Development of flexible polycation-based mRNA delivery systems for *in vivo* applications

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Pros and cons of messenger RNA

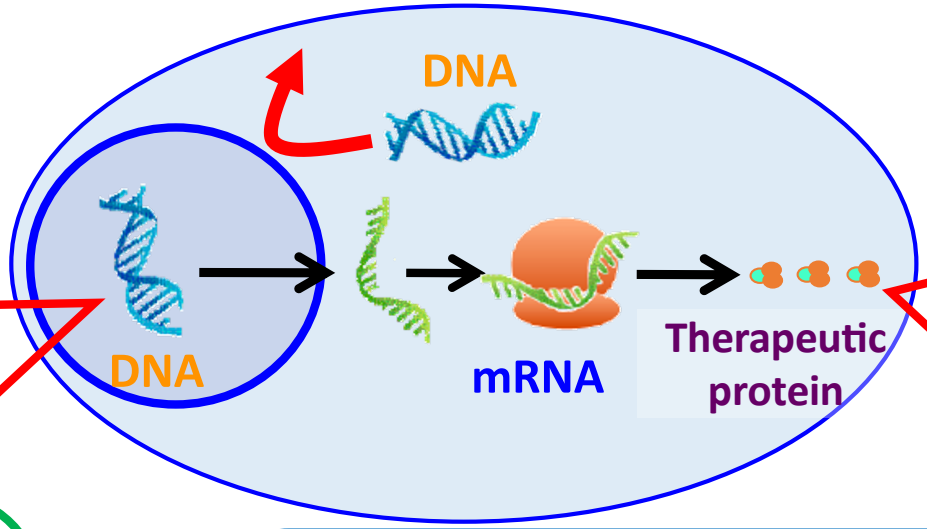
<u>DNA</u>

✔Sustainable effects

✔Low cost

▪Needs to enter nucleus

▪Insertional mutagenesis

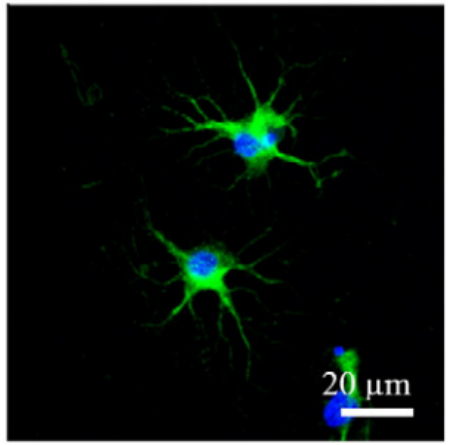


<u>Protein</u>

▪Short effects

▪High cost

Transfection to non dividing cells (Primary neural cells)



C. –Y. Lin et. al., ***J. Control. Release*** **235** (2016) 268-275.

<u>mRNA</u>

✔Efficient production of therapeutic protein

✔Avoidance of insertional mutagenesis

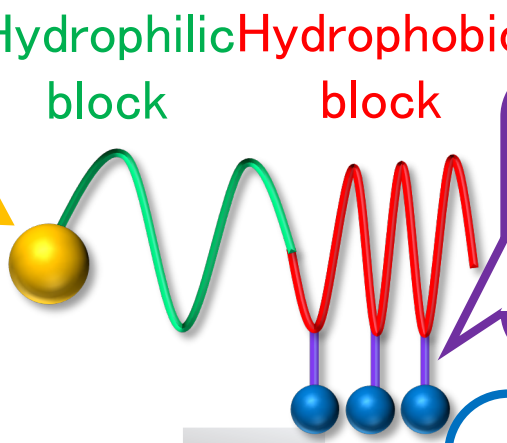
✔Applicability to non dividing cells

- **Rapid degradation by nuclease**
- **Poor cellular uptake**
- **Adverse immune response**

U. Sahin et. al., ***Nat. Rev. Drug Discovery*** **13** (2014) 759-780.

The development of carriers for mRNA is necessary.

Polymeric micelles



Ligand

- **Control biodistribution**

(i.e. tumor targeting)

- **Control microdistribution**

(i.e. macrophage targeting)

Cleavable bonds

- **Sense pathological signals**

(i.e. enzyme, pH, redox, ATP, ROS)

Self assembly

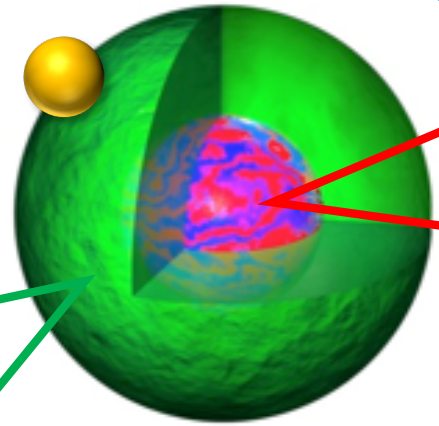
Drug loading

- ***via* covalent bonds**

(i.e. small molecules)

- ***via* electrostatic interactions**

(i.e. RNA, DNA, protein)



Hydrophilic shell

- **Inhibit protein absorption**

→Improve pharmacokinetics

- **Prevent interactions with cells**

→Control biodistribution

Drug loading core

- **Degradable amino acids**

→Increase biocompatibility

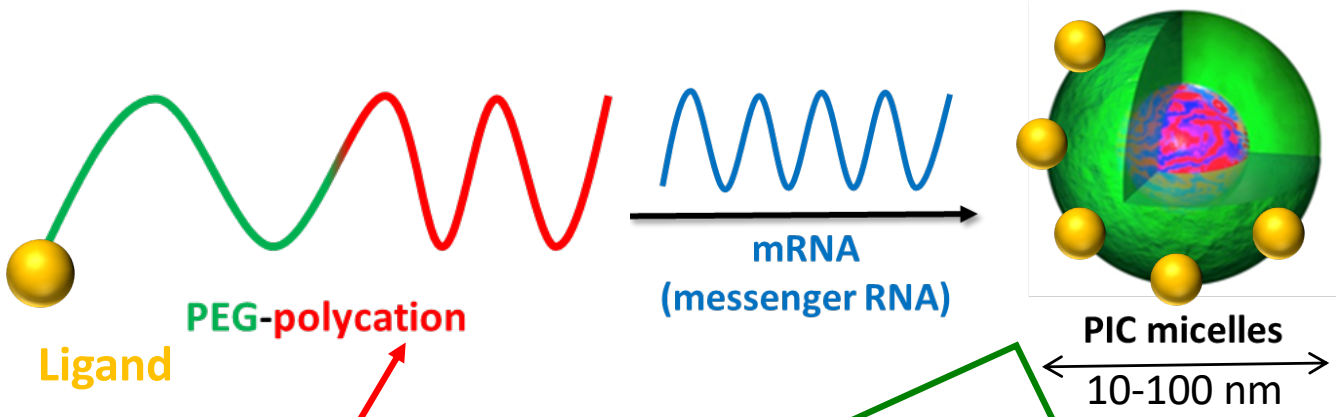
- **Protect drug payload**

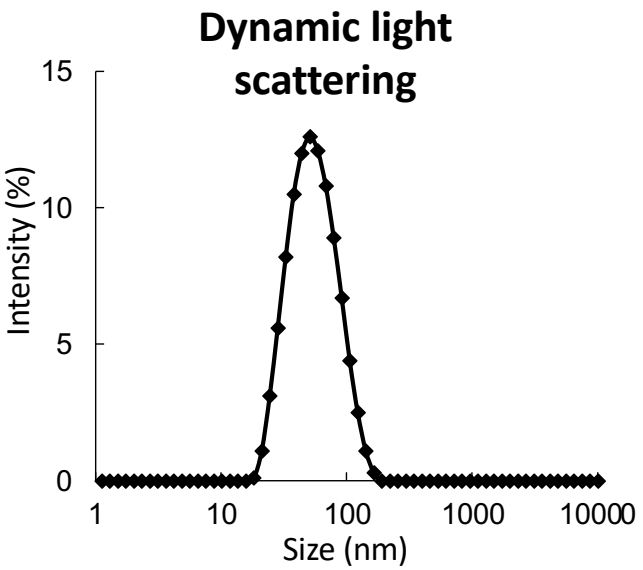
→Prevent degradation by enzymatic attacks

30–100 nm

H. Cabral *et. al.*, ***Chem. Rev.* 118** (2018) 6844-6892.

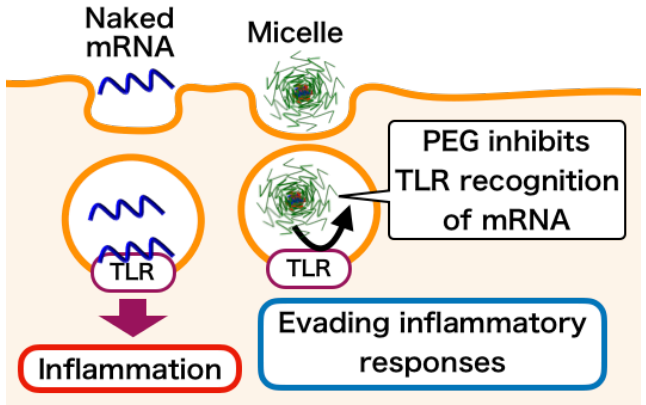
Polyplex micelles loaded with mRNA





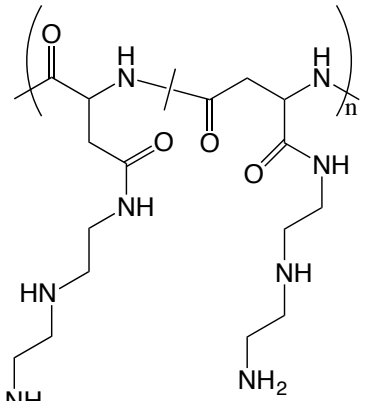
- Enhance cellular uptake
- **Reduce mRNA degradation**
- Decrease mRNA immunogenicity

