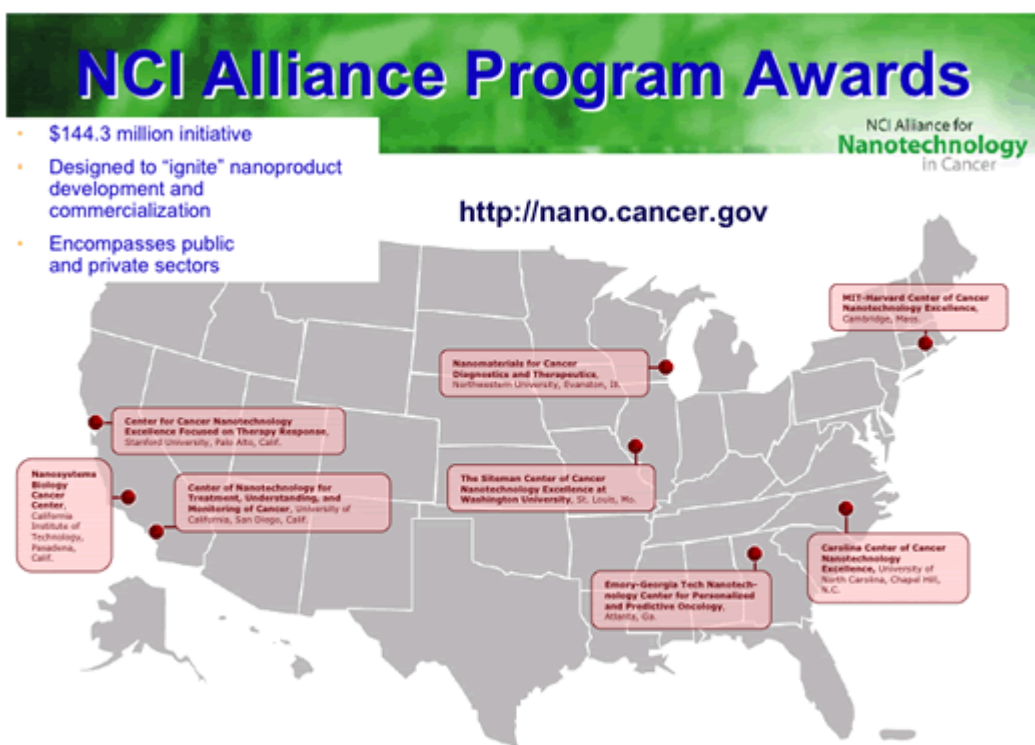


## Life Science

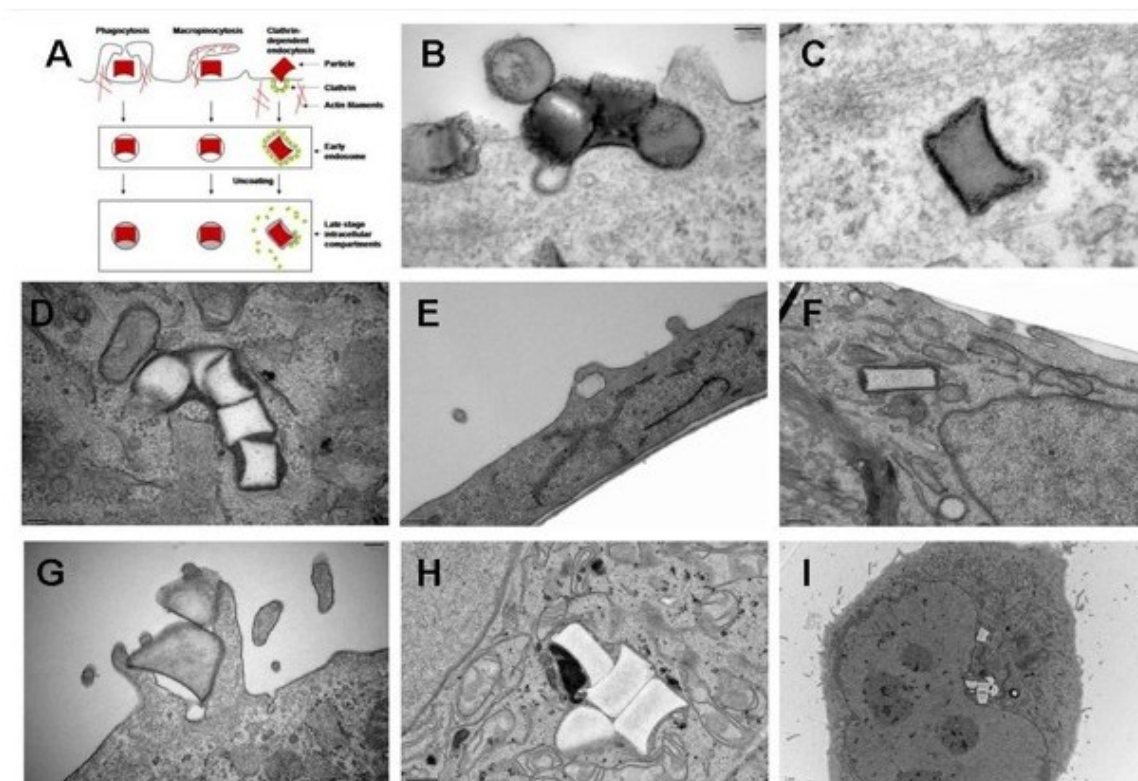
### Cell Biology: Examining the effects of particle size, shape, and surface chemistry

