Novel nanoparticles for Tuberculosis chemotherapy

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Outline

- Challenges in TB treatment
- Nanotechnology based drug delivery
- Status of TB nano drug delivery project
- Networking and HCD
- Market trends

Main Challenges facing South Africa

- TB leading cause of death in SA
  - TB co-infection with HIV/AIDS
    - 68% of TB patients are HIV+
- Patient non-compliance to treatment
  - High dose and dose frequency

Anti-TB drugs

First line drugs	Mode of action	Metabolism	Daily dose	MIC_{90}
RIF (MW = 822.9)	Inhibits assembly of bacterial DNA and protein into mature virus	deacetylation	10-12 mg/kg	0.2 ug/ml
	Inhibits initiation of RNA synthesis			
INH (MW = 137.1)	Inhibits synthesis of cell wall components	Acetylation and hydroxylation	5 mg/kg	0.3 ug/ml
PYR(MW = 123.1)	Disrupts membrane potential	hydrolysis	25 mg/kg	8 ug/ml
	Inhibits membrane transport functions			
ETB(MW = 277.23)	Inhibits cell wall synthesis	Metabolised by hepatic enzymes	15-20 mg/kg	6 ug/ml

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Main Challenges facing South Africa

- TB leading cause of death in SA
  - TB co-infection with HIV/AIDS
    - 68% of TB patients are HIV+
- Patient non-compliance to treatment
  - High dose and dose frequency
  - Length of treatment (6-9 months)
  - Poor bioavailability
    - SAEs
- Emergence of drug resistance
  - MDR and
  - XDR-TB

Main Challenges facing South Africa...

- DOT's program
  - 53% cure rate
  - Logistics are impractical
  - Expensive program
  - Education
- Research to improve treatment is in progress
  - No new drug in the market in almost 50 years

Current TB drug R&D portfolio (TB Alliance)

Drug compound/ analogue/ derivative	Mechanism of action	Stage of development	Ref
Linezolid	Orally active anti-bacterial agents acting through inhibition of protein synthesis. Studied for treatment of MDR, it was the first antibiotic of its class to be approved	FDA approved	[39,69]
TMC-207	Inhibits ATP synthase	Phase I human trials	[69]
Moxifloxacin	Dual inhibition of ATP-dependant DNA-gyrase. Efficacy in pulmonary TB has already been reported.	Pivotal large scale phase III clinical trials in TB patients	[70-73]
PA-824	A prodrug that requires activation by a bacterial F420 dependant glucose-6-phosphate dehydrogenase and nitroreductase to activate components that then inhibit bacterial mycolic acid and protein synthesis. Active against MDR-TB.	Phase II clinical trials	[39,74]
OPC-67683	Chemically related to PA-824 with similar mechanism. Both drugs' mechanism has not been fully elucidated.	Clinical trials	[69,75]
Riminophenazines	Thought to inhibit energy metabolism, which is needed even in latent *M.tb*	Lead identification	[14]
Multifunctional molecules	Chemically linking multiple TB drugs to function as one, ensuring simultaneous optimal concentrations at the site of action	Lead optimization	[14]
Quinolone analogues	Inhibition of DNA-gyrase these 600 synthesized quinolones are being tested for an optimized quinolone	Preclinical	[14]
InhA inhibitors	Resistance to INH occurs as a result of KatG, the enzyme needed to activate INH, becoming nonfuntional. This new group of inhibitors will not need KatG, thus potentially eliminating INH-resistant strains	Lead optimization	[14]
Mycobacterial gyrase inhibitors	Novel inhibtor of DNA-gyrase. May be effective against fluoroquinolone-resistant strains.	Lead optimization	[14]
Pleuromutilins	New class of antibiotics able to selectively inhibit multiple steps in bacterial protein synthesis.	Lead optimization	[14]
Malate synthase inhibtors	Malate synthase thought to be a key enzyme used by *M.tb* to convert its food source to maintain its slow-growing, latent state. Hence inhibition may starve persisters and shorten therapy.	Lead identification	[14]

Main Challenges facing South Africa...

- DOT's program
  - 53% cure rate
  - Logistics are impractical
  - Expensive program
  - Education
- Research to improve treatment is in progress
  - No new drug in the market over 40 years
- Drug delivery system
  - address non compliance, toxicity, bioavailability and emergence of drug resistant strains
- Nanobased drug delivery system
  - Reduce dose, dose frequency and treatment time

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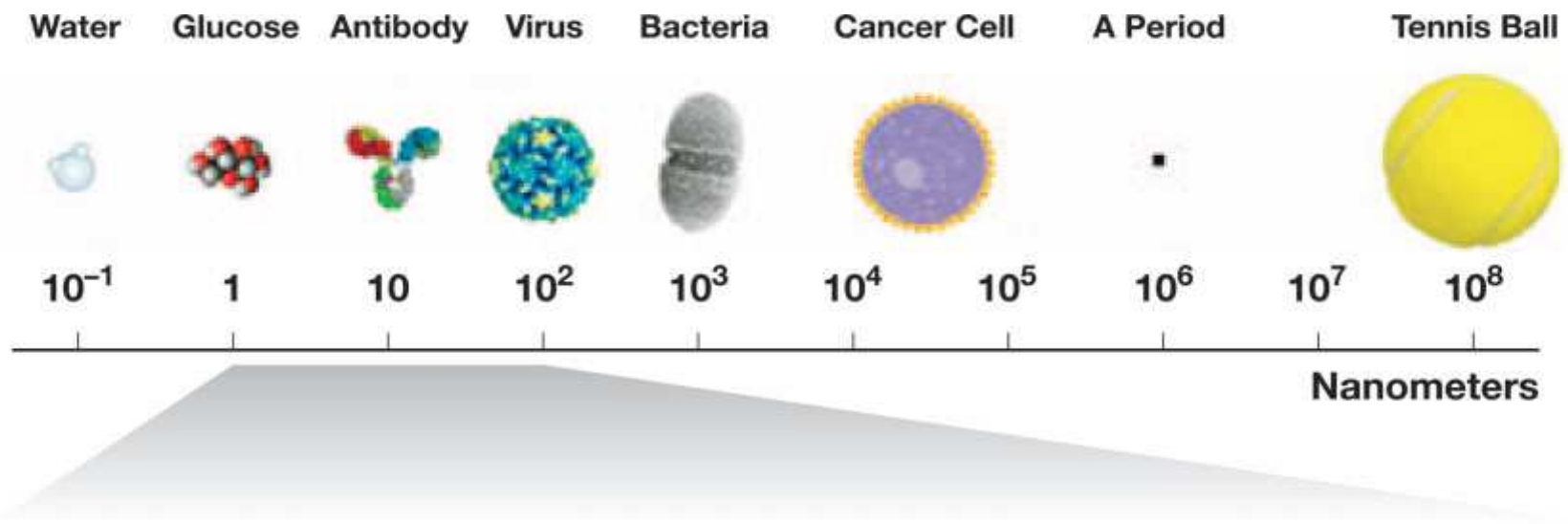
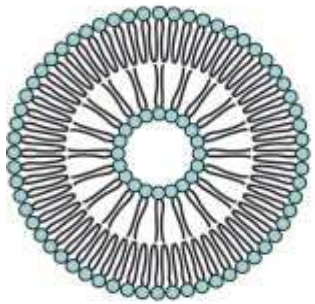
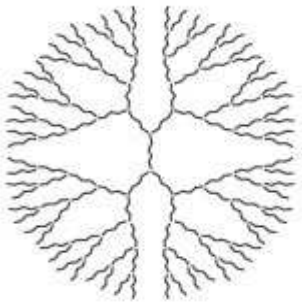
How small?

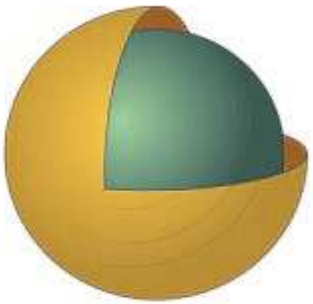




Liposome



Dendrimer



Gold Nanoshell

Quantum Dot

Fullerene

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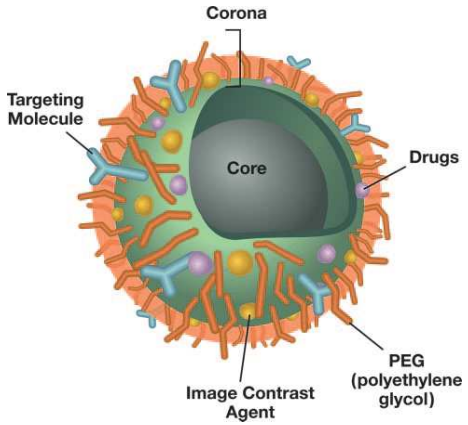
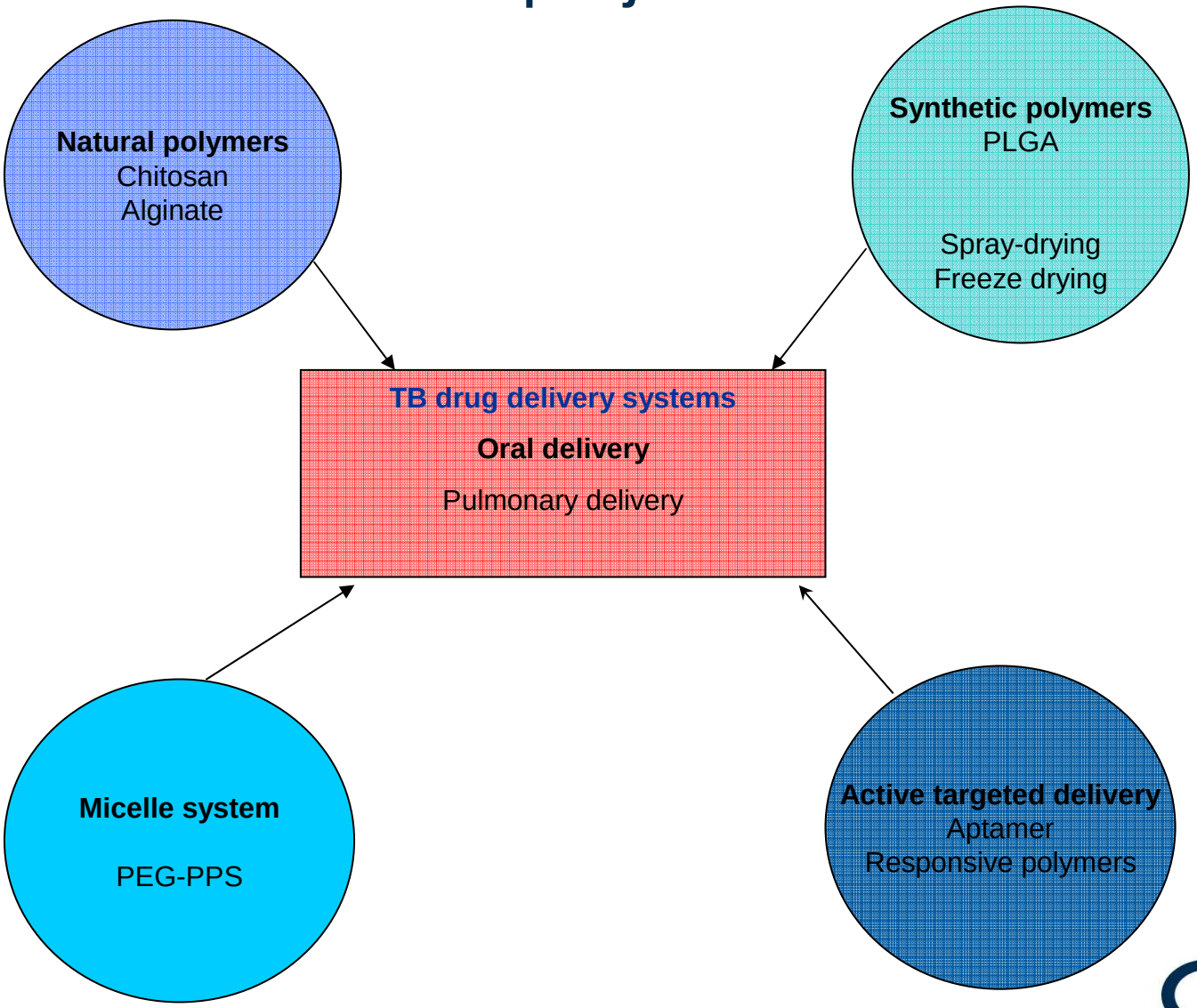
Promise of Nano-based drug delivery systems

