Engineering Biomaterial-based Platform for Immune cells Manipulation

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Acknowledgement

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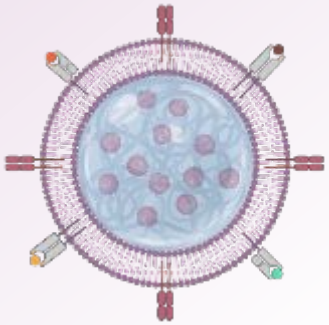
Financial disclosure

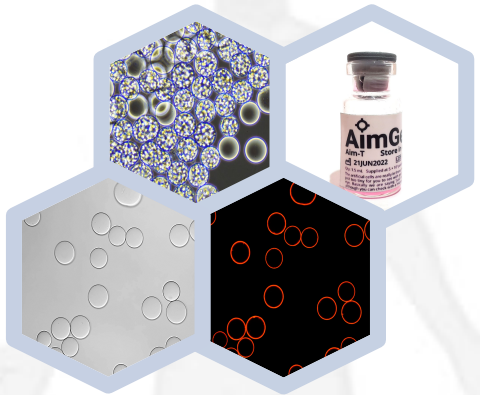
Allegrow
www.allegrowbiotech.com

- Ying Chau, C.M. Laurence Lau and J.T. Chung are co-founders of Allegrow Biotech Ltd

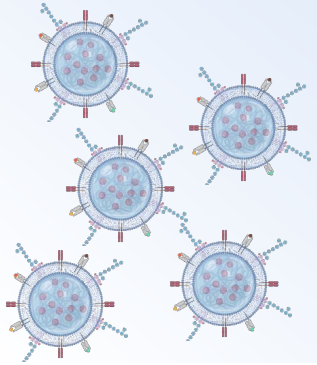
Use biomaterial approaches to manipulate immune cells

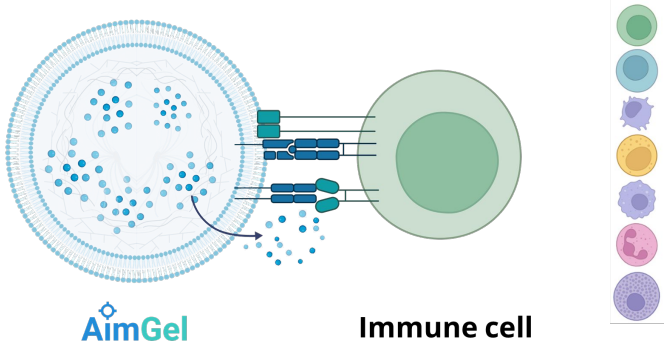
Produce immune cells
Ex vivo





Train and prime immune cells
In vivo



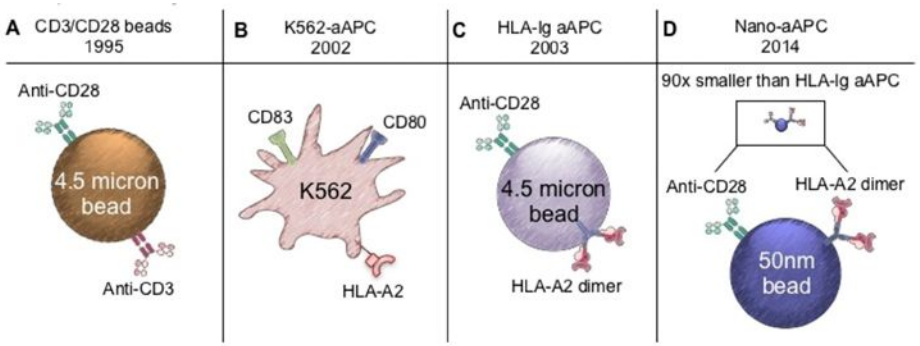


Reagent for cell manufacturing

Personalized therapeutic vaccines

Ex vivo T cell expansion

1



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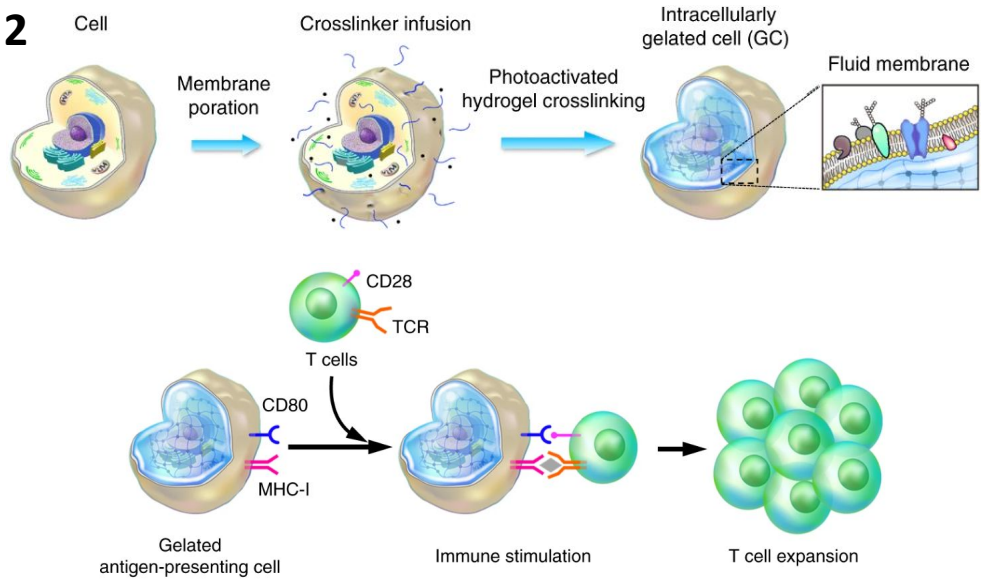

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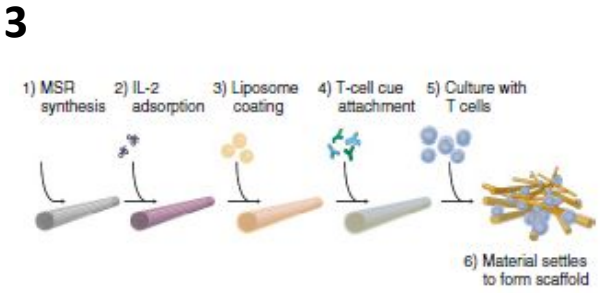

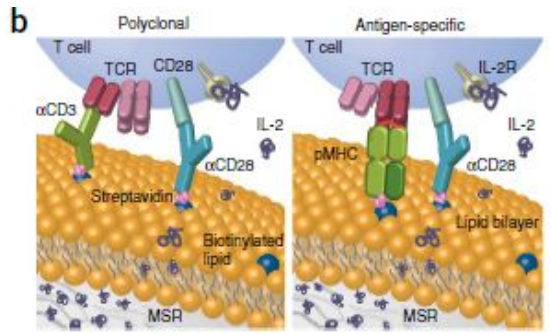

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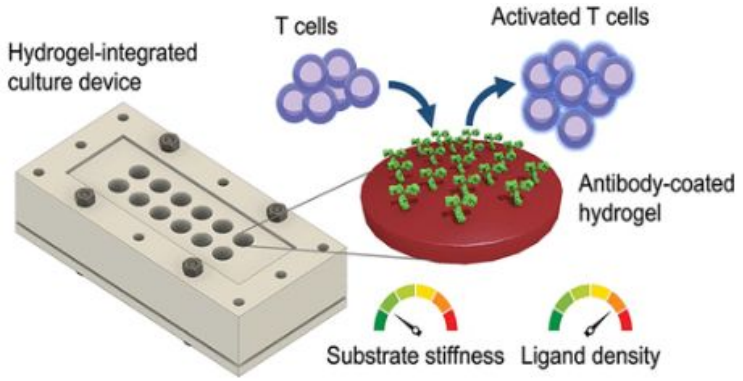

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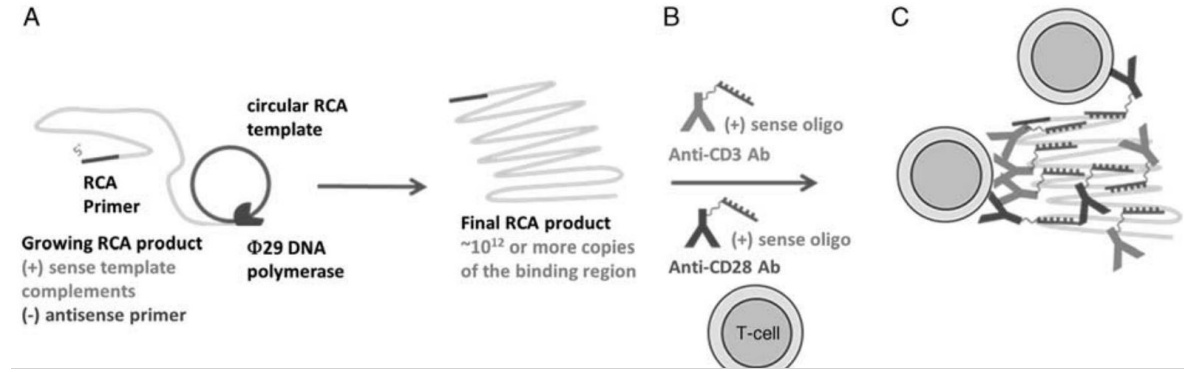
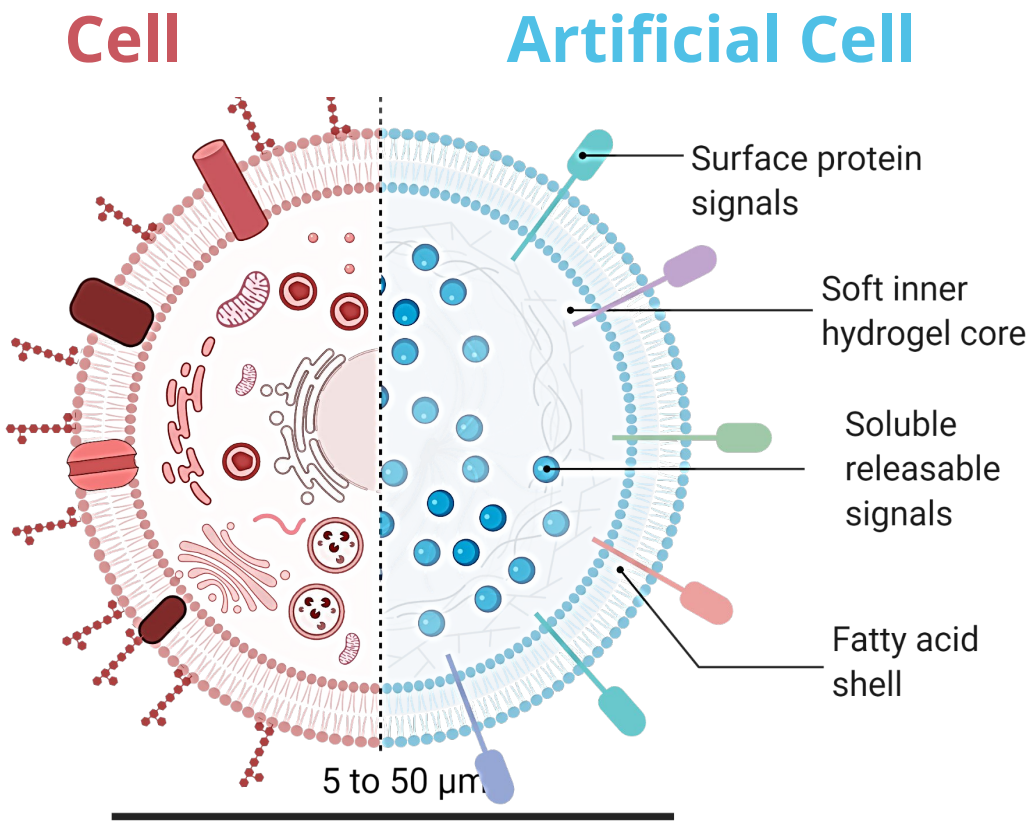
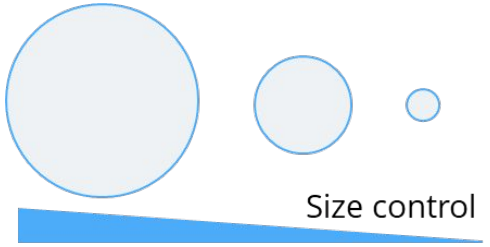


1. Neal, Lillian R., et al. "The basics of artificial antigen presenting cells in T cell-based cancer immunotherapies." *Journal of immunology research and therapy* 2.1 (2017): 68.
2. Lin, JC., Chien, CY., Lin, CL. et al. Intracellular hydrogelation preserves fluid and functional cell membrane interfaces for biological interactions. Nat Commun 10, 1057 (2019).
3. Cheung, Alexander S., et al. "Scaffolds that mimic antigen-presenting cells enable ex vivo expansion of primary T cells." Nature biotechnology 36.2 (2018): 160-169.
4. Chin, Matthew HW, et al. "A hydrogel-integrated culture device to interrogate T cell activation with physicochemical cues." ACS applied materials & interfaces 12.42 (2020): 47355-47367.
5. Keskar, Vandana, et al. "Novel DNA-based T-cell activator promotes rapid T-cell activation and expansion." Journal of Immunotherapy (Hagerstown, Md.: 1997) 43.8 (2020): 231.

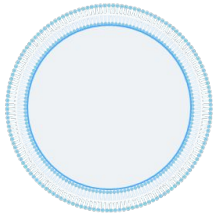
Ex vivo application of AimGel: Hydrogel microparticles (HMPs) as artificial antigen presenting cells (aAPCs)



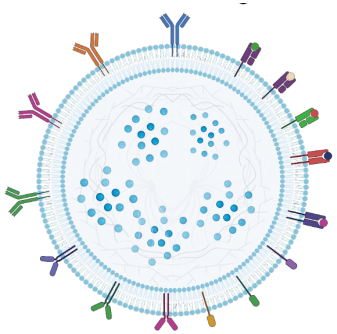
Core hydrogel fabrication



Membrane coating (synthetic or biological)



