplets can exist. Upon a two-fold decrease of ATP (red arrow) the concentration of $P$ increases, thus taking the system inside the phase-separating region (‘△’). SG can form via the nucleation of small droplets of radius $\sim 10$ nm. The nuclei then grow and coarsen (upward black arrow) leading to stable SG with much larger radii, between $\sim 1$ and $\sim 10\, \mu m$ (grey region), depending on the number of droplets nucleated. We thus find that the two biological constraints that SG must form when the ATP concentration decreases by two-fold, and in a switch-like manner, are both satisfied (Sec. 1.1). Furthermore, the quantitative predictions for the stable SG radii are consistent with experimental observations. Therefore, model A reproduces salient experimental observations of SG formation and dissolution based on the ATP level.

A peculiar feature of model A is that under normal condition, ATP is continuously hydrolysed to keep SG from forming. Superficially, it may seem wasteful energetically. However, this is in fact not dissimilar to any insurance schemes that we are familiar with. For instance, we pay a car insurance premium every month so that when an accident occurs, the damage cost is covered. This perspective is particularly pertinent for SG regulation since the timing of environmental stresses can be unpredictable. Furthermore, due to physical constraints such as cell size, storage of ATP for a long period of time is difficult. It may therefore be desirable to have survival mechanisms, such as SG formation, that are spontaneous and do not require additional ATP consumption for the formation of SG. Indeed, there is already experimental evidence suggesting that ATP can promote SG disassembly via ATP-dependent helicases [35], and the activity of dual specificity tyrosine-phosphorylation-regulated kinase
Figure 5. Among the three minimal models proposed, only model A in the $H_A \gg k$ regime can describe SG formation and dissolution upon two-fold variations of the ATP concentration $\alpha$. In this model phase-separating states $P$ are converted into soluble states $S$ at a rate proportional to $\alpha$. During normal conditions $\alpha$ is high (blue arrow) and there are few molecules $P$ so that the system does not phase separate (‘□’ symbol in insert). When $\alpha$ falls by a two-fold during environmental stresses (red arrow) the concentration of $P$ increases, making the system cross the phase boundary and SG assemble by phase separation (‘△’ symbol). SG nucleate from small droplets of radius of about 10 nm then grow and coarsen (black upward arrow) until they reach a stable radius between 1 and 10 $\mu$m (grey region). Parameters: $\phi = 2$ $\mu$M, $P_{\text{out}} = 0.4$ $\mu$M, $P_{\text{in}} = 40$ $\mu$M, $D = 6.5$ $\mu$m$^2$s$^{-1}$, $l_c = 3$ nm, $H_A = 5 \times 10^{-3}$ s$^{-1}$, $K_A = 4/3 \times 10^{-2}$ mM$^{-1}$s$^{-1}$. These parameters are chosen such that $\alpha_c = 1.5$ mM (Eq. 22) is a typical ATP concentration, $D$, $\phi$ and $P_{\text{out}}$ are typical protein diffusion coefficient and concentrations in the cytoplasm, $P_{\text{in}} = 100P_{\text{out}}$ [28, 33] and $l_c$ is according to [34]. $K_A$ and $H_A$ are such that when ATP is varied, the times needed for the concentrations $\bar{P}$ and $\bar{S}$ to reach a steady state ($\simeq 1/k, 1/h$) are smaller than the time scale of SG formation and dissolution ($\simeq 15$ min [14, 15]).
Stress granule formation via phase separation

On the other hand, there is also substantial evidence that at the early stage of stress initiation, the ATP-dependent kinase eukaryotic initiation factor alpha (EIF-alpha) is involved in SG formation. However, SG formation pathways independent of EIF-alpha also exists and ATP depletion have specifically been identified as one of them. Given all these evidence, the cell seems to have multiple mechanisms to ensure SG assembly and disassembly at stressful times and our work may describe a particular pathway of SG regulation. Nevertheless, the virtue of our model is that it leads to specific predictions that can be tested experimentally, which we will now enumerate.

5.1. Predictions

Our model provides the following experimental predictions:

(i) Since the $P \rightarrow S$ reaction is the one that requires the input of ATP, it is natural to relate the conversion to the ubiquitous ATP-driven phosphorylation reaction. In other words, our model suggests that the soluble state of the SG assembling constituents corresponds to the phosphorylated form of these constituents.

(ii) We predict the existence of a concentration gradient of the phase-separating constituent $P$ outside the SG, with the gradient length scale of the form

$$\xi = \sqrt{\frac{D}{\alpha K_A + H_A}},$$

(iii) If the inter-granule distance is much larger than the gradient length scale $\xi$ obtained in ii), then within the SG formation regime we predict a relationship between the maximal SG radius $R_u$ and the ATP level $\alpha$:

$$R_u \sim \alpha^{-1/2}.$$  

(iv) Finally, we predict that there exists a critical value of ATP concentration below which the upper bound on SG radius diverges. Specifically, the critical concentration is

$$\alpha^* = 2 \frac{\phi - \hat{P}_{out}}{P_{in}} \frac{H_A}{K_A}.$$  

The first prediction may be tested by screening the purified constituents of SG in an in vitro setting. The second, third and fourth predictions can be tested using imaging techniques with well regulated ATP concentration either in vivo or in vitro.

