Dendrimer-Peptide Conjugates for Enhanced *in vivo* Tumor Targeting and Efficacy

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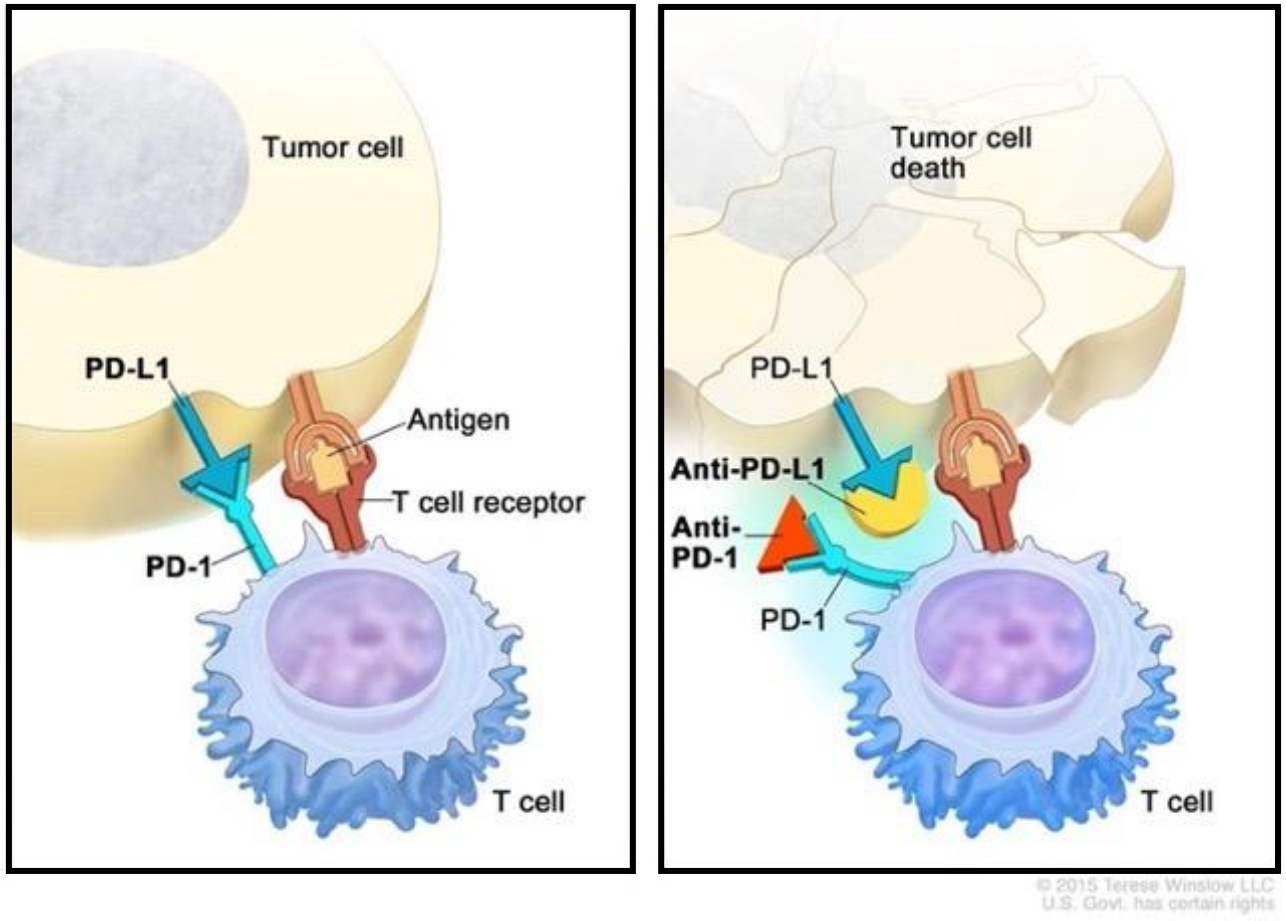
THE FUTURE OF DELIVERY SCIENCE

FDA-approved PD-L1 Immune Checkpoint Inhibitors (ICIs)

- Antibodies binding to programmed death-ligand 1 (PD-L1) allow T cells to kill tumor cells



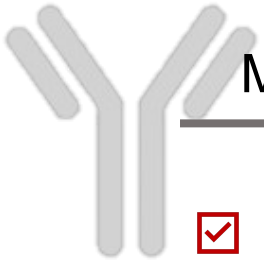
Atezolizumab

Durvalumab

Avelumab

Arakelians, S. Pharmacy Times (2022); Arakelians, S. Pharmacy Times (2021); Bavencio Rx.

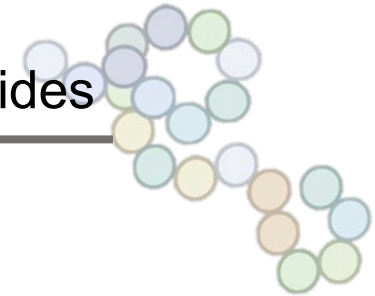
Exploiting peptides as targeting molecules



Monoclonal Antibodies

- ☑ High affinity and selectivity
- ☑ High efficacy in the clinic

- ☒ Immune related adverse events
- ☒ High Cost
- ☒ Poor diffusion and tumor penetration
- ☒ Batch-to-batch variations



Peptides

- ☑ Reduced immunogenicity
- ☑ Precise sequence and structure control
- ☑ Enhanced tumor penetration
- ☑ Minimized steric hindrance

- ☒ Variability in conformational changes
- ☒ Typically lower binding affinity
- ☒ Lower retention / faster clearance

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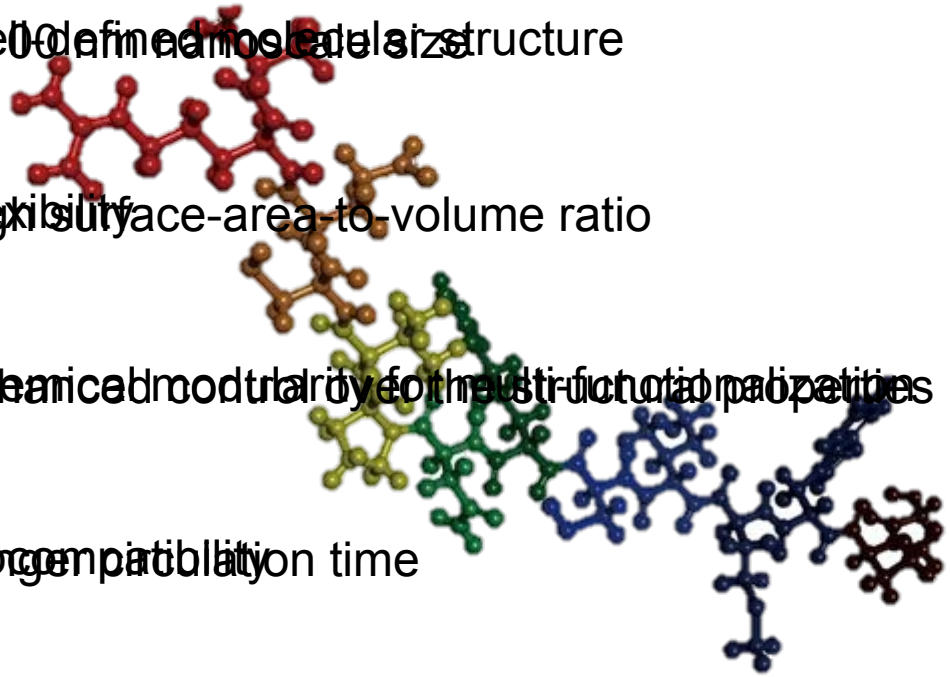
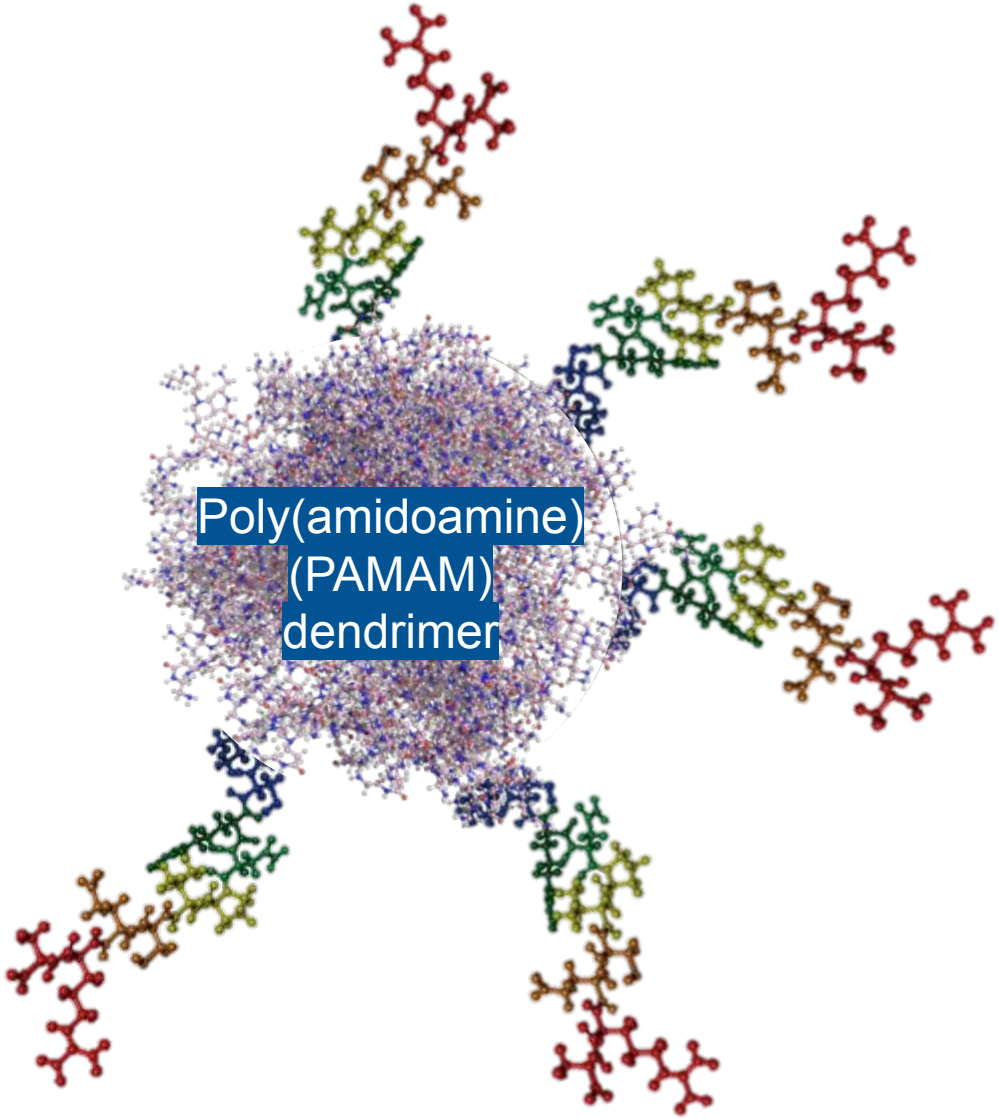
Incorporation of peptides with nanoparticles

- 1-10 nm in size
- Well-defined molecular structure
- High surface-area-to-volume ratio
- Chemical modularity for multifunctionalization
- Longer circulation time
- Multivalency



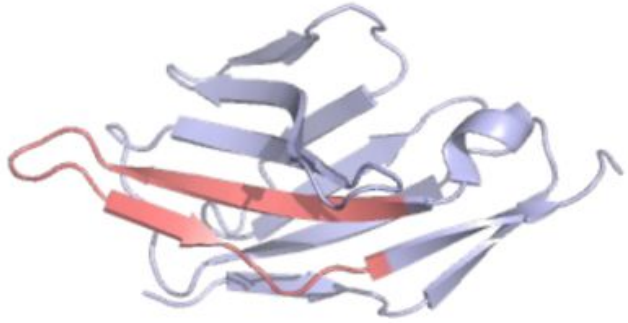


Thus, improving the functionality of peptides

Jeong, W.J.. et al. Nano Convergence. 5:38 (2018).

PD-L1-targeting Dendrimer Peptide Conjugates (DPCs)

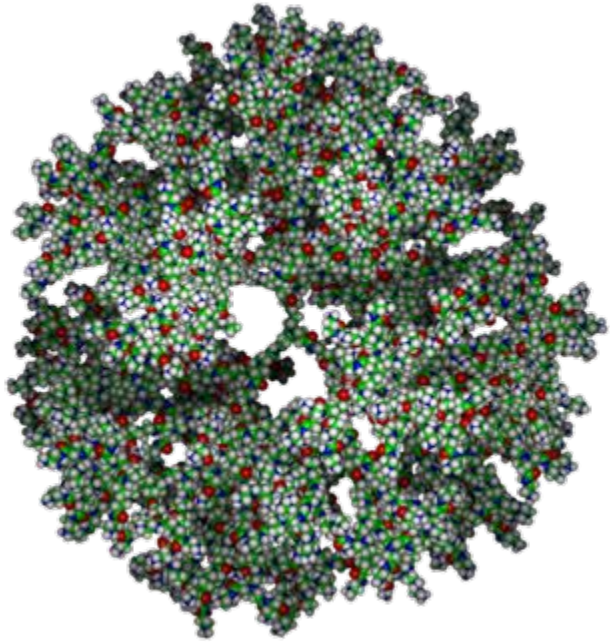
Programmed Cell Death Protein 1
(PDB ID: 3BIK)



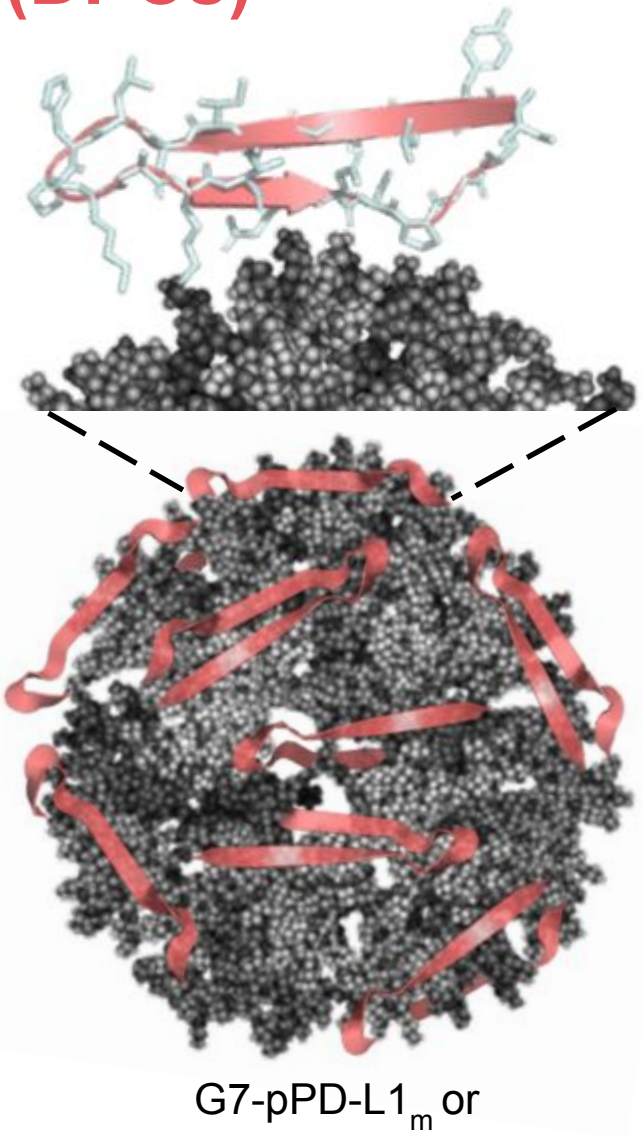
Ac-IYLCGAISLHPKAKIEESSPGA-H

Mouse PD-L1-binding peptide

+



Generation 7 (G7)
PAMAM Dendrimer



G7-pPD-L1$_m$ or
Dendrimer Peptide Conjugates (DPC)

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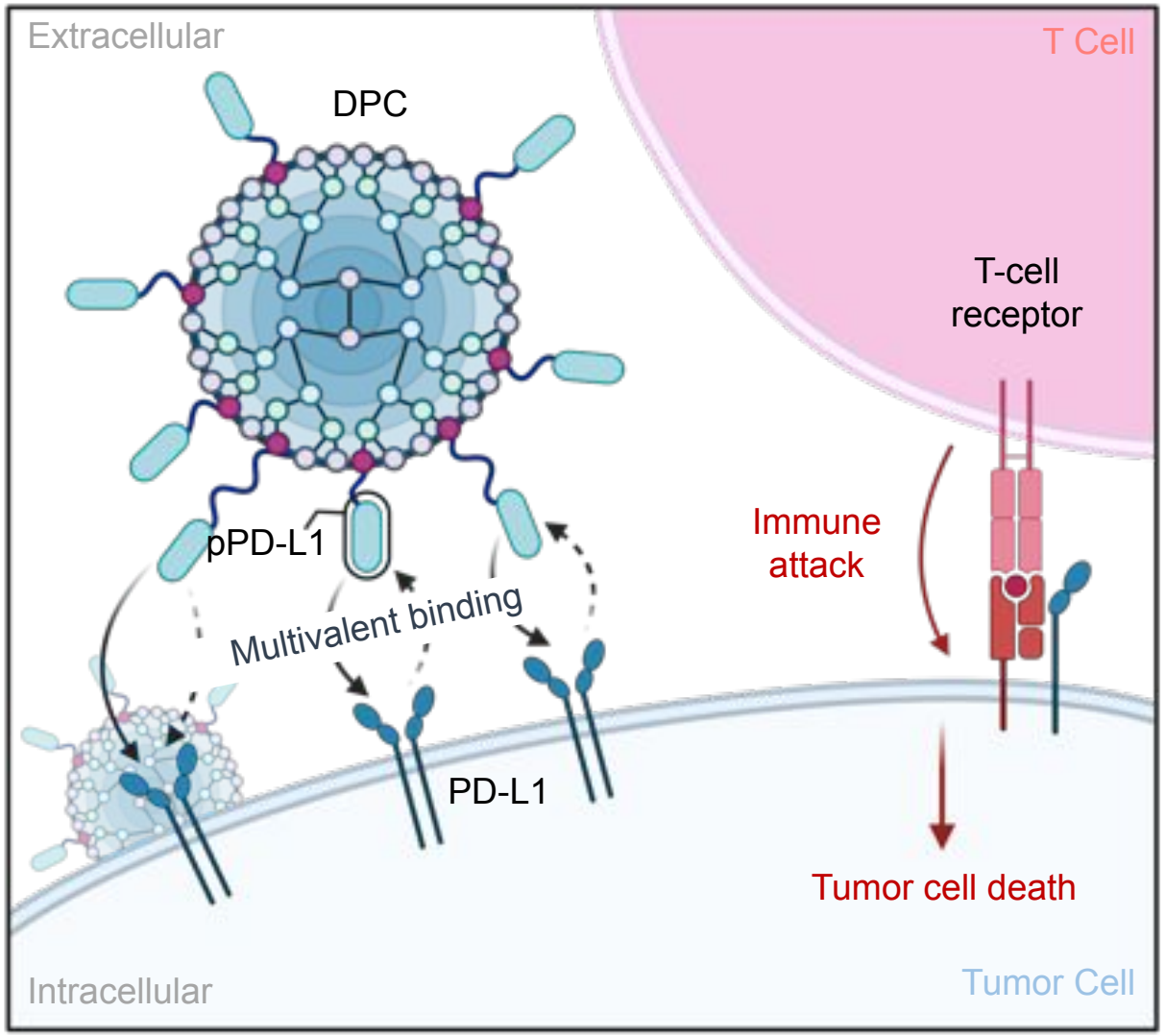
Illustration drawn by Piper Rawding



Goal:

Evaluate *in vivo* binding efficiency and immune cell activation of DPC blocking PD-1/PD-L1 pathway

Hypothesis:

Multivalent binding effects mediated by PD-L1-binding DPCs would enhance binding efficiency, tumor retention, and T cell reactivation.

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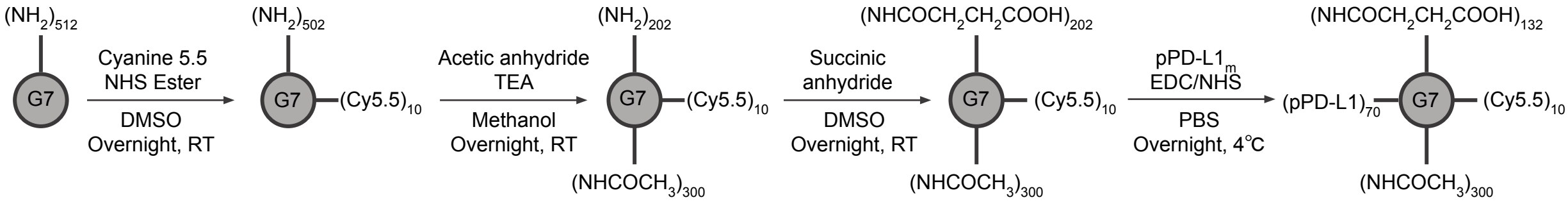
Synthesis and characterization of DPCs

❖ DPC synthesis scheme



Approximately 85 peptides were conjugated to each G7 PAMAM dendrimer

❖ Binding kinetics measurements via biolayer interferometry (BLI)

Sample	k_a (1/Ms)	k_d (1/s)	K_D (M)
aPD-L1	1.29×10^{3}	1.24×10^{-3}	9.65×10^{-7}
pPD-L1	2.84×10^{1}	3.31×10^{-3}	1.16×10^{-4}
DPC	9.86×10^{3}	1.61×10^{-3}	1.63×10^{-7}

~ 3-fold enhancement

G7 PAMAM dendrimers significantly improved the binding of free peptides

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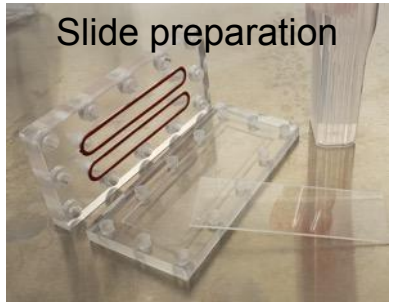
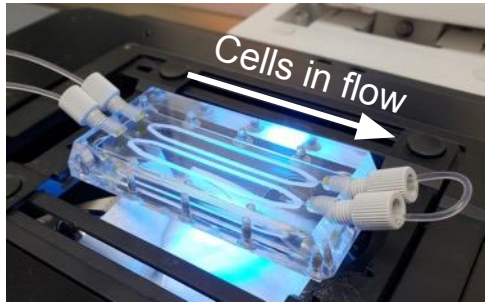
Data obtained by Piper Rawding

High association rate of PD-L1^{High} cells to DPCs in flow condition

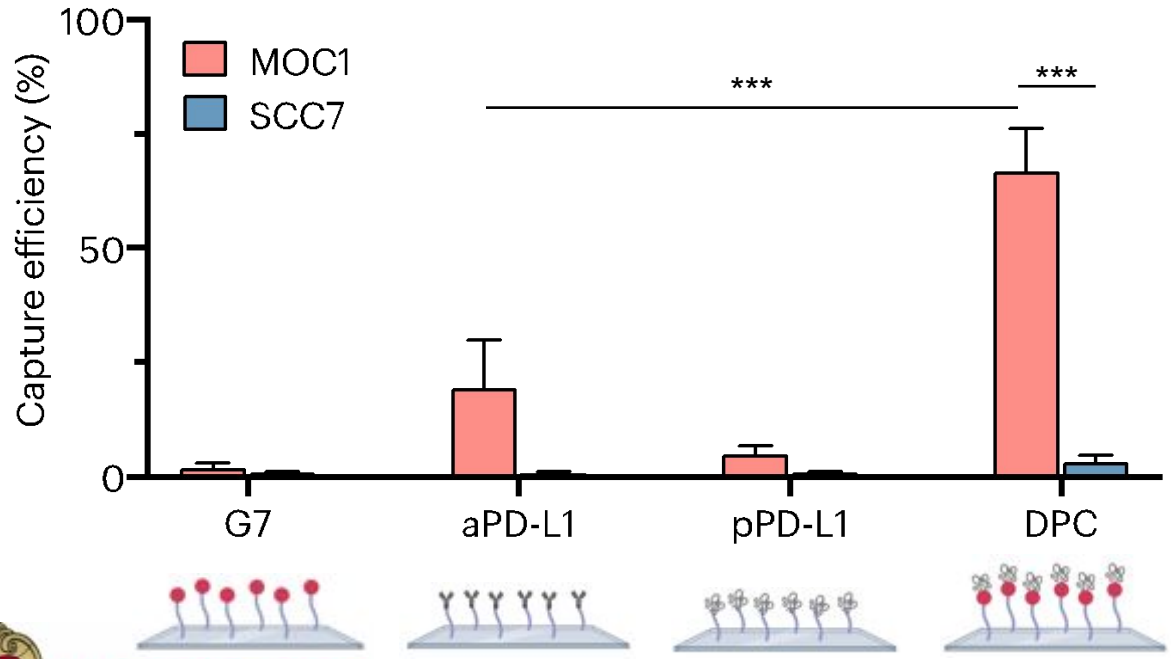
Scheme



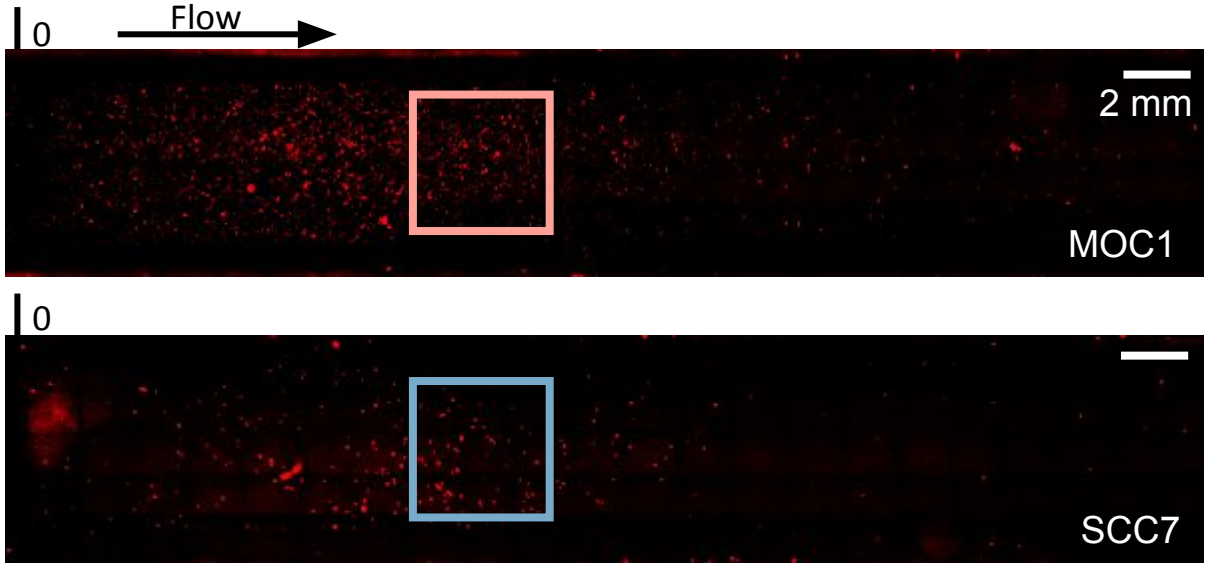
Assemble

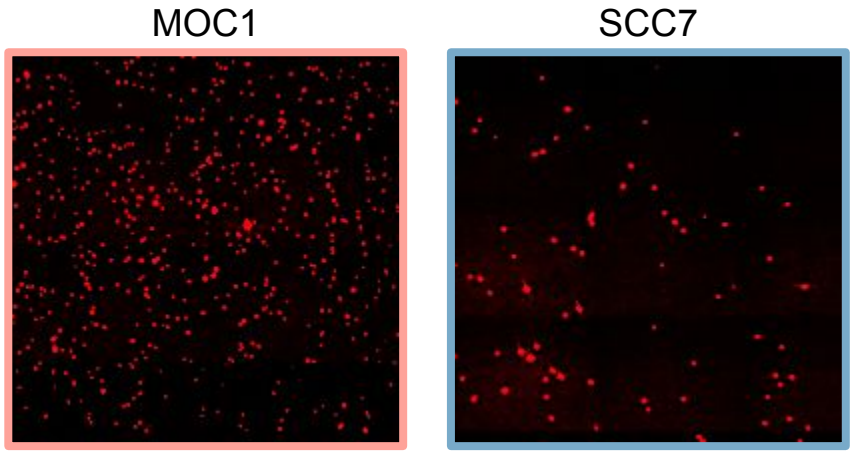


Quantification



Image





DPCs have high capture efficiency to PD-L1^{High} MOC1 compared to PD-L1^{Low} SCC7

