Supporting Information

High-Throughput FRAP Analysis of Solute Diffusion in Hydrogels

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Links to further resources

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DOI Figshare Link to protocols for experiments:

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DOI Figshare Link to R scripts used to analyze data:

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DOI Figshare Link to summary data and GraphPad File used to analyze data:

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DOI Figshare Links to batch-analyzed, manually analyzed, solute, swelling, and partition coefficient data:

https://doi.org/10.6084/m9.figshare.14998644

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https://doi.org/10.6084/m9.figshare.14998668

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Table S1. Hydrogel Formulations and Swelling Characteristics

Formulation	Initial Polym. Vol. Frac. (φ_0)	Deg. Polym. Bet. Jun. (N_j)	Jun. Funct. (f)	Freq. Chain-End Defects (γ)*	Swollen Polym. Vol. Frac. (φ_s)	Mesh Size (ξ, nm)	Mesh Radius (r_m, nm)
PVA-M-50-40	0.050	20	4	0.052	0.079	4.6	3.8
PVA-M-50-60	0.050	30	4	0.078	0.066	6.0	4.9
PVA-M-50-80	0.050	40	4	0.104	0.056	7.3	6.0
PVA-M-50-100	0.050	50	4	0.130	0.053	8.4	6.8
PVA-M-50-120	0.050	60	4	0.156	0.049	9.4	7.7
PVA-M-50-140	0.050	70	4	0.182	0.043	10.6	8.7
PVA-M-75-40	0.075	20	4	0.052	0.128	3.9	3.2
PVA-M-75-60	0.075	30	4	0.078	0.105	5.2	4.2
PVA-M-75-80	0.075	40	4	0.104	0.090	6.3	5.1
PVA-M-75-100	0.075	50	4	0.130	0.080	7.3	5.9
PVA-M-75-120	0.075	60	4	0.156	0.074	8.2	6.7
PVA-M-75-140	0.075	70	4	0.182	0.069	9.1	7.4
PVA-M-100-40	0.100	20	4	0.052	0.185	3.5	2.8
PVA-M-100-60	0.100	30	4	0.078	0.135	4.7	3.9
PVA-M-100-80	0.100	40	4	0.104	0.121	5.7	4.6
PVA-M-100-100	0.100	50	4	0.130	0.111	6.5	5.3
PVA-M-100-120	0.100	60	4	0.156	0.099	7.4	6.1
PVA-M-100-140	0.100	70	4	0.182	0.090	8.3	6.8

*Frequency of chain-end defects was calculated from the measured PVA molecular weight (M_N = 33,900 g/mol, PDI = 1.81) and synthesis-predicted degree of polymerization between junctions (N_j): $\gamma = \frac{fM_rN_j}{(f-2)M_n}$, where *f* is the junction functionality and M_r is the formula weight of the PVA repeating unit (44 g/mol).

Table S2. Solute Characteristics

Name	ID	Hydrodynamic Radius (nm)	Stokes-Einstein Diffusivity ($\mu m^2 s^{-1}$)	FRAP Diffusivity ($\mu m^2 s^{-1}$; $n = 3$)
Fluorescein	FL00	0.5	489	278 ± 13.5
FITC-dextran, 4 kDa	FD04	1.5	160	142 ± 6.8
FITC-dextran, 20 kDa	FD20	3.3	73	85 ± 14.5
FITC-dextran, 70 kDa	FD70	6.1	40	60 ± 1.7
FITC-PEG, 5 kDa	FP05	2.3	108	114 ± 3.9
FITC-PEG, 20 kDa	FP20	4.9	50	61 ± 0.3
FITC-PEG, 40 kDa	FP40	7.2	34	44 ± 2.6

The hydrodynamic radius of fluorescein was taken from previous reports,[1-2] and the hydrodynamic radii of the FITC-dextrans and FITC-PEGs was calculated from their nominal molecular weights according to the method of Armstrong et al.[3]

