An injectable hydrogel particle platform enabling high concentration delivery of amorphous solid and crystalline biologics

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Acknowledgements

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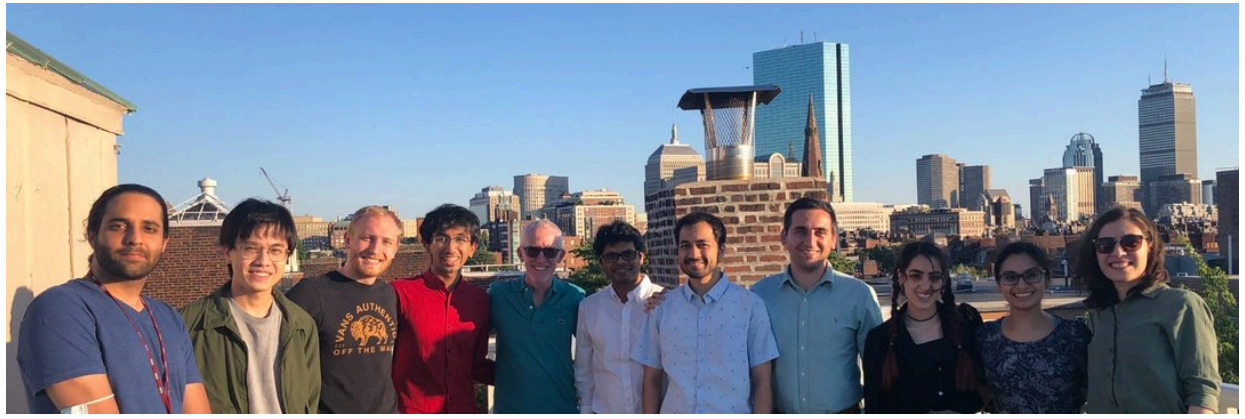
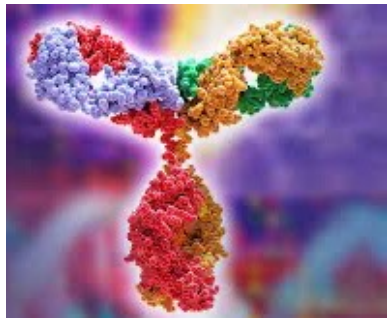
- Vaishali Parab (Merck): *in vivo* studies
- Huiping MA (Merck): *in vivo* studies

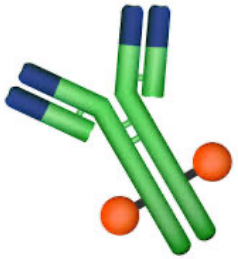


Doyle Group at MIT

Biologics



monoclonal antibodies (mAbs)



antibody drug conjugate (ADC)

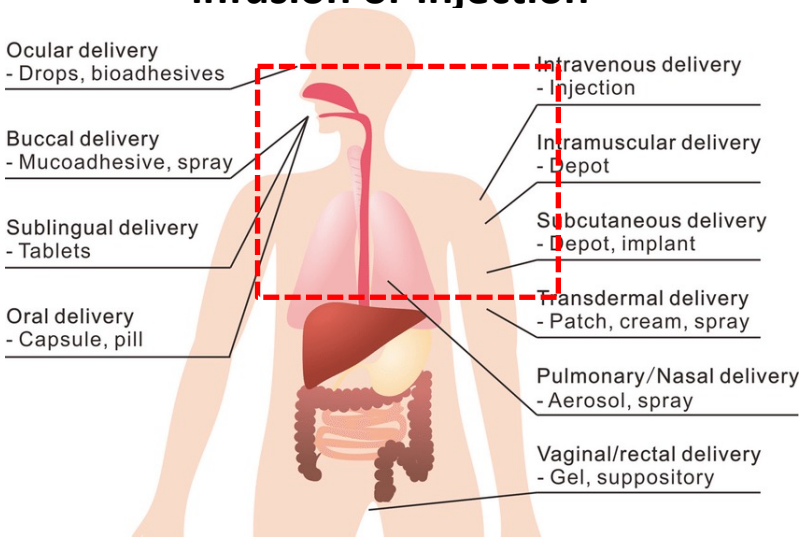
therapeutic enzymes

- Treatment of cancer, auto-immune, and chronic diseases
- Biologics revenue (2030 forecast): $719 B
- 37 novel biologics approved by FDA in 2022

mAb therapies commonly delivered to patients intravenously

Many of the most recent breakthroughs in therapies: monoclonal antibody treatments

mAbs primarily delivered by infusion or injection

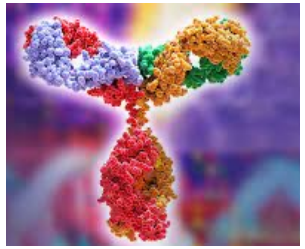


Time of administration depends on route of delivery:

Ex. Rituximab (total time at clinic)

Intravenous ~ 3.7 hours

Subcutaneous ~ 0.8 hour



Monoclonal antibodies (mAbs)
MW ≈150 KDa

Subcutaneous formulation goals:

- Small volume (≤ 2 mL)
- High concentration (≥ 300 mg/mL)
- Low viscosity (ideally ≤ 0.025 Pa.s)

Injection through 27G needle: shear rate ~10^5 - 10^6 s^{-1}

Examples of antibody therapies delivered subcutaneously:

- Herceptin hylecta: 600mg/5 mL (w/ hyaluronic acid)
- Rituximab hycela: 1400mg/12 mL (w/ hyaluronic acid)
- Certolizumab pegol: 200mg/1ml

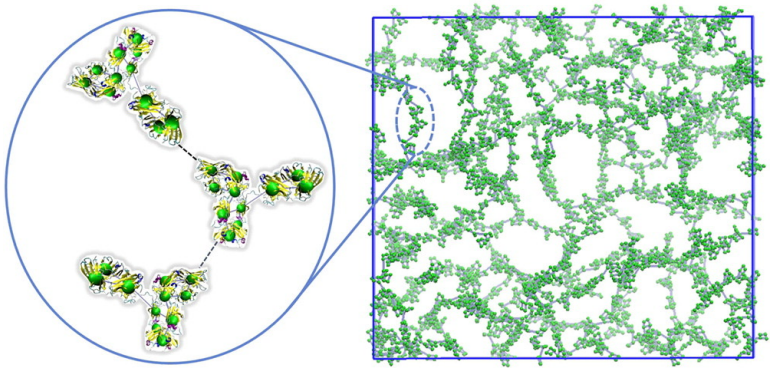
National Pain Centers, Routes of Administration

Rule, S. et al., J. Med. Econ., **17**, 2014, 459-468

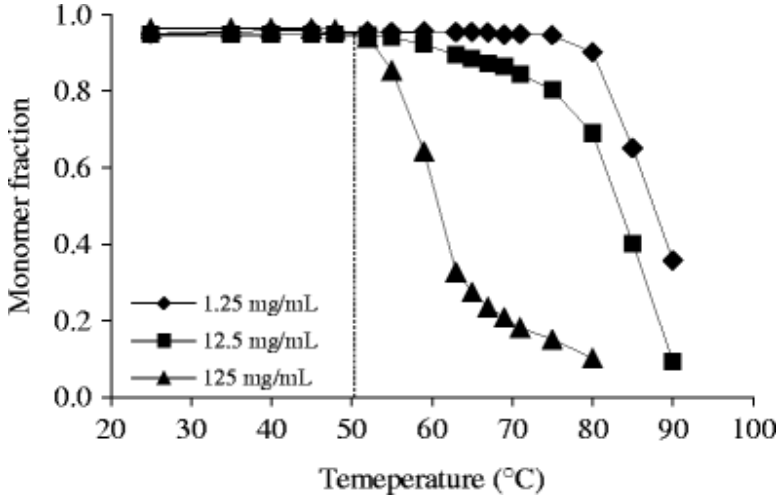
Walsh, 2010, Hutmacher and Neri,2019

Challenges of High Concentration mAb Formulations

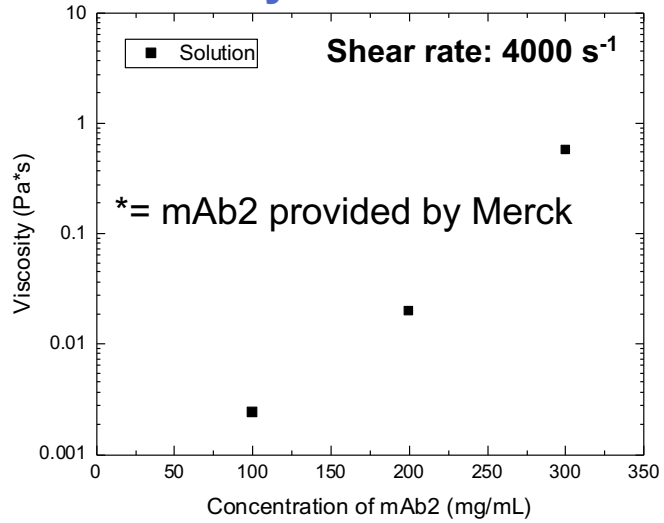
High viscosity through self-association



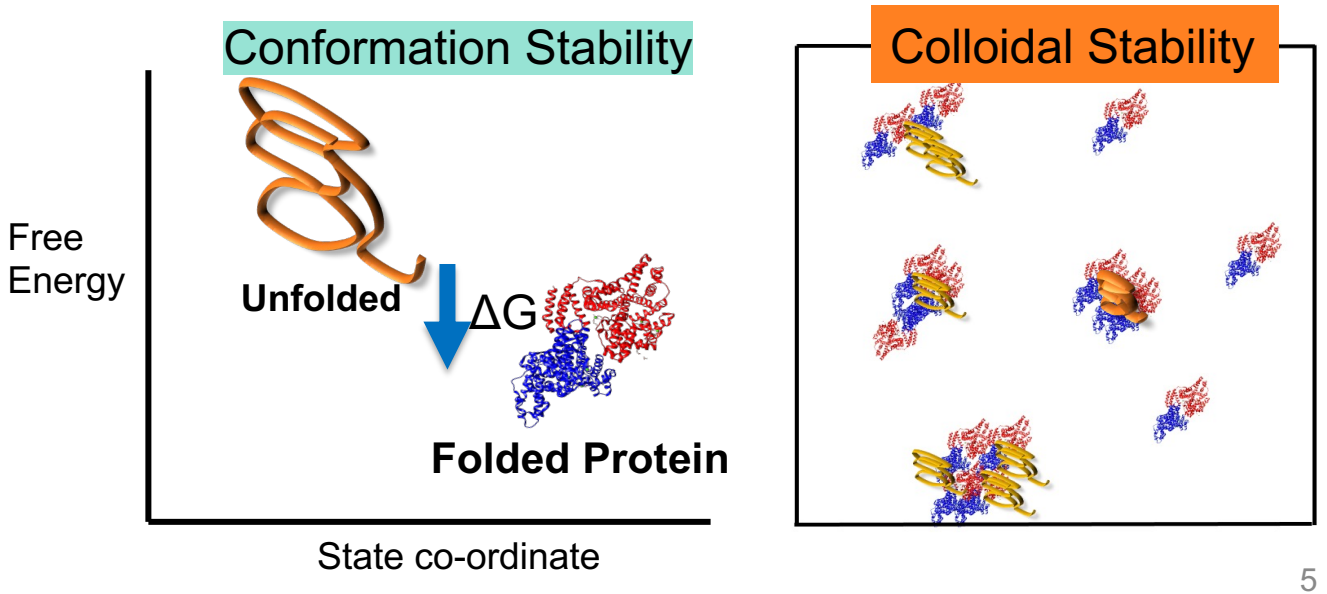
Prone to aggregation, unfolding, degradation



Shear Viscosity of the studied mAb*



Physical stability



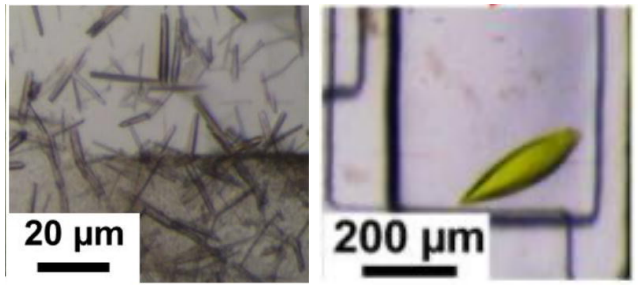
Saluja, A. *et al.*, Pharm. Res., 2015, **12**, 127-139
Buck, P. *et al.*, Mol. Pharmaceutics, 2015, **12**, 127-139

Technological pain points:
High concentration, manufacturability, plug-and-play approach

Leveraging protein crystallization

- Protein crystals form in the presence of certain salts, additives
  - Crystals hydrated, ~50% solvent content
  - Proteins within crystal are typically well-folded, active
- Concentration of protein *within* crystals >500 mg/mL
  - Crystal suspensions can be concentrated by centrifugation, sedimentation
- Crystals dissolve outside of crystallization conditions (*e.g.* PEG, salts)
  - Extant crystals can be soaked in additives (e.g. small molecules, polymers)

Microscopic in size (1 - 100 µm)



