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Supplemental Information

Multivalent Molecules as Modulators of RNA Granule Size and Composition

Cibele Vieira Falkenberg, John H. Carson, and Michael L. Blinov

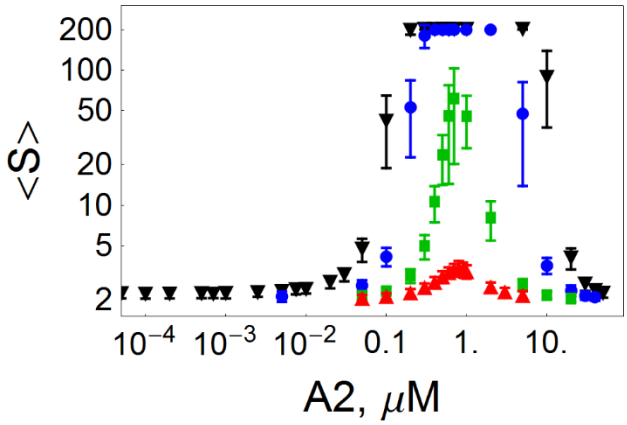

Figure S1. Mean number of RNA molecules per granule <S> as a function of A2 concentration for RNA of different valencies: 100 (black downward triagles), 50 (blue dots), 20 (green squares) and 10 (red upward triangles). Simulation for 100 molecules of A2RE RNA (0.005 µM), 100 molecules of nonA2RE RNA (0.005 µM), 0.1 µM TOG and K_D=50 µM dissociation constant between TOG sites and RNA sites. Each point represents average over 100 simulations.

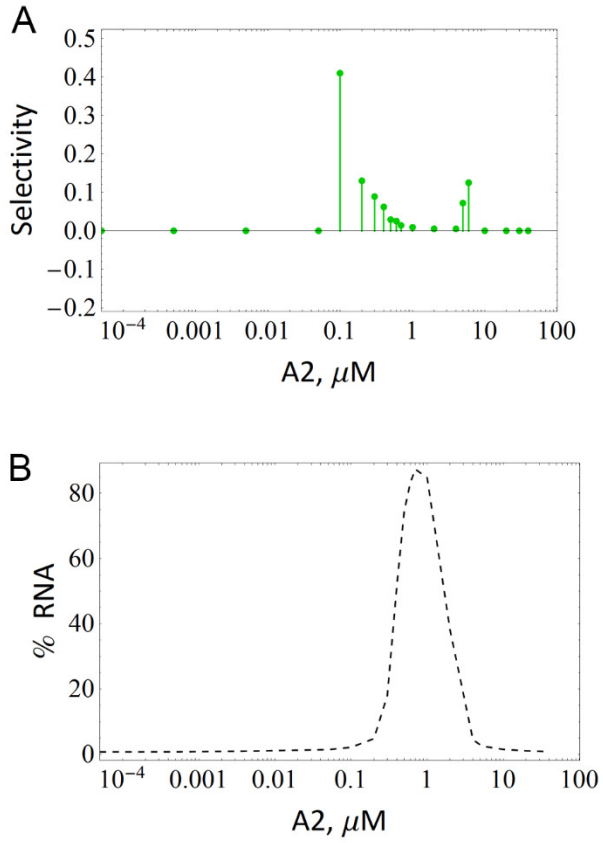

Figure S2. Impact of A2 on selectivity and sequestration for RNA of valency 20. Each A2RE RNA has a single A2RE sequence plus 19 non-specific binding sites. Simulation for 100 molecules of A2RE RNA (0.005 μM), 100 molecules of nonA2RE RNA (0.005 μM), 0.1 μM TOG and K_D=50 μM dissociation constant between TOG sites and RNA sites. Each point represents average over 100 simulations.