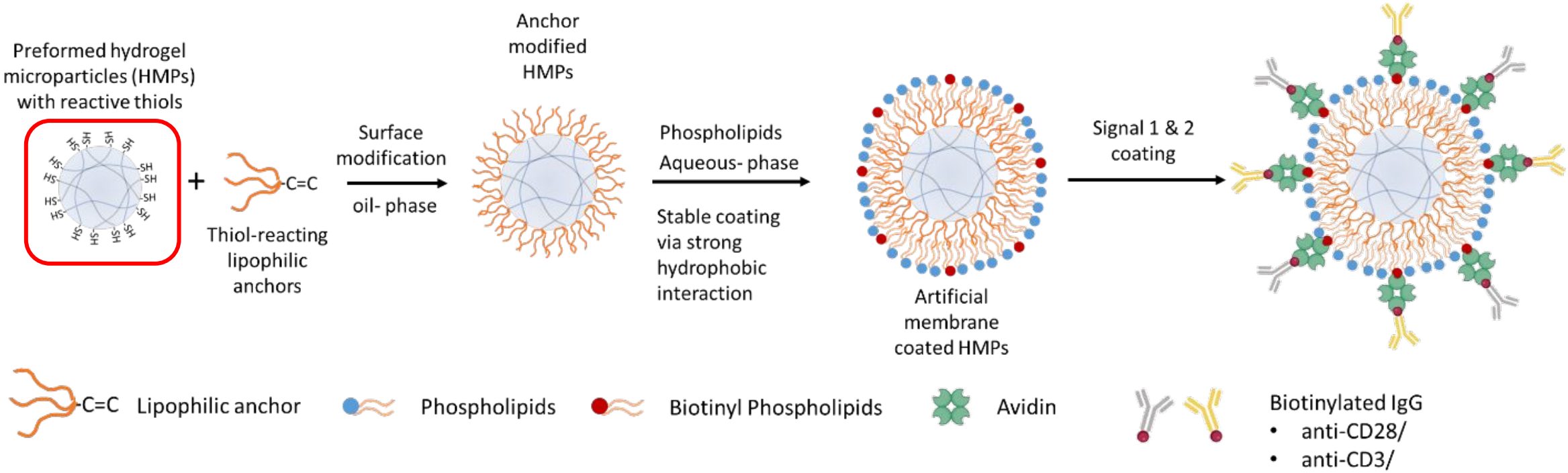
Programmed signal presentation



Favorable features of aAPCs for T cell interactions

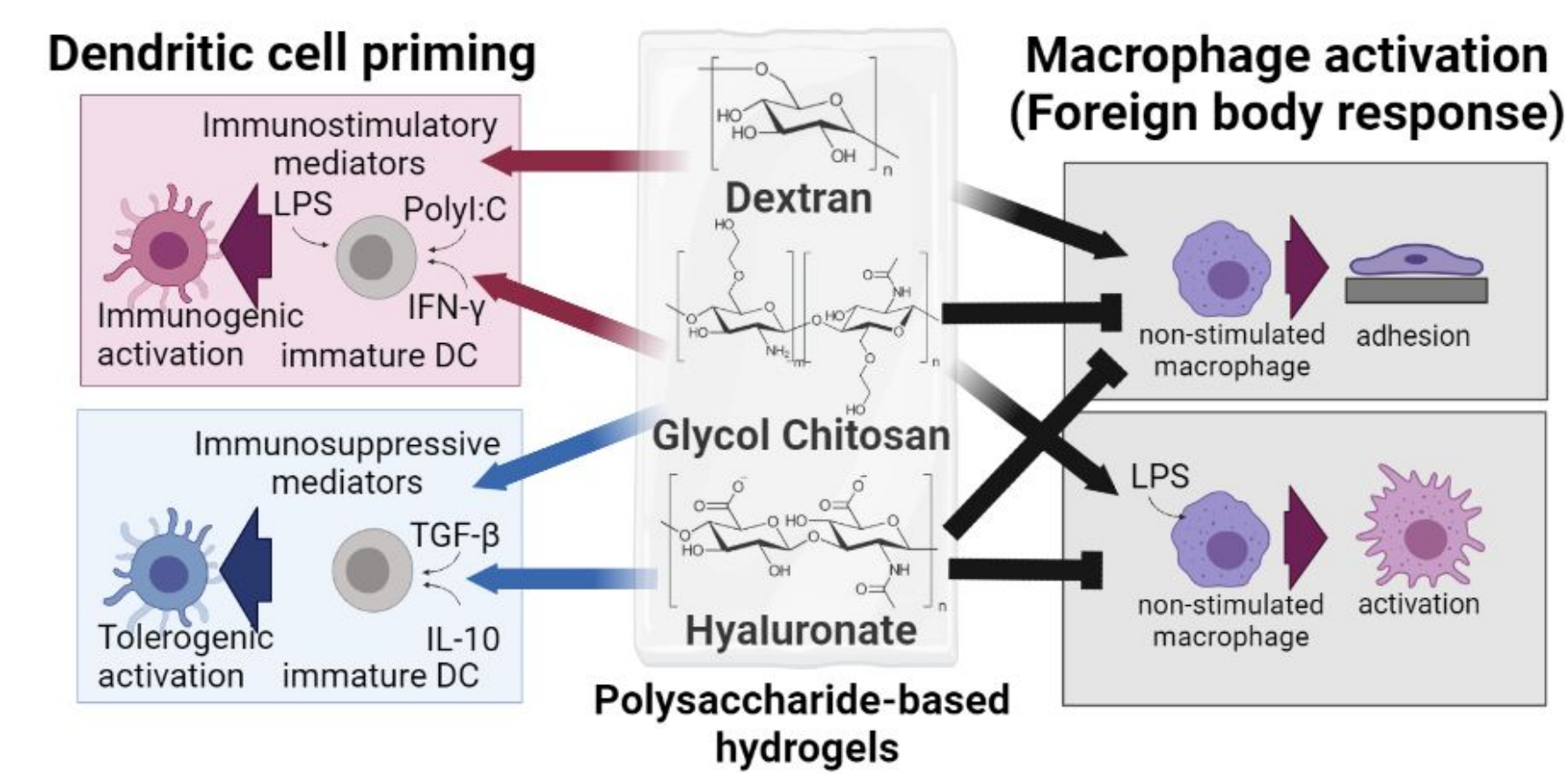
Features	Key parameters	Our approach
Softness	• 7~20 kPa	Chemically crosslinked hydrogel particles with appropriate crosslinking density
Size	• ≥ 300 nm	Hydrogel particles (HMP) with diameters of 5~30 μm
Signal 1 &2 density	• Distance btw signal ligands better **> 50nm and < 500 nm**	Screen for optimal ligand distance
Signal fluidity	• Signal 1 & 2 presented on a **fluidity** membrane	Wrap hydrogel particles with a stable yet fluidic membrane, then dock signal 1 & 2 on the membrane.
Signal 3 source	• Sustained release of signal 3 (e.g.) IL-2 correlated with higher CD8+ T cell proliferation	Neglect
Activation duration	• Sustained signal 1&2 presentation > **5 days**	Target HMP degrdation within 7 days

Design of Programmable Modularity



Signaling molecules	Functions	Presentation method
Anti-CD3	TCR binding (Signal 1)	Bound on HMPs surface
Anti-CD28	Co-stimulation (Signal 2)	Bound on HMPs surface
IL-2	Proliferation signal (Signal 3)	Soluble signal supplemented in medium

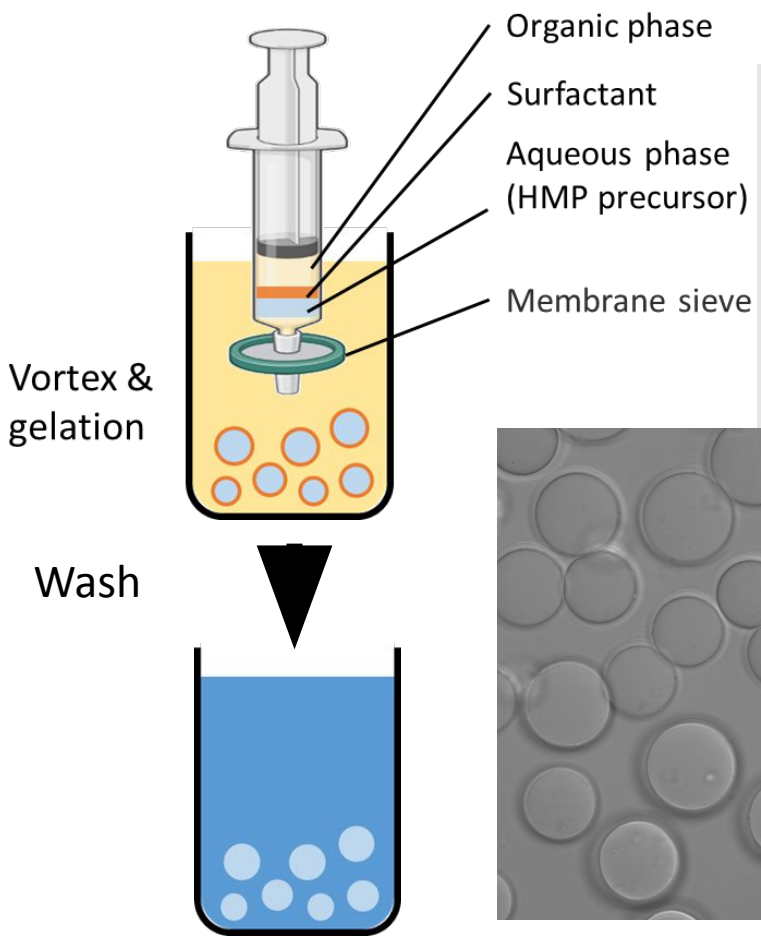
Polysaccharide as the artificial cell core



Chung, J. T., Lau, C. M. L., Chau, Y. (2021), The effect of polysaccharide-based hydrogel on the response of antigen presenting cell line to immunomodulators. Biomater. Sci.

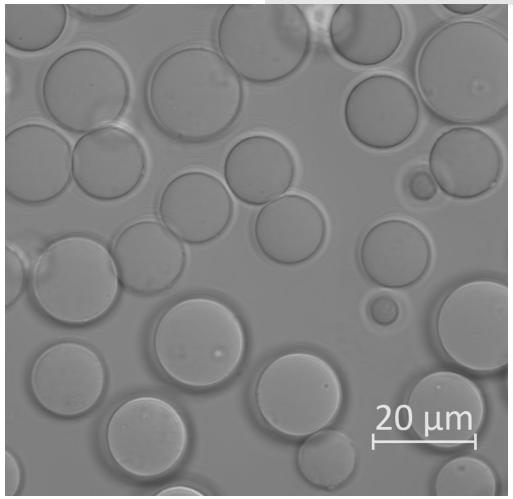
Hydrogel microparticles (HMP) of controllable size

Inverse emulsion (w/o)

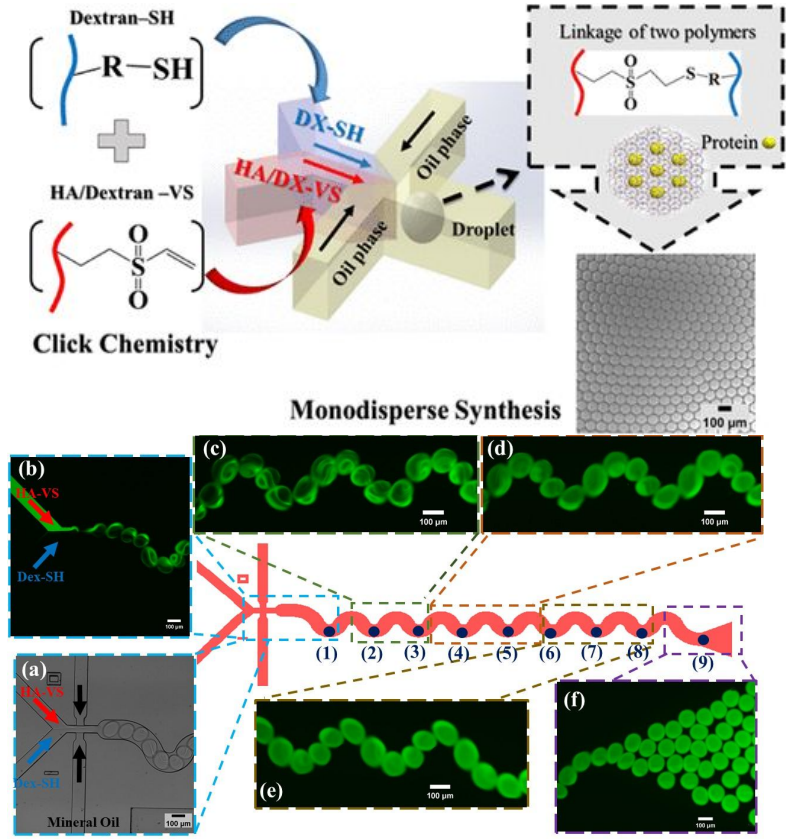


Rough size control

Batch mode



Microfluidic (w/o)



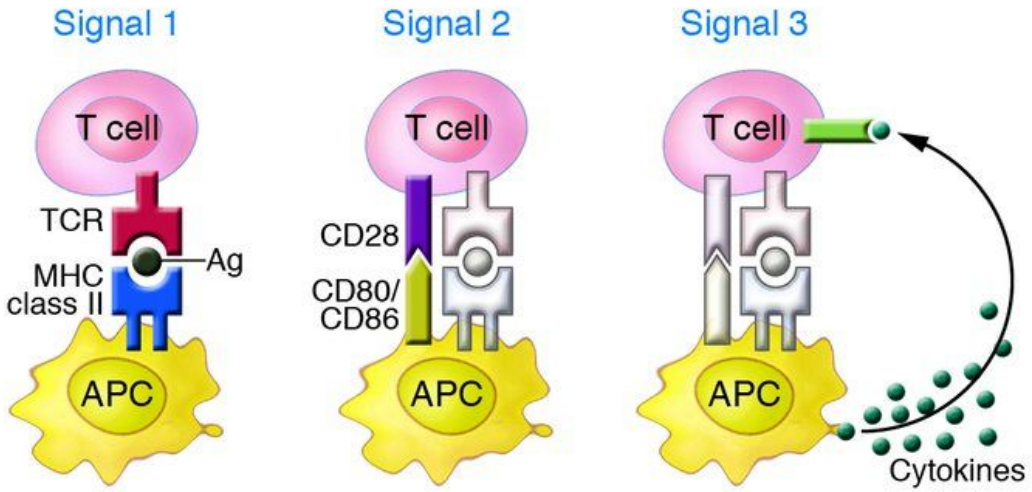
Precise size control

Continuous manufacturing

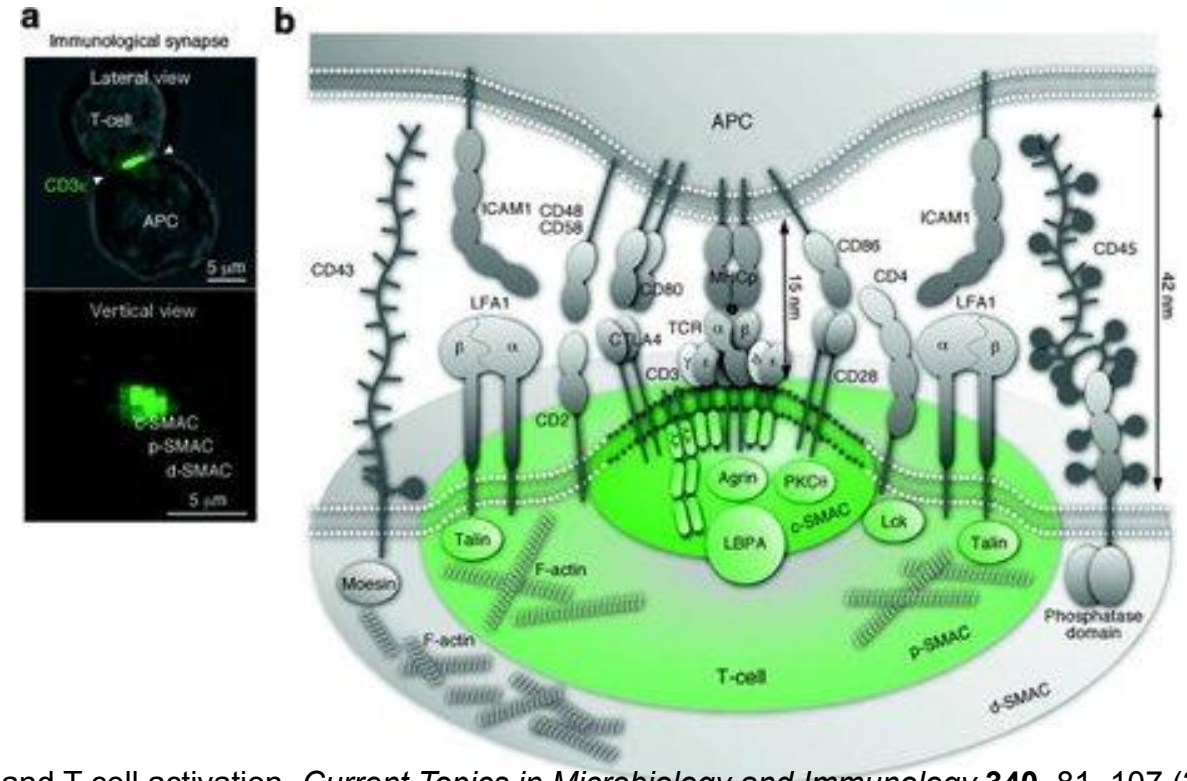
Chung, C. H., Lau, C. M. L., Sin, D. T., Chung, J. T., Zhang, **Y., Chau**, Y., & Yao, S. (2021). Droplet-Based Microfluidic Synthesis of Hydrogel Microparticles via Click Chemistry-Based Cross-Linking for the Controlled Release of Proteins. ACS Applied Bio Materials, 4(8), 6186-6194.

