Supporting information

Amorphous calcium organophosphates nanoshells as potential carriers for drug delivery to Ca^{2+}-enriched surfaces.

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Characterization methods

Wide angle X-ray scattering (WAXS) experiments were performed using a Siemens D500 diffractometer equipped with a conventional X-ray tube operating at a wavelength of $\lambda = 0.15418$ nm (Cu Kα). Alternatively, the CaPLiLX sample was left in contact with 0.05M $CaCl_2$ solution for 24 hs before analysis. *X-Ray Diffraction analysis* (XRD) was performed with a Philips PW 1011/00 diffractometer using CuKα radiation operated at 30 mÅ and 35 kV. XRD patterns were scanned in steps of 0.02°/2s in the range from 5° to 60°. The CaPLiLX sample was deposited on glass slide with and without previously deposited Ca^{2+} and was left to dry on a dissicator.

Transmission electron microscopy images of the CaP covered, and uncovered liposomes samples were taken with a JEOL JEM 1200 EX II microscope. Samples were prepared by dripping on carbon-coated 300-mesh copper grid and water evaporated in air. Alternatively, the samples were stained with 2% phosphotungstic acid.

High-resolution transmission electron microscopy images of CaPLiLX were obtained with a FEI Talos 1162 microscope equipped with an energy-dispersive X-ray spectrometer (EDS, Si(Li) Jeol detector). The microscope was used in scanning TEM mode (STEM). The sample was prepared by deposition of the suspension on carbon- coated 400 mesh copper grid and evaporated under air. The images were analyzed by Image J software.

Surface composition of nanoshells was obtained by Attenuated Total Reflectance – Fourier Transformed Infrared spectroscopy (ATR-FTIR) in an Agilent Cary Series 630 spectrometer equipped with ZnSe accessory. In all cases, samples were dropped on potassium bromide solids and was left to dry on air. Fifty scans over the range of 600–4000 cm^{-1} were performed at a resolution of 2 cm^{-1}.

Nanoshell size distribution and surface charge were determined by dynamic light scattering (DLS) and electrophoretic mobility (μ_e) measurements, respectively. Aqueous 0.1 M KCl samples of LiLX, and CaPLiLX in the absence and in the presence of 0.05M of Ca^{2+} and Mg^{2+} were measured without filtration at 25 °C within a week with a Zetasizer Nano (NanoZSizerZEN3600, Malvern, U.K.) equipped with a He-Ne 633nm laser with a cell drive voltage of 30 V using a monomodal analysis model.

Photoluminescence measurements were performed using a Jobin-Yvon Spex Fluorolog FL3-11 spectrometer equipped with a Xe lamp as the excitation source, a monochromator with 1 nm bandpass gap for selecting the excitation and emission wavelengths, and a red sensitive R928 PM detector. The spectra were corrected for the wavelength-dependent sensitivity of the detector and the source and the emission spectra were corrected for Raman scattering by using the solvent emission spectrum. Fluorescence lifetime and time-resolved anisotropy measurements were completed with a TCSPC (time-correlated single-photon counting) with LED excitation at 341 nm The anisotropy curves were fitted according to $r(t) = r_\infty + r_0\, x\, e^{-t/\theta}$, where r(t) is the anisotropy, r_∞ is the residual anisotropy; r_0 is the fundamental anisotropy and θ is the rotational correlation time.

Time resolved emission spectroscopy (TRES) was performed, and results were fitted to an exponential model until optimal values χ^2 and residuals were obtained. The emission spectrum associated with each fluorescence lifetime may be obtained taking the contribution of each decay lifetime to the overall emission at a given wavelength, weighted by the emission intensity at the emission maximum.

