Providing an address for delivery of nanoencapsulated TB drugs

Yolandy Lemmer

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UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

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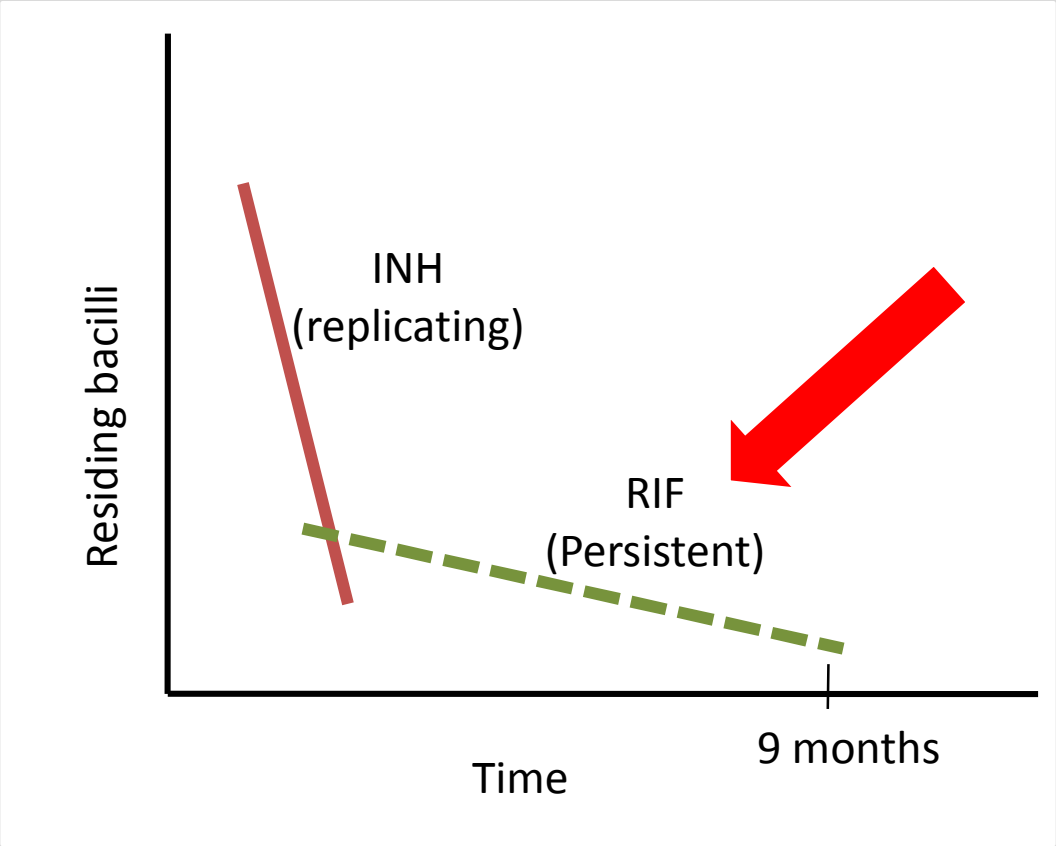
Objective

Reduce the dose frequency of anti-tb drugs and simultaneously shorten duration of treatment by:
nanoencapsulation of drugs and adding a targeting ligand to address persistent *M.tb* infection



Adapted from :Comstock 1999

Background

Nanoparticles:

Refer to: Dr. H.S. Swai poster no. 83

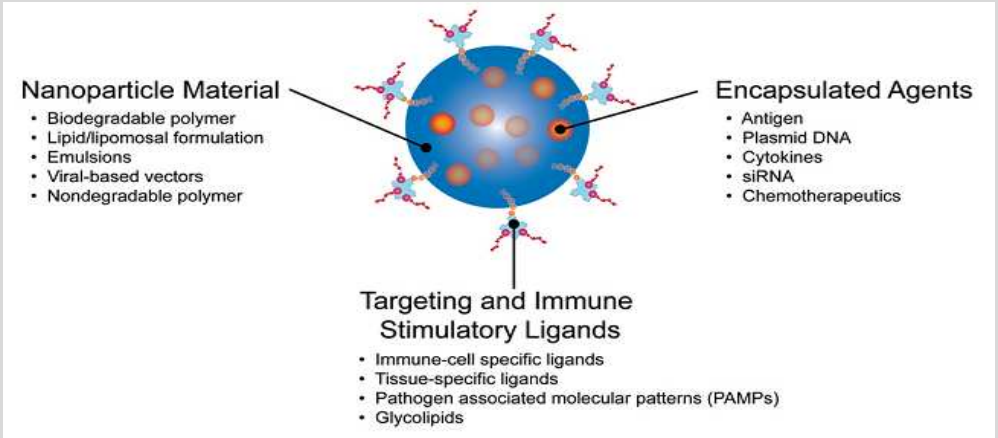
Mr. L. Kalambo presentation: Track 1 session 4 (03/06/10)

