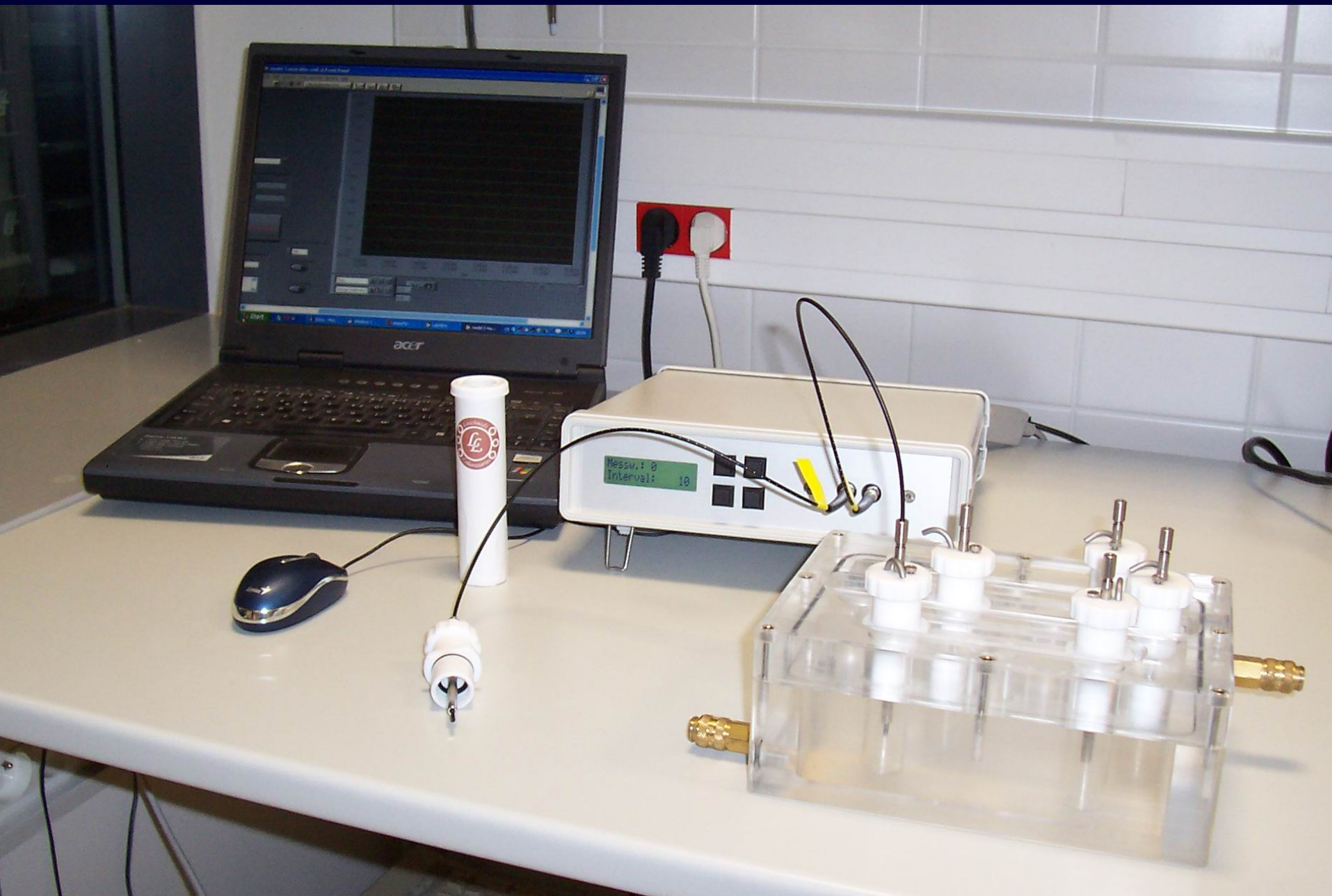
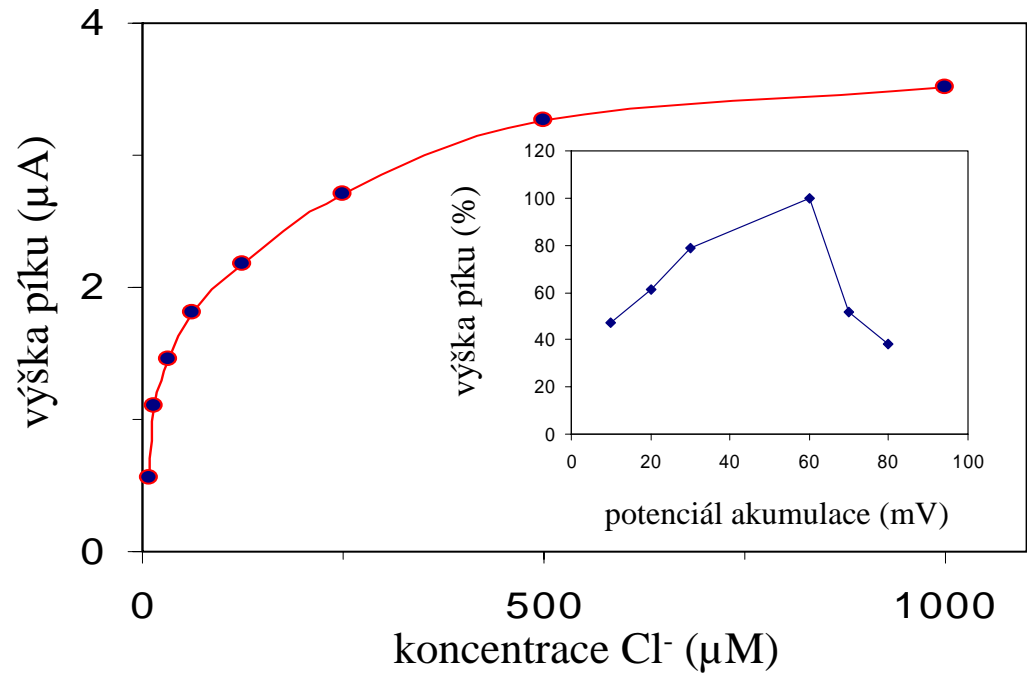
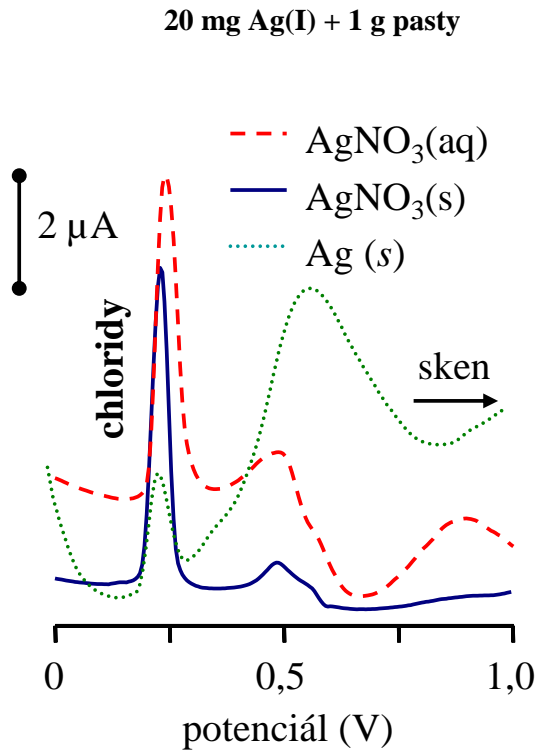


New TCI system installed in Brno



Electrochemical biosensor



Discovery of Stereoselective Halalkane dehalogenase

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Dick B. Janssen³, Yuji Nagata², Toshiya Senda², Jiri Damborsky¹

¹*Loschmidt Laboratories, Masaryk University, Czech Republic*

²*Tohoku University and AIST, Japan*

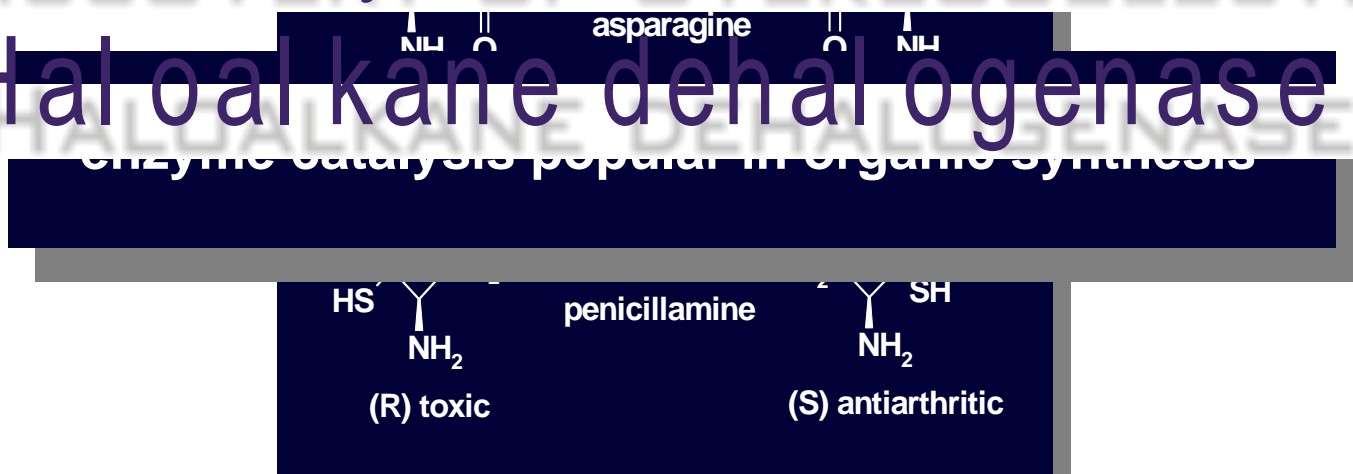
³*University of Groningen, The Netherlands*



Discovery of Stereoselective Halalkane dehalogenase

- ❑ biological effect of enantiomers often different

Discovery of Stereoselective Halalkane dehalogenase



- ❑ high enantiomeric **purity** required (e.e. >98%)
- ❑ **enantioselectivity** of catalyst crucial

Discovery of Stereoselective Halalkane dehalogenase

❑ **advantages** of enzyme catalysis

- **efficient** catalysis (10^8 - 10^{12})
- **selectivity**
- environmentally **acceptable**
- **compatibility** with each other
- catalyse **broad** spectrum of reaction

❑ **disadvantages** of enzyme catalysis

- **narrow** operation parameters
- highest activity in **water**
- **cofactor** requirement
- prone to **inhibitions**
- **allergies**

Discovery of Stereoselective Haloalkane dehalogenase

- ❑ Haloalkane dehalogenase **microbial enzymes**
- ❑ **first** haloalkane dehalogenase **isolated in 1985¹**
- ❑ α/β **hydrolases²**

DEGRADATORS



SYMBIOTIC BACTERIA



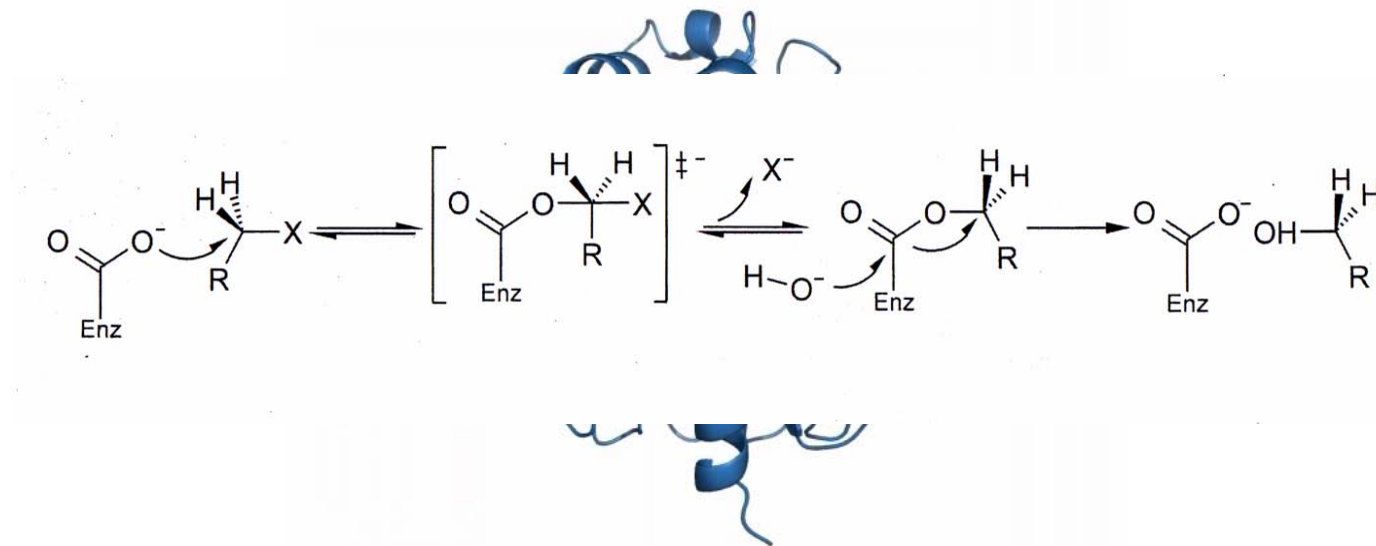
PATHOGENIC BACTERIA



¹ D. B. Janssen et al. / Applied and Environmental Microbiology (1985) 673-677
² Oms et al. / Protein Engineering (1992) 197-211

