Development of Novel Optical Nanosensors for Biological Analysis

김 범 상

홍익대학교 화학공학과

Outline

- *Optical Nano/Biosensors*
- *PEBBLE Nanosensors*
- *Design of PEBBLE Nanosensors for Detecting Oxidative Stress*
- *HRP-loaded PEG Hydrogel Nanospheres*

Optical Nano/Biosensors

Optical sensor

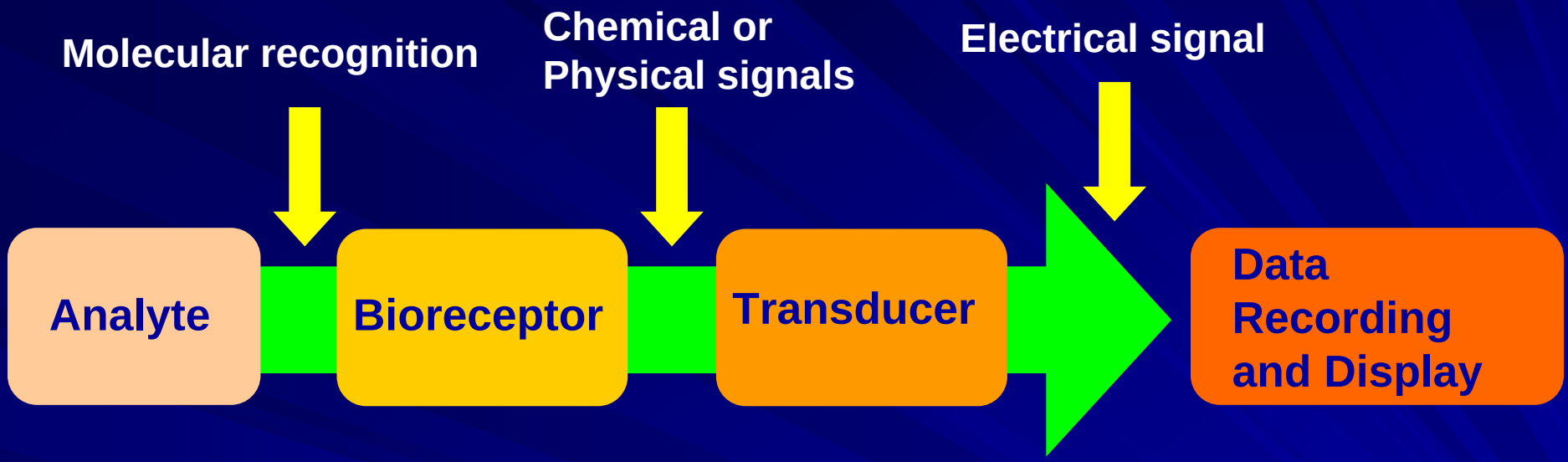
A sensor which is generally based either on measuring an intensity change in one or more light beams or on looking at phase changes in the light beams by causing them to interact or interfere with one another

Nanosensor

A sensor having all dimensions less than 1000 nm

Biosensors

- ✓ A device for detecting physiological signals
- ✓ A device that consists of a biological recognition system (bioreceptor) and a transducer



Bioreceptor

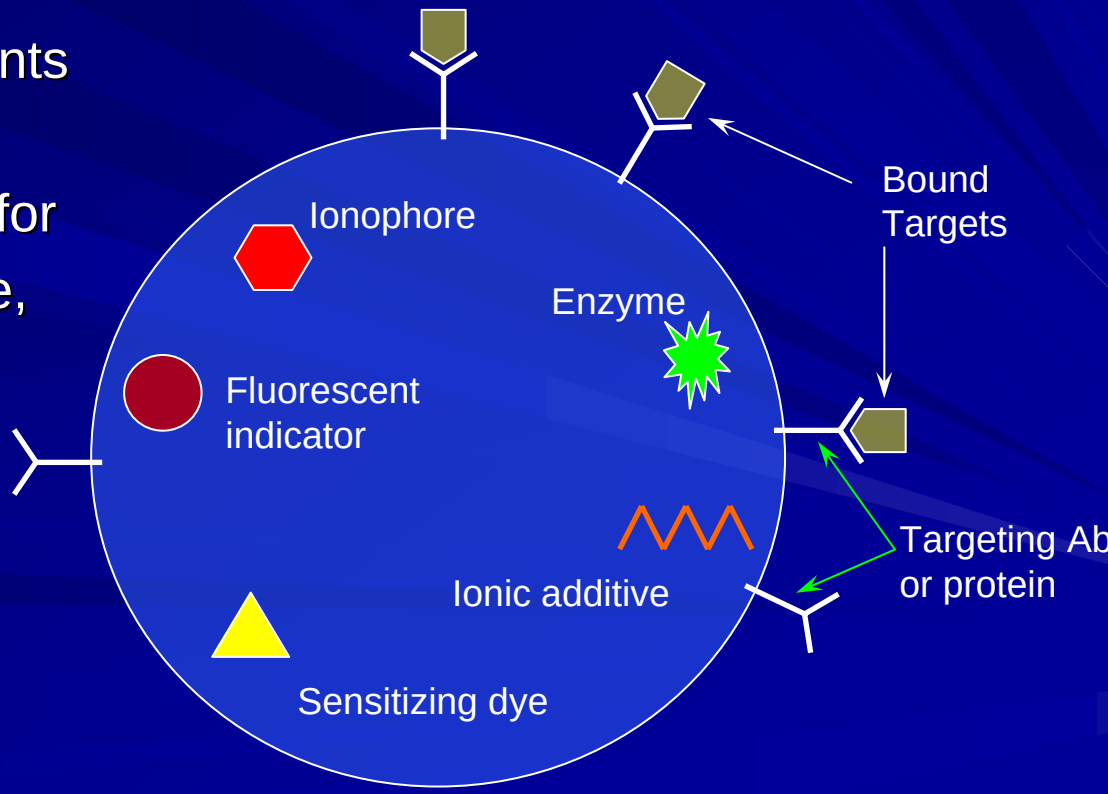
1. Antibody / antigen
2. Enzymes
3. nucleic acids / DNA
4. Cellular structures / cells
5. Biomimetic

