Workshop Argentina-Japan
"Bioscience and Biotechnology for the Promotion of Agriculture and Food Production"
- August 3rd to 7th 2009 -
Buenos Aires

Structural Biology of Carbohydrate-Degrading Enzymes that Contribute to Biotechnology

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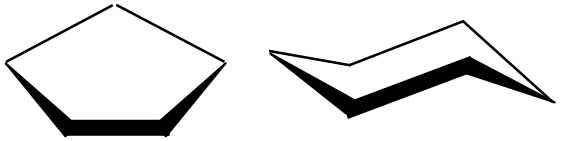
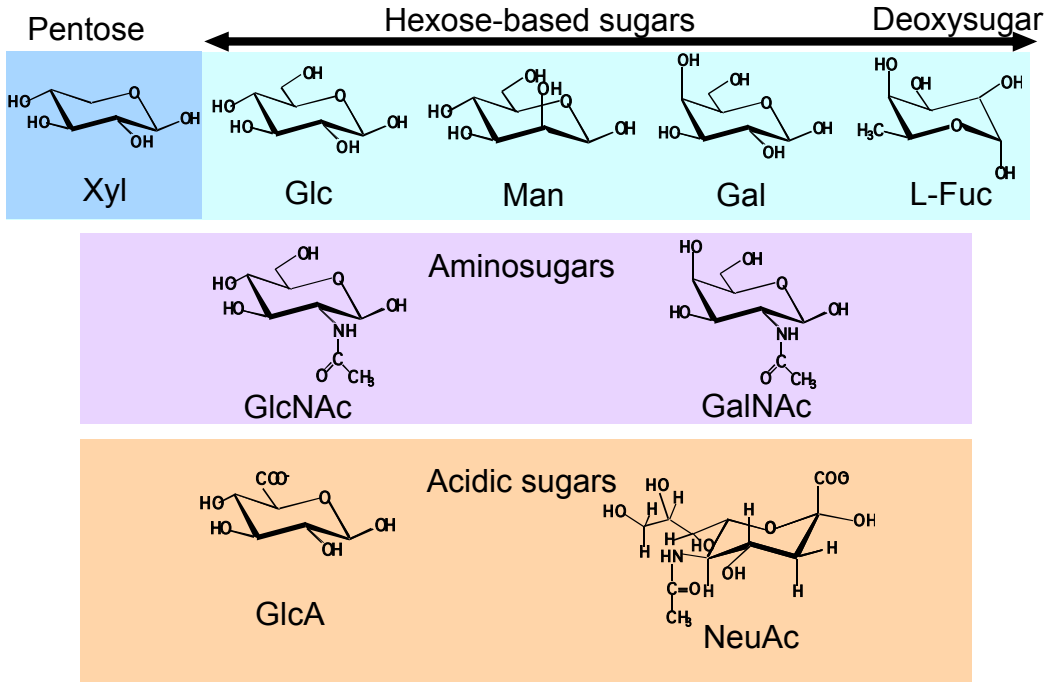
The University of Tokyo

Diversity of carbohydrates

- Function
  - Energy store (starch etc.)
  - Structural materials（cellulose, chitin etc.）
  - Information molecules（glycoconjugates）

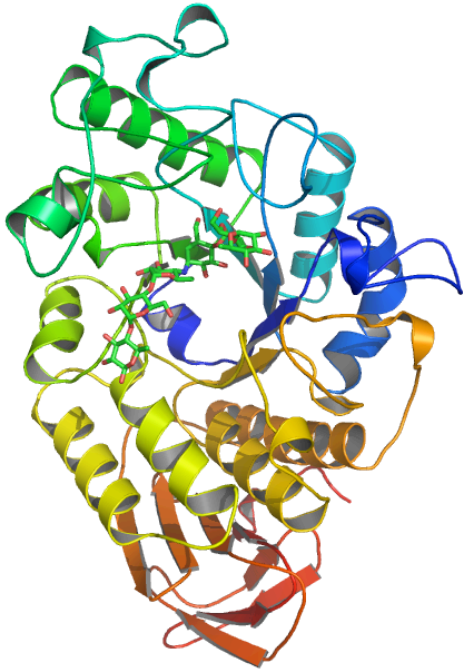


- Structure
  - Building block sugars (Pyranose/Furanose, Aldose/Ketose, stereoisomers etc.)
  - Bonds (α/β anomers, 1,1; 1,2; 1,3; 1,4; 1,6 etc.)
  - Degree of polymerization (1, 2, 3, … 10 … 100 … and branches)

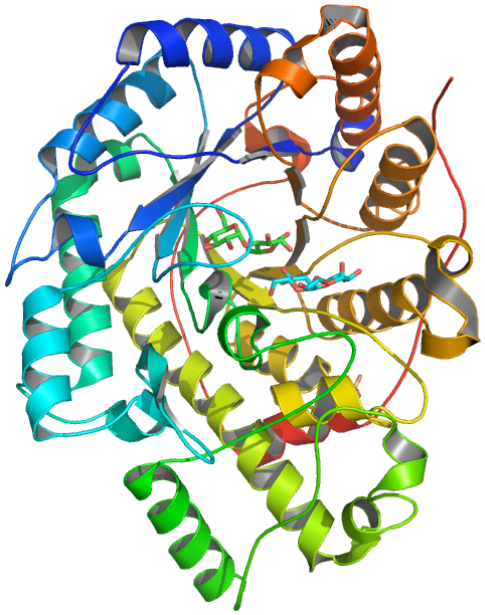


Structures of “Amylases”

$(\beta/\alpha)_8$ barrel

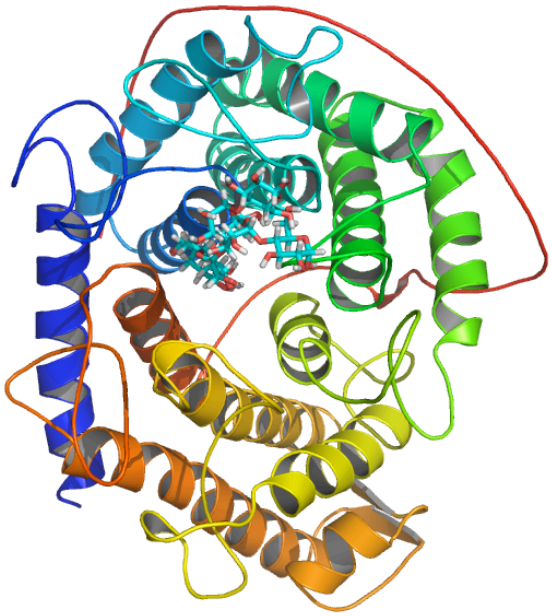


GH13
A. oryzae Taka-amylase



GH14
G. max β-Amylase

$(\alpha/\alpha)_6$ barrel

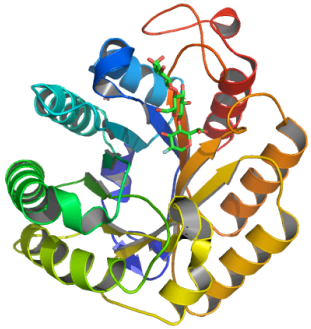


GH15
A. awamori Glucoamylase

Structures of "Cellulases"