Discovery of Stereoselective Haloalkane dehalogenase

- ❑ Haloalkane dehalogenase **microbial enzymes**
- ❑ **first** haloalkane dehalogenase **isolated in 1985¹**
- ❑ **α/β hydrolases²**



³ S. Keuning et al. / *Journal of Bacteriology* (1985) 635-639

⁴ A. N. Kulakova et al. / *Microbiology* (1997) 109-115

² D. L. Ollis et al. / *Protein Engineering* (1992) 197-211

⁵ J. Marek et al. / *Biochemistry* (2000) 14082-14086

Discovery of Stereoselective Halalkane dehalogenase

- ❑ **alcohols valuable building blocks** in organic synthesis
- ❑ no cofactor needed, easy to prepare and store
- ❑ potential HADs as **biocatalyst when enantioselective**

production of optically active alcohols

- ❑ **first kinetic resolution** using DhIA and DhaA ⁷
 - chiral recognition low (*E*-value of max 9)
- ❑ enantioselective HAD major **challenge** of the field ⁸

⁷ R. J. Pieters et al. / *Tetrahedron Letters* 42 (2001) 469-471

⁸ D. B. Janssen / *Current Opinion in Chemical Biology* 8 (2004) 150-159

Discovery of Stereoselective Halalkane dehalogenase

□ kinetic resolution of chiral substrates

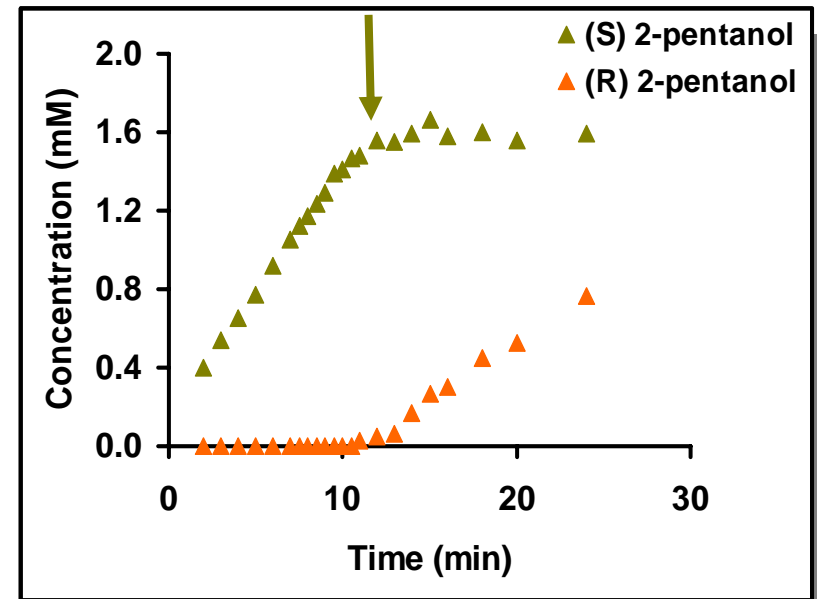
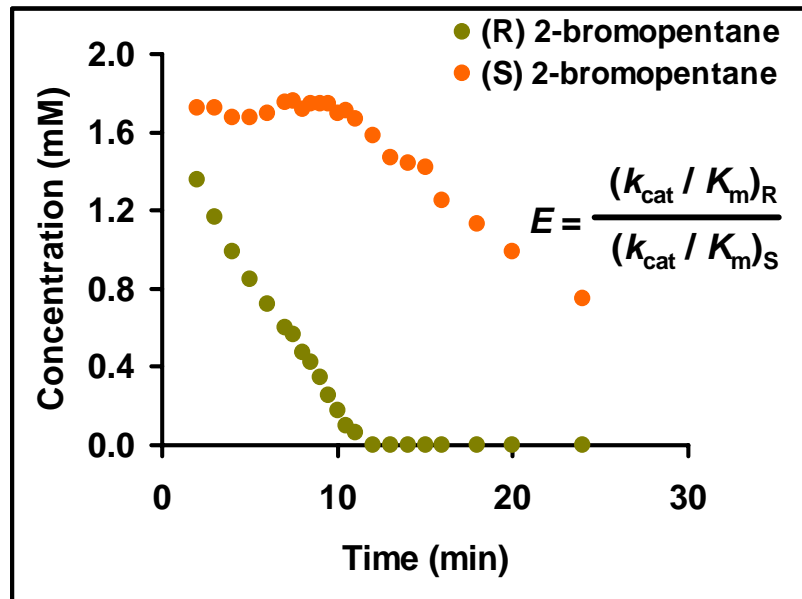
DhIA: *Xanthobacter autotrophicus*

DhaA: *Rhodococcus rhodochrous*

LinB: *Sphingomonas paucimobilis*

DbjA: *Bradyrhizobium japonicum*

e.e. 98% yield 47%, E=145



Discovery of Stereoselective Haloalkane dehalogenase

Substrate	<i>E</i> -value			
	DhIA	DhaA	LinB	DbjA
first enantioselective haloalkane dehalogenase				
PCT/CZ2005/000099				
2-bromohexane	n.a.	4	12	35
2-bromoheptane	2	3	3	28
methyl 3-bromo-2-methylpropionate	n.d.	5	3	20
ethyl 2,3-dichloropropionate	n.d.	n.d.	5	32
ethyl 3-bromo-2-methylpropionate	n.d.	4	1	20
ethyl 2-bromopropionate	n.d.	72	97	>200

n.d. activity not detected
n.a. not analysed

Discovery of Stereoselective Halooalkane dehalogenase

- ❑ **DbjA** isolated from *Bradyrhizobium japonicum*⁸
- ❑ nitrogen-fixing **symbiotic bacterium**



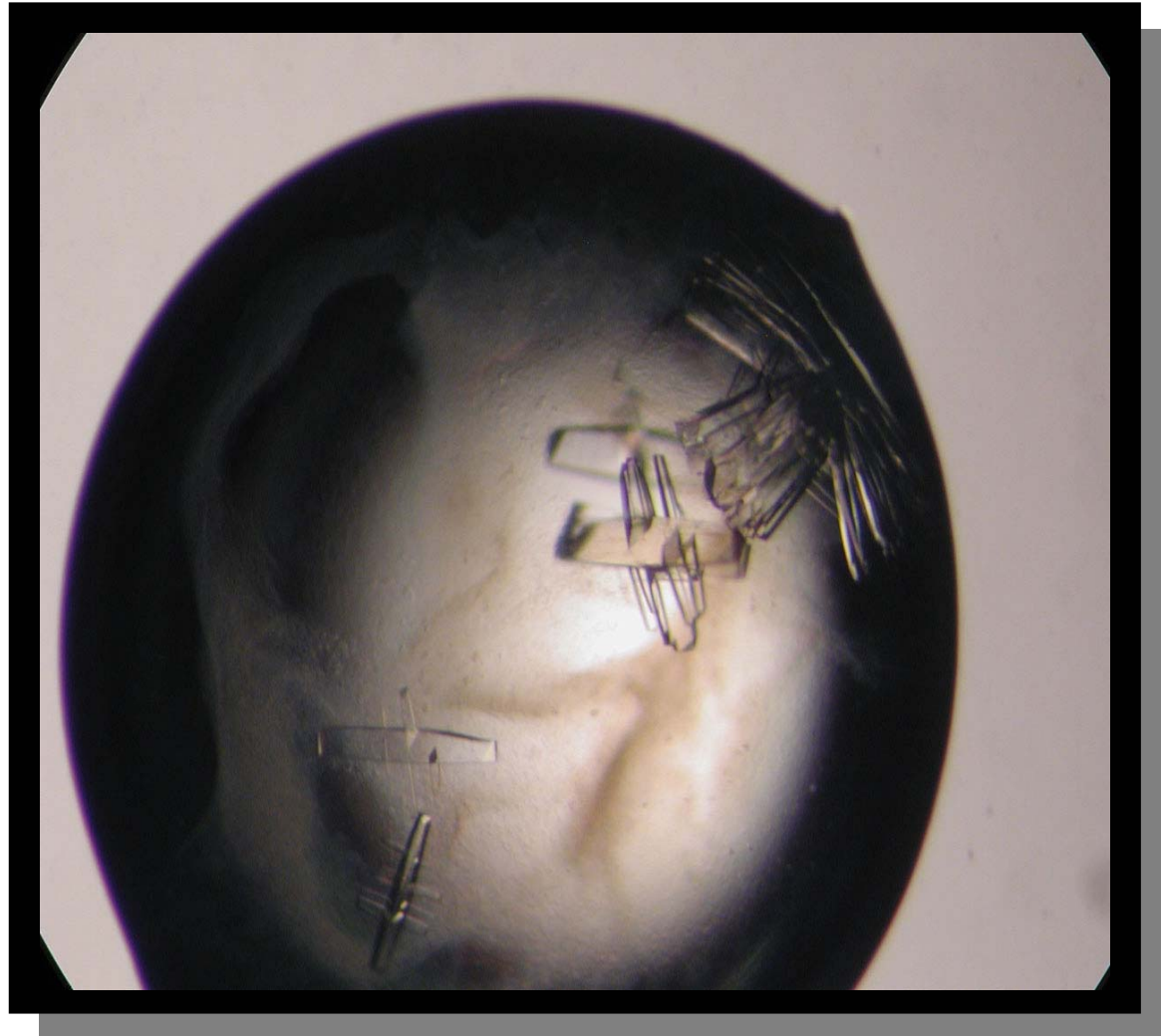
Discovery of Stereoselective Halocane dehalogenase

