Supplementary Materials for:

Molecular crowding accelerates ribozyme docking and catalysis

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1. RNA purification and labeling

A two-piece construct was used to assemble the hairpin ribozyme. Purified and labeled RNAs were purchased from the Keck Foundation Resource Laboratory at the Yale University School of Medicine. The 2'-hydroxyl protection groups were deprotected according to the manufacturer's protocol. The RNAs were purified by denaturing gel electrophoresis (20% wt/vol polyacrylamide and 8 M urea) and diffusion elution against elution buffer (0.5 M NH_4OAc and 0.1 mM EDTA) overnight at 4°C, followed by chloroform extraction, ethanol precipitation and C8 reverse-phase HPLC. The 5' end of the ribozyme is labelled with Cy5 using C6 amino linker in labelling buffer (100 mM Na_2CO_3, pH 8.5) overnight at room temperature. The doubly labelled RNA was purified by ethanol precipitation and reverse-phase HPLC. RNA concentrations were measured by UV-Vis absorbance at 260 nm.

2. Single Molecule FRET

Single molecule experiments were carried out as previously described.[1,2] We annealed the two RNA strands at 0.5 μM HPRZ A and 1 μM HPRZ B in standard buffer (50 mM Tris-HCl pH 7.5, and 10 mM $MgCl_2$ in saturating trolox). We heated a 10 μL solution at 90°C for 45 s before cooling to room temperature over 20 min. We diluted the annealed, biotinylated and fluorophore-labeled complex to 25 pM and immobilized the RNA to a streptavidin-coated quartz microscopic slide via a biotin-streptavidin bridge to generate a surface density of ~0.1 molecules/μm^2. We then excited the donor fluorophore from a home-built total internal reflection microscope with a 532 nm laser. We separated the donor and acceptor emission using appropriate dichroic mirrors (610DCXR, Chroma) and detected them as two side-by-side images on a back-illuminated electron-multiplied CCD camera (Andor I-Xon) at 33 ms time resolution. We obtained measurements at room temperature with an oxygen-scavenging system (OSS) consisting of protocatechuic acid (PCA) and protocatechuate-3,4-dioxigenase (PCD) to reduce photo bleaching of the fluorophores. Different percentages of PEG were mixed in OSS solution for PEG measurements. We then constructed histograms and calculated dwell times for each folding event to determine the folding rate constants, as described. A threshold of 0.5 FRET was used to distinguish between docked and undocked states. The dwell

time histograms were fit to single exponential and multiple exponential equations to determine k_{dock} and k_{undock}, respectively.

3. Cleavage Assays

Single-turnover ensemble cleavage assays were performed in a standard buffer solution (50 mM TRIS-HCl and 10 mM $MgCl_2$). Ribozyme (HPRZ A) labeled with Cy3 and Cy5 was mixed with radioactively labeled substrate (HPRZ B labeled at 5' end) in a 20:1 ratio and pre-annealed by heating to 70˚C for 2 min followed by slow cooling over 15min at room temperature.[3] We performed cleavage assays using 200 nM of the ribozyme and 10 nM of the 5' end radiolabeled substrate. After heat annealing we added PEG in RNA solution and allowed the solution for 10-15 mins to equilibrate. Then, magnesium was added to initiate the reaction. Reaction mixture of 2 µl was quenched using quenching buffer at desired time intervals, and then it was loaded into the 20% denaturing PAGE gel. The cleaved product was scanned through phosphorimager, and resulting fraction cleaved products were fit to single or double exponentials functions of the form, $y(t) = y_0 + A_1(1 - e^{-k_{obs1}*t})$, where A_1 is fraction of cleavage and $y(t) = y_0 + A_1(1 - e^{-k_{obs1}*t}) + A_2(1 - e^{-k_{obs2}*t})$, where A_1+A_2 is the final extent of the cleavage and k_{obs1} and k_{obs2} are the apparent rate constants respectively. All the cleavage assays were performed at room temperature.

