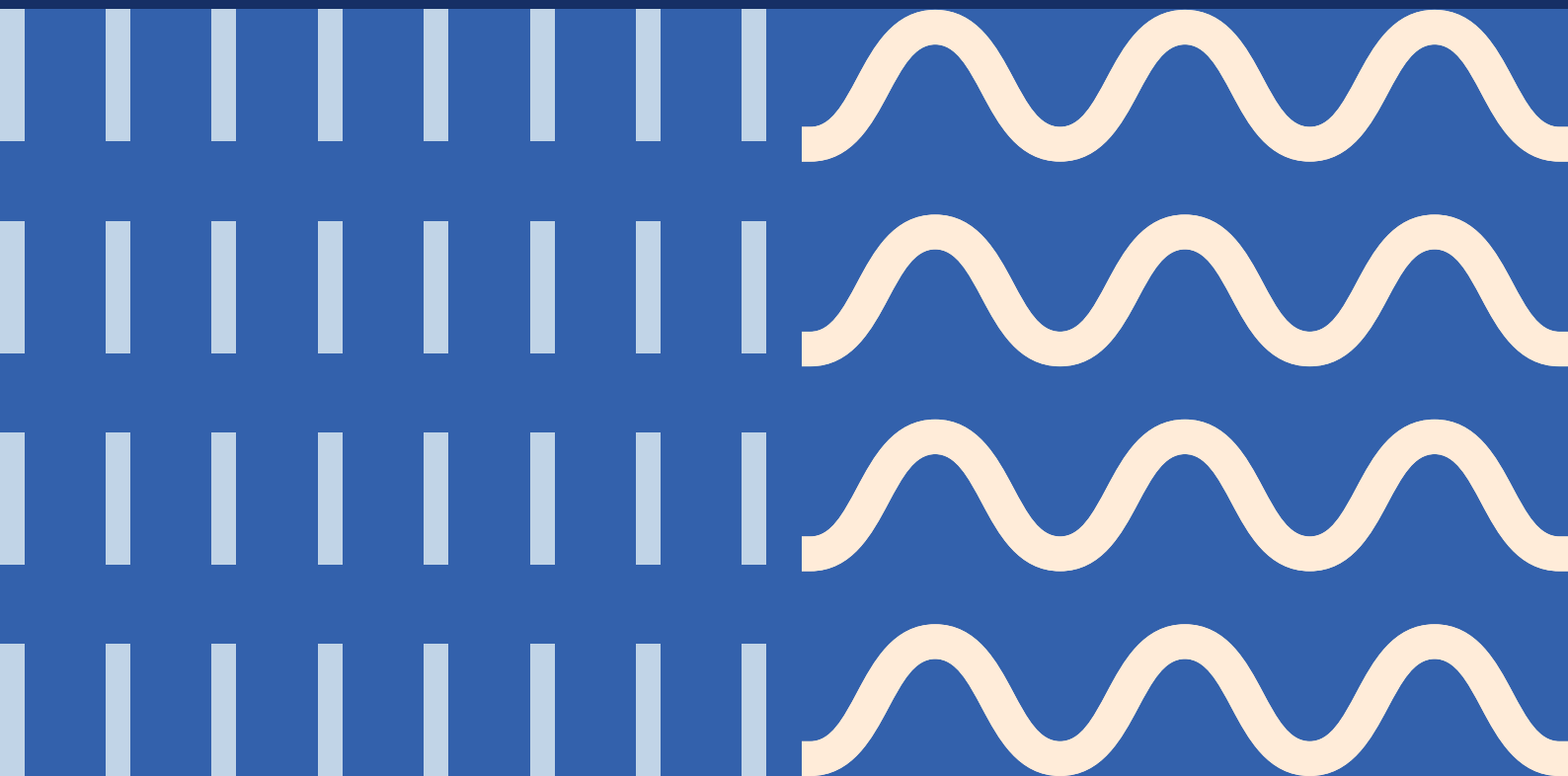
Hongene Biotech

HiXCap™

Novel Cap Analog Designs for Enhanced mRNA Immune Evasion



The chemistry of 5′ end cap is crucial for stability and translation of mRNA drug modalities. Engineered to enhance translation efficiency during immune stress, our HiXCap™ novel cap structures offer a promising solution for improving mRNA-based therapeutics. Efficacy studies carried out in *in vitro* and *in vivo* inflammation models demonstrated their potential to transform treatment outcomes.