- ✓ good sensitivity
- ✓ good specificity
- ✓ good biocompatibility

PEBBLE Nanosensors

What is the PEBBLE nanosensors?

- PEBBLE : Probes Encapsulated By Biologically Localized Embedding
- Nano-scale optical sensors consisting of sensor elements entrapped in a chemically inert and biofrendly matrix for analyte monitoring in viable, single cells.



General cellular analysis technique

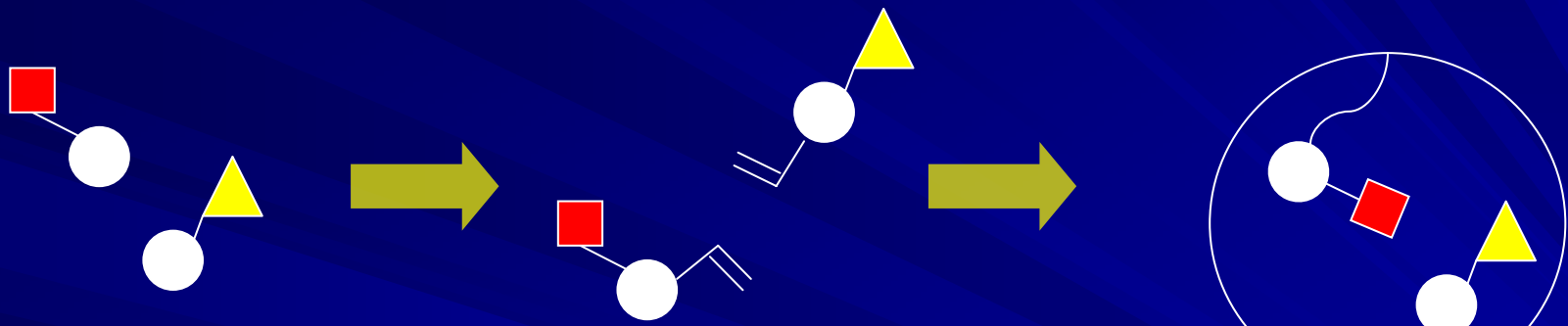
Using molecular fluorescent probes (fluorescent dyes)

- ✓ cytotoxicity
- ✓ dying all cell region
- ✓ heterogeneous intracellular distribution within the cell

Advantages of PEBBLE nanosensors

- ➢ Protection of the intracellular environment from any toxic effects of the sensing elements
- ➢ Protection of the sensing elements from cellular interferents such as protein binding
- ➢ Creation of multi-sensing schemes using more than one sensing element

