Electronic Supplementary Information

Fig. S1 Mass inclusion/exclusion of rhodamine-conjugated proteins using hydrogel posts.

For further characterization of mesh size, PEG-DA/AC-PEG-Biotin posts were dried and then rehydrated by the addition of PBS containing proteins of varying hydrodynamic radii conjugated to rhodamine (β-lactoglobulin A, M_w 18.4 kDa, R_{HYD} 2 nm (Ahmadi et al., 2015); SOD, M_w 32.5 kDa, R_{HYD} 2.54 nm (McCord and Fridovich, 1969); ovalbumin, M_w 43-45 kDA, R_{HYD} 2.92 nm ((Shearwin and Winzor, 1990)). Fluorescent image exposures in column A were acquired 5 min. following the addition of the protein-rhodamine conjugate and images shown in column B were acquired following a subsequent wash with PBS (exposure times taken for each image are indicated). The absence of post fluorescence using ovalbumin and SOD is consistent with the absence of non-specific binding and exclusion from the hydrogel matrix due to their respective molecular dimensions. B-lactoglobulin A, however, shows uniform penetration, which is again predicted by the calculated average mesh size for posts. The retention of fluorescence at the exterior of post surfaces following a brief wash (column B, 40 ms exposure) is enhanced by the longer exposure time and was found to be absent subsequent to extensive washing with PBS.

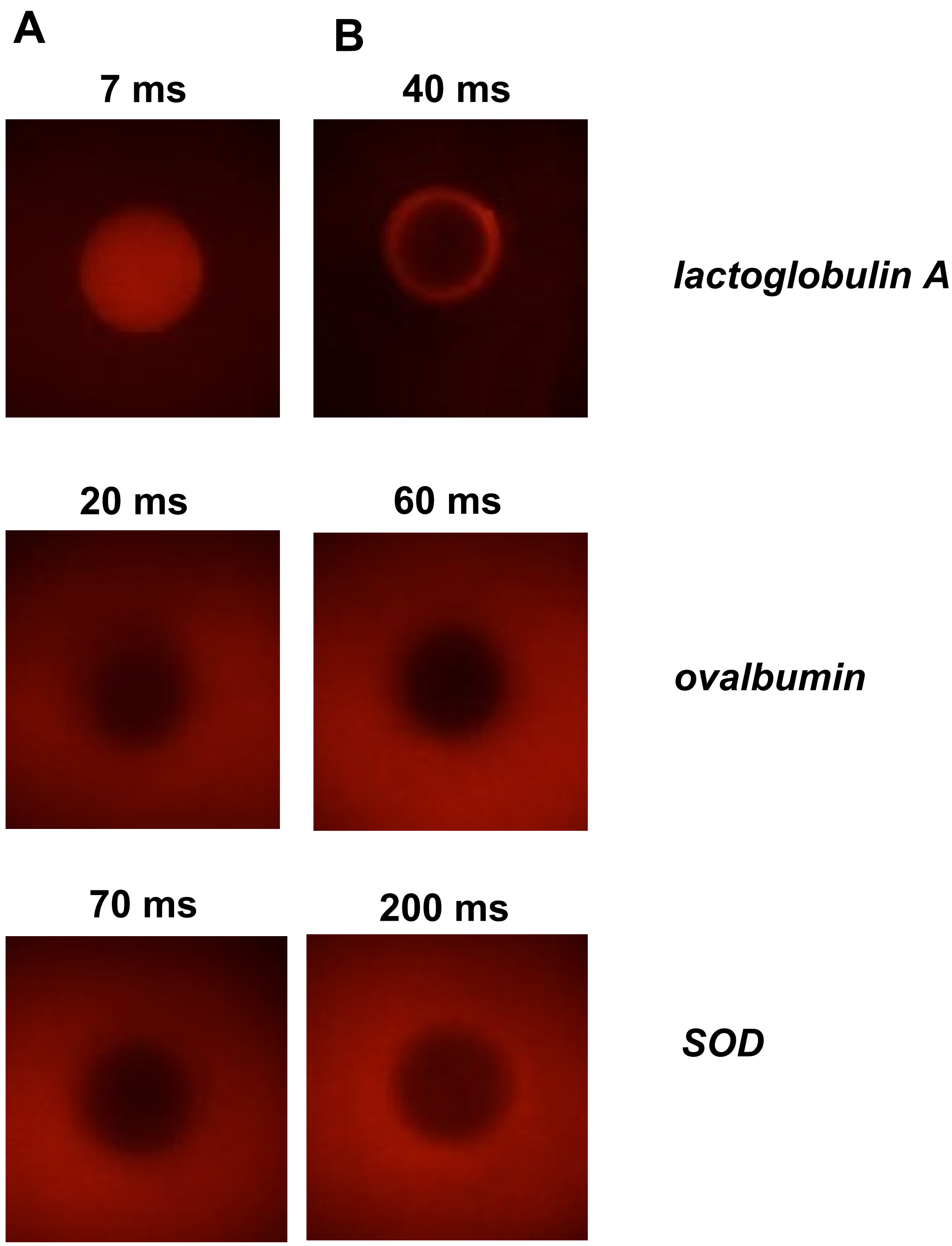
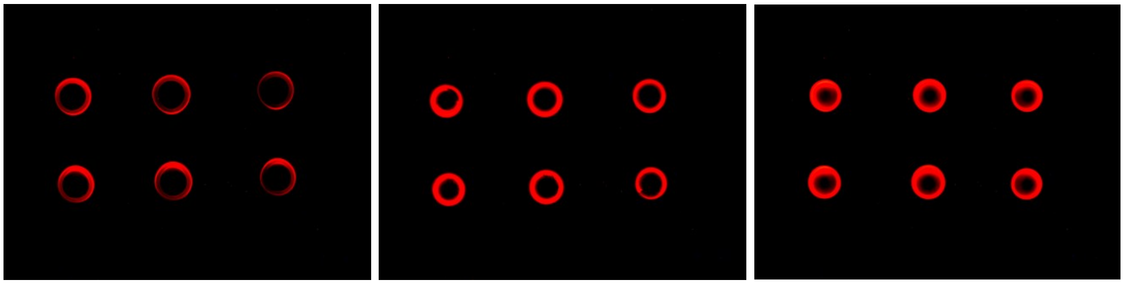