DbjA
→

```
LVITNTAVFPMPMSKKMP-----WQIALGRDWR
LVITNTAAFPLPPEKPMP-----WQIAMGRHWR
IVVLNTAAFLSHPVP-----LRIAAGKLPR
LVVLNTAAFRSTHIP-----LRISLCRAPL
VVLGNTWFWPADTLAMKAF-----SR-VMSSPPV
LVITNTAAFPLPA-EKPMP-----WQIAMGRHWR
VAFFEPVLRNIDSVDLSP-----EFVTRRAKLRQPG
VVHAESVVDVIESWDEWP-----DIEEDIALIKSEE
IVHAESVVDVIESWDEWP-----DIEEDIALIKSE-
VAFMEALVPPALPMPSEYAMGP-----QLGPLFRDLRTAD
LAFMEFIR-PMPTWQDFIHTEVAEEQDHAAARAVFRKFRTPG
IACMEFIR-PIPTWDEWP-----EFARETFQAFRTAD
IACMEFIR-PIPTWDEWP-----EFARETFQAFRTAD
ITYMEAIVGPIESWEDWP-----ENARNIFQGFRSE-
IAYMEAVTMPLWADFP-----EQDRDLFQAFRSQ-
IAYMEGIVRPFANWGEWS-----AAATSVFQGFRSD-
IAFTETIVKPKMA-WAEFP-----EGGRELFRRAIKTRG
VAFFEPVLRNIDSVDLSP-----EFVTRRAKLRQPG
IACMEFIR-PIPTWDEWP-----EFARETFQAFRTAD
IVHMETVSVPMWDDFP-----DEVAQMFRGLRSP-
IAYMEAIAMPIEWADFP-----EQDRDLFQAFRSQ-
IAFMEAIVTPMTWADWP-----PAVRGVFQGFRSP-
IAFMEAIVTPMTWADWP-----PAVRGVFQGFRSP-
LAFFESHIRPTTDWDMLS-----LPVQQLATLLHRPG
IVSQNGNAY----IEGFS-----DEWGTWESYWR
LVTQNGNLY----REGLAR-----PFWAPFEAYWA
LIVQNGNAY----DEGLK-----KFDWPIKQYWA
LIVQNGNAY----EEGLR-----EFWEPIKAYWQ
LIIQNGNAY----EEGIDN-----QFWVPVKAFWN
IVTQNGNGY----TDGFVK-----DFWDGLFAYTD
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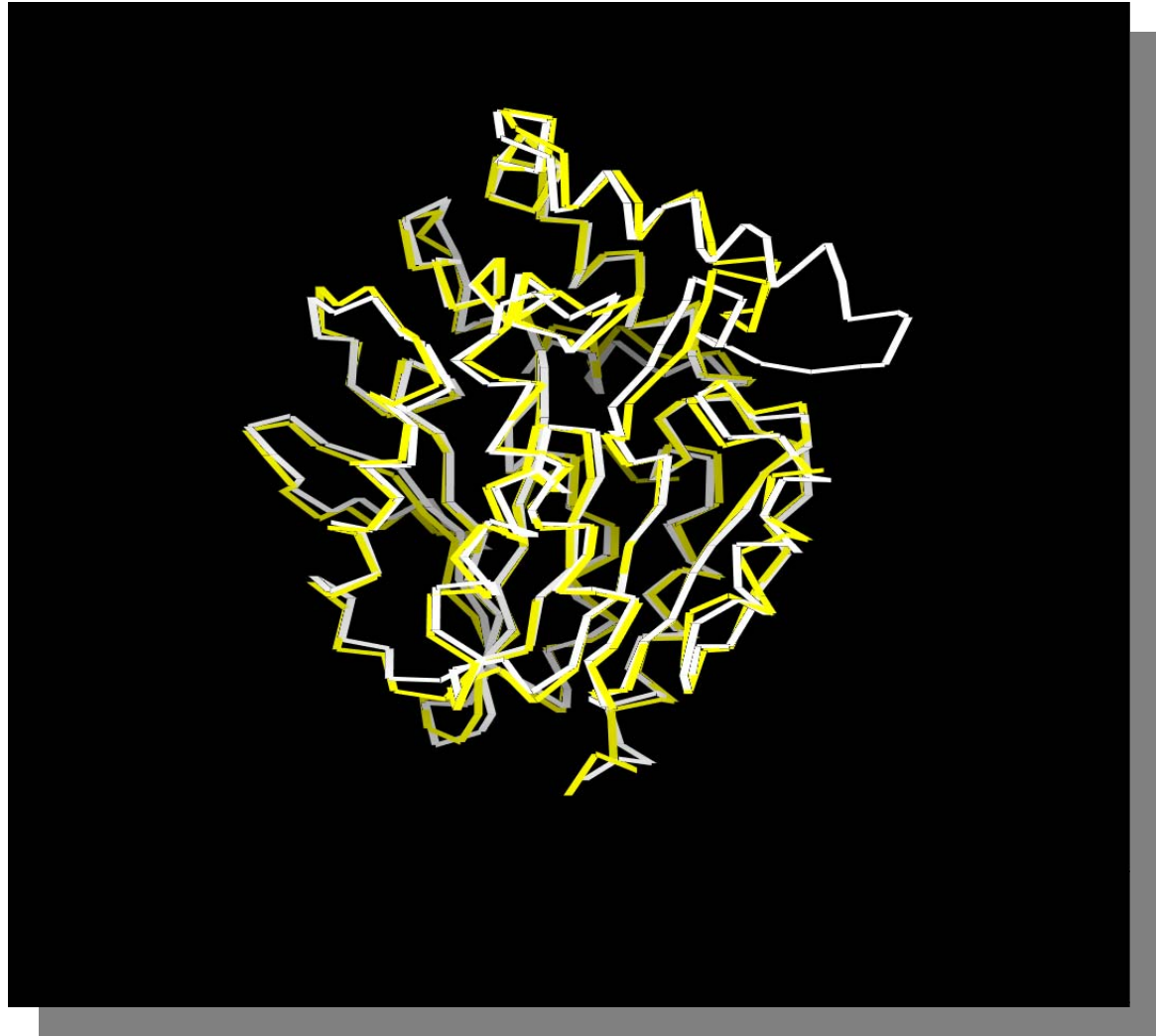
Discovery of Stereoselective Halalkane dehalogenase

DbjA wild type **crystals**



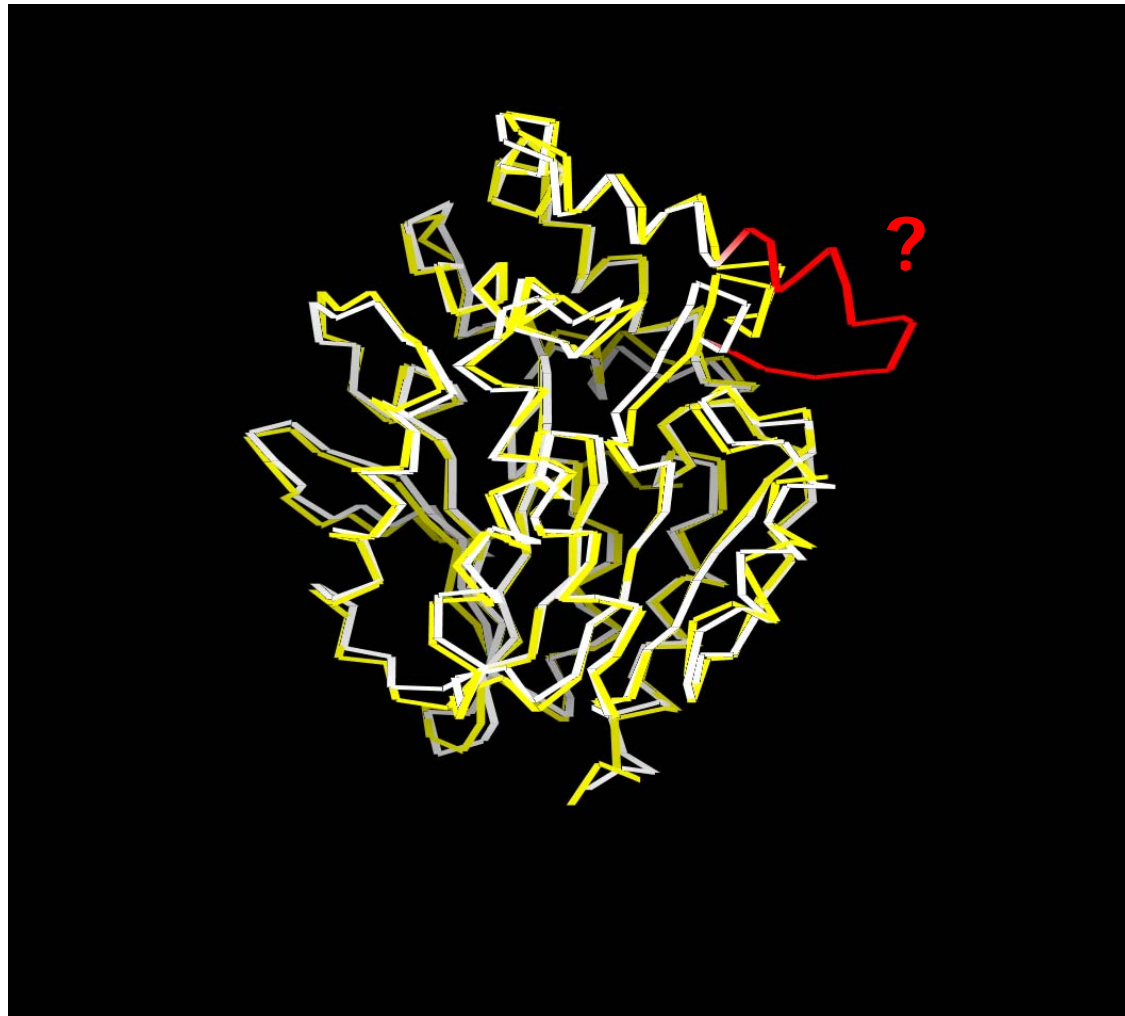
Discovery of Stereoselective Halalkane dehalogenase

□ DbjA X-ray structure



Discovery of Stereoselective Halalkane dehalogenase

- study of DbjA **extra loop effect**



<i>E</i> -value	
DbjA	DbjA delta
	1
5	36

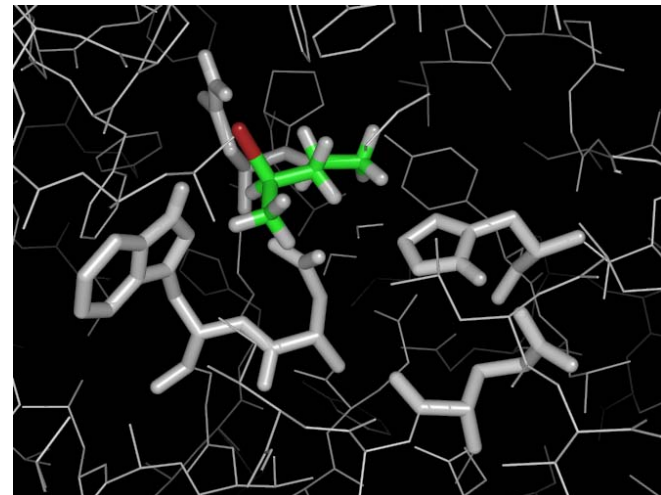
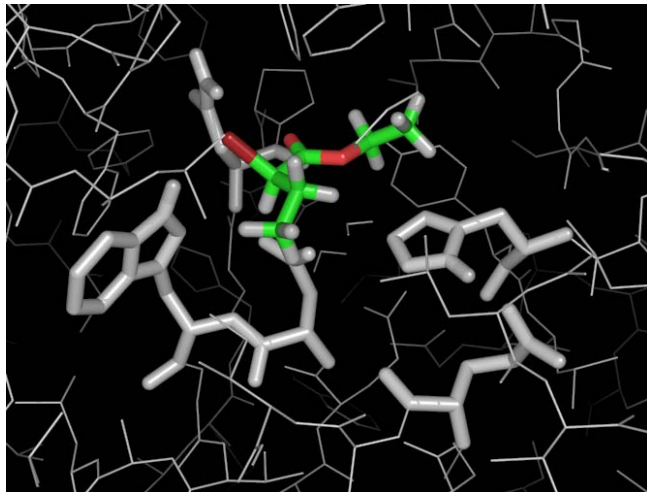
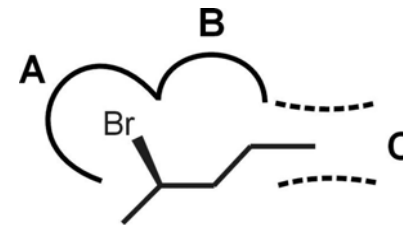
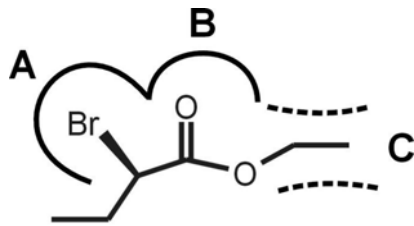
Discovery of Stereoselective Halalkane dehalogenase

Substrate	E-value			
2-bromohexane	n.a.	4	12	35
2-bromoheptane	2	3	3	28
methyl 3-bromo-2-methylpropionate	n.d.	5	3	20
ethyl 2,3-dichloropropionate	n.d.	n.d.	5	32
ethyl 3-bromo-2-methylpropionate	n.d.	4	1	20
ethyl 2-bromopropionate	n.d.	85	97	>200
ethyl 2-bromobutyrate	n.d.	>200	>200	>200
methyl 2-bromopropionate	n.d.	>200	52	>200

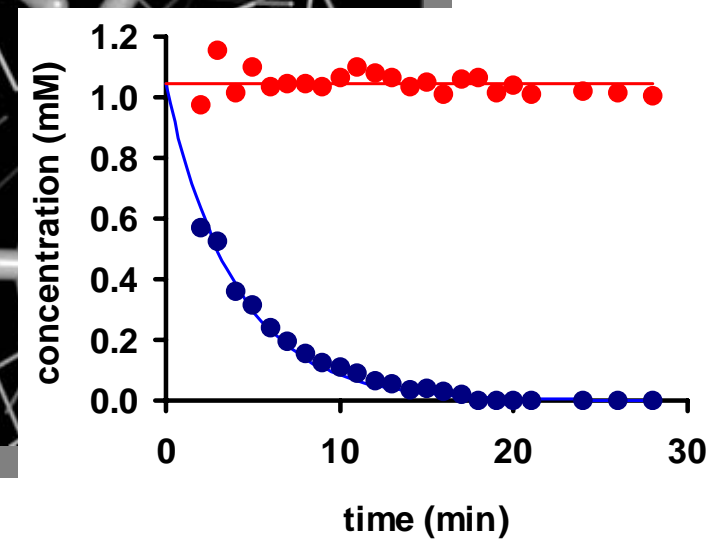
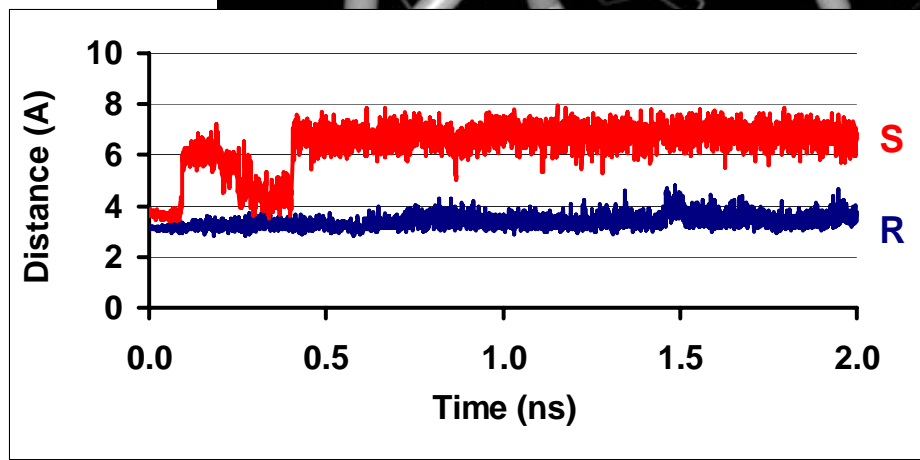
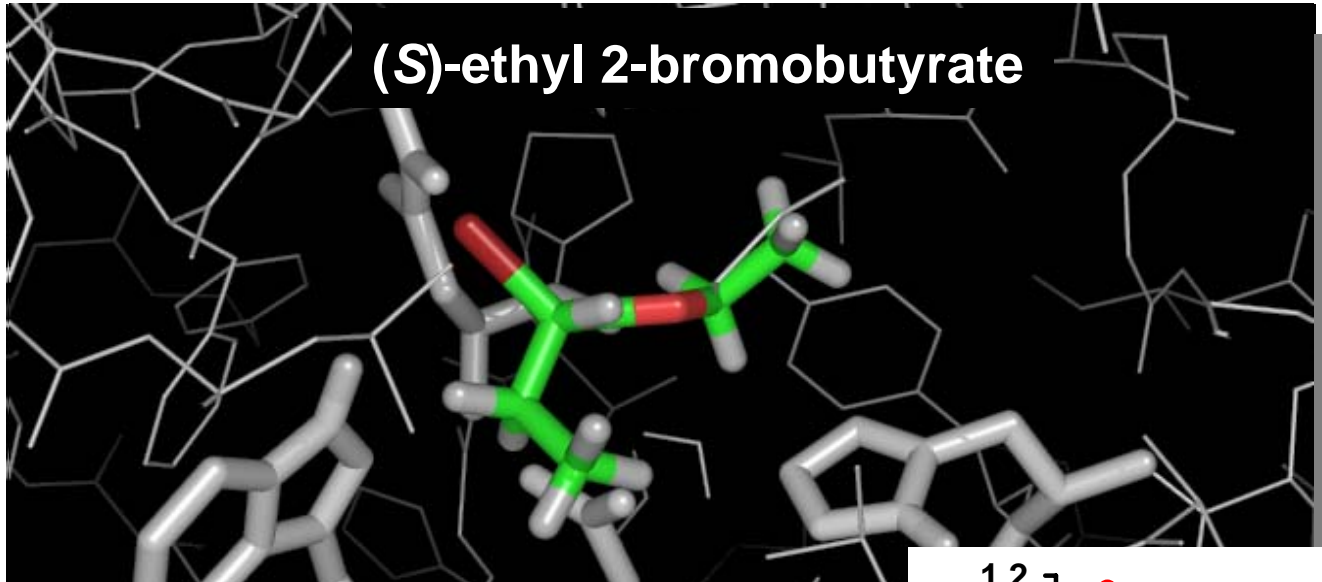
n.d. activity not detected
n.a. not analysed

Discovery of Stereoselective Halalkane dehalogenase

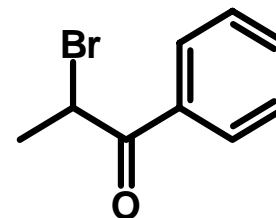
