New Class of Cell-Penetrating Peptides and Its Application to Myotonic Dystrophy Type 1

20161112 Atsushi Hayata

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1-2. Helical Poly(Arginine) Mimics [2)]

2. Application for Myotonic Dystrophy Type I
(Collaboration of Prof. Cheng and Prof. Zimmerman)

(1) Lu, H.; Wang, J.; Bai, Y.; Lang, J. W.; Liu, S.; Lin, Y.; Cheng, J. *Nat. Commun.* **2011**, *2*, 206.
(2) Tang, H.; Yin, L.; Kim, K. H.; Cheng, J. *Chem. Sci.* **2013**, *4*, 3839

1-0. Cell-Penetrating Peptides (CPPs)

Table 1. Six CPPs Described Herein[a] 1)

CPP	sequence	length	net charge	molecular weight	amphipathicity	secondary structure in the presence of phospholipids
Penetratin	RQIKIWFQNRRMKWKK	16	+7	2247	secondary	α-helix, β-sheet
Tat	GRKKRRQRRRPPQ	13	+8	1719	nonamphipathic	random coil
Arg9	RRRRRRRRR	9	+9	1423	nonamphipathic	random coil
R6/W3	RRWWRRWRR	9	+6	1514	secondary	α-helix

[redacted]

low net charge and helix

high net charge and random coil

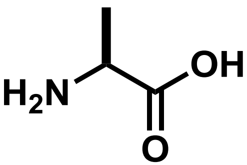
High net charged helical peptides have never been reported.

About Arg-rich peptides: Please see LS Takuya Kaji 150919

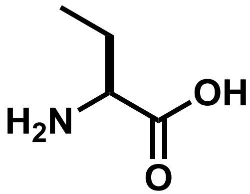
(1) Pisa, N. D.; Chassaing, G.; Swiecicki, J-M. *Biochemistry*, **2014**, *54*, 194.

1-1. Side Chain Hydrocarbons (Non-Charged)[1]

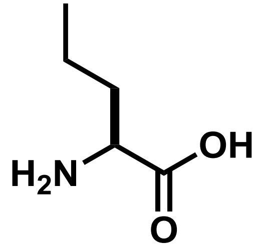
succinyl-Tyr-Ser-Glu_4-Lys_4-(Xaa_3)-Glu_4-Lys_4-NH_2,



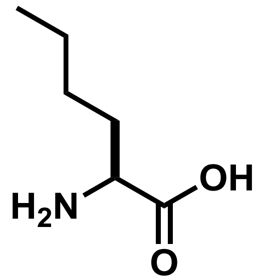
Ala



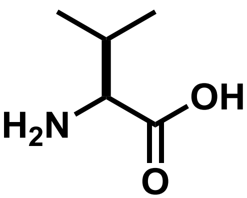
Abu



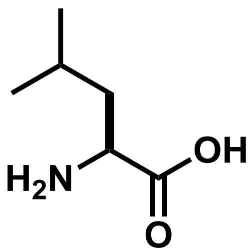
Nva



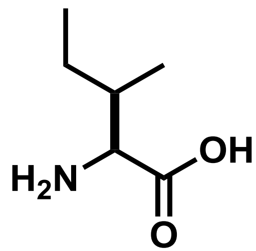
Nle



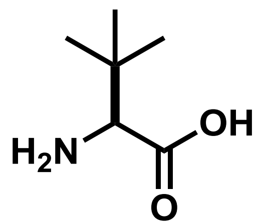
Val



Leu



Ile



Tle

Peptide	$-[\theta]_{222}$
Ala_3	27,300 ± 800
Abu_3	27,000 ± 900
Nva_3	27,520 ± 800
Nle_3	27,370 ± 600
Leu_3	24,100 ± 700
Ile_3	17,800 ± 500
Val_3	16,000 ± 450
Tle_3	4,800 ± 300

Linear elongation of side chain with hydrocarbons does not affect helical structure.

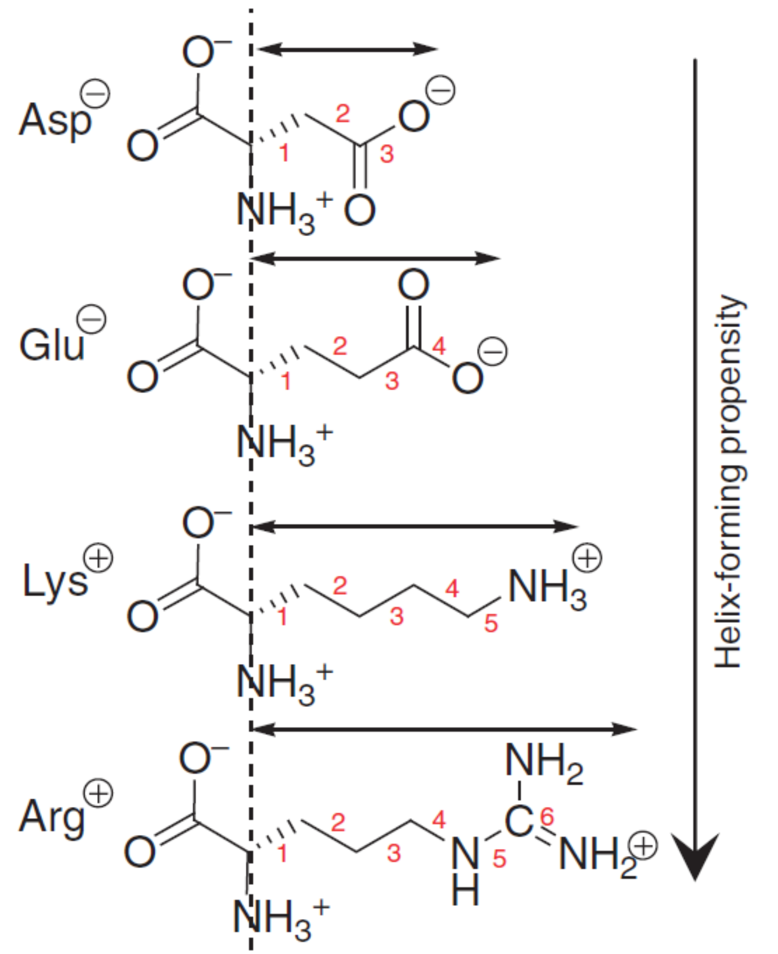
(1) Lyu, P. C.; Sherman, J. C.; Chen, A.; Kallenbach, N. R. *Proc. Natl Acad. Sci. USA*, **1991**, *88*, 5317.

1-1. Side Chain Hydrocarbons (Charged)

1)

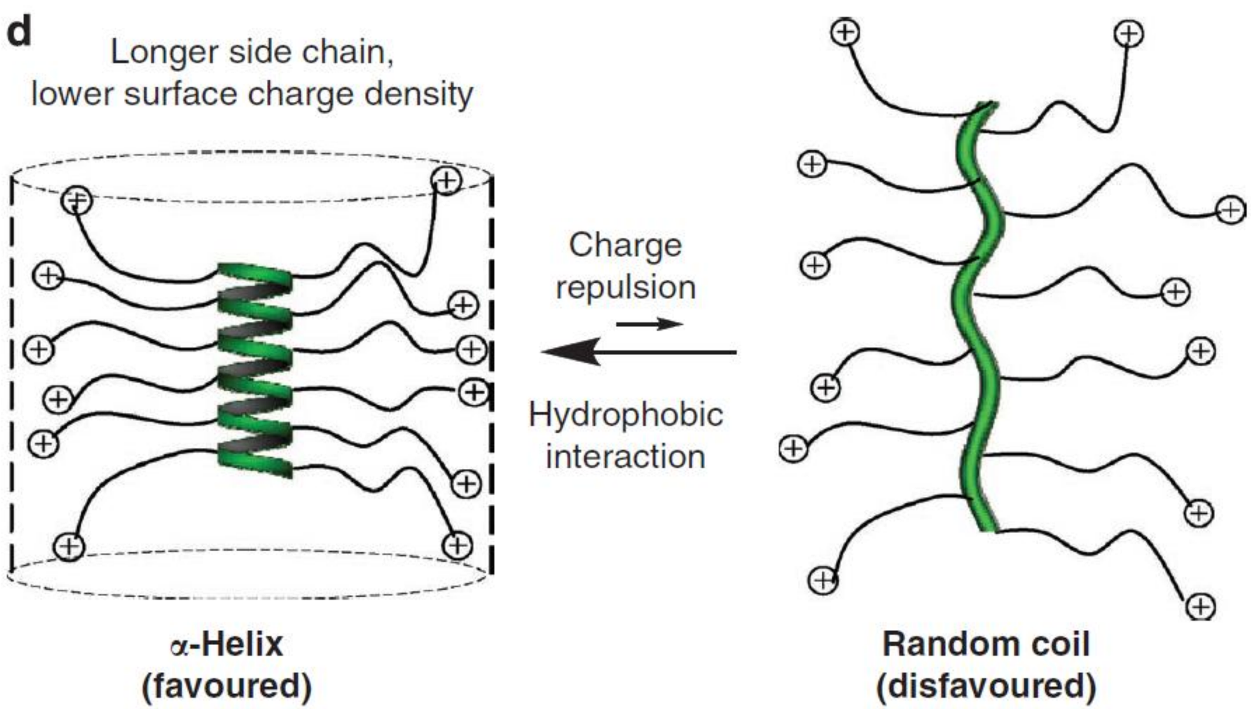
Amino acid	Helix propensity (kcal/mol)
Ala	0.00
Glu^0	0.16
Leu	0.21
Met	0.24
Arg^+	0.21
Lys^+	0.26
Gln	0.39
Glu^-	0.40
Ile	0.41
Asp^0	0.43
Ser	0.50
Trp	0.49
Tyr	0.53
Phe	0.54
Val	0.61
Thr	0.66
His^0	0.56
His^+	0.66
Cys	0.68
Asn	0.65
Asp^-	0.69
Gly	1.00
Pro	3.16

b Distance between the charge and the α-carbon on the backbone

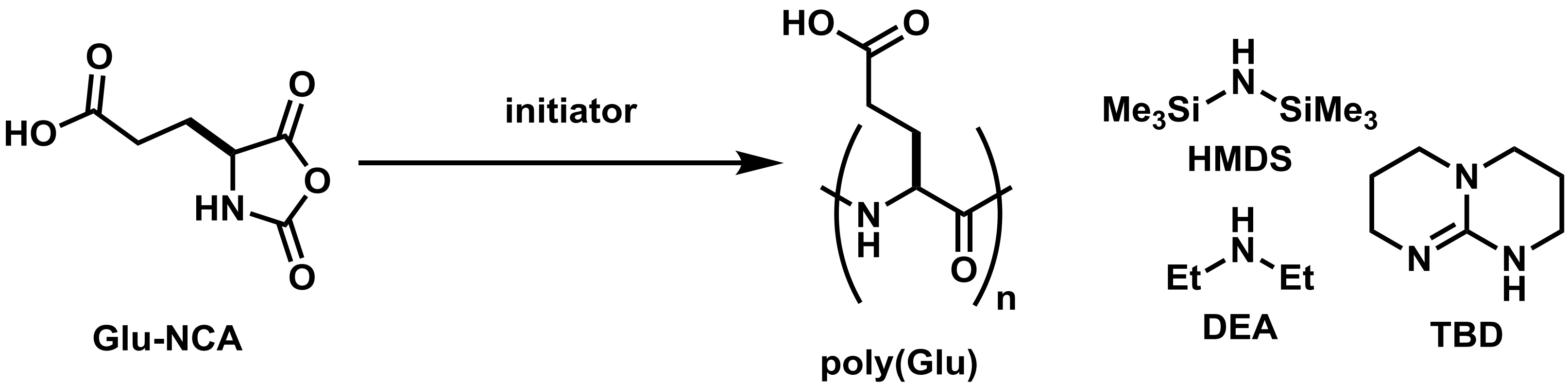


(1) Pace, C. N.; Scholtz, J. M. *Biophys. J.*, **1998**, *75*, 422.

