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Supplementary Materials for

Encapsulation-free controlled release: Electrostatic adsorption eliminates the need for protein encapsulation in PLGA nanoparticles

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This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/2/5/e1600519/DC1)

Supplementary Materials

Supplementary Figures

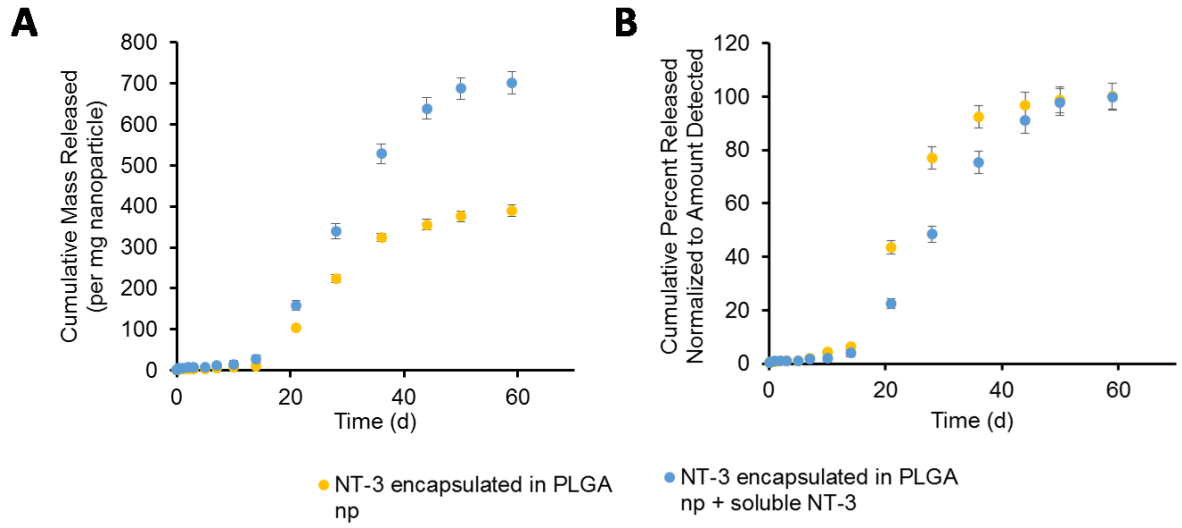

fig. S1. Release of NT-3 from a PLGA np/hydrogel DDS containing encapsulated NT-3 and soluble NT-3. (**A**) Addition of soluble NT-3 increases the cumulative mass of NT-3 released from the PLGA np/hydrogel DDS, but not (**B**) the cumulative percent release. The release profile remains largely unchanged (n = 3, mean ± standard deviation plotted). Data for "NT-3 encapsulated in PLGA np + soluble NT-3" was reproduced from (*17*) with permission from the Royal Society of Chemistry.

