

Project **BIOCAT**
**Modular biocatalyst platform for chiral synthesis of chemical compounds by
structure-based directed evolution**
(the 2008 annual report)
Academy of Finland, project no. 117874

Funding period: 01.01.2007 - 31.12.2010
Site of research: University of Oulu

Project webpage: <http://www oulu fi/bioprocess/biocatalysis.pdf>

Partners:

Professor Dr. Peter Neubauer, Bioprocess Engineering Laboratory, University of Oulu,
project coordinator <http://www oulu fi/bioprocess/personne3.htm#Peter>

Professor Dr. Rik Wierenga, Department of Biochemistry, University of Oulu
<http://www.biochem oulu fi/tutkimus/wierenga/>

Professor Dr. Jouni Pursiainen, Department of Chemistry, University of Oulu
http://www oulu fi/chemistry/english/coord_chem/jp.html

Professor Dr. Marja Lajunen, Department of Chemistry, University of Oulu
<http://www oulu fi/chemistry/english/3.html>

Dr. Sampo Mattila, Department of Chemistry, University of Oulu
<http://www oulu fi/chemistry/english/4.html>

Scientists working in the project:

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| Post-Docs: | Dr. Päivi Pirilä (Biochemistry) Dr. Mari Ylianttila (BPEL, BIOCATKAL) |
| PhD-students: | M.Sc. Markus Alahuhta (Biochemistry) M.Sc. Mikko Salin (Biochemistry) M.Sc. Marco Casteleijn (BPEL) M.Sc. Matti Vaismaa (Chemistry) M.Sc. Nanna Alho. (Chemistry) |
| Dipl. Biol. | Mirja Krause (BPEL) |
| Technical staff: | Ville Ratas (Biochemistry) |

Other collaboration partners: Prof. K. Takkinen (Dept. Physiology, University of Oulu and VTT Biotechnology)

Project abstract and objectives:

BIOCAT is a new approach for the creation of tailor-made engineered biocatalysts to be used for the efficient, environmentally sound, biotransformation of chemical building blocks into highly pure enantiomers. It is based on the scaffold of the monomeric form of triosephosphate isomerase (TIM).

The interdisciplinary consortium consisting of five research groups from three faculties of the University of Oulu, was established during an earlier Academy-of-Finland project where a new approach was developed to create a platform of non-natural enzyme catalysts. This concept was developed using as an example the well-known TIM-barrel framework of TIM which was successfully engineered to accept a wide range of chemical ligands. Now, the extended consortium aims to develop a series of efficient biocatalysts for the cofactor-free synthesis of chirally pure chemical compounds. Therefore emerging techniques such as structure-based modeling and directed evolution of the biocatalyst in connection to chemical synthesis of a library of new compounds are jointly applied.

BIOCAT clearly addresses to strengthen basic research in protein engineering and chemistry with a clearly applied relevance, namely strengthening scientific expertise and further developing research environments in biocatalysis, as a key area supporting sustainable production and products. Despite a solid past in industrial enzymes and chemistry, these areas have been separated in Finland. The project consortium creates a multidisciplinary team intertwining chemistry, biochemistry, molecular biology and bioprocessing. By the use of enzyme catalysts, working in aqueous solutions at intermediate temperatures and pressure, BIOCAT is directly targeting to new eco-efficient production concepts and chemical products. The project is well integrated into national and international networks which are used to further strengthen the area and to disseminate research results. The project is closely connected to the interest in Finland for building up competence in industrial biotechnology and green chemistry.

Results of the research (publications, posters, patents etc)

Publications:

- Alahuhta, M., Casteleijn, M.G., Neubauer, P., Wierenga, R.K. (2008) The A178L mutation in the C-terminal hinge of the catalytic loop-6 of triosephosphate isomerase (TIM) induces a closed-like conformation in dimeric and monomeric TIM, *Acta Cryst*, D64, 178-188.
- Alahuhta, M., Salin, M., Casteleijn, M.G., Kemmer, K., El-Sayed, I., Augustyns, K., Neubauer, P., Wierenga, R.K. (2008) Structure-based protein engineering efforts with a monomeric TIM variant: the importance of a single point mutation for generating an active site with suitable binding properties. *PEDS*, 21, 257-266.
- Matti J.P. Vaismaa, M.J. P. , Yliniemelä, S.M., Lajunen, M.K. (2007) Simple and effective green preparation of 3-alkylthiopropionic acids, *Z. Naturforsch.* 62b, 1317-1323.
- Vaismaa, M.J.P , Leskinen, M.V., Lajunen, M.K. (2009) The microwave-assisted one carbon chain extension in the preparation of terminal α -hydroxy ketones, *Synth. Commun.* in press.

