Potential for treating tuberculosis with nano drug delivery system

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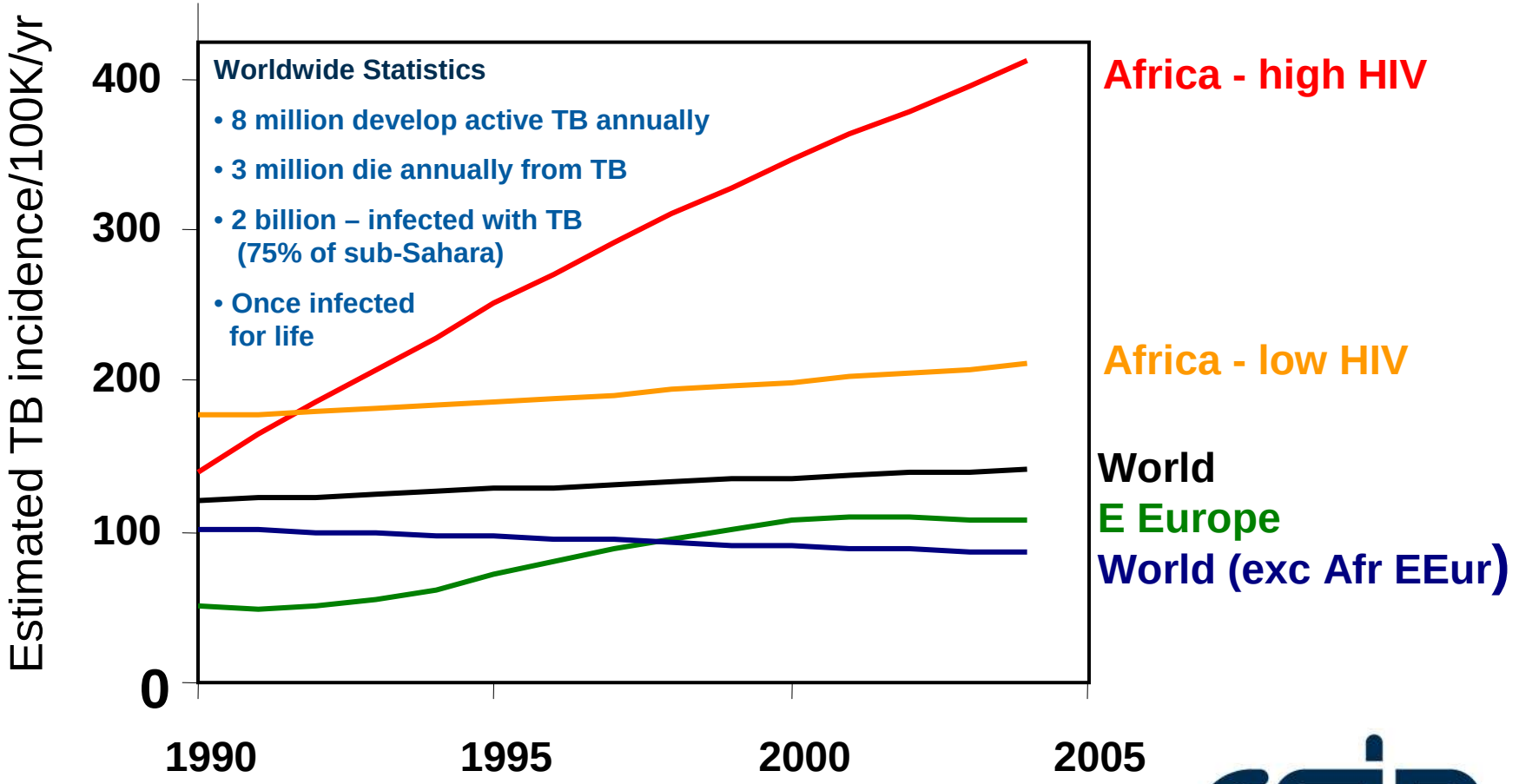
Global incidence is rising at 1% largely due to the African epidemic



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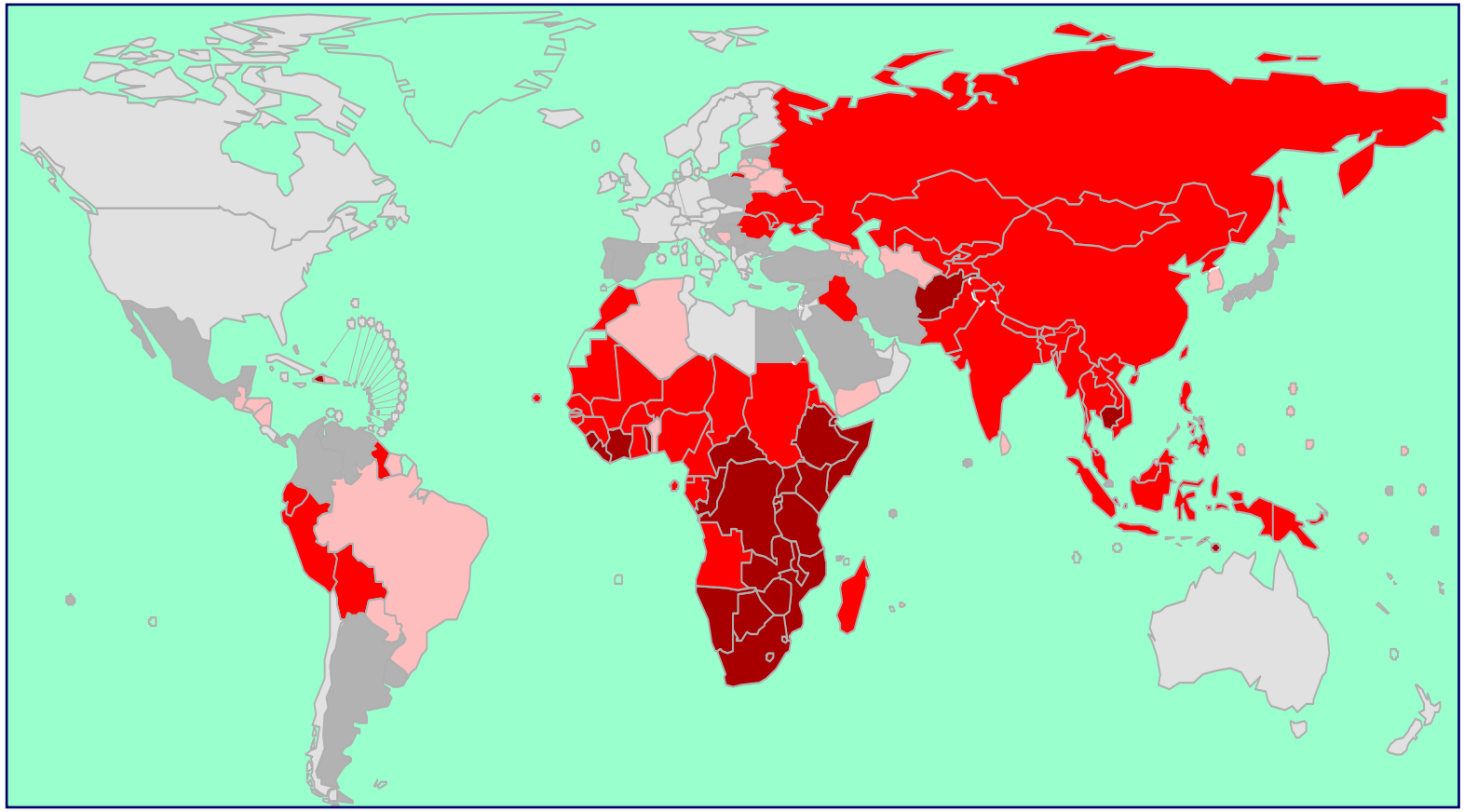
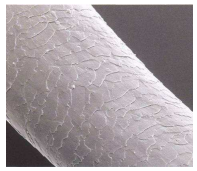
Highest incidence rates per 100 000 in Africa…



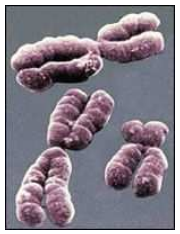
How small?

Ant head - 1mm

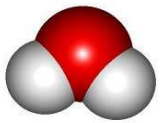


Human hair - 100um , 100 000nm

Red blood cell - 10um, 10 000nm



DNA - 4nm wide



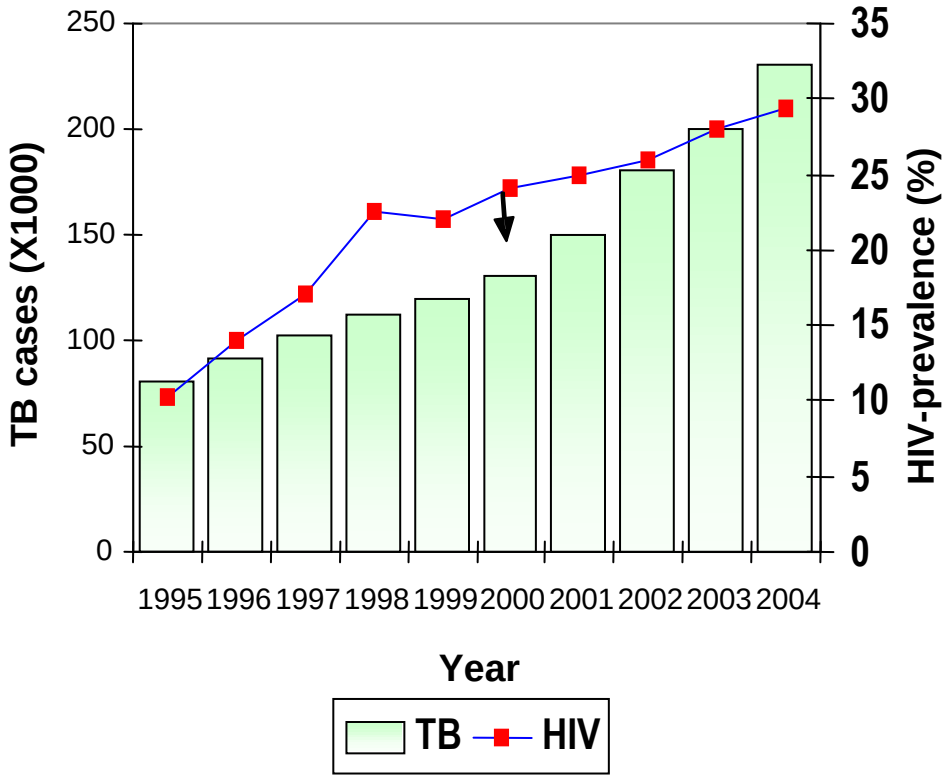
H^2O Molecule - 0.2nm

TB: prevalence

Worldwide statistics

- SA 125,000 (1997)
  256,000 (2004) – doubled
- TB biggest cause of death in SA – 67,000 deaths in 2003 compared to 22,000 in 1997
- Male deaths increase by 60% between 1997 and 2003 and female death by 93%
- Co-infection of HIV and TB in 85% of cases
- Multi-drug resistant TB (MDR)
- XDR-TB

