SUPPORTING INFORMATION

Mathematical model for the analysis of sm-FCS data.

Sm-FCS uses fluorescence intensity fluctuations within the confocal volume to obtain properties as the diffusion coefficient of a fluorescently tagged molecule, as well as fluorescence kinetics due to dark states (triplet-state and 2nd dark state). Fluorescence fluctuations, *I(t)*, were analyzed using an autocorrelation function:

$$G(\tau) = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I(t)\rangle^2} \qquad \text{Eq. (1)}$$

The measured $G(\tau)$ can be written as:

$$G(\tau) = G(\tau)_D \cdot \chi(\tau)_{kinetics} \qquad \text{Eq. (2)}$$

The contribution of diffusion, $G(\tau)_D$, to the $G(\tau)$ can be described, under the assumption of an elliptical Gaussian-shaped confocal volume, by (1):

$$G(\tau)_D = \left[\langle N\rangle\left(1 + \frac{\tau}{\tau_D}\right)\right]^{-1} \qquad \text{Eq. (3)}$$

Here <N> is the average number of particles in the confocal volume and τ_D is the characteristic diffusion time, which depends on the diffusion coefficient, *D*, and the beam waist, ω_{xy} in the x,y-dimensions of the laser focus:

$$\tau_D = \omega_{wy}{}^2/4D \qquad \text{Eq. (4)}$$

The effective hydrodynamic radius, R_h, was determined by using the Stokes-Einstein relation:

$$D = \frac{k_B T}{6\pi\eta R_h} \qquad \text{Eq. (5)}$$

where k_B, T and η are the Boltzmann constant, the temperature and solvent viscosity, respectively.

The measured G(τ) also includes contributions from intra- and/or intermolecular dynamics that give rise to fluorescence fluctuations on time scales much faster than the characteristic diffusion time, τ_D. Such fluctuations can be described by a simple exponential decay for the dark states:

$$\chi(\tau)_{kinetics} = 1 - T + T \cdot e^{-\frac{\tau}{\tau_T}} - B + B \cdot e^{-\frac{\tau}{\tau_B}} \qquad \text{Eq. (6)}$$

Here T and τ_T are the fraction and characteristic lifetime of fluorophores in their triplet state, and B and τ_B are the amplitude and characteristic lifetime of a 2nd dark state (bunching term), with $\tau_T < \tau_B < \tau_D$. The typical values for the lifetime of the triplet-state, 2nd dark state and the diffusion time are 10^{-3}, 10^{-1} and 10^{1}-10^{2} ms, respectively.

When there are two diffusing species with two different quantum yields the autocorrelation curve can be re-expressed as:

$$G(\tau)_D = \frac{\alpha_1^2 N_1 G(\tau)_{D,1} + \alpha_2^2 N_2 G(\tau)_{D,2}}{(\alpha_1 N_1 + \alpha_2 N_2)^2} \qquad \text{Eq. (7)}$$

where $G(\tau)_{D,1}$ and $G(\tau)_{D,2}$ are the diffusive parts of the autocorrelation correlation function for species 1 and 2, respectively, α_2, N_1 and N_2 are the ratio of the quantum yield of species 2 with respect to that of species 1, and the number of particles of species 1 and 2, respectively. By definition $\alpha_1 = 1$.

