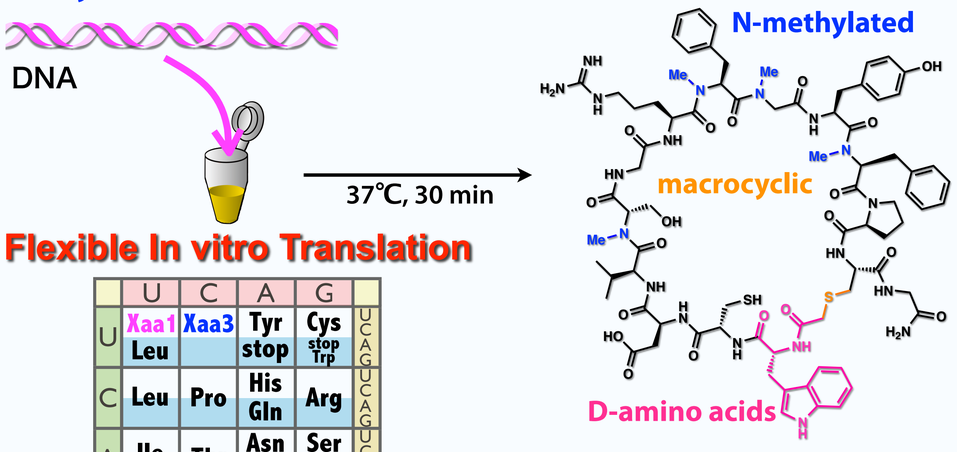
構造を制約したシクロプロパンユニットを含むペプチドライブラリーの試験管内選択による環状ペプチド阻害剤の開発

Yuki Goto (Dept. of Chem., U. Tokyo)

1. Our key technologies to develop artificial bioactive molecules

FIT system



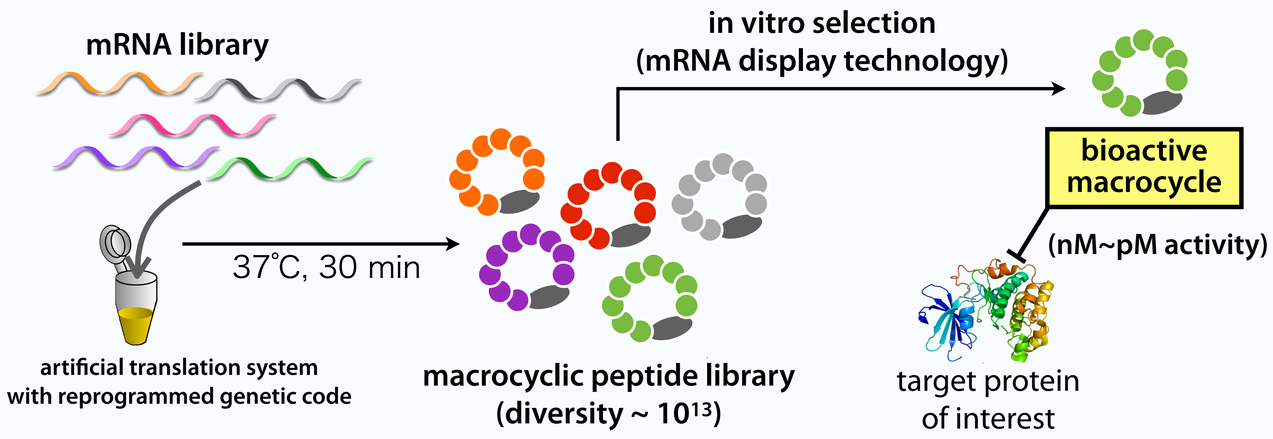
	U	C	A	G	
U	Xaa1 Leu	Xaa3	Tyr stop	Cys stop Trp	UCAG
C	Leu	Pro	His Gln	Arg	UCAG
A	Ile Xaa2	Thr	Asn Lys	Ser Arg	UCAG
G	Val	Xaa4	Asp Glu	Gly	UCAG

reprogrammed genetic code
(Xaa: nonproteinogenic amino acids)

