Highly efficient gene release in spatiotemporal precision approached by light and pH dual responsive copolymers

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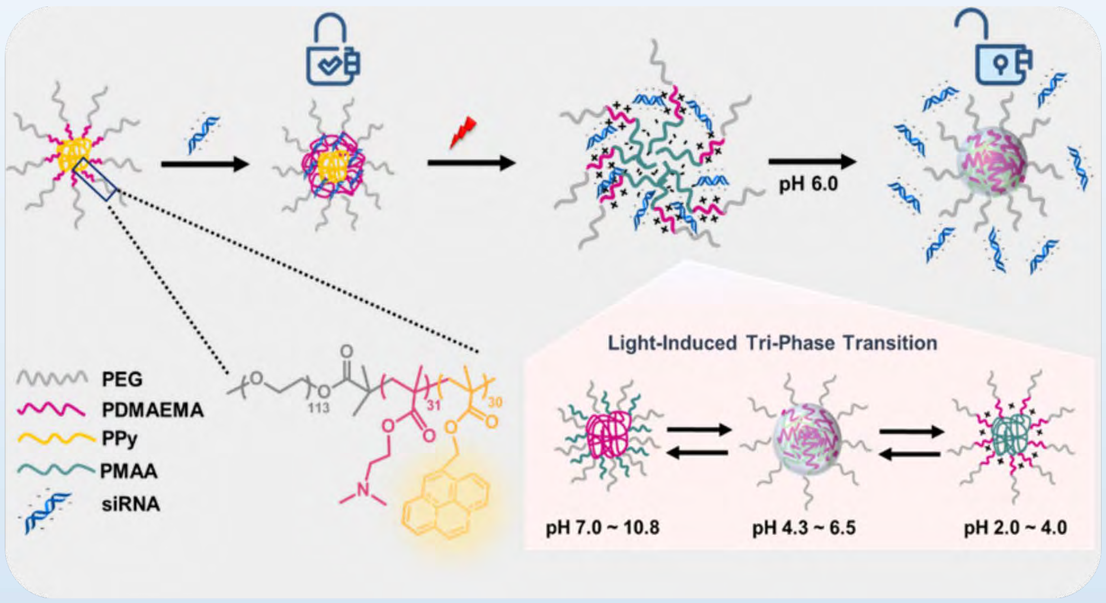
Introduction

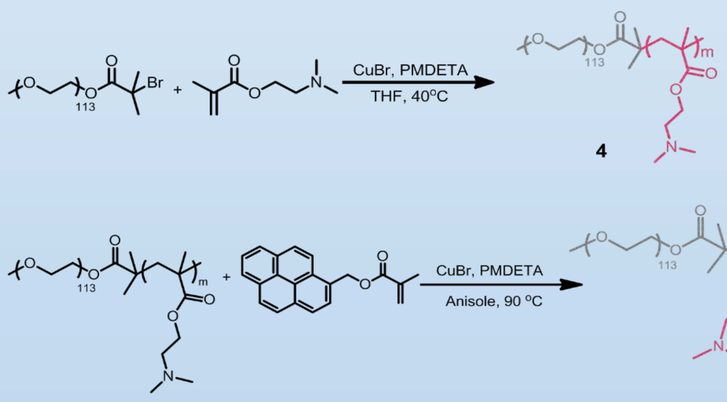
The triblock copolymer of PDMAEMA-b-PPy has been designed as the siRNA carrier, which showed both a high stability in physiological condition and an efficient gene release in mimetic cancer environment via the transformation from PPy to poly(methacrylic acid) (PMAA) after light irradiation.

The substantial increase in the releasing rate before and after the photo-triggering treatment indicated that the triblock copolymer designed in this study is an ideal candidate for gene therapy and could be explained by the joint effects of photo-triggered structural transformation from PPy to PMAA and tri-phase transition under different pH environment (**Scheme 1**).

Scheme 1 Illustration of photoresponsive micelles binding to and releasing siRNA



The synthetic process of PEG_{113}-*b*-$PDMAEMA_{31}$-*b*-PPy_{30}



Scheme 1. Preparation of PEG_{113}-*b*-$PDMAEMA_{31}$ **4** and PEG_{113}-*b*-$PDMAEMA_{31}$-*b*-PPy_{30} **5** via ATRP.

Table 1. Characterization of **4** and **5** copolymers.