History:

- First drug delivery systems described 1960's – liposomes
- First controlled release polymer 1976
- Followed by other drug delivery & targeting ligands

Advantages

- Hydrophobic & hydrophilic drugs
- Targeted delivery in cells & tissue (small size)
- Controlled release
- Improvement of bioavailability

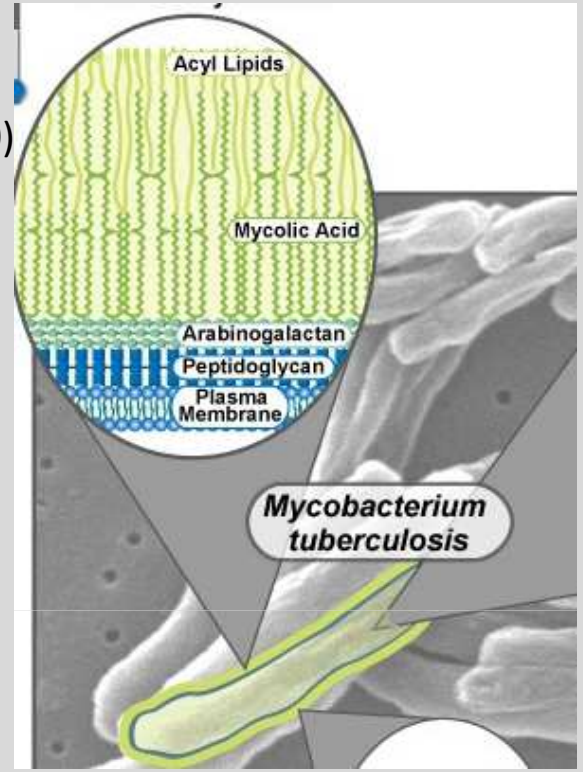


(Look 2009, Farokhzad 2009, Bangham 1965, Langer 1976)

Background

Targeting ligand: Mycolic acids

Refer to: Prof. J.A. Verschoor presentation: track1 session 2 (02/06/10)



www.niaid.nih.gov/.../tuberculosis/tb1.jpg

The wax coat :

Mainly consists of mycolic acids (MA)

