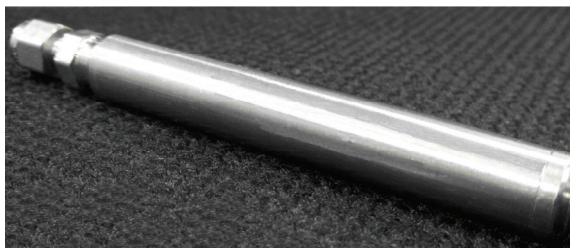
Enzymatic Flow Reactor for Immobilized Enzyme Processes

metals · inorganics · organometallics · catalysts · ligands · custom synthesis · cGMP facilities · nanomaterials

Why Immobilized Enzyme Processes?

The advantages of biocatalysis are well recognized. These green and sustainable processes typically proceed under mild conditions, do not require the use of heavy or toxic metals, in many cases can be conducted in water and may replace the need for highly hazardous reagents. However, enzymes can be quite expensive. Immobilization provides for easy separation of the enzyme from the product so that it can be reused/recycled, thereby lowering the cost.

Why the Enzymatic Flow Reactor?



Strem Catalog No. 96-0900
Tube Reactor–with Nanospring coated support mesh

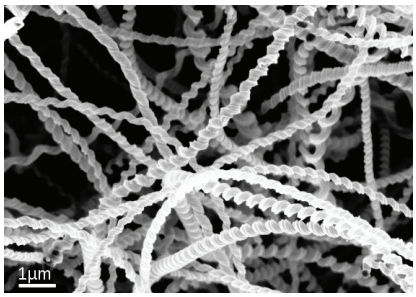
Strem Catalog No. 96-0900

While enzyme immobilization can provide numerous advantages, the commercial adoption of this technology has been somewhat limited due to the lack of availability of appropriate support materials. The chemical, biochemical, mechanical and kinetic properties of the immobilized enzyme are influenced by the interactions between the enzyme and the support. In addition, biocatalytic reactions run in batch processes using immobilized enzymes can experience inhibition due to saturation of the reaction solution.

In the **Enzymatic Flow Reactor**, enzymes are immobilized on the highly accessible surface area of functionalized silica Nanosprings™. The Nanosprings have a high surface area (>350 m2/g), and thus allow for a high density enzyme loading; because they are nonporous. However, they don't experience flow restriction or suffer from the diffusional limitations often associated with porous substrates. Furthermore, with the **Enzymatic Flow Reactor**, the product is removed continuously from the reaction mixture, so catalyst inhibition is not an issue. Furthermore, the enzyme remains stable in the reactor, so it can be reused multiple times.

What are NanoSprings™?

Silica Nanosprings™ are high surface area, one-dimensional nanomaterials made of amorphous silica that are produced using a proprietary atmospheric chemical vapor deposition process. They can be readily functionalized and coated onto a wide variety of substrates, including aluminum, polyimide, glass, silicon, stainless steel and carbon.



TEM image of Nanosprings

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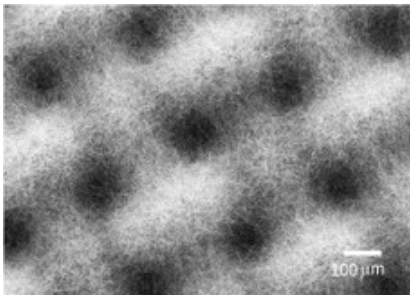
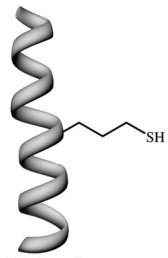
What is the Enzymatic Flow Reactor? Strem Catalog No. 96-0900



SEM image of Nanospring coated catalyst on support mesh

The Nanospring™ **Enzymatic Flow Reactor** is a 2.5 inch long, 0.25 inch i.d. tube that is packed with stainless steel mesh coated with a total of approximately 50 mg of silica Nanosprings.

The surfaces of these Nanosprings are functionalized with free sulfhydryl groups at the end of a three carbon chain that can react with an appropriately activated enzyme.

