Stereoselective bionano-catalysis on gold nanoparticles

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Selected applications of gold nanoparticles

- Biological applications of gold nanoparticles, Ralph A. Sperling, Pilar Rivera Gil, Feng Zhang, Marco Zanella and Wolfgang J. Parak *Chem. Soc. Rev.,* **2008**, *37*, 1896–1908,
- A Review on Functionalized Gold Nanoparticles for Biosensing Applications, S. Zeng K.T. Yong, I. Roy X.Q. Dinh, X. Yu, F. Luan, *Plasmonics* , **2011**, *6,* 491–506,
- Gold Nanoparticles in Chemical and Biological Sensing, K. Saha, S. S. Agasti, C. Kim, X. Li, V. M. Rotello, *Chem. Rev.*, **2012**, *112*, 2739 – 2779,
- The use of gold nanoparticles in diagnostics and detection, Robert Wilson, *Chem. Soc. Rev.*, **2008**, *37*, 2028–2045,
- Synthesis and electrochemical applications of gold nanoparticles, S. Guo, E. Wang, *Analytica Chimica Acta*, **2007,** *598*, 181–192,

- Bio-Inspired Nanocatalysis in Book Bio-Inspired Nanotechnology, R. Coppage, M. R. Knecht, **2014**, 173-219,

Selected applications of gold nanoparticles

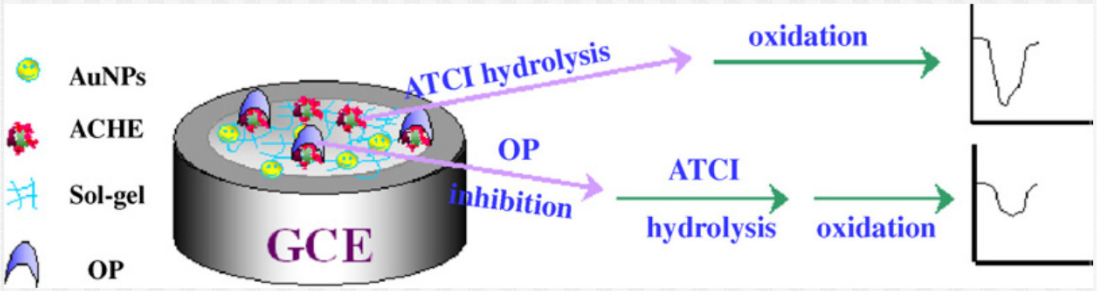
- The gold–sulfur interface at the nanoscale, H. Häkkinen, Nature Chem., **2012,** *4,* 442,
- Application of Thiolated Gold Nanoparticles for the Enhancement of Glucose Oxidase Activity, P. Pandey,S. P. Singh,S. K. Arya, V. Gupta, M. Datta, S. Singh, B. D. Malhotra, *Langmuir* **2007**, *23*, 3333-3337,

- Pepsin-Gold Colloid Conjugates: Preparation, Characterization, and Enzymatic Activity, A. Gole, C. Dash, V. Ramakrishnan, S. R. Sainkar, A. B. Mandale, M. Rao, M. Sastry, *Langmuir,* **2001**, *17*, 1674-1679,
- „The enzyme in the pepsin-Au bioconjugate retained substantial biocatalytic activity and was more stable than the free enzyme in solution".

- Nanoparticle–enzyme hybrid systems for nanobiotechnology, I. Willner, B. Basnar, B. Willner, *FEBS Journal ,* '**2007,** *274,* 302–309,
- „The use of NP–biomolecule hybrid systems, specifically NP–enzyme assemblies, is in the early phases of development. The results already obtained promise exciting future developments in this area of nanobiotechnology."