Essential features of artificial antigen presenting cells (APCs) for effective T cell interactions

Necessary signals for APC to instruct T cell functioning



Supramolecular activation cluster (SMAC)

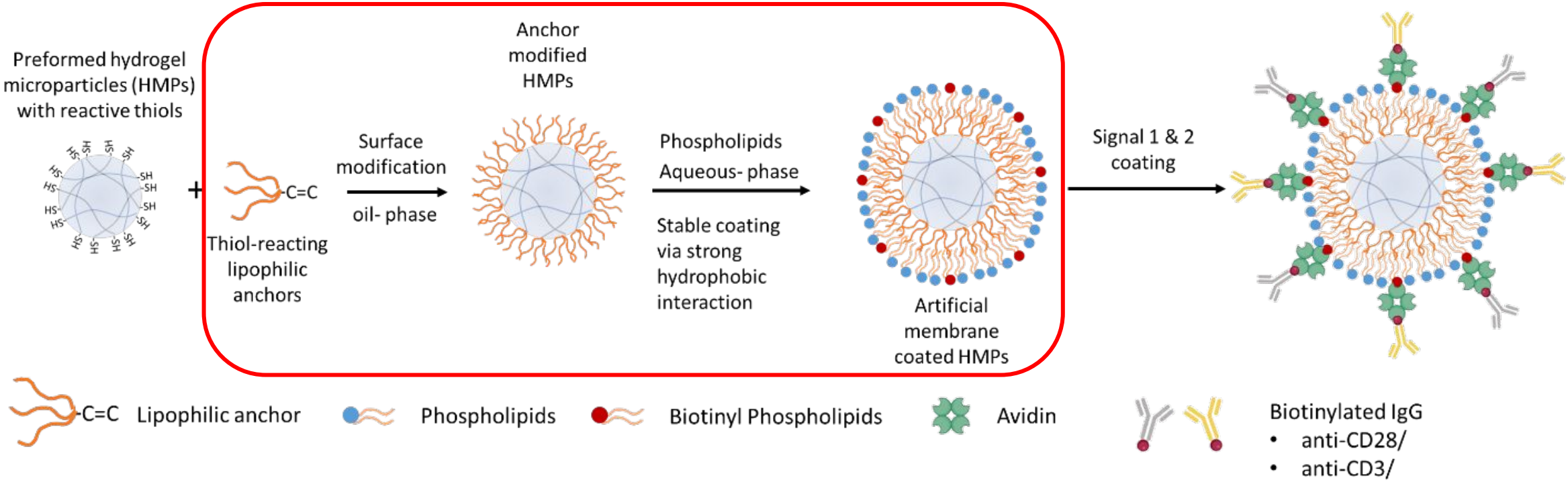


Yokosuka, T. & Saito, T. The immunological synapse, TCR microclusters, and T cell activation. *Current Topics in Microbiology and Immunology* **340,** 81–107 (2010)

Kim, J. K., Shin, Y. J., Ha, L. J., Kim, D. H. & Kim, D. H. Unraveling the Mechanobiology of the Immune System. *Advanced Healthcare Materials* **8,** (2019)

Gutcher, I. & Becher, B. APC-derived cytokines and T cell polarization in autoimmune inflammation. *Journal of Clinical Investigation* **117,** 1119–1127 (2007)

Design of Programmable Modularity



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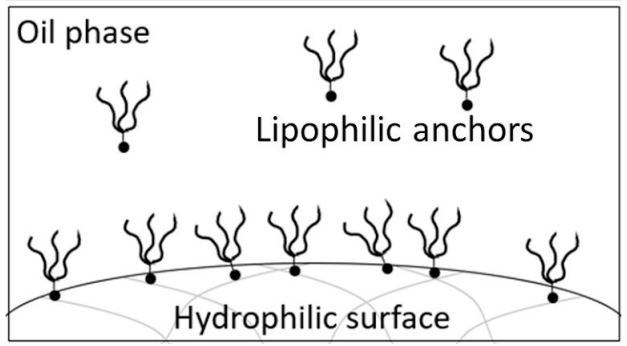
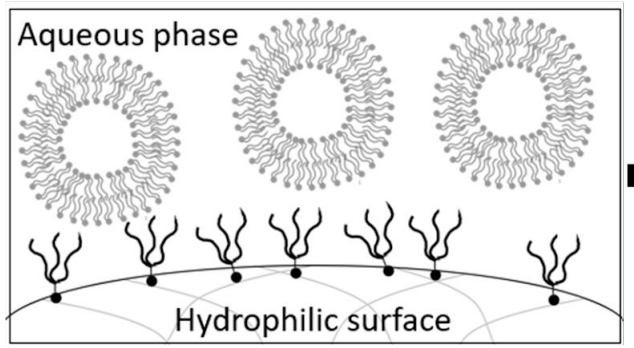
Screening lipophilic linkers for phospholipid coating

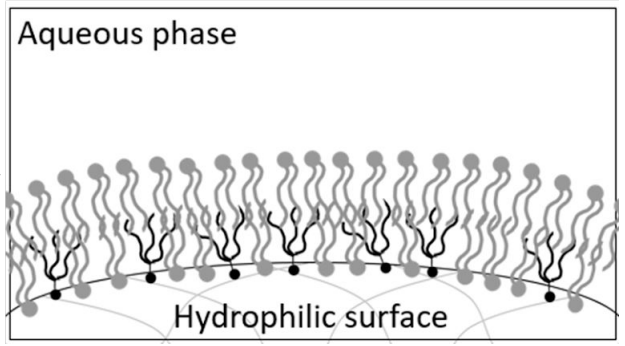
Conjugate lipophilic anchors on a hydrophilic surface



Incubate with liposomes or lipid vesicles



Lipids got adsorbed to the surface and form a stable layer



Selective lipophilic anchors being screened

Tail No.	Tail length				
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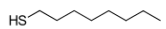
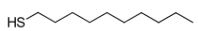
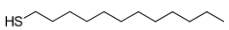
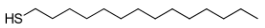
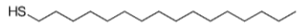
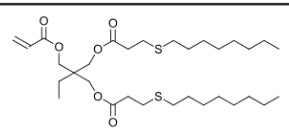
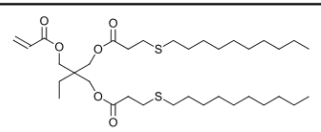
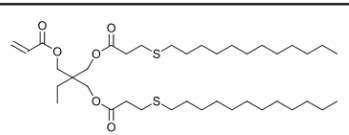
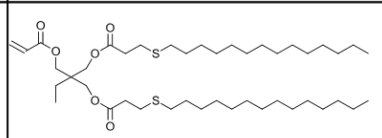
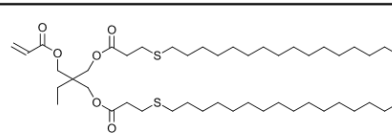
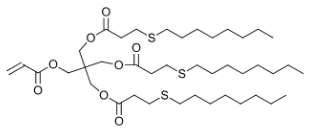
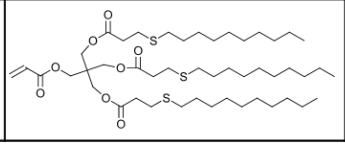
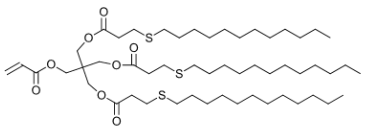
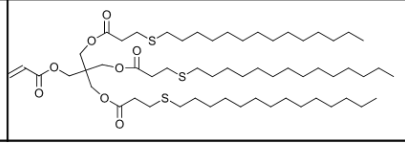
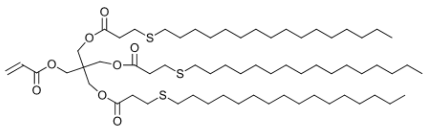
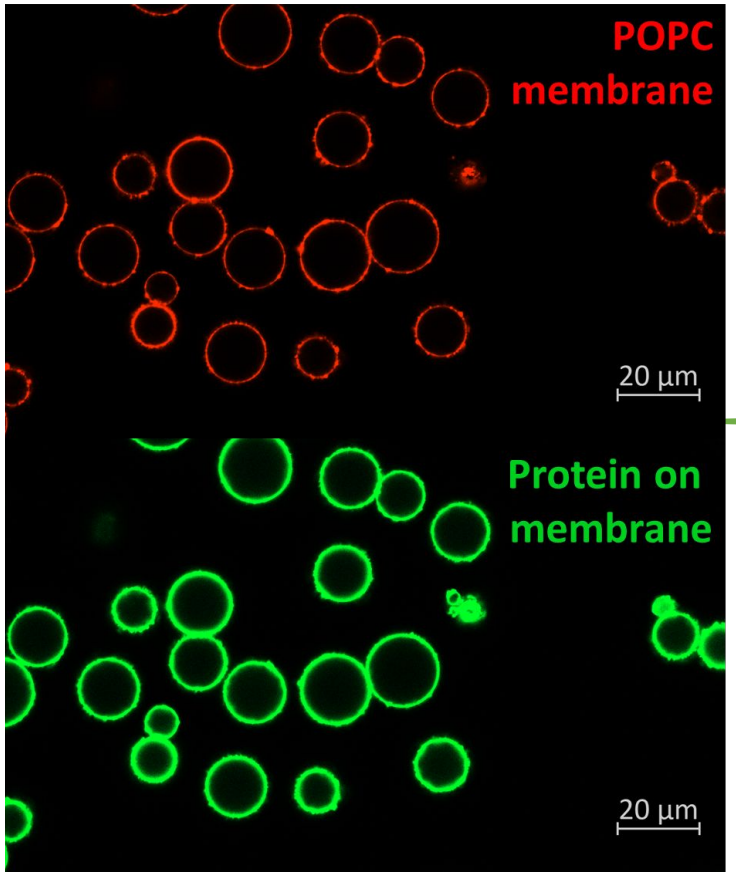
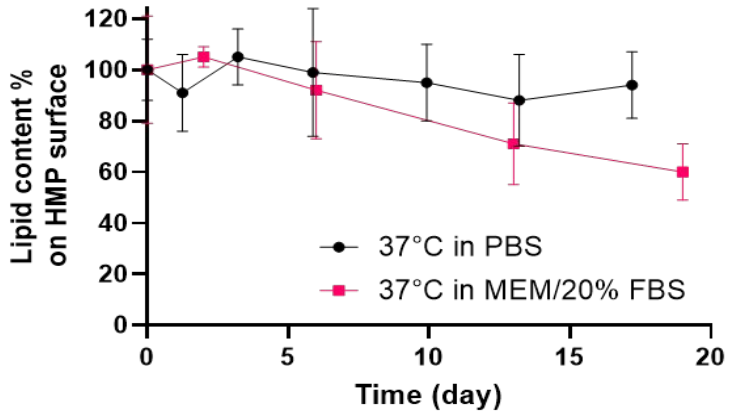
1					
2					
3					

Chau Y, Lau CML, Chung JT, Abesekara MS. Compositions of lipophilic anchors and the use thereof. US provisional Patent Application No. 63/389,897. Pending.

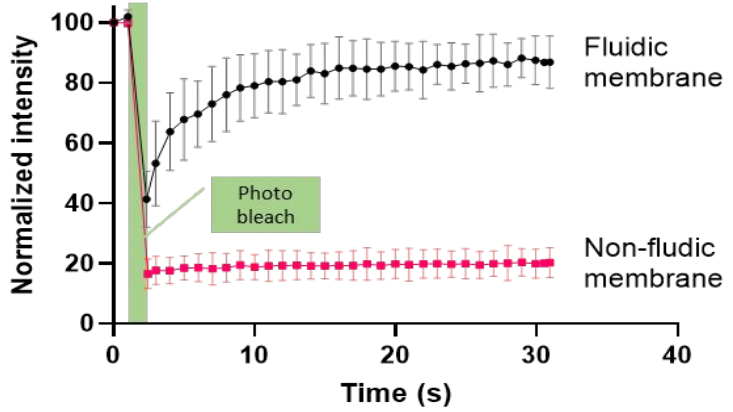
Biomimetic HMP surface: stable and fluidic phospholipid coating

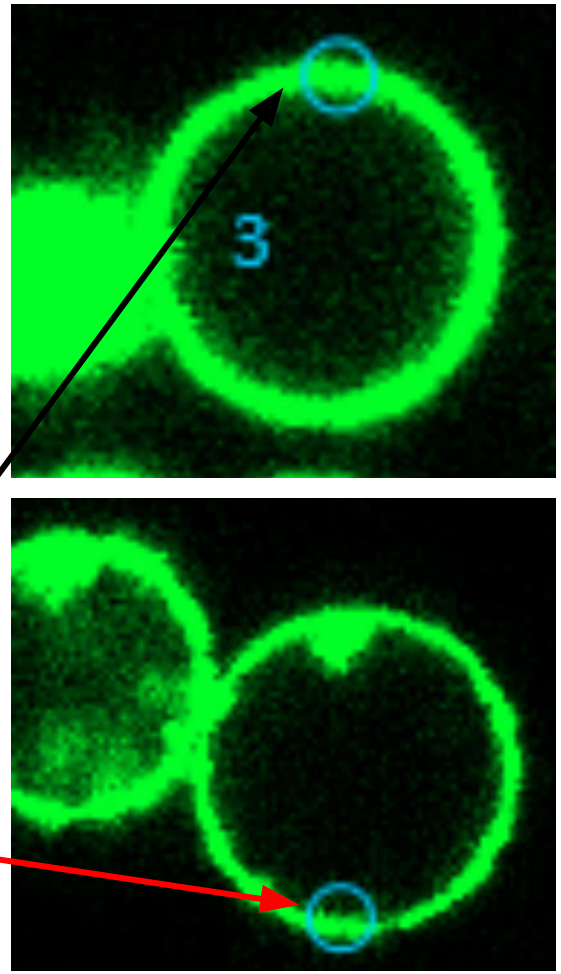


Signals presented on **stable** membrane



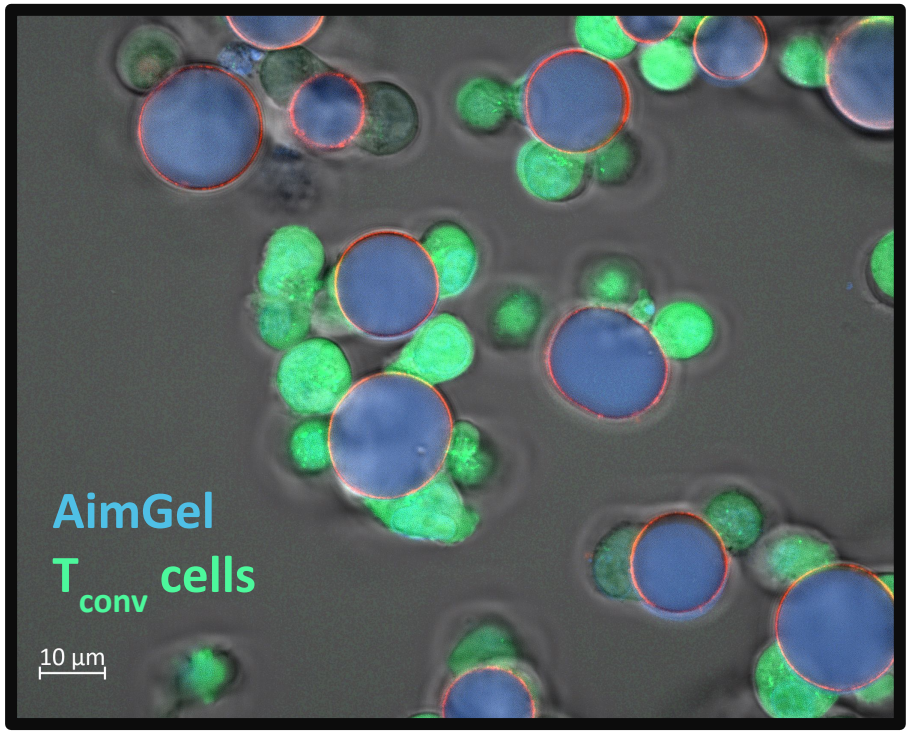
Signals presented on **fluidic** membrane