Supplementary figures

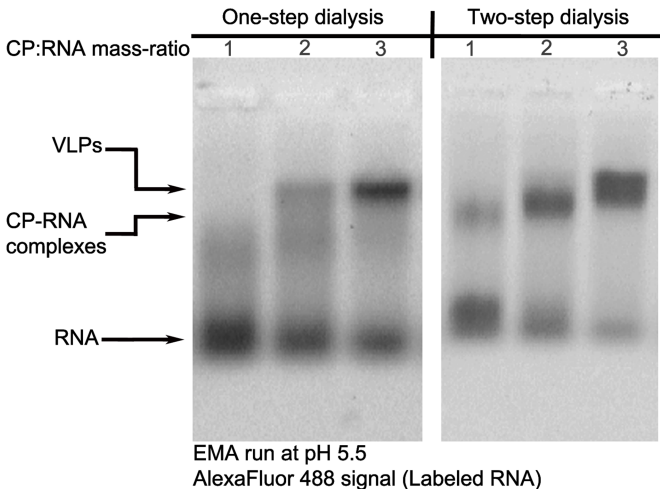

FIG S1. Rapid acidification does not promote cooperativity at low CP:RNA mass ratios. The EMAs show the difference between samples dialyzed only against RAB (one-step dialysis) and those against RAB and VSB (two-step dialysis, see Fig. 1a). For CP:RNA mass ratios ≤ 3, rapid pH acidification due to the conditions at which the EMA are run was not sufficient to promote a two-state system. By comparing both EMAs it is clear that low-molecular weight CP/RNA complexes formed during pH quenching disappear to form higher-molecular-weight complexes if the sample is dialyzed against a low-pH buffer.

