



Use of Silica Nanosprings in an Enzyme-Based Continuous Flow Reactor

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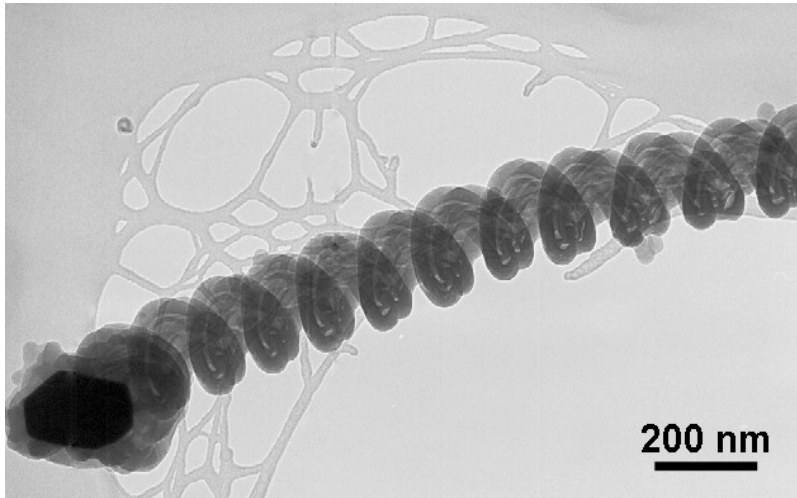
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Advantages of CFR over Batch Processes

- The problem of product inhibition can be avoided because product continuously removed from the reaction
- The enzyme is continuously recycled , so there is no need for a recovery step
- Temperature, pH, and other reaction components can be more precisely controlled
- More convenient scale up of the reaction by adding more reactors

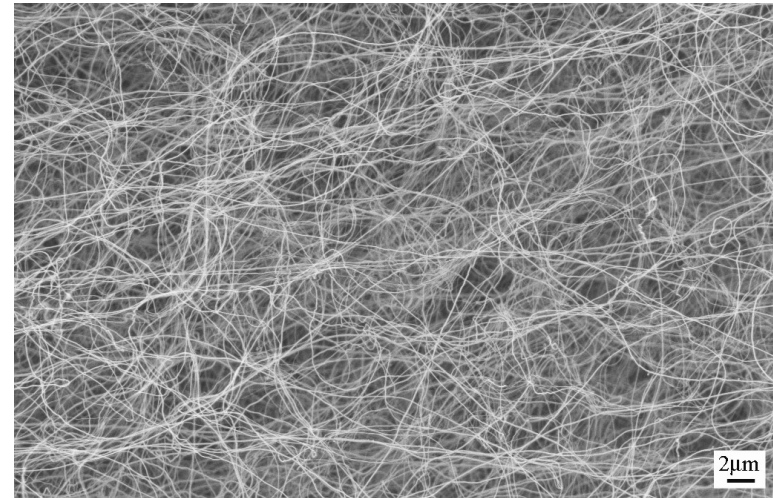
Silica NanospringsTM



An individual SiO₂ Nanospring

Advantages of Nanosprings:

- Low growth temperature (< 350°C)
- Atmospheric pressure process
- Mat thickness 3 – 300µm
- Hydrophilic
- 100% accessible surface area (>350m²/g)
- Easy to functionalize, e.g., using silane chemistry