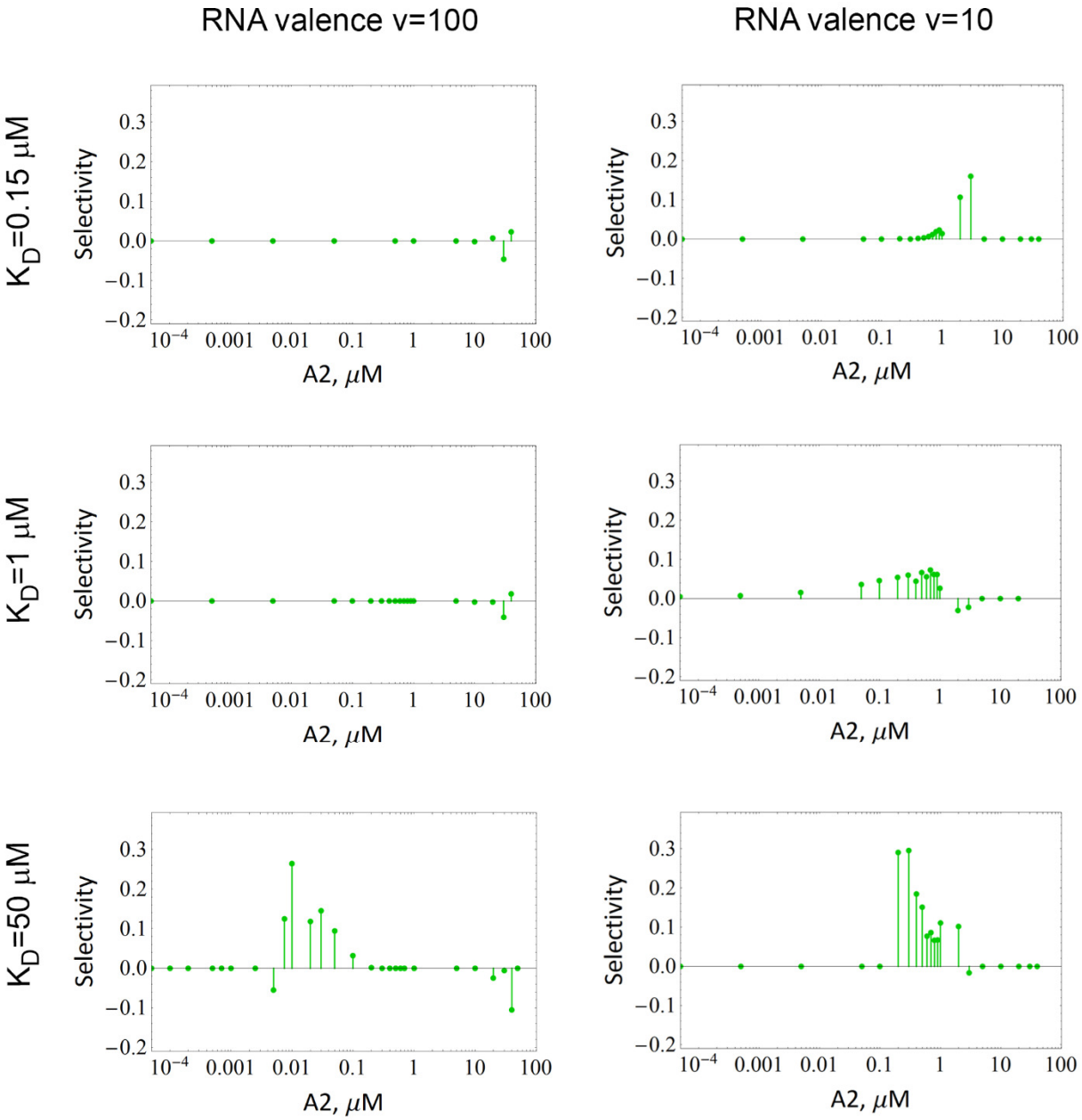

Figure S3. The impact of A2 concentration on granule selectivity for RNA with effective valence 100 (left column) and 10 (right column), for three different values of K_D (0.15 μM, 1 μM and 50 μM, from top to bottom rows) between RNA sites and TOG. Total of 100 simulations, and a visible granule was defined as composed of at least 6 RNA's.