Diploma and PhD theses:

- Alahuhta, M. 2008. Protein crystallography of triosephosphate isomerases: Functional and protein engineering studies. (PhD thesis).

Lectures at scientific meetings:

- Wierenga R.K. Structural enzymological studies of dimeric and monomeric TIMs. June 14, 2008, INPEC-2008. Naantali Spa, Turku, Finland.
- Vaismaa, M. The design, synthesis and evaluation of new substrate candidates based on triosephosphate isomerase, *Strubiocat minisymposium*, Oulu, 08.-09.10.2008.
- Alho, N Utilization of NMR and MS techniques in biocatalysis research. *StruBioCat Minisymposium* 9.10.2008.
- Casteleijn, M. Towards new biocatalytic activity of ATIM by structure based directed evolution. *StruBioCat Minisymposium*, Oulu, Finland October 8th – 9th, October, 2008
- Salin, M. Protein crystallographic characterization of the A-TIM binding properties. *StruBioCat Minisymposium*, Oulu, Finland October 8th – 9th, October, 2008

- Neubauer, P. Bringing fed-batch fermentation into the protein lab straight forward efficient high throughput protein expression and labeling with EnBase™. Workshop "Protein expression and isotope labeling for structural biology" BNMRZ, Garching, July 24-25 2008.

Other lectures:

- Marco G. Casteleijn. Biocatalysis and Future plans. Bioforum Regional meeting, Oulu (Finland) June 3rd, 2008
- Neubauer, P. Kealases - a new structural framework for artificial enzymes. Bio meets Nano and IT conference 2008. Oulu, Finland, December 8-11, 2008.

Posters:

- M. Salin, N. Alho, M. Vaismaa, M. Alahuhta, M. Casteleijn, M. Lajunen, S. Mattila, P. Neubauer, R. Wierenga, Structural Studies with A-TIM: Towards Better Binders and New Enzyme Activity, INPEC2008, Naantali, 12.-15.06.2008, P138.
- N. Alho. "Binding studies of A-TIM" (XXX Finnish NMR Symposium 11.-13.6.2008) The poster was chosen as Best poster
- Casteleijn, M.G., Panula-Perälä, J., Krause, M., Wierenga, R.K., Neubauer, P. High Throughput Protein Expression Optimization of Monomeric TIM Libraries using EnBase™ technology. XXII Paulo foundation symposium: INPEC, Naantale (Finland) 12th – 15th June 2008;
- Casteleijn, M.G., Panula-Perälä, J., Krause, M., Wierenga, R.K., Neubauer, P. High Throughput Protein Expression Optimization of Monomeric TIM Libraries using EnBase™ technology. StruBioCat, Oulu (Finland), 8th – 9th, October, 2008
- Casteleijn, M.G., Panula-Perälä, J., Krause, M., Wierenga, R.K., Neubauer, P. Creating new enzymes - High Throughput Protein Expression Optimization of Monomeric TIM Libraries using EnBase™ technology. StruBioCat Minisymposium, Oulu, Finland October 8th – 9th, October, 2008 (Poster)
- Krause, M., Casteleijn, M.G., Wierenga, R.K., Neubauer, P. Creating new enzymes - Directed Evolution of Triosephosphate Isomerase. INPEC, Naantale (Finland) 12th – 15th June 2008 (Poster)
- Casteleijn, M.G., Markus Alahuhta, M., Peter Neubauer, P., Rik K. Wierenga, R.K. The A178L mutation in the C-terminal hinge of the flexible loop-6 of dimeric and monomeric TIM favours the closed active site geometry. Biocenter day, Oulu (Finland), 3rd April 2008 (Bulletin)
- Casteleijn, M.G., Krause, M., Alahuhta, M., Vaismaa, M., Juvani, R., Mattila, S., Lajunen, M., Pursiainen, J., Wierenga, R.K., Neubauer, P. Creating new enzymes - From Triosephosphate Isomerase to Kealases. Poster at the SusChem Stakeholder and Brokerage event, 29-30 January 2008, Berlin.
- Casteleijn, M.G., Panula-Perälä, J., Krause, M., Wierenga, R.K., Neubauer, P. High Throughput Protein Expression Optimization of Monomeric TIM Libraries using EnBase™ technology. Poster at the Recombinant protein production conference RPP2008, Porto Conte Bay, Sardinia, Italy, 24-28.8.2008.
- Casteleijn, M.G., Krause, M., Alahuhta, M., Vaismaa, M., Juvani, R., Mattila, S., Lajunen, M., Pursiainen, J., Wierenga, R.K., Neubauer, P. TIM-based biocatalyst platform for chiral synthesis of chemical compounds. ESF-EMBO Symposium on Protein Design and Evolution for Biocatalysis, Sant Feliu, Spain: 25-30 October 2008
- Alahuhta, M., Casteleijn, M.G., Neubauer, P., Wierenga, R.K. The A178L point-mutation induces catalytic loop closure in dimeric and monomeric Triosephosphate Isomerase. VAAM/GBM conference, 9-11 March 2008, Frankfurt.
- Salin M, Alahuhta M, Casteleijn M, Vaismaa M, Lajunen M, Mattila S, Alho N, Neubauer P & Wierenga R. Crystallographic docking studies with A-TIM: towards better binders and new enzyme activity. VAAM/GBM conference, 9-11 March 2008, Frankfurt.

