Multifunctional Poly(Methacrylate) Polyplexe Libraries: A Platform for Gene Delivery Inspired by Nature
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Purpose
We describe the generation and testing of a collection of poly(methacrylate) copolymers that include different combinations of functionalities inspired by natural amino acid side chains known to exert specific roles in the biological activity of peptides in oligonucleotide delivery. Next to functionalities for the complexation of oligonucleotides, these include imidazoles for induction of endosomal release, PEGs for control of hydophilicity and a cell-penetrating peptide to enhance cellular uptake.

Methods
The polymers were synthesized by RAFT polymerization, that enables the incorporation of this diverse set of functionalities without the need for side chain protection.
Polymers were tested with a newly established workflow that starts with assays that can be conducted in high throughput, yet have high predictive power for in vivo biocompatibility. Once the lack of hemolytic activity and toxicity are established, more labour and reagent-extensive assays on biological activity are conducted (Scheme 1). This assay represents a reversal of current testing paradigms that typically start with biological activity.

Results
The inclusion of side chains in the methacrylate polymer backbone that facilitate the import of the polyplexes and mediate their endosomal release resulted in transfection efficiencies in the presence of serum and a toxicological profile exceeding the ones of PEI.
For the polyplexes that showed significant activity we investigated in detail intracellular trafficking and heparan sulfate dependence of uptake. Importantly, we show that those polyplexes that are sensitive to decomplexation by heparan sulfates in solution, show higher transfection efficiency after removal of heparan sulfates from the cell surface. This result demonstrates that in contrast to the current paradigm heparan sulfates do not necessarily promote polyplex uptake. Moreover, time-lapse analyses revealed a massive dissipation of endosomal structures, followed by a slow release of material into the cytoplasm. This data reveals that for polyplexes more diverse mechanisms than a recently proposed endosomal burst mechanism exist.

Conclusion
Our study sheds new light onto the interdependence of polymer characteristics and drug delivery capacity, establishes a new workflow for testing, and provides novel insights into the mechanism of cellular uptake and intracellular trafficking of polyplexes.

![Scheme 1](image)

Scheme 1: Workflow for the identification of biologically active polymers from large polymer collections.