Current Situation
Filters for various applications:
Filters for various applications:

Maximum surface on minimum volume
Ultrafiltration membranes

Microbeads
maximum surface on minimum volume

Ultrafiltration membranes

Microbeads

- Uniform polymer particles
- 0.5 to 5 μm
- Function depends on surface properties

Protein coupled to beads
Microbeads

- Uniform polymer particles
- 0.5 to 5 μm
- Function depends on surface properties

http://en.wikipedia.org/wiki/Microbeads
Protein coupled to beads
Current Situation

Filters for various applications:

- Maximum surface area
- Minimum volume

Ultratransmission membranes

Microbeads
The ISSUE

- Synthetic beads
- Biological beads
- Covalent
- Non-covalent
- Convenient self-expression
- Very cheap
- Same size as synthetic beads

Our Gold Sponsors

- BioTek
- DSM
- BioLogics
non-covalent

laborious:
protein expression, binding, washing

expensive

synthetic beads
The ISSue

- Non-covalent
- Covalent
- Convenient self-expression
- Very cheap
- Same size as synthetic beads

synthetic beads ----> covalent ----> convenient self-expression ----> very cheap

Our Gold Sponsors

- Biotech
- DSM
- BIOCASE
- ...
Our Gold Sponsors

We could not have accomplished our project without our Gold Sponsors:
The Issue

- Synthetic beads
  - Not covalent
  - Durable and easy to store

- Covalent
  - Convenient self-expression

- Biological beads
  - Same size as synthetic beads
  - Very cheap

Our Gold Sponsors
The ISSUE

- Synthetic beads
- Biological beads
- Covalent
- Non-covalent
- Convenient self-expression
- Very cheap
- Same size as synthetic beads

Our Gold Sponsors:
- [List of sponsors]

Prezi
The Solution: BEADzilluS

Endospore: dormant, resistant

- Core
- Cortex
- Inner and Outer Coat
- Crust: CgeA and CotZ
The Solution: BEADzilluS

Endospore: dormant, resistant
- Core
- Cortex
- Inner and Outer Coat
- Crust: CgeA and CotZ

Bacillus subtilis life cycle

Germination

Vegetative Cycle

Sporelation

Prezi
The Solution: BEADz

Endospore: dormant, resistant

- Core
- Cortex
- Inner and Outer Coat
- Crust: CgeA and CotZ

Bacillus subtilis life cycle
- Core
- Cortex
- Inner and Outer Coat
- Crust: CgeA and Cotz

Bacillus subtilis life cycle
Bacillus subtilis life cycle

- Vegetative Cycle
- Sporulation
- Germination
Endospore: dormant, resistant

- Core
- Cortex
- Inner and Outer Coat
- Crust: CgeA and CotZ

Bacillus subtilis life cycle
Current Situation

The Issue

The Application

The Sporobeads

The Solution: BEADzilla

The requirements
The requirements

GerminationSTOP
essential for the use of Sporobeads

1) Germination Gene Knockouts:

Wild type

Quadruple mutant

Quadruple-mutant strains yielded no germination in 3x10^8 Spores!

2) Suicideswitch
The requirements

1) Germination Gene Knockouts:
- Quadruple-mutant strains yielded no germination in 3x10^5 spores!

2) Suicide switch
- Toxin produced in spores
- Only kills germinating cells

3) Biosafety
- Are Sporobacillus (SBAC)?
- Are SBAC spores safe? probably not
- Not really... Spores are unethical

BacillusBioBrickBox
essential for work with B. subtilis

5 working vectors!

Germination STOP
essential for the use of Sporobeads

<Diagram of gene knockout experiments>

<Image of wild type and quadruple mutant samples>

<Diagram of suicide switch mechanism>

<Image of discussion group>
Bacillus BioBrickBox
essential for work with B. Subtilis

5 working vectors!

thrC' promoter reporter spec res 'thrC

Amp res E. coli ori

15 promoters evaluated!
3 reporters evaluated!

2) Suicideswitch

Vegetative Cycle
Speciation
Germination (Gene K.O.)
Suicideswitch
BacillusBioBrickBox
essential for work with B. Subtilis

5 working vectors!

15 promoters evaluated!

