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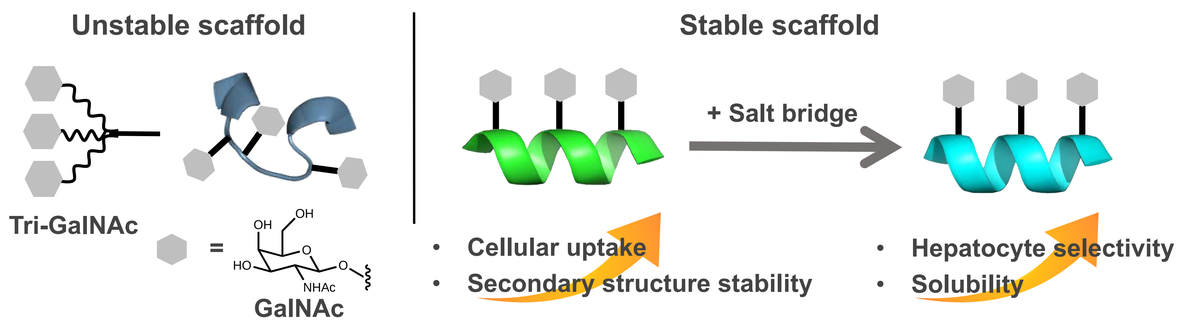
Strategies for designing hepatocyte-selective carriers using helical peptides as stable scaffolds

Takahito Ito,[1,2] Takashi Misawa,[2] Nobumichi Ohoka,[2] Michihiko Aoyama,[2] Takao Inoue,[2] Akiko Ishii-Watabe,[2] Yosuke Demizu[1,2,3]

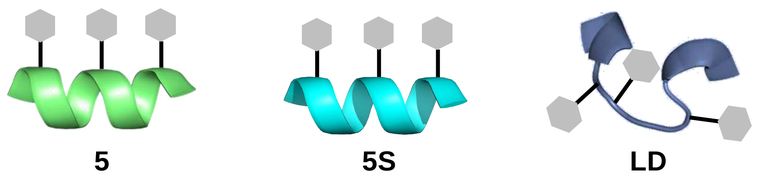
1. Graduate School of Medical Life Science, Yokohama City University
2. National Institute of Health Sciences
3. Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University

1. Abstracts

Asialoglycoprotein receptor (ASGPR) is the representative targets for liver-selective DDS. ASGPR generally forms trimer, which trivalent GalNAc ligand is considered to be effective for hepatocyte-selective DDS. Representative triantennary GalNAc ligand (Tri-GalNAc) is already in medical use. Each GalNAc-binding site exists in the same orientation; however, due to GalNAc being mounted on flexible scaffolds, it faces disadvantages that prevent it from adopting a specific conformation. Helical structures can control the side-chain orientation due to their structural nature. Therefore, we thought to use helical peptide as a rigid scaffold to modulate the orientation of GalNAc and it could be more entropically favored structures. In this study, we designed helical peptides conjugated with GalNAc for hepatocyte selective carriers.



2. Design



Peptide	Sequence
5	FITC-BL1SXSSLL2SXSSLL3SXSSL
5S	FITC-BS1EXSRLS2EXSRLS3EXSRL
LD	FITC-Bs1eXSrLs2eXSrLs3eXSrL

B = β-alanine

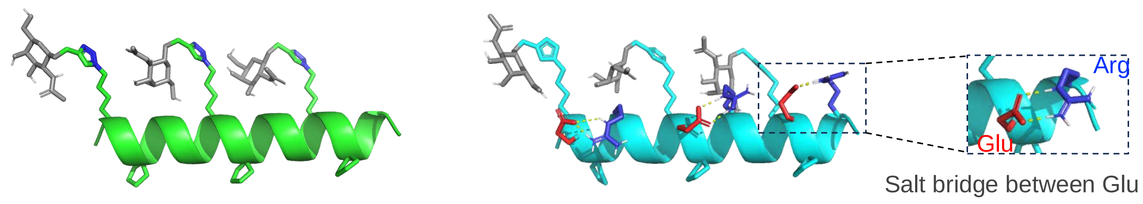
X =
Ac_5c
for stabilizing helix

1,2,3 =
GalNAc

5S Based on **5**, introduced salt bridge to stabilize helix and improving solubility.
LD Based on **5S**, destabilize the secondary structure by introducing L and D-amino acids alternatively.