A mat of Nanosprings

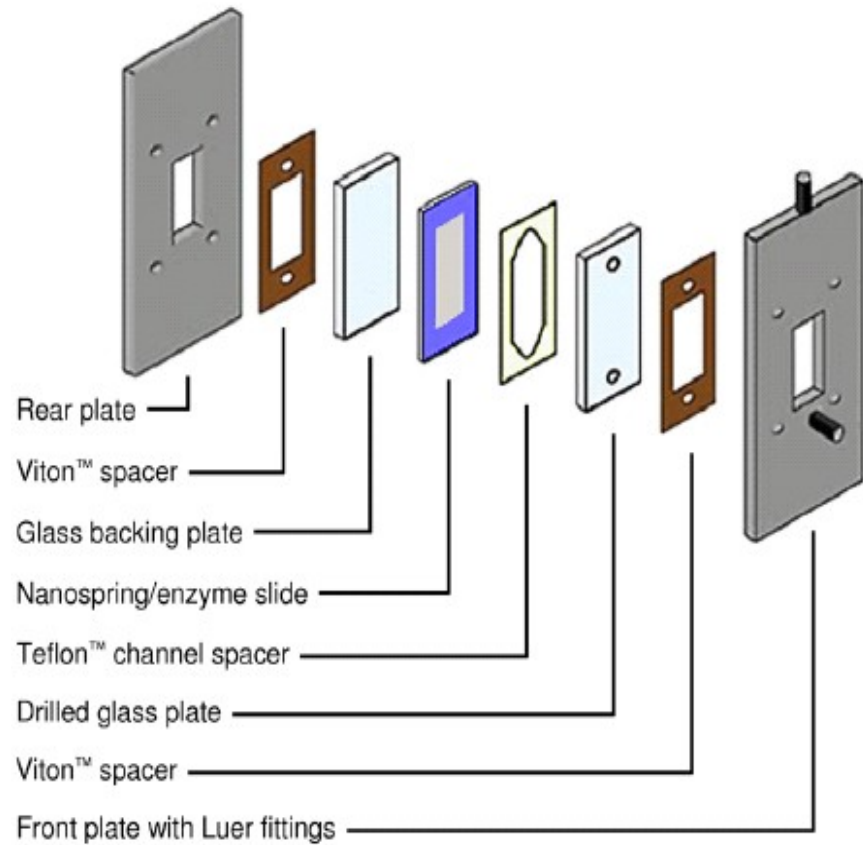
Substrate Flexibility:

- Aluminum foil
- Glass (including Pyrex[®])
- Ceramics
- Fiberglass
- Silica beads
- Stainless steel
- High T_g polymers

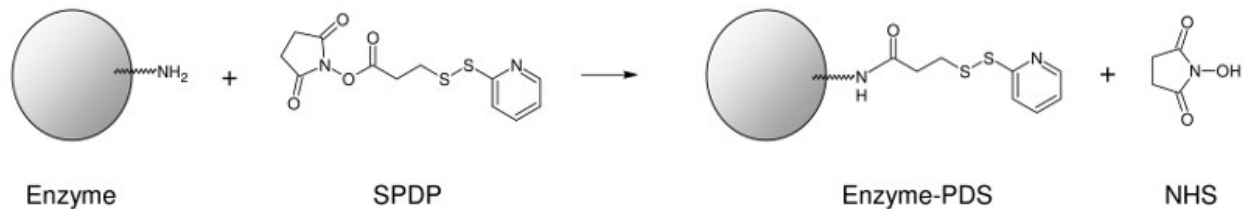
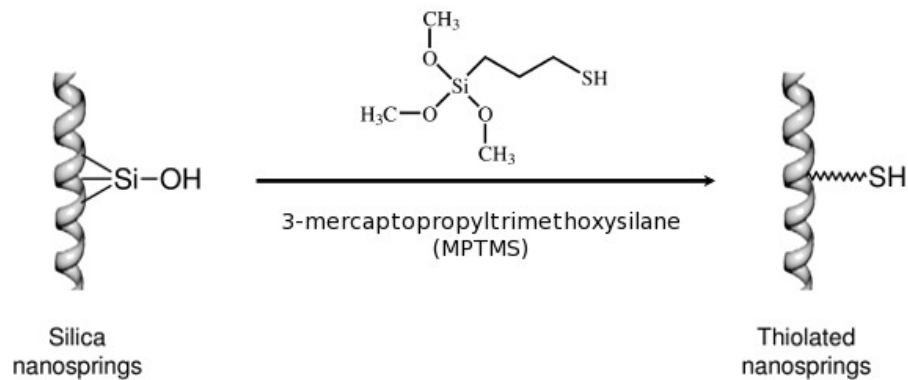
Reactor Geometry