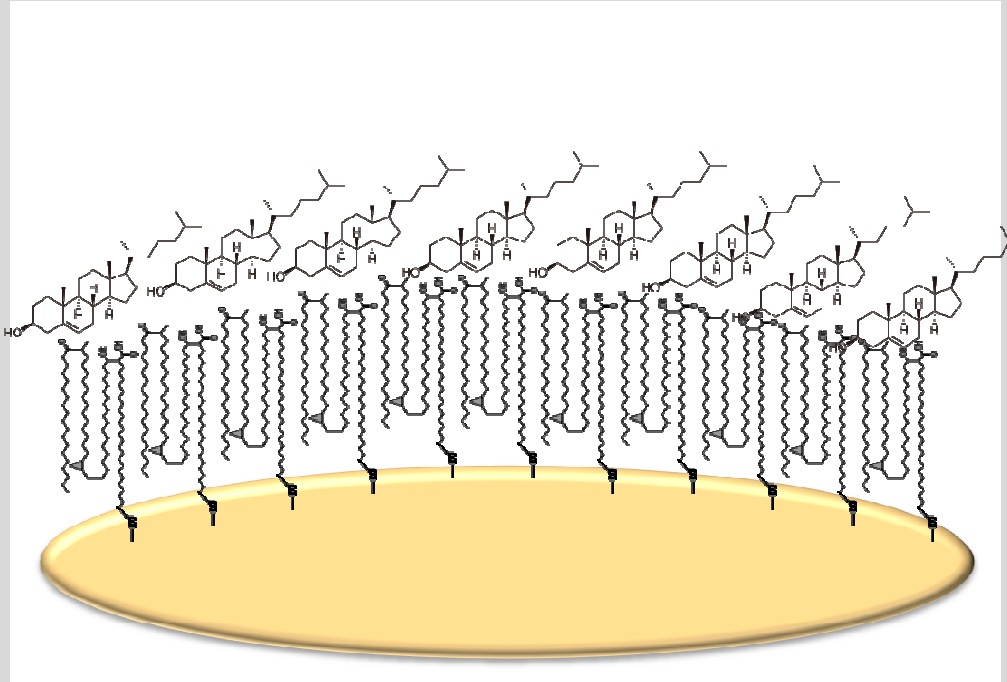
Properties of MA

- Convert macrophages into foam cells (Korf J.E. *et al.,* 2005)
- Assumes cholesteroid nature and attracts cholesterol (Benadie Y. *et al.,* 2008).
- Present in high concentrations in the extracellular matrix of *M tb.* biofilms , contributing to drug tolerance (Ojha A. *et al.,* 2008).

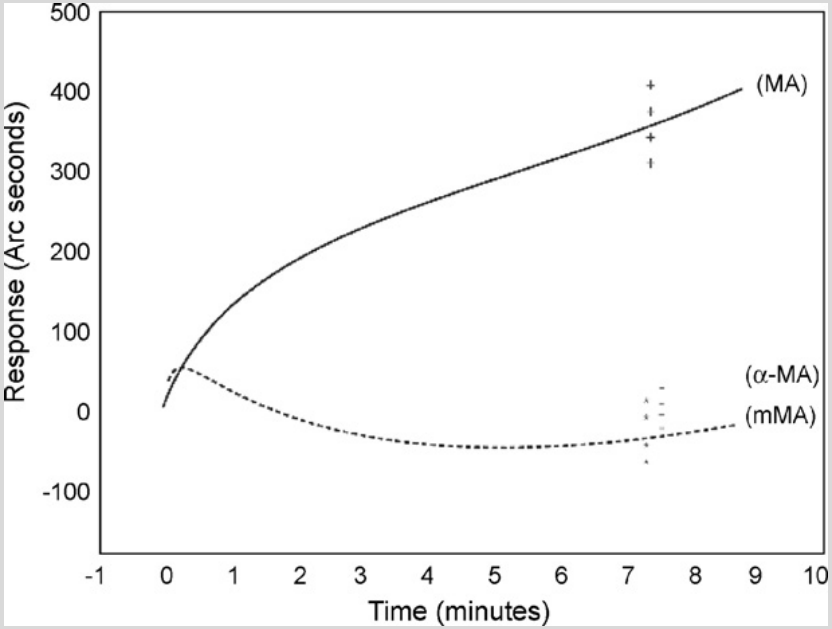
Thus, MA may possibly target the cholesterol enriched infected areas

Methods and Results

The cholesteroid nature of native MA



SPR biosensor – measures mass accumulation on immobilized ligand



- Mycolic acids actually attract cholesterol !

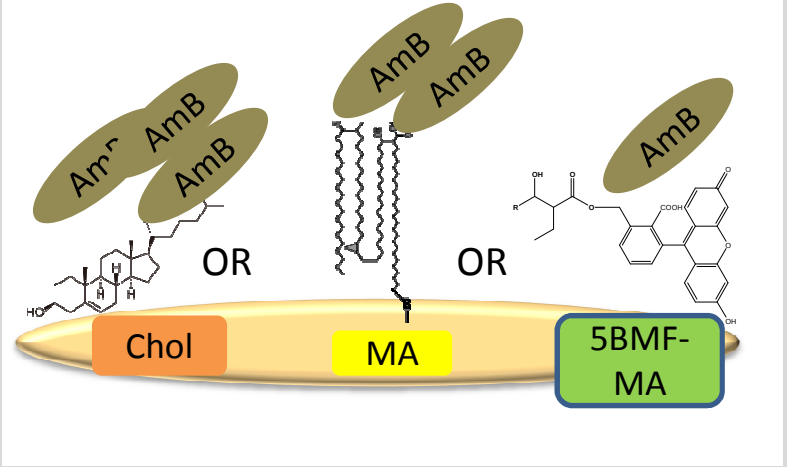
- Thus, use MA as targeting agent in anti-TB NP drug delivery