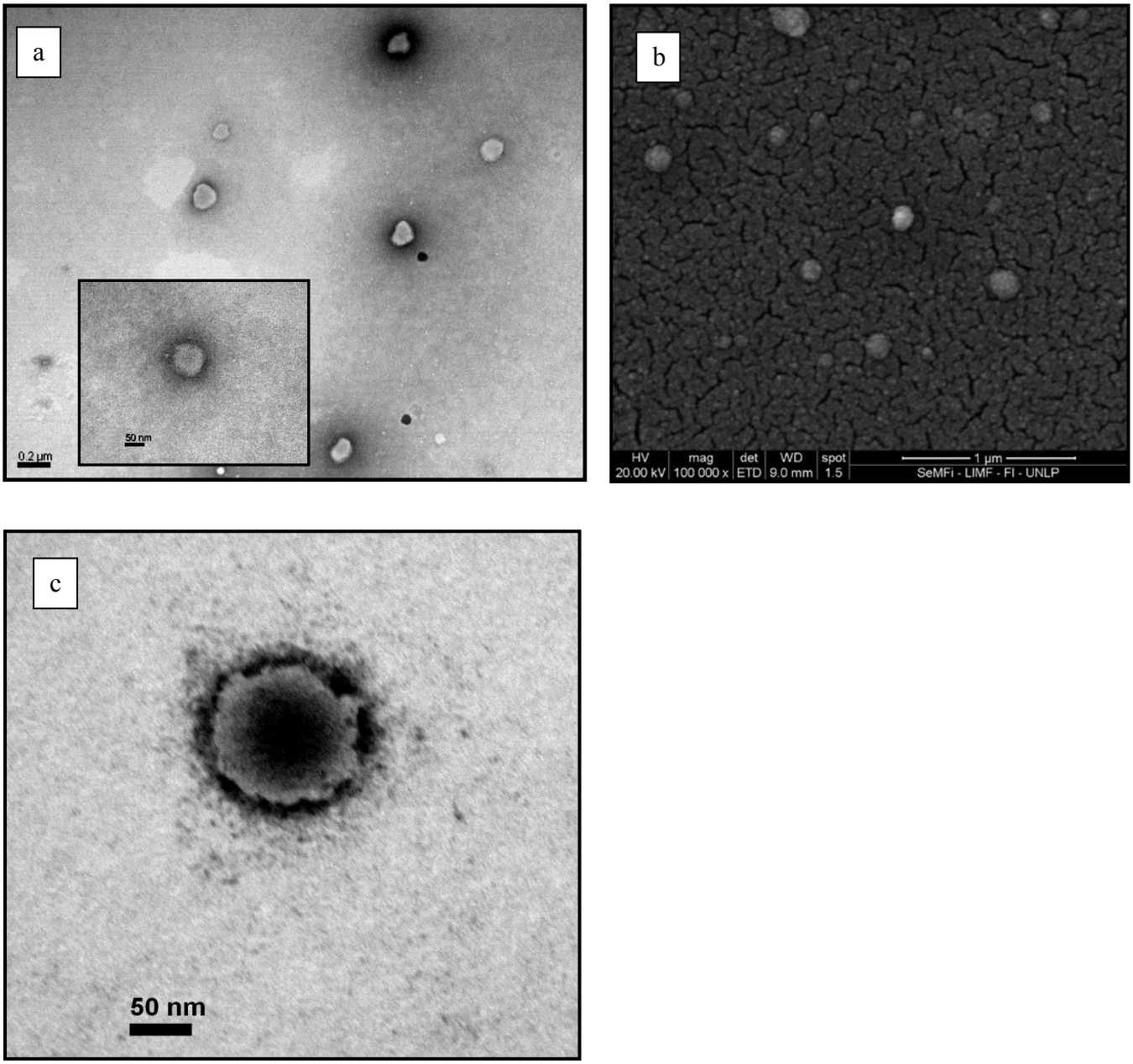
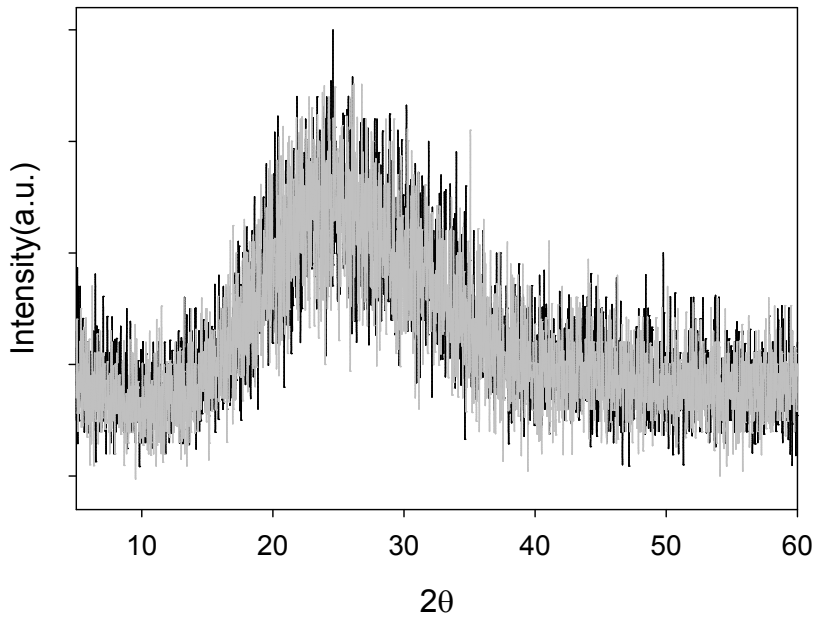


