Supplementary Information for

Dynamic Covalent Dextran Hydrogels as Injectable, Self-adjuvating Peptide Vaccine Depots

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1. Materials Characterization

Figure S1. $^1$H NMR spectra (399.7 MHz) of Dex70k in D$_2$O.
Figure S2. $^1$H NMR spectra (399.7 MHz) of P70-4.2 (DS 4.2) in D$_2$O. Blue area shows dextran anomic protons and red area shows the protons in lipoic acid group.

Figure S3. $^1$H NMR spectra (399.7 MHz) of P20-6.4 (DS 6.4) in D$_2$O.
Figure S4. $^1$H NMR spectra (399.7 MHz) of P500-5.8 (DS 4.8) in D$_2$O.

Figure S5. $^1$H NMR spectra (399.7 MHz) of P70d-4.2 (DS 4.2) in D$_2$O.
**Figure S6.** $^1$H NMR (399.7 MHz, in D$_2$O/ PB8.2, 4 drops of D$_2$O in 950 μL PB8.2) monitoring of reduction of sodium lipoate by DTT at room temperature. Spectrum 1 (bottom) shows the sodium lipoate at 0 minutes and spectrum 2 (top) shows the result 10 minutes after adding DTT. The reaction can be monitored by characteristic protons: the peaks in blue square represent two protons of sodium lipoate, the peaks in yellow square represent two protons of reduced sodium lipoate.
Figure S7. Rheological sweep measurements of Dex P-A hydrogel. a) Time sweep measurements (γ = 0.5 %, ω = 1 Hz); b) Frequency sweep measurement (γ = 0.5 %, ω = 100-0.01 rad/s); c) Strain sweep measurements (γ = 1-1200 %, ω = 1 Hz); d) Step-strain sweep, alternative strain switched from 1% to 1200% twice then back to 1%.

2. Biological Experiments

Figure S8. a) Cumulative percentage SIINFEKL release from Dex P-A hydrogels loaded with 1000 µM SIINFEKL. b) T-cell activation induced by non-loaded Dex P-A hydrogels, measured as beta-galactosidase-directed CPRG hydrolysis. Data represent three independent experiments. Error bars are the standard error from the mean.
Figure S9. Fits of the Korsmeyer-Peppas model to the release data at a) 1 µM, b) 10 µM, c) 100 µM loaded peptide. We use the time-lag modified Korsmeyer-Peppas equation described below. As time-lag $t_{\text{lag}}$ we chose the latest time point where $[\text{peptide}]_{\text{released}} = 0$. The equation was fitted by least squares optimization in MS Excel. We excluded the $t = 48$ h data points from the fitting optimization. Fit parameters are: a) $k = 0.015$ h$^{-n}$; $n = 0.55$; $t_{\text{lag}} = 2$ h; b) $k = 0.033$ h$^{-n}$; $n = 0.49$; $t_{\text{lag}} = 6$ h; c) $k = 0.045$ h$^{-n}$; $n = 0.35$; $t_{\text{lag}} = 4$ h.

$$\frac{M_t}{M_\infty} = k(t - t_{\text{lag}})^n$$

3. Release of OVA$^{323-339}$

In addition to the SIINFEKL peptide, we tested the release of OVA$^{323-339}$ from Dex P-A hydrogels (Figure S10). The more hydrophilic nature of the OVA$^{323-339}$ peptide resulted in an early burst release after washing, followed by gradual release plateauing after 8 hours. On long time scales (>30 hrs), a slight reduction in T-cell activation was observed. This effect may be caused by gel breakdown products impinging on MHC-II restricted antigen processing and T-cell activation.

Figure S10. OVA$^{323-339}$ release from Dex P-A hydrogel. OVA$^{323-339}$ release from Dex P-A hydrogel over a 48 h period. A20 cells were pulsed for 4 h with supernatant released from Dex P-A hydrogel loaded with 10 µM of OVA$^{323-339}$, and then co-cultured with DO11.10 cells to analyze their activation. *Retained indicates OVA$^{323-339}$ retained in the remaining hydrogel after 48 h. Dots represent the mean and whiskers the SD. Data correspond to 3 independent experiments (n=2 replicates per experiment).
**OVA\textsubscript{323-339} release assay:** The indirect release of OVA\textsubscript{323-339} from the Dex P-A hydrogel was evaluated through a T-cell activation assay. Briefly, 100 µL of Dex P-A hydrogel loaded with 10 µM of OVA\textsubscript{323-339} peptide was added per well to a 96-well plate. After washing the Dex P-A hydrogel with PBS 1X, 100 µL of RPMI medium was added per well. The supernatant was removed after 2, 4, 6, 8, 10, and 24 h of incubation at 37 °C, 5% CO\textsubscript{2}, and 95% humidity. Afterward, the A20 B cell lymphoma line presenting I-Ad MHC was seeded in a 96-well plate (50 000 cells/well) and pulsed for 4 h with 100 µL of each supernatant (1:10 diluted). Then cells were spun down at 300xg per 5 min, the medium was removed, and 50 000 DO11.10 T-cells were added per well. The co-cultures were incubated overnight (15h) at 37 °C, 5% CO\textsubscript{2}, and 95% humidity. The supernatants were collected.

The T-cell activation was measured as IL-2 release. The IL-2 concentration in the supernatant was quantified by ELISA following the manufacture protocol (Invitrogen Catalog # 88-7024-88). A standard curve of 0.1-0.8 µM of OVA323-339 peptide was performed to interpolate the concentration of OVA\textsubscript{323-339} available in the supernatants and able to activate the DO11.10 T-cells. All experiments were performed three times with two replicates each. Data were analyzed with Graphpad Prism 9 (La Jolla, CA).