[WHO report: 2000]



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XDR-TB – extensive and 'extreme' TB drug resistance

MMWR
Morbidity and Mortality Weekly Report

Weekly March 24, 2006 / Vol. 55 / No. 11

World TB Day — March 24, 2006

World TB Day is March 24. This annual event commemorates the date in 1882 when Robert Koch announced his discovery of *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis (TB). Worldwide, TB remains one of the leading causes of death from infectious disease. An estimated 2 billion persons (i.e., one third of the world's population) are infected with *M. tuberculosis*. Each year, approximately 9 million persons become ill from TB, and approximately 2 million die as a result. World TB Day provides an opportunity for TB programs, nongovernmental organizations, and other partners to describe TB-related problems and solutions and to support TB control worldwide.

During 1985–1992, after more than 30 years of decline, the number of TB cases reported in the United States increased by 20%. This resurgence generated a renewed emphasis on TB control and prevention during the 1990s, which reversed the trend. Although the 2005 TB rate was the lowest recorded in the United States since national reporting began in 1953, the average annual decline has slowed during the past 3 years, multidrug-resistant TB remains a threat, and disparate rates of TB persist among certain racial, ethnic, and foreign-born populations.

Many states are offering educational programs organized by local TB coalitions in recognition of World TB Day. For example, the Georgia Department of Human Resources, Division of Public Health, Tuberculosis Program is hosting an observance recognizing the activities of a coalition working to reduce disparities in TB among blacks in the Atlanta area. Additional information about World TB Day and CDC TB-elimination activities is available at http://www.cdc.gov/nchstp/tb/worldtbday/2006/activities.htm.

Emergence of *Mycobacterium tuberculosis* with Extensive Resistance to Second-Line Drugs — Worldwide, 2000–2004

During the 1990s, multidrug-resistant (MDR) tuberculosis (TB), defined as resistance to at least isoniazid and rifampin, emerged as a threat to TB control, both in the United States (*1*) and worldwide (*2*). MDR TB treatment requires the use of second-line drugs (SLDs) that are less effective, more toxic, and costlier than first-line isoniazid- and rifampin-based regimens (*3*). In 2000, the Stop TB Partnership's Green Light Committee was created to increase access to SLDs worldwide while ensuring their proper use to prevent increased drug resistance. While assisting MDR TB treatment programs worldwide, the committee encountered reports of multiple cases of TB with resistance to virtually all SLDs. To assess the frequency and distribution of extensively drug-resistant (XDR) TB cases,* CDC and the World Health Organization (WHO) surveyed an international network of TB laboratories. This report summarizes the results of that survey, which determined that, during 2000–2004, of 17,690 TB isolates, 20% were MDR and 2% were XDR. In addition, population-based data

*Defined as cases in persons with TB whose isolates were resistant to isoniazid and rifampin and at least three of the six main classes of SLDs (aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine, and para-aminosalicyclic acid).

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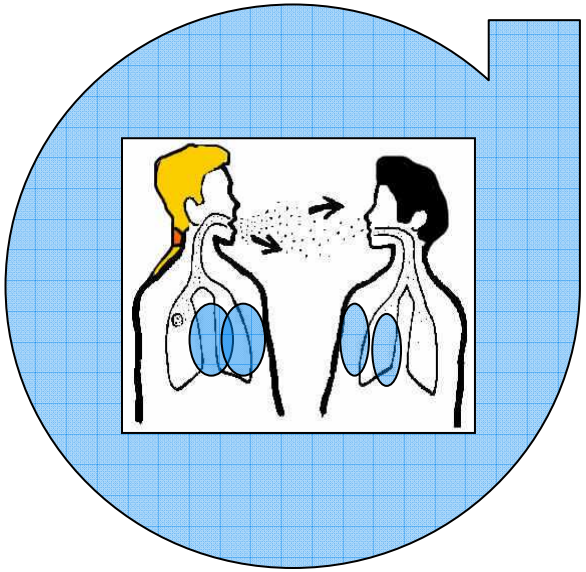
DEPARTMENT OF HEALTH AND HUMAN SERVICES
CENTERS FOR DISEASE CONTROL AND PREVENTION



XDR = MDR-TB plus resistance to at least 3 of the 6 available classes of second line drugs

Of 17,690 isolates during 2000-2004:
20% were MDR and 2% were XDR

Of 544 TB cases in SA during 2006:
40.6% were MDR
and 24% of MDR were XDR.
All XDR tested positive for HIV

XDR found in:
USA: 4% of MDR
Latvia: 19% of MDR
S Korea: 15% of MDR

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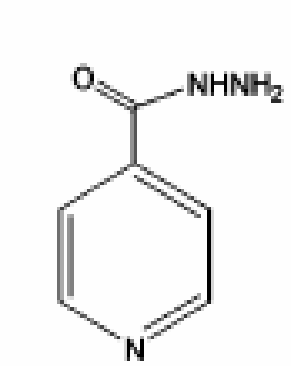
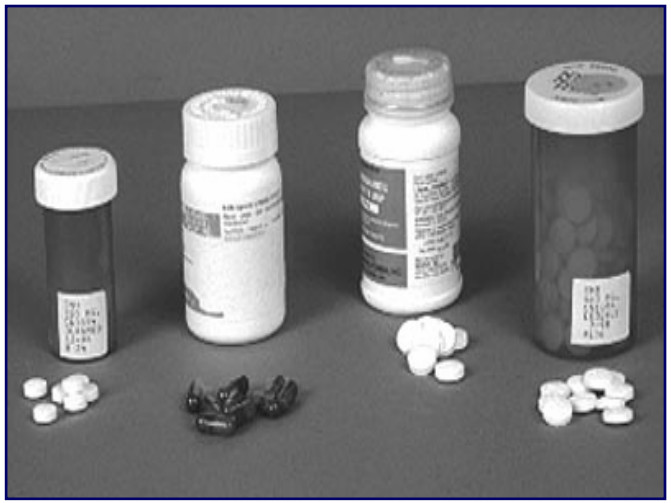
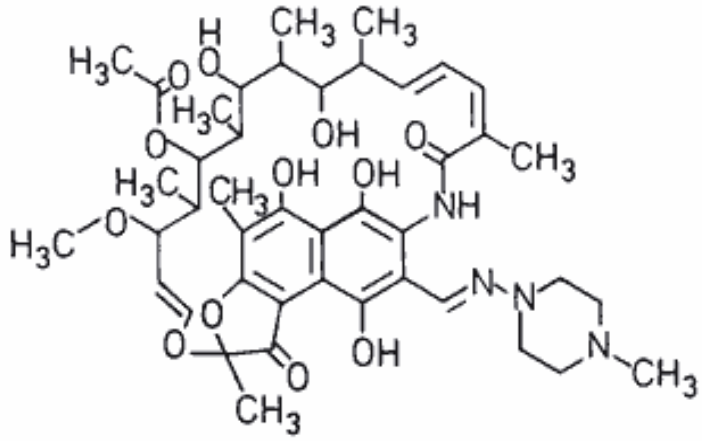
TB drugs / recommended treatment

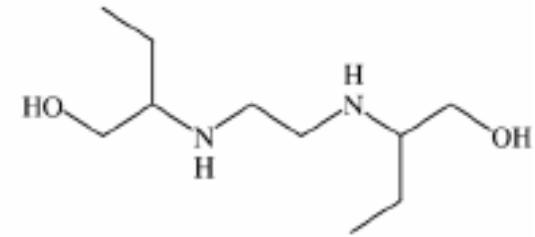


Isoniazid

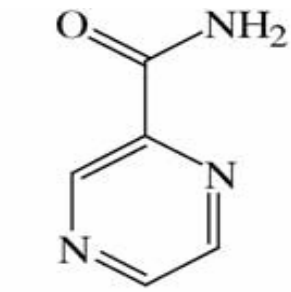




Rifampicin



Ethambutol



Pyrazinamide

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TB: shortfalls of existing treatment

Shortfalls of current therapy

- Extended treatment time
- Degradation of the drugs before reaching their target
- Low permeability and Poor bioavailability / poor bio distribution

Consequences:

- Large doses can cause toxic side effects
- Excretion of native drug
- Patient non compliance due to long treatment period
- Emergence of MDR-TB