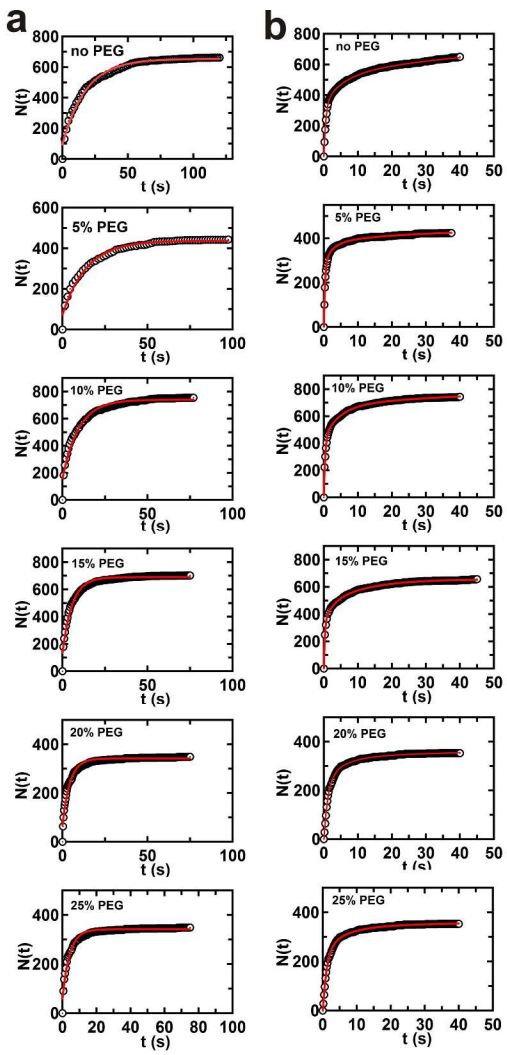

Figure S1. Docking and undocking rate constants in the presence of increasing PEG concentrations. (a) The ribozyme's docking rate constant in increases with increasing PEG concentrations. In 25% PEG, the docking rate constant is ~5-fold higher than in the absence of PEG. (b) The ribozyme exhibits multi-exponential undocking rate constants at all PEG concentrations. The undocking rate constants do not change significantly with increasing PEG concentrations.

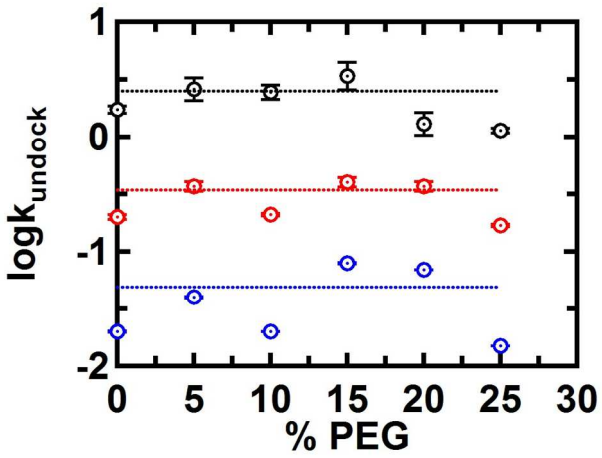

Figure S2. Crowding does not alter undocking rate constants significantly. Three different undocked rate constants were obtained from the fit in Supplementary Figure 1 (blue, red and black markers), in agreement with prior results revealing the folding heterogeneity in ribozyme folding.

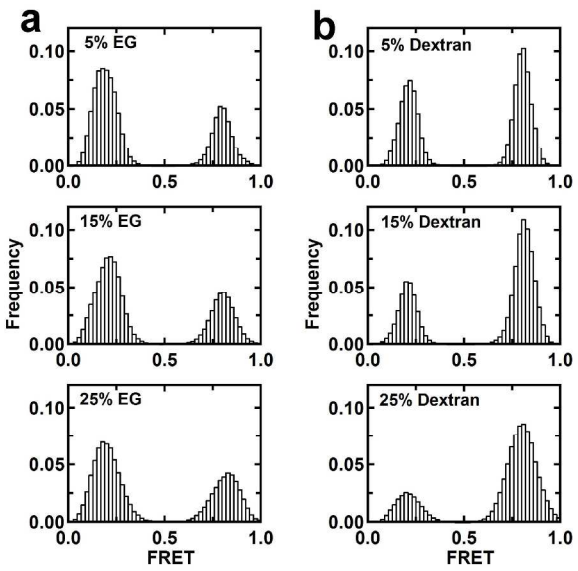

Figure S3. (a) FRET histograms in Ethylene glycol (a PEG monomer). Both the docked and undocked state populations conformations remains unchanged with increasing ethylene glycol concentrations. (b) FRET histograms in dextran (a 10 KDa molecular crowding agent). The docked state population increases with increasing dextran concentration, consistent with the PEG results. At least 103 trajectories were used to construct each histogram.

