Improving CRISPR-Cas Specificity with Chemical Modifications in Single-guide RNAs

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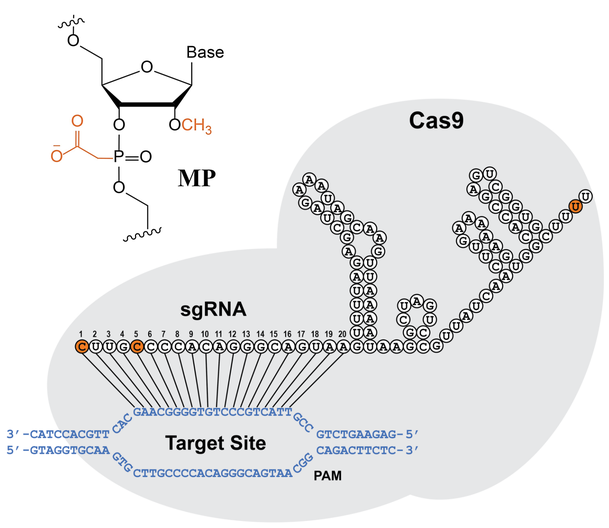
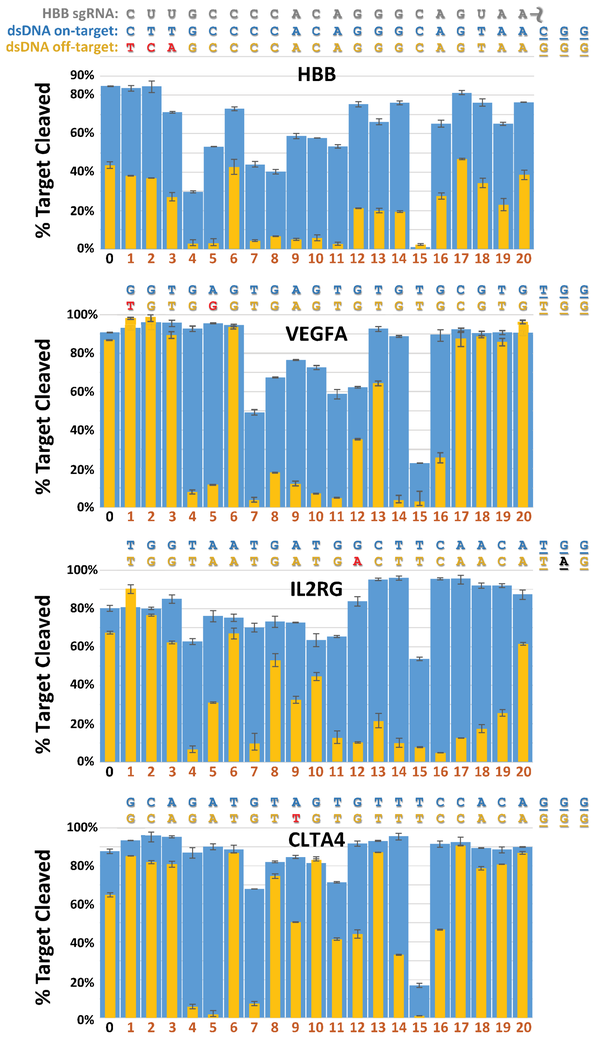
INTRODUCTION

CRISPR systems have emerged as transformative tools for altering genomes in living cells with unprecedented ease, inspiring keen interest in increasing their specificity for perfectly matched targets. We have developed a novel approach for improving specificity by incorporating chemical modifications in guide RNAs at specific sites in their DNA recognition sequence ("guide sequence") and systematically evaluating their on-target and off-target activities in biochemical DNA cleavage assays and cell-based assays. Our results show that a chemical modification (2′-*O*-methyl-3′-phosphonoacetate, or "MP") incorporated at select sites in the ribose-phosphate backbone of gRNAs can dramatically reduce off-target cleavage activities while maintaining high on-target performance, as demonstrated in clinically relevant genes. These findings reveal a unique method for enhancing the target specificity of Cas9 cleavage by selective chemical modification of the guide sequence in sgRNAs.