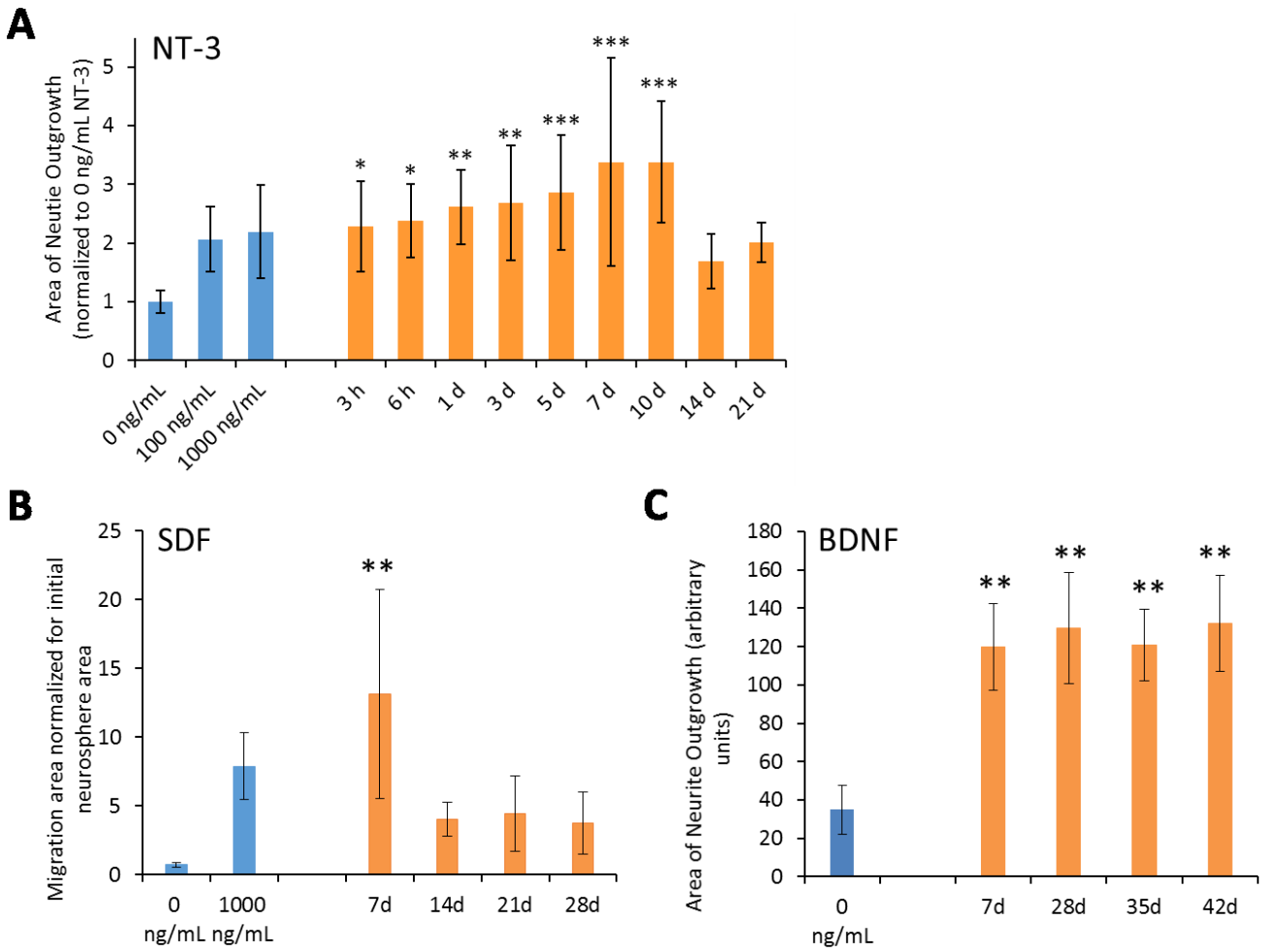

fig. S2. Bioactivity of proteins released from hydrogels with embedded PLGA np. (**A**) Activity of released NT-3 was assessed using a DRG neurite outgrowth assay. Released NT-3 elicits significantly increased neurite outgrowth for up to 10 days compared to a 0 ng/mL control, with a trend towards increased outgrowth for 21 days (*n* = 3, mean ± standard deviation plotted). (**B**) SDF activity was assessed using a neurosphere migration assay. Released SDF caused significantly higher neural stem/progenitor cell migration for up to 7 days compared to a 0 ng/mL control, with a trend towards increased migration for 28 days (*n* = 5 independent releases, mean ± standard deviation plotted). (**C**) Activity of released BDNF was assessed using a DRG neurite outgrowth assay. Released BDNF elicits significantly increased neurite outgrowth for 42 days compared to a 0 ng/mL control (*n* = 3, mean ± standard deviation plotted). (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