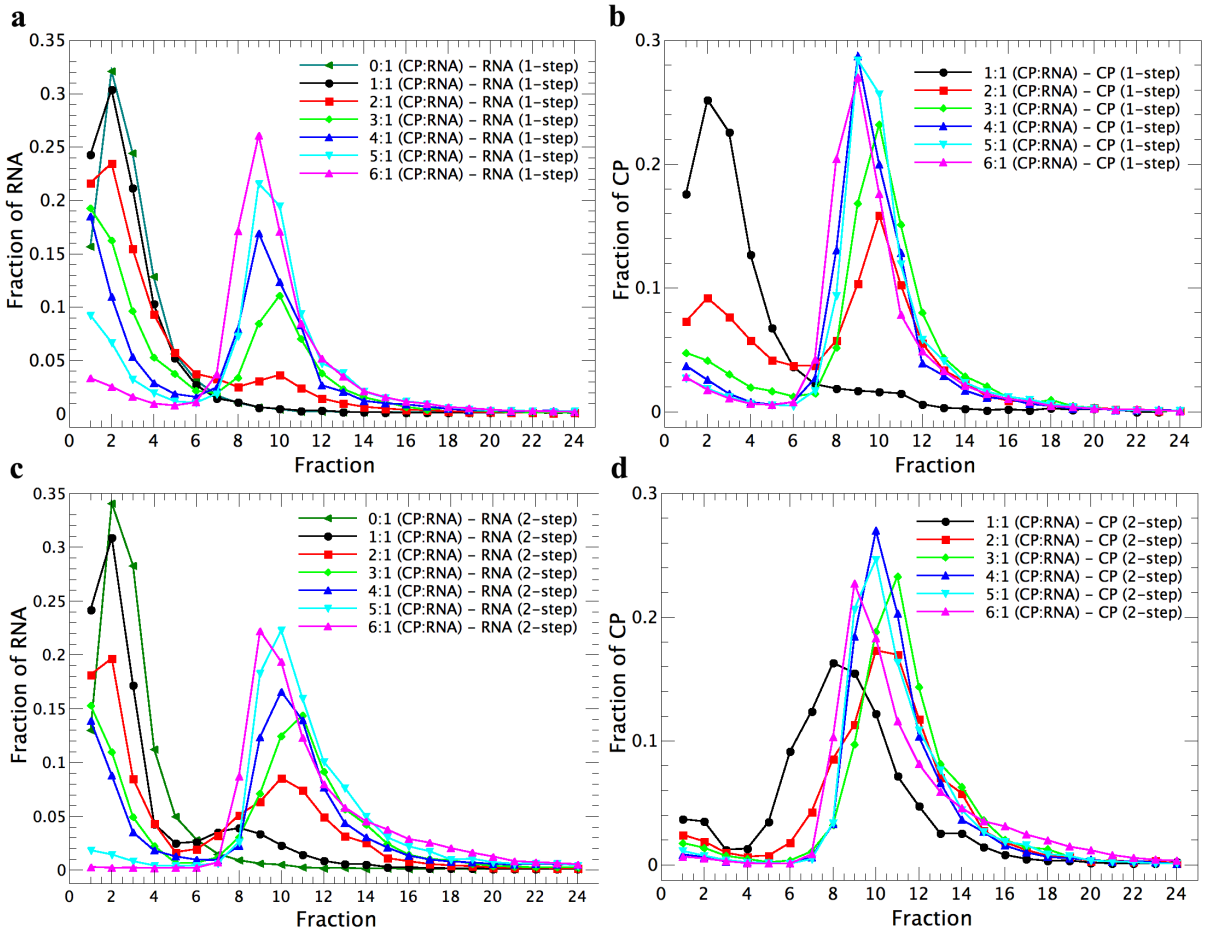

FIG S2 500-nt RNAs form multiple-RNA complexes at neutral pH. Sedimentation profiles in a sucrose gradient of bi-labeled assemblies (AF647-CP and AF488-RNA) at CP:RNA mass ratios ranging from 0:1, 1:1, 2:1, 3:1, 4:1, 5:1 and 6:1. a) and b) Profiles of the RNA (a) and CP (b) for the one-step assembly protocol. c) and d) Sedimentation profiles of the RNA (c) and CP (d) for the two-step assembly protocol.

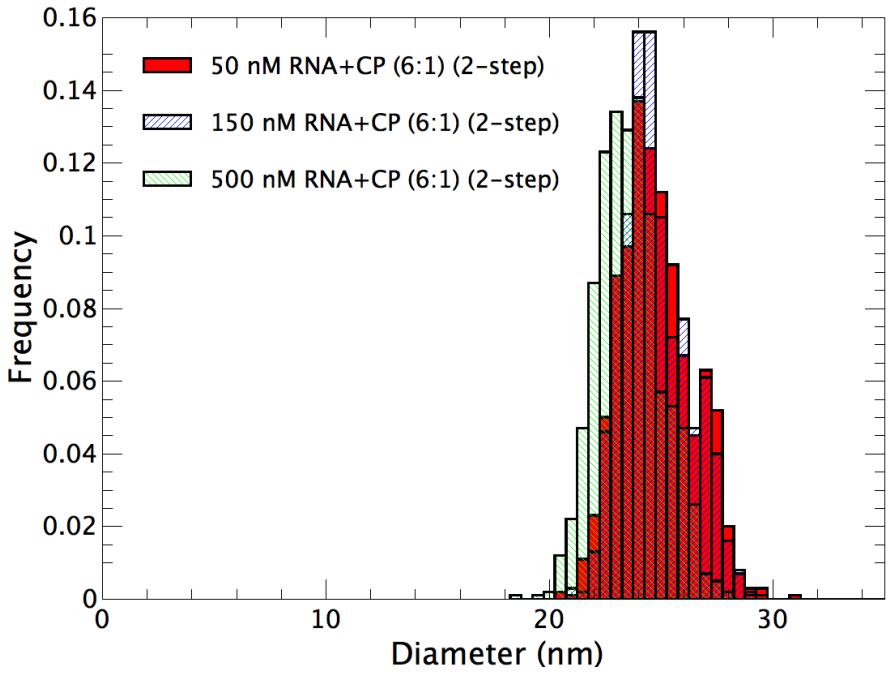

FIG S3 The size of the capsids is independent of the absolute concentration of the RNA. 500-nt RNA assemblies at three RNA concentrations (50, 150 and 500 nM) were carried out at the magic (6:1 CP:RNA mass) ratio by the two-step protocol and then analyzed by negative-stain EM. The absolute concentration of RNA at fixed mass ratio, over a 10-fold concentration interval under the two-step assembly protocols, is seen to have no effect on the size of the capsid.