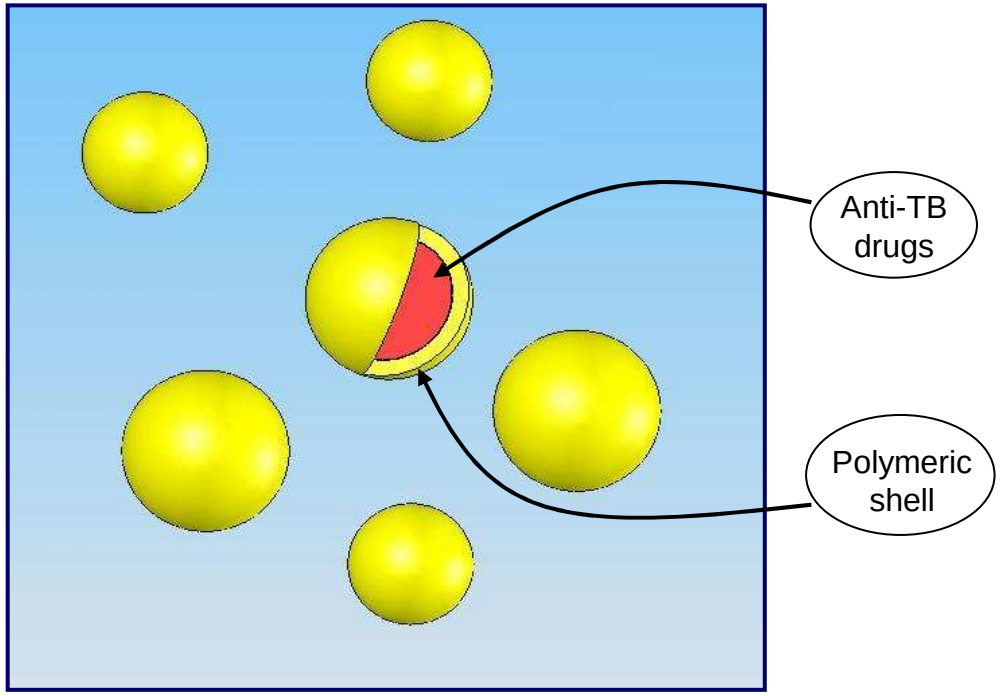
DOTs programme:

- Logistics are impractical and expensive-cure rate is 53%
- Research to improve treatment is in progress, but nothing changed in the last 40 years

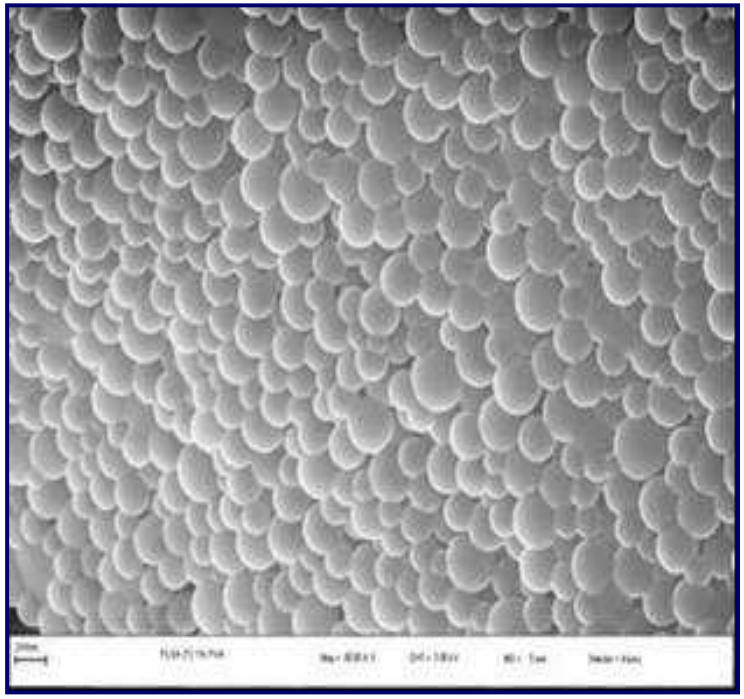
Solution:

- **Polymeric nanoparticles loaded with anti-TB drugs for sustained release**

Nano encapsulated TB drugs



Smallest human cell is 2um
Nanoparticles diameter = 2% of a human hair diameter



INH-loaded PLGA nanoparticles

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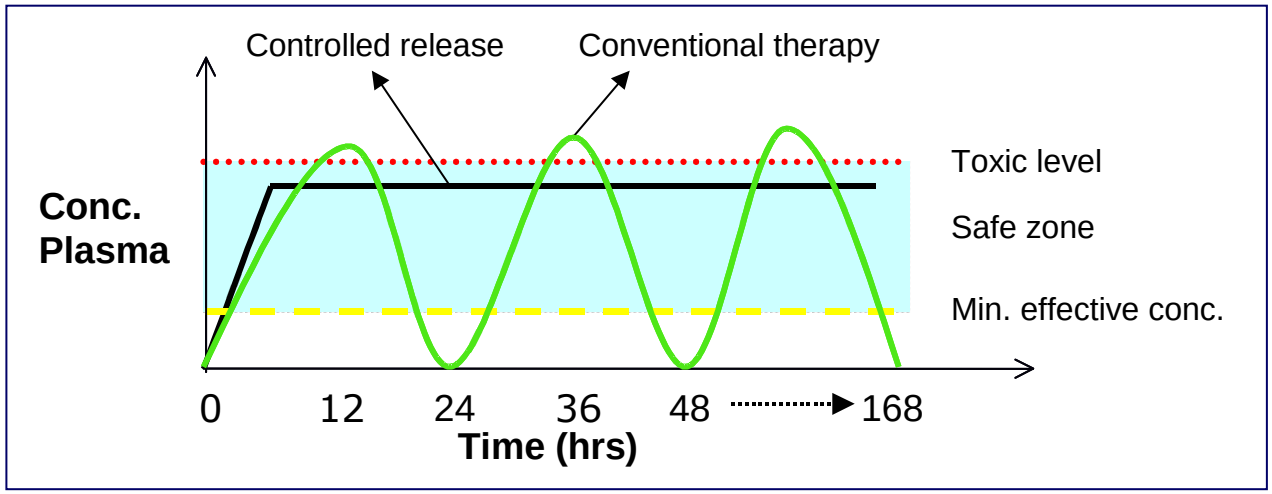
Objectives

Improve the bioavailability of ADTs

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



Reduce the dosage and dosage frequency

- Treatment 4 drugs/day – 4 drugs/week
- Improve patient compliance
- Minimise the toxicity of drugs
- Reduce the cost of TB treatment

Targeting TB in infected Macrophages

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Dosage and pharmacokinetics of anti-TB drugs for 60 kg body weight

Drugs	Adult dose per day	Peak serum conc. (µg/ml)	Usual range MIC (µg/ml)
Isoniazid	0.3 g	3 - 5	0.01 - 1.25
Rifampicin	0.45 – 0.6g	8 – 20	0.06 – 0.25
Pyrazinamide	1.5g	20 - 60	6.2 – 50
Ethambutol	1.2g	3-5	0.5 – 2.0

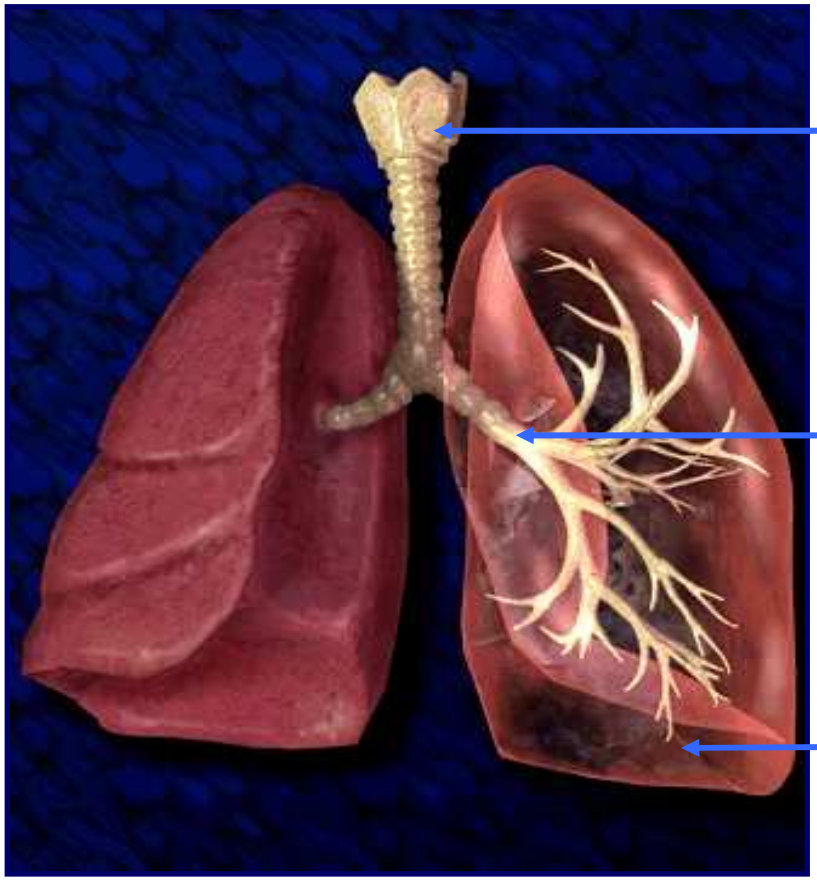
Weekly required dose for unencapsulated and nano-encapsulated drugs (Khuller *et al*. 2004)

Essential ADT	Total weekly dose taken daily of unencapsulated drug (average body weight 60 kg)	Weekly dose nano-encapsulated (average body weight 60kg)
Isoniazid	2.1g	0.6g
Rifampicin	4.2g	0.72g
Pyrazinamide	10.5g	1.5g
Ethambutol	8.4g	0.84g

Advantages of nano drug delivery

- Nano DDS have made it possible to extend the residence time in the GIT to weeks
- High encapsulation efficiency
- Good bioavailability and reduce dose frequency
- Ability to target the drug
  - Smallest capillaries in the body are 5-6μm
  - Typical human cell is 2μm hence, nanoparticles can move easily within the body
- Once Optimised for TB - NDDS can be applied for treatment of:
  - Anti-Malaria drugs
  - Anti-Cancer drugs
  - Anti-Retrovirus
  - Antibiotics
  - Long term pain killers etc

Lung delivery
Size and deposition of inhaled particles



Upper airways:
Particles >10μm impact in the upper ways

Tracheobronchial (TB):
Particles in the size range of 2-5μm deposit by sedimentation in the TB