Synthesis of Polymer Spheres having Fluorophore-labeled Bioreceptors



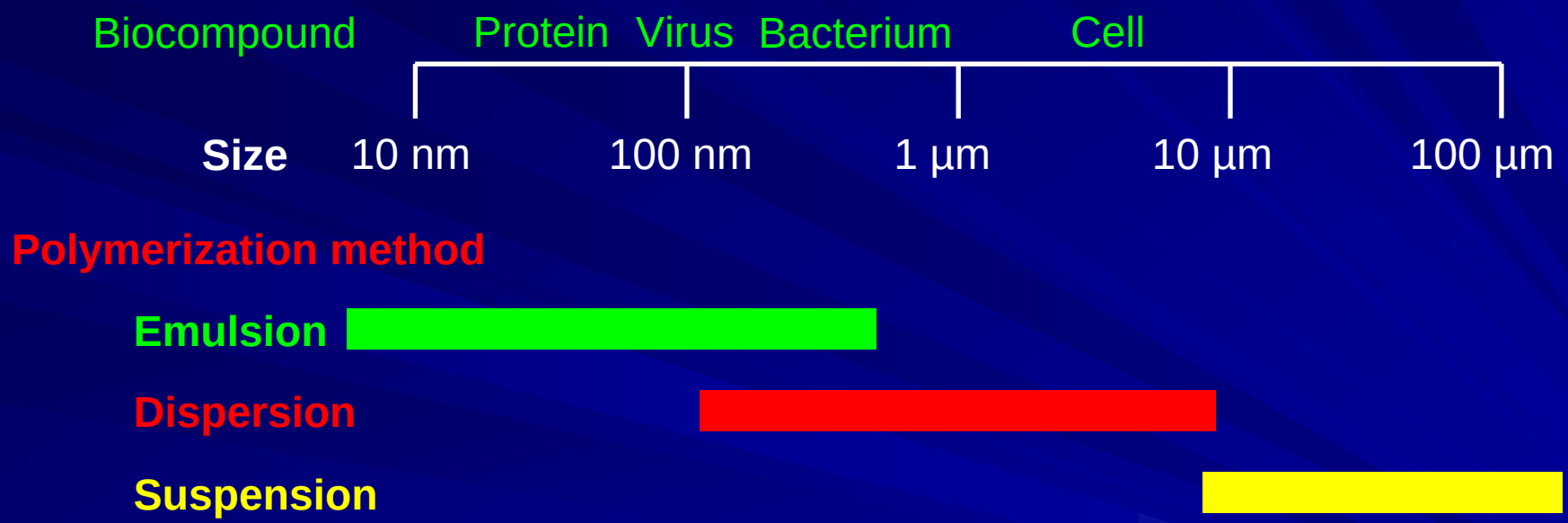
fluorophores

Bioreceptors

Polymer hydrogel (matrix)

Reactive group

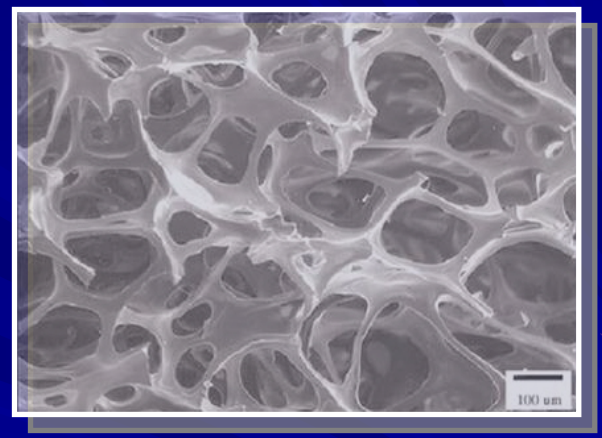
Polymerization techniques for synthesis of polymer spheres



- Maintain the activity of the biorecpetor during the reaction (polymerization)

Polymer Hydrogels as a Matrix

- Should have a good biocompatibility
- Should make analytes transfer through the matrix
- Should incorporate the bioreceptors easily



Polymer Hydrogels

- Hydrophilic polymer networks
- Insoluble due to presence of chemical or physical cross-links
- Excellent biocompatibility
- Easy introduction of functional groups along the polymer chain by use of various monomers
- Use in wide range of biomedical and pharmaceutical applications

Design of Optical Nanoiosensor for Detecting Oxidative Stress

Oxidative stress

- Destruction caused by the reactive oxygen species such as free radicals and peroxide
- Oxygen free radicals are highly reactive with lipids, proteins, and DNA
- Is involved in many human diseases such as cancer, heart failure, neural diseases, general aging processes, and Alzheimer's diseases

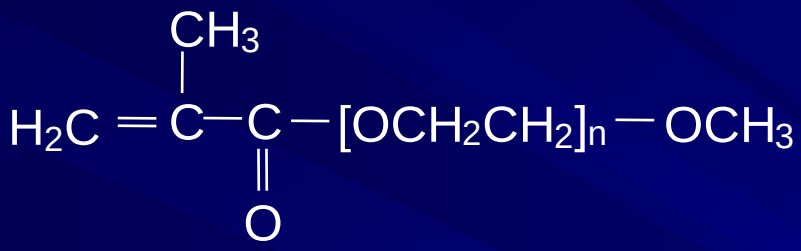
Sensing element: HRP (Horseradish Peroxidase)

- Most useful enzyme in biological essay, especially to detect H_2O_2
- [H2O2-HRP] can oxidize various chromogenic hydrogen donors
- Possible to detect oxidative stress in single cell or organisms
- Amplex red (colorless, nonfluorescent)

Matrix: poly(ethylene glycol) (PEG) hydrogels

- FDA approved materials
- Use in wide range of biomedical and pharmaceutical applications (artificial organs, contact lenses, biosensors, sutures, dental materials, and drug delivery systems)

HRP-loaded PEG Hydrogel Nanospheres



PEGMA (poly(ethylene glycol) monomethyl ether methacrylate)
n=400: monomer

TEGDMA (tetra(ethylene glycol) dimethacrylate) : crosslinking agent

- HRP were treated with PEG-NHS to introduce reactive groups
- Reverse emulsion photopolymerization (water in oil)
- UV initiation
- UV irradiation (reaction) time: < 30 sec

