https://doi.org/10.17952/37EPS.2024.P2223

Molecular Design for Cytosolic Delivery of Antibodies Using an Intracellular Delivery Peptide

○Yoshimasa Kawaguchi[1], Shiroh Futaki[1] [1]Institute for Chemical Research, Kyoto University

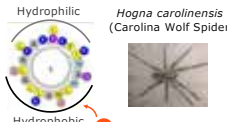
Introduction

Cytosolic Antibody Delivery

☺ High antigen recognition ability
☹ Lack of membrane permeability

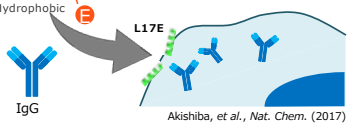
Cytosolic antibody delivery methods are required to expand the target of antibodies in the cells

L17E : Attenuated Cationic Amphiphilic Lytic Peptide



Hogna carolinensis (Carolina Wolf Spider)

Peptide	Sequence	CC50 (μM)
M-Lycotoxin	IWLTALKFLGKHAAKHLAKQQLSKL-amide	1.36
L17E	IWLTALKFLGKHAAKHEAKQQLSKL-amide	> 40

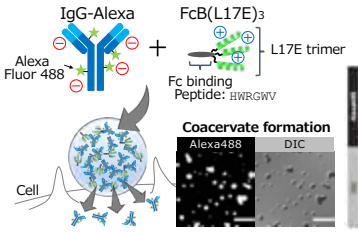


Akishiba, *et al.*, *Nat. Chem.* (2017)

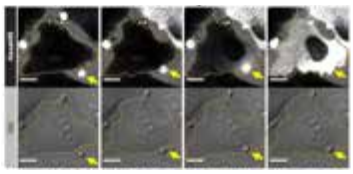
☺ Cytosolic antibody delivery was achieved by mixing L17E and antibodies
☹ Both L17E and antibodies require high concentrations

Created L17E trimer to improve the cytosolic delivery ability

Cytosolic Antibody Delivery by Coacervate



Influx occurs approximately 1 min after attach with the cell.



Iwata, *et al.*, *Angew. Chem. Int. Ed.* (2021)

☹ Requires random fluorescent labeling
- Affects antigen recognition and reproducibility
- Difficult to analyze interaction patterns

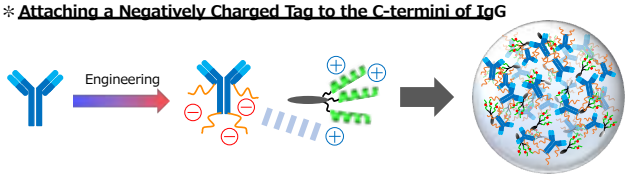
Research aim

Development of antibody molecules that form coacervates and achieve cytosolic delivery without fluorescent labeling.

Strategy

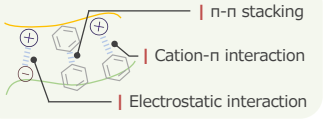
Design of Anion Antibody Scaffold

※ Attaching a Negatively Charged Tag to the C-termini of IgG



※ Design of the Negatively Charged Tag Sequence

Interaction mode for LLPS*



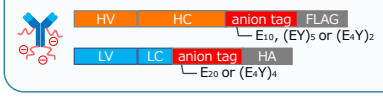
Anionic tag	Sequence
E10	EEEEEEEEEE
E20	EEEEEEEEEEEEEEEEEEEE
$(EY)_{10}$	EYEYEYEYEY
$(E_4Y)_2$	EEEEYEEEEY
$(E_4Y)_4$	EEEEYEEEEYEEEEYEEEEY

*LLPS: liquid-liquid phase separation

Results & Discussion

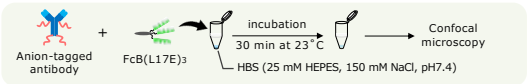
Coacervate Formation

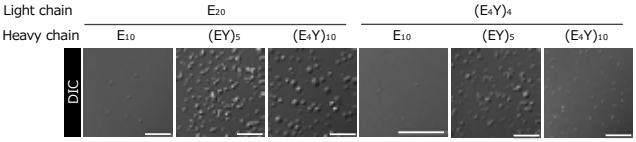
※ Anion-tagged antibody scaffold



Light chain	Heavy chain	Light chain	Heavy chain
E20	E10	(E4Y)4	E10
	(EY)5		(EY)5
	(E4Y)2		(E4Y)2

