

Article

# mRNA Vaccines Encoding the HA protein of Influenza A H1N1 virus delivered by Cationic Lipid Nanoparticles Induce Protective Immune Responses in Mice

Xinyu Zhuang <sup>1,†</sup>, Yanxin Qi <sup>2,†</sup>, Maopeng Wang <sup>3</sup>, Ning Yu <sup>4</sup>, Fulong Nan <sup>5</sup>, He Zhang <sup>1</sup>, Mingyao Tian <sup>1</sup>, Chang Li <sup>1</sup>, Huijun Lu <sup>1</sup> and Ningyi Jin <sup>1,\*</sup>

<sup>1</sup> Key Laboratory of Jilin Province for Zoonosis Prevention and Control, Institute of Military Veterinary Medicine, Academy of Military Medical Sciences, Academy of Military Sciences, Changchun, 130000, Jilin, China; xinyuzhuang367@163.com (X.Z.); hezhangvs@126.com (H.Z.); klwklw@126.com (M.T.); lichang78@163.com (C.L.); huijun\_lu@126.com (H.L.)

<sup>2</sup> State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, China; yxqi@ciac.ac.cn

<sup>3</sup> Institute of Virology, Wenzhou University, Wenzhou, 325000, Zhejiang, China; wangmaopenga@126.com

<sup>4</sup> Department of Veterinary Medicine, College of Agriculture, Yanbian University, Yanji, 133000, Jilin, China; yn8597@126.com

<sup>5</sup> College of Veterinary Medicine, Jilin University, Changchun, 130000, Jilin, China; nflcom@163.com

<sup>†</sup> The authors contributed equally.

\* Correspondence: ningyik@126.com; Tel.: +86-431-86985929

## Supplementary Materials

**Figure S1.** (a) Agarose gel electrophoresis of pGEM-EGFP-n1/n2/n3 digested by *XhoI* enzyme. 1, 3 and 5 represent the intact plasmids of pGEM-EGFP-n1/n2/n3; 2, 4, 6 represent their linearized products of 4215bp, 4406bp and 4364bp, respectively. (b) Agarose gel electrophoresis of pGEM-H3HA-n1/n2/n3 digested by *XhoI* enzyme. 1, 3 and 5 represent the intact plasmids of pGEM-H3HA-n1/n2/n3; 2, 4, 6 represent their linearized products of 5206bp, 5397bp and 5355bp, respectively. M1:  $\lambda$ -EcoT14 I digest (TaKaRa, Tokyo, Japan); M2: DL5,000 DNA Marker (TaKaRa, Tokyo, Japan).

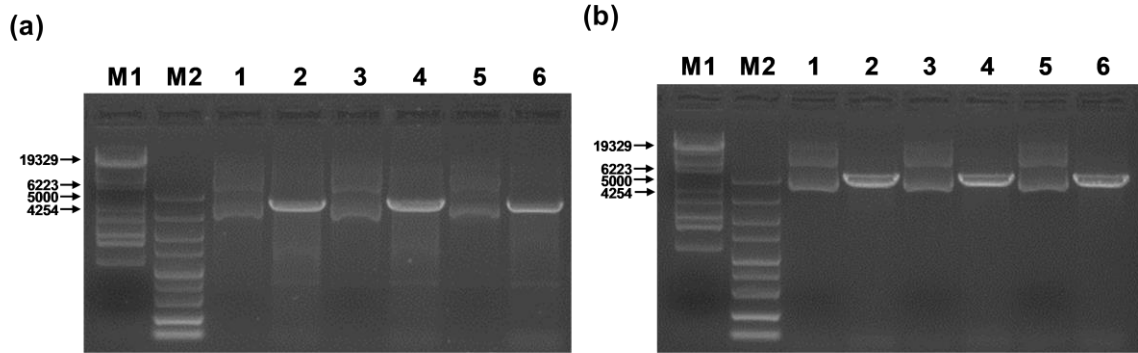
**Figure S2.** The gating strategies of flow cytometry to detect the percentage of EGFP positive cells. (a) The control group. (b) The Cap-mEGFP-n1 group. (c) The Cap-mEGFP-n2 group. (d) The Cap-mEGFP-n3 group.

