“Reducing animal usage through longitudinal *in vivo* fluorescent imaging to monitor delivery of single-injection vaccines”

Purpose: The goal of this project was to validate that longitudinal *in vivo* imaging can be used to monitor functional vaccine release from novel microparticle delivery systems developed in the Stevens group, and to facilitate *in vivo* imaging training for students and CBS staff. We have developed biodegradable 3D-printed hollow microparticles that can provide a delayed release of protein (e.g. model antigen OVA) to refine vaccination techniques, wherein a single injection can deliver both the prime (soluble) and boost (particle-released) vaccine doses. Building on the refinement provided by the single-dose vaccine administration platform technology, this project sought to establish non-invasive imaging techniques as predictive measurements of functional immunity, thereby decreasing reliance of repeated serum sampling and decreasing the number of mice needed by parallelizing biodistribution and immunogenicity experiments.

Results: We performed a longitudinal imaging study while monitoring prime-only immunogenicity of two different delayed-release particle chemistries with 25 µm wall thickness to correlate OVA release kinetics with anti-OVA IgG titers. Microparticle design, light microscopy, chemical composition, and injection site are outlined in **(A)** below, illustrating our comparison of particles chemistries with fast (CEA) and moderate (NVP) degradation rates. *In vivo* imaging revealed decreasing CF647-OVA intensity at the subcutaneous injection site **(B)**, which correlated with resin degradation rates observed *in vitro*. The 25 µm-thick CEA particles release with delayed exponential decay kinetics from week 3-6 and 25 µm NVP particles release with linear kinetics from week 3-8. Both release profiles resulted in similarly high anti-OVA IgG titers, which increased after release began at week 2 and were maintained at significantly higher levels than freely soluble OVA or soluble OVA mixed with empty particles **(C)**. Having validated that imaging-detected release correlates with induction of immunity, our next step is to evaluate whether these differences in release kinetics shape antibody maturation when used to deliver the booster dose of a subunit influenza vaccine.