Appendix A. Largest SG to nucleus size ratio $R_u/R_c$ close to the droplet dissolution point $\alpha \approx \alpha_c$

In model A for $H_A \ll \kappa$ and model C for $K_C/H_C \gg \lambda$ we have seen that there exists a critical ATP concentration $\alpha_c$, beyond which no droplets can exist, and below which droplets can nucleate from a minimal radius $R_c$ and grow until they reach their stable radius that is upper bounded by $R_u$. Therefore one can control droplet formation and dissolution via small variations of $\alpha$ in the vicinity of $\alpha_c$ (Figs 3(a) and 4(a)). From experimental observations we know that during stress $\alpha$ vary by two-fold and $R_u \gg R_c$, providing a constraint on our modelling (Sec. 1.1). Here we examine this constraint by quantifying the size ratio $R_u/R_c$ in the vicinity of $\alpha_c$. 

[96x708]
Stress granule formation via phase separation

Figure A1. Fixed points of the droplet growth rate Eq. (A.1) in an infinite single-droplet system for varying ATP concentration $\alpha_c$ for model C in the $K_C/H_C \gg \lambda$ regime. The stable droplet radius $R_u$ (red continuous line) and the nucleus radius $R_c$ (red dashed line) correspond to the maximal stable radius and nucleus radius in a multi-droplet system (Fig. 3 (a)). No droplets exist for $\alpha$ larger than a critical value $\alpha_c$. For $\alpha \ll \alpha_c$, $R_u \simeq \sqrt{3D\Delta/(\hat{P}_{in}K_C/\alpha_c)}$ (upper black line, Eq. (A.5)) and $R_c \simeq R_{eq}^c$ (lower black line, Eq. (A.4)). When $\alpha \ll \alpha_c$, small variations of $k$ lead to strong variations of $R_u$ and $R_c$. In this strong response regime the ratio $R_u/R_c$ is bounded by $\sqrt{3D\Delta/(\hat{P}_{in}K_C/\alpha_c)}/R_{eq}^c$.

We consider a single droplet of radius $R$ in an infinite system. Its nucleus radius $R_c$ and stable radius $R_u$ are equal to the nucleus radius and maximal droplet radius in a multi-droplet system [13]. When droplets are much smaller than the gradient length scale $\xi$ which is true in the regimes under consideration, the net flux $J$ of molecules $P$ at the droplet’s interface is composed of an in-flux from the medium and an out-flux due to the chemical conversion $P \rightarrow k^i S$ inside the droplet that depletes $P$ [13]:

$$J = 4\pi DR \left( \Delta - \frac{\hat{P}_{out}l_c}{R} \right) - \frac{4\pi R^3}{3} k \hat{P}_{in} , \quad (A.1)$$

where $\Delta$ is the supersaturation set by the chemical reaction rates $k, h$:

$$\Delta = \frac{\phi}{1+k/h} - \hat{P}_{out} . \quad (A.2)$$

The droplet grows when $J > 0$ and shrink otherwise. At equilibrium ($k = h = 0$ but $k/h$ is still defined by Eqs. (2) or (4)) there is a unique fixed point radius ($J = 0$):

$$R_{eq}^c = \frac{\hat{P}_{out}l_c}{\Delta} . \quad (A.3)$$

$R_{eq}^c$ is unstable ($dJ/dR|_{R_c} > 0$) and is the nucleus radius at equilibrium: smaller droplets dissolve while larger droplets grow.
When chemical reactions are switched on \((k, h > 0)\), Eq. \((A.1)\) admits two fixed points, shown in Fig. \(A1\) for varying \(k\). For small \(R\) we can neglect the reaction term \((\propto kR^3)\) and find the unstable fixed point, or nucleus radius,
\[
R_c \simeq R_c^{\text{eq}},
\]
and for large \(R\) we neglect the surface tension term \((\propto l_c/R)\) and find the stable fixed point.
\[
R_u \simeq \sqrt{\frac{3D\Delta}{P_{\text{in}}k}}.
\]
Additionally there exist a critical rate \(k_c\) above which no fixed points exist and \(J < 0\) for all \(R\) meaning that all droplets dissolve.

We will now examine these results in model A and C, seeking for the ratio \(R_u/R_c\) for \(k \simeq k_c\).