Goto *et al.*, *ACS Chem. Biol.*, 2008, *3*, 120.
Goto *et al.*, *RNA*, 2008, *14*, 1390.
Goto *et al.*, *J. Am. Chem. Soc.*, 2009, *131*, 5040.
Goto *et al.*, *Nat. Protoc.*, 2011, *6*, 779.
Goto *et al.*, *ACS Chem. Biol.*, 2013, *8*, 2630.
Iwane *et al.*, *Nat. Chem.*, 2016, *8*, 317.

template-dependent synthesis of macrocyclic peptides

RaPID system

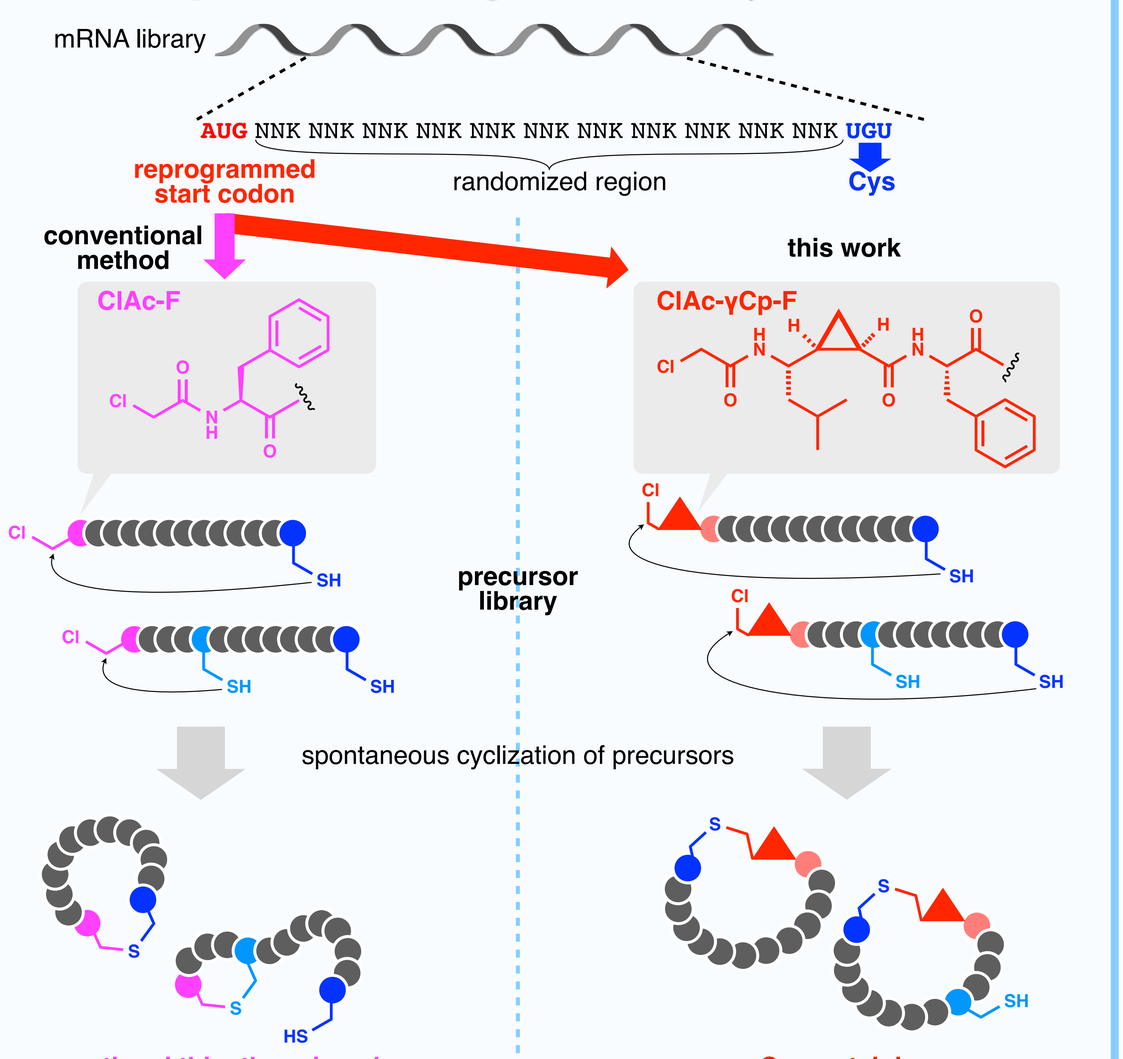


Yamagishi *et al.*, *Chem. Biol.*, 2011, *18*, 1562.
Yamagata *et al.*, *Structure*, 2014, *22*, 345.
Review: Passioura *et al.*, *Annu. Rev. Biochem.*, 2014, *83*, 727.
Review: Morioka *et al.*, *Curr. Opin. Chem. Biol.*, 2015, *26C*, 34.