The exploration and utilization of nanocarriers for the delivery of therapeutics *in vivo* has led to dramatic improvements in the efficacy of various therapies. Over the past few years, intense research and development of novel platforms has resulted in drug delivery vehicles such as polymeric nanoparticles, micelles, immunoconjugates, DNA-polymer conjugates, dendrimers and liposomes. Clinically, the success of these carriers has been limited by the lack of control over size, chemical composition, uniformity, cell targeting and ability to consistently load and release known amounts of cargo. A recent breakthrough from the DeSimone laboratory has led to the production of monodisperse, shape-specific particles from an extensive array of organic precursors. This particle fabrication technology, called **PRINT**<sup>TM</sup> (Particle Replication In Non-wetting Templates) takes advantage of the unique properties of elastomeric molds comprised of a low surface energy perfluoropolyether network.



Understanding the interdependent role of particle size, shape, surface and matrix composition on the intracellular pathway will lead to a deeper knowledge of the fate of organic nanoparticles *in vivo*. The advent of "calibration quality" particles using PRINT allows for the elucidation of mechanisms by which organic particles of controlled size, shape, site-specific surface chemistry, tunable particle matrix composition and tunable modulus undergo endocytosis. Obtaining knowledge on the endocytic pathway used from "calibration quality" particles should lead to crucial information required for not

only enhancing specific cellular internalization, but also manipulating the intracellular location of particles, and minimizing cytotoxic effects. Once the mechanisms of internalization are established, it is then possible to use these findings to better engineer the intracellular release of specific cargos. This information, in combination with ongoing efforts to understand the biodistribution of shape controlled particles, will help to establish rules towards the rational design of nanocarriers for the effective *in vivo* delivery of various cargos, especially those cargos that need to be internalized into cells such as siRNA and antisense oligonucleotides.

