Polysaccharides modified with attenuated cationic lytic peptides: Formation of microparticles and their use in cytosolic antibody delivery

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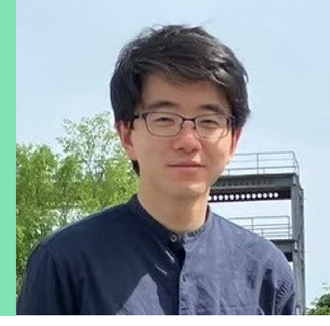

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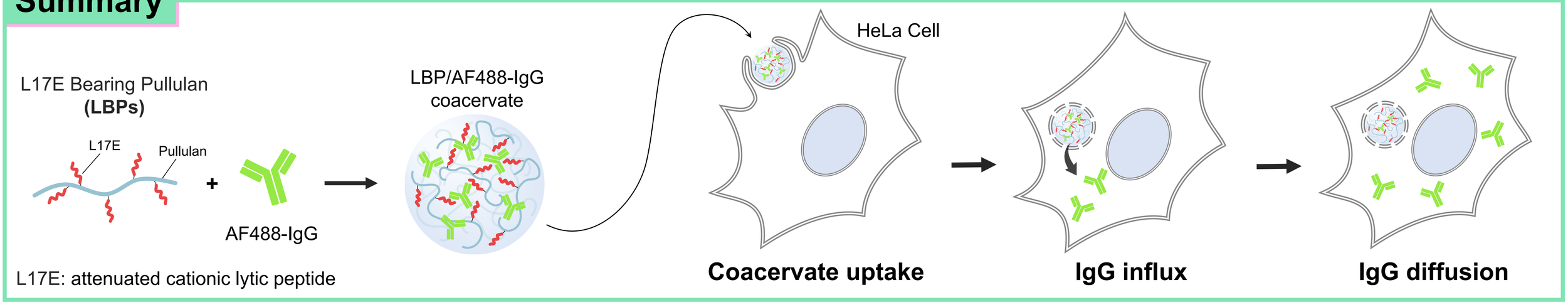
Summary

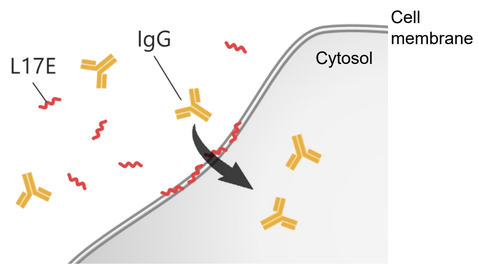


Background

- IgG does not penetrate cell membrane.
- With L17E, IgG can move into cytosol
  → but not efficient enough

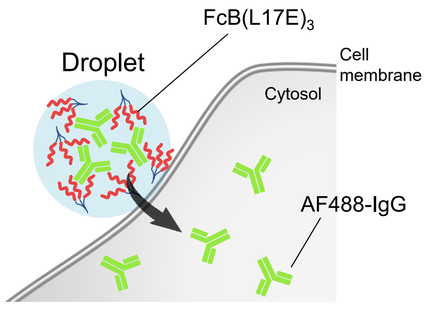
L17E：IWLTALKFLGKHAAKHEAKQQLSKL-NH_2

Akishiba, M. *et al.* (2017) *Nat. Chem.* 9, 751-761.



- $FcB(L17E)_3$, trimer of L17E, can deliver IgG into cytosol more efficiently by forming microdroplets with Alexa Fluor 488 labeled IgG（AF488-IgG）.

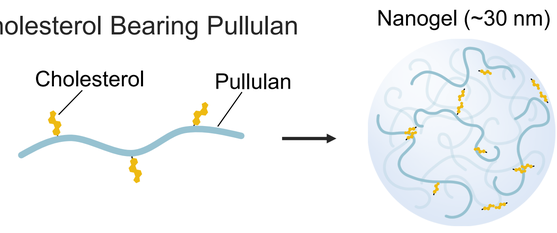
Iwata, T. *et al.* (2021) *Angew. Chem. Int. Ed. Engl.* 60, 19804-19812.



Develop more stable coacervate for future therapeutic application

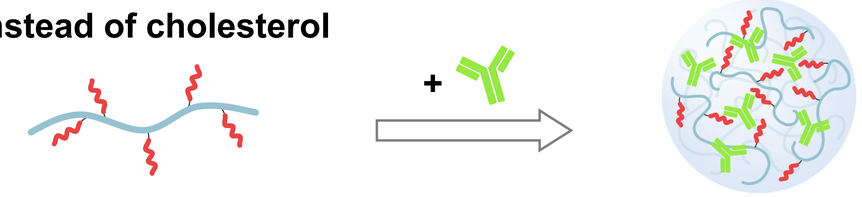
Strategy

- Pullulan, a kind of polysaccharide, was used.
- Cholesterol bearing pullulan forms nanogel.
  Akiyoshi, K. *et al.,* (1997) *Macromolecules.* 30, 857-861.
- Pullulan nanogel is hydrophilic and low toxic.
  Kawasaki, R. *et al.,* (2021) *Adv. Healthc. Mater.* 10, e2001988.