Schieferstein, J. et al., Lab Chip, 2018, 18, 944-954

Densely packed, folded

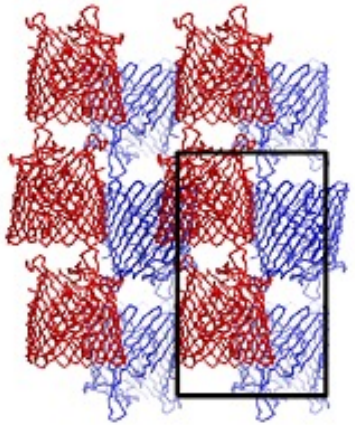
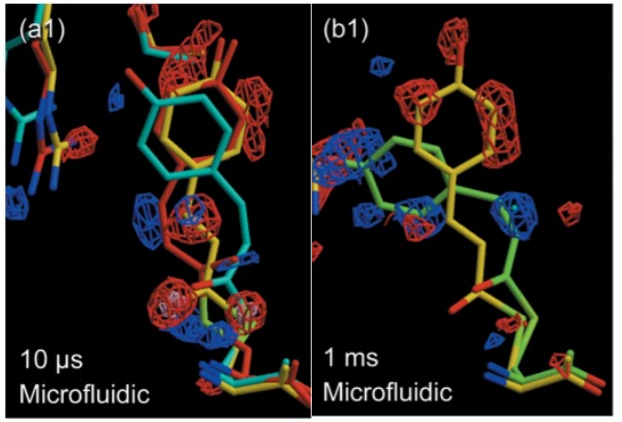


Image from The Cherezov Lab website; PDB: 2GUF

Proteins within crystal are functional



Pawate, A. et al., Acta Cryst, 2015, F71, 823-830

Leveraging Hydrogel Technologies

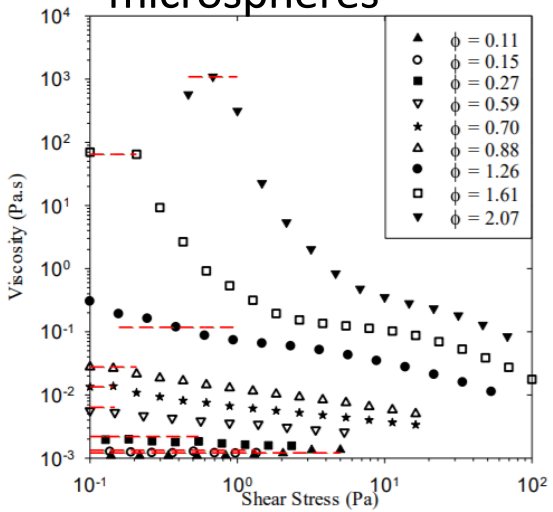
Hydrogels particles:

- High water content, soft & biocompatible
- Modular chemistry, structure, and functionality
- Low polymer content: high theoretical drug loadings
- Good flow properties

Challenges for delivery of biologics with hydrogels:

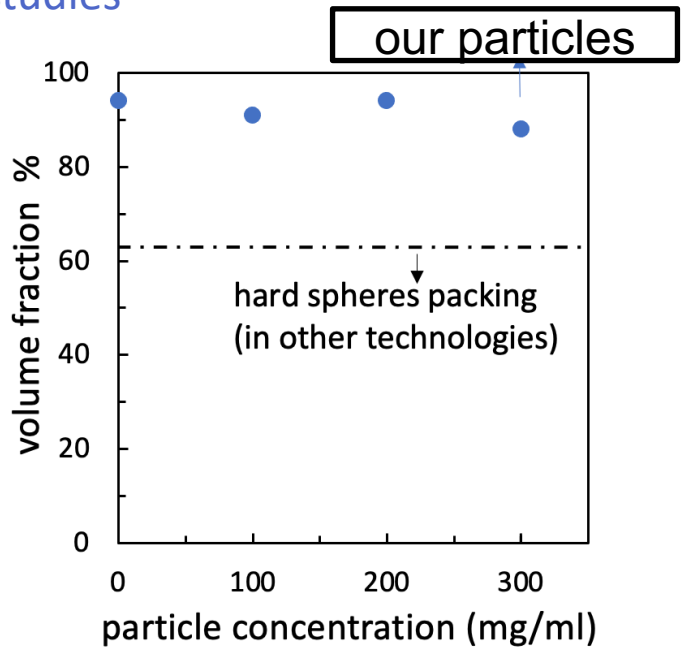
- Protein compatible process
- Chemical reaction can affect protein
- Compatible with translational or clinical studies

Shear thinning hydrogel microspheres

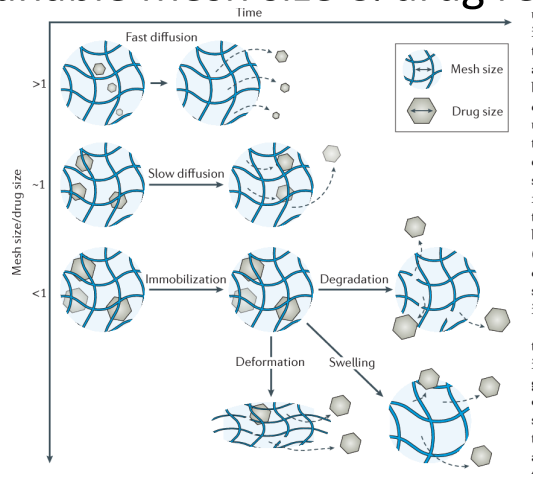


Shewan, H. & Stokes, J. Colloid Interface Sci. 2015

Soft particles pack better than hard spheres:



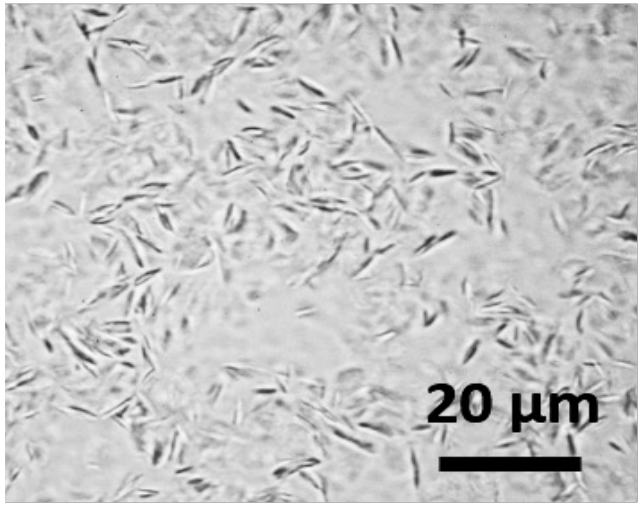
Tunable mesh size & drug release

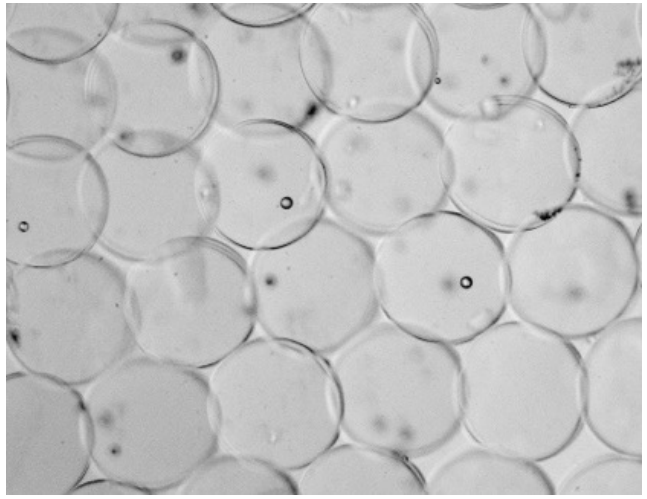


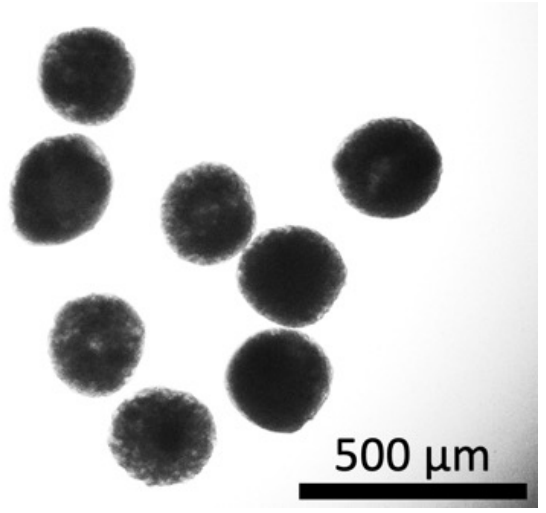
Li, J. & Mooney, D.J., Nat. Rev. Mater., 2016

Leveraging solid forms of antibodies and hydrogel technologies

Encapsulate and stabilize mAb solid forms within crosslinked hydrogel microparticles







solid forms of antibodies:
stable and concentrated

Anti-PD-1 (pembrolizumab) crystals
6% w/v PEG (MW 3350)
2.5% caffeine
~500 mg/mL mAb concentration

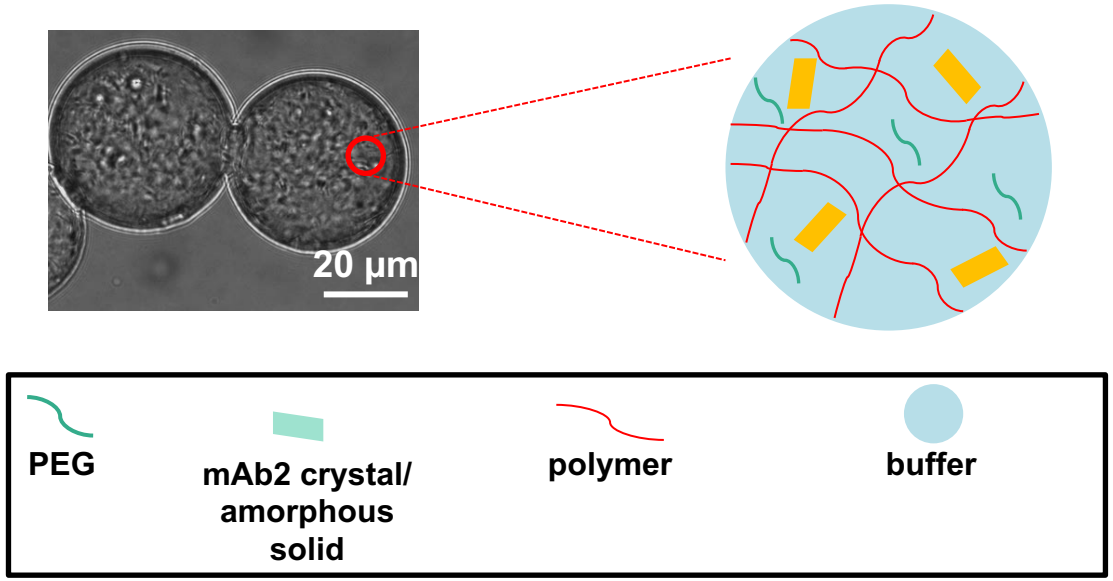
soft hydrogel beads:
good flow properties
high packing fraction

platform, high-concentration, injectable technology

International Patent Application No.: PCT/US2021/043916
Title: COMPOSITIONS INCLUDING SOLID FORMS OF POLYPEPTIDES AND RELATED METHODS
Filing Date: July 30, 2021

Leveraging solid forms of antibodies and hydrogel technologies

Encapsulate and stabilize mAb solid forms within crosslinked hydrogel microparticles

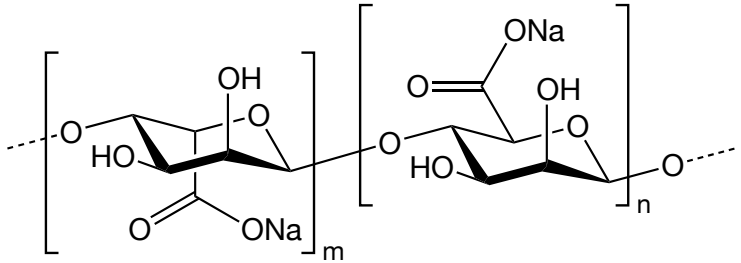


Platform Technology, Enabling New Therapies Through Drug Carrier/Formulation Design

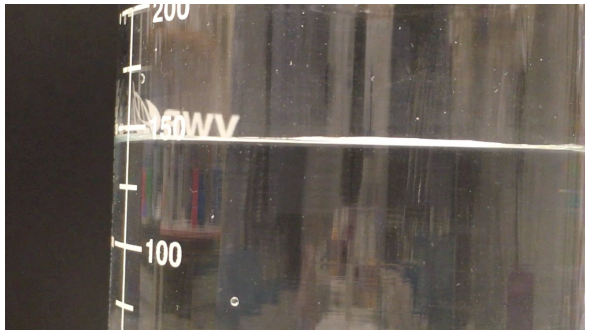
Polymer chemistry

- Monomer chemistry
  - Polyethylene glycols
  - Alginate
- Crosslinking chemistry
  - Free-radical
  - Click
  - Ionic