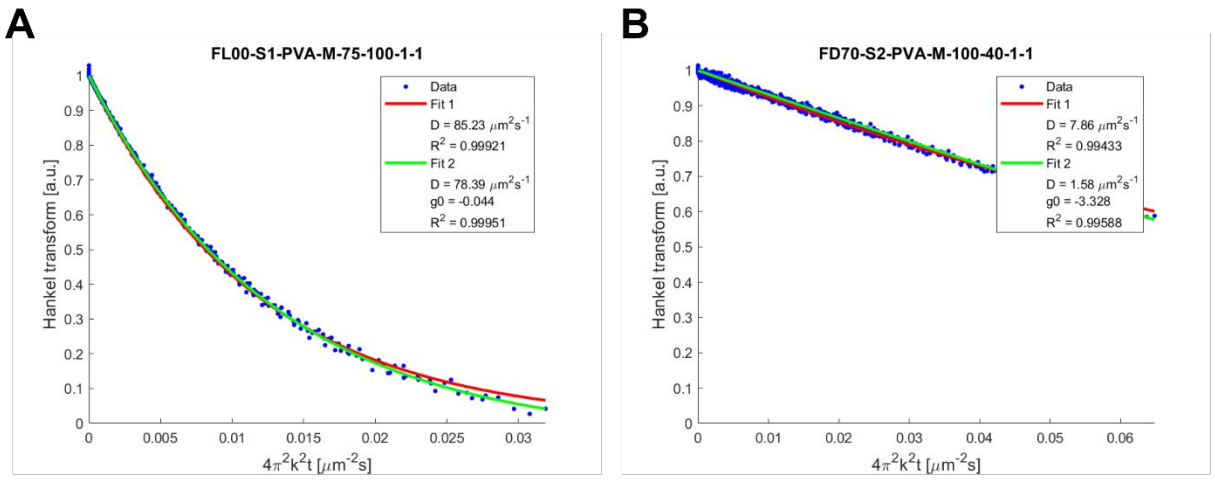

Figure S1. Representative graphs of FRAP experiments that reached equilibrium (A) and did not reach equilibrium (B). Experiments that reach equilibrium also reach an asymptote for the Hankel transform, typically near Hankel transform values of zero. Solute-hydrogel pairings with higher diffusion coefficients were more likely to reach equilibrium within the standard FRAP experiment time. Graphs were produced using the automated FRAP analysis program.

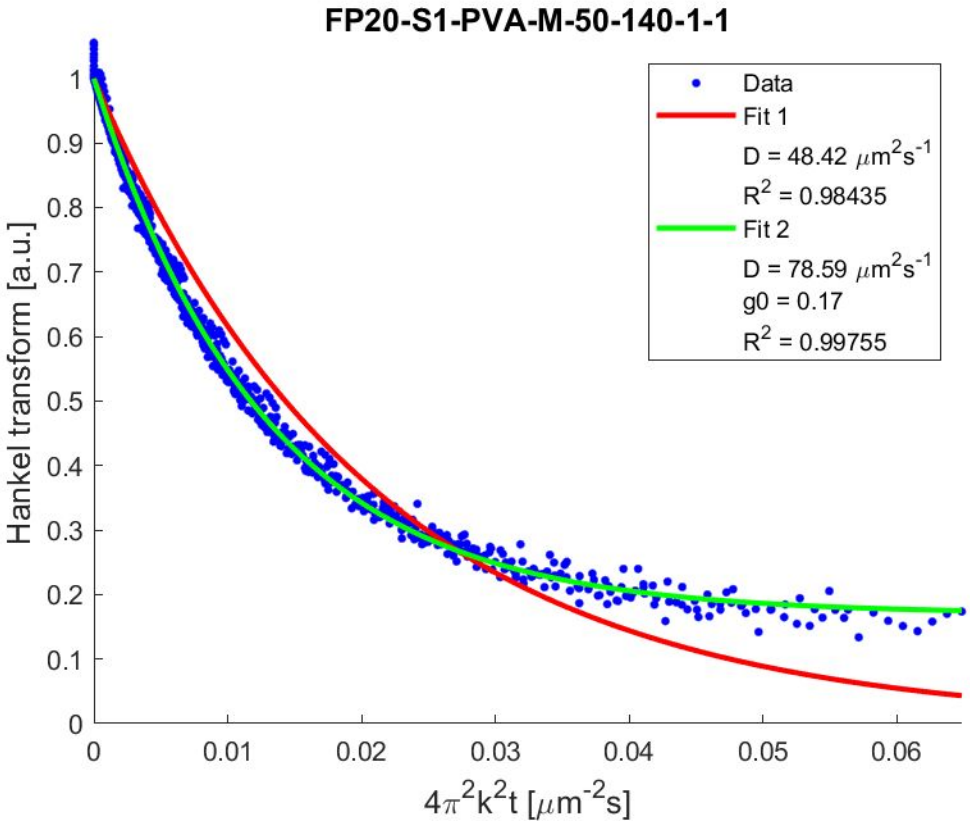

Figure S2. Example of a large deviation between Fit 1 and FRAP experimental data at diffusive equilibrium. The graph was produced using the automated FRAP analysis program.