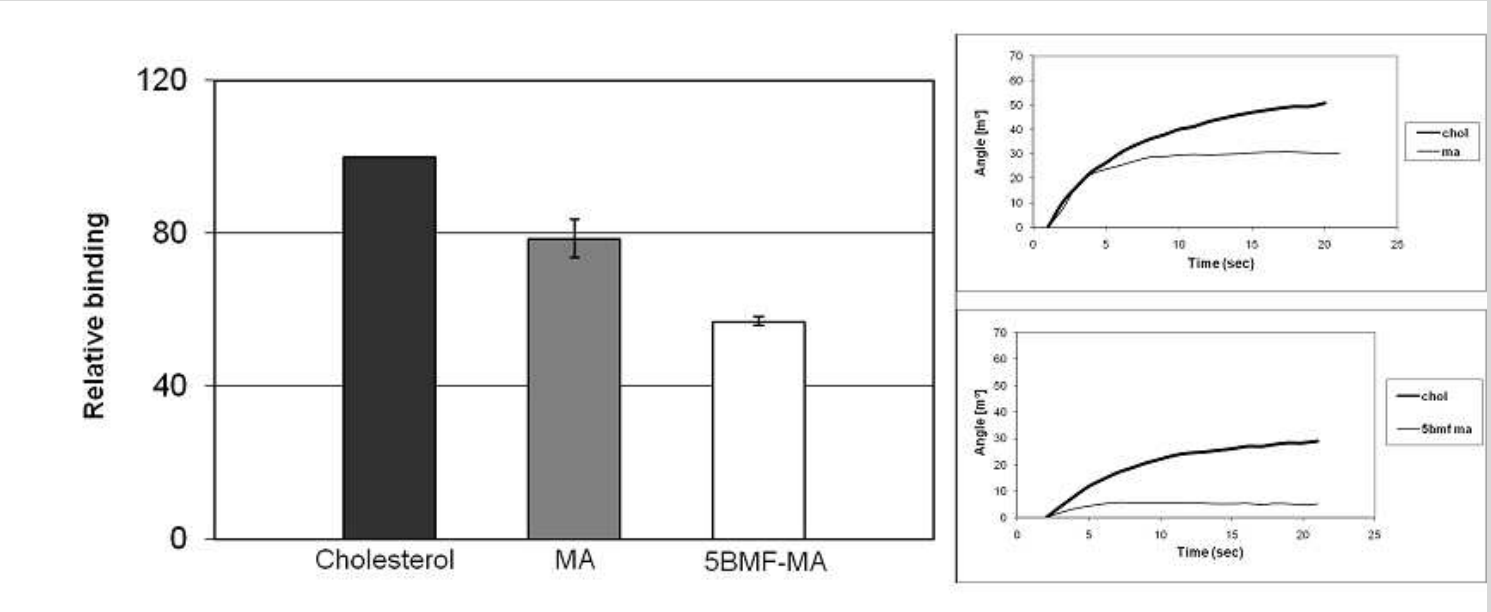
(Benadie Y. *et al.*, 2008)

Methods and Results

The cholesteroid nature of native MA

- Struct relationship & attraction between free MA and cholesterol (Benadie Y. *et al*., 2008).
- Principle confirmed using the ESPRIT biosensor
  - Cholesteroid nature attenuated when MA structure altered





(Lemmer Y *et al.,* 2009)

Methods and Results

Nano encapsulation of mycolic acids

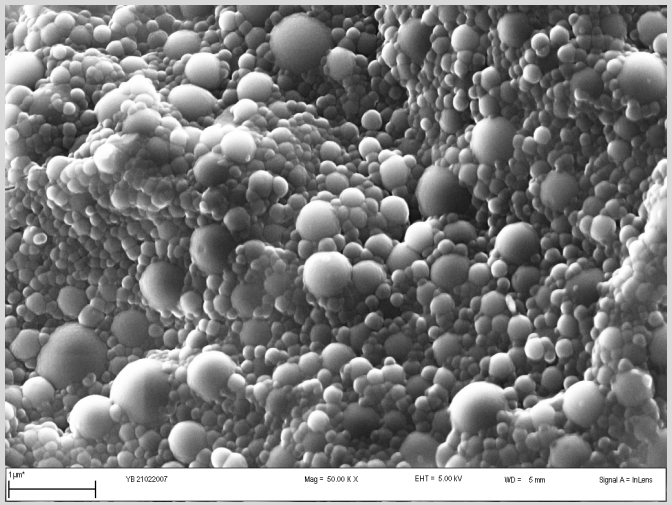
Polymer and / or Targeting ligand and / or Anti-TB drug

Poly lactide-co-glycolide (PLGA)

MA (with or without fluorophore)

INH

Double emulsion evaporation technique (W/O/W).



