Supplementary Electronic Information

Easy Directed Assembly of only Nonionic Azoamphiphile Builds up Functional Azovesicles

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1. Lyotropic behavior of $C_{12}OazoE_3OH$: Pure amphiphiles with rigid segments like azobenzenes with a similar non-ionic head are able to form stable bilayers[1]. An aliquot of $C_{12}OazoE_3OH$ (19 mM, in chloroform) was placed onto non-treated glass and evaporation the solvent at room temperature was allowed. Polarized optical microscopy (POM) observation through crossed polarizers revealed the smectic C phase of $C_{12}OazoE_3OH$ which occurs with typical spherulites and SmC schlieren textures. POM was performed using a Nikon Eclipse E 200 POL polarizing microscope.

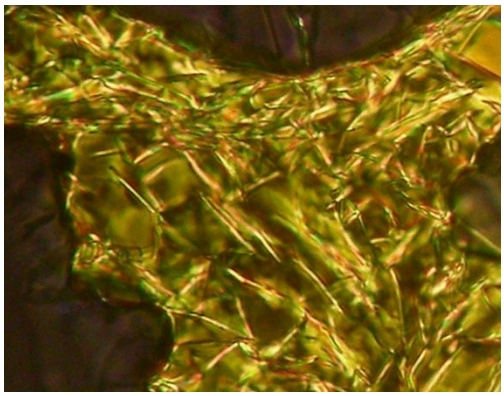

Figure S1. POM photomicrograph of $C_{12}OazoE_3OH$ at 25ºC after $CHCl_3$ evaporation from a 19 mM solution.

2. Critical Aggregation Concentration (C.A.C) in water

[1] T. Shang, K. A. Smith and T. A. Hatton, Self-assembly of a nonionic photoresponsive surfactant under varying irradiation conditions: a small-angle neutron scattering and cryo-TEM study, *Langmuir,* 2006**, 22**(4), 1436-1442.

The compound exhibited poor solubility in water at room temperature, so we determined the Krafft temperature and C.A.C. by Dynamic Light Scattering (DLS) and UV-Vis absorption spectroscopy (Figure S2). The Krafft temperature obtained was 70°C and the CAC $_{70^{o}C}$ determinated was 0, 35 μM.

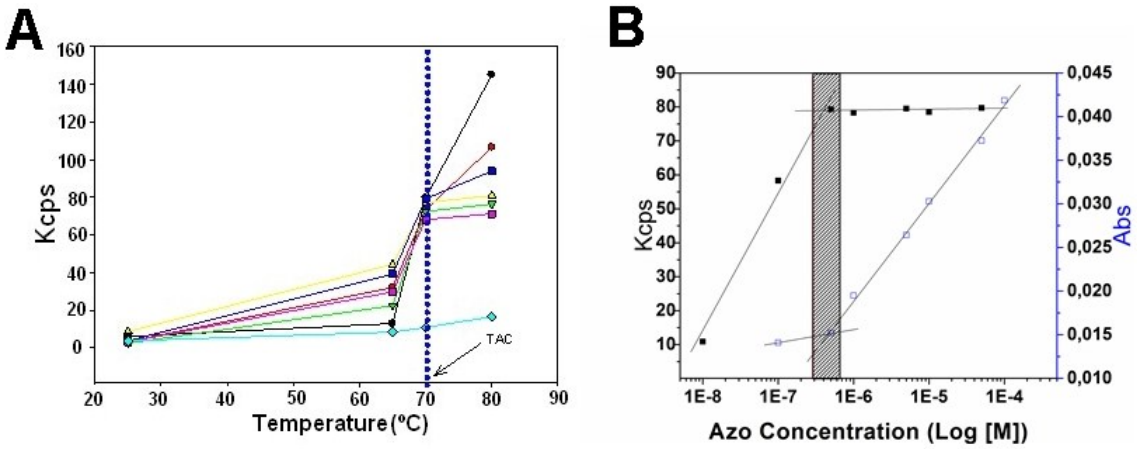

Figure S2. A) Krafft point calculated by DLS. Kilo counts per second (Kcps) as mean count rate (average scattering intensity) vs temperature. **B)** C.A.C. obtained from correlating both DLS and Uv-Vis techniques. The grey bar indicate the region of critical aggregation concentration.