Reduced mRNA immunogenicity



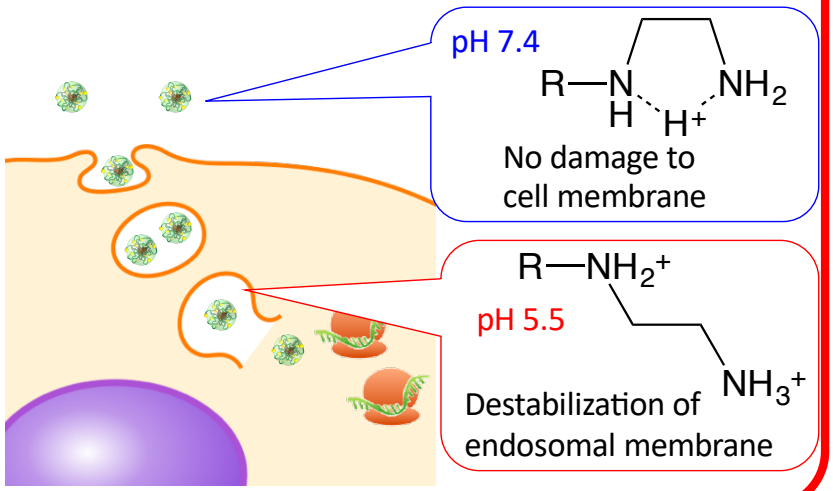
S. Uchida, et. al. ***PLoS One*** 2013, 8, e56220

PAsp(DET)



ChemMedChem (2006)
J Am Chem Soc (2008)
Biomaterials (2010)