Alveoli:
Particles < 3μm deposit in the alveolar regions

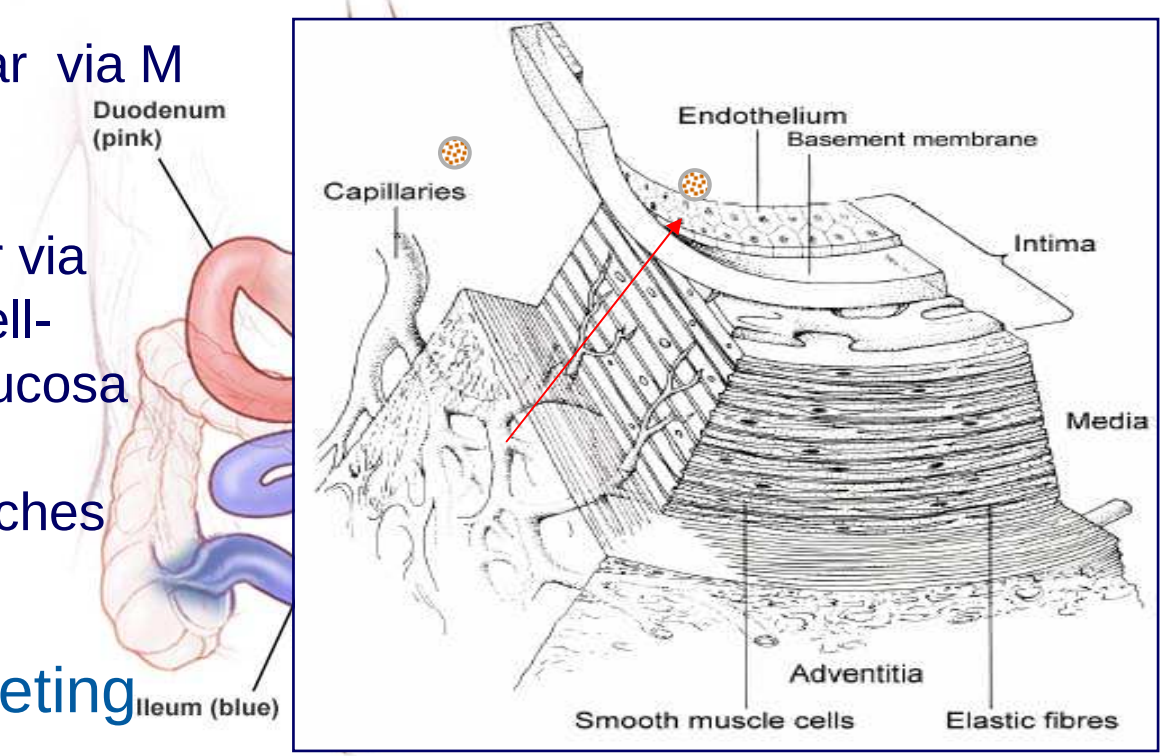
- Large surface area - 140cm^2
- Reduce systemic toxicity-high drug concentration

Biocirculation

Nano particles uptake from the gut

Structure of the GIT Structure of the intestine

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



Drug Targeting

Active targeting-surface modification/ functionalisation

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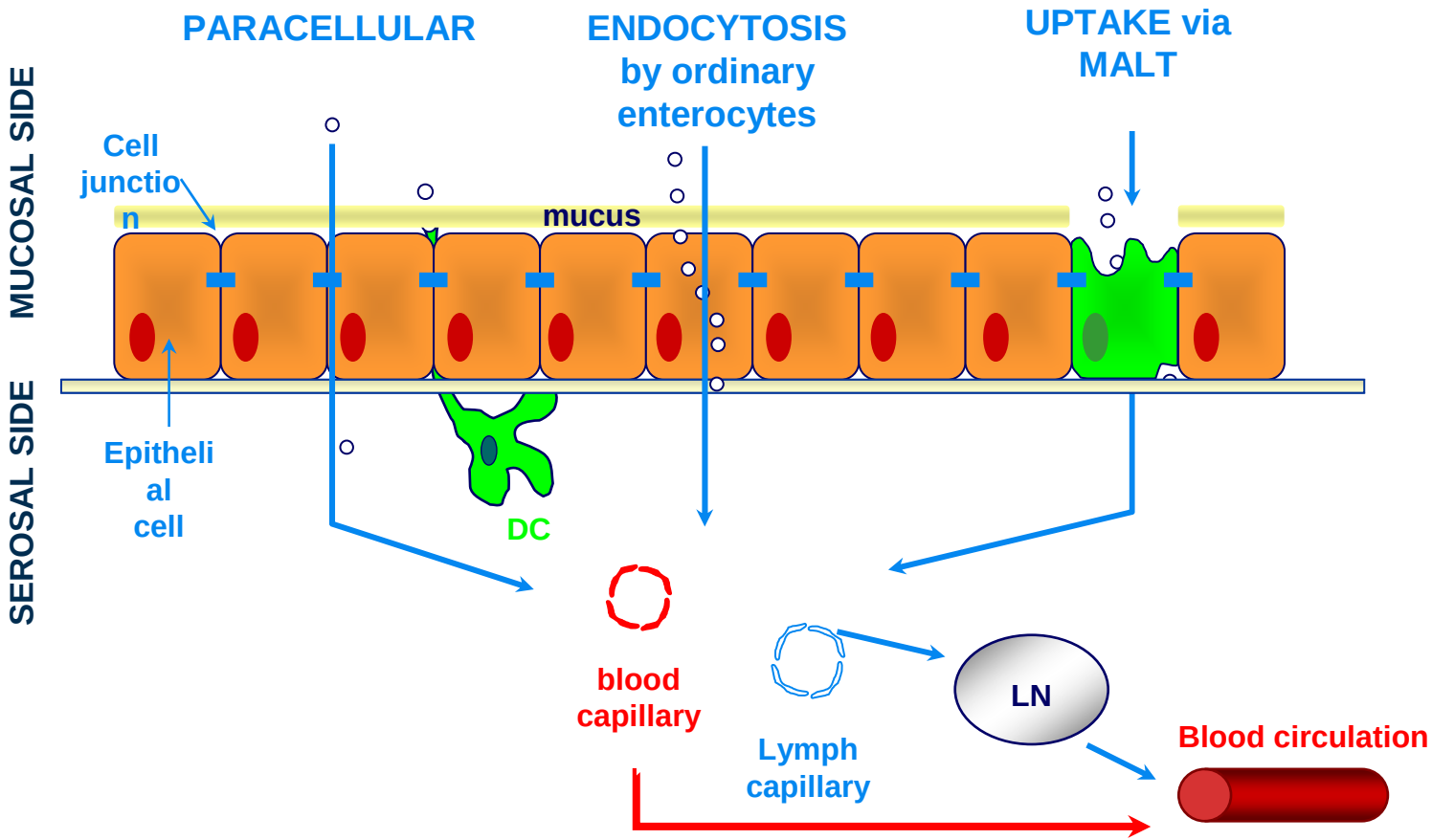
Routes and mechanisms of particle transport across epithelia



Particle diameter (< 1μm, preferably nanometer)
Surface charge, hydrophobicity, ligands

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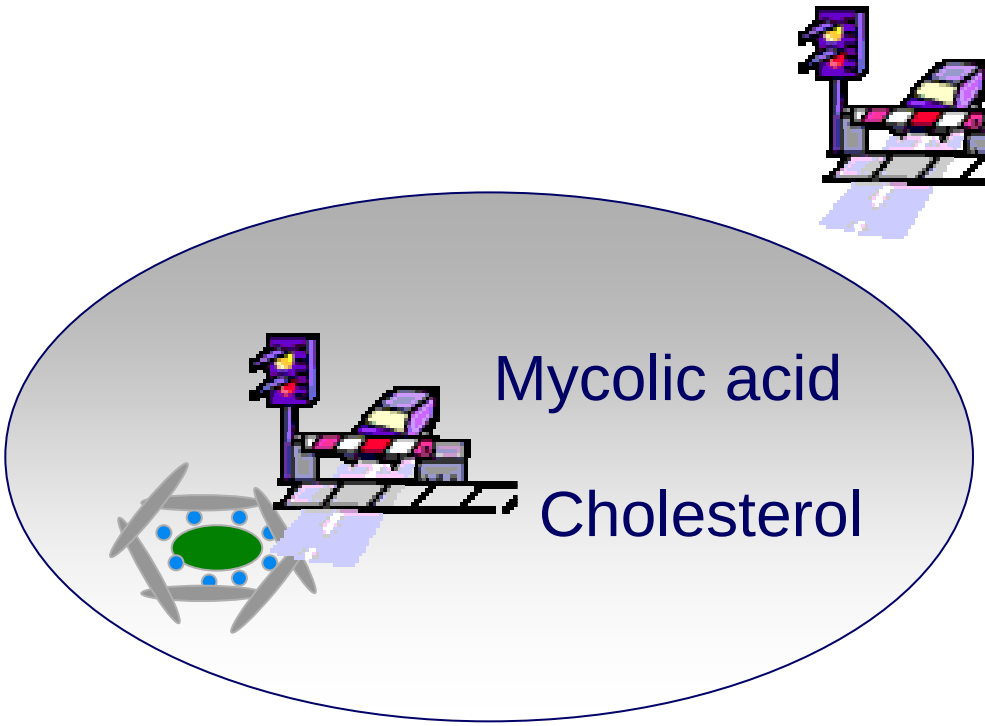
Anti-TB drug carrying nanovehicles that target TB infected macrophages



Research in progress: preparation of nanoparticles

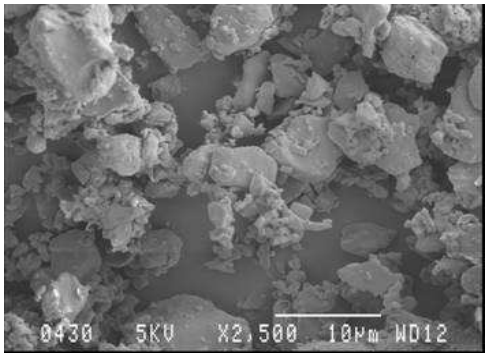
Polymers used

- Poly(lactide-glycolic acid) PLGA(50:50)
  - Biocompatible and biodegradable polymer
  - Hydrolytically degraded to lactic and glycolic acid
- Alginate-Chitosan
- Block copolymers
  - PEG/Pluronic-PPS (Micelles, Vesicles and solid nanoparticles)

