A novel unit poly-ion complex-type siRNA delivery platform which utilizes inherent neomycin-B-RNA binding

West Kristian Paraiso[1,2], Sabina Quader[2], Kotaro Hayashi[2], Kazuko Toh[2], Shigeto Fukushima[2], Kazunori Kataoka[2]

[1] Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universitat Internacional de Catalunya, Barcelona (Spain)

[2] Innovation Center of Nanomedicine, Kawasaki Institute of Industrial Promotion, Kawasaki (Japan)

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BACKGROUND

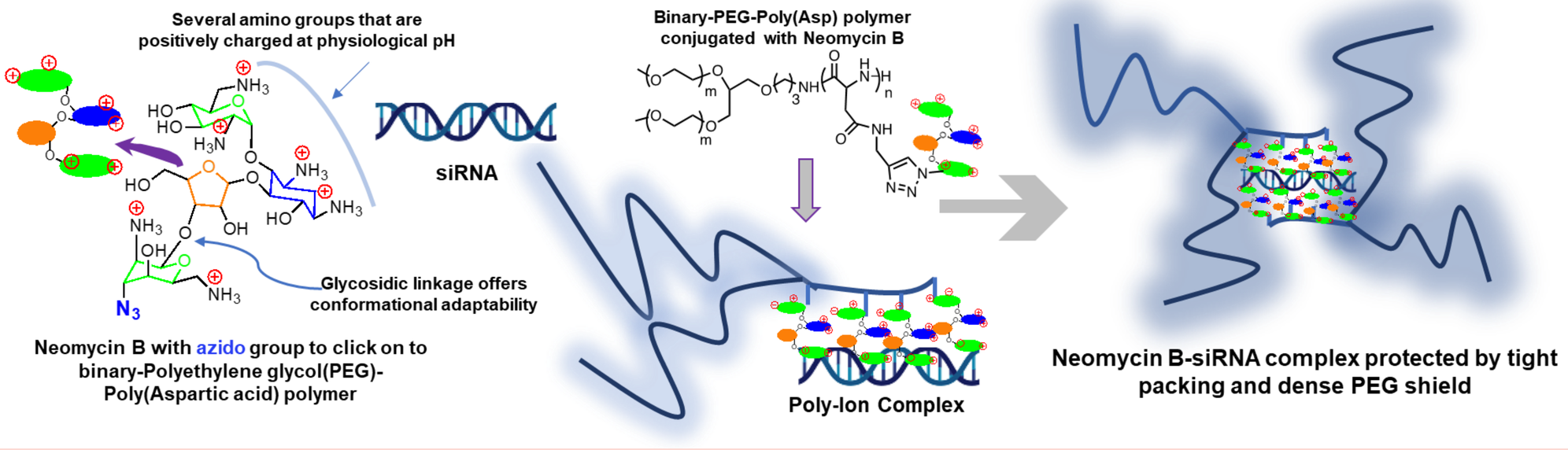
- **Aminoglycosides (AGs)** are antimicrobial oligosaccharides made up of two or more amino sugars glycosidically-linked by to a central aminocyclitol such as 2-deoxystreptamine (1).
- **Neomycin B** binds to RNA through electrostatic and H-bonding between negatively charged phosphate backbone of RNA and positively charged amino groups.
- The six amino groups plus the inherent conformational adaptability through the glycosidic bond permit **optimum structural adjustment** to bind with diverse RNA targets effectively (2).

STRATEGY

- Cellular delivery of therapeutic RNA pose significant challenges, mainly due to their inherently instability to nuclease degradation and dense anionic nature.
- Stabilization of small nucleic acids such as **siRNA** was demonstrated by using a **Y-shaped PEG-block catiomer** in which the number of positive charges is adjusted to match the number of negative charges in each nucleic acid strand, yielding a **unit polyion complex (uPIC)** that is stable in the bloodstream and can penetrate tumors (3).
- Using polycationic segments **with inherent and unique RNA binding abilities** is strategic in effectively complexing and delivering siRNA to the target site.