Applications of enzymes in nanotechnology

- Biosensors

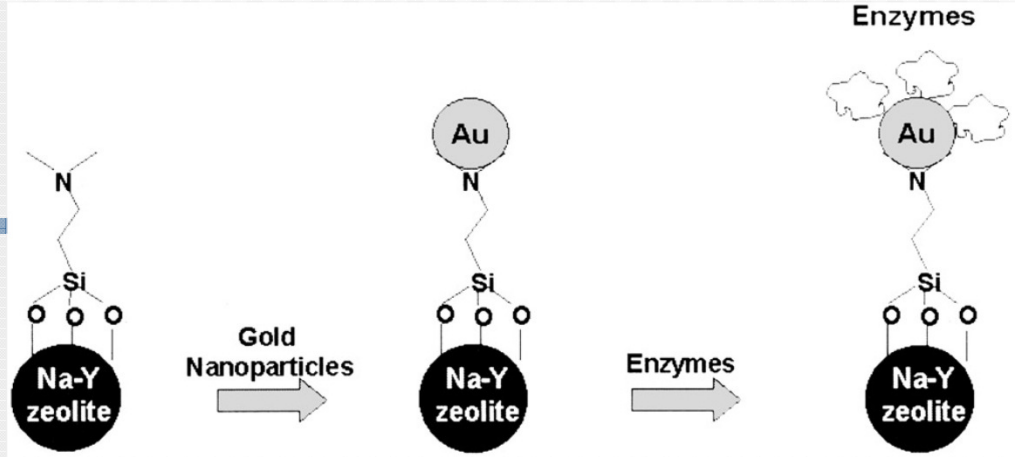


Du D., Chen Sh., Cai J., Zhang A., *Biosens. Bioelectron.*, **2007**, 23, 130-134

- Immunoenzymatic tests

- Biocatalysis!?

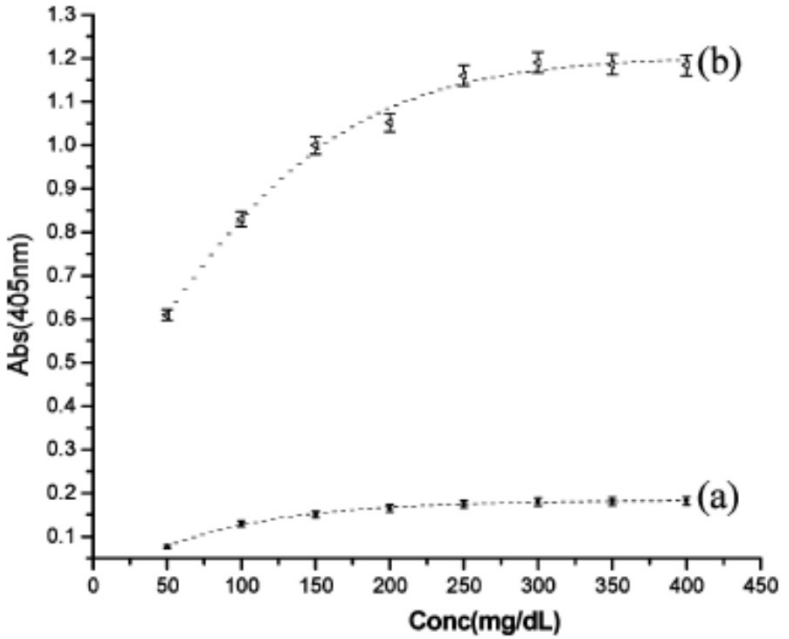
Biocatalysis



No. of cycles	Activity of protease on zeolit [U/mg]	Activity of protease on nanogold-zeolit [U/mg]
1	55	78
2	34	40
3	12	26
4	2	16