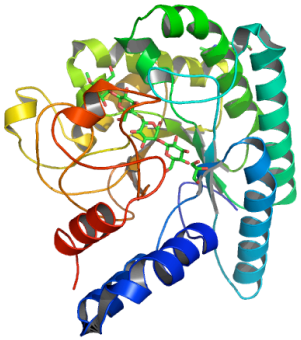
$(\beta/\alpha)_8$ barrel

GH1
P. crysosporium
β-glucosidase



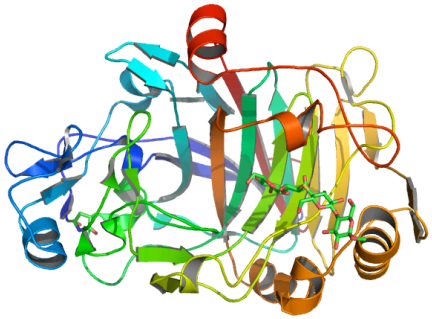
GH5
B. agaradhaerens
endo-β1,4-glucanase (Cel5A)

$(\beta/\alpha)_7$ barrel

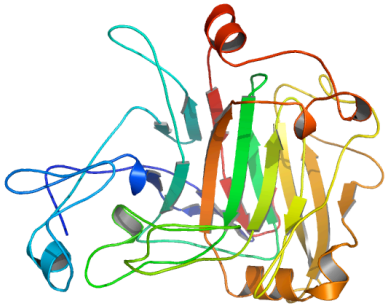


GH6
T. reesei
CBH II (Cel6A)

β-jelly roll

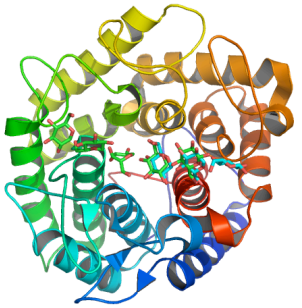


GH7
T. reesei
CBH I (Cel7A)



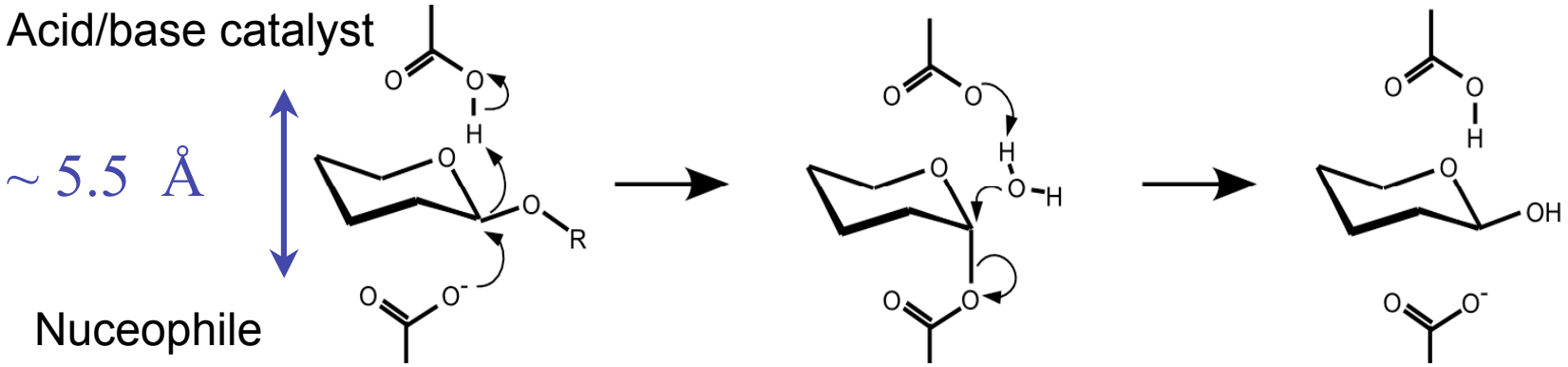
GH7
T. reesei
EG I (Cel7B)

$(\alpha/\alpha)_6$ barrel

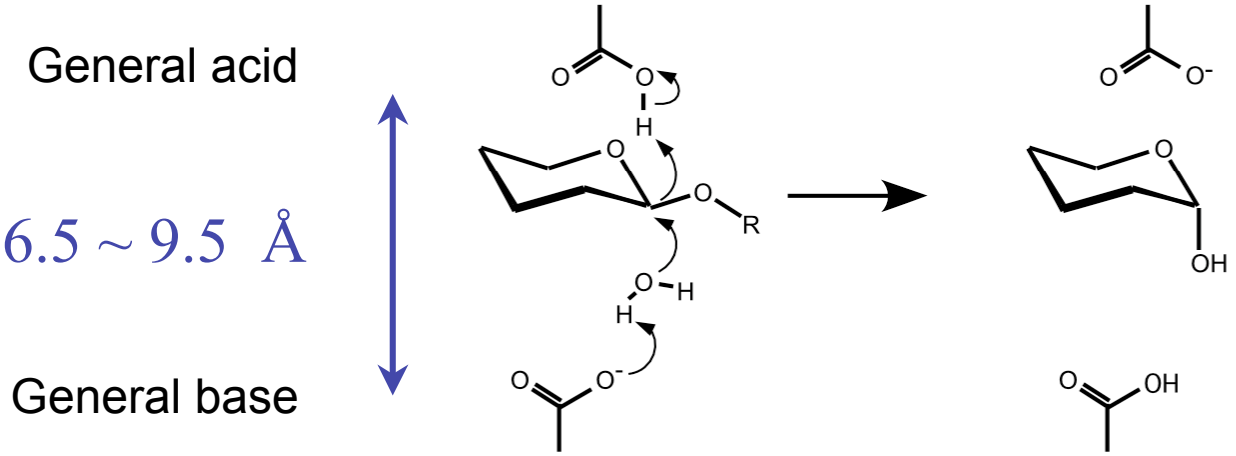


GH8
C. thermocellum
endo-β1,4-glucanase (CelA)