- Enhance drug properties
  - Solubility
  - Rate of dissolution
  - Oral bioavailability
  - Targeting ability
- Enhance dosing requirements
  - Improved dose frequency
  - Minimal side effects
  - More convenient dosage forms
  - Shortened treatment time
- Generic
  - Anti-malarials
  - ARVs
  - Anti-cancer drugs
  - Long term pain killers



Nanoparticulate based drug delivery project



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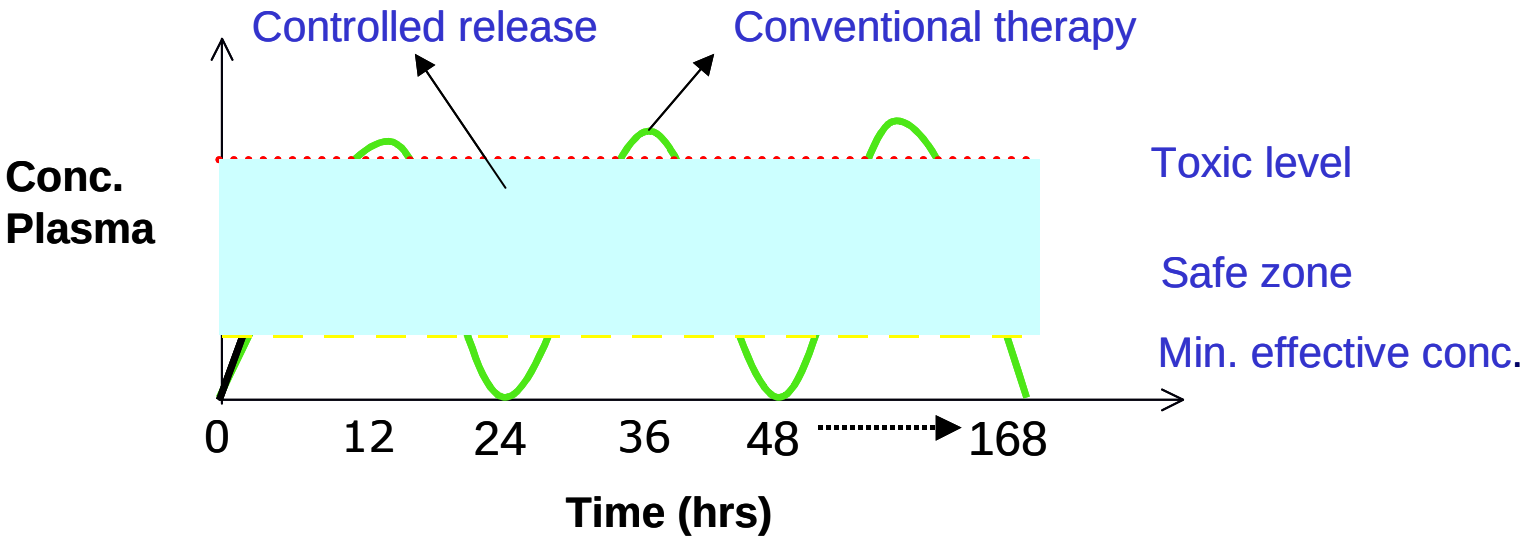
Objectives

- Improve the bioavailability of ATDs
  - Minimise degradation of the drugs in the stomach
  - Steady and controlled release



- Reduce the dosage and dose frequency
  - Treatment 4 drugs/day – 4 drugs/weeks
  - Improve patient compliance
  - Minimise the toxicity of drugs
  - Reduce the cost of TB treatment
- Targeting TB in infected macrophages

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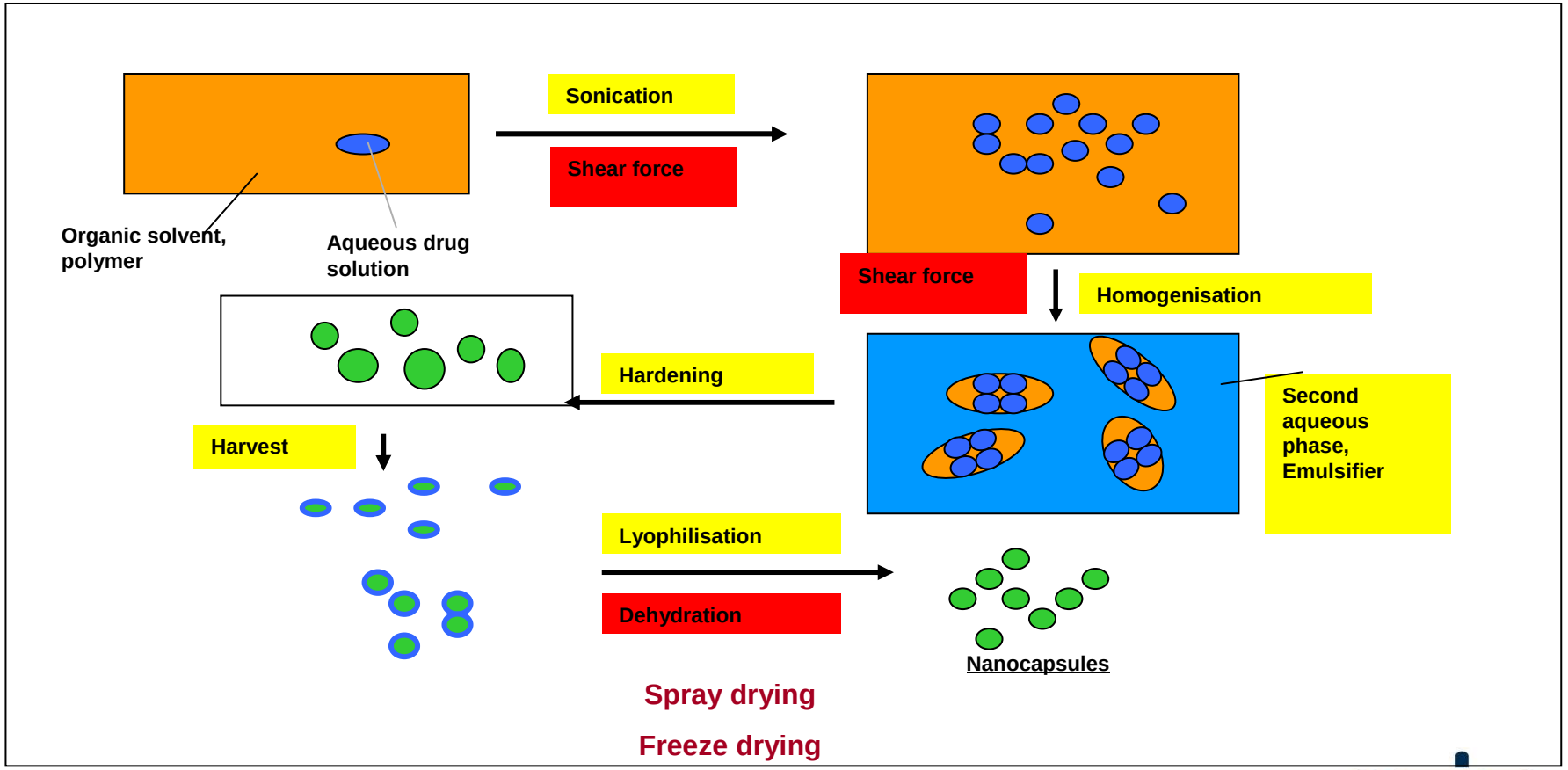
Synthesis/preparation SNP

Multiple emulsion solvent evaporation



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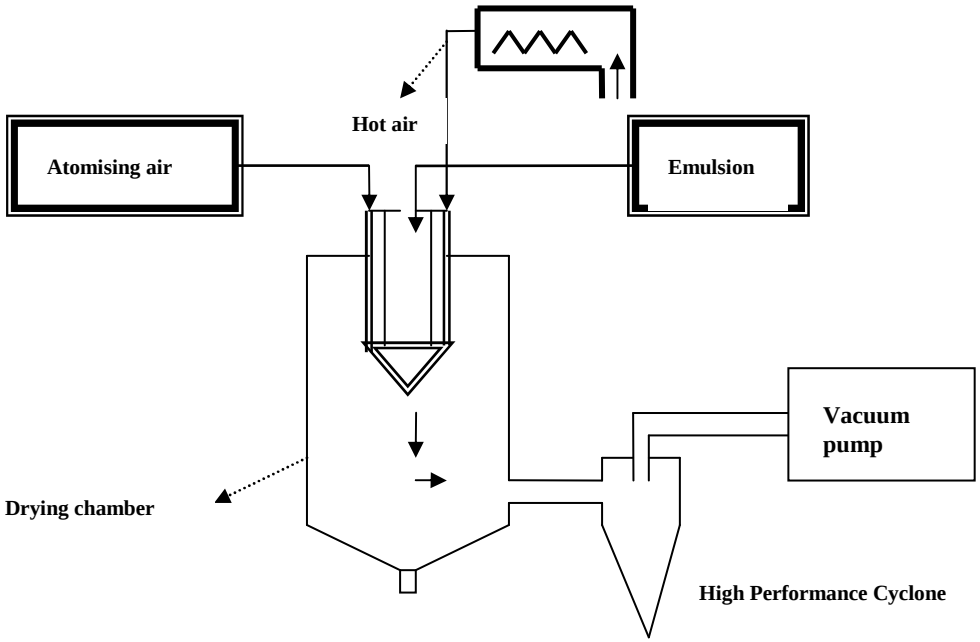
Spray drying of a double emulsion W/O/W