Entry[a]	$M_{n,\ ^bNMR}$ (g mol^{-1})	$Đ$	Size[e]
PEG_{113}-Br	5,000	1.03	--
PEG_{113}-*b*-$PDMAEMA_{31}$	9,900	1.27[c]	--
PEG_{113}-*b*-$PDMAEMA_{31}$-*b*-PPy_{30}	18,900	1.38[d]	83

[a]Degree of polymerization calculated by ^{1}H-NMR
[b]Molecular weight determined by ^{1}H-NMR
[c]Polydipersity measured by GPC using DMF/LiBr as eluent
[d]Polydipersity measured by GPC using THF as eluent
[e]Particle size distribution measured by Dynamic Laser Scattering (DLS, Malvern Nano S), PDI= $(\sigma/d)^2$ whereas, σ is standard deviation, d is measured diameter. DLS analysis of micelles (50 μg/mL) were measured in triplets.

Self-assembling of triblock copolymer

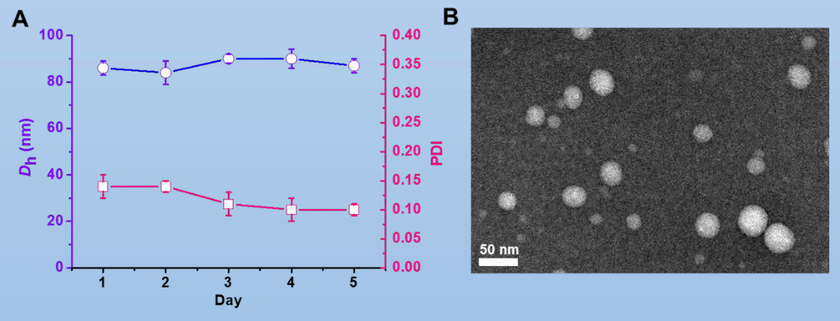


Fig. 1 Self-assembling of **5** triblock copolymer. (A) Time-dependent hydrodynamic diameter (D_h) and particle dispersity (PDI) of **5** self-assemblies (50 μg/mL) measured by dynamic light scattering (DLS) in distilled water. (B) Transmission electron microscope (TEM) image of **5** self-assemblies solution (0.5 mg/mL) casted on carbon/Formvar-coated copper TEM grid and negatively stained by 1% phosphotungstic acid (PTA)

Time-dependent fluorescence spectra

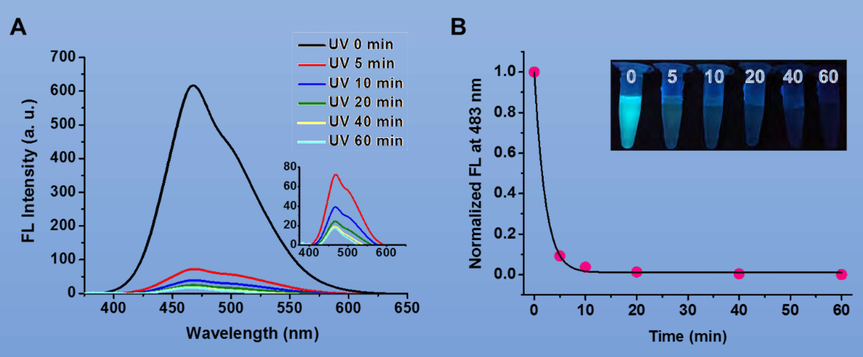


Fig. 2 **5** in distilled water upon UV irradiation (λ = 365 nm). (A) Time-dependent fluorescence spectra of (5) . (B) Normalized time-dependent fluorescent intensity at 468 nm of **5**. Inset: visual expression of blue fluorescence decreasing upon UV irradiation.

siRNA encapsulation and release

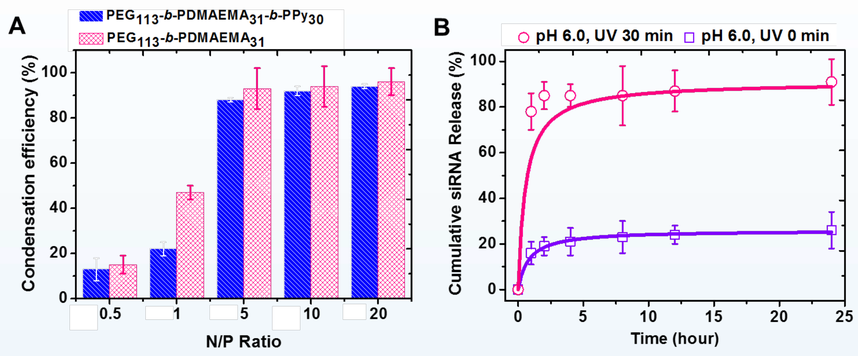


Fig. 3 (A) siRNA condensation efficiency of **4** and **5** micelles determined by ethidium bromide assay at various N/P ratios. (B) Cumulative siRNA release of micelleplexes (N/P = 5) at pH 6.0 with and without light irradiation.

