

Supplementary Information for

Encapsulation of Photosystem I in organic microparticles increases its photochemical activity and stability for ex vivo photocatalysis

Arianna Cherubin,¹ Laura Destefanis¹, Michele Bovi¹, Federico Perozeni¹, Ilaria Bargigia^{2,3}, Gabriel de la Cruz Valbuena^{2,4}, Cosimo D'Andrea^{2,4}, Alessandro Romeo⁵, Matteo Ballottari^{1*}, Massimiliano Perduca^{1*}

¹Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy

²Center for Nano Science and Technology @PoliMi, Istituto Italiano di Tecnologia, via Pascoli 70/3, 20133 Milano, Italy

³Georgia Institute of Technology, School of Chemistry and Biochemistry, 901 Atlantic Drive, Atlanta GA 30332-0400

⁴Department of Physics, Politecnico di Milano, P.za L. da Vinci 32, 20133 Milano, Italy

⁵Department of Computer Science, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy

Corresponding authors: Matteo Ballottari, Massimiliano Perduca
Email: matteo.ballottari@univr.it; massimiliano.perduca@univr.it

Number of pages: S1-S16

Number of Tables: Table S1-S3

Number of Figures: Figure S1-S11

Table S1. HPLC analysis of PSI in detergent and in PLGA MPs. Summary table of HPLC analysis conducted on PSI complexes in detergent and encapsulated in PLGA microparticles (MPs). The names of the pigments extracted are in their short form: Chlorophylls (Chl); Carotenoid (Car); Neoxanthin (Neo); Violaxanthin (Viola); Antheraxanthin (Antera); Lutein (Lute); Zeaxanthin (Zea); β -Carotene (β Car). Standard deviation (s.d.) are reported for each value.

	Chl	Chla/b	Chla	Chlb	Chl/car	Neo	Viola	Antera	Lute	Zea	βcar
PSI in detergent	156.00	6.44	135.05	20.95	5.12	1.22	4.76	0.00	9.25	0.00	15.24
<i>s.d.</i>	<i>0.00</i>	<i>0.01</i>	<i>0.03</i>	<i>0.03</i>	<i>0.07</i>	<i>0.31</i>	<i>0.16</i>	<i>0.00</i>	<i>0.10</i>	<i>0.00</i>	<i>0.98</i>
PSI in PLGA MPs	156.00	6.47	135.09	20.91	6.27	0.52	4.18	0.00	7.52	0.00	12.68
<i>s.d.</i>	<i>0.00</i>	<i>0.39</i>	<i>1.07</i>	<i>1.07</i>	<i>0.31</i>	<i>0.17</i>	<i>0.13</i>	<i>0.00</i>	<i>0.48</i>	<i>0.00</i>	<i>0.79</i>

Table S2. Z-Average value, polydispersity index (PDI) and superficial charge of PLGA and chitosan microparticles. Data reported in the table were obtained by Dynamic Light Scattering measurements. Error range are reported as standard deviation. CS: chitosan

	<i>PSI in detergent</i>	<i>Empty PLGA microparticles</i>	<i>PSI in PLGA microparticles</i>	<i>Empty CS microparticles</i>	<i>PSI in CS microparticles</i>
<i>Z-Average values (nm)</i>	54.87 ±10.0	402.4 ±97.7	1698 ±66.6	196.20 ±59.0	539.5 ±70.4
<i>PDI (× 10⁻³)</i>	55 ±9	16 ±6	542 ±23	212 ±2	518 ±7
<i>Superficial charge (mV)</i>	-9.01 ±3.2	-0.327 ±2.7	-5.48 ±4.9	+25.3 ±4.7	+16 ±3.5

Table S3: Fitting analysis of fluorescence decay integrated kinetics. Fluorescence decay kinetics reported in Fig. 4 were analysed by exponential fitting, two exponential functions were sufficient for best fit in the case of PSI in PLGA MPS, while three exponentials were required in the case of PSI in detergent. τ_{AVG} was calculated as $\tau_{AVG} = \sum A_x * \tau_x / \sum A_x$. The long component in the ns range obtained in the case of PSI in detergent was not used for τ_{AVG} calculation, as previously reported, being related to free chlorophyll or antenna proteins in the sample. The photochemical efficiency of PSI (Φ_{PSI}) was calculated from τ_{AVG} as described in Galka et al, Plant Cell, Vol. 24: 2963–2978, 2012.

