
[dstl]

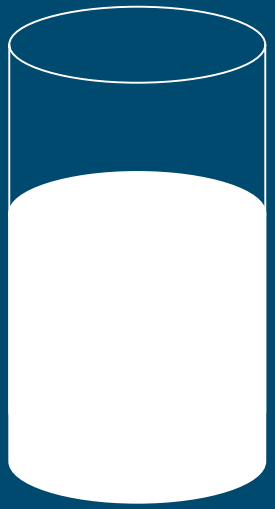
Mucosal immunization against plague and anthrax using microparticles

Jim Eyles

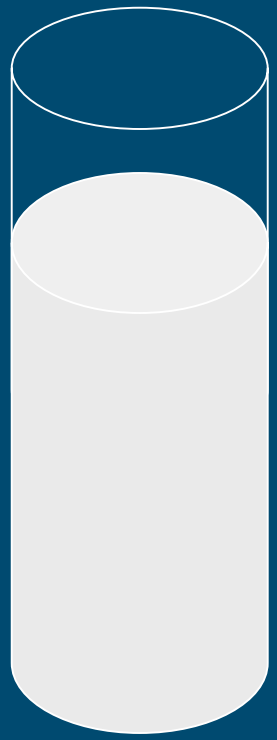
The Science of the technology

- Biodegradable microparticles fabricated using emulsification / solvent evaporation process

Microsphere formulation

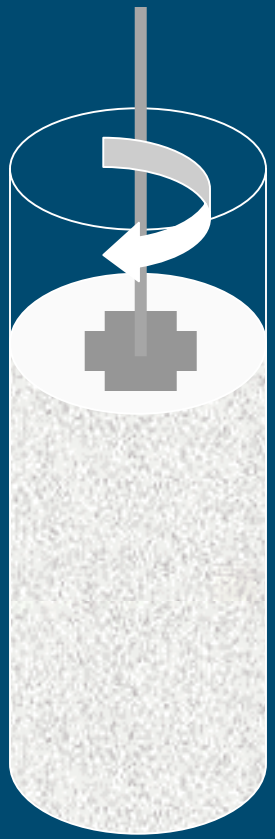


Antigen solution



Polymer in solvent

Microsphere formulation



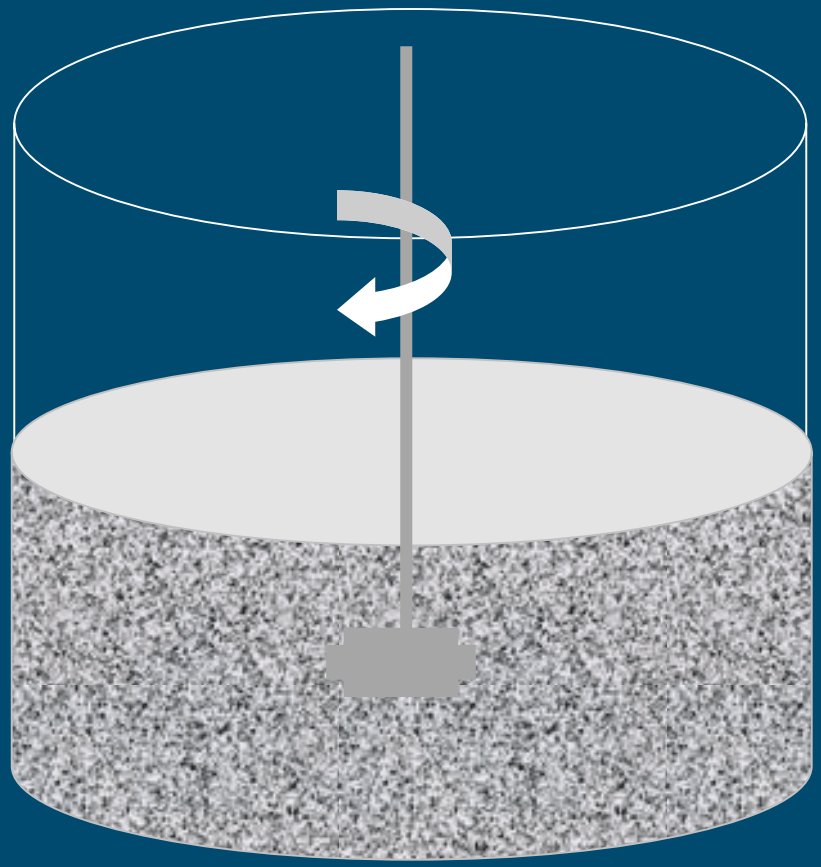
Form Water in Oil emulsion by rapid mixing of two phases

Microsphere formulation

W/O emulsion

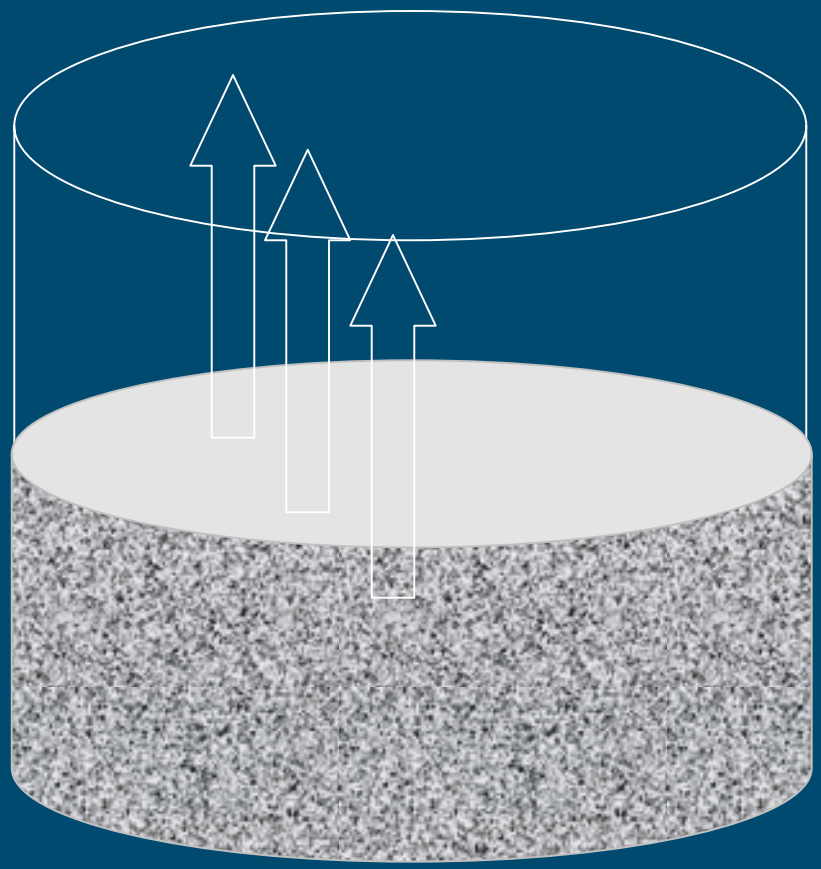
Stabiliser in water

Microsphere formulation



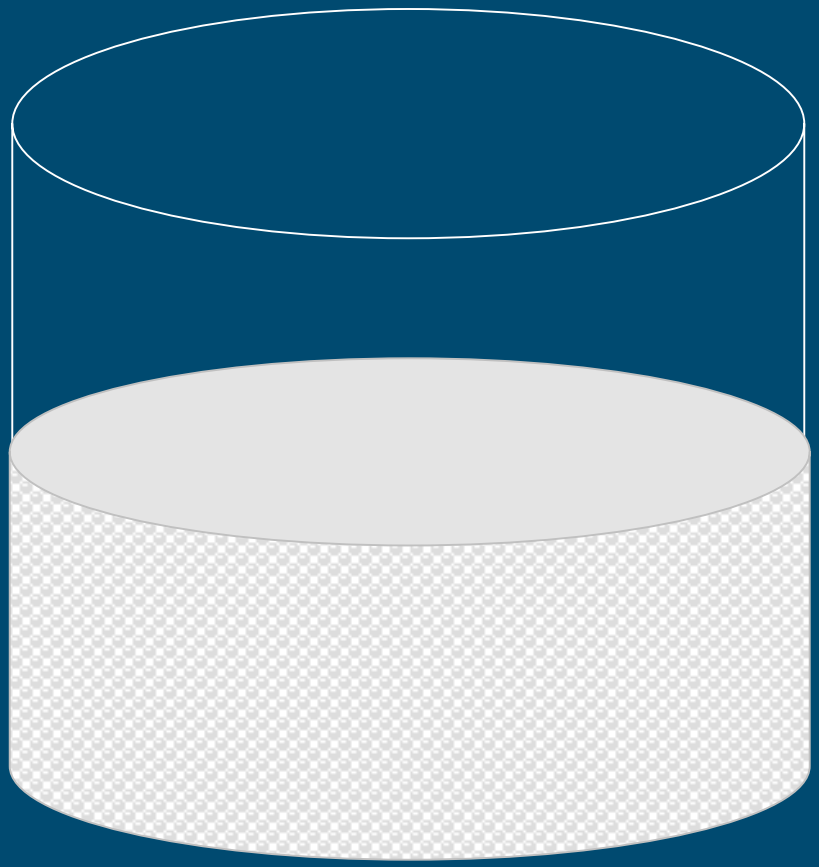
Form water in oil in water emulsion by rapid mixing of W/O emulsion and second aqueous phase