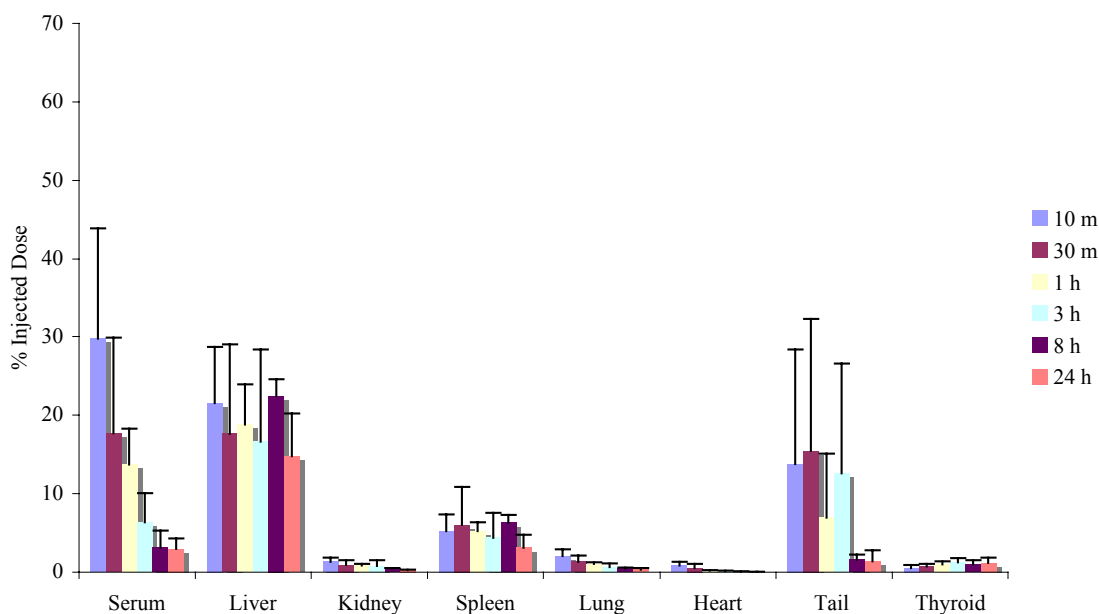


**Figure 1. TEM Images of 1µm, 500nm and 200nm particles being engulfed by HeLa cells.**

## Biodistribution

Definitive biodistribution maps that establish the interdependency of the size, shape, deformability and surface chemistry of nanoparticles *in vitro* and *in vivo* over length scales ranging from cells to tissues to the entire organism are needed by many different research communities. Environmental regulators, pulmonologists, oncologists, pharmaceutical scientists, toxicologists, cell biologists and dermatologists all need definitive answers related to particle biodistributions, particle permeability and transport using “calibration quality” particles. For example, fungal and bacterial pathogens are first and foremost recognized by their form or shape, however the complete understanding of the role and significance of that form and shape is largely lacking. Indeed, some rod-like bacterial pathogens, including the gram-negative bacteria *Salmonella*, *Shigella*, and *Yersinia* and the gram-positive bacterium *Listeria monocytogenes* can induce their entry

