Self Organising NanoStructures: an alternative strategy for directed enzyme-prodrug therapies

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Topics of the presentations

- SONS as therapeutic nanoreactors in our lab
- SONS in other applications

Problems encountered in chemotherapy today:

- Insufficient drug concentrations in the tumour
- Systemic toxicity of drugs
- Lack of selectivity for tumour cells of drugs
- Drug-resistant tumour cells

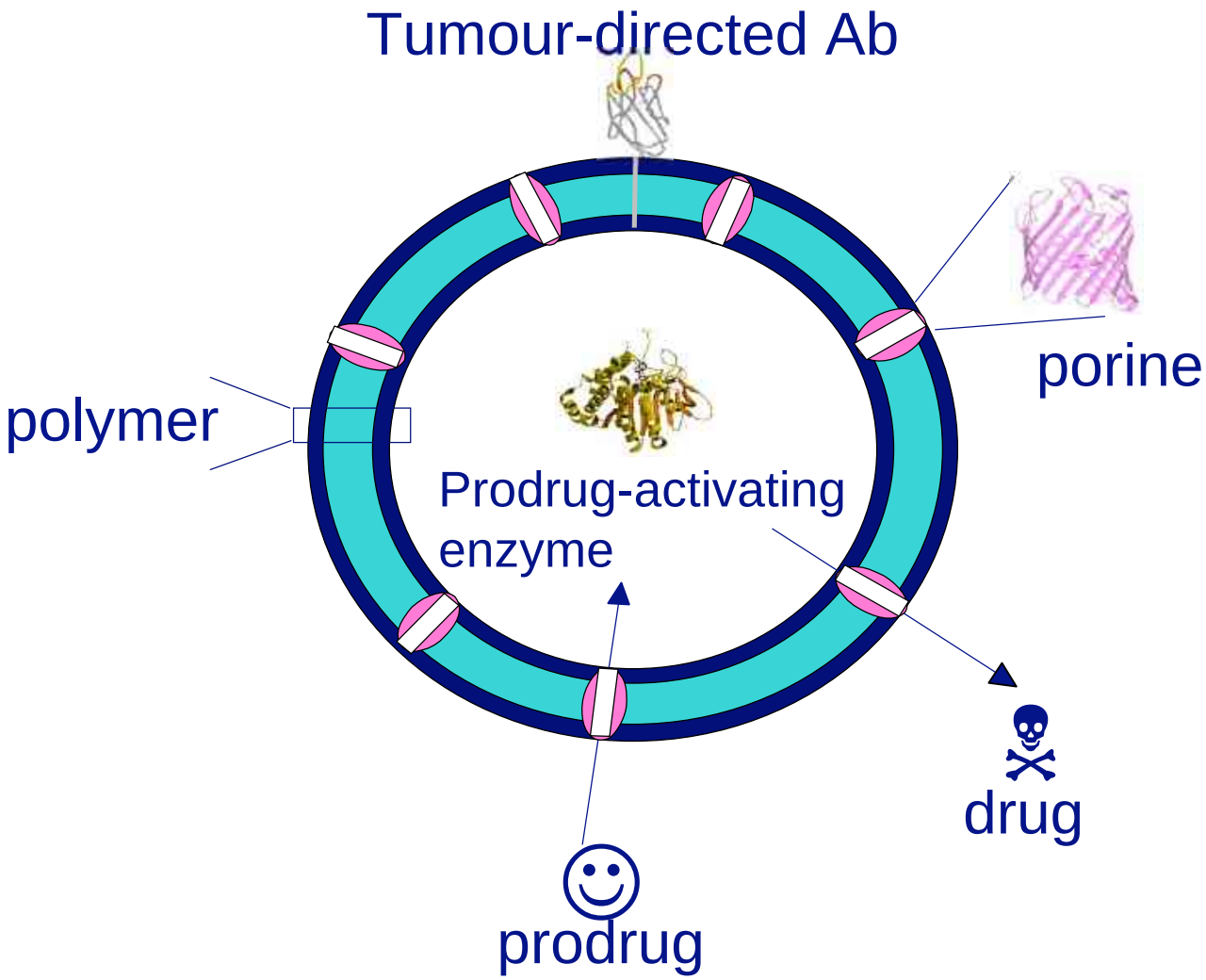
Ways to overcome these problems:

- Alternative drug formulations:
  e.g. PLD
- Resistance modulation:
  e.g. PSC833
- Antidote/toxicity modifiers:
  e.g. Dexrazoxan
- Targeted enzyme-prodrug therapy

Types of enzyme-prodrug therapies:

- Antibody-directed enzyme-prodrug therapy (ADEPT)
- Antibody-directed abzyme-prodrug therapy (ADAPT)
- Gene-directed enzyme-prodrug therapy (GDEPT)
- SONS as therapeutic nanoreactors

SONS as therapeutic nanoreactors:



Advantages over other enzyme-prodrug strategies:

- Shielding of the enzyme from the environment
- High enzyme concentration/bound antibody

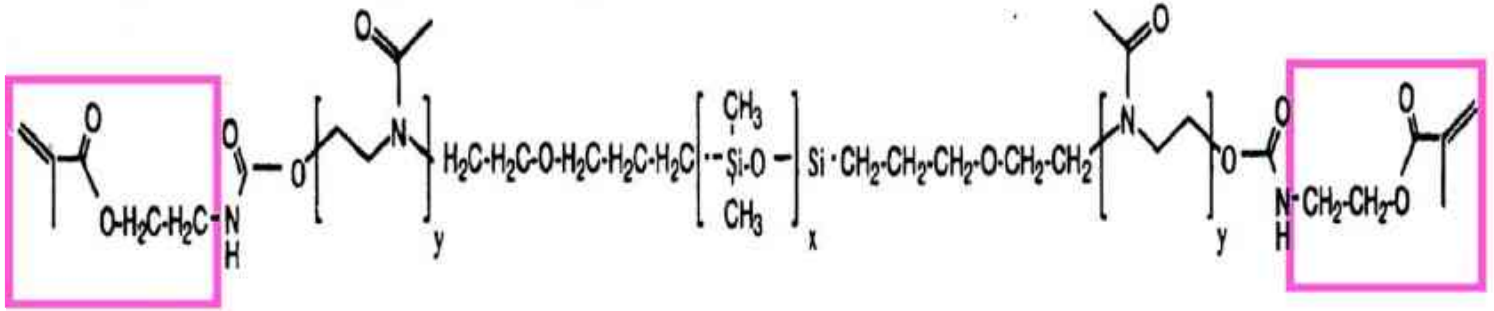
Build-up of the nanoreactor

1. Polymer:

Types of polymers that self assemble in lamelar structures:

- Biopolymers: peptide-based
- Diblock copolymers: lipid-like
- Triblock copolymers: ABA or ABC

pMOXA-pDMS-pMOXA



Build-up of the nanoreactor

1. Polymer:

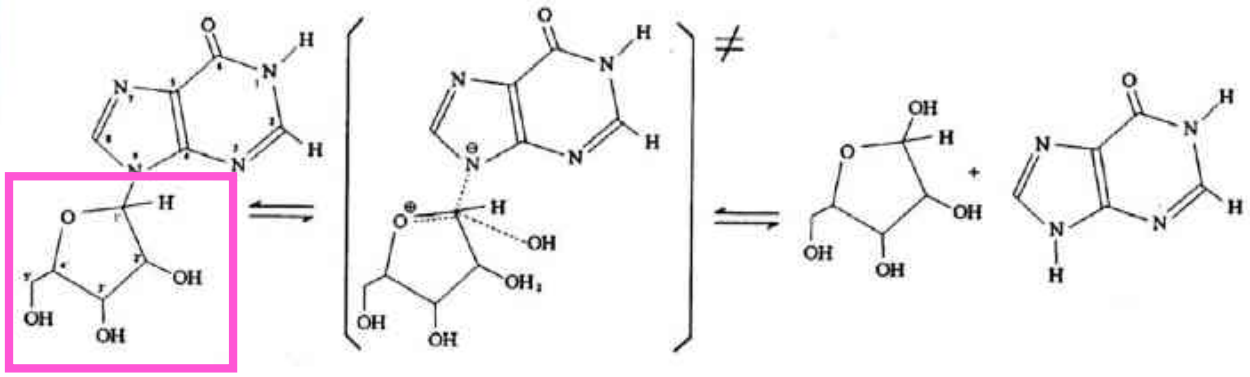
Characteristics of block copolymers:

- Low critical micelle concentration (CMC):
  slow monomer/multimer exchange rate
- long blocks:
  up to 10-20 nm
- Tuneable phase behaviour:
  thermal or pH responsive

Build-up of the nanoreactor

2. Nucleoside hydrolase (NH) as prodrug activating enzyme:

Enzymatic reaction



Highly specific towards the ribose moiety, not present in mammals!!!!

Build-up of the nanoreactor

2. Nucleoside hydrolase (NH) as prodrug activating enzyme:

NH T.vivax: different substrates and their kinetic values

	K_{cat} (s^{-1})	K_M (µM)
Inosine	5.19	5.37
Guanosine	2.31	2.33
Adenosine	2.58	8
2F-adenosine	1.86	39.05
2Cl-adenosine	1.97	2.7
6-methyl purine	4.3	<10

Build-up of the nanoreactor

3. Porines:

Origin:

Outer membrane proteins of gram negative bacteria, e.g. OmpF, PhoE, LamB, Tsx,…

Structure:

β-barrel

Side view

Top view

Build-up of the nanoreactor

3. Porines:

Types of porines:

- Non specific porines: e.g. OmpF

  Serves as a molecular sieve, allowing transport of solutes < 600 Da

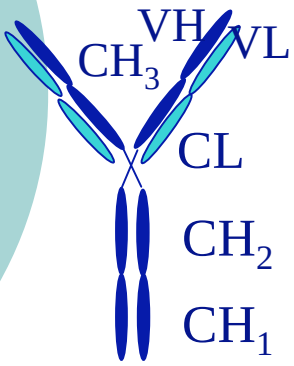
- Specific porines: e.g. Tsx (nucleoside specific)

  Has a binding site for a specific solute, allowing diffusion at very low solute concentrations

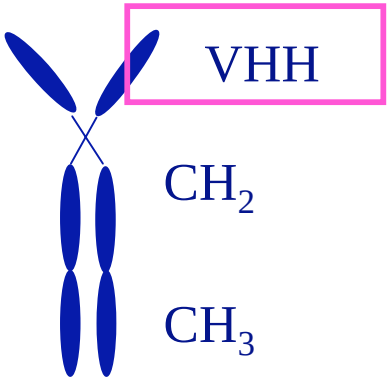
Build-up of the nanoreactor

4. Tumour associated antibodies:

Single chain camelid antibodies



Conventional Ab



= nanobody

Camel Ab

Nanobodies directed against: CEA, EGFR

Experimental data

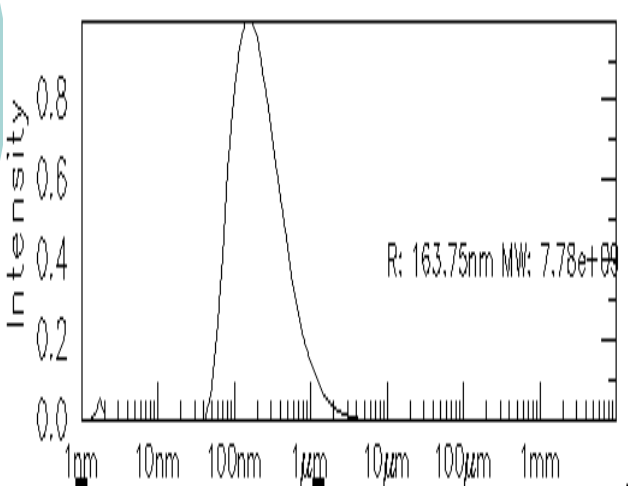
1. Production of the nanoreactors:

- Dissolve 10 % (wt/v) of polymer and porine (10 or 100 μg/ml) in ethanol
- Dry to form a lamellar film
- Rehydrate in NH-solution
- Extrude through 200 nm polycarbonate filter
- Remove non encapsulated NH by gelfiltration or Ni-Nta

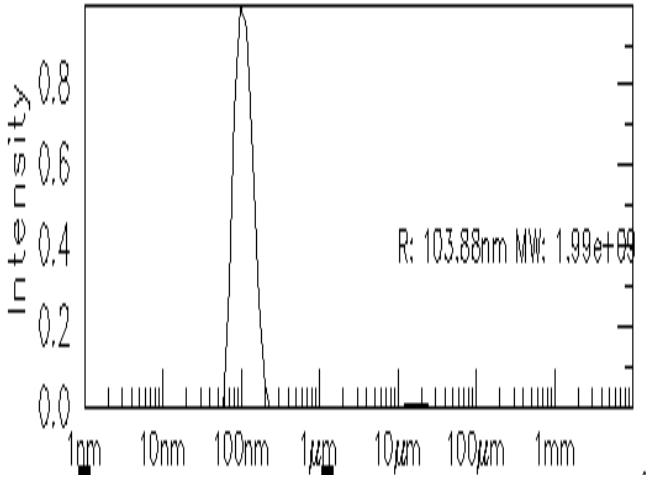
Experimental data

1. Production of the nanoreactors:

DLS measurements



Before extrusion



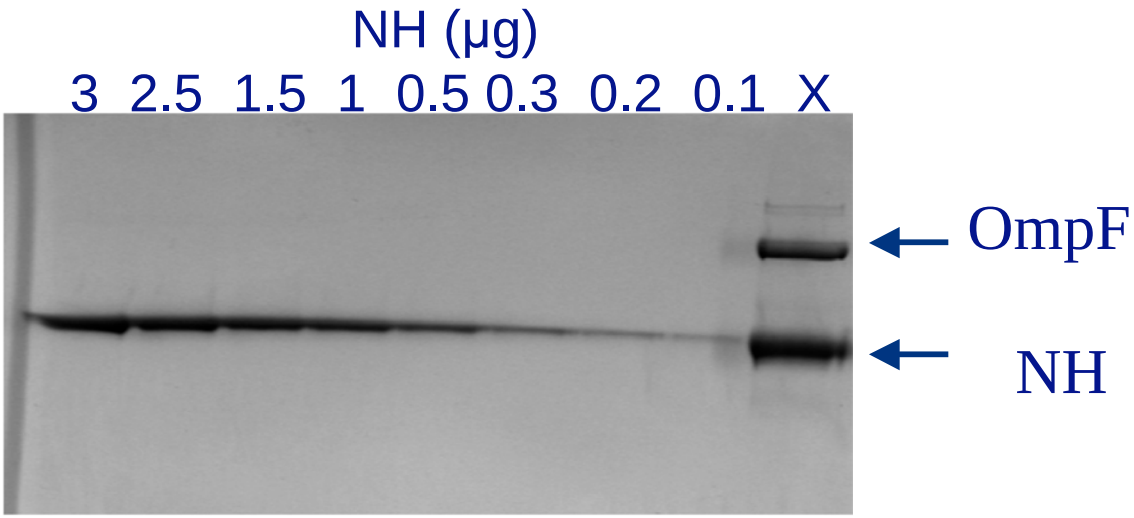
After extrusion