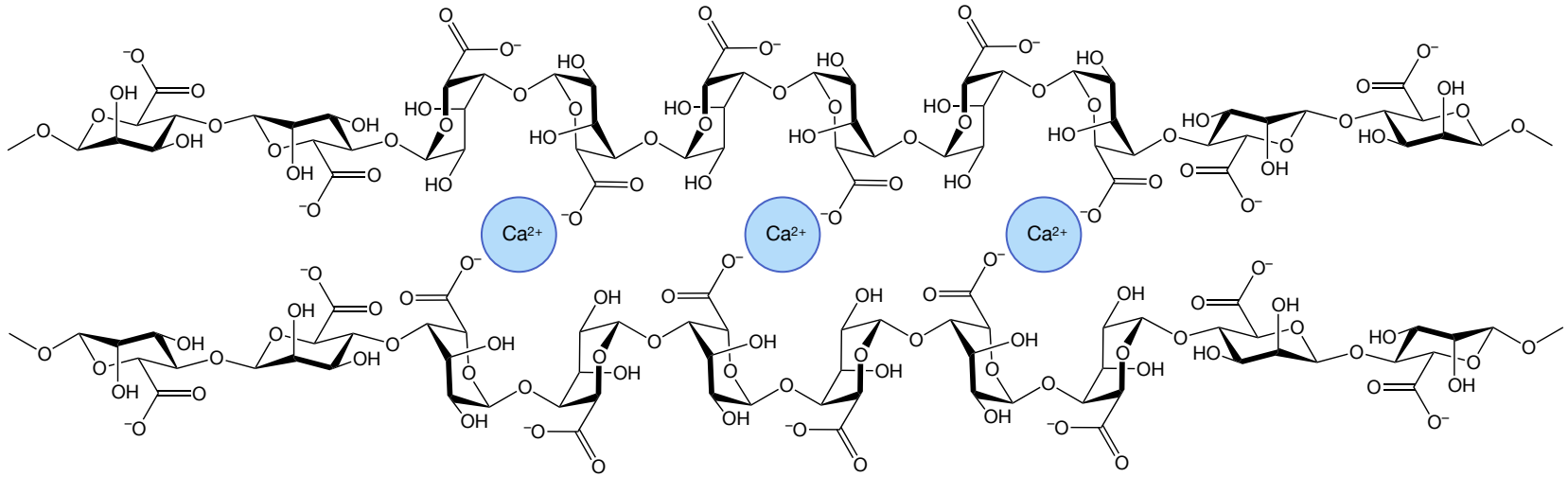
Alginate microparticles



Guluronic (G) block Mannuronic (M) block

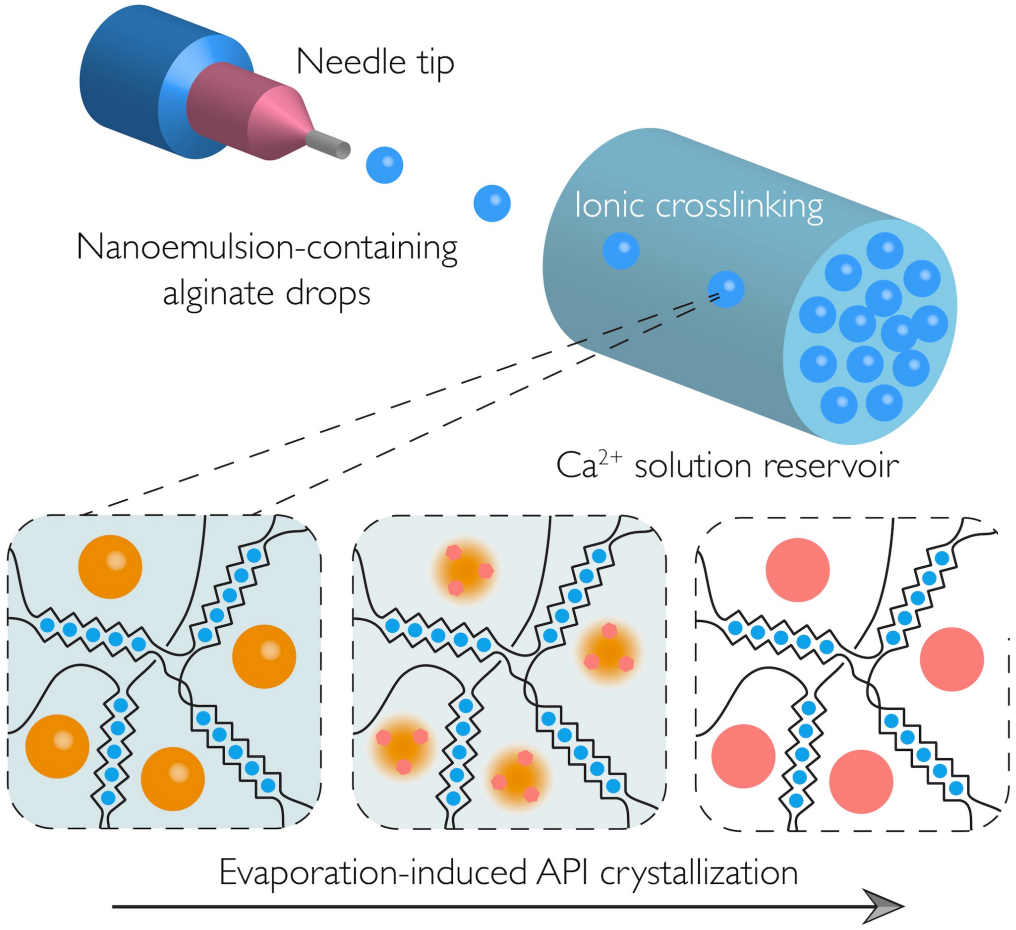


6 wt% $CaCl_2$
in DI H_2O bath

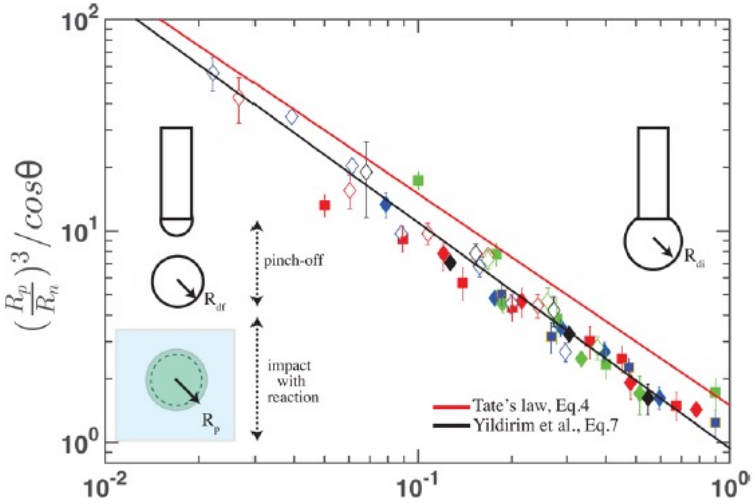


M M G G G G G G M M

Droplet-based alginate particle synthesis



Control gel microparticle size via rotational speed & needle diameter



$$Bo = \frac{\rho a R_n^2}{\gamma}$$

Water

Alginate chains

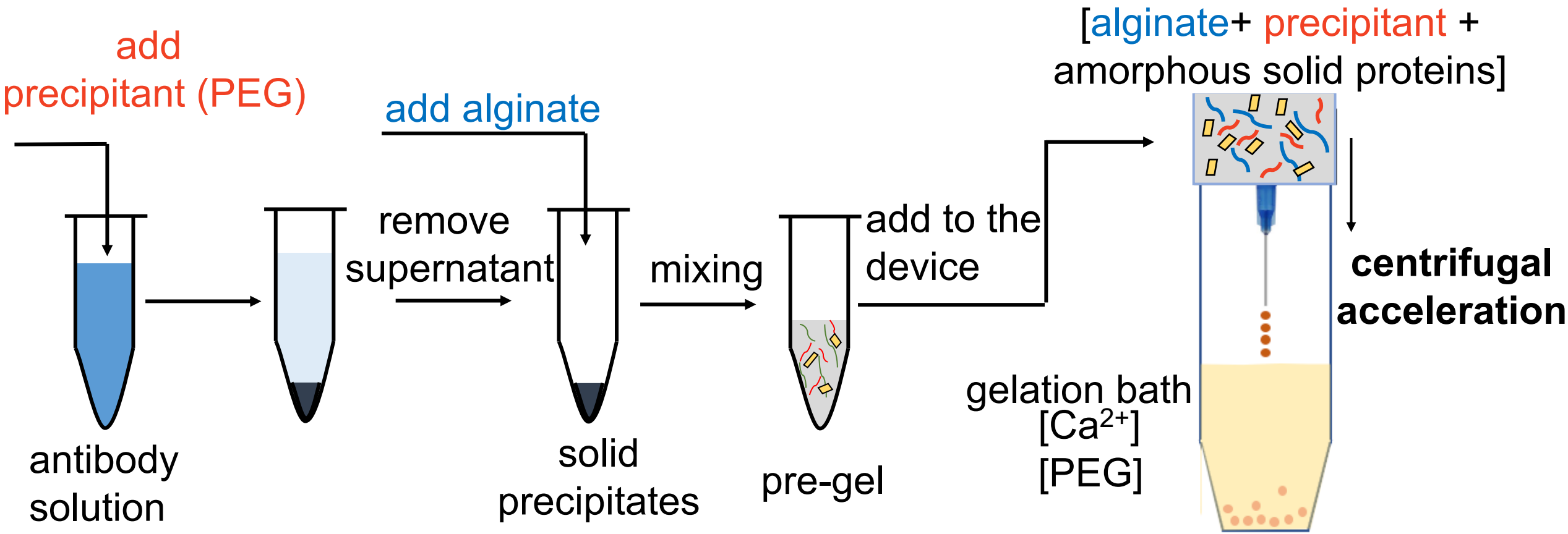
Ca^{2+} cations

API solution droplets

API crystals

Eral et al. Langmuir 2016

Typical process

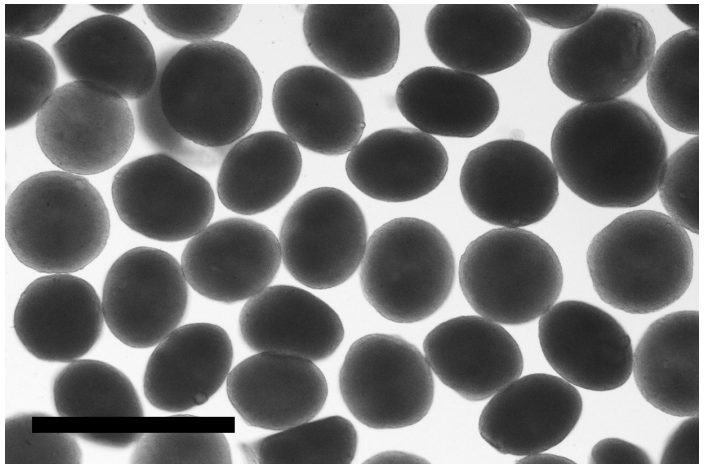


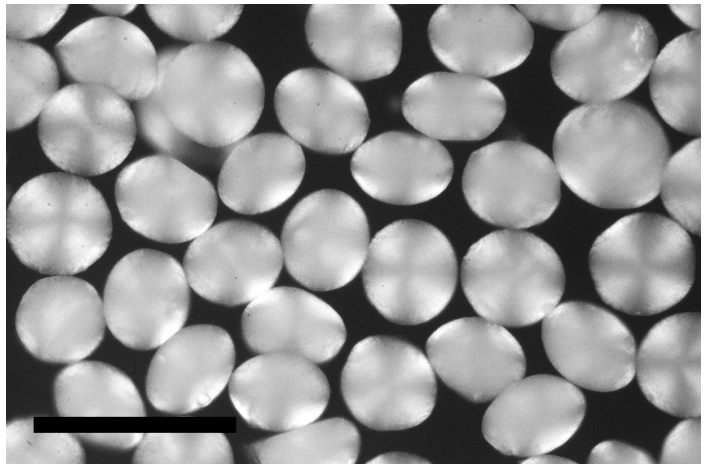
Alginate particles loaded with crystalline pembrolizumab

