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## Encapsulation of Nitrile Hydratases for Improved Conversion of Nitriles to Amides

*A material useful for industrial synthesis of amides or nitrile degradation or remediation.*

### Contact

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### Inventors

Richard C. Holz, Ph.D.  
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### Field

Industrial synthesis  
Nitrile removal

### Technology

Purified nitrile hydratase  
encapsulated in sol-gel materials

### Key Features

- Stereoselective
- Recombinantly purified from *E. coli*

### Key Benefits

- Patent covers the use of a purified enzyme, its encapsulation, and use thereof

### Stage of Development

Recombinantly expressed  
Purified  
Catalytic activity tested  
Encapsulated and tested

### Status

Seeking licensing partner

### Patent Status

Provisional Application

### Bioconversion of nitriles

The ability to degrade nitriles is a common trait of microorganisms. The potential of nitrile-degrading enzymes for biotransformation, waste treatment, and the production of herbicide-resistant plants has been well studied. Stereoselective hydrolysis of nitriles and amides with whole cells or isolated enzymes has been reported for a number of bacterial strains, including those from *Rhodococcus*, *Pseudomonas*, *Brevibacterium*, *Nocardia*, *Psuedonocardia*, and *Arthrobacter*.

### Recombinant nitrile hydratases

The inventors have studied the thermostable Co-type nitrile hydratase from *Psuedonocardia thermophila* JCM 3095 (*PtNHase*) and the Fe-type NHase from *Comamonas testosteroni* (*CtNHase*). They have recombinant expression systems for both of these enzymes in *E. coli* and have purified them to homogeneity. *CtNHase* was chosen because it preferentially hydrates small aliphatic nitriles and *PtNHase* because it preferentially hydrates aromatic and halogenated aromatic nitriles.

### Purified recombinant nitrile hydratases encapsulated in sol-gels

A major issue in the use of enzymes in organic synthetic processes is the difficulty in separating the enzyme from the synthetic reaction mixture. Another complication is that the aprotic solvents so often used in organic synthetic reaction mixtures inactivate most enzymes. One way to overcome these problems is by encapsulating the enzymes in a solid support material that is sufficiently hydrated to protect the protein and sufficiently porous to permit access to small substrates. Some patented technologies use living or encapsulated microbes to perform nitrile hydrations. This allows simple physical removal of the catalysts from the reaction mixture, but has limitations. Since there are other enzymes in the bacterial nitrile degradation pathways, such as nitrilases, catalysis does not always stop with amide production, but can also progress to carboxylate formation. Thus the use of a single purified enzyme is preferable to the use of whole microbes or crude microbial lysate.

### Substrate specificity

The two enzymes, *CtNHase* and *PtNHase*, have been successfully encapsulated in a solid support material and their specificity and activity towards a variety of nitrile substrates has been (and are continuing to be) characterized. This detailed information can be provided by the inventors.

### Enhanced stability of catalytic pellets

Remarkably, these encapsulated enzymes have been proven to retain their catalytic activity weeks after being removed from the reaction vessel, rinsed, and dried. Furthermore, the catalytic pellets can be treated with trypsin, which proteolytically removes all surface accessible proteins, and shown to retain some activity, indicating that active enzyme is trapped inside the pellets (and is not just attached to the pellet surface).

### Opportunity

Loyola University Chicago is looking for commercial licensing partners for this patent.



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### **Inventor**

#### **Richard C. Holz**

Dr. Holz is Professor and Chair of the Chemistry Department at Loyola University Chicago. Dr. Holz received a B.S. degree in Chemistry from Bemidji State University with minors in biology and mathematics, an M.S. degree in Chemistry from the University of Minnesota-Duluth, and a Ph.D. in Chemistry from The Pennsylvania State University under the direction of Dr. William DeW. Horrocks, Jr. He was an NIH Postdoctoral Research Fellow at the University of Minnesota under the direction of Dr. Larry Que who is the 3M/Alumni Distinguished Professor of Chemistry. He joined the Loyola faculty in 2006 as Chair of the Chemistry Department after spending fourteen years at Utah State University. His current research interests include: structure/function studies on metalloenzymes, biophysical methods such as enzyme kinetics, site-directed mutagenesis, UV-VIS, NMR and EPR spectroscopies, nano and micron-sized biologically powered devices, biomolecule recognition at surfaces, and nano molecular detection devices. Dr. Holz is a Member of the American Chemical Society and the Society of Biological Inorganic Chemistry. He has co-authored more than 75 research articles and has lectured at over 50 universities around the world and has been funded by the National Science Foundation, the National Institutes of Health and the Air Force office of Scientific Research. He resides in the northern suburbs of Chicago with his wife Anna and his two daughters Emilia and Marika.

#### **Timothy E. Elgren**

Dr. Elgren is Professor of Chemistry and Chair of the Biochemistry/Molecular Biology Program at Hamilton College in Clinton, New York. He received a B.A. in Chemistry from Hamline University and a Ph.D. in Chemistry from Dartmouth College under the direction of Dr. Dean Wilcox. He did a postdoctoral fellowship at the University of Minnesota with Dr. Larry Que focusing on physical bioinorganic chemistry. Dr. Elgren joined the Hamilton College faculty in 1993. His research efforts have focused on physical characterization of the structure and chemistry associated with metal sites in metalloenzymes. His recent work has centered on the use of silica-derived materials used to encapsulate and stabilize enzymes. These bio-materials catalyze a wide range of reactions including hydrogen production, halogenation and dehalogenation reactions, amine oxidation, and nitril and amide hydrolysis and serve a variety of functions including biosensors, decontamination catalysts, and feed stock production catalysts. His work has been funded by the National Science Foundation and the National Institutes of Health, been published in a variety of professional journals, and presented at conferences and universities domestically and internationally. He lives in upstate New York with his wife Gail and two children, Ruth and Ben.