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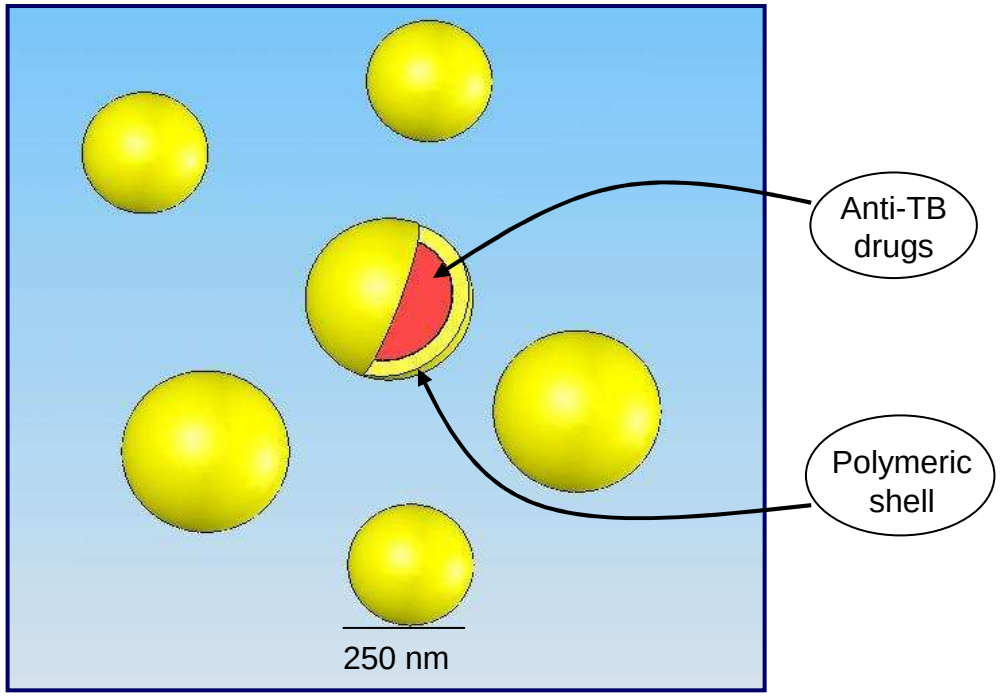
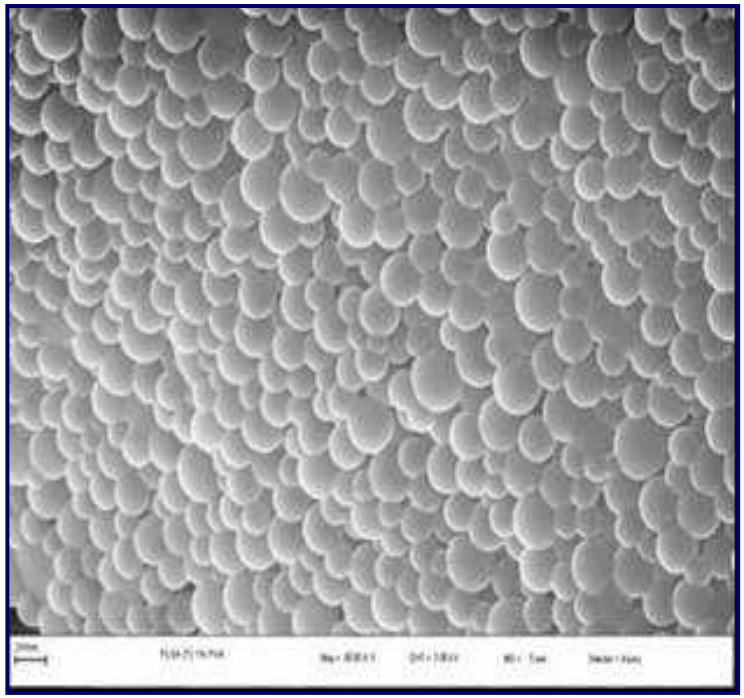
Solid Nanoparticles



Smallest human cell is ~ 2um



INH-loaded PLGA nanoparticles

- Successfully nano encapsulated 4 first line anti-TB drugs
  - Using double emulsion solvent evaporation - spray drying technique
  - PCT patent filed

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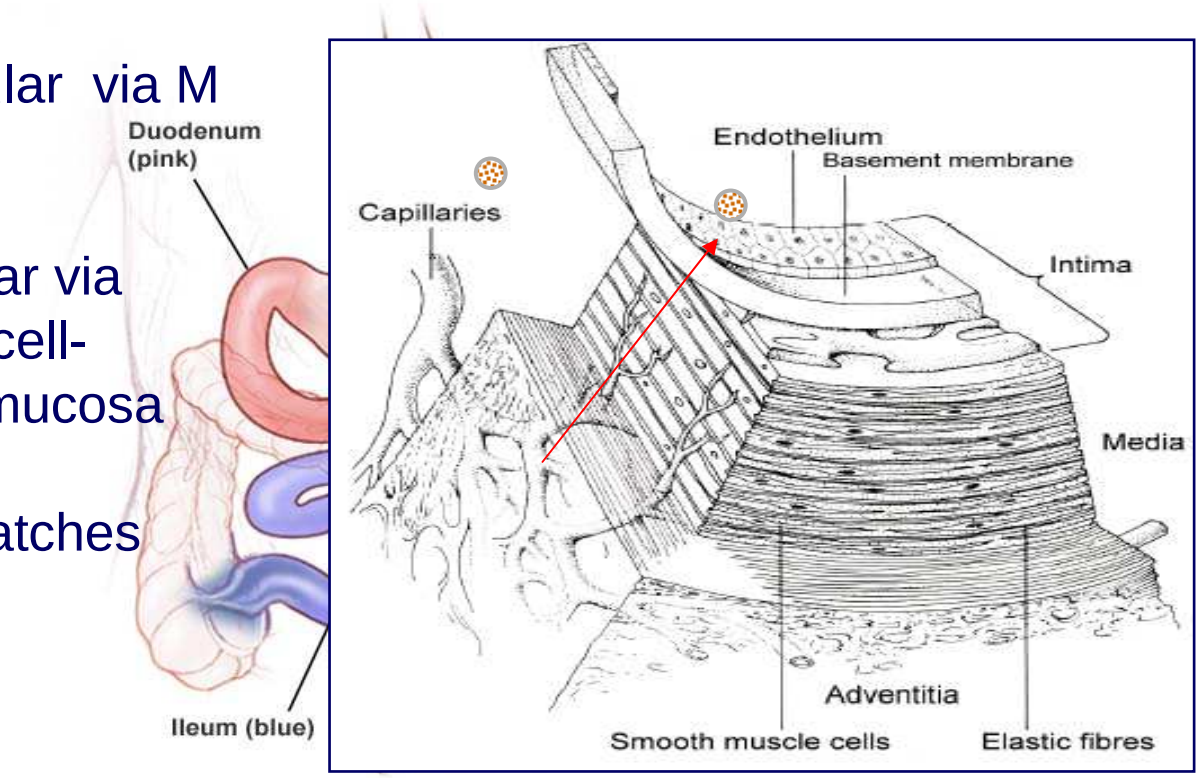
Biocirculation

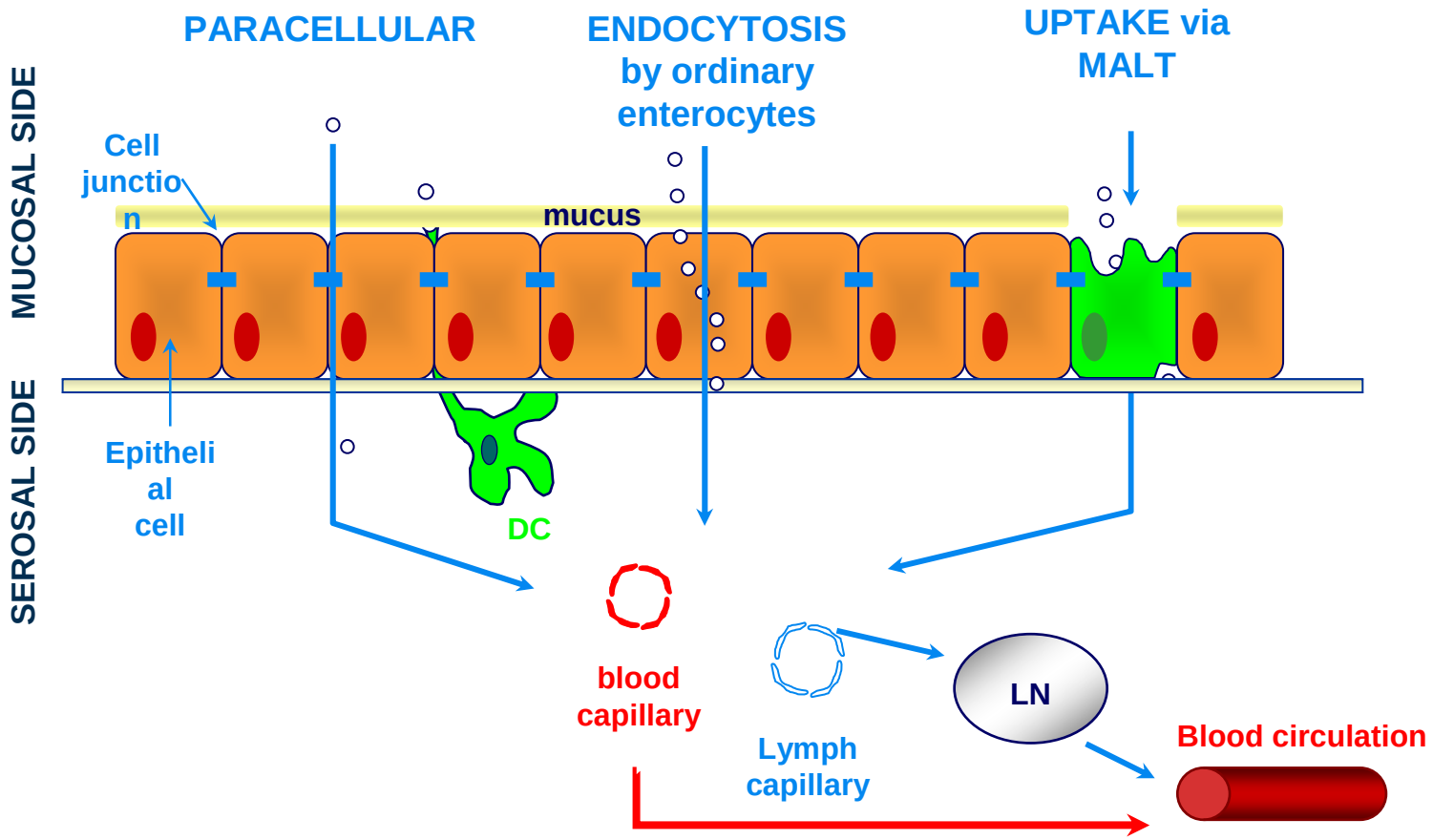
Nano particles uptake from the gut

Structure of the GIT / Internal structure of the intestine

- Para-cellular via M cell
- Intracellular via epithelial cell-intestine mucosa
- Peyer's patches