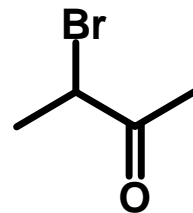
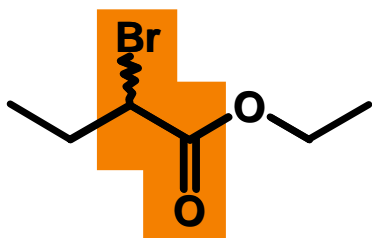
two mechanisms of HADs enantioselectivity



Discovery of Stereoselective Haloalkane Dehalogenase



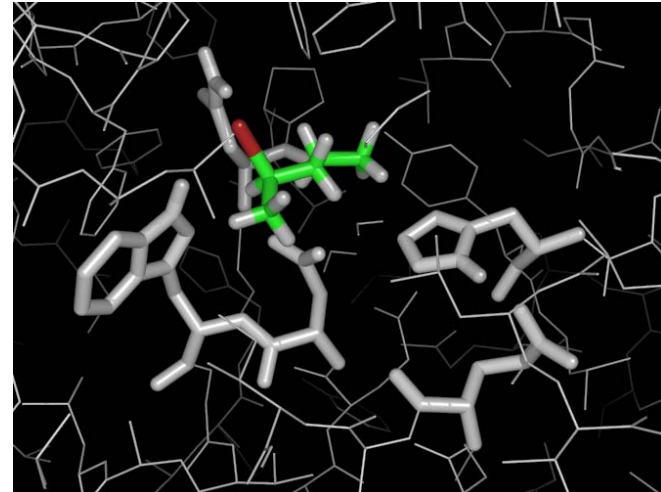
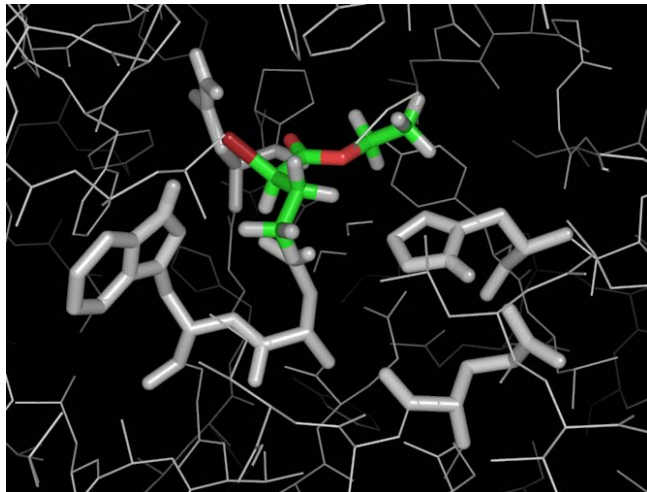
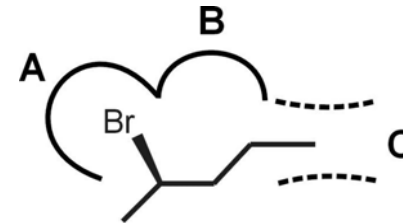
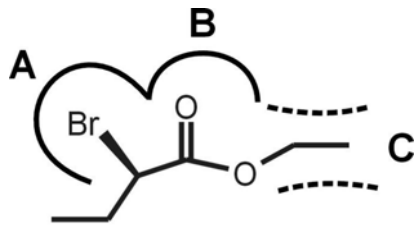
Discovery of Stereoselective Halalkane dehalogenase



Substrate	<i>E</i> -value		
	DhaA	LinB	DbjA
3-bromo-2-butanone	>200	3	>200
2-bromopropiophenone	>200	13	>200
ethyl 2-bromobutyrate	>200	>200	>200
ethyl 2-bromopropionate	72	97	>200
methyl 2-bromopropionate	>200	52	>200

Discovery of Stereoselective Halalkane dehalogenase

two mechanisms of HADs enantioselectivity



ENANTIOSELECTIVITY RECONSTRUCTION

DhaA M S E I G T G F P F - D P H Y V E V L G E R M H Y V D V G P R D G T P V L F L H

DhaA G N P T S S Y L W R N I I P H V A P S H R C I A P D L I G M G K S D K P D L D Y

DhaA F F D D H V R **ENANTIOSELECTIVITY** G F H W A K R N

DhaA P E R V K G I A **RECONSTRUCTION** - - - P E F A R E T

DhaA F Q A F R T A D V G R E L I I D Q N A F I E G A L P K C V V R P L T E V E M D H

DhaA Y R E P F L K P V D R E P L W R F P N E L P I A G E P A N I V A L V E A Y M N W

DhaA L H Q S P V P K L L F W G T P G V L I P P A E A A R L A E S L P N C K T V D I G

DhaA P G L H Y L Q E D N P D L I G S E I A R W L P A L

ENANTIOSELECTIVITY

RECONSTRUCTION

DhaA M S E I G T G F P F - D P H Y V E V L G E R M H Y V D V G P R D G T P V L F L H
DbjA - - - - M S K P I E I E I R R A P V L G S S M A Y R E T G A Q D A P V V L F L H

DhaA G N P T S S Y L W R N I I P H V A P S H R C I A P D L I G M G K S D K P D L D Y
DbjA G N P T S S H I W R N I L P L V S P V A H C I A P D L I G F G Q S G K P D I A Y

DhaA F F D D H V R Y L D A F I E A L G L E E V V L V I H D W G S A L G F H W A K R N
DbjA R F F D H V R Y L D A F I E Q R G V T S A Y L V A Q D W G T A L A F H L A A R R

DhaA P E R V K G I A C M E F I R P I P T W D E W - - - - - - - - - P E F A R E T
DbjA P D F V R G L A F M E F I R P M P T W Q D F H H T E V A E E Q D H A E A A R A V

DhaA F Q A F R T A D V G R E L I I D Q N A F I E G A L P K C V V R P L T E V E M D H
DbjA F R K F R T P G E G E A M I L E A N A F V E R V L P G G I V R K L G D E E M A P

DhaA Y R E P F L K P V D R E P L W R F P N E L P I A G E P A N I V A L V E A Y M N W