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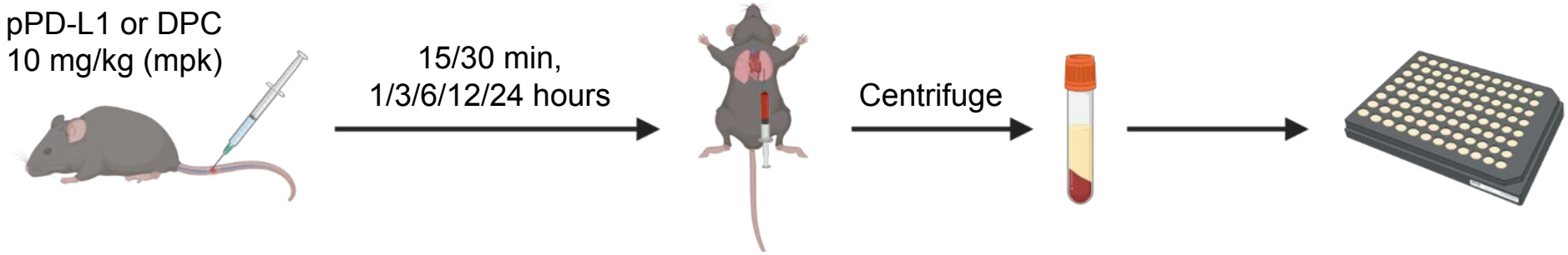
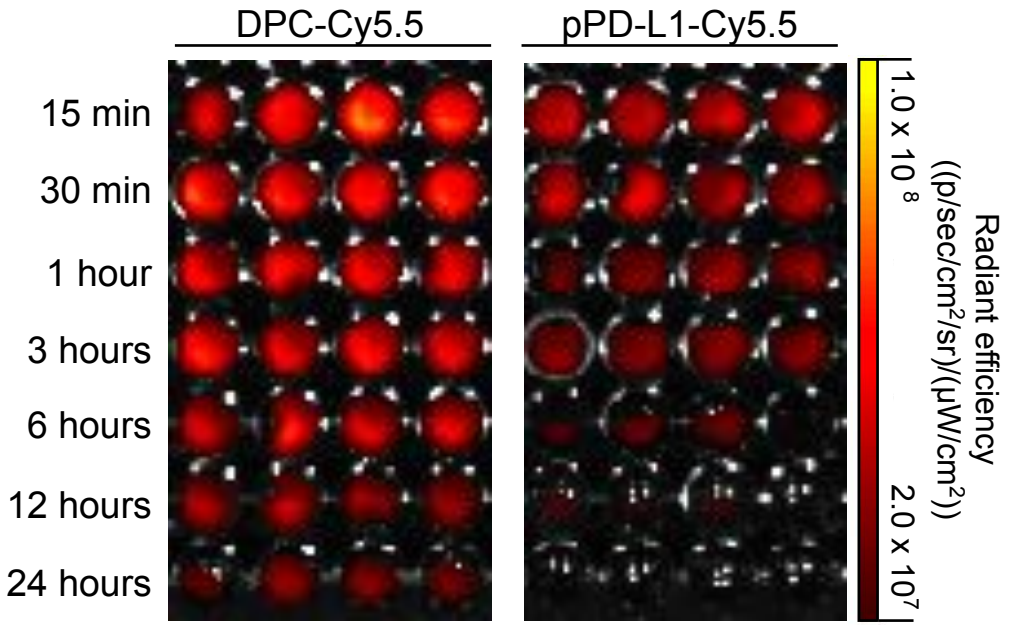
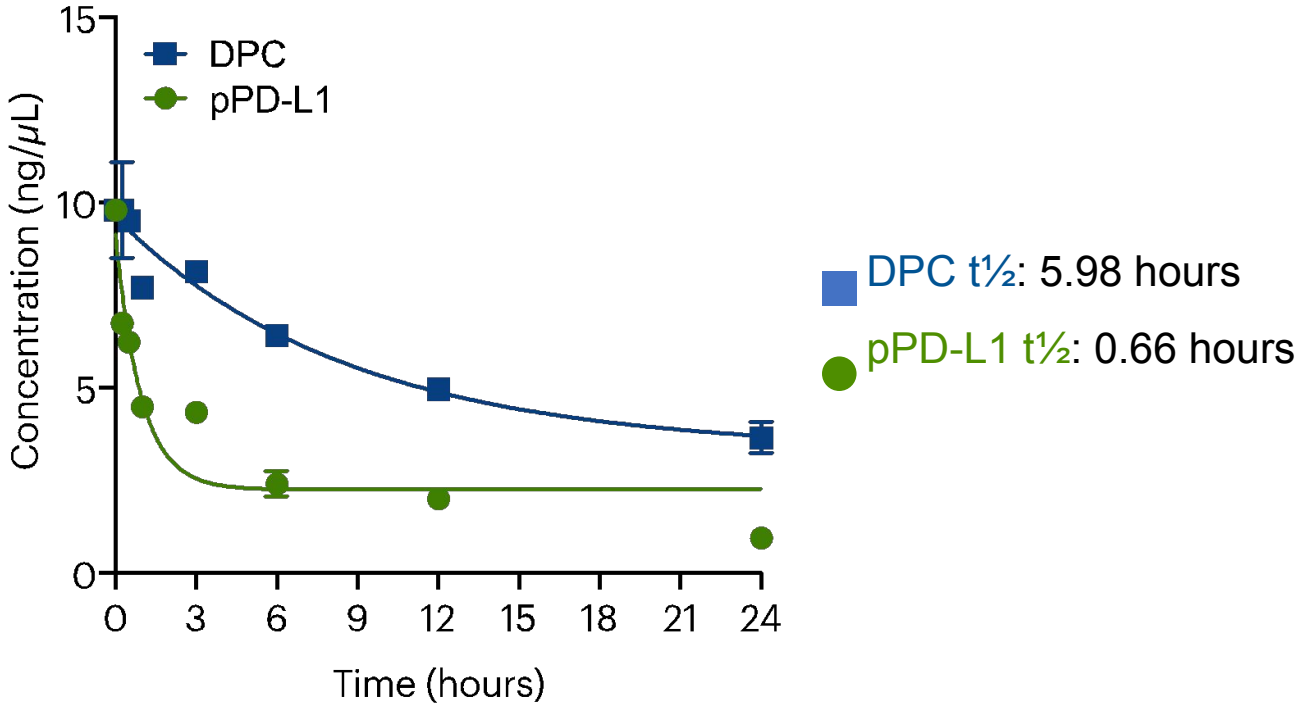
Two-way AVONA using Bonferroni's multiple comparisons test

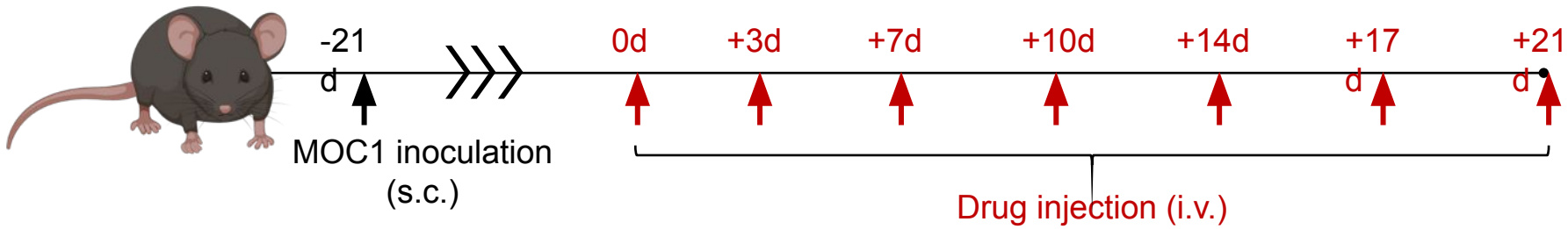
Increased drug half-life of DPCs



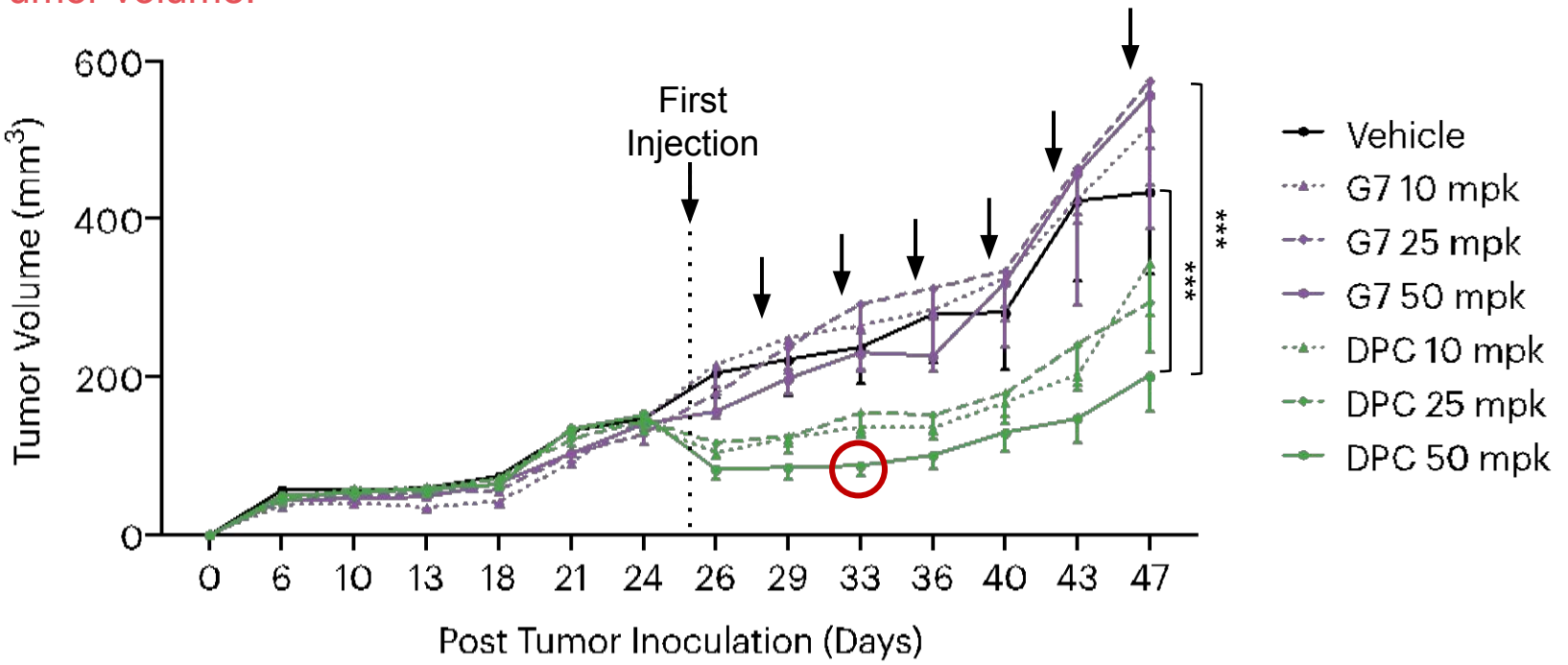




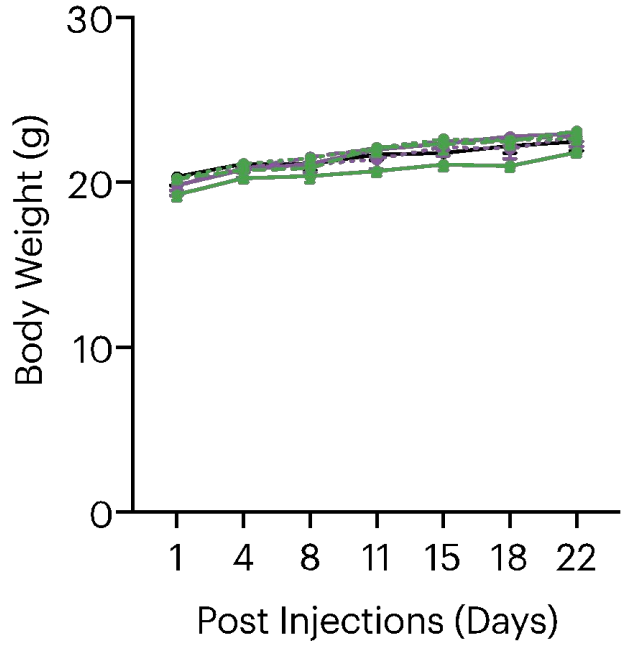
The Half-life of DPC is prolonged by nine-fold compared to peptide alone



❖ Tumor volume:



❖ Body weight:



Tumor volume decreased by ~42% after three injections of DPC at 50 mpk

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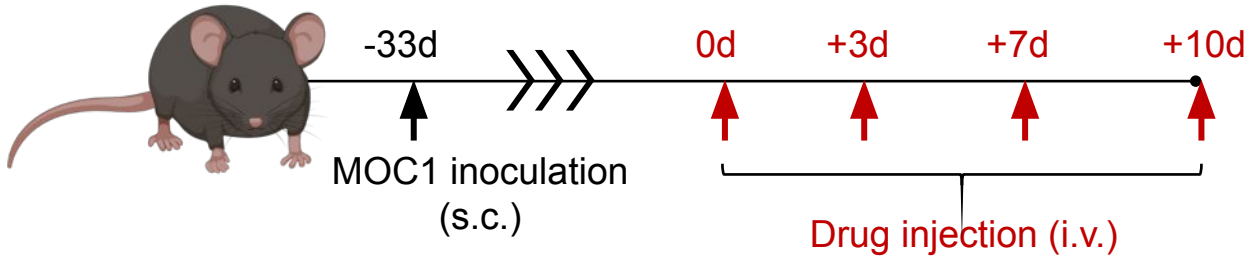
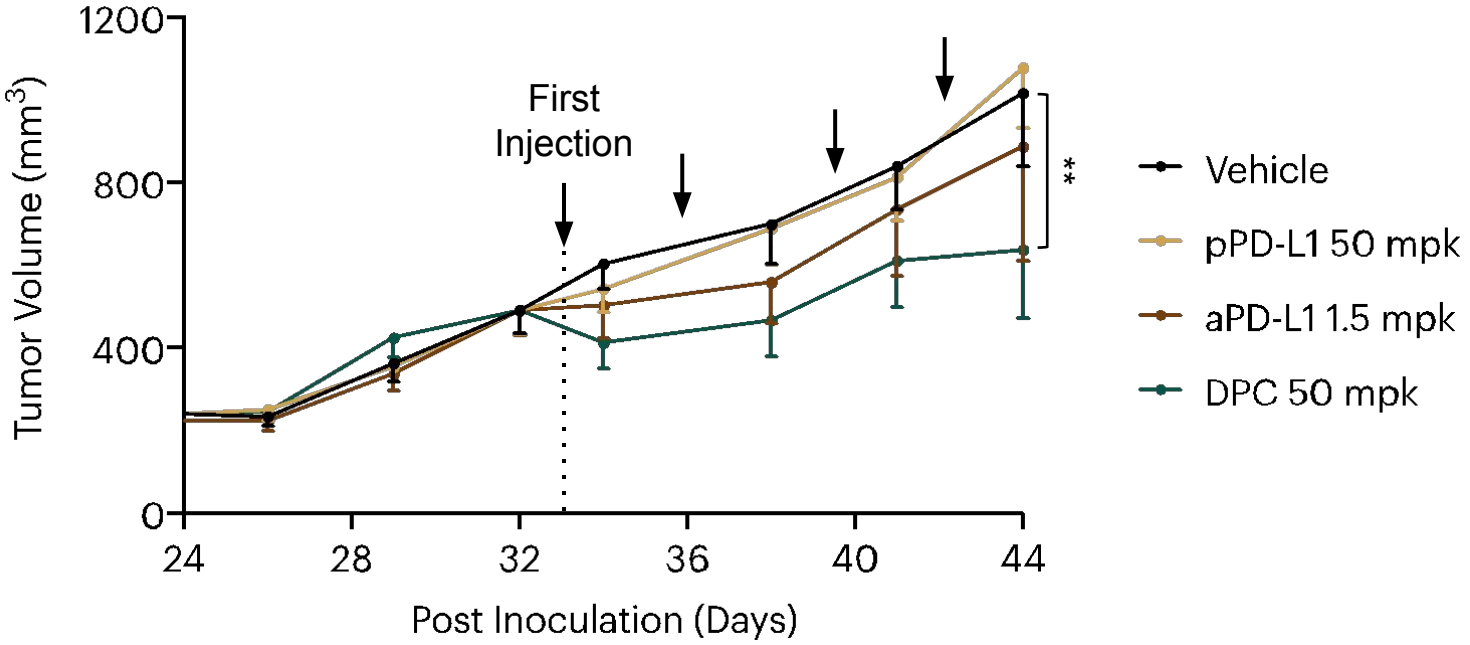
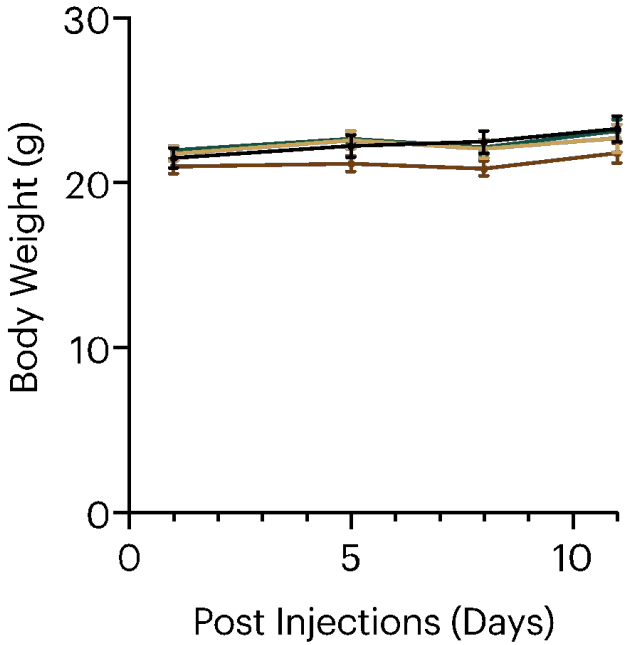
Two-way AVONA using Bonferroni's multiple comparisons test; Mean with SEM; In Collaboration with Wheeler Research Group



❖ Tumor volume:



❖ Body weight:



DPC <u>delayed</u> the tumor growth more efficiently than aPD-L1

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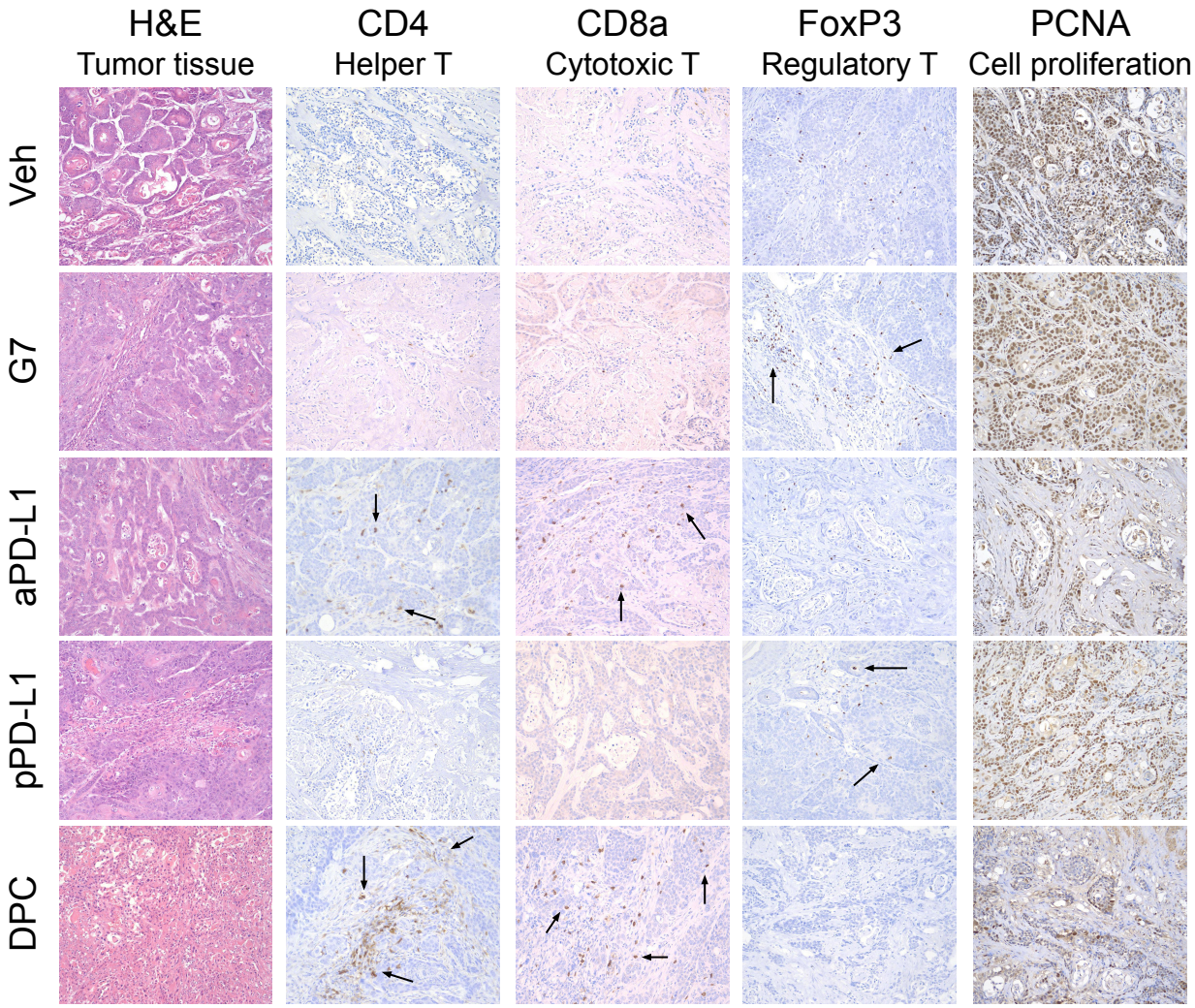
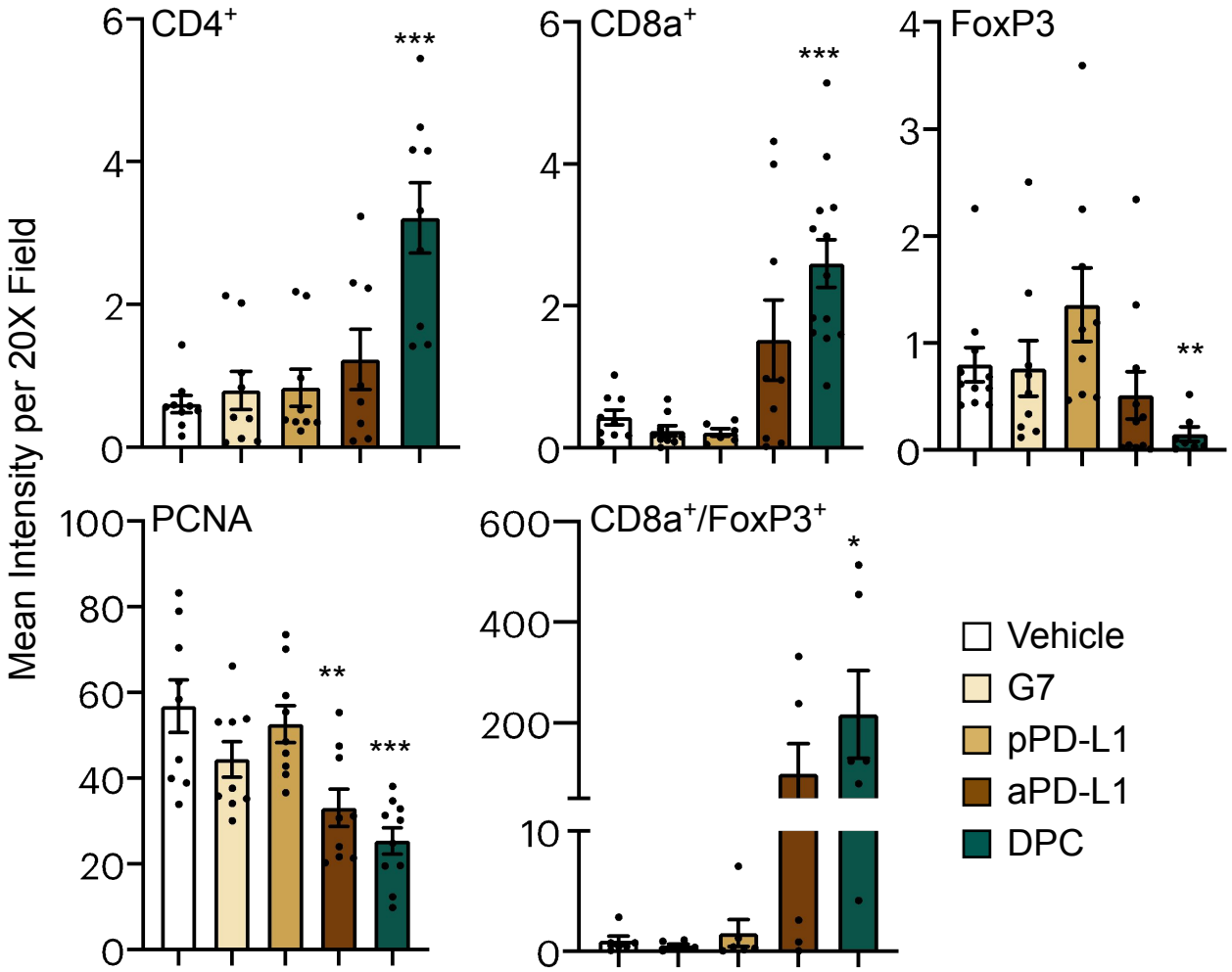
* FoxP3 = Forkhead box P3
* PCNA = Proliferating cell nuclear antigen