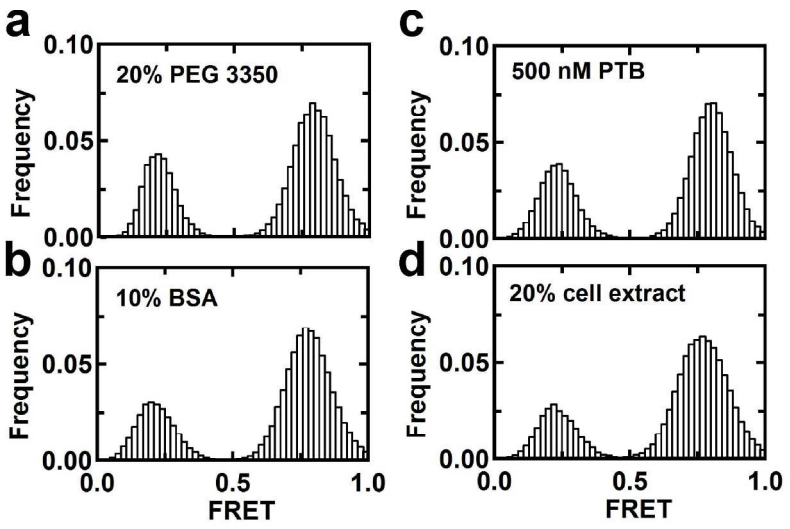

Figure S4. Effect of various crowding agents in ribozyme folding. (a) A smaller crowding agent (PEG 3350) yields less stabilization of the docked state. (b) A non-RNA binding protein (BSA) also favours the docked conformation. (c) A non-specific RNA binding protein (PTB) also favours the docked conformation. Higher concentrations of PTB could not be used due to surface crashing. (d) Yeast cellular extract also stabilizes the most compact sate. At least 47 trajectories were used to construct each histogram.

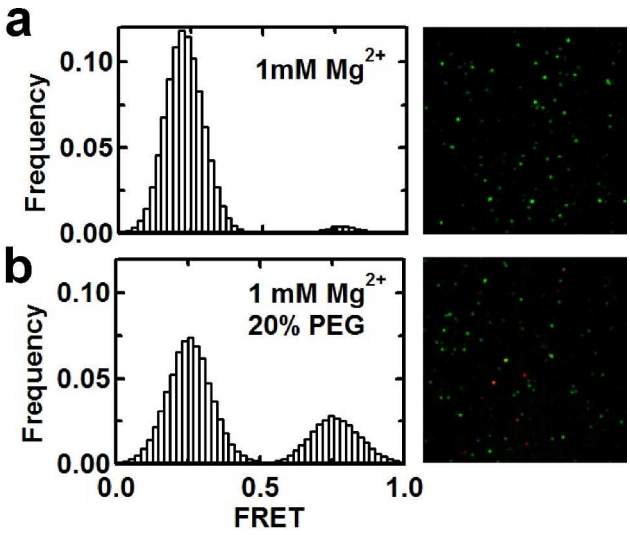

Figure S5. Crowding agents reduce the requirement for Mg^{2+} ions. (a) FRET histograms showing the distribution of the docked and the undocked state population in dilute solutions. At 1 mM Mg^{2+} ions, the majority of the molecules remains at the undocked (0.2 FRET) state, and the majority of molecules appear green in the single molecule image (right). (b) FRET histograms showing the distribution of the docked and the undocked state population in 20% PEG. Almost ~10-fold more molecules switch to the docked state, and more molecules appear red in the single molecule image (right). At least 105 trajectories were used to build each FRET histogram.

