Supplemental on-line material for

Hindered diffusion in polymeric solutions studied by Fluorescence Correlation Spectroscopy

Silviya P Zustiak, Ralph Nossal, Dan Sackett

Supplemental Materials and Methods

Viscosity measurements

Viscosity measurements were performed with an Oswald viscometer (Fisher Scientific, Pittsburg, PA) in a water bath at 22^{o}C. The measured solution viscosity, η, was normalized by that of water, η_0. The scaled quantities η_0/η are thus reported.

Succinylation of Ribonuclease A (RNase)

Ribonuclease A (RNase) was converted to an acidic protein, succinylated RNase (scRNase), by reacting with succinic anhydride following a well-established procedure (1). Briefly, RNase was dissolved in 10X Phosphate Buffered Saline (PBS), pH 7.4. Solid succinic anhydride was added in the same weight amount as the RNase every 15 min for 75 min total. The reaction was maintained at pH 7.4 by titration with 1 *N* NaOH. The product was dialyzed (Float-A-Lyzer, MWCO: 500-1000 Da, Spectrum Laboratories, Inc., Rancho Dominguez, CA) overnight in PBS with 3 changes of the dialysis buffer, concentrated via ultrafiltration (Microcon YM-10, Millipore, Billerica, MA) and stored at 4^{o}C until further use.

Electrophoresis

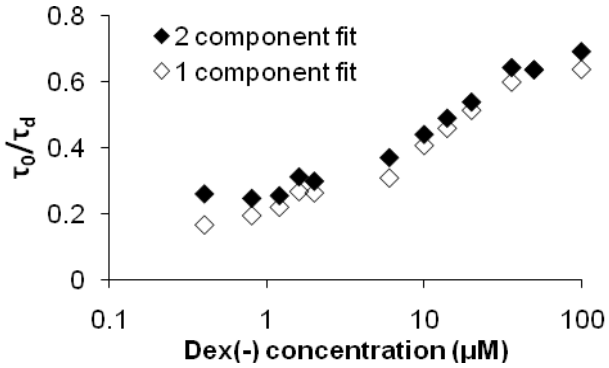
Conversion of RNase charge was confirmed via electrophoresis in 3 mm deep, 1% w/v agarose gels, in 10 mM Imidazole/HEPES buffer, pH 7.4 (HEPES; MP Biomedicals, Solon, OH) (2). Azoalbumin, horse cytochrome c, and un-modified RNase A were used as controls. All samples were diluted to 0.5 mg/ml in loading solution (20% v/v glycerol, 20% w/v Imidazole/HEPES buffer, 1% w/v BromPhenol Blue) and 20 µl aliquots were loaded in each well. Samples were run for 30-40 min at 90 V, constant voltage, and 22^{o}C. The gels were fixed in 5% acetic acid, 50% methanol overnight. After rinsing with 10% acetic acid, the gels were stained with Coomassie brilliant blue R-250 (Piercenet, Rockford, IL) for 15 min, de-stained in 5% acetic acid, 25% methanol overnight or until the background was clear. scRNase was labeled with DyLight 488 the same way as RNase, as described in the Materials and Methods Section of the paper.

Supplemental References:

1. Epstein, C. J., and R. F. Goldberger. 1964. The Reoxidation of Reduced Ribonuclease Derivatives. J Biol Chem 239:1087-1089.
2. McLellan, T. 1982. Electrophoresis buffers for polyacrylamide gels at various pH. Anal Biochem 126:94-99.
3. Kosar, T. F., and R. J. Phillips. 1995. Measurement of Protein Diffusion in Dextran Solutions by Holographic Interferometry. AIChE Journal 41:701-711.

