Supplementary Information

Polyanion-Assisted Ribozyme Catalysis Inside Complex Coacervates

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Table 1. Composition of Coacervates at 1:1 Charge

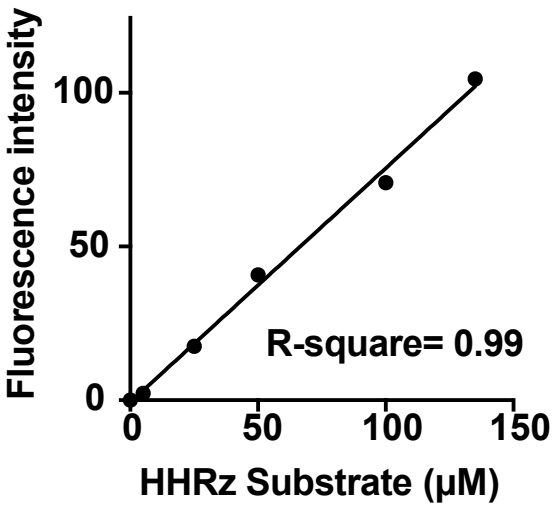
Polyion	Conc. of macromolecule added (mM)	Total charge (mM)
PDAC	0.19	10
D_{10}	1.00	10
D_{30}	0.33	10
D_{50}	0.20	10
D_{100}	0.10	10

Supplementary Table 1. Concentrations of polymers to generate complex coacervates.

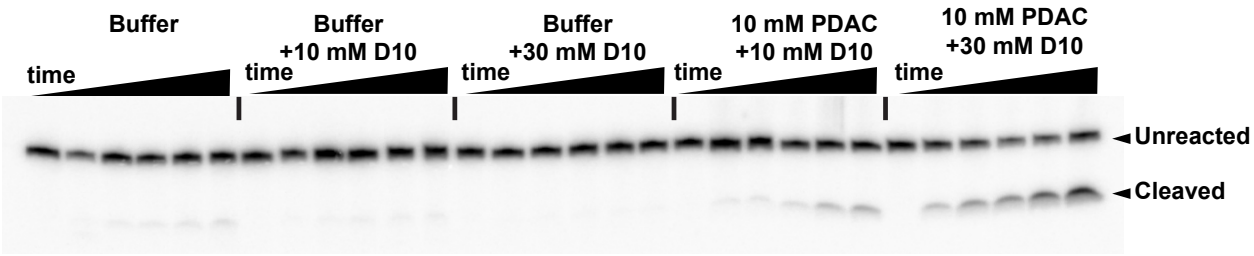
Table 2. Bulk pH of coacervate solutions

Coacervate Composition	Total – (mM)	pH
–	–	8.04
PDAC(only)	–	8.11
D_{10}	10	8.07
D_{30}	10	8.01
D_{50}	10	8.05
D_{100}	10	8.10
D_{10}/PDAC	10	8.02
D_{30}/PDAC	10	8.09
D_{50}/PDAC	10	8.07
D_{100}/PDAC	10	8.00
D_{10}	20	8.02
D_{10}	30	7.96
D_{10}	50	7.82
D_{10}/PDAC	20	8.12
D_{10}/PDAC	30	8.00
D_{10}/PDAC	50	7.86
D_{50}	20	8.00
D_{50}	30	7.99
D_{50}	50	7.82
D_{50}/PDAC	20	8.02
D_{50}/PDAC	30	7.98
D_{50}/PDAC	50	7.85

