

1 ORIGINAL RESEARCH

2 Homsirikamol et al

3 **Inclusion of IR-820 into Soybean-Phosphatides-Based**
4 **Nanoparticles for Near-Infrared-Triggered Release and**
5 **Endolysosomal Escape in HaCaT Keratinocytes at**
6 **Insignificant Cytotoxic Level**

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Supplementary Tables

Table S1 Methods and specifications for the quantitative determination of the substances encapsulated in the nanoparticles

Substance	Technique	Wavelength (nm)	Disrupting agent
IR-820	UV-Vis-NIR spectroscopy	820	Methanol
TPC	UV-Vis-NIR spectroscopy	550	0.1% Triton X-100
BCIP	UV-Vis-NIR spectroscopy	292	1% Triton X-100
TRITC-Dex40	Fluorescence spectroscopy	Excitation/Emission = 551/582	1% Triton X-100

Abbreviations: TPC, total phenolic content; BCIP, 5-bromo-4-chloro-3-indolyl phosphate disodium salt; TRITC-Dex40, tetramethylrhodamine isothiocyanate-dextran (molecular weight = 40kDa); UV, ultraviolet; Vis, visible; NIR, near infrared.

Table S2 Near-infrared fluence corresponding to the irradiation time using an 830-nm LED array providing the irradiance at 0.0118 W/cm²

Irradiation time (minute)	Fluence (J/cm ²)
2.0	1.4
2.8	2.0
5.0	3.5
7.1	5.0
10.0	7.1
14.1	10.0
20.0	14.2

28.2	20.0
30.0	21.2
45.0	31.9
60.0	42.5

Abbreviation: LED, light-emitting-diode.

Table S3 Colocalization metrics and parameters

Metric	ImageJ plugin	Parameter
Van Steensel's cross-correlation coefficient (<i>CCF</i>)	JACoP v.2.0	Pixel shift (Δx) = ± 20 pixel
Threshold overlap score at the highest threshold (<i>TOS_h</i>)	EzColocalization	Threshold = 10% highest signal for both GFP and TRITC channels
Intensity correlation quotient (<i>ICQ</i>)	EzColocalization	-

Abbreviations: GFP, green fluorescent protein; TRITC, tetramethylrhodamine isothiocyanate.

Table S4 Aligned Ranks Transformation ANOVA for the *ICQ* and *TOS_h* ratios according to the nanoparticle formulation, the time after irradiation, and their interactions

Colocalization metric		Degree of freedom	Residual degree of freedom	<i>F</i>	<i>P</i>
<i>ICQ</i> _{(+)<i>irr</i>} / <i>ICQ</i> _{(-)<i>irr</i>}	Formulation	1	18	0.79688	0.3838
	Time after irradiation	2	18	6.32207	0.0083

	Formulation × Time after irradiation	2	18	3.37523	0.0569
$TOS_{h,(+)irr} /$ $TOS_{h,(-)irr}$	Formulation	1	18	2.9403	0.1036
	Time after irradiation	2	18	14.3680	0.0002
	Formulation × Time after irradiation	2	18	10.9500	0.0008

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50 **Note:** Bolded numbers indicate statistically significant differences with $P < 0.05$.

51 **Abbreviations:** ANOVA, analysis of variance; ICQ , intensity correlation quotient; TOS_h , threshold
52 overlap score at the highest threshold; irr , irradiation.