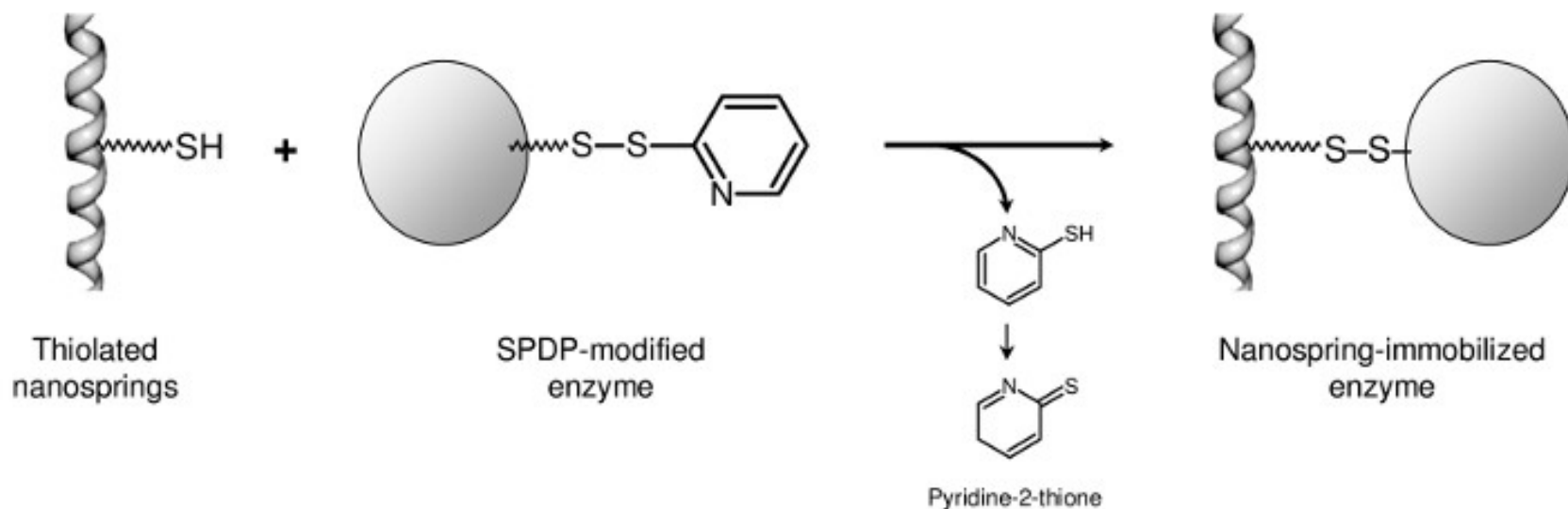
- Nanospring mat formed on fiberglass
- Mat thickness $\sim 80\mu\text{m}$
- Simple plate reactor geometry



