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| **Tracking Intracellular Transport of Self-Reporting Nanomaterials** |
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| **Background:**  Oligonucleotide therapies have the potential to selectively treat a range of life-limiting disorders by modulating expression of disease associated pathways. They operate exclusively in the cytosol, but their hydrophilicity and negative charge limit their translocation across cell membranes, requiring them to be packaged in delivery vectors. Cationic polymers show potential as vectors, but existing strategies do not possess the synthetic tuneability to meet therapeutic needs such as organ- and cell-specific targeting.  In this work, we exploited the Passerini 3-component reaction (P3CR) to synthesise polymers with far greater functionality than possessed by conventional polycation vectors. The P3CR is a high yielding and versatile multicomponent reaction that combines a carboxylic acid with an aldehyde and an isocyanide to produce an α-acyloxy amide in an efficient, single step, one-pot reaction. By incorporating bifunctional monomers into the reaction, it can progress as a rapid, step growth polymerisation technique. |
| **Methods:**  By combining dialdehyde, dicarboxylic acid and isocyanide monomers, we synthesised a range of amine containing polymers, with an ester backbone and alkene or alkyne side chains. Utilising high yielding azide click and thiol/ene reactions allowed us to rapidly functionalise the resultant polymers, to produce a broad polymer library.  Complexing the polymers with Poly(A) via pipetting in acidic buffer at w/w ratios ranging from 512 to 1 produced polyplex nanoparticles that we characterised with DLS and TEM. We then studied the transfection capabilities of selected nanoparticles against HEK293T cells by both luciferase assays and fluorescent imaging of Cy5 labelled particles. |
| **Results:**  We synthesised and characterised a core set of ‘template’ polymers and by functionalising these with a wide range of modalities were able to modify polymer and polyplex properties via a rapid, iterative design and modification process. This resulted in a library of over 20 novel P3CR derived polymers and by tailoring reaction conditions were able to control the polymer Mw to between 3500 and 9000.  By adapting formulation conditions and the polymer structure, it proved possible to produce polyplex nanoparticles with a diameter of between 100 and 200nm, and a PDI between 0.1 and 0.2 across a wide range of polymer/RNA w/w ratios. On studying the transfection capabilities of these polyplexes, two polymers proved capable of significantly outperforming the free RNA control. Although no polymer outperformed the commercially available positive control of lipofectamine. |
| **Conclusions:**  By utilising the robust and high yielding P3CR we have synthesised a versatile polymer template that led us to a wide library of bespoke, multifunctional materials. Initial formulation and transfection results show promise for further studies.  This polymer modality is easily customised and so can be optimised to reach a range of therapeutic needs. The high degree of functionality of these polymers could allow for the development of effective cell- and organ- specific targeting in the future. |