	PSI in PLGA MPs	PSI in detergent
A₁ (%)	41.4%	15.3%
τ_1 (ps)	9.0	9.2
A₂ (%)	58.6%	81.3%
τ_2 (ps)	47.3	58.1
A₃ (%)	/	3.4%
τ_3 (ps)	/	1000.0
τ_{AVG} (ps)	31.5	48.7
Φ_{PSI}	99.1%	98.5%

Fig. S1. PSI encapsulation in PLGA MPs. The modified Solvent Displacement Method is represented.

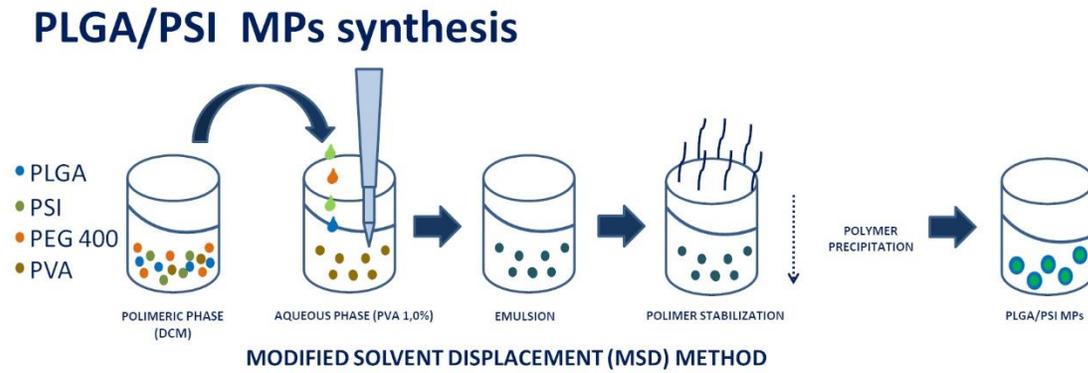


Fig. S2. Atomic Force Microscopy (AFM) observation of PLGA empty microparticles and particle size distribution. PLGA MPs visualized in semi-contact (A) and in 3D mode (B). The images have been acquired in dry mode. The images acquired were then used to determine the distribution of the particles size using Gwyddion software as reported in (C) and (D) for empty PLGA MPs and PLGA MPs containing PSI respectively.