1-1. Working Hypothesis



1-1. Ring-Opening-Polymerization (ROP)

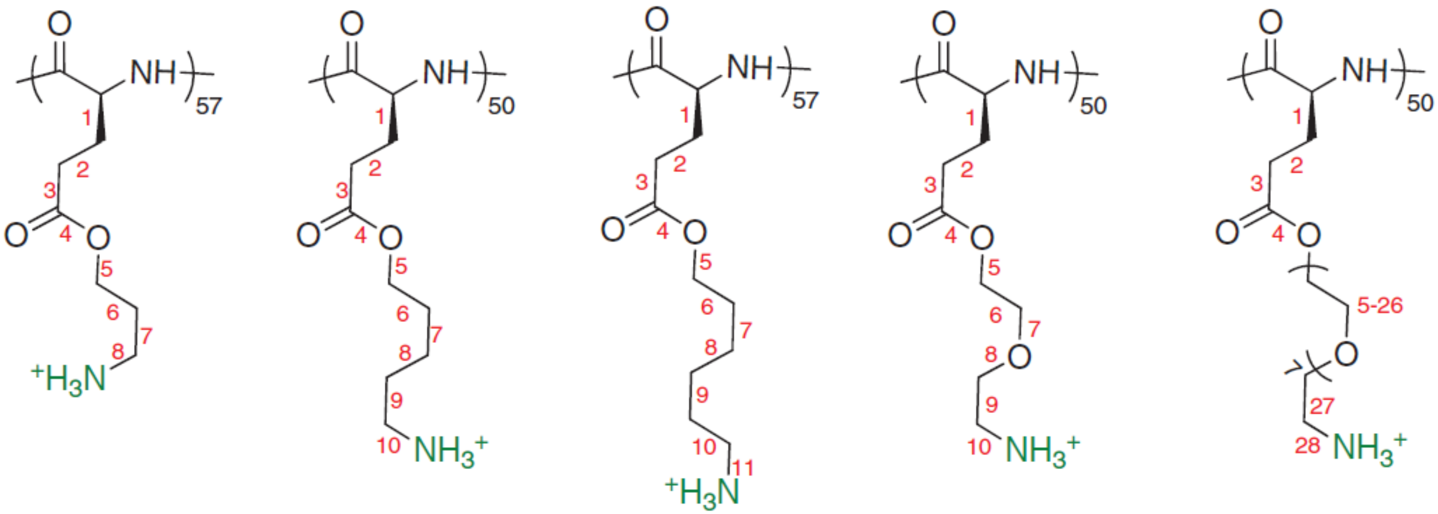


entry	initiator (equiv of NCA)[a]	obtained M_n (g/mol)	expected M_n (g/mol)	M_n (obt.)/ M_n (exp.)	M_w/M_n	conv.[b]
1	HMDS (50 Glu)	8500	10950	0.78	1.26	>99
2	HMDS (100 Glu)	21800	21900	0.99	1.2	>99
3	HMDS (200 Glu)	42800	43800	0.98	1.19	>99
4	DEA (50 Glu)	36600	10950	3.34	1.78	98
5	TBD (100 Glu)	98800	21900	4.51	1.33	86
6	TBD (150 Glu)	102300	32850	3.11	1.3	86

- controlled MWs
- narrow MWDs
- quantitative monomer conversion

(1) Lu, H.; Cheng, J *J. Am. Chem. Soc.* **2007**, *129*, 14114.

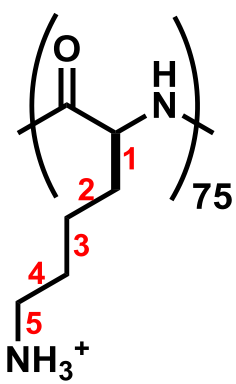
1-1. Poly Amino Alkyl Glutamate



Polymer: PAPG$_{57}$ PAPTG$_{50}$ PAHG$_{57}$ PAEG$_{50}$ PAHEG$_{50}$

pentanyl hexyl etyleneglycol heptaetyleneglycol

Poly(γ–(3-aminopropyl)-L-glutamate)



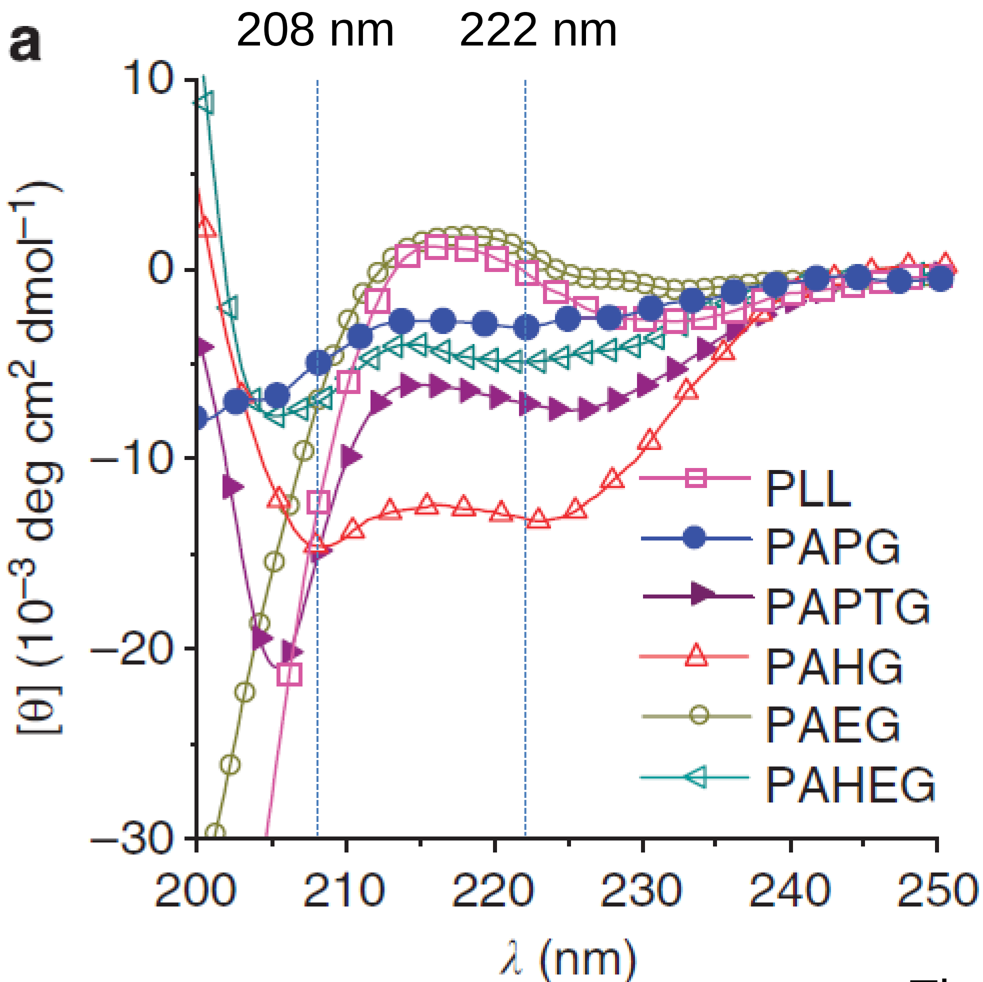
PLL$_{75}$

[PLL: poly(L-lysine)]

Control polymer

Raondom coil

1-1. CD Spectra of Poly Amino Alkyl Glutamate

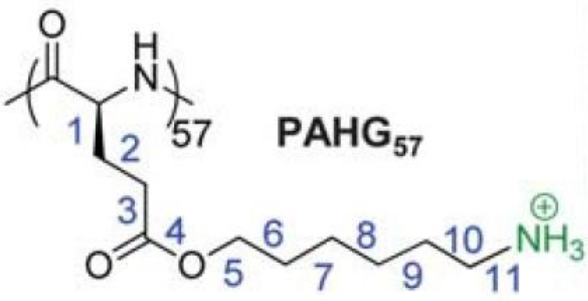


CD spectra at pH 3

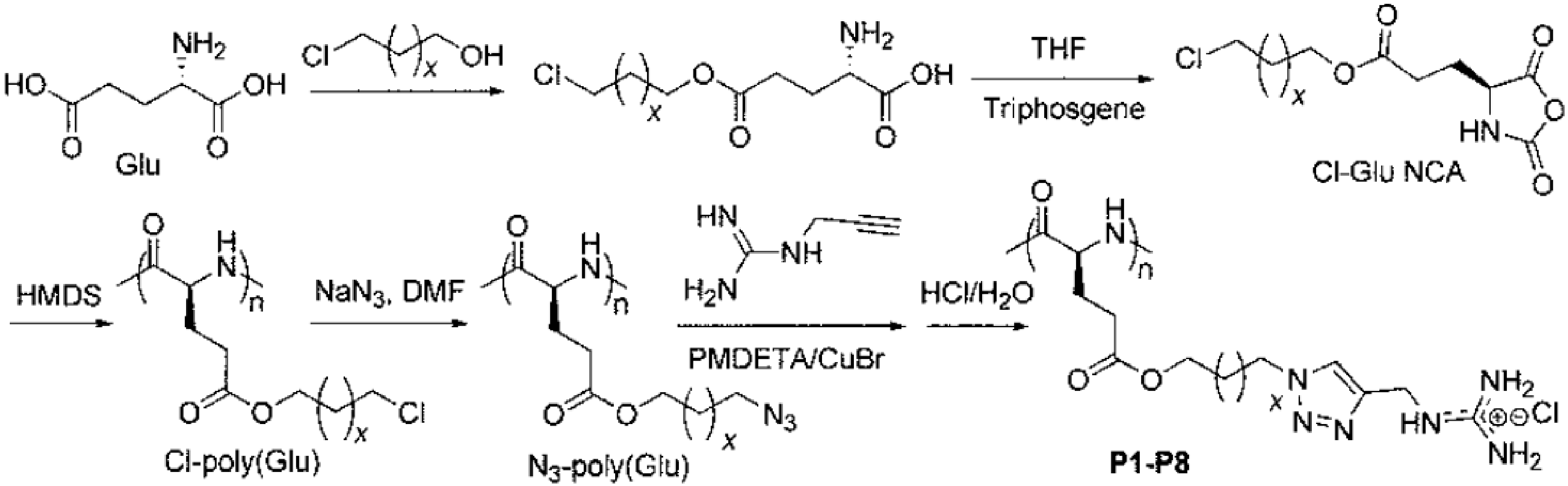
polymer	conformation
PLL_{75} (control)	random coil
$PAPG_{57}$ (8-σbonds)	α-helix (helical content ~16%)
$PAPTG_{50}$ (10-σbonds)	α-helix (helical content ~29%)
$PAHG_{57}$ (11-σbonds)	α-helix (helical content 45%)
$PAEG_{50}$ (10-σbonds)	random coil
$PAHEG_{50}$ (28-σbonds)	α-helix (helical content not mentioned)

- The longer the hydrocarbon side chains, the higher the helix-forming propensity.
- Hydrophobicity of the side chain is important.

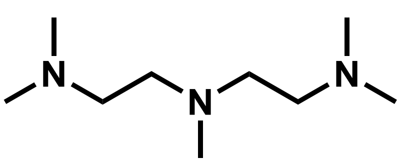
1-2. Helical Poly(Arginine) Mimics (HPRMs)



High net charged helical peptide.
Cell permeability?

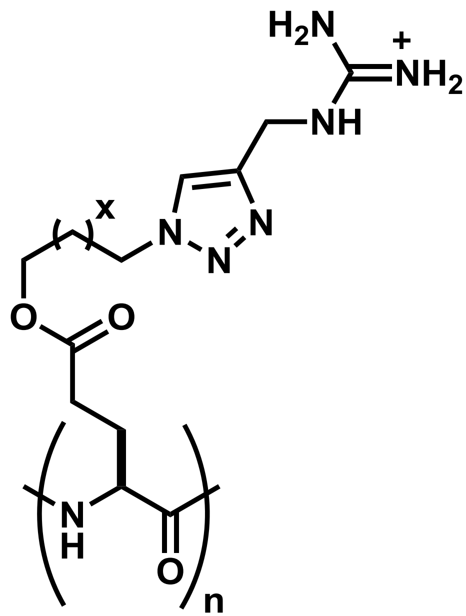


Helical poly(arginine) mimics (HPRMs)



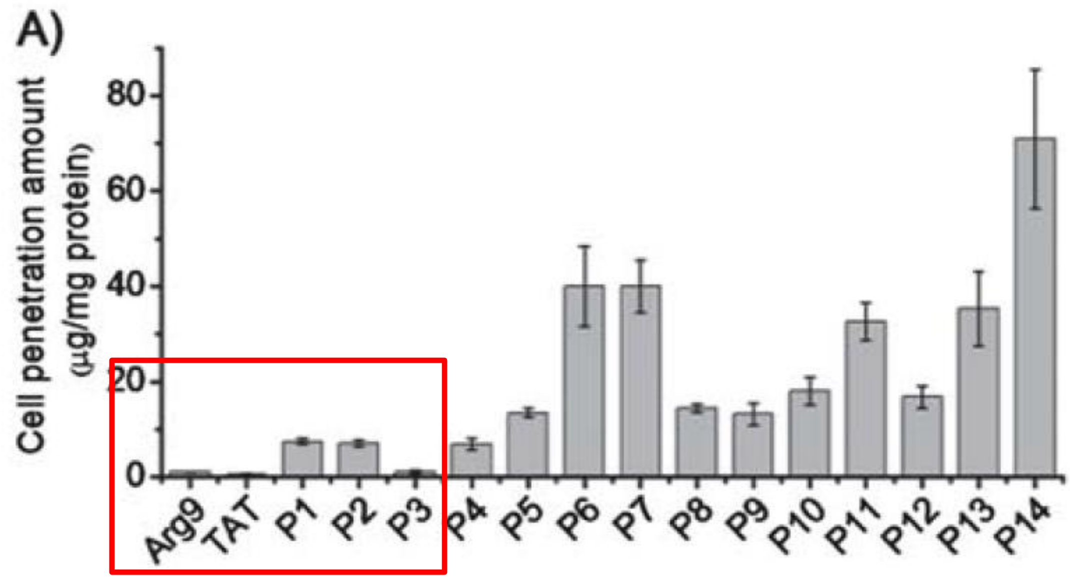
PMDETA

1-2. Helicity and Cell Permeability of HPRMs (1)



HPRMs (P1-P8)

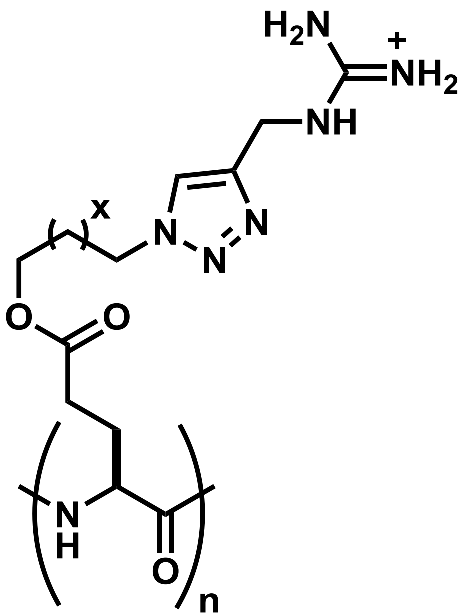
HPRM	Monomer	M_n [a]	M_w/M_n [b]	DP [c]	x [d]	Helicity (%)
P1	L-Cl-Glu NCA	17 600	1.11	51	1	30.0
P2	D-Cl-Glu NCA	18 700	1.17	54	1	31.6
P3	D,L-Cl-Glu NCA	18 000	1.16	52	1	—
P4	L-Cl-Glu NCA	23 900	1.10	69	1	32.0
P5	L-Cl-Glu NCA	19 400	1.25	50	4	55.7
P6	L-Cl-Glu NCA	22 900	1.26	55	6	64.9
P7	L-Cl-Glu NCA	29 900	1.11	72	6	69.8
P8	L-Cl-Glu NCA	4 200	1.16	10	6	50.7



- Helical structure is important for cell permeability.