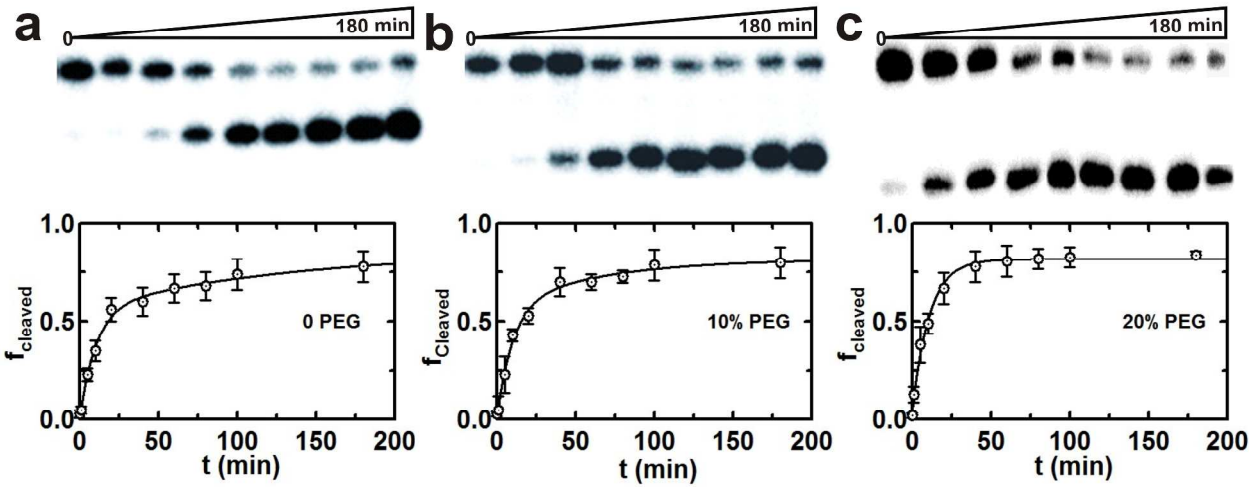

Figure S6. Cleavage of 5'-end radiolabelled substrate analysed in 20% PAGE in (a) dilute solution, (b) 10% and (c) 20% PEG. Top band is uncleaved substrate and bottom band is cleaved product, which increases over time. The increase of the cleaved fraction with time yields two observed cleavage rate constants (fast and slow). As the PEG concentration increases, the slow fraction disappears.

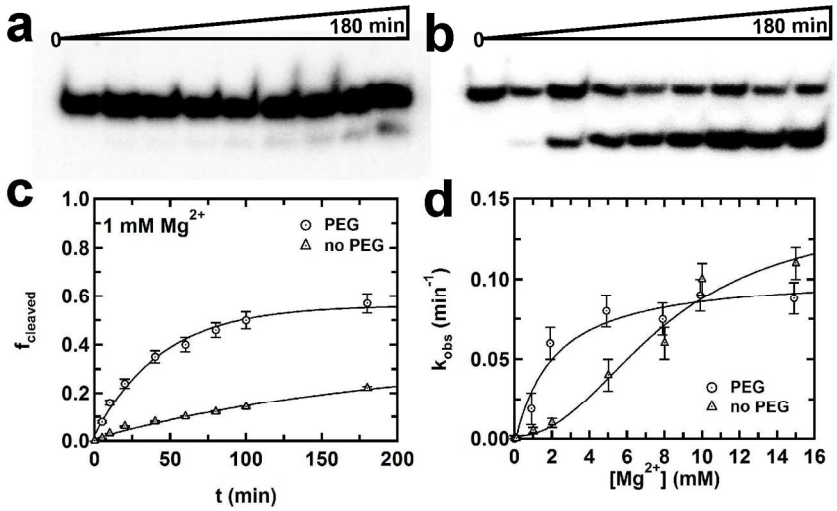

Figure S7. Cleavage assays for the 5'-end radiolabelled substrate at various magnesium concentration and in presence and absence of crowding agents. (a) In 1 mM Mg^{2+} and in the absence of PEG, the ribozyme exhibits very little activity. (b) Addition of Cleavage 20% PEG increases the activity significantly. (c) Fraction cleaved in 1 mM Mg^{2+} in presence and absence of 20% PEG. (d) The plot of observed rate constants in presence and absence of crowding agents as a function of Mg^{2+} ions. The ribozyme activity is accelerated in presence of crowding agents at near physiological concentration of Mg^{2+} ions. The K_{Mg}^{2+} decreases from 8 ± 2 mM to 2 ± 1 mM in presence of crowding agents.

References

(1) Roy, R.; Hohng, S.; Ha, T. *Nature methods* 2008, *5*, 507.
(2) Zhao, R.; Rueda, D. *Methods* 2009, *49*, 112.
(3) Walter, N. G.; Burke, J. M. *Curr Opin Chem Biol* 1998, *2*, 24.