3. Vesicles Synthesis

Directed assembly protocol of $C_{12}OazoE_3OH$ vesicles: Evaporation-Induced Self-Assembly (EISA)

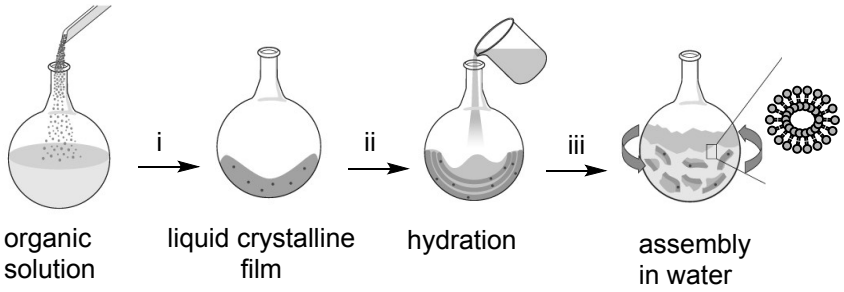

Figure S3. Schematic EISA protocol: i) solvent removal via rotatory evaporation to form an homogeneous liquid crystal film , ii) hydration under heating (70°C), iii) shaking and subsequent ageing for 2 days at 4°C.

The synthesis of vesicles assemblies was conducted following the technique of obtaining a thin film. The procedure involves the formation of a homogeneous organic film by vacuum evaporation at room temperature of a chloroform solution of the $C_{12}OazoE_3OH$ amphiphile (*Z-E*: 5-95, 19 mM) exposed to white light. The film was thoroughly dried to remove residual organic solvent by placing the flask on a vacuum pump for 6 hrs.To hydrate the film, Milli-Q water (38 ml) at Krafft temperature (70ºC) was added to the round bottom flask, and slow rotatory movement was applied for 2 hrs. Next, vigorous shaking using a vortex, and then sonication of the sample was performed. Finally, it was necessary at least two days aging to achieve the formation of the yellowish vesicles (Final volume 38 ml, aprox. 0.5 mM in water).

4. $C_{12}OazoE_3OH$ Vesicles Characterization

4.1. **Polarized Optical Microscopy**: Vesicles were centrifuged and the pellet was placed between glass and cover and observed by a Nikon Eclipse E200 POL microscope. The characteristic birefringence of lamellar phases were detected by optical microscopy. The lamellar phase was observed between two crossed polarizers in the optical microscope with polarizers oriented in the x and y direction respectively, then for a dislocation defect whose edge was oriented along the y direction, no light emerged from the second polarizer and we only saw darkness under the microscope. However, the defect edge was easily observed when the material was rotated, because the director was no longer perpendicular to the polarizers. In addition, spherical multilamellar vesicles were identified because they disappeared under polarized light.

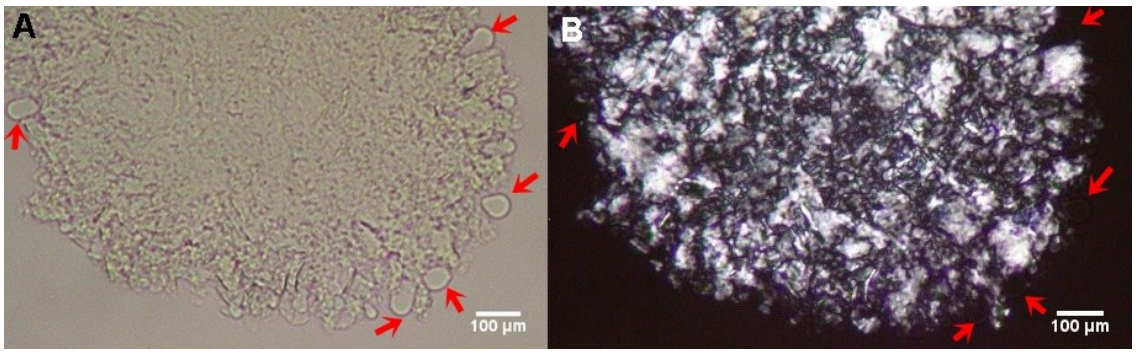

Figure S4. Vesicular membranes dispersion of $C_{12}OazoE_3OH$ (E) at 25°C **A)** Optical photomicrograph B) POM photomicrograph (crossed polarizers). In both, multilamellar vesicles are shown by arrows.