Impact of the research

- In 2008 we initiated the formation of a Center-of-Expertise for promoting the research in the field of biocatalysis, highlighting also its benefits to structure based biotech developments: StruBioCat.
- In the context of the StruBioCat initiative a finnish infrastructure roadmap (StruBioCat) was prepared and we also organized a minisymposium (Oct 8-9, 2008) in Oulu. (<http://www.biochem oulu.fi/StruBioCat/>).

- In the context of this StruBioCat symposium a meeting of the Finnish Biocatalysis working group was also organized. The StruBioCat symposium was attended by approximately 45 registered participants, coming from the Science and Medical faculty of Oulu, as well as from other Universities and biotech companies in Finland. The meeting was very important for exchange of information on the very advanced technologies that we are using in the BIOCAT project.
- Generally the BIOCAT research work in 2008 has considerably enhanced our expertise in this field, highlighting the crucial interplay of genetic methods, protein characterization, X-ray, NMR and MassSpec. The research has also been published in important journals and presented in important lectures.

The progress of the research versus the original plan

- Further screening of the 16 randomized libraries of the A-TIM variants. The design of these 16 libraries was guided by structural information.
- For the screening three different minus strains, obtained from other labs, have been used: D-ribosephosphate isomerase minus strain, D-xylose isomerase minus strain and an L-arabinose isomerase minus strain. Four initial hits, obtained from the L-arabinose isomerase minus strain, are currently characterized further.
- New minus strains are generated currently in the lab by ourselves. In this way the minus strains will be precisely defined.
- Many more crystal structures are available of complexes of A-TIM with a range of molecules. Some of these molecules were synthesized by the Lajunen group, but also the mode of binding of a range of sugar molecules is now being studied.
- The X-ray studies are complemented by thermal shift assays, done by studying the effect of these compounds on shifting the CD-melting curves.
- Further NMR and ESI-MS binding studies with the compounds synthesized by the Lajunen group have been carried out.
- Further NMR studies with N15-labeled and N15/C13-double labeled A-TIM towards determining the solution structure of A-TIM have been done.
- Alternative methods for the creation of cheaper N15-labeled A-TIM variants are being developed using the BioSilta EnBase technology.

BIOCAT will continue to focus on new binders and ligands, while understanding the underlying mechanisms, in so called proof of principle studies of A-TIM. In parallel BIOCAT will transform the monomeric A-TIM variant from binding molecules to active enzymes, based on its original isomerisation reaction, using directed evolution approaches. Originally the proposed sugar isomerase activity concerned the D-ribosephosphate isomerase activity, which could lead to obtaining non-natural enzymes for the synthesis of ribose analogues, which are known to be important building blocks of anti-viral nucleosides. In 2008 we became aware of the importance of developing biocatalytic pathways which are able to increase the bioavailability of pentose sugars. Pentose sugars are an important component of biomass, such as wood. This is an important research topic in Finland as well as world wide, and our directed evolution screening experiments have been expanded to include now the search of D-xylose isomerase and L-arabinose isomerase catalytic activities for the randomised A-TIM variants.