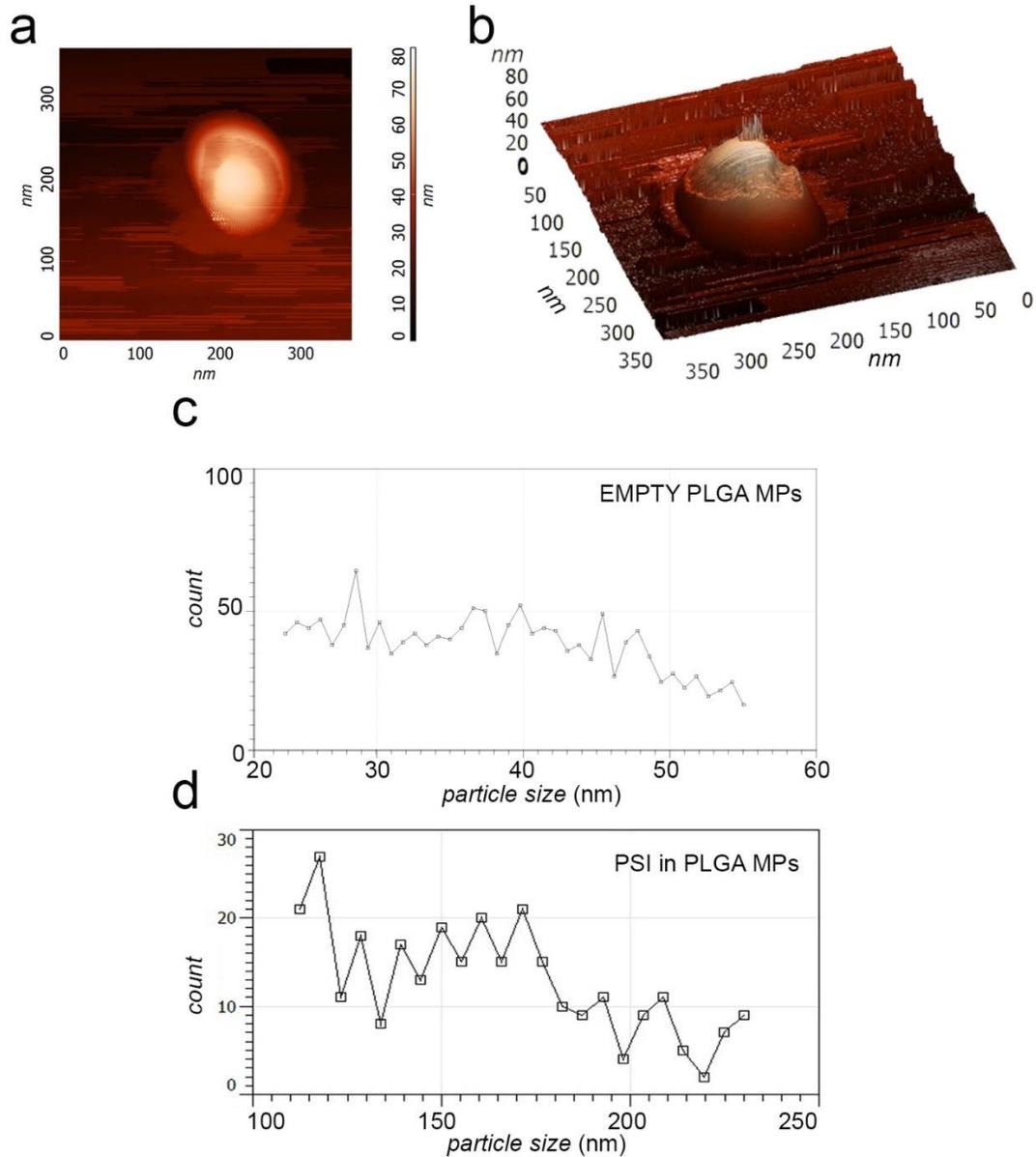


Fig. S3. Power dependency of fluorescence kinetics of PSI in PLGA MPs. In order to evaluate possible effects of annihilation in fluorescence decay kinetics of PSI in PLGA MPs, time resolved fluorescence was measured at different laser power intensities.

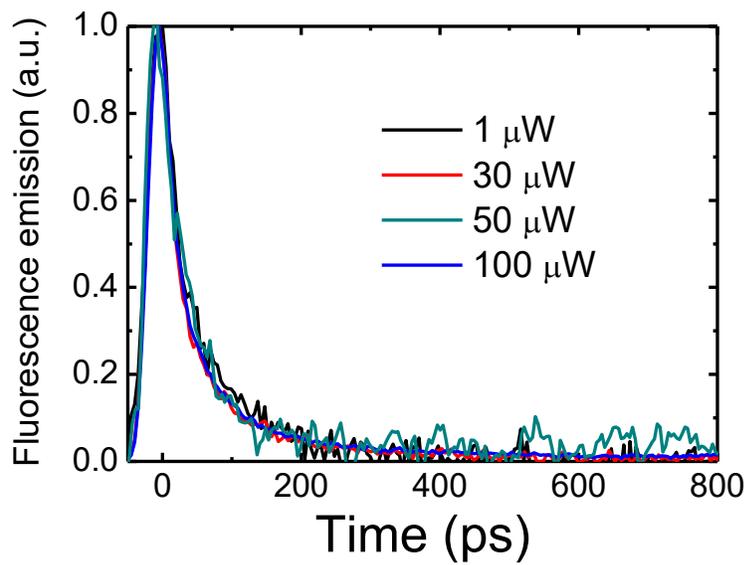


Fig. S4. Isothermal DSC analysis. Experiment temperature: 4°C. The blue line represents the PSI in buffer; the grey line represents the PSI in PLGA microparticles.