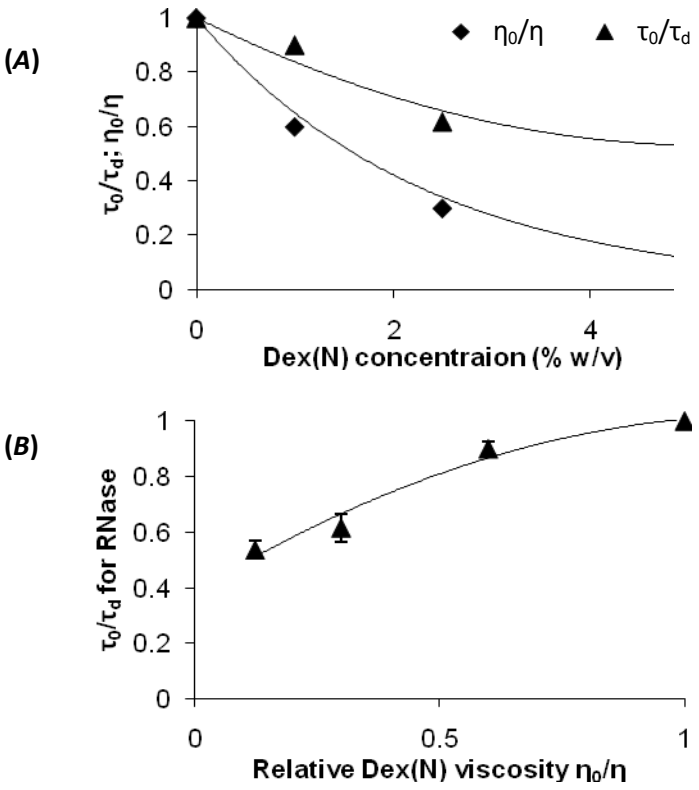
Supplemental Figures

Figure S1



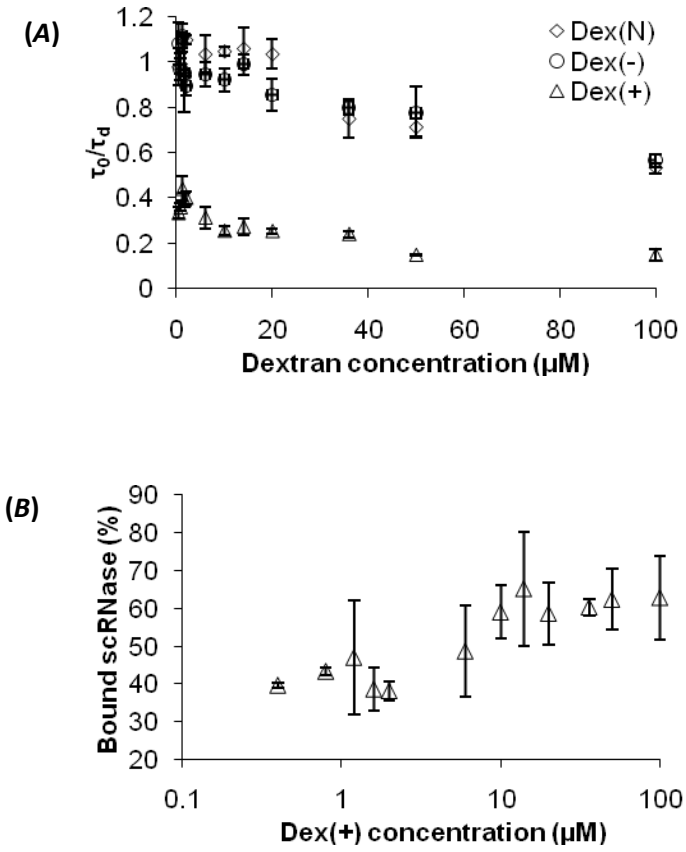
Compound RNase diffusivity in solutions of Dex(-) of increasing concentration: Normalized diffusivities were obtained from fitting the FCS autocorrelation function with a single component fit, or with a two-component fit, where RNase compound diffusivity was the average of the fast and slow components weighted by their contribution to the experimental signal - $\tau_d = f\tau_{d1} + (1-f)\tau_{d2}$. There was no significant difference between the two data sets (t-test p = 0.53), indicating that, for the chosen conditions, a single component fit was well suited to describe the RNase compound diffusivity in the presence of binding.

Figure S2



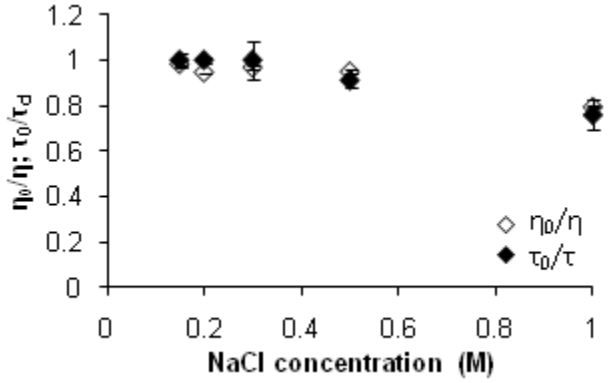
FCS: (*A*) Diffusivity of RNase in solutions of Dex(N). The RNase diffusivity data is based on our own measurements (n = 9). The data on viscosity of the dextran solution is based on measurements by Kosar and Phillips (3); (*B*) Diffusivity of RNase as a function of Dex(N) viscosity, showing a negative deviation from the Stokes-Einstein relationship, $D = kT/6\pi\eta r$. The lines are provided to guide the eye.