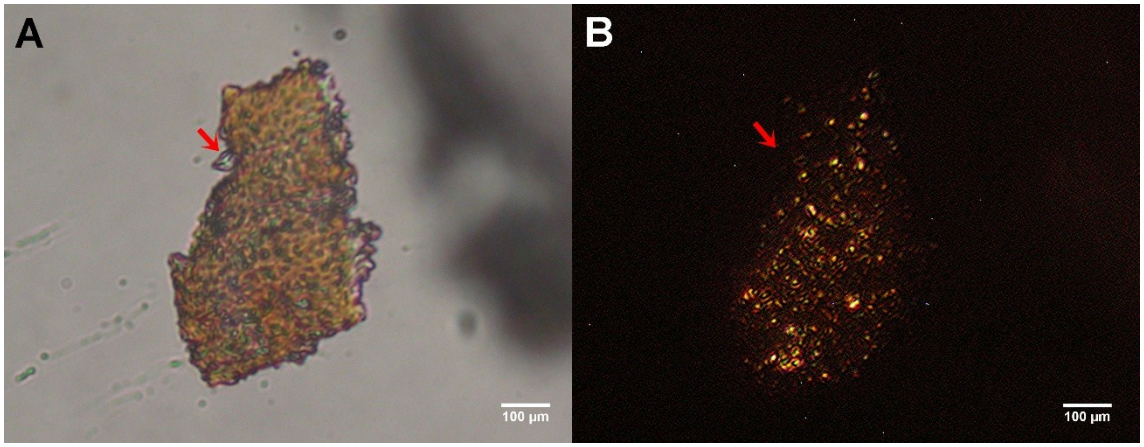

Figure S5. Vesicular membranes dispersion of $C_{12}OazoE_3OH$ (Z) at 25°C **A)** Optical photomicrograph B) POM photomicrograph (crossed polarizers). Multilamellar vesicles are shown by arrows.

4.2. **<u>Electron Microscopy</u>:** Specimens were prepared in duplicate, and grids were thoroughly examined to get an overall statistical evaluation of the structures present in the sample. Control experiments of water alone were performed showing no vesicles or spheres. Statistical evaluation was completed using Image J software.

4.2.1 **<u>TEM Experiment</u>**: Each sample aliquot (5 µL) was deposited onto the copper grid (200 mesh) coated with Formvar. After 5 min of interaction, the excess fluid was removed. The sample was negatively stained with 2% of uranyl acetate in water. After 2 min, excess fluid was removed, let dry, and observed in a JEOL 100CX II microscope operating at 100 kV (Fig. S6 and S7). TEM images revealed that there is an obvious

contrast between the periphery and the center in the sphere, characteristic of the projection images of hollow spheres or vesicles.

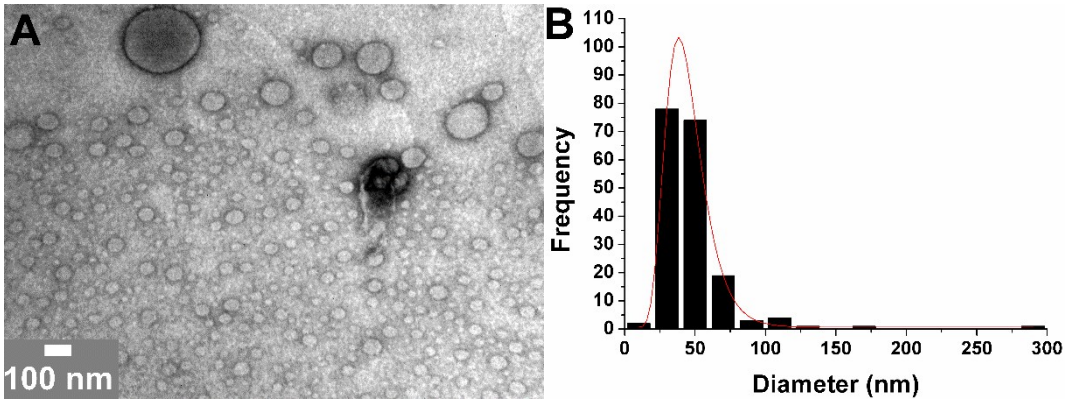

Figure S6. Vesicular membranes dispersion of $C_{12}OazoE_3OH$ (E) at 25°C **A)** TEM photomicrograph. B) Diameter distribution of the particles observed in Image A.