Bright field

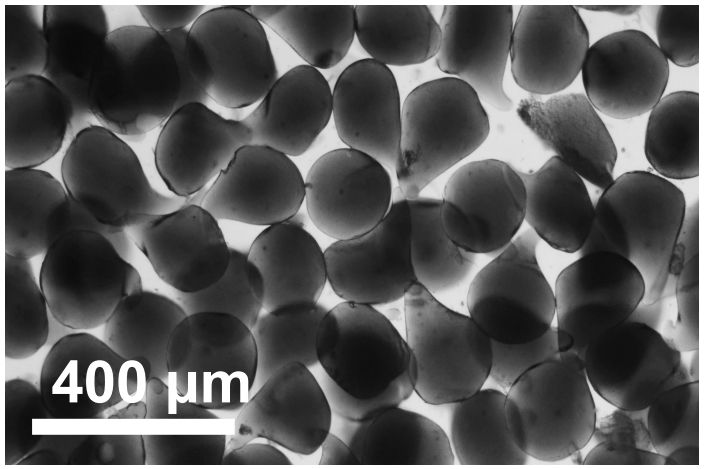
Cross polarized

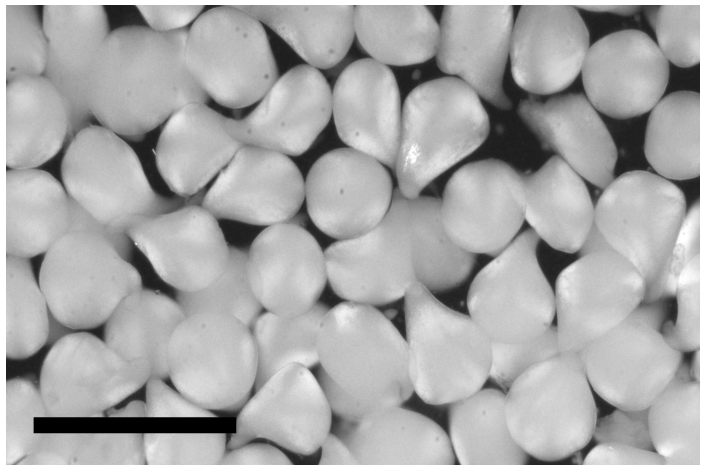
100 mg/ml mAb loading





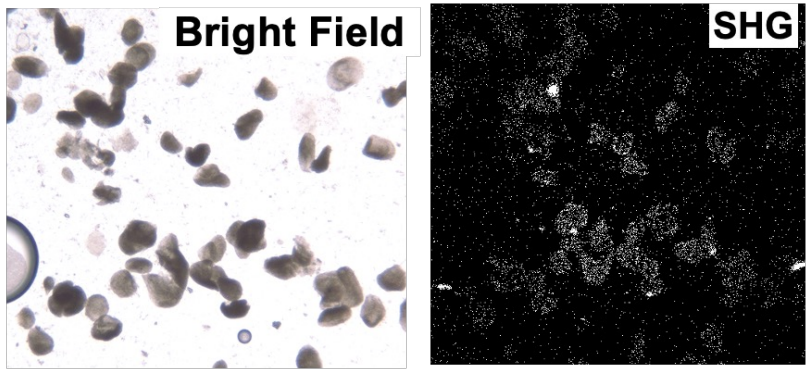
350 mg/ml mAb loading



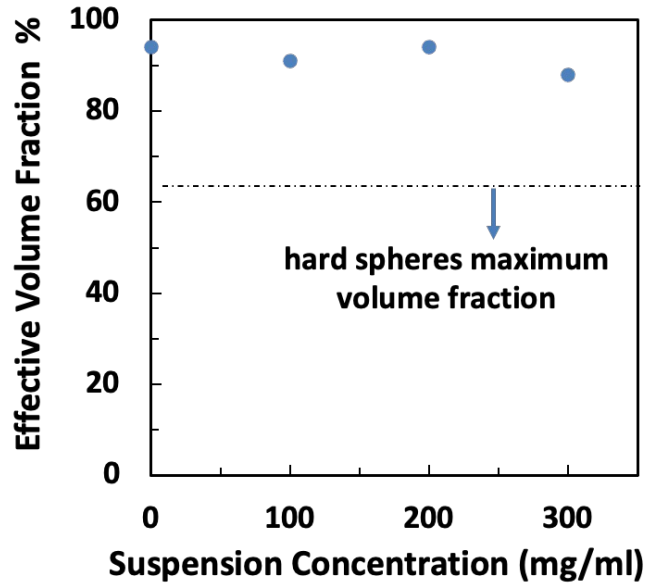


Particles filled in a syringe for SC injection

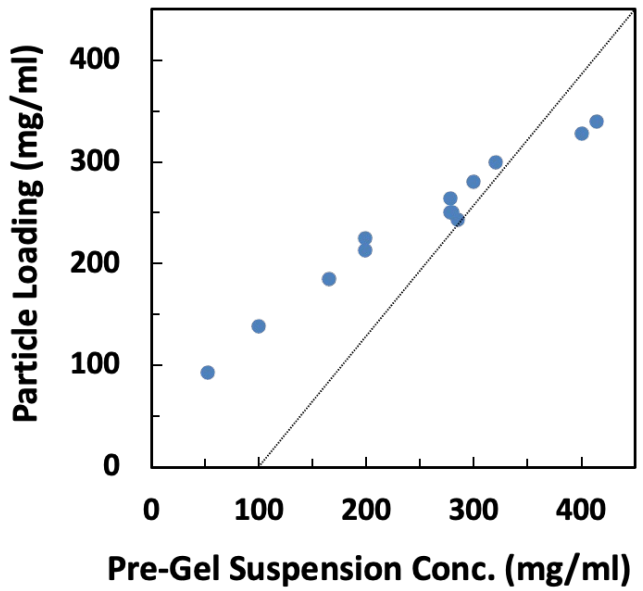
Second harmonic generation microscopy confirms chiral crystals



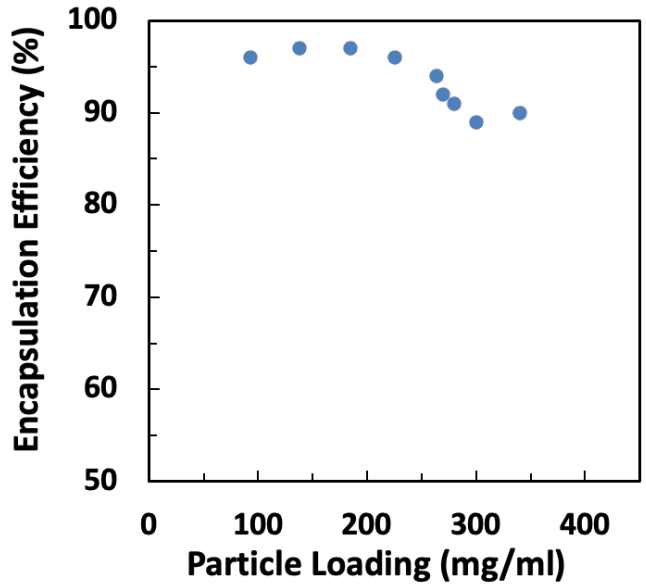
Soft particles allow high volume fractions



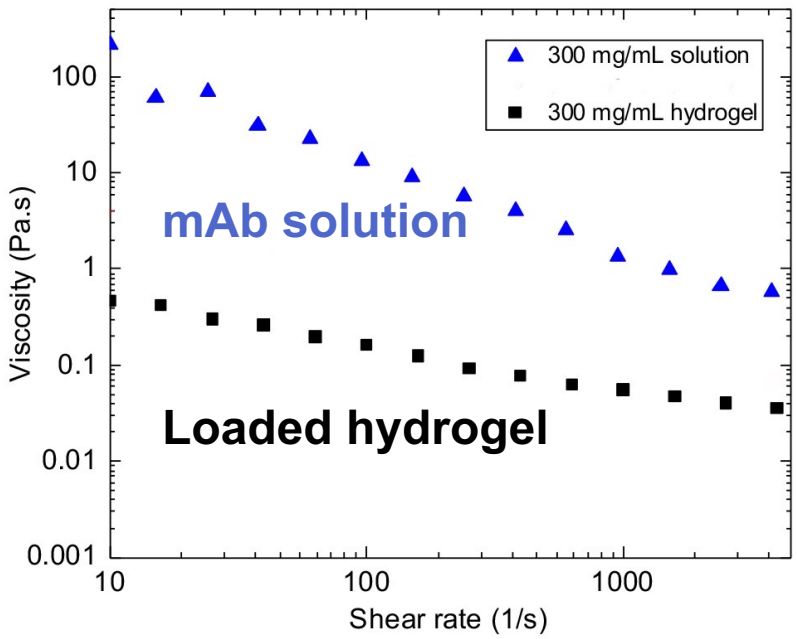
Crystalline suspension allows high particle loading



High encapsulation efficiency was achieved

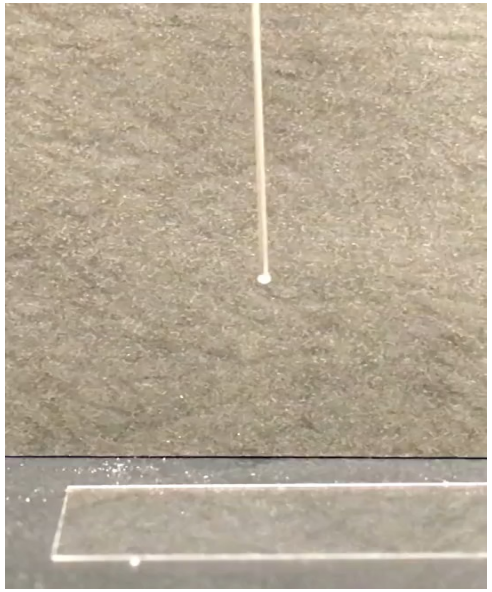


Enhanced flow properties of formulations



injection force (solution): 130 N
(@ 300 mg/ml)

injection force (hydrogel particles): 9 N
(@ 300 mg/ml)



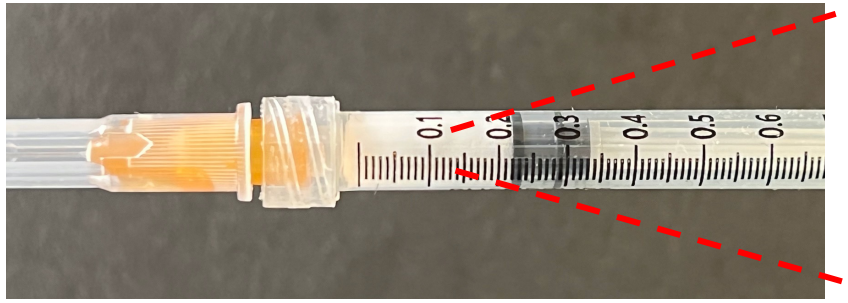
flow from 27 gauge needle

Viscosity reduction is a combination of:

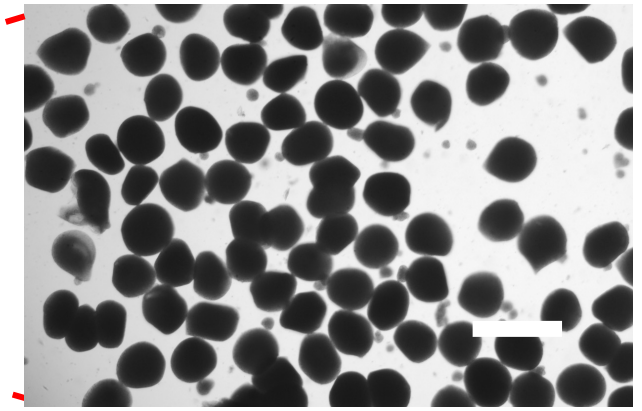
- Hydrogel ‘cloaking’ mAb-mAb interactions (crystals embedded in hydrogel)
- Particle shape minimizes surface area – decreases mAb-mAb interaction in flow
- Hydrogels are ‘soft’, leading to different nature of flow & shear thinning

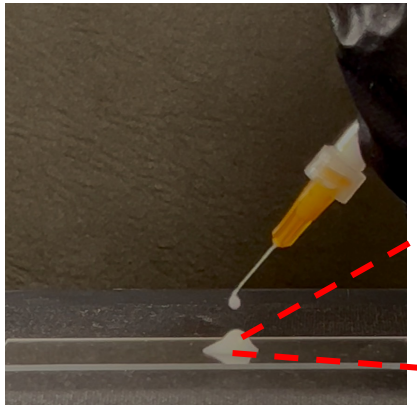
Particles withstand high shear-rates of injection

Engineered our particles to withstand shear rate of injection and maintain their physical attributes upon injection using 27 G needle