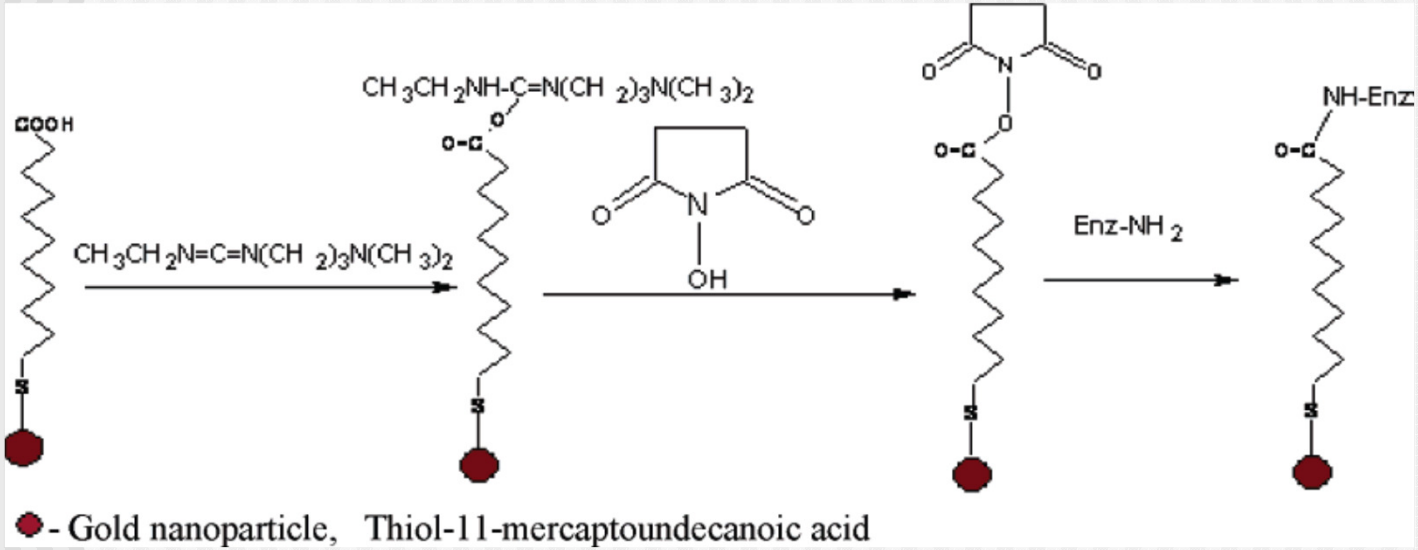
Phadtare S., Vinod V.P., Mukhopadhyay K., Kumar A., Rao M., Chaudhari R.V., Sastry M., *Biotechnology and Bioengineering*, **2004**, 85 (6), 629-637