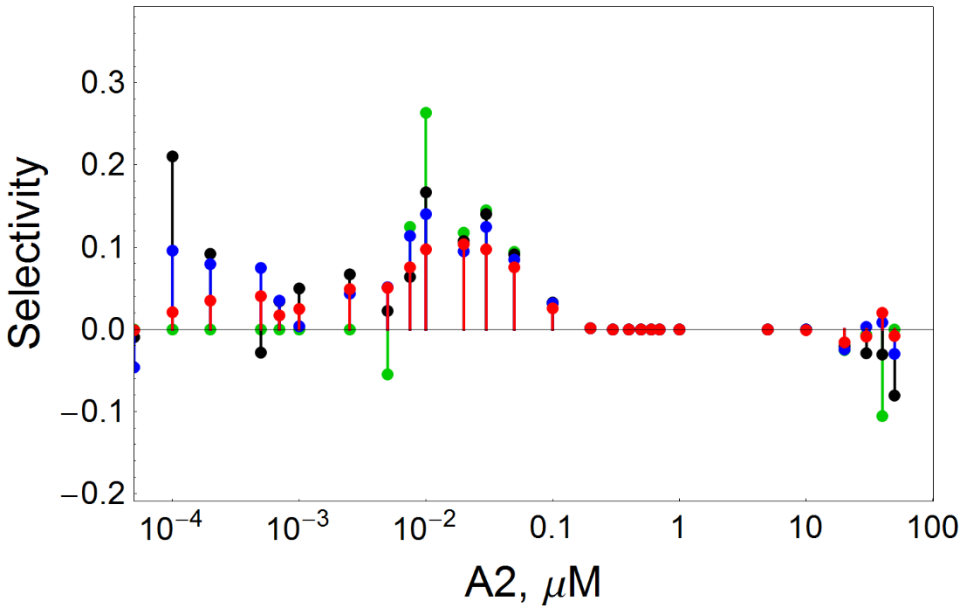

Figure S4. Selectivity of visible RNA granules under different experimental conditions. The minimum number of RNA molecules that must belong to a granule so that it is experimentally visible depends on the experimental setup and methodology. For resolutions of 2 (red), 3 (blue), 4 (black) and 6 (green) RNA molecules per granule, selective granules occur in the concentration range where A2 functions as a granule enabler (below 0.1 μM), and mildly negative selectivity may occur in the concentration range where A2 functions as a granule disruptor (above 10 μM).

Equations

System of equations for 13 concentrations (for each of the 6 binding sites plus 7 types of bonds). See Tables S1 and S2 for nomenclature.

Non-specific RNA sites ($\mathrm{RNA_n}$) are consumed through interactions with TOG site ($\mathrm{TOG_b}$) and RNA-binding site of A2 ($\mathrm{A2}_r$), and sites become available by breaking bonds between $\mathrm{RNA_n}$ and $\mathrm{TOG_b}$ (n.b) and between $\mathrm{RNA_n}$ and $\mathrm{A2_r}$ (n.a):

$$\frac{d(\mathrm{RNA_n})}{dt} = -k_{+,\mathrm{b}}(\mathrm{RNA_n})(\mathrm{TOG_b}) + k_{-,\mathrm{b}}(n.b) - k_{+,\mathrm{n}}(\mathrm{RNA_n})(\mathrm{A2}_r) + k_{-,\mathrm{n}}(n.a)$$

Non-specific RNA sites of A2RE RNA ($\mathrm{A2RERNA_n}$) are consumed through interactions with TOG site ($\mathrm{TOG_b}$) and RNA-binding site of A2 ($\mathrm{A2}_r$), and become available by breaking bonds between $\mathrm{A2RERNA_n}$ and $\mathrm{TOG_b}$ (sn.b) and between $\mathrm{A2RERNA_n}$ and $\mathrm{A2_r}$ (sn.a):

$$\frac{d(A2RE\mathrm{RNA_n})}{dt} = -k_{+,\mathrm{b}}(A2RE\mathrm{RNA_n})(\mathrm{TOG_b}) + k_{-,\mathrm{b}}(sn.b) - k_{+,\mathrm{n}}(A2RE\mathrm{RNA_n})(\mathrm{A2}_r) + k_{-,\mathrm{n}}(sn.a)$$

Specific RNA sites of A2RE RNA ($\mathrm{A2RERNA_s}$) are consumed through interactions with TOG site ($\mathrm{TOG_b}$) and RNA-binding site of A2 ($\mathrm{A2}_r$), and become available by breaking bonds between $\mathrm{A2RERNA_s}$ and $\mathrm{TOG_b}$ (ss.b) and between $\mathrm{A2RERNA_s}$ and $\mathrm{A2_r}$ (ss.a):

$$\frac{d(\mathrm{A2RERNA_s})}{dt} = -k_{+,\mathrm{b}}(A2RE\mathrm{RNA_s})(\mathrm{TOG_b}) + k_{-,\mathrm{b}}(ss.b) - k_{+,s}(A2RE\mathrm{RNA_s})(\mathrm{A2}_r) + k_{-,s}(ss.a)$$