Routes and mechanisms of particle transport across epithelia



- Modified surface
  - Increase circulation time: PEG
  - Enhance particle uptake: Chitosan

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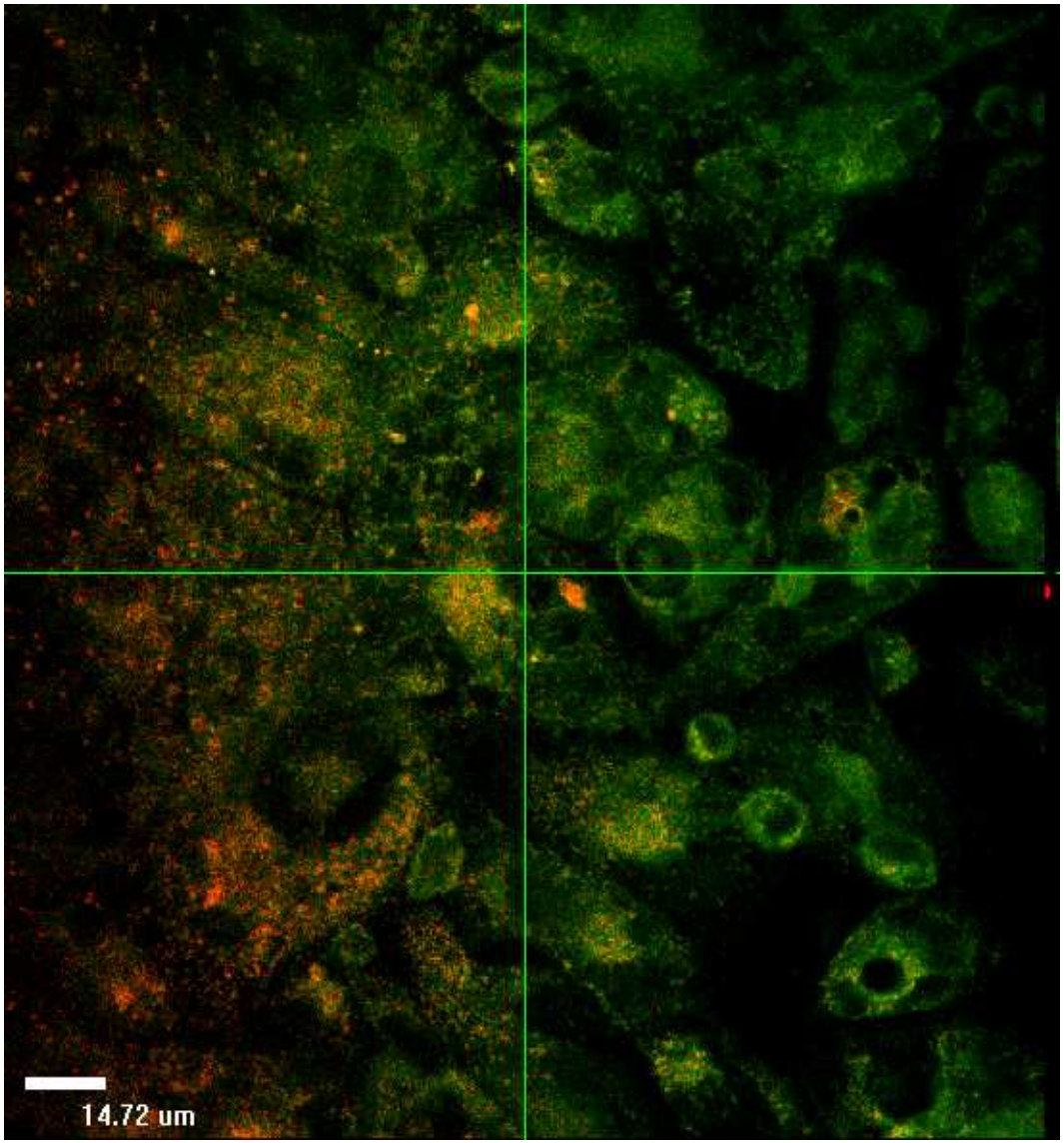
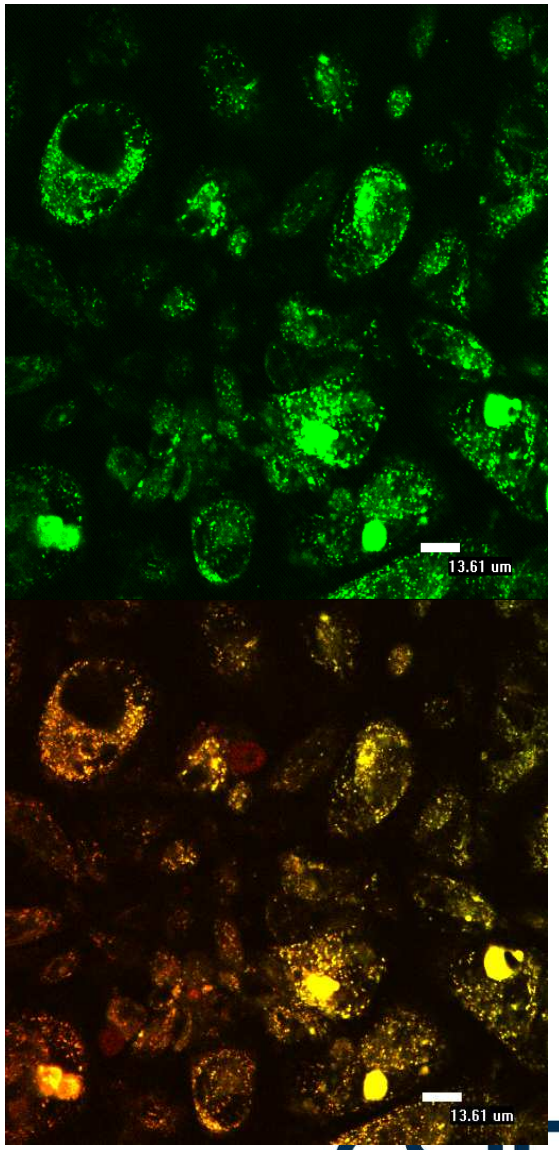
Uptake studies in CaCo-2 cells: PLGA



Z-stack 30 min incubation



60 min incubation

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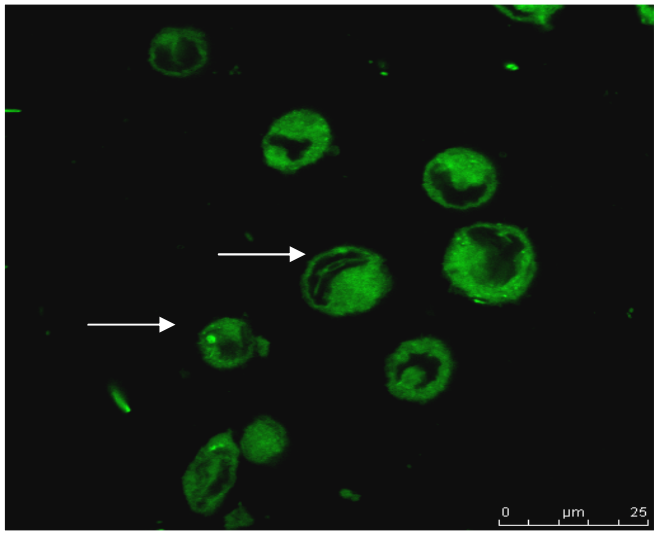
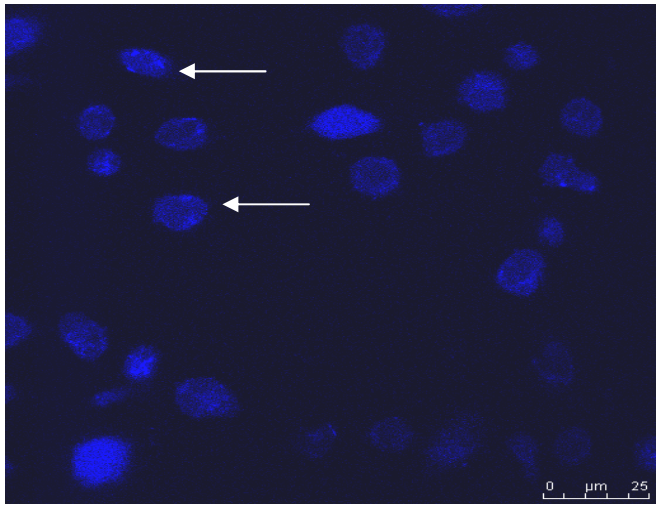
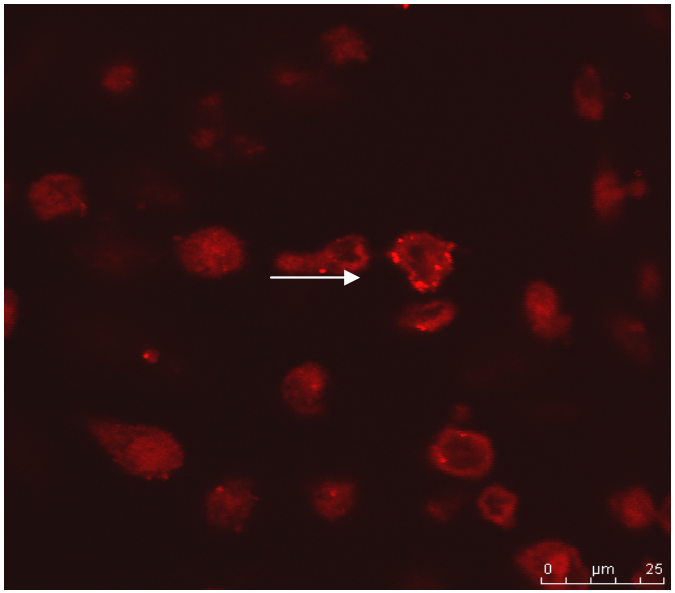
Particle uptake in THP-1 cells