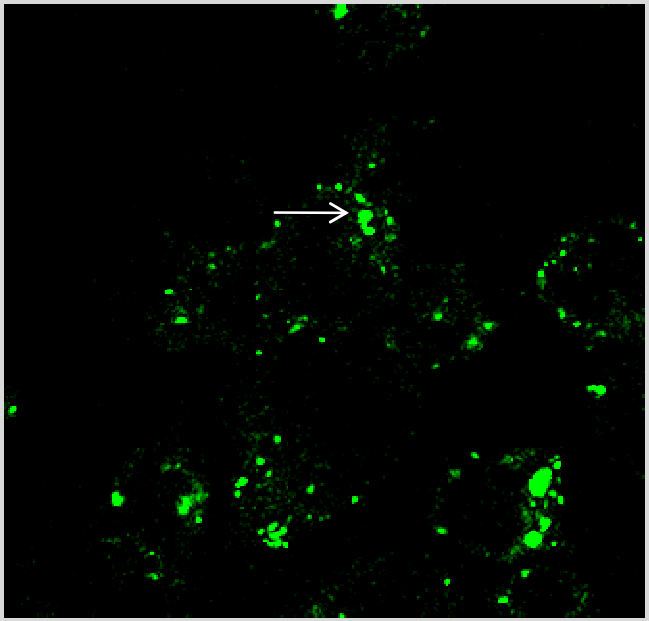
~ 500nm

(Penco, M. et al.,1998; Tsung, M.J. et al., 2001)

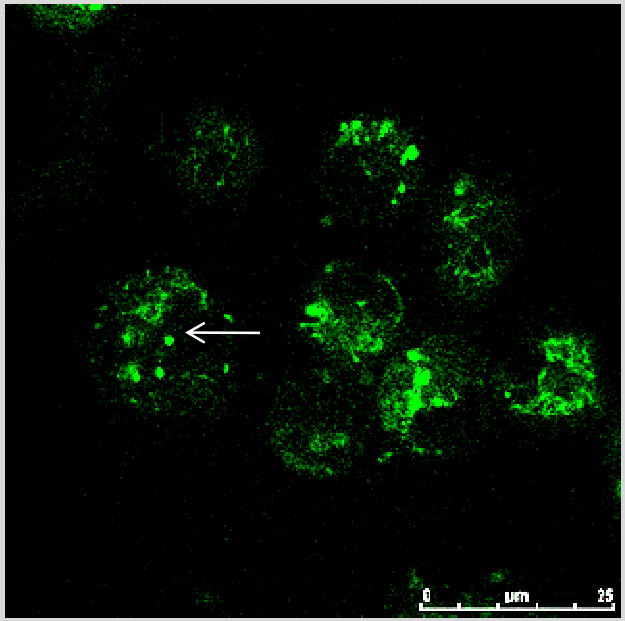
Methods and Results

Nanoparticle uptake in U937 and THP-1 macrophages

- Live cell images of fluorescently labeled MA PLGA NP uptake after 3 hours in macrophage cell lines.