Procedure for Immobilizing β -galactosidase on Nanosprings - I



Procedure for Immobilizing β -galactosidase on Nanosprings - II

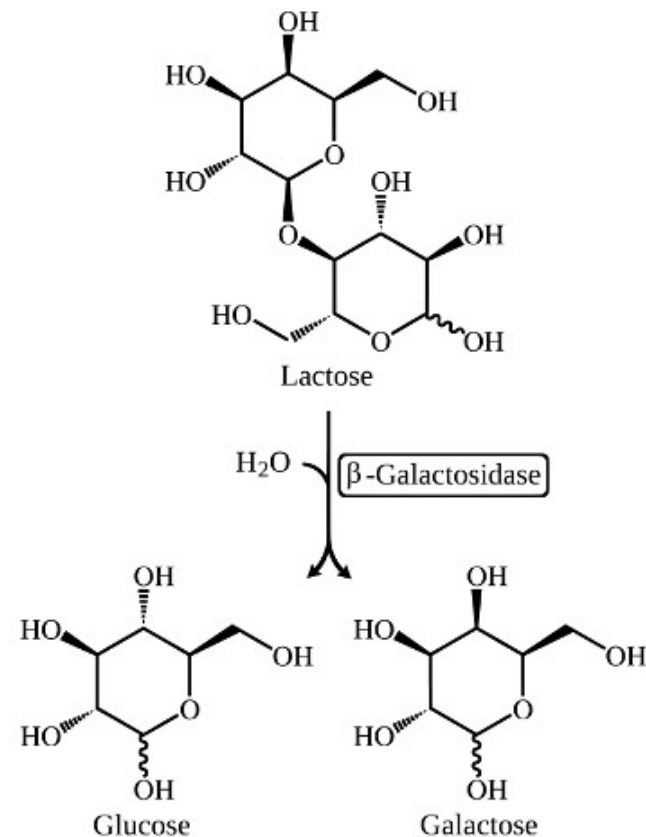


β -Galactosidase from *Aspergillus oryzae*

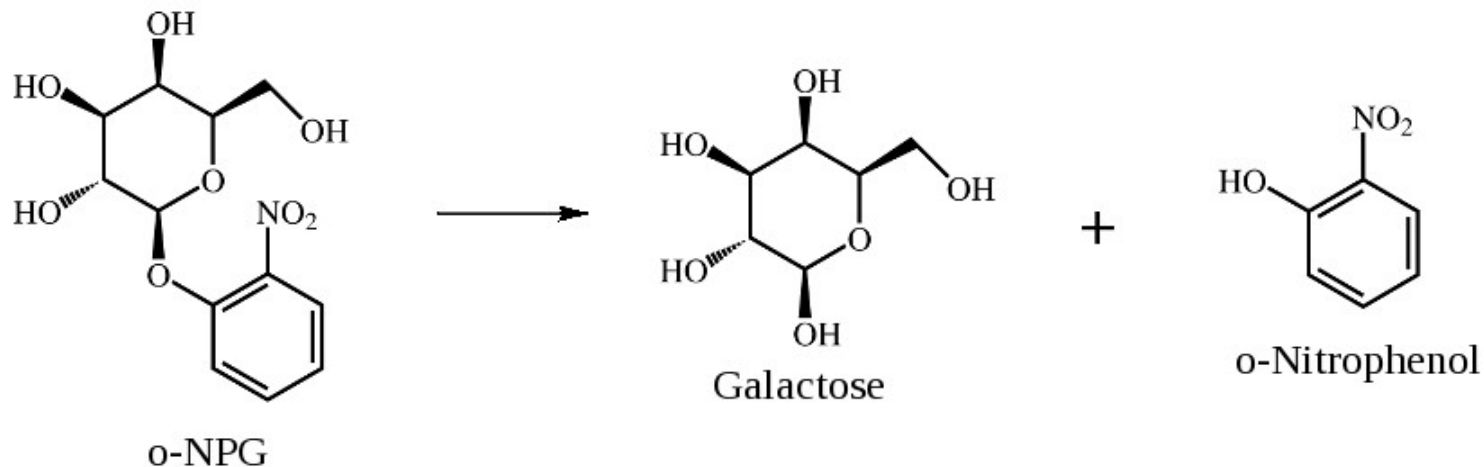
This is the natural reaction for the model enzyme, β -galactosidase

The reaction cleaves lactose into glucose and galactose

This enzyme is useful for method development because its reaction can be followed spectrophotometrically



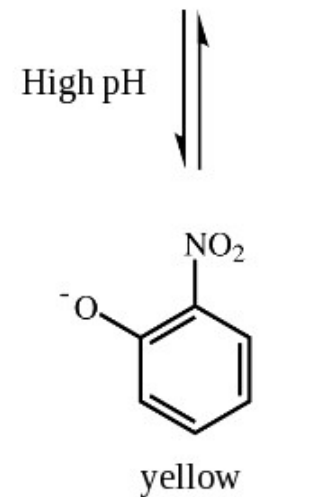
Model Reaction



Beta-galactosidase cleaves *o*-(nitrophenol β -D-galactosylpyranosid)NPG into galactose and *o*-nitrophenol.

At high pH, *o*-nitrophenol is converted to a colored compound with an absorbance maximum at 415 nm.

The reaction can be monitored by spectrophotometrically measuring the amount of yellow compound produced.