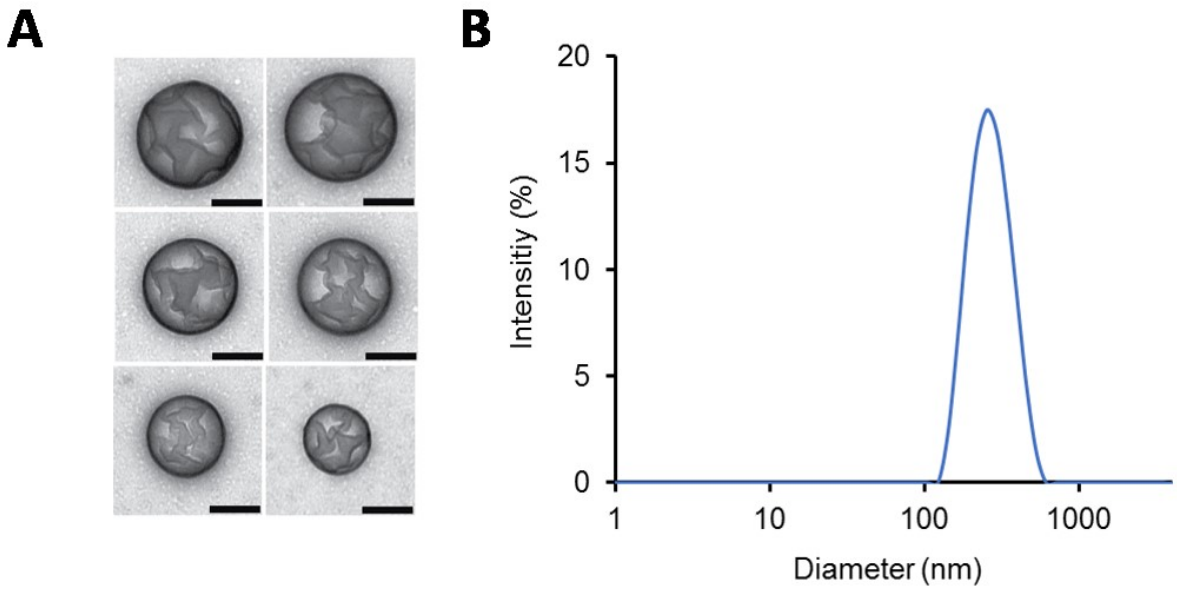

fig. S3. Characteristics of PLGA np used in this study. (**A**) Representative TEM images of PLGA np stained with uranyl acetate. Scale bar is 100 nm. (**B**) Representative dynamic light scattering trace. Nanoparticles have an average diameter of ~300 nm with a PDI of ~0.2.

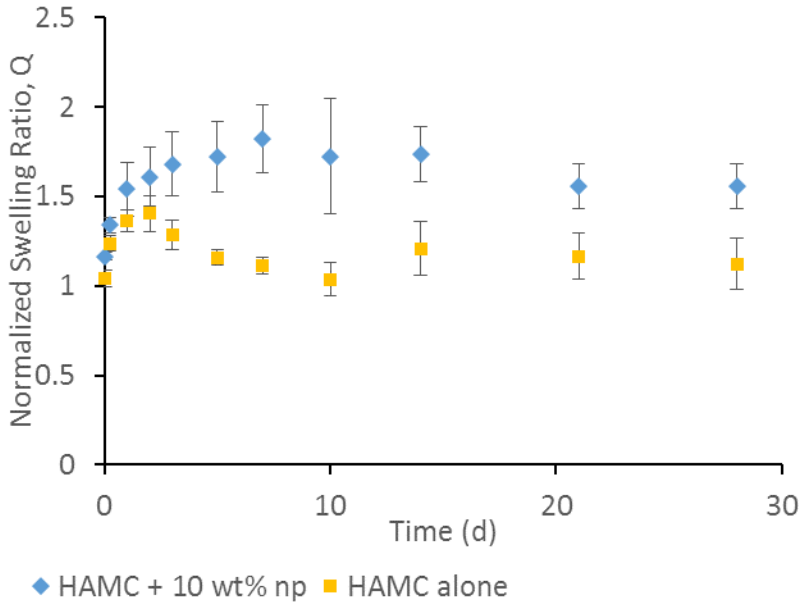

fig. S4. Swelling of HAMC hydrogel with and without PLGA np. HAMC with 10 wt% PLGA np has a significantly higher swelling ratio than HAMC alone between 3 and 28 days ($p < 0.05$, $n = 3$, mean ± standard deviation plotted).

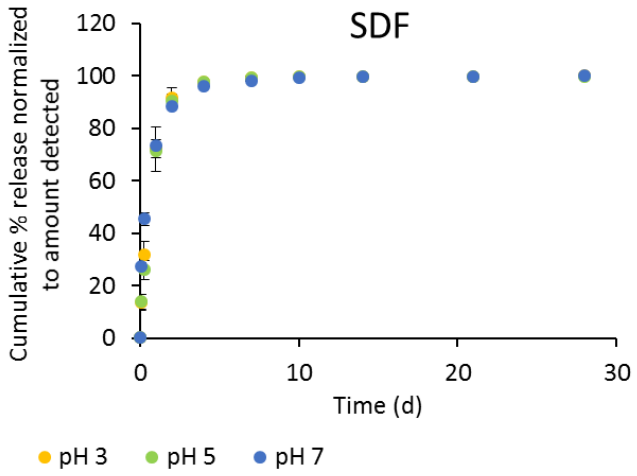

fig. S5. Release of soluble SDF from XMC alone into aCSF at pH 3, pH 5, or pH 7. Release results indicate no effect of varying pH on protein detection by ELISA (*n* = 3, mean ± standard deviation plotted).

