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SUPPLEMENTARY INFORMATION

LIGHT-INDUCED MOLECULAR ROTATION TRIGGERS ON-DEMAND RELEASE FROM LIPOSOMES

Authors: Laís Ribovski[1,2], Qihui Zhou[3], Jiawen Chen[4], Ben L. Feringa[4], Patrick van Rijn*[1], Inge S. Zuhorn*[1]

[1]University of Groningen, University Medical Center Groningen, Department of Biomedical Engineering, Groningen, the Netherlands. A. Deusinglaan 1, 9713 AV Groningen, The Netherlands
[2]Nanomedicine and Nanotoxicology Group, Physics Institute of São Carlos, University of São Paulo, CP 369, 13560-970 São Carlos, SP, Brazil
[3]Institute for Translational Medicine, Department of Periodontology, The Affiliated Hospital of Qingdao University, Qingdao University, Qingdao 266021, China.
[4]Center for Systems Chemistry, Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 4, 9747AG Groningen, Netherlands

*Corresponding authors: Patrick van Rijn; Inge S. Zuhorn
E-mail address: p.van.rijn@umcg.nl; i.zuhorn@umcg.nl

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MATERIALS AND METHODS

Ultrapure water (MQ) with conductivity of 18.5 x MΩ cm^{-1} was used for the preparation of buffers. For the preparation of lipids round-bottom Kimax® tubes (Sigma Aldrich) pre-washed with aqua regia and MQ were used. All volumes were measured with capillary tubes.

Preparation of liposomes with molecular motors: Liposomes with 10 mM 1,2-dioleoyl-sn-glycero-3-phosphocholine (#850375P, 18:1 (Δ9-Cis) PC (DOPC)), Avanti Polar Lipids, Inc.) were prepared by hydration method followed by extrusion through polycarbonate 100 nm pore membrane. A solution of DOPC in chloroform (50 mg mL^{-1}) was mixed with pure methanol (#1060092511, Emsure® Merck) or methanol containing molecular motors (MM; synthesis of 5,5'-(9-(2-methyl-2,3-dihydro-1H-cyclopenta[a]naphthalen-1-ylidene)-9H-fluorene- 3,6-diyl)diisophthalic acid, has previously been described[1]) in a ratio 1:1 (v/v), typically 150 µL of each solution. Liposomes containing MM were prepared at two different mixing ratios, 50:1 (MM1) and 25:2 (MM2) of lipid to MM (lipid: MM, w/w). The solvents were evaporated with a stream of N_2 followed by drying in vacuum overnight. The dry lipid films were hydrated with 954 µL of 100 mmol L^{-1} solution of calcein (#C0875, Sigma-Aldrich) in 10 mM HEPES (prior to use the pH of calcein was adjusted to 7.4 with 5M of NaOH). After addition of calcein, the mixtures were vortexed for 15 sec, followed by vigorous shaking for 2-3 hours to generate liposomes. Liposomes were extruded 17 times using an Avestin LiposoFast - Basic extruder with two gas tight glass syringes and assembled with two filter supports (#610014, Avanti) and one 100-nm pore polycarbonate membrane (Avestin) prewetted in HEPES buffer. Liposome purification was performed using a gel filtration resin Sephadex® G-100 (#17006001, GE Healthcare) with HEPES (10 mM, 7.4) buffer. During the purification, the setup was protected from light. The purified liposomes were used within two days.

Liposome characterization: The size of liposomes after purification was determined in 10 mM HEPES, pH 7.4 at 20°C by a Zetasizer Nano ZS (Malvern Instruments) with the scattering detector positioned at the fixed angle 173 °. To assess size alterations due to irradiation samples were measured before and after 30 minutes UV-irradiation.

The zeta potential (ζ-potential) was determined under similar conditions to the size measurements. Electrophoretic mobilities of the particles were measured and converted into ζ-potentials using the Smoluchowski equation with the software supplied by the manufacturer. The ζ-potentials were reported as the average of three successive measurements.