HPRMs were conjugated with rhodamine, and cell penetration amout was estimated from rhodamine spectra.

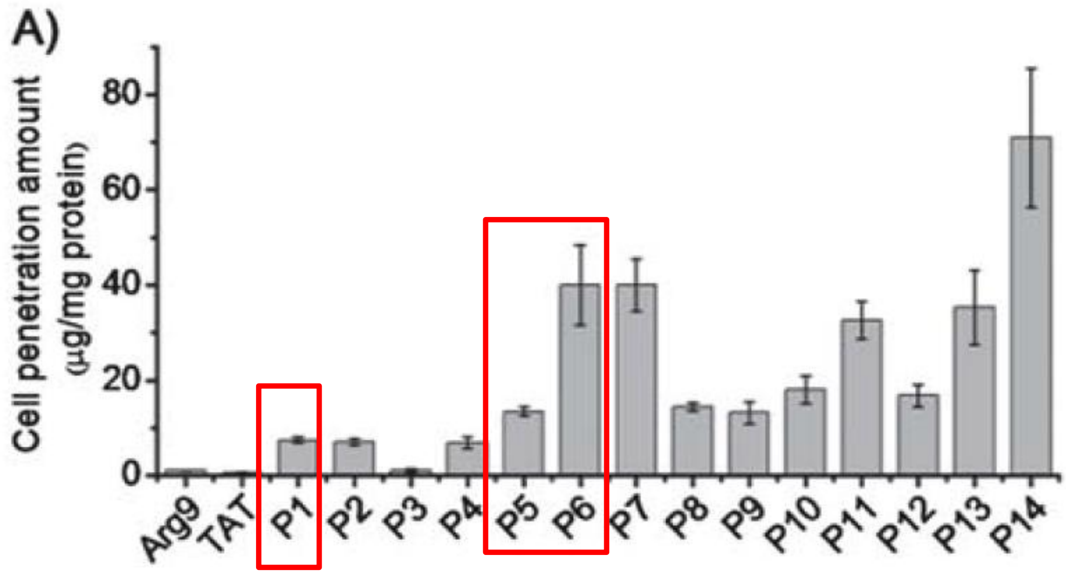
1-2. Helicity and Cell Permeability of HPRMs (2)



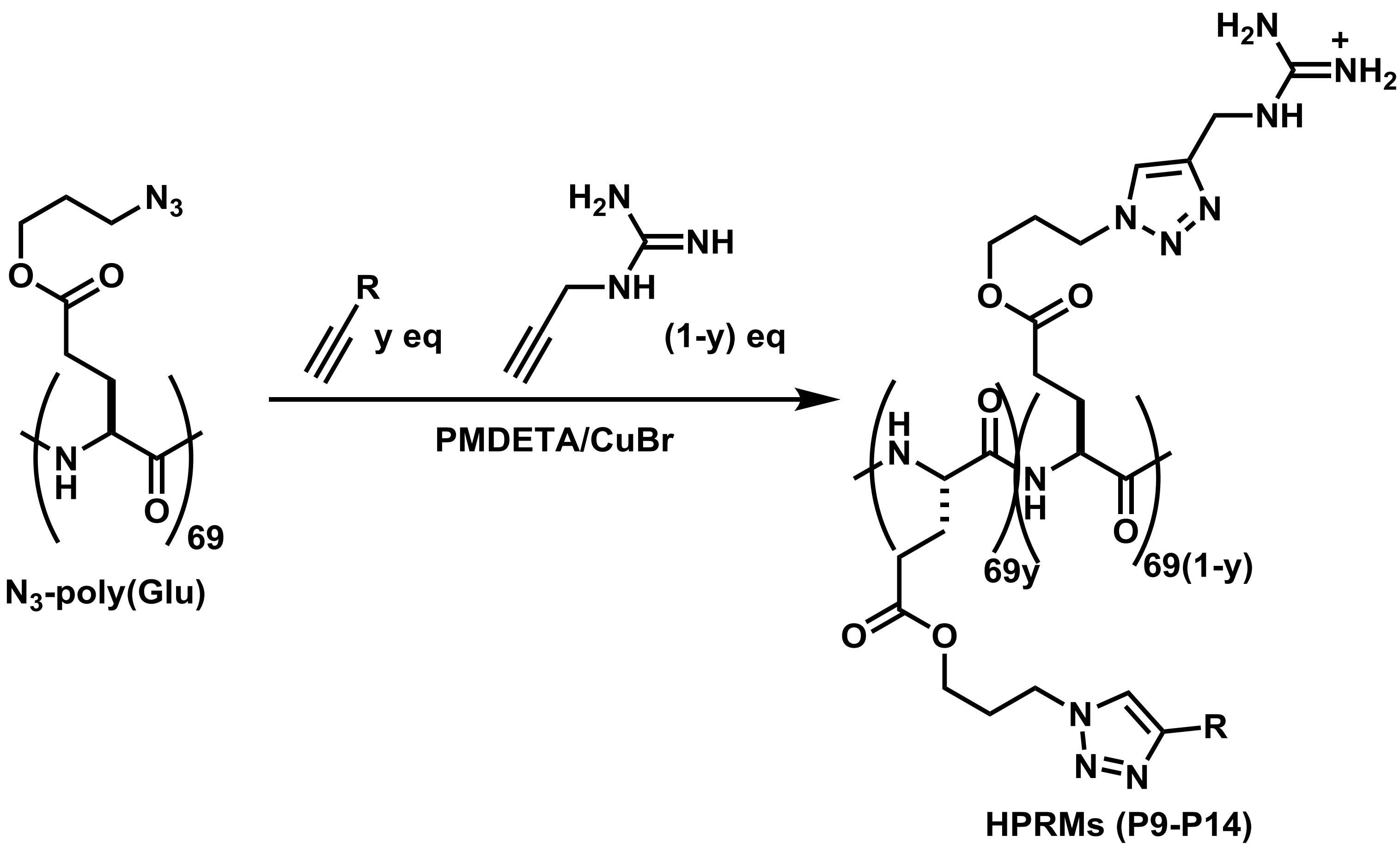
HPRMs (P1-P8)

HPRM	Monomer	M_n [a]	M_w/M_n [b]	DP[c]	x[d]	Helicity (%)
P1	L-Cl-Glu NCA	17 600	1.11	51	1	30.0
P2	D-Cl-Glu NCA	18 700	1.17	54	1	31.6
P3	D,L-Cl-Glu NCA	18 000	1.16	52	1	—
P4	L-Cl-Glu NCA	23 900	1.10	69	1	32.0
P5	L-Cl-Glu NCA	19 400	1.25	50	4	55.7
P6	L-Cl-Glu NCA	22 900	1.26	55	6	64.9
P7	L-Cl-Glu NCA	29 900	1.11	72	6	69.8
P8	L-Cl-Glu NCA	4 200	1.16	10	6	50.7

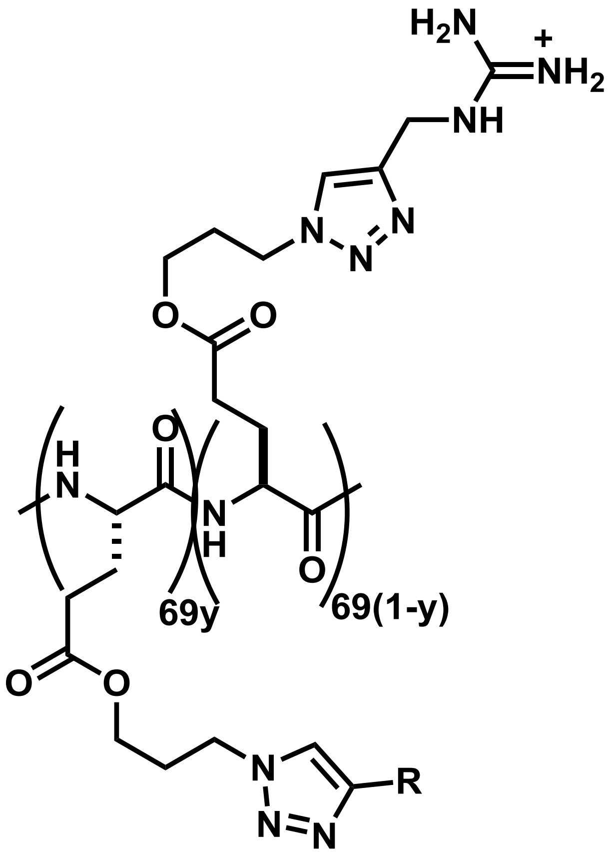
- Increasing hydrophobicity of side chains improved cell permeability.



1-2. Random Copolymers of HPRMs (1)



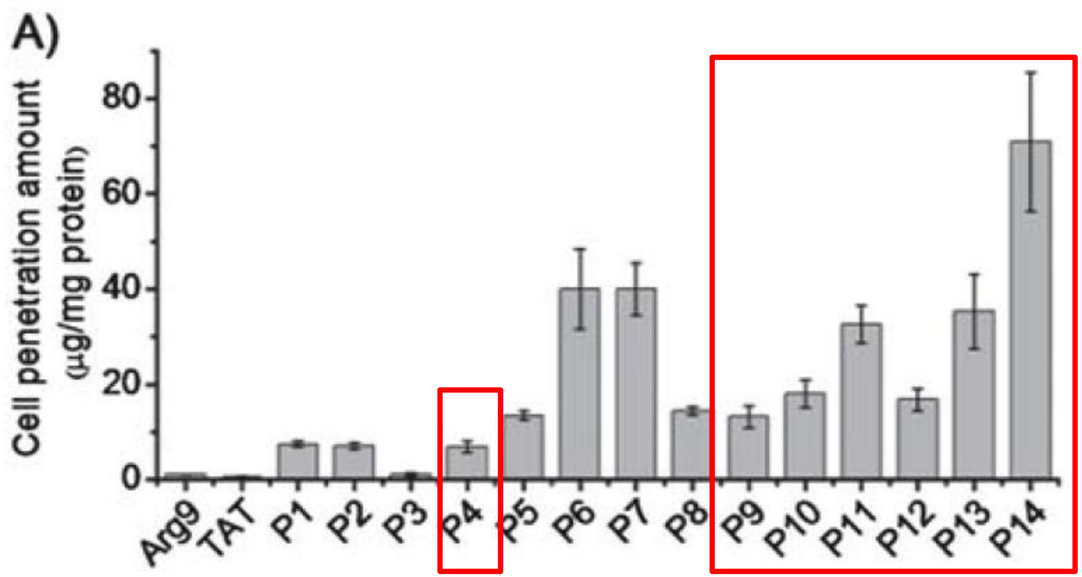
1-2. Random Copolymers of HPRMs (2)



HPRMs (P9-P14)

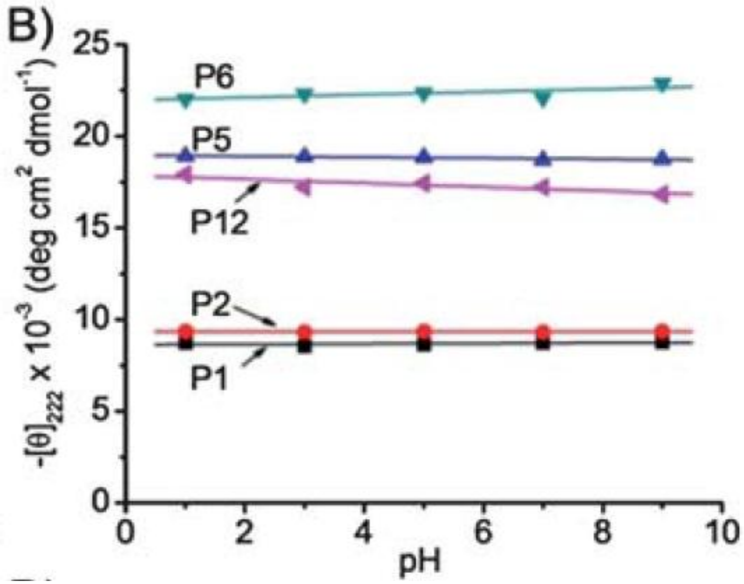
Polymer	R	y^b	Helicity (%)
P9	n-C_3H_7–	0.5	53.9
P10	n-C_4H_9–	0.5	54.7
P11	n-C_5H_{11}–	0.5	54.0
P12	n-C_6H_{13}–	0.5	51.9
P13	n-C_5H_{11}–	0.2	53.5
P14	n-C_5H_{11}–	0.1	54.6

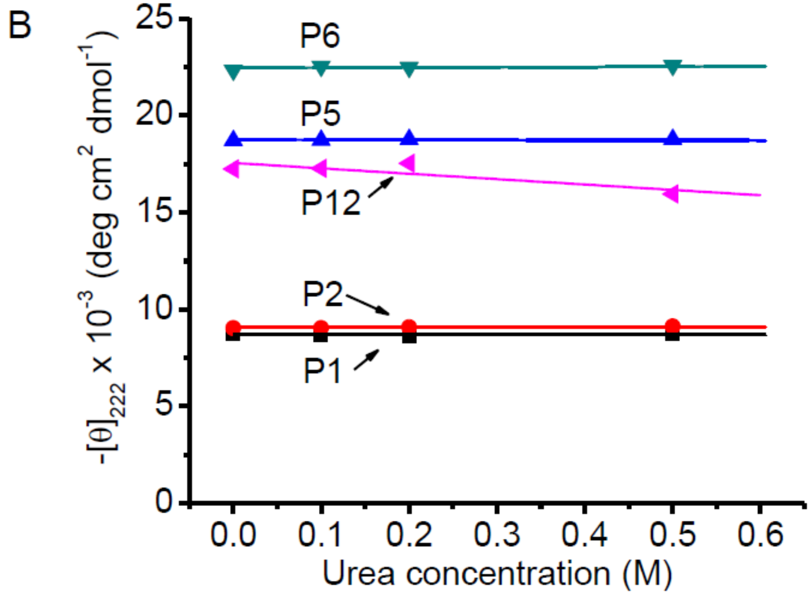
[a] The DP and polydispersity of the polypeptides are 69 and 1.10. [b] The molar content of hydrophobic alkyl side-groups.