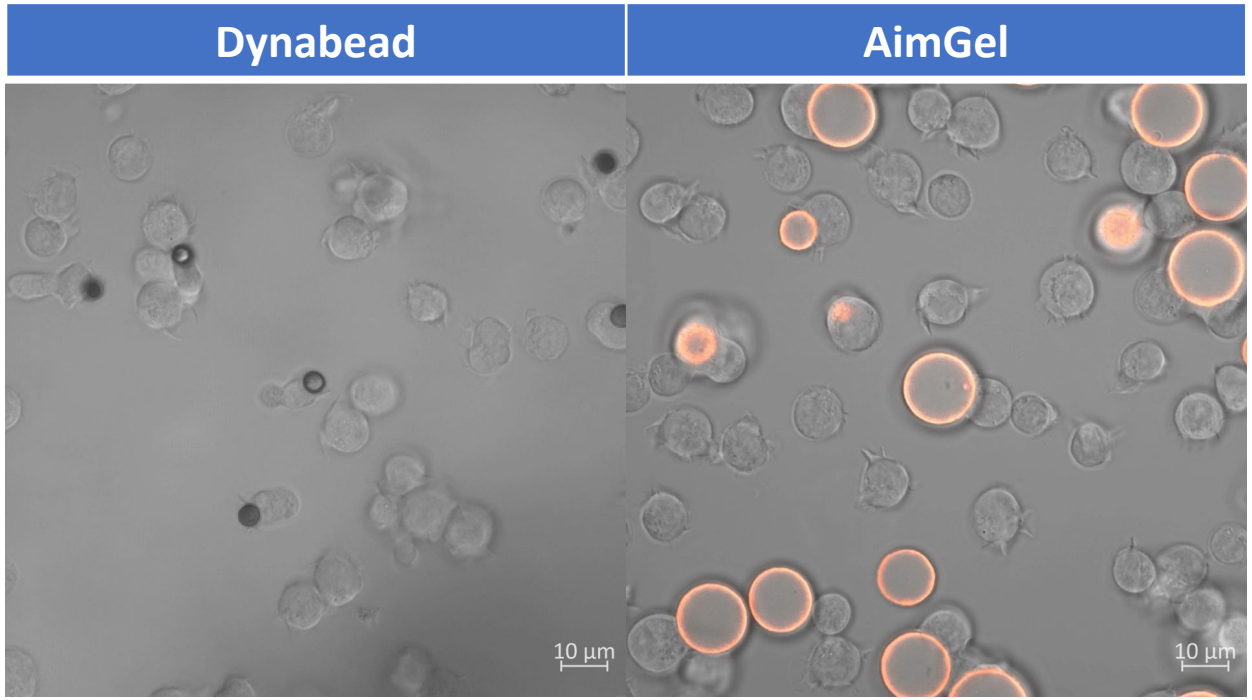




Manuscript in preparation
Patent pending

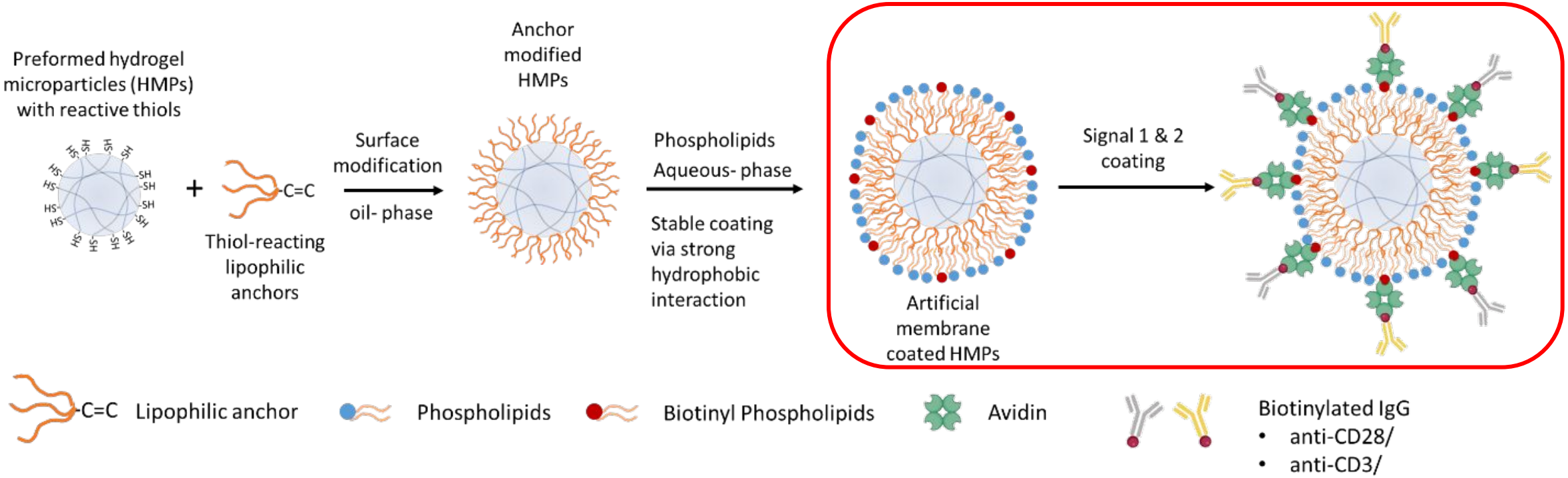
AimGel:
Hydrogel microparticles (HMPs) as aAPCs effectively interact with T cells





Manuscript in preparation /Patent pending

Design of Programmable Modularity



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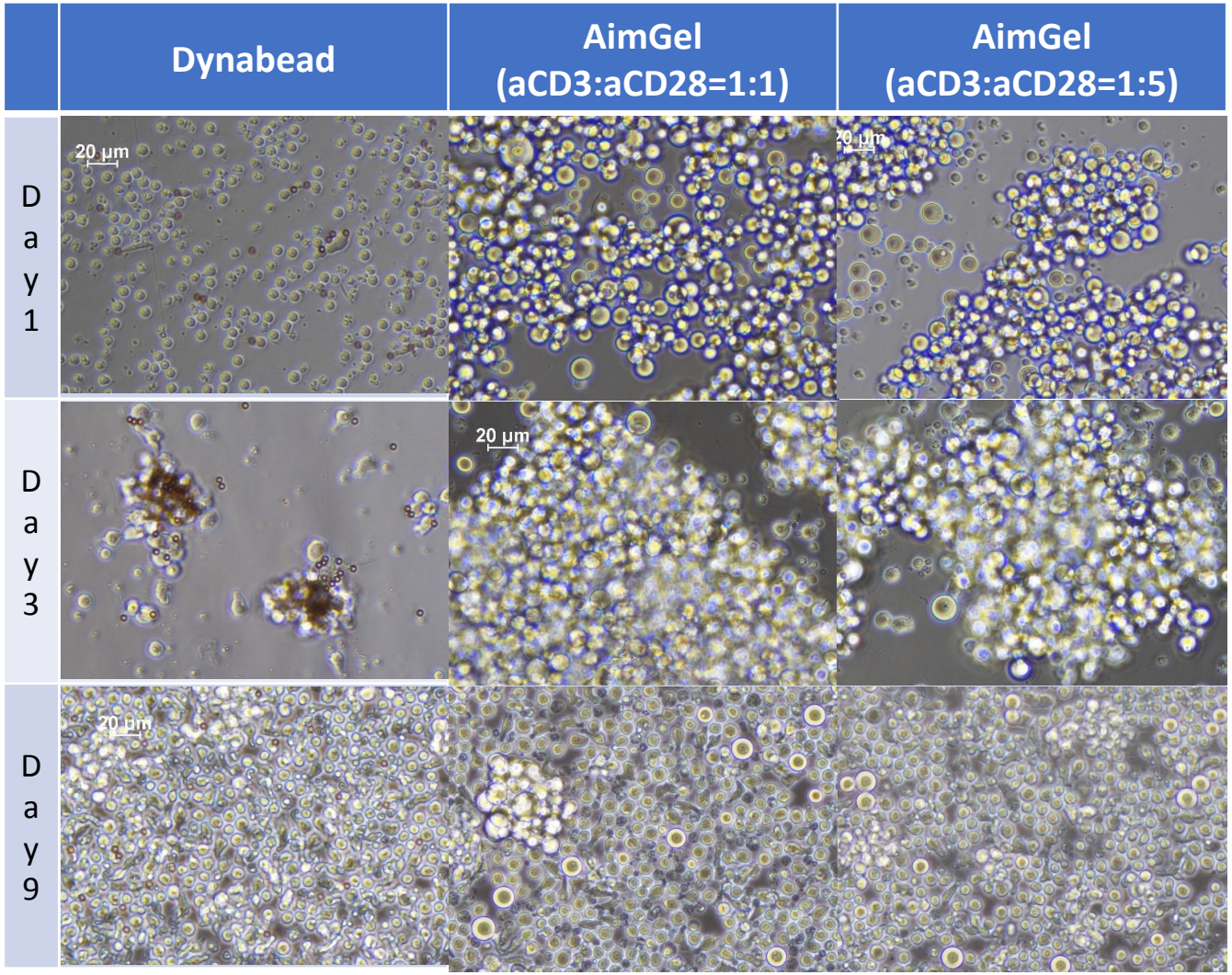
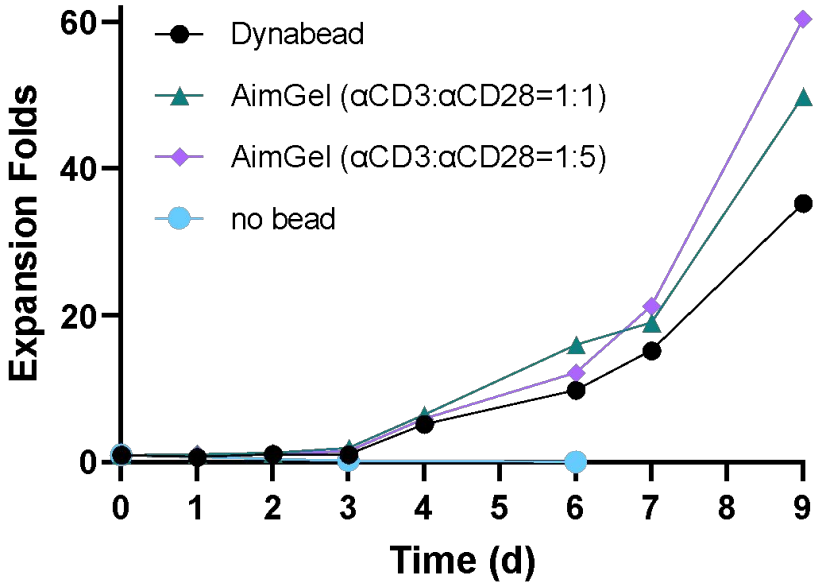
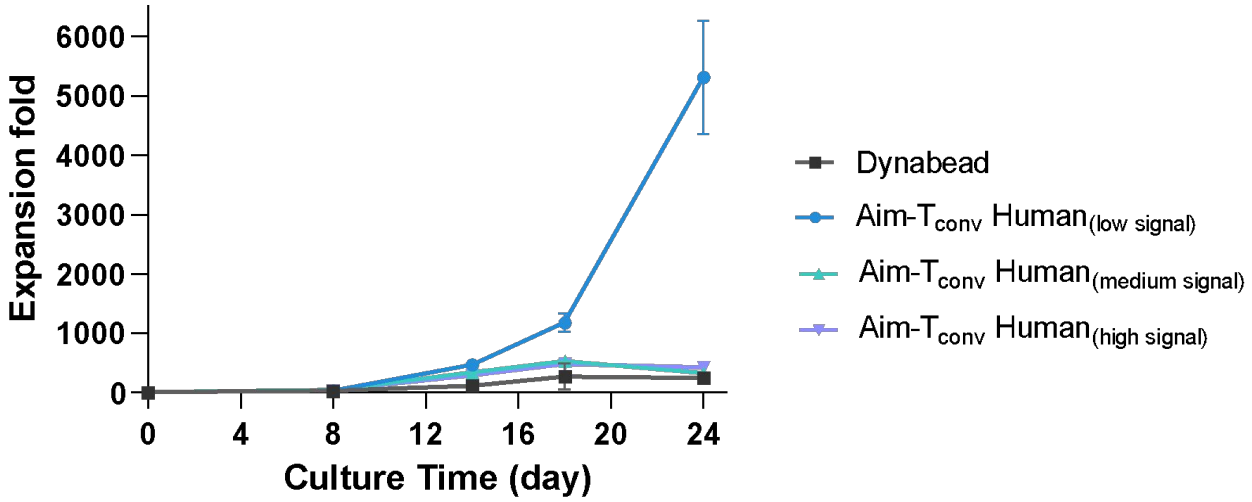
Optimizing signal ratio for expansion of mouse pan T cells

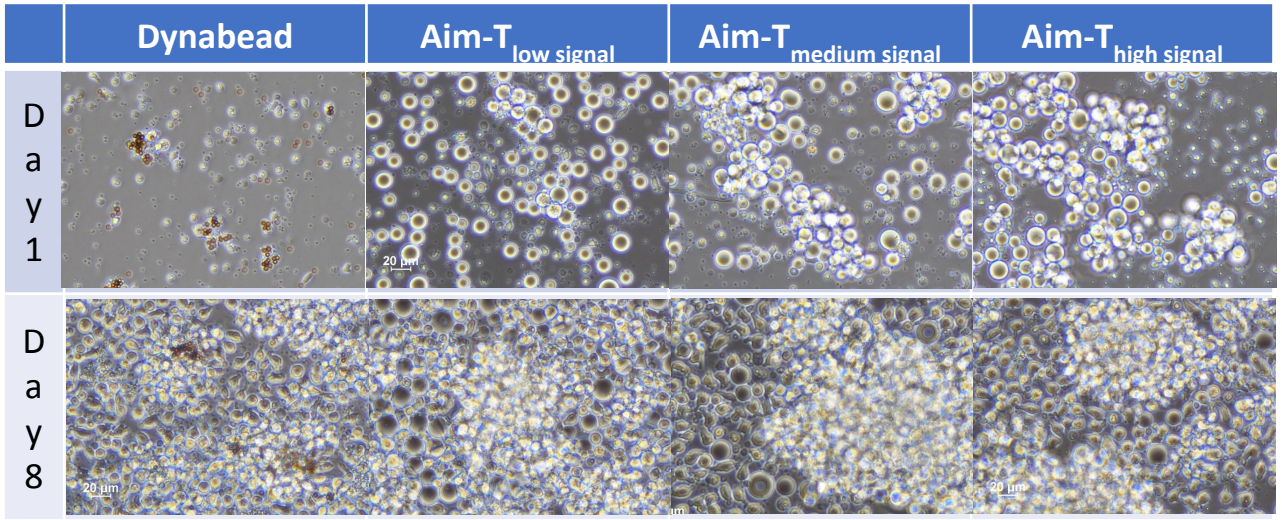


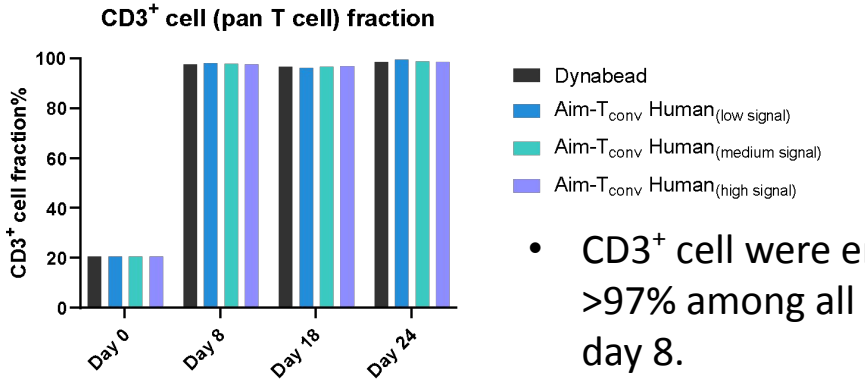


Manuscript in preparation

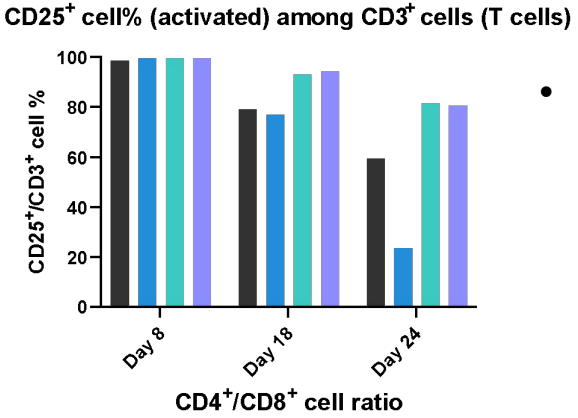
Optimizing signal density for enrichment and expansion of human pan T cells from PBMC



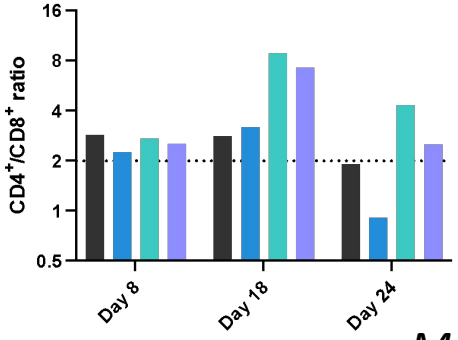




- $CD3^+$ cell were enriched to >97% among all groups at day 8.