**Figure S3.** Size distributions of (a) LNP. (b) LNP-Man. (c) LNP/mH3HA. (d) LNP-Man/mH3HA (N/P=10:1).

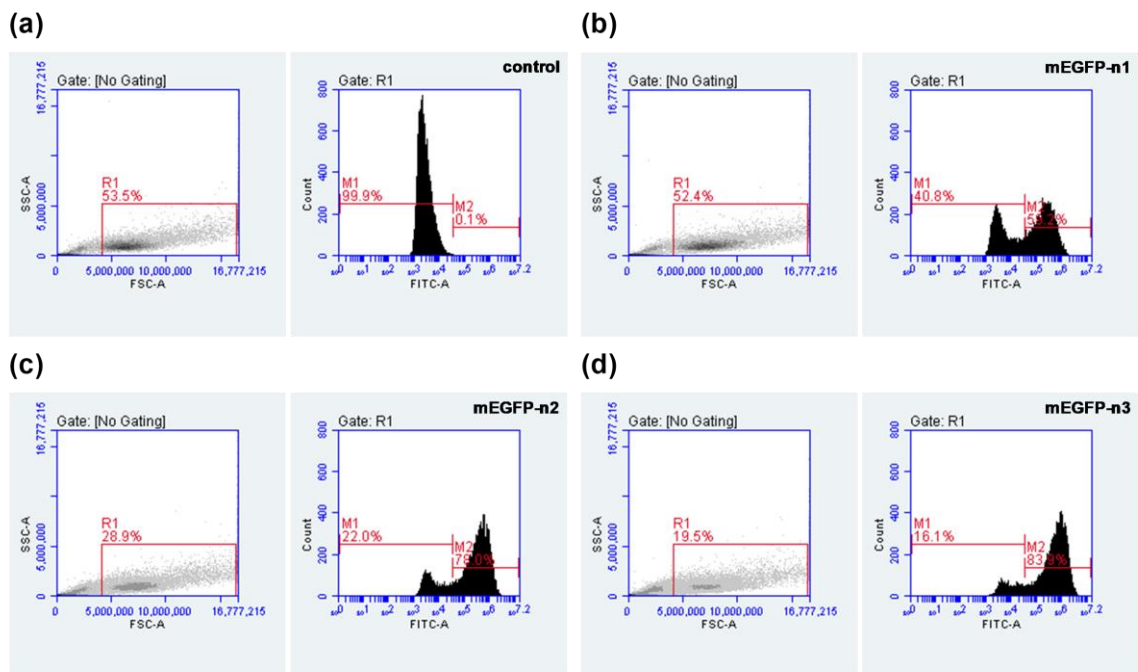
**Table S1.** Sequences of tested UTR configurations.