a) Coumarin labelled

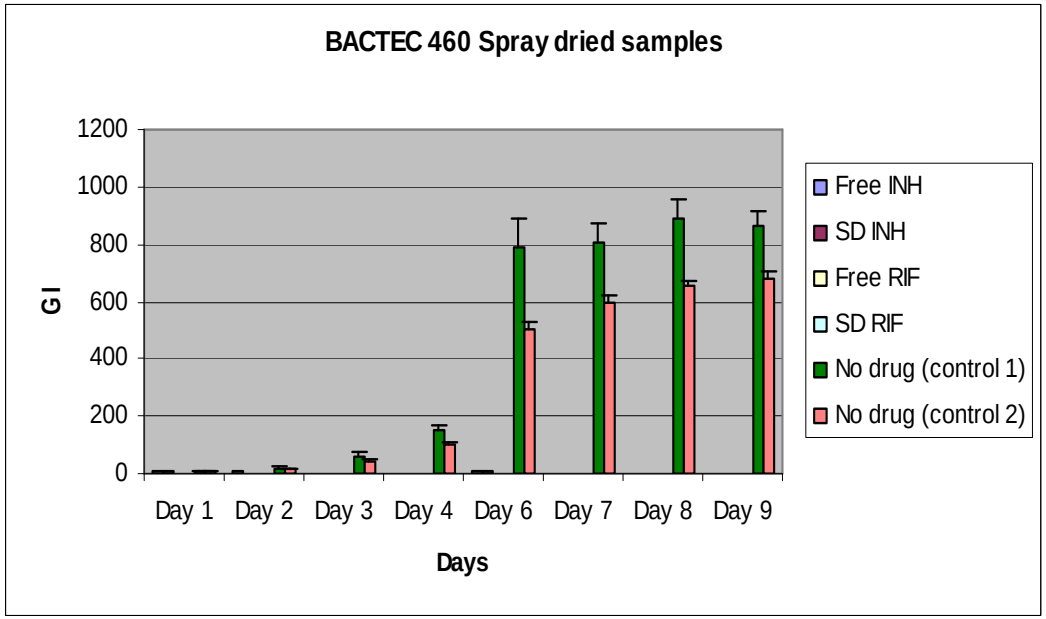


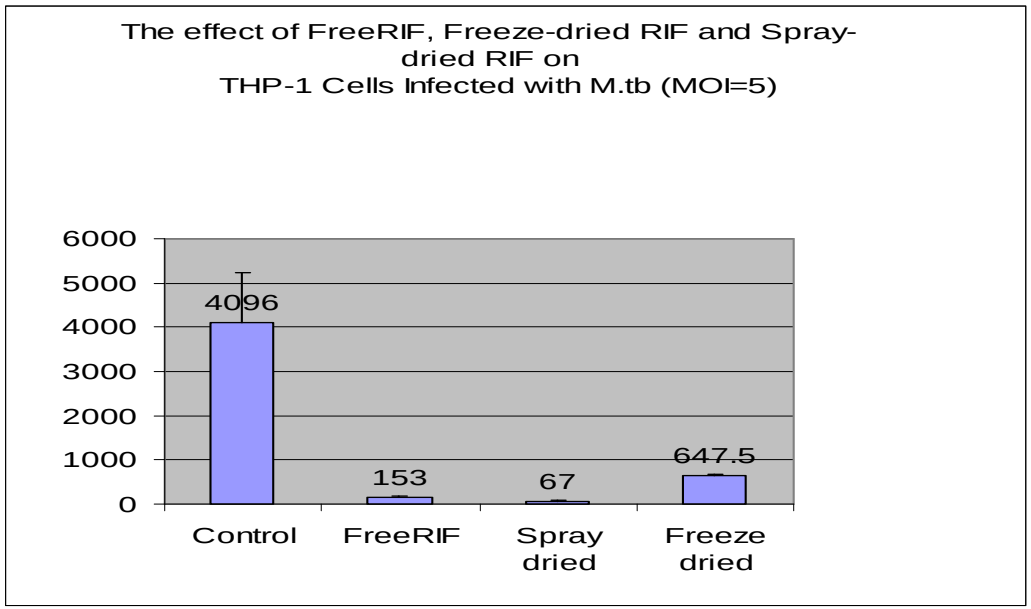
b) INH-PLGA



c) Rhodamine labelled

In vitro efficacy: BACTEC 460





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In vivo assays (Prof Khuller: Laca mice)

- Objectives:
  - Determine plasma concentration of encapsulated RIF, INH and PZA
  - Compare three encapsulation techniques
    - Spray-drying
    - Freeze-drying
- Method
  - 6 unchallanged Laca mice per group (20-25g)
  - Resuspended in UHQ
  - Administered orally to mice via gavage
  - Collect blood daily for 5 days

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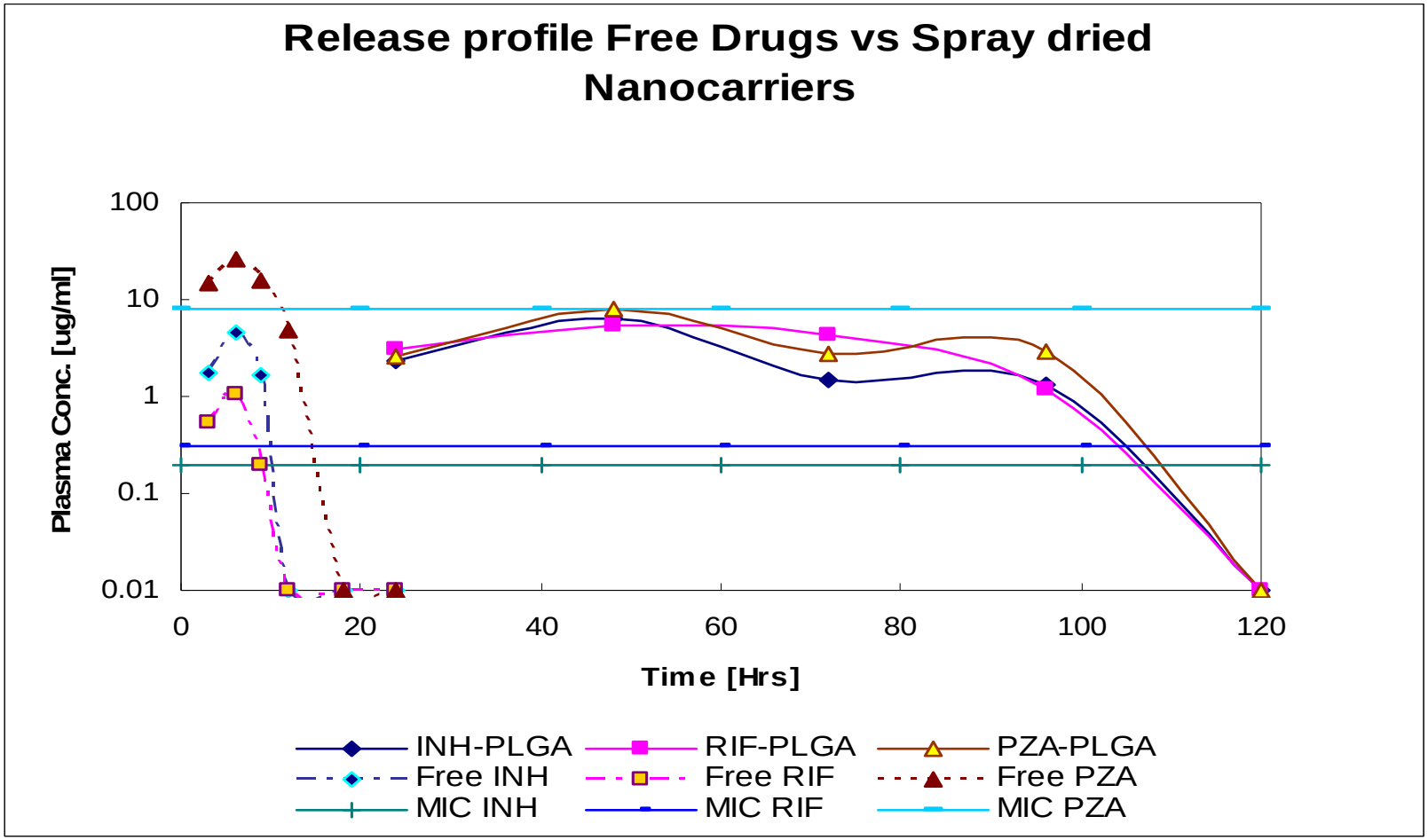
Results: Spray-dried formulation



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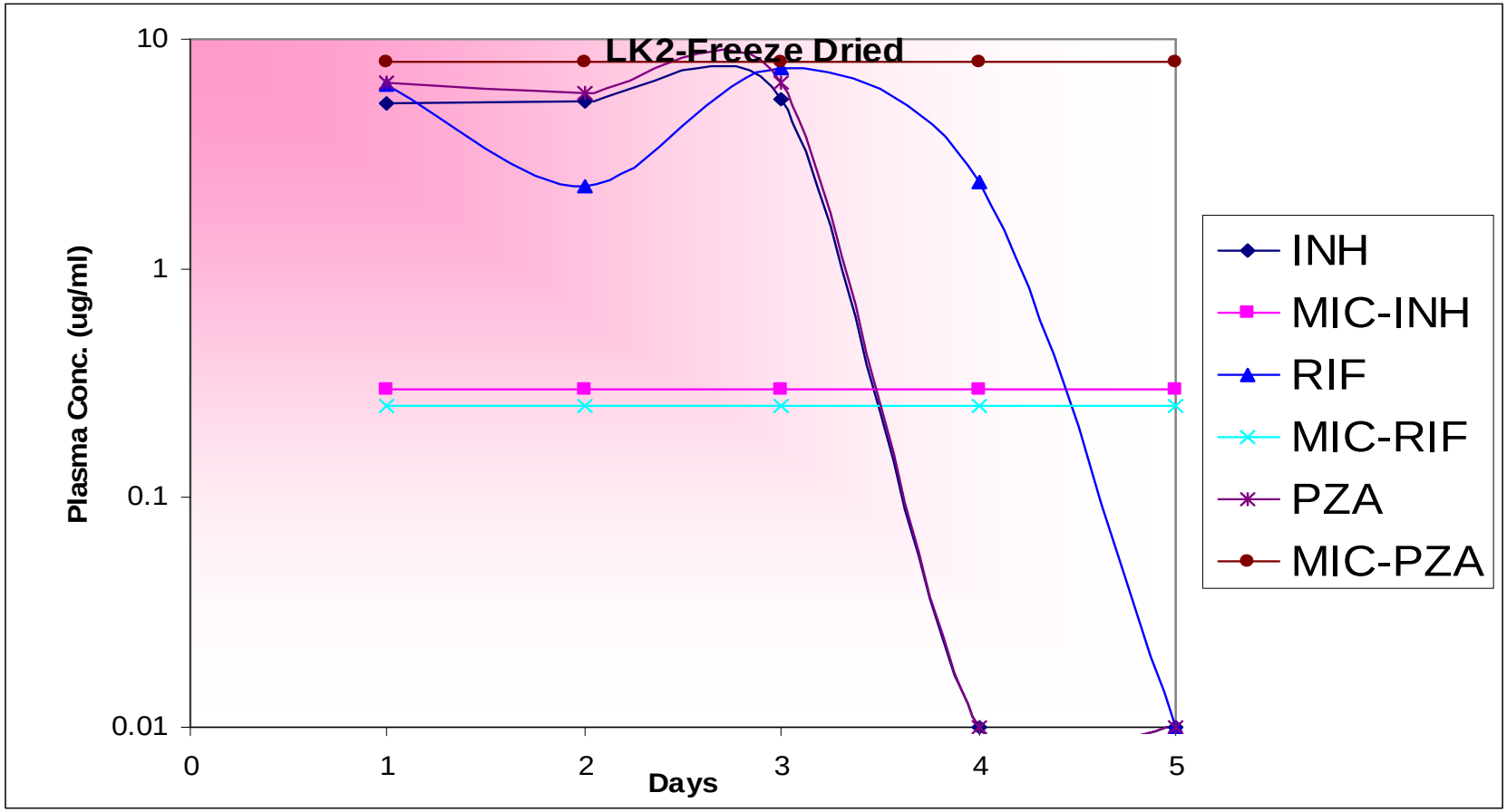
Results: Freeze-dried formulation