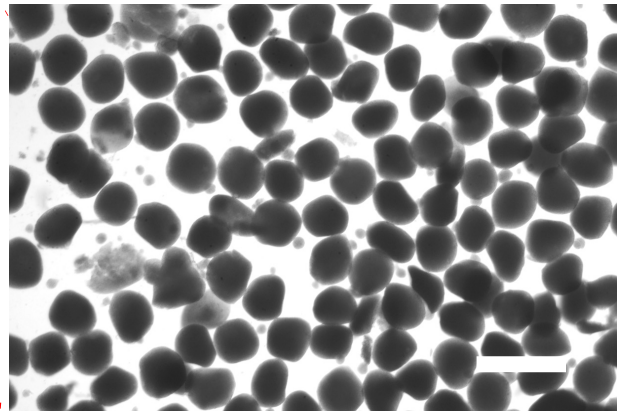


before injection



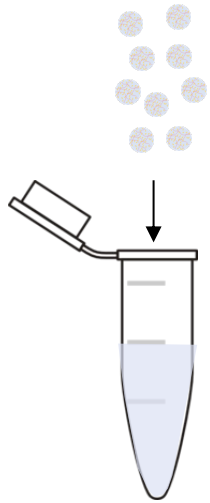


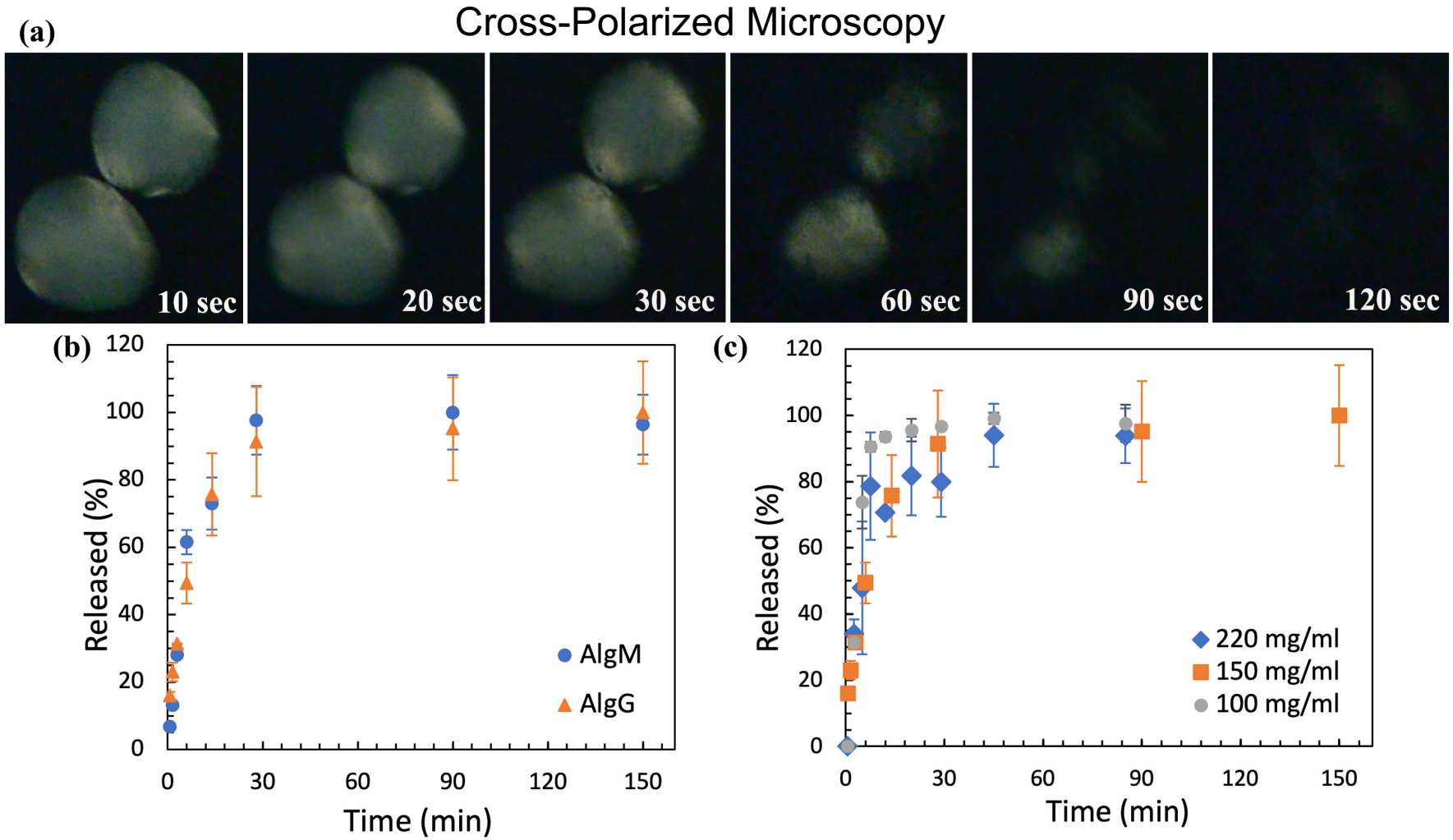
after injection



In Vitro Release

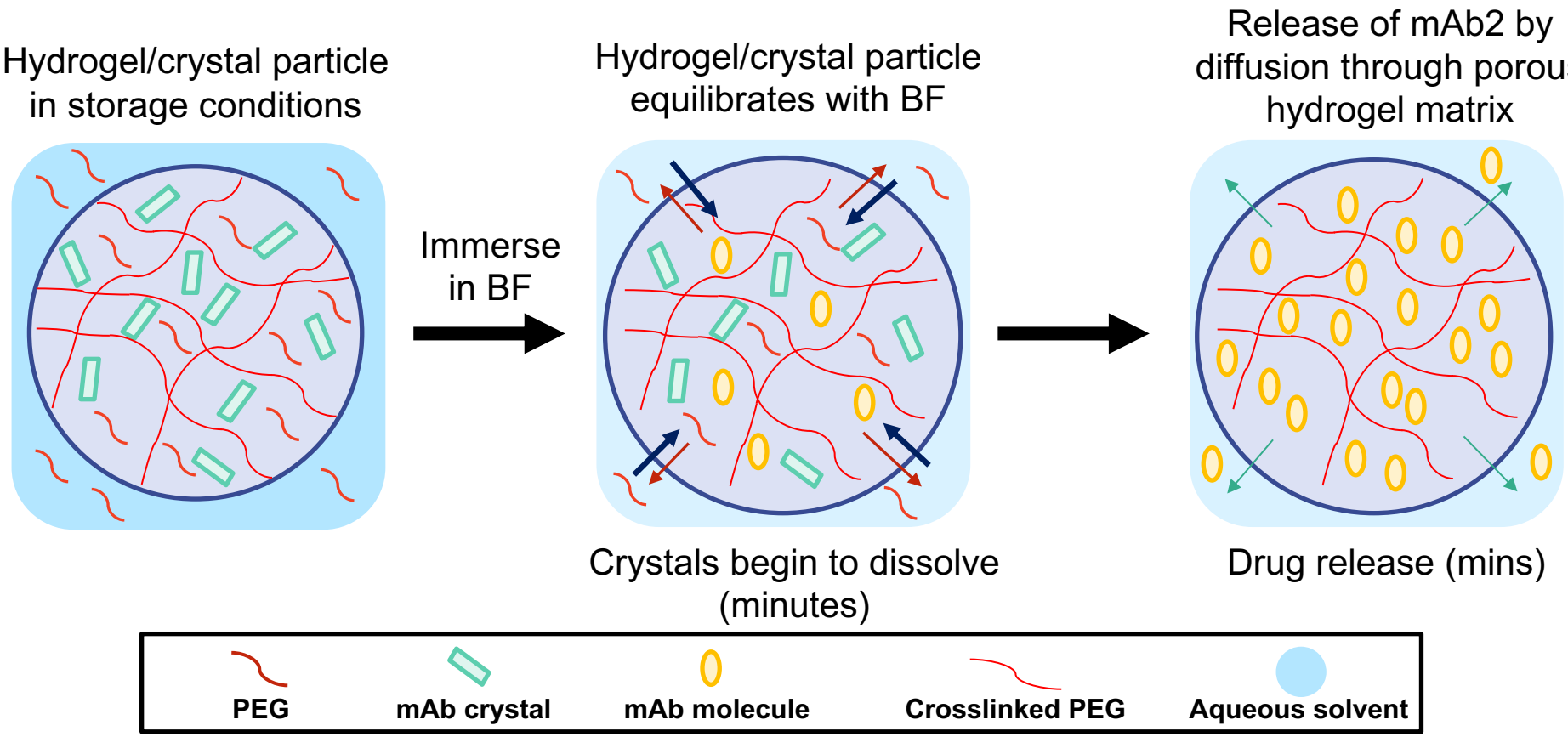
dissolution in simulated body fluid @ 37° C





- The encapsulated antibody was completely released.
- Release profiles were independent of the crystalline mAb concentration.

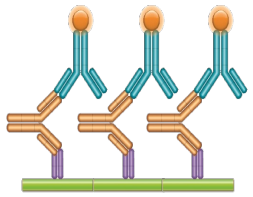
In Vitro Release



Released antibody quality confirmed through bio-analytical tests (stored 5 months 4 °C)

Determining the Potency

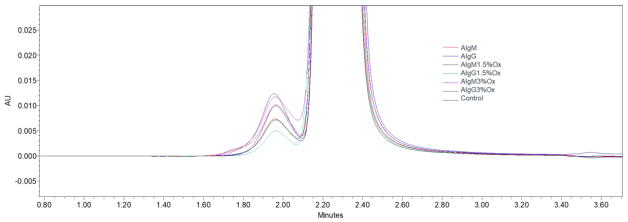
ELISA



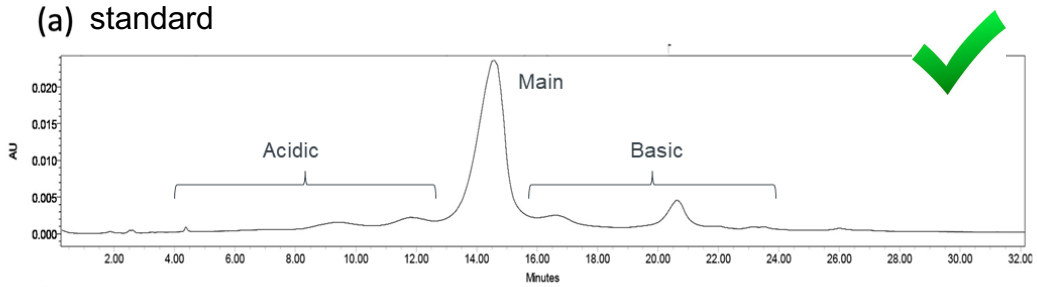
Sample	ELISA		Size Exclusion Chromatography
	% Normalized activity	Geometric Standard deviation	% Monomer
Control	--	--	98
AlgM	95	1	97.3
AlgM1%Ox	89	2	97.9

