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Characterization of CRISPR RNPs by Ion-Exchange Chromatography

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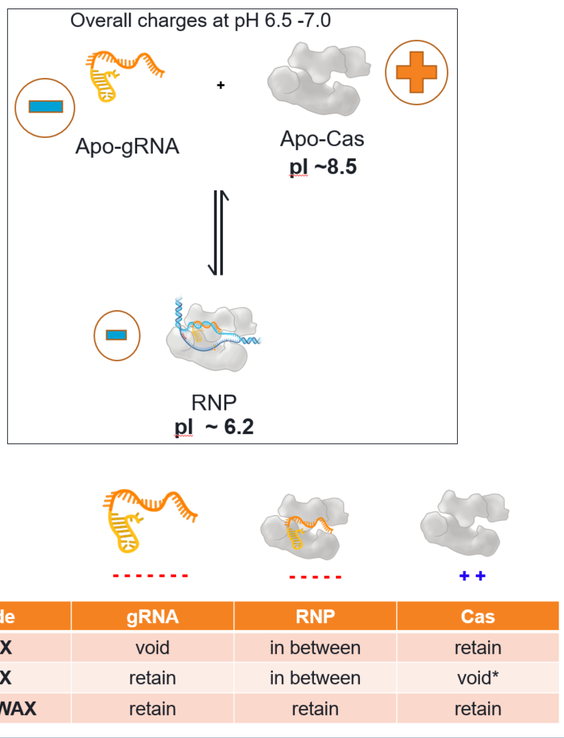
OBJECTIVE

- Characterize and quantitate the individual components of ribonucleoprotein complexes

INTRODUCTION

- CRISPR cell genome editing is carried out using ribonucleoproteins (RNP), which are complexes of a guide ribonucleic acid (gRNA) and a Cas protein
- RNPs are composed of 3 main ionic species in equilibrium at physiological pH, RNP, apo-Cas and apo-gRNA. Therefore, ion-exchange chromatography (IEX) is theoretically well suited for RNP analysis. However, while apo-Cas will be positively charged, both apo-gRNA and RNP are expected to have net negative charges in solution
- Thus, when using weak cation-exchange (WCX) chromatography, only apo-Cas will be retained while apo-gRNA and RNP will elute in the void volume. The opposite will occur when using weak anion exchange (WAX) chromatography where RNP and apo-gRNA will be retained while apo-Cas would be expected to elute in the void
- WAX and WCX were combined to leverage both modes of chromatography in one analysis

Ribonucleoprotein (RNP) Complex Formation



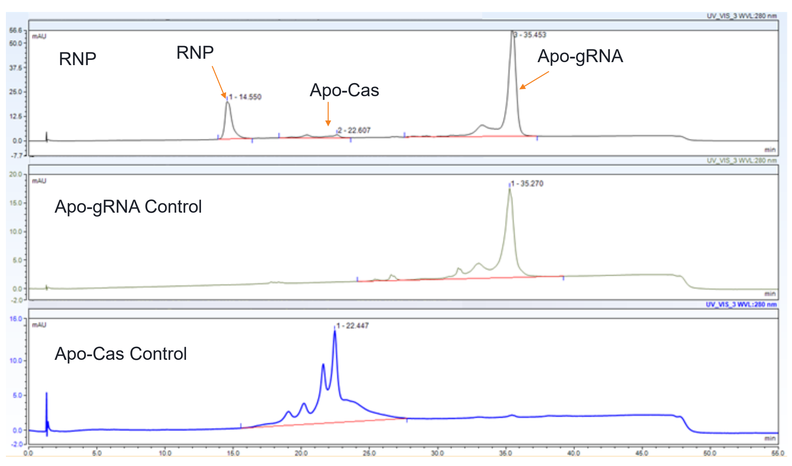
mode	gRNA	RNP	Cas
WCX	void	in between	retain
WAX	retain	in between	void*
WCX-WAX	retain	retain	retain

METHOD PARAMETERS

- Columns: Agilent BioWAX 3 µm, 4.6 x 50 mm
  Agilent BioWCX 3 µm, 4.6 x 50 mm
- Column Temperature = 25 °C
  Flow Rate = 0.5 mL/min
  Detection Wavelength: 280 nm
- MP A = 10 mM HEPES, 150 mM NaCl, pH 6.5
- MP B = 10 mM HEPES, 1 M NaCl, pH 6.5
- Gradient: 10 %B to 100%B in 42 min