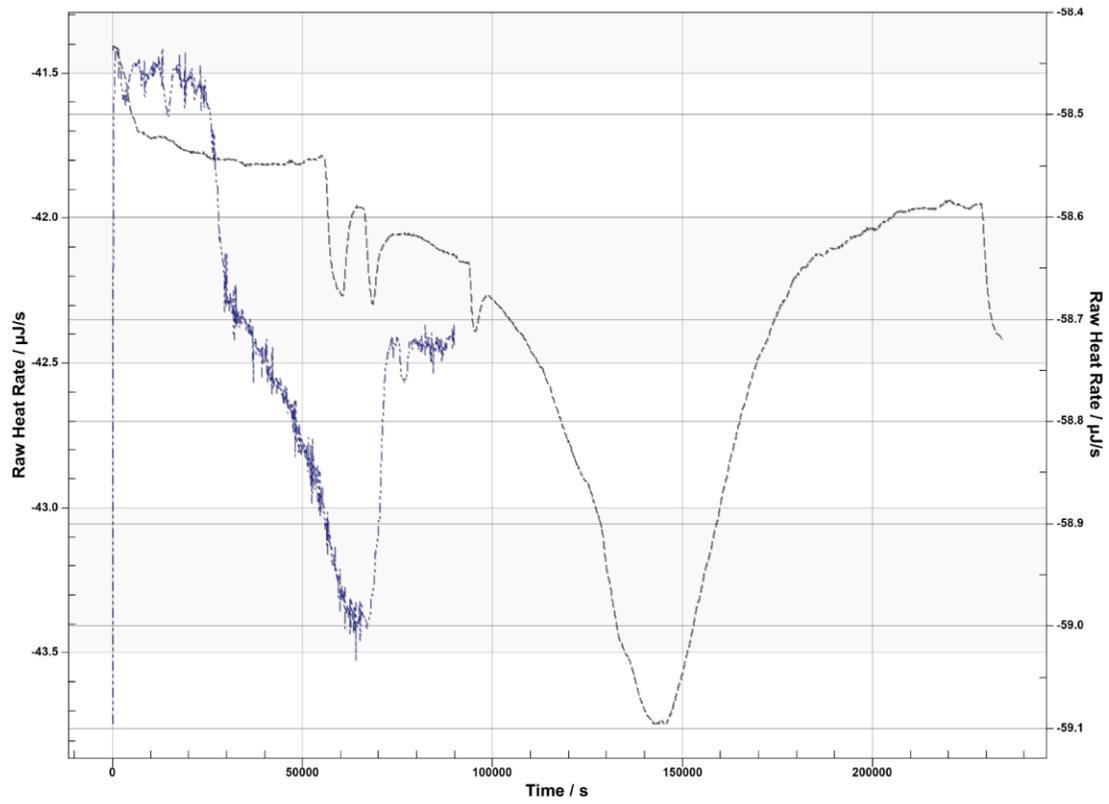


Fig. S5. PSI encapsulation in chitosan MPs. The method for PSI encapsulation in chitosan (CS) microparticles is represented.