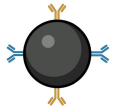
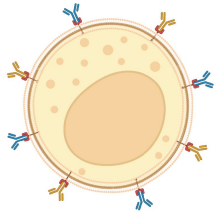
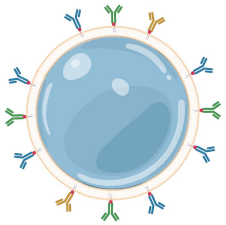
- Restimulation led to persistent stronger activation



- Higher signal density biased pan T cells towards $CD4^+$ lineage

Manuscript in preparation

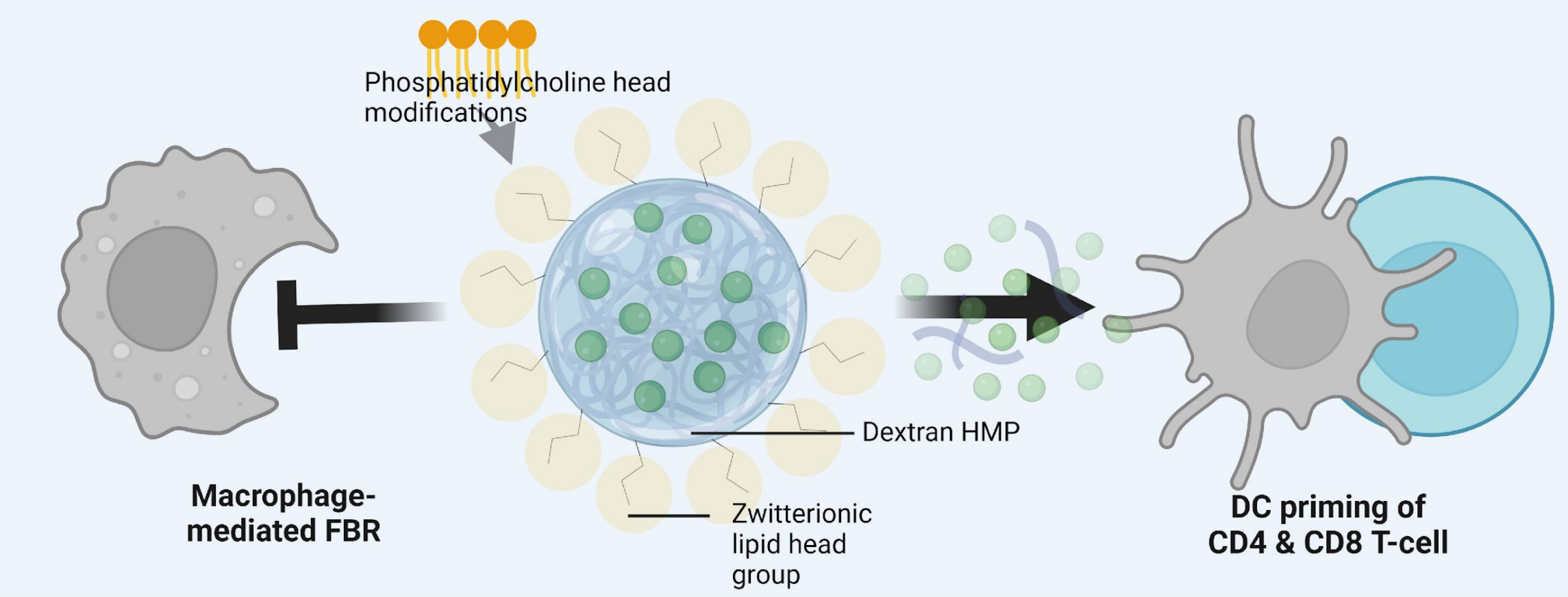
Ex vivo cell growth and activation: AimGel to mimic Artificial Presentation Cell

	Bead-based platform	Cell-based platform	AimGel platform
Size	3.5~4.5 μm	10~20 μm	10~30 μm
Stiffness	> 30 MPa	1~8 kPa	Adjustable, 1~30 kPa
Type of surface signals	αCD3; αCD28;	B7 proteins (CD80 or CD86) αCD3	αCD3; αCD28;
Lateral mobility of surface signals	No	Yes	Yes
Ratio of surface signals	Fixed	Fixed	Variable
Epigenetic controlling signals, e.g. rapamycin	supplemented and replenished periodically in medium		supplemented in soluble form and nanoparticle encapsulated form

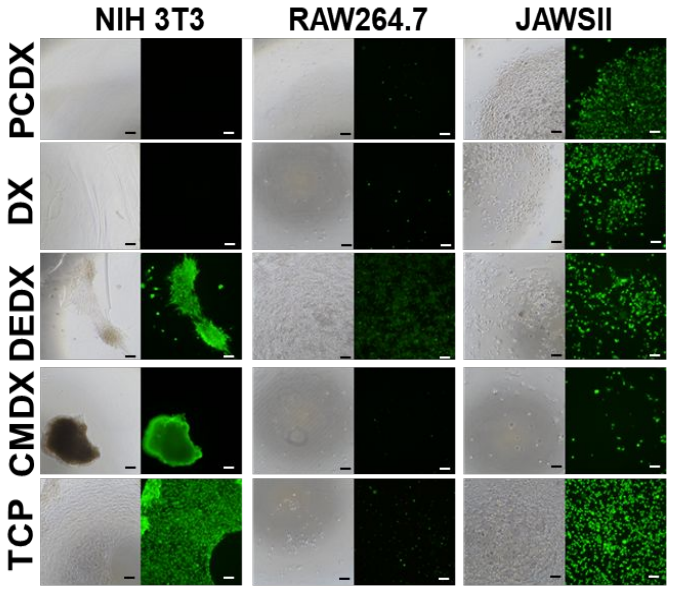
Please check out our poster #3909677

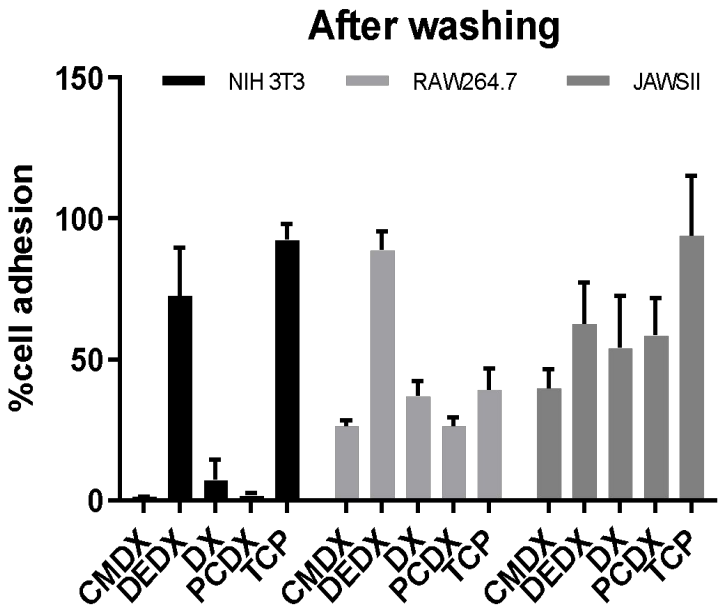
In vivo application of Aimgel: Zwitterionic HMPs for long-term antigen delivery

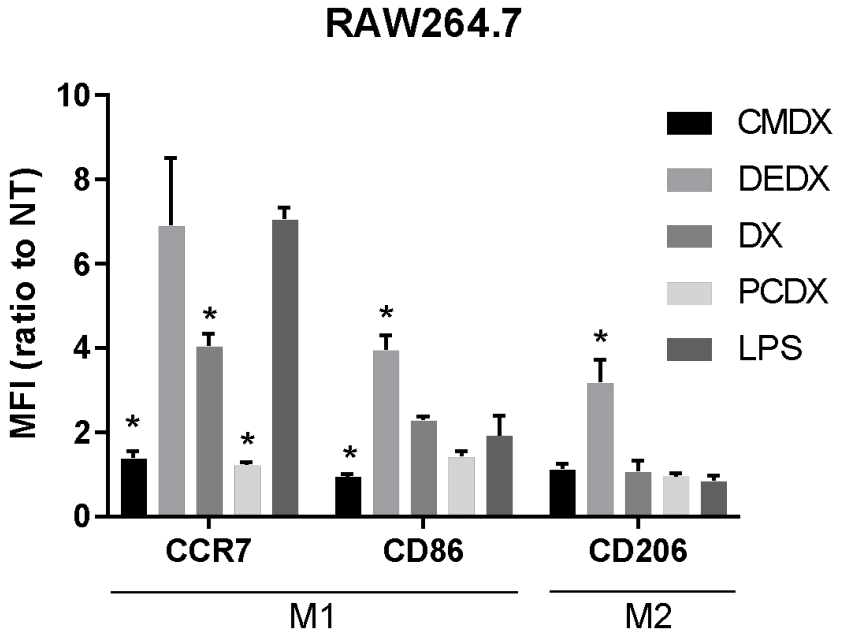


Chung, J. T. M., Lau, C. M. L., Chung, C. H., Rafiei, M., Yao, S., & Chau, Y. (2023). Vaccine delivery by zwitterionic polysaccharide-based hydrogel microparticles showing enhanced immunogenicity and suppressed foreign body responses. *Biomaterials Science*.

Zwitterionic PCDX HMPs suppress activation of fibroblast and macrophage



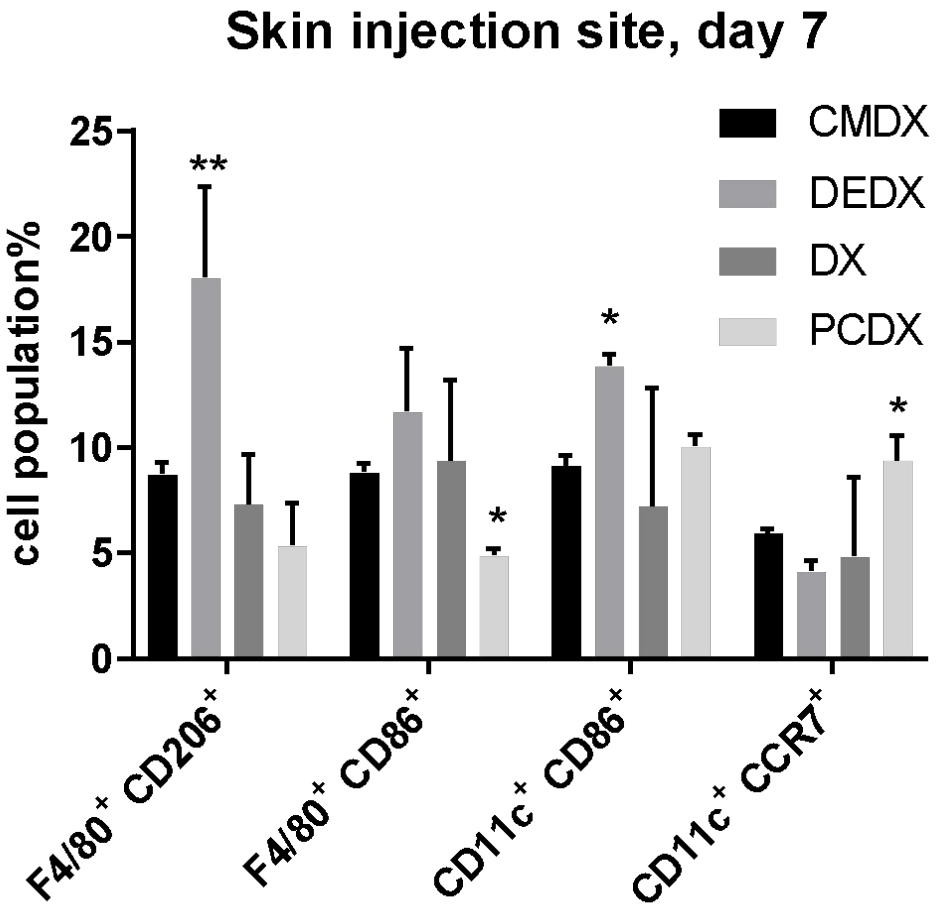


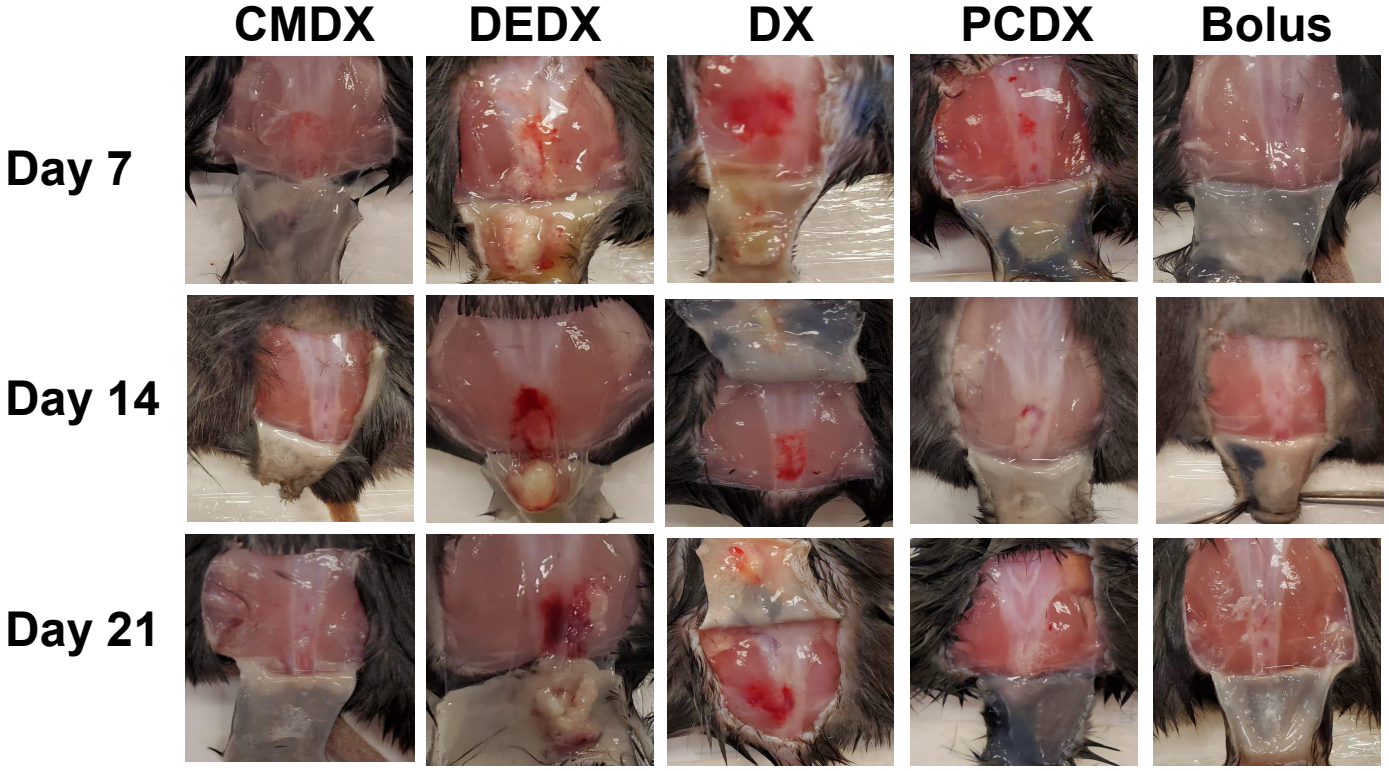


PCDX: phosphorylcholine modified dextran (+/-)
DX: dextran (o)
DEDX: diethylaminoethyl dextran (+)
CMDX: carboxymethyl dextran (-)
TCP: tissue culture plate

Ref: Chung, J. T. M., Lau, C. M. L., Chung, C. H., Rafiei, M., Yao, S., & Chau, Y. (2023). Vaccine delivery by zwitterionic polysaccharide-based hydrogel microparticles showing enhanced immunogenicity and suppressed foreign body responses. *Biomaterials Science*.