One-pot construction of highly diverse macrocyclic peptide libraries

Rapid screening of potent peptide ligands binding target protein of interest

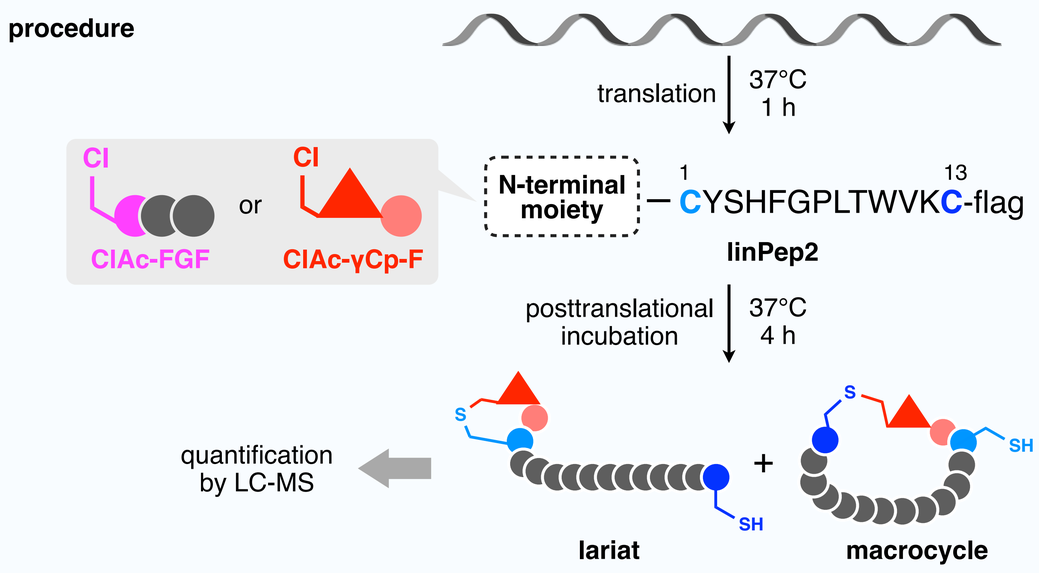
2. Aim and experimental design of this study



The conventional ClAc-α-initiator generally dictates the spontaneous cyclization with the nearest upstream Cys residue to form undesignated lariat-shaped peptides.

The newly designed ClAc-initiator bearing structurally constrained cyclopropane unit may change topological selectivity of the cyclization and preferencially yield macrocyclic peptide libraries.