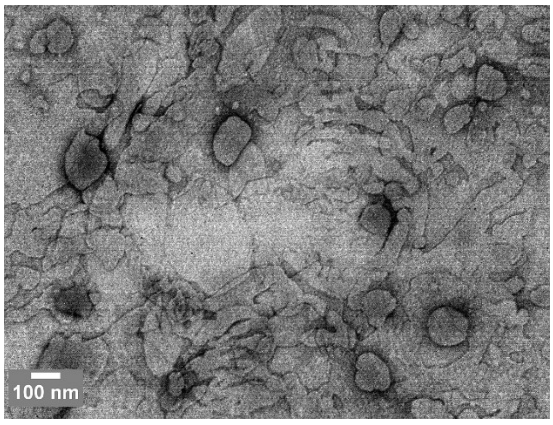

Figure S7. Vesicular membranes dispersion of $C_{12}OazoE_3OH$ (Z) at 25°C**.** TEM photomicrograph.

TEM images of (Z) azovesicles did not provide information about the size of the new vesicles, because their coalescence on grid. This result is expected if we considered that at the interface both E and Z isomers possess a substantial difference in bending

[2] L. A. Benedini, M. A. Sequeira, M. L. Fanani, B. Maggio, V. I. Dodero, Development of a non-ionic azobenzene amphiphile for remote photocontrol of a Model Biomembrane, *Phys. Chem. B*. 2016, **120**, 4053-4063.

[3] T. Kunitake and Y. Okahata, A totally synthetic bilayer membrane, *Journal of the American Chemical Society,* 1977, **99**(11), 3860-3861.

elasticity (100 mN/m for $C_{12}OazoE_3OH$ (E) and 60 mN/m for $C_{12}OazoE_3OH$ (Z)). [2] In other experiment, the specimen was sonicated in the presence of 2% of uranyl acetate (1:1 volume) and treated as above. This protocol was employed to detect the external structure (Figure 1C in main text).[3]

4.2.2 **SEM Experiment**: Each sample aliquot (20 µL) was deposited on uncoated cover slips. After 5 min of interaction, the excess of fluid was removed and let dry in a Petri dish. The resulting specimens were metalized with Au(0) using sputter coater Pelco 91000 and observed via a JSM-35 CF equipped with secondary electron detector (EVO 40).

5. General method for observation of photoinduced changes and vesicles stability

5.1 **UV-Vis Spectroscopy**: Switching experiments were done with 150 watts mercury arc lamp from Pleuger antwerp Brussels with filter for 360 nm and a white light bulb of 60 watts. Both experiments have been done maintaining the sample temperature at 20 ±5°C using a cool water bath. UV-Vis spectroscopy data were recorded on a JASCO V-630BIO Spectrophotometer equipped with EHCS-760 peltier. After switching experiments, the sample were lyophilized and analyzed by mass spectrometry. Mass calculated for $C_{30}H_{47}N_2O_5$: 515.3485; obtained 515.3497. No degradation products were detected.