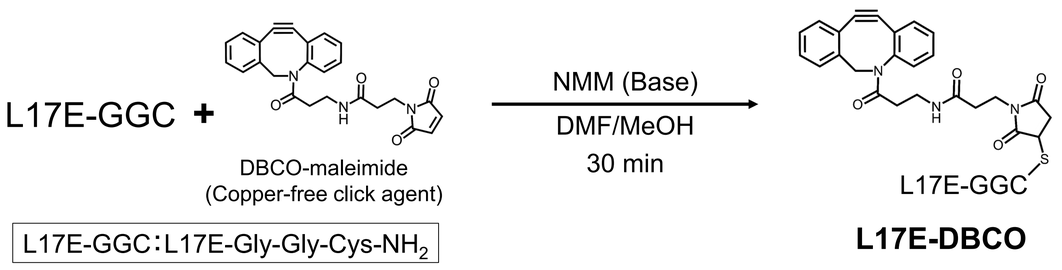


Pullulan modified with L17E instead of cholesterol

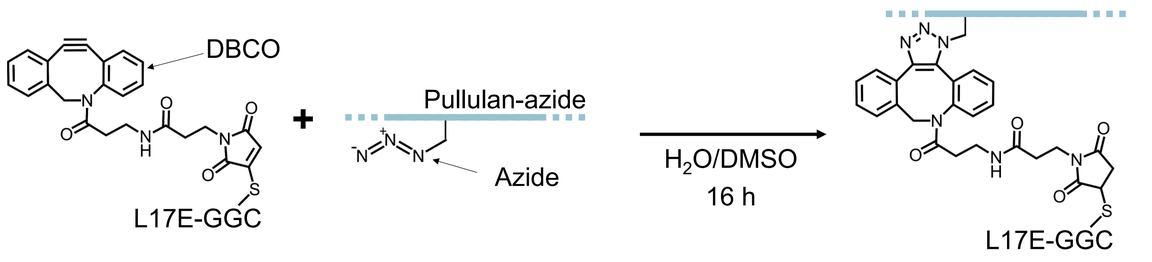
Coacervate formation?



Synthesis



L17E-GGC：L17E-Gly-Gly-Cys-NH_2

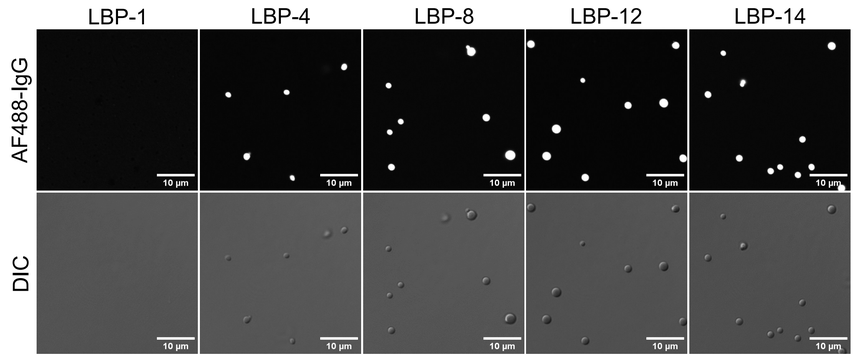


	Equivalence of added L17E-DBCO to 100 glucose units	Actual modification ratio to 100 glucose units
LBP-1	1	0.66
LBP-4	4	3.5
LBP-8	8	7.4
LBP-12	12	11.4
LBP-14	14	12.2

Actual modification ratio was measured by ^{1}H NMR.