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55 **Table S5** Tukey post-hoc test for the ICQ and TOS_h ratios on the statistically significant main

56 effect and the interactions between the nanoparticle formulation and the time after irradiation

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Colocalization metric	$ICQ_{(+)irr} / ICQ_{(-)irr}$	$TOS_{h,(+)irr} / TOS_{h,(-)irr}$
	P	P
<i>Main effect: time after irradiation</i>		
0 hr – 2 hr	0.0123	0.0018
0 hr – 4 hr	0.9429	0.6381
2 hr – 4 hr	0.0245	0.0002
<i>Interactions</i>		
TRITC-Dex40-IR-NP × 0 hr – TRITC-Dex40-IR-NP × 2 hr	-	0.0654
TRITC-Dex40-IR-NP × 0 hr – TRITC-Dex40-IR-NP × 4 hr	-	0.9841
TRITC-Dex40-IR-NP × 2 hr – TRITC-Dex40-IR-NP × 4 hr	-	0.2149
TRITC-Dex40-IR-NP × 0 hr – TRITC-Dex40-NP × 0 hr	-	0.1723
TRITC-Dex40-IR-NP × 0 hr – TRITC-Dex40-NP × 2 hr	-	0.9515

TRITC-Dex40-IR-NP × 0 hr – TRITC-Dex40-NP × 4 hr	-	0.7334
TRITC-Dex40-IR-NP × 2 hr – TRITC-Dex40-NP × 0 hr	-	0.9942
TRITC-Dex40-IR-NP × 2 hr – TRITC-Dex40-NP × 2 hr	-	0.0114
TRITC-Dex40-IR-NP × 2 hr – TRITC-Dex40-NP × 4 hr	-	0.5772
TRITC-Dex40-IR-NP × 4 hr – TRITC-Dex40-NP × 0 hr	-	0.4608
TRITC-Dex40-IR-NP × 4 hr – TRITC-Dex40-NP × 2 hr	-	0.6566
TRITC-Dex40-IR-NP × 4 hr – TRITC-Dex40-NP × 4 hr	-	0.9759
TRITC-Dex40-NP × 0 hr – TRITC-Dex40-NP × 2 hr	-	0.0340
TRITC-Dex40-NP × 0 hr – TRITC-Dex40-NP × 4 hr	-	0.8653
TRITC-Dex40-NP × 2 hr – TRITC-Dex40-NP × 4 hr	-	0.2651

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59 **Note:** Bolded numbers indicate statistically significant differences with $P < 0.05$.

60 **Abbreviations:** *ICQ*, intensity correlation quotient; *TOS_h*, threshold overlap score at the highest

61 threshold; *irr*, irradiation; TRITC-Dex40, tetramethylrhodamine isothiocyanate-dextran (molecular

62 weight = 40kDa); IR, IR-820.

