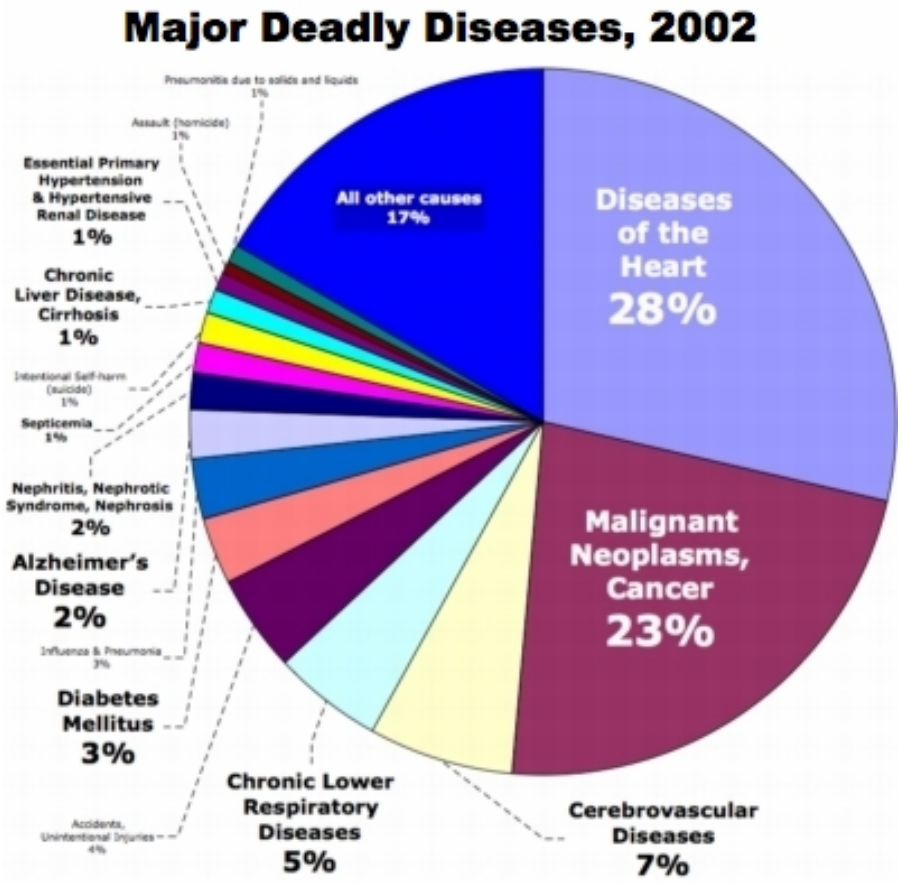
Nanodevices for Treatment of Hyperlipidemia

Vladimir Reukov, Daniel Carey and Alexey Vertegel

Bioengineering Department, Clemson University, SC, USA

Atherosclerosis

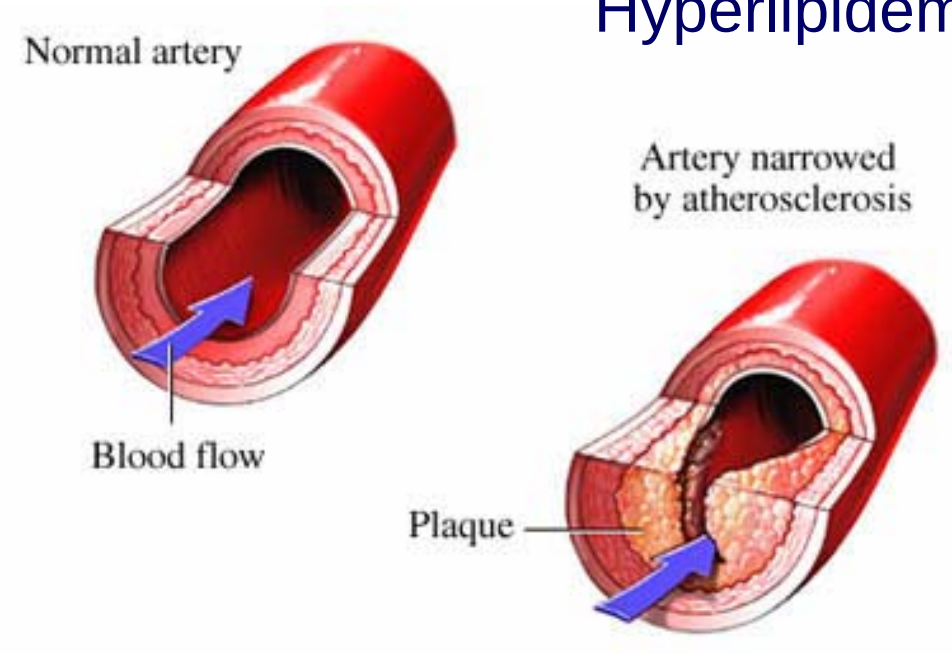
Atherosclerosis is the leading cause of death in the developed world, is responsible for more than half of the yearly mortality in the United States.