Microsphere formulation



Allow solvent to evaporate

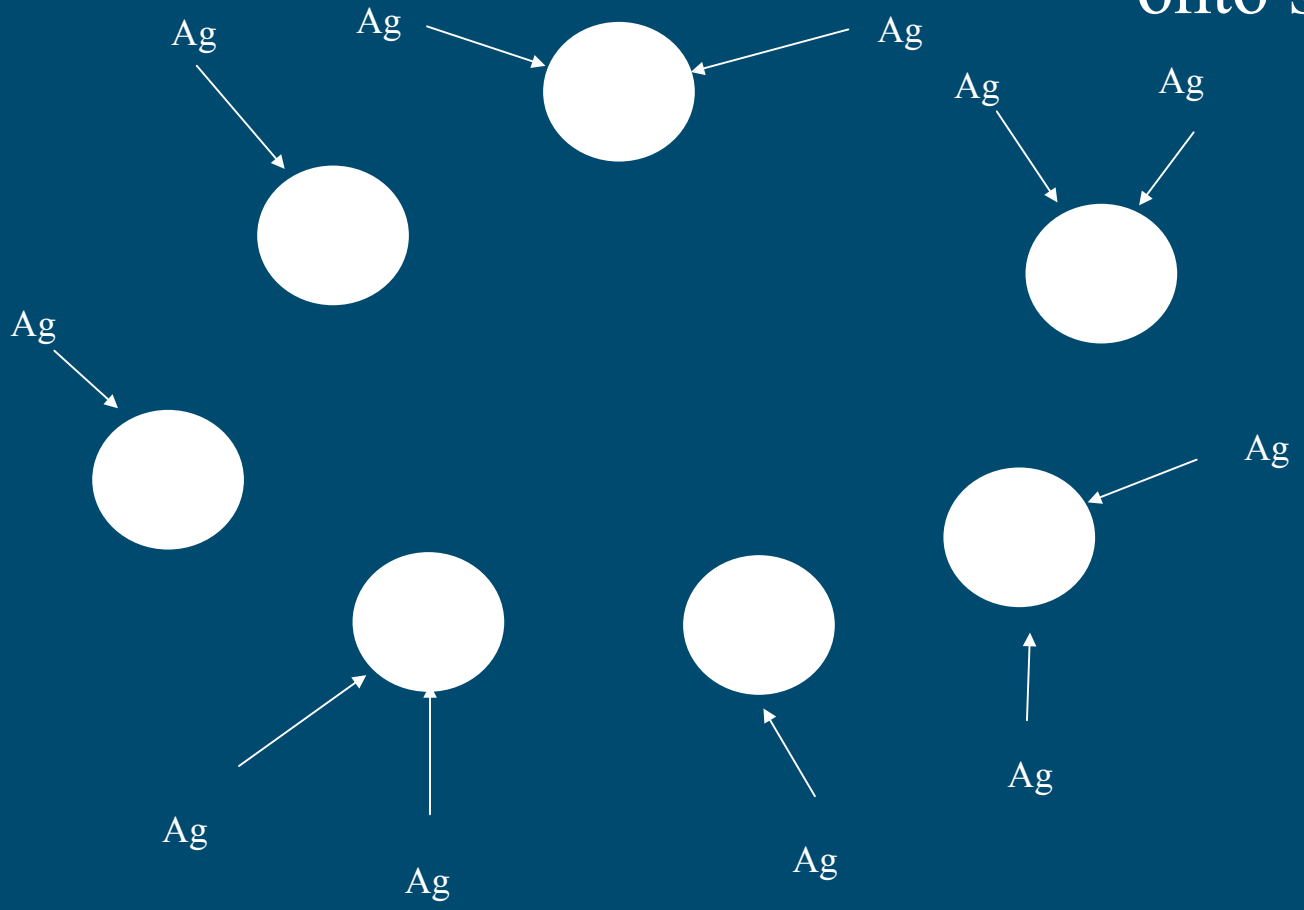
Microsphere formulation



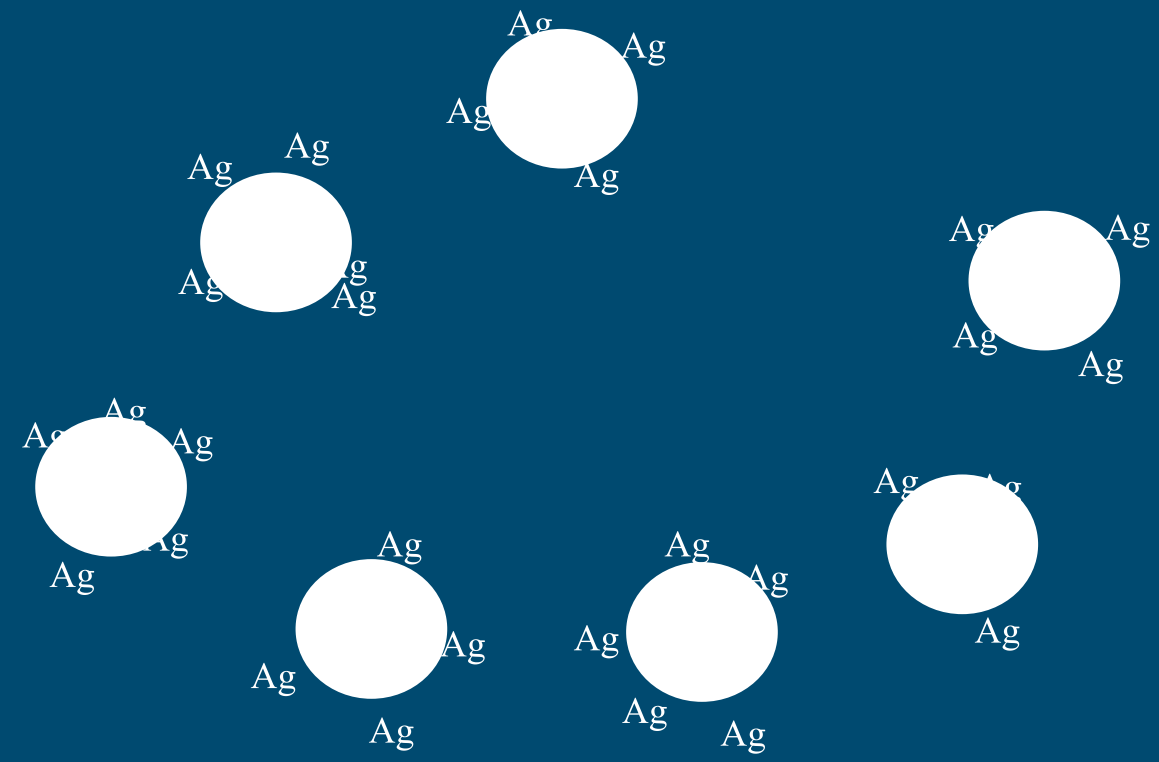
Microspheres containing antigen form as solvent evaporates