Model of β -galactosidase

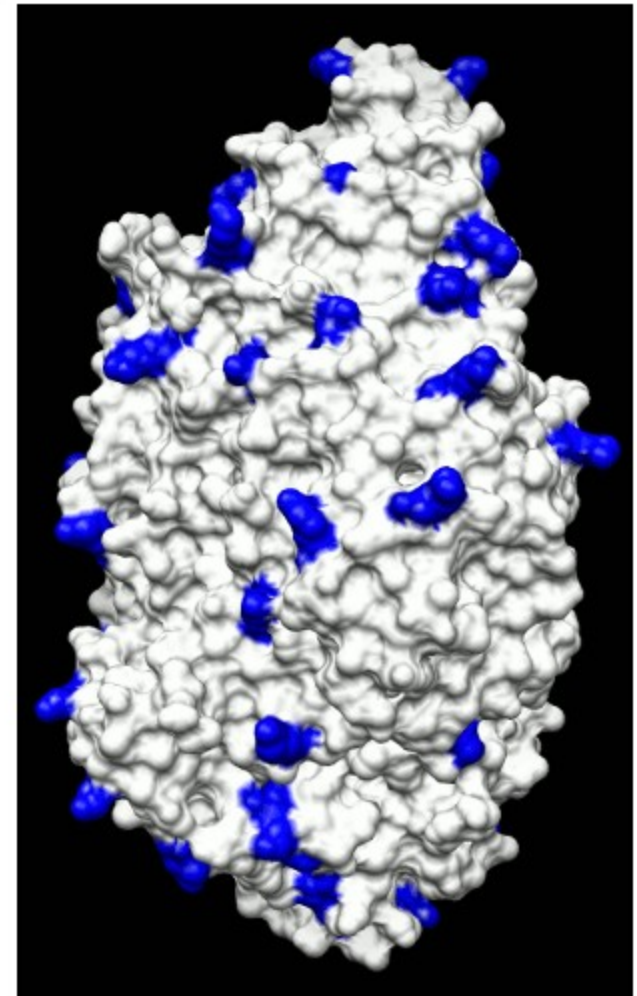
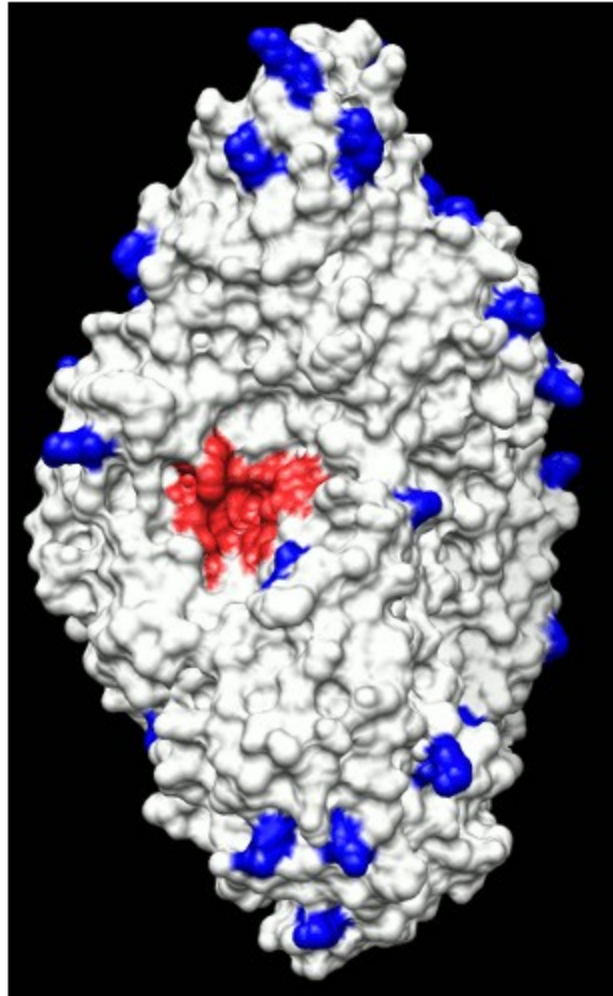
The red spot is the active site

The active site needs to remain accessible upon immobilization

The attachment points are the lysine side chains, shown in blue

Most attachment sites are on the side opposite to the active site

This enzyme is well suited for immobilization studies



Efficiency of Enzyme CFR with β -galactosidase

Current efficiency of enzyme reactor prototype

- 2.5 mM substrate (*o*-NPG)

