Nanopore sequencing grants detailed insights into a small molecule's impact on encapsidated DNA composition during manufacturing platform development

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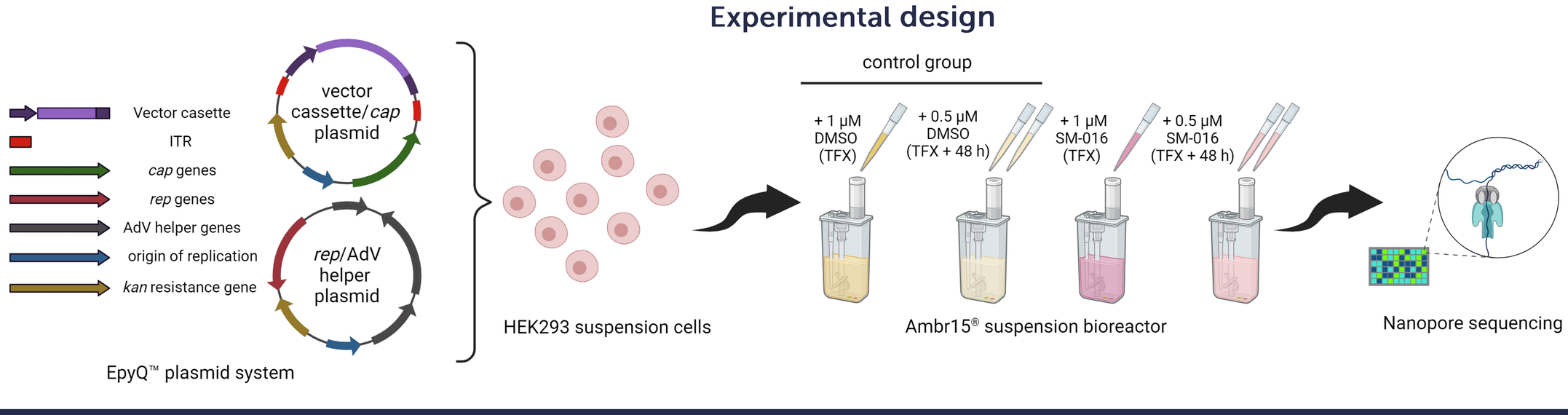
P0059

Abstract

One of the major challenges associated with recombinant adeno-associated virus (rAAV) gene therapy is the cost-efficient supply of large vector amounts. Ascend previously identified a small molecule (SM-016) that can increase viral vector titers, however its influence on vector quality has not been fully characterized. A fast, but comprehensive characterization of rAAV vector batches is crucial for platform and process development. Its low input requirements and fast turnaround time predetermine nanopore sequencing to guide development of rAAV based gene therapies.

We present here data on vector length distribution, encapsidated DNA impurities and vector integrity. Overall, we observed no negative impact of SM-016 on vector length distribution, integrity and host cell DNA impurities, but a slight increase of vector plasmid backbone derived impurities. This increase was caused by elevated ITR read-through/bidirectional packaging, providing a starting point for future mitigation strategies. Taken together, we highlight how nanopore sequencing can guide iterative vector production platform development.