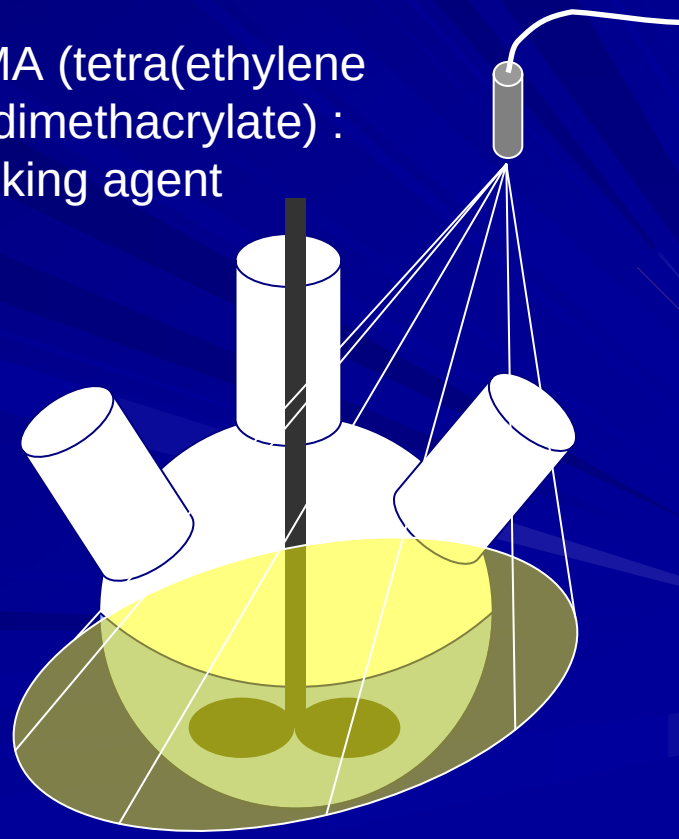
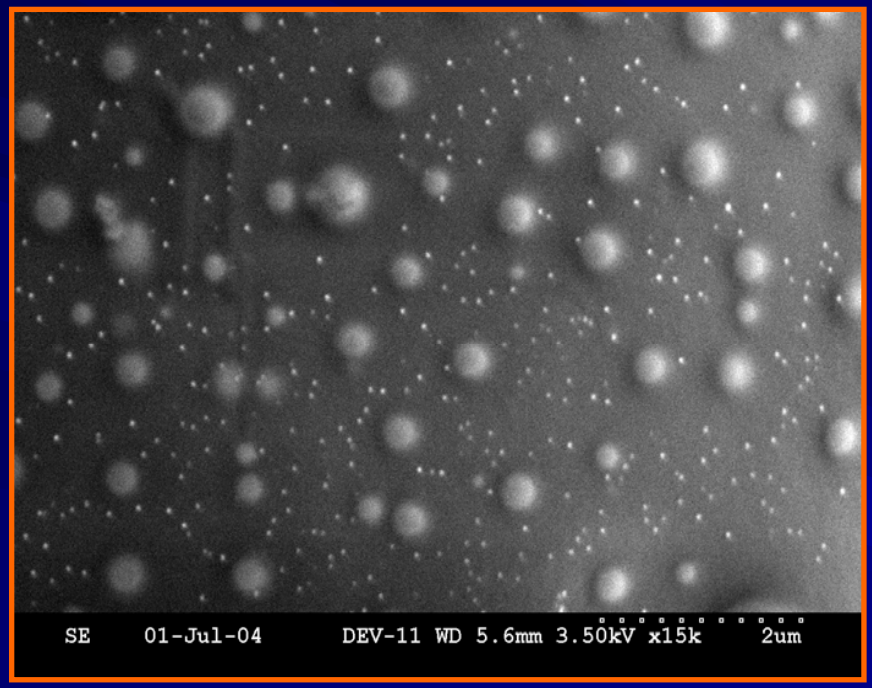
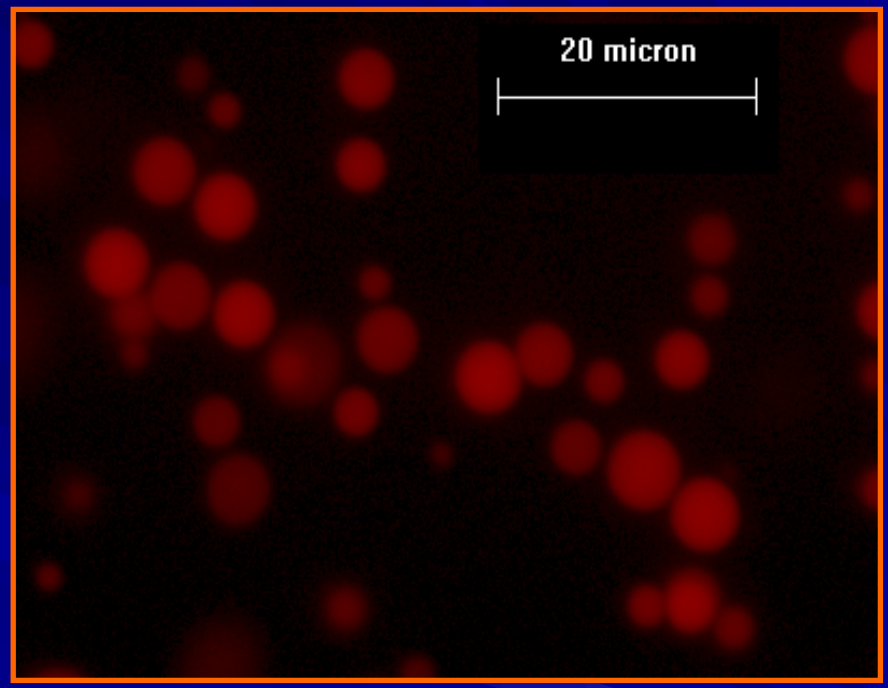