Narrow size distribution, mean size 100 nm

Experimental data

2. Encapsulation efficiency:

SDS-PAGE analysis



Encapsulation efficiency = 15.4 %

Experimental data

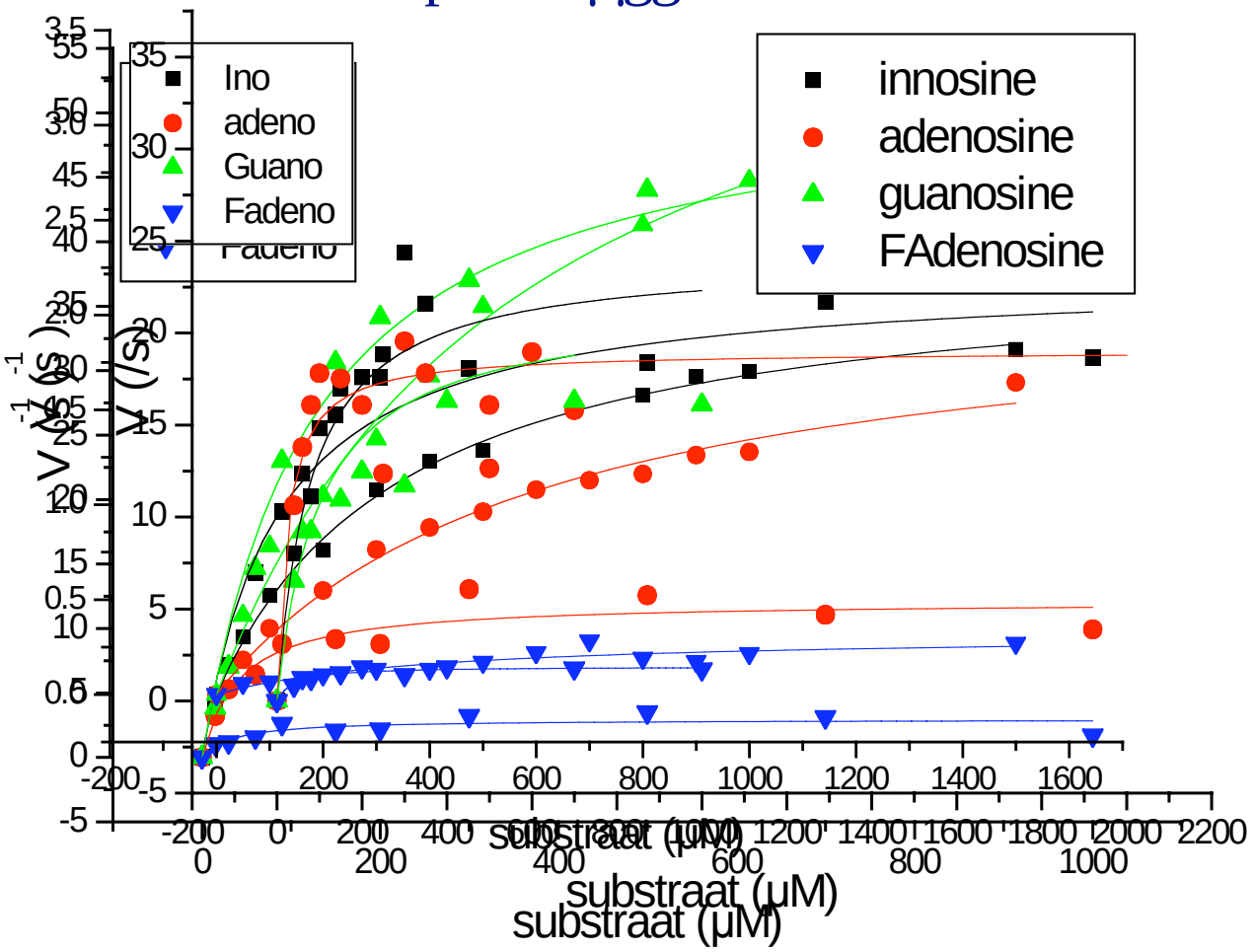
3. Activity of the nanoreactors:

Colorimetric assay

- Measure the turn over rate from substrate to product
- Plot the turn over rate in function of the substrate concentration
- Fit to a Michaelis-Menten equation