General reaction mechanisms of glycoside hydrolases



Retaining (double displacement) mechanism



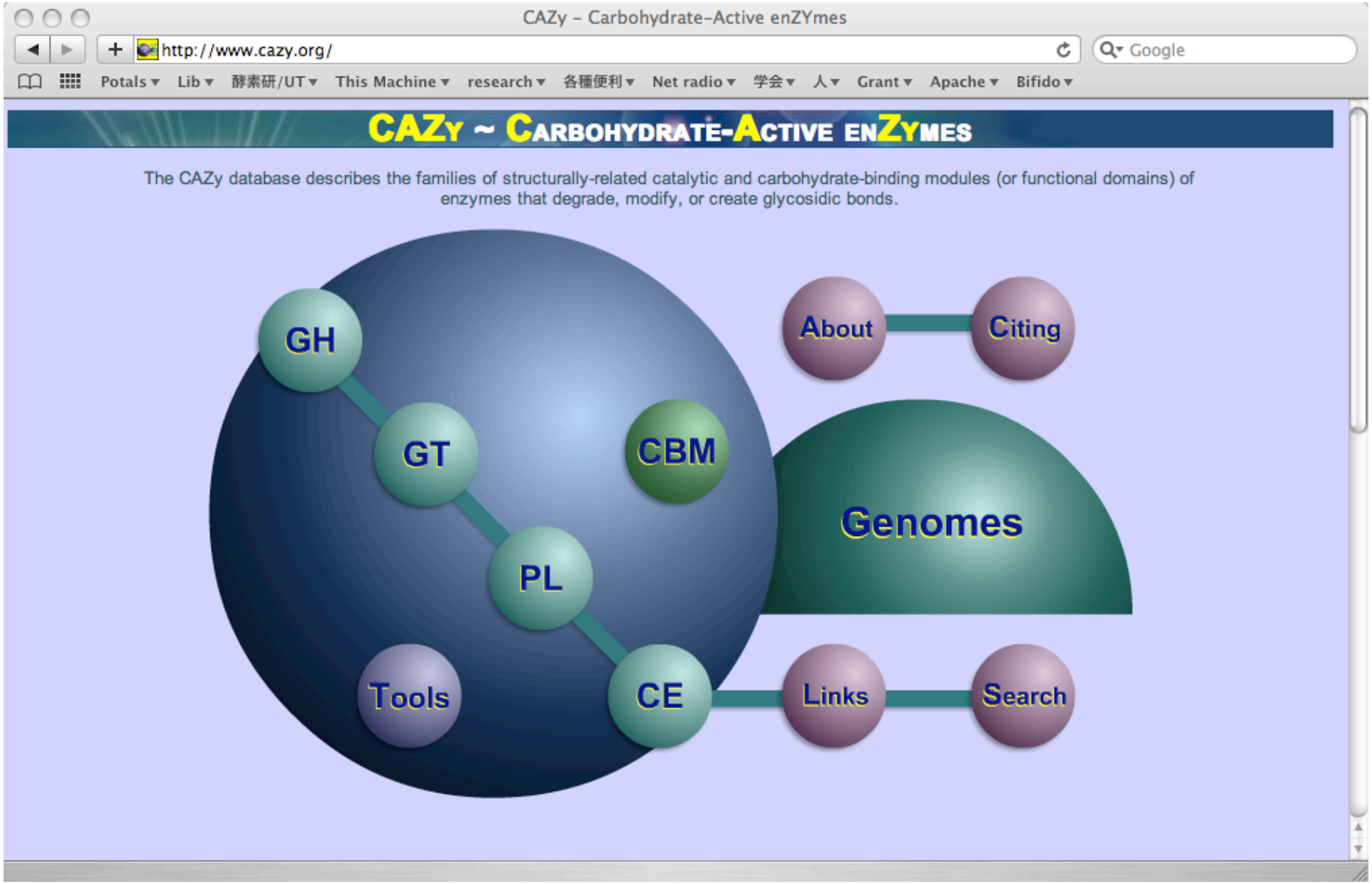
Inverting (single displacement) mechanism

McCarter & Withers, *Curr. Opin. Struct. BIol.* **4**, 885-892, 1994

How carbohydrate-acting enzymes evolved?

- The 3D structural scaffolds (folds) of Glycoside Hydrolases varies – Multiple origins!
- Protein folds are highly conserved during molecular evolution – Structure determination gives insights their liniages
- Sugar binding sites are prone to change
- Specificities for α- or β-bonds, and the reaction mechanisms (retaining/inverting, hydrolase/phosphorylase) can also change?

CAZy: Carbohydrate-Active enZYmes



CAZy: Carbohydrate-Active enZYmes

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	
61	62	63	64	65	66	67	68		70	71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90		92	93	94	95	96	97	98	99	100
101	102	103	104	105	106	107	108	109	110	111	112	113	114	115					

3D available

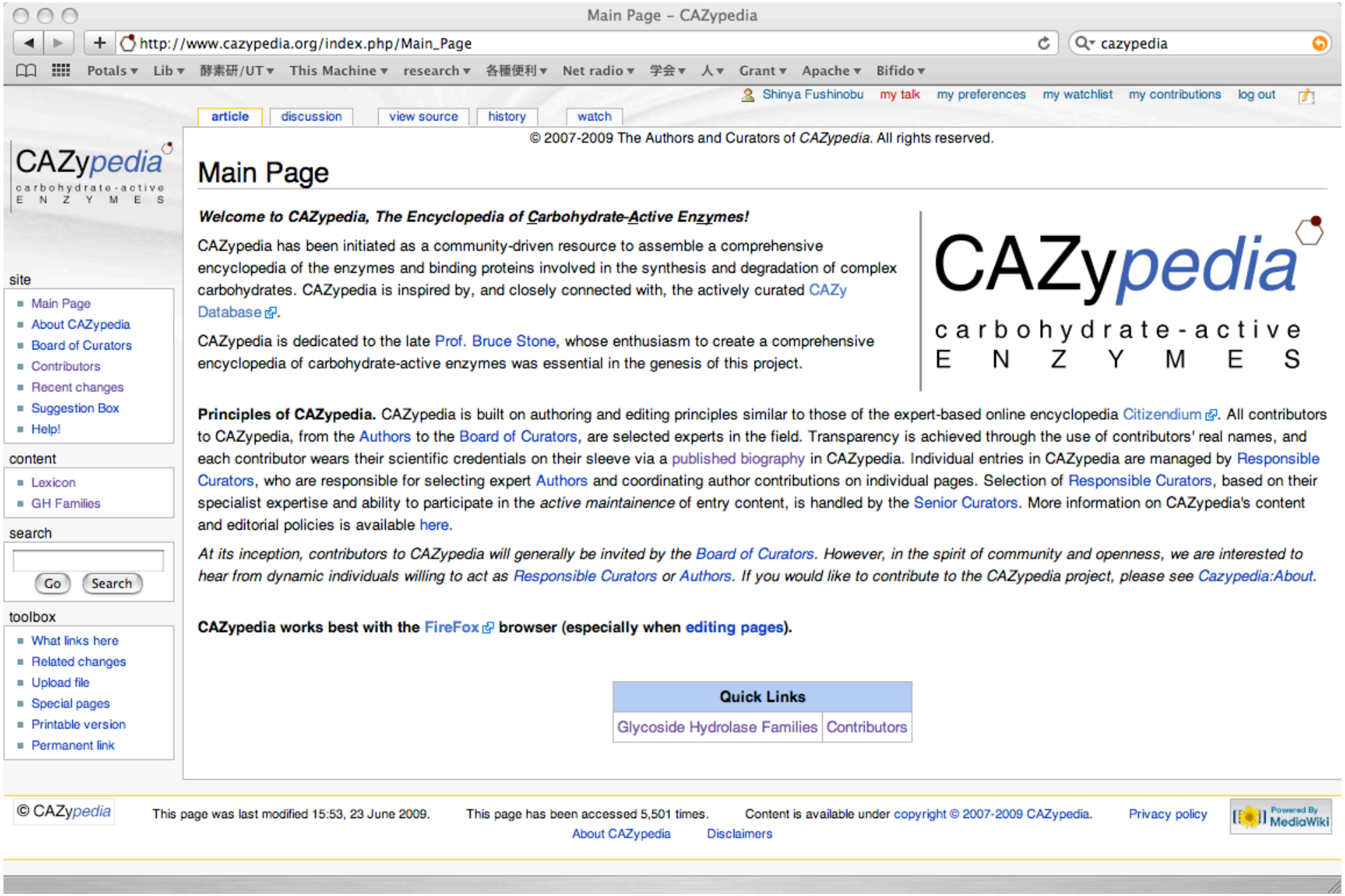
3D unknown

(at July, 2009)

Class	Catalytic activity	Max. Family No.	Deleted Families
GH (Glycoside Hydrolase)	EC 3.2.1.* and EC 2.4.*.*	115	21, 40, 41, 60, 69, 91
GT (Glycosyltransferase)	EC 2.4.*.*	91	36
PL (Polysaccharide Lyase)	EC 4.2.2.*	21	-
CE (Carbohydrate Esterase)	EC 3.1.1.*	16	-
CBM (Carbohydrate-Binding Module)	Not Enzyme	55	7

http://www.cazy.org/

CAZypedia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CAZypedia is dedicated to the late Prof. Bruce Stone, whose enthusiasm to create a comprehensive encyclopedia of carbohydrate-active enzymes was essential in the genesis of this project.
CAZypedia carbohydrate-active ENZYMES
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α- and β- glycosidases