Image of HRP-loaded PEG Hydrogel Spheres

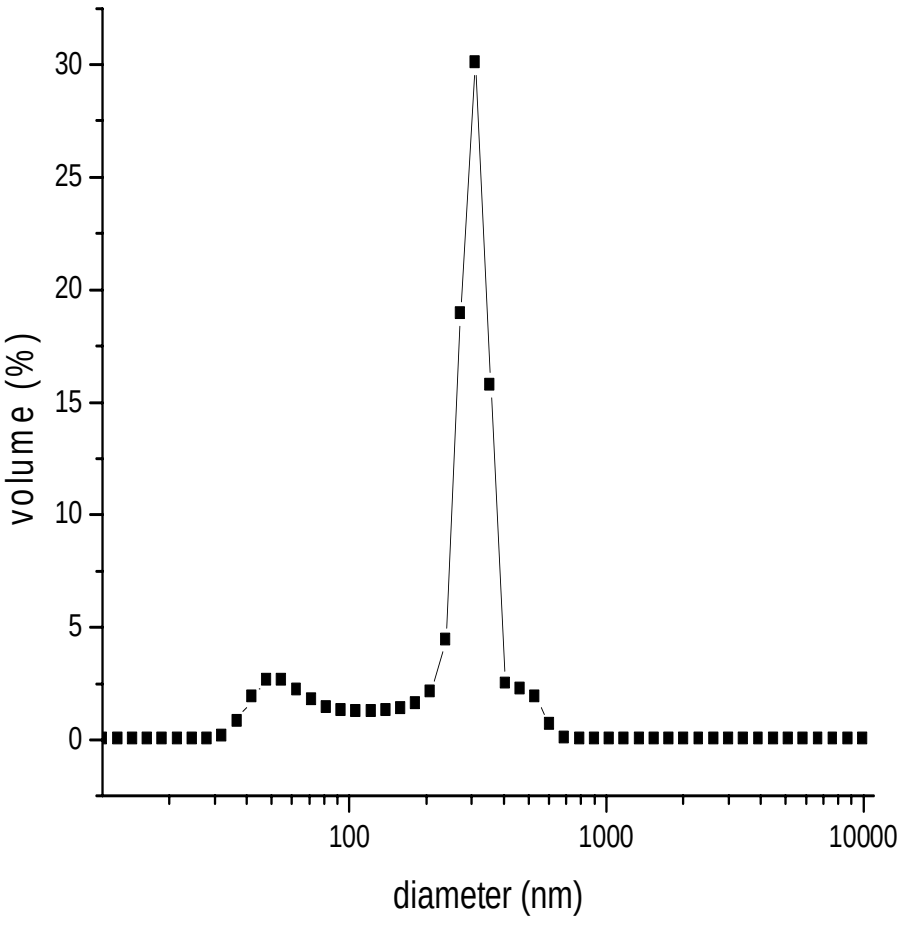


SEM image



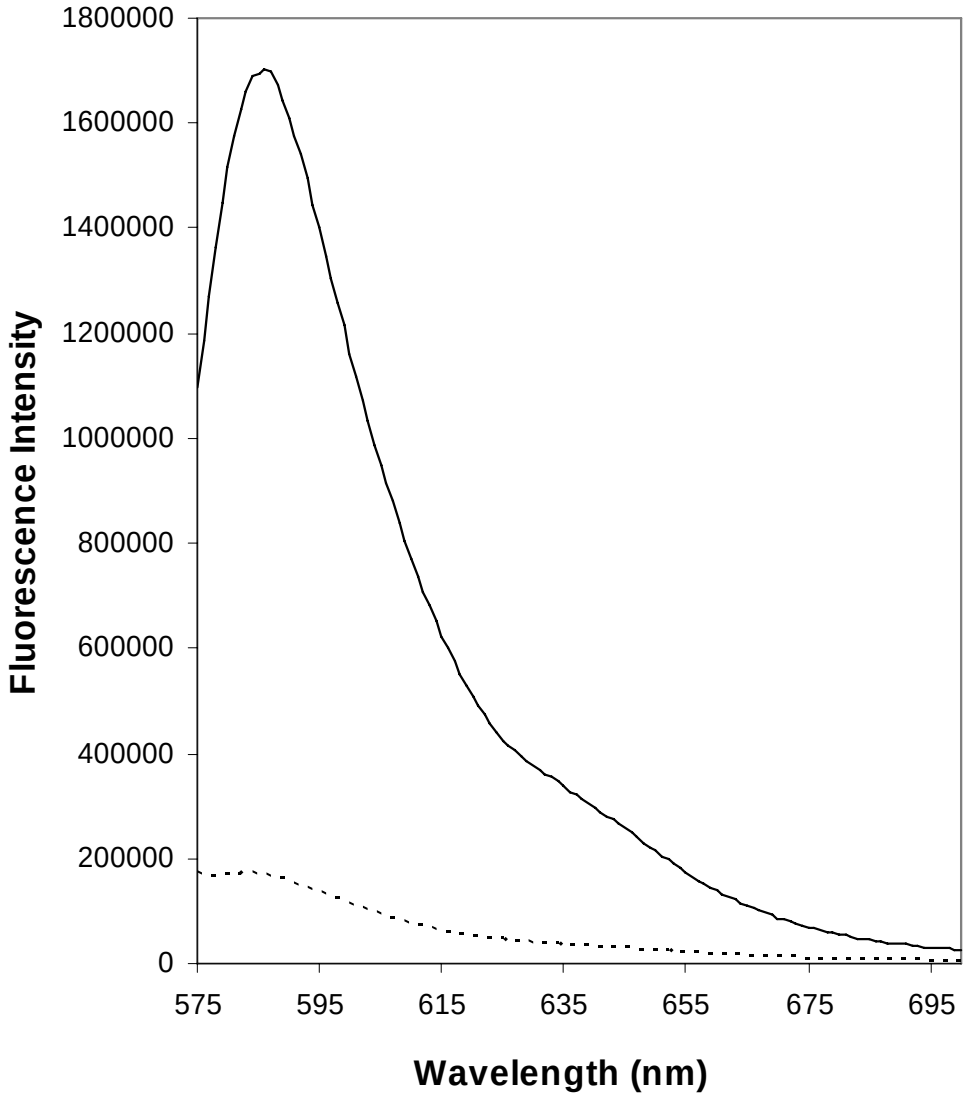
Fluorescence image after H_2O_2 and Amplex Red were introduced