DPC treatment induced exclusion of regulatory T cells, recruitment of $CD4^+$ and $CD8a^+$ tumor-infiltrating T cells, and inhibition of tumor cell proliferation

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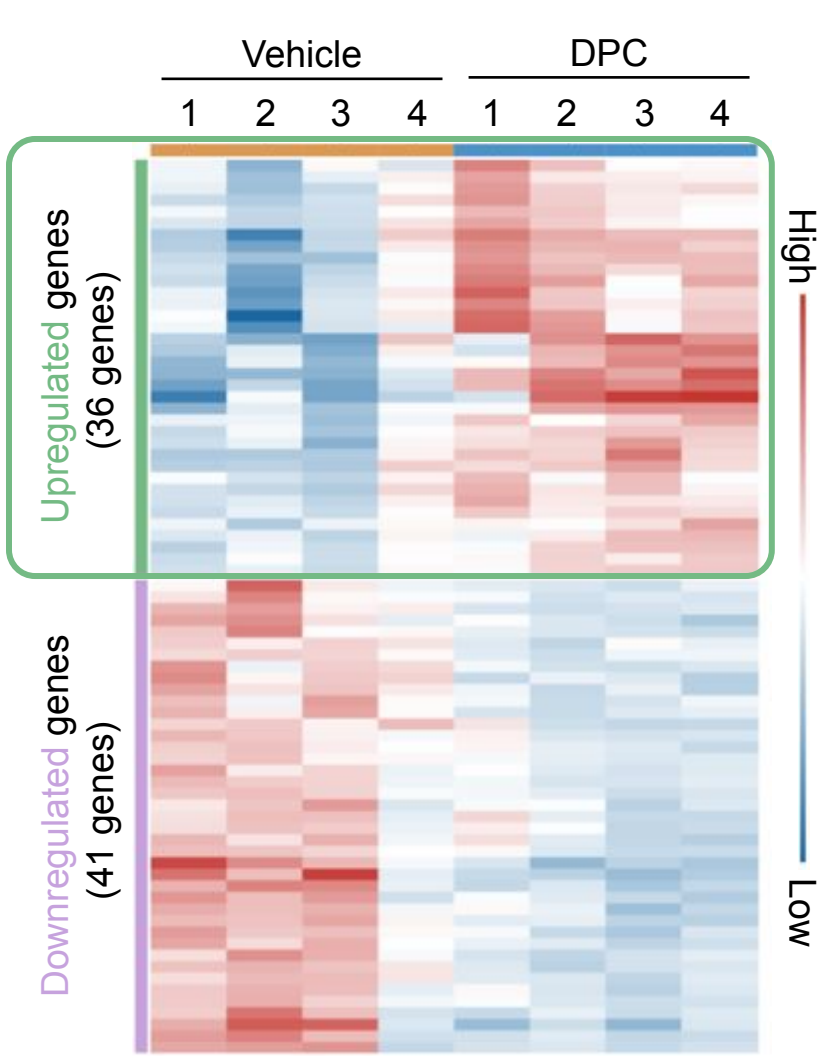
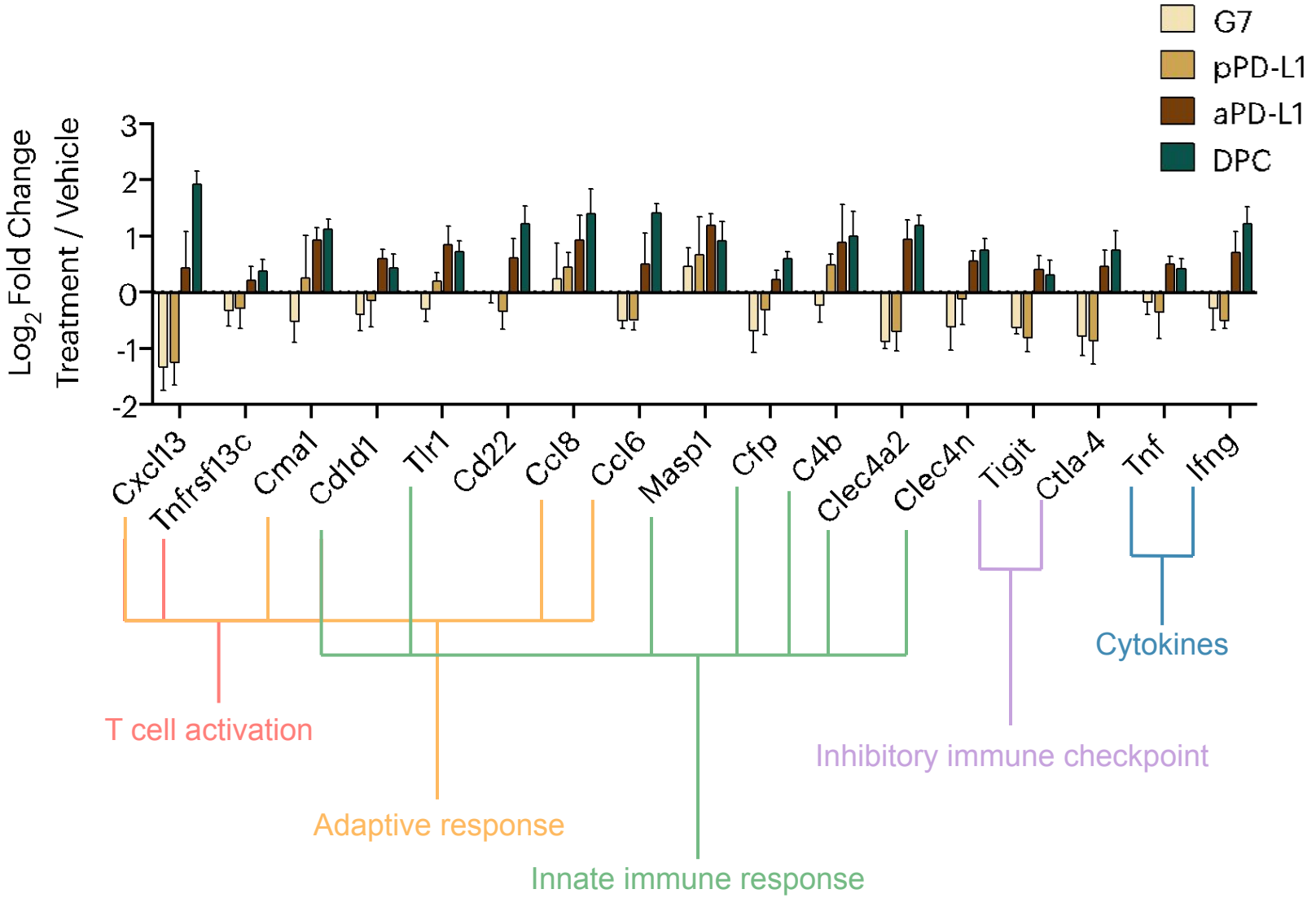
One-way AVONA using Bonferroni's multiple comparisons test; In Collaboration with Wheeler Research Group





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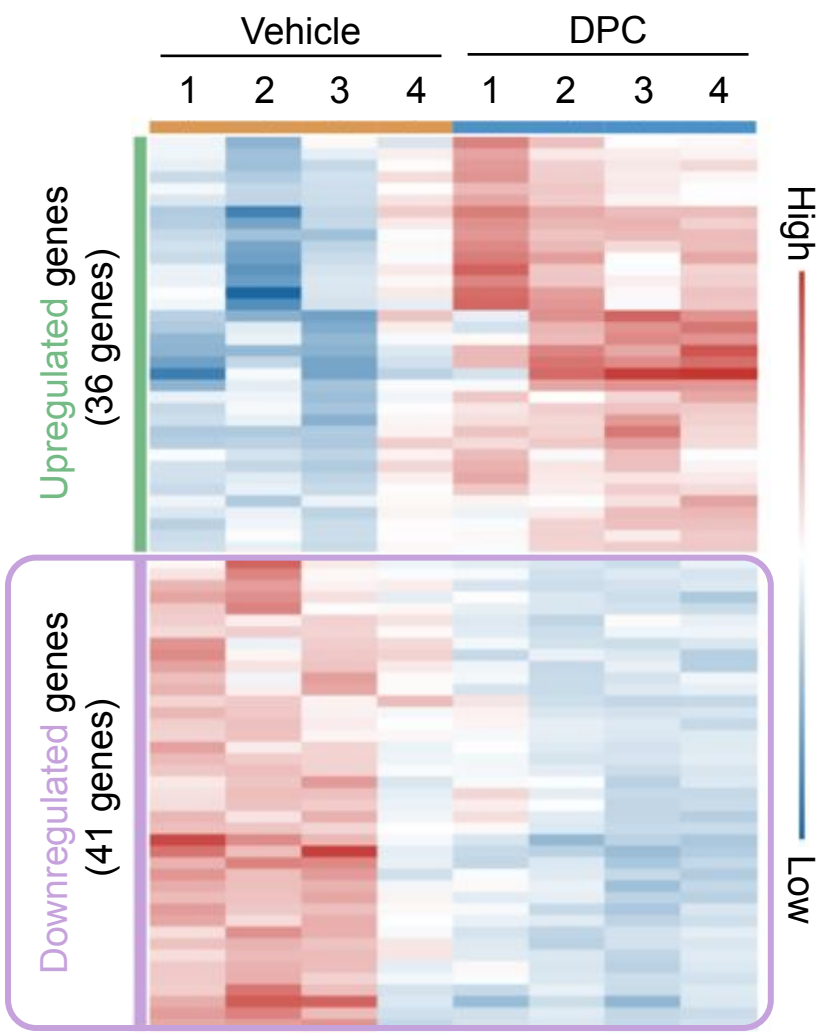

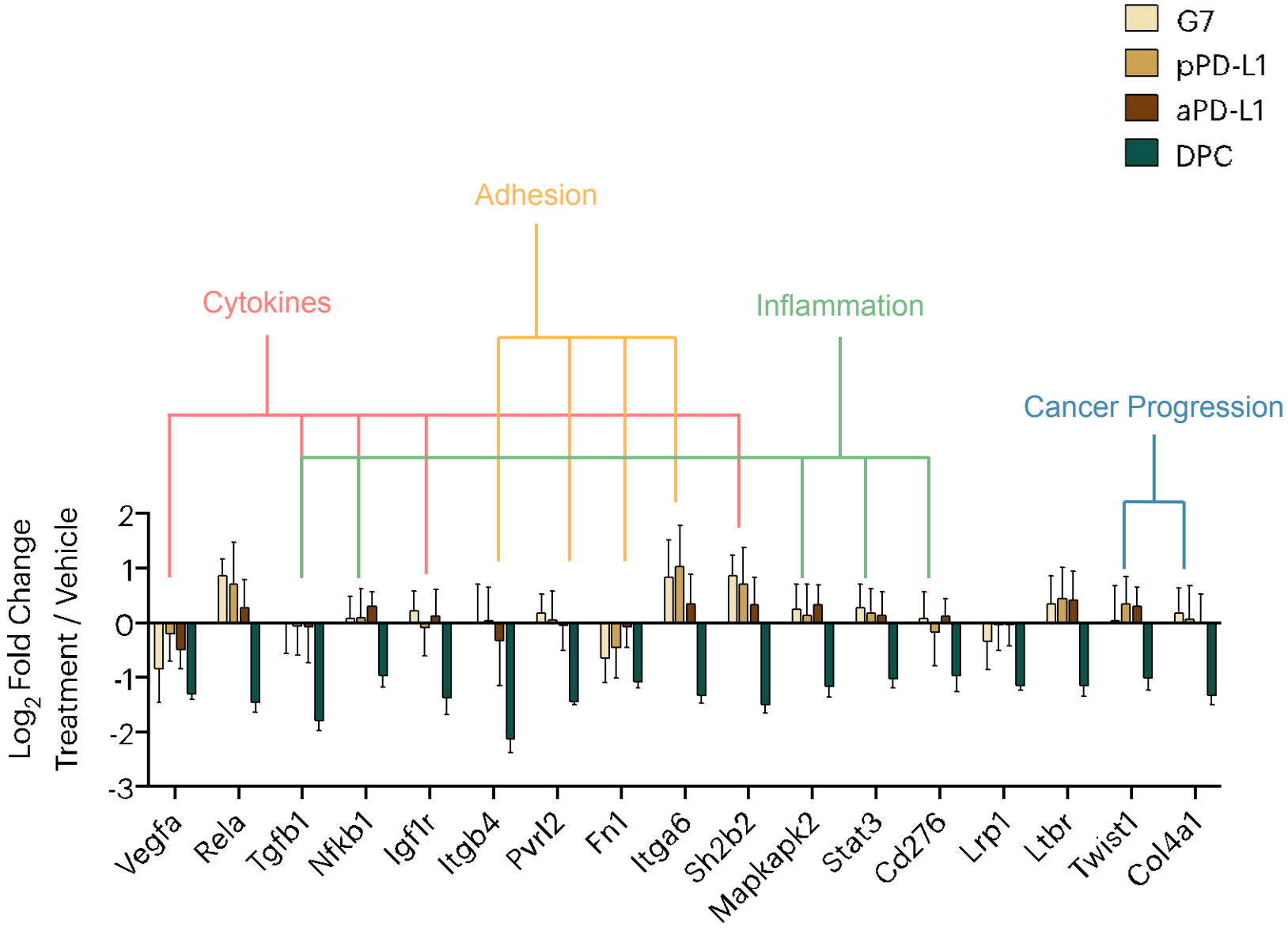

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Summary of the Study

- DPCs enhanced binding affinity three-fold higher than peptide alone
- DPCs showed high association rate to PD-L1^{High} MOC1 cells
- DPCs improved drug half-life by nine-fold compared to peptide alone
- DPC treatment resulted in 42% reduction in tumor volume at 50 mpk
- DPC treatment delayed tumor growth more effectively than monoclonal antibody
- DPC-treated tumors had higher populations of $CD4^+$ and $CD8^+$ lymphocytes and lower populations of regulatory T cells and proliferating cells
- DPC treatment influenced the expression of immunoregulatory genes

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Dr. Weiping Tang

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Kourtney Kostecki
Bridget Mehall

Previous Hong Lab:
Dr. Jiyoon Bu
Dr. Woo-jin Jeong

Current Hong Lab:
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Dr. JinWoong Lee
Dr. Narsimha Mamidi
Caroline Hopkins
Kaila Javius-Jones
Piper Rawding
Erika Kay-Tsumagari
Emily Walker
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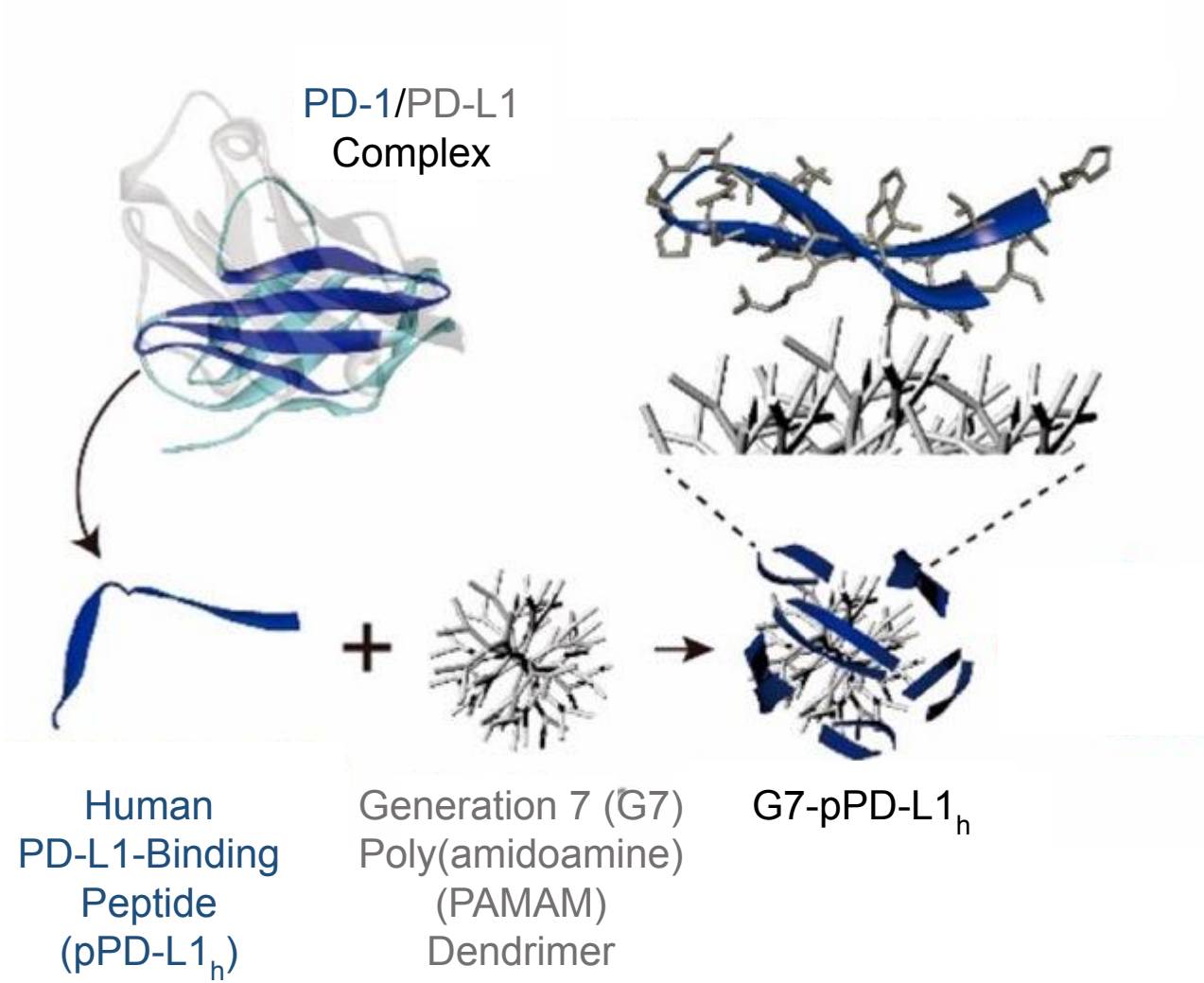
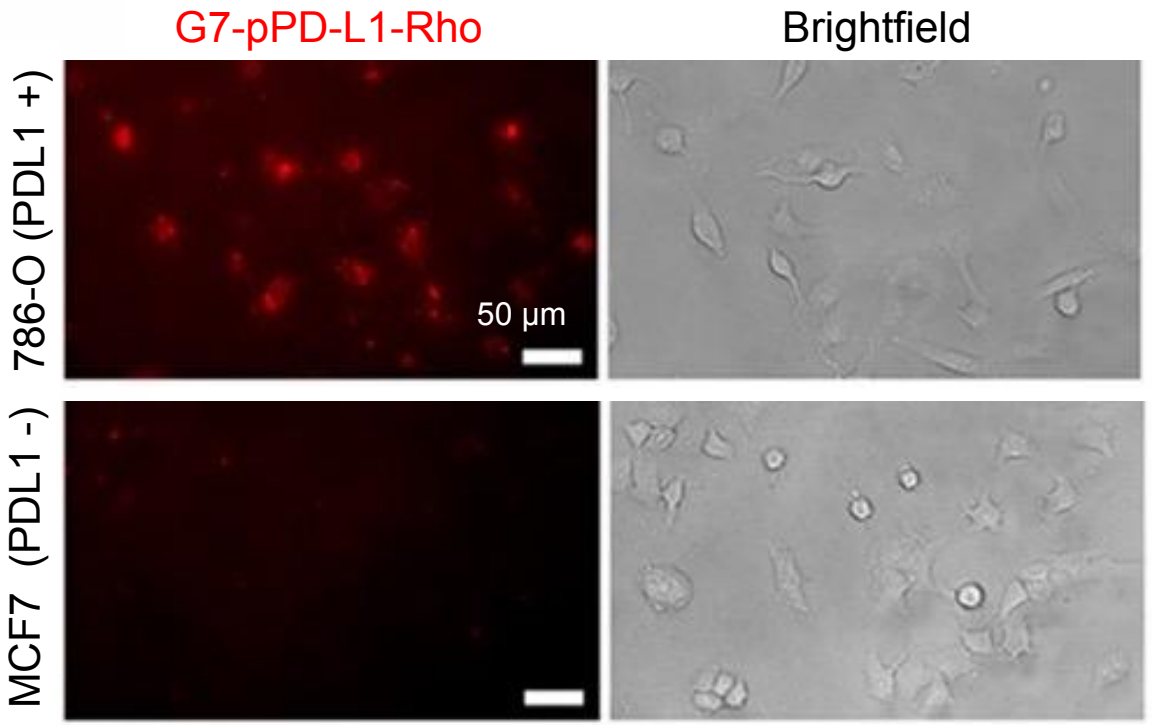
NIH National Institutes of Health

NSF

Dongkook

Dendrimer-Peptide Conjugates (DPCs) for Drug Delivery





Conjugation of PD-L1-binding peptides to dendrimer enhanced *in vitro* efficiency

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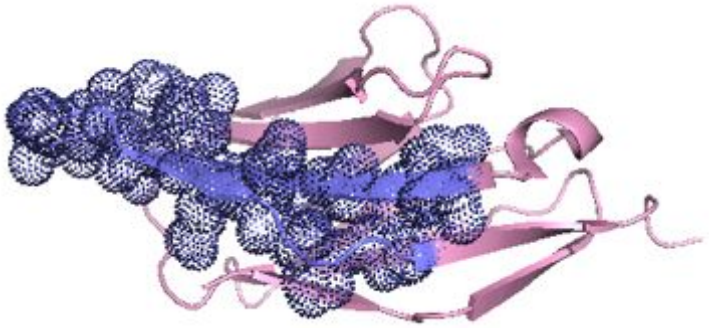
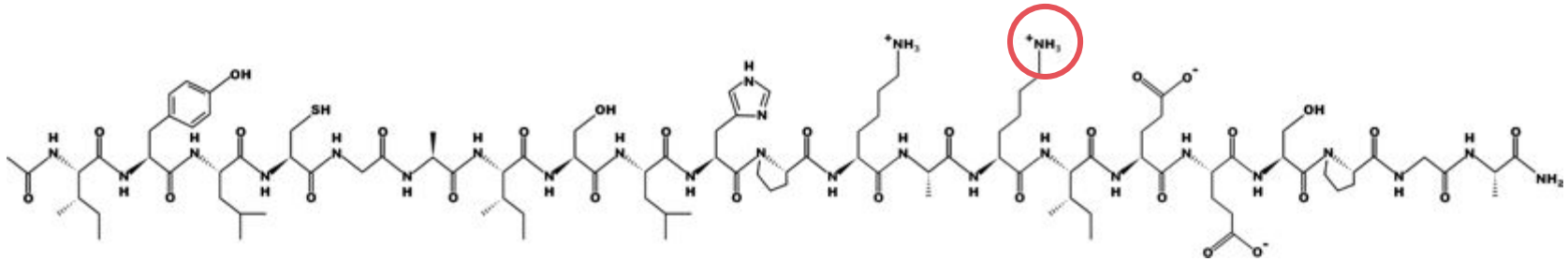
Synthesis of Mouse PD-L1-binding peptide (pPD-L1$_m$)

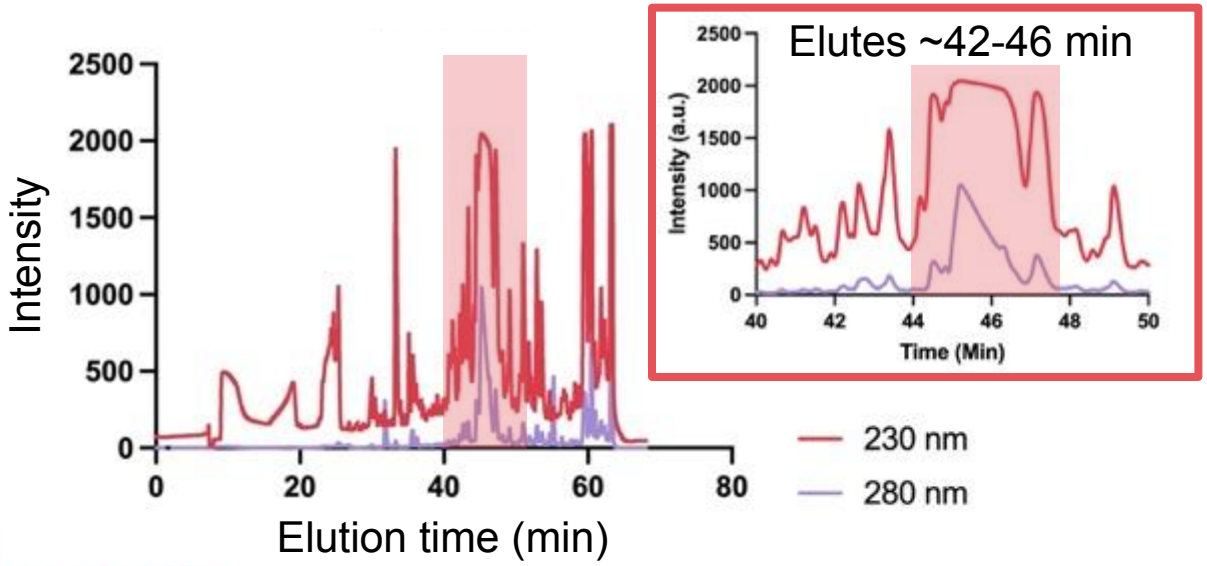
- **Mouse pPD-L1 (pPD-L1$_m$)**



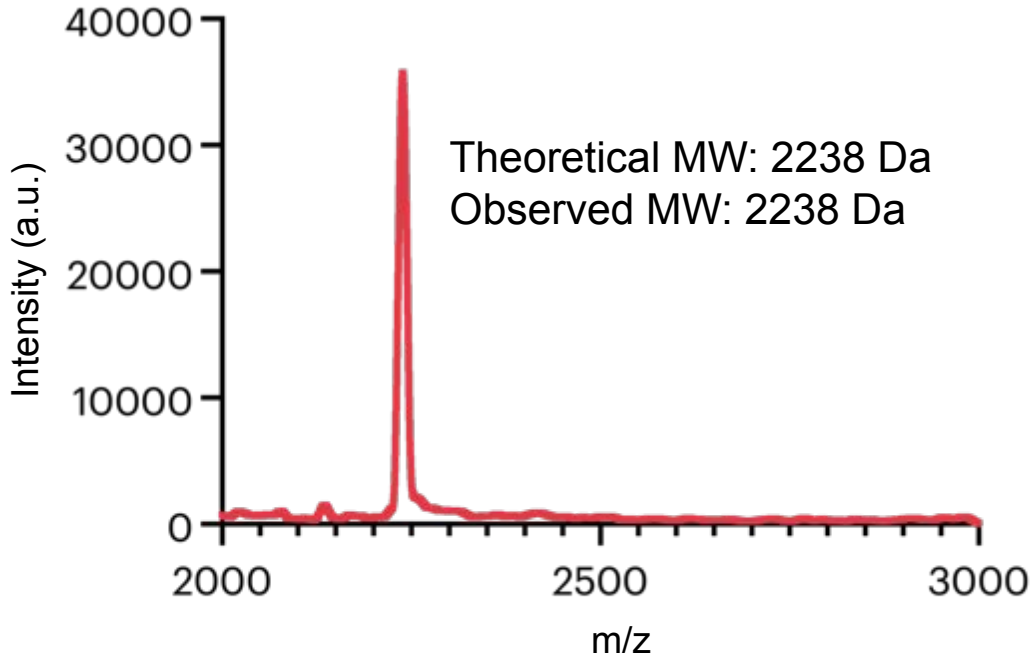


pPD-L1m: Ac-IYLCGAISLHPKAKIESSPGA-H ◯ = Dendrimer Conjugation Site

- **High performance liquid chromatography (HPLC)**



- **MALDI-TOF MS Spectra**



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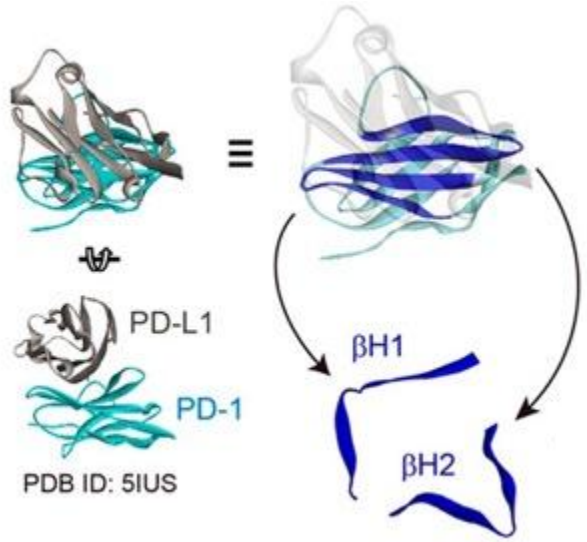
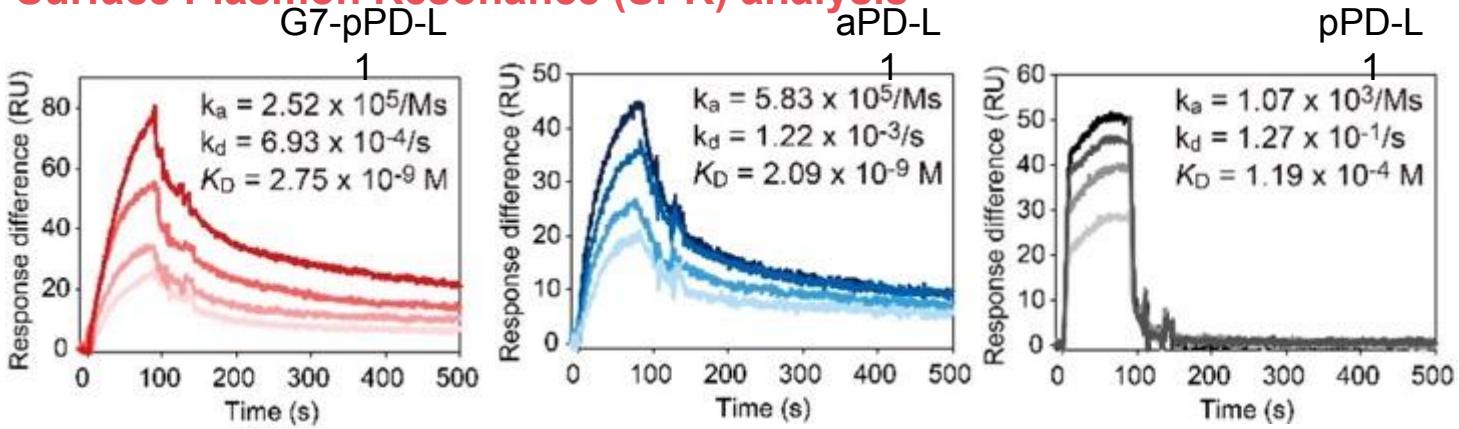
Data obtained by Piper Rawding; Lin, D.Y. et al. PNAS. 105(8): 3011-3016 (2008).