Experimental design



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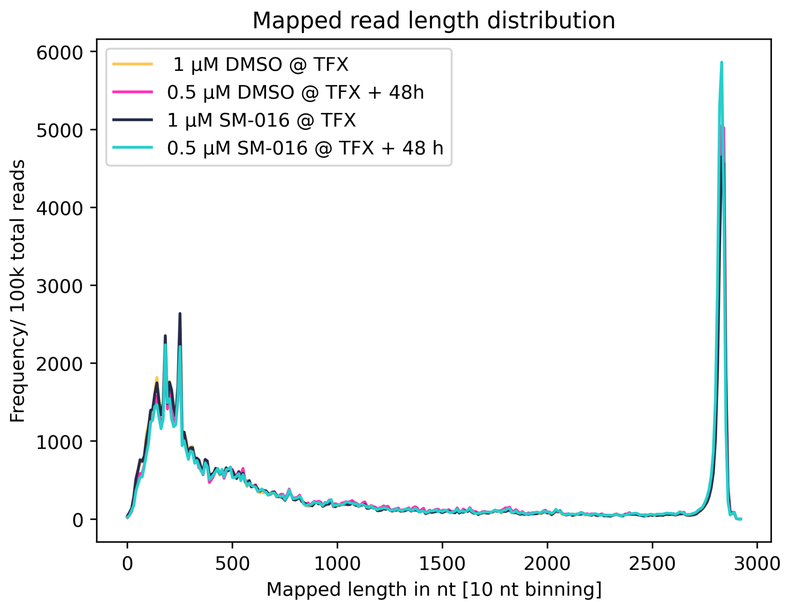
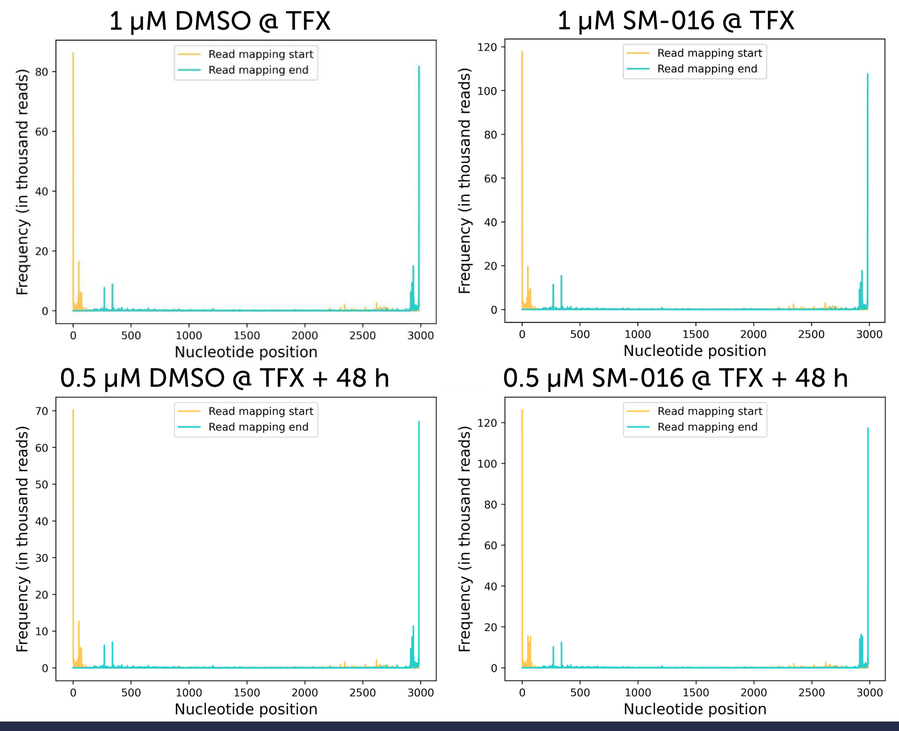
SM-016 has no influence on vector length distribution

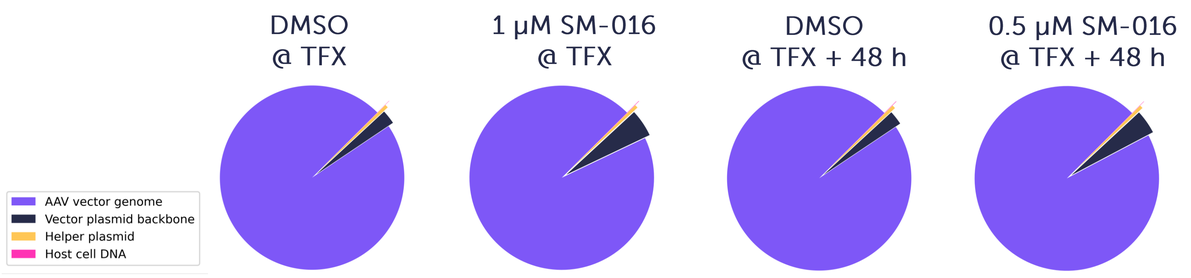




SM-016 does not impact vector integrity

- After mapping to the reference gene, all read border positions were counted, and their frequency was plotted using a custom pipeline
- The vast majority of reads started at the left ITR and ended at the right ITR
- Two minor premature read end points were observed in the transgene promoter
- The pattern was consistent in all samples and not influenced by the addition of the yield enhancer SM-016