Fig. S1. TEM (a) and SEM (b) images of CaPLiLX and LiLX (inset (a)). Core-shell structure of CaPLiLX observed by TEM (c).



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70 **Fig. S2.** XRD patterns of CaP nanoshells deposited on glass slides in the presence (black line) and in the

71 absence (grey line) of Ca^{2+}.

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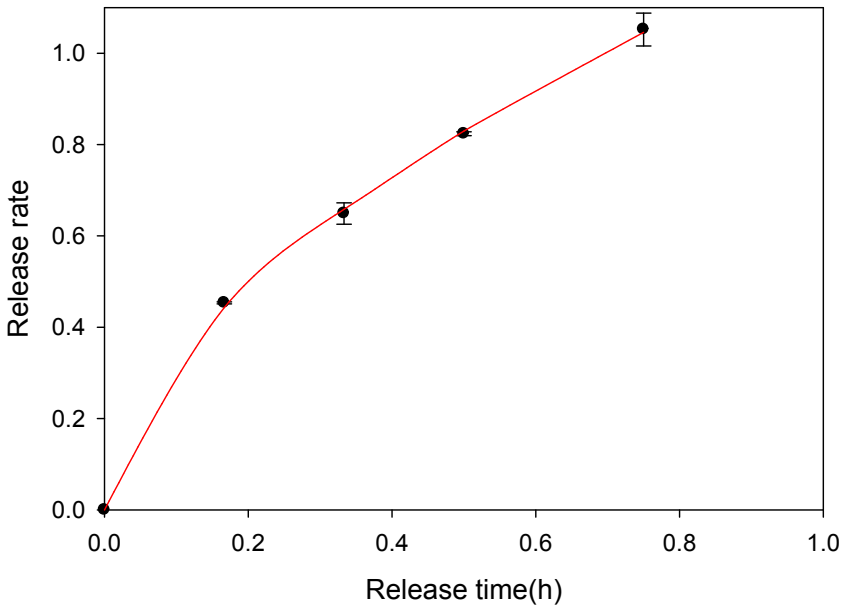

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74 **Fig. S3**. Release profile for free LX in acetate buffer solution. Line stands for the fitting to Kosmeyer-

75 Peppas model. Error bars stands for SD.

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Table S1. Parameters obtained from data fitted to Korsmeyer-Peppas and Weibull models drug release models for the release kinetics of LX from CaPLiLX in Simulated body fluid (SBF), phosphate buffer (PBS), and acetate buffer.