TOG sites ($\mathrm{TOG_b}$) are consumed by establishing bonds with A2 sites for TOG ($\mathrm{A2_t}$), and sites in specific ($\mathrm{A2RERNA_n}$ and $\mathrm{A2RERNA_s}$) and non-specific RNAs ($\mathrm{RNA_n}$), and become available by breaking such bonds:

$$\begin{aligned}\frac{d(\mathrm{TOG_b})}{dt} = &-k_{+,\mathrm{a}}(\mathrm{A2_t})(\mathrm{TOG_b}) + k_{-,\mathrm{a}}(a.b) - k_{+,\mathrm{b}}(\mathrm{RNA_n})(\mathrm{TOG_b}) + k_{-,\mathrm{b}}(n.b) - k_{+,\mathrm{b}}(A2RE\mathrm{RNA_n}) \\ &+ k_{-,\mathrm{b}}(sn.b)(\mathrm{TOG_b}) - k_{+,\mathrm{b}}(A2RE\mathrm{RNA_s})(\mathrm{TOG_b}) + k_{-,\mathrm{b}}(ss.b)\end{aligned}$$

A2 sites for RNA ($\mathrm{A2}_r$) are consumed by establishing bonds with sites in specific ($\mathrm{A2RERNA_n}$ and $\mathrm{A2RERNA_s}$) and non-specific RNAs ($\mathrm{RNA_n}$), and become available by breaking such bonds:

$$\begin{aligned}\frac{d(\mathrm{A2_r})}{dt} = &-k_{+,\mathrm{n}}(\mathrm{RNA_n})(\mathrm{A2}_r) + k_{-,\mathrm{n}}(n.a) - k_{+,\mathrm{n}}(A2RE\mathrm{RNA_n})(\mathrm{A2}_r) + k_{-,\mathrm{n}}(sn.a) \\ &- k_{+,s}(A2RE\mathrm{RNA_s})(\mathrm{A2}_r) + k_{-,s}(ss.a)\end{aligned}$$

A2 sites for TOG ($\mathrm{A2_t}$) are consumed by establishing bonds with sites in TOG ($\mathrm{TOG_b}$), and become available by breaking such bonds:

$$\frac{d(\mathrm{A2_t})}{dt} = -k_{+,\mathrm{a}}(\mathrm{A2_t})(\mathrm{TOG_b}) + k_{-,\mathrm{a}}(a.b)$$

The number of bonds between TOG and A2 (a.b) is governed by the corresponding interactions:

$$\frac{d(a.b)}{dt} = k_{+,\mathrm{a}}(\mathrm{A2_t})(\mathrm{TOG_b}) - k_{-,\mathrm{a}}(a.b)$$

The number of bonds between non-specific RNA and A2 (n.a) is governed by the corresponding interactions:

$$\frac{d(n.a)}{dt} = k_{+,\mathrm{n}}(\mathrm{RNA_n})(\mathrm{A2}_r) - k_{-,\mathrm{n}}(n.a)$$

The number of bonds between non-specific RNA and TOG (n.b) is governed by the corresponding interactions:

$$\frac{d(n.b)}{dt} = k_{+,\mathrm{b}}(\mathrm{RNA_n})(\mathrm{TOG_b}) - k_{-,\mathrm{b}}(n.b)$$

The number of bonds between specific RNA and A2 (sn.a) is governed by the corresponding interactions:

$$\frac{d(sn.a)}{dt} = k_{+,\mathrm{n}}(A2RE\mathrm{RNA_n})(\mathrm{A2}_r) - k_{-,\mathrm{n}}(sn.a)$$

The number of bonds between A2RERNA non-specific sites and TOG (sn.b) is governed by the corresponding interactions:

$$\frac{d(sn.b)}{dt} = k_{+,\mathrm{b}}(A2RE\mathrm{RNA_n})(\mathrm{TOG_b}) - k_{-,\mathrm{b}}(sn.b)$$