Source: "Deaths: Final Data for 2002", Kenneth D. Kochanek, M.A., Sherry L. Murphy, B.S, Robert N. Anderson, Ph.D., and Chester Scott, Division of Vital Statistics, Center for Disease Control and Prevention, U.S. Dept. of Health Services, National Vital Statistics Reports, Vol. 53, No 5, October 12, 2004.

http://www.level1diet.com/inflammation.html

Hyperlipidemia



One of the most important risk factors for atherosclerosis is **hyperlipidemia**, characterized by elevated blood levels of low-density lipoproteins (LDLs). Excessive LDLs can accumulate in the vicinity of a small vascular injury, forming so-called fatty streaks, the first manifestation of atherosclerotic plaque.

http://www.upmc.com/HealthManagement/ManagingYourHealth/HealthReference/Diseases/?chunkiid=11767

Hyperlipidemia

Hyperlipidemia is often related to the **lack of LDL-receptors** in hepatocytes, which consequently couldn't recognize low-density lipoproteins and make impossible further metabolism of "bad" cholesterol located in LDLs.

Drug treatment

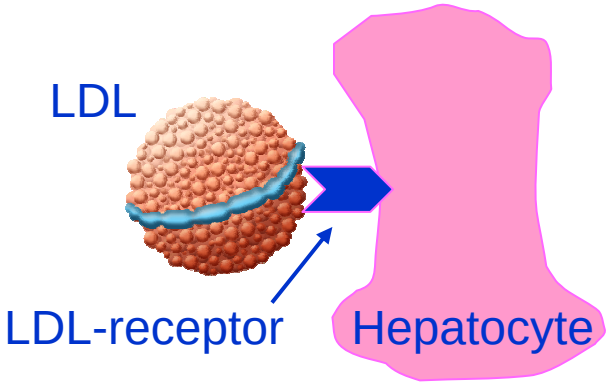
Four major classes of medications are used to treat hyperlipidemia:

- HMG-CoA reductase inhibitors (statins)
- Bile acid sequestrants
- Nicotinic acid
- Fibric acids

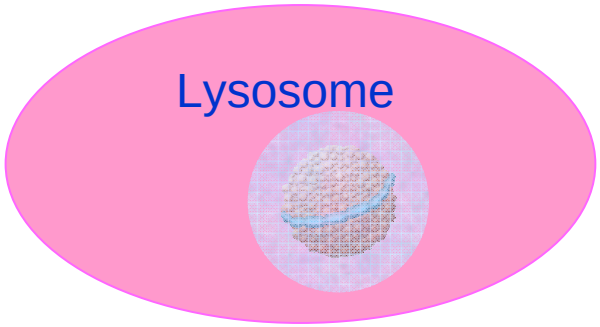
Such treatments result in up to 50% decrease in blood LDL level.

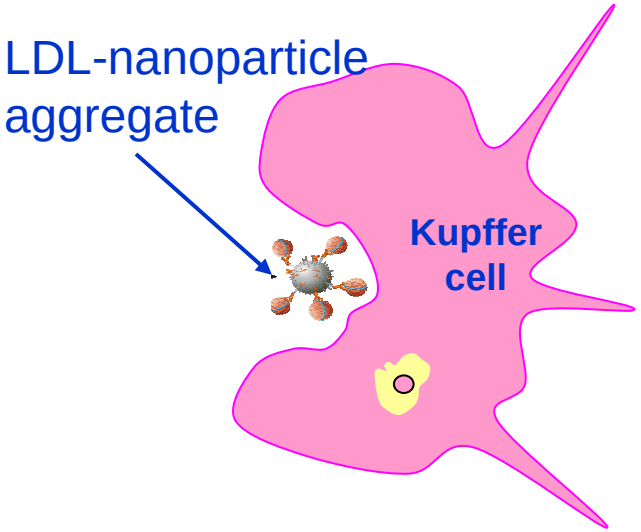
However, in many cases it is desirable to further decrease this index (an average LDL level in an adult Western man is 7-8 times higher than that of a newborn infant; the latter is often considered the optimum).

This necessitates development of new strategies for periodically cleaning up excessive LDLs.



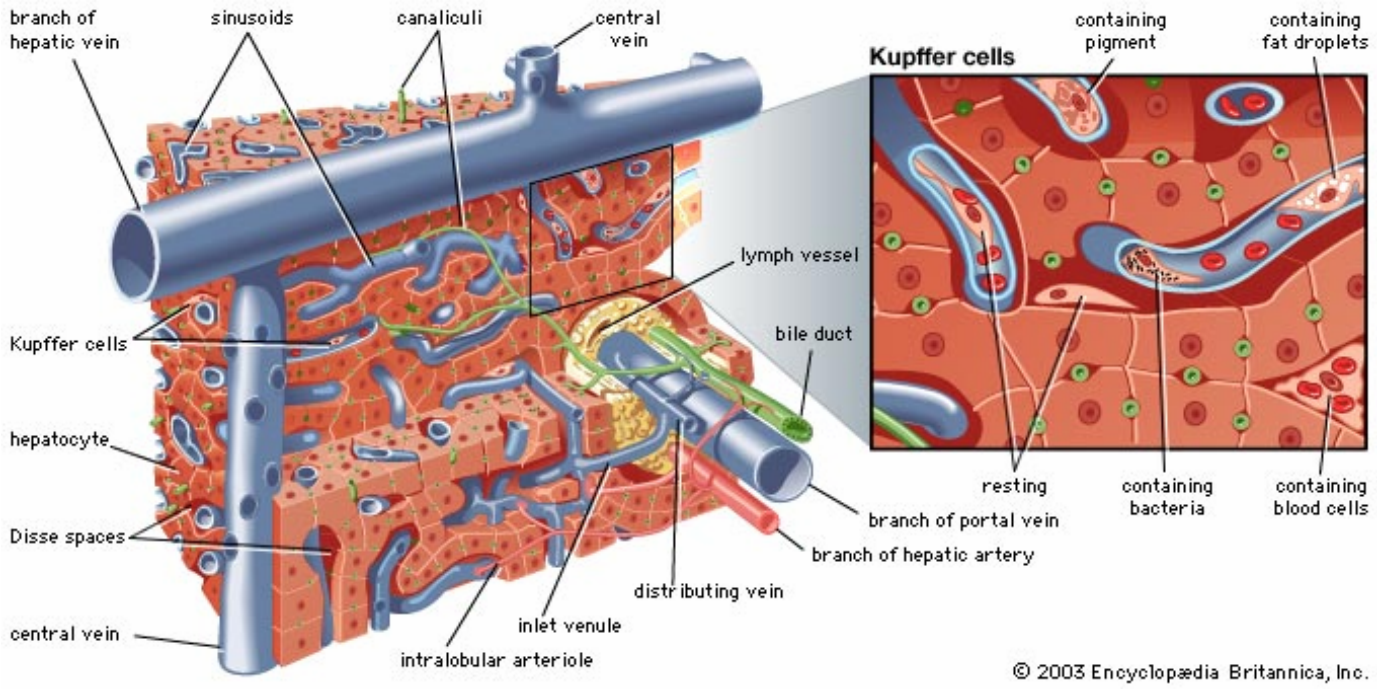
During endocytosis, LDLs fuse with lysosomes in hepatocytes and are digested by lysosomal acid hydrolases.