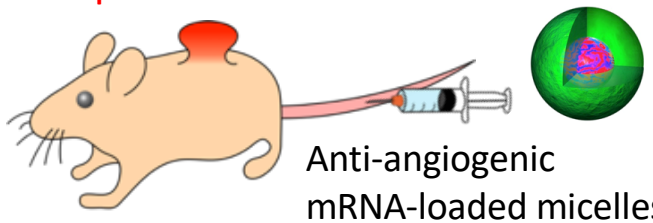
Efficient endosomal escape



mRNA-loaded micelles in disease models

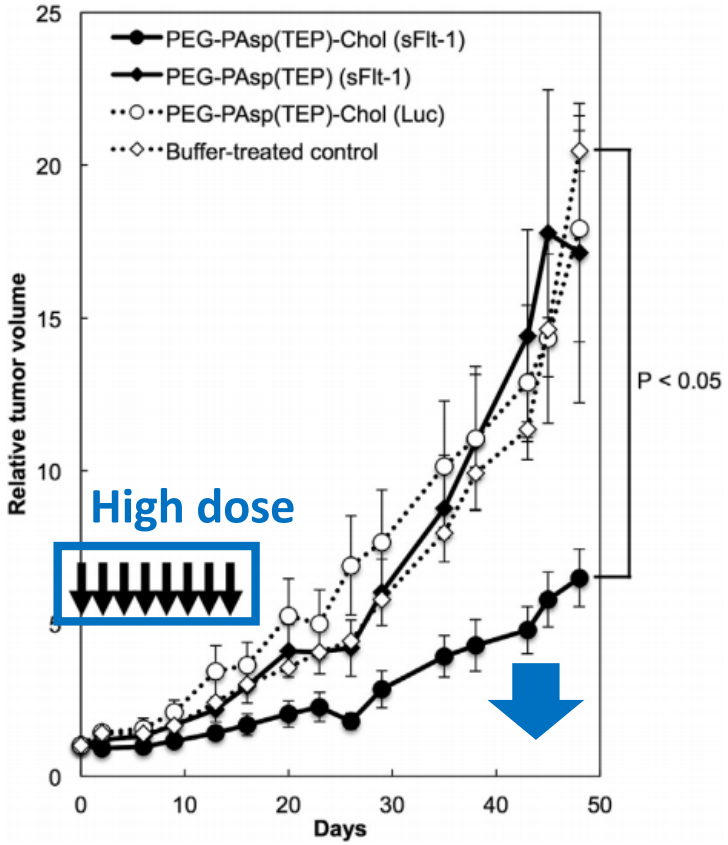
Pancreatic cancer

Human pancreatic cancer

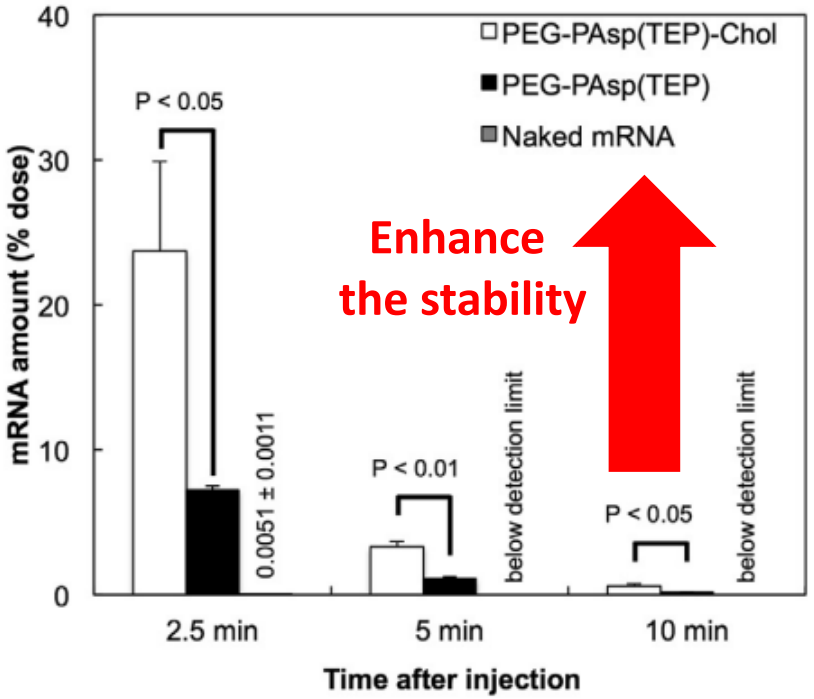


Anti-angiogenic
mRNA-loaded micelles

Anti tumor effect of mRNA-loaded micelles



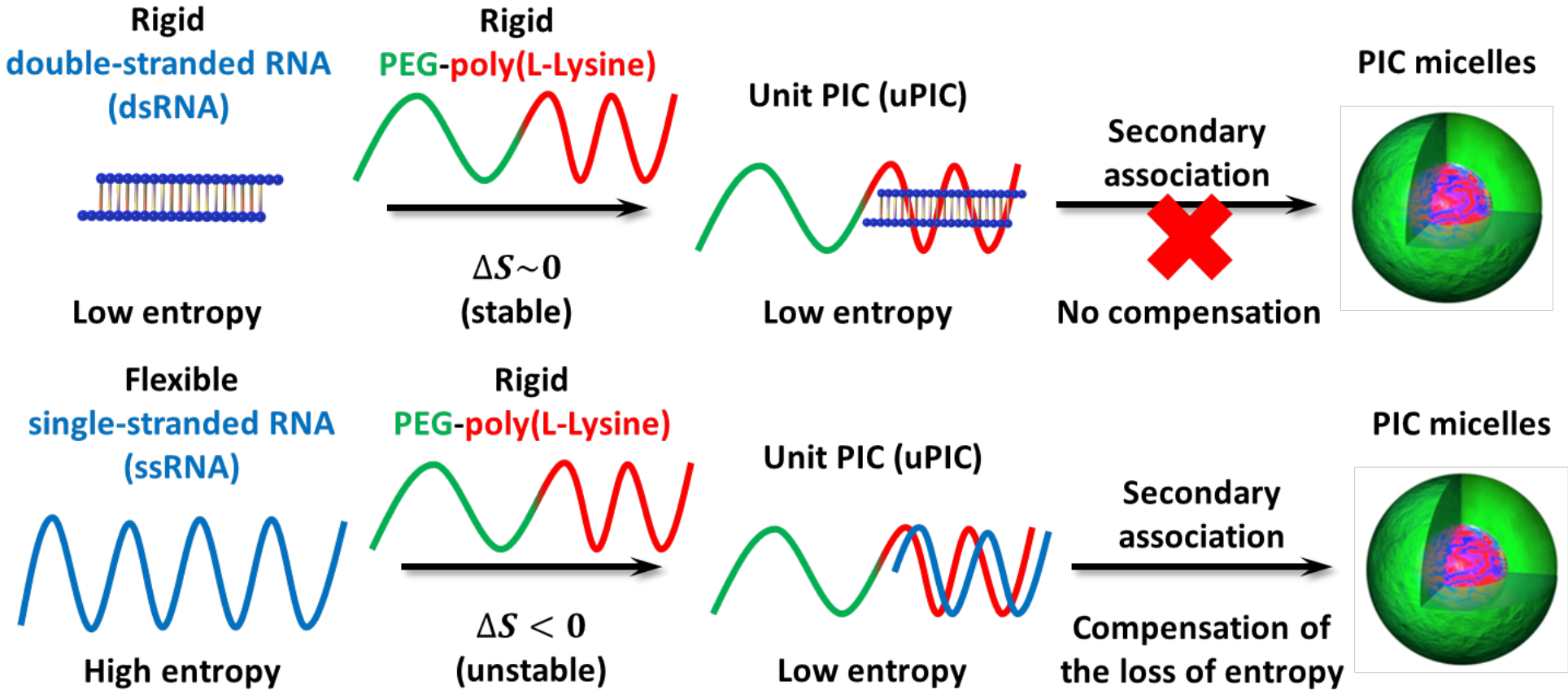
Blood circulation profile in mice



Enhance the stability
→Decrease dose,
Increase therapeutic outcomes

S. Uchida, et. al. ***Biomaterials*** 82, 221-228 (2016)

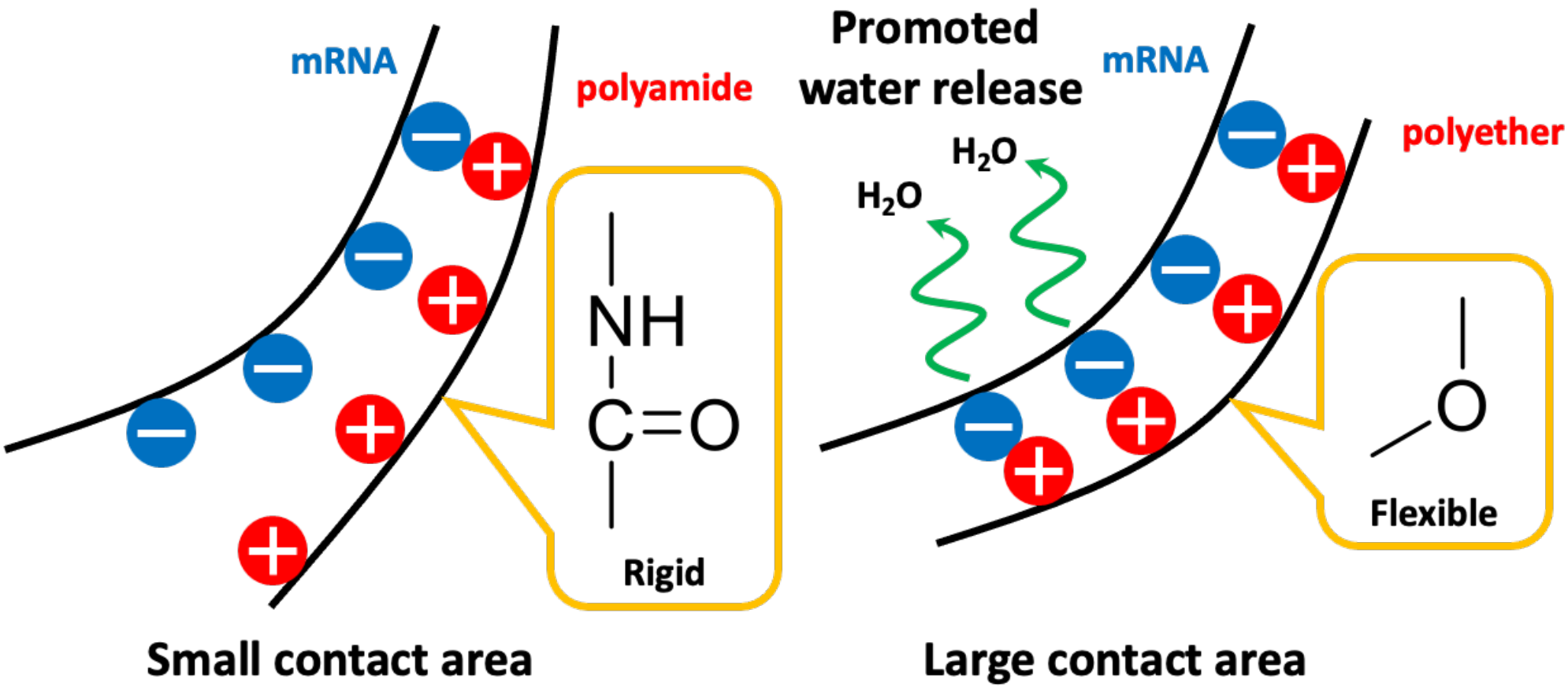
The flexibility of polymers and nucleic acids



Rigid block copolymers form unstable PIC with flexible RNA because of the loss of conformational entropy.

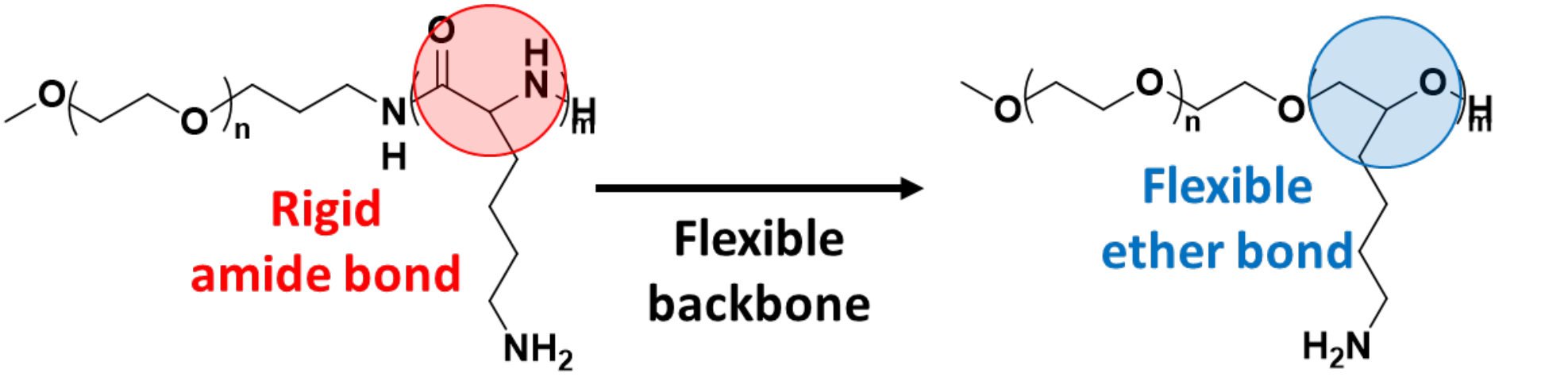
K. Hayashi *et. al.*, ***Macromol. Rapid Commun.*** **37** (2016) 486-493.

The flexibility of polymers and mRNA



Flexible polyether may increase contact area and promote water release during PIC formation

The design of the flexible block copolymers

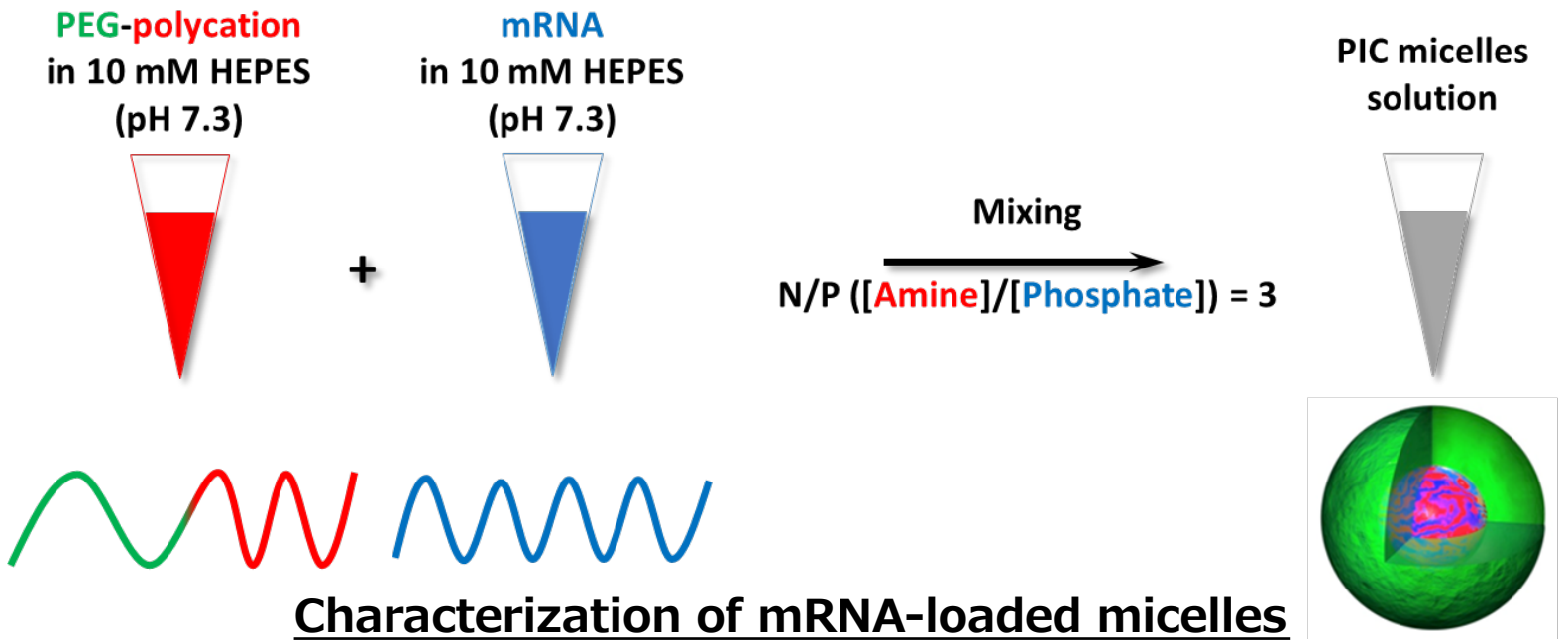


PEG-poly(L-Lysine)
(PEG-PLL)

PEG-poly(Glycidyl Butyl Amine)
(PEG-PGBA)

PEG-PGBA may decrease surface area ($\Delta H<0$) and promote the release of free water ($\Delta S>0$), resulting in strong binding with mRNA

Preparation of mRNA-loaded micelles



Characterization of mRNA-loaded micelles
(DLS, FCS, 25°C)

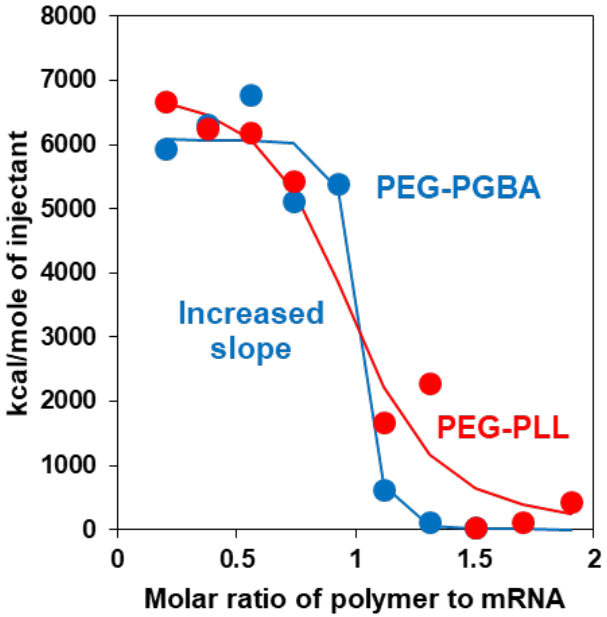
Sample	Cumulant diameter [nm]	Polydispersity index	Association number of mRNA
PEG-PLL/mRNA	52	0.18	1.6
PEG-PGBA/mRNA	56	0.16	1.4

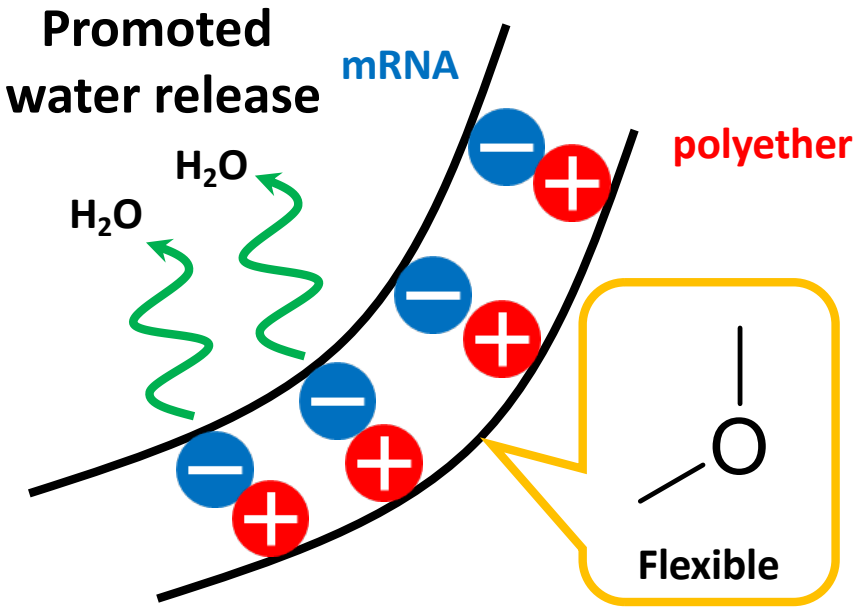
The size and the association number of mRNA of PEG-PGBA was comparable with that of PEG-PLL.