Detecting the Aggregates

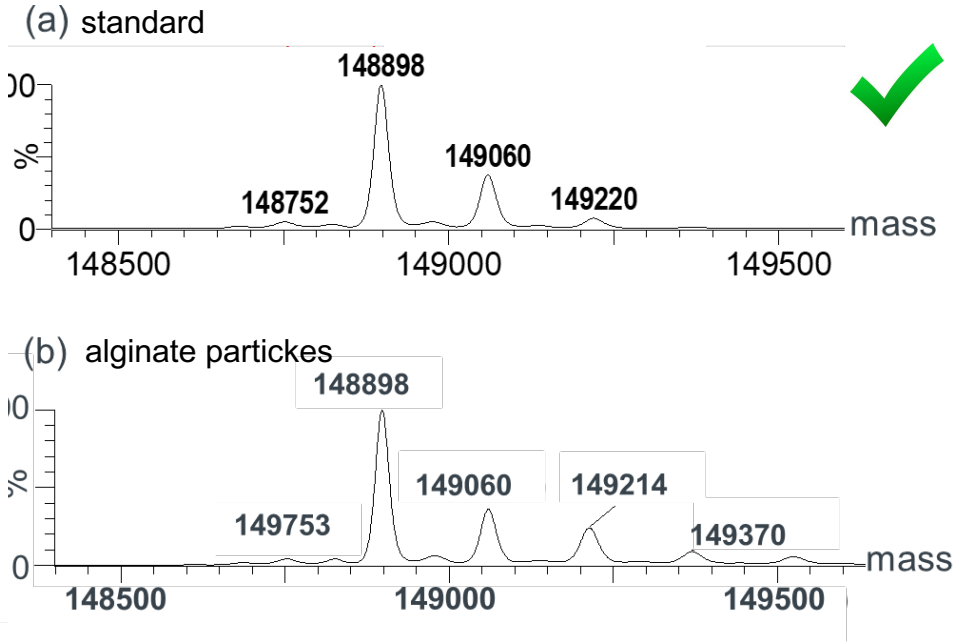
Size exclusion chromatography



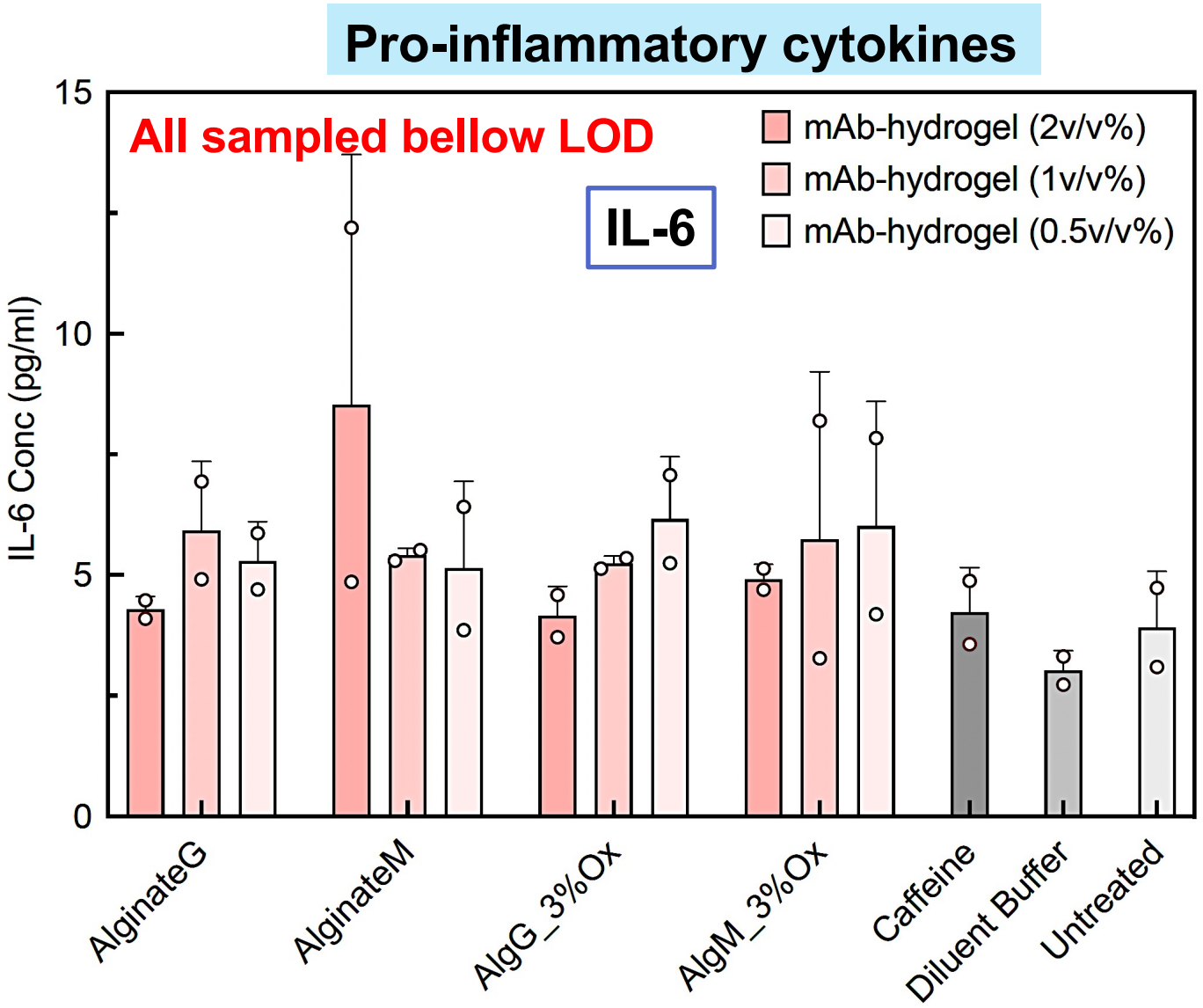
IEX: No Charge Variant detected



LC-MS: No Mass Shift Detected



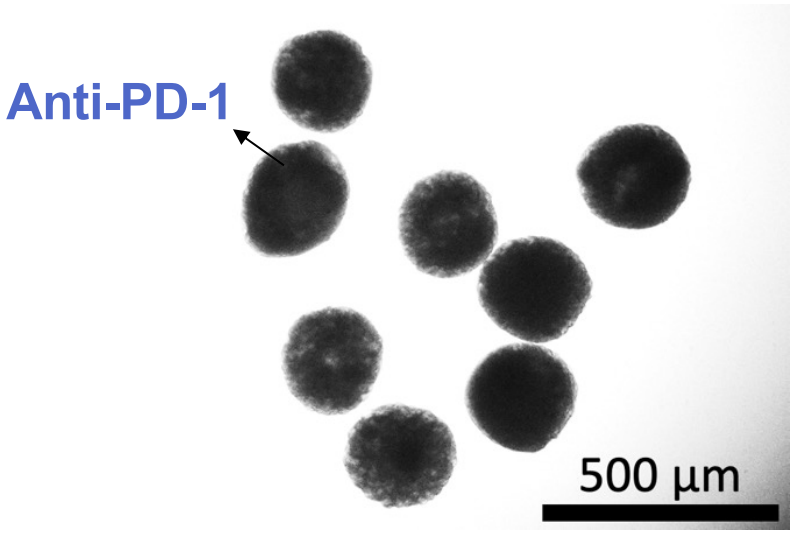
In vitro cell-based cytotoxicity and immunogenicity of mAb-laden alginate particles



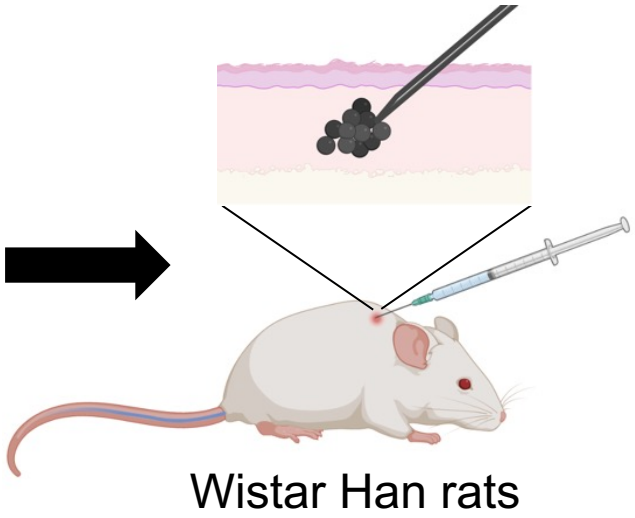
RAW 264.7 (mouse macrophage)

- Complete cell viability was observed.
- No indication of pro-inflammatory cytokines IL-6 and TNF-α (data not shown) secretion.

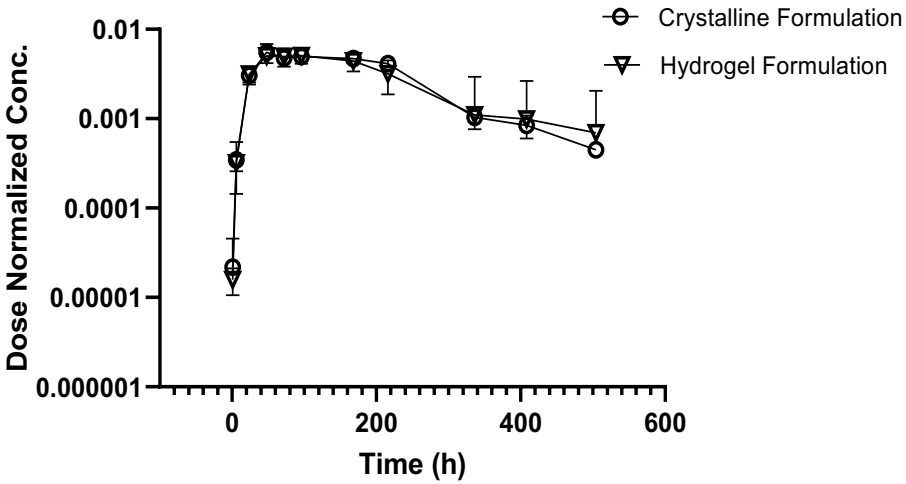
in vivo PK studies with Pembrolizumab



injectable particles



high concentration subcutaneous injection

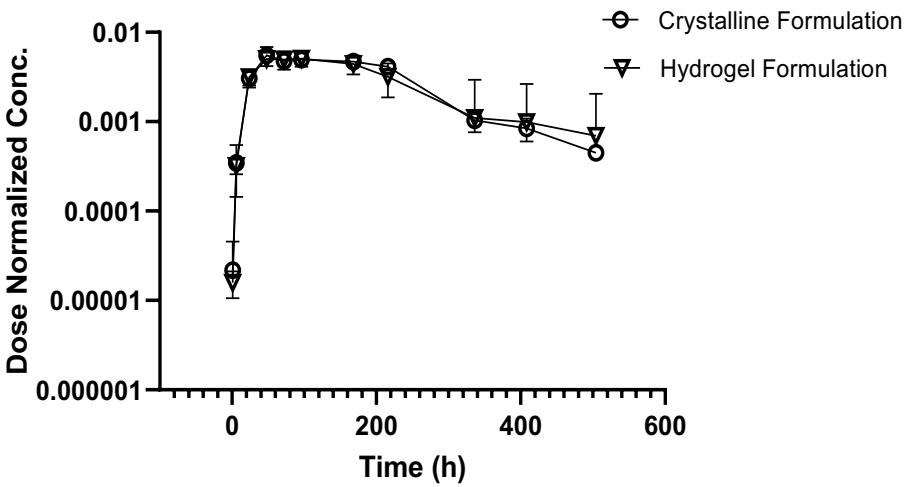


bioequivalence in rats
(Same pharmacokinetics as non-particulate formulation)

Erfani, Amir, Jeremy M. Schieferstein, Paul Reichert, Chakravarthy N. Narasimhan, Cinthia Pastuskovas, Vaishali Parab, Denarra Simmons et al. "Crystalline Antibody-Laden Alginate Particles: A Platform for Enabling High Concentration Subcutaneous Delivery of Antibodies." *Advanced Healthcare Materials* (2023): 2202370.

in vivo PK studies with Pembrolizumab

Formulation	Crystalline formulation	mAb-laden ALG particles formulation
C_{max}/Dose	0.005	0.005
T_{max} (hour)	48	48
AUC/Dose	1.4	1.4
Vz-F [mL/kg]	94	123
CL [mL/h/kg]	0.7	0.7
Mean Residence Time [hr]	171	174
AUC_{inf} [h*µg/mL]	62222	57495



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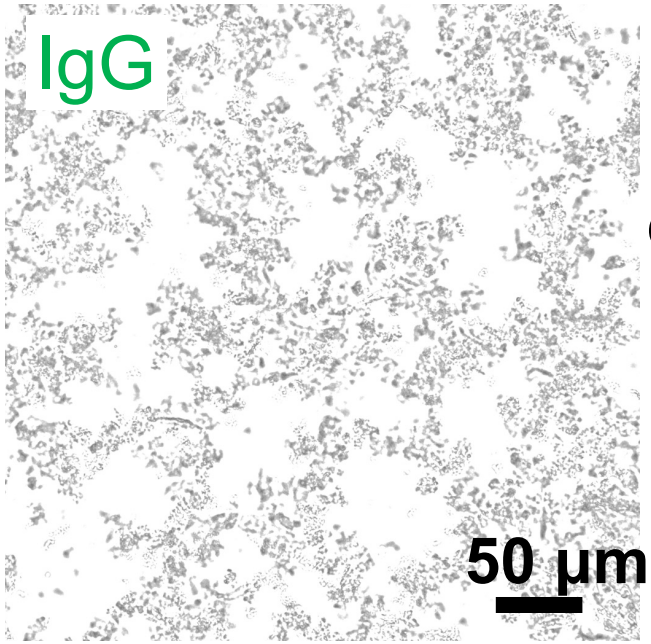
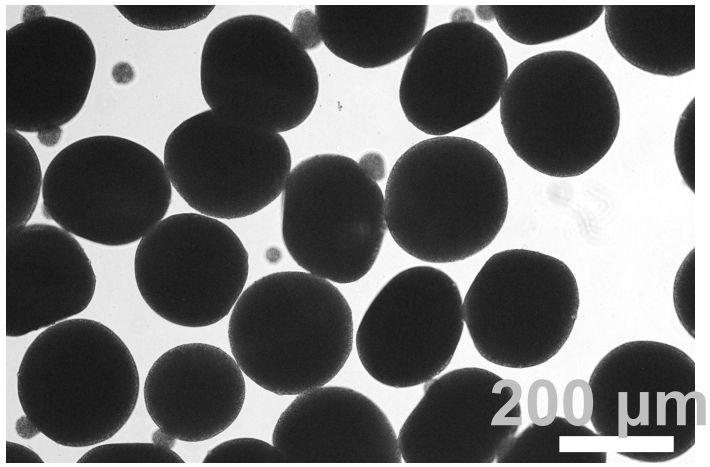
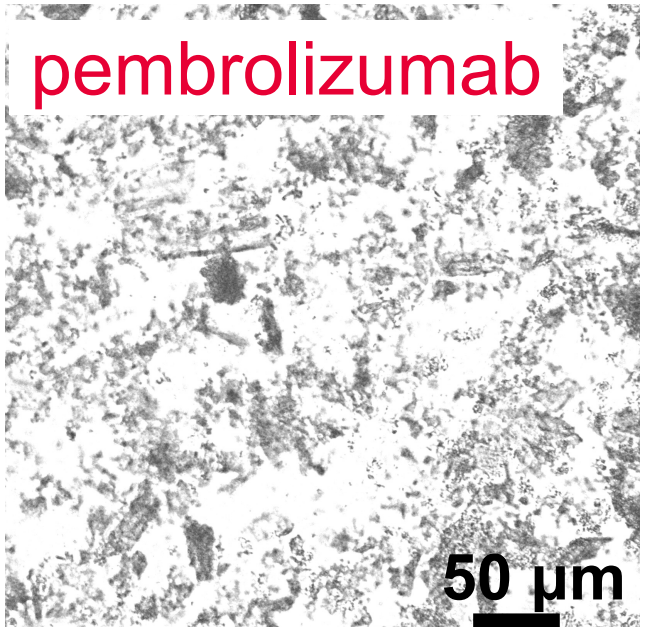
Extending approach to amorphous solid mAbs