Polymeric nanoparticles are known to be actively uptaken by Kupffer cells in liver with the half life of several minutes. We propose to use biodegradable polymeric nanoparticles to enhance delivery of low-density lipoproteins to liver.

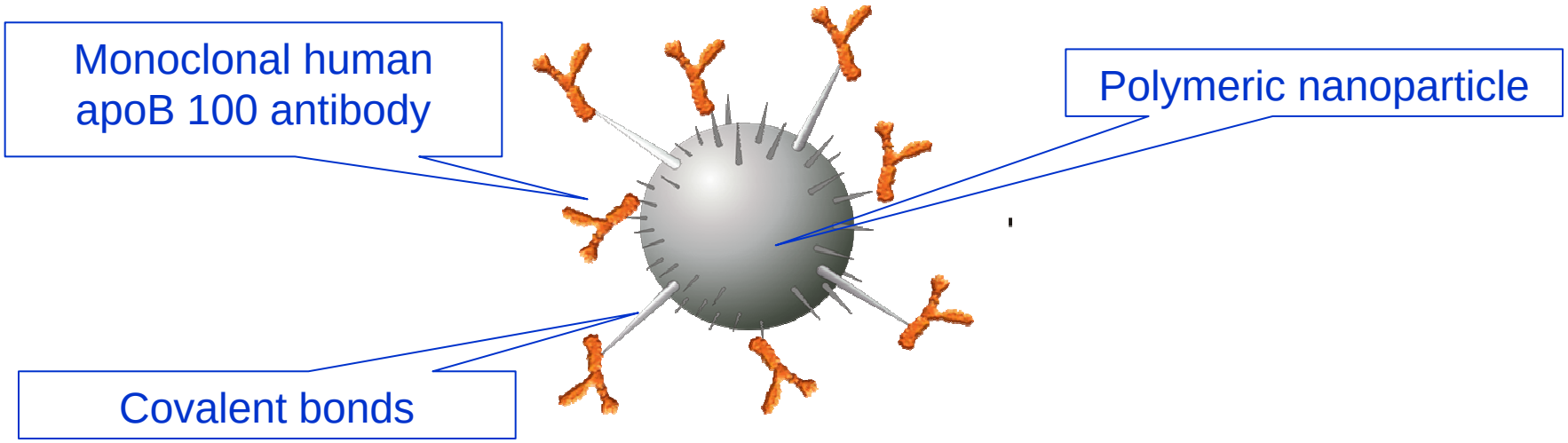
Development of new strategies for **periodically cleaning up** excessive LDLs.



Kupffer cells are specialized macrophages located in the liver that form part of the reticuloendothelial system (aka: mononuclear phagocyte system).