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Supplementary Figures

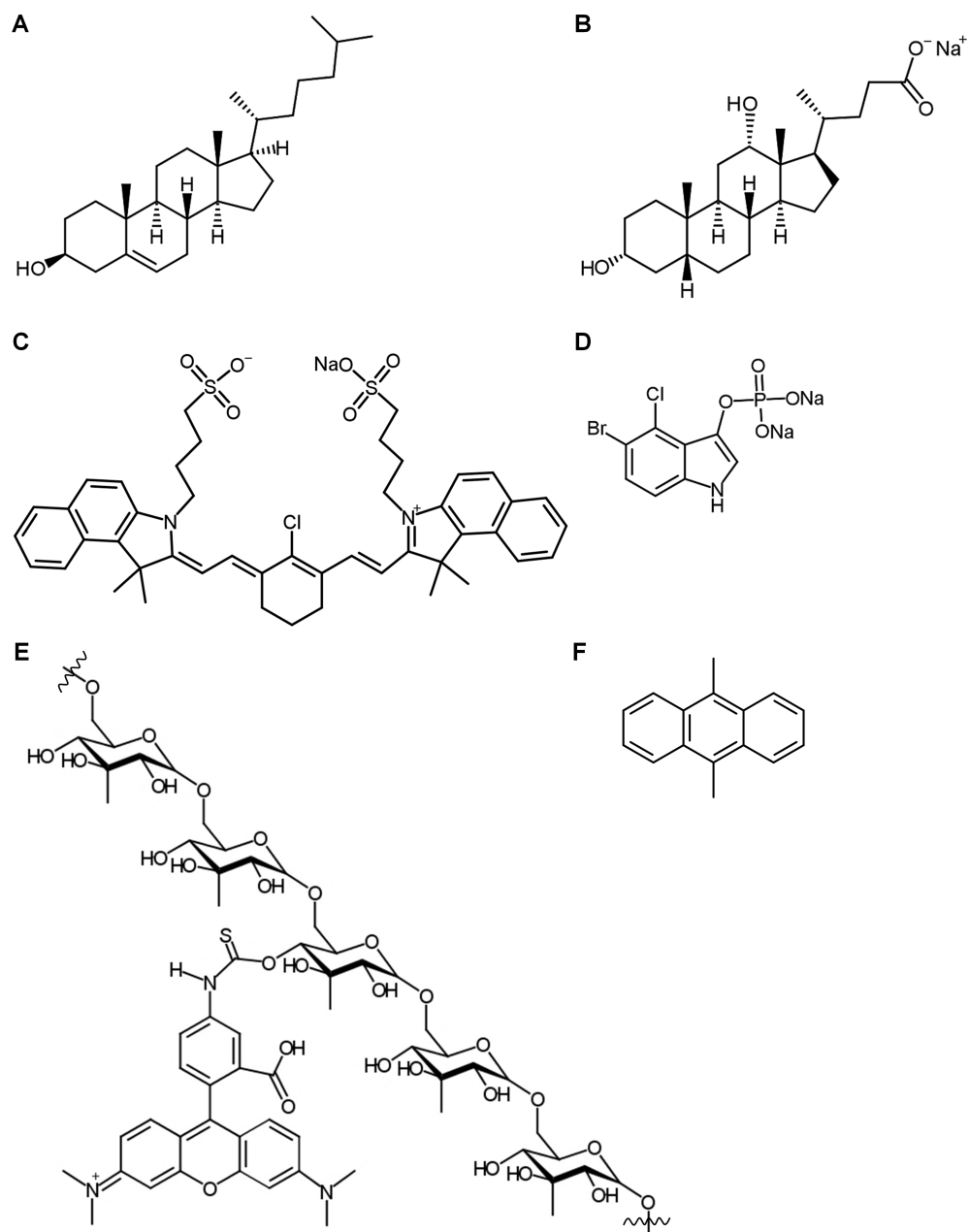
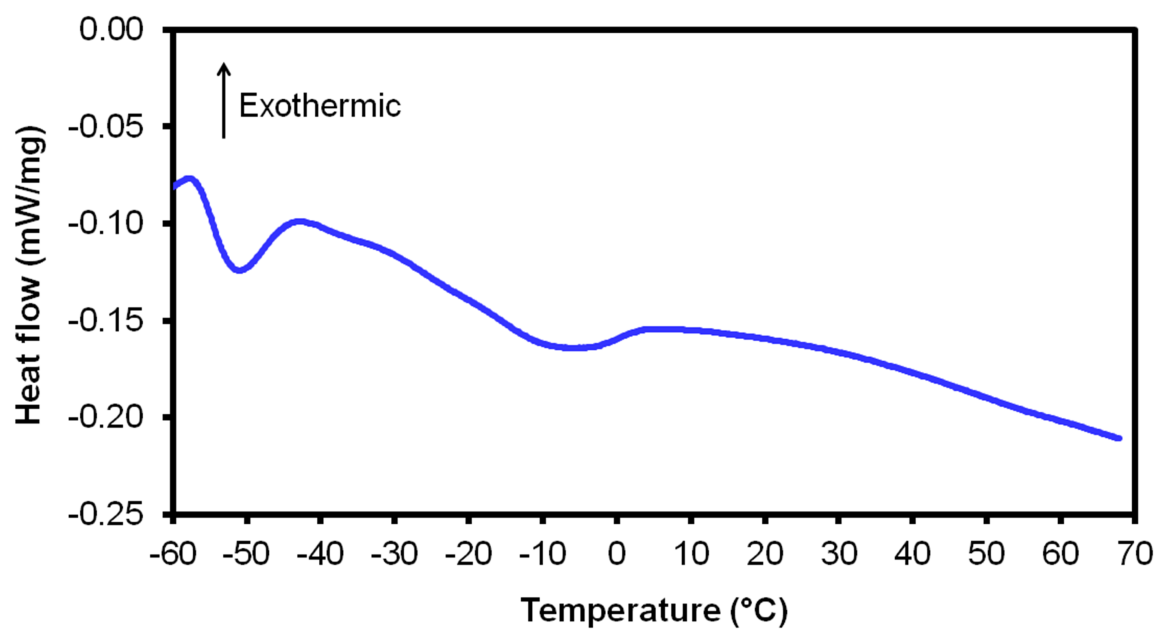