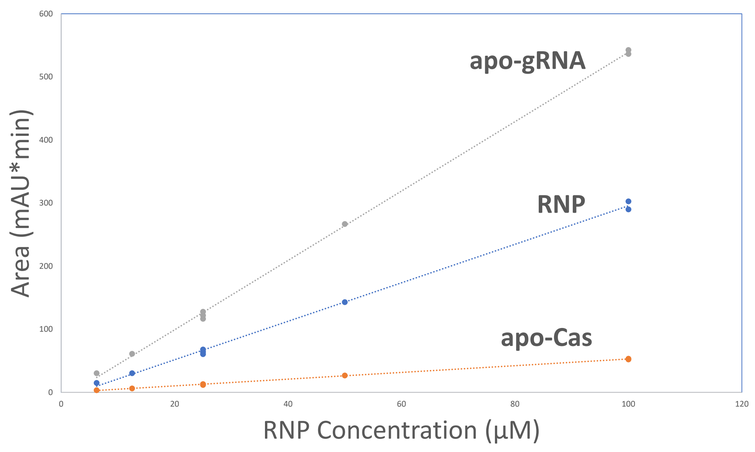
RESULTS

Figure 1. RNP Analysis by WAX-WCX



All 3 species are retained and resolved

Figure 2. RNP Linearity



Linear response observed for all 3 species

Figure 3. RNP Dissociation at Low Concentrations

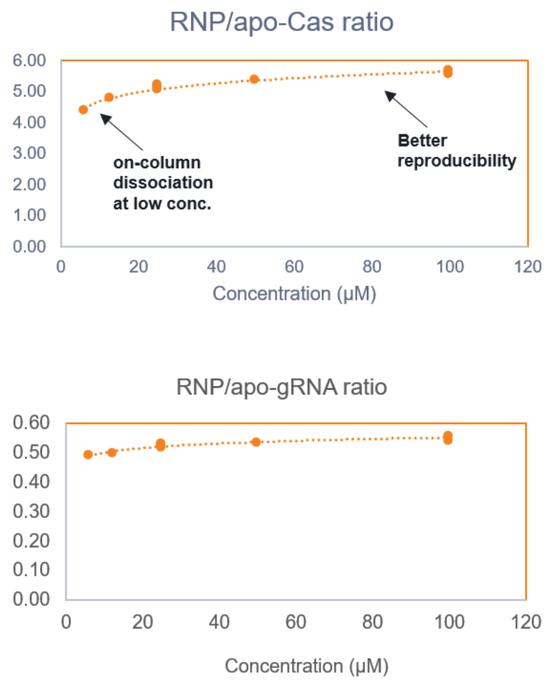
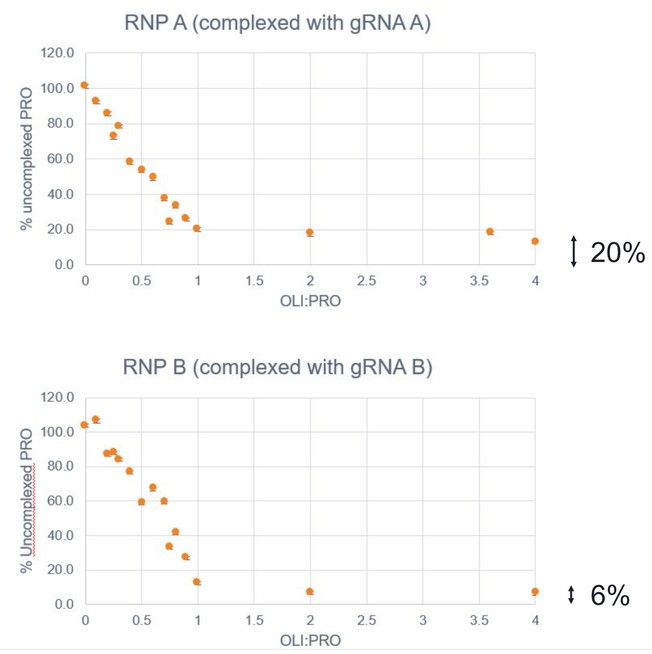
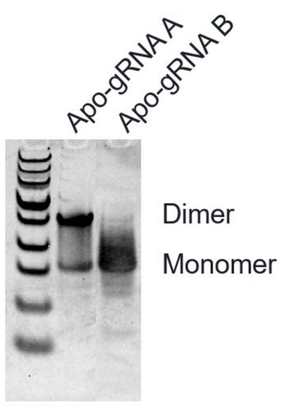


Figure 4. % Uncomplexed Cas vs gRNA:Cas Ratio



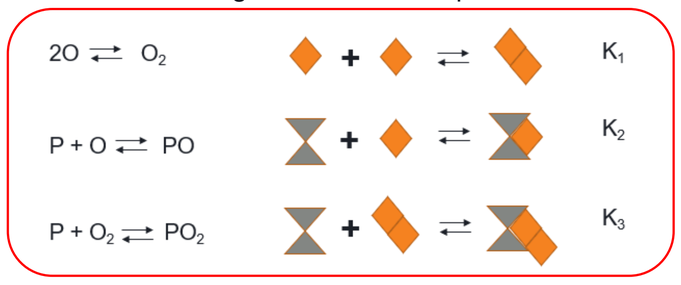


Different plateaus observed at gRNA:Cas >1 for Monomer vs. Dimer apo-gRNAs

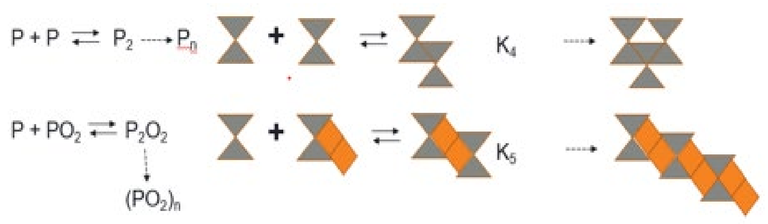
Figure 5. Modelling % Uncomplexed Cas vs gRNA:Cas Ratio

Possible Explanations:

- kinetically driven
  - *on-column dissociation* $k_{-3} > k_{-2}$
- thermodynamically driven
  - *e.g.*, $K_1, K_2 >> K_3$
- Initial modelling insufficient to explain observed trends



Likely more complex:



CONCLUSIONS

- We here describe the development and fit-for-purpose qualification of an ion-exchange chromatography analytical method to help characterize RNP complexation
- The amount of uncomplexed protein reached different plateaus for monomeric vs. dimeric apo-gRNAs at gRNA:Cas ratios ≥ 1
- Understanding the relationship between the structure and function of these non-covalent RNP complexes is key to optimizing the cell editing process as well as characterizing these compounds as therapeutics

DISCLOSURES

Jean-Noel Lemercier is an employee and a stock holder of Editas Medicine, Inc.

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