The number of bonds between A2RERNA specific sites and A2 (ss.a) is governed by the corresponding interactions:

$$\frac{d(ss.a)}{dt} = k_{+,\mathrm{s}}(A2RE\mathrm{RNA_s})(\mathrm{A2}_r) - k_{-,\mathrm{s}}(ss.a)$$

The number of bonds between A2RERNA specific sites and TOG (ss.b) is governed by the corresponding interactions:

$$\frac{d(ss.b)}{dt} = k_{+,\mathrm{b}}(A2RE\mathrm{RNA_s})(\mathrm{TOG_b}) - k_{-,\mathrm{b}}(ss.b)$$

Table S1: Nomenclature for model variables. Both RNA and A2RERNA have the same total valence v. Molecule names in parenthesis represent total concentration, μM. Bond concentration is also in μM.

Variable	**Description**	**Initial condition**
RNA_n	Site of non − specific RNA	$(RNA) * \mathrm{v}$
$\mathrm{A2RERNA}_n$	Non-specific site of A2RE RNA	$(A2RERNA) * (\mathrm{v}-1)$
$\mathrm{A2RERNA}_s$	high affinity A2RE RNA site for A2	$(A2RERNA)$
TOG_b	Binding site of TOG for either RNA or A2	$(TOG) * 7$
$\mathrm{A2}_r$	Binding site of A2 for RNA	$(A2)$
$\mathrm{A2}_t$	Binding site for of A2 for TOG	$(A2)$
$(n.a)$	Bond between RNA_n and $\mathrm{A2}_\mathrm{r}$	0
$(n.b)$	Bond between RNA_n and TOG_b	0
$(sn.a)$	Bond between $\mathrm{A2RERNA}_n$ and $\mathrm{A2}_\mathrm{r}$	0
$(sn.b)$	Bond between $\mathrm{A2RERNA}_n$ and TOG_b	0
$(ss.a)$	Bond between $\mathrm{A2RERNA}_s$ and $\mathrm{A2}_\mathrm{r}$	0
$(ss.b)$	Bond between $\mathrm{A2RERNA}_s$ and TOG_b	0
$(a.b)$	Bond between $\mathrm{A2}_t$ and TOG_b	0

Table S2: Nomenclature for reaction rate constants. $K_{D,b}$ ranges from 0.15 to 50 μM, as described in the text.

Interaction	**Binding**	**Unbinding**
RNA_n - $\mathrm{A2}_\mathrm{r}$	$k_{+,\mathrm{n}} = 1.5 \times 10^{-3}/\mathrm{s}$	$k_{-,\mathrm{n}} = 2 \times 10^{-3}/(\mu\mathrm{M\ s})$
RNA_n - TOG_b	$k_{+,\mathrm{b}} = k_{-,\mathrm{b}}/\mathrm{K}_{D,\mathrm{b}}$	$k_{-,\mathrm{b}} = 1.46 \times 10^{-3}/(\mu\mathrm{M\ s})$
$\mathrm{A2RERNA}_n$ - $\mathrm{A2}_\mathrm{r}$	$k_{+,\mathrm{n}} = 1.5 \times 10^{-3}/\mathrm{s}$	$k_{-,\mathrm{n}} = 2 \times 10^{-3}/(\mu\mathrm{M\ s})$
$\mathrm{A2RERNA}_n$ - TOG_b	$k_{+,\mathrm{b}} = k_{-,\mathrm{b}}/\mathrm{K}_{D,\mathrm{b}}$	$k_{-,\mathrm{b}} = 1.46 \times 10^{-3}/(\mu\mathrm{M\ s})$
$\mathrm{A2RERNA}_s$ - $\mathrm{A2}_\mathrm{r}$	$k_{+,s} = 7 \times 10^{-3}/\mathrm{s}$	$k_{-,s} = 5 \times 10^{-5}/(\mu\mathrm{M\ s})$
$\mathrm{A2RERNA}_s$ - TOG_b	$k_{+,\mathrm{b}} = k_{-,\mathrm{b}}/\mathrm{K}_{D,\mathrm{b}}$	$k_{-,\mathrm{b}} = 1.46 \times 10^{-3}/(\mu\mathrm{M\ s})$
$\mathrm{A2}_t$ - TOG_b	$k_{+,\mathrm{a}} = 0.12/\mathrm{s}$	$k_{-,\mathrm{a}} = 0.003/(\mu\mathrm{M\ s})$