Figure S1 Chemical structures of **(A)** cholesterol, **(B)** sodium deoxycholate, **(C)** IR-820, **(D)** BCIP, **(E)** TRITC-Dex40, and **(F)** DMA.

Abbreviations: BCIP, 5-bromo-4-chloro-3-indolyl phosphate disodium salt; TRITC-Dex40, tetramethylrhodamine isothiocyanate-dextran (molecular weight = 40kDa); DMA, 9,10-dimethylantracene.

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Figure S2 DSC thermogram of the NP nanoparticles.

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Abbreviation: DSC, differential scanning calorimetry.

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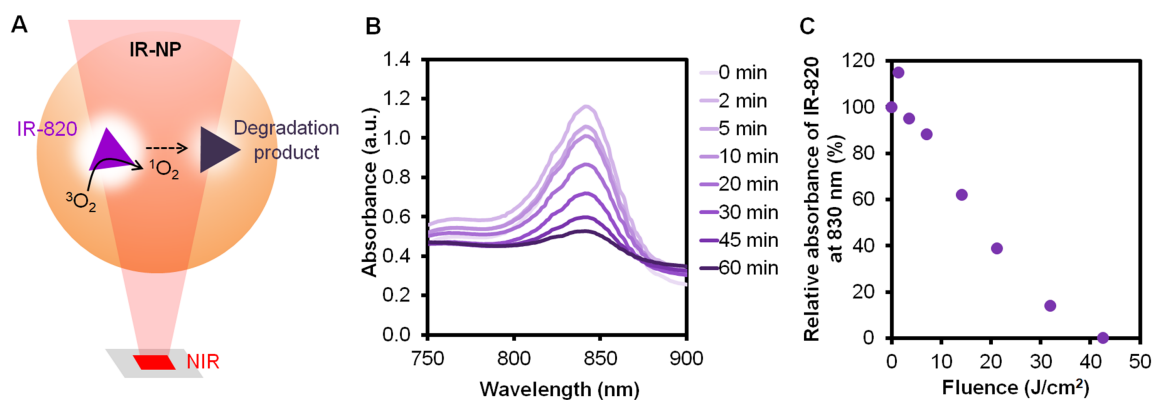


Figure S3 (A) Interpretive illustration of the possible event resulting in **(B)** absorption spectra of the IR-NP irradiated with 830-nm NIR at $0.0118 \text{ W}/\text{cm}^2$ at different time points, and **(C)** relative absorbance percentages of IR-820 in IR-NP at 830 nm corresponding to NIR fluence.

Abbreviations: IR, IR-820; $^3\text{O}_2$, triplet oxygen; $^1\text{O}_2$, singlet oxygen; NIR, near infrared.