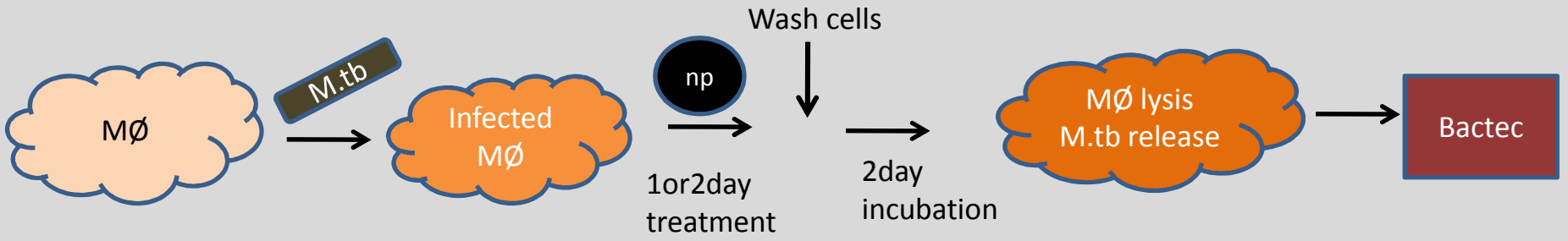
a) THP-1

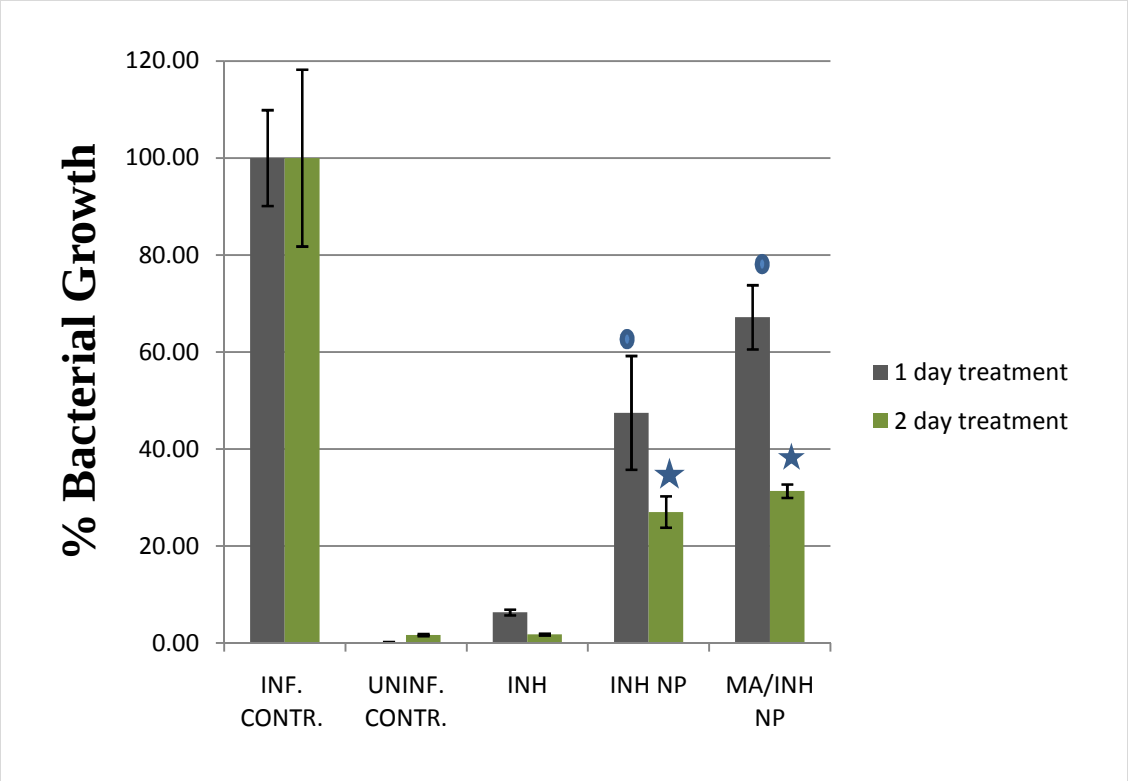


b) U937 macrophages.

Methods and Results

Assess antibacterial effect of isoniazid (INH) containing nanoparticles vs free drug