T. Miyazaki *et. al.*, ***Adv. Healthc. Mater.*** (2020)

Characterization of mRNA-loaded micelles

Isothermal titration calorimetry (ITC)
(0.1 mg/mL, 1 mg/mL, 25°C)





Large contact area

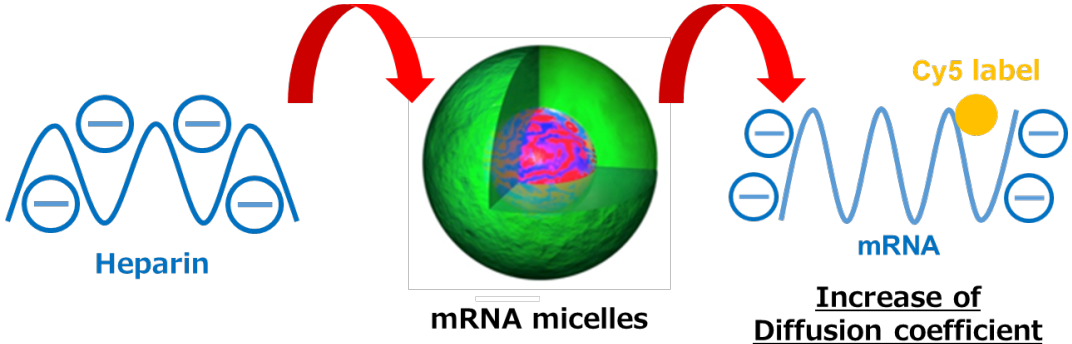
Sample	Binding ratio	ΔH [kcal/mol]	ΔS [cal/(mol·K]	K [M^{-1}]
PEG-PLL/mRNA	0.917	7.0	52.8	2.6×10^{6}
PEG-PGBA/mRNA	0.925	6.1	57.7	1.4×10^{8}

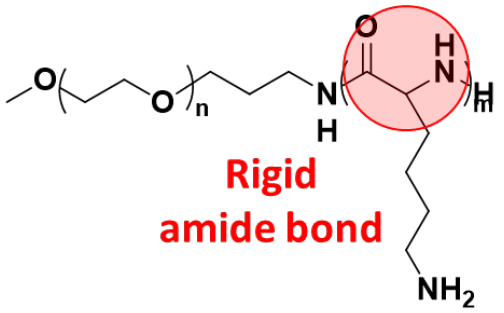
50x

Lower enthalpy, higher entropy→Higher binding affinity

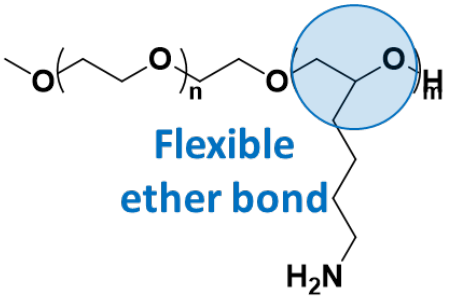
T. Miyazaki *et. al.*, ***Adv. Healthc. Mater.*** (2020)

Micelle stability against polyanion





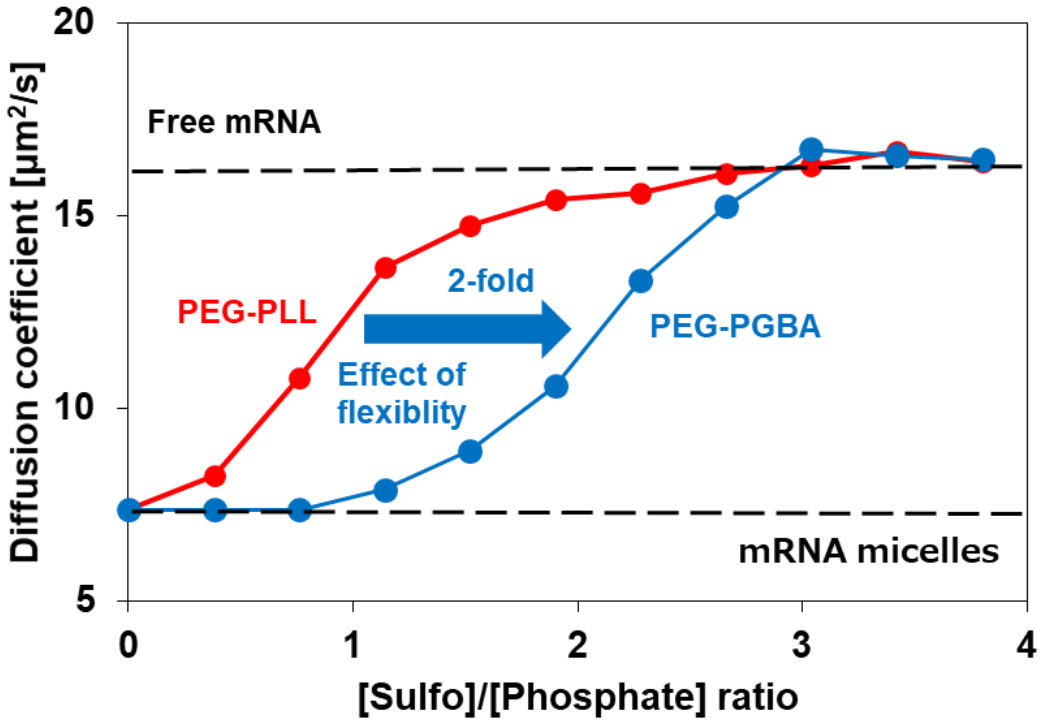
PEG-poly(L-Lysine)
(PEG-PLL)



PEG-poly(Glycidyl Butyl Amine)
(PEG-PGBA)

Release of mRNA

Release of mRNA
(FCS, 6h, 25°C)

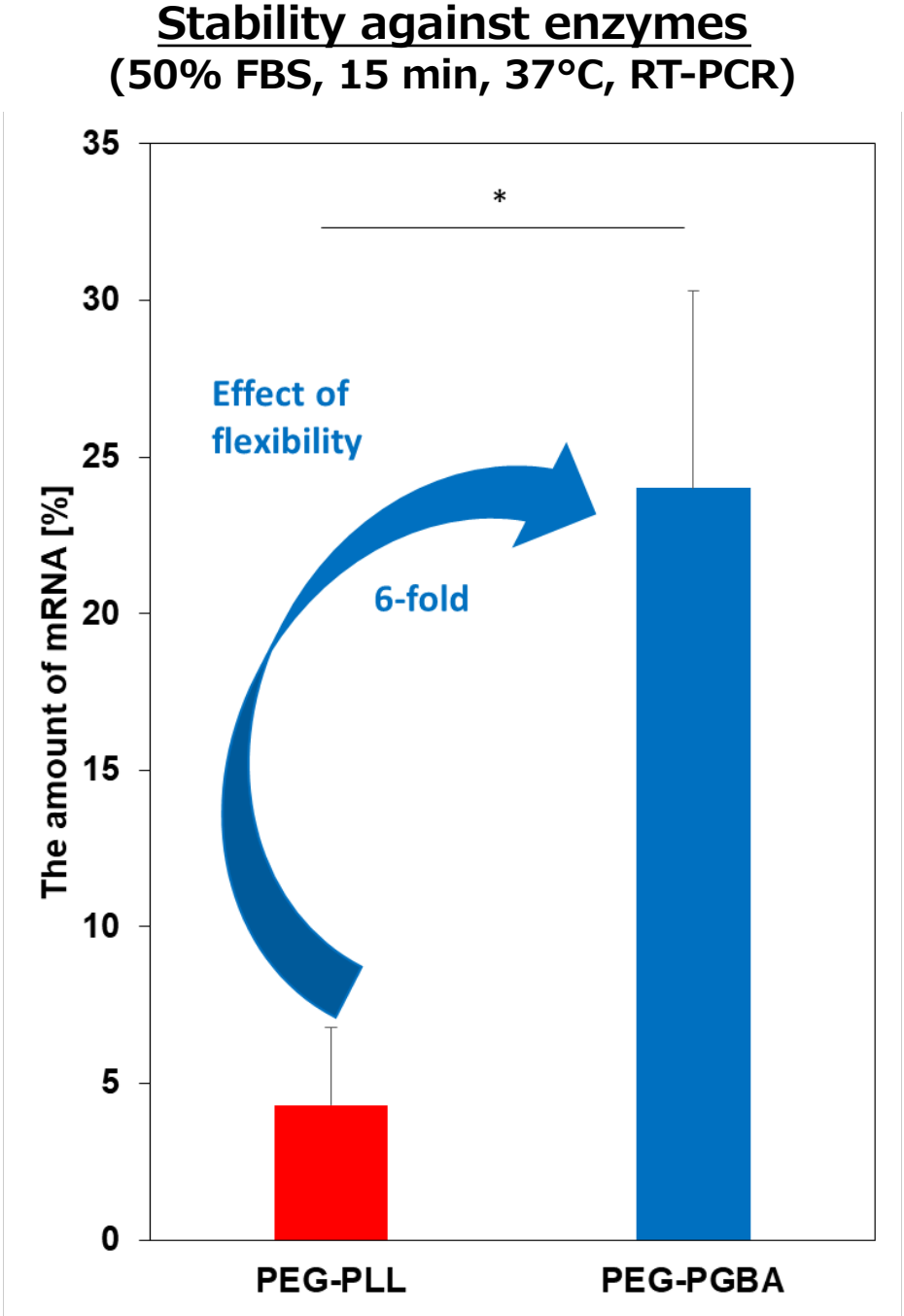


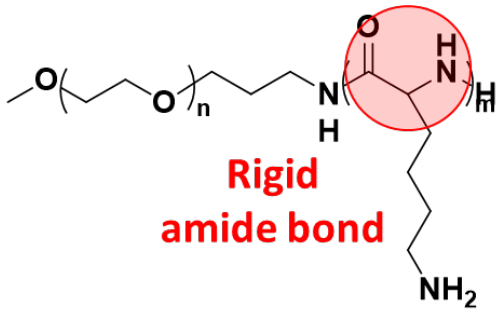
Increase of heparin

The stability was enhanced by flexible PGBA chain.

