Supporting Information

PERCHLORATE REDUCTION USING FREE AND ENCAPSULATED *AZOSPIRA ORYZAE* ENZYMES

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Supporting Information

Methods

Vesicle preparation. Vesicles were formed using the previously published method of film rehydration [1]. Twenty mg of lipid (9:1 ratio of soy asolectin and cholesterol (Sigma-Aldrich, St. Louis, MO)) or 12 mg of polymer (poly-(2-methyloxazoline)-poly-(dimethylsiloxane)-poly-(2-methyloxazoline) $PMOXA_{15}$-$PDMS_{110}$-$PMOXA_{15}$) [2] was dissolved in a 100 mL round bottom flask with 2 mL of chloroform and evaporated to form a thin film. For rehydration, 2 mL of *Azospira oryzae* soluble protein fraction, 1:200 molar ratio of purified OmpF where specified, and 0.3% n-octyl-oligo-oxyethylene (Enzo Life Sciences, Ann Arbor, MI) were added to the round-bottom flask. The solution was mixed by alternating 30 s bath sonication and vortexing for 5 minutes. The mixture was stored at 4°C with stirring for 12 hours, sonicated and vortexed for an additional 5 minutes, and stored at 4°C with stirring for an additional 24 hours. This treatment resulted in a cloudy, red mixture that was extruded through a 1μm track-etched filter (Nucleopore, Whatman, GE Healthcare, Piscataway, NJ) in a LIPEX extruder (Northern Lipids, Burnaby, British Columbia, Canada). To produce unilamellar vesicles, the solution was extruded an additional ten times through a 0.4 μm pore-sized track-etched filter. To break up protein aggregates, the extruded vesicles were treated with 5% (v/v) of 20 mg mL^{-1} Proteinase K (Roche Applied Science, Indianapolis, IN) for one hour at room temperature. Size exclusion chromatography was used to separate non-inserted OmpF, non-encapsulated protein and vesicles, on a Tricorn 10/300 filled column with Sephacryl 500-HR (GE Life Sciences, Piscataway, NJ) and an ÄKTAprime plus system (GE Life Sciences, Piscataway, NJ) using a maximum column pressure of 0.38 mPa and a flow rate of 0.5 ml min^{-1}.

Dynamic light scattering (DLS). Lipid and polymer vesicle sizes (diameter) and size distribution (polydispersivity index – PDI) were analyzed by dynamic light scattering (DLS) on a Zetasizer Nano ZS90 (Malvern Instruments Ltd., Malvern, UK) using a 632.8 nm He-Ne gas laser at 12.8° and 90°.

Samples were analyzed at room temperature using a refractive index of 1.47 and an absorption value of 0.10.

Transmission Electron Microscopy (TEM). To confirm formation of polymer vesicles, images were captured using a JEM-2100F TEM with LaB6 emitter (JEOL USA, Inc., Peabody, MA) located at the University of Illinois Material Research Laboratories (Urbana, IL.) Ultrathin holey carbon grids supported on gold mesh (Ted Pella, Inc., Redding, CA) were prepared by charging for 45 s under a Denton DPG-1 glow-discharge system (Denton Vacuum Inc., Moorestown, NJ) at 200 mA. Vesicle dilutions of 1:2 by volume in 50 mM phosphate buffer at pH 6.0 were incubated on the charged carbon grids for 2 min, blotted, stained with 1% uranyl acetate dye (SPI, West Chester, PA), and immediately blotted again. Grids were stained a final time for 1 min, blotted, and air dried for 5 min. Grids were viewed at 200 kV and 110 μA e-beam current, and several images were acquired per grid.

Verification of Incorporation of OmpF. Incorporation of OmpF was confirmed by measuring retention of encapsulated fluorescence in polymer vesicles. During vesicle formation, 2.5 mM of 5,6-carboxyfluorescein dye (Life Technologies Molecular Probes, Carlsbad, CA) was included. Following separation of vesicles from unencapsulated dye by size exclusion chromatography as detailed under vesicle preparation, fluorescence was measured with excitation at 492 nm and emission at 517 nm using a SPECTRAmax PLUS microplate spectrophotometer (Molecular Devices Corporation, Sunnyvale, CA). Leakage of carboxyfluorescein through the lipid membrane prevented similar analysis in lipid vesicles.

Results

Characterization of Vesicles. The average size and PDI, a measure of size distribution, of the vesicles were measured by dynamic light scattering (Table S1). For lipids, the average size of OmpF vesicles was not statistically different from vesicles without OmpF (Table S1, n=3 P=0.254). The PDI was relatively high in these preparations. This is due to the limited number of extrusions and the use of a 0.4 μm pore size for extrusion; these decisions were designed to minimize vesicle lysis and the accompanying release

of the perchlorate-reducing enzymes. The PDI for the two sets is significantly different (OmpF = 0.452, No OmpF = 0.268, n=3 P=.018). For polymer vesicles, the size and PDI were similar with and without OmpF. Transmission electron microscope imaging also indicates formation of polymer vesicles (Figure S1). As reported previously, vesicle size in TEM appears smaller than with DLS due to the vacuum conditions used in TEM [1].