100 μ L/min:	91% conversion	0.23 μ mole/min
200 μ L/min:	87% conversion	0.44 μ mole/min

At lower (2.5 mM) substrate concentrations we observe almost complete conversion

- 5 mM substrate

At higher substrate concentrations, the amount of material converted per minute is high

100 μ L/min:	88% conversion	0.44 μ mole/min
200 μ L/min:	75% conversion	0.75 μ mole/min
500 μ L/min:	50% conversion	1.25 μ mole/min

Linking reactors in series, or making the reactor larger, could achieve complete conversion with a large quantity produced per minute

- 20 mM substrate

100 μ L/min:	63% conversion	1.26 μ mole/min
200 μ L/min:	47% conversion	1.86 μ mole/min
500 μ L/min:	23% conversion	2.30 μ mole/min

Series Reactors

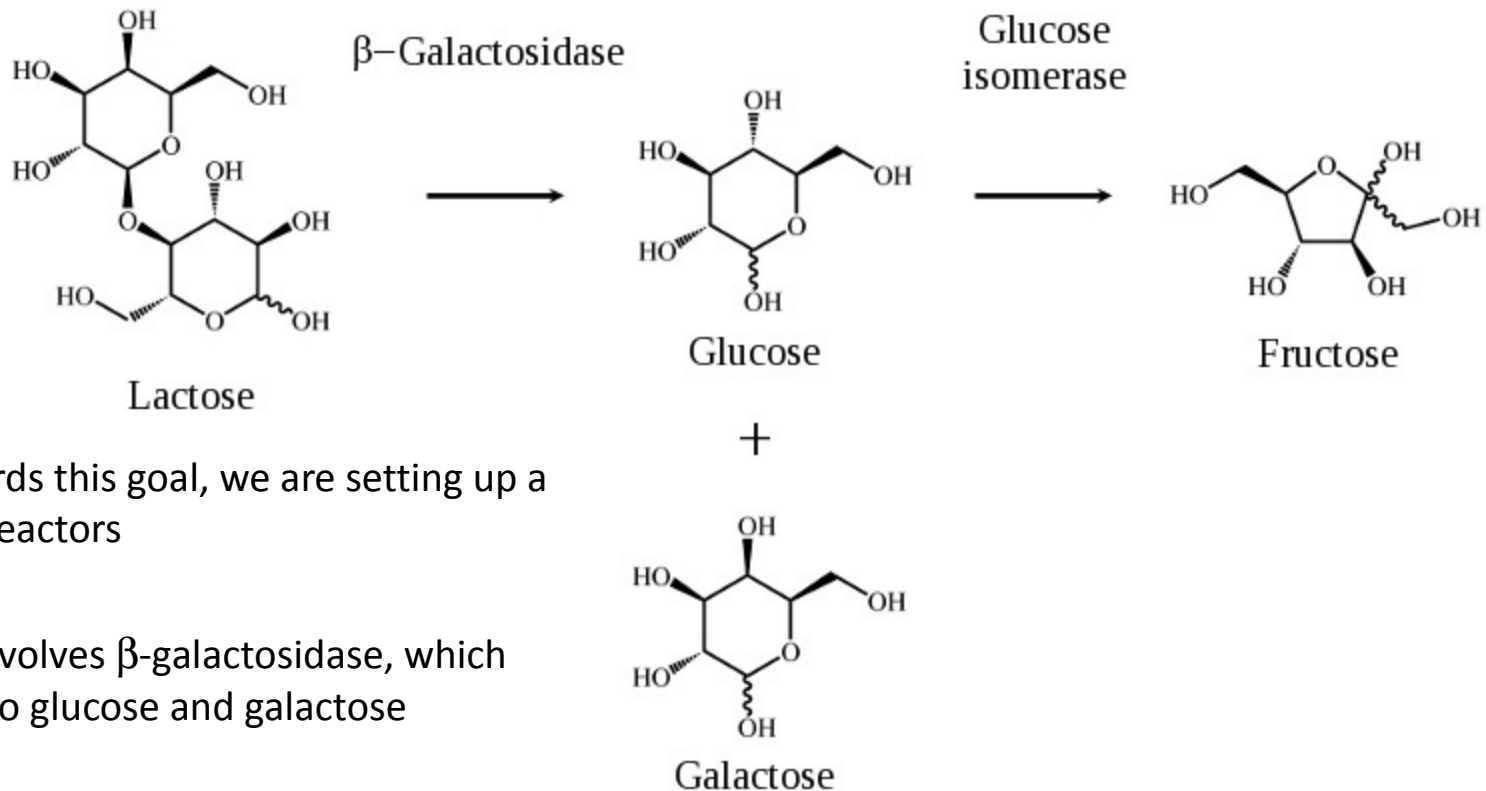


We are working on linking reactors in series, each with a different enzyme and a different reaction

The compact nature of Nanospring enzyme reactors makes this feasible