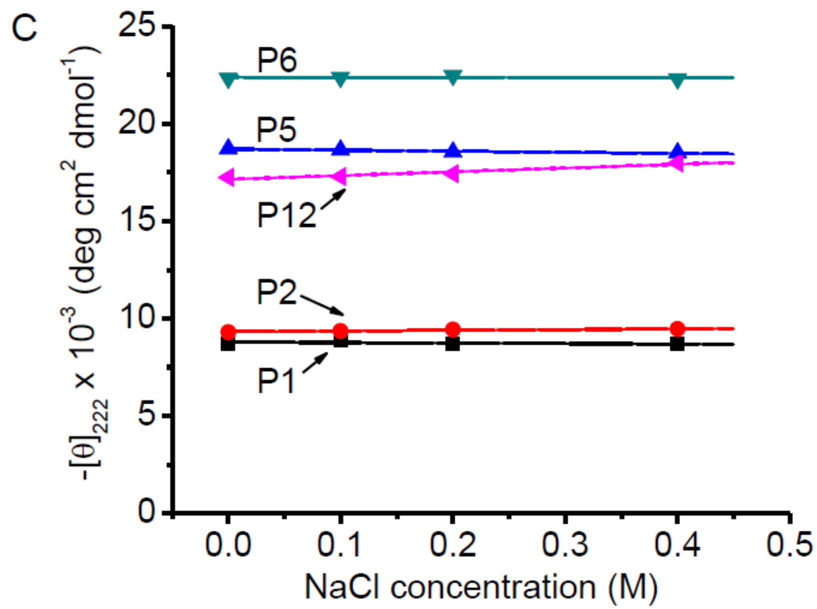


- A proper balance between the cationic charge and the hydrophobic domain is important.

1-2. Stability of HPRMs







- HPRMs forms highly stable helical structure.

Contents

1. Cell-Penetrating Peptides (Prof. Cheng)

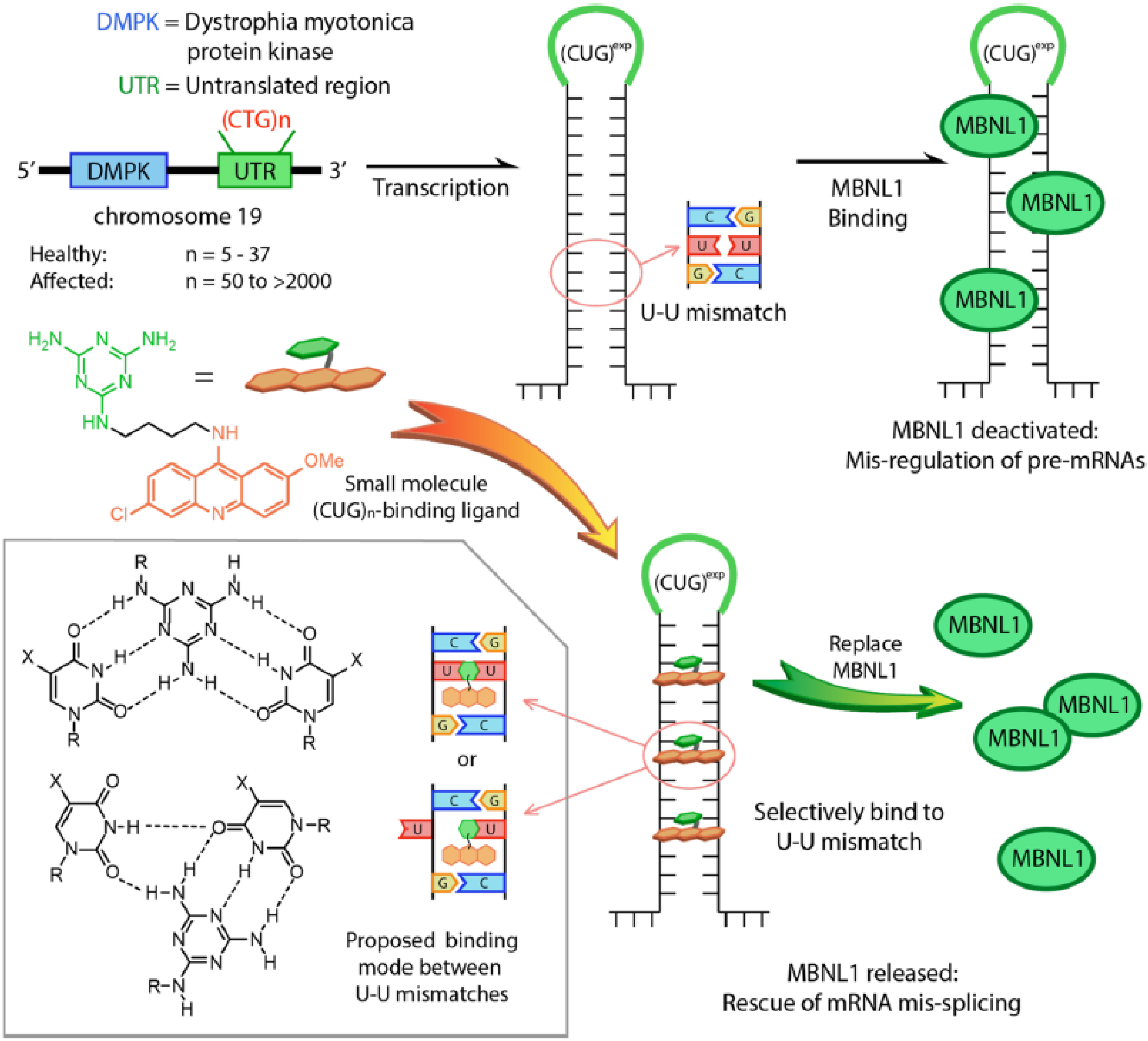
1-1. Poly Amino Alkyl Glutamates

1-2. Helical Poly(Arginine) Mimics

2. Application for Myotonic Dystrophy Type I [1)]
(Collaboration of Prof. Cheng and Prof. Zimmerman)

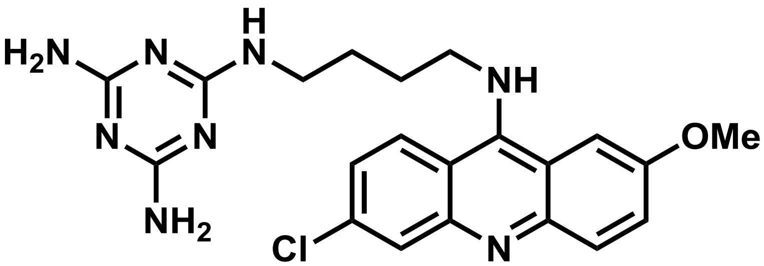
(1) Bai, Y.; Nguyen, L.; Song, Z.; Peng, S.; Lee, J.; Zheng, N.; Kapoor, I.; Hagler, L. D. Cai, K.; Cheng, J; Chan, H. Y. E.; Zimmerman, S. C. *J. Am. Chem. Soc.* **2016**, *138*, 9498.

2. Myotonic Dystrophy Type I (DM1)

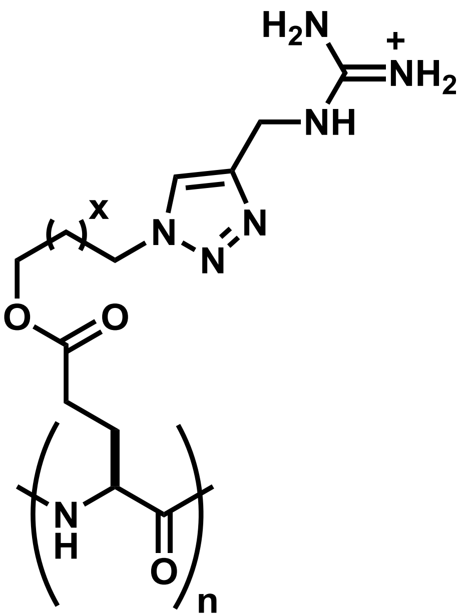


MBNL1: muscleblind-like 1 protein

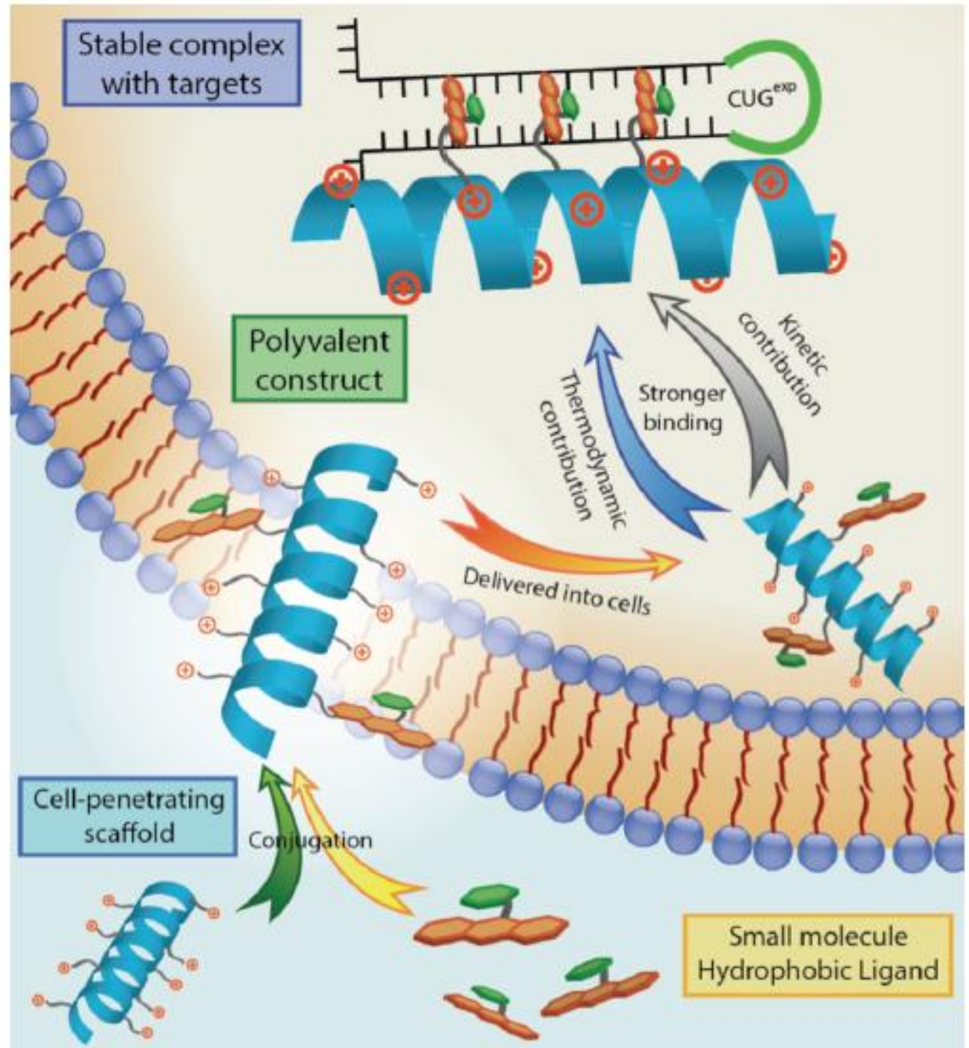
2. Working Hypothesis



Ligand (cell-impermeable)