Calcein release assay: UV light irradiation (λ_{max} = 365 nm) of the liposomal formulations was performed with a Spectroline lamp model ENB-280C/FE kept ≈10 cm from the 96-

well plate with a delivery intensity of ≈ 0.2 mW cm^{-2} for 30 or 60 sec. Fluorescence of calcein-loaded liposomes with and without motors (MM1, 1:50 (w/w)) was measured before and after UV light irradiation in a Synergy HTX Multi-mode plate reader (BioTek Instruments Inc.) with excitation 485/20 nm and emission 528/20 nm using 96-well black flat bottom plates. Number of excitation flashes was set at 3 flashes per well to reduce photobleaching. The fluorescence intensity was determined at 25°C, and the measurements were acquired every 5 min over a course of 70 min, after which 10% solution of Triton X-100 (BioXtra, Sigma Aldrich) in HEPES (10 mM, pH 7.4) was added to each well to the final concentration of 0.1% and the samples were incubated for 10 min at RT. The percentage of the released calcein was calculated according to the equitation below:

$$\%Release = 100 * \frac{F(\Delta t) - F(0)}{Fmax - F(0)}$$

where FΔt is fluorescence at the certain time, F(0) is fluorescence measured before the exposure to the UV light and F max is fluorescence measured after incubation of liposomes with Triton X-100.

References

1 J. Chen, K. Chen, G. T. Carroll and B. L. Feringa, *Chem. Commun.*, 2014, 12641–12644.

Supplementary Information 1

Mechanism of rotation of molecular motor around double bond.

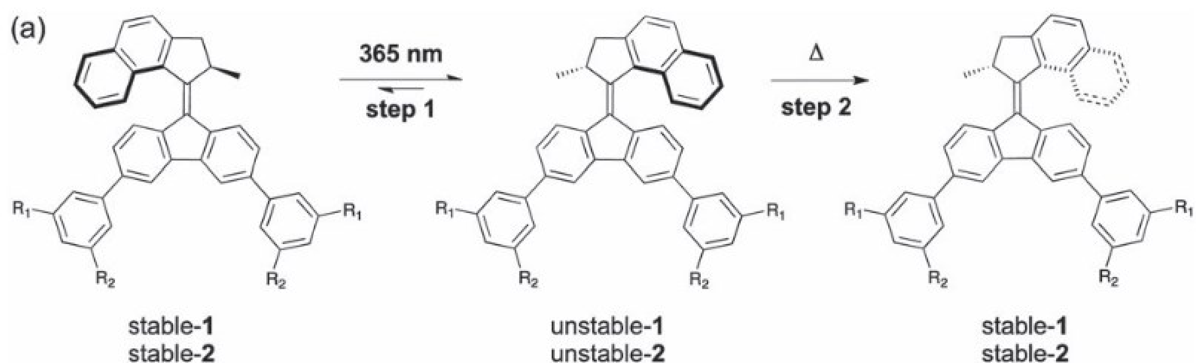

Figure SI1 – The mechanism of MM rotation around its double bond. The first step is light induced followed by thermal relaxation (step 2). This process is repeated to come back to the original molecular motor configuration. Image obtained from (1) J. Chen, K. Chen, G. T. Carroll and B. L. Feringa, *Chem. Commun.*, 2014, 12641–12644.