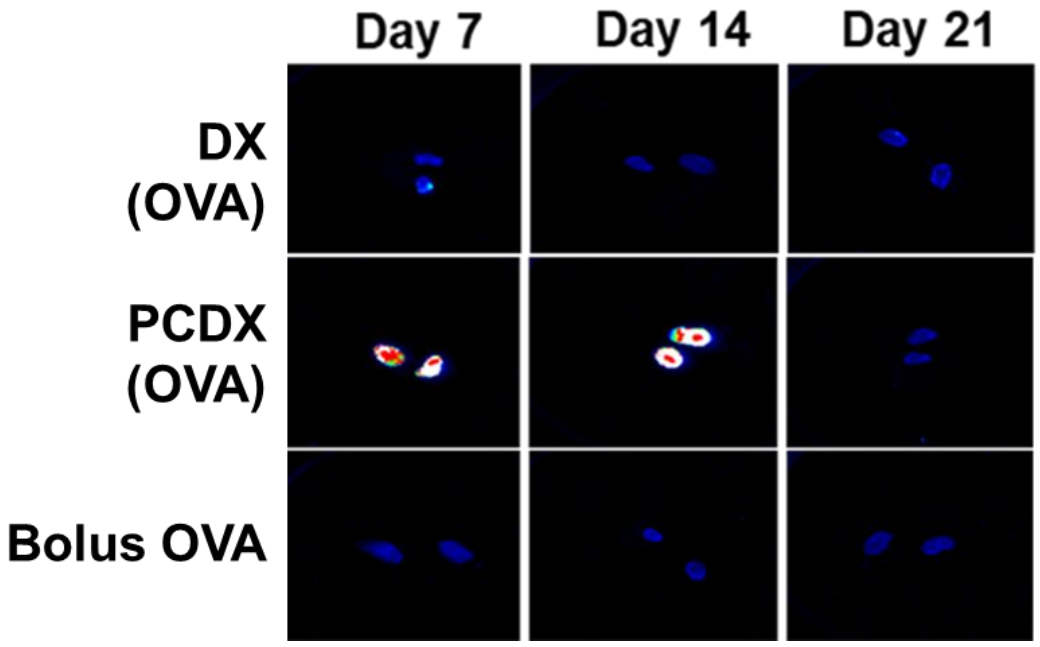
PCDX HMPs suppress foreign body response mediated by F4/80+ cells, while promoting $CD11c^{+}CCR7^{+}$ population

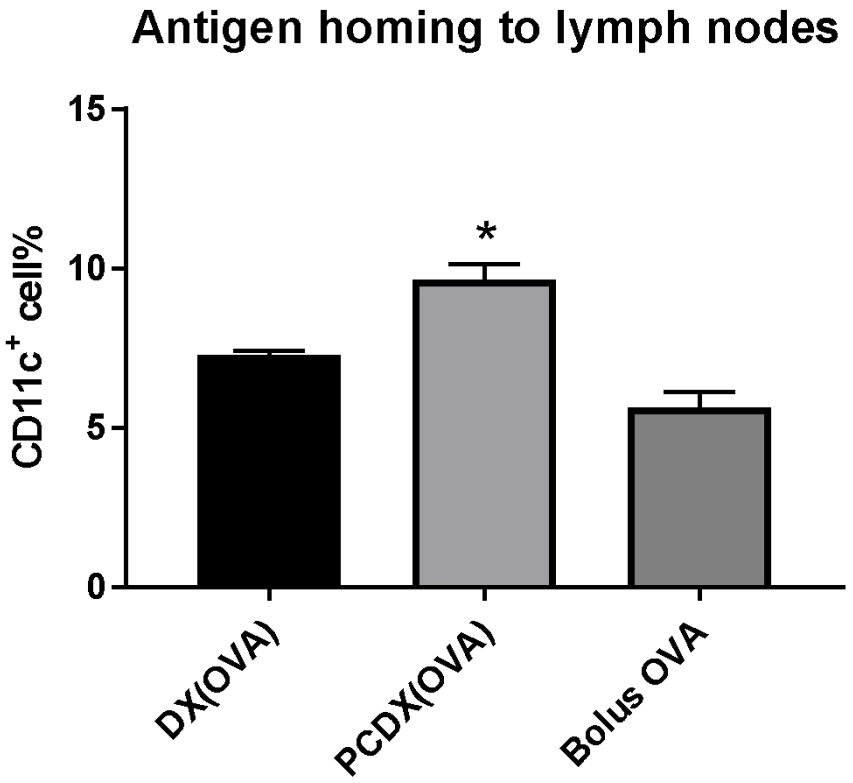




Chung, J. T. M., Lau, C. M. L., Chung, C. H., Rafiei, M., Yao, S., & Chau, Y. (2023). Vaccine delivery by zwitterionic polysaccharide-based hydrogel microparticles showing enhanced immunogenicity and suppressed foreign body responses. *Biomaterials Science*.

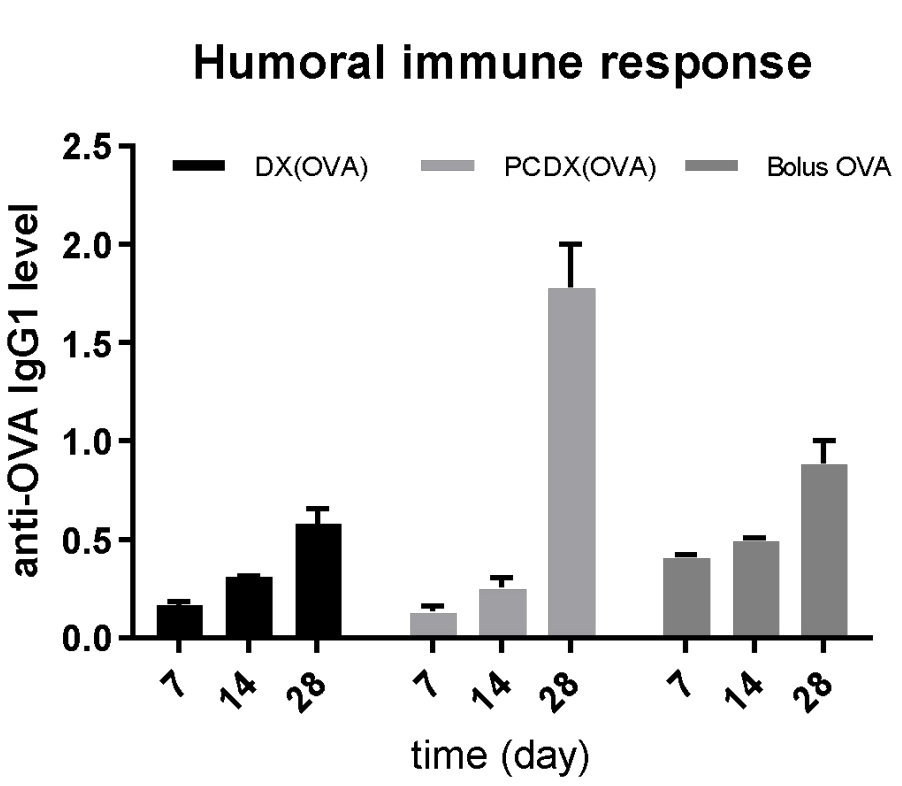
PCDX HMPs provide sustained antigen distribution to lymph nodes via efficient CD11c$^+$ lymph node homing

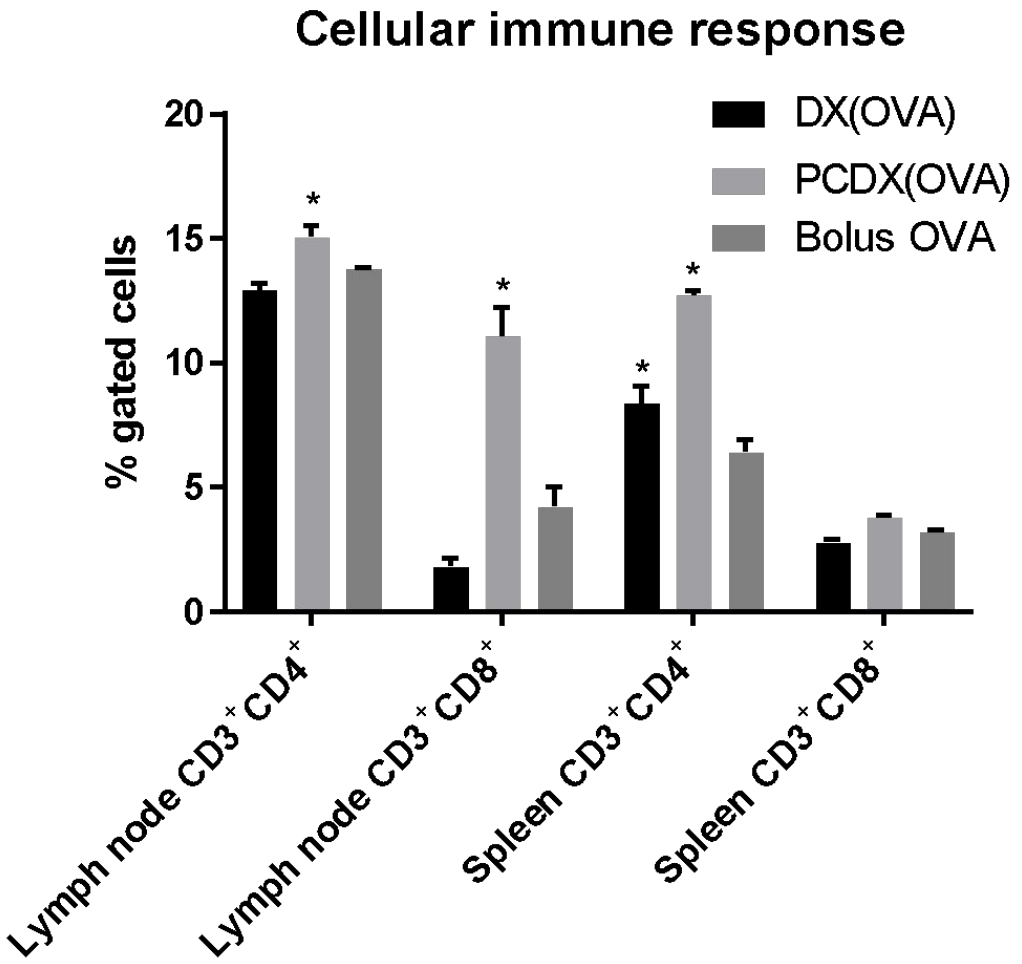




Chung, J. T. M., Lau, C. M. L., Chung, C. H., Rafiei, M., Yao, S., & Chau, Y. (2023). Vaccine delivery by zwitterionic polysaccharide-based hydrogel microparticles showing enhanced immunogenicity and suppressed foreign body responses. *Biomaterials Science*.

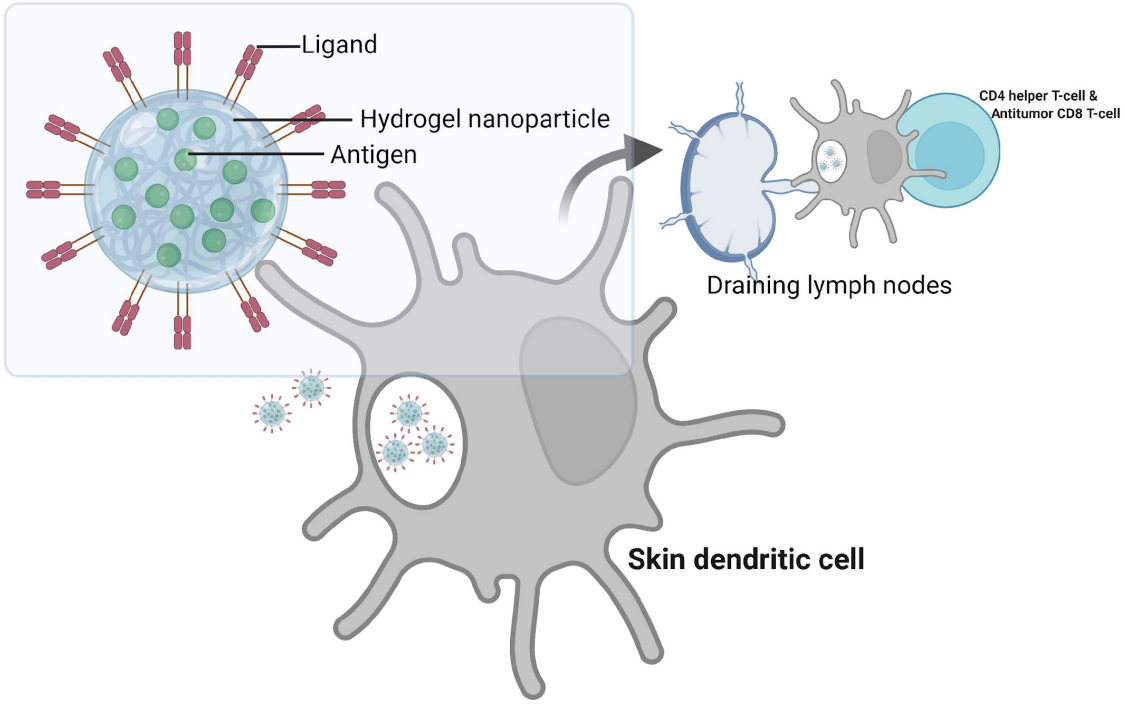
PCDX HMPs enhance both cellular and humoral responses with greater $CD4^+$ and $CD8^+$ T-cell populations



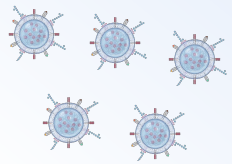


Chung, J. T. M., Lau, C. M. L., Chung, C. H., Rafiei, M., Yao, S., & Chau, Y. (2023). Vaccine delivery by zwitterionic polysaccharide-based hydrogel microparticles showing enhanced immunogenicity and suppressed foreign body responses. *Biomaterials Science*.

In vivo application of Aimgel: amino-acid coated dextran hydrogel nanpoparticles HNPs as self-adjuvanted vaccine delivery



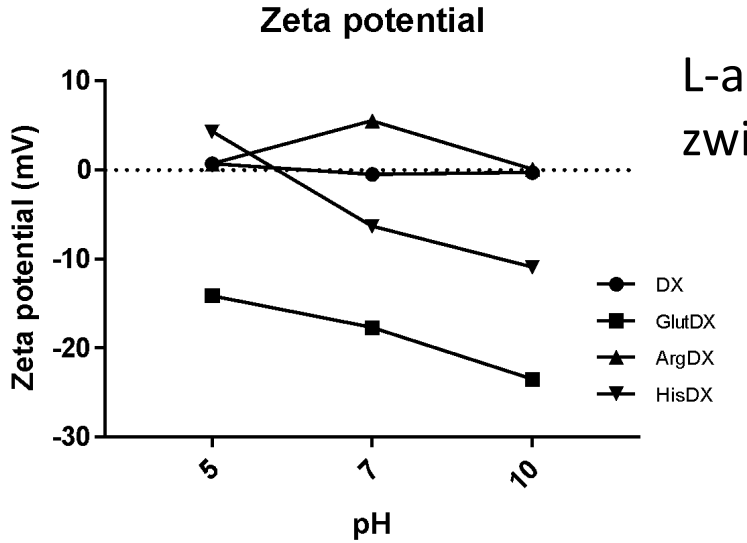
Therapeutic vaccines



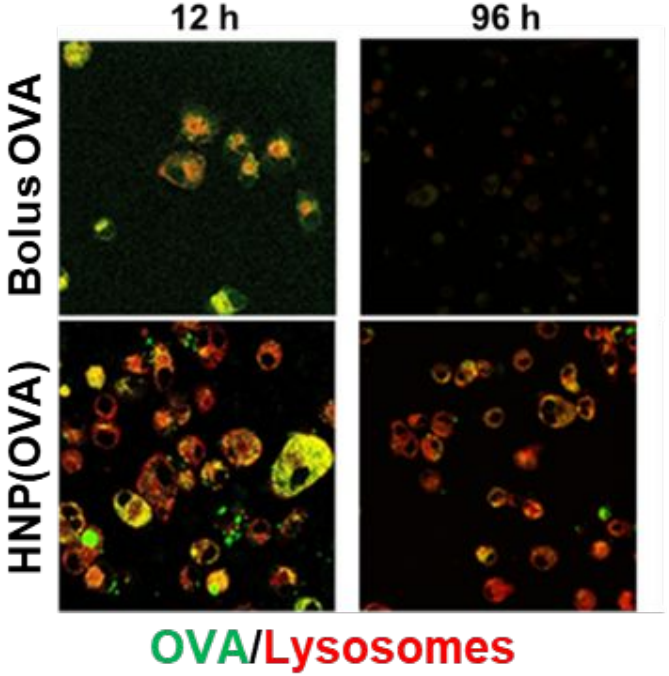
Train and prime immune cells **in the body**