Experimental data

3. Activity of the nanoreactors:



Future work

1. Coupling of the antibodies

S-S bridges between Ab and the nanoreactors

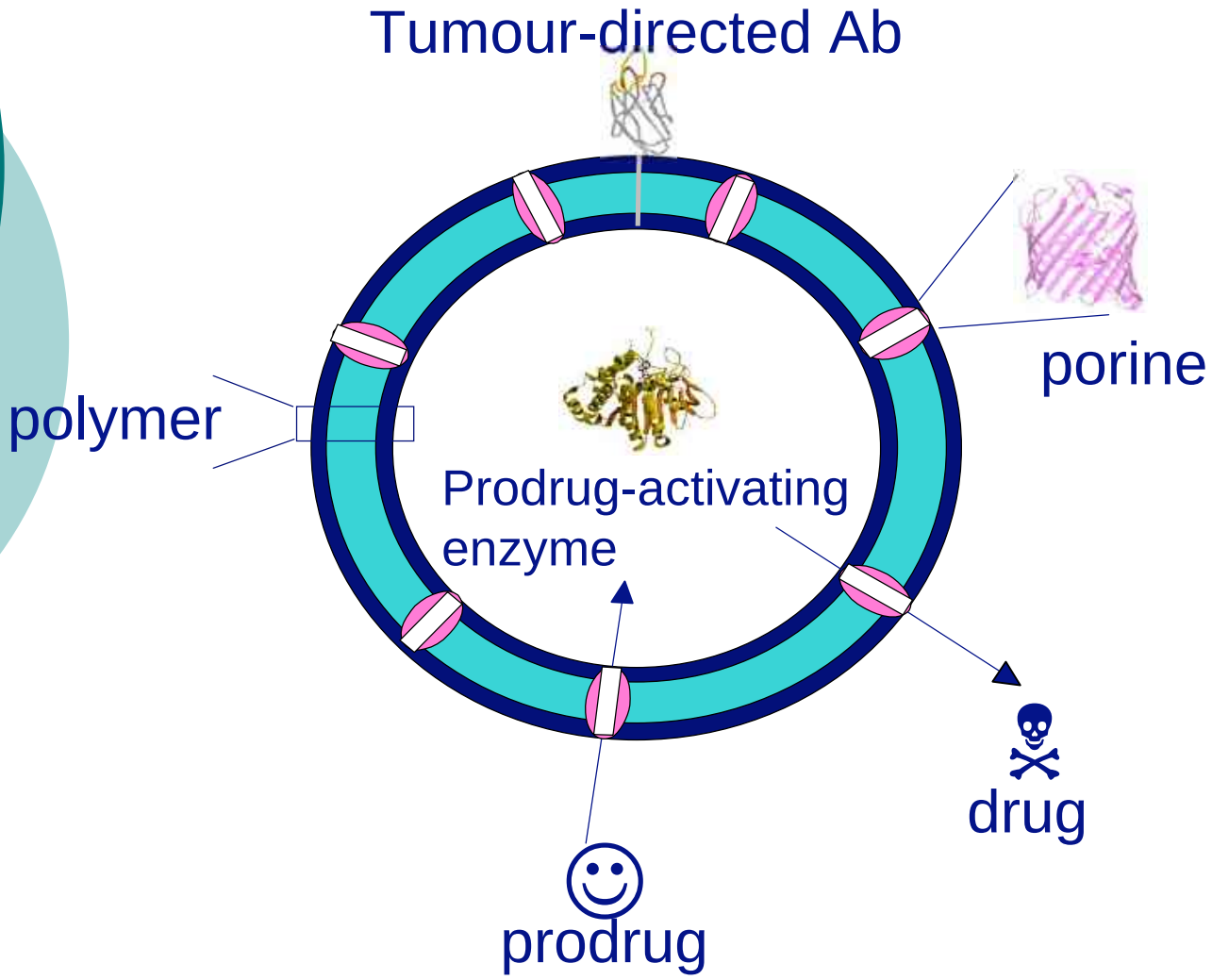
2. In vitro

- Activity in serum
- Fagocytosis by macrophages
- Proliferation of tumour cells

3. In vivo

- Biodistribution
- Immunogenicity
- Tumour proliferation

Towards a modular system



SONS in other applications

1. Drug carriers:

- Load SONS with drugs
- (optional) Target SONS with Ab

→ Higher bioavailability and specificity

2. Induction of apoptosis:

- Encapsulation of caspases
- Targeting to tumorcells

SONS in other applications

3. As a diagnostic tool:

Classic immunoassay

- For the detection of antigens involved in bacterial/viral infections and tumour development
- Ab directed against these antigens used for detection
- Enzyme (horseradish peroxidase) used to amplify the signal