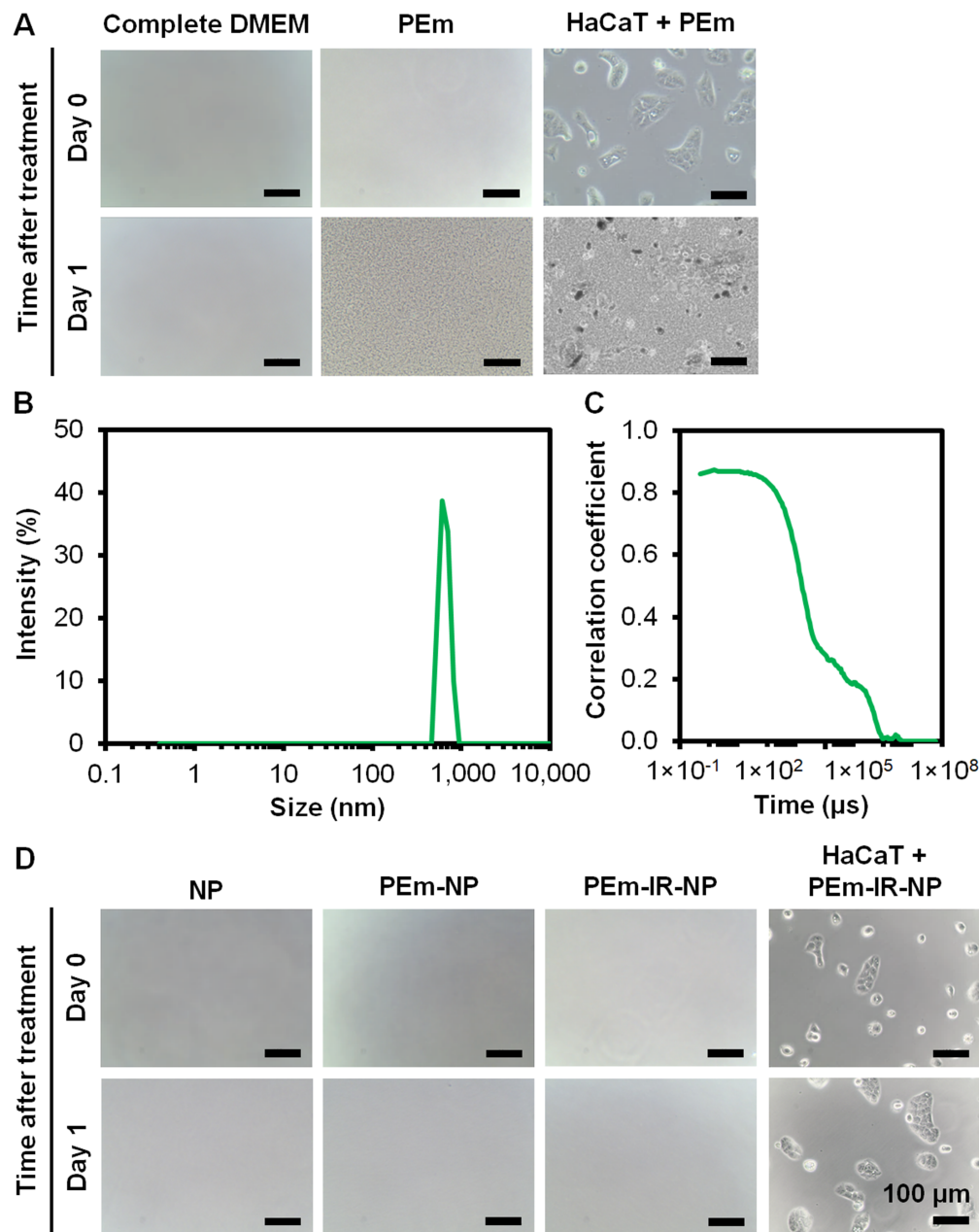
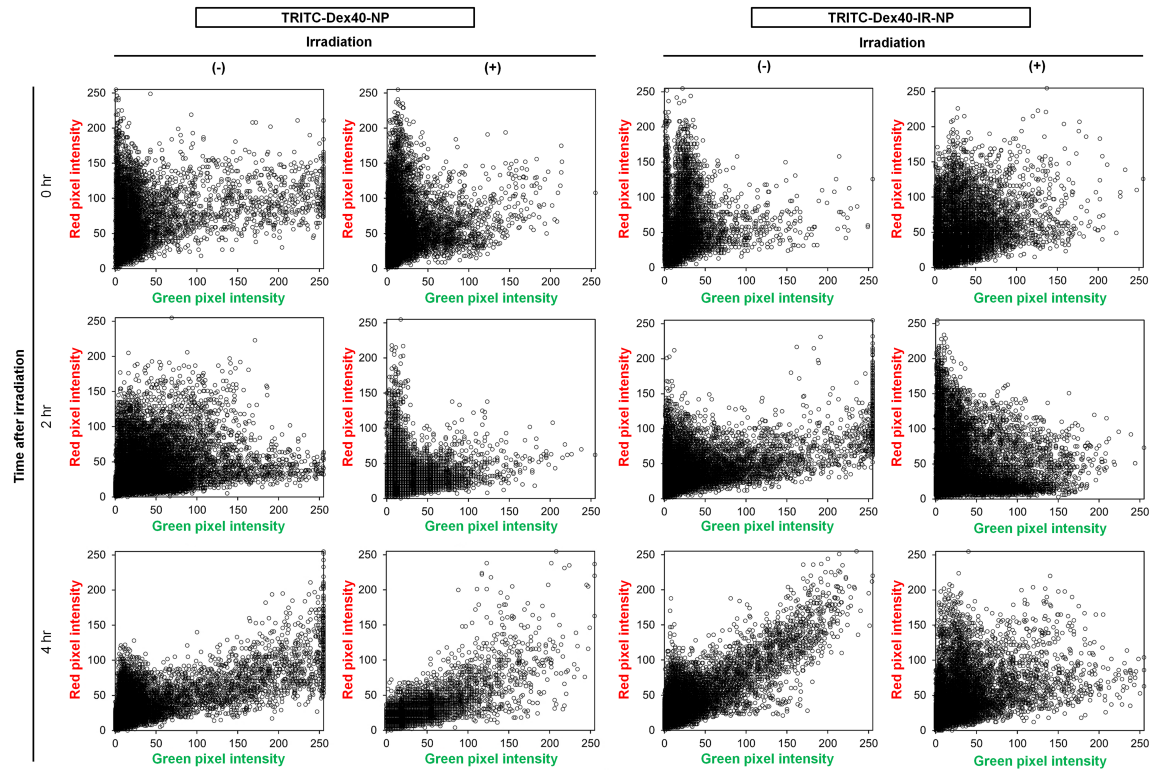