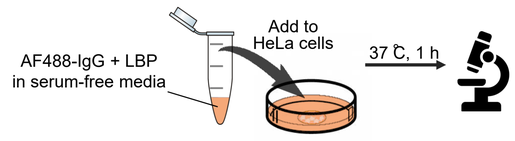
Result

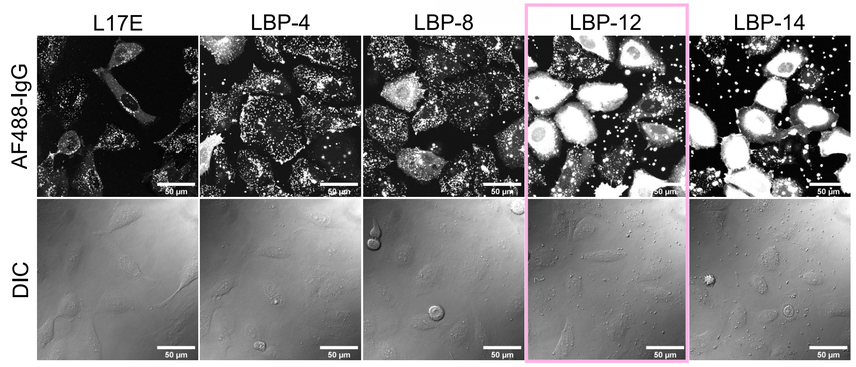
❑ LBPs can form 1-3 μm particles with AF488-IgG and multimerization of L17E is important for particle formation



LBPs: 3 μM
AF488-IgG: 56 μg/mL
※ Concentrations of LBPs are written as concentration of L17E.

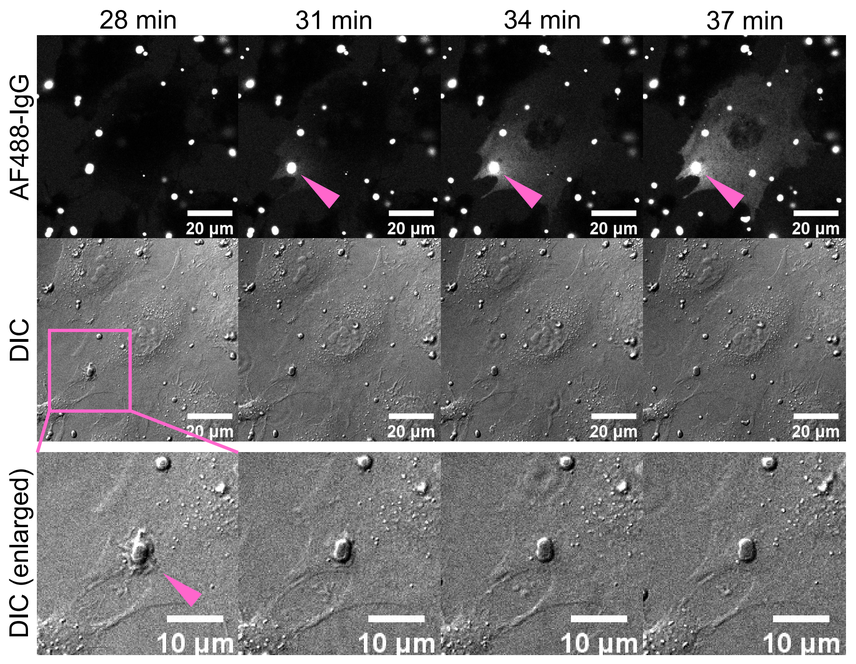
❑ LBPs efficiently delivered AF488-IgG into cells





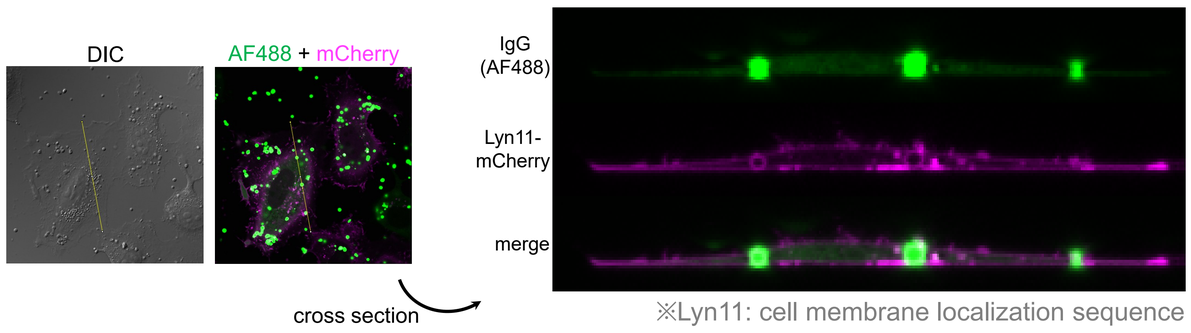
L17E: 40 μM
with 770 μg/mL AF488-IgG
LBP-4 and LBP-8: 1 μM
with 19 μg/mL AF488-IgG
LBP-12 and LBP-14: 3 μM
with 56 μg/mL AF488-IgG
※ Concentrations of LBPs are written as concentration of L17E.

❑ AF488-IgG diffusion started from the particle and the particle induces the structural change of cell membrane



❑ The particle was inside the cell after delivery and it was wrapped by membrane

➢ Non-canonical or atypical mode of cellular uptake?



※Lyn11: cell membrane localization sequence