Supplemental Information

Materials and Methods

Polymer synthesis and characterization

Briefly, DL–lactide was precipitated twice into anhydrous diethyl ether, before mixing with different MW mPEG in stoichiometric ratios to achieve desired molecular weights. Reactants were purged with dry nitrogen and sealed in a round bottom flask before heated to 140°C while stirring for two hours to remove trace water from samples. The temperature was reduced to 130°C and stannous octoate (1 wt%) was added to catalyze the ring opening polymerization (ROP) of lactide with mPEG serving as the initiator. The polymerization was allowed to continue for six hours. The diblock copolymer was then dissolved in dichloromethane (DCM) and twice precipitated in cold diethyl ether. Residual solvent was then removed by first drying via rotary evaporation (Safety Vap 205, Buchi, Switzerland), followed by lyophilization (VirTis BenchTop SLC, SP Industries, Gardiner, NY).

Number average molecular weights ($\overline{M_n}$) of bulk copolymers were determined using proton nuclear magnetic resonance (^{1}H-NMR). The weight average molecular weights ($\overline{M_w}$) and polydispersity indices (PDI) were also determined by gel permeation chromatography (HPLC-GPC), with a Binary HPLC pump (1525, Waters, Milford, MA), a Refractive Index Detector (2414, Waters) and three serial 7.8 x 300 mm Styragel columns (Waters) using tetrahydrofuran (THF) as the mobile phase. Chromatograms were analyzed using Breeze version 3.3 software with polystyrene standards used for calibration.

Filamentous polymer nanocarrier (f-PNC) formulation

A freeze-thaw double emulsion solvent evaporation technique was used as previously outlined[15, 18] and is also outlined in detailed in the supplement. The primary emulsion consists of the organic phase (1 ml of a 25 mg/ml polymer-DCM solution) and the aqueous phase (100 µl of a 1 mg/ml catalase-PBS solution) homogenized at 15,000 rpm for 1 minute in a dry ice-acetone bath with a 7 mm–generator tissue homogenizer (Kinemetica Polytron 3100 with a PDTA3007/2 generator, Brinkmann Instruments,

Westbury, NY). The primary emulsion is then added to 5 ml of a 2 wt% polyvinyl alcohol (PVA, 2 grams PVA in 100 ml of PBS) surfactant solution (87-89% hydrolyzed, $\overline{M_w}$ =13,000-23,000) and homogenized at 15,000 rpm for 1 minute. The resultant mixture is added to 10 ml of PVA solution and stirred overnight to allow residual solvent evaporation. The microparticle fraction is removed by a primary centrifugation at 1,000 g for 10 minutes and the nanoparticle fraction is collected by subsequent centrifugation at 20,000 g for 30 minutes. The supernatant is then removed and the *PNC* pellet is re-suspended in PBS and purified again by further centrifugation.

Enzyme loading determination

Enzyme loading was determined via isotope tracing and enzymatic activity. Loading via radiolabeling was determined as described before, by formulating *PNC* with ^{125}I-labeled catalase[18]. Catalase was radiolabeled with Na^{125}I (Perkin Elmer, Boston, MA) via the Iodogen method (Pierce Biotech., Rockford, IL). Unbound ^{125}I was removed from the enzyme using Biospin 6 columns in accordance with the manufacturer's instructions (Bio-Rad labs, Hercules, CA). Total solution ^{125}I-catalase content was measured before centrifugation, and then radioactivity of the ^{125}I-catalase/*PNC*-composed pellet after centrifugation was measured with a Wizard 1470 gamma counter (Wallac, Oy, Turku, Finland).

Enzymatic activity loading was determined via a catalase activity assay,[29, 30] both for the total sample before and after centrifugation. The assay, based on substrate (H_2O_2) consumption, was performed by combining 990 µl of 5 mM H_2O_2 in PBS and 10 µl of enzyme-loaded *PNC* in a quartz cuvette. A spectrophotometer set to measure absorbance at 242 nm was used to determine the kinetics of H_2O_2 degradation (absorbance at this wavelength corresponds to the H_2O_2 concentration and thus catalase activity, U/mg = 23.0(ΔA_{242}/min)/mg of catalase).