Figure S4 (A) Phase-contrast images of complete DMEM as a blank, PEm, and PEm-treated HaCaT keratinocytes on Day 0 and Day 1 post treatment, (B) intensity-weighted hydrodynamic size distribution of PEm dispersed in PBS (pH 7.4), (C) corresponding correlogram, and (D) the absence of precipitates in complete DMEM with NP, PEm-NP, PEm-IR-NP, and the HaCaT cells treated with PEm-IR-NP; Scale bar = 100 μ m.

Abbreviations: DMEM, Dulbecco's modified Eagle medium; PEm, *Phyllanthus emblica* L. fruit extract; PBS, phosphate-buffered saline; IR, IR-820.



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97 **Figure S5** Scatterplots of the red (TRITC-Dex40) and green (LAMP1-GFP) pixel intensities of
 98 deconvoluted fluorescence images of the HaCaT keratinocytes at 0, 2, and 4 hours after the
 99 treatment with TRITC-Dex40-NP and TRITC-Dex40-IR-NP for six hours with and without 830-nm
 100 irradiation at fluence of 20 J/cm².

101 **Abbreviations:** TRITC-Dex40, tetramethylrhodamine isothiocyanate-dextran (molecular weight =
 102 40kDa); LAMP1, lysosomal-associated membrane protein 1; GFP, green fluorescent protein; IR,
 103 IR-820.