Preliminary Data Using Human G7-pPD-L1 Conjugates

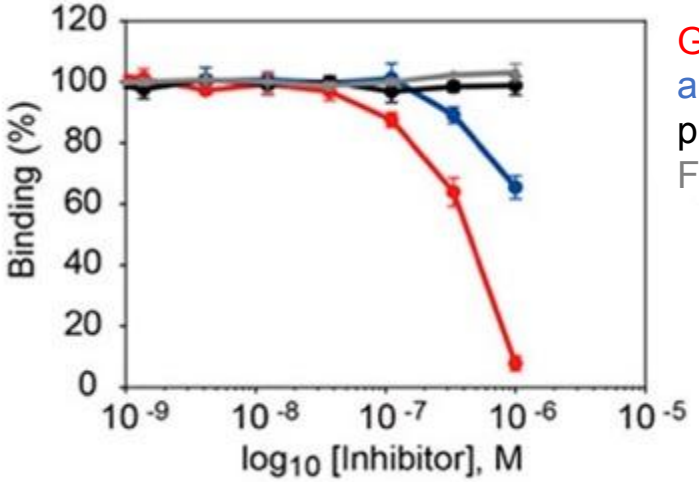
Scheme for DPC development



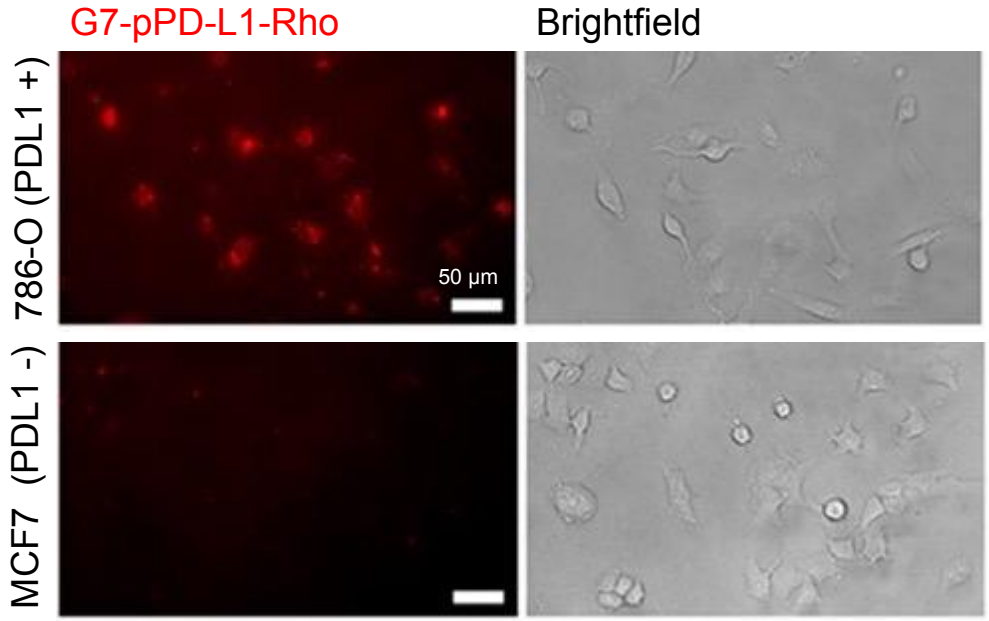
Surface Plasmon Resonance (SPR) analysis



Competition assay



Cell binding

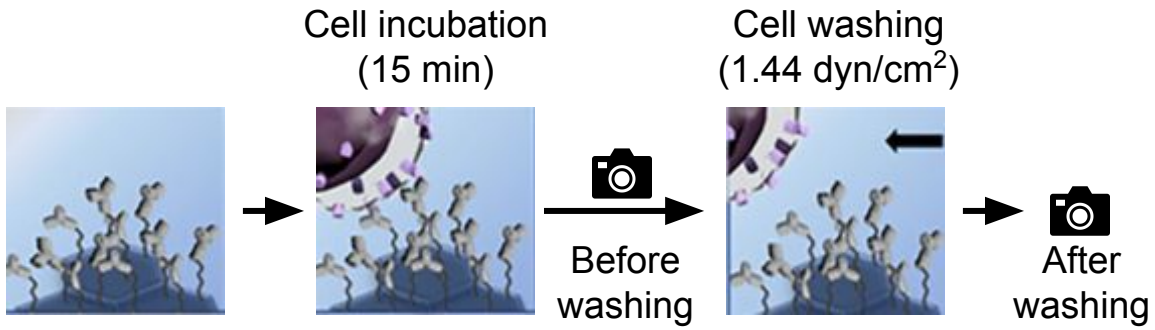


G7-pPD-L1s high target affinity translates to high *in vitro* efficiency

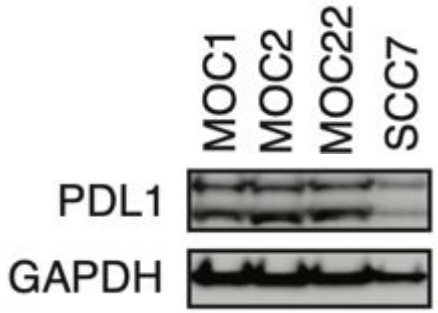
Jeong, W.J.. et al. J Am Chem Soc, 142:1832-1837 (2020).

Low dissociation rate of PD-L1^{High} cells to DPCs

Cell retention test scheme

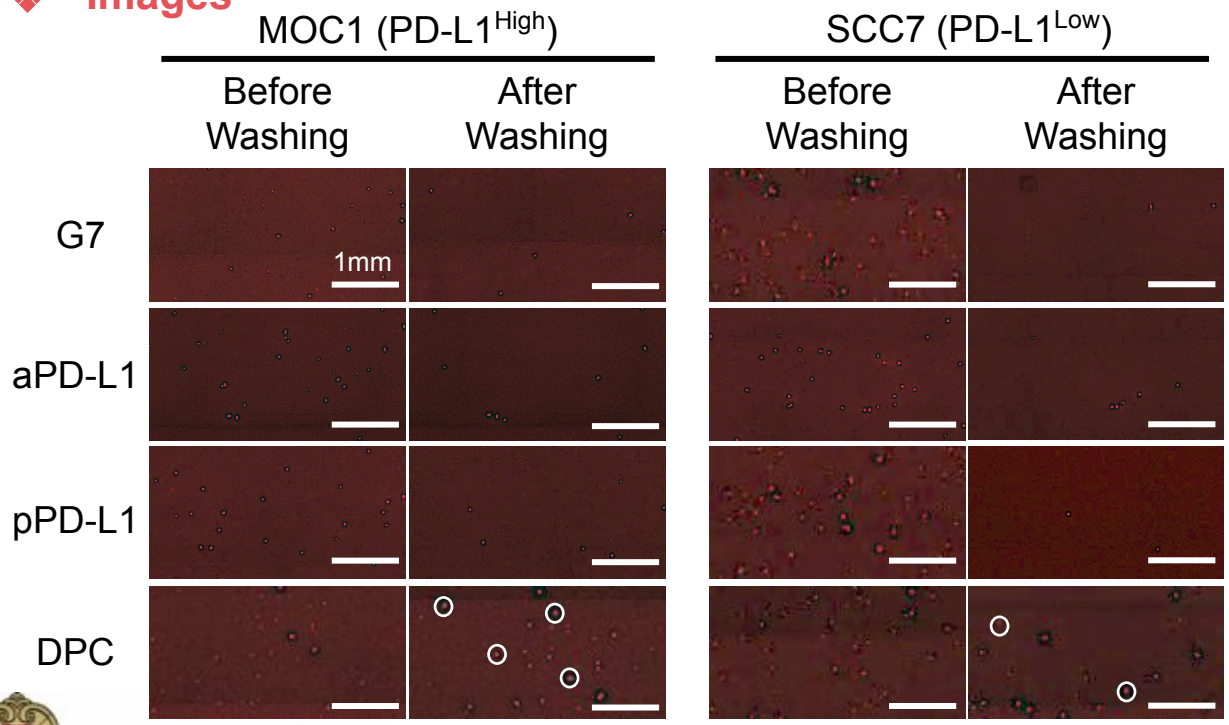


Western blot

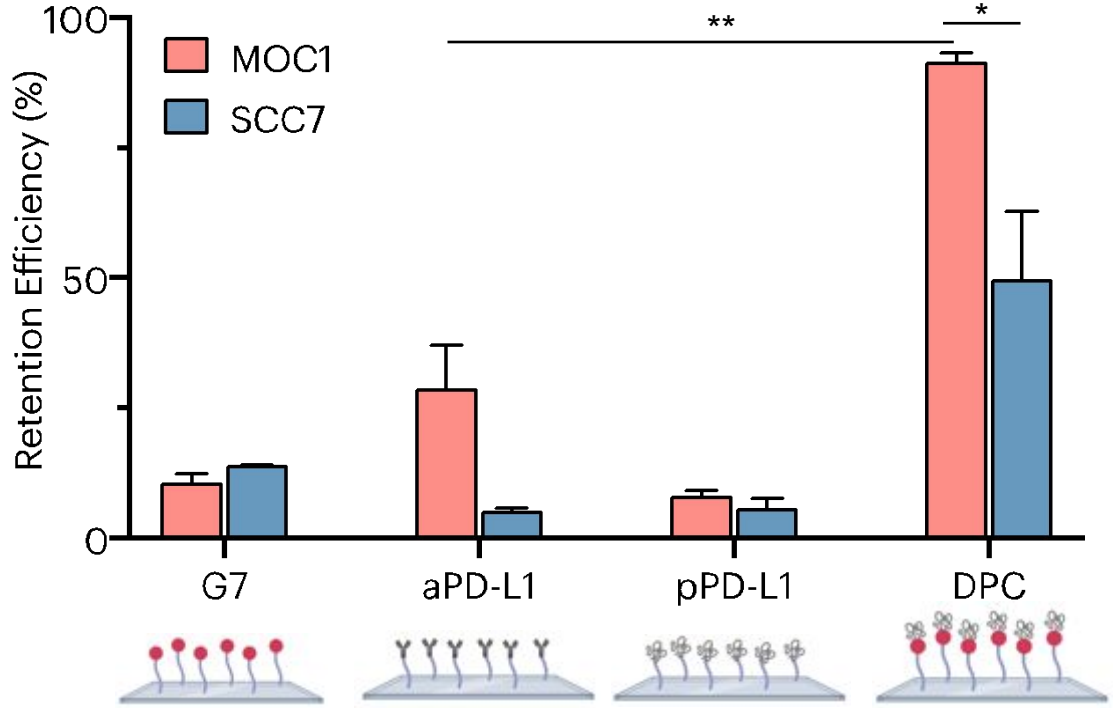


DPCs have
higher retention efficiency to
PD-L1^{High} MOC1
compared to PD-L1^{Low} SCC7

Images



Quantification

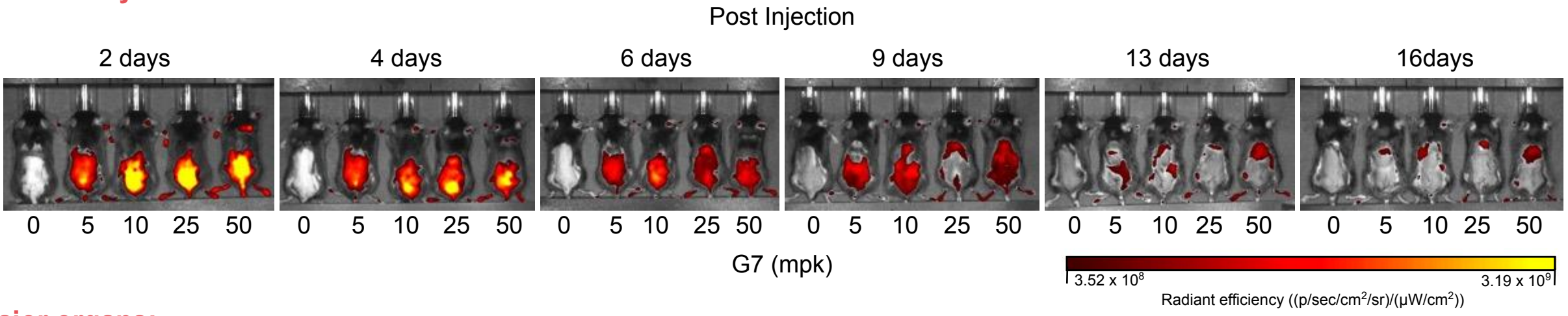


Western blot done by Dr. Mari Iida; Two-way AVONA using Bonferroni's multiple comparisons test

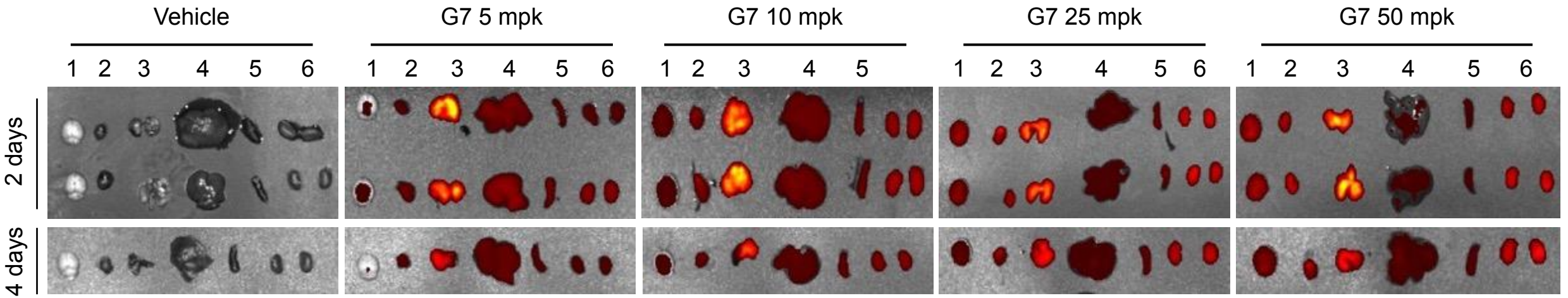
Bu, J. et al. Nano Lett. 20, 4901–4909 (2020).

G7 Biodistribution in Body and Major Organs Post Injections

❖ Whole Body:



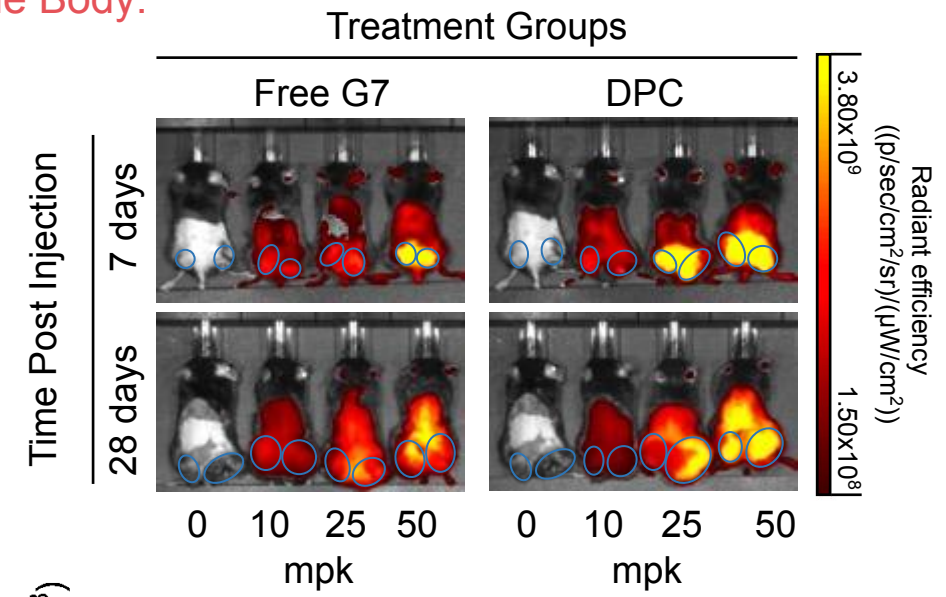
❖ Major organs:

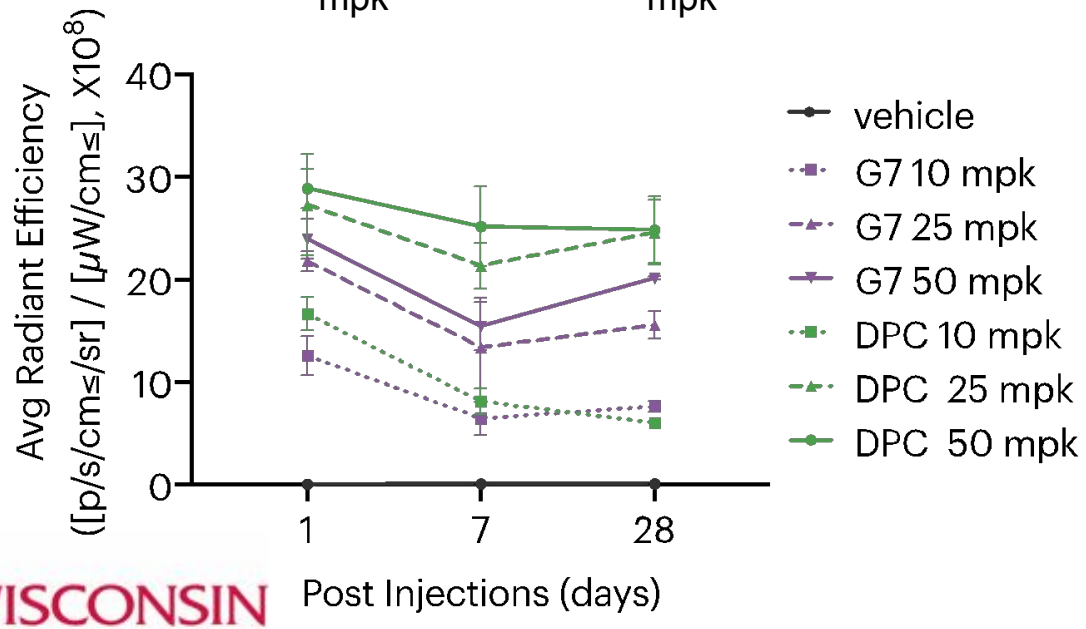


G7 is retained in the body at least
<u>two weeks</u> after the last administration

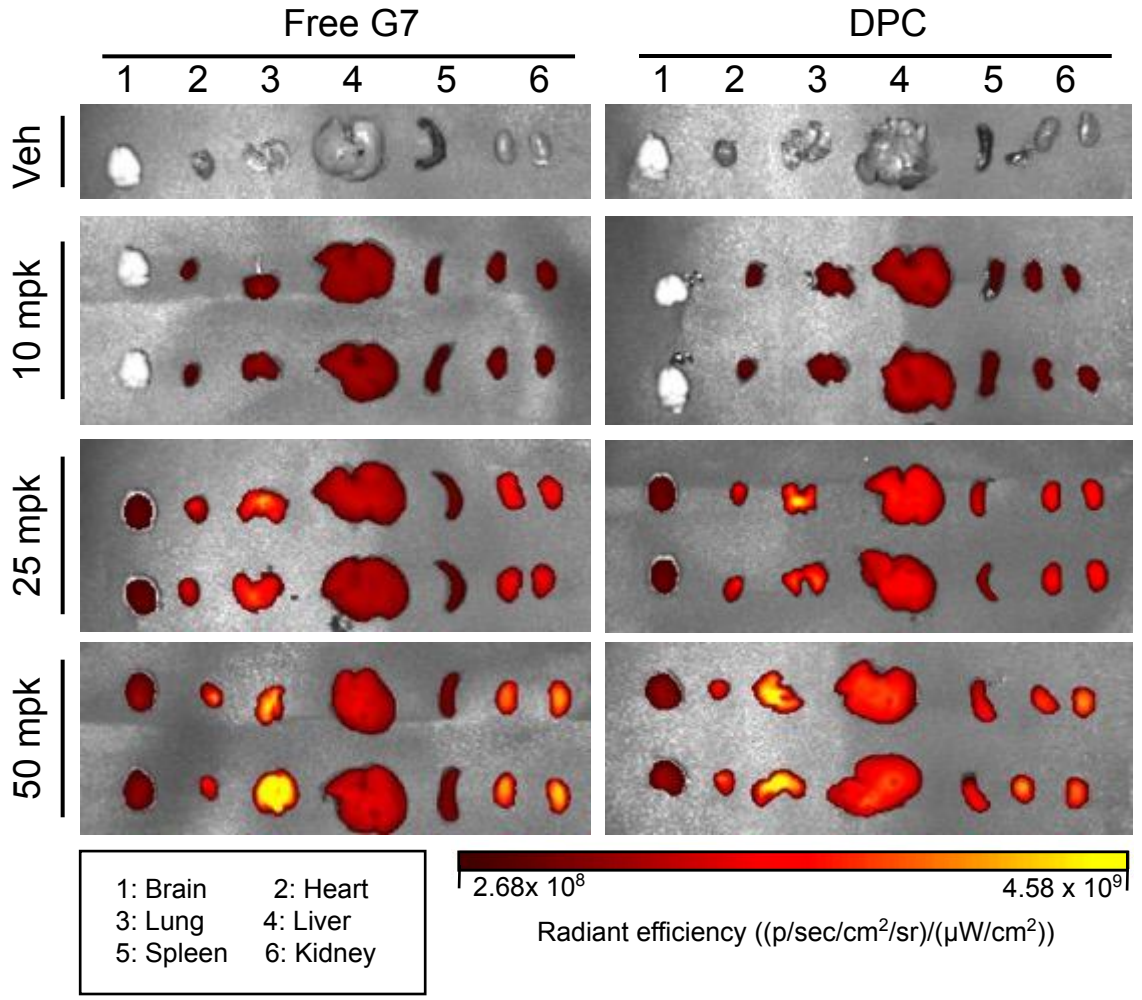
DPC Biodistribution in Body and Major Organs Post Injection

- Whole Body:



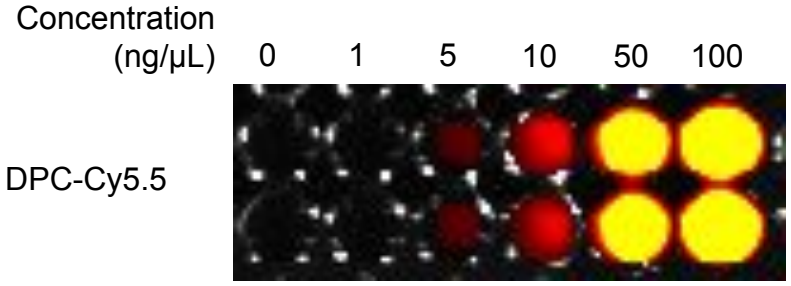


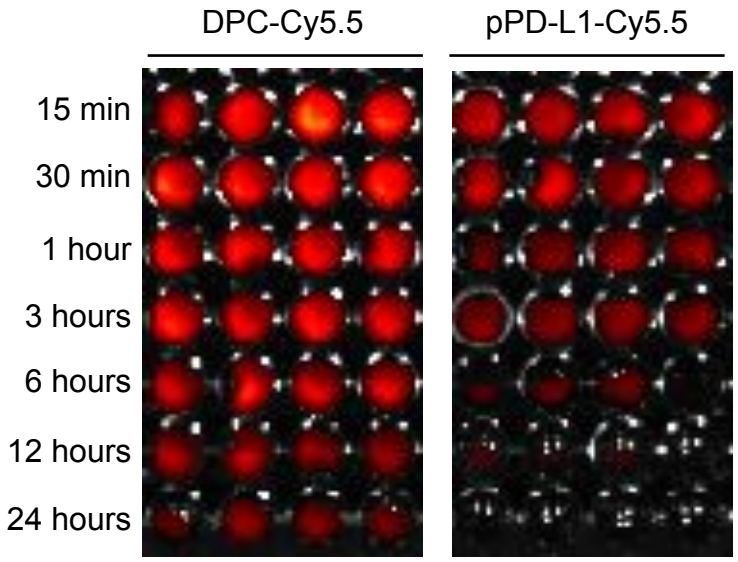
- Major Organ:

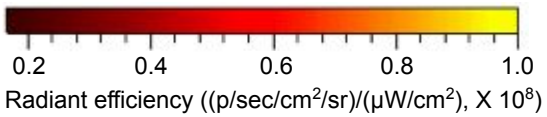


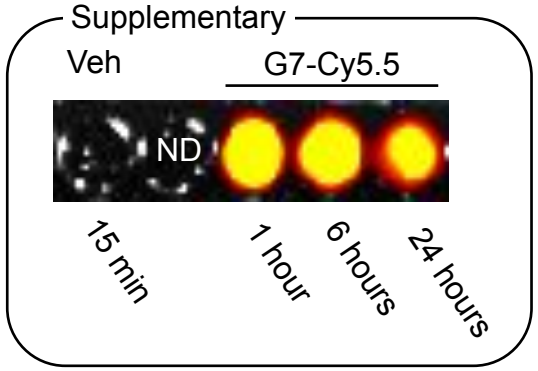
DPC is retained in the body for at least four weeks with higher accumulation than free G7

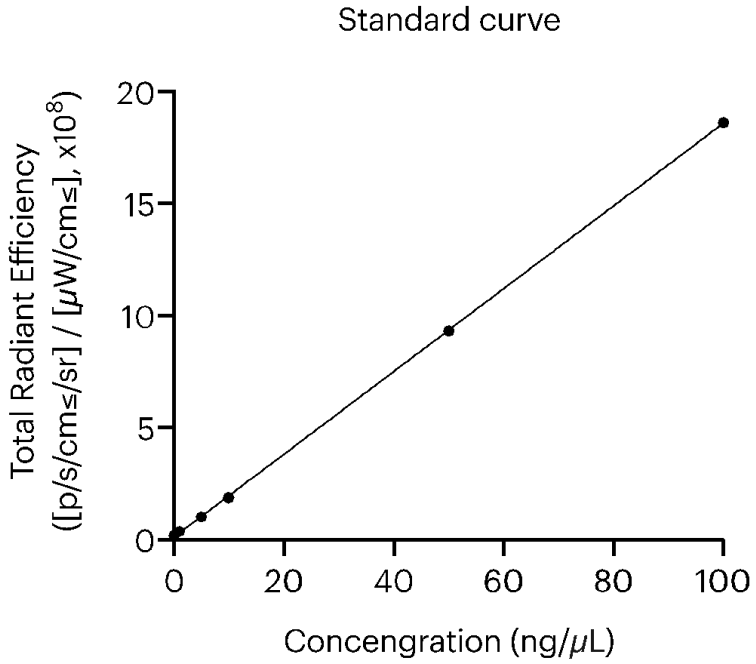
G7 Biodistribution in Body and Major Organs Post Injections

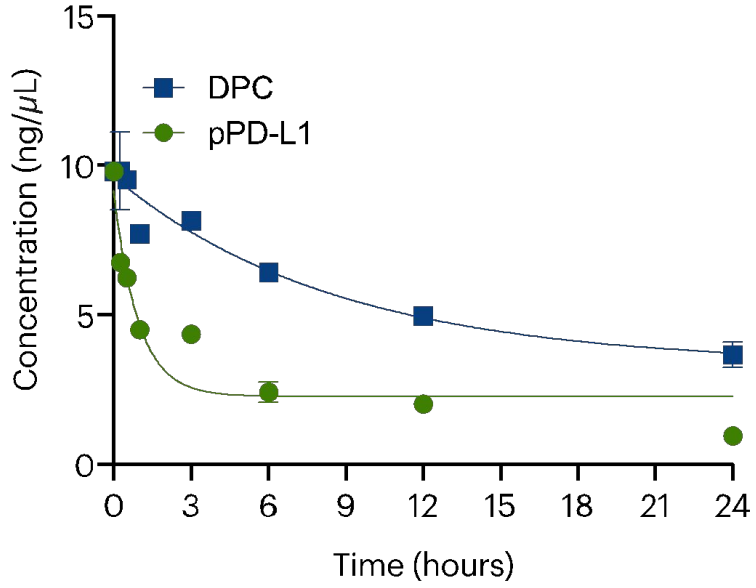








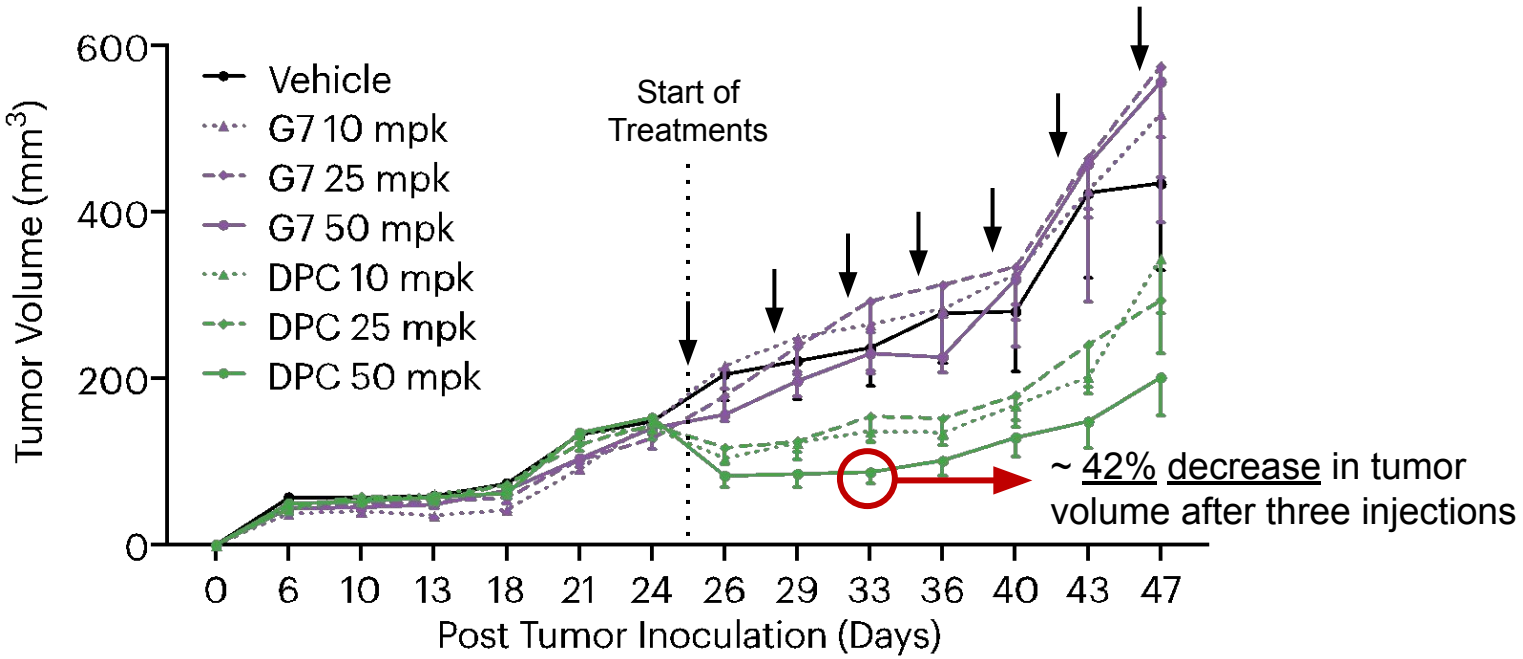




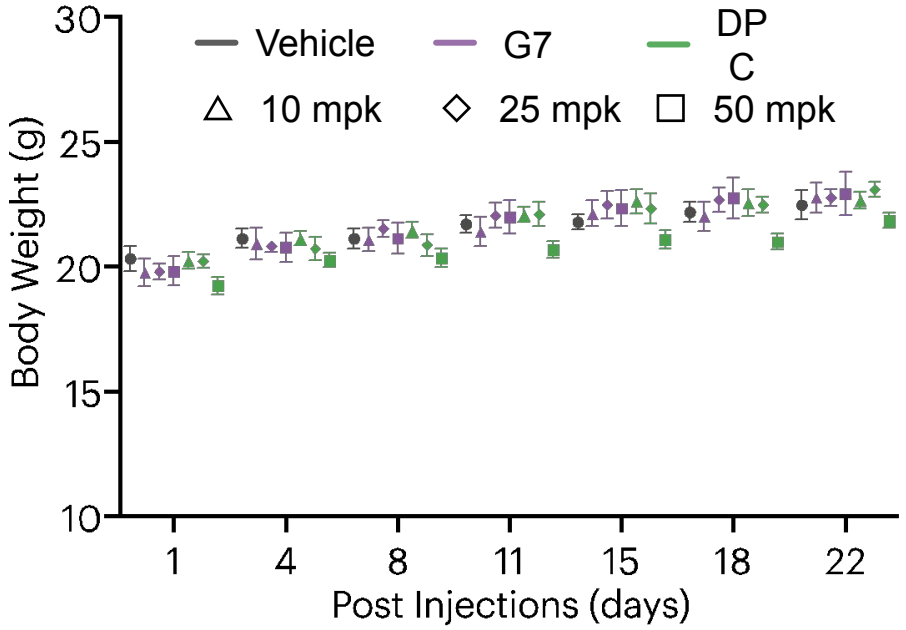
One phase decay graph

- Equation for the model:
  Y=(Y0 - Plateau)*exp(-K*X) + Plateau
- Half-life:
  - pPD-L1: 0.66 hours (about 40 min)
  - DPC: 5.98 hours (about 6 hours)

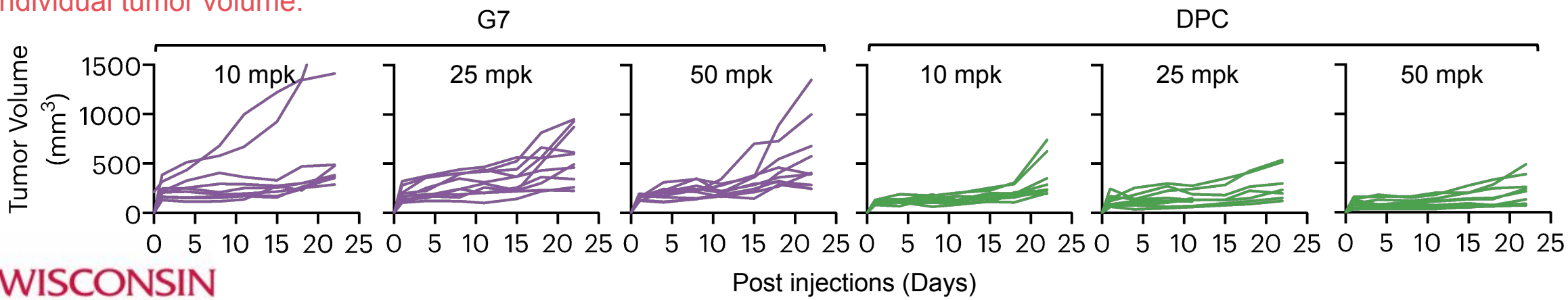
❖ Tumor volume:



❖ Body weight:



❖ Individual tumor volume:



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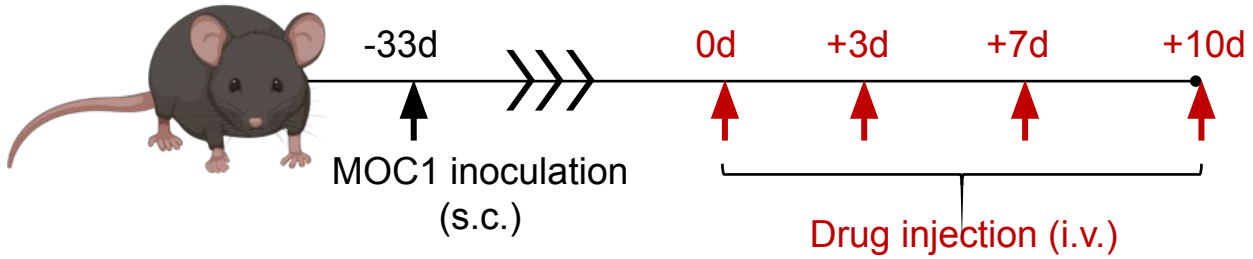
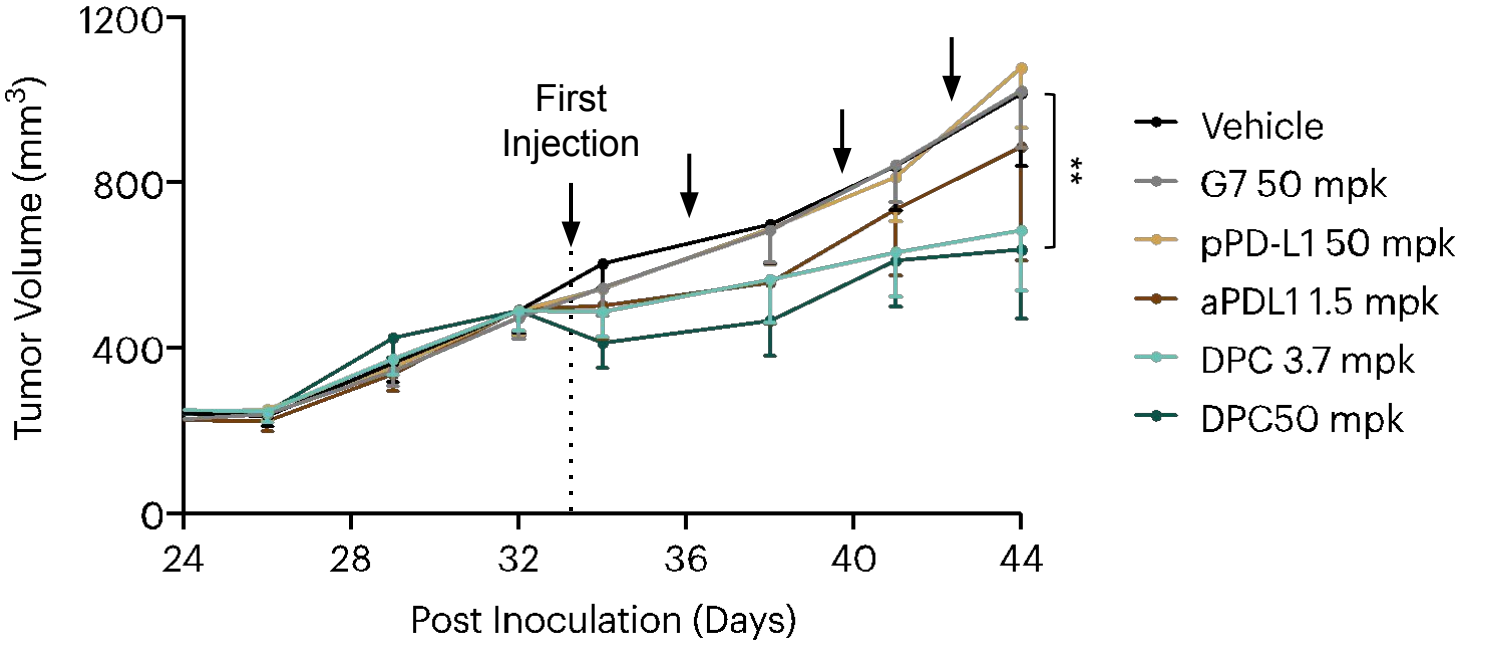
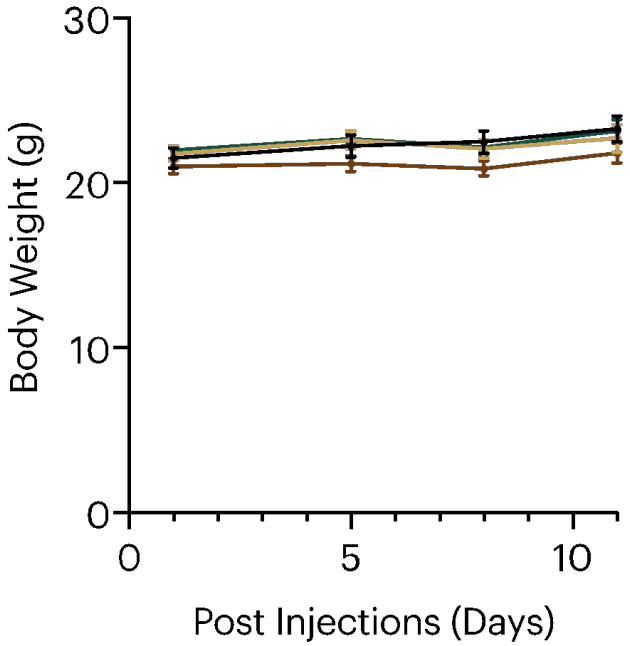
*Mean with SEM; In Collaboration with Wheeler Research Group



❖ Tumor volume:



❖ Body weight:



DPC <u>delayed</u> the tumor growth more efficiently than aPD-L1

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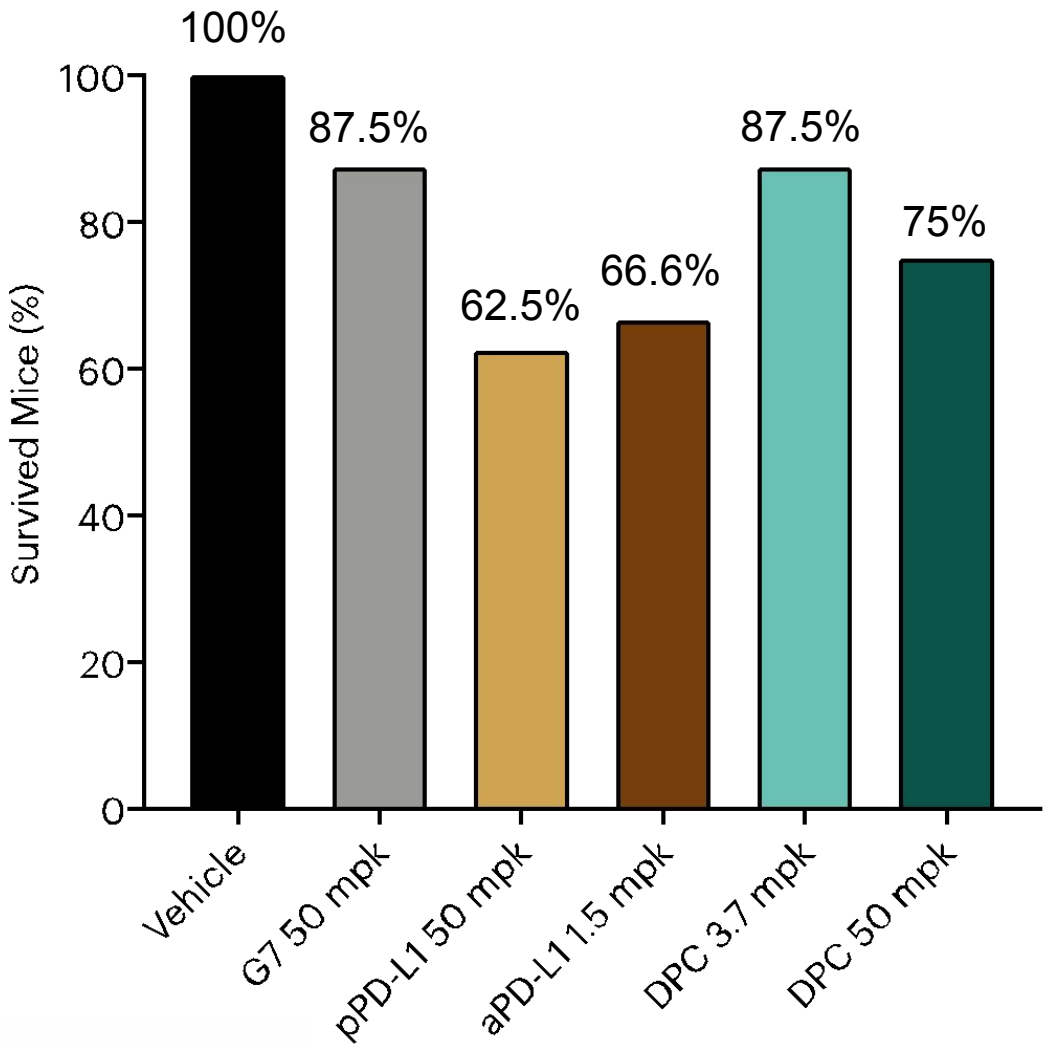

Number of mice dead throughout the study:

- Vehicle: none
- G7: 1 (out of 8) mice dead
- pPD-L1 50 mpk: 3 (out of 8) mice dead
- aPD-L1 1.5 mpk: 4 (out of 12) mice dead
- DPC 3.7 mpk: 1 (out of 8) mouse dead
- DPC 50 mpk: 2 (out of 8) mice dead

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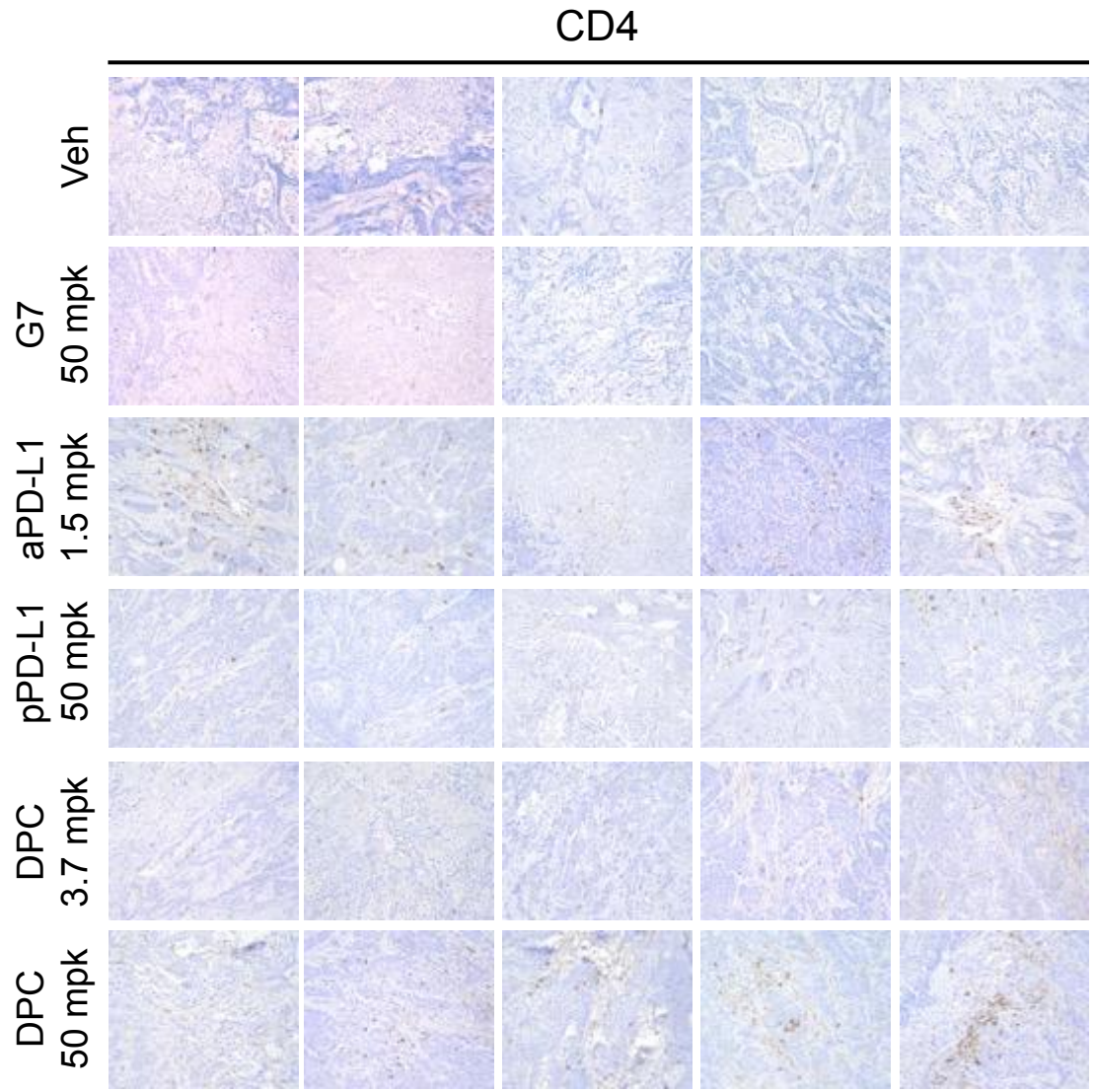
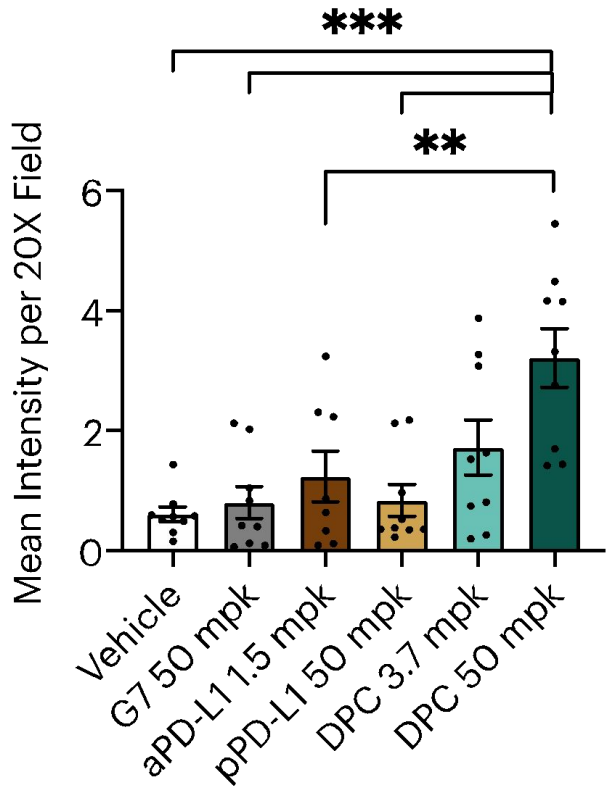
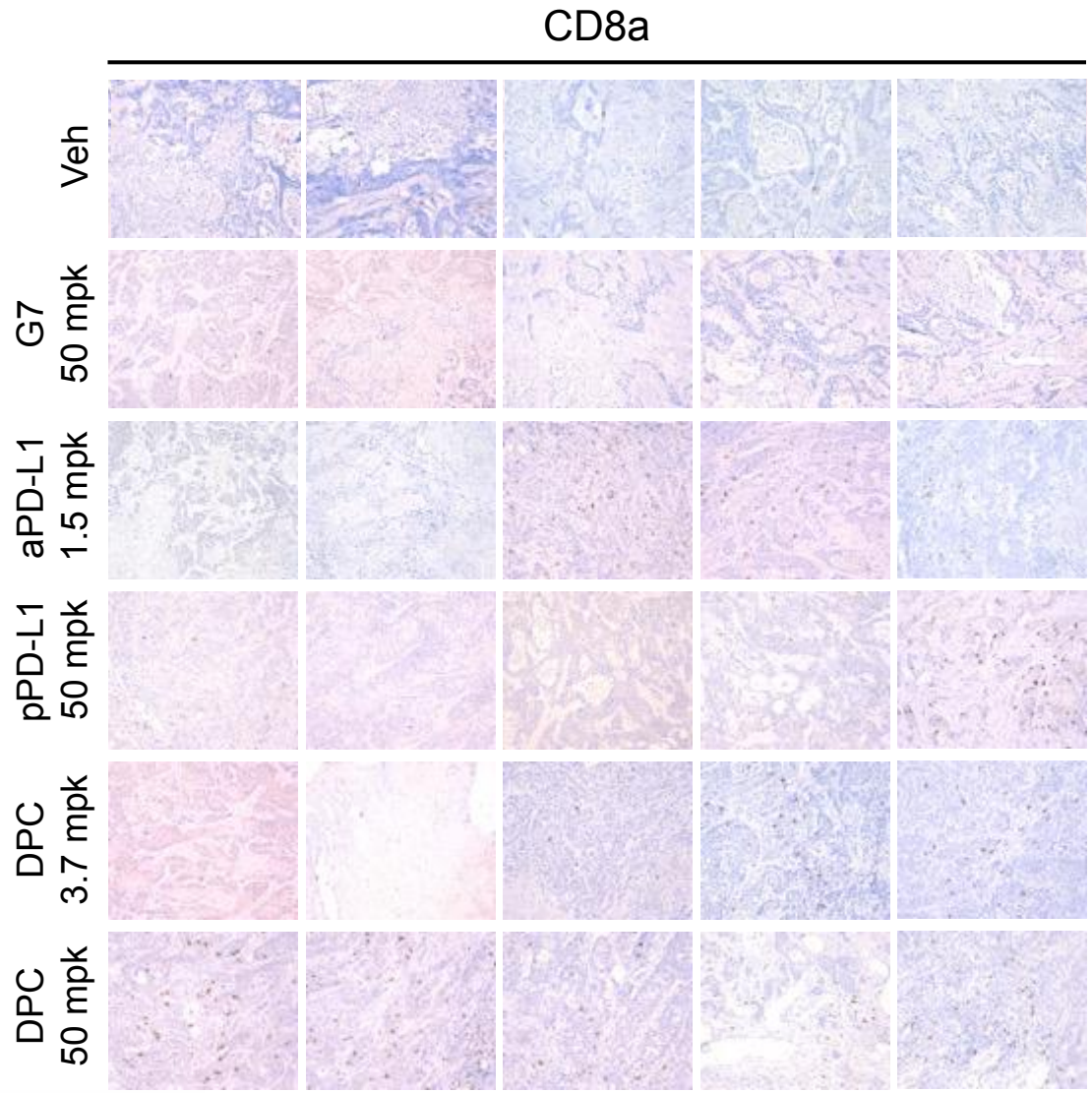