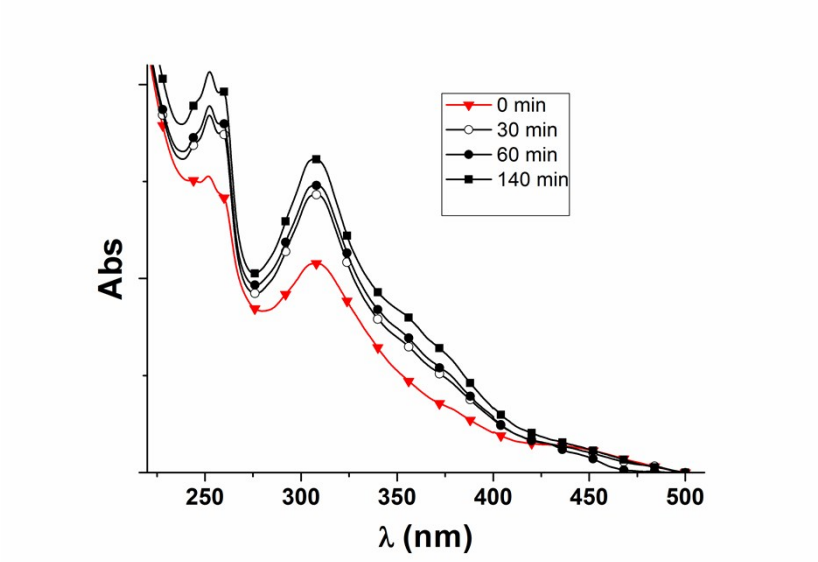

Figure S8: UV/Vis Experiment: Photo-conversion of $C_{12}OazoE_3OH$ from (Z) vesicles to (E) vesicles promoted by white light in water (60 watts).

5.2 **Thermal Stability**: The stability of the (E) azovesicles were evaluated by heating and cooling experiments monitoring the absorption bands at λ 305 nm and λ 341 nm or 360 nm which correspond to azobenzene H-aggregates and “free -type“ azobenzene, respectively.

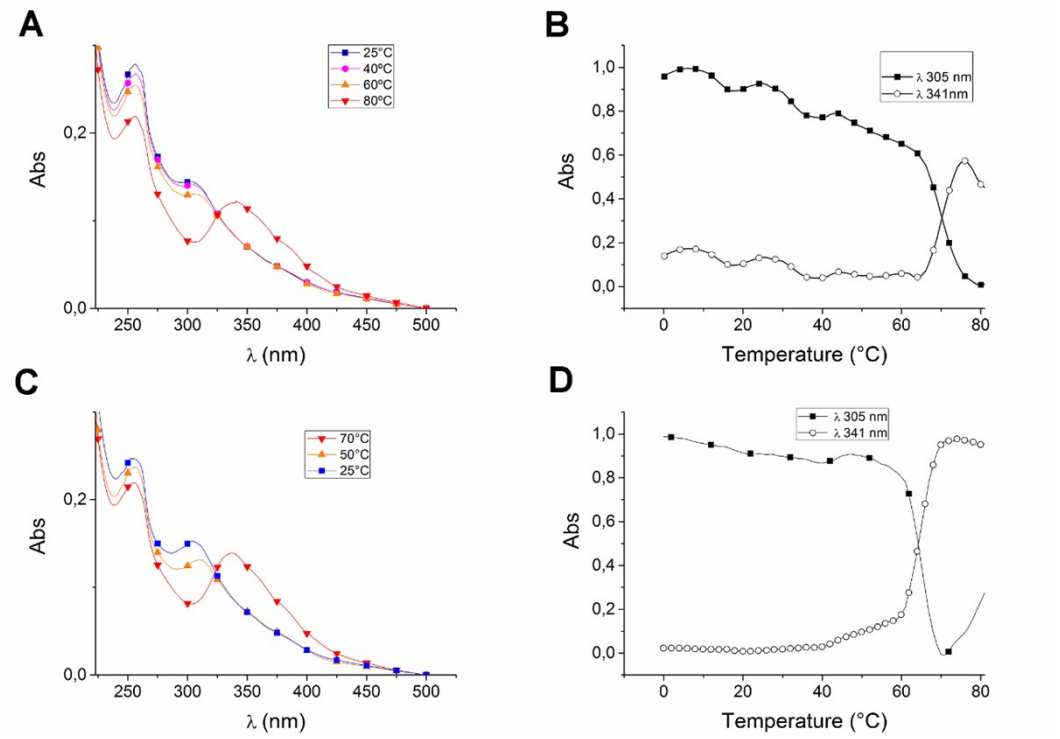

Figure S9: **A)** UV-Vis spectra of (E) $C_{12}OazoE_3OH$ on heating; **B)** Temperature dependence experiment on heating monitoring the sample at λ 305 nm and λ 341 nm, respectively; **C)** UV-Vis spectra of (E) $C_{12}OazoE_3OH$ on cooling; **D)** Temperature dependence experiment on cooling monitoring the sample at λ 305 nm and λ 341 nm, respectively.