Key takeaways:

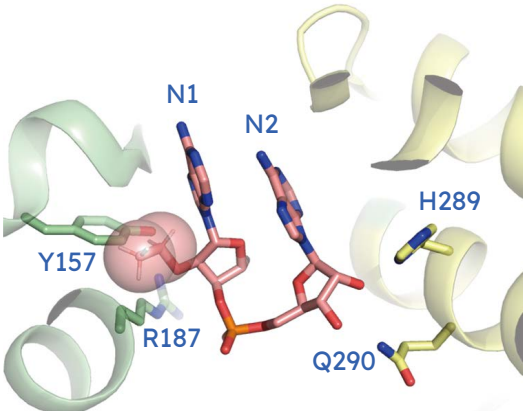
mRNA binding to the translation suppressor protein IFIT1 can be abrogated by chemical modification of Cap 1 structures.

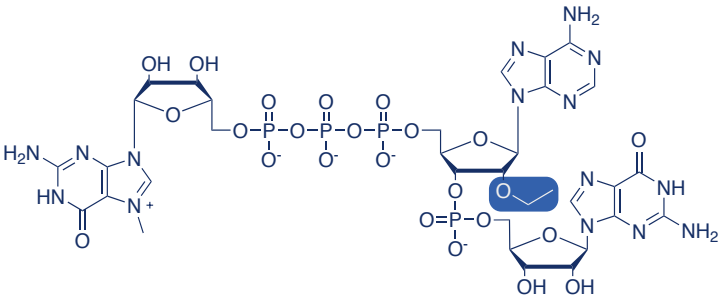
Substitution of methyl for ethyl at the 2′ ribose of N1 improves mRNA translation efficiency under conditions of immune stress.

HiXCap™ reagents are available now and suggested for use with all mRNA drug modalities.

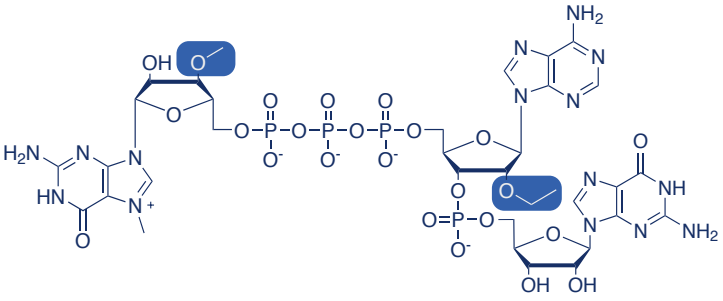
Our structure-based rational 5’ cap design

Improved translation by evading IFIT1 binding[1]





HiXCap™ E1
m7G(5′)ppp(5′)(2′-OEtA)pG



HiXCap™ E2
(3′-OMe-m7G)(5′)ppp(5′)(2′-OEtA)pG

We hypothesized that a bulkier group on the ribose of N1 would better evade IFIT1 binding, leading to increased translation under immune stress.

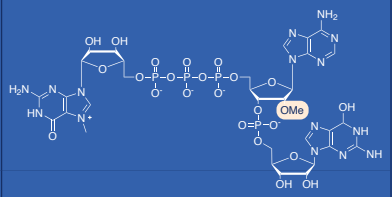
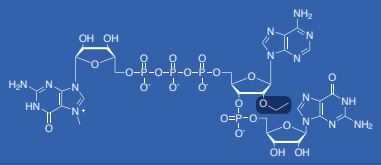
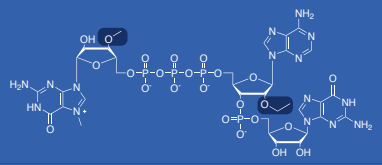
Our *in silico* analysis of the cap binding pocket of IFIT1 in complex with HiXCap™ E1 predicted a severe steric clash between N1 2′-O-Ethyl and Y157 and R187 of IFIT1.

This steric clash results reduced binding of HiXCap™ to IFIT1, leading to enhanced translation efficiency under conditions of immune stress.

Comprehensive quality control

Each HiXCap™ structure underwent rigorous identity testing, confirmed by MS and NMR analysis. Measurements with HPLC consistently demonstrated purity levels exceeding 99%.