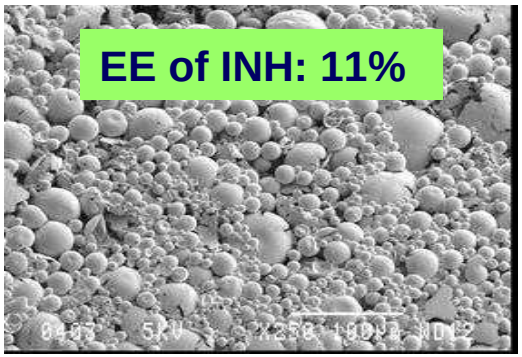
Encapsulation techniques

- Double emulsion-solvent evaporation
- Double emulsion spray drying technique
- Nanoprecipitation
- Ionotropic gelation of Alignate-Chitosan
- Supercritical CO_2

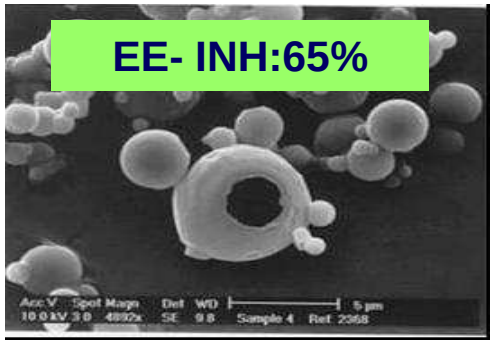
Results of different techniques of encapsulation



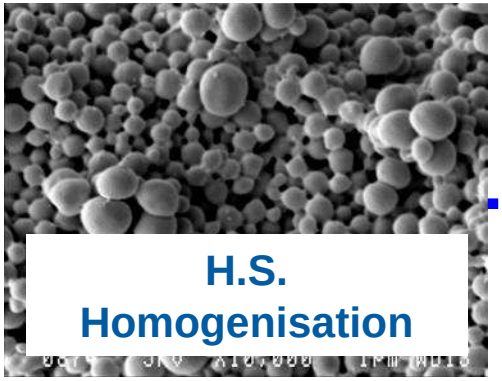
1. Supercritical CO_2

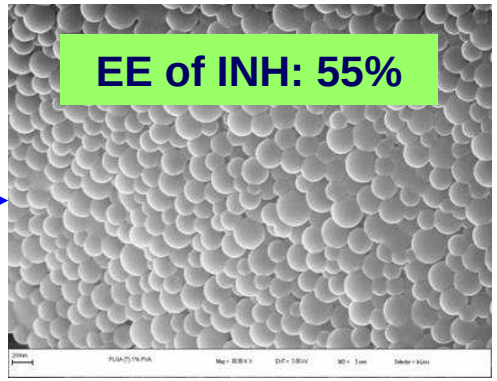


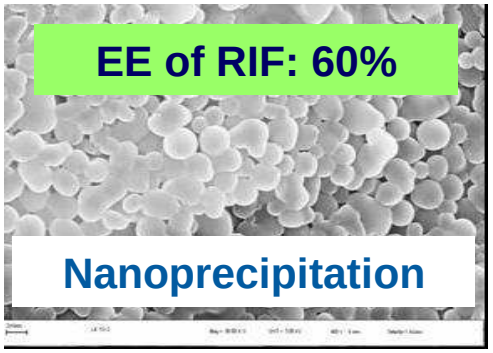
2. Sonication



Spray drying of emulsion







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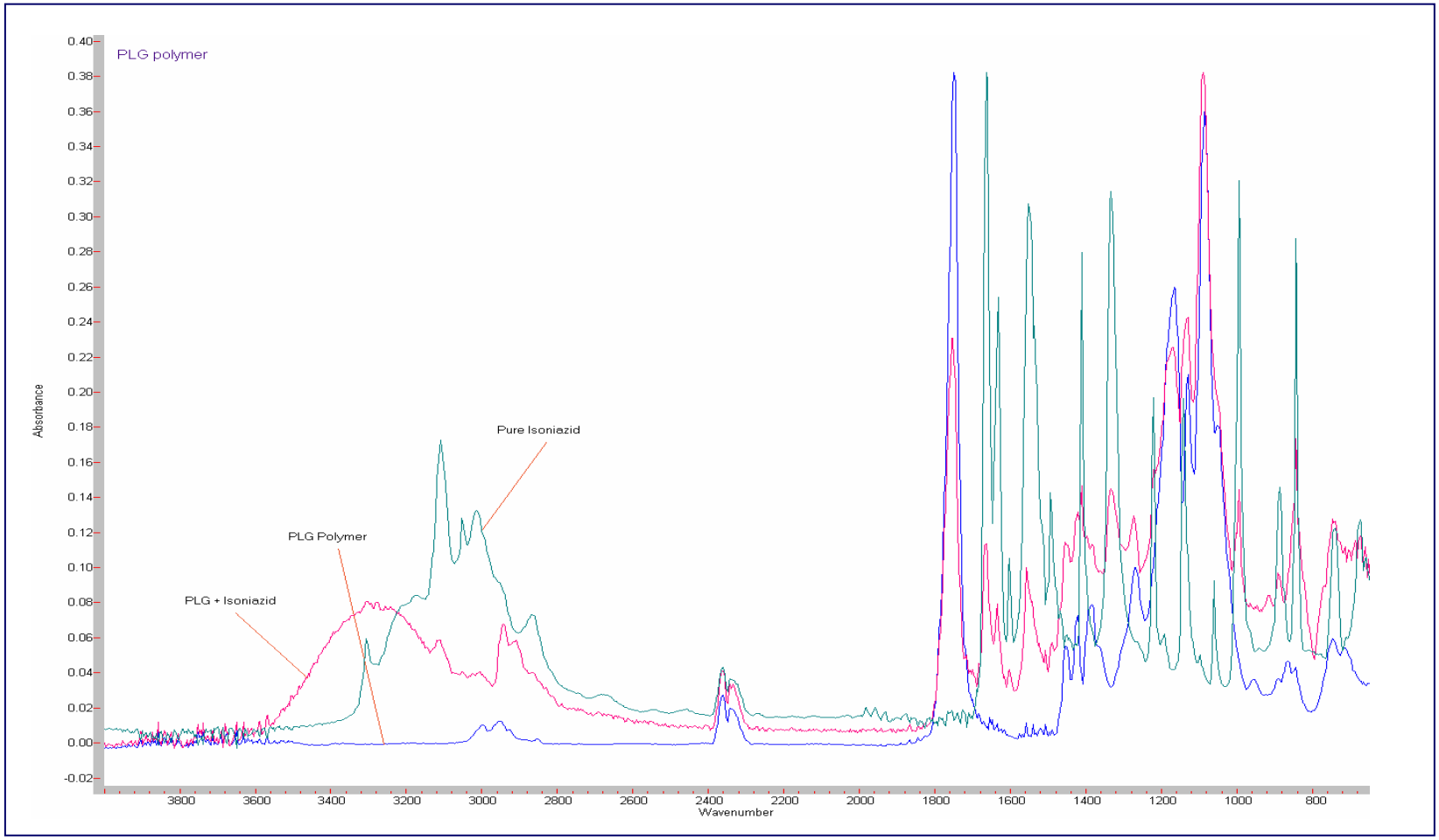
FTIR-ATR results



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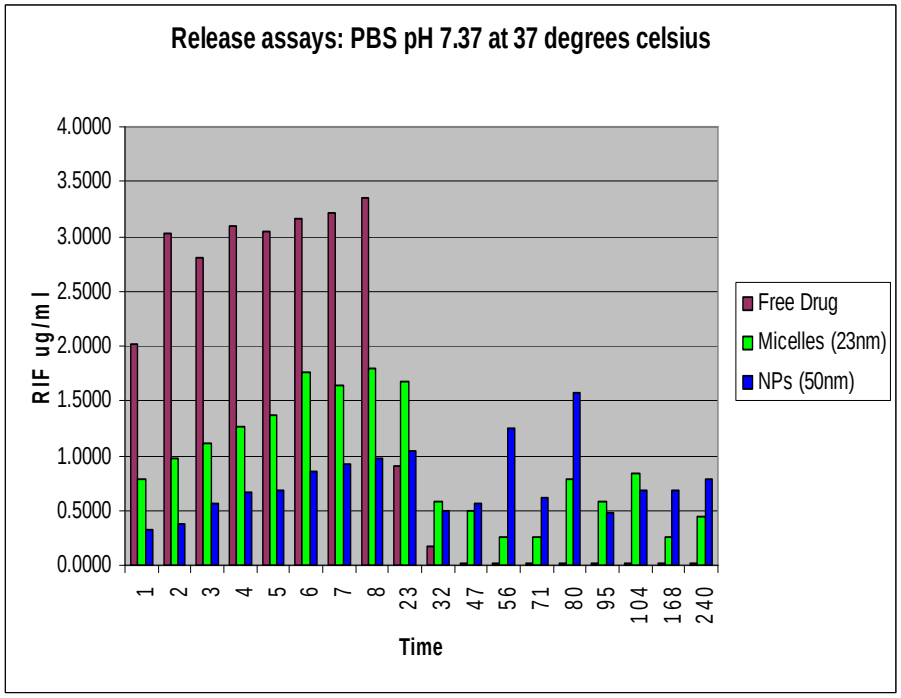
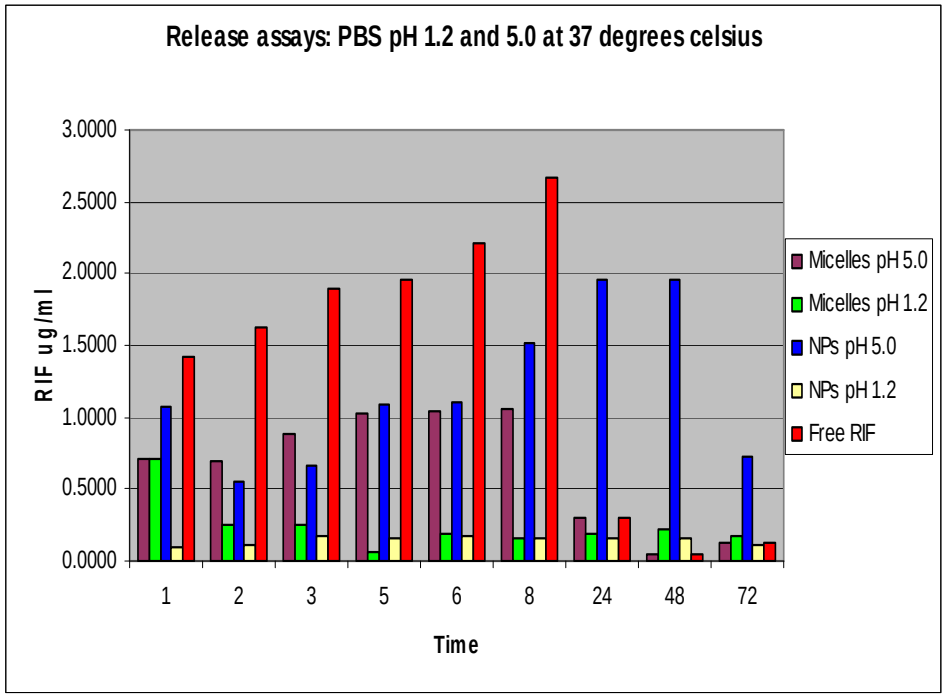
In vitro RIF release assays