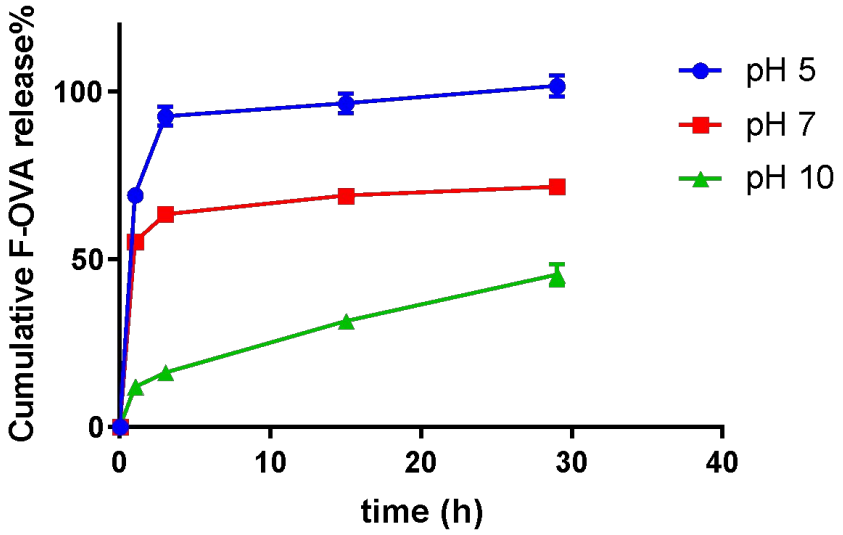
Chung, J. T., Chau, Y. (2022). Self-adjuvanted L-arginine modified dextran-based nanogel for sustained local antigenic protein delivery to antigen presenting cells and enhanced cellular and humoral immune responses. (Manuscript submitted)

Characterization of ArgDX HNPs



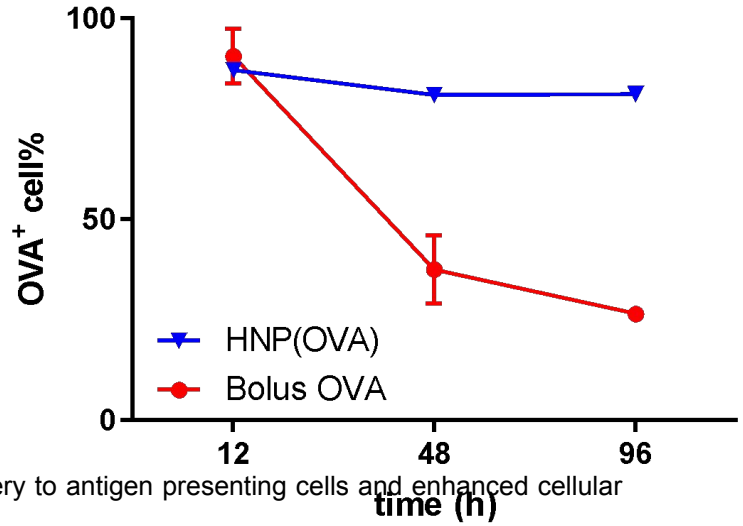
L-arginine (Arg) decorated HNP is zwitterionic





pH sensitive release of antigen from hydrazone crosslinked HNP

ArgDX HNPs prolong intracellular storage and release of antigen



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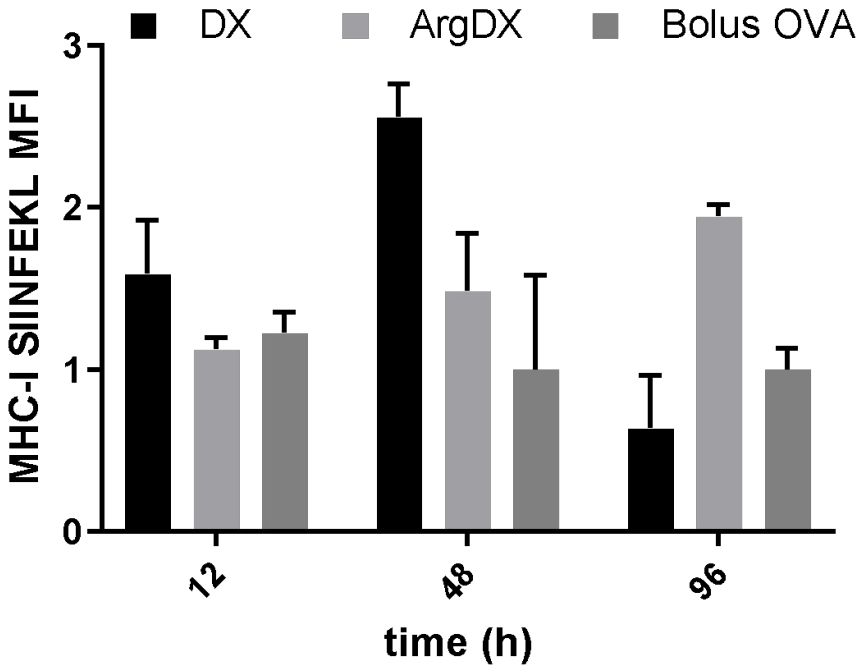
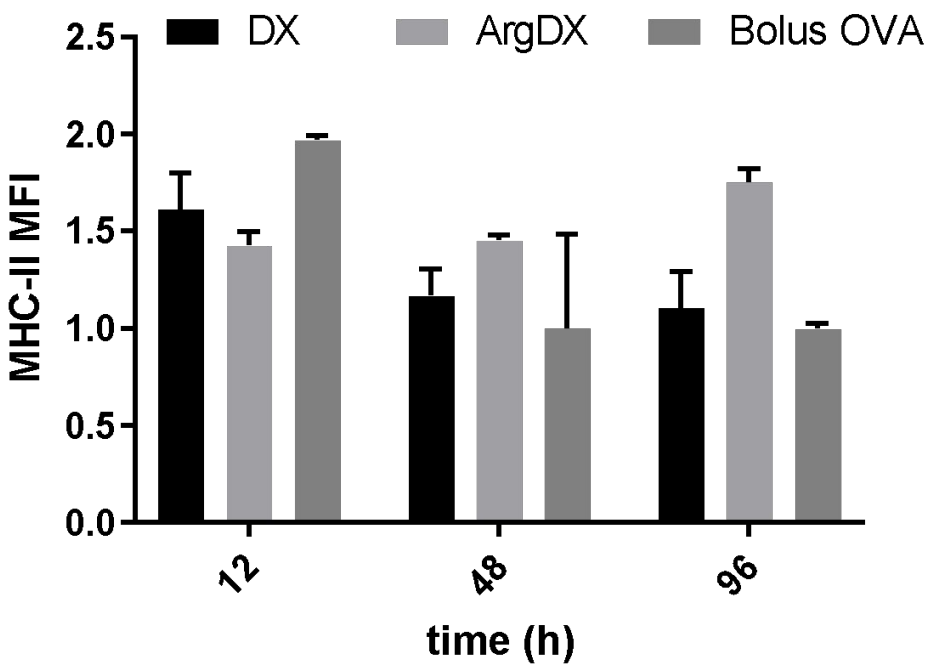
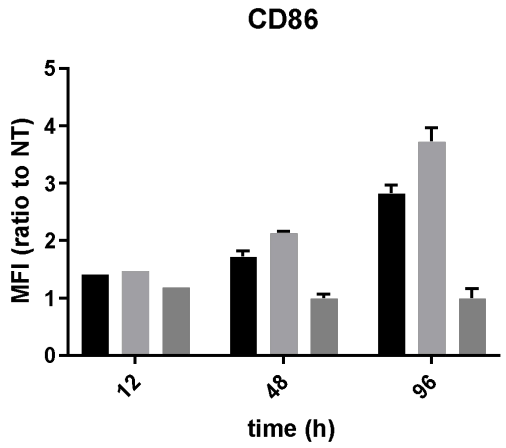
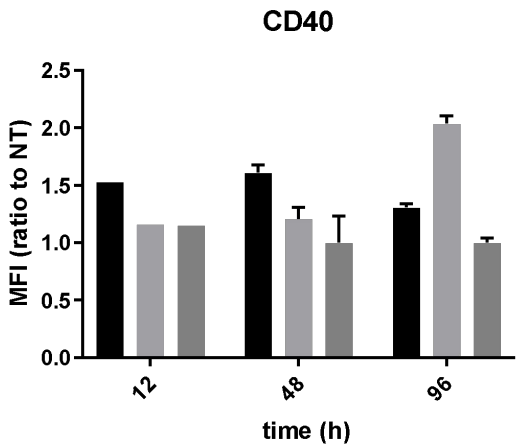
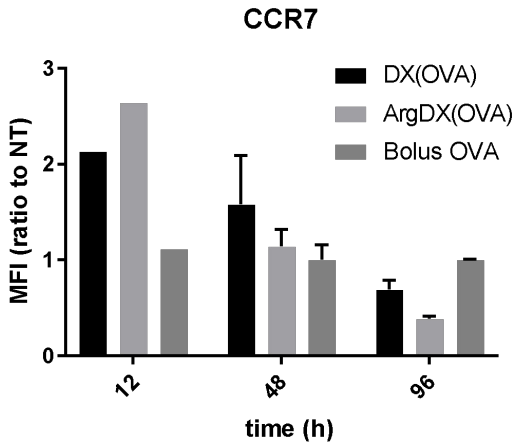
ArgDX HNPs promote antigen presentation and cell activation

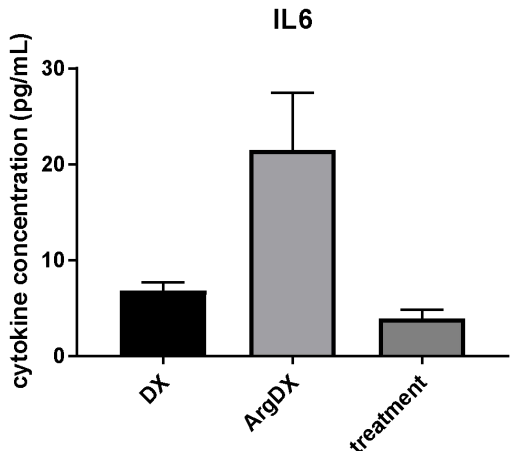






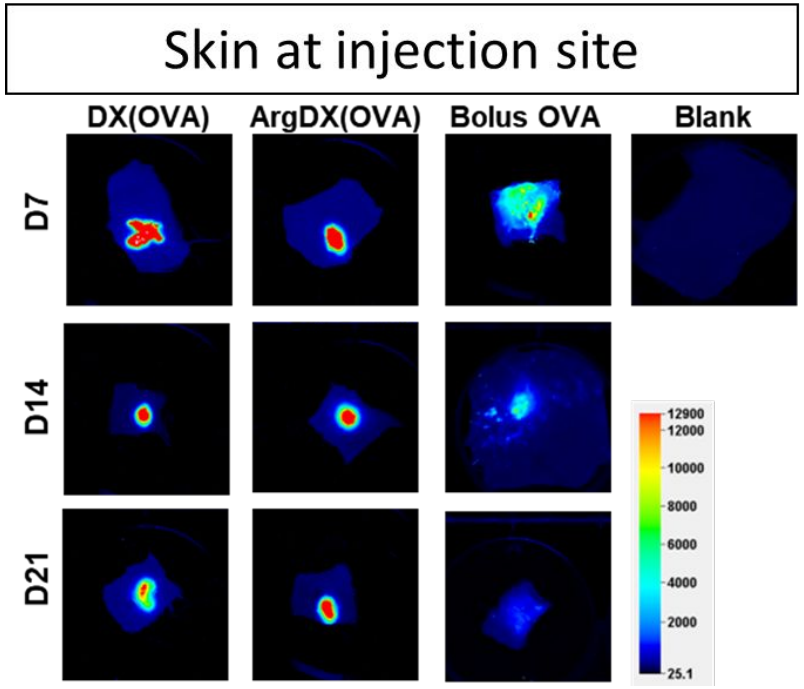


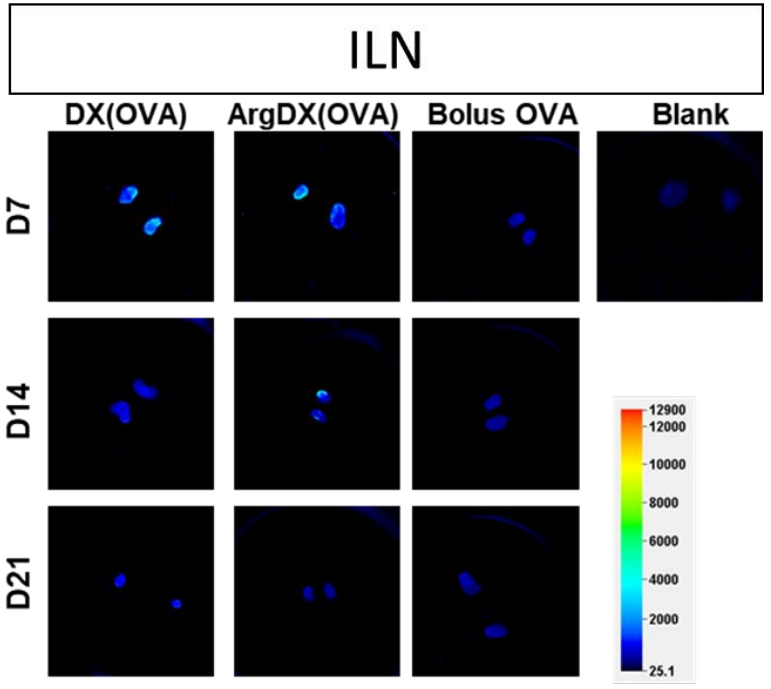


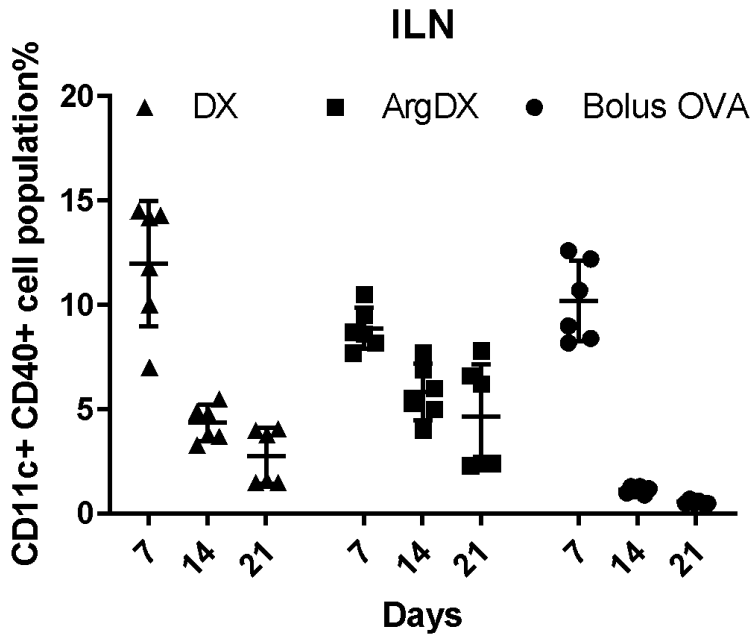


Chung, J. T., Chau, Y. (2022). Self-adjuvanted L-arginine modified dextran-based nanogel for sustained local antigenic protein delivery to antigen presenting cells and enhanced cellular and humoral immune responses. (Manuscript submitted)