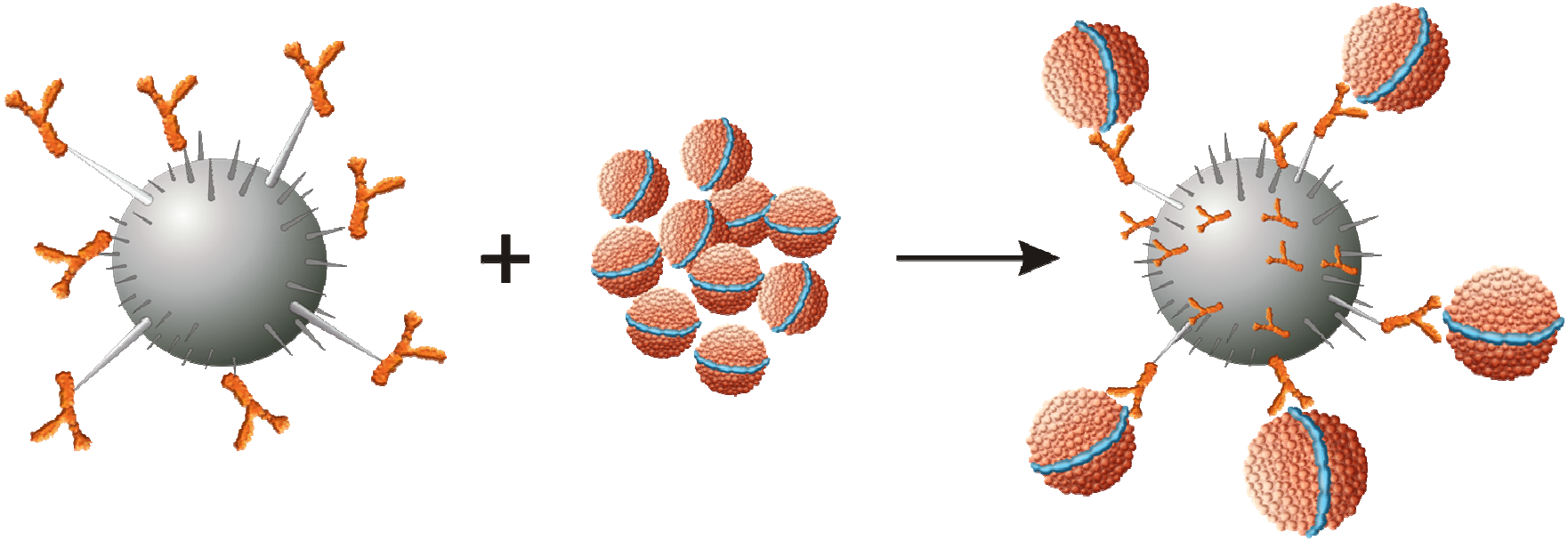
http://www.britannica.com/ebc/art-60419/Microscopic-structure-of-the-liver-Liver-cells-or-hepatocytes-have

Nanodevice



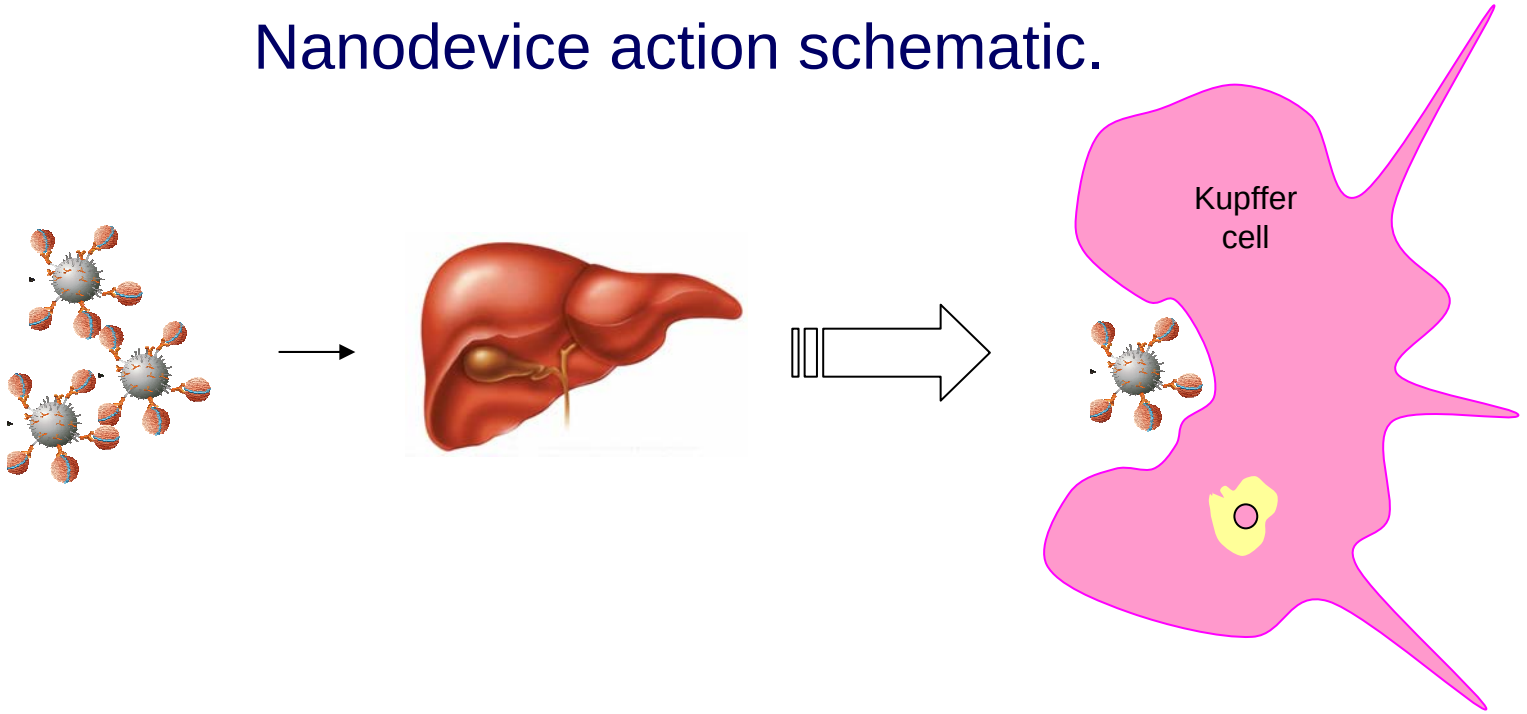
We propose to use monoclonal antibody to a major LDL component, human apolipoprotein B-100, covalently attached to biocompatible polymeric nanoparticles to remove excess of LDL from blood.