Size and Size Distribution of HRP-loaded PEG Hydrogel Nanospheres



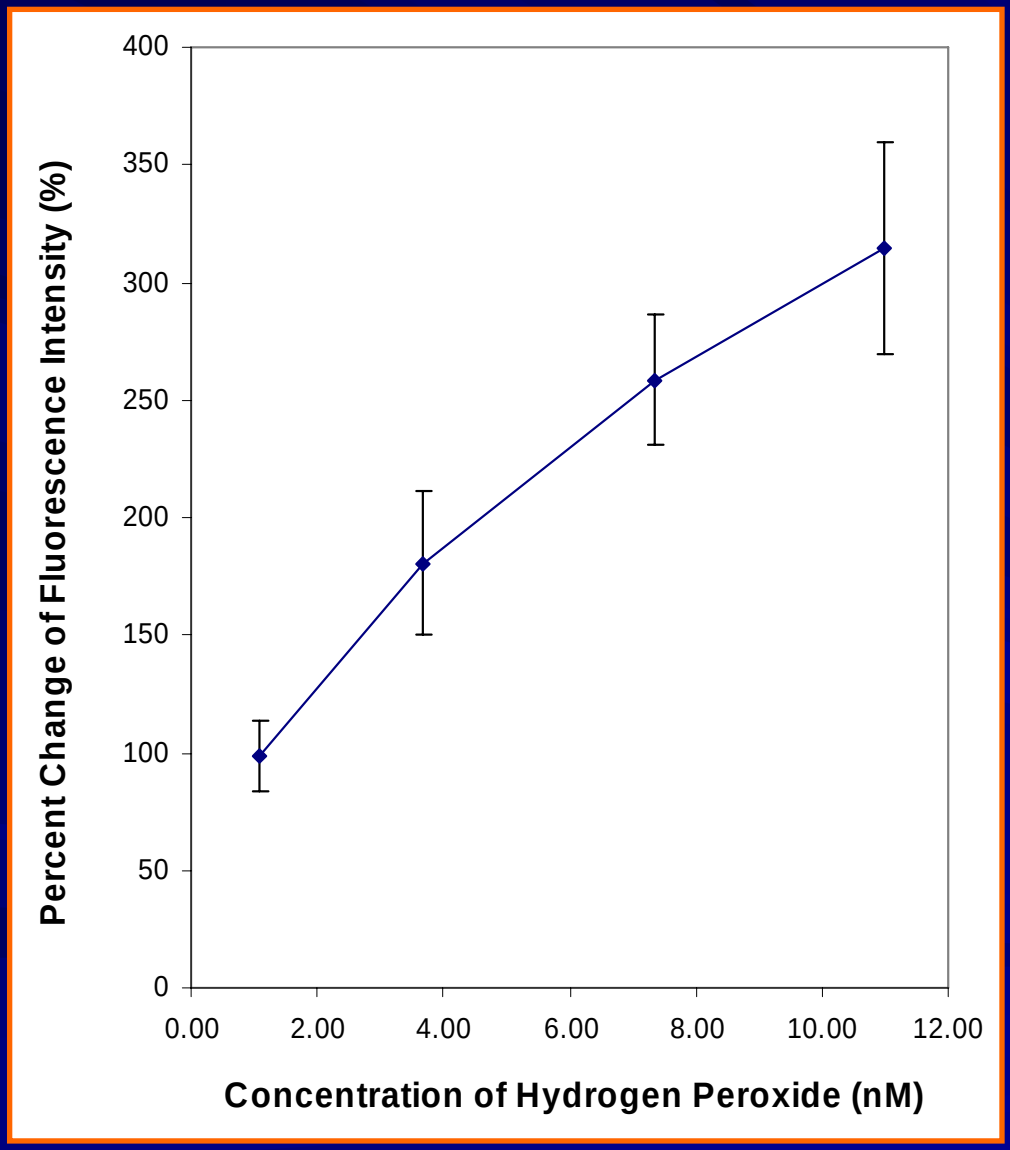
Average size = 300 ±50 nm

Fluorescence Spectra of the HRP-loaded Nanospheres



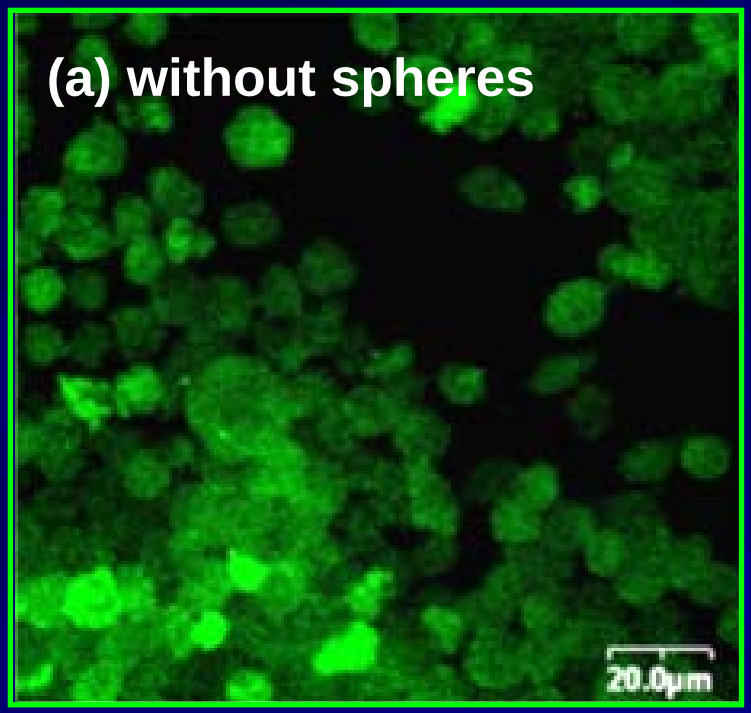
Fluorescence spectra of HRP-loaded PEG hydrogel sphere suspension; sphere suspension after adding