ArgDX HNPs promote antigen retention at injection site and lymph node homing







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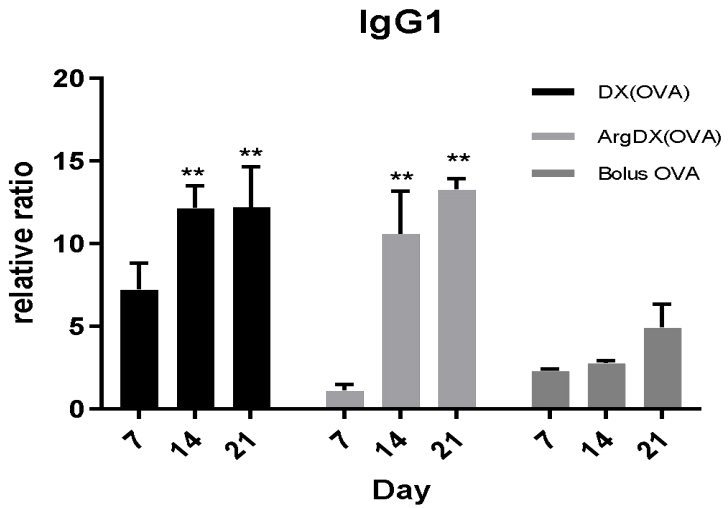
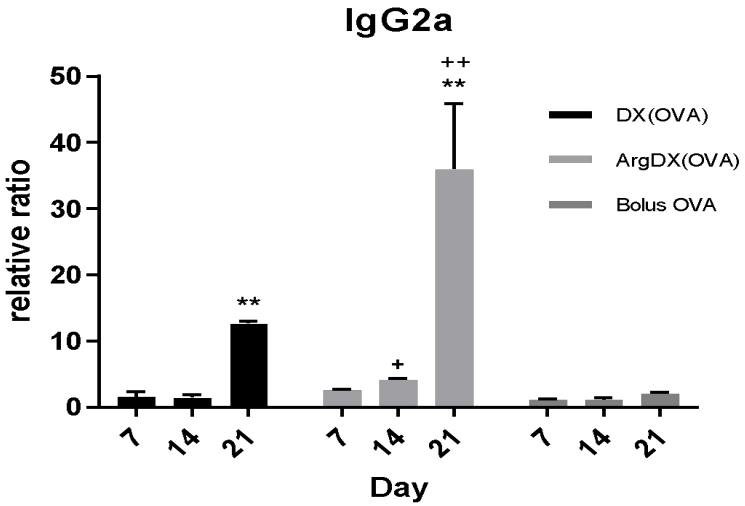
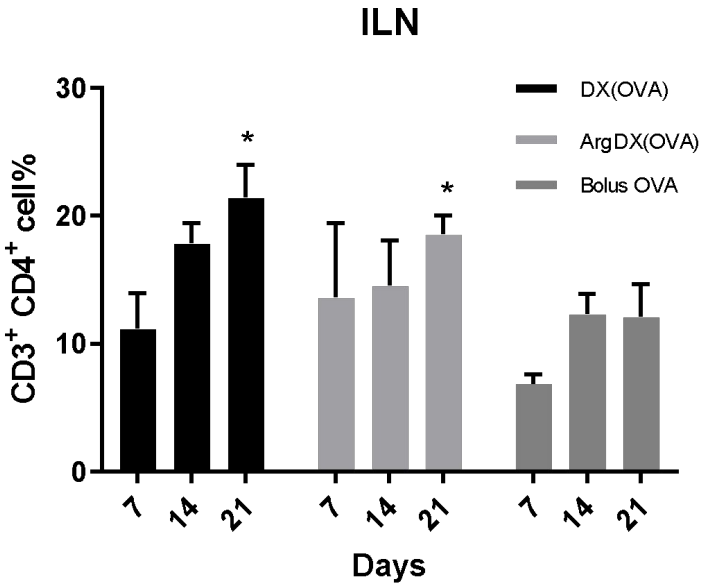
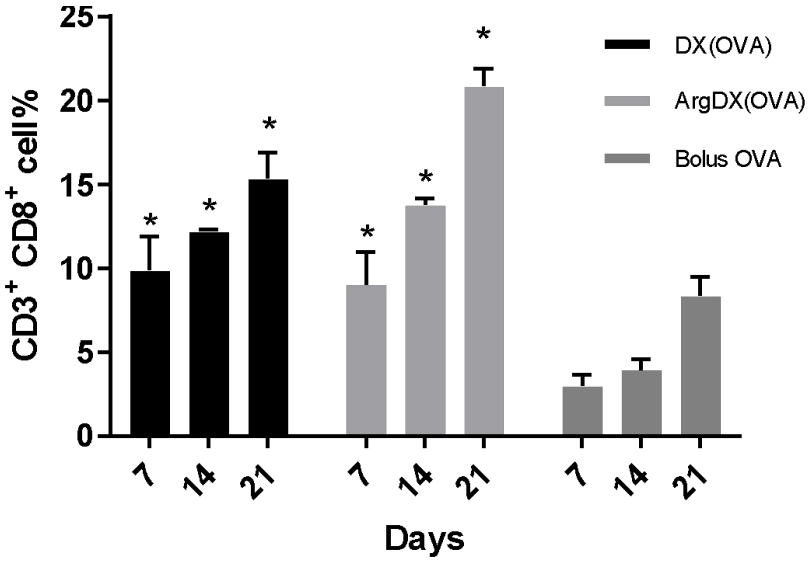
ArgDX HNPs enhance humoral and cellular responses









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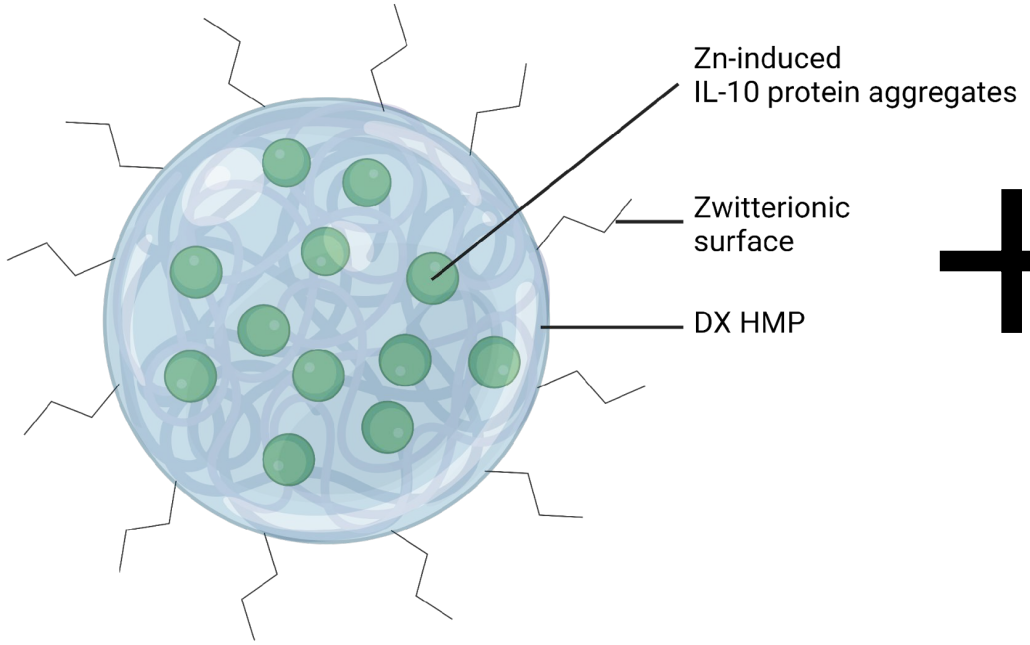
In vivo application AimGel:
HMP + HNP delivery system for tolerogenic vaccines

HMP system

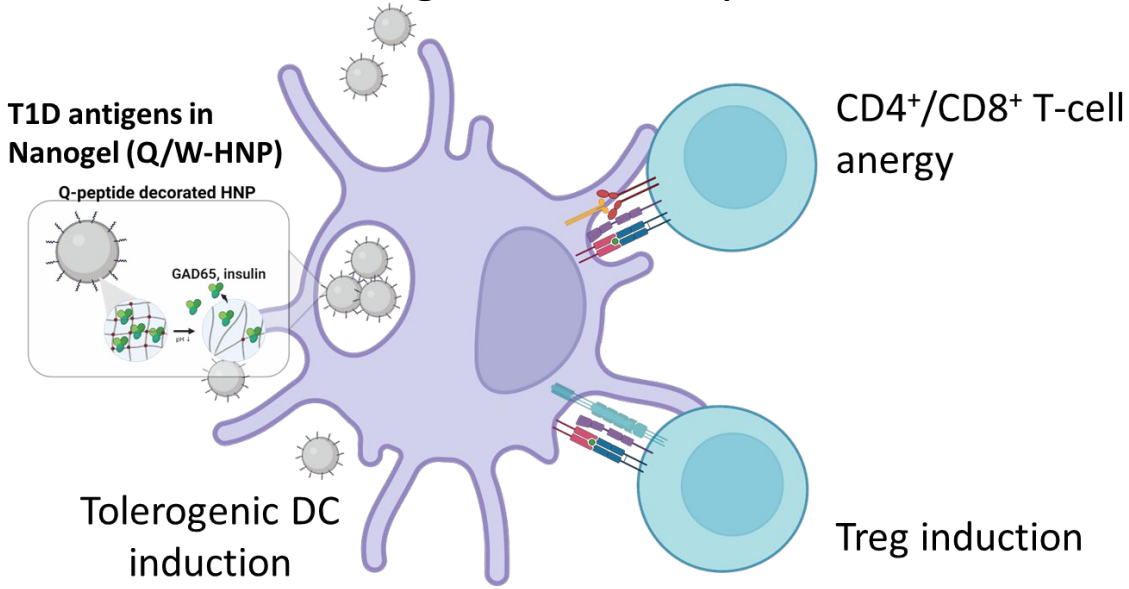
extended extracellular IL-10 delivery



+

HNP system

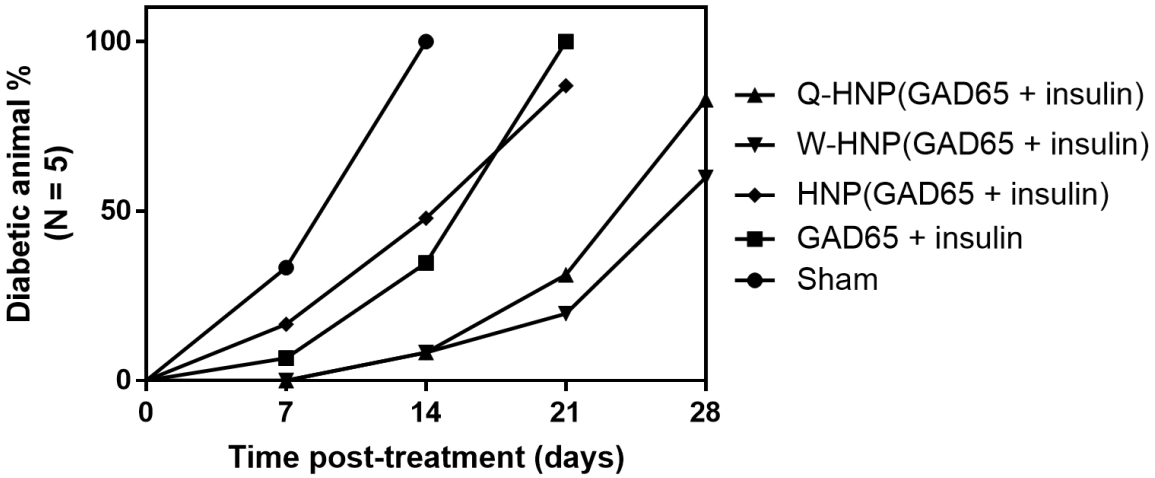
pH-controlled intracellular antigen
storage and delivery

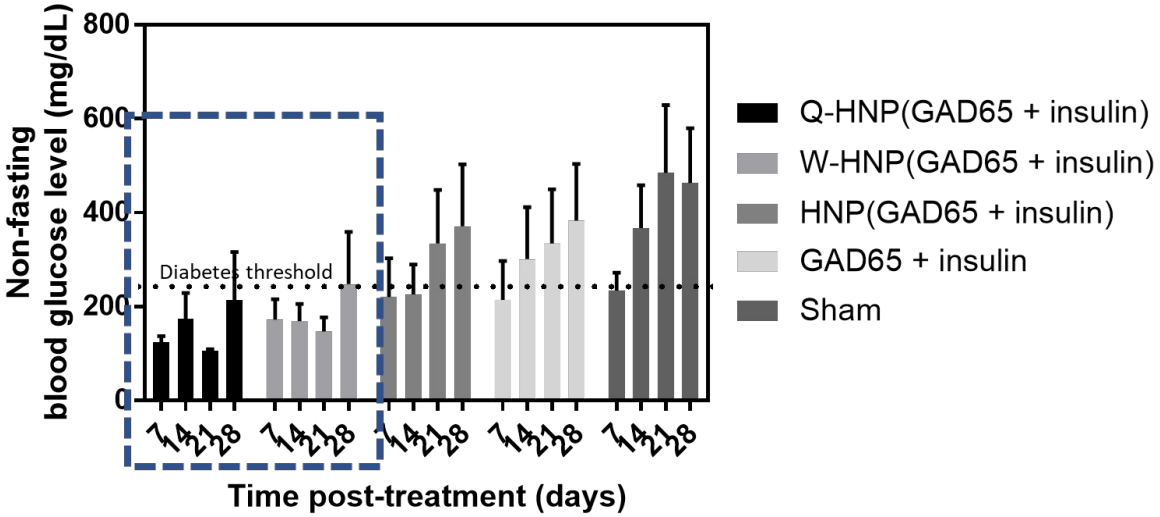


Q-peptide (derived from Angiopoietin-1)
W-peptide (derived from FPR2 agonist)

Patent pending / Manuscript in preparation

HMP + HNP delivery system delays onset of T1 diabetes in NOD/ShiLtJ mouse model



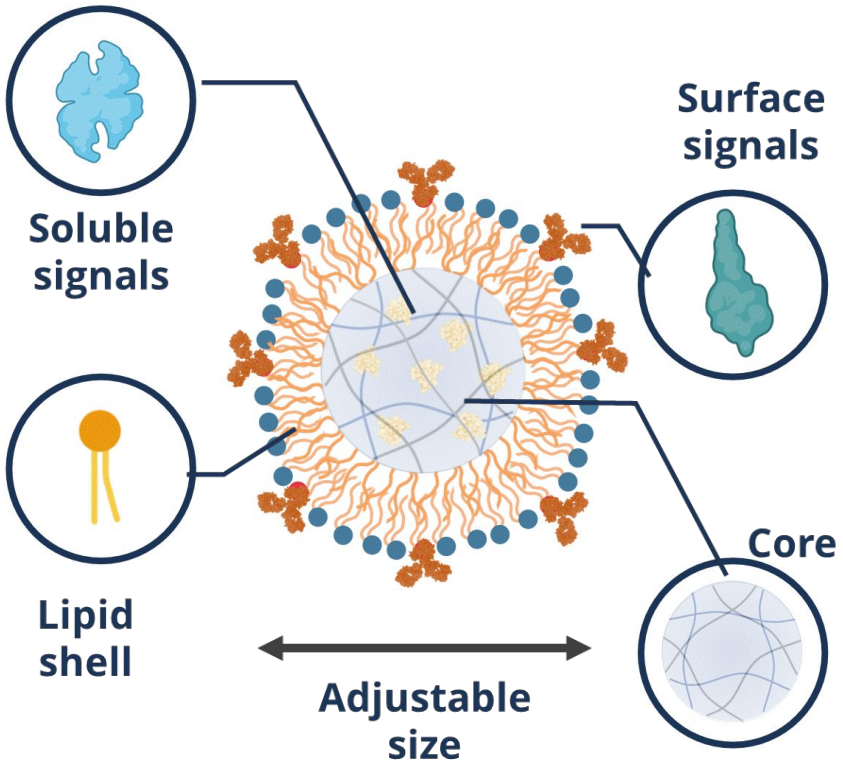


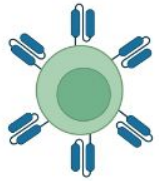
Please check out our poster #3911894

Manuscript in preparation

AimGel:

Versatile hydrogel particle platform to accelerate immune cell therapy



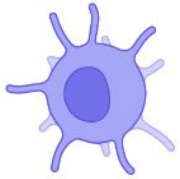


Aim-T$_{conv}$ & Aim-T$_{reg}$

Activate & expand T cells ex vivo

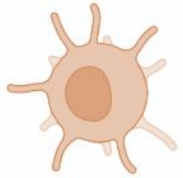
Aim-NK

Activate & expand NK cells ex vivo



Aim-DC$_{Act}$

personalized immune-activating vaccines for cancer and infectious diseases treatment



Aim-DC$_{Supp}$

personalized tolerogenic vaccines for autoimmune diseases treatment

Allegrow