SONS in other applications

3. As a diagnostic tool:

Improving the immunoassay by using SONS

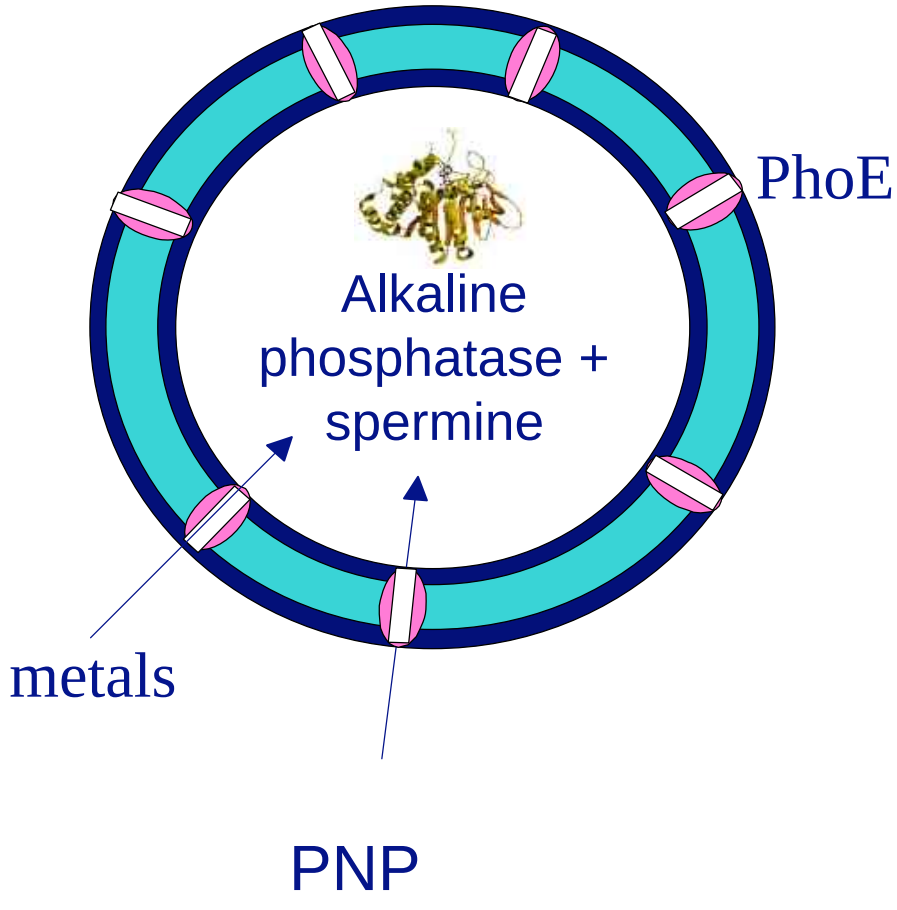
- SONS are coupled to the detecting Ab
- SONS are permeabilised by porines
- SONS are loaded with signal amplifying enzyme

→ Higher amplification of the signal

→ Early detection possible

SONS in other applications

4. Precipitation of heavy metals:



SONS in other applications

5. Gene and RNAi delivery vectors:

Health risks of viral delivery vectors

- Isertional mutagenesis
- Viral infections
- Host immune response

Targeting of SONS to organs

- Antibodies
- Ligands to organ specific receptors
- Bacterial adhesins

SONS in other applications

6. As an Imaging tool:

- Incorporate fluorescent dyes in SONS
- Target the SONS through Ab
- Imaging through fluorescent microscopy

  → Lower detection limit

7. Immunotherapy:

- Load SONS with tumour-specific antigen
- Target to dendritic cells for correct presentation in combination with MHCI/MHCII
- Stimulation of CD8+ en CD4+ cells

Acknowledgements

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