Tri-Phase Transition of PEG_{113}-*b*-$PDMAEMA_{31}$-*b*-PPy_{30} Micelles

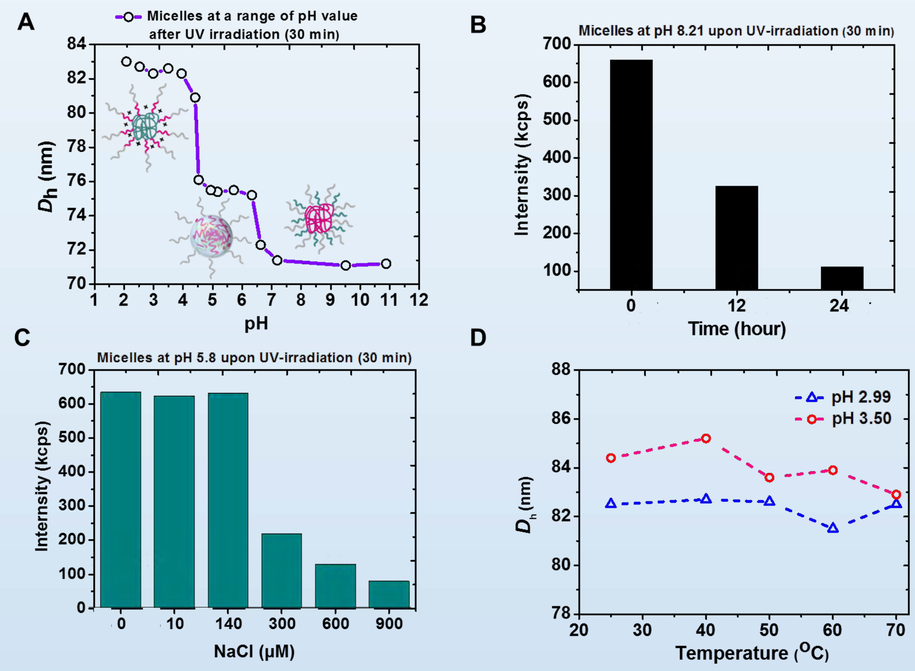


Fig. 4 Characterization of PEG_{113}-*b*-$PDMAEMA_{31}$-*b*-$PMAA_{30}$ (**6**) particles generated from **6** via UV irradiation (30 minutes) in aqueous solution. (A) The change of LIAHD of **6** under varied pH environment. (B) The decreasing of LSI** of **6** at pH 8.21 with time. (C) Variation of LSI** of **6** with various ionic strengths at pH 5.8. (D) Temperature effect to the size of **6** particle at pH 2.99 (red line) and pH 3.50 (blue line).

*Light-intensity-average hydrodynamic diameter (LIAHD) ; ** Light-scattering intensity (LSI)

The cytotoxicity of material

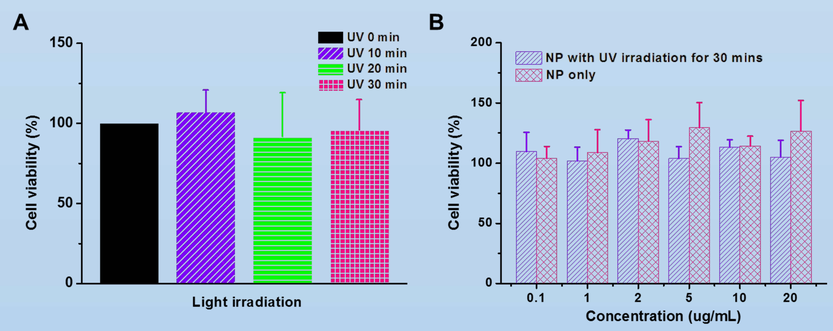


Fig. 5 (A) Cell viability of MDA-MB-231 cancer cells upon UV irradiation for 10, 20, and 30 minutes. (B) Material cytotoxicity of micelles with or without UV irradiation for 30 minutes and further incubation for 24 hours evaluated by cell viability of MDA-MB-231 cancer cells using MTT assay.

Conclusions

The siRNA release was triggered by UV irradiation that convert PPy to PMAA. The formation of PMAA segment at pH 4.3 to 6.5 not only removed the hydrophobic core but also competed for the protonated amine groups with siRNA using the anionic carboxylic groups to push out the siRNA and caused an ultrahigh release efficiency of 91%.

The triblock copolymer self-assembled micelles have shown the superior biocompatibility and been verified their cytotoxic concern to detached pyrenemethanol molecules at an effective concentration for further in vitro and in vivo transfection.

PEG_{113}-*b*-$PDMAEMA_{31}$-*b*-PPy_{30} which is photo- and pH-responsive material, has thus been demonstrated as an ideal siRNA carrier having not only the remarkable siRNA condensation with high stability but also an efficient siRNA release capability.

Reference

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Acknowledgements

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