Microsphere formulation

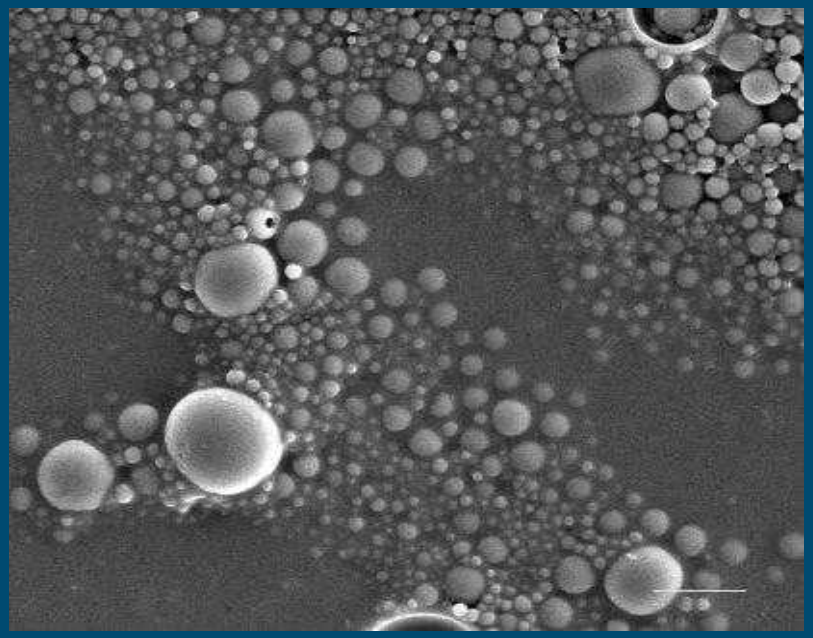
Adsorb antigen onto surface



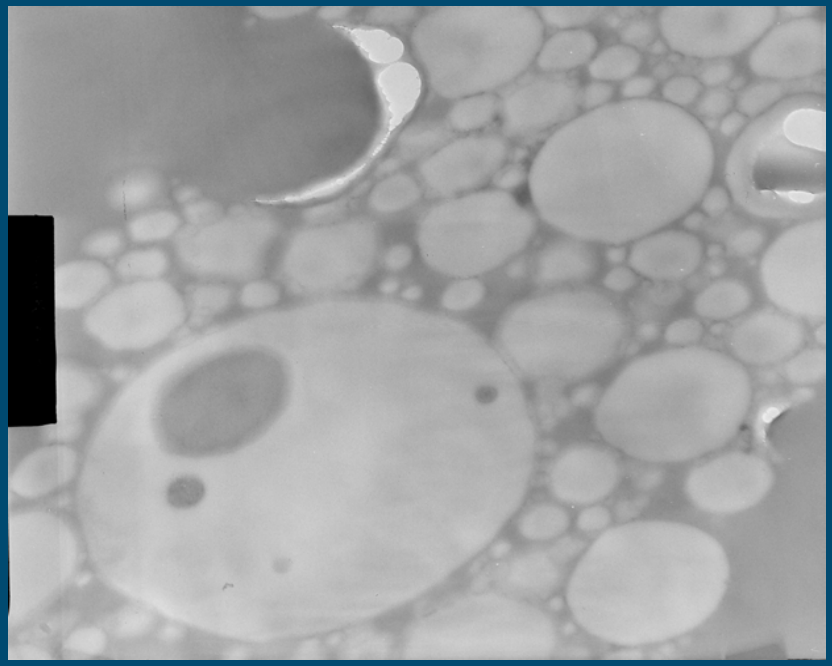
Microsphere formulation



Particle characteristics: morphology

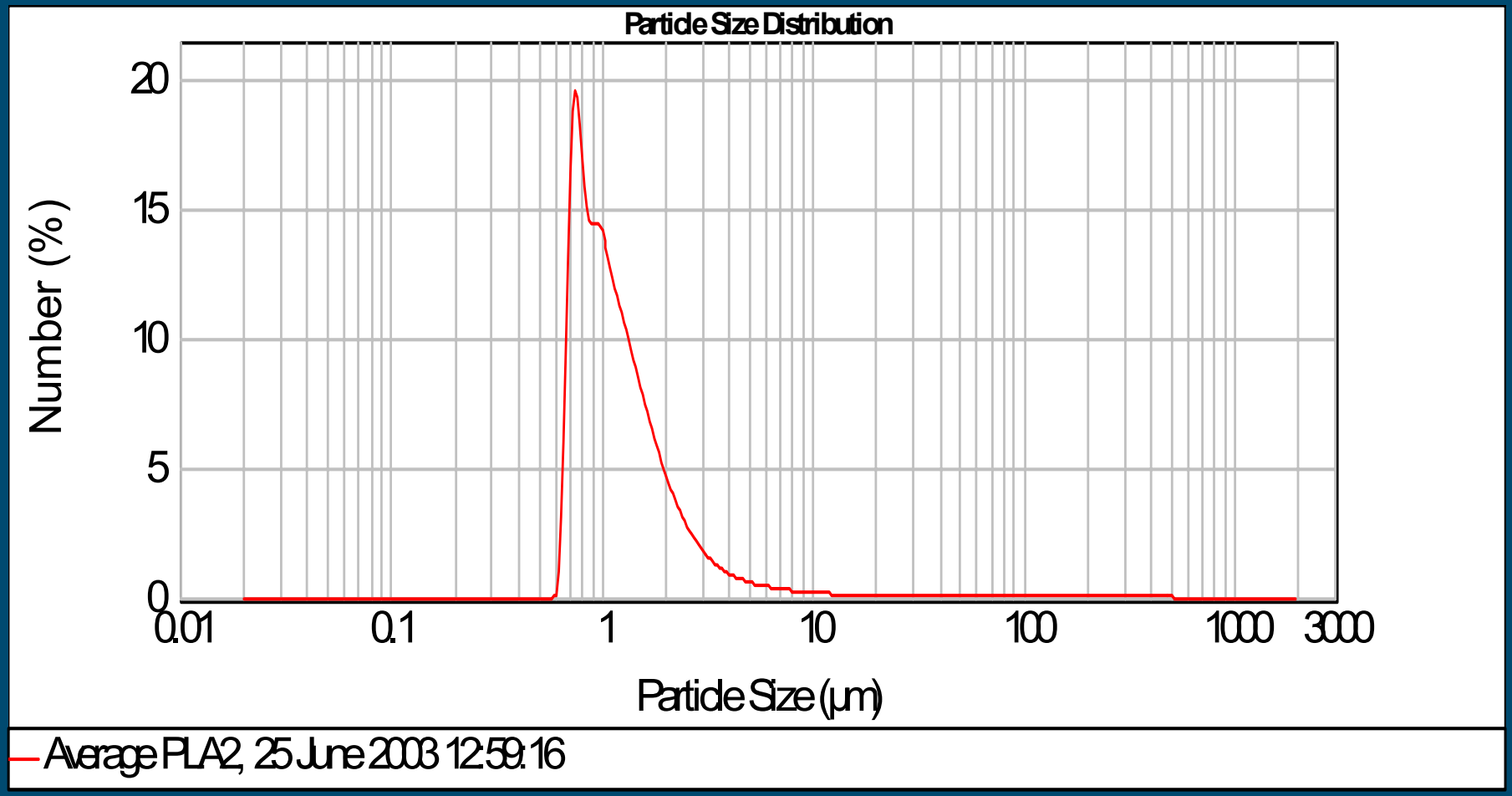


SEM

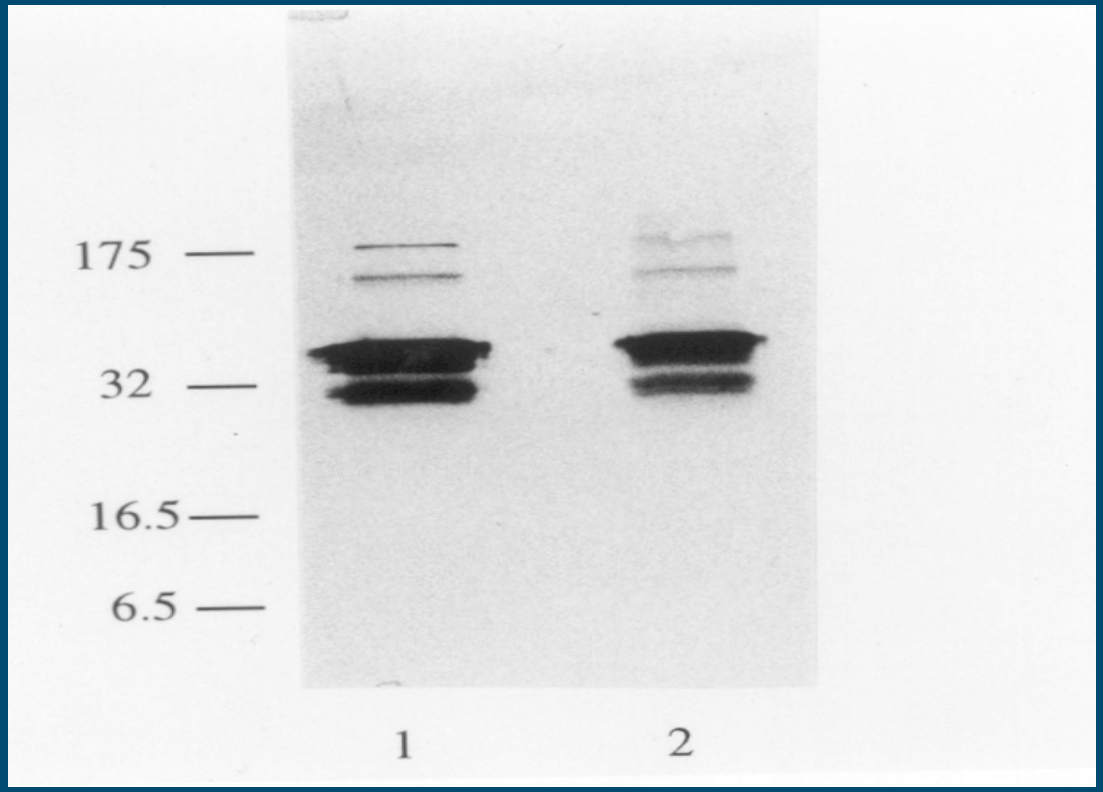


TEM

Particle characteristics: size



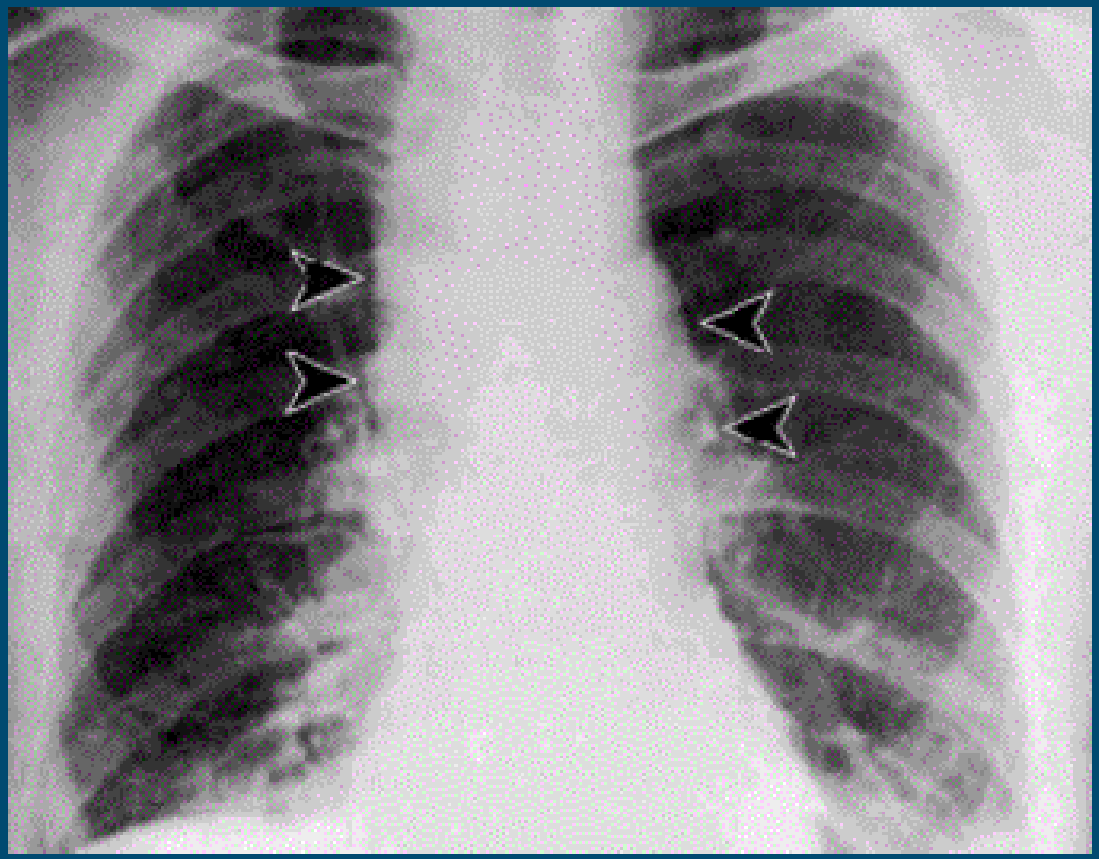
Particle characteristics: bioactivity of encapsulated material retained



