**"MOBILE GalNAc" Boosts Drug Targeting for the Antibody Chimera and the Genome-Editing Molecule**

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**Background and aims.** *N*-Acetylgalactosamine (GalNAc) is a widely used ligand for liver-targeted drug delivery due to its affinity for the asialoglycoprotein receptor (ASGPR), which is highly expressed on hepatocytes. Trivalent GalNAc (*tri*GalNAc), designed to match the ASGPR trimer via a forked linker, has been validated as a platform technology with six approved oligonucleotide drugs.1) However, its synthesis is technically challenging and costly, and recent studies suggest that interaction with multiple ASGPR oligomers may be more effective than a trivalent binding.2)

Polyrotaxane (PRX), a supramolecular polymer with cyclic molecules that can move along a linear axle molecule, offers dynamic mobility.3, 4) In this study, we hypothesized that PRX modified with monovalent GalNAc (*mono*GalNAc) could adaptively cluster in response to ASGPR distribution, thereby facilitating enhanced multivalent interactions (**Figure 1**).

**Results and discussion.** *mono*GalNAc- and *tri*GalNAc-modified PRXs were synthesized along with immobile dextran-based controls (*mono*GalNAc-DEX, *tri*GalNAc-DEX). *tri*GalNAc-DEX showed higher hepatocyte uptake than *mono*GalNAc-DEX, supporting current *tri*GalNAc designs. Importantly, *mono*GalNAc-PRX and *tri*GalNAc-PRX showed comparable uptake and ASGPR binding, and both outperformed *tri*GalNAc-DEX by 4- to 5-fold, suggesting that PRX mobility enables dynamic clustering of *mono*GalNAc to mimic trivalent binding and efficient multivalent interaction (**Figure 1**).

The lysosome-targeting antibody chimaera (LYTAC)-conjugated *mono*GalNAc-PRX demonstrated superior delivery and pharmacological efficacy *in vitro* and *in vivo* compared to conventional *tri*GalNAc-LYTAC. In addition, genome-editing nanoparticles functionalized with *mono*GalNAc-PRX exhibited improved hepatic accumulation and editing efficiency.

**Conclusion.** In summary, *mono*GalNAc-PRX, namely "MOBILE GalNAc", offers a promising alternative to conventional *tri*GalNAc systems. By exploiting the inherent molecular mobility, "MOBILE GalNAc" offers a sophisticated yet facile strategy to enhance drug targeting, with the potential to improve therapeutic efficacy and broaden the applicability of liver-targeted drug delivery platforms.



**Figure 1.** Schematic image of *mono*GalNAc-modified PRX (*mono*GalNAc-PRX).

**References:**

(1) J.K. Nair, *et al*., ***J. Am. Chem. Soc.***, 136, 16958-16961 (2014).

(2) K. Schmidt *et al.*, ***Nucleic Acid. Res.***, 45, 2294-2306 (2017).

(3) A. Harada *et al.*, ***Nature***, 356, 325-327 (1992).

(4) Y. Yasuda *et al.*, ***J. Am. Chem. Soc.***, 141, 9655-9663 (2019).