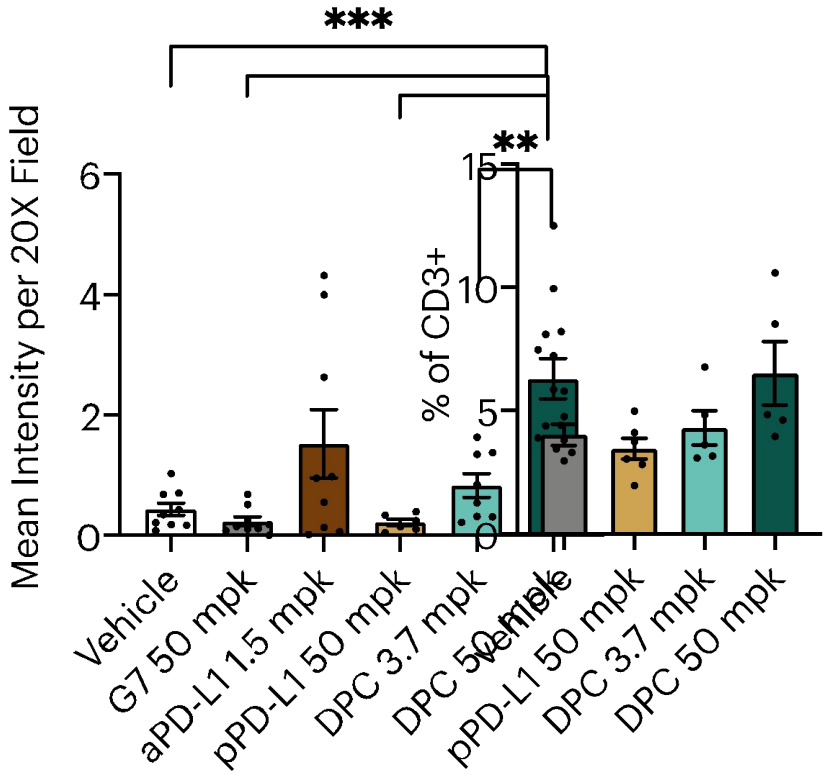
- $CD4^+$ staining = Helper T cells including Th1, Th2, Th9, Th17, regulatory T cell, follicular helper T cell, each contributing to immune function



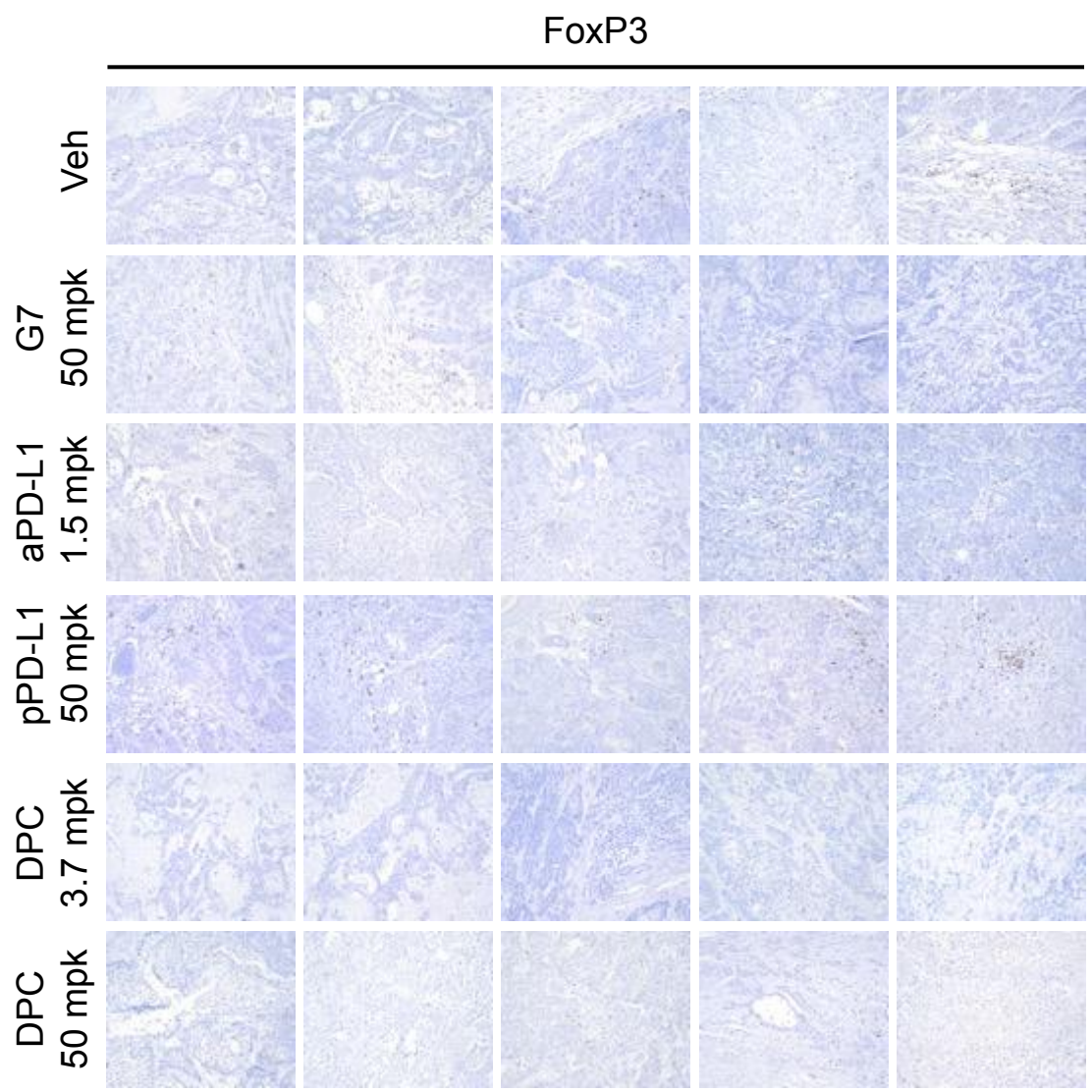
One-way AVONA using Bonferroni's multiple comparisons test; In Collaboration with Wheeler Research Group

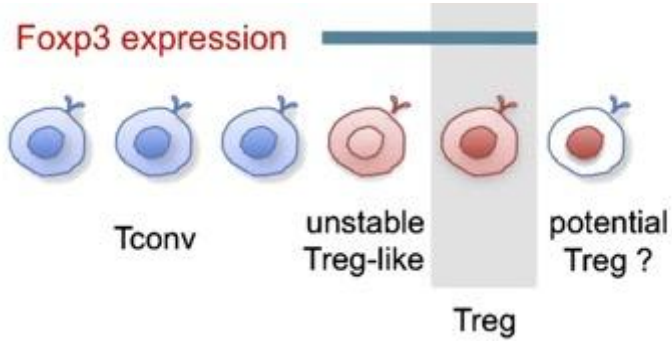


- CD8a^{+} staining = A transmembrane glycoprotein expressed mainly on the surface of cytotoxic T lymphocytes.

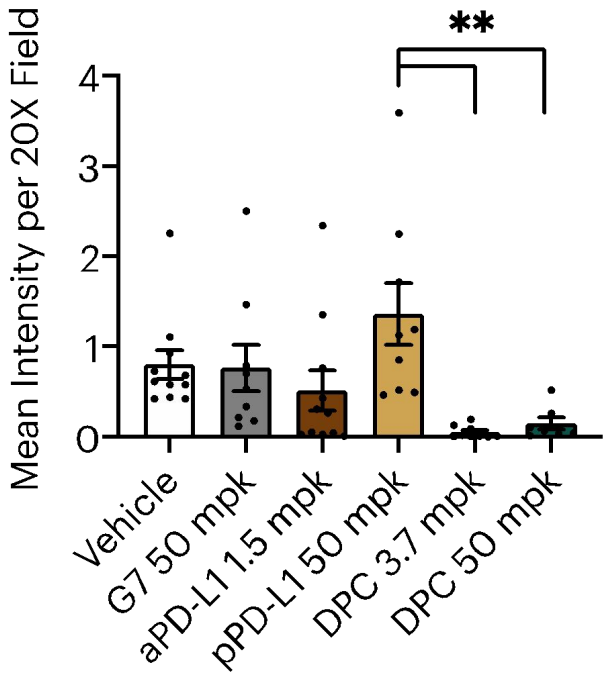


One-way AVONA using Bonferroni's multiple comparisons test; In Collaboration with Wheeler Research Group





- Forkhead transcription factor (FoxP3) staining = Identification of regulatory T cells (Tregs)



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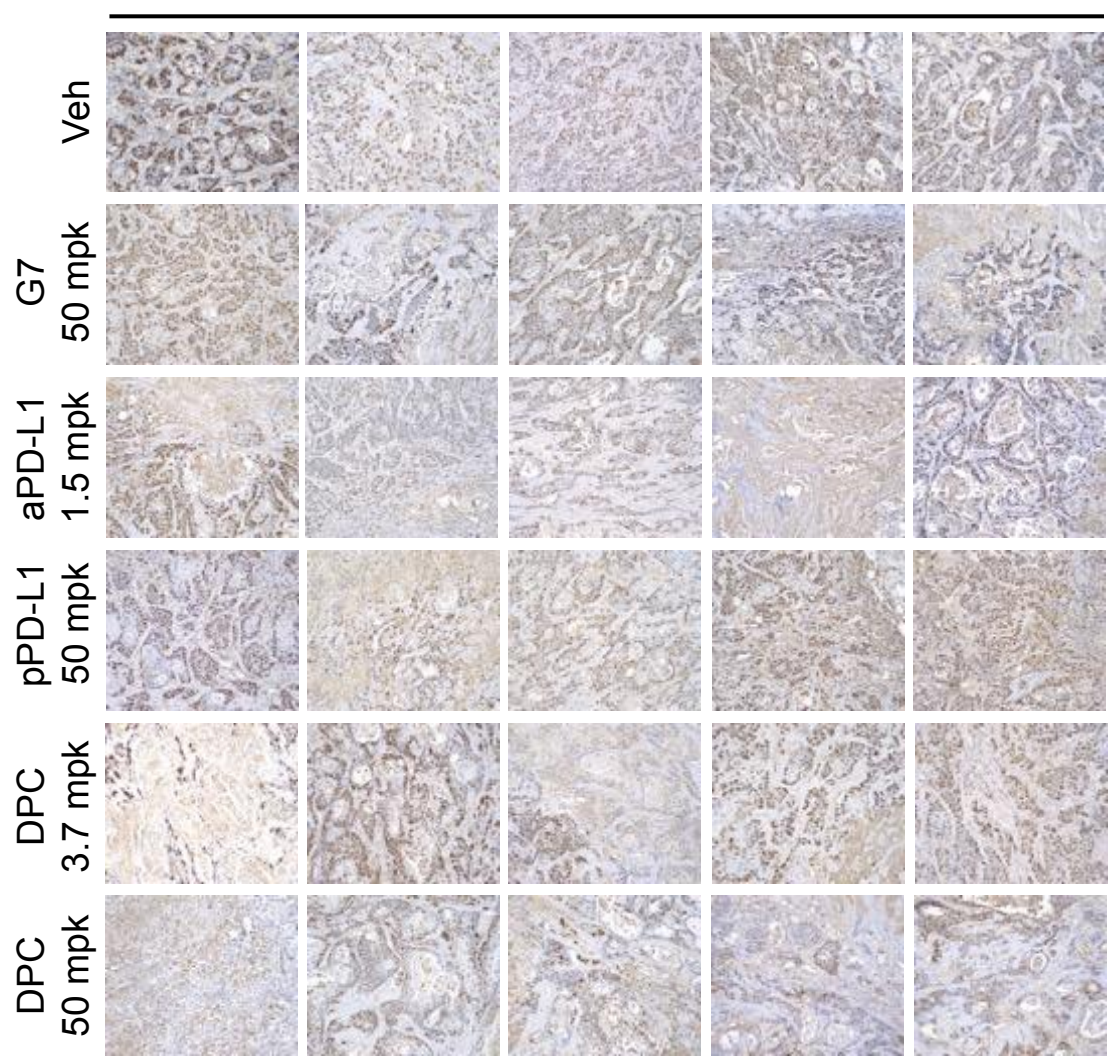
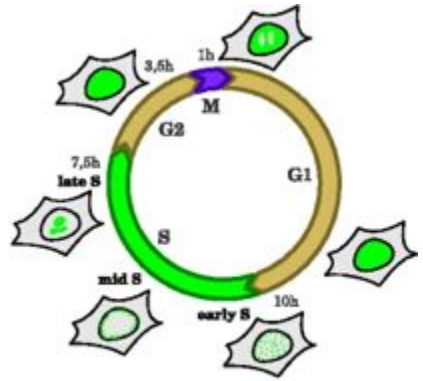
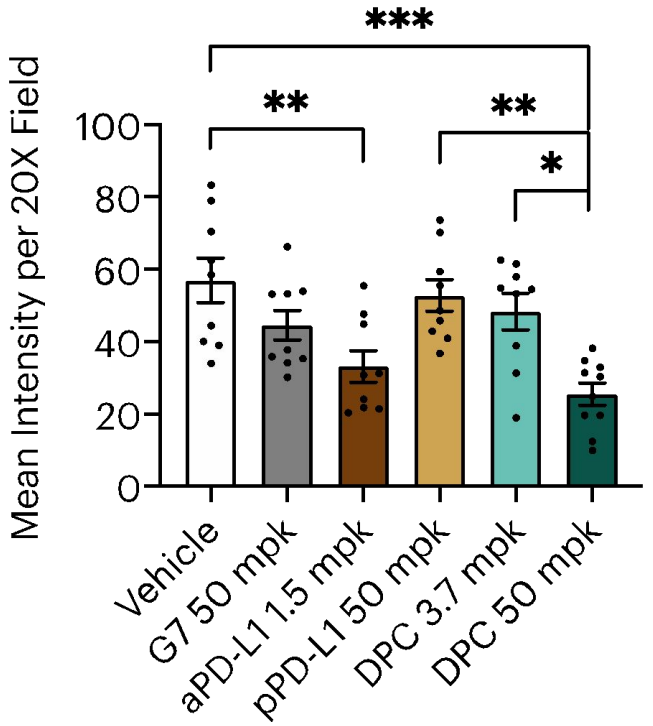
Ohkura, N. et al. Immunity, 37(5):785-799. (2012); One-way AVONA using Bonferroni's multiple comparisons test; In Collaboration with Wheeler Research Group





- Proliferating cell nuclear antigen (PCNA) staining= Nuclear protein synthesized in late G1 and S phase of cell cycle, thus proliferating cells



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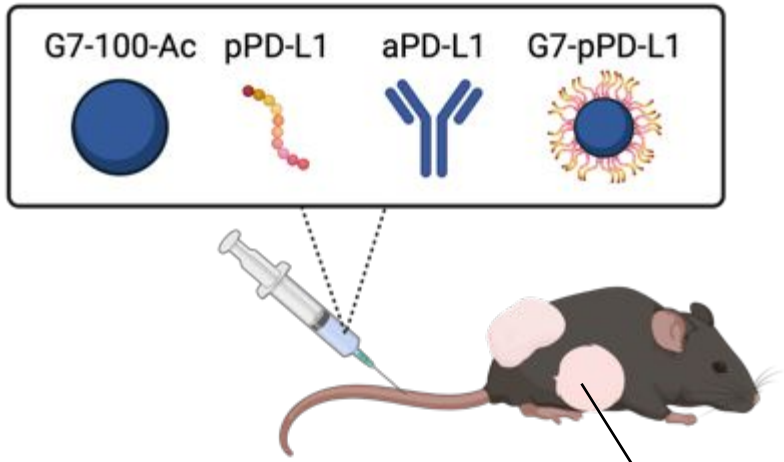
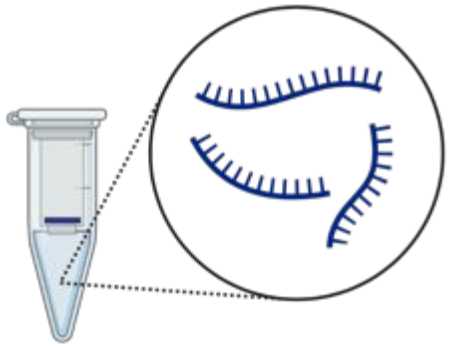
Schonenberger, F. et al. BMC Bioinformatics, 16: 180. (2015); One-way AVONA using Bonferroni's multiple comparisons test; In Collaboration with Wheeler Research Group

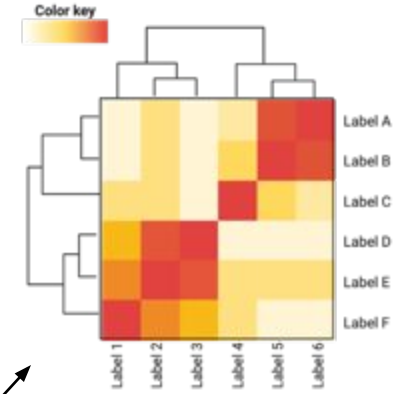
Drug administration

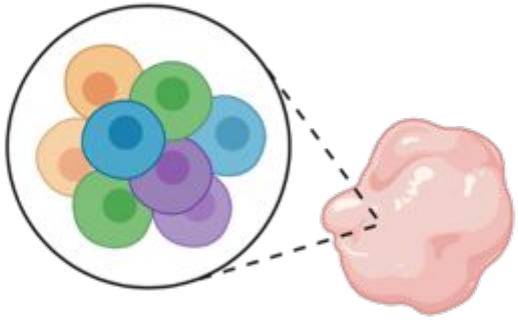
RNA extraction

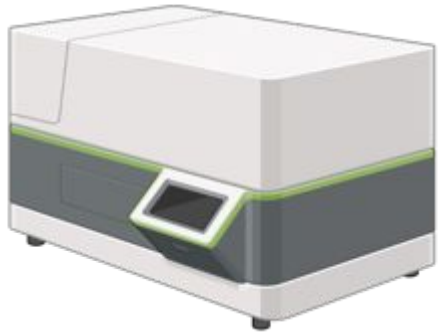
Data analysis











Tumor collection

NanoString nCounter

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Future Directions: Multi-specific DPCs

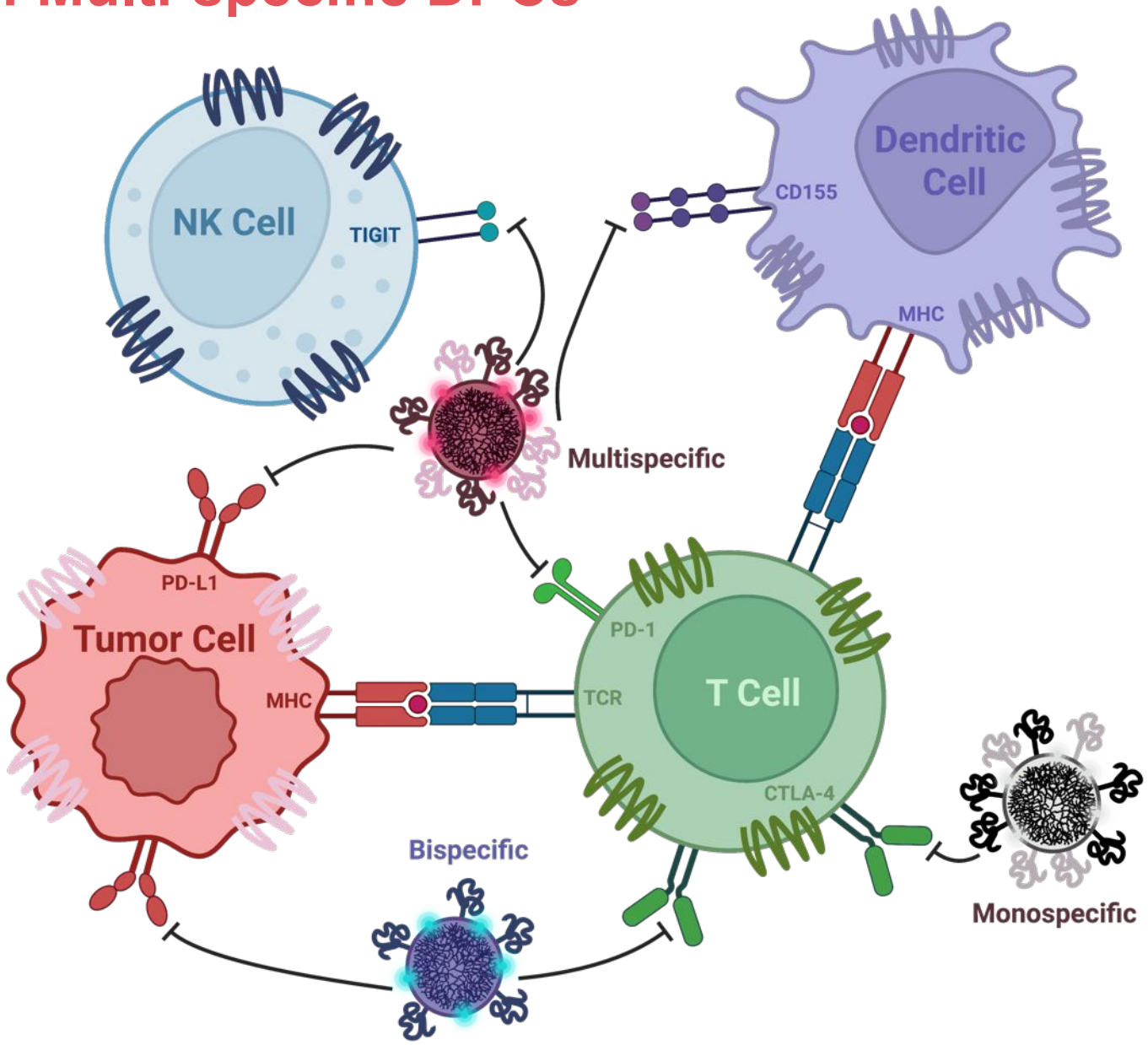
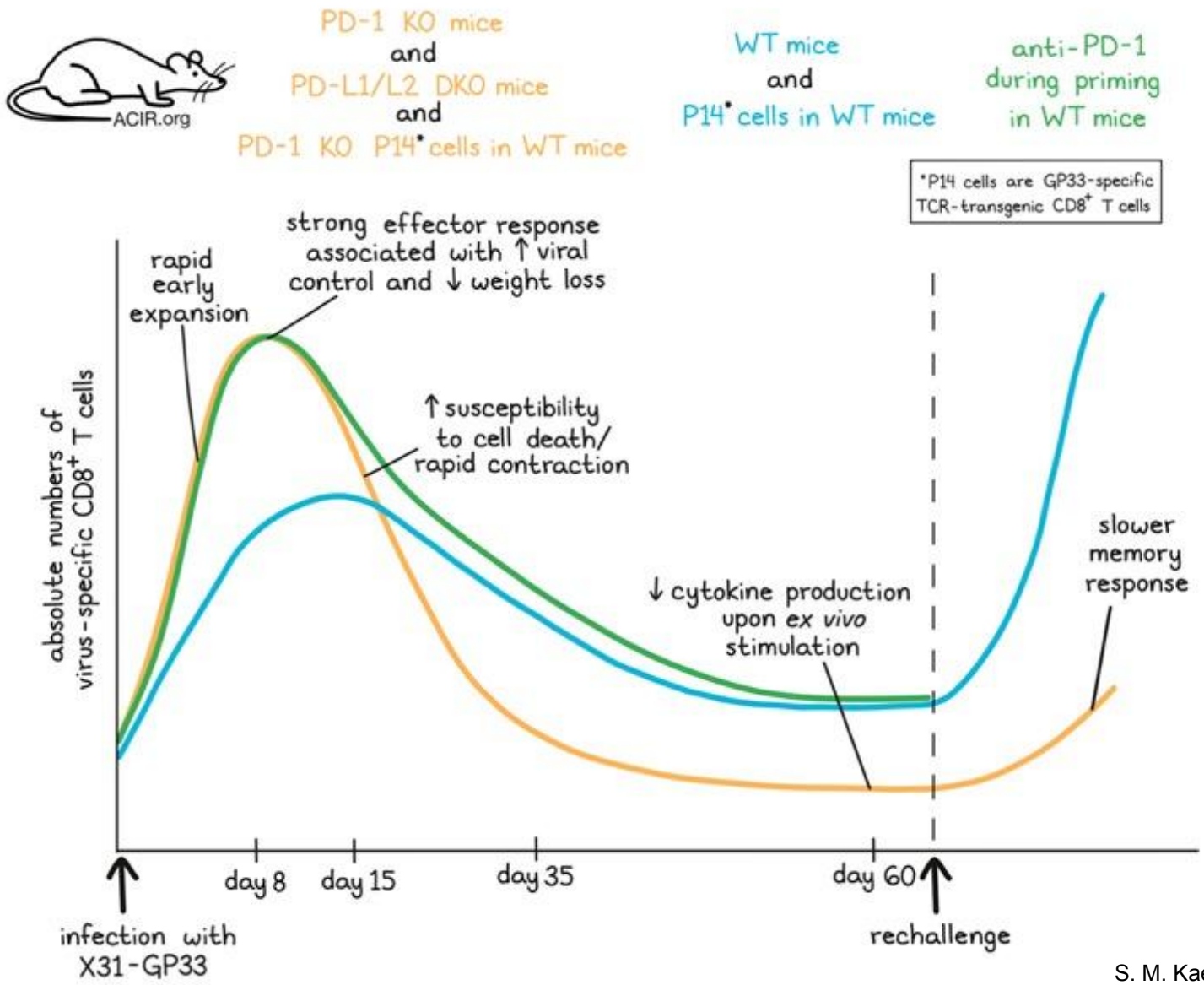
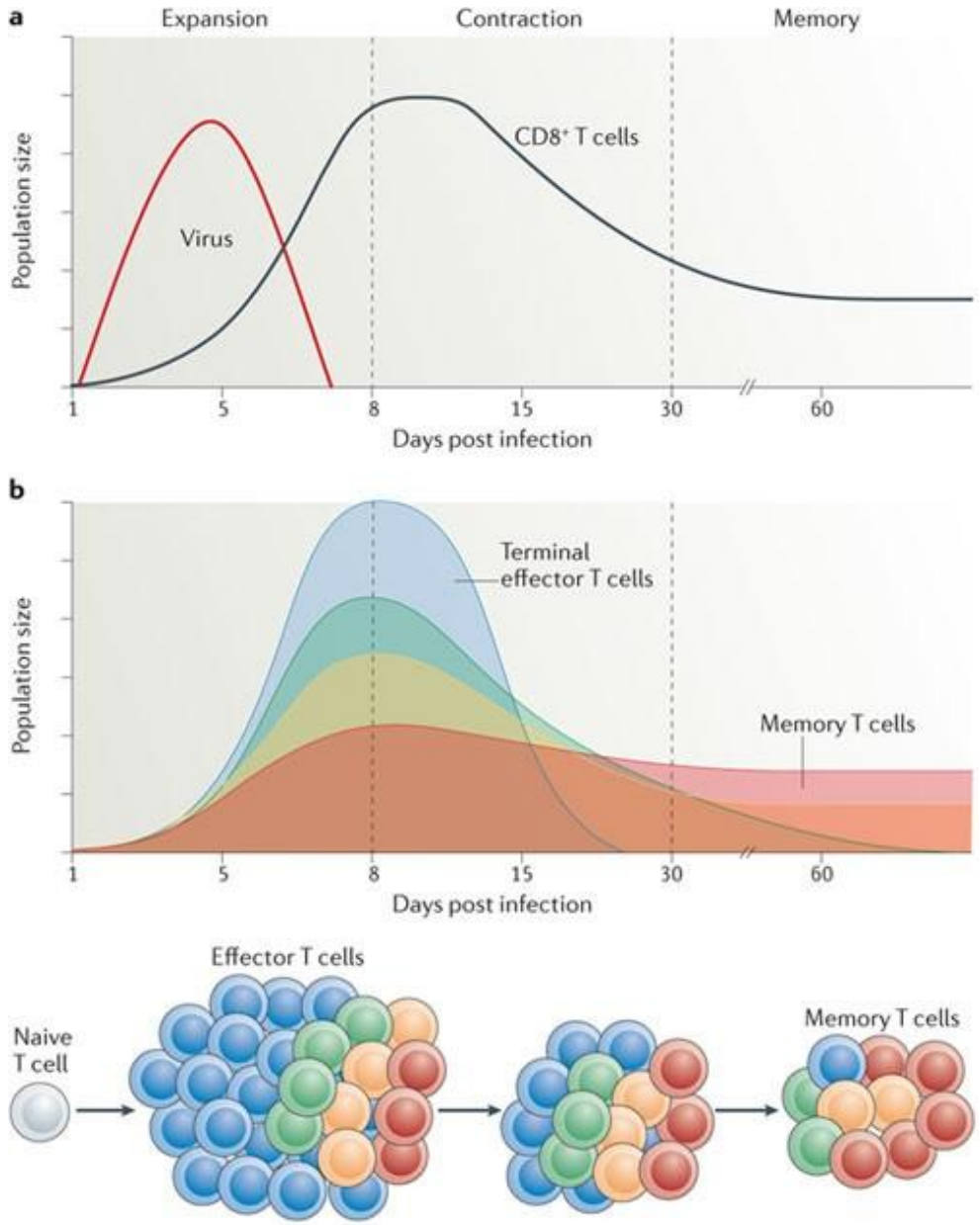