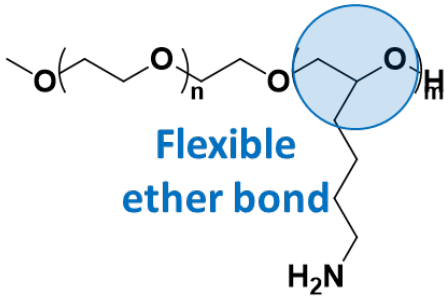
T. Miyazaki *et. al.*, ***Adv. Healthc. Mater.*** (2020)

Micelles stability against enzymes in serum





PEG-poly(L-Lysine)
(PEG-PLL)



PEG-poly(Glycidyl Butyl Amine)
(PEG-PGBA)

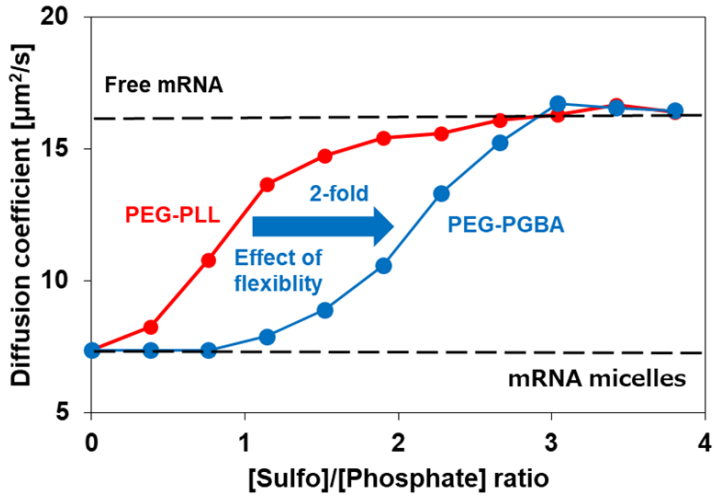
The stability was enhanced by flexible PGBA chain.

T. Miyazaki *et. al.*, ***Adv. Healthc. Mater.*** (2020)

In vitro gene expression

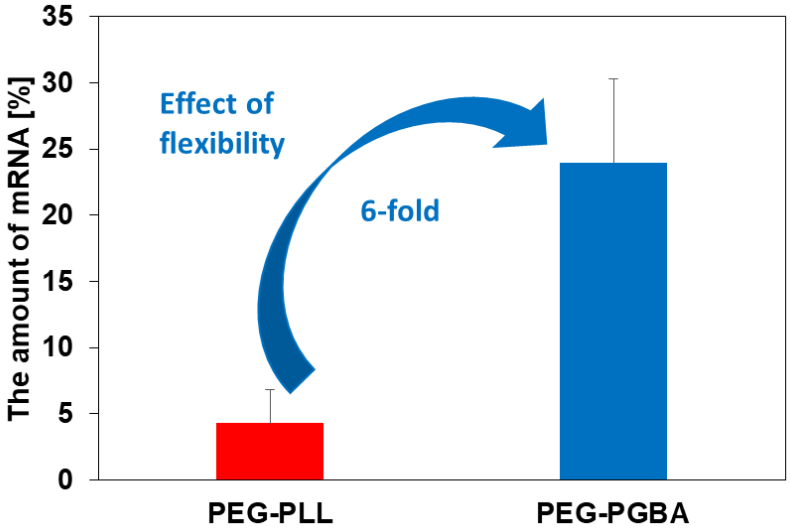
Stability against polyanion

Glycosaminoglycan (GAG) as polyanion on the cell surface (*Nature* 446 (2003) 1030-37).



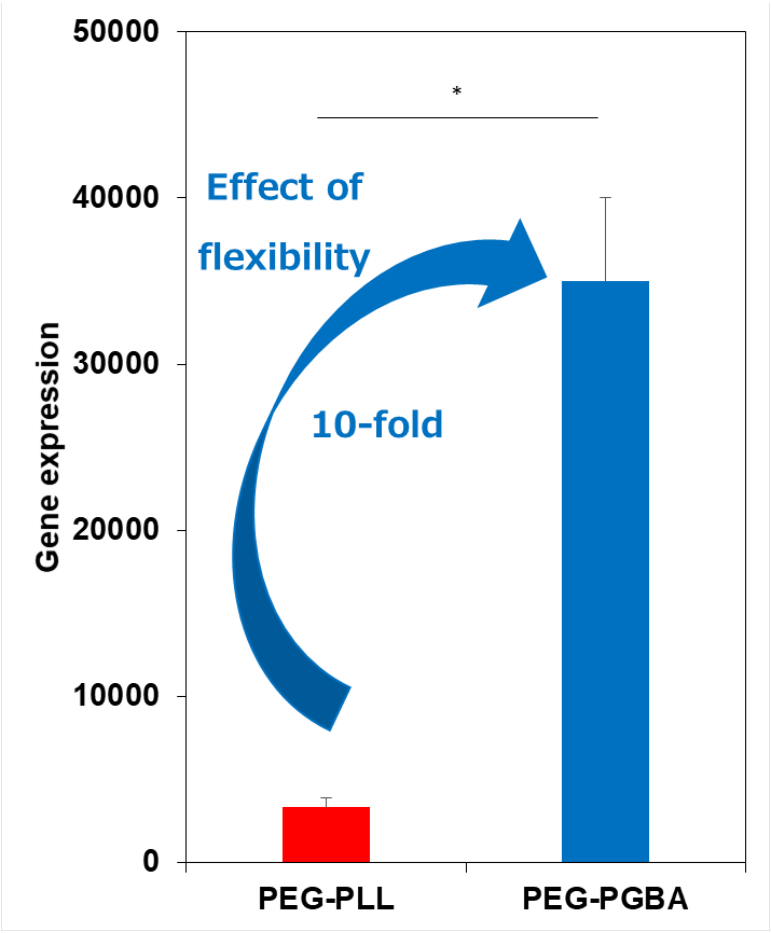
Stability against enzymes

RNase in cell culture medium.



Gene expression

(Huh-7 cells, 24h, luminometer)

