Phosphodiester-backboned Molecular Brushes as Aptamer Enhancers *in Vivo*

Yuyan Wang[1], Dali Wang[1], Jiachen Lin[1], Zidi Lyu[1], Peiru Chen[1], Tingyu Sun[1], Mehrnaz Mojtabavi[2], Armin Vedadghavami[2], Sijia Dong[1], Lei Zhang[1], Christopher Park[1], and Ke Zhang[1]*

1. Department of Chemistry and Chemical Biology, Northeastern University, Boston, Massachusetts 02115, USA.
2. Department of Bioengineering, Northeastern University, Boston, Massachusetts 02115, USA

ABSTRACT: Aptamers face challenges for use outside the ideal conditions in which they are developed. These difficulties are most palpable for *in vivo* applications due to nuclease activities, rapid clearance, and off-target binding. Herein, we demonstrate that a biohybrid of a thrombin-binding aptamer and a phosphodiester-backboned bottlebrush polymer can reduce nuclease digestion while retaining the target binding affinity of the aptamer. Importantly, these biohybrids suppress non-specific cellular uptake, enable long blood circulation times, and successfully rescue the bioactivity of the aptamer *in vivo*, producing a potent anticoagulation effect. The bottlebrush polymer is assembled via solid-phase synthesis, which also constructs the aptamer portion of the conjugate, followed by derivatization with poly(ethylene glycol) (PEG) side chains via graft-onto methodology using a two-step process with near quantitative efficiency (90%-100%). The synthesis allows for precise control over the size and molecular architecture of the bottlebrush polymer. Consisting entirely of building blocks that are generally recognized as safe for therapeutic purposes, this novel bottlebrush polymer system is expected to provide a highly translatable route for aptamer-based therapeutics.