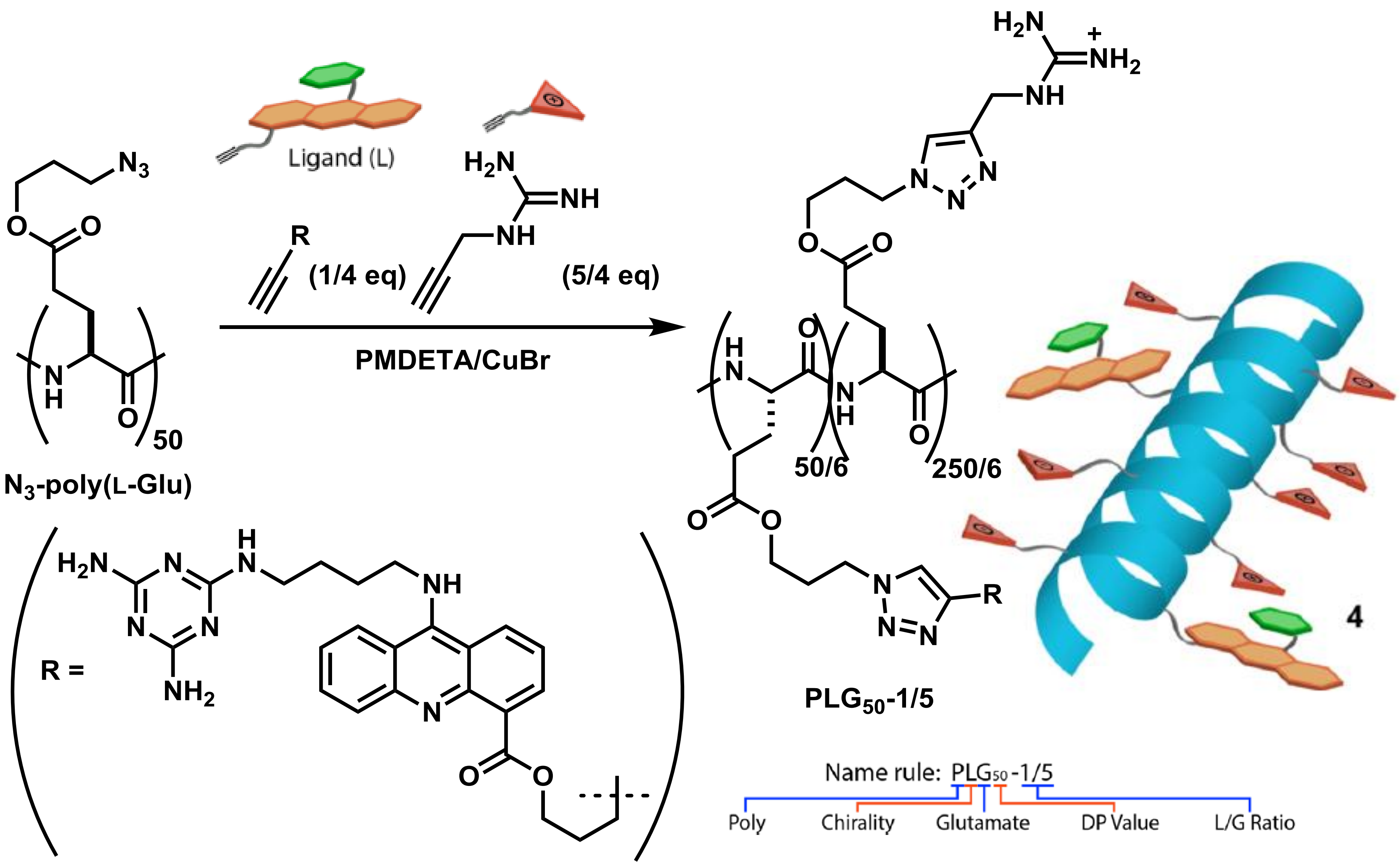


HPRMs (cell-permeable)



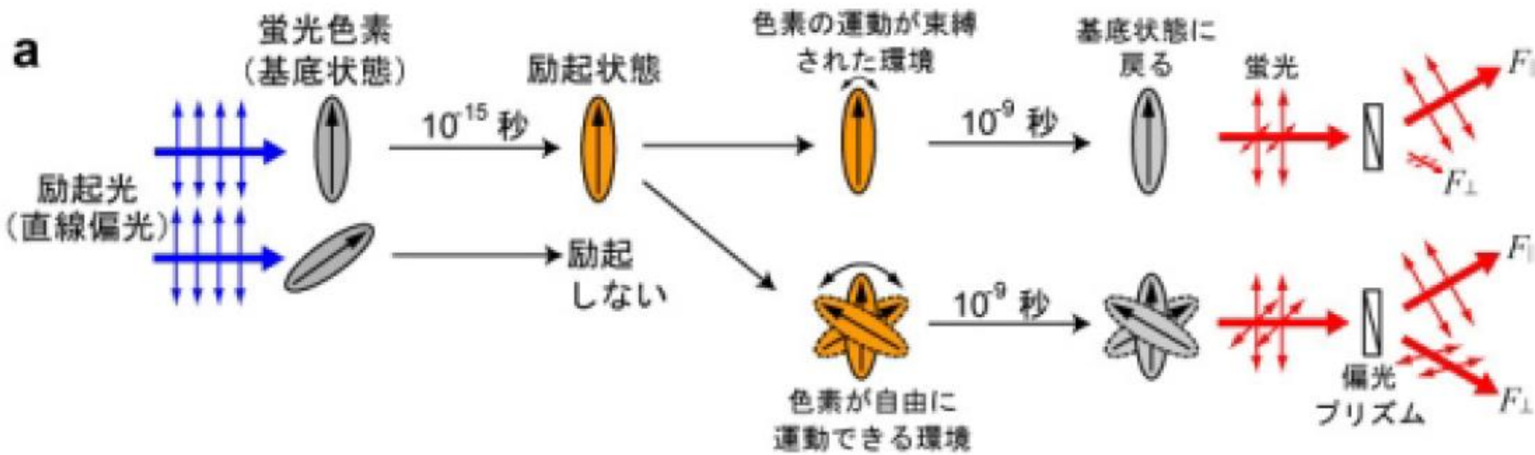
① Delivery cell-impermeable ligand inside the cell.
② Delivery ligand around the target. (Highly cationic peptides possess high binding affinity to anionic DNA or RNA.)
③ Multi copies of ligand is displayed simultaneously. (Target is CUG repeat.)

2. Polypeptide-based DM1 Polymeric Ligands



2. In Vitro Inhibitory Assay -Fluorescence Anisotropy-

[TARMA-r(CUG)$_{16}$][MBNL]complex



TARMA-r(CUG)$_{16}$

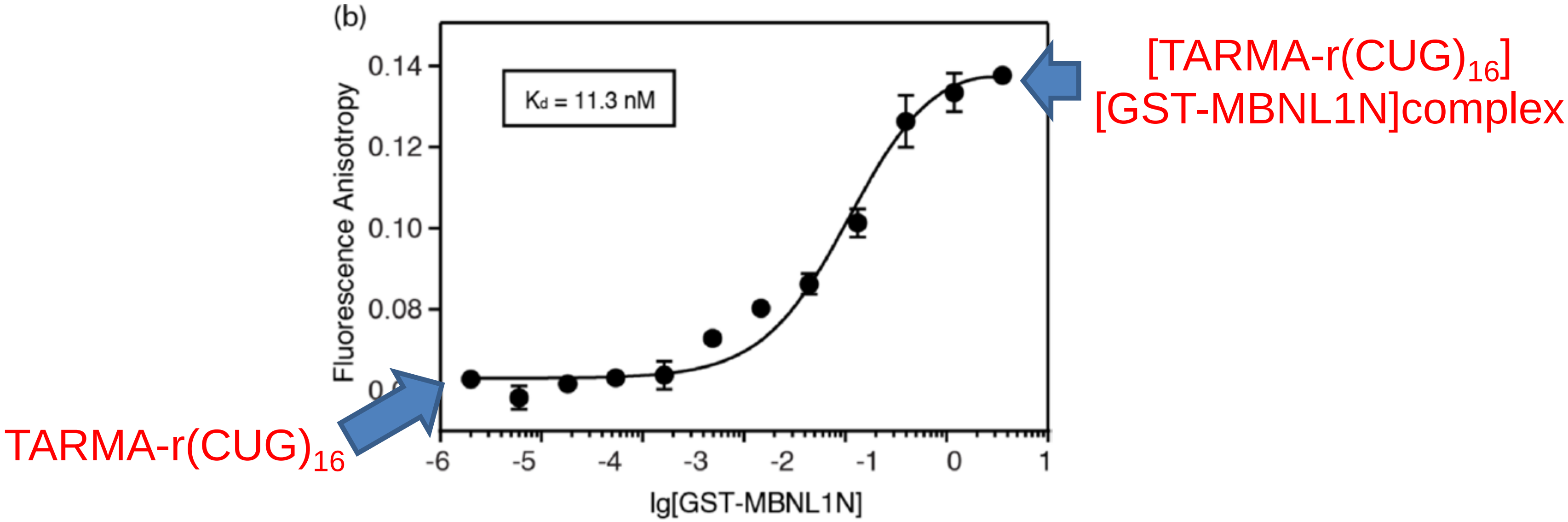
Fluorescence Anisotropy = (F$_{parallel}$ – G x F$_{orthogonal}$)/ F$_{all}$

G: apparatus parameter

(1) 日本生物物理学会HP http://www.biophys.jp/highschool/D-16.html
(2) Gradinaru, C. C.; Marushchak, D. O.; Samim, M.; Krull, U. J. *Analyst* **2010**, *135*, 452.

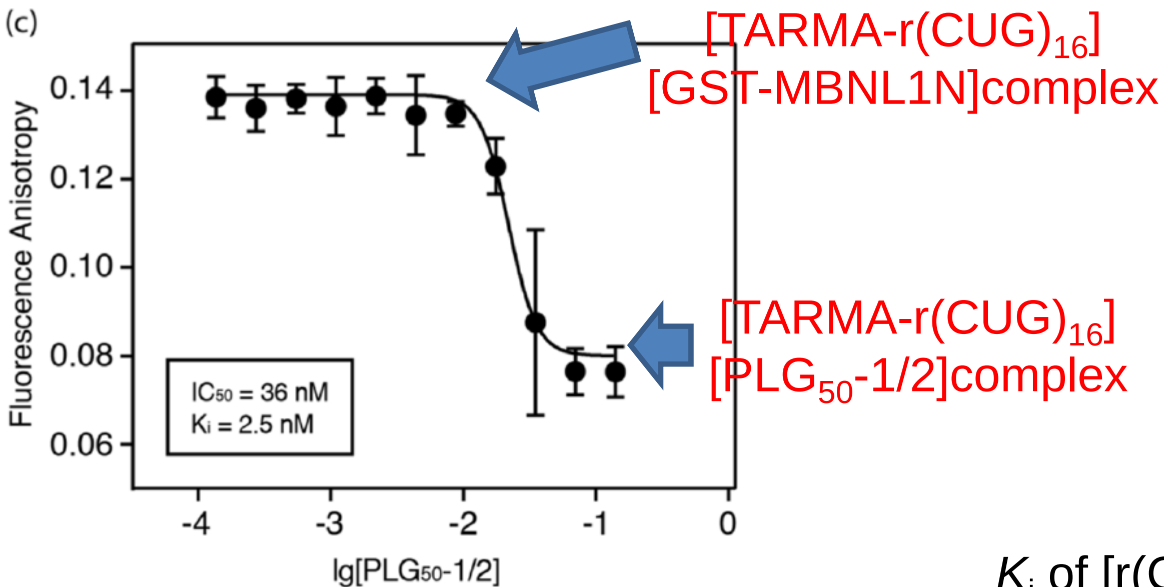
2. In Vitro Inhibitory Assay (1)

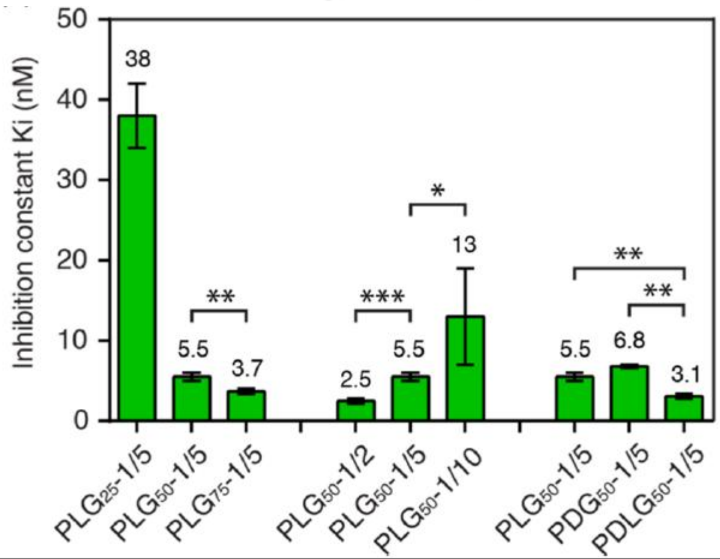
Dissociation of TARMA-r$(CUG)_{16}$ and GST-MBNL1N
(MBNL1N : 272-mer truncated peptide of MBNL1)



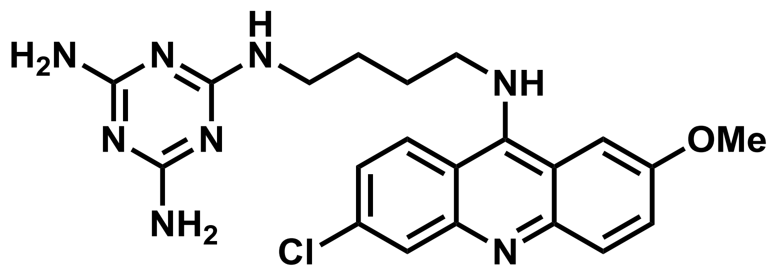
2. In Vitro Inhibitory Assay (2)

Inhibition of [TARMA-r(CUG)$_{16}$][GST-MBNL1N]complex by polymeric ligands





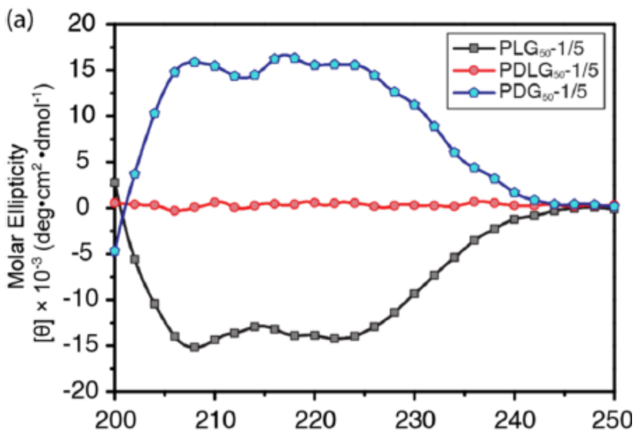
K_i of [r(CUG)$_{12}$][MBNL1N]complex [1)]
by ligand **1** is 7 μM.