DbjA Y R T P F P T P E S R R P V L A F P R E L P I A G E P A D V Y E A L Q S A H A A

DhaA L H Q S P V P K L L F W G T P G V L I P P A E A A R L A E S L P N C K T V D I G
DbjA L A A S S Y P K L L F T G E P G A L V S P E F A E R F A A S L T R C A L I R L G

DhaA P G L H Y L Q E D N P D L I G S E I A R W L P A L
DbjA A G L H Y L Q E D H A D A I G R S V A G W I A G I E A V R P Q L A A

ENANTIOSELECTIVITY

RECONSTRUCTION

DhaA M S E I G T G F P F - D P H Y V E V L G E R M H Y V D V G P R D G T P V L F L H
DbjA - - - - M S K P I E I E I R R A P V L G S S M A Y R E T G A Q D A P V V L F L H

DhaA G **N** P T S S Y L W R N I I P H V A P S H R C I A P D L I G M G K S D K P D L D Y
DbjA G **N** P T S S H I W R N I L P L V S P V A H C I A P D L I G F G Q S G K P D I A Y

DhaA F F D D H V R Y L D A F I E A L G L E E V V L V I H **DW** G S A L G F H W A K R N
DbjA R F F D H V R Y L D A F I E Q R G V T S A Y L V A Q D W G T A L A F H L A A R R

DhaA P E R V K G I A C M E F **T** R P I P T W D E **W** - - - - - P E F **A** R E I
DbjA P D F V R G L A F M E F T R P M P T W Q D F H H T E V A E E Q D H A E A A R A V

DhaA **F** Q A F R T A D V G R E L I I D Q N A **F** I E **GA** L P **KC** V V R P L T E V E M D H
DbjA F R K F R T P G E G E A M I L E A N A F V E R V L P G G I V R K L G D E E M A P

DhaA Y R E P F L K P V D R E P L W R **FP** N E **L** P I A G E P A N I V A L V E A Y M N W
DbjA Y R T P F P T P E S R R P V L A F P R E L P I A G E P A D V Y E A L Q S A H A A

DhaA L H Q S P V P K L L F W G T P G **VL** I P P A E A A R L A E S L P N C K I V D I G
DbjA L A A S S Y P K L L F T G E P G A L V S P E F A E R F A A S L T R C A L I R L G

DhaA P G L **HY** L Q E D N P D L I G S E I A R W L P A L
DbjA A G L H Y L Q E D H A D A I G R S V A G W I A G I E A V R P Q L A A

ENANTIOSELECTIVITY

RECONSTRUCTION

DhaA M S E I G T G F P F - D P H Y V E V L G E R M H Y V D V G P R D G T P V L F L H
DbjA - - - - M S K P I E I E I R R A P V L G S S M A Y R E T G A Q D A P V V L F L H

DhaA G **N** P T S S Y L W R N I I P H V A P S H R C I A P D L I G M G K S D K P D L D Y
DbjA G **N** P T S S H I W R N I L P L V S P V A H C I A P D L I G F G Q S G K P D I A Y

DhaA F F D D H V R Y L D A F I E A L G L E E V V L V I H **DW** G S A L G F H W A K R N
DbjA R F F D H V R Y L D A F I E Q R G V T S A Y L V A Q **DW** G T A L A F H L A A R R