In the case of (Z) $C_{12}OazoE_3OH$ azovesicles (Fig. S10), the thermal behavior is quiet complex. This is because, Z-E thermal isomerization is accelerated on heating, thus the initial E:Z is changed during thermal evaluation. Simultaneously, disassembly of the initial (Z) azovesicles is taking place displaying a red-shift of the maximum wavelength to 360 nm, showing that (Z) isomers are more ”free” in water after disassembly than the (E) isomers which maximum wavelength was observed at 341 nm. This behavior is consistent with the increase of dipole moment of Z isomer and the increase of solubility of Z isomer, previously reported.[2] On cooling, the assembly of the thermal stationary state occurs, which is reflected in the final spectrum (Compare Fig S10 A and C)

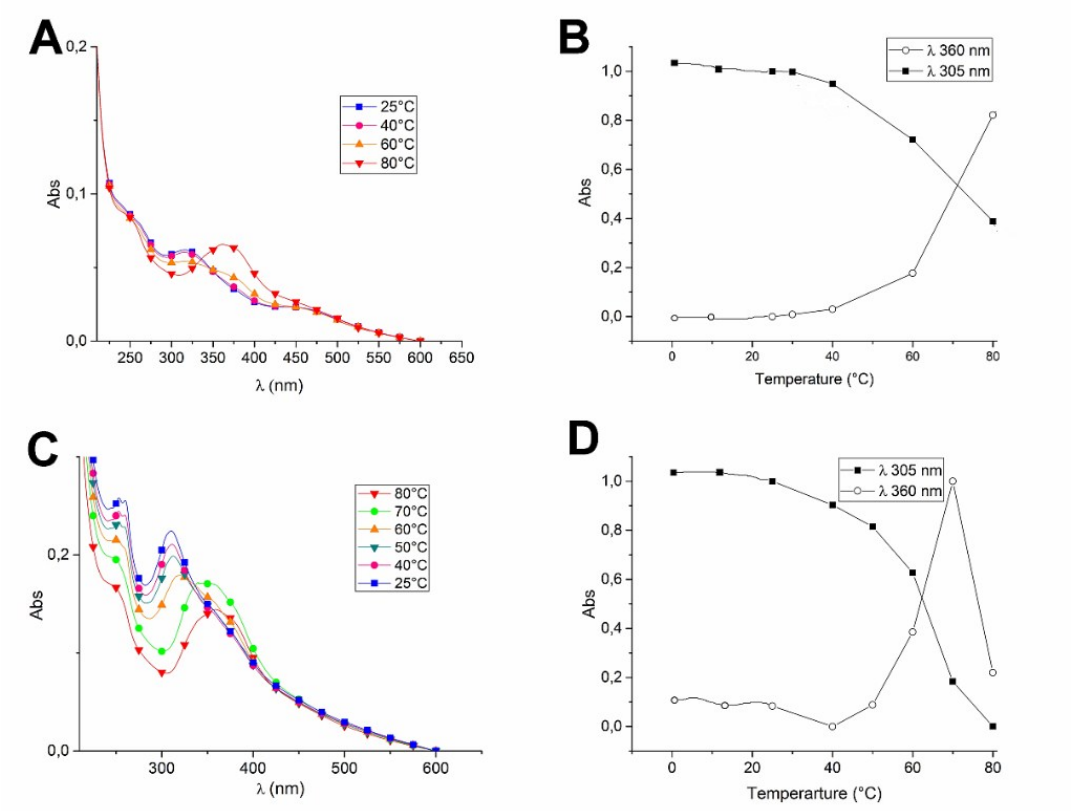

Figure S10: **A)** UV-Vis spectra of (Z) $C_{12}OazoE_3OH$ on heating; **B)** Temperature dependence experiment on heating monitoring the sample at λ 305 nm and λ 360 nm, respectively; **C)** UV-Vis spectra of initial (Z) $C_{12}OazoE_3OH$ on cooling; **D)** Temperature dependence experiment on cooling monitoring the sample at λ 305 nm and λ 360 nm, respectively.