Chitosan/PSI MPs synthesis

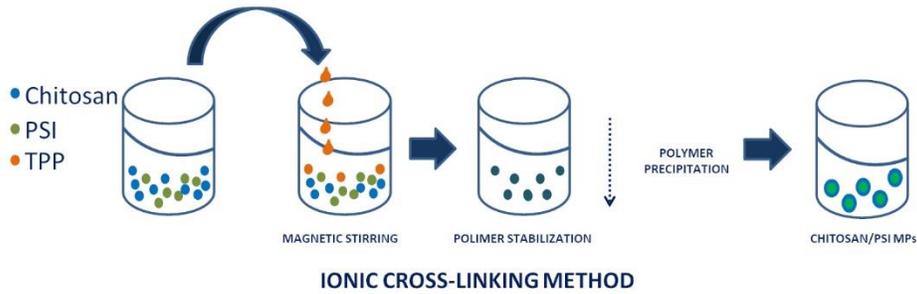


Fig. S6. Atomic Force Microscopy (AFM) observation of PSI in chitosan microparticles. PSI chitosan MPs visualized in three different modes: (A, B) Error-mode; (C) Semi-contact; (D) 3D. The images have been acquired in dry mode

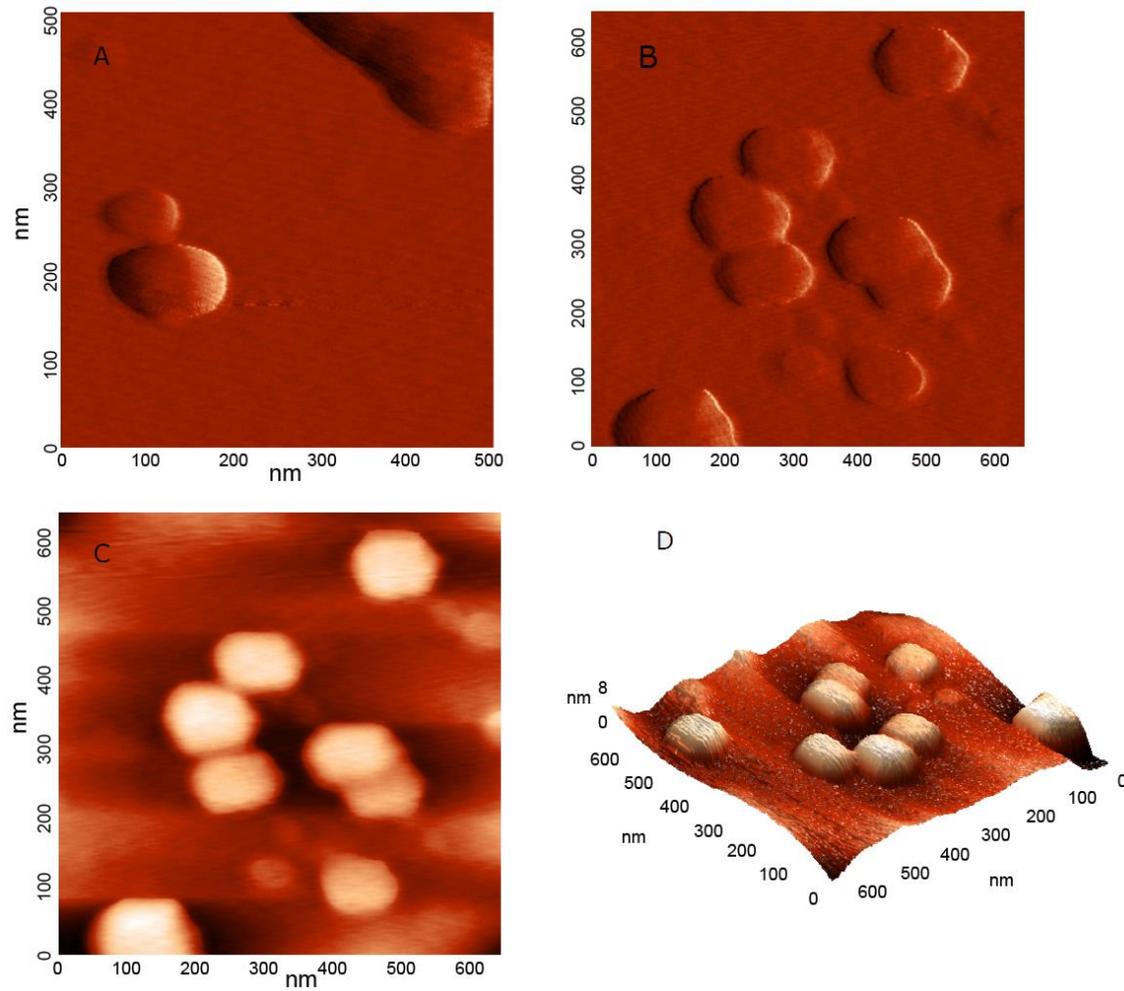


Fig. S7. Absorption and fluorescence emission spectra of PSI in detergent and in chitosan MPs. (A) Absorption spectra of isolated PSI in detergent solution (black) or in chitosan (CS) MPs (orange). (B) Fluorescence emission spectra of PSI in detergent and in chitosan MPs