DhaA P E R V K G I A C M E F **T** R P I P T W D E **W** - - - - - P E F **A** R E I
DbjA P D F V R G L A F M E F **T** R P M P T W Q D **F** H H T E V A E E Q D H A E A **A** R A V

DhaA **F** Q A F R T A D V G R E L I I D Q N A **F** I E **GA** L P **KC** V V R P L T E V E M D H
DbjA **F** R K F R T P G E G E A M I L E A N A **F** V E R V L P G G I V R K L G D E E M A P

DhaA Y R E P F L K P V D R E P L W R **FP** N E **L** P I A G E P A N I V A L V E A Y M N W
DbjA Y R T P F P T P E S R R P V L A **FP** R E **L** P I A G E P A D V Y E A L Q S A H A A

DhaA L H Q S P V P K L L F W G T P G **VL** I P P A E A A R L A E S L P N C K T V D I G
DbjA L A A S S Y P K L L F T G E P G A **L** V S P E F A E R F A A S L T R C A L I R L G

DhaA P G L **HY** L Q E D N P D L I G S E I A R W L P A L
DbjA A G L **HY** L Q E D H A D A I G R S V A G W I A G I E A V R P Q L A A

ENANTIOSELECTIVITY

RECONSTRUCTION

DhaA M S E I G T G F P F - D P H Y V E V L G E R M H Y V D V G P R D G T P V L F L H
DbjA - - - - M S K P I E I E I R R A P V L G S S M A Y R E T G A Q D A P V V L F L H

DhaA G **N** P T S S Y L W R N I I P H V A P S H R C I A P D L I G M G K S D K P D L D Y
DbjA G **N** P T S S H I W R N I L P L V S P V A H C I A P D L I G F G Q S G K P D I A Y

DhaA F F D D H V R Y L D A F I E A L G L E E V V L V I H **DW** G S A L G F H W A K R N
DbjA R F F D H V R Y L D A F I E Q R G V T S A Y L V A Q **DW** G T A L A F H L A A R R

DhaA P E R V K G I A C M E F **T** R P I P T W D E **W** - - - - - P E F **A** R E I
DbjA P D F V R G L A F M E F **T** R P M P T W Q D **F** H H T E V A E E Q D H A E A **A** R A V

DhaA **F** Q A F R T A D V G R E L I I D Q N A **F** I E **GA** L P **KC** V V R P L T E V E M D H
DbjA **F** R K F R T P G E G E A M I L E A N A **F** V E **RV** L P **GG** I V R K L G D E E M A P

DhaA Y R E P F L K P V D R E P L W R **FP** N E **L** P I A G E P A N I V A L V E A Y M N W
DbjA Y R T P F P T P E S R R P V L A **FP** R E **L** P I A G E P A D V Y E A L Q S A H A A

DhaA L H Q S P V P K L L F W G T P G **VL** I P P A E A A R L A E S L P N C K T V D I G
DbjA L A A S S Y P K L L F T G E P G **AL** V S P E F A E R F A A S L T R C A L I R L G

DhaA P G L **HY** L Q E D N P D L I G S E I A R W L P A L
DbjA A G L **HY** L Q E D H A D A I G R S V A G W I A G I E A V R P Q L A A