Nanodevice action schematic.



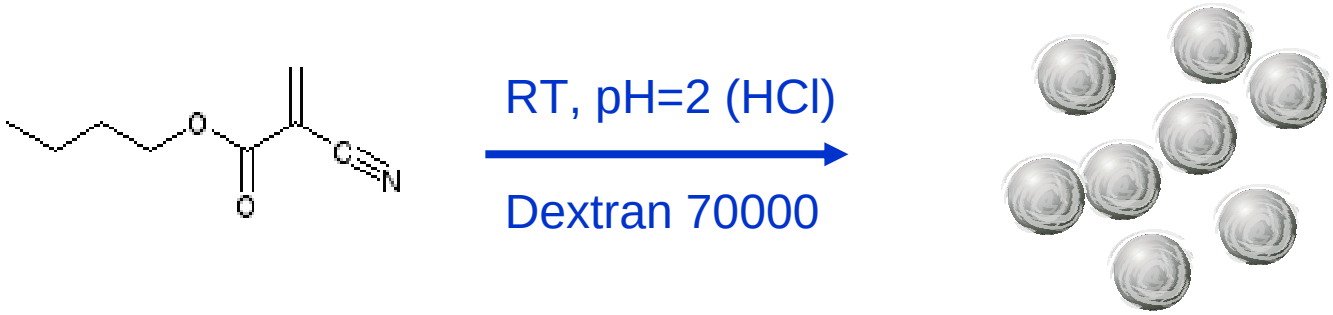
After injection into circulation, such nanoparticles will adsorb LDLs via antibody-antigen interactions

Nanodevice action schematic.

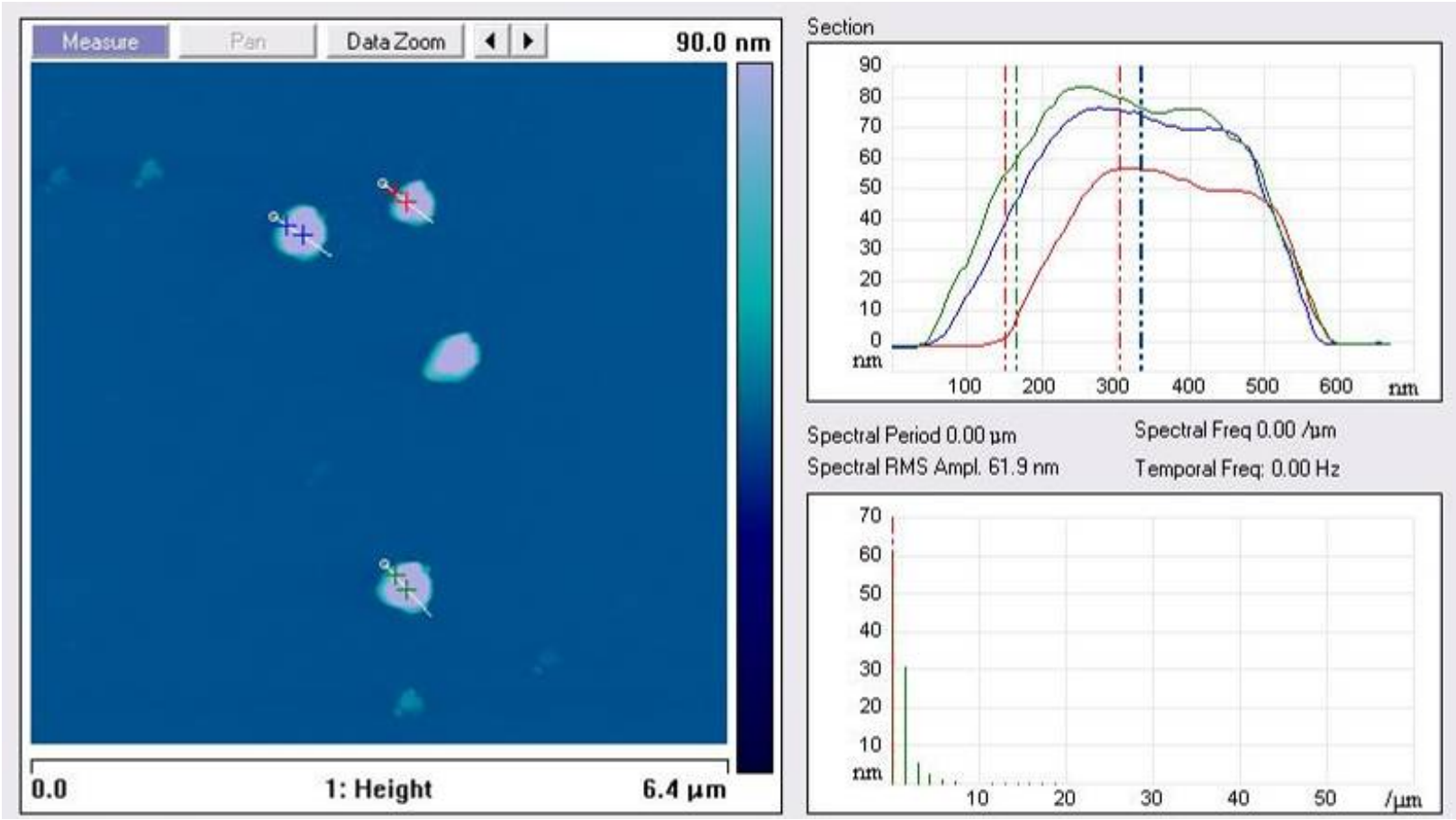


These complexes will be quickly uptaken by Kupffer cells. The Kupffer cells attempt to digest the uptaken material by directing it to lysosomes, thus providing a biochemical pathway similar to that for normally uptaken LDLs.