Observation of Coacervate





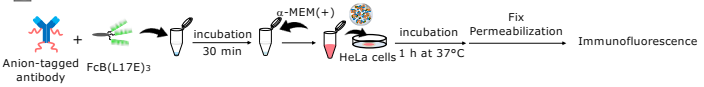
Peptide/Ab; **1**, scale bars; 5 μm

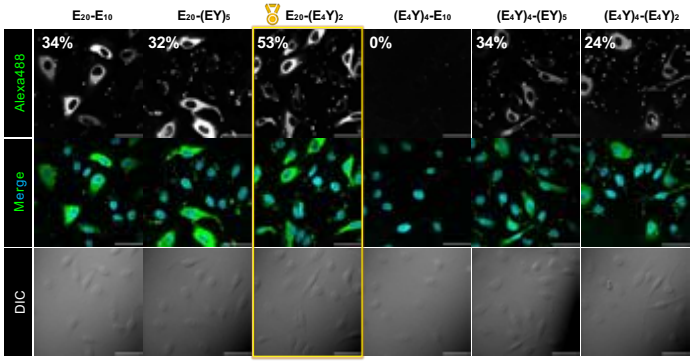
Formation of stable coacervates in HBS by introducing negatively charged tags into the heavy and light chains.

Results & Discussion

Intracellular Delivery of Antibodies by Coacervate

Cytosolic Antibody Delivery



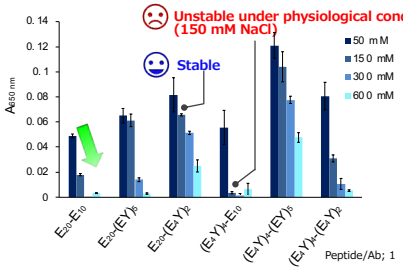


FcB(L17E)3 final conc. 1 μM, IgG final conc. 1 μM, (Peptide/Ab; **1**), scale bars; 50 μm

※ The delivery efficiency differed depending on the tag sequence
※ Coacervate composed of E20-$(E_4Y)_2$ demonstrated the highest delivery efficiency

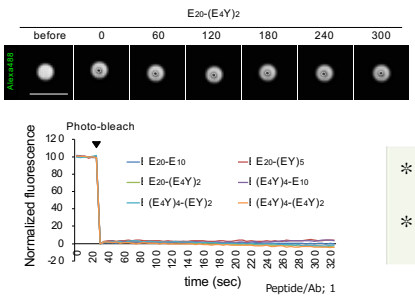
Physical Properties of Coacervates

Turbidity: Stability



※ Electrostatic interactions are important for coacervate formation
※ Coacervate formation under physiological condition is crucial for cytosolic delivery of Antibodies.

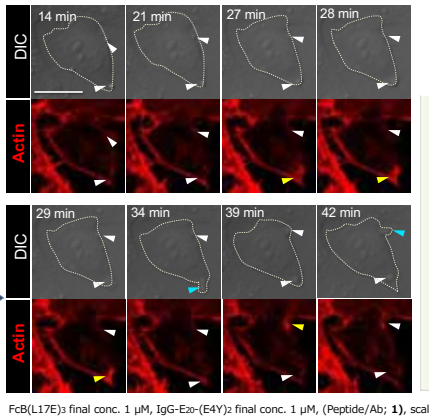
Fluorescence Recovery After Photobleaching: Fluidity



※ Fluorescence recovery was not observed.
※ Coacervates may be gel-like particles with low fluidity

Unique Intracellular Uptake of Coacervate

Timelapse imaging: lifeact-mCherry-expressing cells (Actin stain)



※ Observation of accumulation of actin around particles after being taken up by cells (Yellow arrowheads)
※ Following the actin acculation, an arm-like structure emerged. (blue arrowheads)

New mechanism of cellular uptake ?

FcB(L17E)3 final conc. 1 μM, IgG-E20-(E4Y)2 final conc. 1 μM, (Peptide/Ab; **1**), scale bars; 50 μm

Summary

※ A negatively charged antibody scaffold was created to form a coacervate with FcB(L17E)3 and achieve intracellular delivery of antibodies.
※ The stability of coacervate at physiological salt concentrations was important for intracellular delivery by the coacervates.
※ Intracellular uptake of the coacervate may be a novel uptake mechanism.