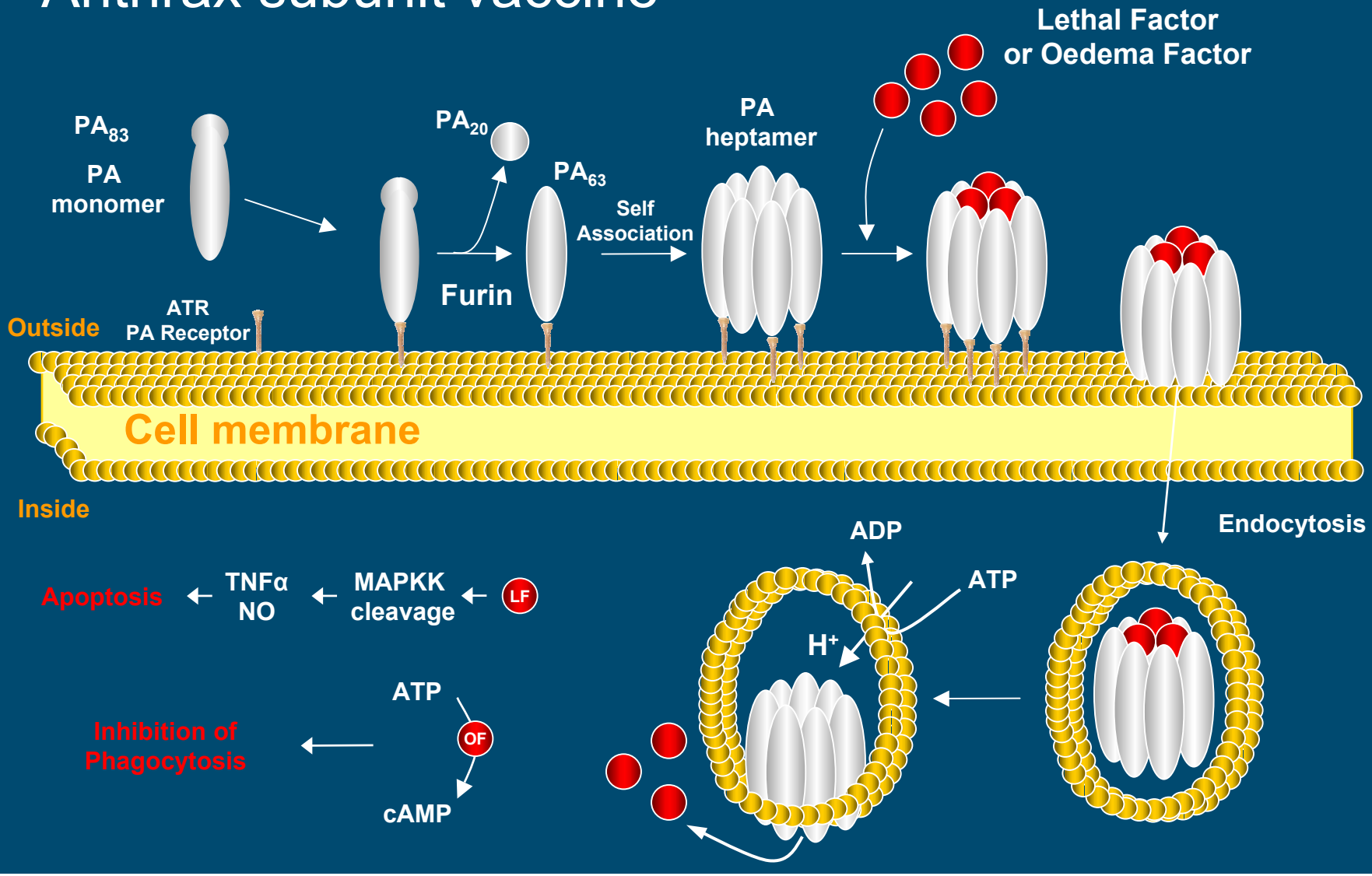
Distinguishing features of formulation

- Crystalline polymer (PLLA)
- Particle size (1-5 μm)
- Pronounced burst followed by protracted release of antigen
- High doses achievable (if necessary)

Microparticles for mucosal immunization against inhalational anthrax



Anthrax subunit vaccine



Targeted immune response

- Anti-PA neutralizing antibody correlates with protection

however

Targeted immune response

- Anti-PA neutralizing antibody correlates with protection

however

- Evidence that cell mediated immunity is important in the early stages of anthrax infection
  - clearance and destruction of spores and vegetative cells by macrophages in the lung milieu

How technology facilitates immune response

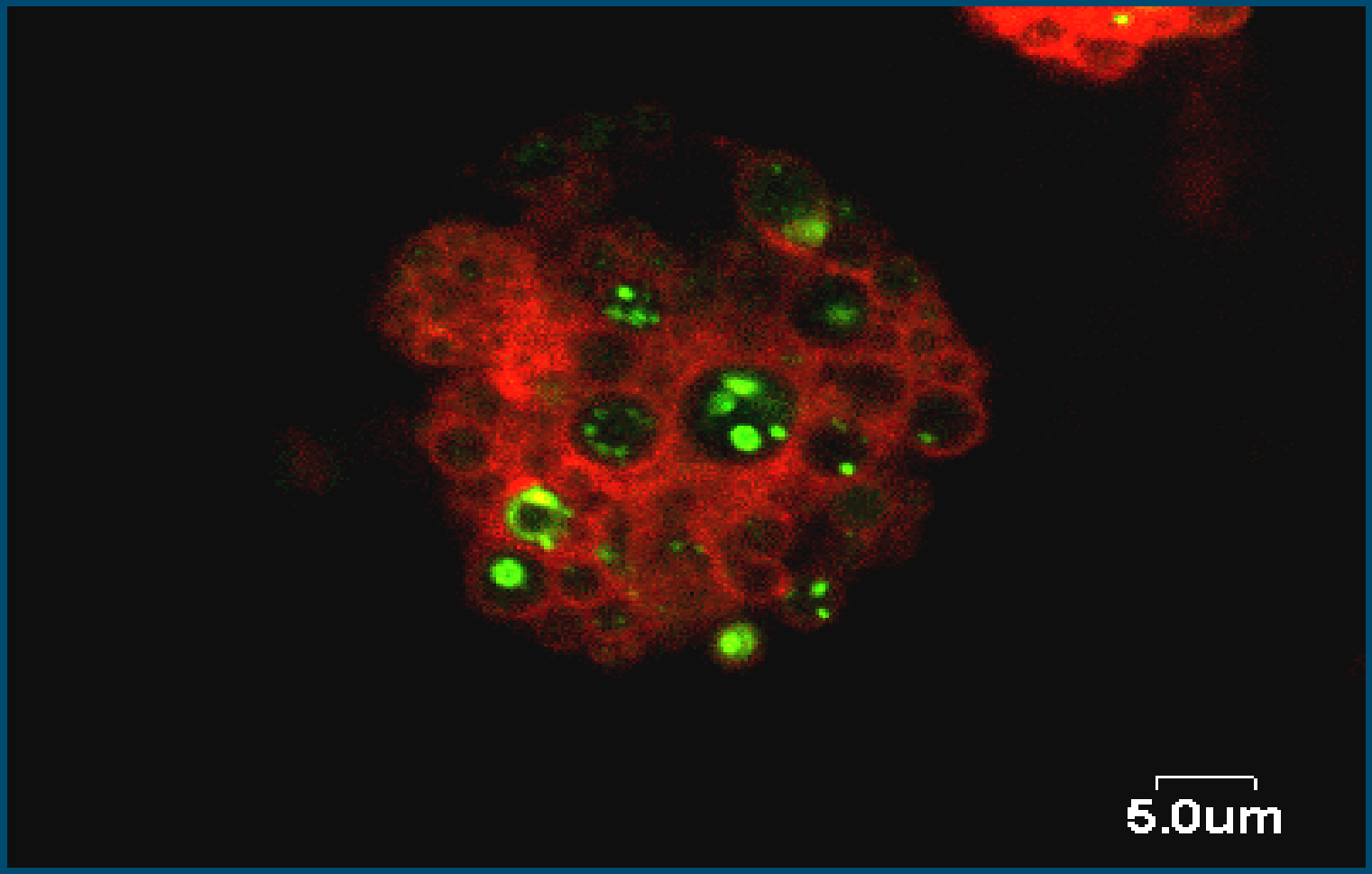
- Protein subunits (such as PA) are normally poor immunogens

How technology facilitates immune response

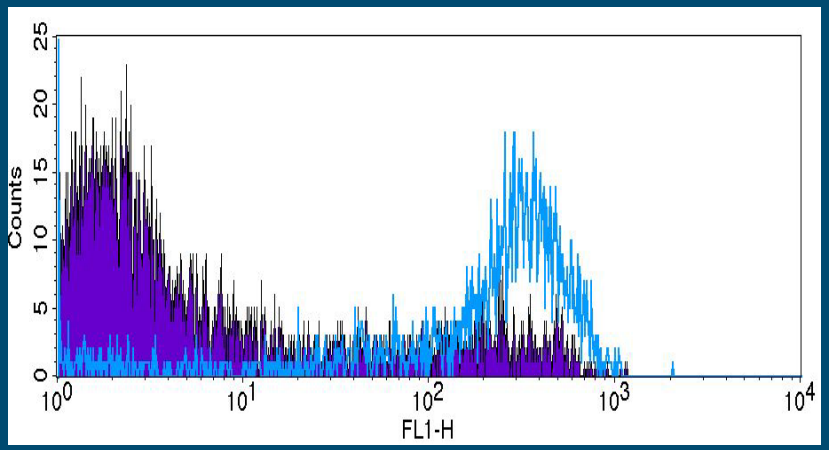
- Protein subunits (such as PA) are normally poor immunogens

- Microparticle formulation:
  - Improves targeting to APCs
  - Activates APCs
  - Enhances presentation to T cells
  - Elicits mixed Th1 and Th2
  - Mucosal administration engenders response in appropriate compartment for protection