1

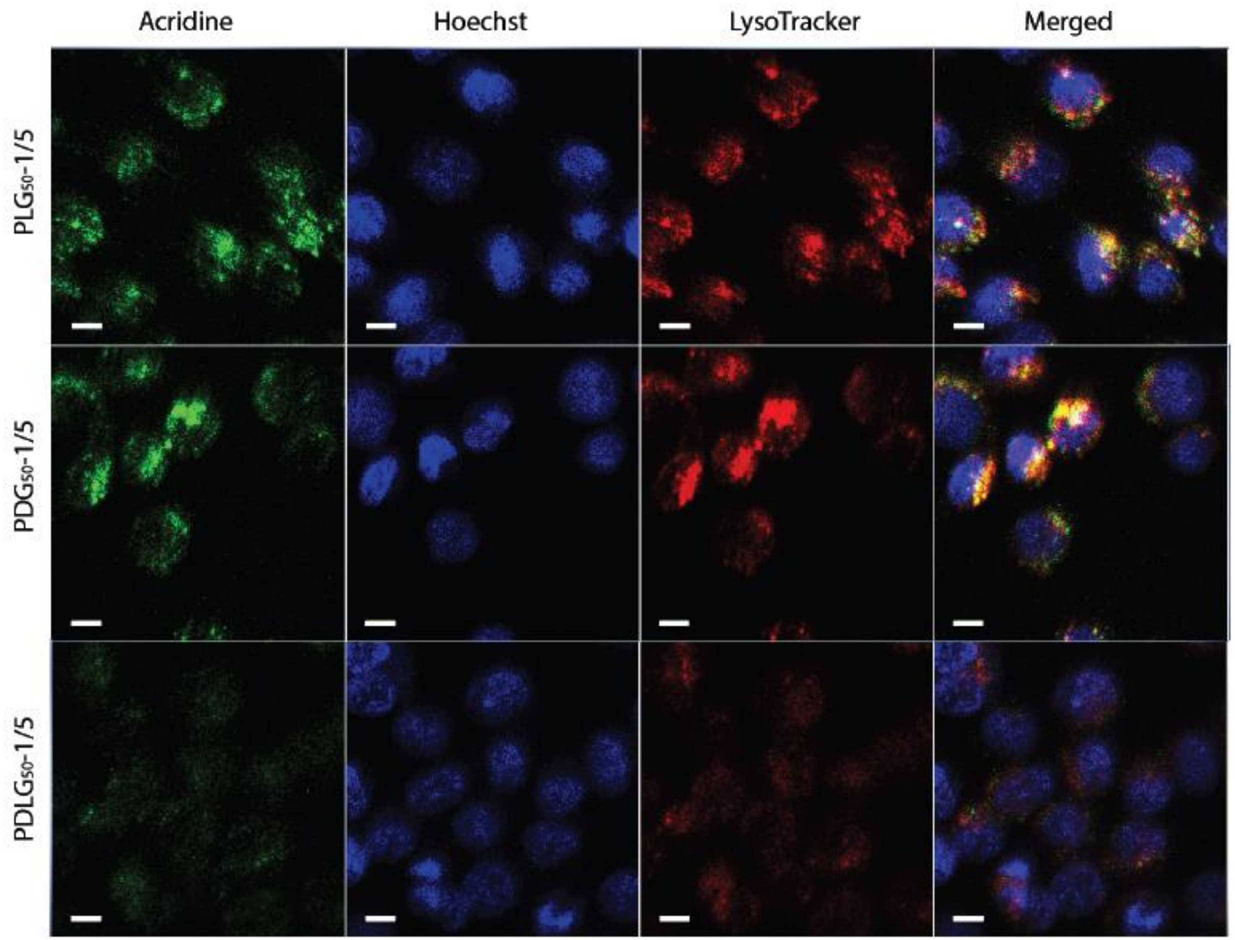
(1) Arambula, J. F.; Ramisetty, S. R.; Baranger, A. M.; Zimmerman, S. C. *Proc. Natl Acad. Sci. USA*, **2009**, *106*, 16068.

2. Helicity and Cell Uptake



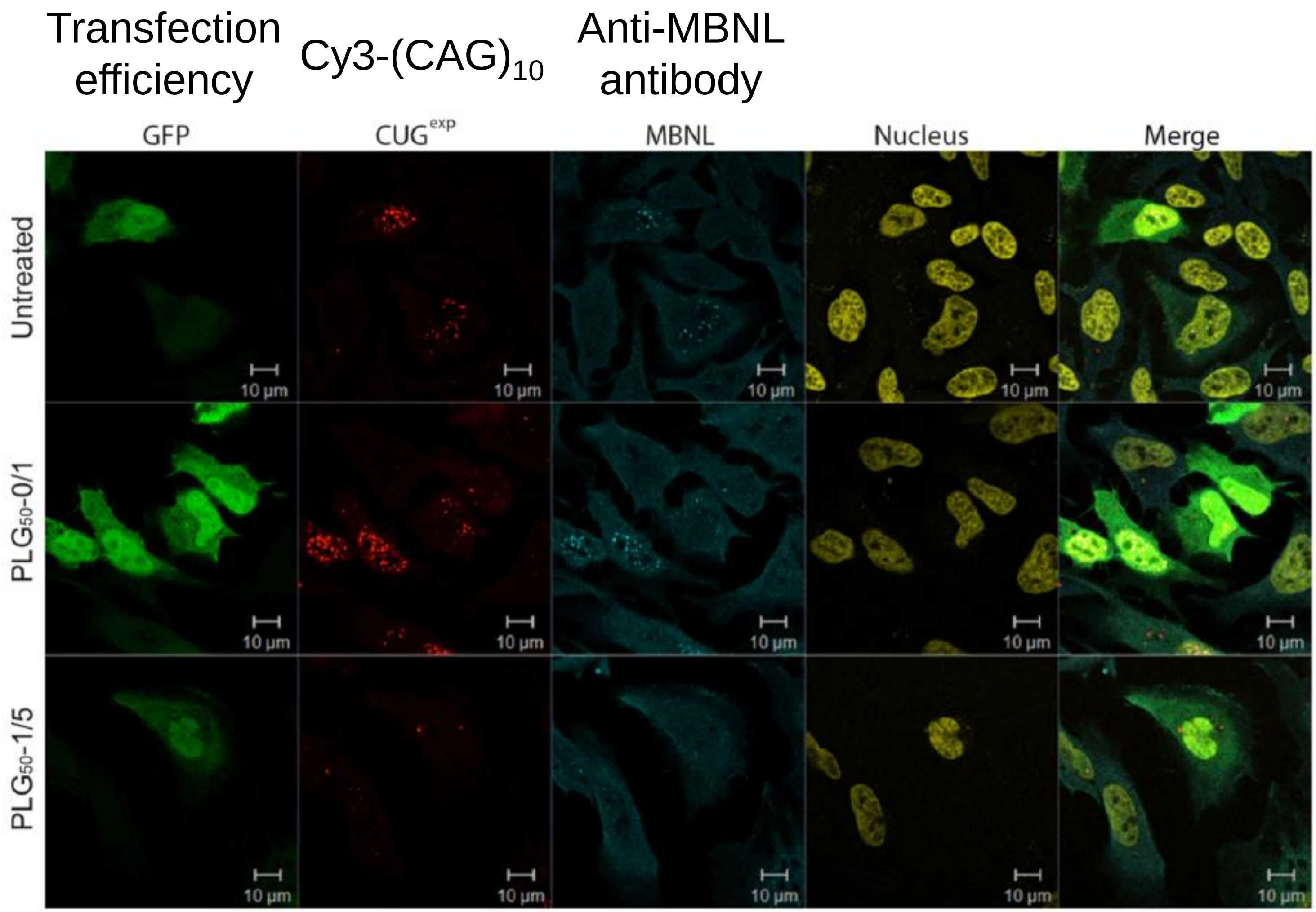
CD spectra at pH 7
Helicity of PLG_{50}-1/5 was approximately 50%

Ligand-polymer complex Nucleus Endosome



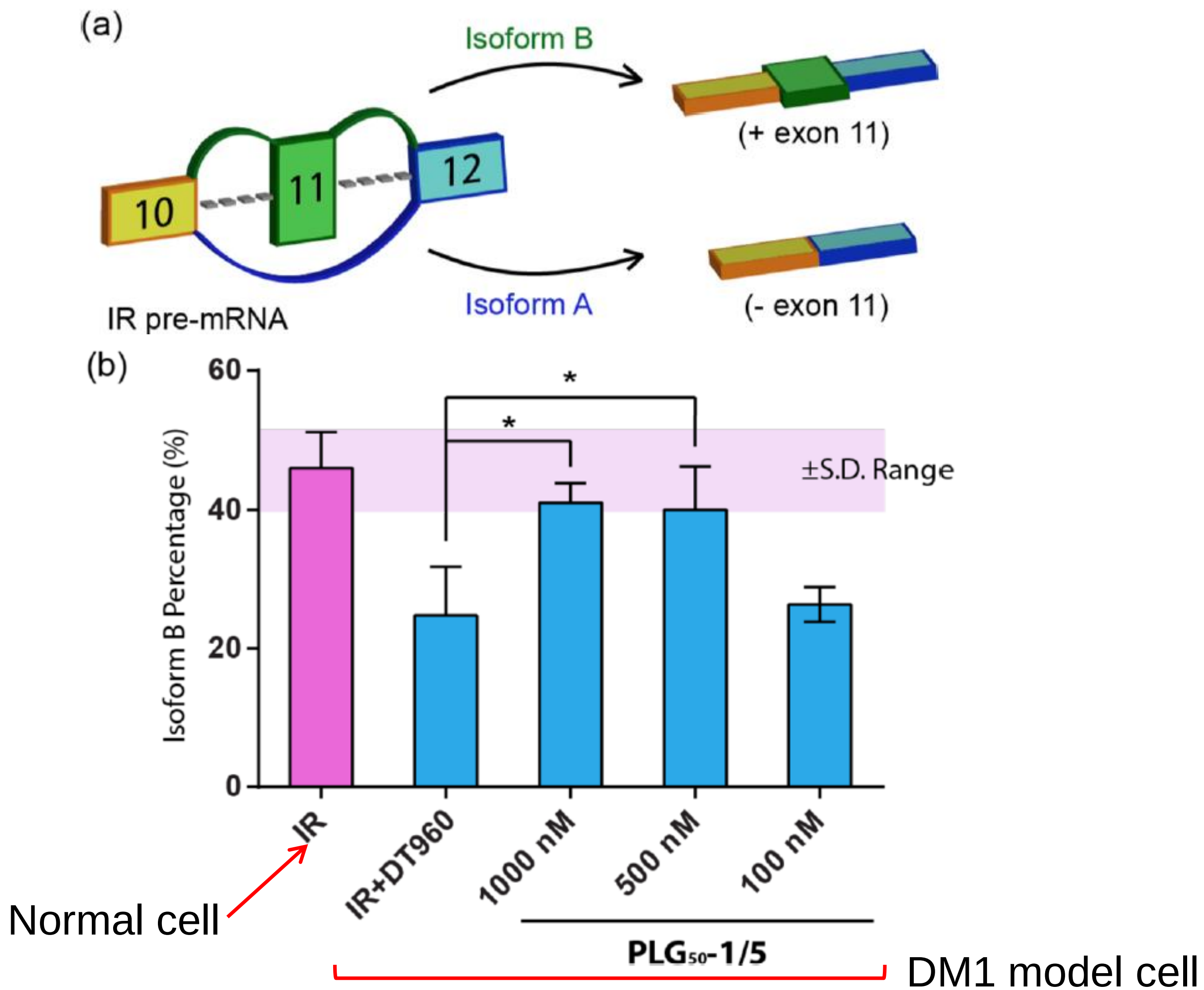
Hela celluar uptake
Incubation time: 4h
Ligand concentration: 500 nM