Chemical modifications in sgRNAs enhance the target specificity of Cas9 cleavage by an order of magnitude in biochemical assays

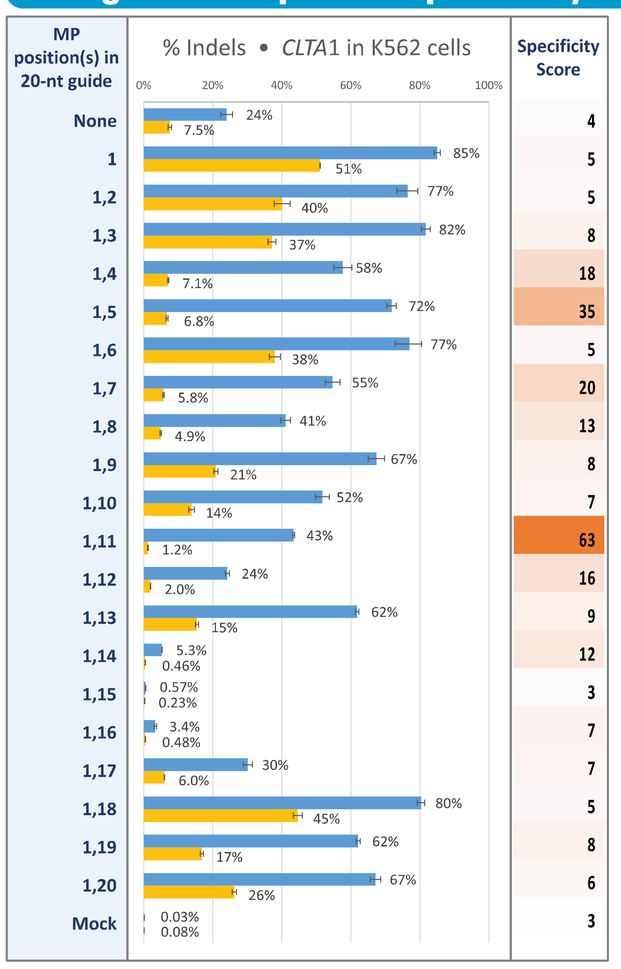


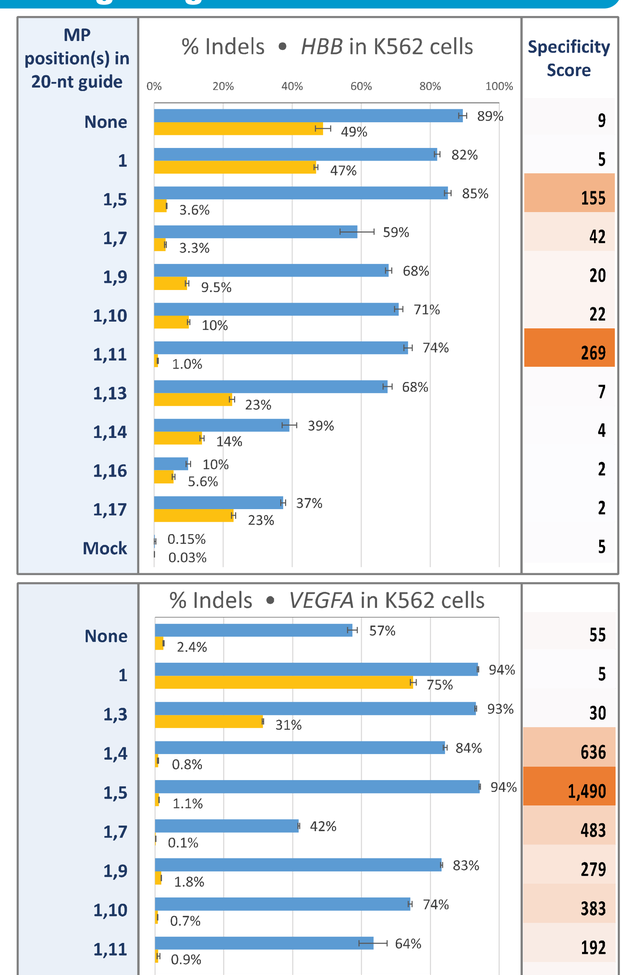


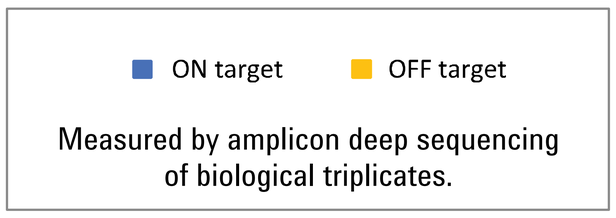
Target	Sequence
HBB ON	CTTGCCCCACAGGGCAGTAACGG
HBB OFF1	TCAGCCCCACAGGGCAGTAAGGG
HBB OFF5	GCTGCCCCACAGGGCAGCAAAGG
HBB OFF9	ATTGCCCCACGGGGCAGTGACGG
VEGFA ON	GGTGAGTGAGTGTGTGCGTGTGG
VEGFA OFF2	TGTGGGTGAGTGTGTGCGTGAGG
IL2RG ON	TGGTAATGATGGCTTCAACATGG
IL2RG OFF2	TGGTGAGGATGGCTTCAACACGG
IL2RG OFF3	TGGTAATGATGACTTCAACATAG
CLTA4 ON	GCAGATGTAGTGTTTCCACAGGG
CLTA4 OFF1	GCAGATGTAGTATTTCCACAGGG
CLTA4 OFF2	CCAGATGTAGCGTTTCCACAGGG
CLTA4 OFF3	GCAGATGTTGTGTTTCCACAGGG
CLTA1 ON	AGTCCTCATCTCCCTCAAGCAGG
CLTA1 OFF3	ACTCCTCATCCCCCTCAAGCAGG