### Appendix A.1. Model C

In model C, \(k = \alpha K_C\) and \(h = \alpha H_C\) so the ratio \(k/h\) and \(\Delta\) are constant. Therefore \(R_u \propto \alpha^{-1/2}\) and we recover Eq. \((18)\). Since this scaling is sub-linear we saw that it cannot explain SG formation and dissolution upon small variations of \(\alpha\). However when \(\alpha\) approaches \(\alpha_c\) the separation between \(R_c\) and \(R_u\) becomes small so the above approximations cease to be valid and a strong response regime exists: small variations of \(\alpha\) lead to strong variations of \(R_u\) and \(R_c\) (Fig. \(A1\)).

Qualitatively, it can be seen from Eq. \((A.1)\) that since we omitted the term \(\propto l_c/R\) in the determination of \(R_u\) we have overestimated \(R_u\), and since we neglected the term \(\propto kR^3\) in the determination of \(R_c\) we have underestimated \(R_c\). Therefore the exact value of \(R_u\) is bounded from above by \(\sqrt{3D\Delta/(P_{\text{in}}k)}\) while the exact value of \(R_c\) is bounded from below by \(R_c^{\text{eq}}\). The ratio \(R_u/R_c\) in the strong response regime is therefore also bounded (see Fig. \(A1\)):
\[
\frac{R_u}{R_c} < \frac{3\sqrt{3}D\Delta/(P_{\text{in}}k)}{R_c^{\text{eq}}}
\]

At \(\alpha = \alpha_c\) the two fixed points \(R_c\) and \(R_u\) intersect at the radius \(R^*\) and since \(R_c\) and \(R_u\) are unstable and stable fixed points, respectively, we have
\[
J(\alpha_c, R^*) = 0
\]
\[
\frac{dJ}{dR}|_{\alpha_c, R^*} = 0,
\]
and solving for \(\alpha_c\) gives
\[
\alpha_c = \frac{4D\Delta^3}{9l_c^2P_{\text{out}}^2P_{\text{in}}K_C}.
\]
We then find
\[
\frac{R_u}{R_c} < \frac{3\sqrt{3}}{2}.
\]

In other words, the size of the stable droplets and nuclei are of the same order. This shows independently of the system parameters that the strong response regime in model C cannot account for the switch-like response observed experimentally.
Appendix A.2. Model A

We now concentrate on model A. Here the supersaturation $\Delta$ is no more constant since only the backward rate is constant (i.e. $k = \alpha K_A$, $h = H_A$):

$$\Delta = \frac{\phi}{1 + \alpha \frac{K_A}{H_A}}.$$  \hfill (A.10)

Therefore there exist a critical ATP concentration $\alpha_c$ above which $\Delta = 0$ and all droplets dissolve ($R_u = 0$). From this equation the expression of $\alpha_c$ is:

$$\alpha_c = \frac{\phi - \hat{P}_{\text{out}} H_A}{\hat{P}_{\text{out}} K_A}.$$  \hfill (A.11)

We define $\alpha_{\text{stress}} = 2\alpha_c/3$ and $\alpha_{\text{normal}} = 4\alpha_c/3$ the ATP concentrations during stress and normal condition, respectively, in agreement with the biological constraint that $\alpha_{\text{normal}} = 2\alpha_{\text{stress}}$ (Sec. 1.1). Moreover we assumed these concentrations to be equidistant from $\alpha_c$ for simplicity. During normal conditions $\alpha = \alpha_{\text{normal}} > \alpha_c$ so no droplets can exist. During stress condition $\alpha = \alpha_{\text{stress}} < \alpha_c$ and using Eqs (A.4), (A.5), (A.10) and (A.11), we find the size ratio $R_u/R_c$ during stress conditions:

$$\frac{R_u(\alpha_{\text{stress}})}{R_c(\alpha_{\text{stress}})} = 3 \left[ \frac{D}{4 \hat{P}_{\text{out}}^2} \left( \frac{\phi}{\hat{P}_{\text{out}} - 2} \right) \left( \frac{\phi}{\hat{P}_{\text{out}} + 1} \right)^3 \right]$$

(A.12)

Therefore we find that in model A and contrarily to model C, the size ratio between stable droplets and nuclei is function of the system parameters and can be arbitrarily large. This can potentially provide the switch-like response observed experimentally which we discuss quantitatively in Sec. 5 using physiologically relevant parameters.