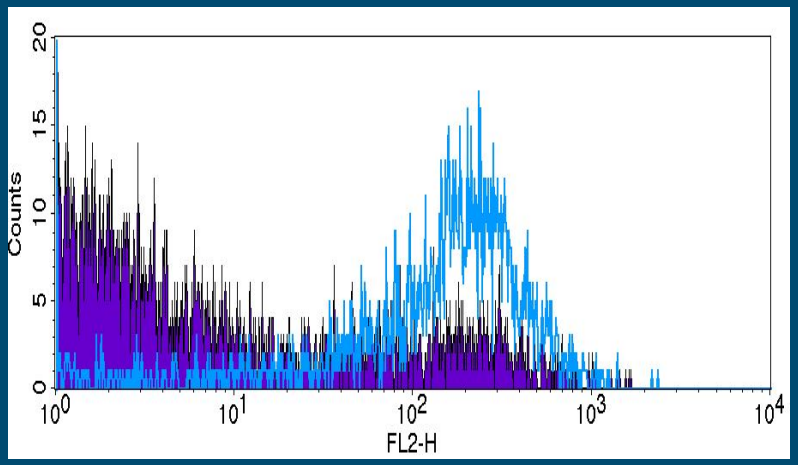
Microparticle uptake into dendritic cells



DC activation at 0hrs / 48hrs post exposure to PA loaded spheres

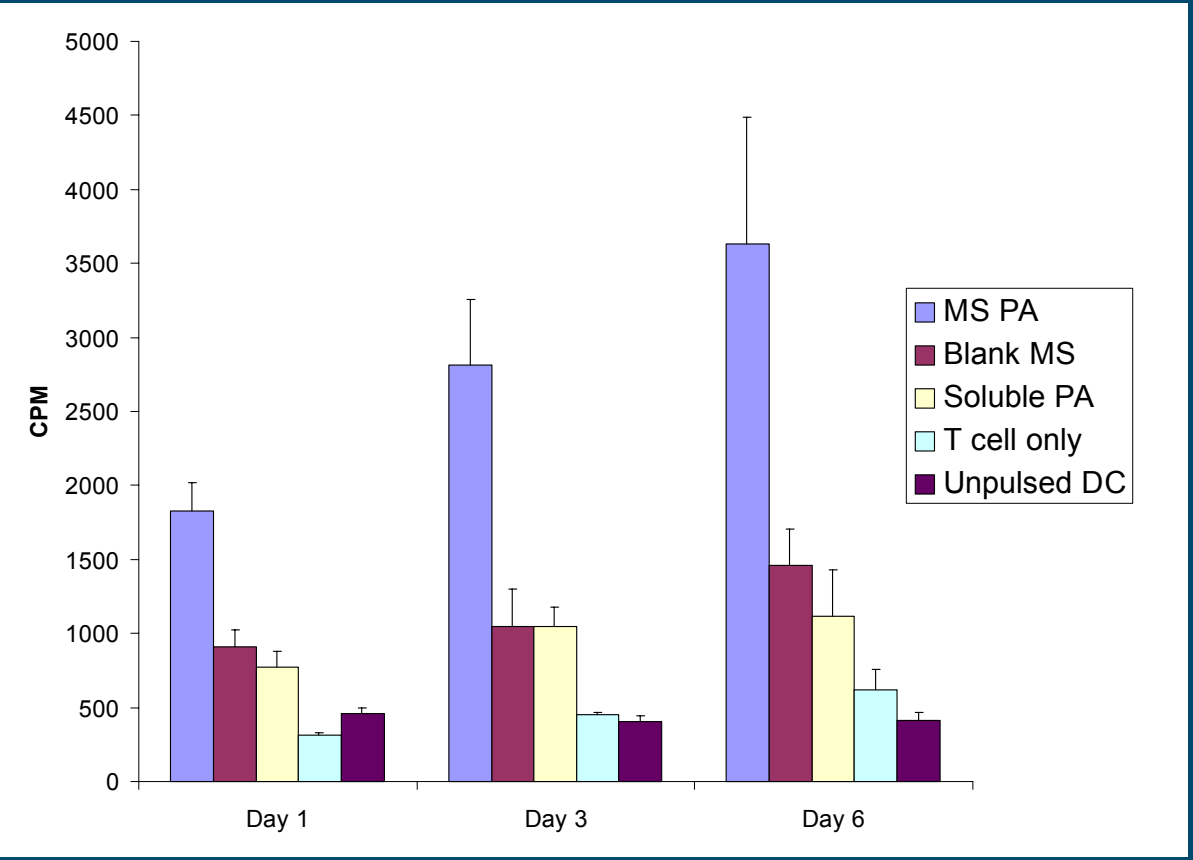


MHC class II

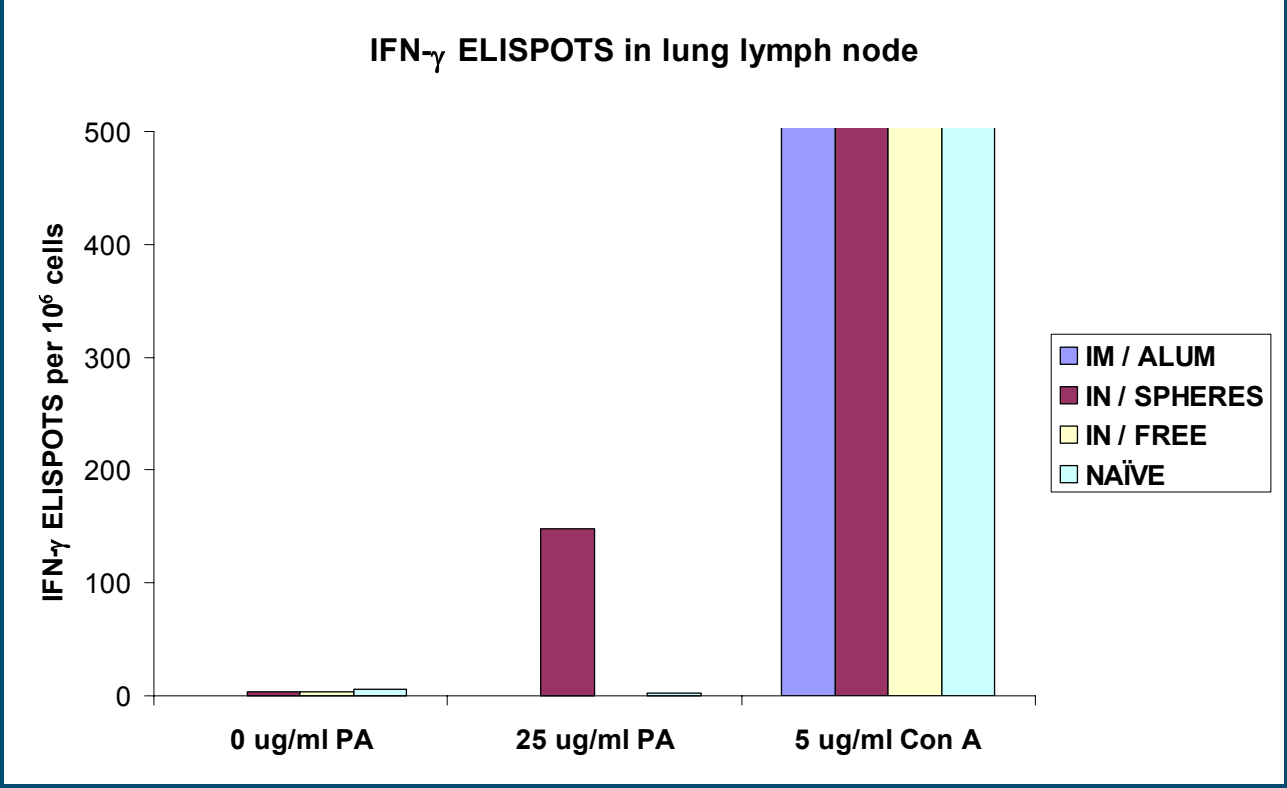


CD86

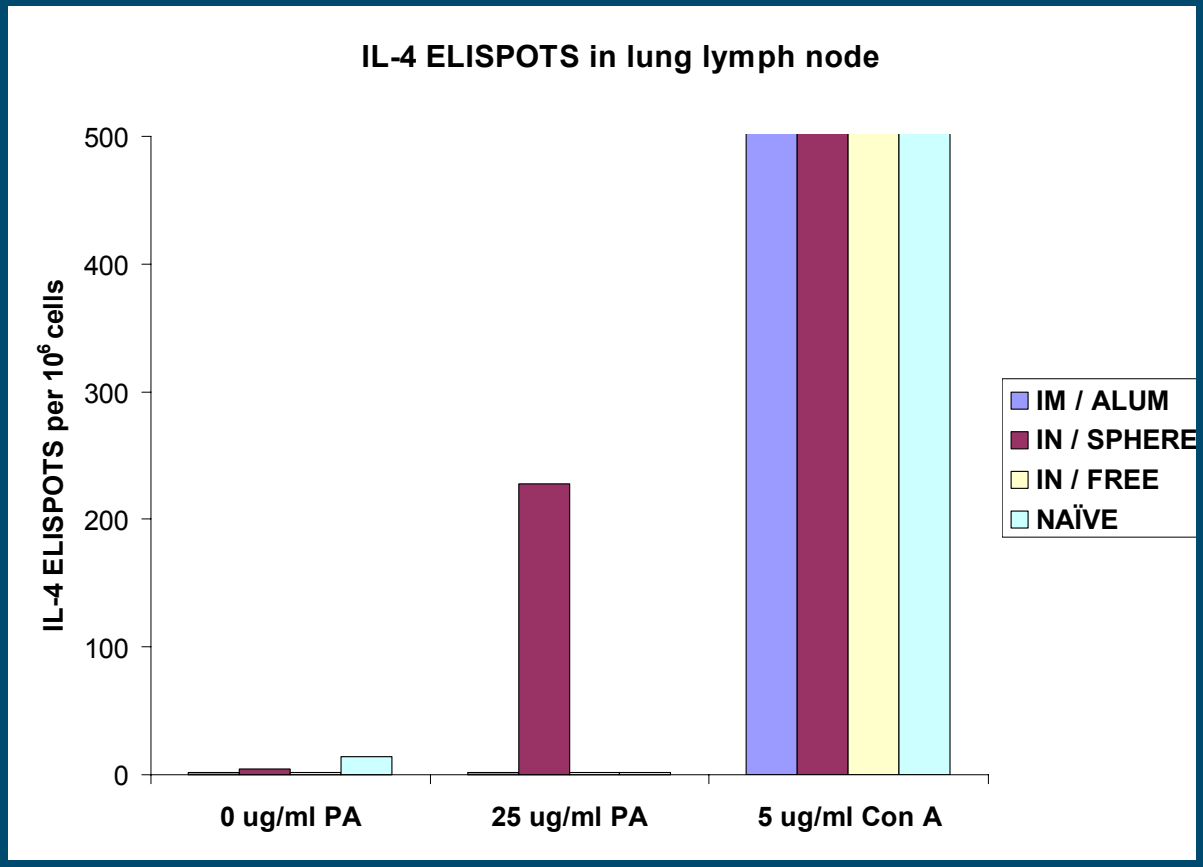
DCs pulsed with encapsulated PA stimulate enhanced and more protracted proliferation of T-cells