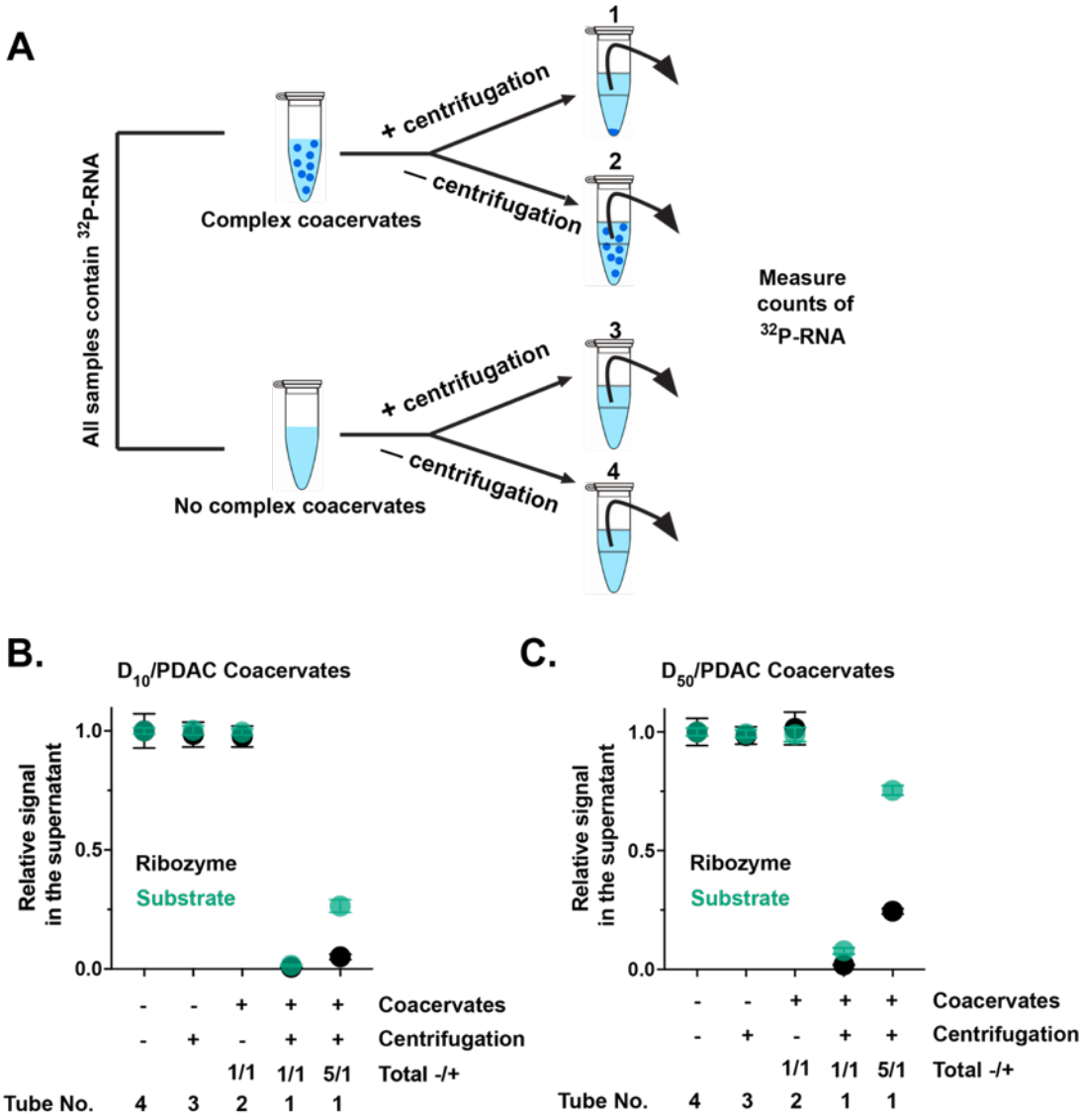
Supplementary Table 2. pH measurements of bulk coacervate solutions. Bulk coacervate samples were prepared in 300 µL volume which contained 25 mM Tris•HCl at pH 8.0, 2.5 mM KCl and 1 mM $MgCl_2$. All coacervates contained 10 mM total positive charge from $PDAC_{53}$ and varying amounts of negative charge.



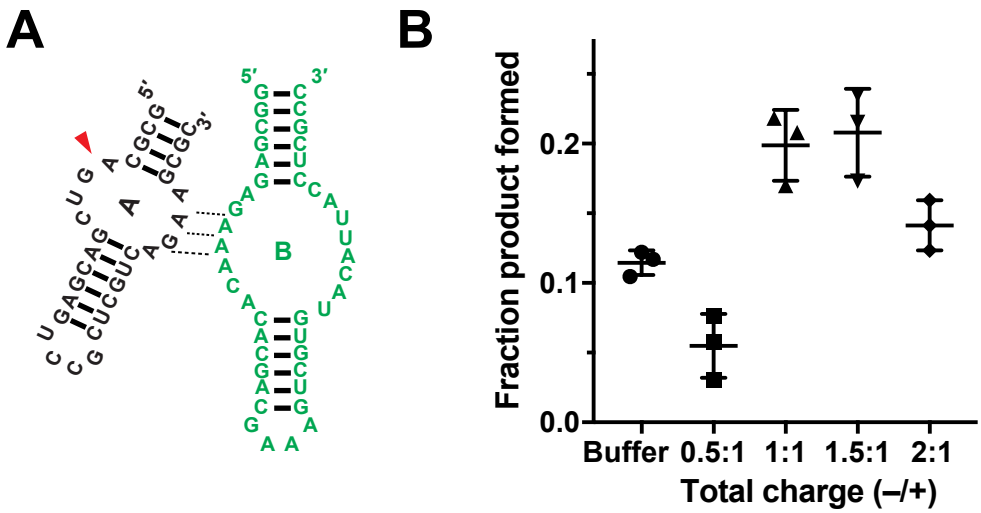
Supplementary Figure 1. Standard curve used to calculate concentration of the HHRz substrate inside complex coacervates showed in Figure 2, 3, and SI Figure 5.