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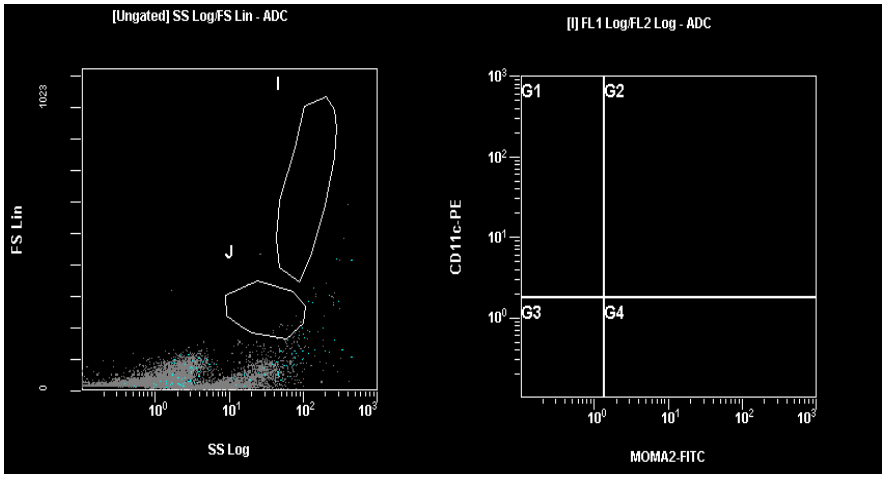
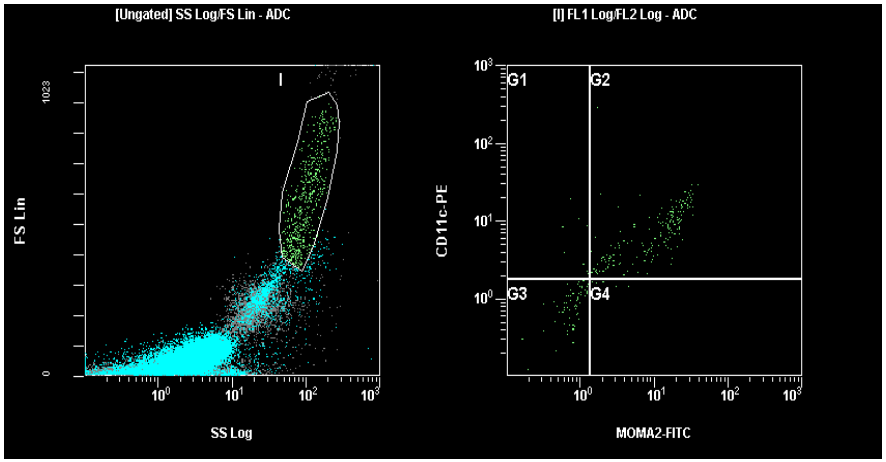
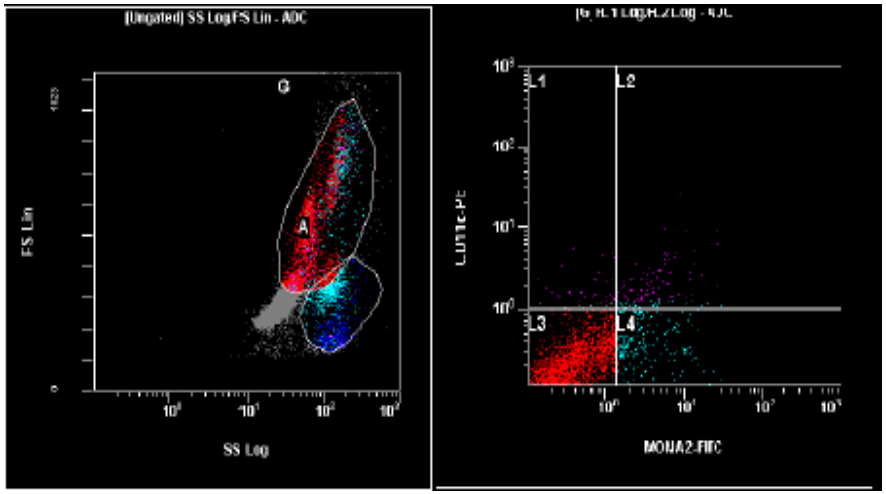
FACS: Peritoneal lavage cells: anti-MOMA-2 and CD11c



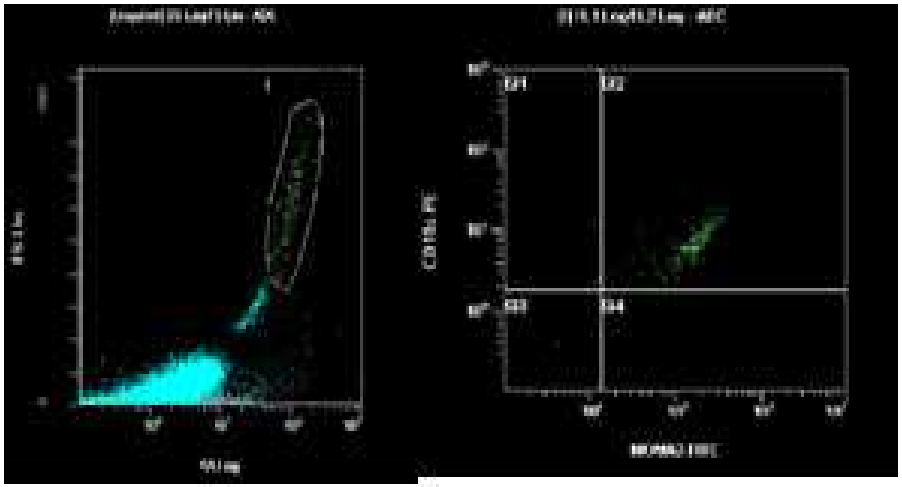
Control: Saline



IP administration of PLGA particles



Oral administration of PLGA particles



IP administration of Rhodamine labelled particles

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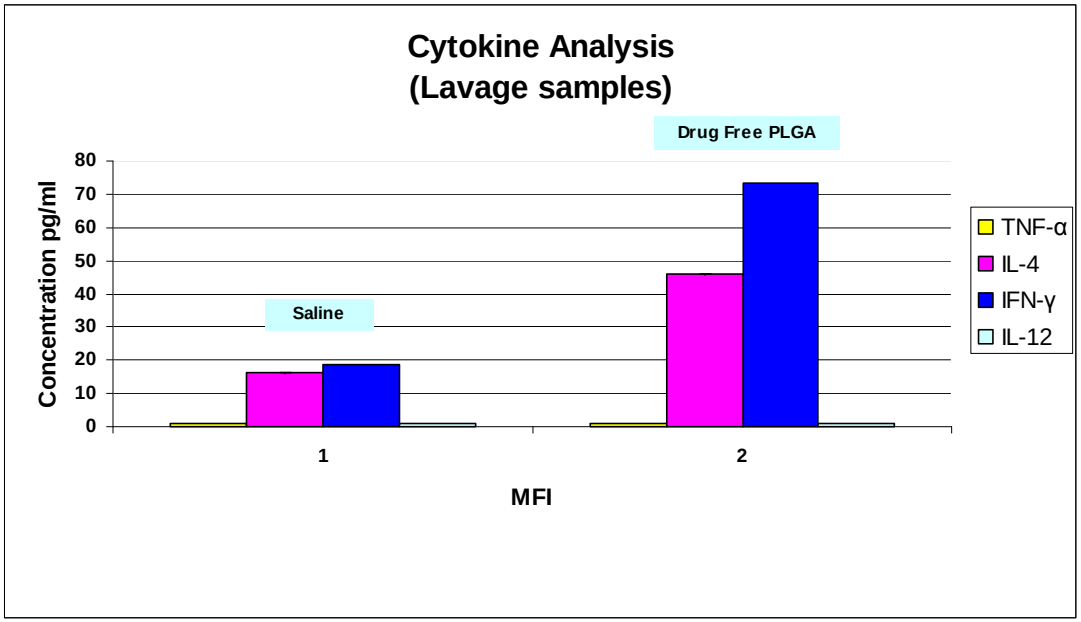
FACS: Peritoneal lavage…

- Determine macrophage activation or phagocytosis



- Low TNF production
  - indicates no infection/inflammation
- Low IL-12p70 production
  - Generally produced in response to antigen stimulation
- Higher levels of IL-4 and IFN gamma
  - Activation of mononuclear phagocytes

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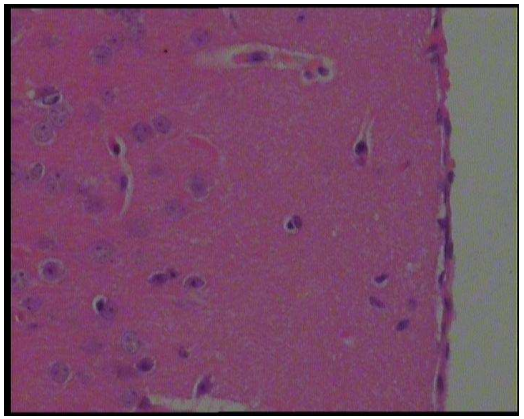
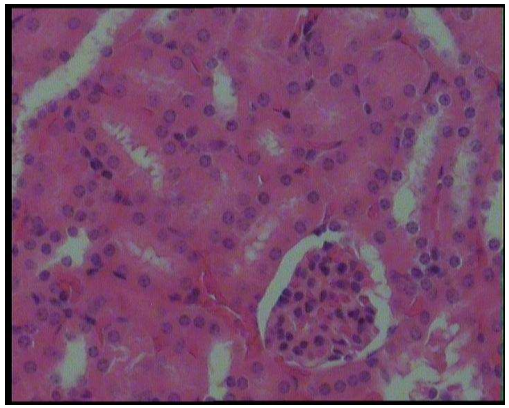
In vivo toxicity assays

- Histopathology
  - Various doses
    - Therapeutic
    - above therapeutic and
    - overdosing
  - Formalin preservation method and H/E staining
  - Liquid nitrogen preservation and H/E staining
    - best of the two methods
  - Heart, brain, kidney, liver, lung and spleen
    - Four doses: 4, 8,16 and 60 mg over 24 hrs
    - No abnormalities