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
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101	102	103	104	105	106	107	108	109	110	111	112	113	114	115					

α-glycosidases (axial)

β-glycosidases (equatorial)

Mixed

Retaining and Inverting glycosidases

1	2	3	4*	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	
61	62	63	64	65	66	67	68		70	71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
101	102	103	104	105	106	107	108	109*	110	111	112	113	114	115					

Retaining

Inverting

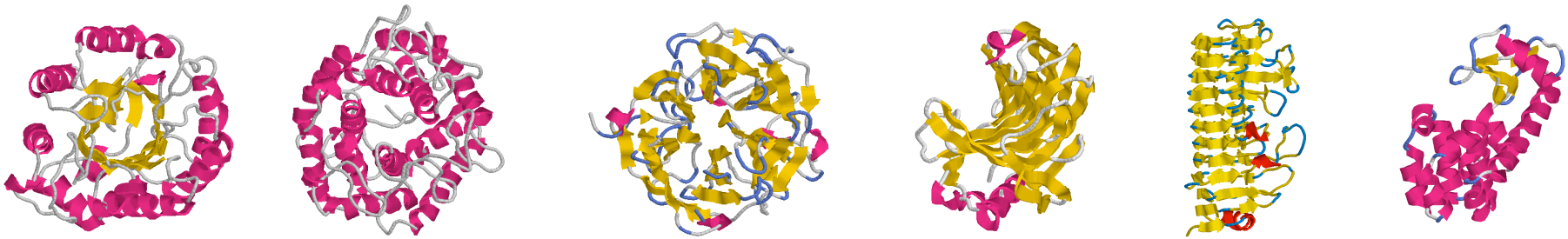
Mixed

Unknown

*NAD^+-dependent

Fold diversity of GH families

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	
61	62	63	64	65	66	67	68		70	71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
101	102	103	104	105	106	107	108	109	110	111	112	113	114	115					



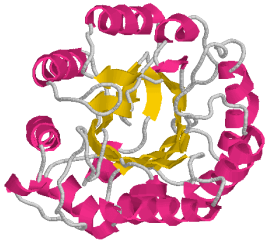
$(\beta/\alpha)_8$ barrel | $(\alpha/\alpha)_6$ barrel | 6-fold β-propeller | β-jelly roll | β-helix | Lysozyme-like

$(\beta/\alpha)_7$ barrel | 5-fold β-propeller | α + β | IG-like

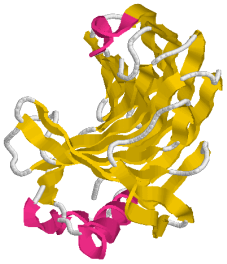
7-fold β-propeller | Others | Unknown

GH Clans

GH-A: $(\beta/\alpha)_8$ 1 2 5 10 17
26 30 35 39 42 50 51
53 59 72 79 86



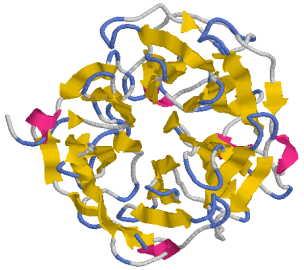
GH-B: β-jelly roll 7 16



GH-C: β-jelly roll 11 12

GH-D: $(\beta/\alpha)_8$ 27 31 36

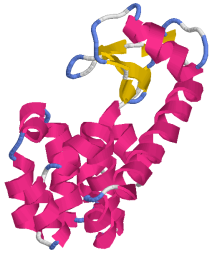
GH-E: 6-fold β-propeller 33 34 83



GH-F: 5-fold β-propeller 43 62

GH-G: - 37 63

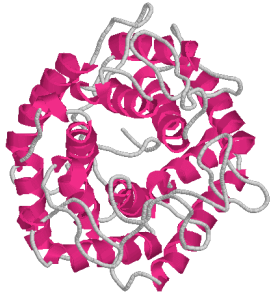
GH-H: $(\beta/\alpha)_8$ 13 70 77



GH-I: (α+β) 24 46 80

GH-J: 5-fold β-propeller 32 68

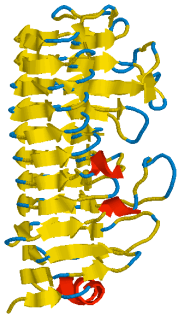
GH-K: $(\beta/\alpha)_8$ 18 20



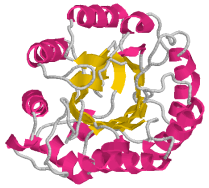
GH-L: $(\alpha/\alpha)_6$ 15 65

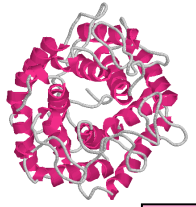
GH-M: $(\alpha/\alpha)_6$ 8 48

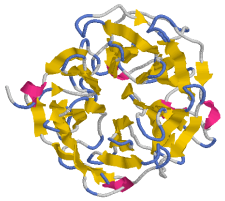
GH-N: β-helix 28 49



Possible GH Lineages





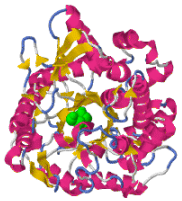


- $(\beta/\alpha)_{(8)}$
  - $(\beta/\alpha)_8$
    - GH-A 1 2 5 10 17 26 30 35 39 42 50 51 53 59 72 79 86
    - 14
    - 112
  - $(\beta/\alpha)_7$
    - 6
    - 38 57
    - 47
    - 56
  - $(\beta/\alpha)_5(\beta)_3$
    - 25
- $(\alpha/\alpha)_{(6)}$
  - $(\alpha/\alpha)_6$
    - GH-L 15 65
    - 94
    - 37 63 78 92 95
    - GH-M 8 48
    - 9
  - GH-D 27 31 36
  - GH-H 13 70 77
  - GH-K 18 20 85
  - 3 29 44 67 89
- β-propeller
  - 5-fold
    - GH-F 43 62
    - GH-J 32 68
  - 6-fold
    - GH-E 33 34 83
  - 7-fold
    - 74
    - 58
- β-jelly roll
- (α+β)
  - Etc.
- β-helix
- Lysozyme-like

- Increase and decrease of repeating units
- Similar structure with different mechanism

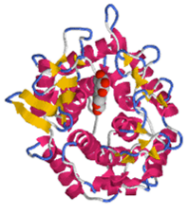
Structures of CAZymes determined by our group