2. Inhibition of Nuclear Foci Formation



Imaging study of DM1 model cells (Hela cells transfected with GFP-DT960 plasmids)
GFP-DT960 plasmids containing $(CTG)_{960}$ in a truncated *DMPK* gene
Incubation time: 2 d, Ligand concentration: 500 nM

2. Rescue of mRNA Mis-Splicing



Splicing of insulin receptor (*IR*) with DM1 model cells

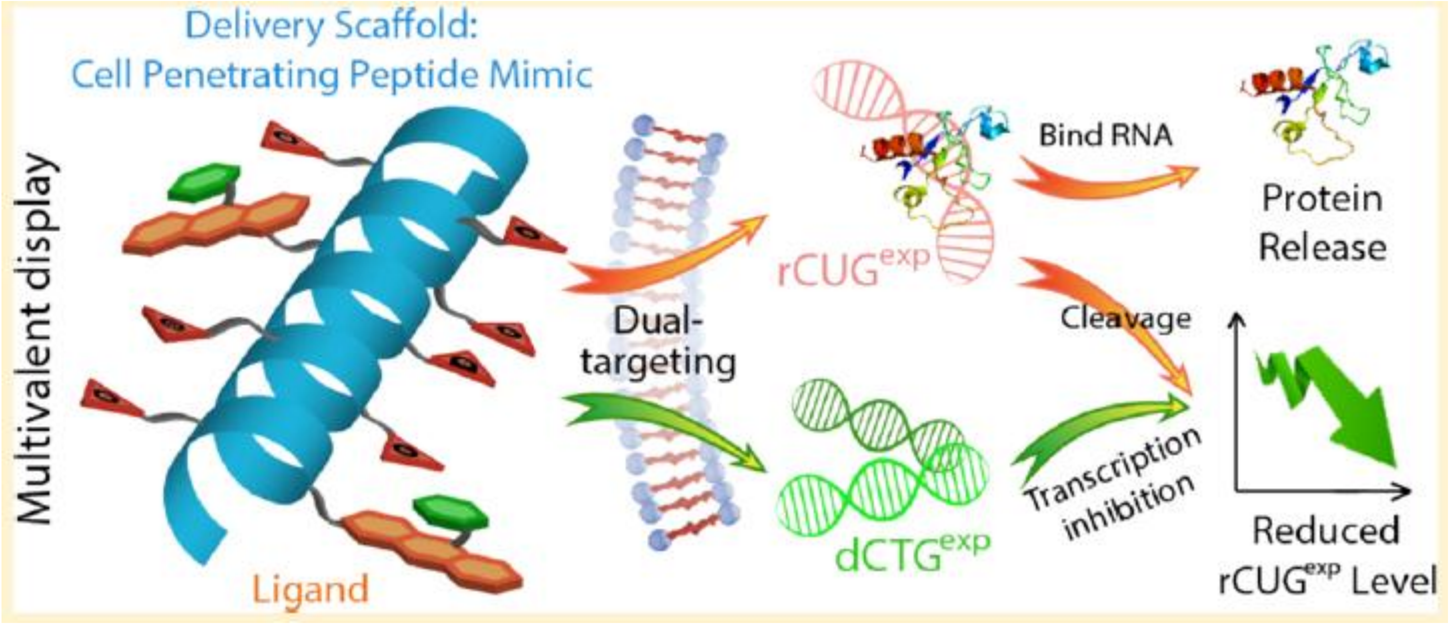
[Hela cells transfected with plasmids containing $(CTG)_{960}$ (DT960) and the *IR* minigene]

Incubation time: 3 d

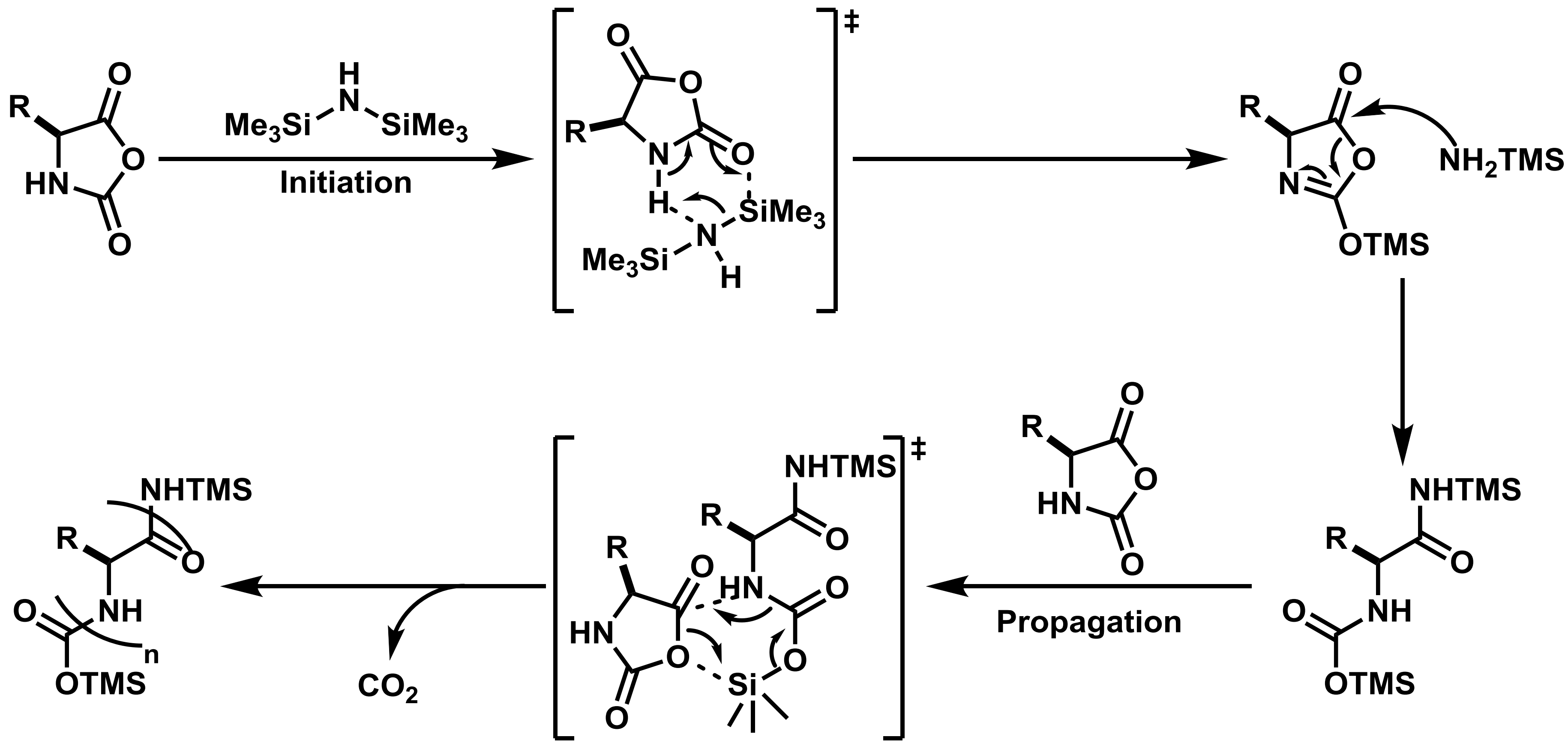
Summary

Development of high net charged and helical cell-penetrating peptides.

Cell-penetrating peptides function as both
a delivery vehicle
and
a scaffold to display multiple copies of ligand.



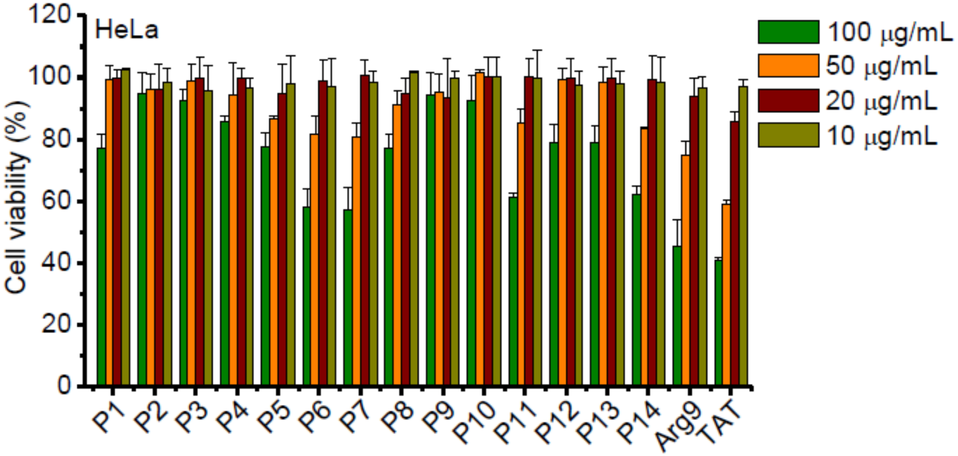
A1. Proposed Mechanism of ROP



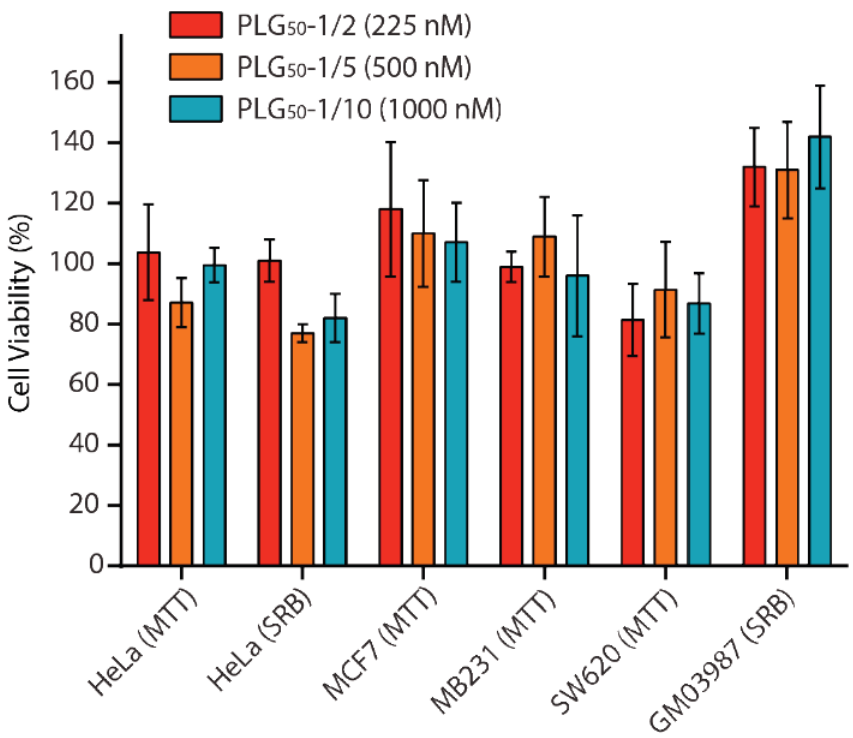
(1) Lu, H.; Cheng, J *J. Am. Chem. Soc.* **2007**, *129*, 14114.

A2. Cytotoxicity of HPRMs and PLG_{50}-1/n

1)



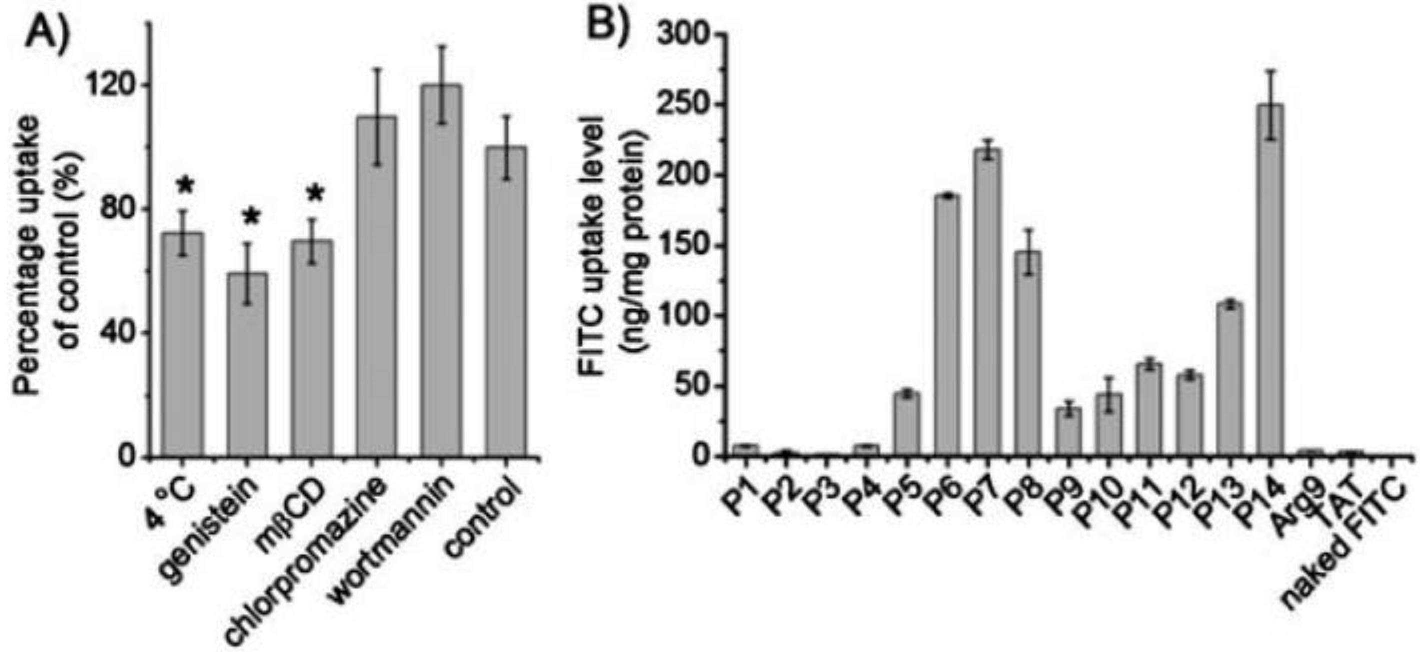
2)