A
B
7 ms
40 ms
lactoglobulin A
20 ms
60 ms
ovalbumin
70 ms
200 ms
SOD

Fig S2. Fluorescent images of biotin/NA rhodamine assay with 0.2%, 0.5% 1% (w/w) of biotin.



0.2% **0.5%** **1%**

Average Mesh Size calculation

The hydrogel mesh size is estimated based on Flory-Rehnor Equation. Where the 20% (w/w) PEGDA 700 solution was used to fabricate hydrogel structures. If we assume the mass swelling ratio $Q_m = 4$ (where the water has evaporated completely). We can get the polymer volume fraction in the swollen state $v_{2,s}$, which is $v_{2,s} = \frac{\frac{1}{\rho_{polymer}}}{\frac{Q_m}{\rho_{solvent}} + \frac{1}{\rho_{polymer}}} = 0.183$. Where $\rho_{solvent} = 1g/mL$ and $\rho_{polymer} = 1.12g/mL$

Then we inserted the value of $v_{2,s}$ into equation 1 to get the average molecular weight between two adjacent crosslinks $\overline{M}_c = 250\ g/mol$

$$\frac{1}{\overline{M}_c} = \frac{1}{\overline{M}_n} - \frac{\frac{\bar{v}}{V_1}[In(1 - v_{2,s}) + v_{2,s} + \chi_1 v_{2,s}^2]}{(v_{2,s}^{1/3} - \frac{v_{2,s}}{2})} \qquad (1)$$

Here $\overline{M}_n = 700$, which is the molecular weight of the polymer chains without crosslinking agent. χ_1 is a polymer-solvent interaction parameter, the value is 0.494 for PEG according to polymerdatabase.

The average mesh size can be calculated by equation (2) as,

$$\xi = \alpha(\bar{r}_0^2)^{1/2} \qquad (2)$$

α in equation (2) is the elongation ratio of polymer chains in any directions and $(\bar{r}_0^2)^{1/2}$ represents the distance of the polymer chains between two adjacent crosslinks, which is the average root distance and unperturbed.

α and $(\bar{r}_0^2)^{1/2}$ can be further represented as,

$$\alpha = v_{2,s}^{-1/3} \tag{3}$$

$$(\bar{r}_0^2)^{1/2} = l(C_n N) = l\left(\frac{2C_n\overline{M}_c}{M_r}\right)^{1/2} l \tag{4}$$

Here, l represents the length of the bond along polymer backbone and C_n is the Flory characteristic ratio. M_r is the molecular weight of repeating units in the composing polymer chains. If equations (3) and (4) are combined, the mesh size can be expressed as,[71]

$$\xi = v_{2,s}^{-1/3}\left(\frac{2C_n\overline{M}_c}{M_r}\right)^{1/2} l \tag{5}$$

In our system $M_r = 44\ g/mol$, l for or vinyl polymers is 1.54 Å and C_n is 6.9 according to the polymerdatabase. When we insert all those values into equation (5) we can get the average mesh size of the hydrogel in our system is about 2.4 nm.

time (min.)	**FI of Convection**	**FI of Mass Diffusion**
0	0	0
1	18.96733	0.17933333
3	32.40033	0.184
5	40.58233	0.14
7	45.934	0.29
10	54.13267	3.43333333
20	78.746	6.92666667
30	85.89733	9.80533333
60	97.259	9.07333333
120	107.389	15.999
240	113.3967	19.6993333

Table T1. Fluorescent signal of convection assay V.S. mass diffusion assay recorded as a function of time.

Table T2. First derivatives (*f'*(x)) of convection and mass diffusion plots for absolute fluorescence units recorded as a function of time. Convection (hyperbolic plot, where y = 19.247*ln*(x) + 14.126), R^2 = 0.975). Mass Diffusion (second-order polynomial plot, where y = -0.0005x^2 + 0.195 + 0.654, R^2 = 0.930).

time (min.)	**Convection**	**Mass Diffusion**
1	19.25	0.184
3	6.42	0.182
5	3.85	0.180
7	2.75	0.178
10	1.92	0.175
20	0.96	0.165
30	0.64	0.155
60	0.32	0.125
120	0.16	0.065
240	0.08	0.055