3. Secondary structure analysis

In silico conformational search (MD)

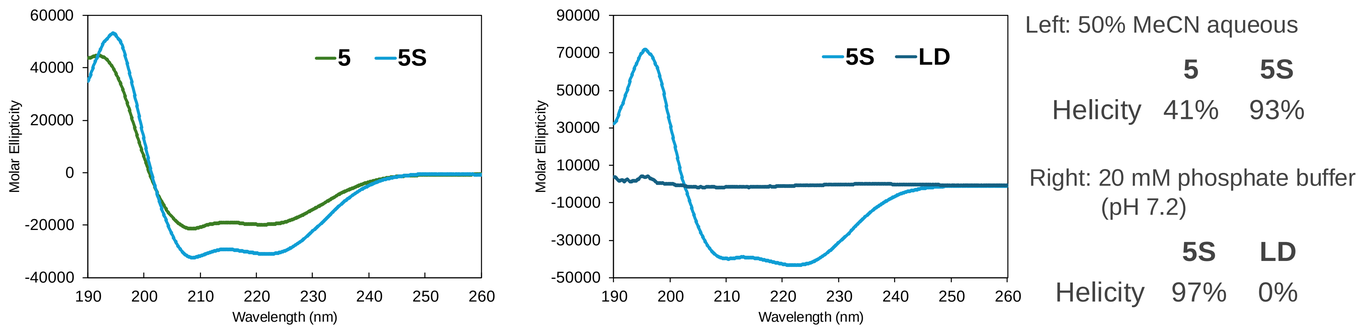


-297.5354 kcal/mol for **5**

-1020.4069 kcal/mol for **5S**

Salt bridge between Glu and Arg

CD spectra



Left: 50% MeCN aqueous

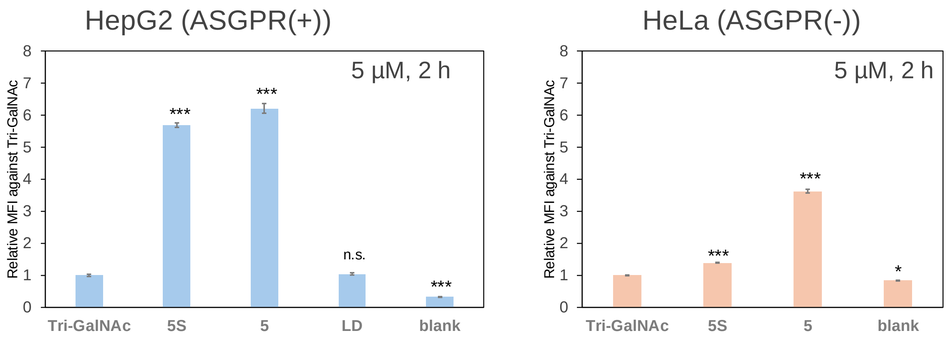
	5	5S
Helicity	41%	93%

Right: 20 mM phosphate buffer (pH 7.2)

	5S	LD
Helicity	97%	0%

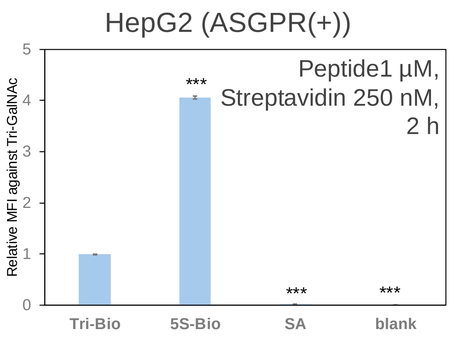
5S Secondary structure stability was confirmed by both MD and CD spectra by introducing salt bridge on peptide.
LD Did not showed specific secondary structure.

4. Cellular uptake studies



5 Better uptake than Tri-GalNAc but tissue-selectivity was low.
5S Equal activity to **5**, better tissue-selectivity.
LD Lower uptake than **5S** due to unstable secondary structure.

5. Cellular uptake of streptavidin



Peptide	Sequence
5S-Bio	Biotin-BS1EXSRLS2EXSRLS3EXSRL

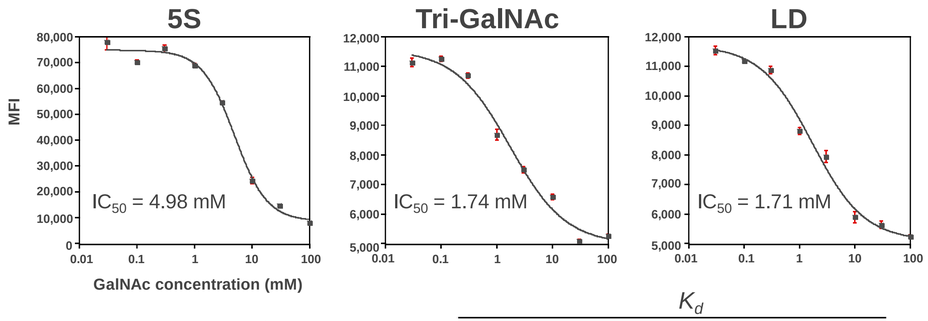
Biotin =

Tri-Bio

5S-Bio 4-folds better than **Tri-Bio**.
5S-Bio delivered protein into the hepatocytes.