Biocatalysis



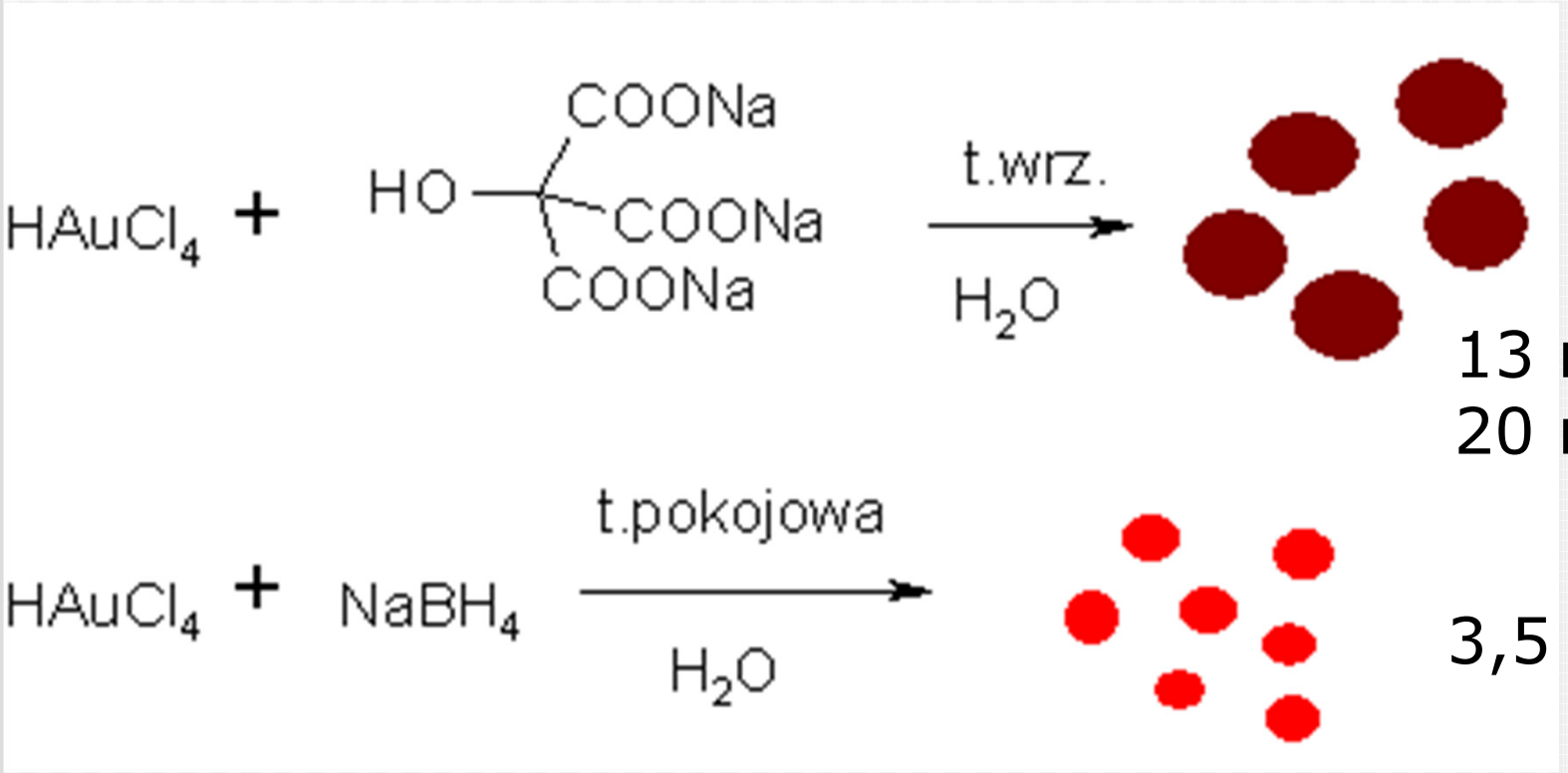
a – free enzyme

b – glucose oxidase on AuNP

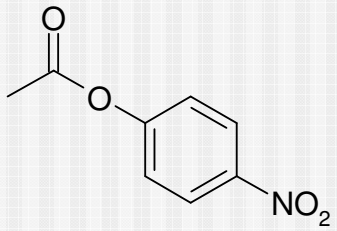


Pandey P., Singh S.P., Arya S.K., Gupta V., Datta M., Singh S., Malhotra B.D., *Langmuir*, **2007**, 23, 3333-3337

The synthesis of gold nanoparticles



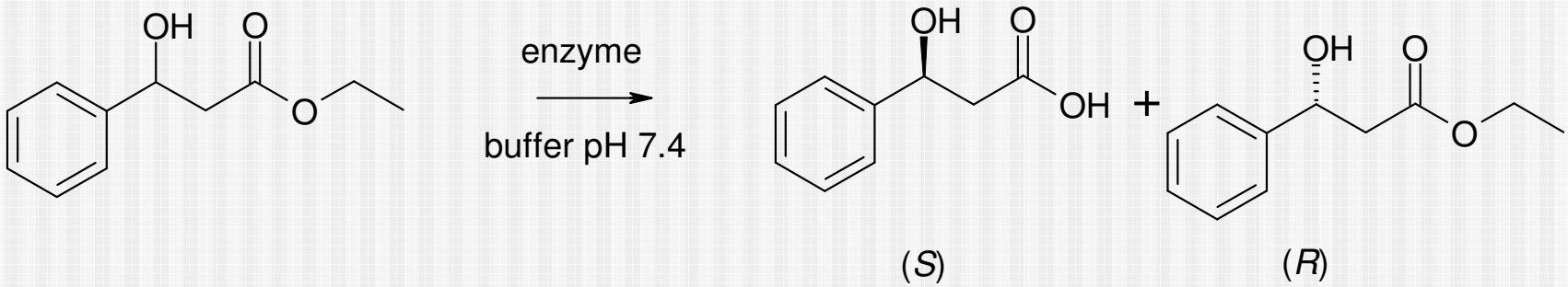
Kinetic parameters of enzymes



sample	V_{max} [mM/min]	K_m [mM]	k_{cat}/K_m [$M^{-1}s^{-1}$]
PLE	0,0101	0,114	$5,96*10^4$
PLE + 3,5 nm AuNP	0,0099	0,095	$7,06*10^4$
PLE + 20 nm AuNP	0,0107	0,116	$6,27*10^4$

sample	V_{max} [mM/min]	K_m [mM]	k_{cat}/K_m [1/min]
C. Cylindracea lipase	0,0080	0,131	0,061
lipase + 3,5 nm AuNP	0,0096	0,177	0,054
lipase + 20 nm AuNP	0,0106	0,198	0,053