upon excitation at 440 nm (similar results were obtained upon excitation at 475 or 500 nm).

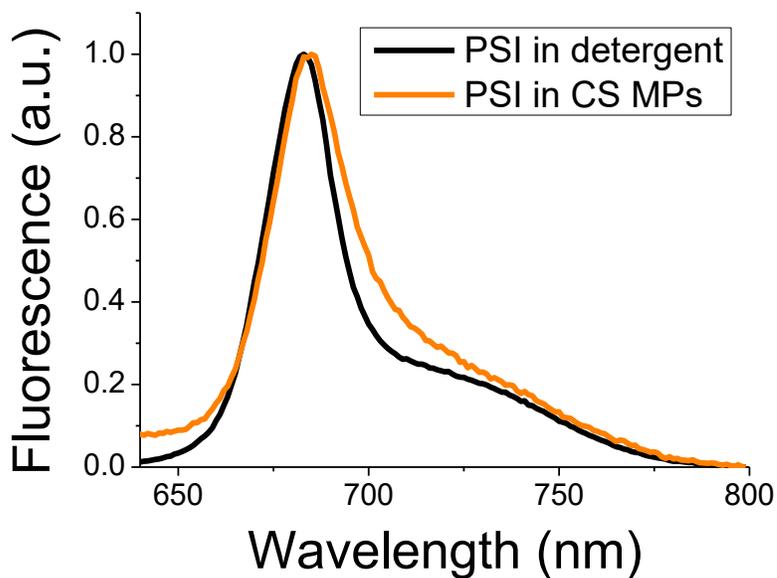
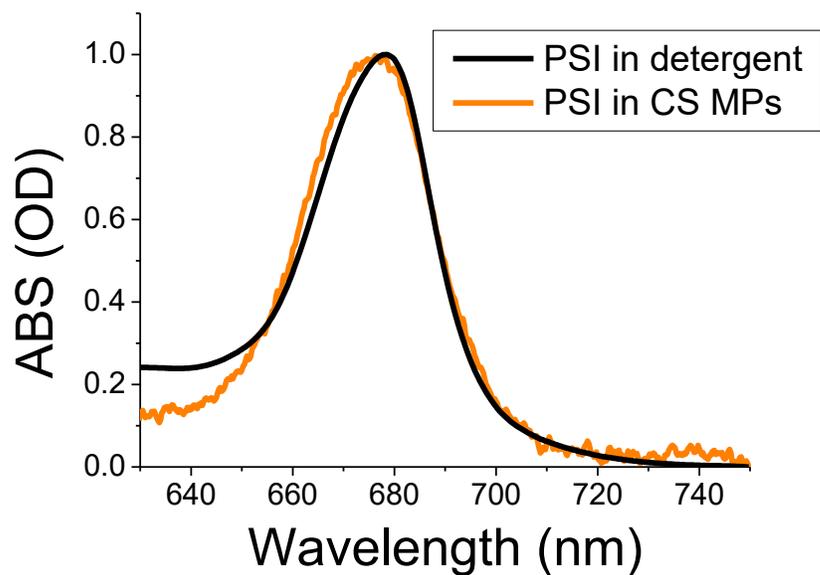


Fig. S8. Photochemical activity of PSI in detergent vs. PLGA or chitosan microparticles. Light dependent P700 oxidation of PSI in detergent or in PLGA or chitosan (CS) MPs were measured as transient absorption at 705 nm. Ascorbate (Asc) and Methyl-viologen (MV) were added where indicated as electron donor and acceptor respectively. Orange actinic light at $940 \mu\text{mol m}^{-2}\text{s}^{-1}$ were used to induce P700 oxidation.