P. chrysosporium
β-glucosidase 1A



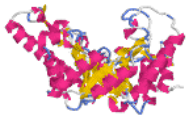
GH1

B. halodurans
"Rex"



GH8

C. stercorarium
xylanase B

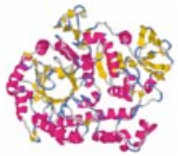


GH10

A. kawachii
xylanase C

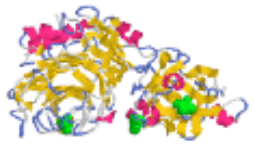
GH11

T. thermophilus
β-galactosidase



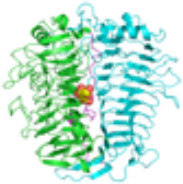
GH42

A. kawachii
α-L-arabino-
furanosidase



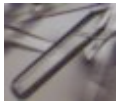
GH54+
CBM42

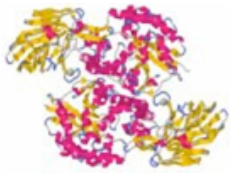
P. chrysosporium
Lam55A



GH55

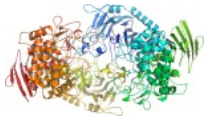
T. litoralis
4-α-glucano-
transferase





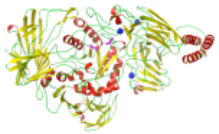
GH57

V. proteolyticus
Chitobiose
phosphorylase
C. gilvus
Cellobiose
phosphorylase



GT36 -> GH94

B. longum
endo-α

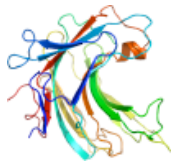


GH101

B. longum
GNB/LNB
phosphorylase

GH112

T. reesei
glucuronan
lyase



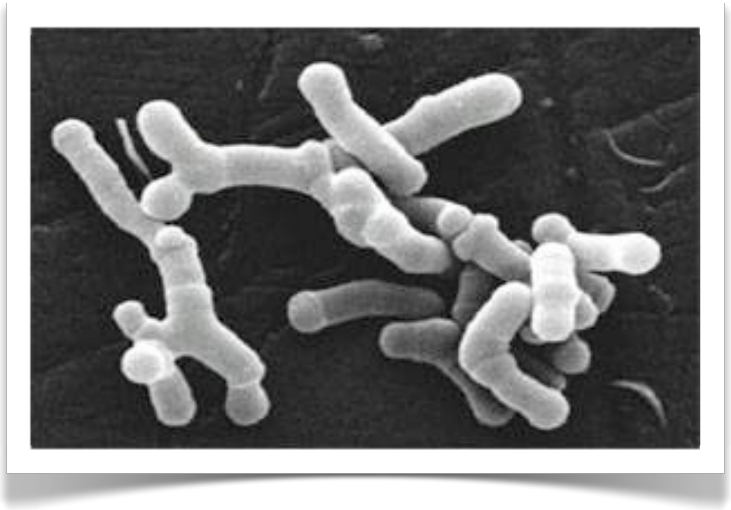
PL20

… and more!

Enzymatic production of prebiotic oligosaccharide and its structural basis

Bifidobacteria

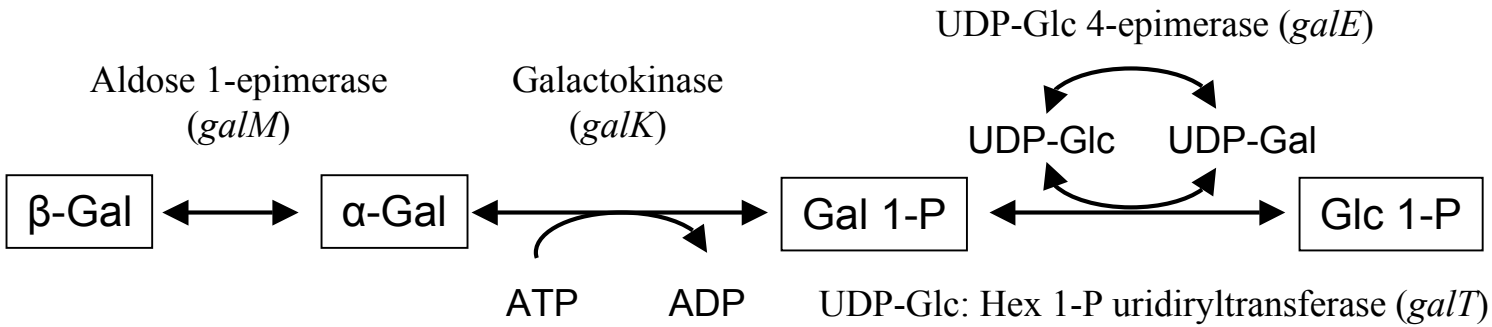
- Gram-positive intestinal anaerobic bacteria
- Rapidly colonize in breast-fed infants' intestine
- Beneficial to human health as "probiotics"
- Prevents infection of pathogenic bacteria and diarrhea

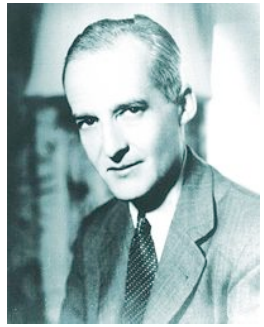


Bifidobacterium longum
(Mark Schell, MicorbeWiki)

Unique galactose metabolism of Bifidobacteria

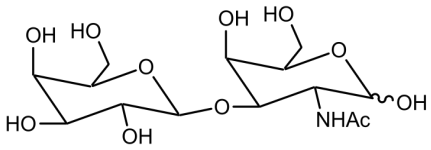
Authentic galactose pathway (Leloir pathway)



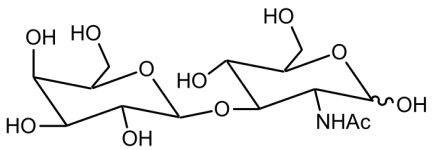


Luis Leloir
Nobel Prize 1970

Bifidobacterial galactose pathway (GNB/LNB pathway)



GNB (Gal-β1,3-GalNAc)
LNB (Gal-β1,3-GlcNAc)



GNB/LNB phosphorylase (BL1641)

Pi

GlcNAc/ GalNAc

UDP-Glc 4-epimerase (BL1644)

UDP-Glc UDP-Gal

Gal 1-P

Glc 1-P

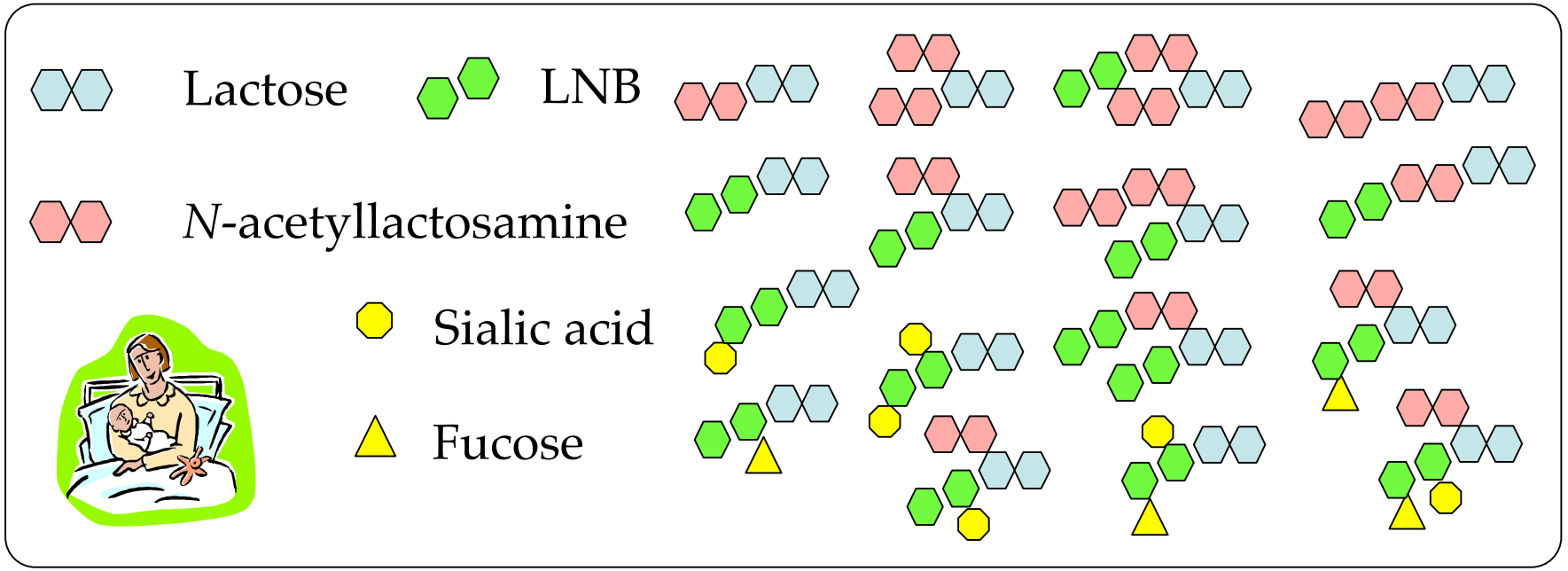
UDP-Glc: Hex 1-P uridiryltransferase (BL1643)