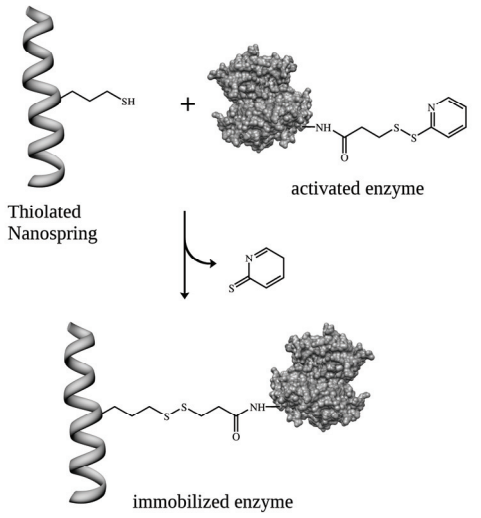


thiolated Nanospring

The maximum theoretical capacity of the **Enzymatic Flow Reactor** is 1 mg enzyme / 1 mg Nanosprings for average enzymes. Loading up to 40% of theoretical maximum has been observed.

Using the Enzymatic Flow Reactor

The **Enzymatic Flow Reactor** comes ready for enzyme immobilization. The first step is to incubate the desired enzyme with, for example, N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), which is a crosslinking agent that reacts with the free amino groups on the enzyme surface. A solution of the SPDP-modified enzyme is then slowly pumped through the reactor, during which time the thiolate groups on the Nanosprings react with the crosslinker on the enzyme to yield a covalently bound enzyme on the Nanospring surface.



Attachment of modified enzyme to Nanosprings

Key Features of the NanoSprings™ Enzymatic Flow Reactor

- Controllable processing for biocatalysis
- Continuous flow configuration
- No need for large capital expenditures or specialized equipment
- Can be connected in series for multi-step synthesis
- Can be functionalized for immobilization of enzymes, DNA, cells or proteins
- High density enzyme loading with increased stability due to silica Nanosprings support
- Easy and continuous separation of the product from the reaction mixture avoids inhibition
- Strong enzyme attachment eliminates need for recovery
- Reactor can be reused multiple times

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Detailed Instructions for Use
Nanospring™ Enzymatic Flow Reactor (Strem Cat. No. 96-0900)

Additional materials required

Syringe or peristaltic pump
Desalting column (BioRad Econo-Pac® 10DG column or equivalent)
DMSO
Buffer components:
- DTT
- Potassium Phosphate
- Sodium Borate
- Sodium Chloride
- Sodium Phosphate

Abbreviations

DTT: dithiothreitol
SPDP: N-succinimidyl 3-(2-pyridyldithio) propionate
DMSO: dimethyl sulfoxide
PBS: phosphate buffered saline (140 mM NaCl, 8 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.5)

Storage of SPDP

Recommended storage conditions for SPDP is -20 °C.

Completion of the immobilization process

For best results, all immobilization steps should be completed in one day. The entire procedure takes about one full workday to complete. Steps 1-2 can be done simultaneously with steps 3-6.

Directions for enzyme immobilization

1. Restore the sulfhydryl groups on the Nanospring surface (Fig 1) to reduced form by pumping 25 mL of the following buffer solution through the reactor: 50 mM Sodium Borate, 50 mM DTT, pH 8.5 (suggested flow rate: 0.5 mL/min).
2. Flush the reactor by pumping 25 mL of H_2O through followed by 25 mL of PBS (suggested flow rate: 0.5 mL/min).
3. Prepare the crosslinker solution: The reactor is shipped with a vial containing 2 - 3 mg of SPDP (F.W. = 312.36). Add enough DMSO to make a 20 mM solution (for example, if the vial contains 2.5 mg of SPDP, add 400 µL DMSO).
4. Dissolve the enzyme to be immobilized in PBS. The reactor has been developed using 10 mL of a 10 mg/mL enzyme solution; however, immobilization of smaller enzyme quantities should be successful.

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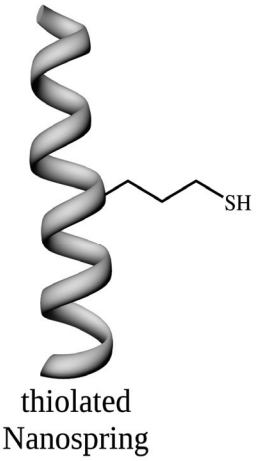

Figure 1. Free sulfhydryl groups on the Nanospring surface.