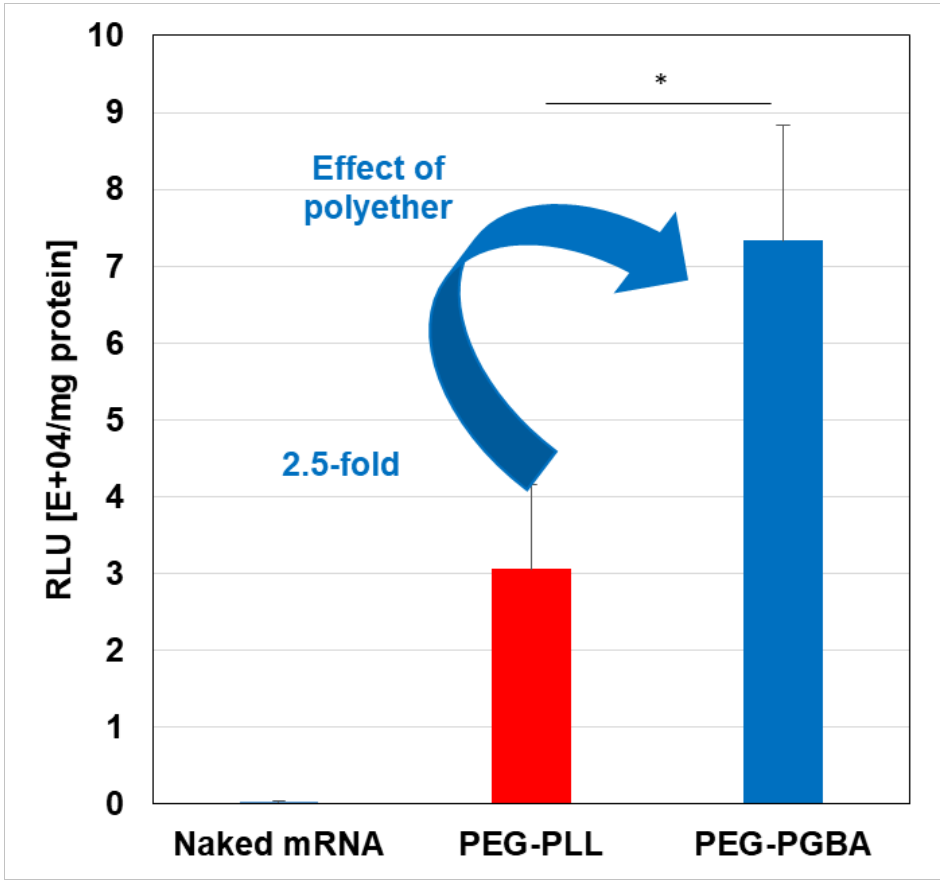


Enhanced stability
→Increased gene expression

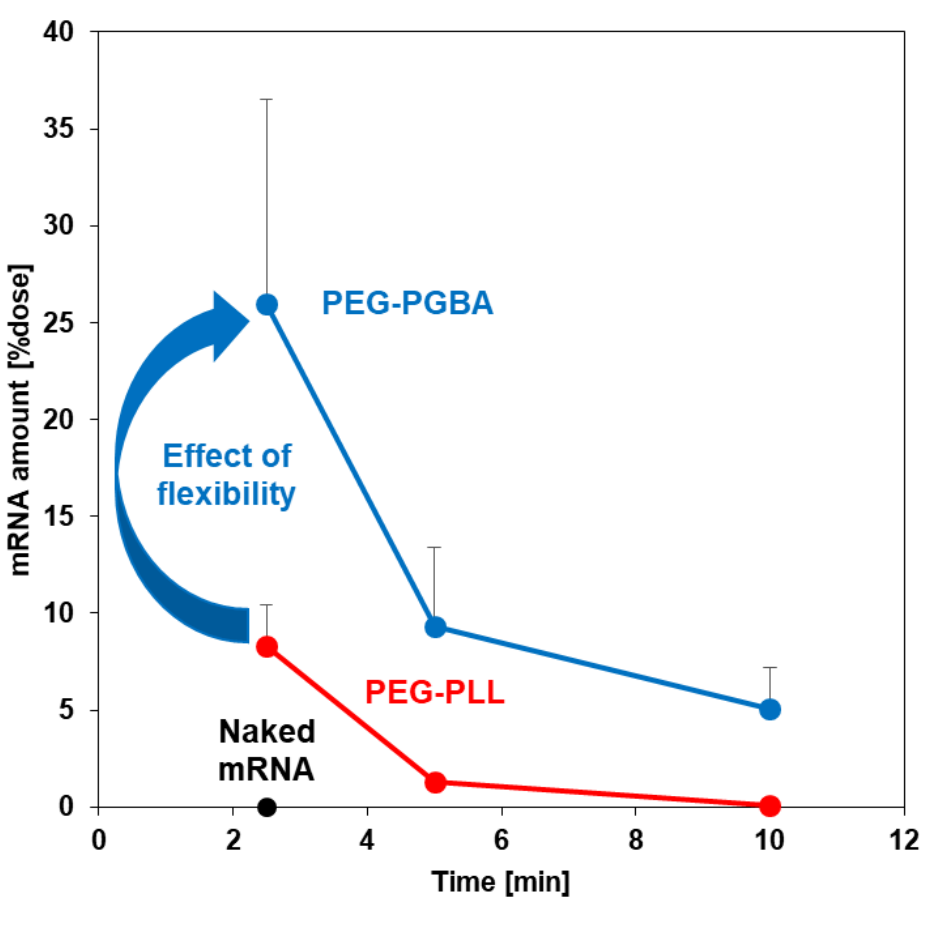
T. Miyazaki *et. al.*, ***Adv. Healthc. Mater.*** (2020)

In vivo gene expression

In vivo Gene expression
(Pulmonary administration, 24h, luminometer)



Blood circulation in mice
(RT-PCR)

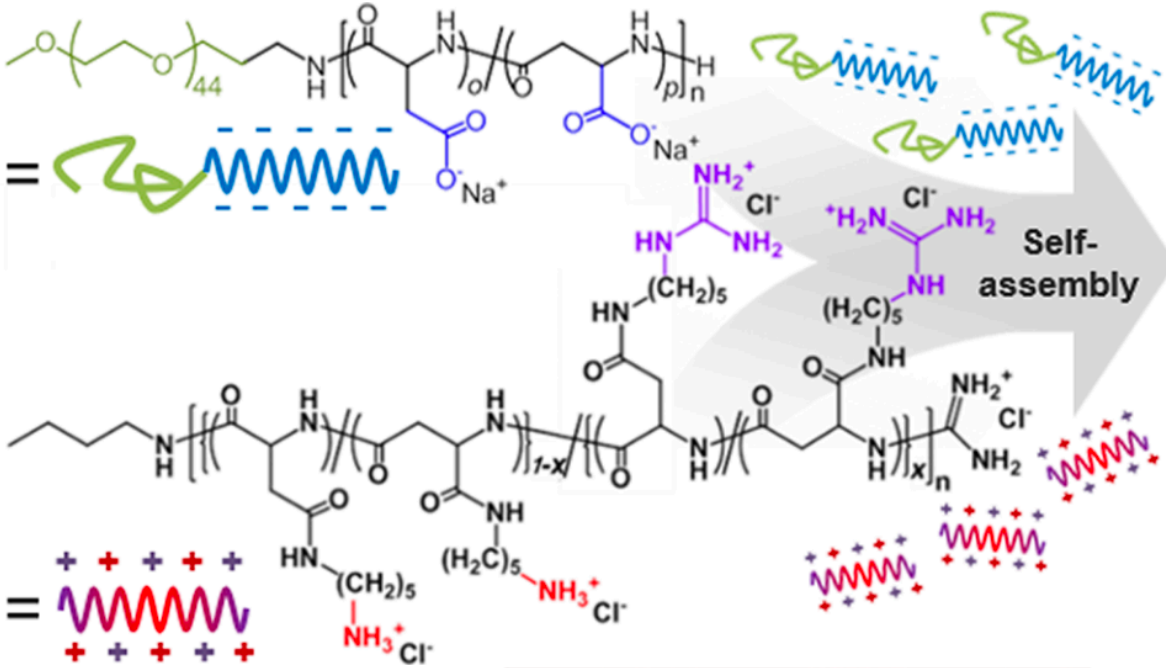


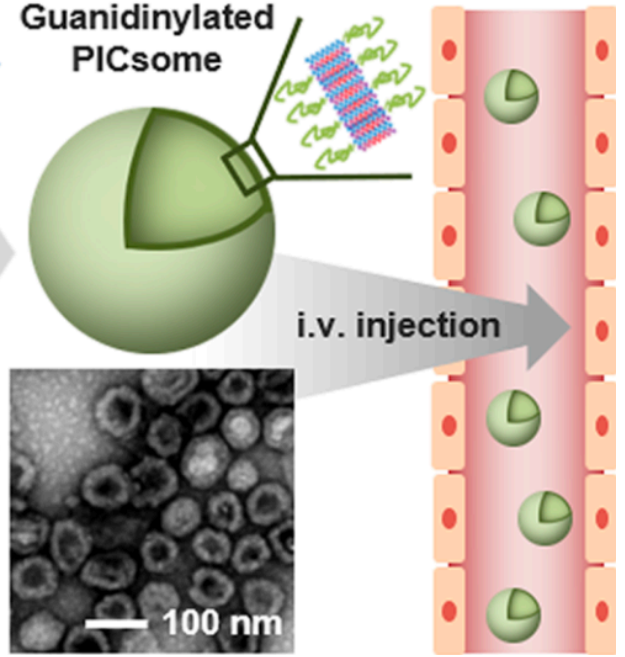
Enhanced stability
→Increased gene expression

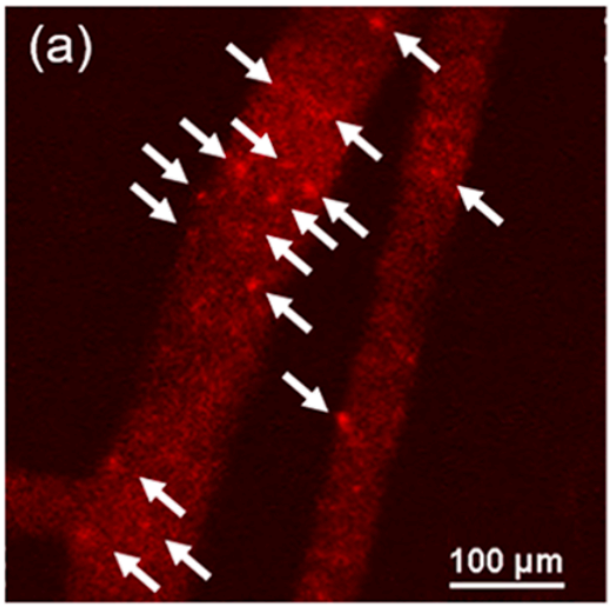
The stability was enhanced by flexible PGBA chain.

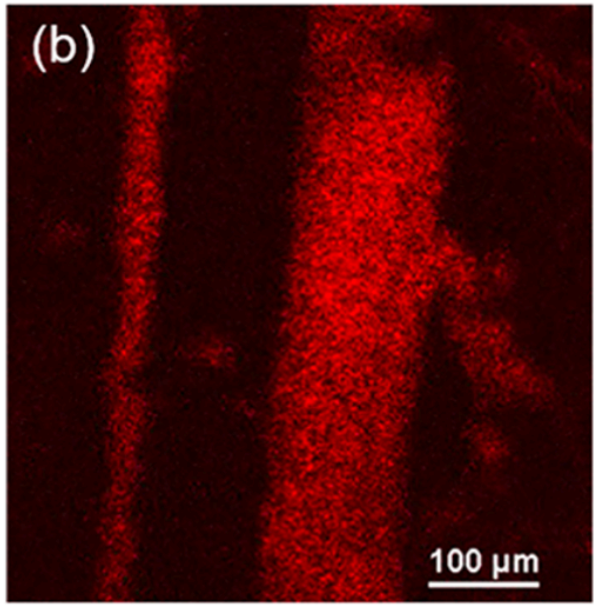
T. Miyazaki *et. al.*, ***Adv. Healthc. Mater.*** (2020)