Supplementary Information 2

Leakage from MM2 Liposomes

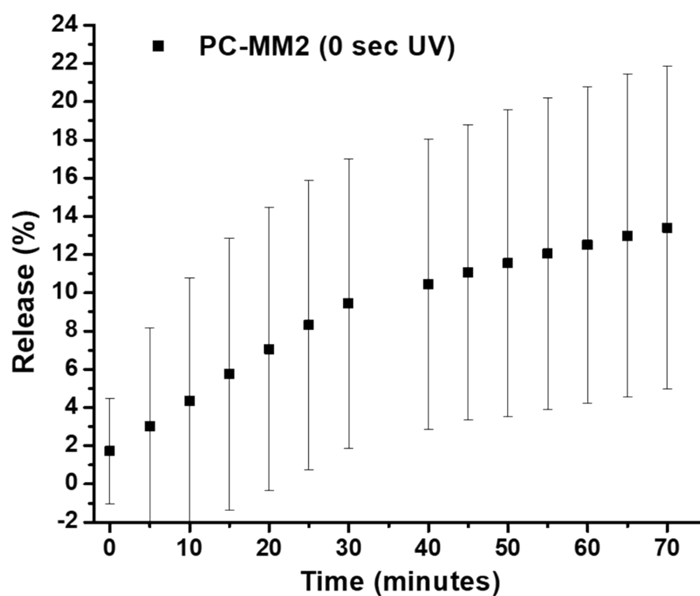

Figure SI2 – The leakage of calcein from (Δ9-cis)PC liposomes with a molecular motor : lipid ratio 2:25 (MM2), after purification with Sephadex® G100, without UV-irradiation. Leakage profile depicted as release (%) shows significant leakage from the liposomes without molecular motion, which indicates poor membrane integrity with higher motor loading within the lipid bilayer.