Sample	Diameter (nm)	PDI
Lipid OmpF	371.6	0.45
Lipid	320.3	0.27
Polymer OmpF	185.2	0.15
Polymer	193.3	0.14

Table S1: Diameter and Size Distribution for Lipid and Polymer Vesicles with and without OmpF.

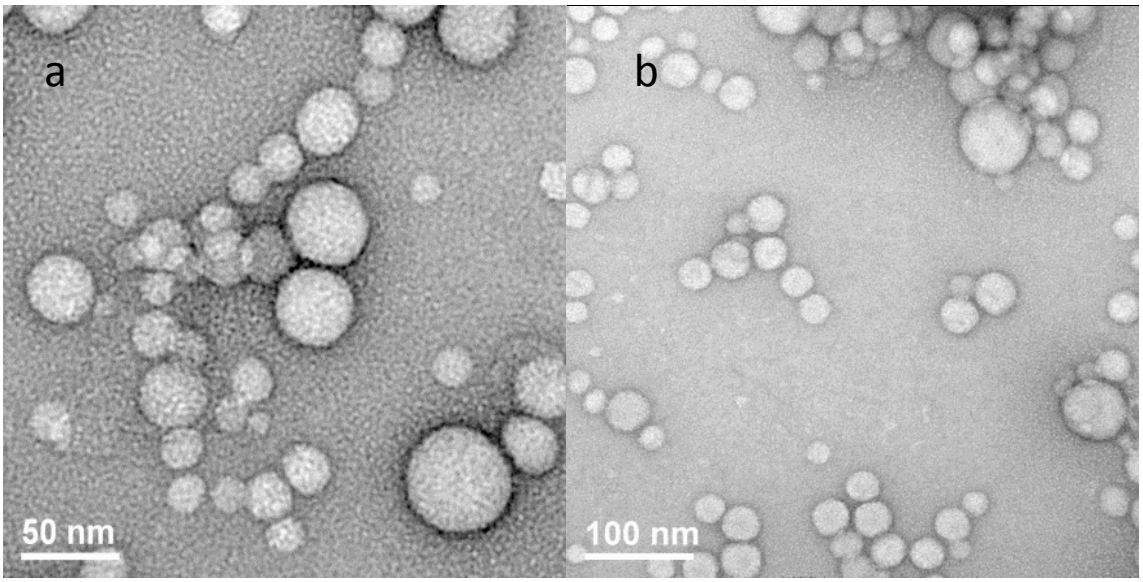

Figure S1: Examination of polymer vesicles using transmission electron microscopy. (a) Micrograph of the No OmpF vesicles (Scale bar: 50nm) (b) Micrograph of the OmpF vesicles (Scale bar: 100nm),

Incorporation of OmpF. The incorporation of OmpF in the polymer vesicles was confirmed by testing retention of carboxyfluorescein in vesicles with and without OmpF. The low fluorescence signal observed in vesicles with OmpF, compared to vesicles without OmpF, supports the functional incorporation of OmpF (Figure S2) [3]. Lipid vesicles did not retain the carboxyfluorescein even in the absence of OmpF.

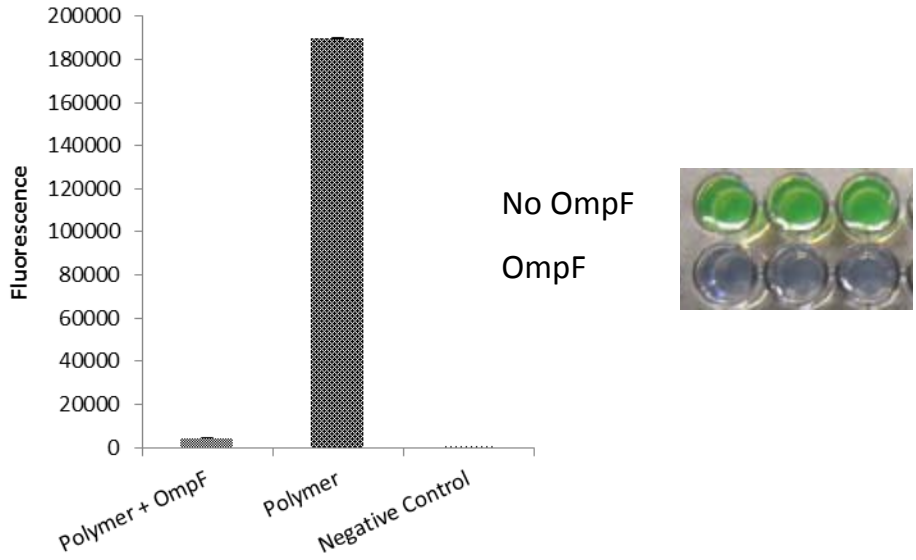

Figure S2: Incorporation of OmpF prevents retention of the fluorescence dye, carboxyfluorescein, as shown by the decrease in fluorescence in the presence of OmpF for polymer vesicles, illustrated here visually and through fluorescence measurements taken at 517nm emission wavelength. The negative control was buffer.