Assessments of concentration, pH, ammonium ion content and endotoxin levels for each solution showed exceptional and consistent reagent quality.

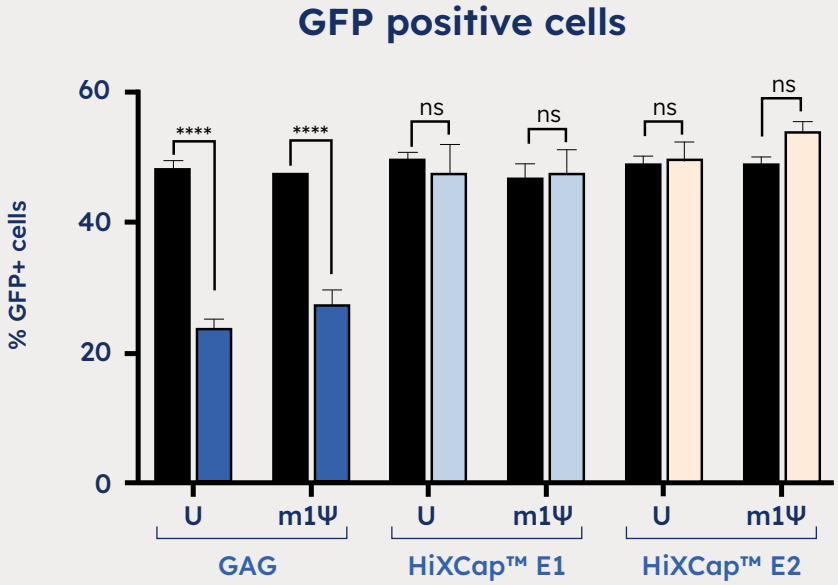
			
Product name	**GAG**	**HiXCap™ E1**	**HiXCap™ E2**
Appearance	Clear to slightly yellow solution	Clear to slightly yellow solution	Clear to slightly yellow solution
ID Mass Spec (free acid)	1145.7	1159.7	1173.7
ID (1H NMR)	Conforms to structure	Conforms to structure	Conforms to structure
ID (31P NMR)	Conforms to structure	Conforms to structure	Conforms to structure
Concentration	98 mM	102 mM	103 mM
Purity (HPLC)	99.5%	99.1%	99.7%
Unspecified Impurity	<1.0%	<1.0%	<1.0%
pH (22~25°C)	6.3	6.2	6.2
Ammonium Ion Content	0.33%	0.48%	0.54%
Bacterial Endotoxin	<1 EU/mL	<1 EU/mL	<1 EU/mL

Robust IVT performance and mRNA quality

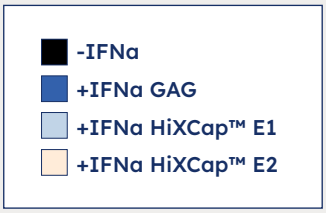
Performance of each HiXCap™ structure was evaluated under standard *in vitro* transcription (IVT) reaction conditions by synthesizing Fluc and eGFP mRNAs containing either uridine (U) or N1 methylpseudouridine (m1Ψ). Quantification of IVT yield and analyses of mRNA integrity, capping efficiency and double-stranded RNA (dsRNA) content demonstrated consistent IVT performance across the differently capped mRNAs with each exhibiting high yield and integrity and very low dsRNA content, essential for minimizing unwanted immune responses.