Enzymatic kinetic resolution – model reaction



Ps.cepacia lipase E = 37

Boaz N.W., *J. Org. Chem*., **1992**, 57, 4289-4292

Ultrasounds + *Ps.cepacia* lipase E = 458

Ribeiro C.M.R., Passaroto E.N., Brenelli E.C.S., *Tetrahedron Lett*., **2001**, 42, 6477-6479

Kinetic resolution: native enzymes with or without nanoparticles

Enzyme	time [h]	Conv. [%]	ee_s [%]	E^a
TLAP (Turkey liver acetone powder)	5	40	9	1.4
TLAP + 3.5 nm AuNPs	5	40	8	1.4
TLAP + 20 nm AuNPs	5	40	10	1.6
Wheat Germ lipase	3	40	1	-
Wheat Germ lipase + 3.5 nm AuNPs	3	40	3	-
Wheat Germ lipase + 20 nm AuNPs	3	40	4	-
Rhizopus arrhizus lipase	2,5	40	12	1.6
Rhizopus arrhizus lipase +3.5nmAuNPs	2,5	40	11	1.6
Rhizopus arrhizus lipase + 20nmAuNPs	2,5	40	10	1.5

Kinetic resolution: native enzymes with or without nanoparticles

Enzyme	time [h]	Conv. [%]	ee_s [%]	E^a
PLE (Pig liver esterase)	8	45	38	3,9
PLE + 3,5 nm AuNP	8	45	49,9	6,7
PLE + 20 nm AuNP	8	45	43,9	5,0
PPL (Porcine pancreatic lipase)	2	50	28,8	2,3
PPL + 3,5 nm AuNP	2	50	37,1	3,1
PPL + 20 nm AuNP	2	50	43,4	3,8
Ps.cepacia lipase	5	55	99,9	72,1
Ps.cepacia lipase + 3,5 nm AuNP	5	55	100	117
Ps.cepacia lipase + 20 nm AuNP	5	55	100	117

[a]calculated from $E = [\ln((1-c)*(1-ee_s))]/[\ln((1-c)*(1+ee_s))]$

Kinetic resolution catalyzed by enzymes adsorbed on gold nanoparticles

Enzyme	time [h]	Conv. [%]	ee_s [%]	Ea
***Ps.cepacia* lipase**	**5**	**55**	**99,9**	**72,1**
Ps.cepacia lipase on 3.5 nm AuNPs	2	20	8	2.1
Ps.cepacia lipase on 13 nm AuNPs	2	20	10	2.7
Ps.cepacia lipase on 20 nm AuNPs	2	30	14	2.2
PLE	**8**	**45**	**38**	**3,9**
PLE on 3.5 nm AuNPs	2	25	*rac*	-
PLE on 13 nm AuNPs	2	25	*rac*	-
PLE on 20 nm AuNPs	2	25	*rac*	-

[a]calculated from $E = [\ln((1-c)*(1-ee_s))]/[\ln((1-c)*(1+ee_s))]$

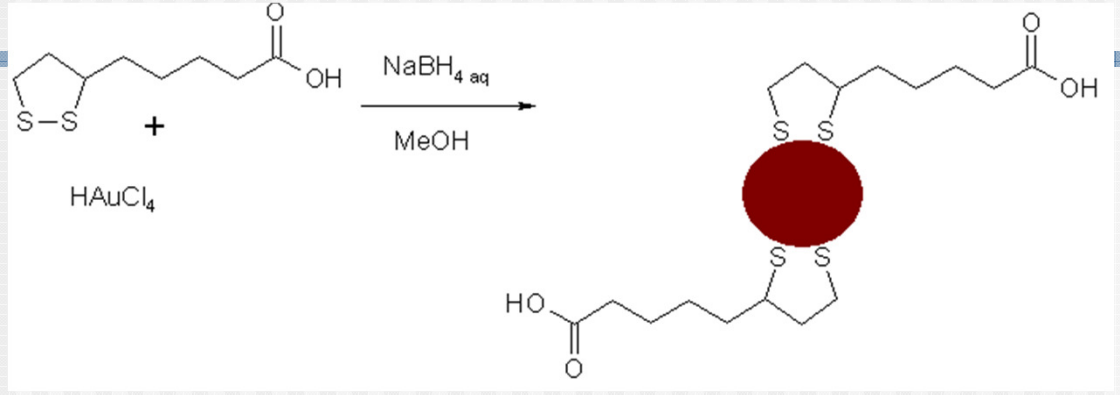
The influence of incubation time on enantioselectivity for *Ps.cepacia* lipase