PBCA nanoparticles synthesis.



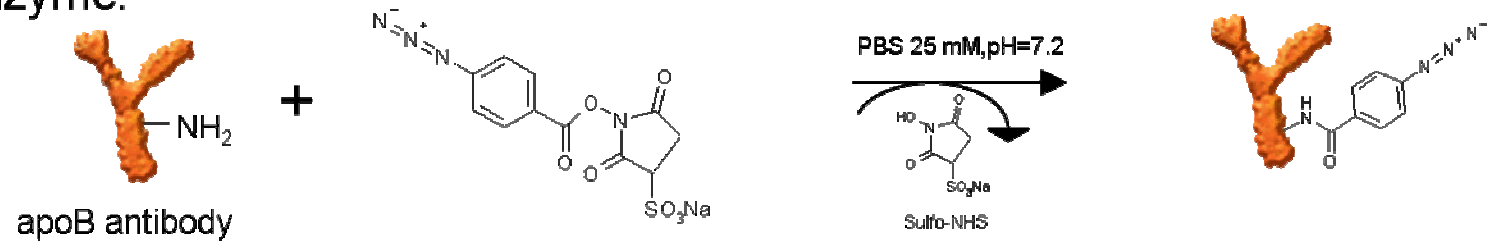
PBCA nanoparticles were prepared by PBCA nanoparticles were prepared by polymerization in acidic medium (HCl, pH 2) in presence of Dextran 70000 as a stabilizer.



Tapping mode AFM image of PBCA nanoparticles prepared at pH=2.0 using 1 % Dextrane 70,000 as a stabilizer. Image size is 6 µm x 6 µm. Z scale is 90 nm; height of the nanoparticles is 80±10 nm.

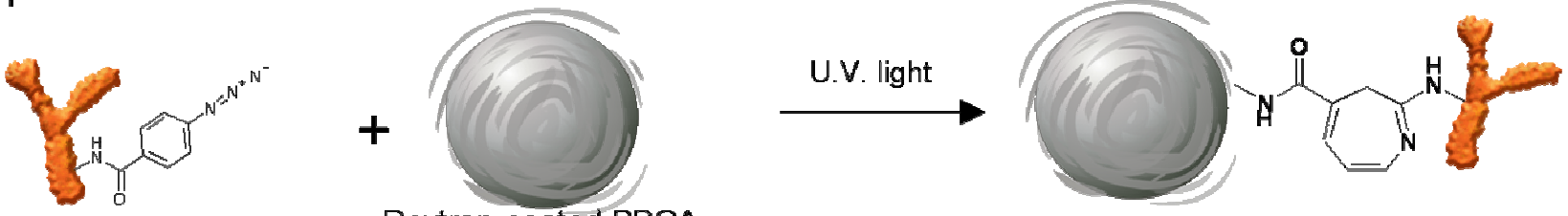
Schematic of the protein attachment to PBCA nanoparticles.

1. NHS-ester end of sulfo-HSAB cross-linker reacts with the amine groups of the enzyme.



apoB antibody

2. After photolysis, the phenyl azide group can react with nucleophile containing compound to create a covalent bond.



Dextran-coated PBCA nanoparticle

Efficiency of protein binding to PBCA nanoparticles

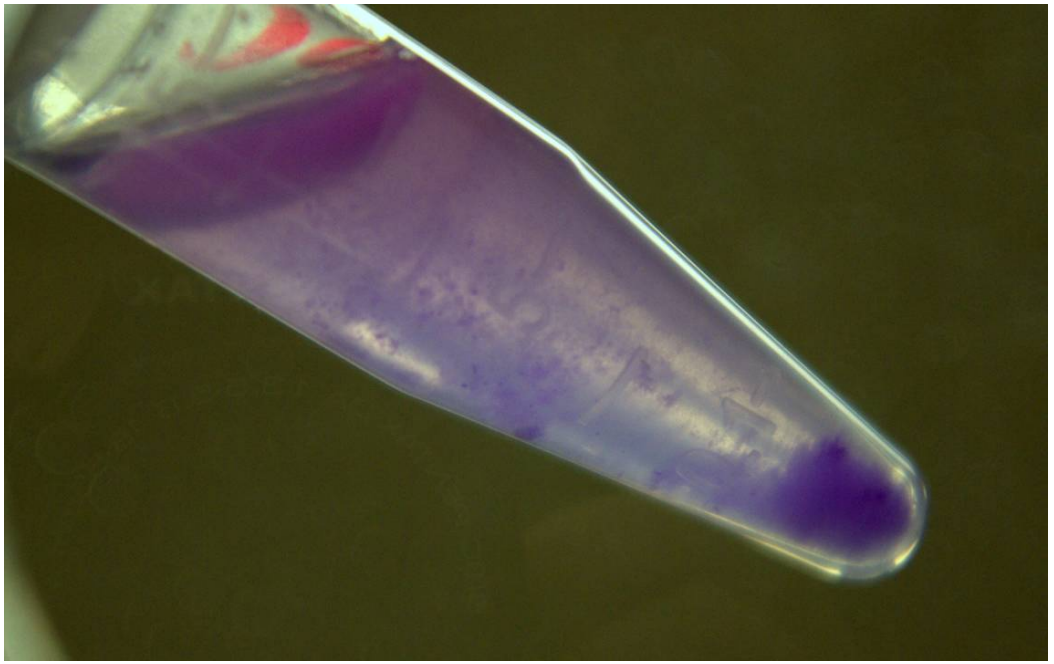
NP:Antibody ratio	Binding efficiency, %
1:100	9.34
1:50	10.66
1:25	11.25
1:10	13.86