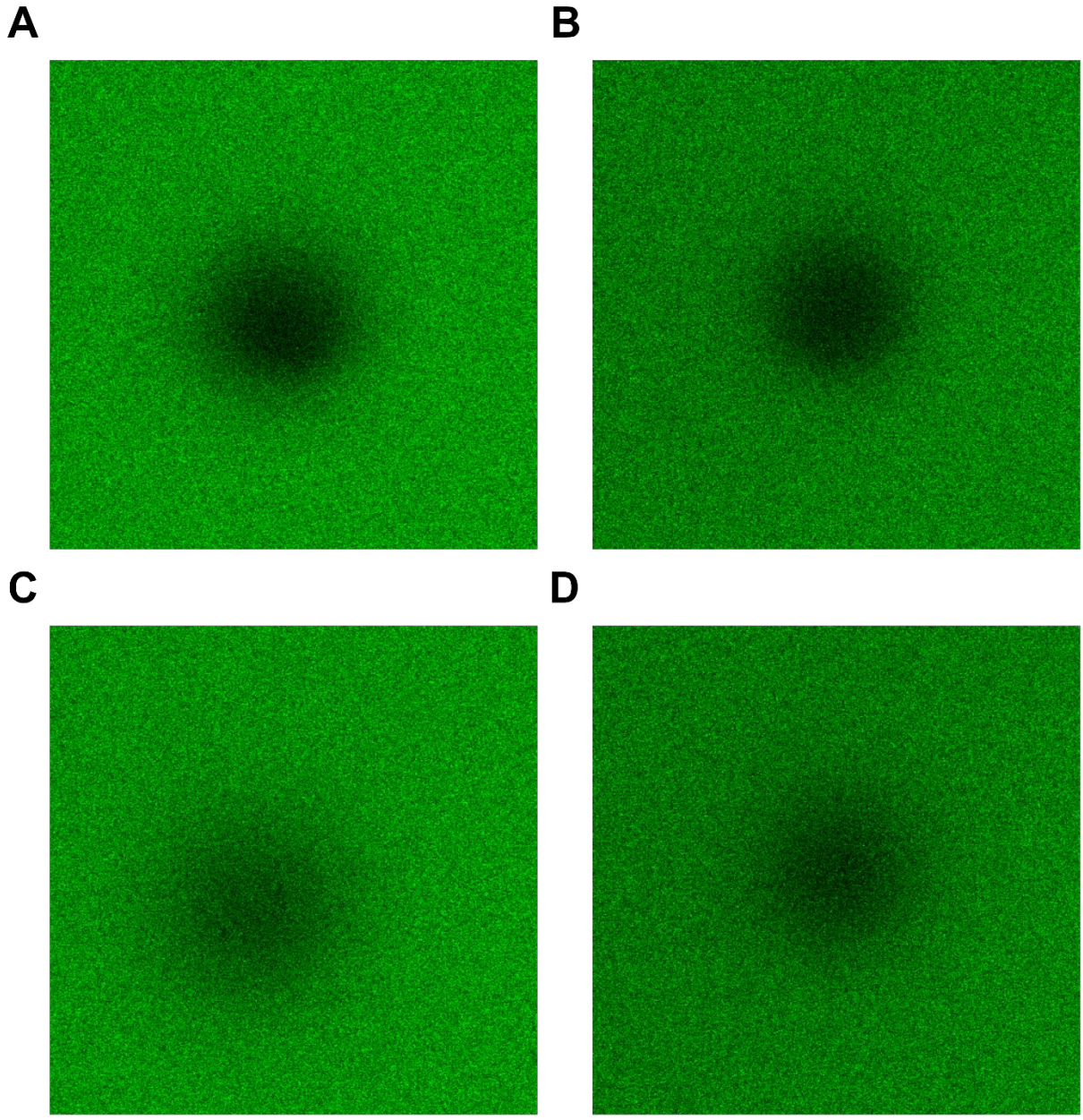

Figure S3. Comparison of bleaching spot stability in solution and in hydrogels. In the first post-bleach frame, the FP20 (20 kDa FITC-PEG) bleach spot is centered in both the solution (A) and in a hydrogel (B). By the 10th post-bleach frame, the bleach spot in solution has drifted toward the bottom-left (C), while the bleach spot in the hydrogel remained centered (D). The rate of in-solution bleach spot drift varied between experiments and with different solutes, but bleach spots in hydrogels remained consistently centered, suggesting that negligible convection occurred within the hydrogels.

Partition Coefficient Measurements in Three PVA Hydrogel Formulations

Methods: To further investigate the unusual behavior of PEG solutes in the PVA hydrogels, partition coefficients of five of the seven solutes into three hydrogel formulations were measured. The five solutes were FL00, FP05, FP20, FP40, FD70; lower molecular weight FITC-dextran solutes (FD04, FD20) were not available due to COVID-related backorders. The three hydrogel formulations were ("Lo" $\varphi_0 = 0.050$, $N_j = 70$, "Me" $\varphi_0 = 0.075$, $N_j = 50$, and "Hi" $\varphi_0 = 0.100$, $N_j = 30$. Since the sample material was limited for the "Me" formulation, three samples (~80 mg, 5 mm diameter punch) per solute were used for Lo and Hi hydrogel formulations, but only two samples per solute were used for the Me hydrogel formulations.