Supplementary Information 3

Quantified release of calcein at fixed time points

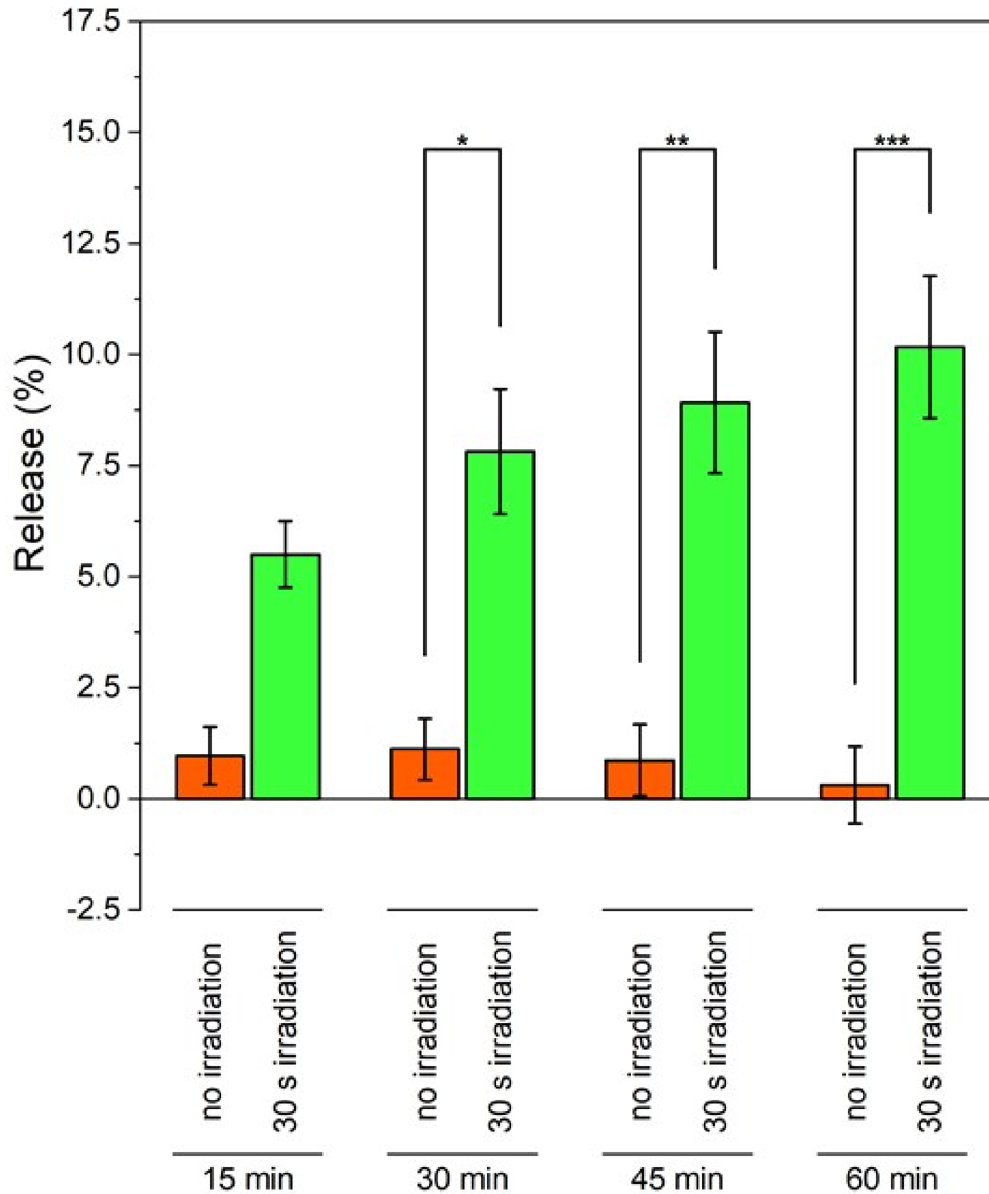

Figure SI3 - Liposomes without and with MM (1:50) with irradiation for 30 seconds of which the calcein release was studied at fixed time-points using fluorescence spectroscopy. Measurements are average ± SE of three independent experiments. Data was analyzed by analysis of variance (ANOVA) and Tukey's test. Significances are indicated with * for p-value < 0.05, ** for p-value < 0.01 and *** for p-value < 0.001.

Supplementary Information 4

Dynamic Light Scattering of irradiated liposomes and MM1 liposomes

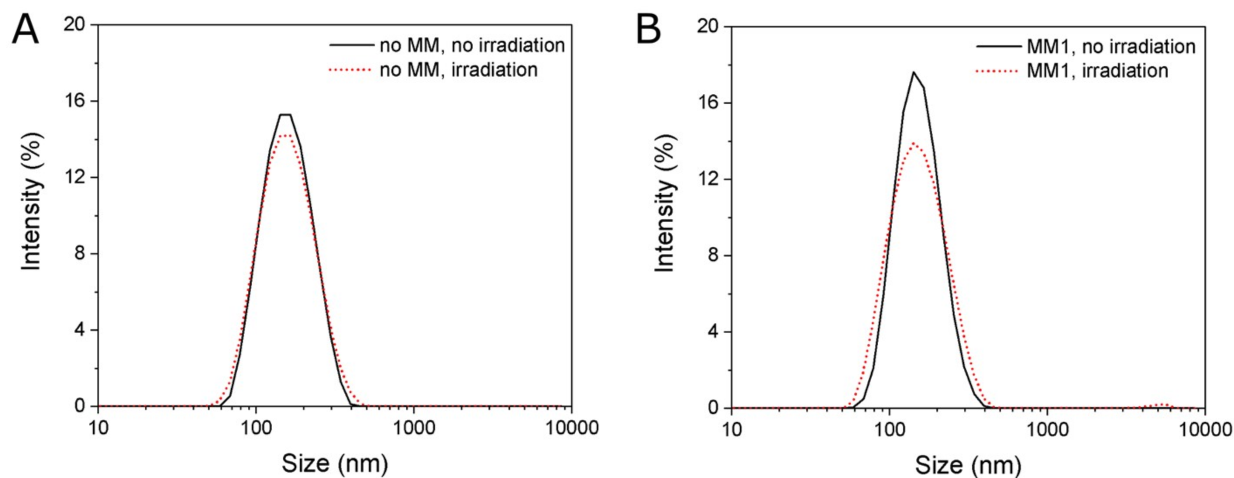

Figure SI4 - Dynamic Light Scattering of of (Δ9-cis)PC liposomes (A) without MM (no MM), and (B) with MM at mixing ratio 50:1 (MM1) after purification with Sephadex® G100. Size control was induced via extrusion through a polycarbonate filter (pore-size 100 nm) and the measurements were performed at 20°C. Samples measured before and after 30 minutes UV-irradiation showed no significant size alterations due to irradiation.