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5.3 **Dynamic Light Scattering (DLS)**: measurements were performed using Zetasizer-Nano® spectrometer from Malvern. All the DLS measurements were performed at a fixed scattering angle (θ) of 90º at 25.0 ± 0.1 °C. Figure was performed using Image J software.

6. **Calcein encapsulation and release:** To evaluate the capability of $C_{12}OazoE_3OH$ to generate photo-switchable vesicles, calcein release experiment is presented. For this experiment, the (E) azovesicles were obtained by EISA as described before, and in this case the hydration procedure was performed with 100 mM calcein solution in buffer Hepes 5 mM, 50 mM NaCl, pH 7. After sonication, the azovesicles were separated from

the free fluorescent probe by gel exclusion chromatography using Sephadex G-100, equilibrated with an iso-osmotic buffer HEPES 5 mM, 150 mM NaCl, at pH 7.0. Passive release was less than 10% after two days.

Fluorescence measurements were performed using a Jasco FP 8300 Spectrofluorometer provide with Xe Lamp with Shielded Lamp Housing, 150 W and a peltier cell changer for temperature control. To evaluate the photo-induced calcein release, the sample was irradiated at 310 nm at different times in the spectrofluorometer at 25°C. Then, calcein release was detected by its typical fluorescence emission at 515 nm, the excitation wavelength were 495 nm. The bandwidths employed were of 5 nm for both excitation and emission.

7. Total Calcein Release by Triton X-100 release: To evaluate the total calcein release, 2 µL Triton X-100 was added to calcein-loaded (E) azovesicles and calcein fluorescence was acquired after addition. The calcein fluorescence intensity obtained was used to calculate the calcein release after each irradiation cycle by the following equation (1)

$$\frac{(I_0 - I_i)}{(I_0 - I_t)} \times 100 = Percentage\ of\ Calcein\ Release$$

Where I_0 is the initial fluorescence before the irradiation of the sample, I_i is the emission intensity after the irradiation at each time analyzed, and I_t is the total fluorescence obtained after the treatment of the sample with Triton X-100 (Fig S11).

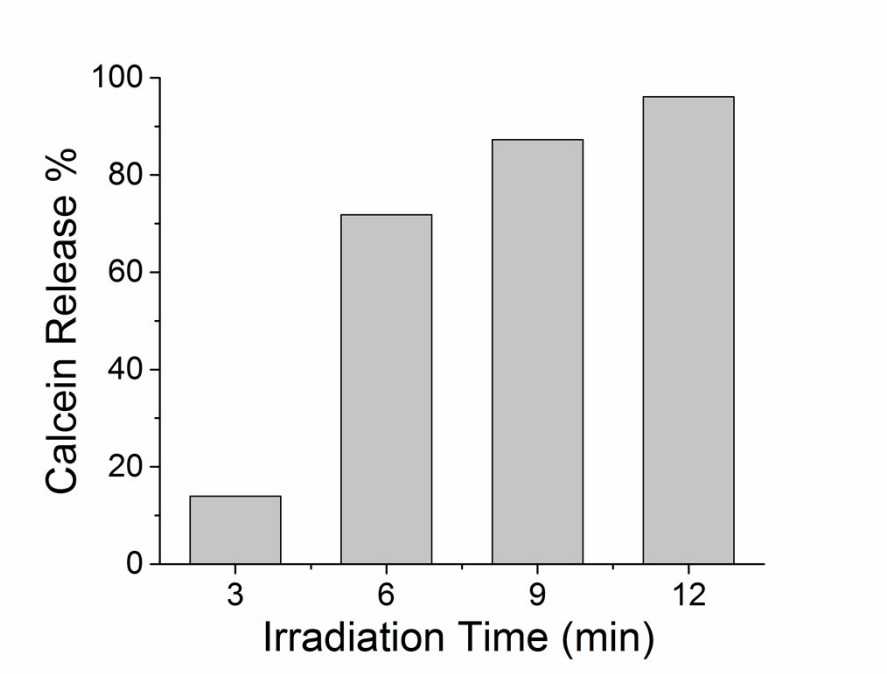

Figure S11. Cumulative Calcein release percentages at 0, 3, 6, 9 and 12 minutes irradiation at 310 nm. Calcein fluorescence at each time was detected by exciting at 495 nm and the emission was registered at 515 nm. Chart obtained by equation (1)