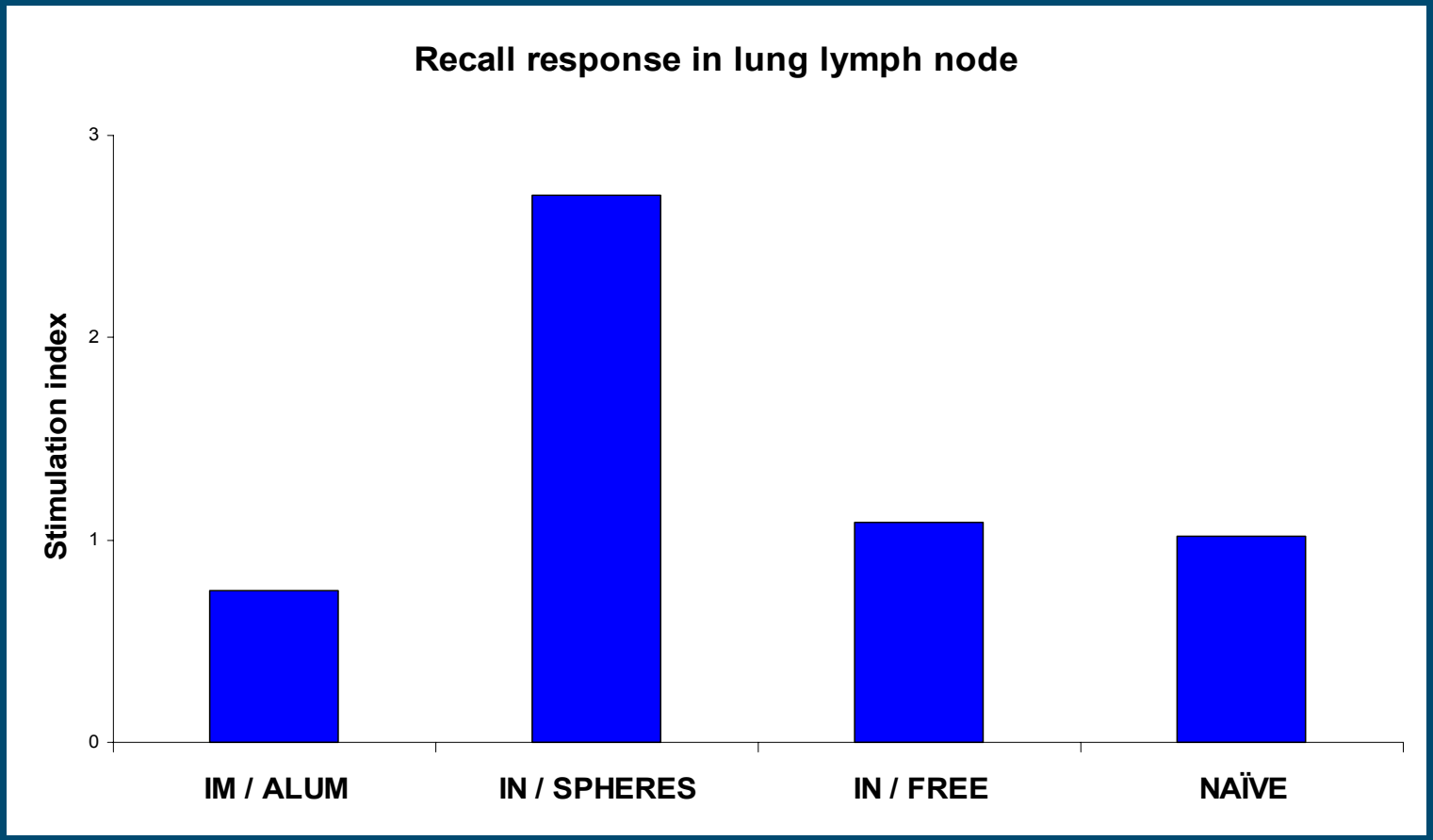
Microparticles engender PA specific CMI in lung milieu



Microparticles engender PA specific CMI in lung milieu



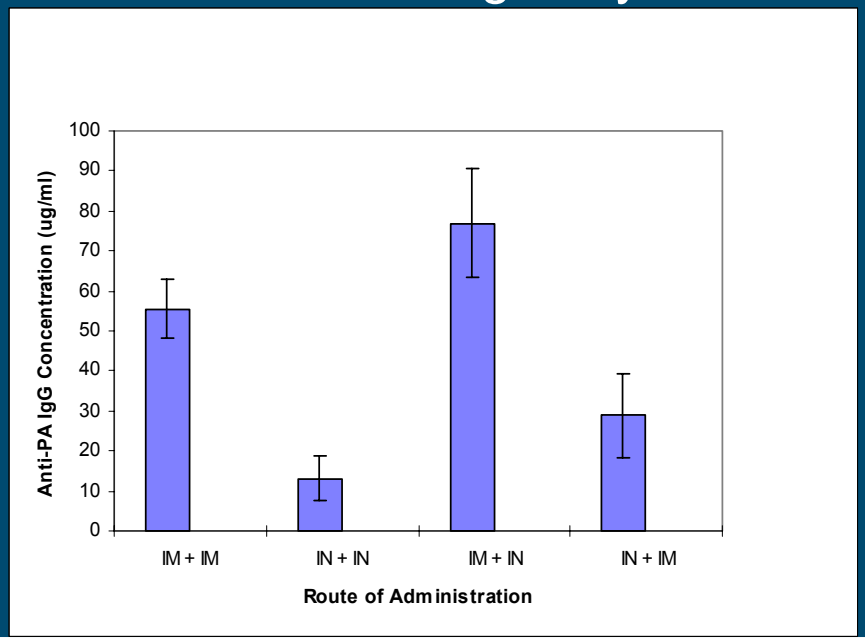
Microparticles engender PA specific CMI in lung milieu

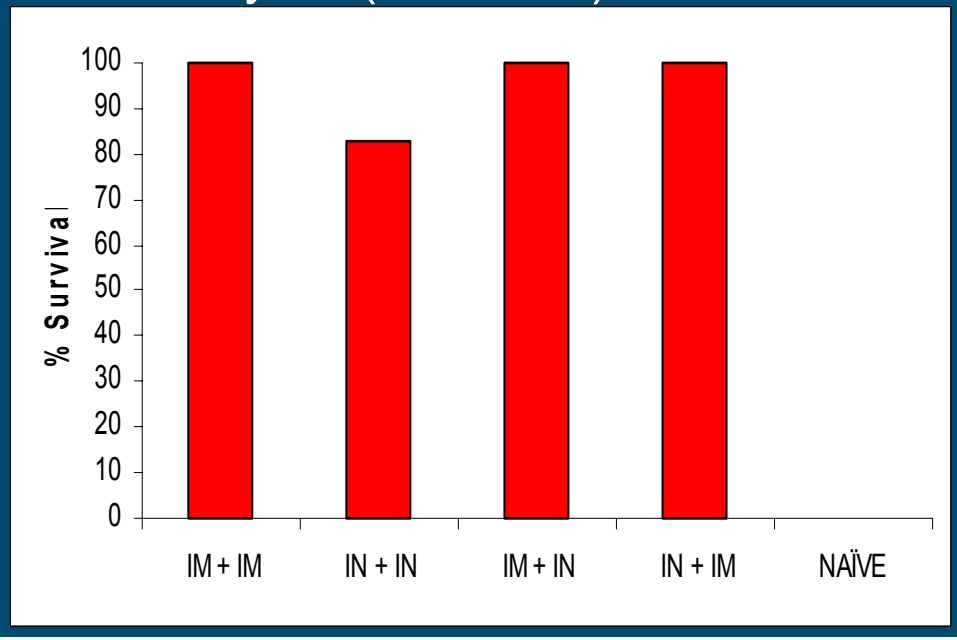


Humoral immunity and protection from inhalational anthrax

AJ mice immunized on day 1 and 21 with 10 μg of PA in microparticles

Mice challenged by aerosol route on day 90 (30 MLDs)