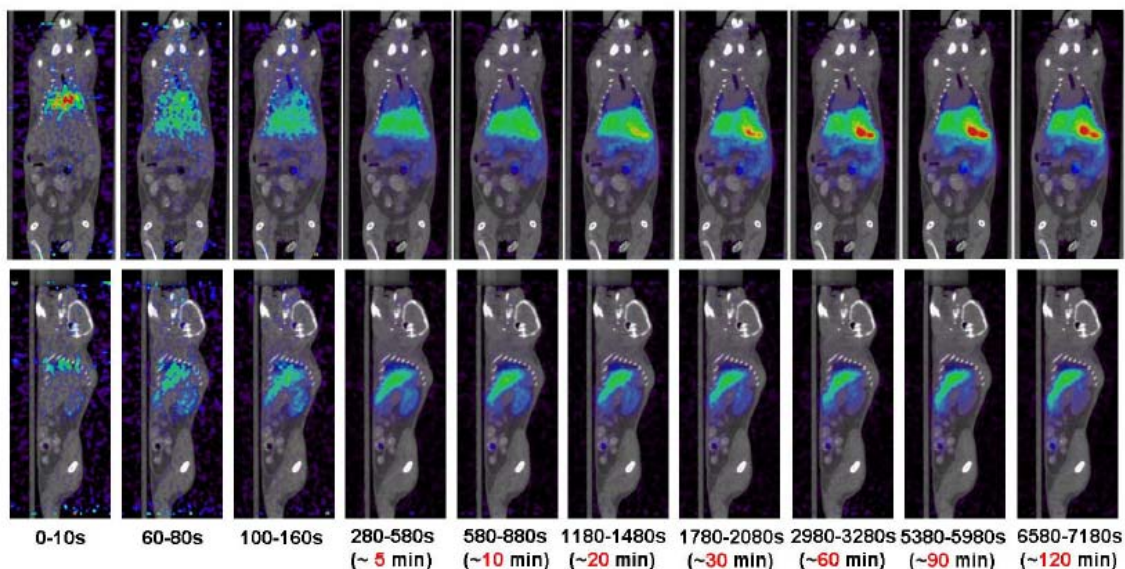
into non-phagocytic mammalian cells. Likewise, red blood cells and neutrophils are able to deform and undergo over 100 % strain (double in length) in order to navigate through various biological barriers that would prevent non-flexible objects from crossing. As such, nanofabricated tools (e.g. precisely defined particles) hold significant promise to provide insight into the fundamentals of cellular and biological processes. These tools can also yield essential insights into the design of effective vectors for use in nanomedicine, especially for the design of nanoparticles for use as targeted therapeutics and imaging agents. Indeed, very little is known how the interdependency of size, shape, deformability and surface chemistry can influence the biodistribution, cell-uptake, and intra-cellular trafficking of micro- and nanoparticles. Beyond understanding the biodistribution of particles delivered via parenteral routes, particle size, shape, deformability and surface chemistry should play a very significant role for understanding the mechanisms associated with particles that are inhaled, either intentionally for use as a therapeutic or during environmental exposure. Understanding the role that mechanobiology plays as a function of size, shape and surface chemistry certainly lies at the core of how biological particles like neutrophils and red blood cells navigate their barriers. Ascertaining definitive biodistribution maps through the use of precisely defined particle probes containing appropriate imaging beacons useful for quantification will undoubtedly lead to a set of rules that will be of immense use to science and to the application of nano-carriers to improved human health, treatment and diagnosis.



**Figure 2. Biodistribution of  $^{125}\text{I}$ -labeled 200 nm particles over 24 hours in healthy mice.**

## Imaging Modalities for a Dynamic View of Biodistribution

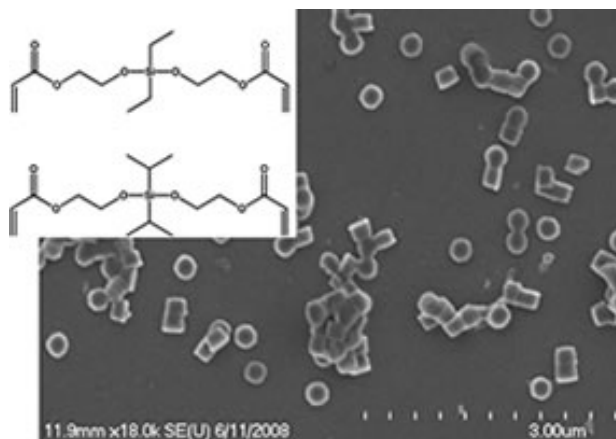
We have successfully designed PRINT particles that can be conjugated to  $^{64}\text{Cu}$ , a long-lived positron emitter useful for micro-PET/CT imaging. This work, in collaboration with the Stanford Center for Cancer Nanotechnology Excellence Focused on Therapy Response and the CalTech/UCLA/Institute for Systems Biology Nanosystems Biology Cancer Center, allows us to monitor the biodistribution of our PRINT particles *in vivo*, in real time. Currently the group is working on the incorporation of MR contrast agents as a cargo within PRINT particles to complement the PET/CT results described above.



**Figure 3. MicroPET imaging with  $^{64}\text{Cu}$ -DOTA PRINT particles. Time resolved PET images consisting of a two hour dynamic scan. The PET/CT images are overlaid. Mouse was injected with  $136.2 \mu\text{Ci}$  of  $^{64}\text{Cu}$ -labeled DOTA-nanoparticle. Both the coronal view (top), and sagittal view (bottom) are presented.**

## Degradation

Degrading PRINT Particles using Trojan horse ideas are being developed. These particles generally incorporate a reductively-labile or pH-sensitive crosslinker to achieve activated release of active cargo once internalized by cells. Specific focus is on the synthesis and evaluation of disulfide and pH sensitive crosslinkers. These novel cross linkers are integrated with the PRINT technology to form degradable particles of specific shape and size. Examination of the rates of release of various cargos encapsulated within these particles is also investigated. Both *in vitro* and *in vivo* experiments are on-going.