Yield of enzyme covalent binding was estimated by comparing fluorescence intensity of the initial solution and resuspended nanoparticles. Antibody demonstrated constant binding yield of ~11±2% independednt of its initial concentration

Immunoprecipitation of low-density lipoprotein solution with Ab-nanoparticle conjugates.

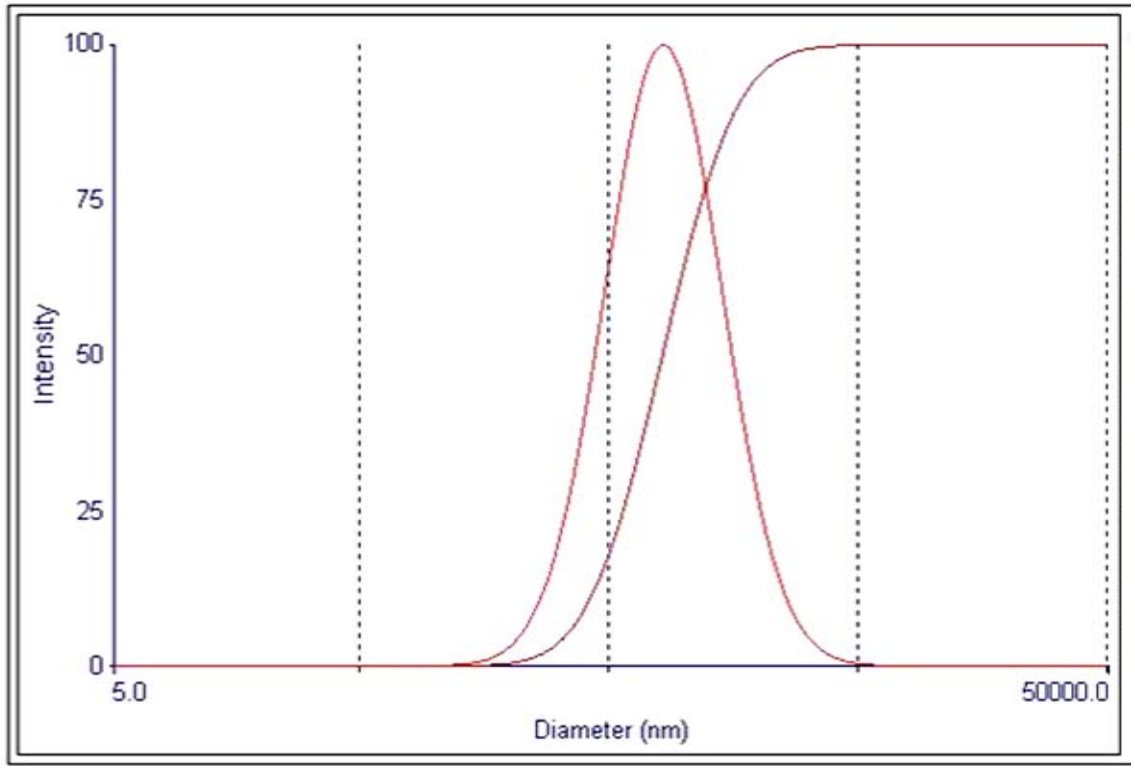
To study binding of nanoparticles to LDL, LDLs were labeled by AlexaFluor®594, mixed with the suspension of antibody-nanoparticle conjugates, and incubated for 30 min at 37°C.

LDL concentration was 500 mg/dL (normal adult range 62 – 130 mg/dL).
It was found that LDL concentration in the supernatant dropped almost 10-fold indicating that at least 90% of LDLs were adsorbed onto nanoparticles.



pbca_apoB_ldl (Combined)

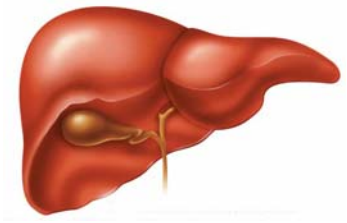
Effective Diameter:	**818.0 nm**
Polydispersity:	**0.337**
Avg. Count Rate:	**21.8 kcps**
Baseline Index:	**0.0/ 50.01%**
Elapsed Time:	**01:40:00**



Diameter of nanoparticle conjugates increases eight-fold after immunoprecipitation (particle size analysis using Dynamic Light Scattering).