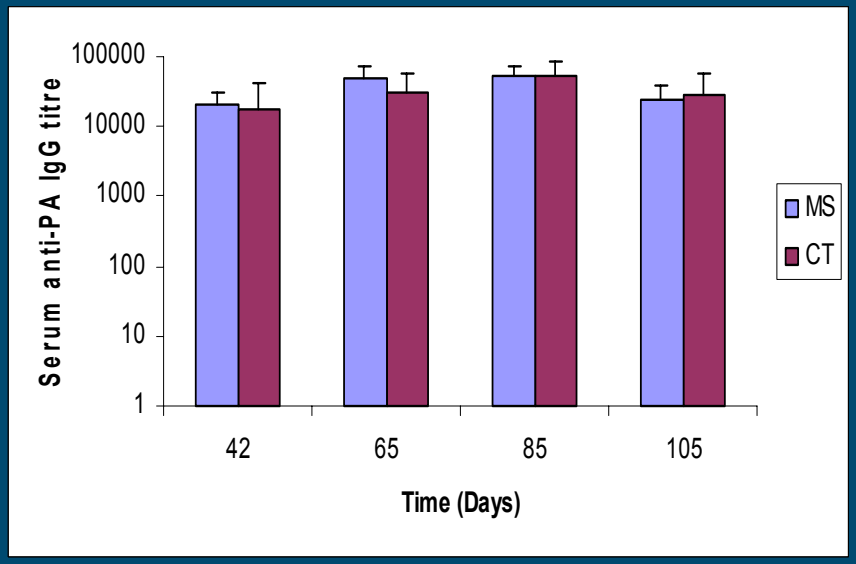


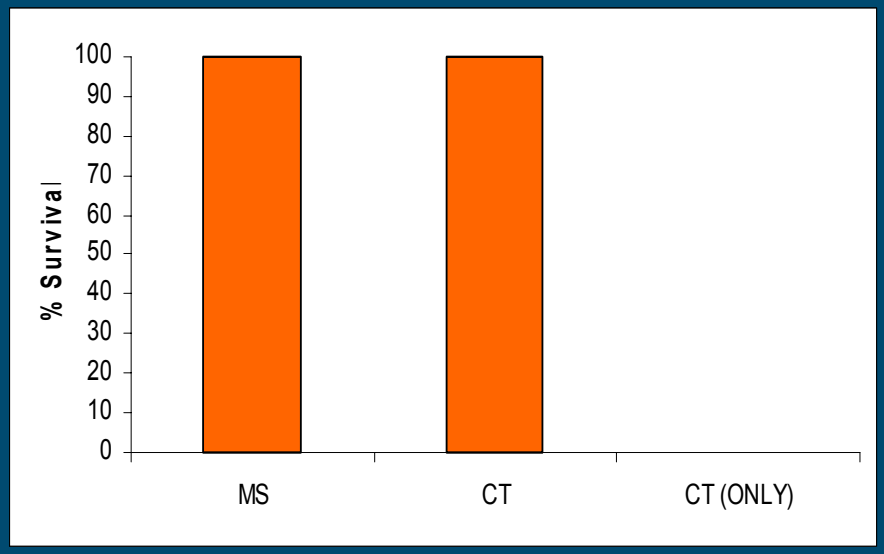


Protection from anthrax following a single mucosal immunization

AJ mice immunized intranasally with single 75 μg dose of PA (either microencapsulated or admixed with cholera toxin)

Mice challenged by IP route on day 128 (1000 MLDs)





Adaptability of the technology

- Protection following a single mucosal administration of formulated subunits:
  - rPA (anthrax)
  - rF1 and rV (plague)
  - MBP-FHc fusion (botulinum)

- Corroborate the tenet that this approach is broadly applicable to many subunits

Adaptability of the technology

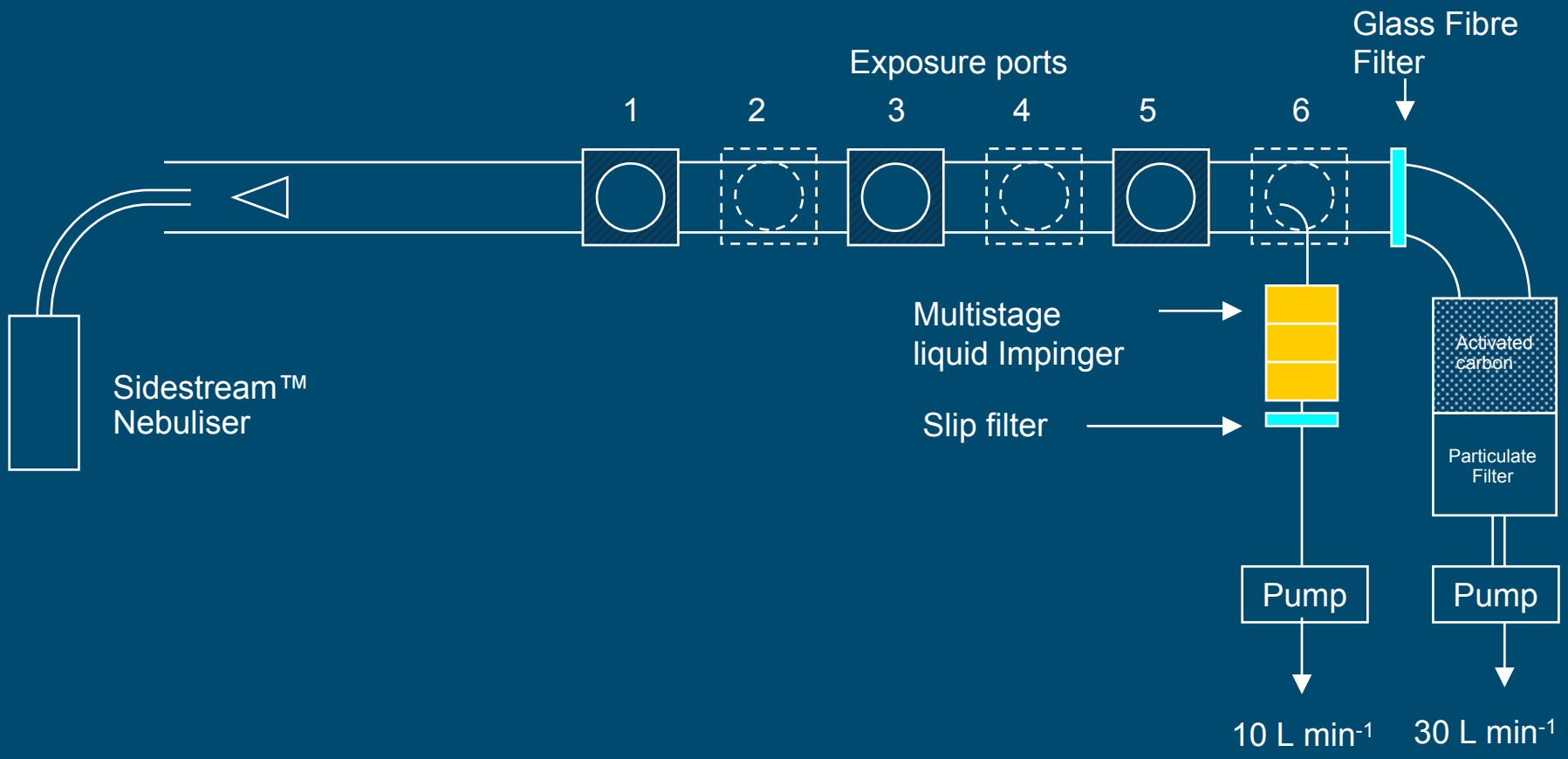
- Protection following a single mucosal administration of formulated subunits:
  - rPA (anthrax)
  - rF1 and rV (plague)
  - MBP-FHc fusion (botulinum)

- Corroborate the tenet that this approach is broadly applicable to many subunits
- Other bioactives (cytokines) can be formulated

Delivery of microparticles - aerosolisation

- Assess feasibility of using aerosol to deliver microencapsulated plague vaccine
  - Stability of microspheres in an aerosol
  - Stability of encapsulated antigen in aerosol
  - Aerosol particle size (Respirable?)
  - Immunogenic ?

Biodegradable microparticles - aerosolisation



Biodegradable microparticles - aerosolisation

Line 2 - exposure (W9098), generation (W9099) and preparation (W9096) cabinet

Biodegradable microparticles - aerosolisation

Sample Port	Sample Period (minutes)	Particle Concentration (number ml^{-1})	MMAD (μm)	GSD (σ_g)	% Mass < 1.0 μm	% Mass < 3.0 μm
1	1-2.5	5.67×10^4	2.01	3.26	12.4	55.0
	3-4.5	5.58×10^4	1.38	1.52	19.5	90.8
	5-6.5	5.43×10^4	1.41	1.63	18.6	88.0
	7-8.5	5.99×10^4	1.47	1.73	17.3	84.3
6	1-2.5	5.83×10^4	1.40	1.56	18.2	88.4
	3-4.5	5.48×10^4	1.67	2.65	14.2	63.7
	5-6.5	5.86×10^4	1.49	1.80	16.2	82.1
	7-8.5	6.09×10^4	1.90	2.70	11.8	59.3

MMAD is Mass Median Aerodynamic Diameter

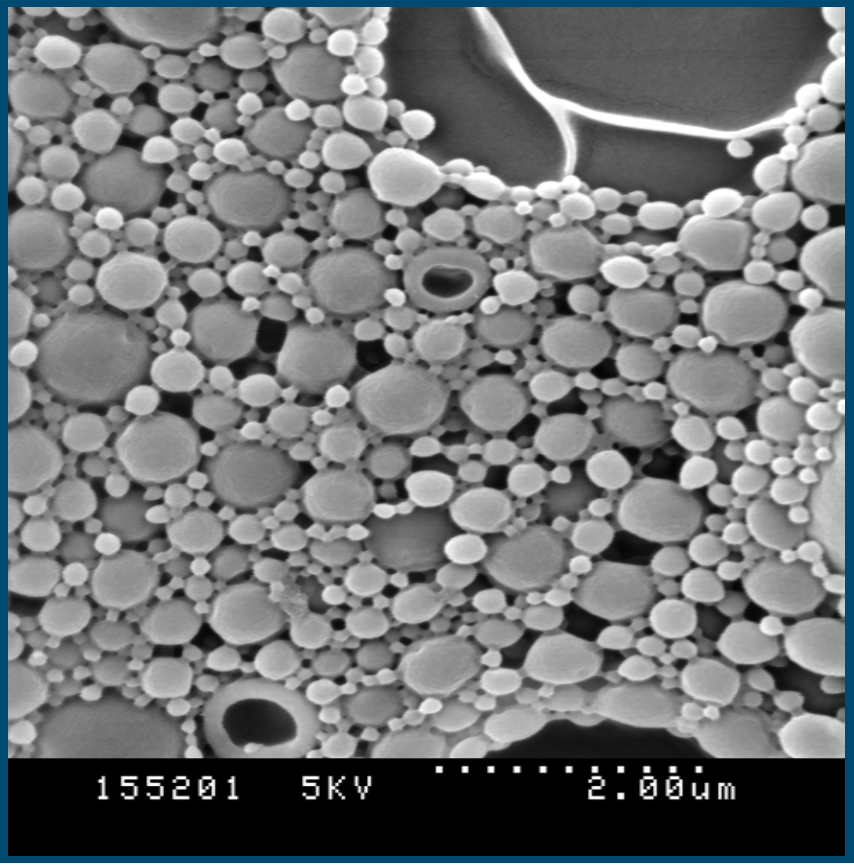
GSD (σ_g) is Geometric Standard Deviation