3. Posttranslational cyclization of model peptides bearing two Cys residues



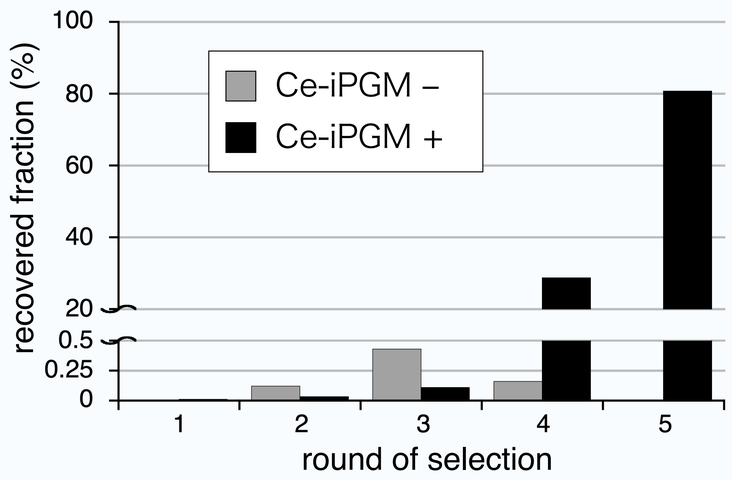
name	N-terminal moiety	peptide sequence	macrocycle (%)	lariat (%)
linPep2	ClAc-FGF	CYSHFGPLTWVKC-flag	35	65
	ClAc-γCp-F		92	8
linPep3	ClAc-FGF	TYSHCGPLTWC-flag	not detected	~100
	ClAc-γCp-F		58	42
linPep4	ClAc-FGF	CYSHFGPLTWC-flag	21	79
	ClAc-γCp-F		85	15
linPep5	ClAc-FGF	TCSHFGPLTWC-flag	not detected	~100
	ClAc-γCp-F		40	60
linPep6	ClAc-FGF	TYCHFGPLTWC-flag	not detected	~100
	ClAc-γCp-F		52	48
linPep7	ClAc-FGF	TCSHFGPLTWVC-flag	not detected	~100
	ClAc-γCp-F		46	54
linPep8	ClAc-FGF	TCSHFGPLTWVKC-flag	not detected	~100
	ClAc-γCp-F		56	44

The ClAc-γCp-F-precursors favorably underwent cyclization with the downstream Cys to yield macrocycles, compared with the corresponding ClAc-FGF-precursors in all other tested sequences

4. *In vitro* selection of γCp-containing macrocyclic peptides against CeiPGM

target protein: cofactor-independent phosphoglycerate mutase from *C. elegans* (CeiPGM)

- ✓ A species-selective drug target in filarial parasites
- ✓ Previous conventional RaPID selection yielded lariat-shaped peptide ligands



ranking	selected peptide sequence	abundance
1	γCp-F-SISGIEWNGPEIC	44%
2	γCp-F-SISGIEWNGPETC	1.0%
3	γCp-F-SISGIEWNGPEVC	0.51%
4	γCp-F-SISGIEWNGPELC	0.26%
5	γCp-F-SISGIEWNSPEIC	0.057%

The in vitro selection using the ClAc-γCp initiator successfully successfully led to the discovery of macrocyclic peptide sequences without a Cys-containing tail region

5. Inhibitory activity of the obtained peptides against to CeiPGM

structure	Kd	IC50
FSISGIEWNGPEIC-NH_2 **iCeiPGM-γCp(*RSS*)**	0.3 nM	9.5 nM
FSISGIEWNGPEIC-NH_2 **iCeiPGM-γCp(*SRR*)**	0.8 nM	15 nM

The obtained peptides exhibited potent inhibitory activity to CeiPGM.

6. Conclusion

The newly designed cyclopropane-containing initiator yielded a topologically different type of peptide libraries from conventional initiators.

Such a cyclopropane-containing macrocycle library would allow us to access mechanistically distinct macrocycle ligands.

Acknowledgements

Prof. Satoshi Shuto (Hokkaido U.)
Prof. Mizuki Watanabe (Hokkaido U.)
Dr. James Inglese (NIH)
Dr. Patricia Dranchak (NIH)
JSPS KAKENHI:
Middle molecular strategy