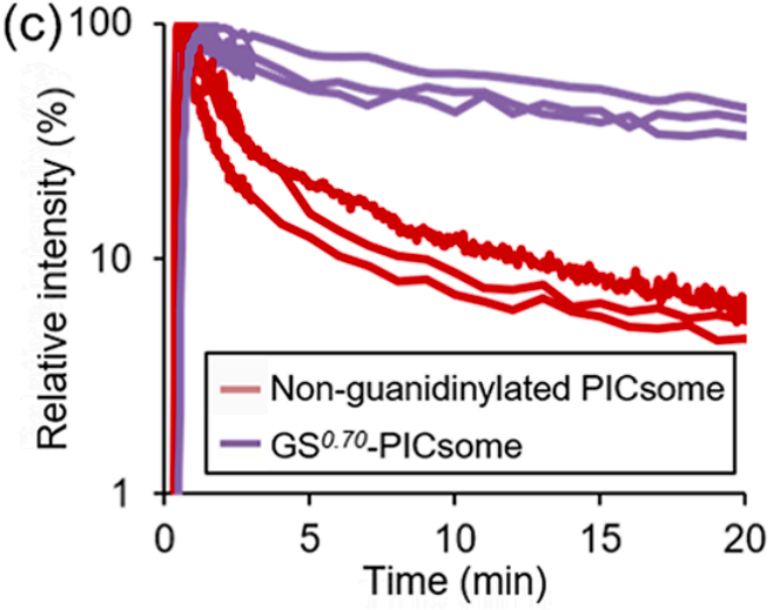
The stabilization by guanidine groups





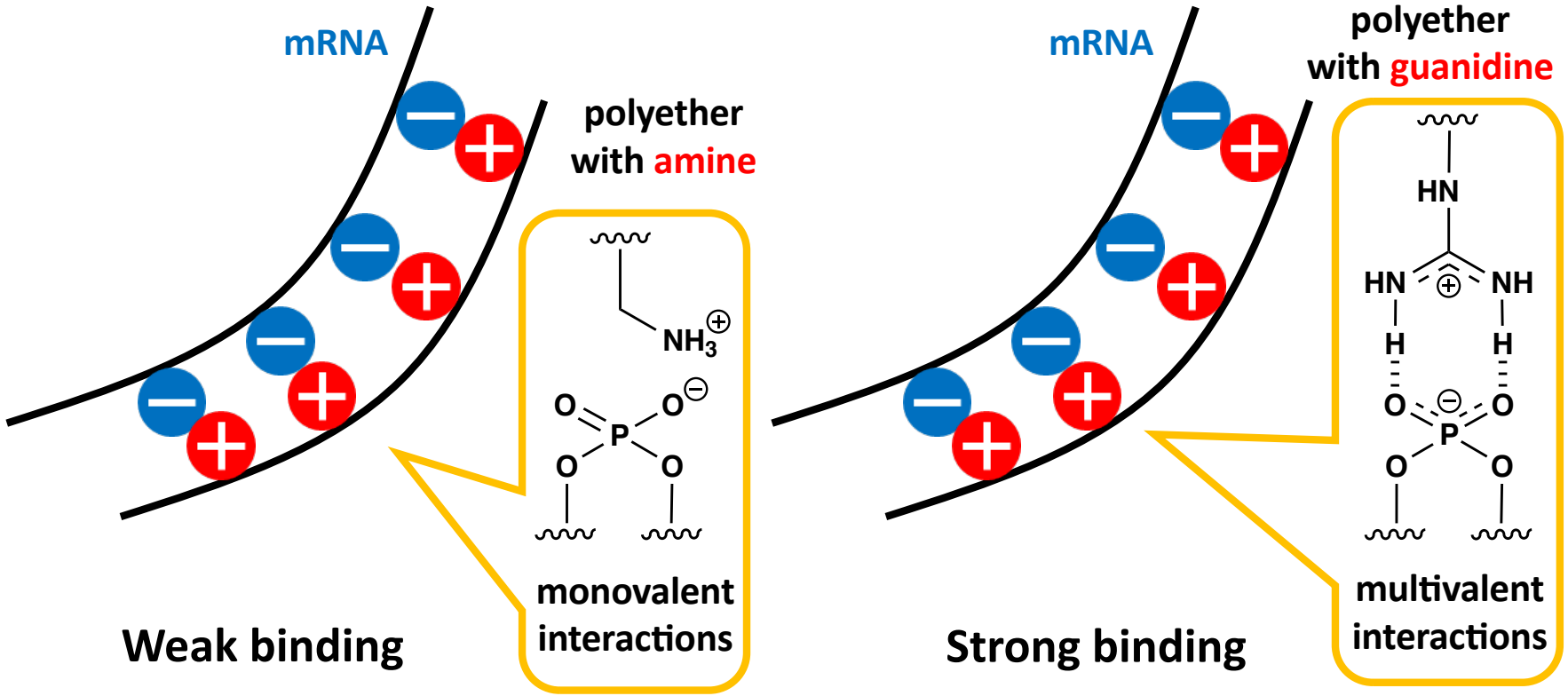






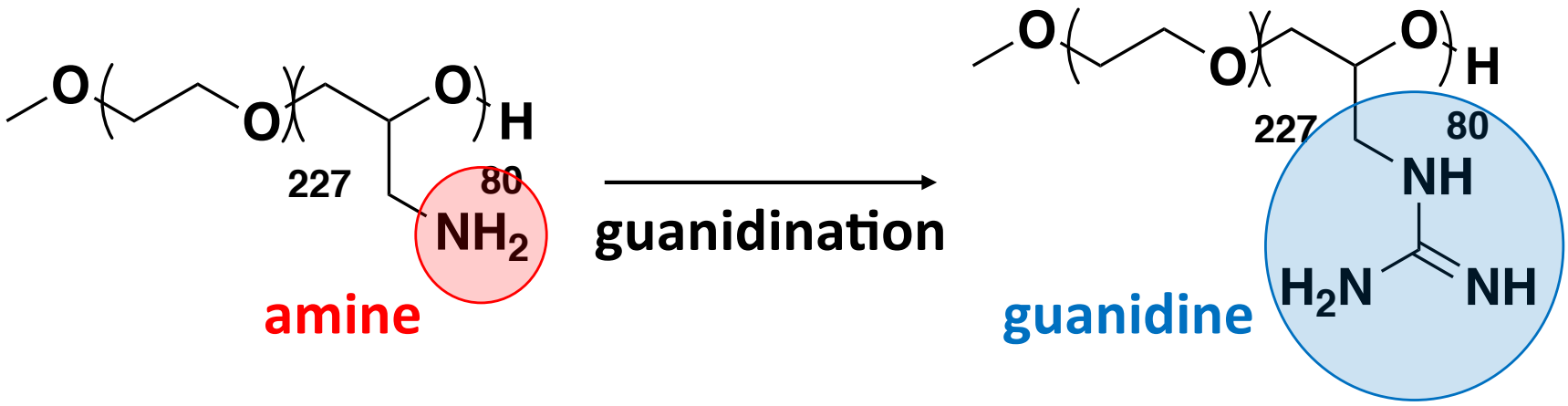
M. Hori *et. al.*, ***Biomacromolecules 19*** (2018) 4113-4121.

The multivalent interactions with mRNA



Guanidinated polymers may strongly bind to mRNA by multivalent interactions.

The design of flexible polymers with guanidine



PEG-poly(Glycidyl Methyl Amine)
(PEG-PGMA)

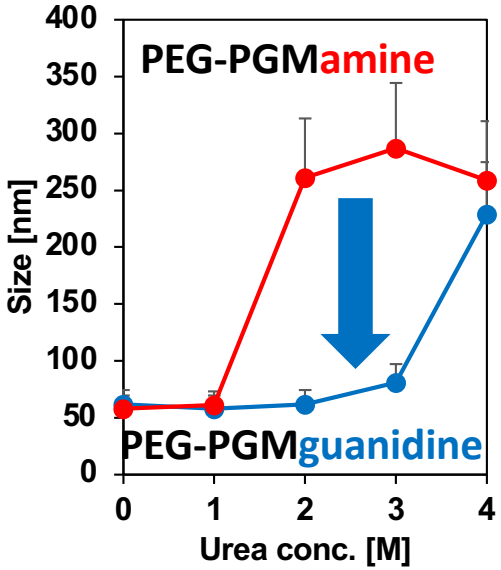
PEG-poly(Glycidyl Methyl Guanidine)
(PEG-PGMG)

PEG-PGMG may show multivalent binding to mRNA, resulting in stabilization of micelles

T. Miyazaki *et. al.*, ***Eur. Polym J.*** (2020)

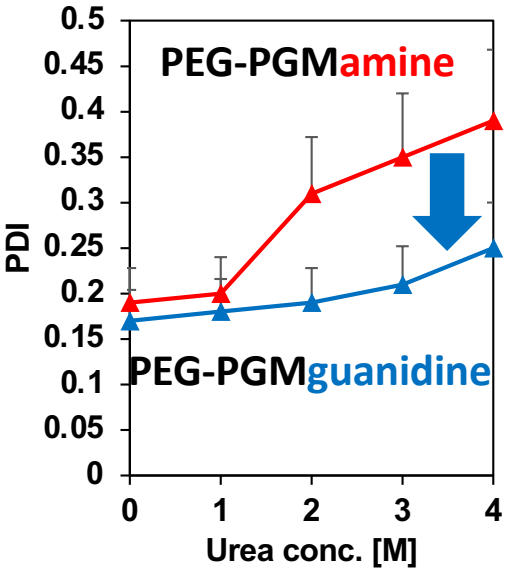
Guanidine stabilized mRNA-loaded micelles