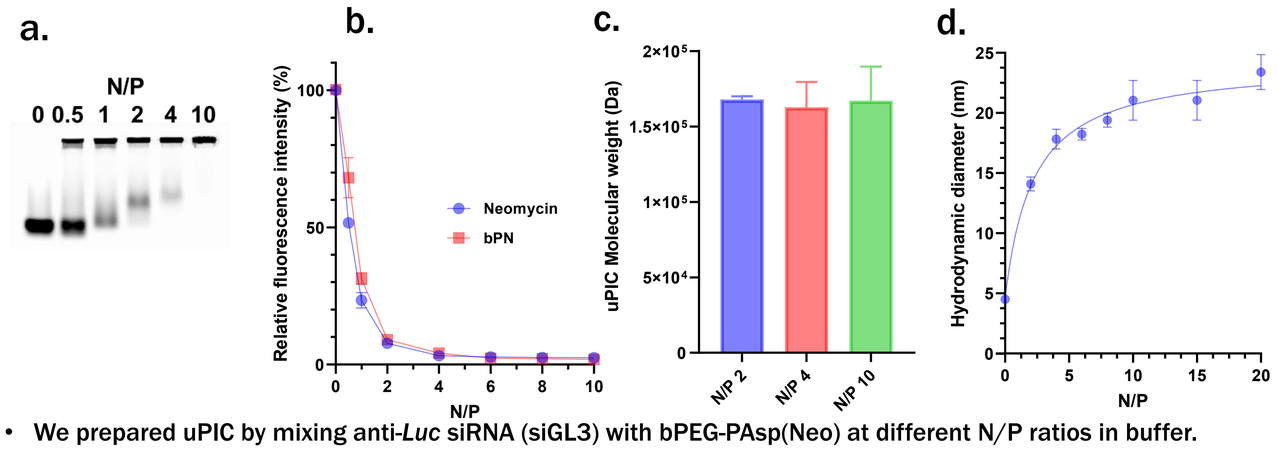
PREPARATION OF UNIT POLY-ION COMPLEXES

Azide-functionalized neomycin B was prepared through Quader et al, 2007 (4). This newly introduced azide function was then used to click with the base polymer, a Y-shaped binary-PEG-block-poly(β-benzyl-L-aspartate) (bPEG-pBLA), (MW_{PEG} = 78k, DP = 6); We introduced an alkyne functionality to the polymer by aminolysis with propargylamine, then we conjugated azido neomycin B (3-4 units) to the polymer using copper-catalyzed click reaction. Amine deprotection using TFA afforded bPEG-pAsp(Neo), **herein bPN.**



CHARACTERIZATION AND EVALUATION IN BIOLOGICAL SYSTEMS

Figure 1: Physicochemical properties of uPICs



- We prepared uPIC by mixing anti-*Luc* siRNA (siGL3) with bPEG-PAsp(Neo) at different N/P ratios in buffer.
- Retardation of siGL3 electrophoretic migration in agarose gel (1a) confirmed its complexation with bPN while EtBr displacement assay demonstrated that Neo units in bPN have retained their inherent RNA-binding ability (1b).
- Through analytical centrifugation, MW_{ave} of uPIC was found to be around **166,000** (1c). This approximates the MW_{calc} of 176,000 for a 2:1 polymer/siRNA complex between bPN (MW = 81,000) and siGL3 (MW = 14,000).
- Fluorescence correlation spectroscopy of AF647-labeled uPICs revealed the hydrodynamic diameter (D_H) **20 nm** (calculated using the Stokes-Einstein equation) (1d). The two-armed PEG prevents further secondary association through steric hindrance

Figure 2: *In vitro* gene silencing

- uPICs prepared from bPN (N/P 4) decreased Luc expression to one-third in HeLa-Luc cells (2a), most notable among other treatments
- Cell viability assays confirmed that treatment did not drastically cause toxicity even after 48 h incubation (2b).