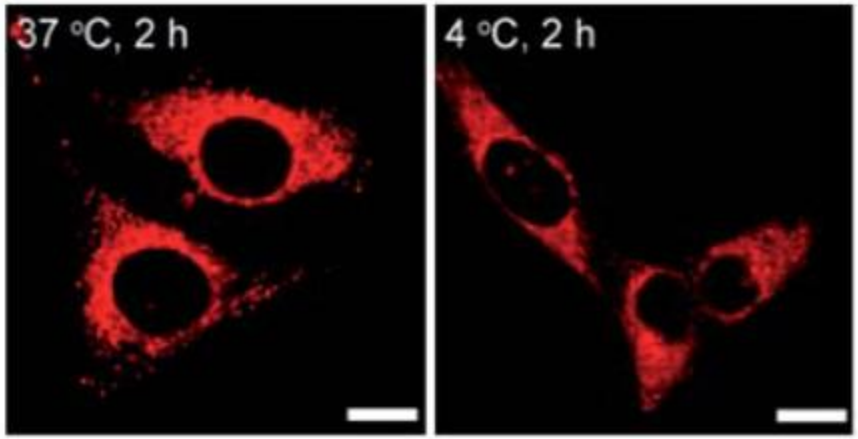


(1) Tang, H.; Yin, L.; Kim, K. H.; Cheng, J. *Chem. Sci.* **2013**, *4*, 3839.

(2) Bai, Y.; Nguyen, L.; Song, Z.; Peng, S.; Lee, J.; Zheng, N.; Kapoor, I.; Hagler, L. D. Cai, K.; Cheng, J; Chan, H. Y. E.; Zimmerman, S. C. *J. Am. Chem. Soc.* **2016**, *138*, 9498.

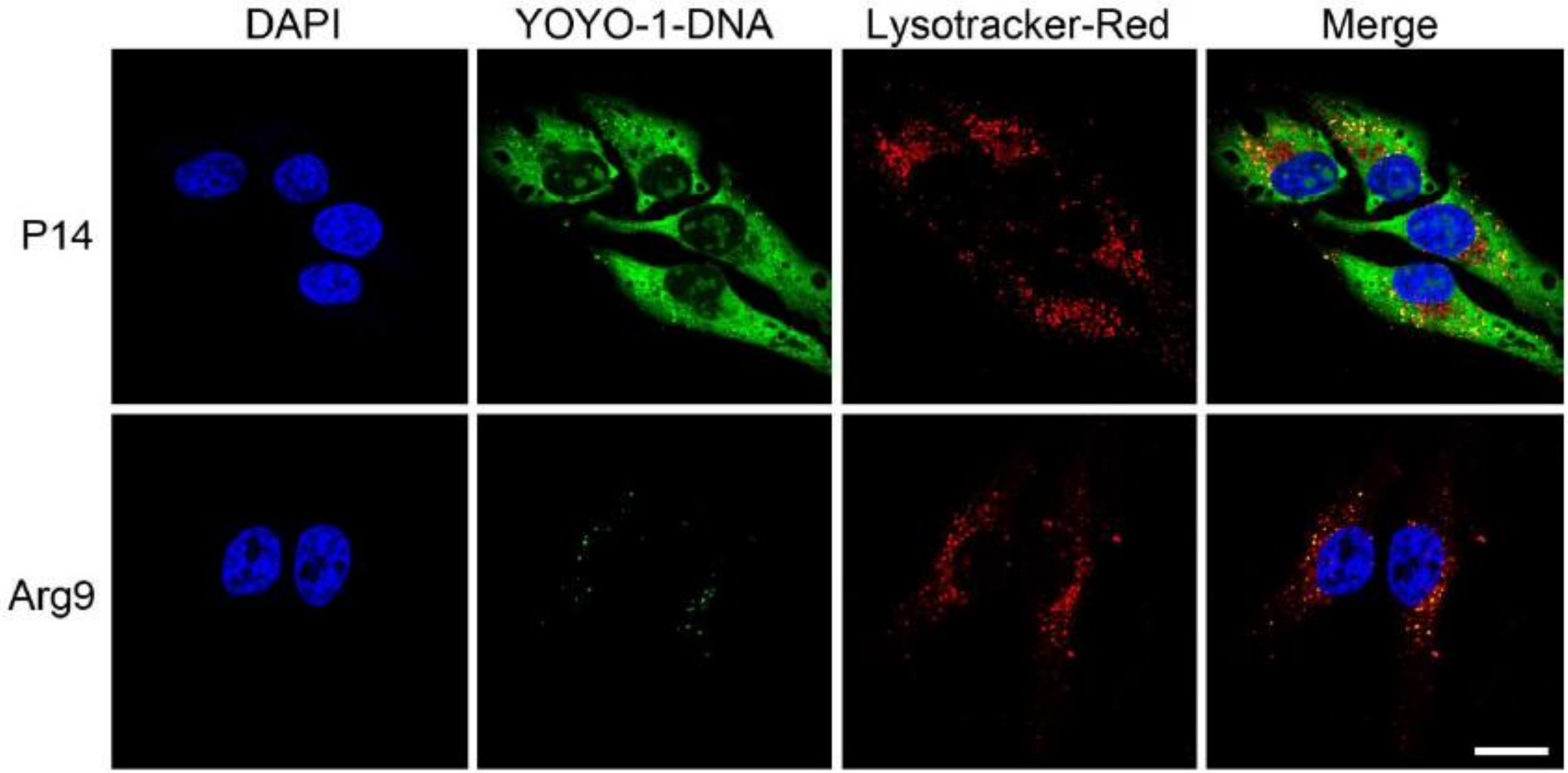
A3. Cell Uptake of HPRMs





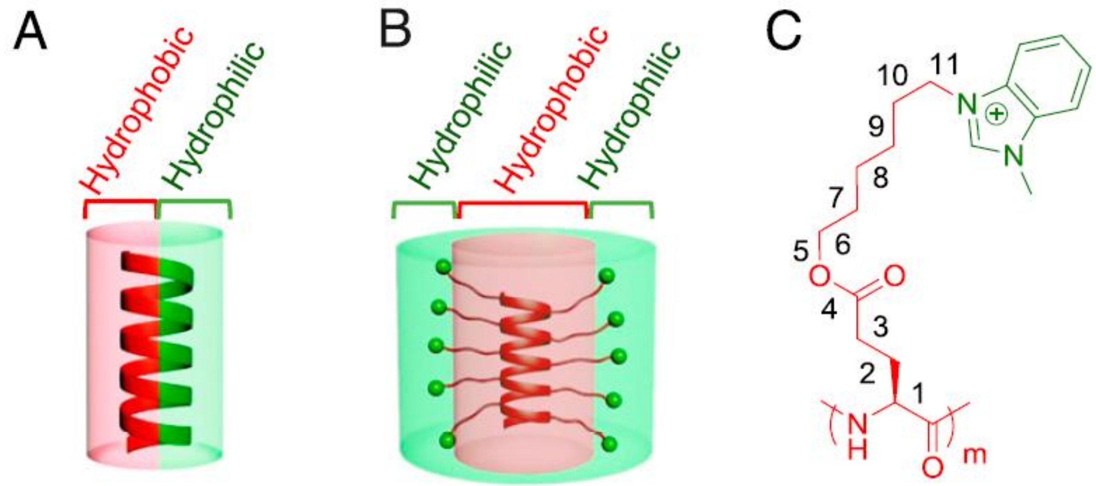
(1) Tang, H.; Yin, L.; Kim, K. H.; Cheng, J. *Chem. Sci.* **2013**, *4*, 3839.

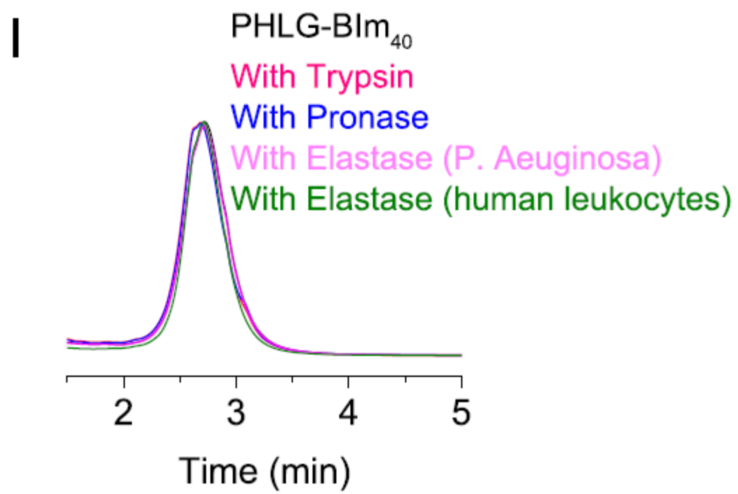
A4. Localization of HPRM-DNA complex



(1) Tang, H.; Yin, L.; Kim, K. H.; Cheng, J. *Chem. Sci.* **2013**, *4*, 3839.

A5. Stability of Poly Helical L-Glutamate (PHLG)

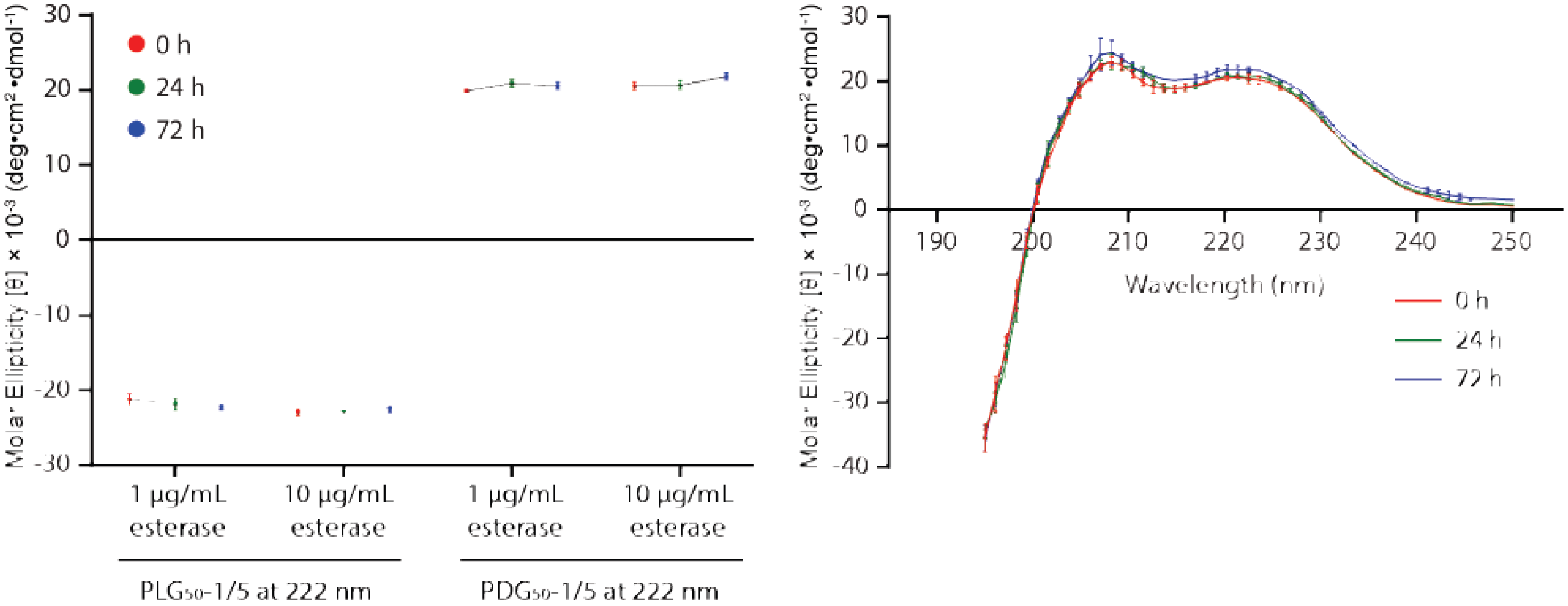




HPLC chart of PHLG after 8h
incubation with protease.

(1) Xiong, M.; Lee, M. W.; Mansbach, R. A.; Song, Z.; Bao, Y.; Peek Jr., R. M.; Yao, C.; Chen, L-F.; Ferguson, A. L.; Wong, G. C. L.; Cheng, J. *P Natl. Acad. Sci. U.S.A.* **2015**, *112*, 13155.

A6. Stability of PLG_{50}-1/5 and PDG_{50}-1/5



(1) Bai, Y.; Nguyen, L.; Song, Z.; Peng, S.; Lee, J.; Zheng, N.; Kapoor, I.; Hagler, L. D. Cai, K.; Cheng, J; Chan, H. Y. E.; Zimmerman, S. C. *J. Am. Chem. Soc.* **2016**, *138*, 9498.