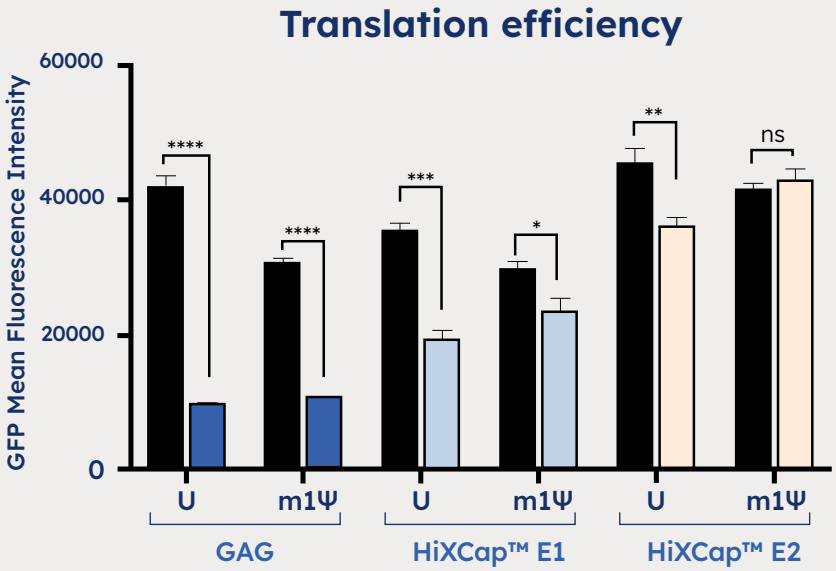
mRNA	nt	U chemistry	Cap structure	IVT yield (mg/mL)	Integrity	Capping efficiency	Rel dsRNA
eGFP	1,003	Uridine	GAG	5.3	96.4%	97%	<0.01%
			HiXCap™ E1	5.3	97.3%	97%	<0.01%
			HiXCap™ E2	5.2	96.0%	94%	<0.01%
		m1Ψ	GAG	5.1	96.6%	98%	<0.01%
			HiXCap™ E1	5.2	95.8%	98%	<0.01%
			HiXCap™ E2	5.1	97.3%	94%	<0.01%
Fluc	1.939	Uridine	GAG	5.6	91.4%	99%	<0.01%
			HiXCap™ E1	5.6	91.7%	99%	<0.01%
			HiXCap™ E2	5.5	91.1%	95%	<0.01%
		m1Ψ	GAG	5.4	91.9%	99%	<0.01%
			HiXCap™ E1	5.4	92.2%	99%	<0.01%
			HiXCap™ E2	5.3	92.9%	95%	<0.01%

HiXCap™ enhances mRNA translation in IFNα stimulated JAWS II cells[2]



Flow cytometry analysis of JAWS II cells transfected with eGFP mRNAs showed that IFNα treatment significantly decreased % of GFP+ cells in GAG capped and had no significant effect on HiXCap™ E1 and E2 capped mRNAs transfected cell.

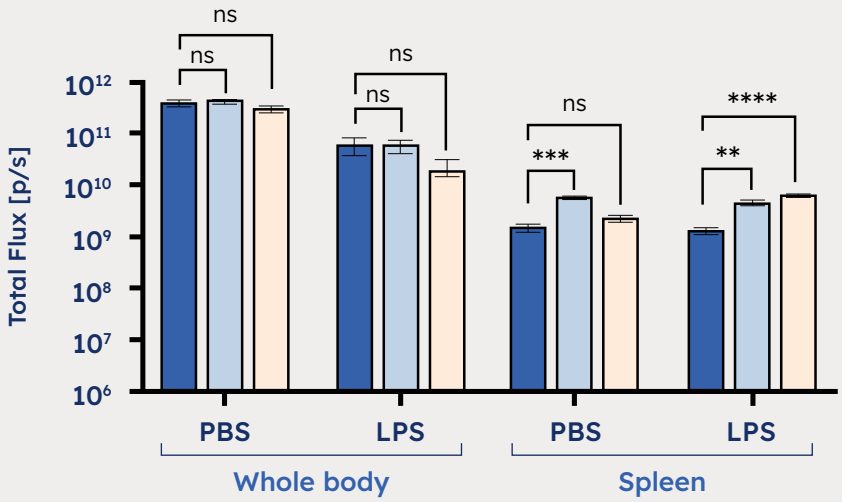




Flow cytometry analysis of JAWS II cells transfected with eGFP mRNAs showed that IFNα treatment significantly suppressed the translation of GAG, and to a lesser extent HiXCap™ E1 capped mRNAs, but HiXCap™ E2 mRNA remained largely unaffected.