The eventual goal of this work is to load a simple precursor material at one end of the reactor series and retrieve a multistep reaction product at the other end

Two-Step Reactor

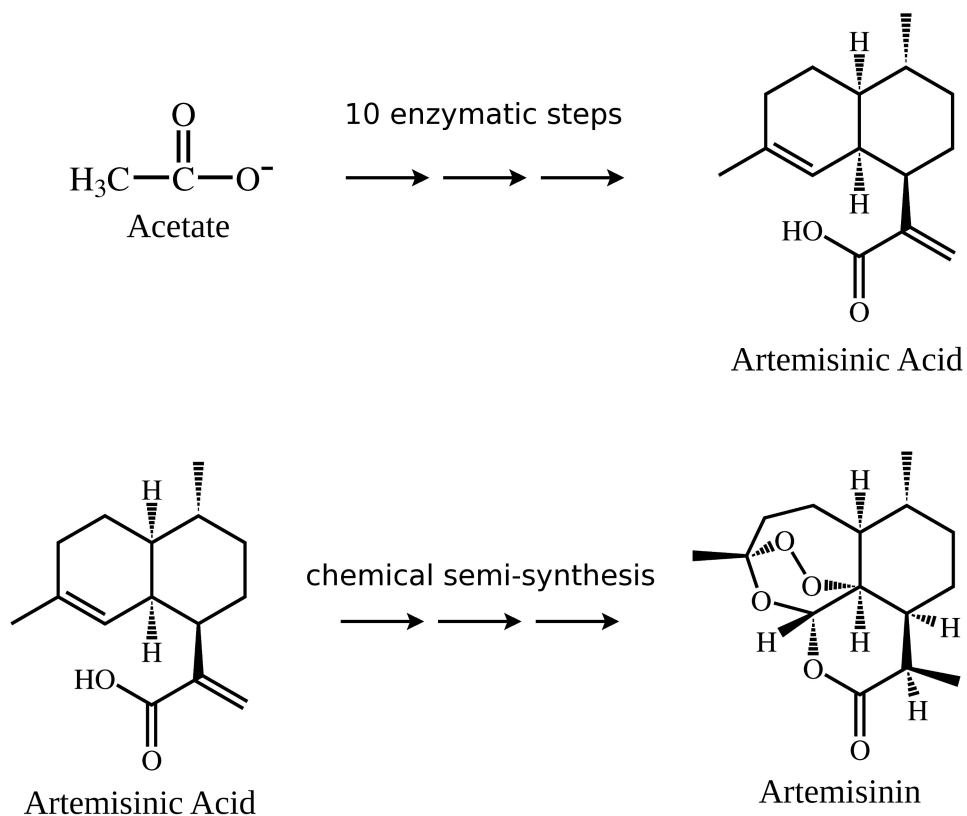


As a first step towards this goal, we are setting up a two-step series of reactors

The first reaction involves β -galactosidase, which converts lactose into glucose and galactose

The second reaction involves glucose isomerase, which converts glucose into fructose

Multi-Step Reactor Concept



Conclusions

Nanosprings provide many benefits as a support material for immobilized enzymes in continuous flow reactors:

- High surface area → increased reaction kinetics
- Miniaturization
- Multifunctionalization Capability
- Inverse Porous → Immediately accessible surface
- Non-agglomerating / fixed to substrate
- Compatible with multiple substrate materials and geometries