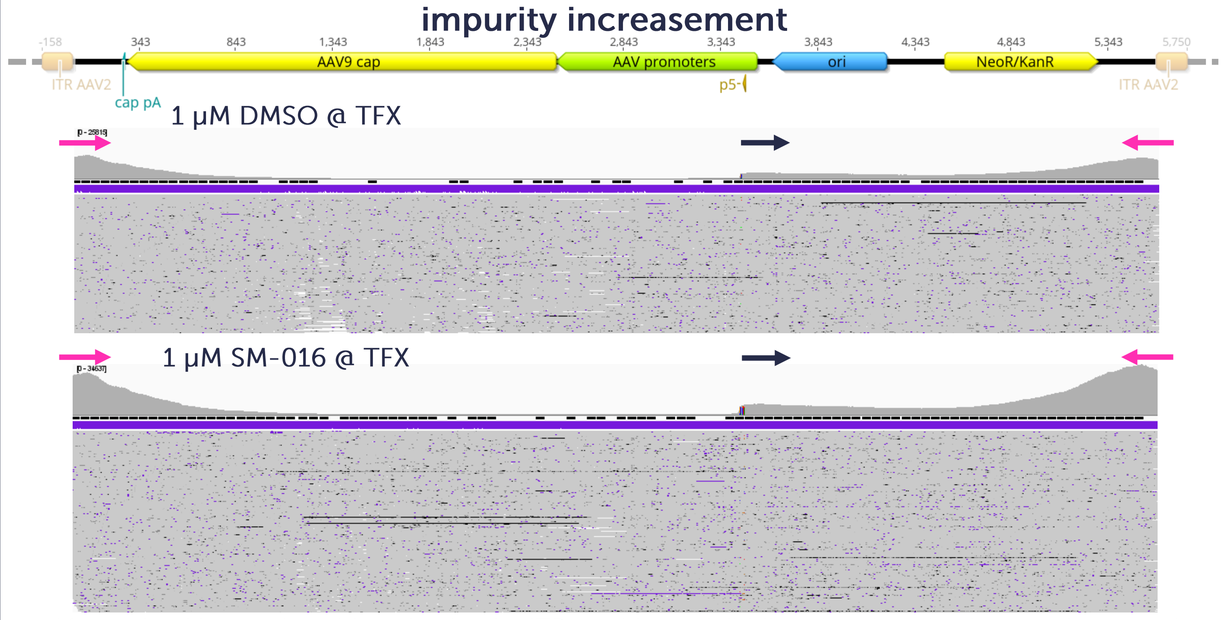
The addition of SM-016 increases vector plasmid backbone impurities



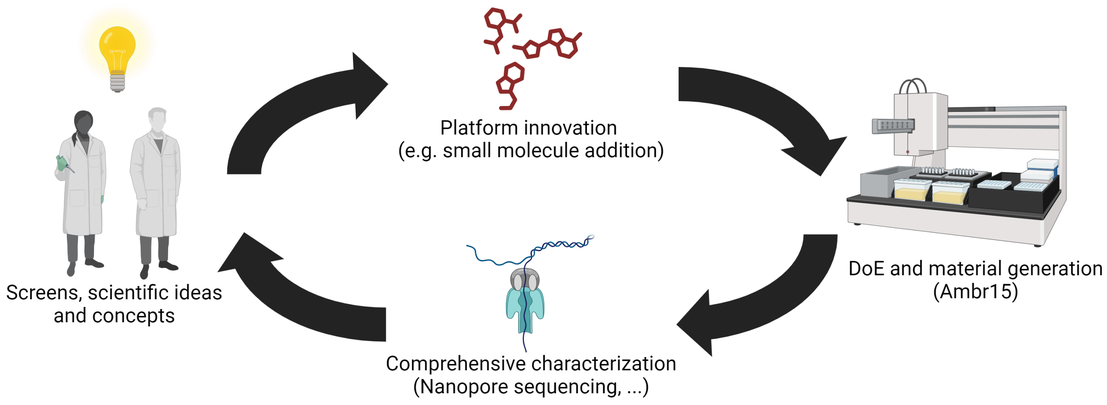
	1 µM DMSO @ TFX		1 µM SM-016 @ TFX		0.5 µM DMSO @ TFX + 48h		0.5 µM SM-016 @ TFX + 48h	
Reference sequence	Absolute reads	Mapping %	Absolute reads	Mapping %	Absolute reads	Mapping %	Absolute reads	Mapping %
AAV vector genome	337009	96.86	441553	94.58	268703	96.81	449162	95.21
Vector plasmid backbone	8765	2.52	22418	4.80	6991	2.52	19719	4.18
Helper plasmid	2024	0.58	2573	0.55	1675	0.60	2643	0.56
Host cell DNA	155	0.04	313	0.07	191	0.07	224	0.05
Total	347953	100	466857	100	277560	100	471748	100

- > 94% of reads mapped to the AAV vector genome in all samples
- The addition of SM-016 increased vector plasmid backbone impurities around two-fold
- The split addition of SM-016 reduced the impurities slightly compared to the single dose
- Addition of SM-016 had no impact on helper plasmid and host cell DNA impurities
- Results align with ddPCR data (not shown)

ITR-mediated mispackaging identified as cause of vector backbone impurity increasement



- Vector backbone plasmid packaging by ITR readthrough/bidirectional packaging was increased (pink arrows) (alignments normalised)
- P5 promoter reverse packaging was less affected (dark blue arrows)



Summary

Nanopore sequencing elucidated the impact of the addition of a small molecule (SM-016) on AAV vector quality. Whereas the vector length distribution and truncation hotspots remained unaffected, we detected a slight increase of packaged vector plasmid backbone DNA fragments. Together with other analytics available at Ascend (ELISA, ddPCR, full/empty, ...), nanopore sequencing data are crucial to guide iterative manufacturing platform development throughout the entire process.

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