Illustration drawn by Piper Rawding

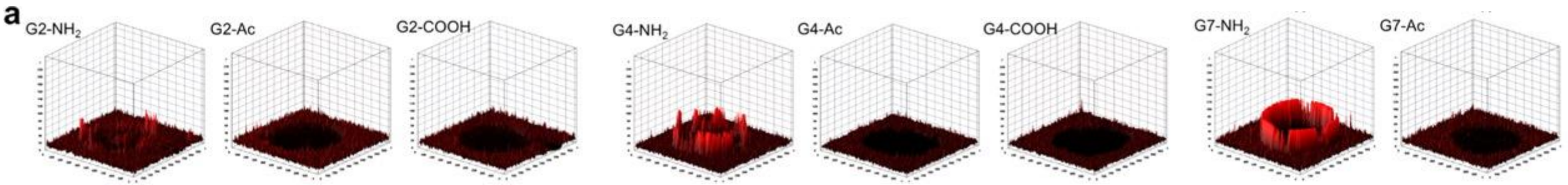
T cell Frequency Timeline

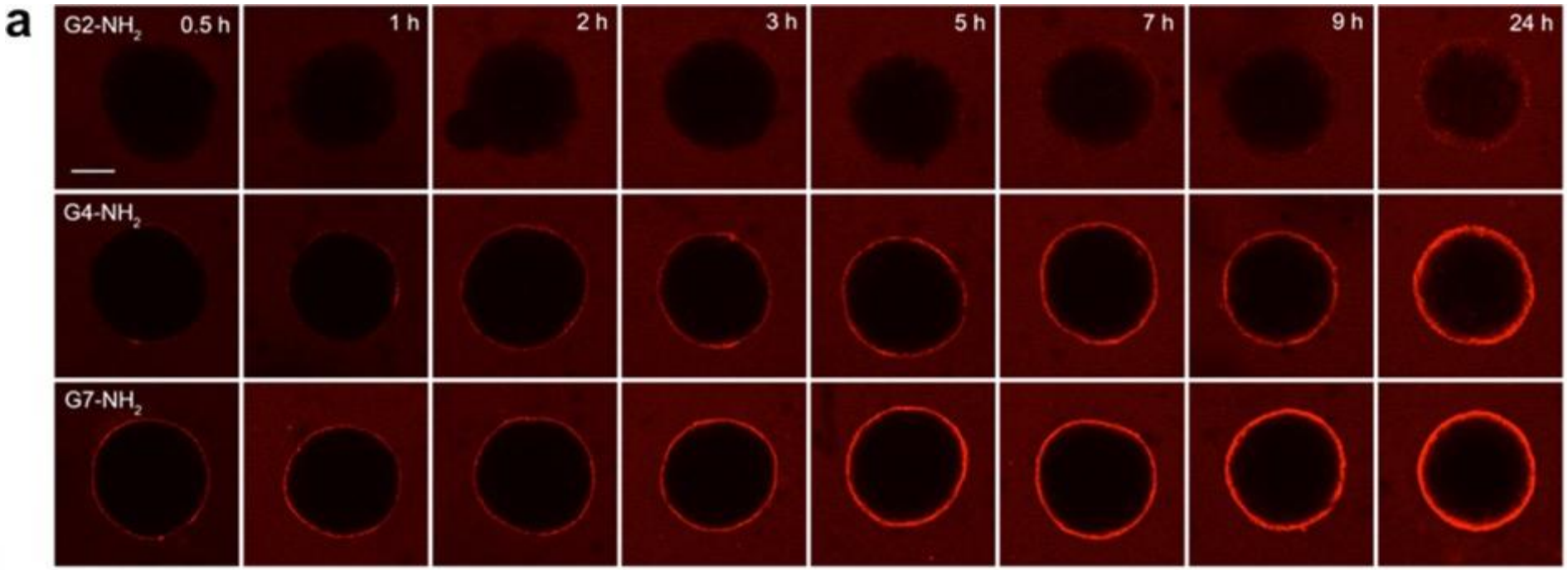




S. M. Kaech et al., Nature Reviews Immunology, 12, 749–761 (2012) Nature Reviews | Immunology

Why G7 PAMAM Dendrimers?





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J. Bugno et al., Mol. Pharmaceutics 13, 7, 2155–2163 (2016).

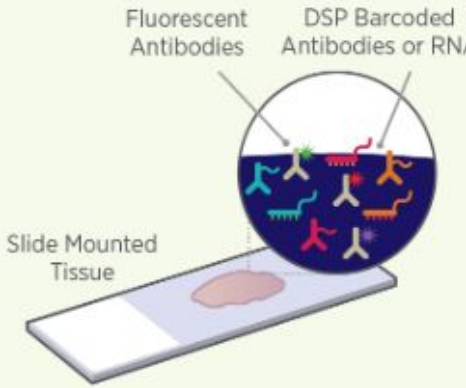
What is Nanostring GeoMx?

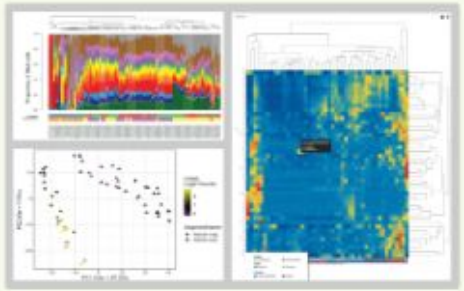
How it **Works**

The GeoMx DSP workflow seamlessly integrates with current histology or genomics workflows to help researchers obtain robust and reproducible spatial multiomics data quickly.

Whole tissue sections, FFPE or fresh frozen, can be imaged and stained for RNA or protein. Researchers can then precisely select which tissue compartments or cell types to profile based on the biology, and subsequently count expression levels using either the nCounter Analysis System or an Illumina Sequencer.

Sample Prep	Image and Profile	Count	Analyze





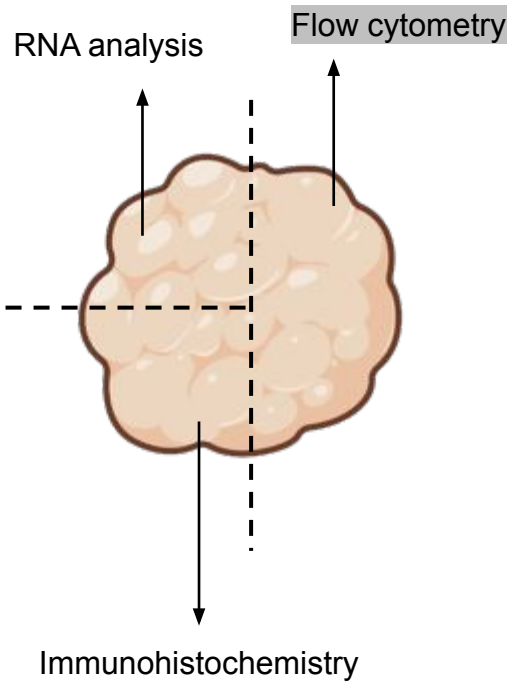
Any sample, FFPE or fresh frozen, use any morphology marker, detect RNA and/or Protein.

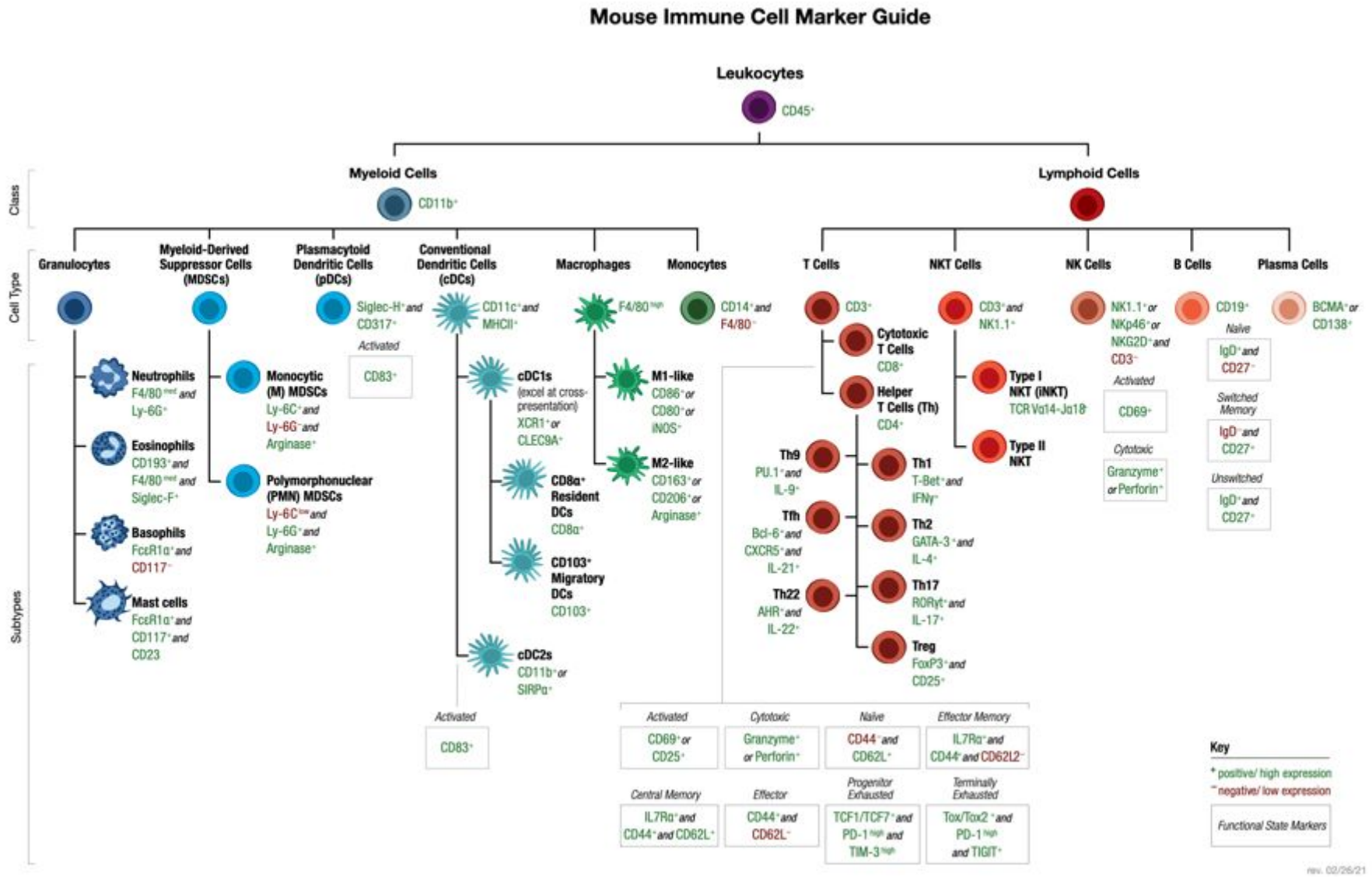
Image and profile RNA and Proteins with GeoMx DSP.

Count on nCounter or NGS.

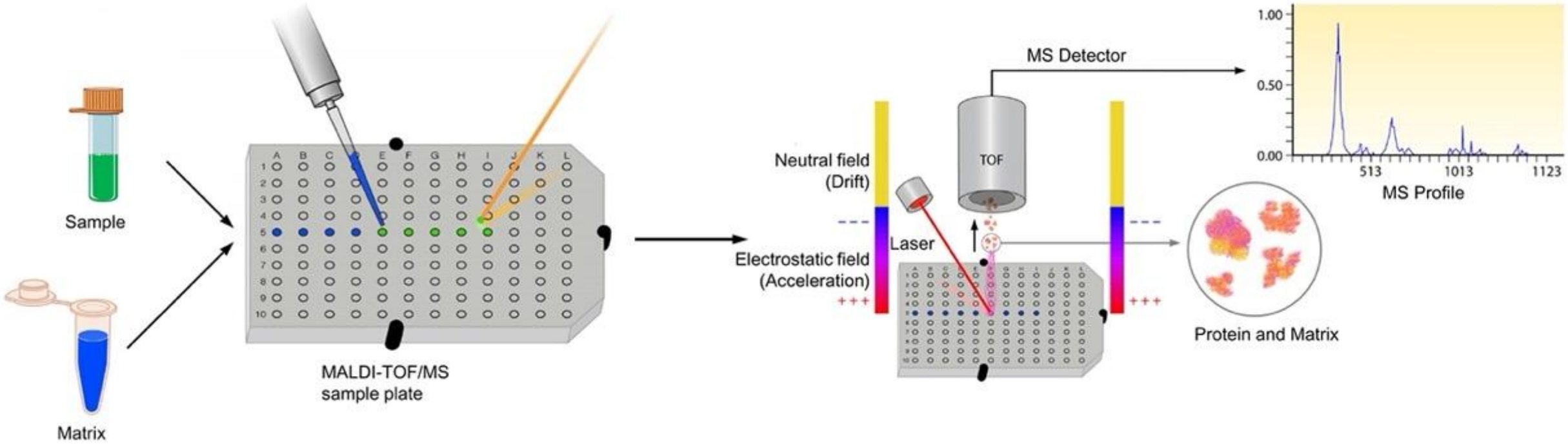
Pre-defined data processing pipeline, interactive data analysis and accessible biological insights.

Objective: Identify the T cell subpopulation reactivated by G7-pPD-L1



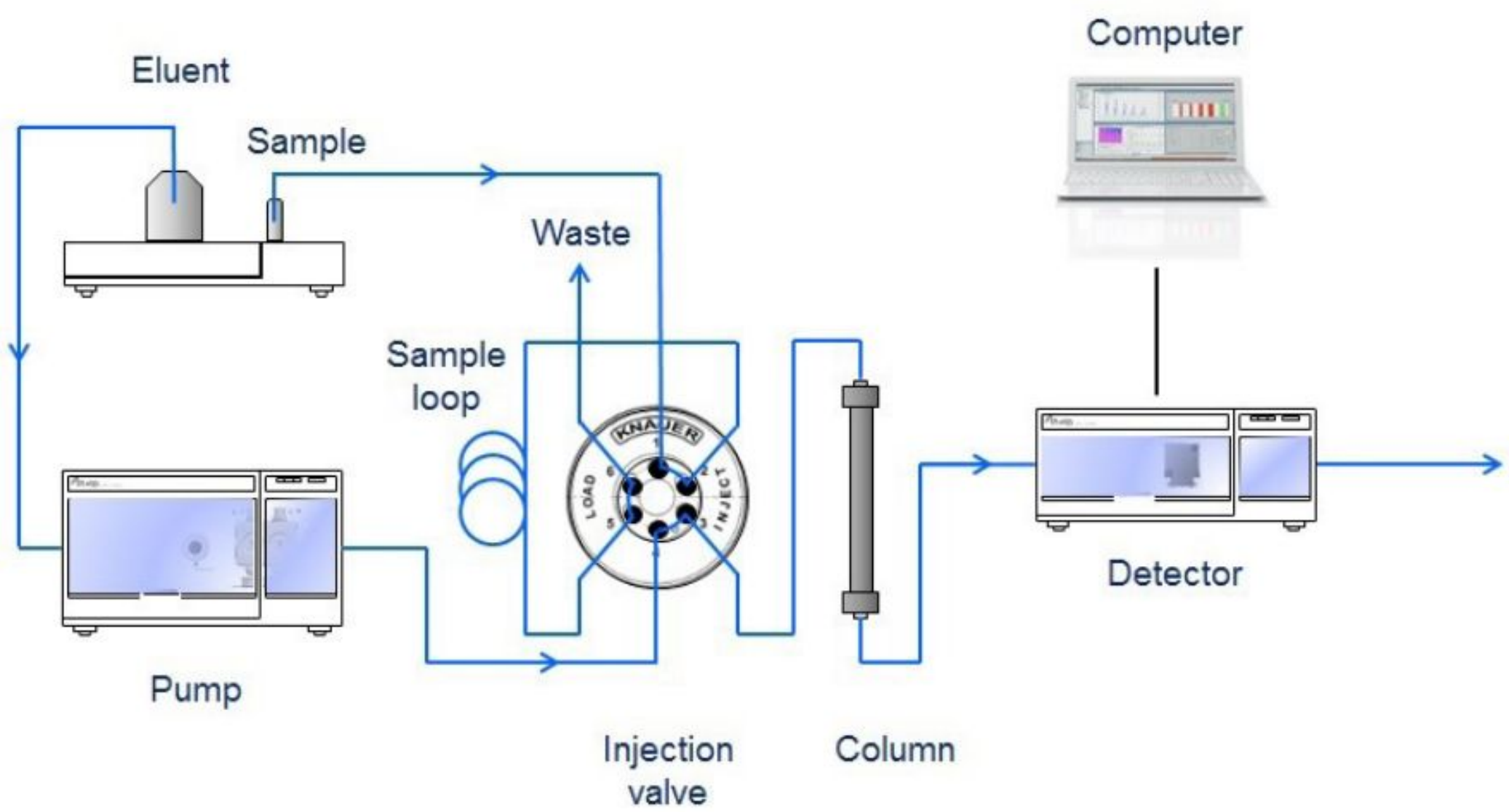


Principles of Matrix Assisted Laser Desorption Ionization (MALDI)

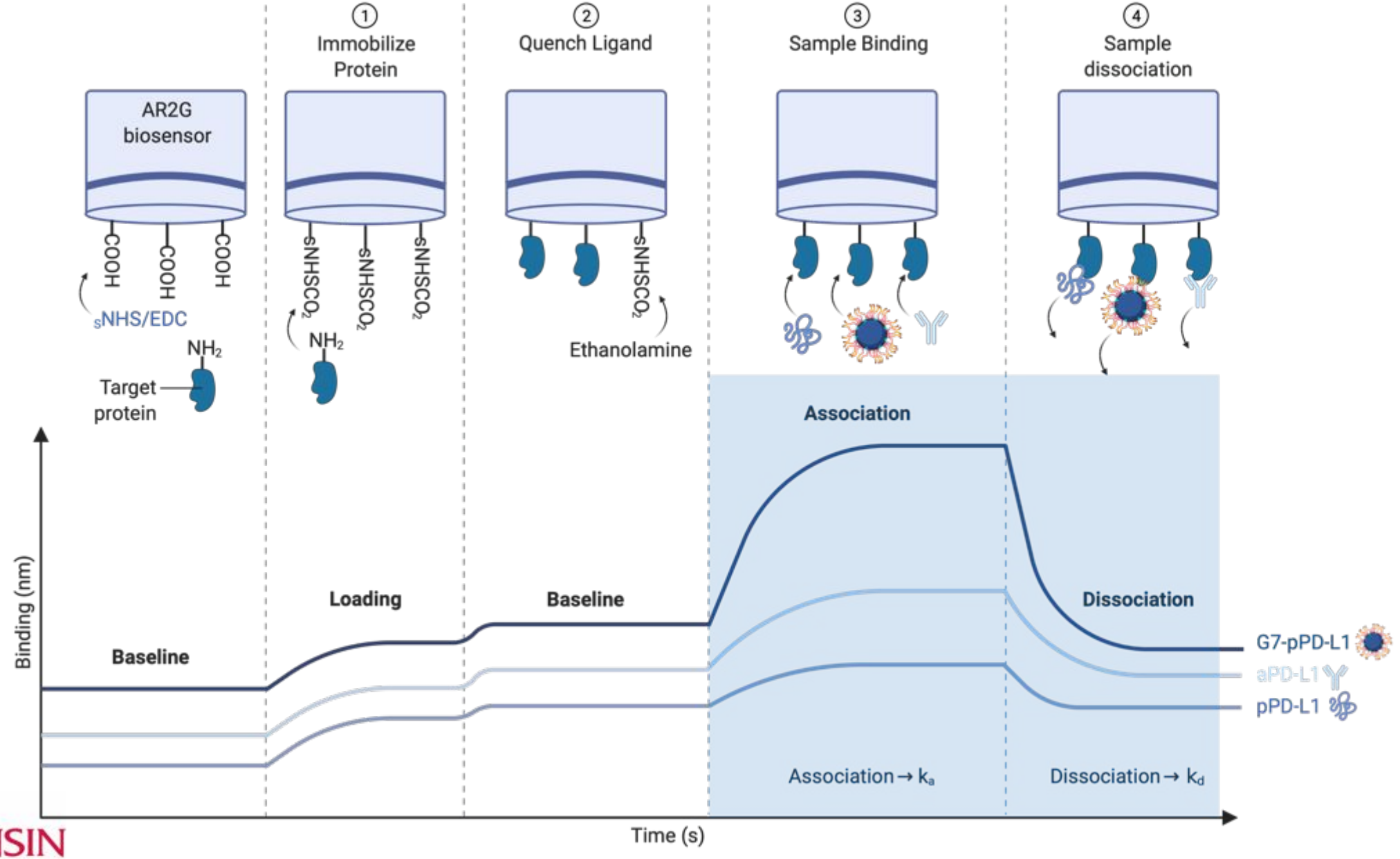


Clark, A.E., et al., Clin Microbiol Rev. 26, 547-603 (2013).