HiXCap™ improved mRNA translation in LPS *in vivo* inflammation model[3]

Translation efficiency of m1Ψ mRNA

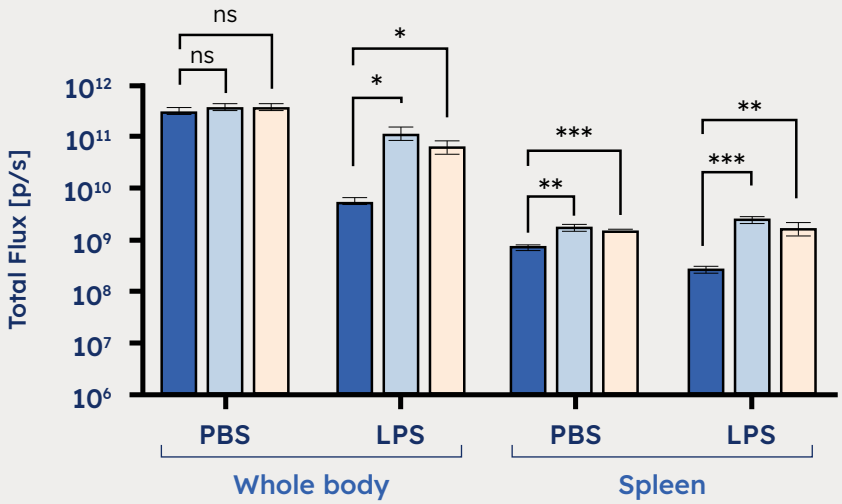


Mice are relatively resilient to inflammatory challenges. We hypothesize that the differences in translation efficiency will be more pronounced in NHP and humans.

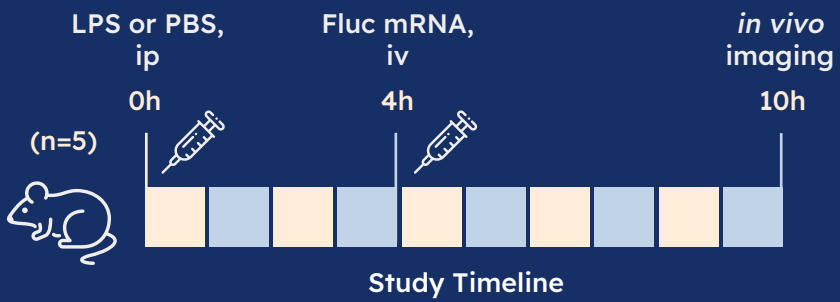
Both HiXCap™ designs significantly improved the translation of Fluc mRNA compared to GAG in spleen in the LPS model and could be successfully used instead of GAG in mRNA drugs containing m1Ψ.

GAG
HiXCap™ E1
HiXCap™ E2

Translation efficiency of U mRNA



Both HiXCap™ designs significantly improved the translation of Fluc mRNA compared to GAG in whole body and spleen in the LPS model, indicating that either HiXCap™ design could be successfully employed in mRNA vaccines containing uridine.



C57BL/6 mice were dosed with LPS (3 mg/kg) by IP. Fluc mRNA (10 µg) in LNP was dosed by IV. Whole body live imaging (2.25 mg of luciferase substrate) was performed using the Perkin-Elmer IVIS Lumina Series III Imaging System. The mice were sacrificed to gather the data for experiments on spleen.

References

1. Y. M. Abbas et al., Proceedings of the National Academy of Sciences. 114, E2106–E2115 (2017).
2. K. Drazkowska et al., Nucleic Acids Research. 50, 9051–9071 (2022).
3. H. Parhiz et al., Journal of Controlled Release. 344, 50–61 (2022).

Bring your RNA to life.

About Hongene

Founded in 1998, we are a world leading manufacturer of nucleic acid raw materials including nucleosides, modified nucleosides, nucleotides, phosphoramidites, GalNAc delivery molecules and enzymes.

We are trusted by global pharmaceutical and biotech companies developing mRNA and oligonucleotide drugs and Hongene products are integral to their pipelines.

Powered by more than 1,600 employees globally, we have established comprehensive **end-to-end mRNA and oligonucleotide CDMO capabilities**, offering a seamless one-stop solution that encompasses everything from raw materials to final drug product. Our services support preclinical research through to full commercialization.

Comprehensive and fully integrated platform for mRNA vaccines and therapeutics

Efficient reactions
High purity
High yield
Competitive cost

Vertical integration
Total solution
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mRNA drug substance
Fill and finish

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Small-scale
Large-scale
Full in-house QC

mRNA raw materials
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Enzymes

25+ Years

of Experience in the RNA industry

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support world-leading capacity for raw materials, and GMP oligonucleotide and mRNA drug substance and drug product CDMO services.

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Employees

2000+

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- GalNAc
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