1) Does NP release INH?
Yes, but slower

2)Does MA influence efficiency of Mtb inhib?
No clear indication at this point

Graph: [INH] = 0.2 ug/ml (2 ug/ml = out of range) ★ ● = P<0.01

Kisich K. *et al.*, 2007

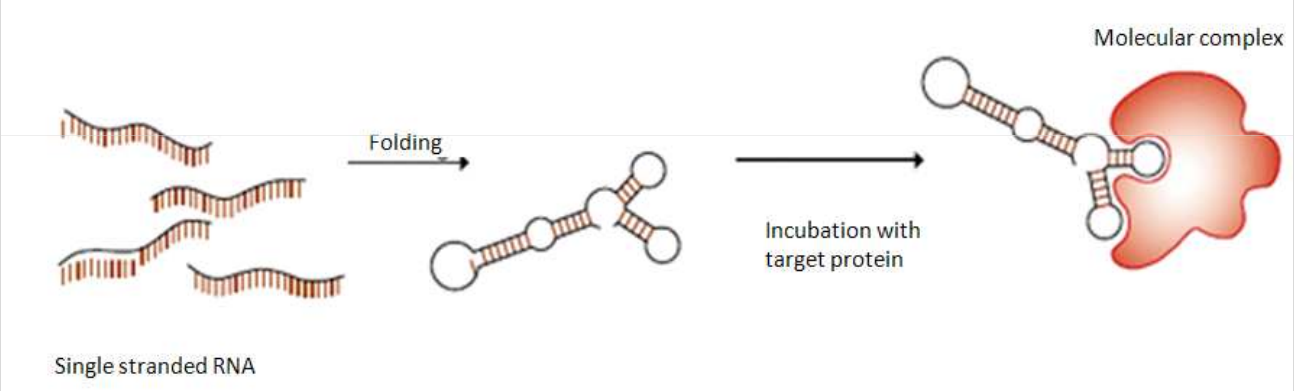
Background

Targeting ligand: Aptamer against Mannose receptor

Refer to: Dr. B. Semete-Makokotlela email: Bsemete@csir.co.za

Aptamers:

- Ab like molecules made up from nucleic acids
- Directed against ligand
- Used as targeting molecule on surface of NP



www.ufz.de/data/11525.gif

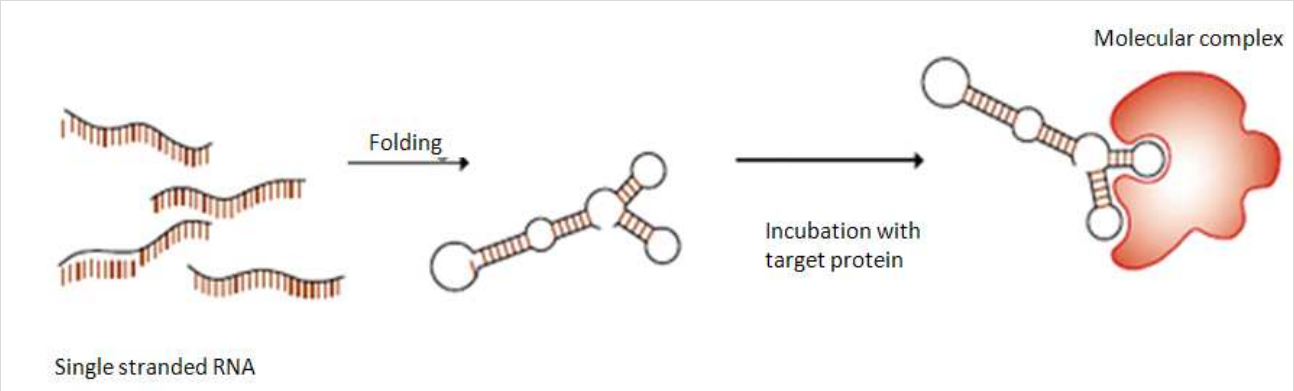
Mannose receptor :

- Transmembrane receptor protein
- Over expressed in infected macrophages (acute stage only?)

Methods and Results

Synthesis of aptamers:

SELEX process:



Random RNA pool

Mannose receptor ligand

Partitioning with NanoSep 100kDa MWCO,
Selection of aptamers against target receptor using size exclusion

Challenges:

Could not enrich beyond 52% recovery
100 bp primer dimers

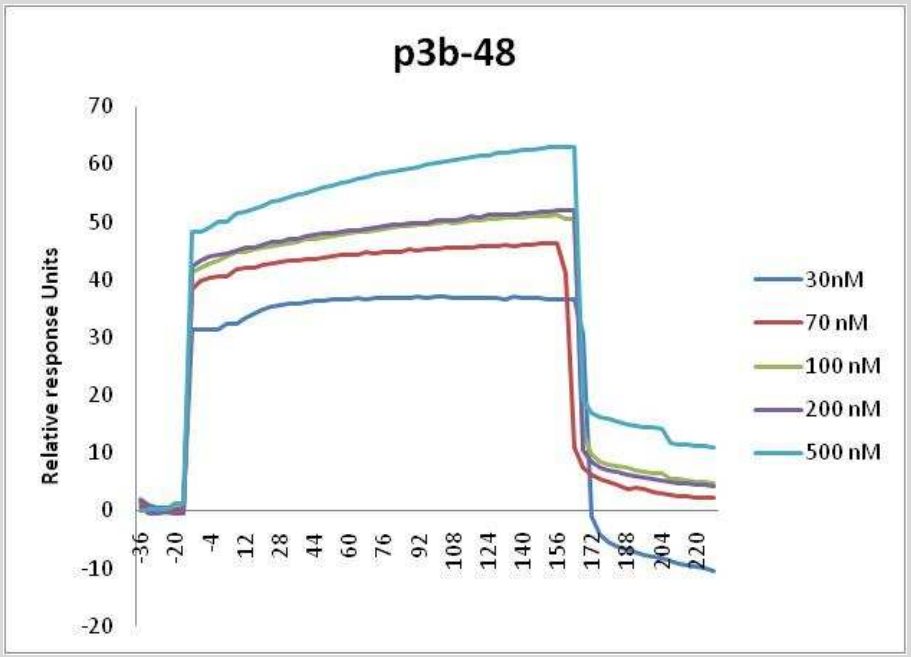
www.ufz.de/data/11525.gif

Methods and Results

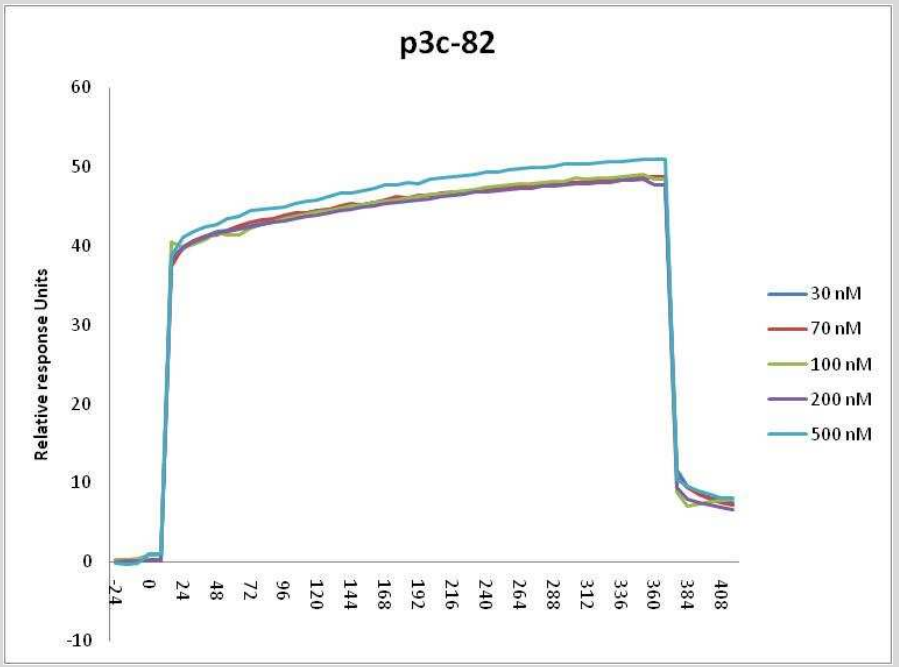
Testing for binding affinity on Biacore biosensor:

- Mannose receptor bound to surface on chip
- Test clones for binding affinity

Binding kinetics (10 clones)



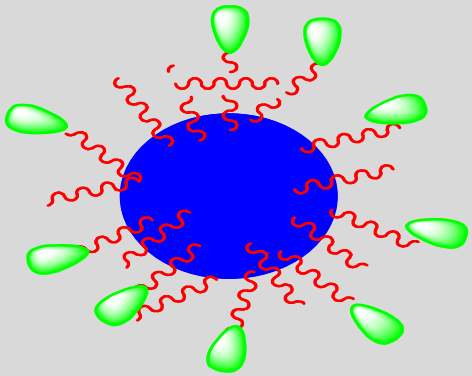
KD: 1.3uM±2.5



KD: 60 nM±0.15

Methods and Results

Conjugate aptamers to nanoparticles:



= PEG- COOH

=Aptamer

- Succinamide coupling of PEG to NP
- Derivatise aptamer to form NH2 – couple to NP

Conclusion and future perspective

- ✓ MA (chol) and Aptamers (against the mannose receptor) could be used as targeting tools

- ✓ MA:

  - ✓ Attract cholesterol
  - ✓ Successful production of MA NP ave size ~ 500nm
  - ✓ *In vitro* uptake into macrophages
  - ✓ *In vitro* localization possibly cytoplasm?
  - ✓ *In vitro* drug testing via BACTEC indicated:
    - ✓ INH released from NP
    - ✓ MA does not show advantage in early replication phase as expected

- ✓ Aptamers:

  - ✓ Against mannose receptor
  - ✓ Coupling to NP
  - ? *In vitro* drug testing via BACTEC
  - ? Confocal imaging of THP-1 cells
  - ? FACS to obtain quantitative data

Acknowledgements

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