Calculations of the volume limitations imposed by the polymer vesicles

Encapsulation should lower the perchlorate reducing activity due to the volume limitations imposed by the vesicles, and might also introduce diffusion limitations. To predict the encapsulated activity, the total volume activity (TVA) restriction of the vesicle, as determined by the encapsulation volume of the perchlorate-reducing vesicles, and 2) the diffusion limited activity (DLA), as determined by the perchlorate diffusion across OmpF in the polymer membrane, were calculated as follows.

1. ENCAPSULATION VOLUME RESTRICTION ON ACTIVITY

The total volume activity (TVA) was calculated using Equation 1.

$$TVA = \frac{TEV * APF}{TVV} \quad (1)$$

Equation 1: Volume Restriction on Vesicle Activity.
The theoretical total volume activity (TVA) was calculated using the total vesicle encapsulation volume (TEV), the activity of the soluble protein fraction (APF), and the total suspension volume of vesicle (TVV) after preparation using size exclusion chromatography.

The TEV was calculated by determining the total number of vesicles (TNV) and the encapsulation volume of one vesicle (EV). The TNV was difficult to determine from dynamic light scattering results due to their small size. So, the TNV was calculated using the mass of polymer, the surface area (SA) of a polymer molecule estimated at 350 $Å^2$ [4, 5], and the surface area of a vesicle using a radius of 90 nm. This calculation assumed that all polymer molecules were incorporated into vesicles. This value was converted to the total number of polymer molecules using the molecular weight of 11,048 mg $mmol^{-1}$.

The EV of a vesicle was determined from the geometrical equation of volume for a sphere. The inner diameter of the vesicle was used. The inner diameter of the vesicle was determined from light scattering and the thickness of the polymer, 15 nm. The inner diameter used in these calculations was 150

nm. For the activity of the soluble protein fraction (APF), the average of activities from soluble protein fraction was used. These calculations resulted in a TVA of approximately 95 U L^{-1}.

PERCHLORATE DIFFUSION RESTRICTION ON ACTIVITY

The diffusion limiting activity (DLA) was calculated using Equation 2.

$$DLA = \frac{F * SAV}{TVV} \quad \textbf{(2)}$$

Equation 2: Diffusion Limiting Activity.
The diffusion limiting activity was determined using Fick's First Law of diffusion (F), the surface area of the vesicles (SAV), and the total volume of the vesicle suspension (TVV).

$$F = -D * n * \frac{dC}{dx} \quad \textbf{(3)}$$

Equation 3: Fick's First Law for Diffusion.
The flux of perchlorate was determined from the diffusion coefficient (D), the porosity of the vesicles (n), and the perchlorate concentration gradient (dC/dx).

For perchlorate, the published diffusion coefficient (D) 2.25 X 10^{-5} cm^2 s^{-1} was used [6]. The equations were adapted to account for the pore structure of the vesicles. Perchlorate was assumed to diffuse into the vesicles only through the pores, and the flux of perchlorate into the pore was assumed not to be hindered by steric effects. The calculations also assumed 100% insertion efficiency of the 1:200 molar ratio of OmpF to polymer. The porosity of the vesicle, a dimensionless number, was therefore determined from the ratio of OmpF surface area to vesicle surface area and molar ratio. The dimensions of the pore were given as 7Å by 11Å [7]. The surface area of the elliptical OmpF was 60.5 $Å^2$. The concentration gradient (dC/dx) was determined by the perchlorate concentration used in the experiments (0.83 µmole cm^3) over the radius of the vesicle. The vesicle radius was used as the diffusion length to approximate the boundary layer thickness. The flux of perchlorate was converted to µmoles of methyl

viologen (MV) consumed per minute for comparison with activity measurements by applying the known reaction stoichiometry (complete reduction of perchlorate consumes eight moles of MV).

Having determined the flux of perchlorate (F), the total surface area of the vesicles (SAV) was calculated using the surface area of one vesicle and the total number of vesicles as previously calculated. The diffusion limited activity was determined to be 4,940,000 U L^{-1}. As the DLA value is much greater than the TVA, diffusion was not found to be limiting under experimental conditions. However, at lower perchlorate concentrations, diffusion could become limiting.

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