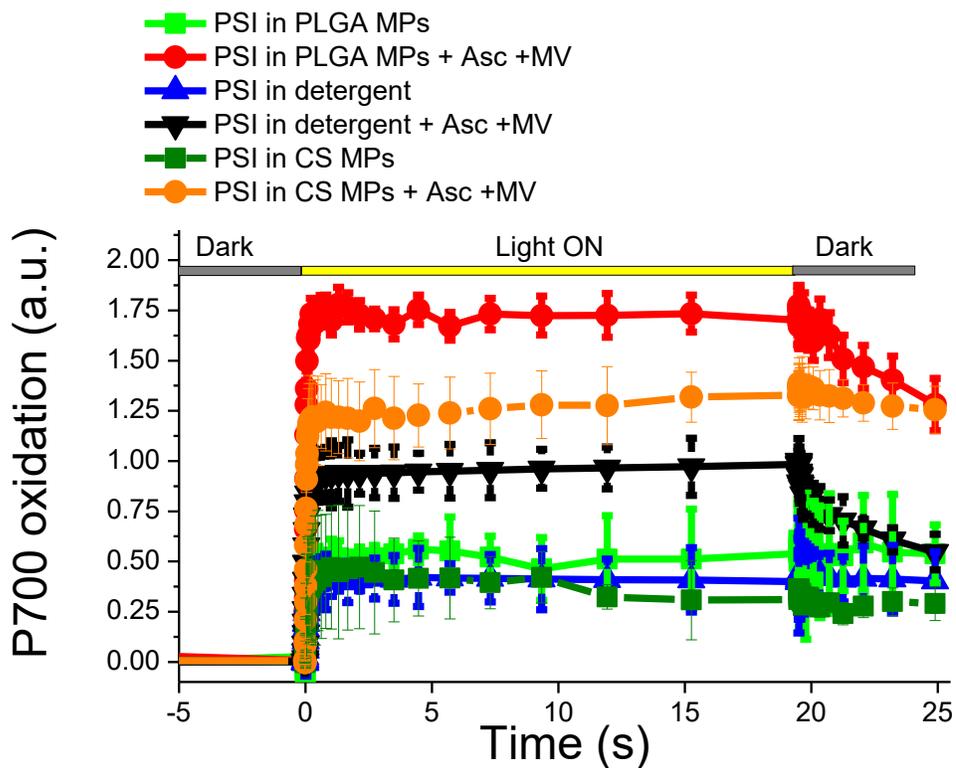


Fig. S9. Stability of PSI to light exposure. Maximum P700 activity of PSI in detergent or in PLGA or chitosan (CS) measured at different time (days) of exposure to $1500 \mu\text{mol m}^{-2}\text{s}^{-1}$.