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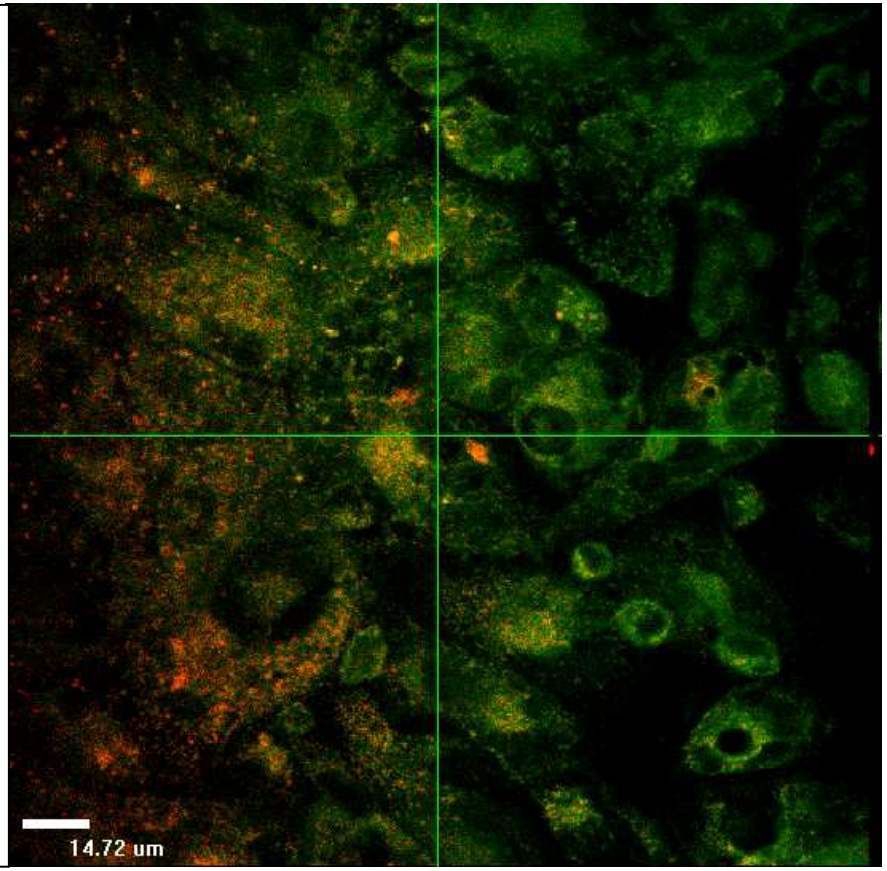
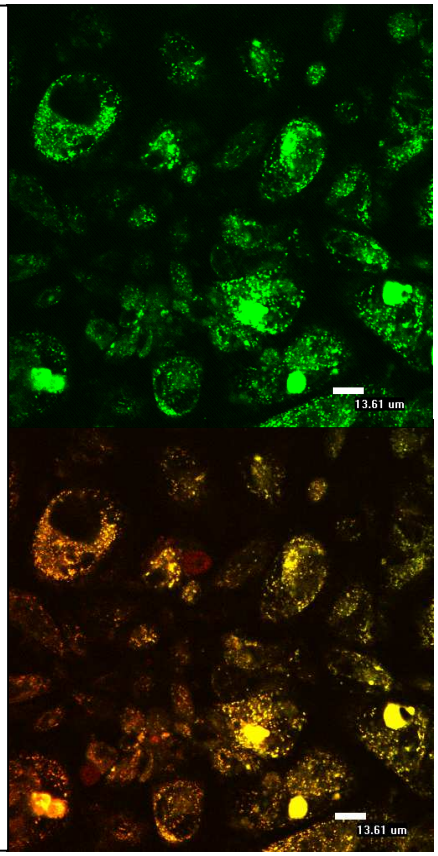
Transport/uptake study: confocal microscopy

Z-stack 30 min incubation



60 min incubation



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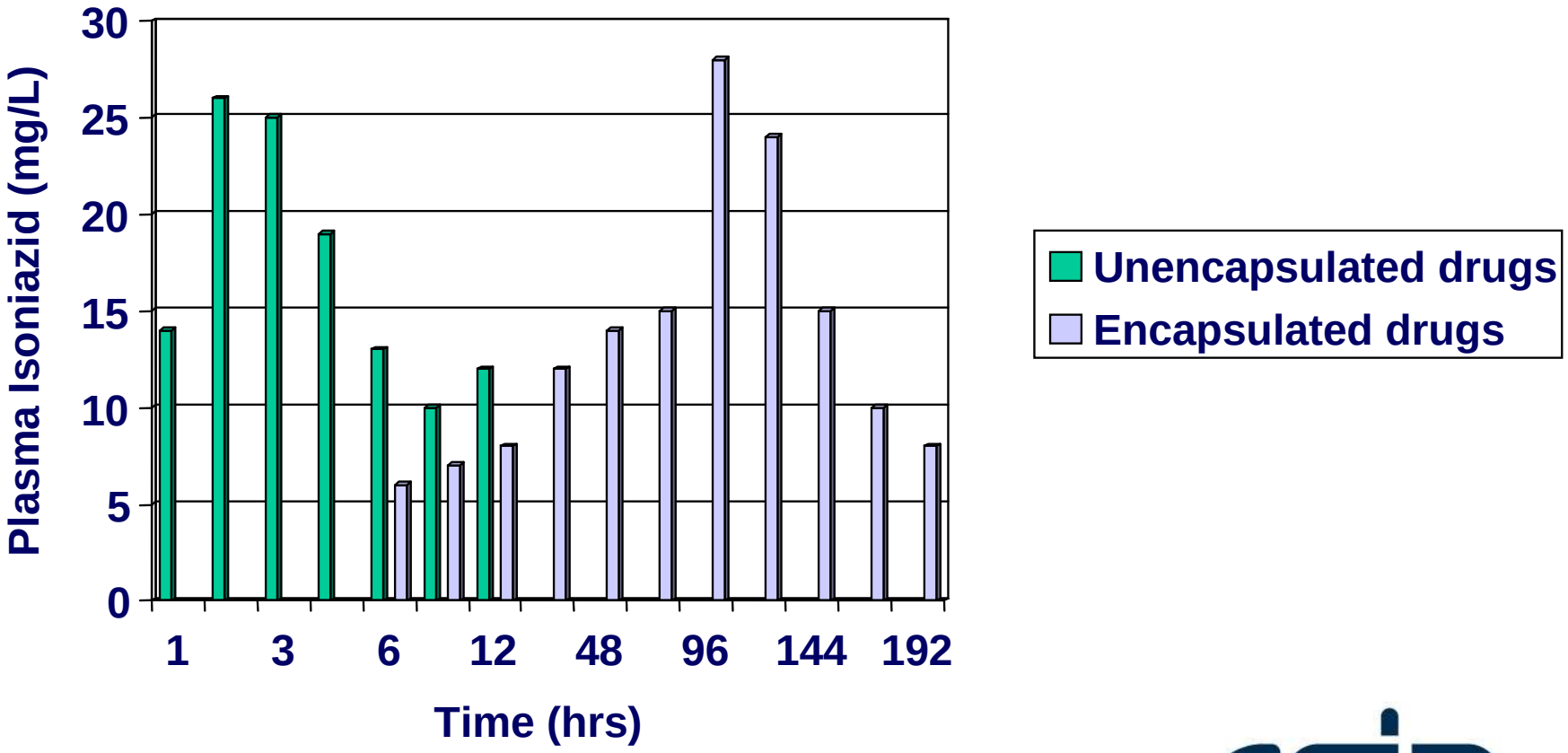
Oral nano-encapsulated ATD delivery system
(Khuller *et al*. 2003)



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Tissue levels of ATDs at day 10 following oral admin of NP formulation to mice

Drug	MIC (μg/ml)	Lung (μg/ml)	Liver (μg/ml)	Spleen (μg/ml)
RIF	0.25	0.6	0.9	0.3
INH	0.1 – 0.2	3	5.5	1
PYZ	8 - 20	15	25	23

Toxicology studies

- Performed over 28 days in mice
  - Three drug formulation
  - Four drug formulation
- Drugs administered orally
- No side effects detected in lung and spleen