Amplex Red (…) and sphere suspension after adding Amplex Red and H_2O_2 (—)

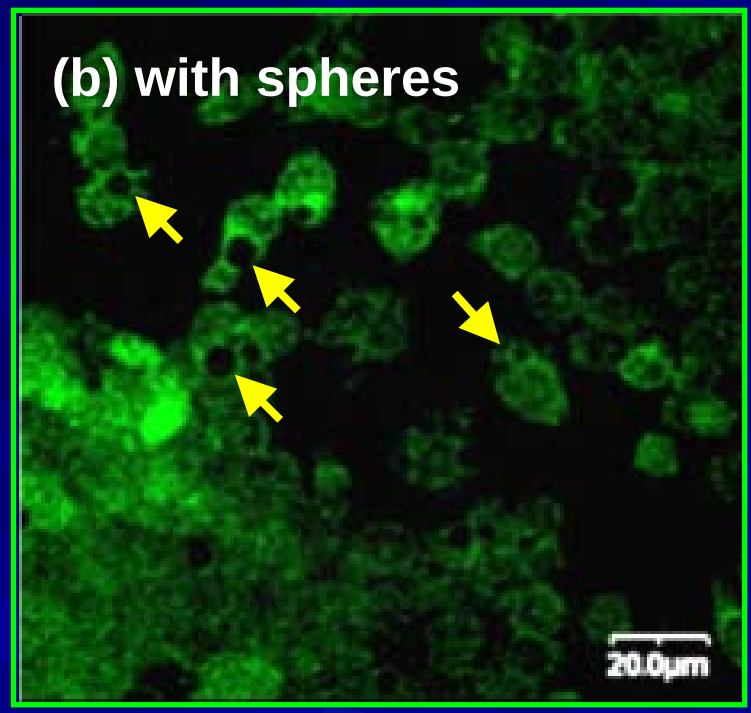
Hydrogen Peroxide Response of HRP-loaded Nanospheres



Change of fluorescence intensity of the HRP-loaded spheres as a function of the concentration of hydrogen peroxide in the presence of 1 µL of 10 mM Amplex Red.