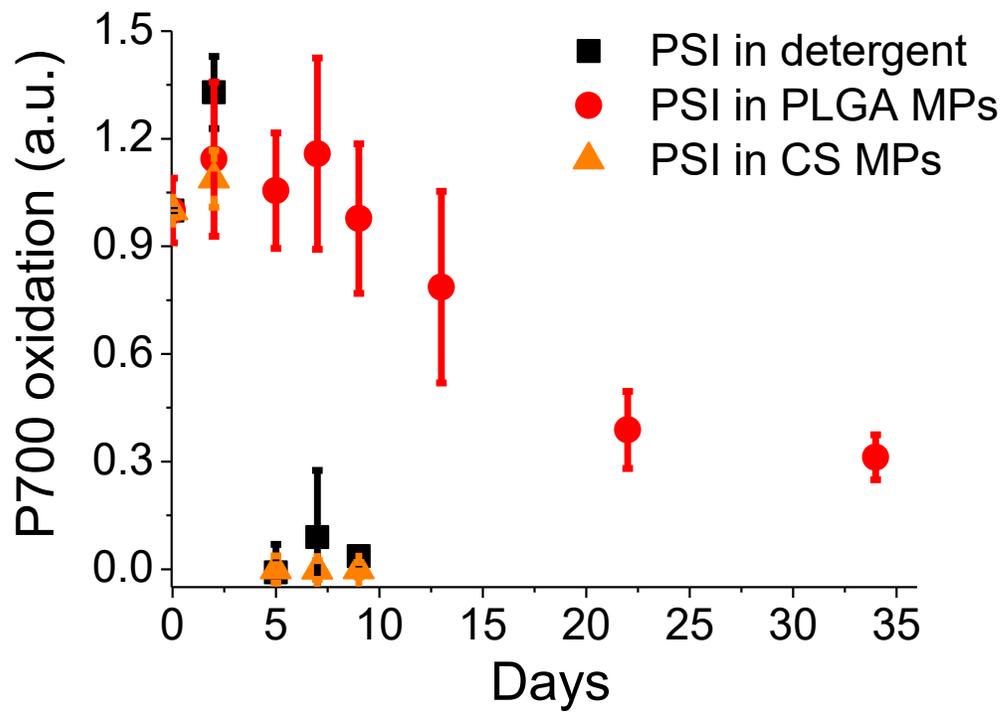


Fig. S10. P700 oxidation of lyophilized microparticles containing Photosystem I.
P700 oxidation kinetic was measured depositing the lyophilized MPs on a 1mm quartz cuvette.

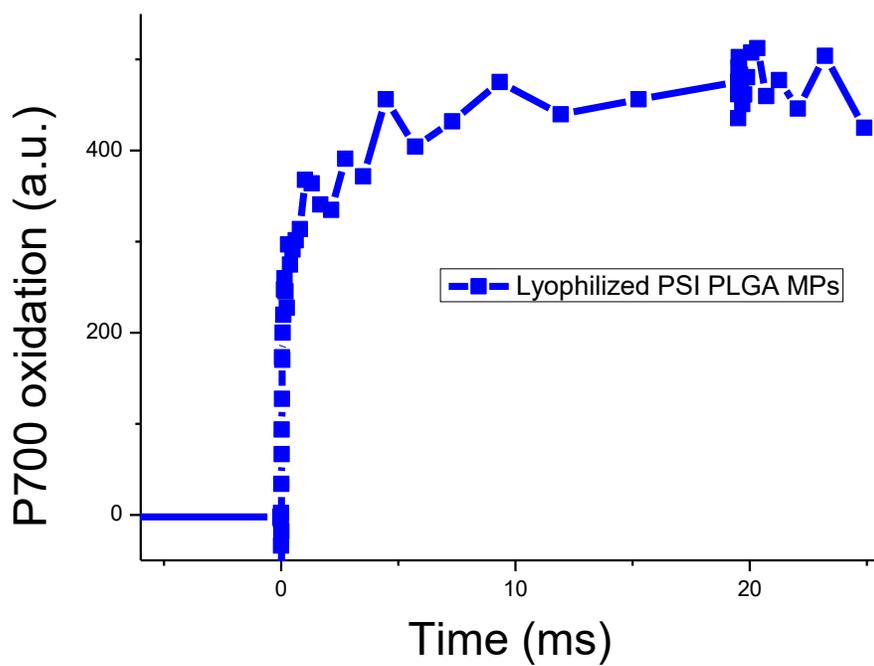


Fig. S11. Absorption spectrum of PSI microparticles before and after lyophilization. Absorption spectra of PSI in MPs are reported after subtraction of the scattering obtained by measuring the scattering traces of empty MPs.