Toxicology studies

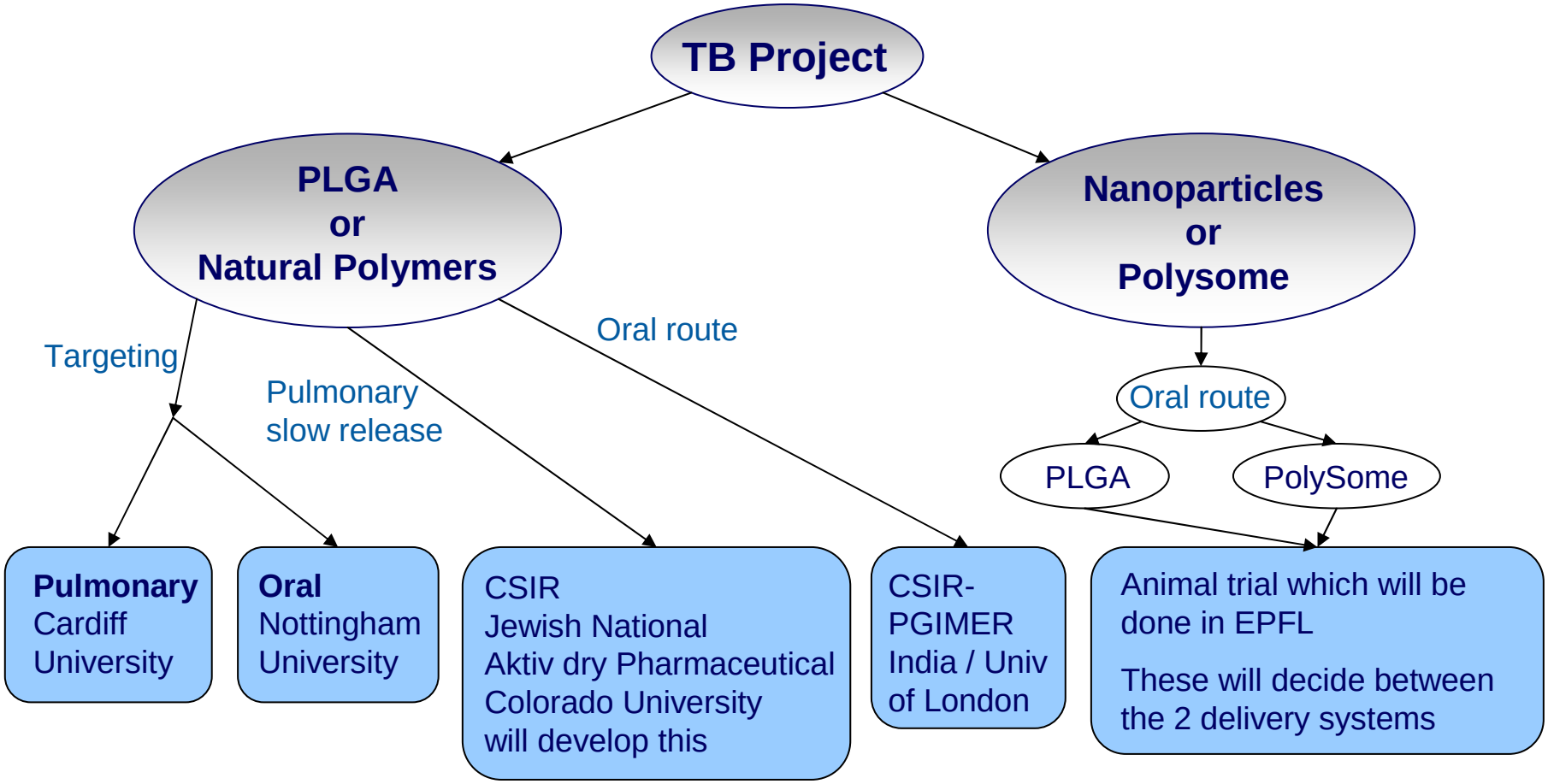
- Performed in guinea pigs
  - Similar pathophysiology to humans
  - Drugs administered orally
  - Analyzed in lung, spleen and liver
- ATD loaded PLGA detectable up to 11 days
- Free ATD cleared in 1-2 days
- No toxic side effects

Primate studies
to clearly establish the uptake mechanism

- MRC, US (Prof Seier)

- Objectives
  - Tissue distribution
  - Degradation profile
  - Bioavailability and bioequivalence
  - Determine dose level to be administered per kg/bwt
  - Pharmacokinetic (UCT)and histopathology

TB project



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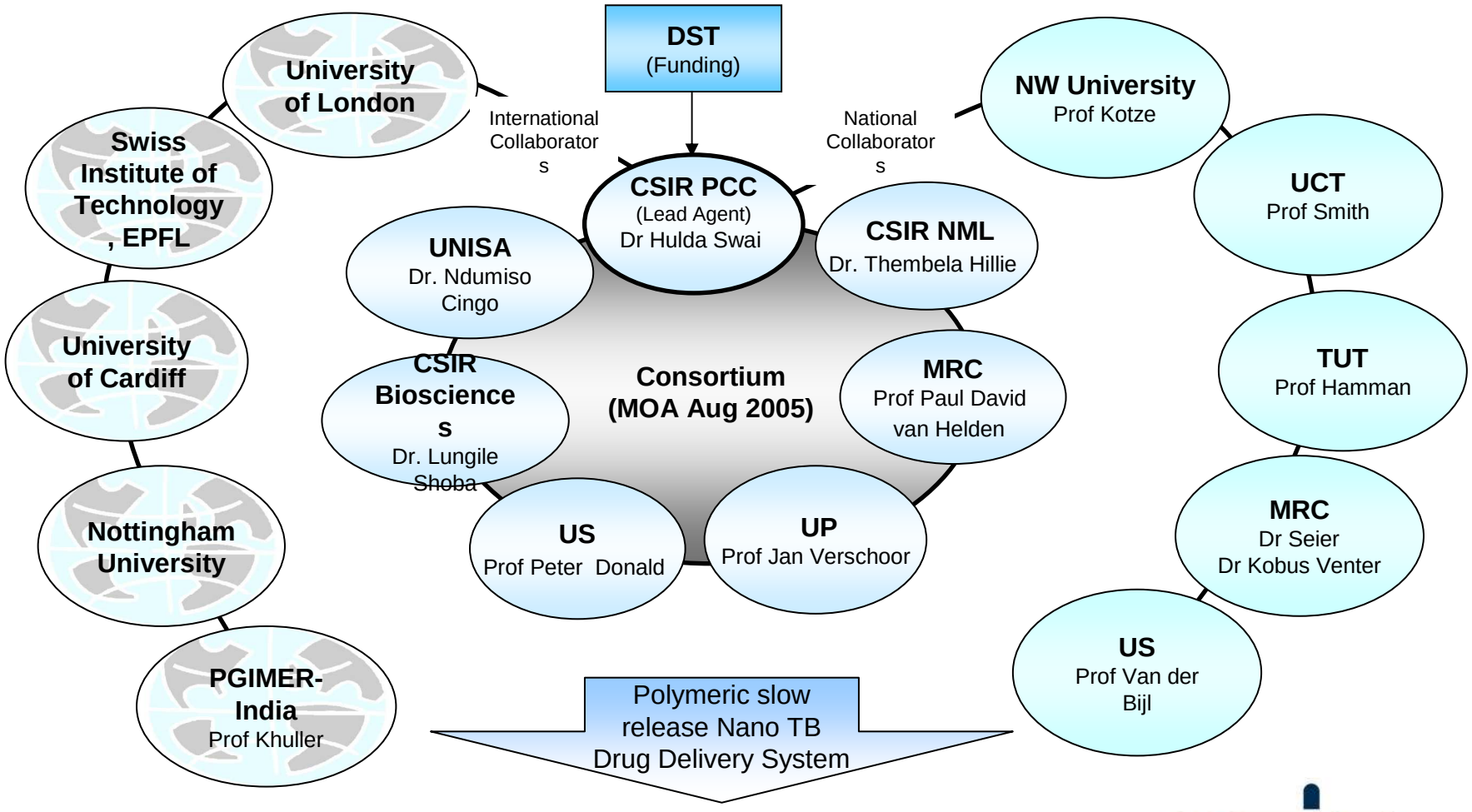
Project organisational overview



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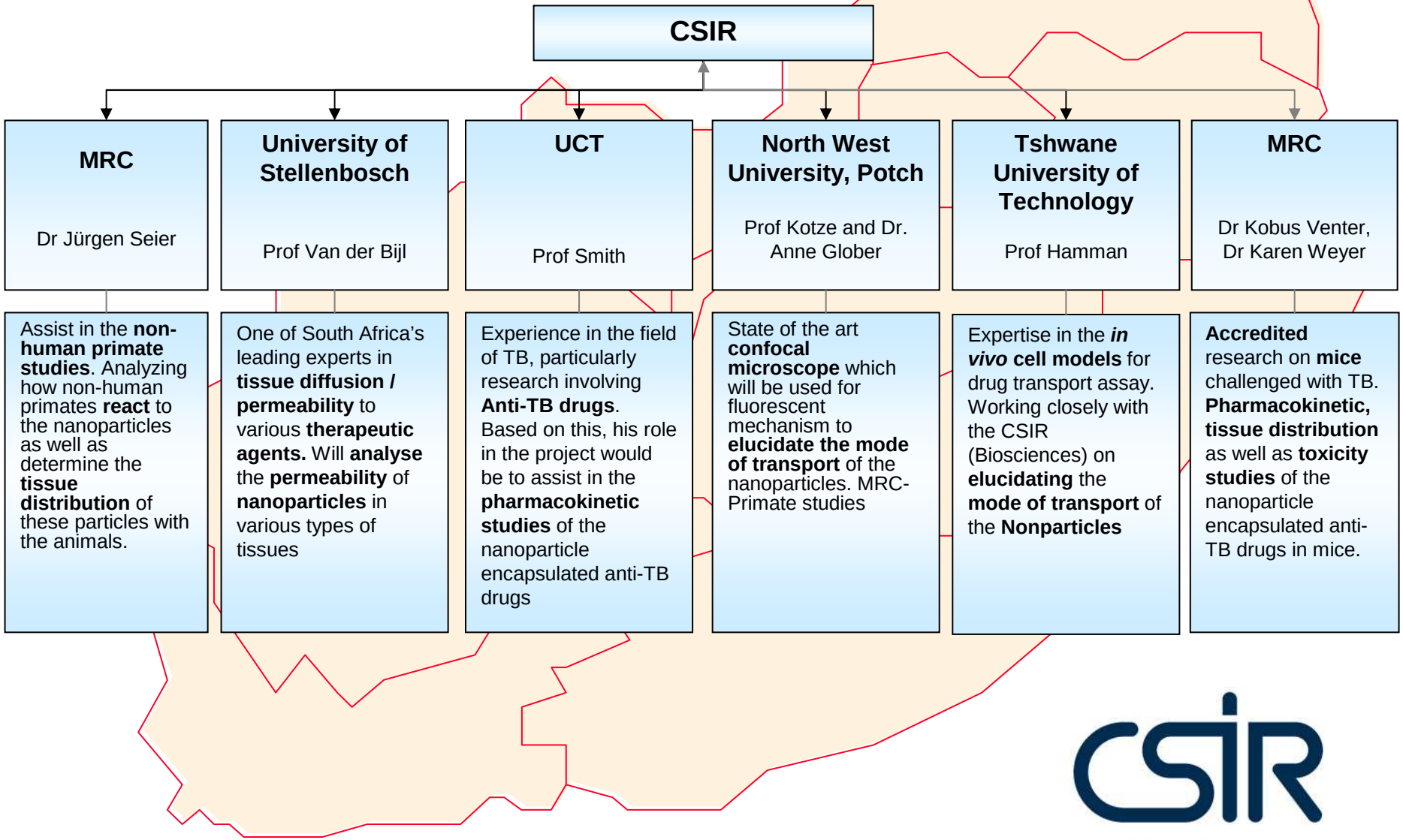
National collaborators