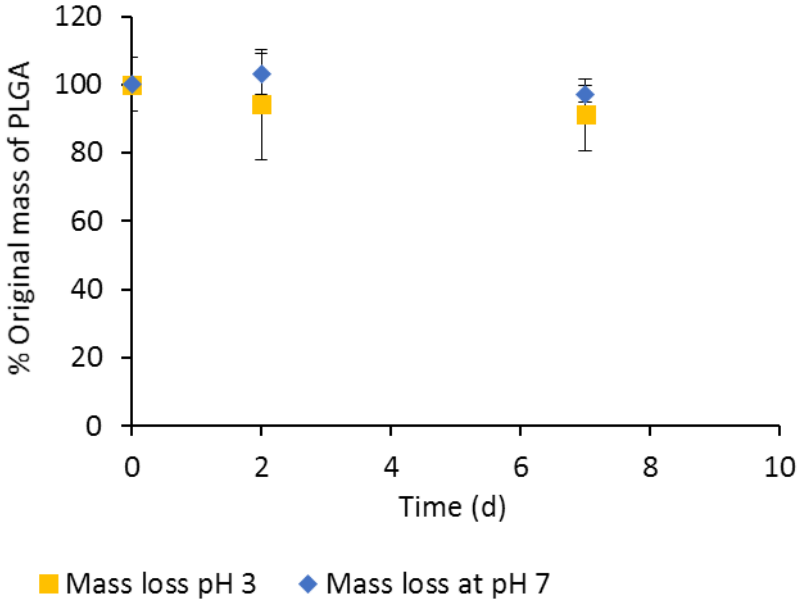

fig. S6. Mass loss of PLGA from the release system at pH 3 and pH 7. There is no significant mass loss of PLGA from the hydrogel over the first 7 days of release, regardless of pH ($p > 0.05$ at all timepoints, *n* = 3, mean ± standard deviation plotted).

movie S1. Two-dimensional Monte Carlo simulations of protein release from a hydrogel with embedded cubic degrading nanoparticles. (**A** to **C**) 2D simulations corresponding to the same fraction of occupied Monte Carlo lattice points as select 3D cases presented in Fig. 4B, i: (A) 0%wt, (B) 1%wt, and (C) 10%wt. The initial average protein concentration is $\langle n_0 \rangle$. The initial barrier to adhesion is ε_0=1.0 and the attractive well is u_0=-10.0. These potential energies decay following the logistic-population growth of nanoparticle degradation products, which increase with a growth rate r_0=0.001 and have a carrying capacity per unit area K=1.4. The maximum concentration shown (red) is the initial maximum and any greater concentrations, common on the surface of nanoparticles, are not differentiated. (D) The cumulative percent of protein released as a function of time. It is important to note that these release profiles will not correspond to those in Fig. 4B, i since these simulations are in 2D not 3D and are simply meant to help visualize the simulation scheme.

Calculation of the relative surface area for PLGA np of different sizes

Assuming spherical nanoparticles, the mass of nanoparticles within the gel is given by

$$m = \rho N \frac{4}{3}\pi r^3$$

where N is the total number of nanoparticles within the gel.

Given equal masses of two different nanoparticle populations where $r_2=3r_1$ and assuming equal nanoparticle densities

$$\rho N_1 \frac{4}{3}\pi r_1^3 = \rho N_2 \frac{4}{3}\pi (3r_1)^3$$

So

$$N_1 = 27N_2$$

The total nanoparticle surface area within the gel is

$$N4\pi r^2$$

Therefore

$$S_1 = N_1 4\pi r_1^2$$

$$S_2 = \left(\frac{N_1}{27}\right) 4\pi (3r_1)^2$$

$$S_2 = \frac{9}{27} S_1 = \frac{S_1}{3}$$

So for a nanoparticle 3 times the radius, we obtain three times less surface area for the same mass.

Calculation for maximum surface coverage

These calculations are done using SDF (MW ~ 8000 Da) as an example. Assuming a spherical (globular) protein with hydrodynamic radius of approximately 2 nm and a nanoparticle radius of approximately 150 nm (based on our DLS measurements in fig. S2), we have

$$A_{protein} = \pi(2)^2 = 12.57\ nm^2$$

$$SA_{particle} = 4\pi(150nm)^2 = 2.83 \times 10^5\ nm^2$$

This means there would be approximately

$$\frac{2.83 \times 10^5\ nm^2}{12.57\ nm^2} = 22513$$

proteins per nanoparticle given complete surface coverage.

The mass of this number of proteins is

$$m_{protein} = \frac{22513 \times MW}{N_A} = \frac{22513\left(8000\frac{g}{mol}\right)}{6.02 \times 10^{23}} = 2.99 \times 10^{-16} g$$

The mass of a nanoparticle is

$$m_{particle} = \frac{4}{3}\pi r^3 \rho = \frac{4}{3}\pi(0.000015cm)^3 \left(1.5\frac{g}{cm^3}\right) = 2.12 \times 10^{-14} g$$

Assuming a density of 1.5 g/cm^3 *(30)*. So the ratio of protein to polymer by mass that gives 100% coverage is

$$\frac{2.99 \times 10^{-16} g}{2.12 \times 10^{-14} g} \times 100\% = 1.41\%$$