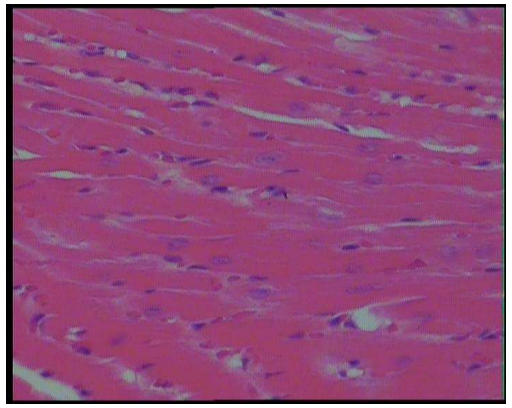
Tissue sections @ 60 mg of PLGA particles



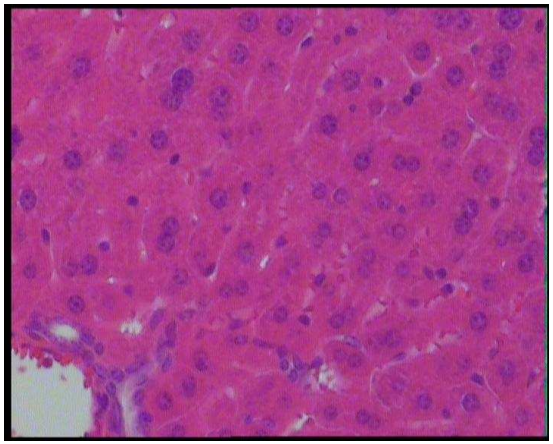
Brain



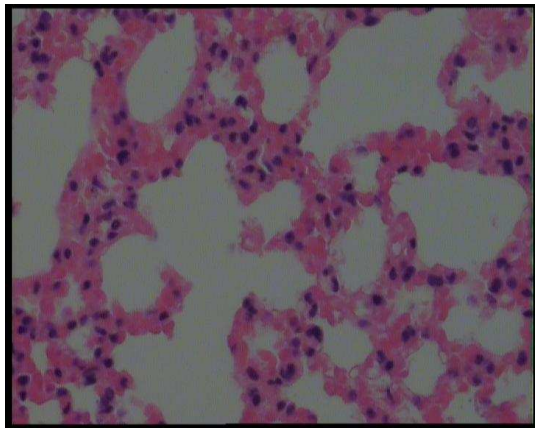
Kidney



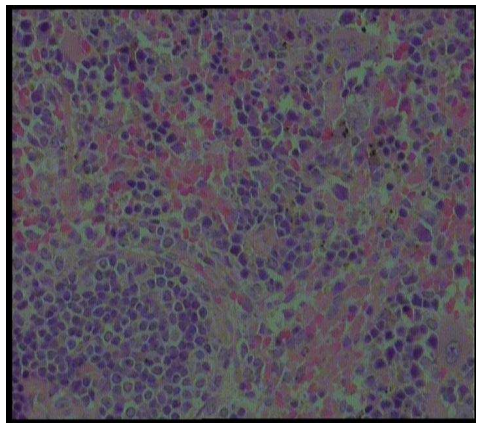
Heart muscle



Liver



Lung



Spleen

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Summary

- Encapsulated first line ATDs
  - PCT patent filed
  - 2 further invention disclosers
  - Publications
- *In vitro* assays
  - *In vitro* efficacy
  - *In vitro* stability and slow release profile
  - Particle uptake
- *In vivo* assays
  - Macrophage uptake
  - No abnormalities in tissues
  - No inflammatory response
  - Sustained release profile over 5 days

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Current phase

Pre-Clinical Trial (MRC PTA and UCT)
- Characterise particle uptake
- Determine safety
- Determine PK/PD and bioavailability
- Determine efficacy

Pre-Clinical trial in Non-human Primates
- Characterise particle uptake in higher primates
- Determine dose tolerance
- Determine PK/PD and bioavailability
- Tissue distribution

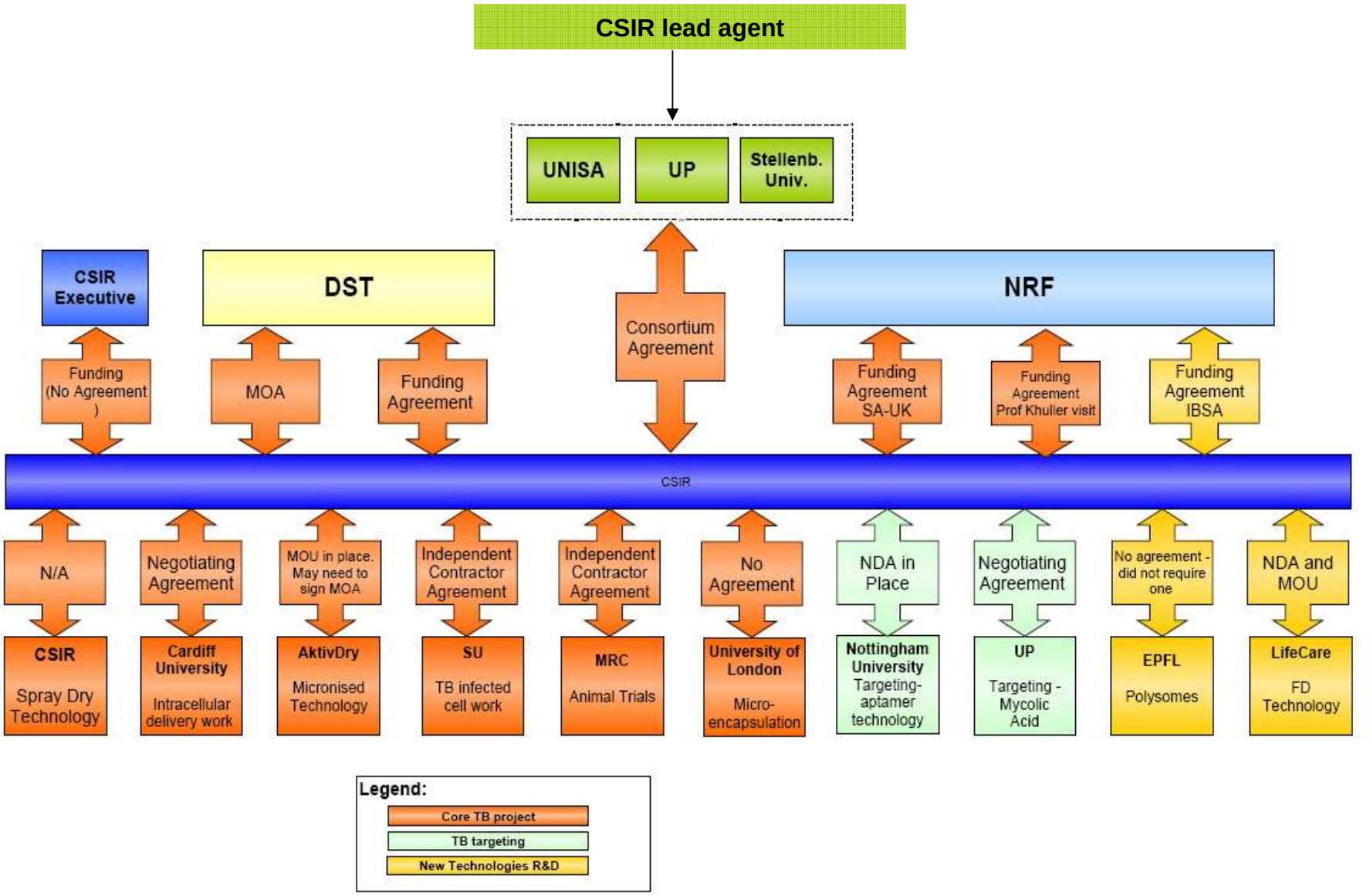
Proof concept clinical trial (Early Bacterial Activity)
- Establish the safety of the delivery system
- Establish the PK and PD of the encapsulated drugs
- Illustrate the efficacy of the delivery system

Clinical trials
- Phase I, II,III

Stakeholder engagement/funding

- TB Alliance
- EDCTP
  - Dr Swai (DCCC)
- DoH

TB Nano Drug Delivery group



Human Capacity Development

- Post doctoral training
  - Post doc (EPFL, Switzerland and UK, Nottingham University , 2006)
  - Post doc (UK, Nottingham University, 2007)
- PhD exchange programme
  - PhD student (UK, University of London, 2005 and 2008)
  - PhD student (UK, Cardiff University and University of Liverpool, 2008)
- Students/Researchers
  - 3 Post doc fellows
  - 4 PhD Students (UP,MWU, and TUT)
  - 2 MSc (UNISA)
  - 1 Hons (UP)
  - 4 Internship students (TUT)
- Further training planned for 2009/10
  - Dosage form design and PK/PD studies
  - GMP production
  - Pulmonary drug delivery systems
  - Microdialysis

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Current applications of nanoparticle delivery systems

Many cosmetics, sunscreens and parenterals have been formulated using nanotechnology.

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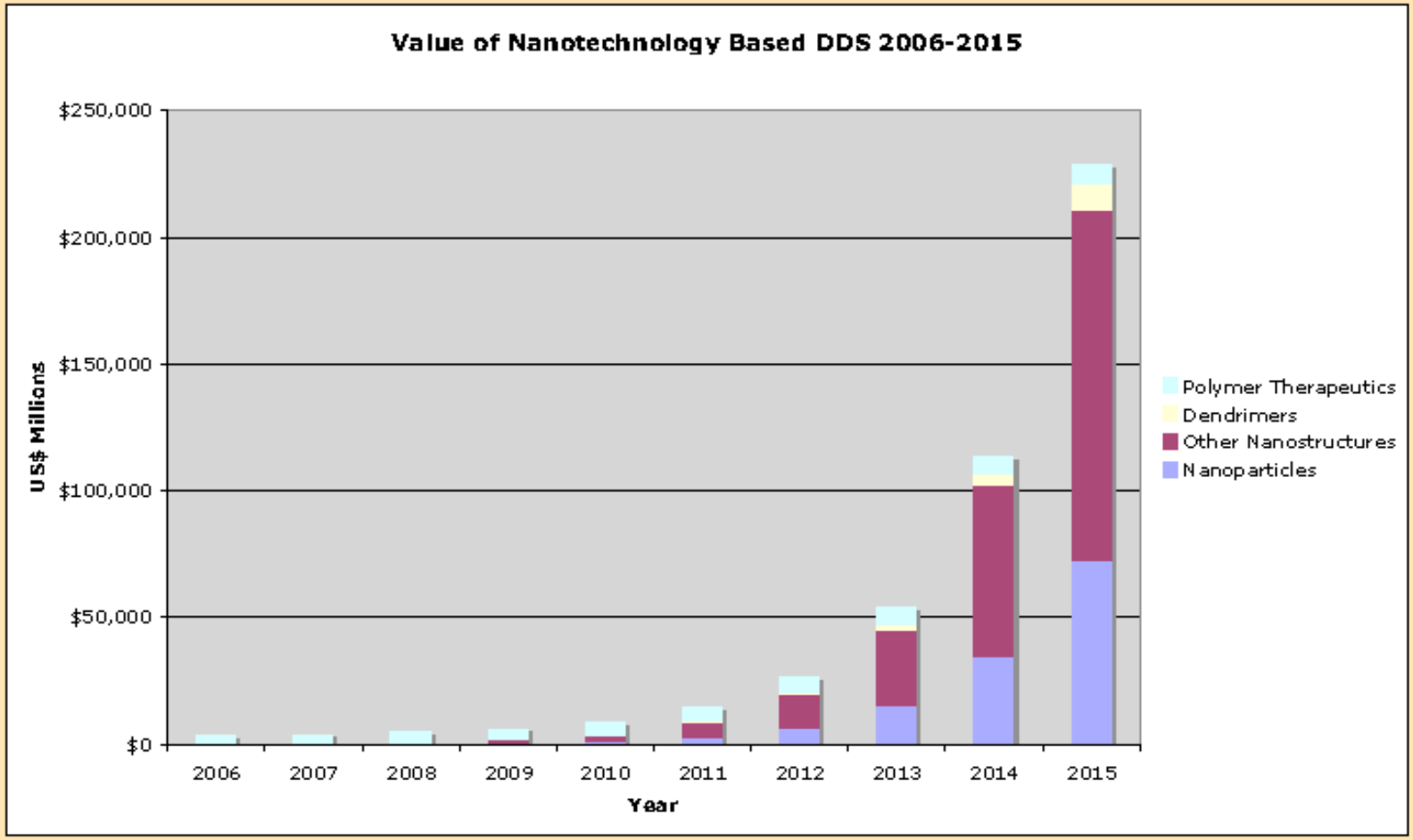
Nanotechnology based drug delivery