SA: Alexafluor-647-labeled streptavidin without carriers.

6. Cell-based competitive binding study



$$K_d = Ki \times \frac{Compound\ concentration}{IC_{50} - Ki}$$

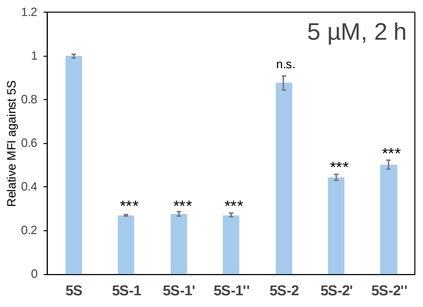
K_i = 150 μM

	K_d
5S	155 nM
Tri-GalNAc	472 nM
LD	481 nM

Evaluation of the affinity against HepG2 by varying GalNAc concentration.
5S exhibited 3-folds better affinity than others.

7. Effects of number and position of GalNAc

Peptide	Sequence
5S-1-FITC	FITC-BS1EXSRLSAEXSRLSAEXSRL
5S-1'-FITC	FITC-BSAEXSRLS2EXSRLSAEXSRL
5S-1''-FITC	FITC-BSAEXSRLSAEXSRLS3EXSRL
5S-2-FITC	FITC-BS1EXSRLS2EXSRLSAEXSRL
5S-2'-FITC	FITC-BS1EXSRLSAEXSRLS3EXSRL
5S-2''-FITC	FITC-BSAEXSRLS2EXSRLS3EXSRL

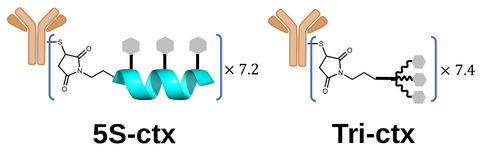


Peptides were designed to identify which GalNAc units contributes ASGPR bindings.

Single GalNAc Exhibits similar uptake trends.
Double GalNAc **5S-2** maintained the activity.

The pair of position 1 and 2 of GalNAc contributes to cellular uptake.

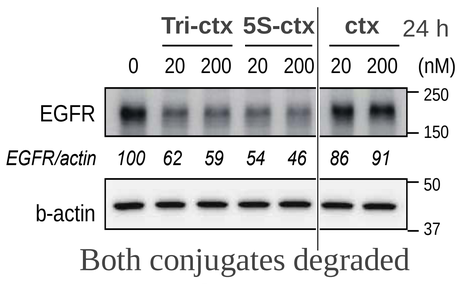
8. Application to protein degradation



Evaluated the activity of membrane protein degradation (application as LYTACs).

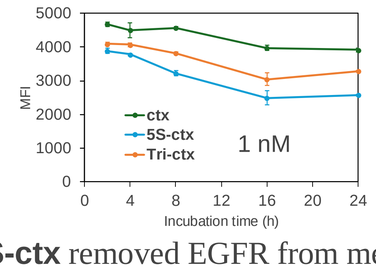
Ctx: anti-epidermal frowth factor receptor (EGFR) antibody

Western blot



Both conjugates degraded EGFR in low concentration.

EGFR clearance from membranes



5S-ctx removed EGFR from membranes efficiently than Tri-GalNAc.

9. Conclusion

GalNAc conjugated helical peptides were more effectively taken up into hepatocytes than flexible ligands such as Tri-GalNAc and peptide LD. Peptide 5S exhibited high hepatocyte selectivity by introducing a salt bridge. Also, we clarified the effects of the number and position of GalNAc on cellular uptake. Furthermore, stronger binding to HepG2 cells was found to be associated with stronger uptake activity. Peptide 5S could be applicable to protein degradation and it facilitates endocytosis of EGFR efficiently than Tri-GalNAc. The results demonstrate a molecular design strategy using helical peptides as stable templates for ligands with specific orientations.

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We declare no conflict of interests.