ENANTIOSELECTIVITY

RECONSTRUCTION

DhaA M S E I G T G F P F - D P H Y V E V L G E R M H Y V D V G P R D G T P V L F L H
DbjA - - - - M S K P I E I E I R R A P V L G S S M A Y R E T G A Q D A P V V L F L H

DhaA G **N** P T S S Y L W R N I I P H V A P S H R C I A P D L I G M G K S D K P D L D Y
DbjA G **N** P T S S H I W R N I L P L V S P V A H C I A P D L I G F G Q S G K P D I A Y

DhaA F F D D H V R Y L D A F I E A L G L E E V V L V I H **DW** G S A L G F H W A K R N
DbjA R F F D H V R Y L D A F I E Q R G V T S A Y L V A Q **DW** G T A L A F H L A A R R

DhaA P E R V K G I A C M E F **T** R P I P T W D E **W** - - - - - P E F **A** R E I
DbjA P D F V R G L A F M E F **T** R P M P T W Q D **F** **H H T E V A E E Q D H** A E A **A** R A V

DhaA **F** Q A F R T A D V G R E L I I D Q N A **F** I E **GA** L P **KC** V V R P L T E V E M D H
DbjA **F** R K F R T P G E G E A M I L E A N A **F** V E **RV** L P **GG** I V R K L G D E E M A P

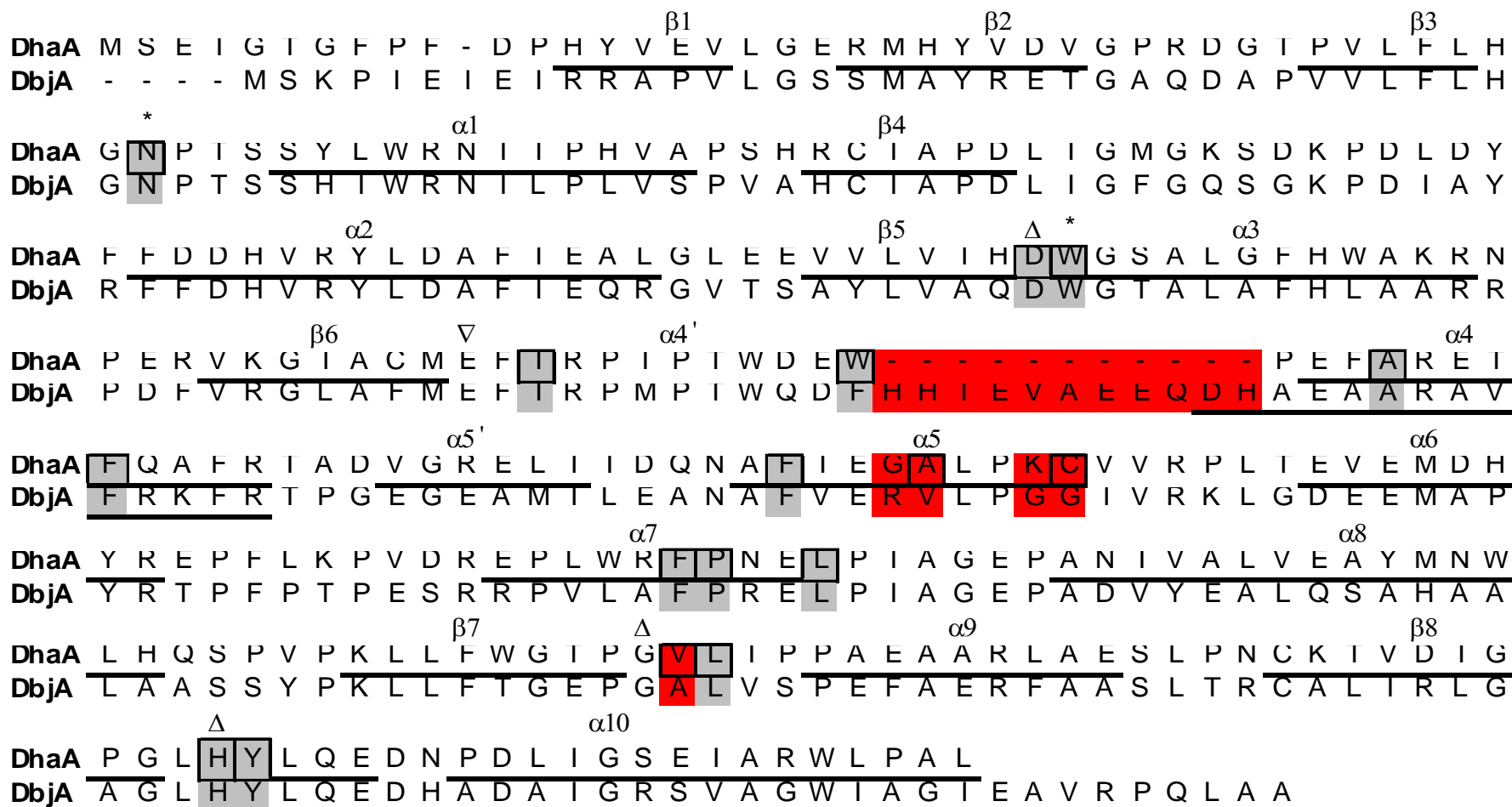
DhaA Y R E P F L K P V D R E P L W R **FP** N E **L** P I A G E P A N I V A L V E A Y M N W
DbjA Y R T P F P T P E S R R P V L A **FP** R E **L** P I A G E P A D V Y E A L Q S A H A A

DhaA L H Q S P V P K L L F W G T P G **VL** I P P A E A A R L A E S L P N C K T V D I G
DbjA L A A S S Y P K L L F T G E P G **AL** V S P E F A E R F A A S L T R C A L I R L G

DhaA P G L **HY** L Q E D N P D L I G S E I A R W L P A L
DbjA A G L **HY** L Q E D H A D A I G R S V A G W I A G I E A V R P Q L A A

ENANTIOSELECTIVITY

RECONSTRUCTION



ENANTIOSELECTIVITY

RECONSTRUCTION

									$\beta 1$								$\beta 2$							$\beta 3$																						
DhaA	M	S	E	I	G	I	G	F	P	F	-	D	P	H	Y	V	E	V	L	G	E	R	M	H	Y	V	D	V	G	P	R	D	G	I	P	V	L	F	L	H						
DbjA	-	-	-	-	M	S	K	P	I	E	I	E	I	R	R	A	P	V	L	G	S	S	M	A	Y	R	E	T	G	A	Q	D	A	P	V	V	L	F	L	H						
DhaA	G	N	P	T	S	S	Y	L	W	R	N	I	I	P	H	V	A	P	S	H	R	C	I	A	P	D	L	I	G	M	G	K	S	D	K	P	D	L	D	Y						
DbjA	G	N	P	T	S	S	H	T	W	R	N	T	L	P	L	V	S	P	V	A	H	C	T	A	P	D	L	I	G	F	G	Q	S	G	K	P	D	I	A	Y						
		*																																												
DhaA	F	F	D	D	H	V	R	Y	L	D	A	F	I	E	A	L	G	L	E	E	V	V	L	V	I	H	D	W	G	S	A	L	G	F	H	W	A	K	R	N						
DbjA	R	F	F	D	H	V	R	Y	L	D	A	F	T	E	Q	R	G	V	T	S	A	Y	L	V	A	Q	D	W	G	T	A	L	A	F	H	L	A	A	R	R						
DhaA	P	E	R	V	K	G	I	A	C	M	E	F	T	R	P	I	P	I	W	D	E	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	E	F	A	R	E	I		