Gal 1-P is directly produced from GNB/LNB by phosphorylase without ATP

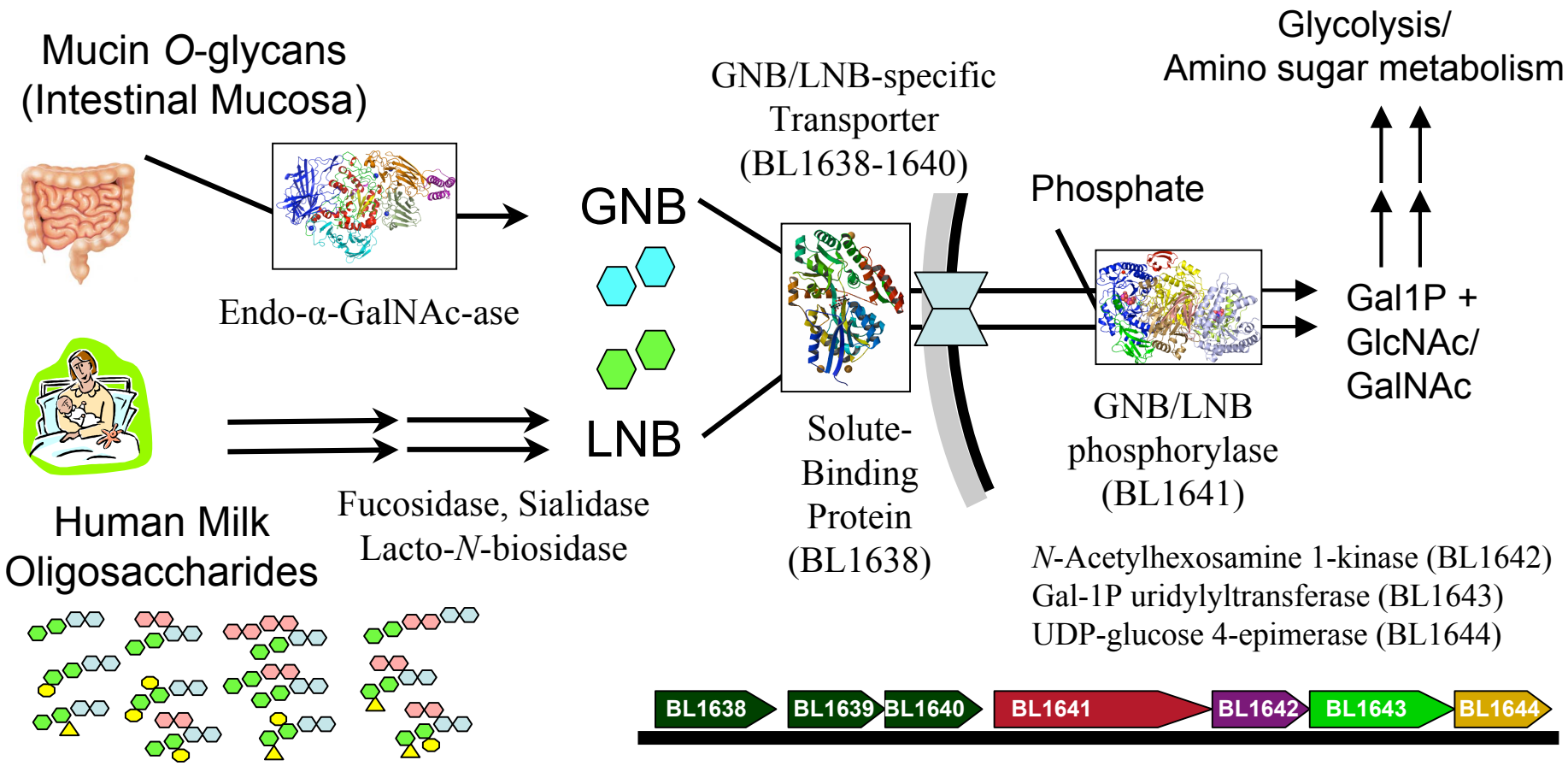
Kitaoka, M. et al. *Appl. Envron. Microbiol.* **71**, 3158-3162, 2005

Human Milk Oligosaccharides contain LNB

- Oligosaccharides in human milk other than lactose (Gal-β1,4-Glc)
- Include more than 100 kinds of molecules (> trisaccharides)
- Predominantly contain LNB as a building block: Exclusive feature of Human Milk (Urashima et al., 2007)
- LNB-containing oligosaccharides are "Bifidus Factor"



The GNB/LNB pathway of Bifidobacteria



Crystallography

- Wada *ActaF* **63**, 751, 2007
- Suzuki *JBC* **283**, 13165, 2007
- Hidaka *JBC* **284**, 7273, 2009
- Suzuki *J. Biochem.*, in press

Enzymology

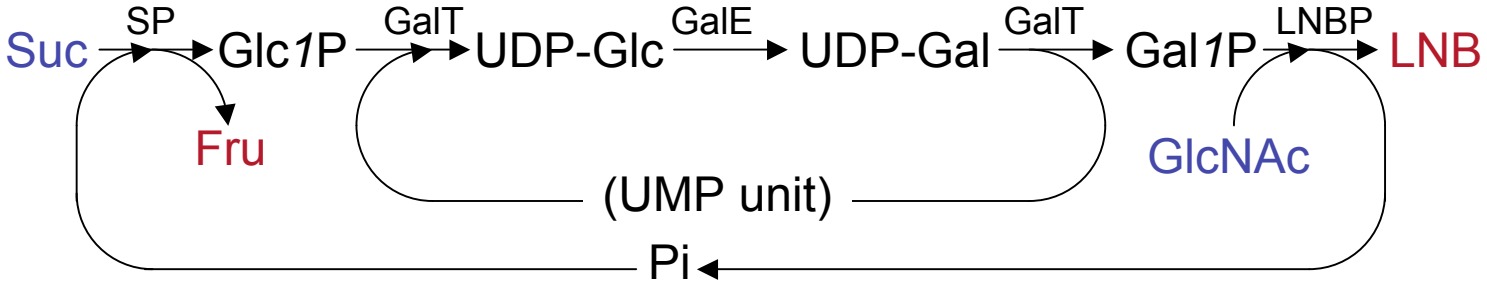
- Kitaoka *AEM*, 2005
- Nishimoto *BBB*, 2007
- Wada *AEM*, 2008
- Nakajima *AEM*, 2008
- Nakajima *AMB*, 2008
- Ashida *Glycobiol.*, 2008
- Nakajima *JBC*, 2009
- Ashida *Glycobiol.*, 2009