	Korsmeyer-Peppas $RR = k_{KP} \times t^n$		**Weibull** $RR = 1 - exp(-t^b/a)$	
	k_{KP}	n	a	b
SBF	0.55±0.01	0.14±0.02	1.23±0.06	0.13±0.02
Acetate	0.39±0.02	0.11±0.02	2.0±0.1	0.15±0.02
PBS	0.35±0.02	0.11±0.02	2.4±0.2	0.17±0.03
Free LX in PBS	0.59±0.05	0.6±0.1	--	--
Adsorbed LX on CaPLi	0.39±0.01	0.88±0.02	--	--

The fitted parameters were obtained with a coefficient of determination $r^2 > 0.90$.

The complete set of data was also fitted to the Weibull model which considers the diffusion of drugs through porous materials.[44] In this case, the relation $RR = 1 - exp(-t^b/a)$ was used, where the value of parameter b is indicative of the type of release mechanism and a is a scale factor. Fitting the release data to the Weibull model yields $r^2 > 0.91$ for all media. However, b values < 0.35 obtained for all the samples (Table S1) are not frequently found in the literature and are attributed to highly disordered materials.

Table S2. Diameter and electrophoretic mobility of LiLX and CaPLiLX in the absence and the presence of Ca^{2+} and Mg^{2+}.

Sample	*Diameter(nm)(±SD)*	*Electrophoretic mobility(**$cm^2V^{-1}s^{-1}$**) (±SD)*	*PDI*
LiLX	100.6 ± 0.6	-4.6 ± 0.2	0.259 ± 0.004
CaPLiLX	171.8 ± 0.6	-4.1 ± 0.1	0.204 ± 0.004
CaPLiLX+Ca^{2+}	832 ± 86	-0.49 ± 0.06	0.75 ± 0.05
CaPLiLX+Mg^{2+}	1182 ± 134 (64%) 228 ± 33 (36%)	--	0.79 ± 0.04

CaPLiLX electrophoretic mobility of -4.1 ± 0.1 $cm^2V^{-1}s^{-1}$ indicates that the nanocarriers are negatively charged at pH 7.4. Moreover, the diminution of μ_e upon LiLX coating is indicative of CaP deposition over the liposomes.

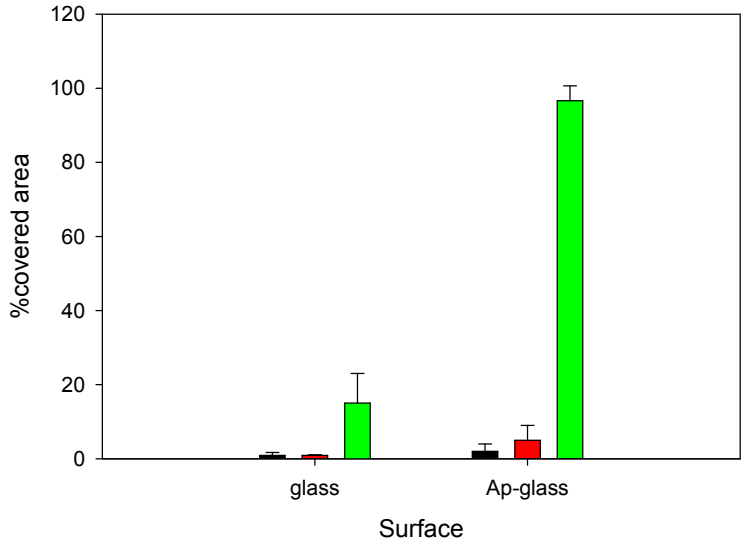

Fig. S4. Analysis of fluorescence covered area on unmodified glass and Ap-modified glass slides as a function of contact time: 0 min (black), 10 min (red) and 180 min (green). Error bars stands for SD.