DbjA	P	D	F	V	R	G	L	A	F	M	E	F	T	R	P	M	P	I	W	Q	D	F	H	H	I	E	V	A	E	E	Q	D	H	A	E	A	A	R	A	V						
DhaA	F	Q	A	F	R	T	A	D	V	G	R	E	L	I	I	D	Q	N	A	F	I	E	G	A	L	P	K	C	V	V	R	P	L	T	E	V	E	M	D	H						
DbjA	F	R	K	F	R	T	P	G	E	G	E	A	M	T	L	E	A	N	A	F	V	E	R	V	L	P	G	G	I	V	R	K	L	G	D	E	E	M	A	P						
DhaA	Y	R	E	P	F	L	K	P	V	D	R	E	P	L	W	R	F	P	N	E	L	P	I	A	G	E	P	A	N	I	V	A	L	V	E	A	Y	M	N	W						
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DhaA	L	H	Q	S	P	V	P	K	L	L	F	W	G	T	P	G	V	L	I	P	P	A	E	A	R	L	A	E	S	L	P	N	C	K	I	V	D	I	G							
DbjA	L	A	A	S	S	Y	P	K	L	L	F	T	G	E	P	G	A	L	V	S	P	E	F	A	E	R	F	A	A	S	L	T	R	C	A	L	T	R	L	G						
DhaA	P	G	L	H	Y	L	Q	E	D	N	P	D	L	I	G	S	E	I	A	R	W	L	P	A	L																					
DbjA	A	G	L	H	Y	L	Q	E	D	H	A	D	A	I	G	R	S	V	A	G	W	I	A	G	T	E	A	V	R	P	Q	L	A	A												

⁹ Stratagene, La Jolla, USA

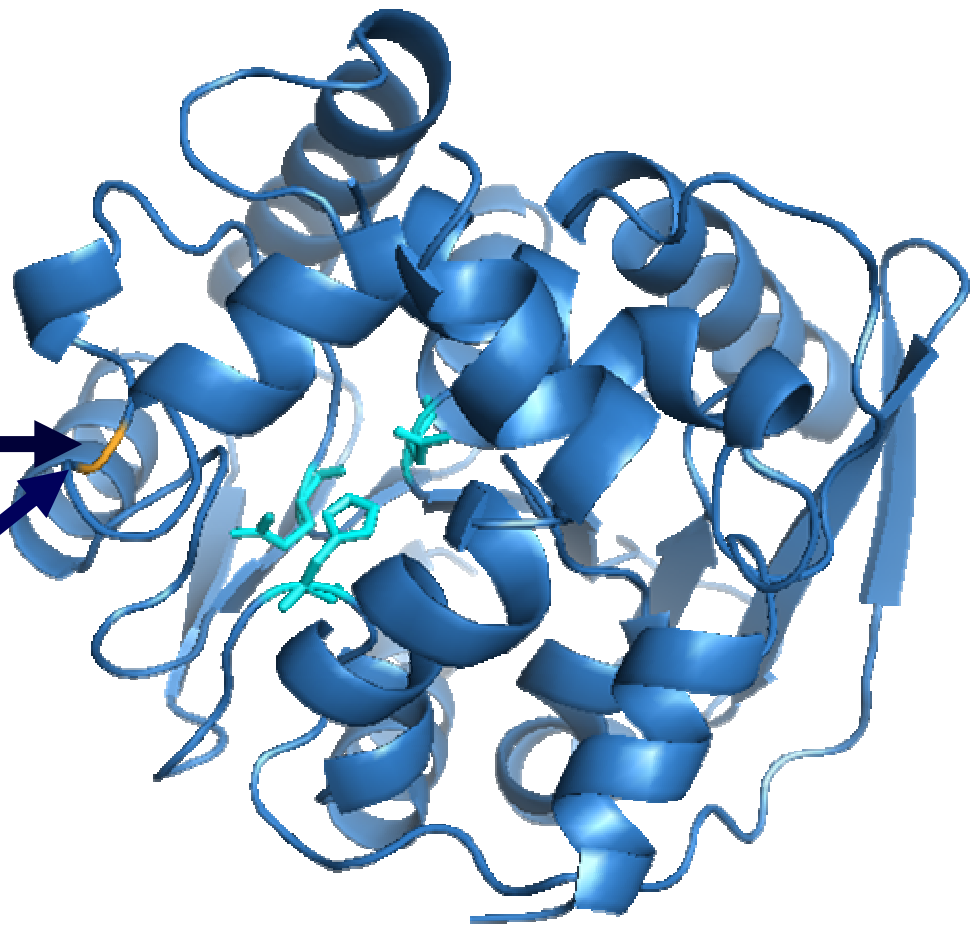
**ENANTIOSELECTIVITY
RECONSTRUCTION**

M1

**insertion
of loop**

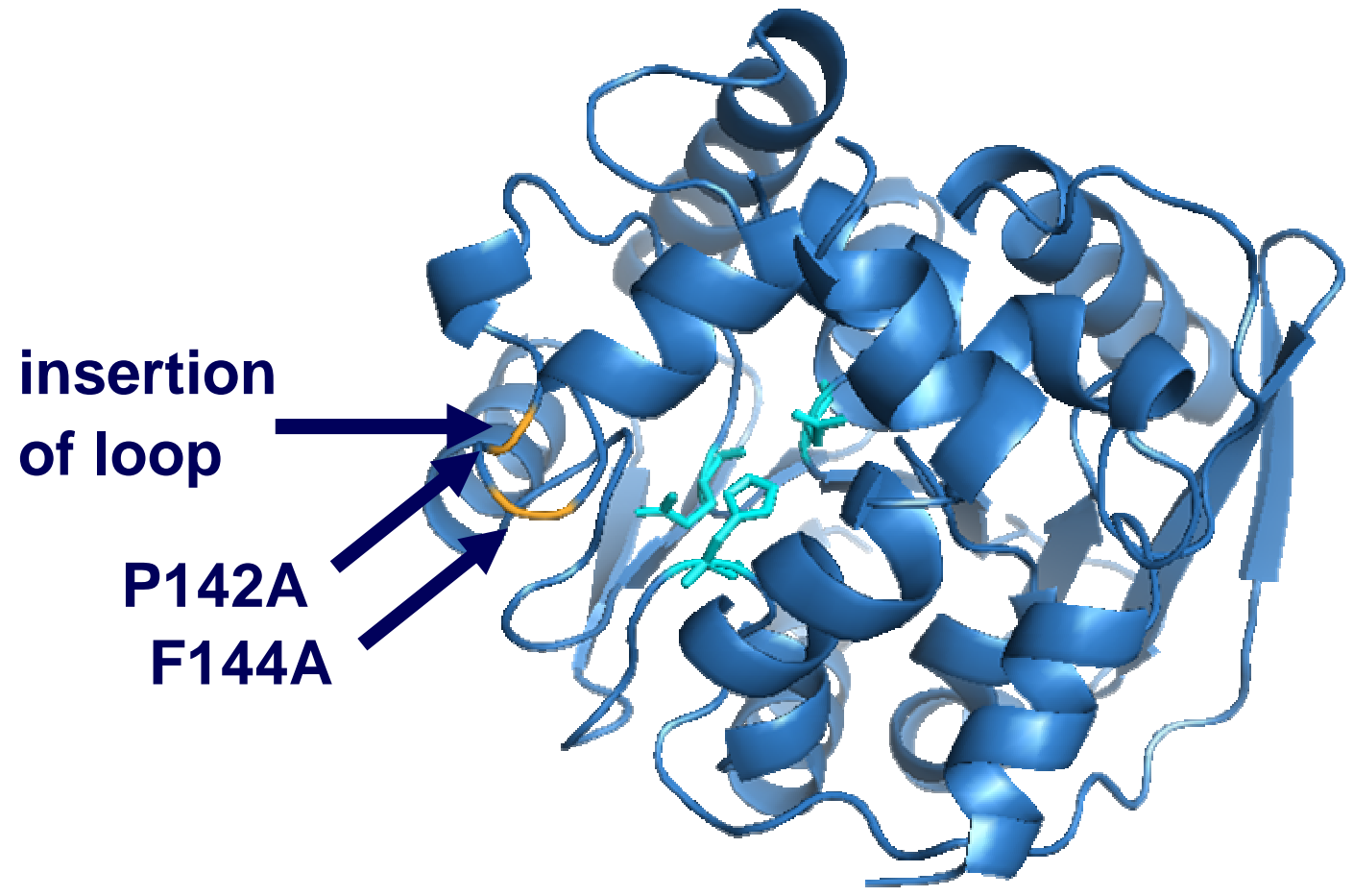


P142A



ENANTIOSELECTIVITY
RECONSTRUCTION

M2

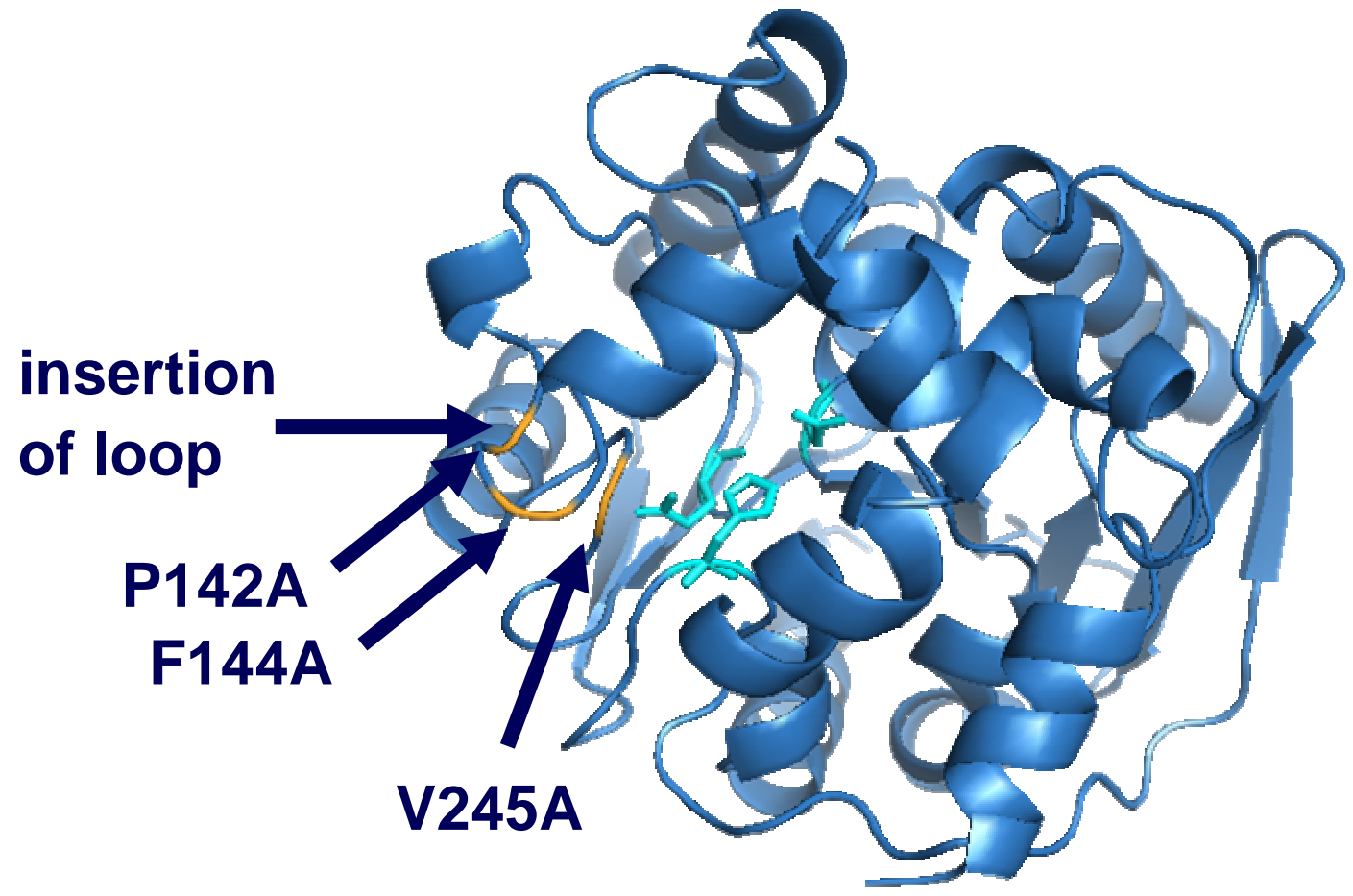


insertion
of loop

P142A
F144A

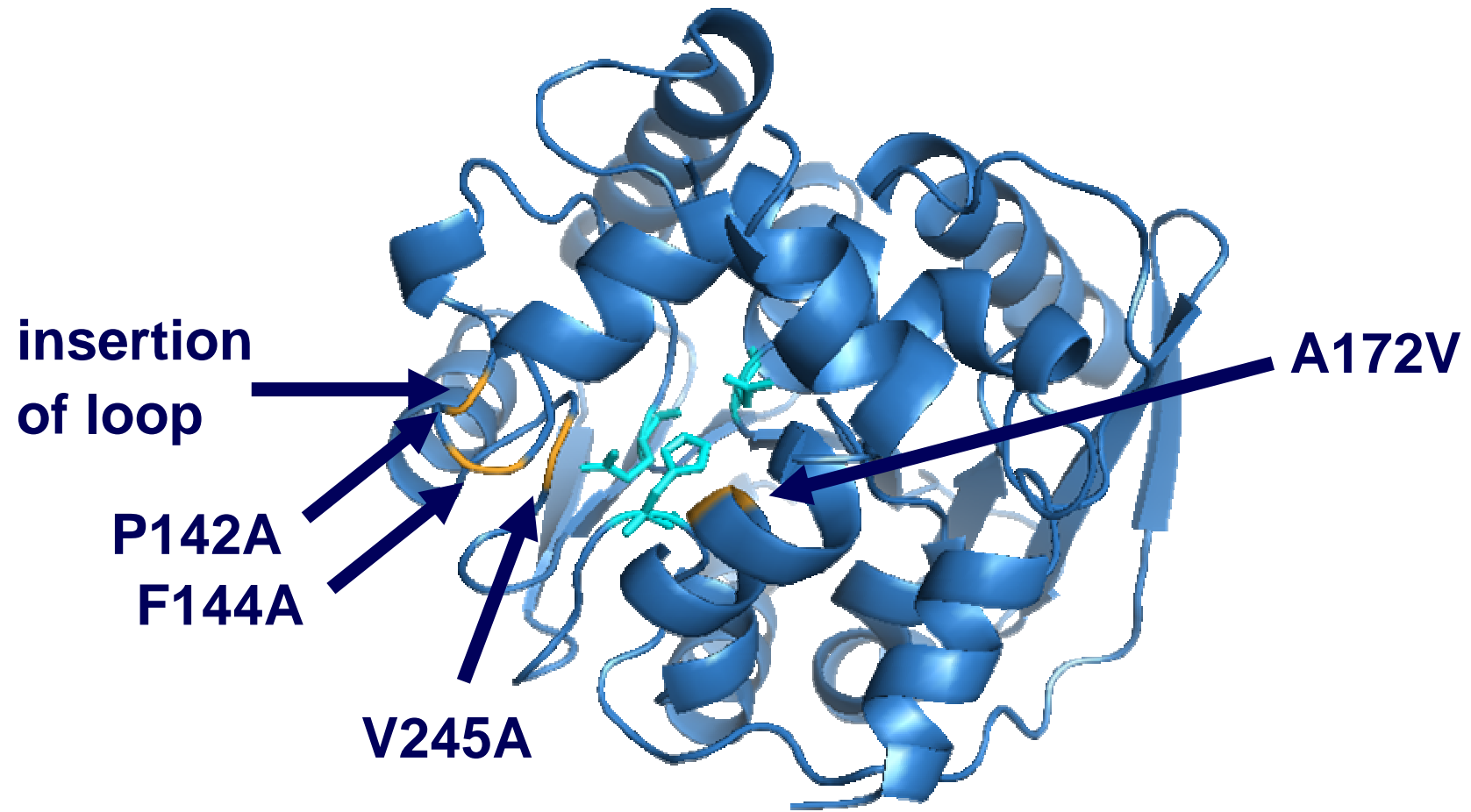
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RECONSTRUCTION**

M3



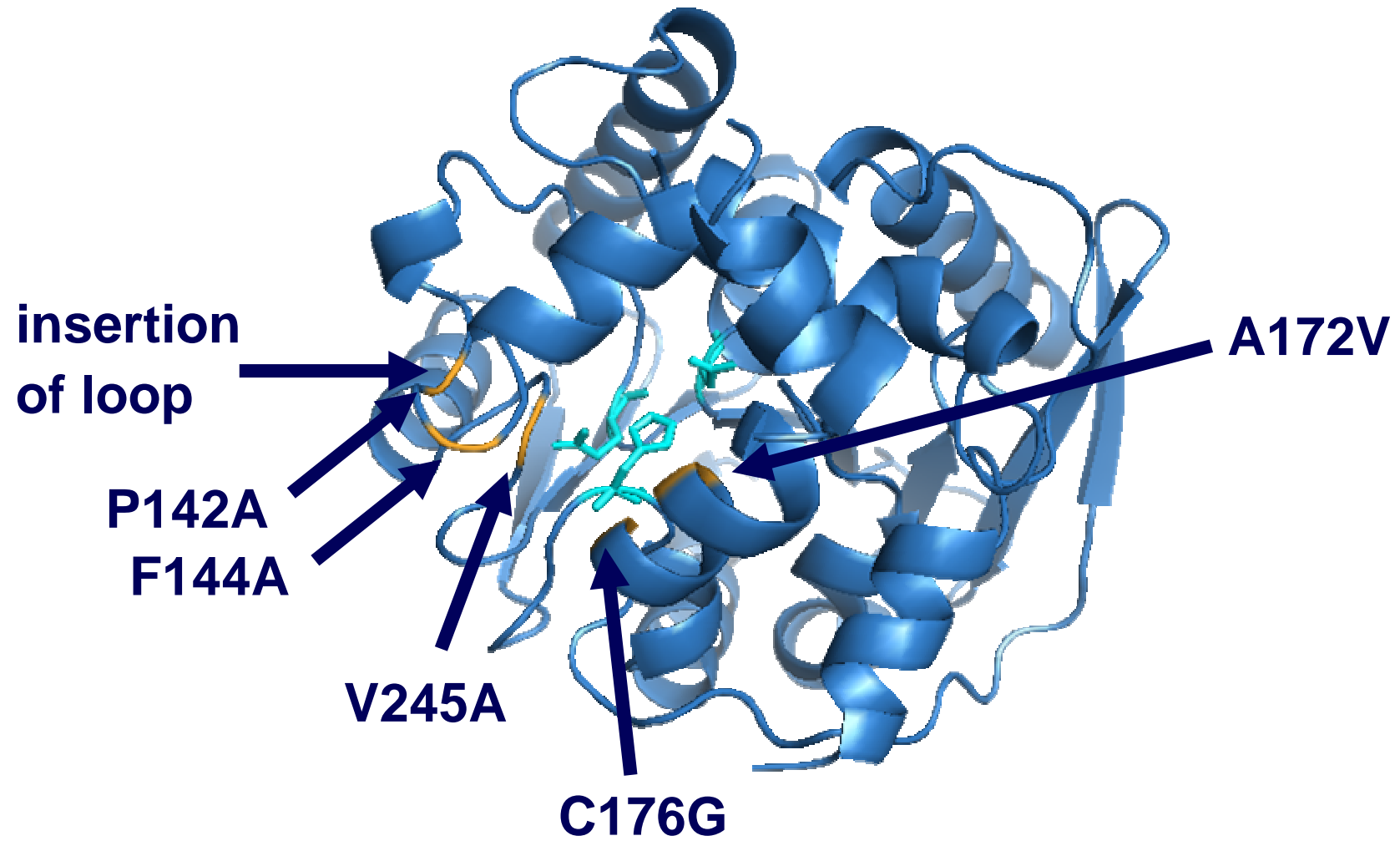
ENANTIOSELECTIVITY
RECONSTRUCTION

M4



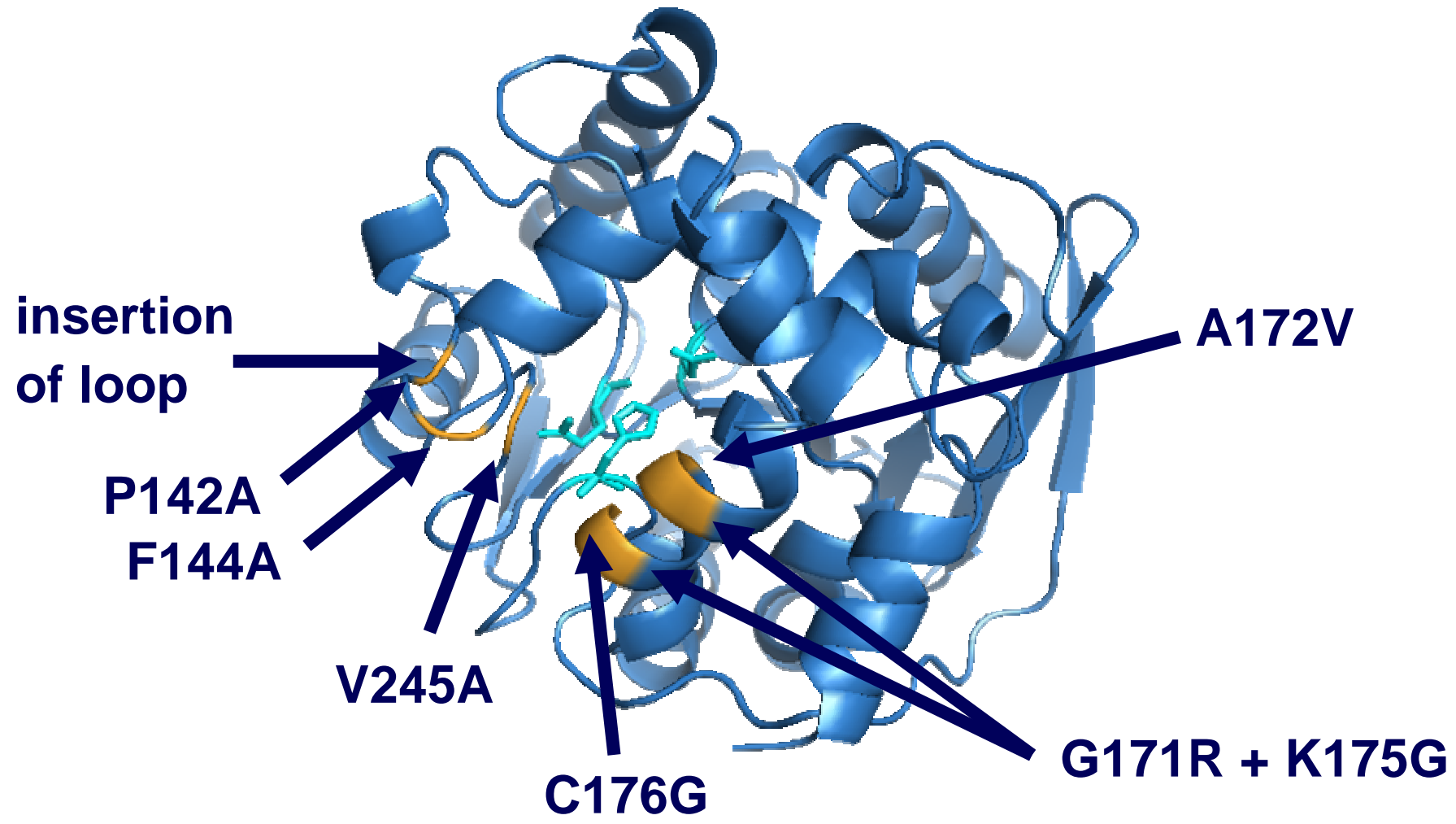
**ENANTIOSELECTIVITY
RECONSTRUCTION**

M5

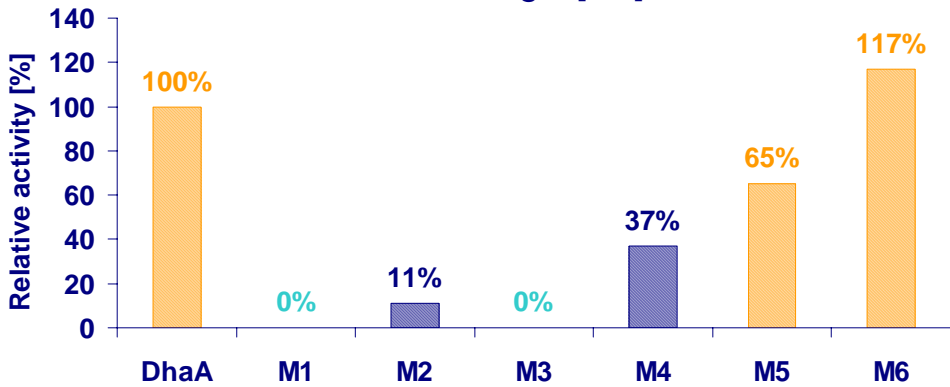
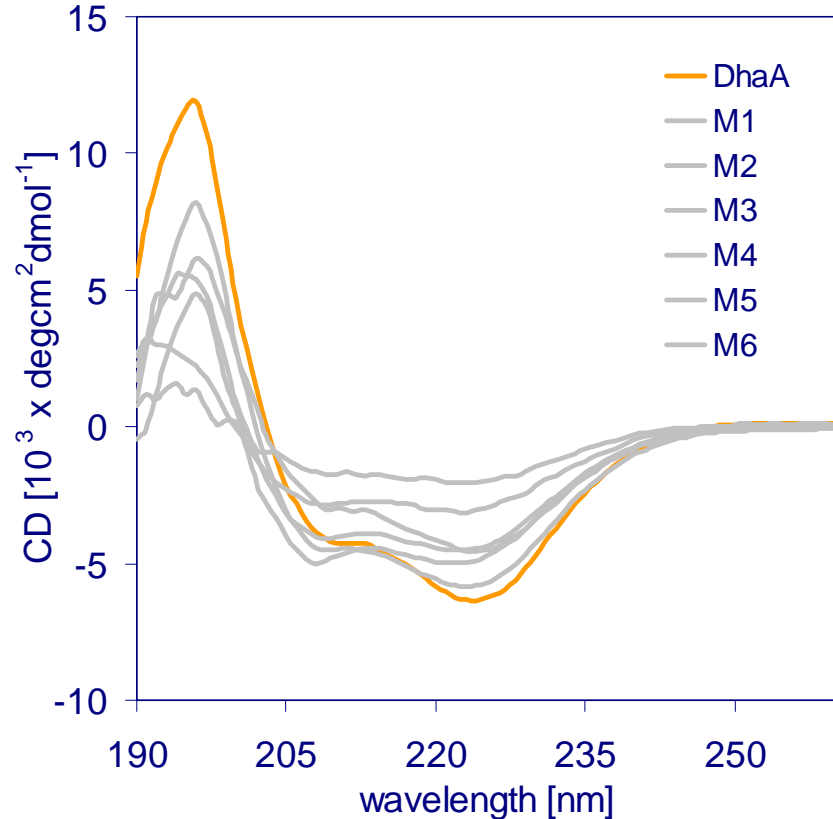


ENANTIOSELECTIVITY
RECONSTRUCTION

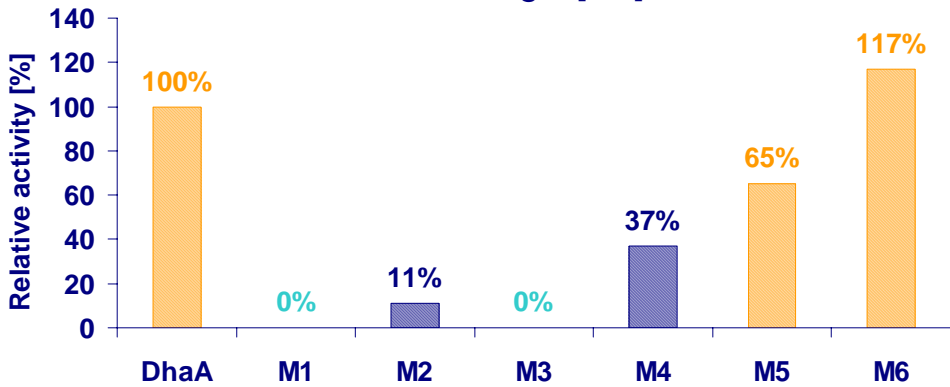
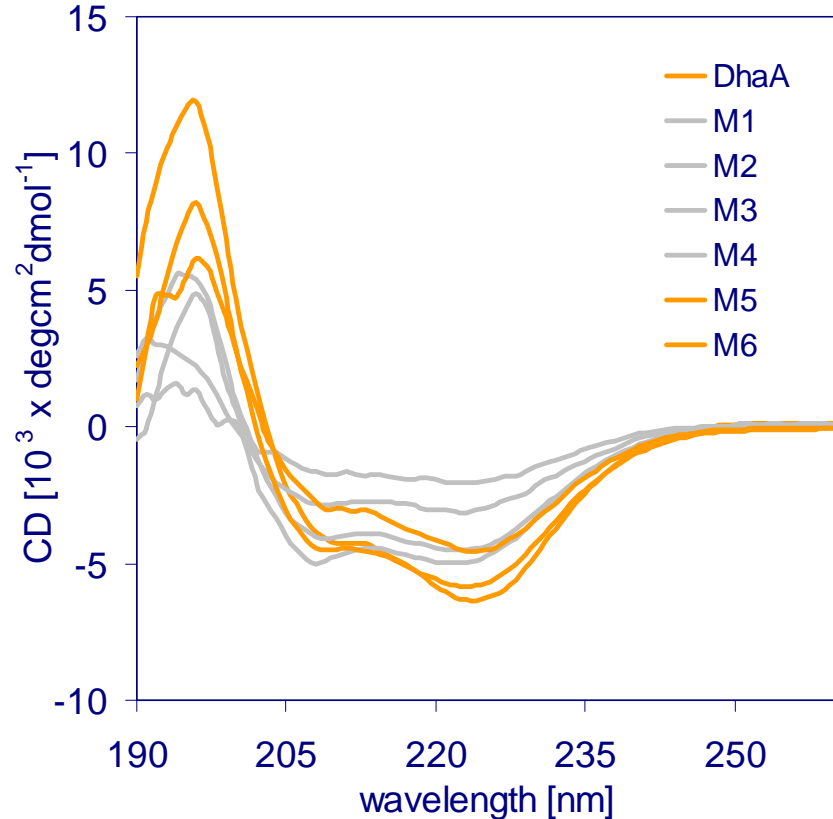
M6



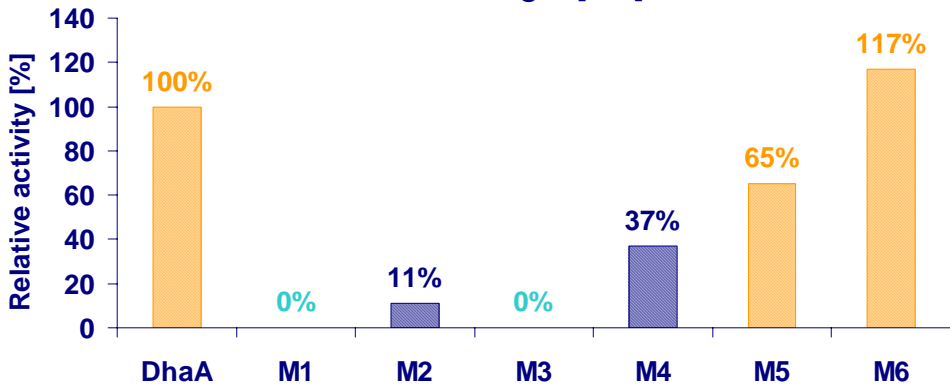
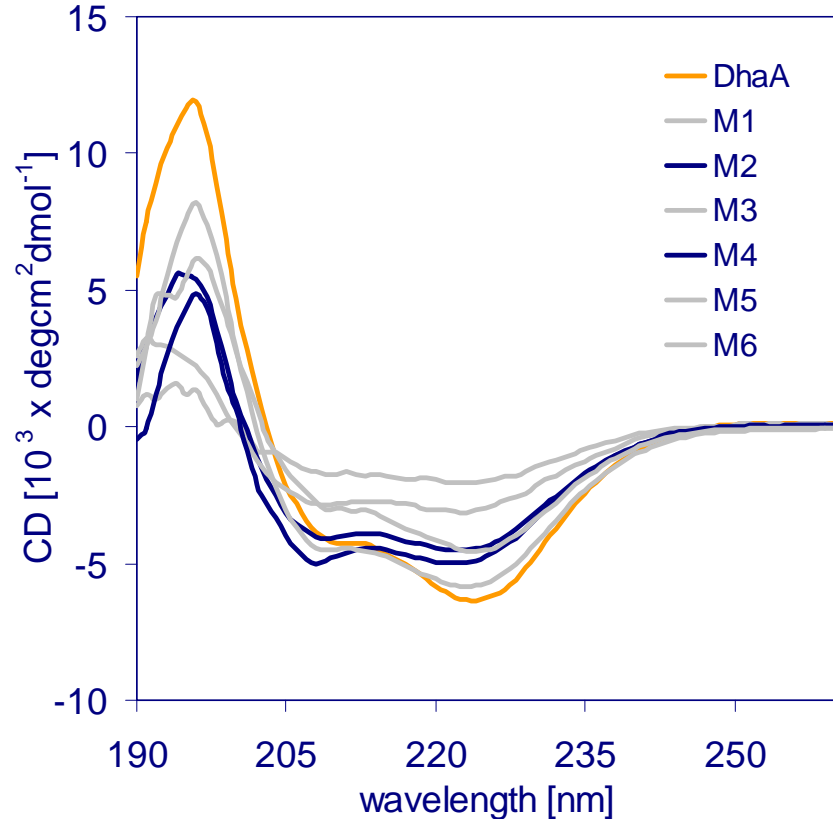
ENANTIOSELECTIVITY RECONSTRUCTION



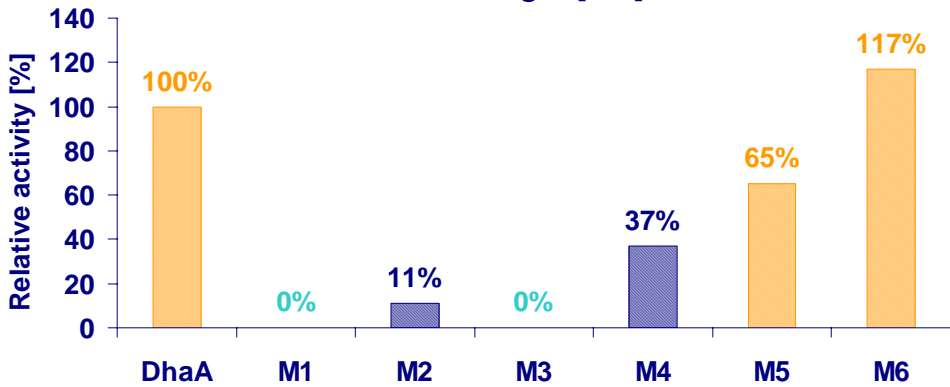
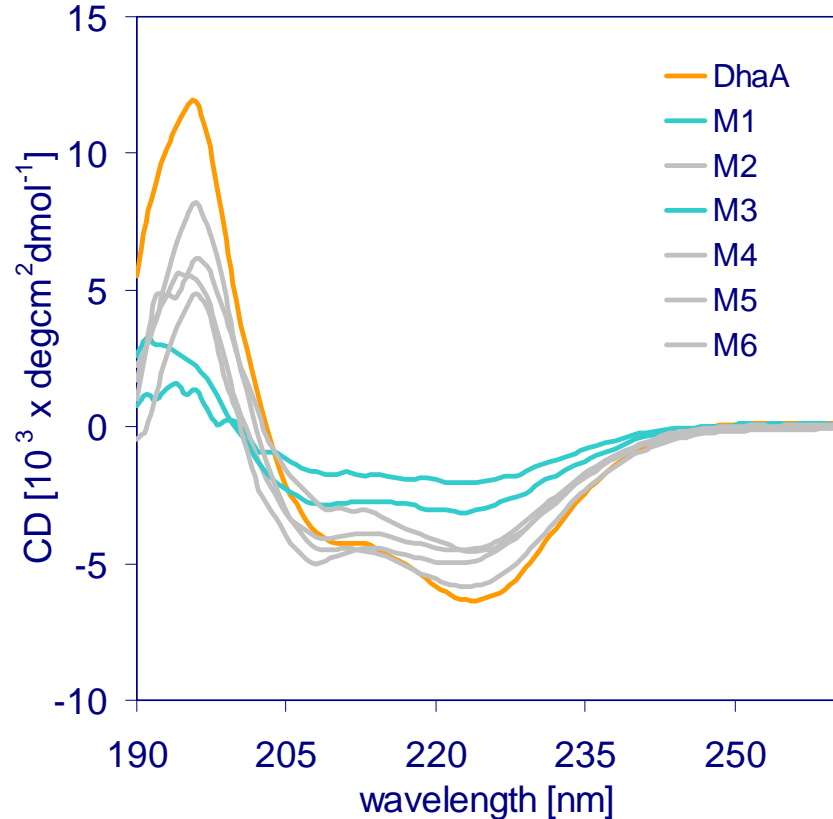
ENANTIOSELECTIVITY RECONSTRUCTION



ENANTIOSELECTIVITY RECONSTRUCTION



ENANTIOSELECTIVITY RECONSTRUCTION



ENANTIOSELECTIVITY RECONSTRUCTION

Substrate	<i>E</i> -value				
	DbjA	DhaA	M4	M5	M6
2-bromopentane	145	8	9	8	7
2-bromohexane	35	4	11	n.t.	31
2-bromoheptane	28	2	3	n.t.	6
ethyl 2-bromopropionate	>200	72	n.t.	n.t.	>200

n.t. not tested

ENANTIOSELECTIVITY RECONSTRUCTION

Substrate	<i>E</i> -value				
	DbjA	DhaA	M4	M5	M6
2-bromopentane	145	8	9	8	7
2-bromohexane	35	4	11	n.t.	31
2-bromoheptane	28	2	3	n.t.	6
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n.t. not tested