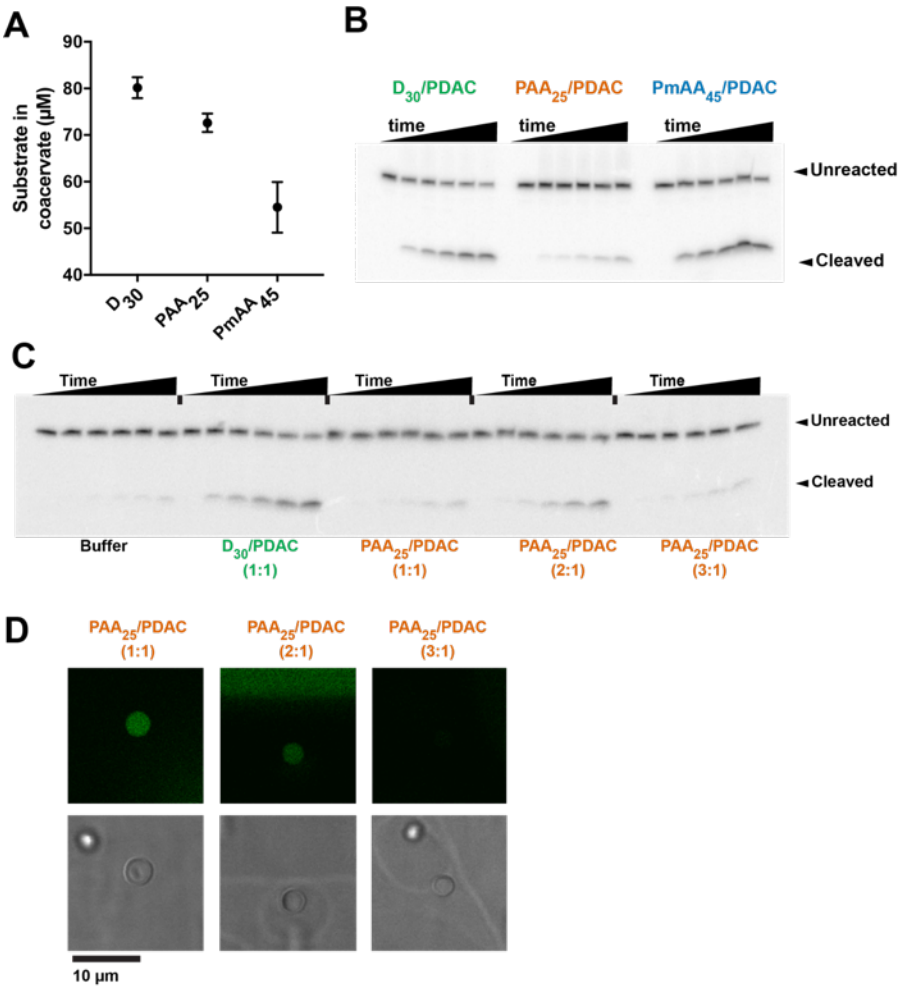
Supplementary Figure 2. Stimulation of ribozyme activity by D_{10} requires coacervates. Reactions contained indicated amounts of total negative charge from D_{10} and PDAC. Reactions were stopped at 0, 7.5, 15, 30, 60, and 90 minutes.



Supplementary Figure 3. Effect of increasing polyanions concentration on ribozyme partitioning (A) Cartoon depiction of the experiment. Complex coacervates were first generated to which 5' end-labeled ribozyme or substrate were added. Counts of ^{32}P were measured either with (sample 1) or without (sample 2) centrifugation. Lack of counts in the supernatant (sample 1) indicate retention of RNA in the condensed phase (top). Similar method was used in the absence of coacervates as controls (samples 3 and 4). (B,C) Counts of ^{32}P in 10 µL volume (20 µL total) in (B) D_{10}/PDAC coacervates and (C) D_{50}/PDAC coacervates. Mean values from n=3, error bars represent the S.D



Supplementary Figure 4. Polyanion enhancement enhance hairpin ribozyme in PDAC-containing coacervates. (A) Structure of the hairpin ribozyme. The enzyme strand is shown in green (loop B) and the substrate RNA is shown in black (loop A). The site of substrate cleavage is indicated by red triangle. (B) Fraction product formed by hairpin ribozyme after 2 hours of reaction. All reactions contained 25 mM Tris•HCl, 2.5 mM $MgCl_2$, 2.5 mM KCl, 40 nM enzyme strand and <1 nM substrate. Coacervates contained PDAC and spermine at 5 mM total positive charge each and varying amounts of D_{30} to give indicated total –/+. Mean values from n=3, error bars represent the S.D



Supplementary Figure 5. Ribozyme reactions in complex coacervates containing PDAC and various polyanions. (A) Concentration of 5' Alexa488 substrate in different complex coacervates (1:1 charge-matched). 0.25 μM Alexa488-labeled HHRz substrate was added to different coacervates. Mean values of three replicates are shown error bars representing the S.D (B) Representative gel images showing hammerhead ribozyme reactions in different coacervates. (C) Gel images showing hammerhead ribozyme reaction in complex coacervates containing indicated compositions. Time points were taken in 0, 7.5, 15, 30, 60 and 90 min (B) and (C). Quantifications for (A) and (B) are shown in Figure 4B. (D) 0.25 μM Alexa488-labeled HHRz substrate was added to the indicated coacervate compositions for confocal microscopy. A large contiguous condensed phase is visible in PAA_{25}/PDAC (2:1) (top) due to coalescence of smaller droplets. Scale bar is 10 μm.