Precipitated using
PEG, 3350 Kda
15 % w/v

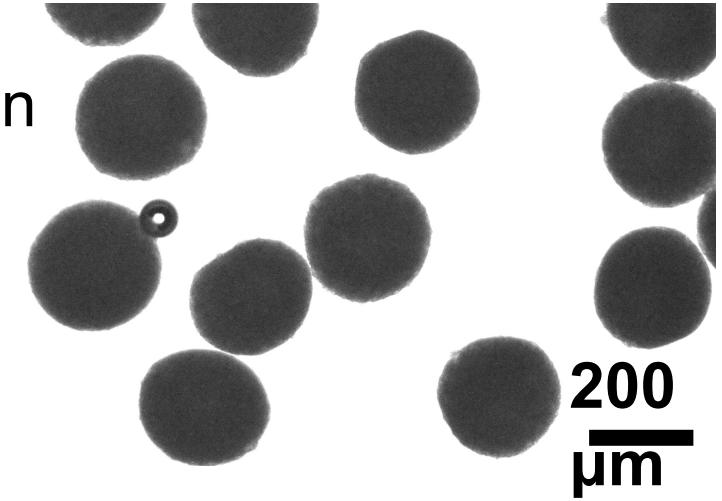


encapsulation

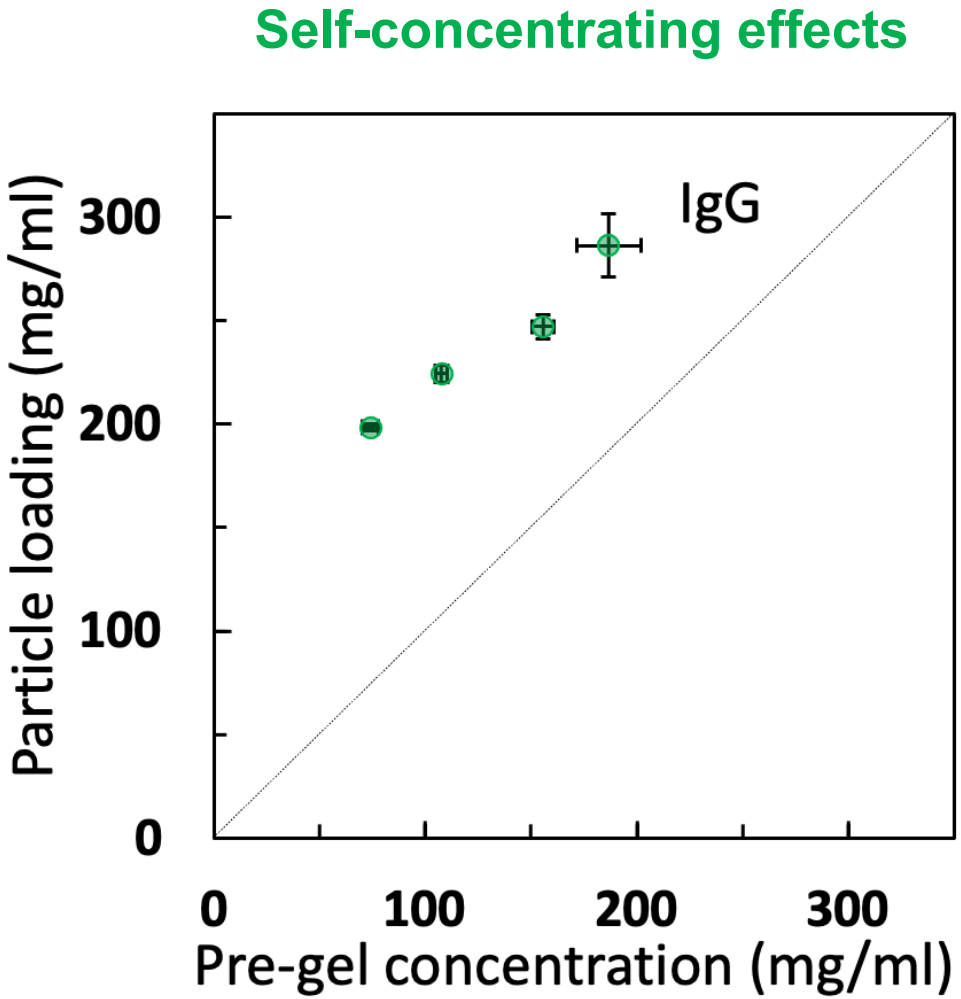


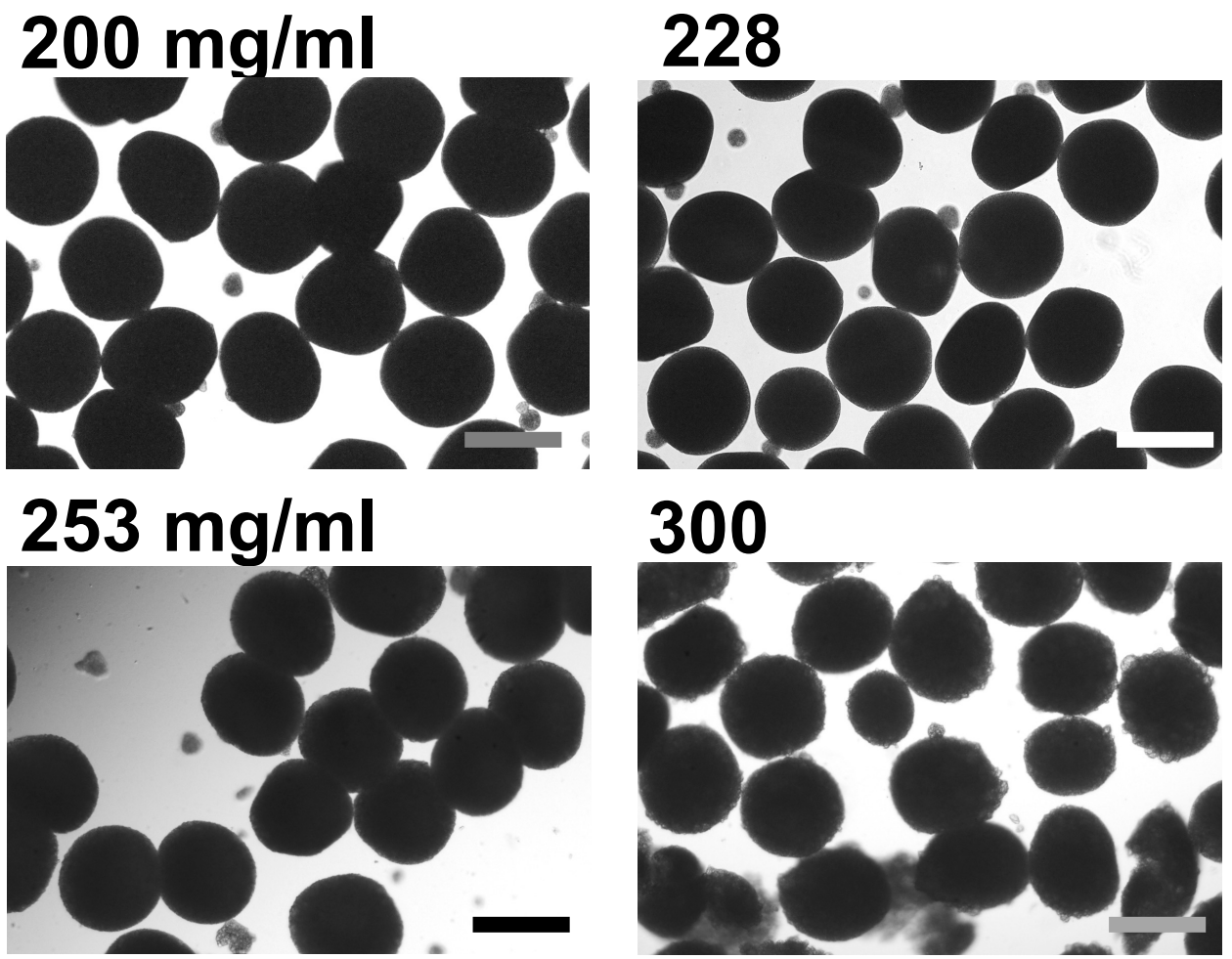


encapsulation

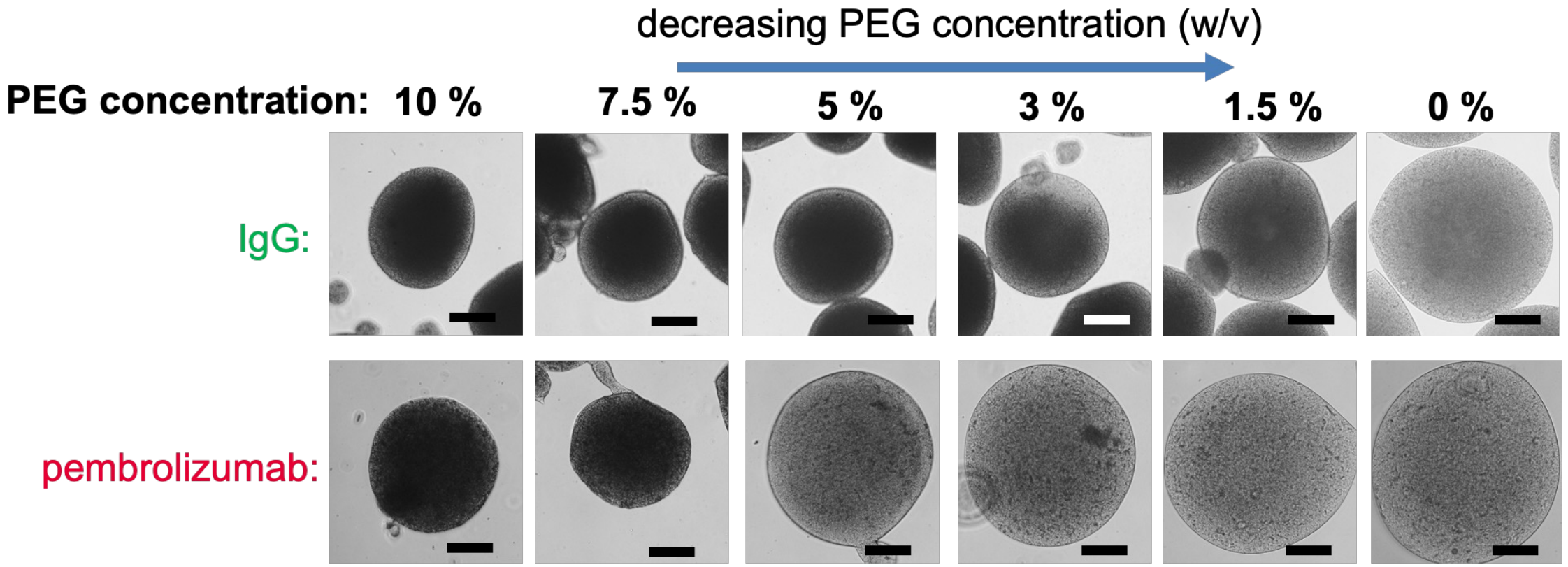


Extending approach to amorphous solid mAbs





Antibody is maintained as amorphous solid precipitate in the presence of PEG



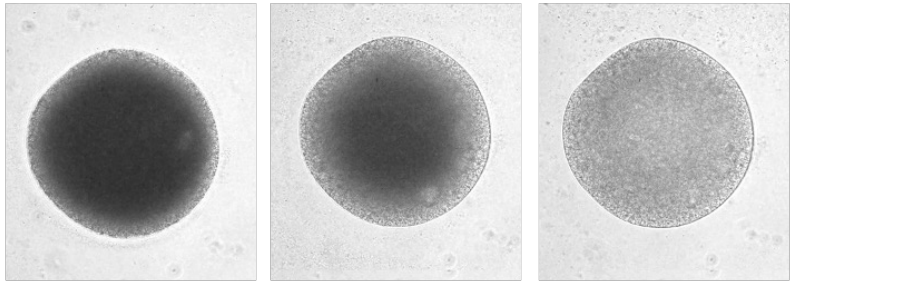
in vitro release in simulated body fluid

amorphous solid precipitates

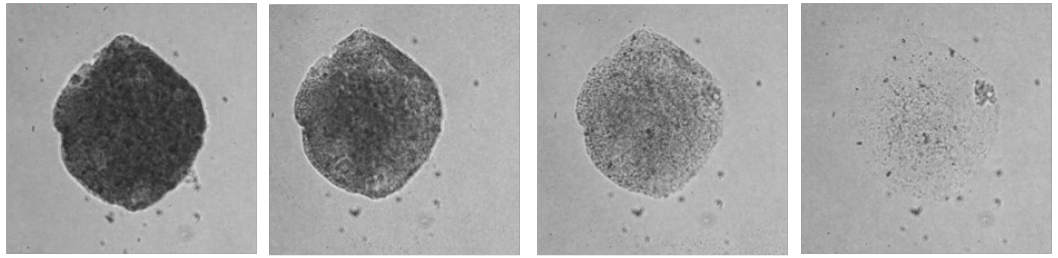
mechanism:
1. dissolution
2. diffusion of the dissolved antibody