10 mg ml^{-1} PLA microspheres were aerosolised for 10 minutes by a Sidestream® nebuliser

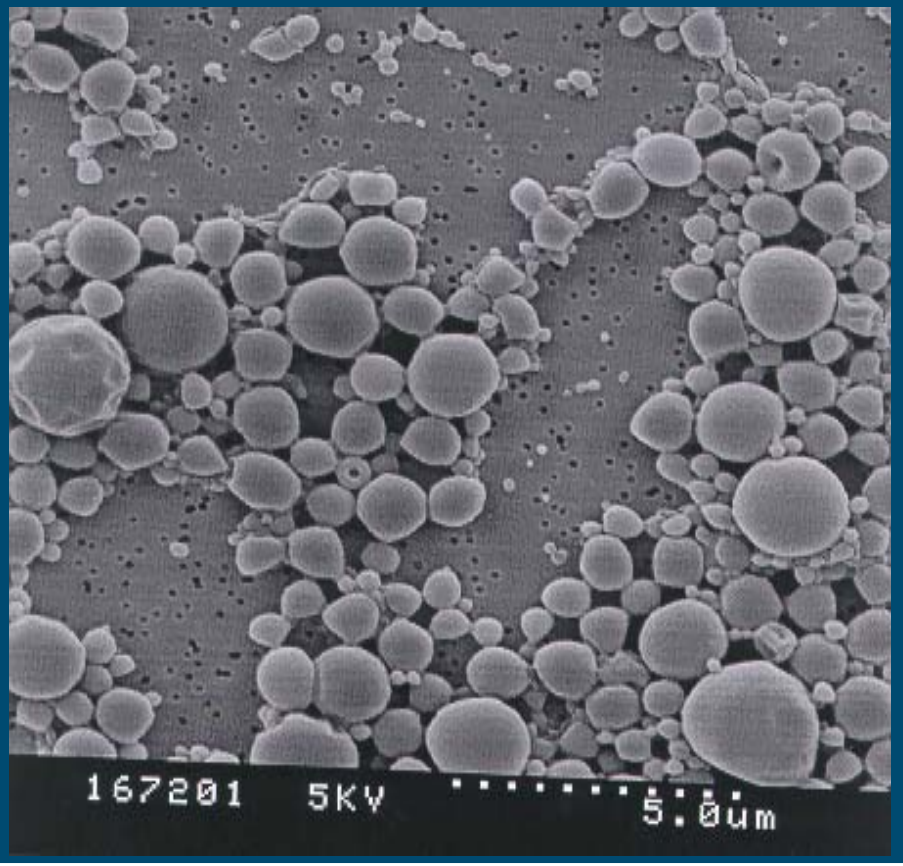
Aerosol dilution ratio was 10000: 1

Biodegradable microparticles - aerosolisation

(Before aerosolisation)



(After aerosolisation)



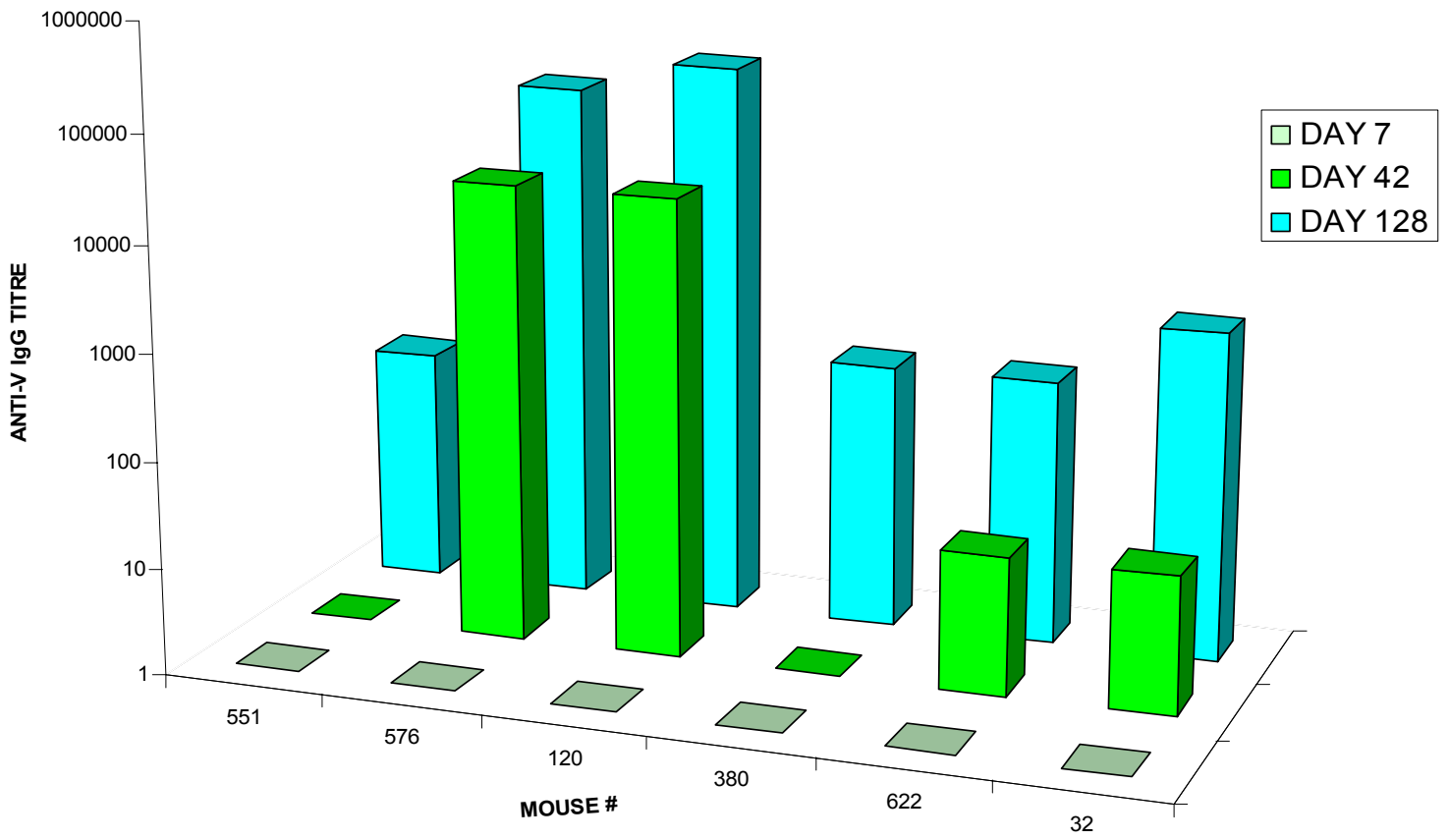
Biodegradable microparticles - aerosolisation

- Assess response to *Y. pestis* V antigen following immunisation of mice with aerosolised microspheres (containing V antigen)

Biodegradable microparticles - aerosolisation

- Assess response to *Y. pestis* V antigen following immunisation of mice with aerosolised microspheres (containing V antigen)
  - BALB/c mice
  - Mice exposed to aerosolised microencapsulated V antigen on days 0, 21 and 107
  - Mice bled on days 7, 42 and 128

Biodegradable microparticles - aerosolisation



Immunology: summary

- Can induce robust humoral and cell mediated responses (in the lung) following mucosal delivery of formulated subunit vaccines
- Formulation in microparticles circumvents requirement for enterotoxin adjuvants (cholera toxin)
- Can protect experimental animals from high levels of injected and inhalational challenge with virulent pathogens (anthrax, plague) and toxins (botulinum)

Other issues

- Stability
  - Freeze dried formulation is stable at ambient temperature
  - No cold chain needed

Other issues

- Stability
  - Freeze dried formulation is stable at ambient temperature
  - No cold chain needed

- Can be administered non-invasively
  - Formulation can be administered without the need for trained medical staff
  - Potential for self administered inhalational vaccine

Other issues

- Simple manufacturing system amenable to ‘scale up’

Other issues

- Projected regulatory timeline
  - Preclinical safety testing
    - Rodent
    - Rabbit
    - NHP

Acute & repeat dose tox testing

using same dose as anticipated clinical trials

Animal models already established

Other issues

- Projected regulatory timeline
  - Apply efficacy model
    - Rabbit
    - NHP

Animal models already established

BSL3 facilities at Dstl

Other issues

- Projected regulatory timeline
  - Derive surrogate marker assays
    - mouse
    - NHP

Already established

Other issues

- Projected regulatory timeline
  - Apply for IND to allow clinical trial

Other issues

- Projected regulatory timeline
  - Apply for IND to allow clinical trial
  - Using injected formulation as precedent
  - Estimate 2-3 years to CT

Acknowledgements

Di Williamson

Helen Flick Smith

Angie Westwood

Gareth Healey

Emma Waters

Chris LeButt

Steve Elvin

Julie Miller

Tony Stagg

Nicki Walker

Gary Phillips

Michael Maidment