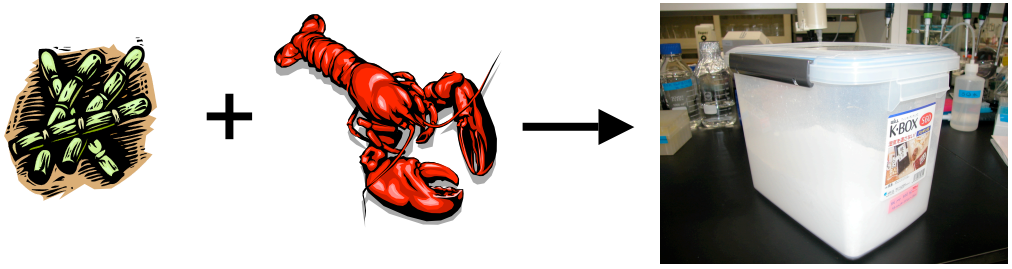
LNB production and effect as prebiotics

- Nishimoto *BBB*, 2007
- Kiyohara *BBB*, 2009

Large scale preparation of LNB

Sucrose + Pi	⇌	Glc1*P* + Fru	SP (BL0536)
Glc1*P* + UDP-Gal	⇌	UDP-Glc + Gal1*P*	GalT (BL1211)
UDP-Glc	⇌	UDP-Gal	GalE (BL1644)
Gal1*P* + GlcNAc	⇌	LNB + Pi	GLNBP (BL1641)
Suc + GlcNAc	⇌	LNB + Fru	(Cat. UDP-Glc, Pi)





1.4 kg LNB, Purity >99% (Yield 73%)

Catalog price of LNB = $369/25 mg

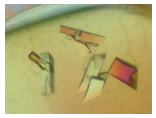
$20,664,000/1.4 kg

Nishimoto & Kitaoka *BBB*, **71**, 2101, 2007

Crystal structure of GNB/LNB phosphorylase (GLNBP)

Solved by Se-MAD
The 1st GH112 structure

Open

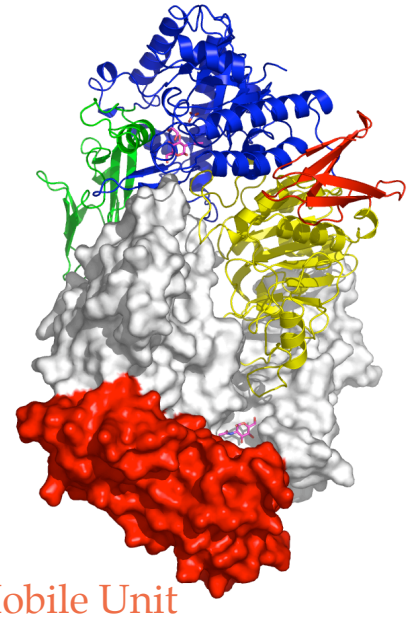


+ Sugar

Semi-closed

+ Phosphate

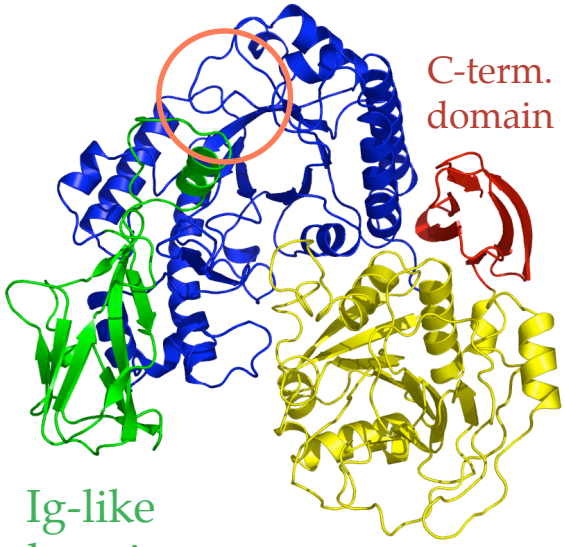
Closed



Mobile Unit

Broken TIM barrel domain

C-term. domain



Ig-like domain

α / β domain

*EG: Ethylene Glycol

	Resolution (Å)	R/R_{free} (%)	Conformation
Ligand-free	2.11	17.0/22.5	Open
GalNAc	1.90	16.1/20.4	Semi-closed
GlcNAc	2.30	17.0/23.1	Semi-closed
GlcNAc-NO_3-EG*	1.85	14.9/19.2	Semi-closed + Closed
GlcNAc-SO_4	2.11	16.9/22.5	Semi-closed + Closed

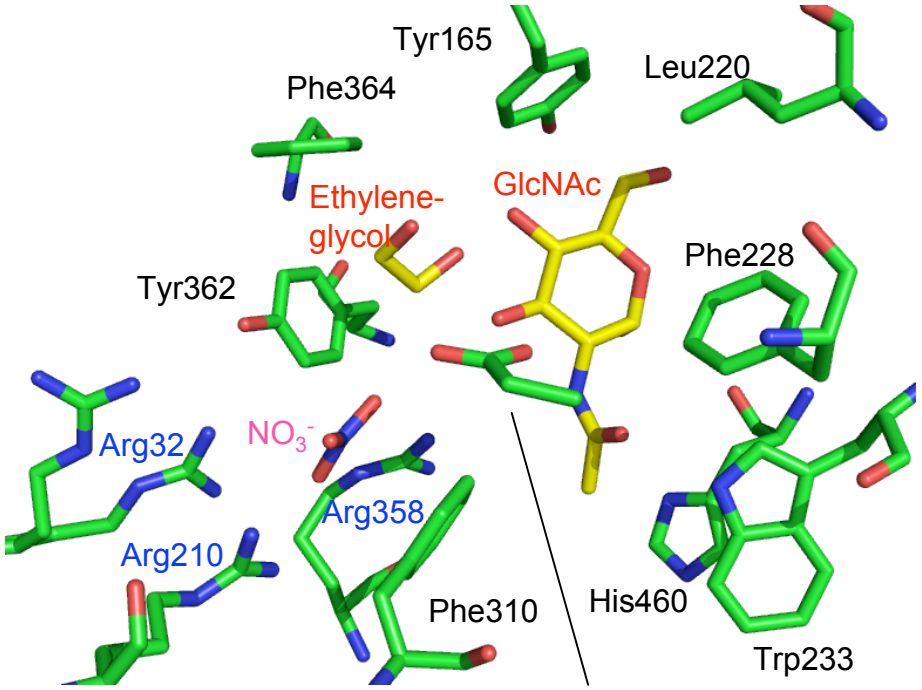
Active Site

LNB (Gal-β1,3-GlcNAc) + Pi → Gal-1-P + GlcNAc

GNB (Gal-β1,3-GalNAc) + Pi → Gal-1-P + GalNAc

	Sites			State
	Gal (-1)	GlyNAc (+1)	Pi (anion)	
1	-	-	-	open
2	-	GalNAc	-	semi-close
3	-	GlcNAc	SO_4^{2-}	close
4	EG	GlcNAc	NO_3^-	close