MP position(s) in 20-nt guide	Guide Sequence Portion	ON:OFF TARGET CLEAVAGE RATIOS						SPECIFICITY SCORES					
		CLTA4 ON:OFF1	CLTA4 ON:OFF2	CLTA4 ON:OFF3	HBB ON:OFF1	VEGFA ON:OFF2	IL2RG ON:OFF3	CLTA4 ON:OFF1	CLTA4 ON:OFF2	CLTA4 ON:OFF3	HBB ON:OFF1	VEGFA ON:OFF2	IL2RG ON:OFF3
1	NNNNNNNNNNNNNNNNNNN-	1.0	0.9	1.1	2.2	1.0	0.9	0.6	0.2	2.5	8.3	0.3	0.5
1,2	NNNNNNNNNNNNNNNNNNN-	1.0	1.0	1.2	2.3	1.0	1.0	1.1	0.5	5.5	9.4	0.5	1.2
1,3	NNNNNNNNNNNNNNNNNNN-	1.0	1.0	1.2	2.6	1.1	1.4	0.4	1.2	4.9	6.7	2.8	3.5
1,4	NNNNNNNNNNNNNNNNNNN-	1.3	1.9	15	11	12	9.8	3.3	8.2	110	15	150	25
1,5	NNNNNNNNNNNNNNNNNNN-	1.1	2.3	52	18	8.2	2.5	1.6	14	530	38	160	7.2
1,6	NNNNNNNNNNNNNNNNNNN-	1.0	0.9	1.0	1.7	1.0	1.1	0.6	0.1	1.2	3.6	1.3	1.5
1,7	NNNNNNNNNNNNNNNNNNN-	1.1	1.0	9.2	10	13	7.3	1.2	1.1	26	17	25	22
1,8	NNNNNNNNNNNNNNNNNNN-	1.0	0.9	1.1	6.1	3.7	1.4	0.8	0.5	1.6	9.5	9.3	2.4
1,9	NNNNNNNNNNNNNNNNNNN-	1.0	0.9	1.7	12	6.2	2.3	1.0	0.2	5.5	28	23	5.6
1,10	NNNNNNNNNNNNNNNNNNN-	1.0	1.2	1.0	10	9.7	1.4	1.1	2.0	0.9	23	33	2.2
1,11	NNNNNNNNNNNNNNNNNNN-	1.2	1.1	1.7	21	12	5.2	1.8	1.3	3.6	43	27	13
1,12	NNNNNNNNNNNNNNNNNNN-	1.0	1.0	2.1	3.6	1.8	8.3	0.8	0.6	14	11	3.0	47
1,13	NNNNNNNNNNNNNNNNNNN-	1.0	0.9	1.1	3.3	1.5	4.5	0.9	0.1	2.1	7.9	7.2	74
1,14	NNNNNNNNNNNNNNNNNNN-	1.0	1.0	2.9	3.9	22	9.9	0.5	1.6	45	13	190	230
1,15	NNNNNNNNNNNNNNNNNNN-	1.1	3.7	17	0.4	7.1	7.0	1.1	4.3	20	0.4	9.0	14
1,16	NNNNNNNNNNNNNNNNNNN-	1.0	1.0	2.0	2.4	3.5	20	0.7	1.0	13	5.0	25	440
1,17	NNNNNNNNNNNNNNNNNNN-	0.9	0.9	1.0	1.7	1.1	7.7	0.3	0.1	1.3	5.0	1.8	150
1,18	NNNNNNNNNNNNNNNNNNN-	1.0	0.9	1.1	2.2	1.0	5.4	0.8	0.3	2.4	6.2	1.3	56
1,19	NNNNNNNNNNNNNNNNNNN-	1.0	0.9	1.1	2.9	1.1	3.6	1.0	0.3	1.9	6.3	1.7	34
1,20	NNNNNNNNNNNNNNNNNNN-	1.0	0.9	1.0	2.0	0.9	1.4	1.0	0.1	1.4	5.2	0.4	4.4