Stability against urea



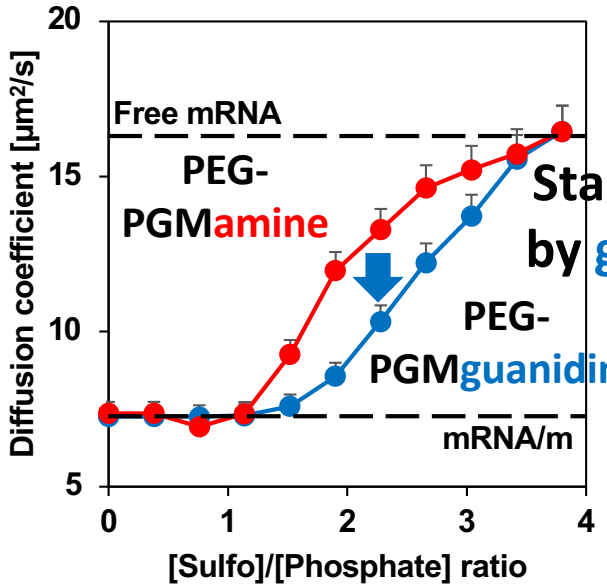
Stabilization by guanidine

Stability against urea



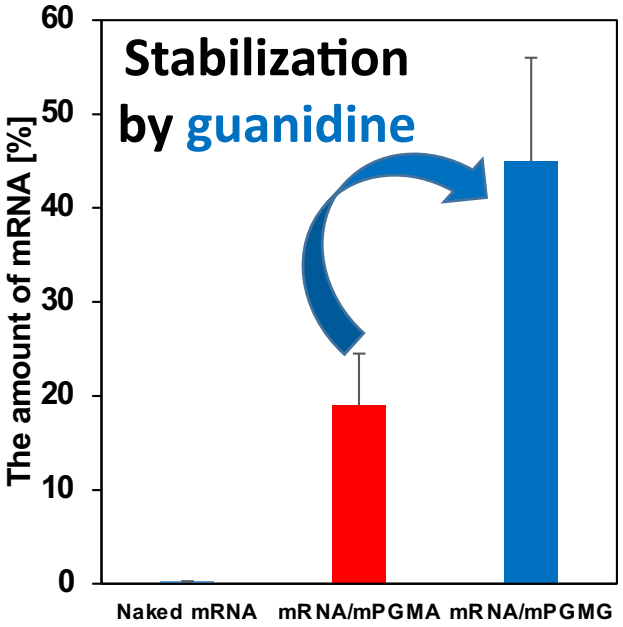
Stabilization by guanidine

Stability against polyanion



Stabilization by guanidine

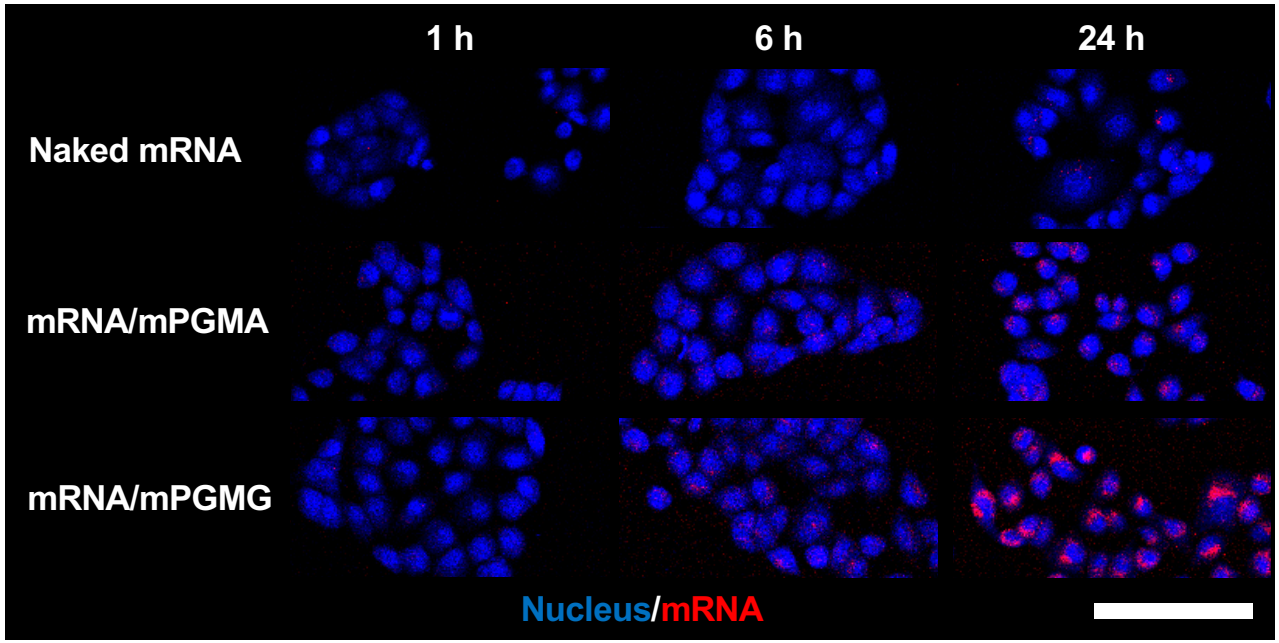
Stability against nucleases

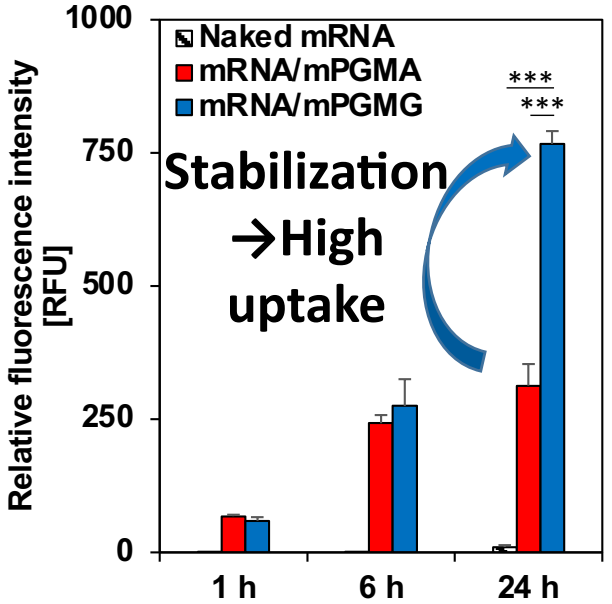


T. Miyazaki
Eur. Polym J.
(2020)

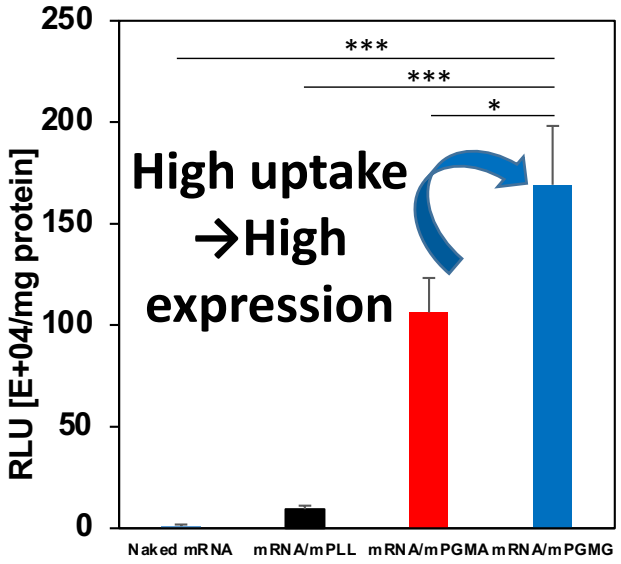
High performance in cultured cells

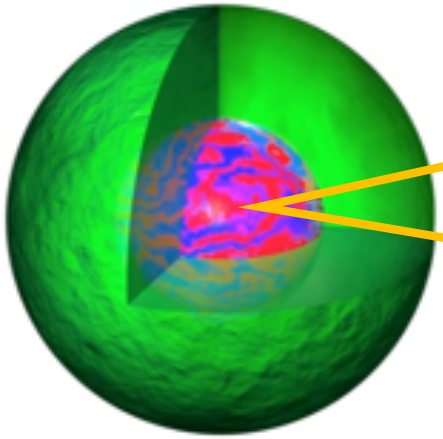
Cellular uptake

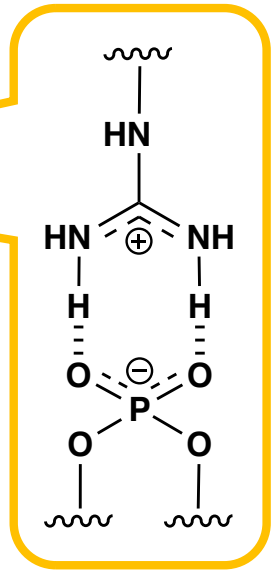




mRNA expression



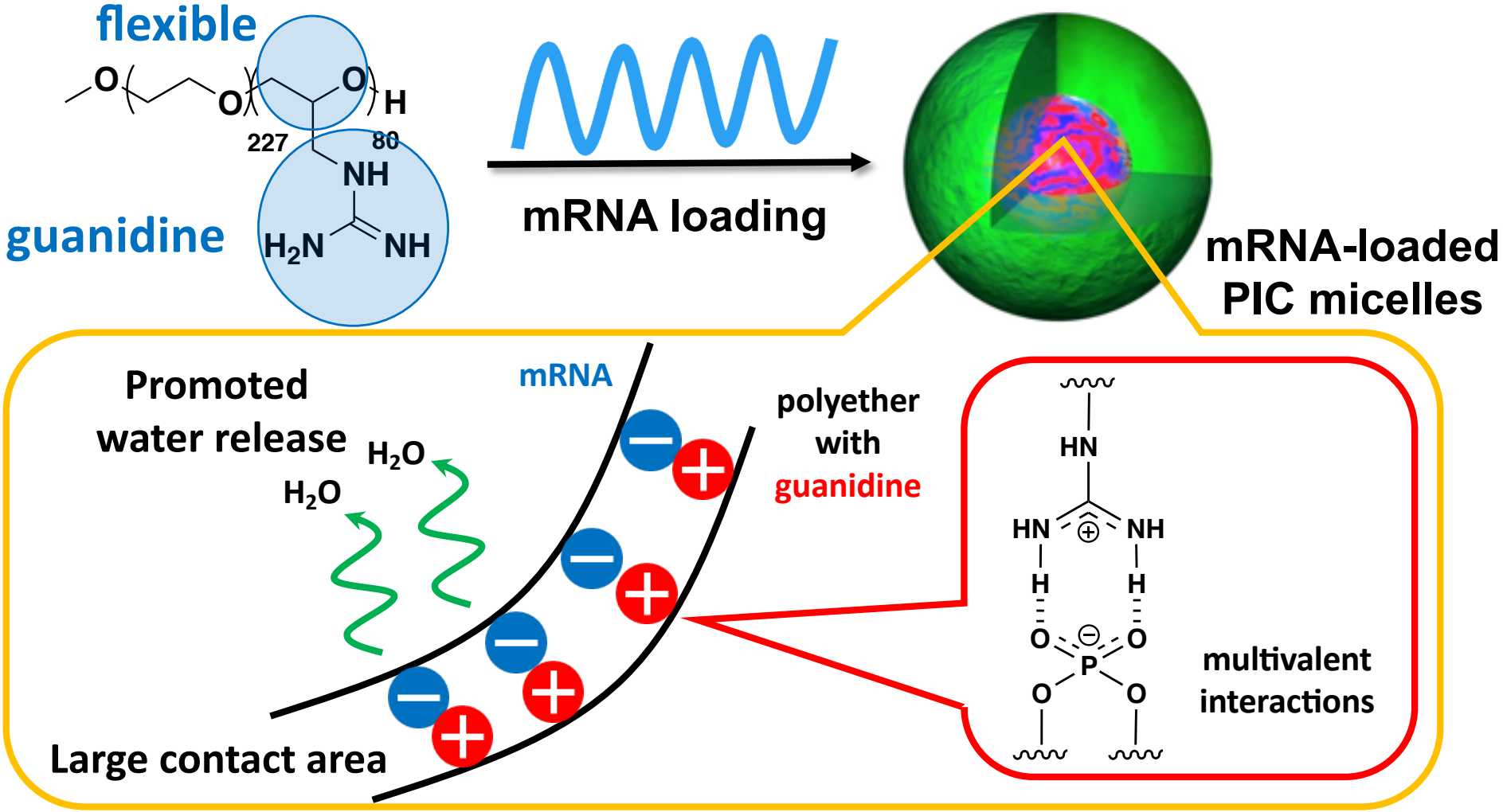




mRNA-loaded micelles with high performance

T. Miyazaki
Eur. Polym J.
(2020)

Flexible polycation-based mRNA delivery



Flexible polycation-based systems showed high performance *in vitro* and *in vivo*

PCT/JP2018/016798 T. Miyazaki *et. al.*, ***Eur. Polym J.*** (2020) T. Miyazaki *et. al.*, ***Adv. Healthc. Mater.*** (2020)

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Prof. Dr. Kouhei Tsumoto
Prof. Dr. Satoru Nagatoishi

iCONM
Innovation Center of NanoMedicine

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