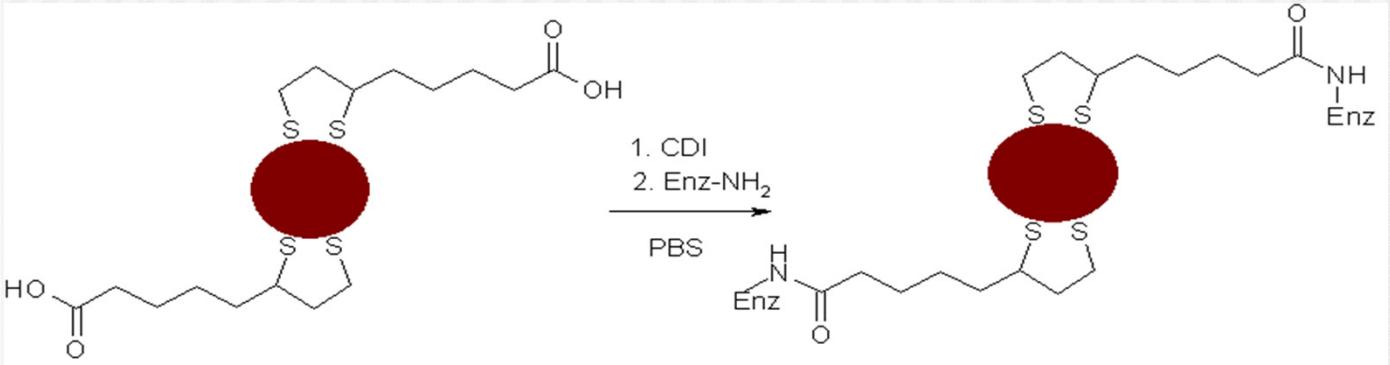
incubation time	Time [h]	Conv. [%]	ee [%]	E
1 min	48	20	7,8	2
10 min	48	19	8	2,2
30 min	48	15	6,5	2,3
1 h	48	20	10,4	2,7
4 h	48	20	8,3	2,2
24 h	48	19	9,5	2,6

The loading of enzyme on nanoparticles

enzyme/nanoparticles	Time [h]	Conv. [%]	ee [%]	E
0,01ml/0,5ml	48	27	19,2	3,8
0,025ml/0,5ml	48	23	17,6	4,6
0,05ml/0,5ml	48	22	13,1	3,1
0,1ml/0,5ml	48	30	16,7	2,7
0,2ml/0,5ml	48	25	14	2,8
0,4ml/0,5ml	48	29	16,7	2,8

Enzymes covalently immobilized on nanoparticles





- Linker: α-lipoic acid
- Coupling agent: 1,1'-carbonyldiimidazole
- Modified procedure from:

Pandey P., Singh S.P., Arya S.K., Gupta V., Datta M., Singh S., Malhotra B.D., *Langmuir*, **2007**, 23, 3333-3337

Pseudomonas cepacia lipase

- About 10% of the enzyme added was immobilized on nanoparticles
- The same quantity of the native enzyme gives the same conversion