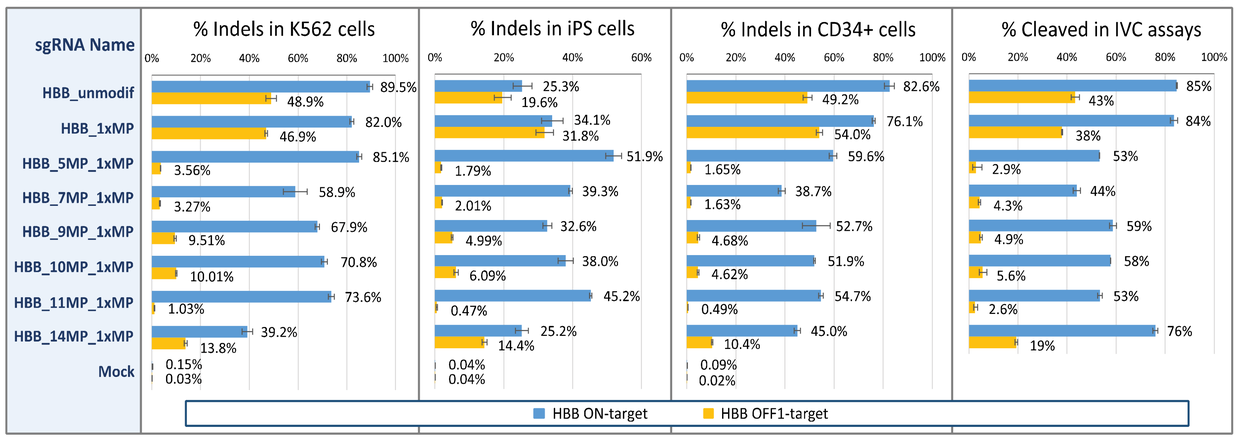
Two hotspots where an MP modification in 20-nt guide sequences in sgRNAs improves specificity for targeting indels in human cells



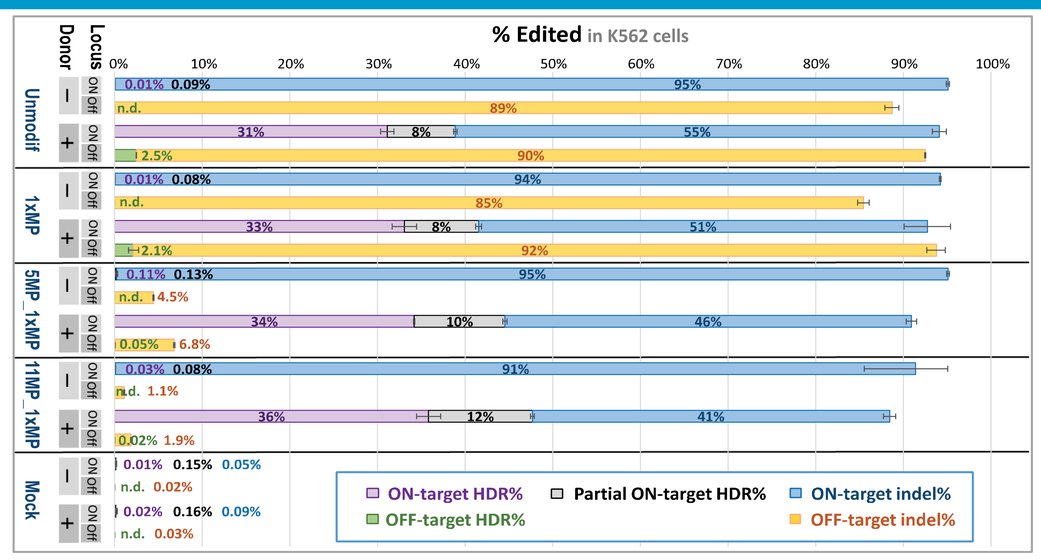




MP modifications in HBB sgRNA enhance specificity for targeting indels in multiple human cell types



Enhanced specificity of editing at the sickle cell locus in *HBB*



CONCLUSIONS

For further information, see our paper in *Nucleic Acids Research*: published online on 04 December 2017, doi.org/10.1093/nar/gkx1199

- Chemical modifications incorporated at select sites in the ribose-phosphate backbone of gRNAs can reduce off-target cleavage activities substantially, in several cases by an order of magnitude or more, without sacrificing on-target activity.
- There are specific positions in the 20-nt guide sequence in sgRNAs at which MP modification improves targeting specificity across multiple guides, suggesting that this approach can be broadly useful for improving CRISPR-Cas9 specificity when synthetic gRNAs are employed for gene editing.
- Our approach reveals a versatile tool for augmenting the performance of CRISPR systems for research, industrial and therapeutic applications.