Primary rat Kupffer cell culture

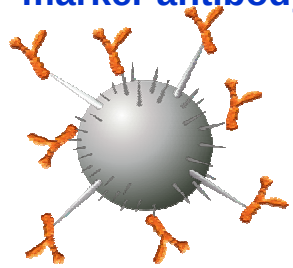
Rat liver



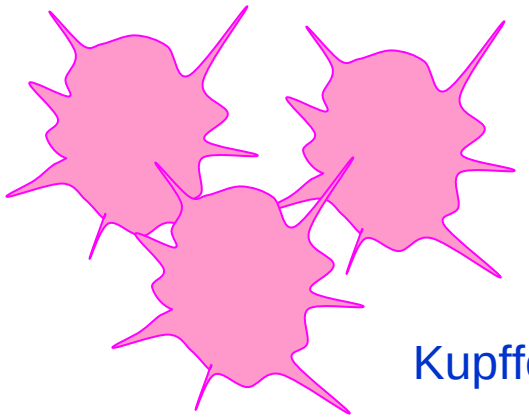
Collagenase, 37°C

Parenchymal and non-parenchymal cells mixture

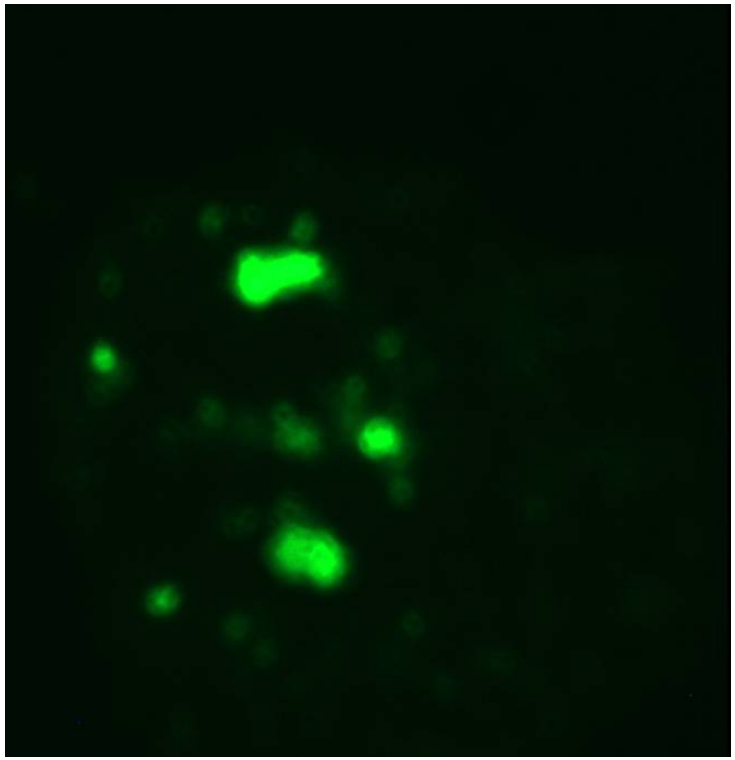
Magnetic beads with CD68/Macrophage marker antibody

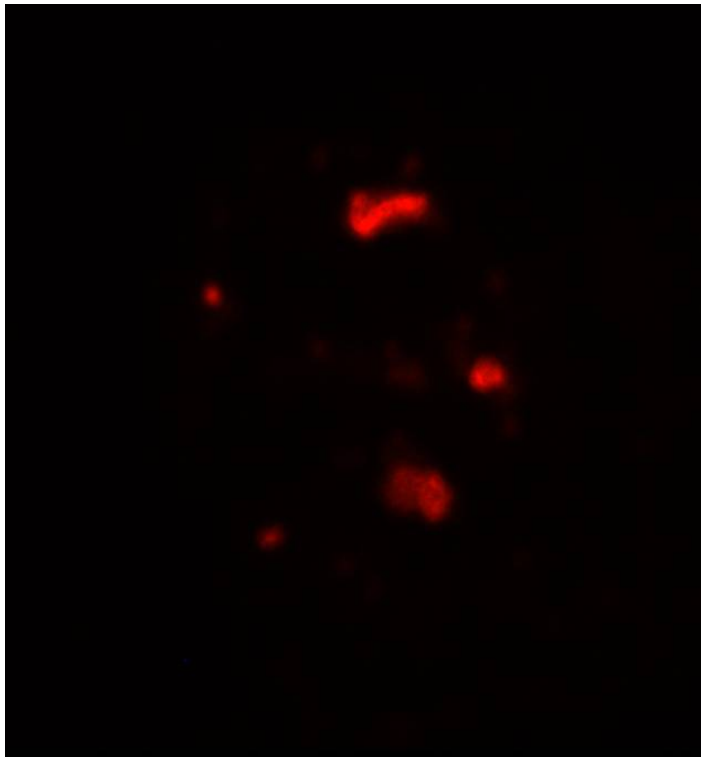


Cells were isolated by incubating mixed non-parenchymal cells, which were obtained by collagenase digestion of the rat liver, with CD68-conjugated superparamagnetic polystyrene beads.



Kupffer cells





Kupffer cells stained with FITC labeled CD68 (left) and treated with **f**luorescently-labeled LDL-nanoparticle complexes (right).

Conclusions

In conclusion, we found that antibody to human apolipoprotein-B can be covalently attached to 100 nm PBCA nanoparticles without considerable changes in receptor-binding ability.

Antibody-nanoparticle conjugates reacted with low-density lipoprotein forms ~800 nm aggregates and such aggregates can be uptake by Kupffer cells.

Future work will focus on utilization of more biodegradable nanoparticles (such as PLA or polyketals) and nanodevices uptake by liver and their toxicity in animal models.

Acknowledgments

Supervisor: Dr. Alexey Vertegel

Students:

John Barry
Daniel Carey
Rohan Satishkumar
Gary Lee Thompson

We also thank Cassie Gregory for cell culture assistance, Jiro Nagatomi for possibility to use the microscope.

Funding was provided by Clemson University Research Grant committee.