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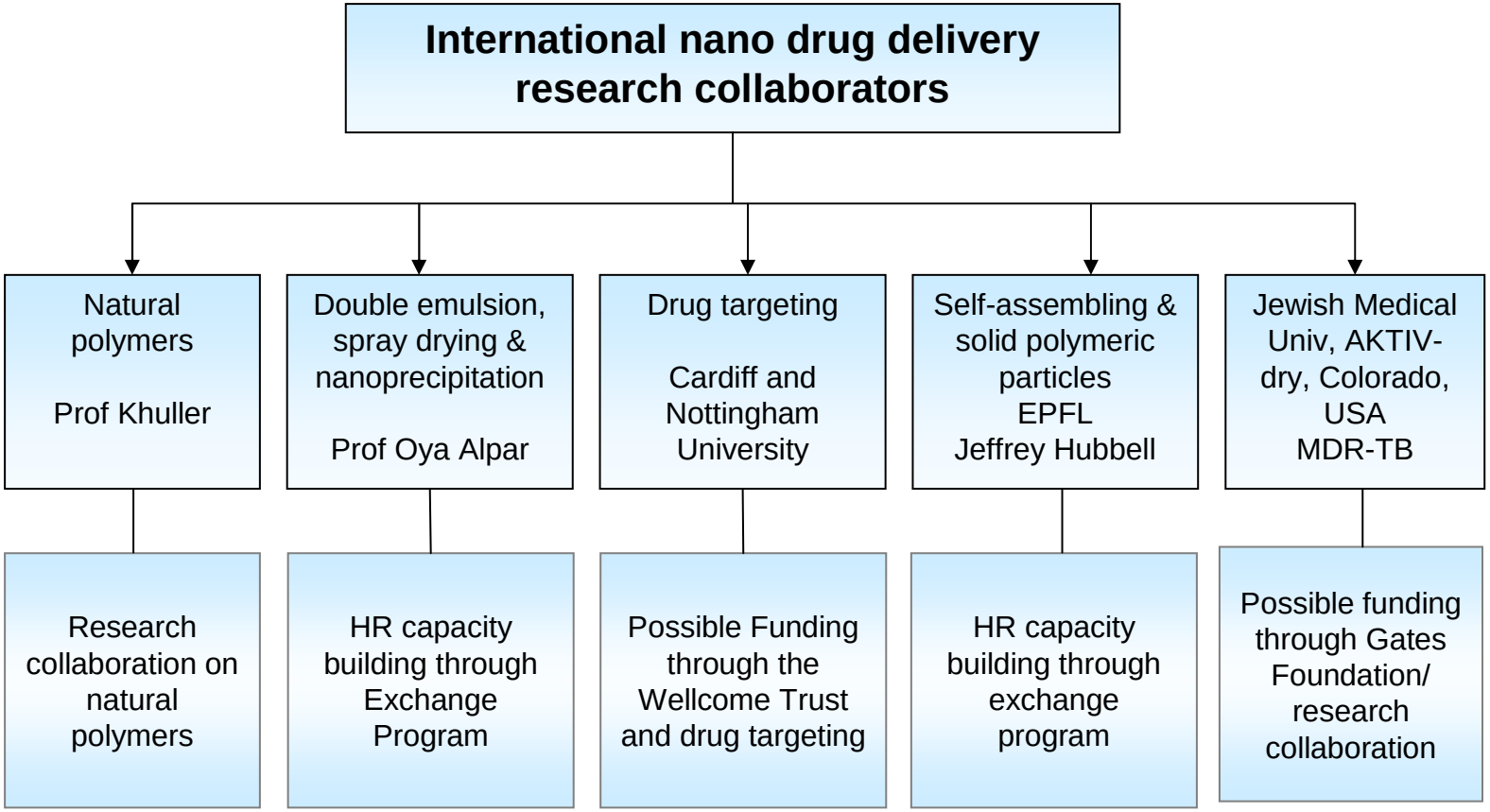
International collaborators



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The project team

Acknowledgements

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- TB consortium
  - Dr Shoba (CSIR, BIoSciences)
  - Dr Cingo (TUT)
  - Dr Hille (CSIR, NML)
  - Prof Vershoor (UP)
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Acknowledgements (continued)

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- International collaborators
  - EPFL
    - Prof Hubbell
  - Nottingham University
    - Dr Stolnik
    - Dr Alexander
    - Prof Shakesheff
  - University of London
    - Prof Alpar
  - Cardiff University
    - Dr Jones
    - Prof Duncan

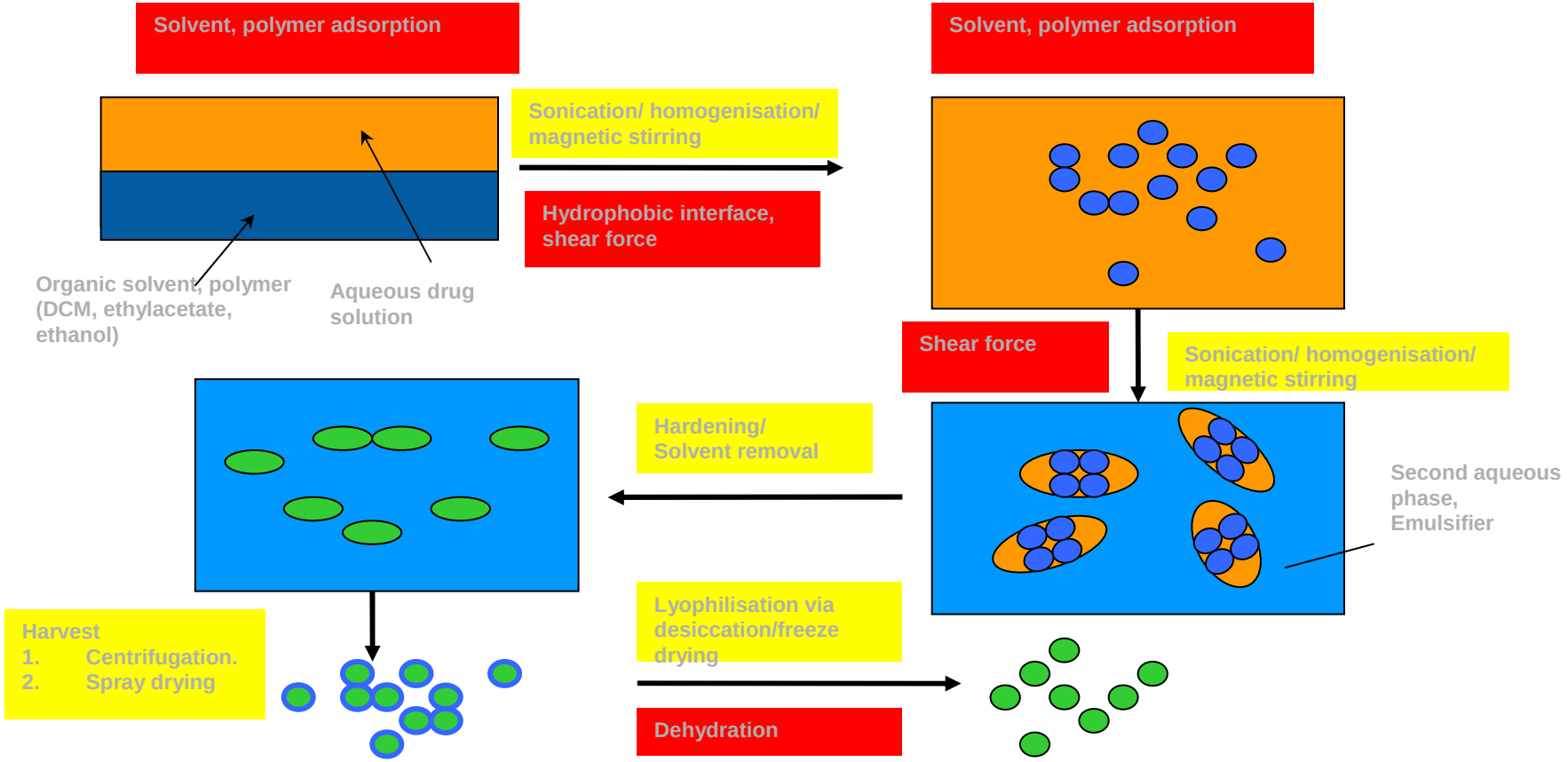
Thank you

Questions?

What is nano?

- A **nanometre** (nm) is a unit of measurement equal to a billionth of a metre, tens of thousands of times smaller than the width of a human hair. The prefix “nano” comes from the Greek word meaning “dwarf”.
- A **micrometre** (µm) is a unit of length equal to one thousandth (10-3) of a millimetre or one millionth (10-6) of a metre.
- **Nanoscience** is the study of the fundamental principles of molecules and structures with at least one dimension roughly between 1 and 100 nm. It is concerned with materials and systems of which the structures and components exhibit novel and significantly improved physical, chemical and biological properties, phenomena and processes, due to their nanoscale size.
- **Nanotechnology** is the application of nanoscience in technology devices. The essence of nanotechnology is the ability to work at the molecular level, atom by atom, to create large structures with fundamentally new molecular organisation.

Preparation of IHN-PLGA nanoparticles via the double emulsion solvent evaporation



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