The *in vitro*, *ex vivo* and *in vivo* effect of polymer hydrophobicity on charge reversible vectors for self-amplifying RNA

Pratik Gurnani¹, Anna K. Blakney², Roberto Terracciano¹,³, Joshua E. Petch¹, Andrew J. Blok¹, Clément R. Bouton², Paul F. McKay², Robin J. Shattock², Cameron Alexander¹

¹Division of Molecular Therapeutics and Formulation, School of Pharmacy, University of Nottingham, NG7 2RD, UK. ²Department of Infectious Disease, Imperial College London, School of Medicine, St Mary’s Hospital, Praed Street, London W2 1NY. ³Drug Delivery Laboratory, Department of Pharmacy, University of Napoli Federico II, Via Domenico Montesano 49, 80131 Napoli, Italy

Contact: pratik.gurnani@nottingham.ac.uk

RNA vaccines

RNA (ribonucleic acid) is the code for proteins, and can be engineered to instruct muscle cells to produce antigens for vaccination.

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<th>Advantages:</th>
<th>Formulation solutions:</th>
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<td>- RNA manufacture the same regardless of target pathogen.</td>
<td>- To protect RNA from degradation and improve cytotoxic delivery, it is typically formulated with cationic polymers, lipids or in viruses</td>
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<td>- Only takes 2 weeks to produce doses</td>
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<td>- No cells required</td>
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<td>- Can be manufactured in poorer nations</td>
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<td>- RNA automatically stimulates the immune system for better immunisation</td>
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Aim

- Polymer hydrophobicity is key factor in polyplex (polymer-RNA complexes) gene delivery as it modulates cell membrane interaction.
- What role does hydrophobicity play in polymer vectors to deliver self-amplifying RNA in models specifically designed to test vaccination?

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**In vitro** - hydrophobic is best

Membrane interaction and performance in HEK293T

Hydrophobic polymers yield the highest transfection efficiency but also cause highest toxicity and membrane interaction

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**Ex vivo** - hydrophobic is best

Transfection in human skin per cell type

Hydrophobic polymers again induce the highest transfection efficiency in human skin explants.

Most hydrophobic polymers improve transfection by enhancing saRNA expression in epithelial cells and also some immune cell types

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**Conclusions**

- *In vitro*: hydrophobic best
- *Ex vivo*: hydrophobic best
- *In vivo*: hydrophilic best

Our data will help shape future polymer designs for the most efficient saRNA expression

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**Polymer synthesis**

![Polymer synthesis diagram](image)

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**In vivo** - hydrophilic is best

Transfection in mice

- None of the polymers outperformed PEI
- Interestingly, the most hydrophilic polymers induced the highest saRNA expression
- We hypothesise this is due to the toxicity of the hydrophobic polymers, possibly inducing inflammation type response, thus shutting down RNA expression pathways

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![In vivo - hydrophilic is best diagram](image)

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