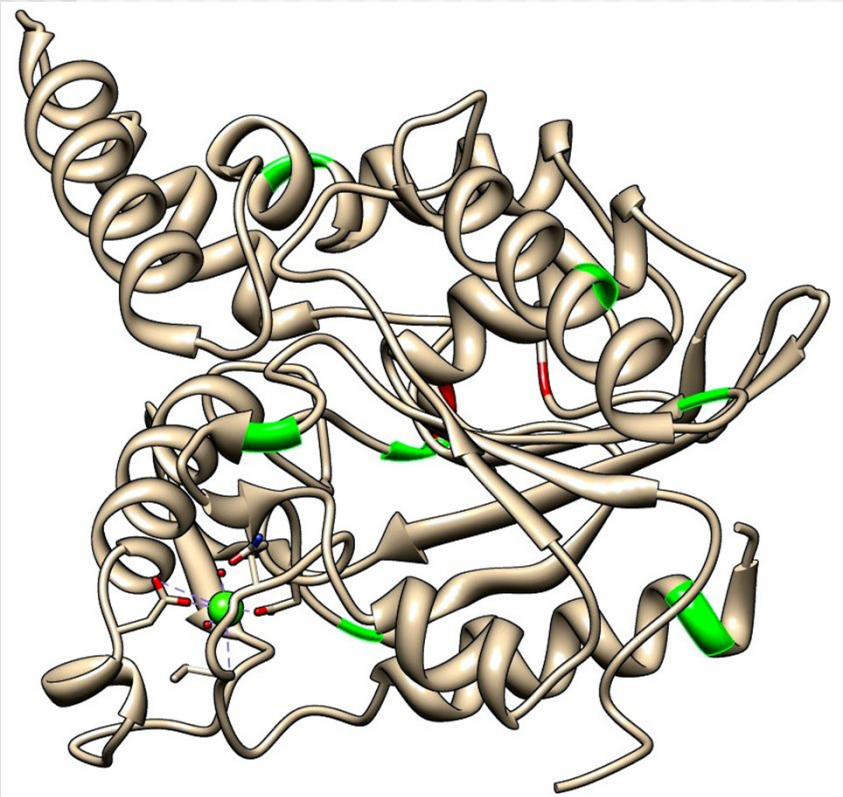
Kinetic resolution catalyzed by enzymes immobilized covalently

Enzyme	time [h]	conv. [%]	ee_s [%]	E^a
Ps.cepacia lipase – native enzyme	2	47	68	15.6
Ps. cepacia lipase on thiol-AuNPs	2	48	72	16.7
PPL – native enzyme	3	28	13	2.3
PPL on thiol-AuNPs	3	23	9	2.0
PLE – native enzyme	2	19	2	-
PLE on thiol-AuNPs	2	10	*rac*	-
Wheat Germ lipase – native enzyme	5	23	2	-
Wheat Germ lipase on thiol-AuNPs	5	19	4	1.5
C.antarctica lipase – native enzyme	2	22	*rac*	-
C.antarctica lipase on thiol-AuNPs	2	28	7	1.5

Pseudomonas cepacia lipase - 5 catalytic cycles

Entry	time [d]	Conv. [%]	ee_S [%]	E
native enzyme	2	47	68	15,6
enzyme on AuNP 1st cycle	2	48	71,5	16,7
2nd cycle	2	42	53,4	11,2
3rd cycle	2	37	40,4	8,0
4th cycle	2	25	16	3,3
5th cycle	2	22	7	1,9

The structure of *Pseudomonas cepacia* lipase From Protein Data Bank



Results obtained for *Pseudomonas cepacia* lipase were significantly better than for any other enzyme. There are seven lysine residues near the protein surface. Therefore immobilization through the amide bond was effective for this enzyme. The structure of enzyme is an explanation of the fact that only small nanoparticles were good base for immobilization. Small AuNPs had size similar to the enzyme and therefore they could connect through one or two lysine residues, which did not cause significant deformation of the lipase. Bigger nanoparticles could bind more lysine residues of one enzyme molecule and it could deactivate the lipase.

Conclusions

- Various enzymes were successfully immobilized on gold nanoparticles.
- Obtained bionanocatalysts were active and catalysed model reaction similarly to native enzymes.
- Size of nanoparticles is important and influence enantioselectivity.
- Only enzymes immobilized on small nanoparticles were active biocatalysts.
- Immobilized enzymes can be used in a few catalytic cycles.

H. Jedrzejewska, R. Ostaszewski, J. Mol. Cat. B: 90 **2013,** *90*, 12– 16

- Acknowledgments:
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