**Table S2.** Mean size (d. nm) of LNPs and LNPs/mRNA.

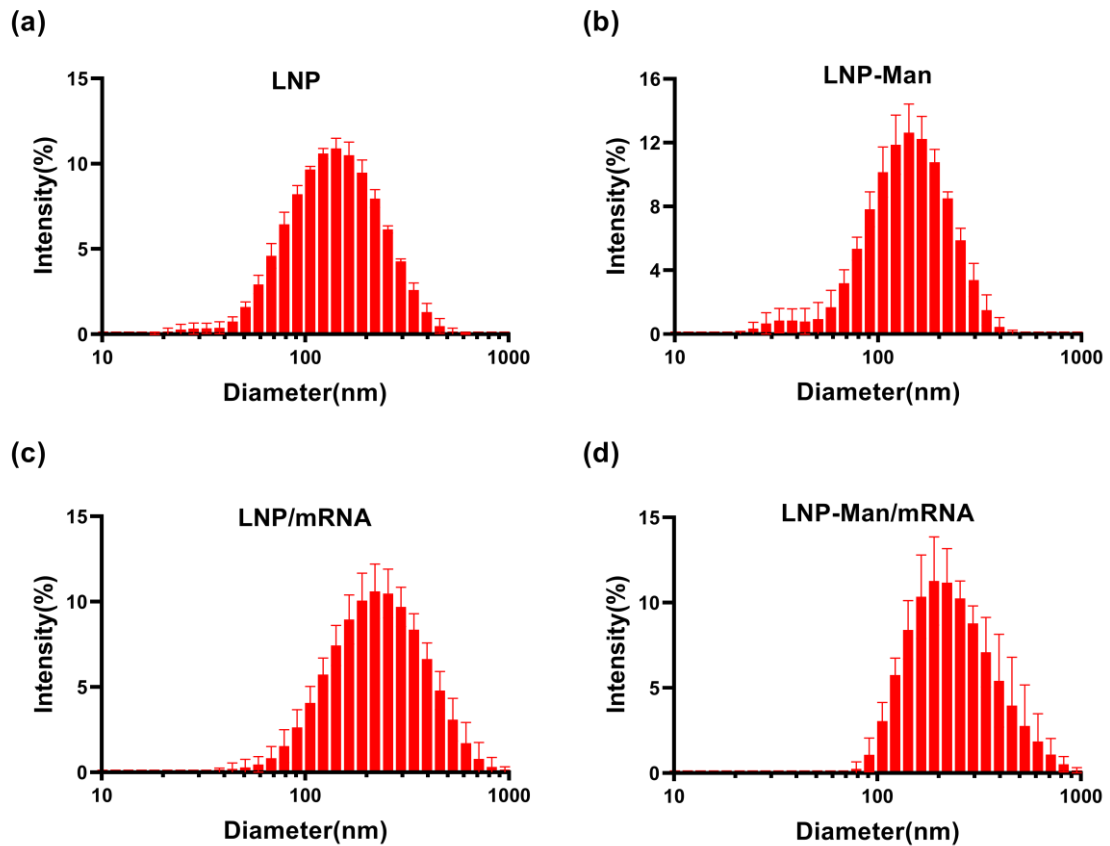
**Table S3.** Zeta potential (mV) of LNPs and LNPs/mRNA.



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**Table S1.** Sequences of tested UTR configurations.

IVT-mRNA-n1

- 5'UTR ( $\alpha$ -globin):

AAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA

- 3'UTR ( $\alpha$ -globin):

GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTGGGCCTCCCCCAGCCCC  
TCCTCCCCTTCCTGCACCCGTACCCCGTGGTCTTTGAATAAAGTCTGAGTG  
GGCGGC

IVT-mRNA-n2

- 5'UTR ( $\beta$ -globin-1):

CAGGGCAGAGCCATCTATTGCTTACATTTGCTTCTGACACA ACTGTGTTAC  
TAGCAACCTCAAACAGACACC

- 3'UTR (2 $\beta$ -globin):

AGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCCTTGTTCCTAAGT  
CCAACACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCT  
GCCTAATAAAAACATTTATTTTCATTGCAGCTCGCTTCTTGCTGTCCAATT  
TCTATTAAAGGTTCCCTTGTTCCTAAGTCCAACACTAAACTGGGGGATAT  
TATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAACATTTATTTTC  
ATTGC

## IVT-mRNA-n3

- 5'UTR ( $\beta$ -globin-2):

AGAGCGGCCGCTTTTTTCAGCAAGATTAAGCCCAGGGCAGAGCCATCTATTG  
CTTACATTTGCTTCTGACACAACCTGTGTTCACTAGCAACCTCAAACAGACA  
CC

- 3'UTR (2 $\beta$ -globin):

AGCTCGCTTTCTTGCTGTCCAATTTCTATTAAGGTTCCCTTGTTCCTAAGT  
CCAACACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCT  
GCCTAATAAAAAACATTTATTTTCATTGCAGCTCGCTTTCTTGCTGTCCAATT  
TCTATTAAAGGTTCCCTTGTTCCTAAGTCCAACACTAAACTGGGGGATAT  
TATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTC  
ATTGC

**Table S2.** Mean size (d. nm) of LNPs and LNPs/mRNA.

Materials	—	+ mRNA	Change folders
LNP	119.8	203.5	1.70 ↑
LNP-Man	118.3	231.5	1.96 ↑

**Table S3.** Zeta potential (mV) of LNPs and LNPs/mRNA.

Materials	—	+ mRNA	Change folders
LNP	35.5	18.3	0.52 ↓
LNP-Man	39.3	18.4	0.47 ↓