

Supplementary Information

Microfabrication of Mesoporous Silica Encapsulated Enzymes using Deep X-Ray Lithography

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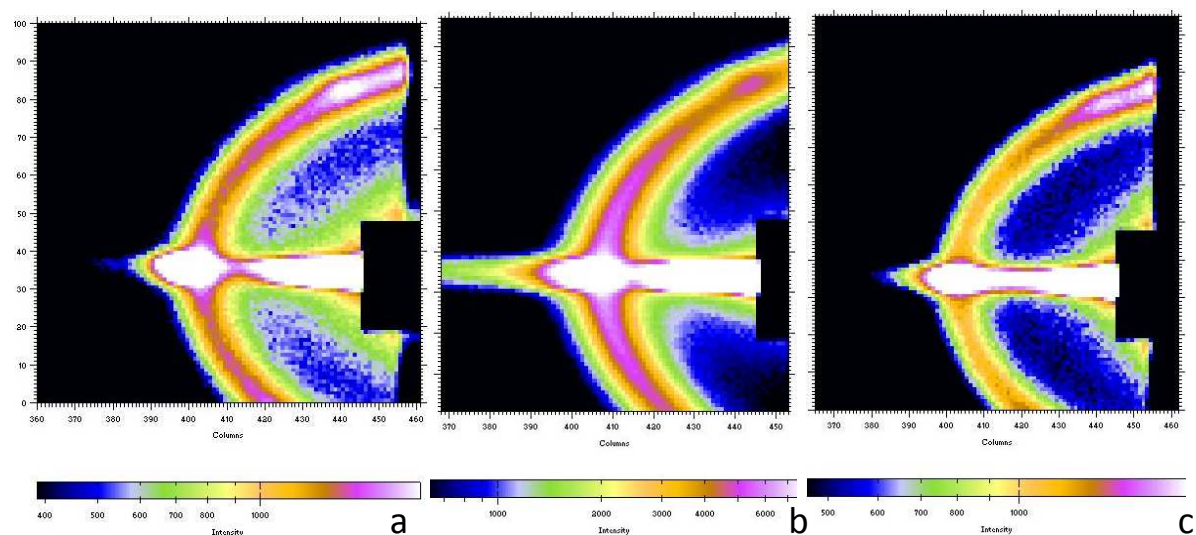


Figure S1. Grazing Incidence Small Angle X-Ray Scattering (GISAXS) patterns of (a) Unexposed mesoporous silica encapsulated enzyme film, (b) Pre-exposed (Route 1) amino-silane functionalized mesoporous silica film with enzyme encapsulation after exposure and (c) Post-exposed (Route 2) mesoporous silica encapsulated enzyme film.

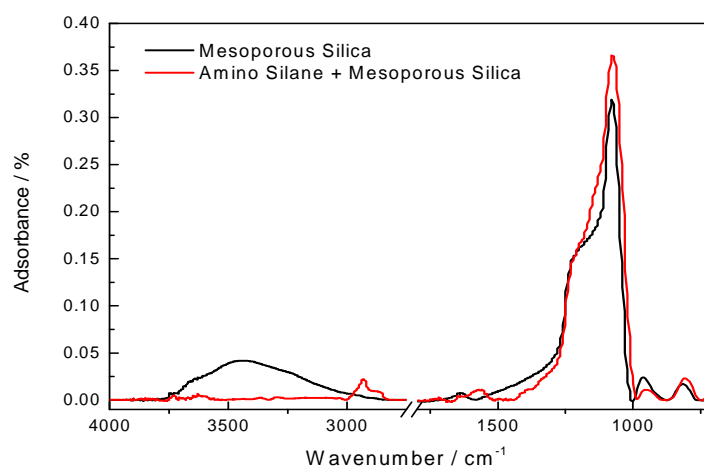


Figure S2. Fourier transform infrared spectroscopy (FTIR) was performed in transmission mode on the mesoporous silica film and the amino-silane functionalized mesoporous silica film prior to x-ray exposure.

Bioconjugation of the Fluorescent Protein

The fluorescent protein (streptavidin and R-phycoerythrin) was immobilized onto the amino silane functionalized mesoporous silica substrate via coupling with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysulfosuccinimide (S-NHS). Here we create a covalent bond between the amino group on the mesoporous silica substrate, introduced using the APTES precursor, and the carboxyl group present at the C-terminal of the polypeptide chain on the protein surface.¹

As described by Hermanson,¹ the formation of a covalent bond between carboxylic and amino functionalities requires a preliminary activation step of the carboxylic group, creating an intermediary which is more reactive towards the amino group. The most common strategy is to use a carbodiimide, where the carbon atom is electrophilic. The carbodiimide used, EDC, is highly water soluble and reacts with carboxylic acids to form an o-acylisourea intermediate, which is highly reactive and short-lived in aqueous environments. The attack of a nucleophile such as a primary amine on the carbonyl group of this ester results in the loss of an isourea derivative and the formation of an amide bond. The EDC coupling can be improved with the addition of N-hydroxysulfosuccinimide (S-NHS), which results in the formation of another intermediate, the sulfo-NHS ester. The formation of this second ester results in a more stable intermediate in aqueous solutions resulting in higher yields.¹ Nucleophiles, such as primary amine, attack the electron-deficient carbonyl group of the sulfo-NHS ester with the release of the sulfo-NHS leaving group and the creation of a stable amide linkage with the amine compound.¹

¹ G. T. Hermanson. *Bioconjugate Techniques*, second edition. Academic Press, 2008.