Source: The Nanoparticle Drug Delivery Report

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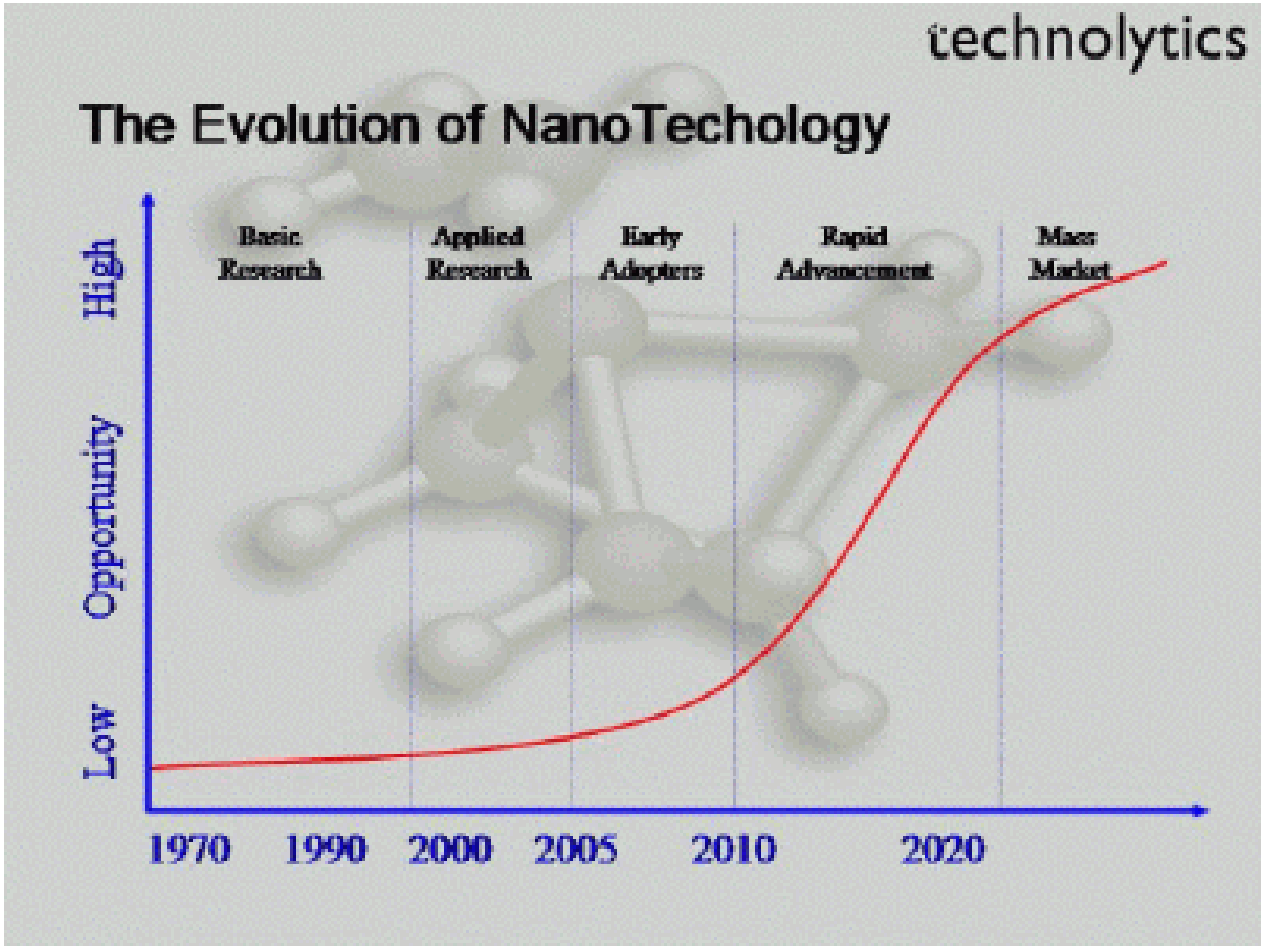
‘The nano-enabled drug discovery market will generate revenues of $1.3 billion by 2009 and $2.5 billion by 2012, predicts a new report, "The Impact of **Nanotechnology** in Drug Discovery: Global Developments, Market Analysis and Future Prospects", by the US consultancy, NanoMarkets, Mark Phillips, 2005



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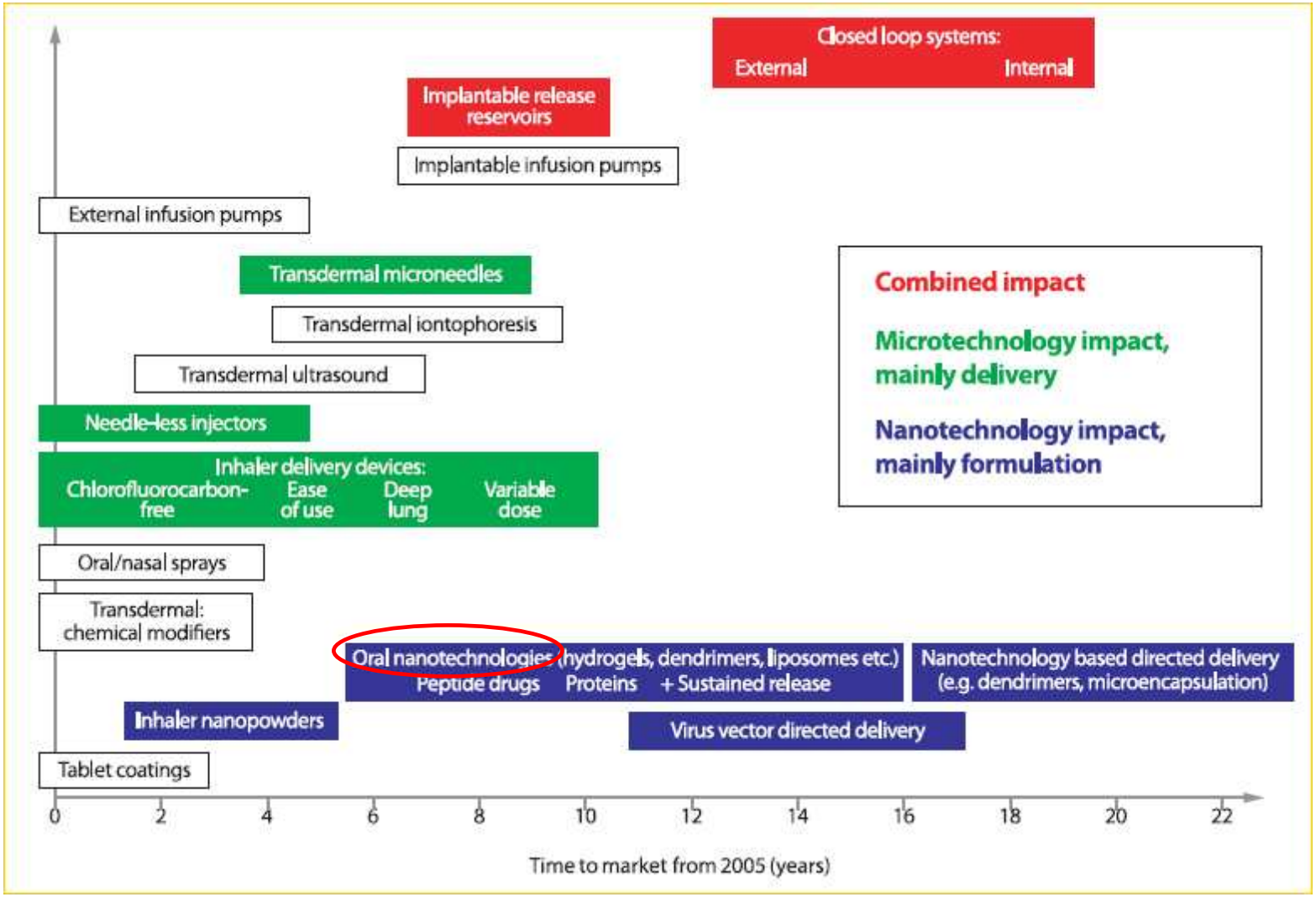
Future Trends in drug delivery systems



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Collaborators

- Consortium members
  - CSIR
  - University of Stellenbosch
  - UNISA
  - TUT
  - University of Pretoria
- National collaborators
  - Dr Anna Glober, Prof Kotze (North-West University, Potch Campus)
  - Dr Karen Weyer, Mr Kobus Venter, (MRC Pretoria)
  - Prof Peter Smith and Dr Jacobs (UCT)
  - CSIR, Biosciences

Collaborators

- International collaborators
  - EPFL
    - Prof Hubbell
  - Nottingham University
    - Dr Alexander
  - University of London
    - Prof Alpar
  - Cardiff University
    - Dr Jones
    - Prof Duncan
  - Nation Jewish Medical Research Centre and Aktiv-dry LCC
    - Dr Kisich
    - Dr Seivers
  - PGIMER
    - Prof Khuller

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Funding

- SA Department of Science and Technology
  - 2005/6: R4M: Infrastructure and HCD
  - 2006/7: R4M: Establishing the technology and HCD
  - 2007/8: R3M: Optimisation and HCD
  - 2008/9: R6M: Pre-clinical studies
- CSIR
  - 2007/8: R2.5M: Preliminary preclinical studies
- NRF: Bilateral;
  - UK-SA
  - IBSA

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