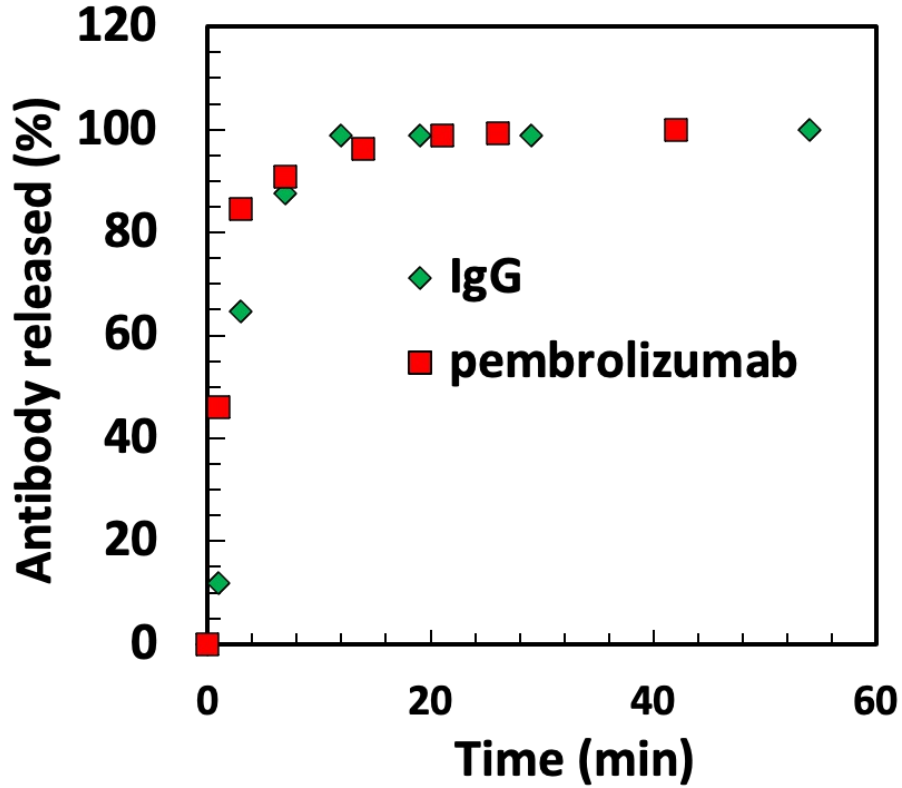
release time: 10 sec **30 sec** **1 min** **2 min**

IgG:



pembrolizumab:

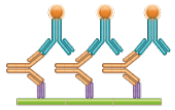




Quality of mAb is maintained through processing, storage & release

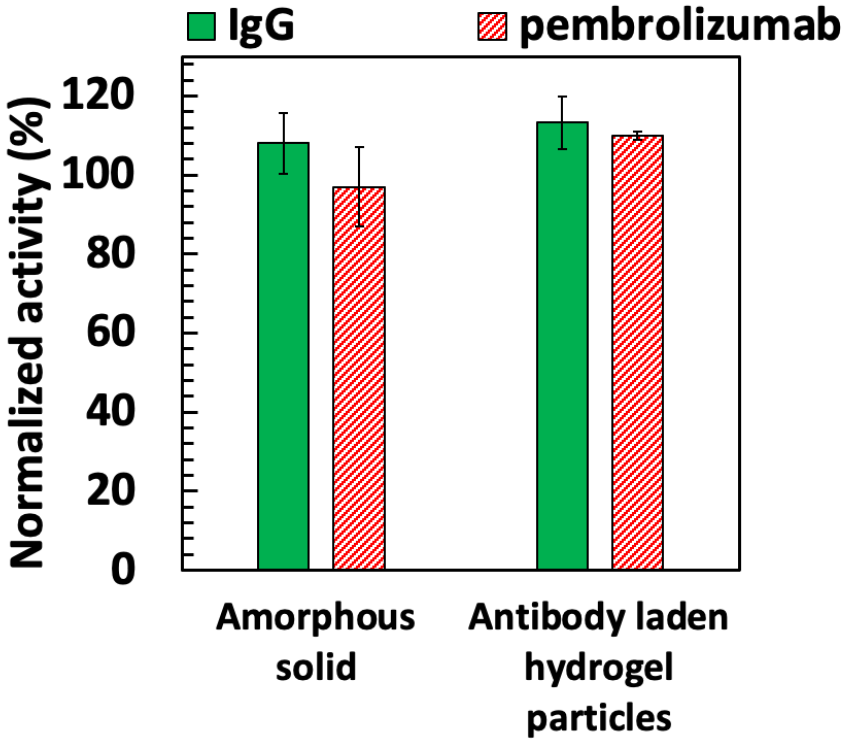
amorphous solid precipitates

Determining the activity **ELISA**



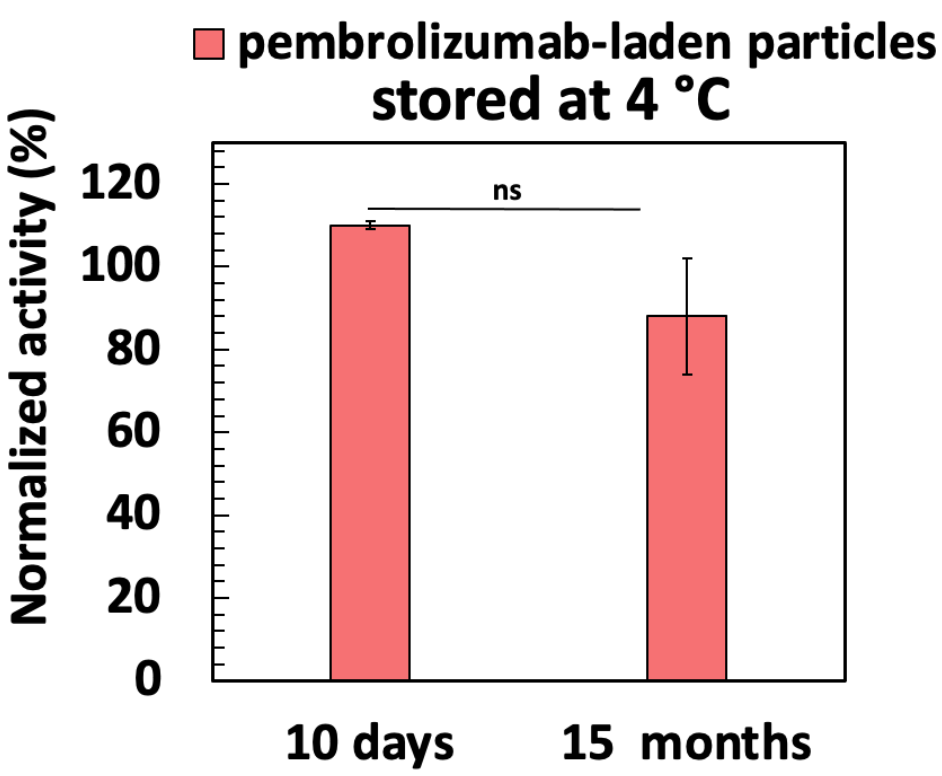
Detecting the aggregates **Size exclusion chromatography**

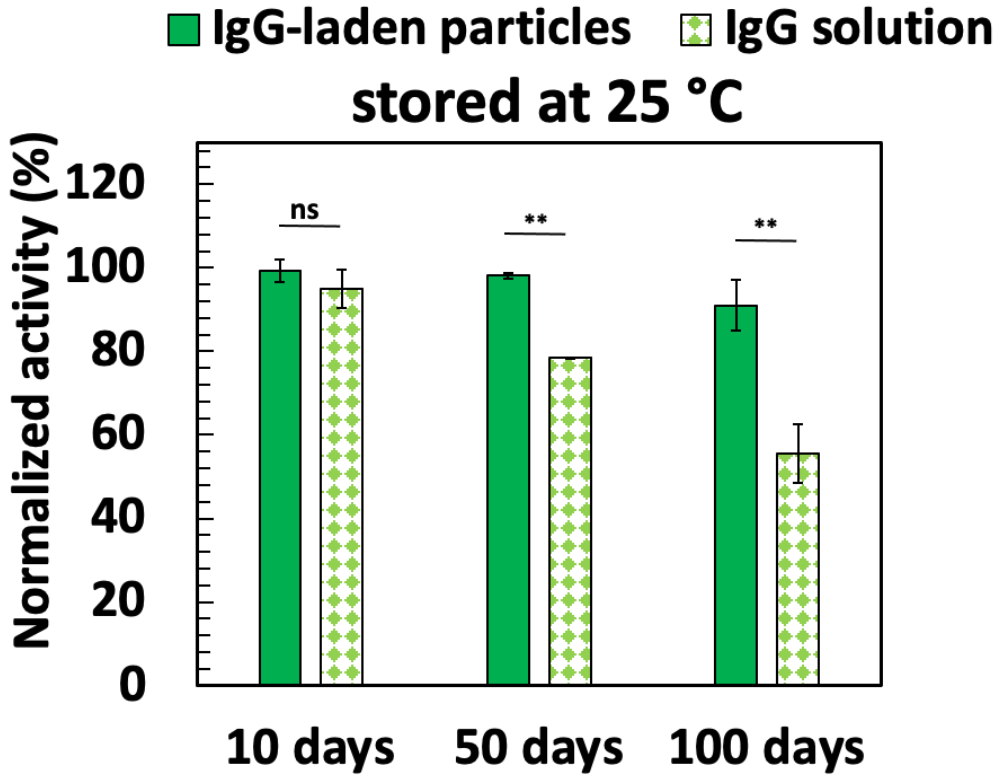
Sample	High molecular weight %	Monomer %	Low Molecular weight %
Fresh	4.4	95.4	0.2
Stored for 15 months	4.6	95.2	0.2



Quality of mAb is maintained through processing, storage & release

amorphous solid precipitates





Formulation benefits

- High-concentration, stable, and injectable formulation of antibodies
- No chemical reactions, organic phase, oil or solvent is used
- Aqueous formulation
- Every single component has a safe track record of clinical use
- Enables drug carrier design

Processing benefits

- Broadly applicable process
- No need for a drying process (ease of manufacturing)
- Substantially smaller footprint compared to spray drying
- Compatibility to very labile biologics with stability similar to solid formulations

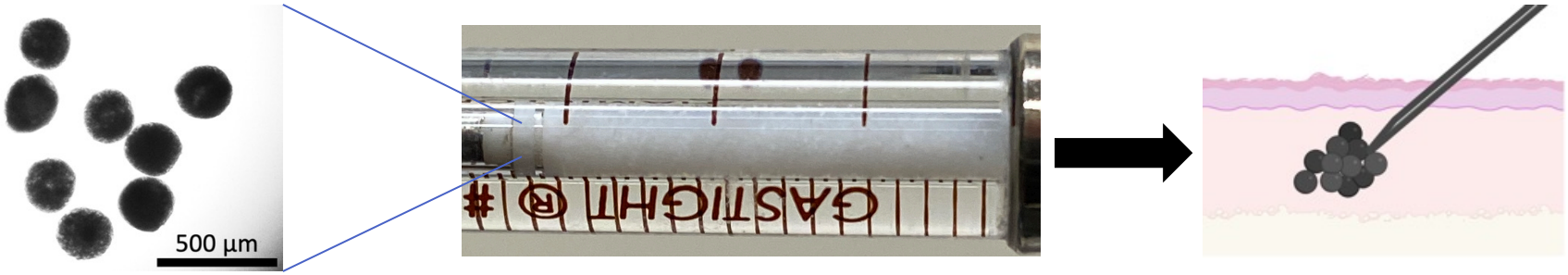
Approach works with other polymer chemistries

Crystalline mAb-Laden Hydrogel	**Case 1**	**Case 2**	**Case 3**
Crosslinking	**Free Radical Reaction**	**Click Chemistry**	**Ionic Crosslinking**
Hydrogel monomer	PEGDA	PEG vinyl sulfone -dithiol Chemistry	Alginate
Production method	Microfluidic	Batch Emulsion	Centrifugal Extrusion
Initiator	UV	None (slow polymerization)	Ca^{2+}
Crystallinity	Y	Y	Y
Decrease in activity?	N	-	N
Aggregation	6 %	6 %	<1 %
Charge variants detected?	N	N	N
Mass shift detected	Y	N	N

Summary

- Technology developed to embed solid forms of mAbs in hydrogel particles
- Enables stable, high concentration, injectable formulations
- Formulation and process advantages

- Schieferstein, J.M., Reichert, P., Narasimhan, C.N., and Doyle, P.S. " Hydrogel Microsphere Encapsulation Enhances the Flow Properties of Monoclonal Antibody Crystal Formulations" Advanced Therapeutics, 2000216, 2021.
- Erfani, A., Diaz, A.E. and Doyle, P.S. "Hydrogel-enabled, local administration and combinatorial delivery of immunotherapies for cancer treatment" Materials Today, 2023.
- Erfani, A., Schieferstein, J.M., Reichert, P., Narasimhan, C.N., Pastuskovas, C., Parab, V., Simmons, D., Yang, X., Shanker, A., Hammond, P. and Doyle, P.S. "Crystalline Antibody-Laden Alginate Particles: A Platform for Enabling High Concentration Subcutaneous Delivery of Antibodies" Advanced Healthcare Materials, 2023.
- Erfani, A., Reichert, P., Narasimhan, Doyle, P.S., Injectable Hydrogel Particles for Amorphous Solid Formulation of Biologics, iScience, in press.



high-concentration antibody loaded particles

particle loaded in prefilled syringe

subcutaneous injection