**Figure 4. SEM micrograph of 200 nm degradable particles.**

## Drug Delivery

Many peptides and proteins have biological activity, which can be used as potential therapeutics. Delivery of these peptide/protein drugs is one of the biggest obstacles to its clinical application. Taking advantage of the PRINT technology, we are exploring how to deliver peptide/protein drugs into cells and maintain its biological activity.

## Nano-molding of Protein Particles

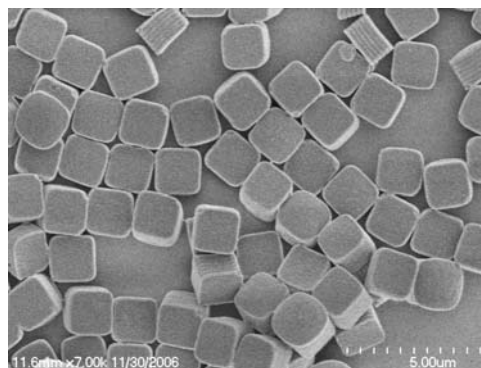
This research involves the nano-molding of proteins for the fabrication of protein PRINT particles of monodisperse size and shape. Lyophilized protein particles are generally highly dispersed in particle size, aggregated, and often made through costly and complicated processes. Attempts to engineer monodisperse, discrete protein particles using wet-milling, spray-freeze-drying, microemulsion, or super critical fluid methods have realized little success. The PRINT technology enables a gentle, facile route to monodisperse particles of 100% protein as small as 200 nm cylinders. Protein PRINT particles of any shape and size are effortlessly achievable. Our research efforts include making PRINT particles composed of albumin and albumin 0.5 wt % siRNA, and Abraxane, the gold standard therapeutic used in metastatic breast cancer.

## Iontophoretics

Numerous diseases are localized in the body; not the least of which are cancer and cardiovascular disease. Though exquisite drug therapies have been developed, they cannot reach their potential without providing a sustained, therapeutically relevant dose at the specific site of disease. PRINT™ is a versatile technology platform that allows for the production of monodisperse, shape and size specific nanoparticles, which can easily encapsulate therapeutics and be tailored for a variety of release profiles thereby providing local, sustained, and controlled therapeutic dosing. To deliver these particles to specific sites of disease we have combined this technology with iontophoresis, which uses electrical fields to enhance the movement of charged molecules and particles into tissue. We are investigating several embodiments of this therapeutic system; one in specific for the intraluminal application of nanoparticles to prevent bypass failure.

## Functional, Bioabsorbable Nanoparticles via PRINT™

Poly(L-lactic acid) (PLLA) and Poly(lactic acid-co-glycolic acid) (PLGA), first used for sutures, have more recently received attention as drug delivery matrices. PLLA and PLGA are bioabsorbable polymers that degrade hydrolytically at physiological pH and are then metabolized by the Krebs cycle. This makes them very attractive for drug delivery because there are no residuals after treatment. Both PLLA and PLGA particles are, for the most part, currently fabricated by either emulsion solvent evaporation methods or supercritical processing



**Figure 5: 2 μm cubic PLLA particles fabricated using PRINT.**

techniques. With these methods, spherical particles are generated and while there is some basic control over size, the particles created are disperse. Encapsulation of cargos is also a challenge with these methods because the cargo typically has some affinity for the secondary phase and partitions out of the polymer phase before solidification. Finally these methods tend to be water and/or solvent intensive, which is harmful for the environment and increases processing costs. Using the PRINT™ technology platform, we have developed new, cleaner methods for making bioabsorbable particles with complete control over size and shape. These particles can be surface functionalized and can easily encapsulate a wide variety of cargos. Currently we are looking at these particles for delivery of biological cargos such as antisense oligonucleotides.