First, a serial dilution for each solute was made, establishing a linear relationship between intensity and concentration up to 2 μM for each solute. Second, stock solutions of each solute at approximately 5 μM were made. Their initial concentrations (C_0) were measured by diluting a small sample of each solution 1:10 to enter the linear intensity-concentration range and measuring intensity in a Cytation 3 spectrophotometer with excitation/emission at 490/525 nm. From the intensity data, initial concentrations were back-calculated using the standard curve and a 10x multiplication factor to account for the dilution.

For each solute-hydrogel pairing, hydrogel samples were separately incubated in 1.5 mL black tubes with 1 mL of the solution for 24 hours. After 24 hours, the supernatant was removed from each tube, diluted 1:10, and fluorescently measured to calculate the equilibrium concentration (C_e) as described above. Partition coefficients for each sample were calculated according to the following equation:[4]

$$K = \frac{C_h}{C_e} = \frac{V_s(C_0 - C_e)}{V_h C_e}$$

Where K is the partition coefficient, C_h is the concentration inside the hydrogel, V_s is the volume of the solution, and V_h is the volume of the hydrogel. Notably, since the concentration within the hydrogels was not measured directly, it is unclear how C_h is affected by surface interactions.

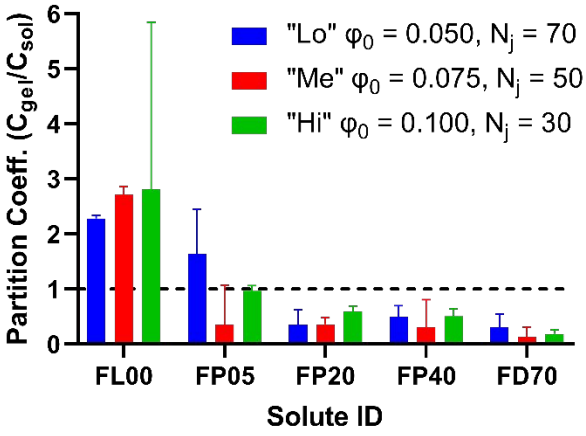

Figure S4. Partition coefficients of fluorescent solutes in three PVA hydrogel formulations with low, medium, and high equilibrium polymer concentrations. FL00: Fluorescein, FP05: FITC-PEG, 5 kDa, FP20: FITC-PEG, 20 kDa, FP40: FITC-PEG, 40 kDa, FD70: FITC-dextran, 70 kDa, φ_0: Initial polymer volume fraction, N_j: Degree of polymerization between junctions.

References

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