Figure S3:



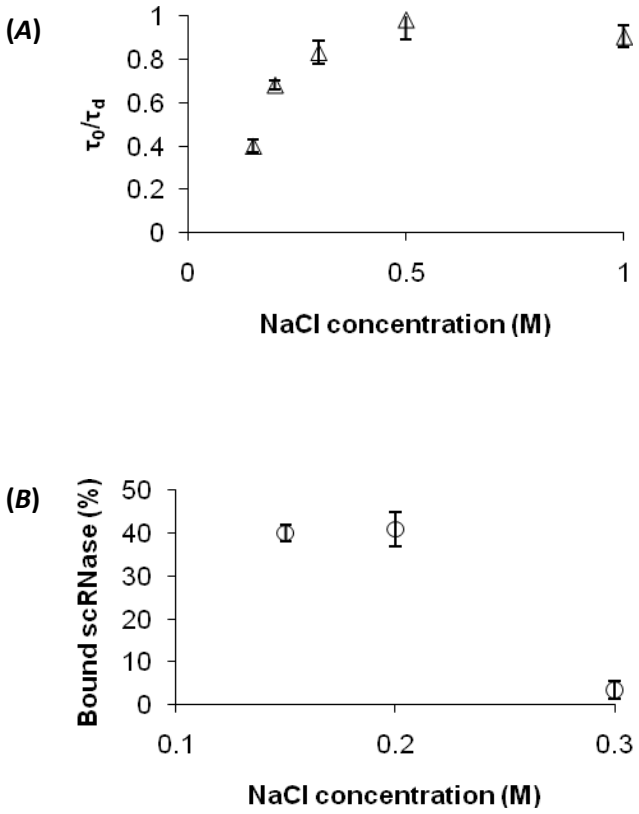
FCS: Effect of reversing the charge of RNase via succinylation (scRNase has a net negative charge): (*A*) Normalized characteristic diffusion time of 0.35 μM scRNase in dextran solutions of indicated charges and concentrations; (*B*) Binding of scRNase to Dex(+). All data shown are the average ± *SD* (n=9).

Figure S4:



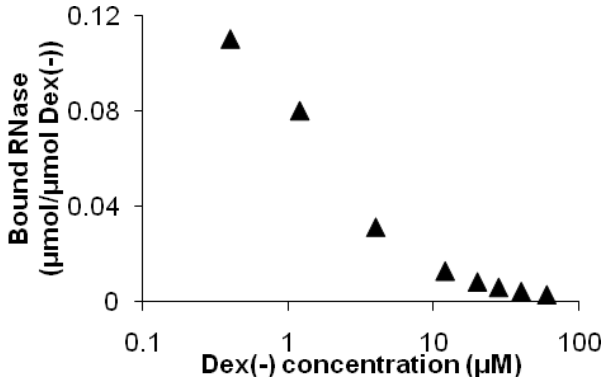
Increasing the concentration of NaCl from 0.15 M to 1 M, resulted in an increased viscosity of the dextran solutions, which led to a decrease in RNase diffusivity. All solutions were prepared in PBS, pH 7.4 (0.15 M NaCl) with 2 μM Dex(N) and increasing concentrations of NaCl. Fluorescent RNase at 0.2 μM was added to the solution only for the diffusivity measurements but not for the viscosity measurements. Relative solution viscosity, η_0/η, where η_0 is the viscosity of water, and RNase normalized diffusivity, τ_0/τ, exhibited similar dependence on NaCl concentration.

Figure S5:



FCS: Increased ionic strength prevented electrostatic binding between scRNase and Dex(+): (*A*) Normalized characteristic diffusion time and binding of 0.35 μM scRNase in 2 μM Dex(+) solutions as a function of NaCl concentration; (*B*) Binding of scRNase to Dex(+) as a function of NaCl concentration. All data shown are the average ± *SD* (n=9).

Figure S6:



Ultrafiltration: Titration experiment for binding of RNase (0.2 µM) to Dex(-) (0.4-60 µM as indicated in the x-axis). By plotting µmol bound RNase to µmol Dex(-) as a function of Dex(-) concentration we observed that binding of RNase to Dex(-) changed from 0.110 to 0.002 µmol of bound RNase per µmol of Dex(-).All data shown are the average ± *SD* (n=3).