8. Nile Red Interaction with $C_{12}OazoE_3OH$ vesicles: (E) $C_{12}OazoE_3OH$ vesicles obtained as described before, were treated with 10 μM final concentration of Nile Red and incubated for 10 minutes. Then the photoinduced transition of the vesicles was performed by irradiating the sample during 12 minutes at 310 nm. Before and after the irradiation, the emission spectra of Nile red was obtained between 580 and 740 nm by exciting the system at 552 nm. These experiments were carried out on a Shimadzu RF spectrofluorophotometer at 25°C, using slits of 5 nm for both excitation and emission. As control, Nile Red alone in buffer HEPES 5 mM, 150 mM NaCl, at pH 7.0 was treated in the same conditions as the sample. No changes on NR emission spectra was observed after 310 nm irradiation. The fluorescence spectra obtained were normalized. The second derivative of each spectra was calculated to analyze changes in the position of the maximum wavelength (Figure S12).

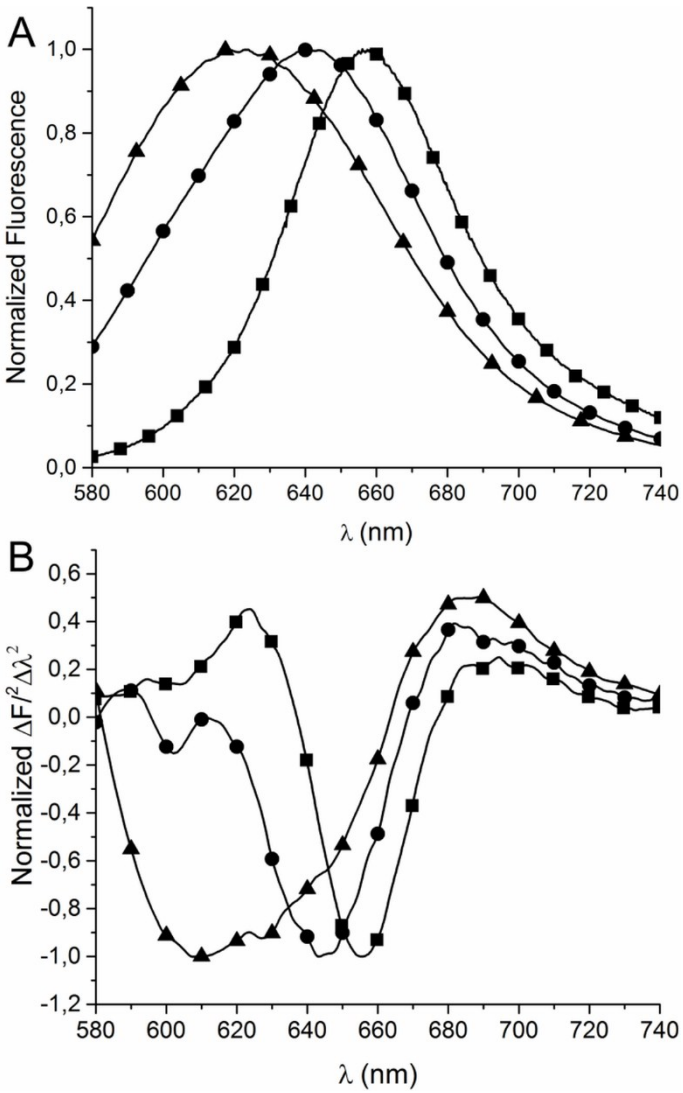

Figure S12. **A)** Normalized Fluorescence Emission spectrum of $C_{12}OazoE_3OH$ vesicles- Nile Red system in buffer Hepes 5 mM, 150 mM NaCl, at pH 7.0 before (●) and after 12 minutes of irradiation at 310 nm (①). As control, Hepes 5 mM, 150 mM NaCl, at pH 7.0 was treated with the same concentration of Nile Red (■). The fluorescence was acquired by exciting the samples at 552 nm. **B)** Second derivative spectra of spectra in Panel A, respectively.