References

[1] Protter D S and Parker R 2016 Trends in Cell Biology 26 668–679
[2] Updike D and Strome S 2010 Journal of andrology 31 53–60
[3] Brangwynne C, Eckmann C, Courson D, Rybarska A, Hoege C, Gharakhani J, Jülicher F and Hyman A 2009 Science 324 1729–1732
[4] Lee C F, Brangwynne C P, Gharakhani J, Hyman A A and Jülicher F 2013 Physical Review Letters 111 88101
[5] Hyman A A, Weber C A and Jülicher F 2014 Annual review of cell and developmental biology 30 39–58
[6] Brangwynne C P, Tompa P and Pappu R Y 2015 Nature Physics 11 899–904
[7] Bray A J 2002 Advances in Physics 51 481–587
[8] Ivanov P A, Chudinova E M and Nadezhdina E S 2003 Experimental Cell Research 290 227–233
[9] Zwicker D, Decker M, Jaensch S, Hyman A and Jülicher F 2014 Proceedings of the National Academy of Sciences of the United States of America 111 E2636–45
[10] Zwicker D, Hyman A A and Jülicher F 2015 Physical Review E - Statistical, Nonlinear, and Soft Matter Physics 92 1–12
[11] Saha S, Weber C A, Nousch M, Adame-Arana O, Hoege C, Hein M Y, Osborne-Nishimura E, Mahamid I, Jahnel M, Jawerth L, Pozniakovski A, Eckmann C R, Jülicher F and Hyman A A 2016 Cell 166 1572–1584
[12] Weber C A, Lee C F and Jülicher F 2017 New Journal of Physics 19 53021
[13] Wurtz J D and Lee C F 2017 (Preprint 1707.08433) URL http://arxiv.org/abs/1707.08433
[14] Wheeler J R, Matheny, T, Jain, S, Abrisch, R and Parker, R 2016 eLife 5 e18413
[15] Ohshima D, Arimoto-Matsuzaki K, Tomida T, Takekawa M and Ichikawa K 2015 PLOS Computational Biology 11 e1004326
Stress granule formation via phase separation

[16] Anderson P and Kedersha N 2009 Current Biology 19 397–398
[17] Aulas a, Caron G, Gkogkas C G, Mohamed N V, Destroismaisons L, Sonenberg N, Leclerc N, Parker J a and Vande Velde C 2015 The Journal of Cell Biology 209 73–84
[18] Buchan J R and Parker R 2009 Molecular Cell 36 932–941
[19] Kedersha N, Stoecklin G, Ayodele M, Yacono P, Lykke-Andersen J, Fritzler M J, Scheuner D, Kaufman R J, Golan D E and Anderson P 2005 The Journal of cell biology 169 871–84
[20] Ramaswami M, Taylor J P and Parker R 2013 Cell 154 727–736
[21] Hofmann S, Cherkasova V, Bankhead P, Bukau B and Stoecklin G 2012 Molecular Biology of the Cell 23 3786–3800
[22] Kedersha N, Gupta M, Li W, Miller I and Anderson P 1999 The Journal of Cell Biology 147 1431–1441
[23] Kedersha N, Chen S, Gilks N, Li W, Miller I J, Stahl J and Anderson P 2002 Molecular biology of the cell 13 195–210
[24] Lilly M B, Durant J R, Ng T C, Evanochko W T, Kumar N G, Elgavish G A, Glickson J D, Hiramoto R, Ghanta V and Katholi C R 1984 Cancer Research 44 633–638
[25] Chang J, Knowlton a a, Xu F and Wasser J S 2001 American journal of physiology. Heart and circulatory physiology 280 H426–H433
[26] Jacobs W M and Frenkel D 2017 Biophysical Journal 112 683–691
[27] Bah A and Forman-Kay J D 2016 Journal of Biological Chemistry 291 6696–6705
[28] Li P, Banjade S, Cheng H C, Kim S, Chen B, Guo L, Llaguno M, Hollingsworth J V, King D S, Banani S F, Russo P S, Jiang Q X, Nixon B T and Rosen M K 2012 Nature 483 336–40
[29] Lifshitz I and Slyozov V 1961 Journal of Physics and Chemistry of Solids 19 35–50
[30] Sigia E D 1979 Physical Review A 20 595–605
[31] Weiss M, Elsner M, Kartberg F and Nilsson T 2004 Biophysical journal 87 3518–24
[32] Glotzer S C, Marzio E A D and Muthukumar M 1995 Phys. Rev. Lett. 74 2034–2037
[33] Nott T J, Petsalaki E, Farber P, Jervis D, Fussner E, Plochowietz A, Craggs T D, Bazett-Jones D P, Pawson T, Forman-Kay J D and Baldwin A J 2015 Molecular Cell 57 936–947
[34] Shin Y, Berry J, Pannucci N, Haataja M P, Toettcher J E and Brangwynne C P 2017 Cell 168 159–171
[35] Jain S, Wheeler J R, Walters R W, Agrawal A, Barsic A and Parker R 2016 Cell 164 487–498
[36] Wippich F, Bodenmiller B, Trajkovska M G, Wanka S, Aebersold R and Pelkmans L 2013 Cell 152 791–805
[37] Dang Y, Kedersha N, Low W K, Romo D, Gorospe M, Kaufman R, Anderson P and Liu J O 2006 Journal of Biological Chemistry 281 32870–32878