3 reporters evaluated!
BacillusBioBrickBox
essential for work with B. Subtilis

5 working vectors!
- BBa.K823021
- BBa.K823022
- BBa.K823023
- BBa.K823025

15 promoters evaluated!
3 reporters evaluated!

2) Suicideswitch
Germination is essential for the use of Sporobeads.

1) Germination Gene Knockouts:

- Wild type
- Quadruple mutant

Quadruple-mutant strains yielded no germination in $3 \times 10^8$ spores!
2) Suicideswitch

- Toxin produced in spores
- Only kills germinating cells
3) Biosafety

Are Sporo\textit{beads} GMOs?

Are Sporo\textit{beads} organisms?

Probably not!

Not really... Spores are vehicles!
The Sporobeads

Sporobeads
platform for fusion proteins

Crust protein operon

Finding the best constructs
Sporobeads
platform for fusion proteins

Crust protein operon

Finding the best constructs

Crust: CgeA and CotZ

The proof of principle
glowing spores!
Sporobeads platform for fusion proteins
Crust: CgeA and CotZ
Crust protein operon

A  cotVWXYZ cluster

B  cgeABCDE cluster

Finding the best constructs
Finding the best constructs

integration locus fwd

sporulation promoter

spore crust protein

cotZ

cgeA

gfp

term

integration locus rev

amyE'
thrC'
P_{cotYZ}

P_{cotV}
P_{cgeA}

'amyE'

'thrC'

otz
**Sporobeads**

**Platform for fusion proteins**

**Crust protein operon**

- **cotVWXYZ cluster**
- **cgeABCDE cluster**

**Finding the best constructs**

**Crust: CgeA and CotZ**

**The proof of principle**

**Glowing spores**
The proof of principle

glowing spores!

wild type

B 53
The proof of principle
glowing spores!
Time lapse
The proof of principle
glowing spores!

Time lapse
Sporovector
create your own Sporobead

Gene of interest (RFC25)

EcoRI
XhoI
NgoMIV

thrC' P_cotYZ
MCS
cgeA
E. coli ori
Amp res
term
spec res
'thrC'
PstI
Sporovector
create your own Sporobead

Gene of interest
(RFC25)

EcoRI
XbaI
NgoMIV

thrC'  P_cotYZ  MCS  cgeA  term  spec res  'thrC

Amp res  E. coli ori

PstI
The Application

Sporofilters

Purification
- Polystyrene
- Heavy Metals
- Toxins

Conversion
Enzymatic reactions like:
- Degradation
- Processing

Sporoevolution

Degradation by Laccase-Sporobeads
Sporofilters

Purification
- Polystyrene
- Heavy Metals
- Toxins

Conversion
Enzymatic reactions like:
- Degradation
- Processing
Sporofilters

Purification
★ PolyStyrene
★ Heavy Metals
★ Toxins

Conversion
Enzymatic reactions like:
★ Degradation
★ Processing
The Application

Sporofilters

Purification
- Polystyrene
- Heavy Metals
- Toxins

Conversion
Enzymatic reactions like:
- Degradation
- Processing

Degradation by Laccase-Sporobeads

Sporoevolution
perfect enzyme
Degradation by Laccase-Sporobeads

ABTS assay

OD(420)/OD(600)

wt | Ecol | Bhal
Sporofilters

Purification
- Polystyrene
- Heavy Metals
- Toxins

Conversion
Enzymatic reactions
- Degradation
- Processing

Sporoevolution
find the perfect enzyme
Sporoevolution

find the perfect enzyme

Gene of interest  Amplification & Mutagenesis  Transformation  Sporobead library  Functional screening  Spore germination  Easy sequencing

Sporodisplay

find the perfect binding partner
Sporoevolution
find the perfect enzyme

Gene of interest → Amplification & Mutagenesis → Transformation → Sporobead library → Functional screening → Spore germination → Easy sequencing

Sporodisplay
find the perfect binding partner

Gene of interest → Amplification & Mutagenesis → Transformation → Sporobead library → Functional screening → Spore germination → Easy sequencing
Sporodisplay
find the perfect binding partner
Conclusion

- BacillusBioBrickBox → B. subtilis as a powerful chassis
- Sporobeads → Spores as protein display platform
- Human Practise → Engage with the public and officials regarding SynBio and safety concerns
Acknowledgements

• Our generous Sponsors

• Our devoted advisors

• The entire Mascher Lab Group