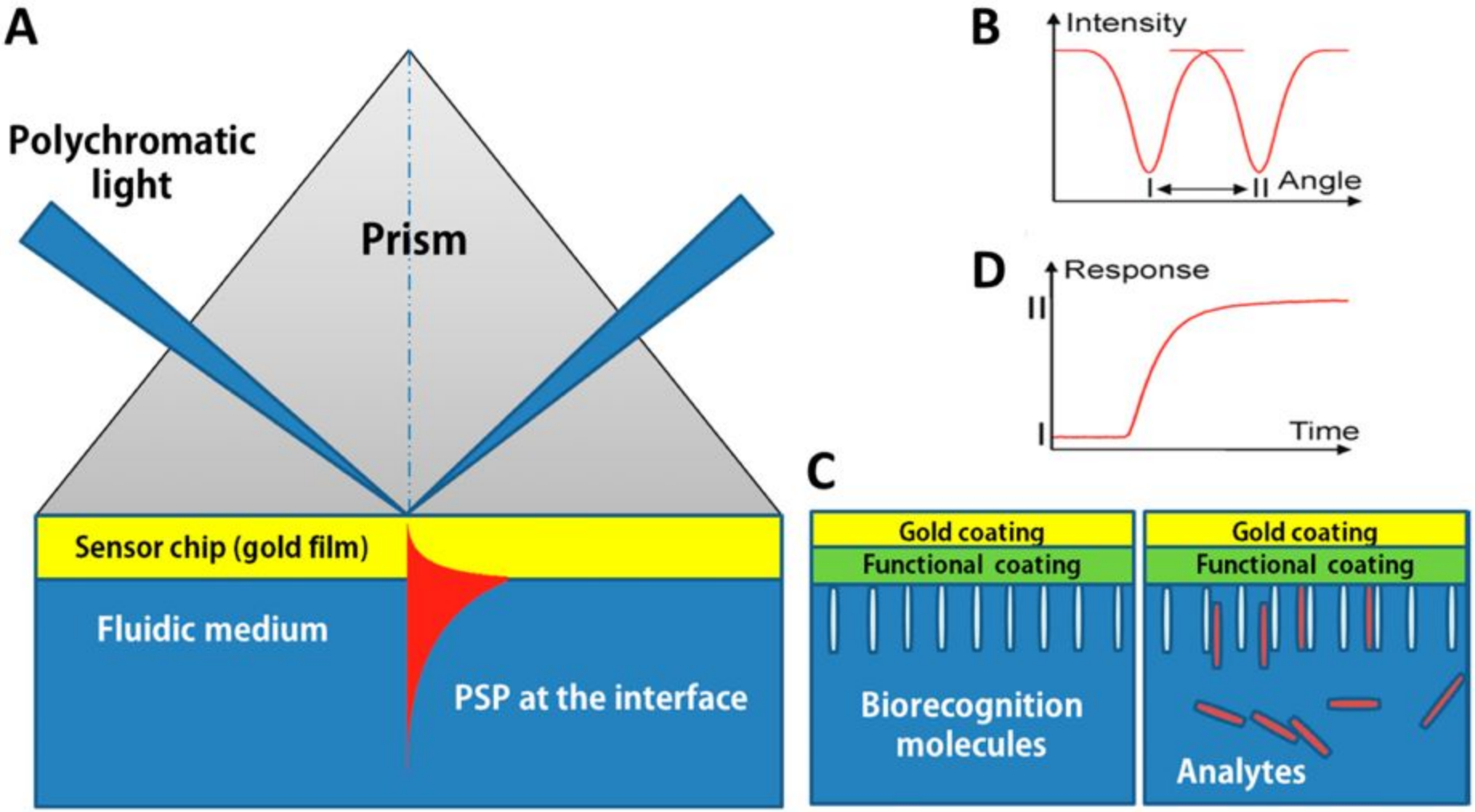
Principles of High-Performance Liquid Chromatography (HPLC)



Principles of Bio-Layer Interferometry (BLI)

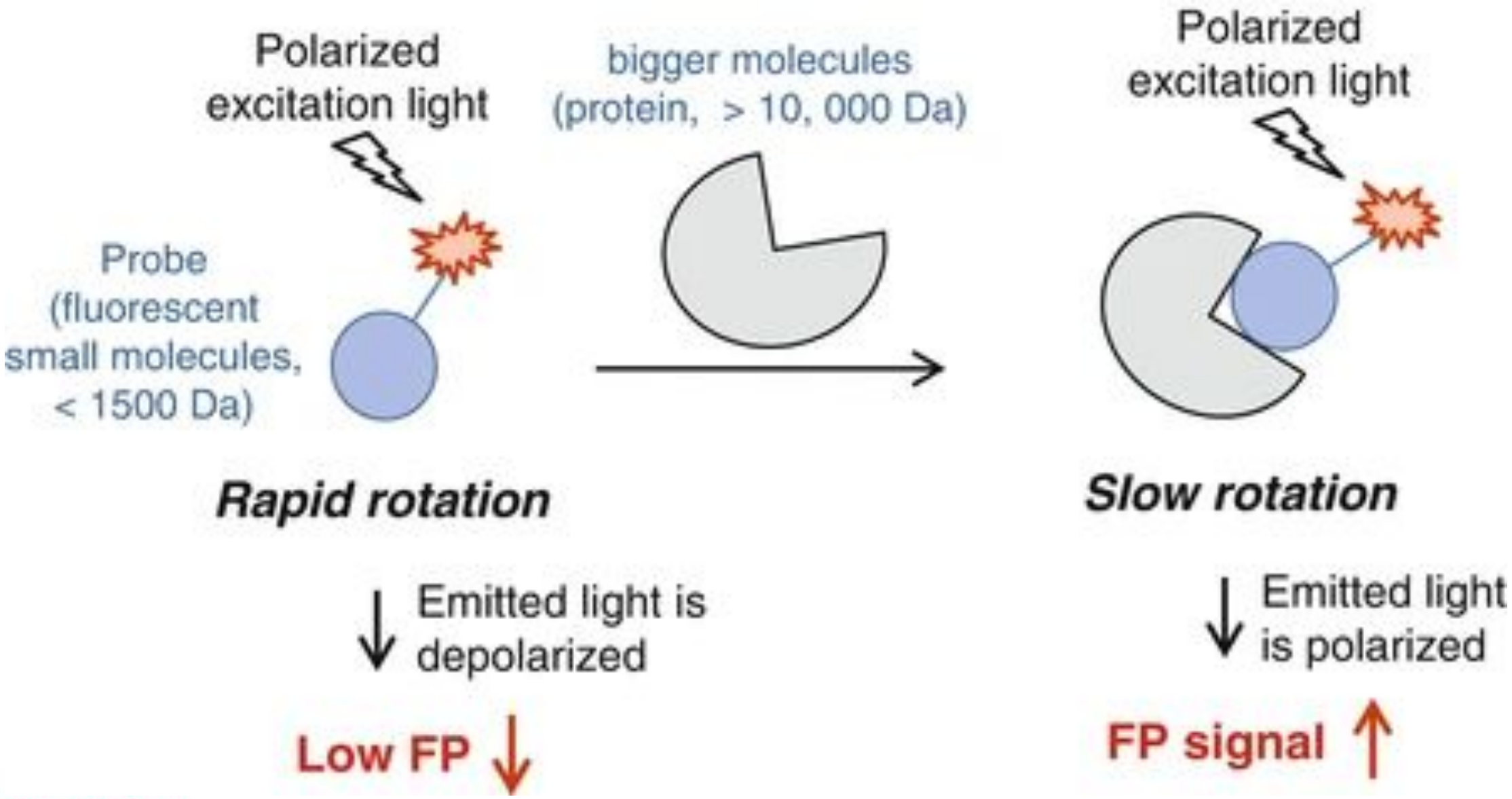


Principles of Surface Plasmon Resonance (SPR) Spectroscopy



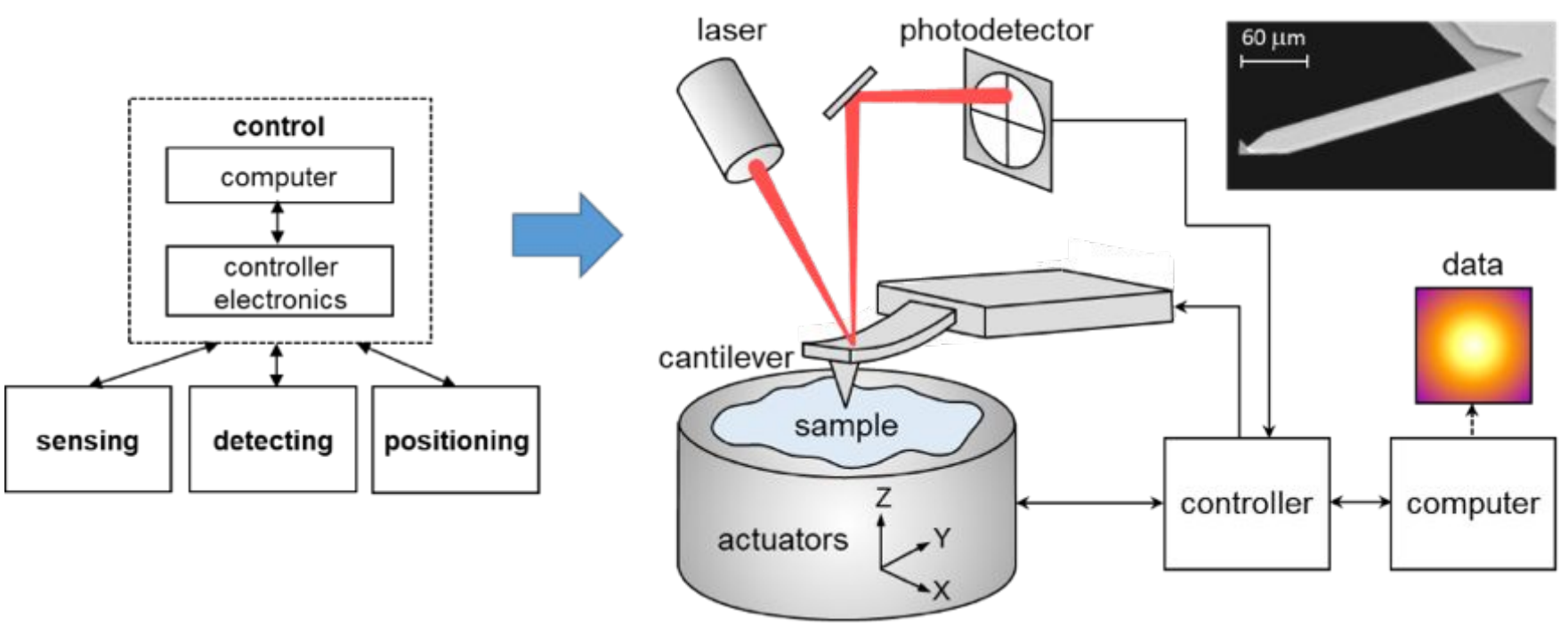
Nguyen, H.H., et al., Sensors. 15, 10481-10510 (2015).

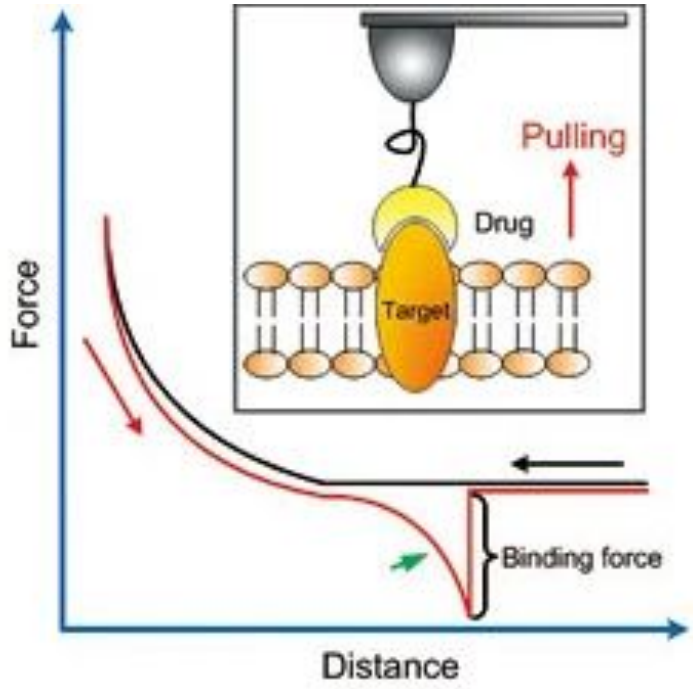
Principles of Fluorescence Polarization (FP) Spectroscopy



Du, Y. (2015). Fluorescence Polarization Assay to Quantify Protein-Protein Interactions in an HTS Format. In: Meyerkord, C., Fu, H. (eds) Protein-Protein Interactions. Methods in Molecular Biology, vol 1278.

Principles of Atomic Force Microscopy (AFM)





Li, M., et al., Acta Pharmacol Sin. 36, 769-782 (2015).

III. Surface Modifications of PAMAM G7 Dendrimers

Surface Amines

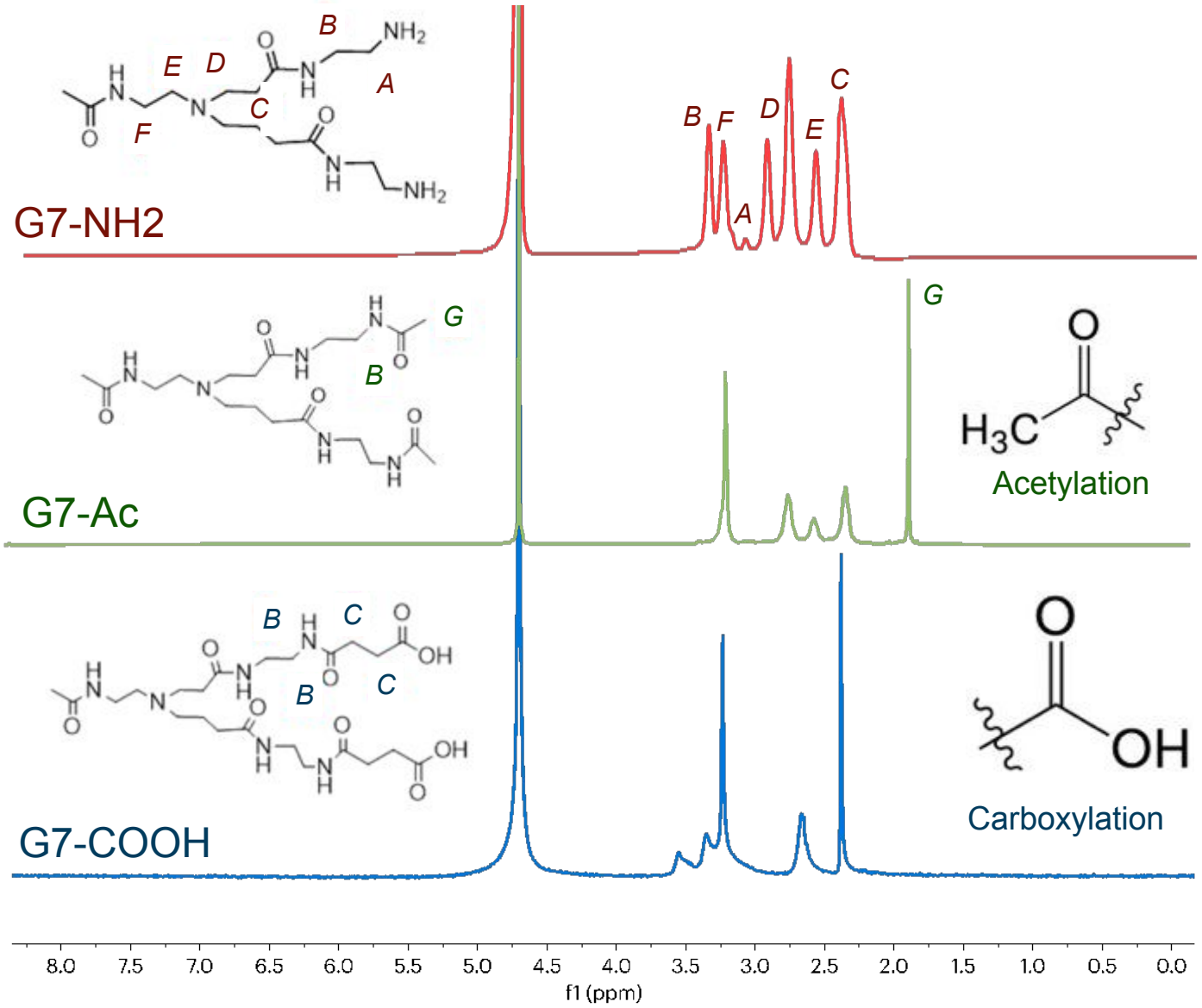
- Cationic surface charge
- Cytotoxic effects
- Can be utilized for conjugation chemistry through SMCC reactions

Surface Acetylation

- Provides a more neutral surface charge
- Reduction in cellular toxicity

Surface Carboxylation

- Can be utilized for conjugation chemistry through EDC/NHS reactions



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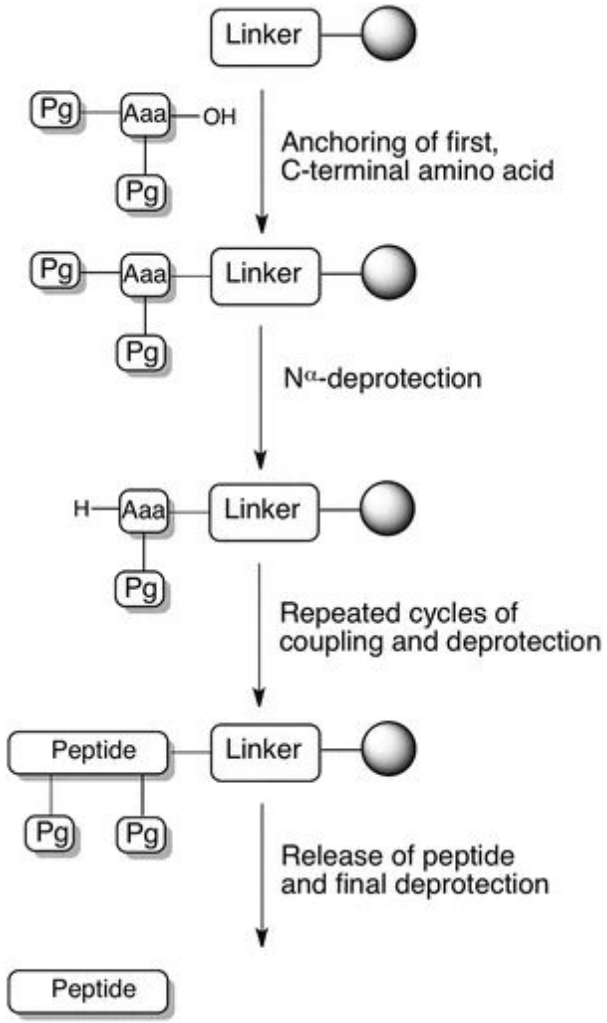
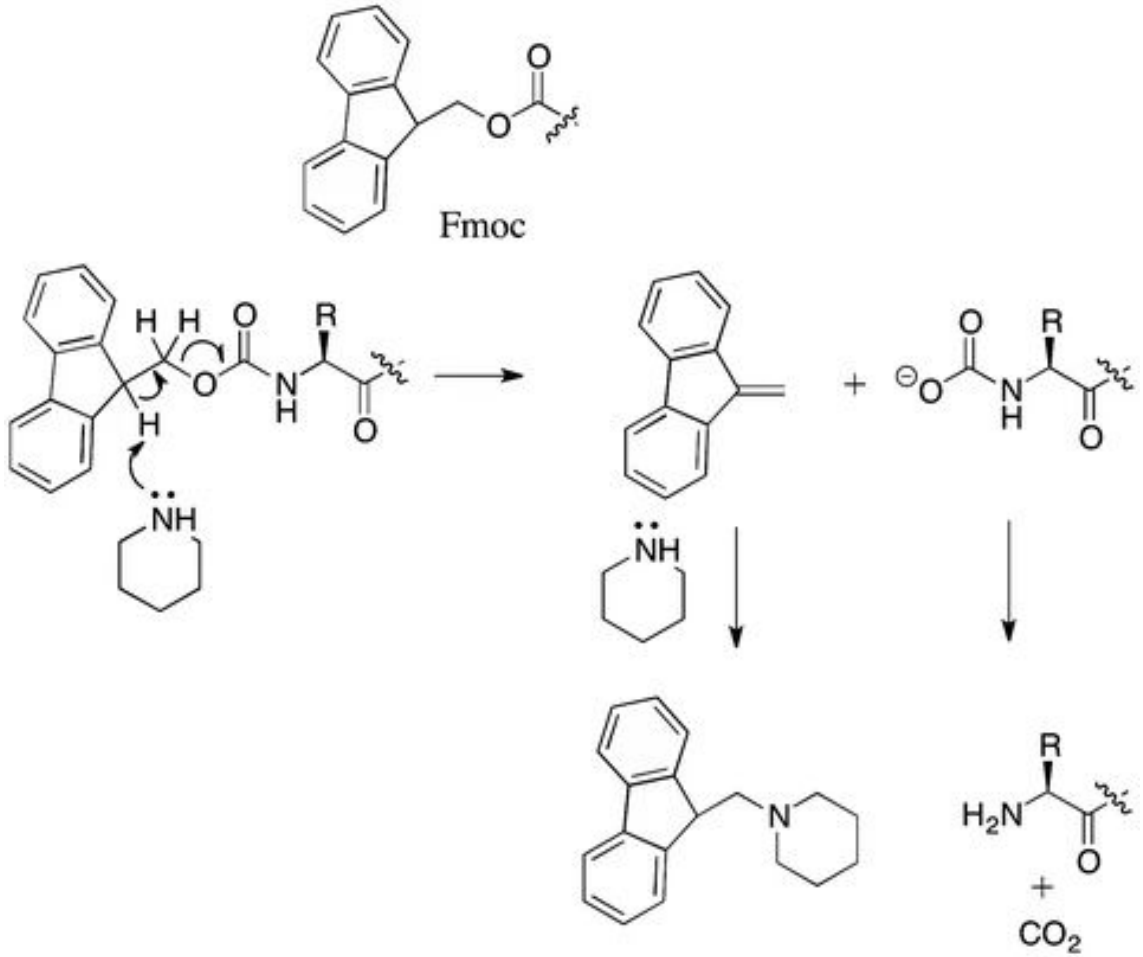
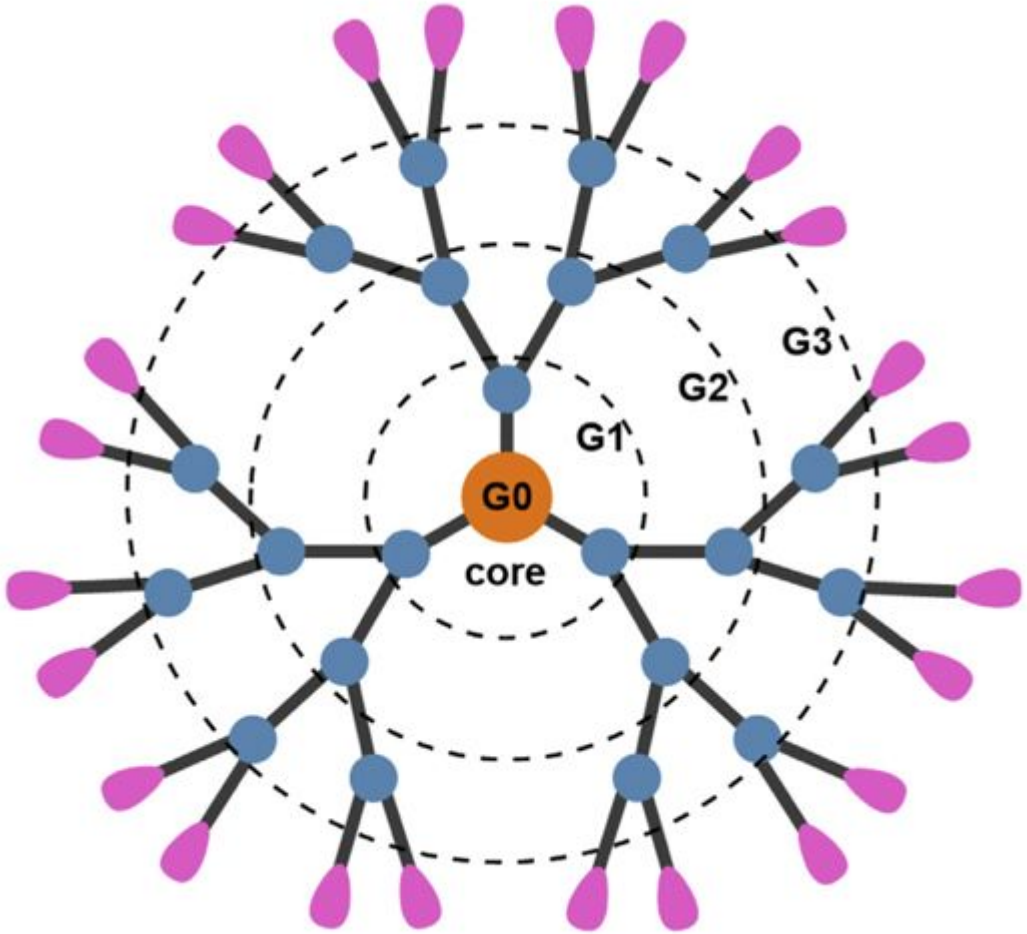
Data obtained by Piper Rawding

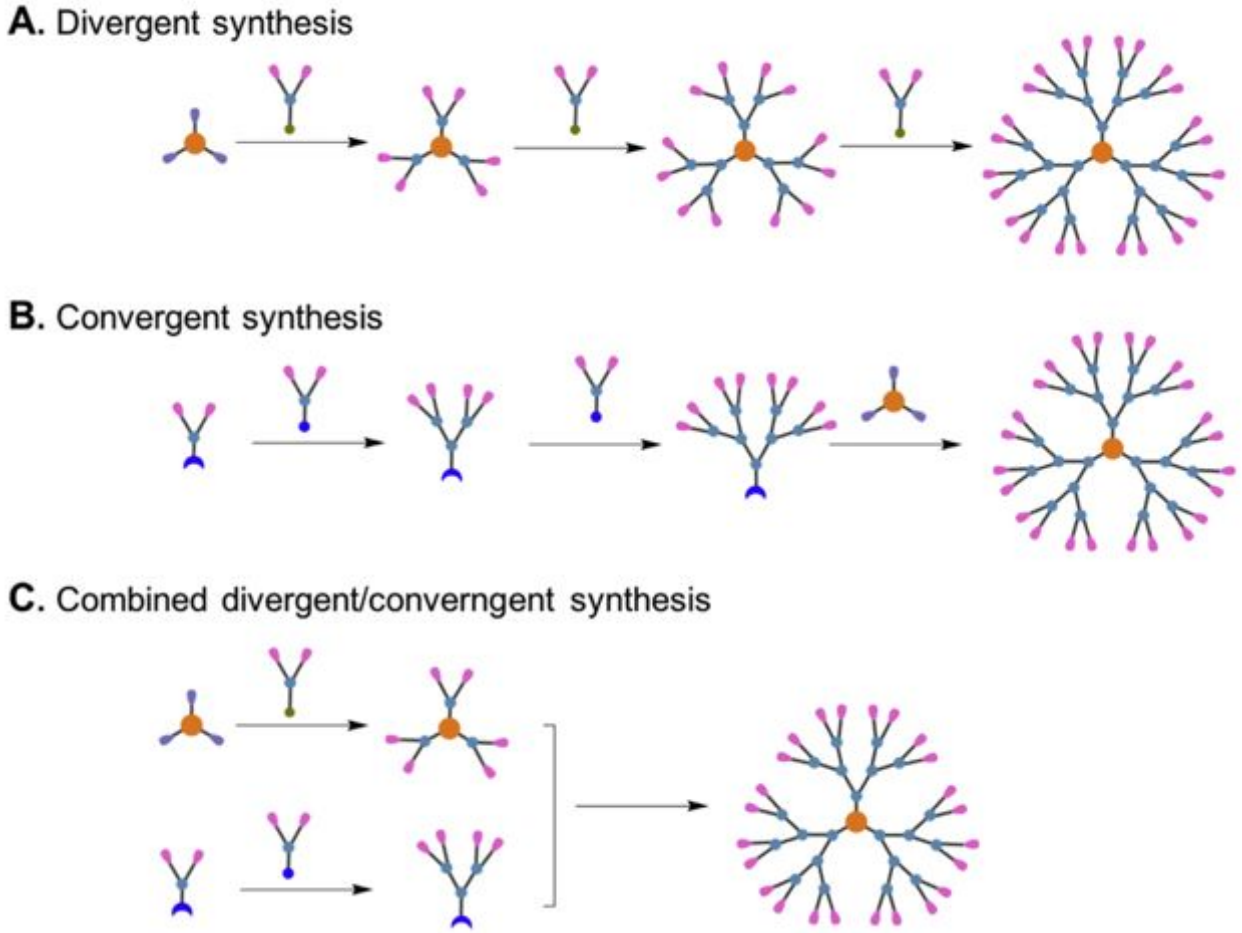
Solid Phase Peptide Synthesis





PAMAM Dendrimer Synthesis





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