Catalase resistance to proteolysis

Resistance to protease degradation was tested as described previously.[15, 18] Briefly, *PNC* preps loaded with ^{125}I-catalase were incubated with a 0.2 wt% pronase (a potent, non-specific protease)

solution at 37°C in a shaker bath set at 60 rpm for 1 hour. Samples were removed and centrifuged at 16,000 g for 20 minutes. Supernatant containing degraded protein and pellet containing intact protein encapsulated within *PNC* were collected and counted. This measure correlates linearly with preservation of enzymatic activity.[18, 27]

PNC concentration determination

A colorimetric PEG assay based on the PEG-Barium Iodide complex was utilized to determine *PNC* concentration.[15] Two solutions were prepared: solution A, consisting of 2.4 g of Barium chloride, 8.0 ml of 6 M HCl and 32 ml of deionized (DI) water, and solution B, consisting of 800 mg of potassium iodide, 500 mg of iodine, and 40 ml of DI water. Aliquots of the *PNC* samples were added to a 96 well plate and diluted to a 170 µl total volume with DI water. Subsequently, 40 µl of solution A and 1/5 diluted solution B were then added to each well. After a 10 minute incubation at 25°C, absorbance of the colored product was measured at 550 nm using a microplate reader (Model 2550-UV, Bio-Rad Labs, Hercules, CA).[31] Standard solutions of PEG of known MW were used for calibration.

Results

Polymer filaments of different MW load active enzyme that is resistant to protease degradation upon encapsulation

Loading of catalase into *f-PNC* and subsequent enzymatic activity was characterized, along with the resistance of the enzyme to degradation by proteases, via established methods.[15] First, ^{125}I-labeled catalase was traced in order to determine the mass loading of the enzyme into the nano-fraction of formulated *f-PNC* (any larger micron sized aggregates were removed during the first purification step, Section 2.4). The loading efficiency, or percent of loading by enzyme mass (defined as the percent of catalase added to the initial emulsion that is loaded into *f-PNC*), is indicated in Fig. 5A as a function of MW. While all filamentous preparations loaded comparable amounts of catalase, relative to previous filament loading efficiencies,[15] the loading dependencies on polymer MW were inversely related. The highest loading efficiency occurred with EL2-10 at 6.1±1.0 %, or approximately 6.1 µg of catalase per

f-PNC preparation, while the lowest efficiency was 3.1±0.7 %, or 3.1 µg per *f-PNC* preparation for EL10-40. EL19-70 *f-PNC* loaded at a similarly low efficiency, near half that of EL2-10 *f-PNC*.

Formulation-induced catalase inactivation, i.e., retention of enzymatic activity post-preparation of the nanofilaments, was determined via UV spectroscopy of substrate depletion, Fig. 5B. This degree of apparent enzyme inactivation during the encapsulation within *f-PNC* was similar for each polymer MW, and was relatively mild compared to previous formulations.[15] Post-formulation specific enzymatic activities, i.e., Units of activity/mg of protein, ranged from 76.2±2.0% (EL5-27) to 96.1±4.1% (EL10-40) of the original stock catalase activity, before processing.

Assessment of resistance of the encapsulated enzyme to protease (e.g., pronase) degradation represents another means to quantify the quality of catalase encapsulation.[15, 18, 27] In other words, *f-PNC* protection of catalase helps differentiate truly *encapsulated* drug from drug that is merely surface adsorbed, or near the surface of the particle and potentially susceptible to external protease denaturation. Percent protection, or the amount of protein resistant to protease degradation relative to the total protein loaded into *f-PNC*, after a one hour incubation with pronase at 37°C ranged between 15.4±2.1% for EL10-40 and 29.7±4.0% for EL5-27, Fig. 5C. This is in contrast to non-*PNC* encapsulated catalase preparations where loading was through pure surface adsorption and protective effect was less than 5%.[15]

Supplemental Figure captions:

Supplement Figure 1. Therapeutic enzyme loading and resistance to proteolytic degradation upon *f-PNC* encapsulation. Loading efficiency or percent mass loading denotes the amount of catalase loaded relative to the amount added (A). Percent initial activity indicates the amount of enzymatic activity retained, post formulation (B). Percent protection indicates the amount of *f-PNC*-encapsulated catalase that is resistant to proteolytic degradation after one hour incubation with the non-specific protease, pronase (C). It should be noted that non-encapsulated, i.e., surface adsorbed, catalase exhibits less than 5% protection within one hour.[15]