5. Mix the SPDP/DMSO solution with the enzyme solution (Fig 2): The optimal SPDP:enzyme ratio varies for different enzymes, but 3-30 µL of SPDP solution per mL of enzyme solution is appropriate for enzyme concentrations of 1-10 mg/mL (a molar ratio of 5:1 SPDP:enzyme is a good starting point). The reaction will be complete after 30 minutes at room temperature.

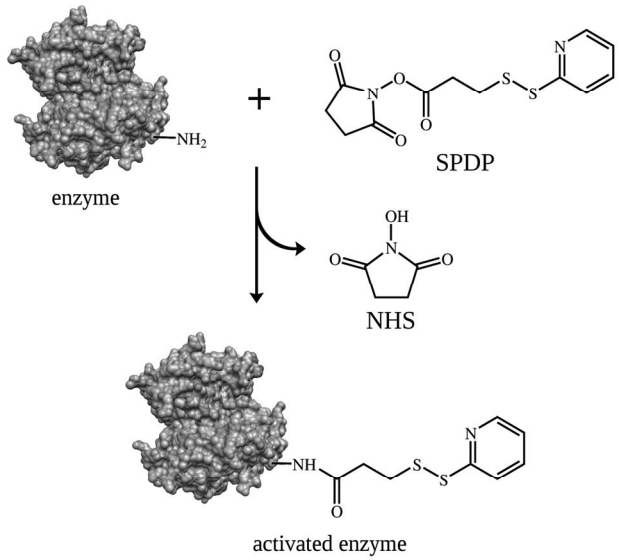

Figure 2. Reaction of the enzyme with the crosslinking reagent.

6. Exchange the modified enzyme into fresh PBS to remove unreacted SPDP. This can be done either by dialysis or with a desalting column.

7. Apply the SPDP-modified enzyme by slowly pumping it through the reactor (suggested flow rate: 0.1 mL/min). This allows the crosslinker on the enzyme to react with the free sulfhydryls on the Nanospring surface, resulting in a covalently attached enzyme (Fig 3). To quantify enzyme loading, aliquots of the enzyme solution can be taken before and after passing through the reactor.

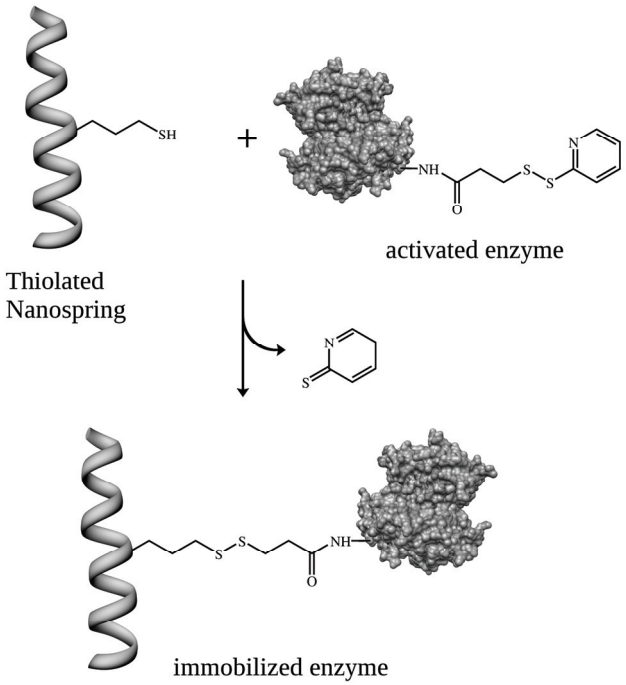

Figure 3. Attachment of modified enzyme to Nanosprings.

8. Wash the reactor with a buffer appropriate for the enzyme reaction. Pump several volumes through to remove any enzyme that is not covalently attached (suggested flow rate: 0.5 mL/min).

9. The immobilized enzyme reactor can be assayed by dissolving the substrate molecule in an appropriate reaction buffer and pumping it through the reactor. Optimal substrate concentrations and flow rates will vary depending on the enzyme (0.05 - 2.00 mL/min).

Storage conditions

After immobilization of the enzyme, the reactor should be kept wet with a compatible buffer solution.

Chemical compatibility of Nanosprings

Nanosprings are silica-based are thus incompatible with strong bases (NaOH, KOH), hydrofluoric acid, or other compounds that dissolve glass.