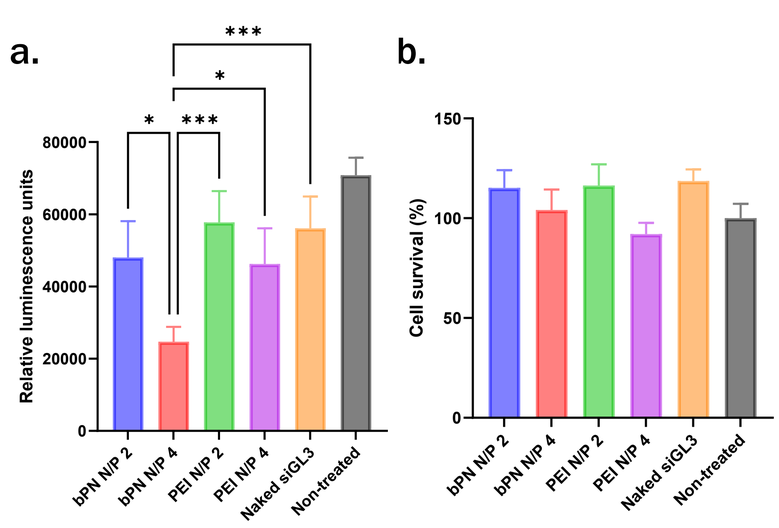
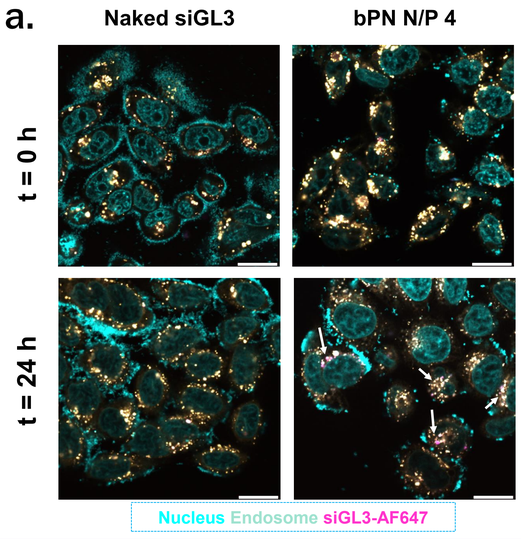
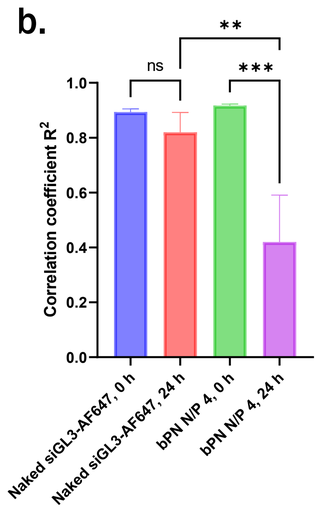


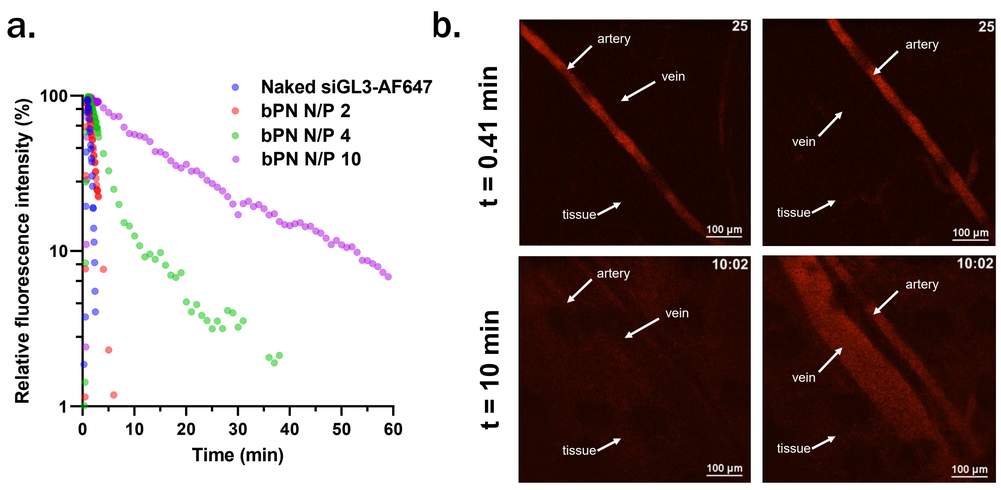
Figure 3: Endosomal escape





- bPN has amino groups whose pK_as match that of low endosomal pH, which means it has high potential for endosomal escape.
- We observed intracellular distribution of siGL3-AF647 and quantitatively evaluated its colocalization ratio with LysoTracker Red signals using Pearson's R correlation.
- Colocalization decreased after 24 h for bPN-treated cells as compared to those treated with naked siGL3-AF647 (3a-b).

Figure 4: uPIC stability *in vivo*



- Fluorescent uPICs were injected into the tail vein of BALB/c mice while the fluorescence intensity from the ear vein was followed using intravital microscopy.
- Stability in the blood is enhanced with increasing N/P ratio, judged by the sustained fluorescence intensity (4a-b).
- $T_{1/2}$ of siGL3-AF647 is increased by almost 7-fold when N/P 10 is used, suggesting that an excess of blood polymer concentration is advantageous in increasing its *in vivo* longevity.

CONCLUSION

We developed a new uPIC platform that utilizes Neo as a natural cationic siRNA captor. This Neo-siRNA uPIC system has an ideal size, excellent RNA binding and complexation, and effective gene silencing ability due to endosomal escape. It is expected to be effective in vivo, as evidenced by the stability in blood circulation and has the potential to be diversified to other genetic payloads from siRNA to antisense oligonucleotides (ASOs), messenger RNA (mRNA), and aptamers with appropriate modification of the base bPN polymer.

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