Phagocytosis of HRP-loaded Nanospheres in Macrophage



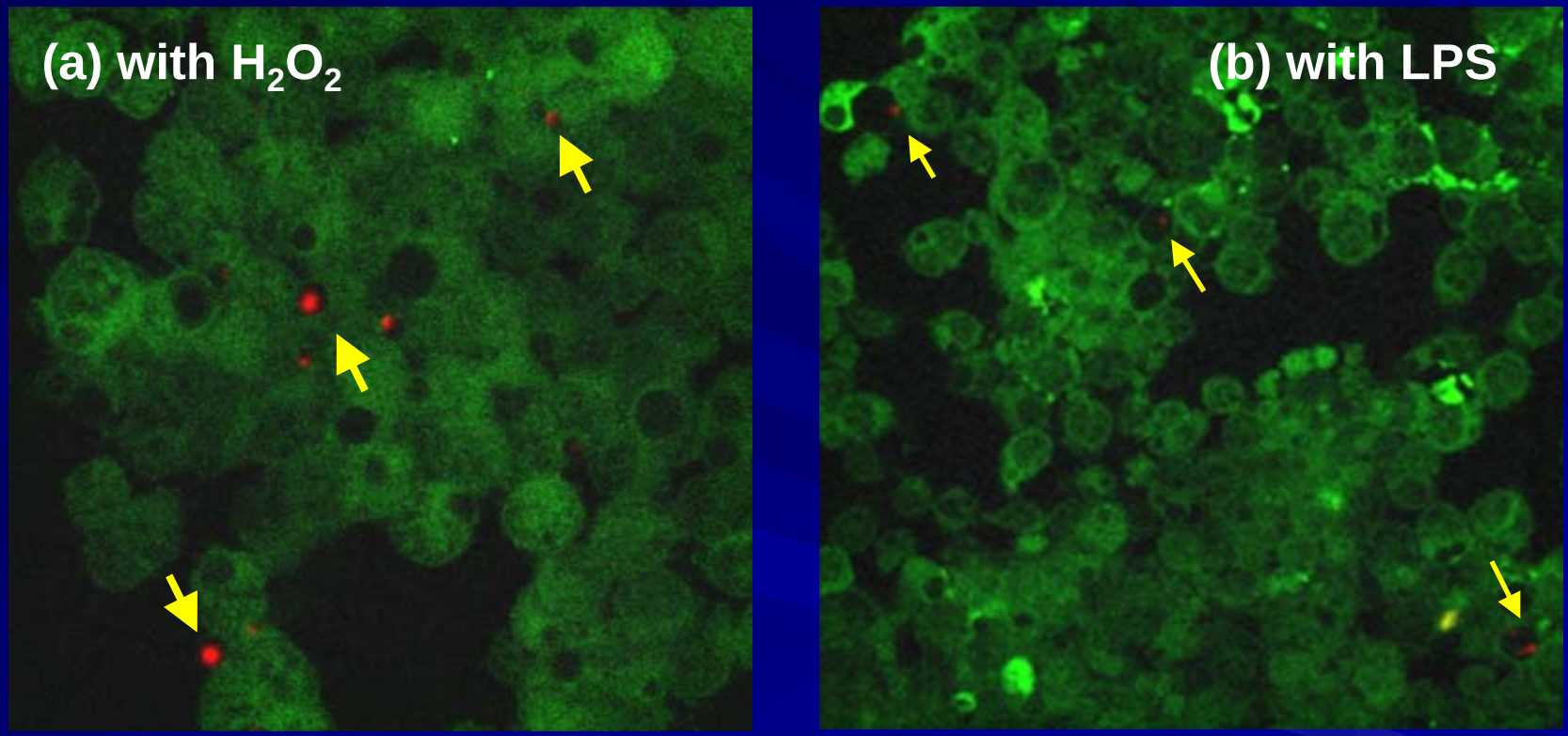


Confocal images of macrophages

(a) Cells were incubated for 24 hrs without HRP-loaded spheres.

(b) Cells were incubated for 24 hrs with HRP-loaded spheres. The phagocytic macrophages were observed.

Application of HRP-loaded Nanospheres In Vitro



Confocal images of macrophages treated with H_2O_2 and LPS

(a) Sphere-containing cells treated with H_2O_2

(b) Sphere-containing cells treated with LPS (lipopolysaccaride)