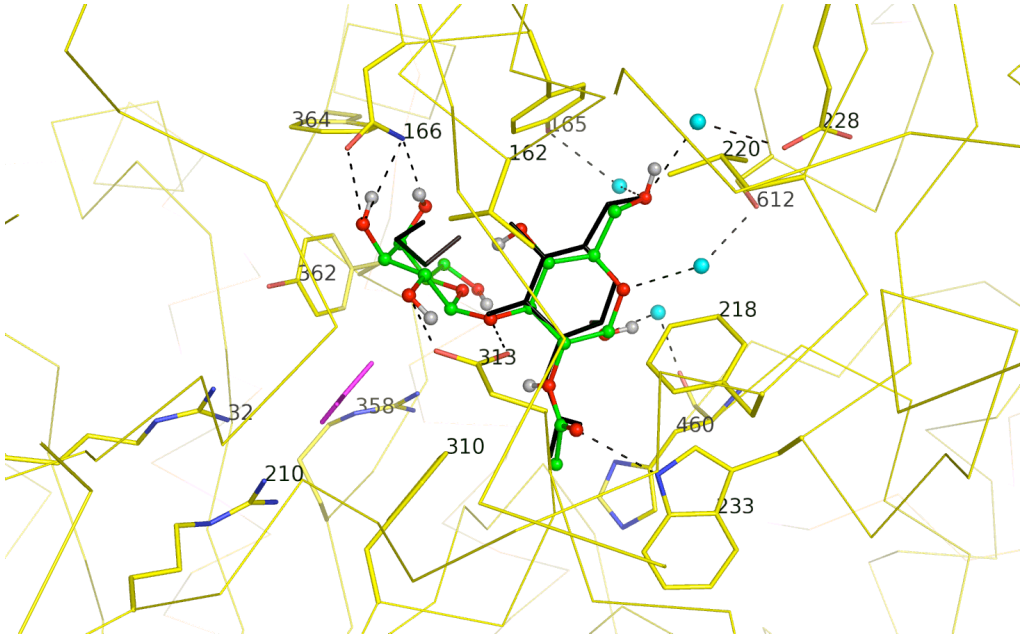
EG: Ethyleneglycol



Arg x 3

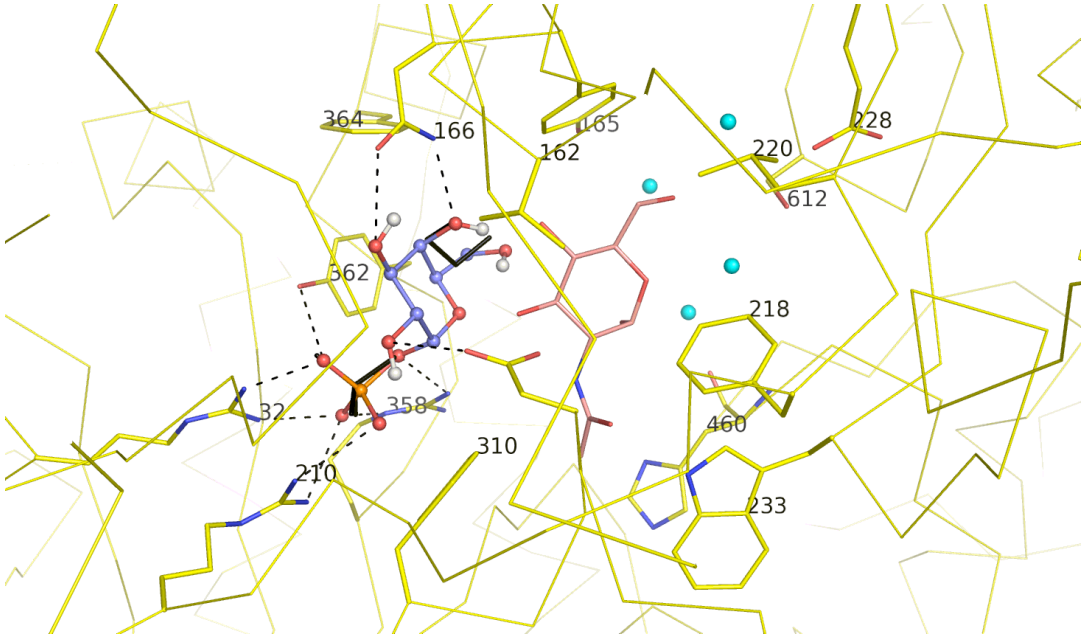
Ligand binding estimated by molecular docking

LNB (Substrate)

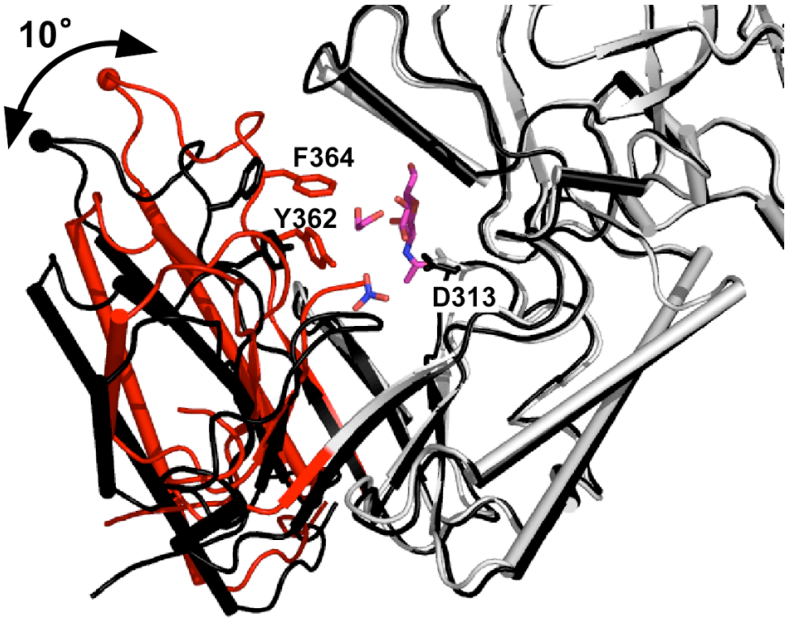


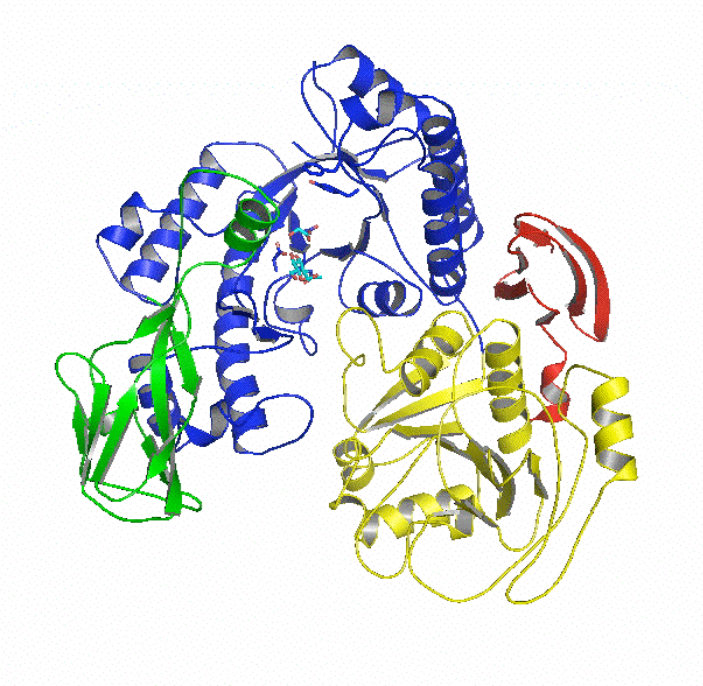
Ligand binding estimated by molecular docking

Galactose 1-phosphate (product)



Movement on substrate binding





Open -> Closed (Side)

Open -> Semi-closed -> Closed (Top)

Adaptation to substrates by deformation of the TIM barrel scaffold
An unique example of molecular evolution
GLNBP homologues are limited to human-related microbes: Relatively “newly evolved” enzymes in relationship with animals, which abundantly display glycoconjugates containing LNB or GNB?

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