Structural studies of lysosomal storage disorders

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15 Mar 2010
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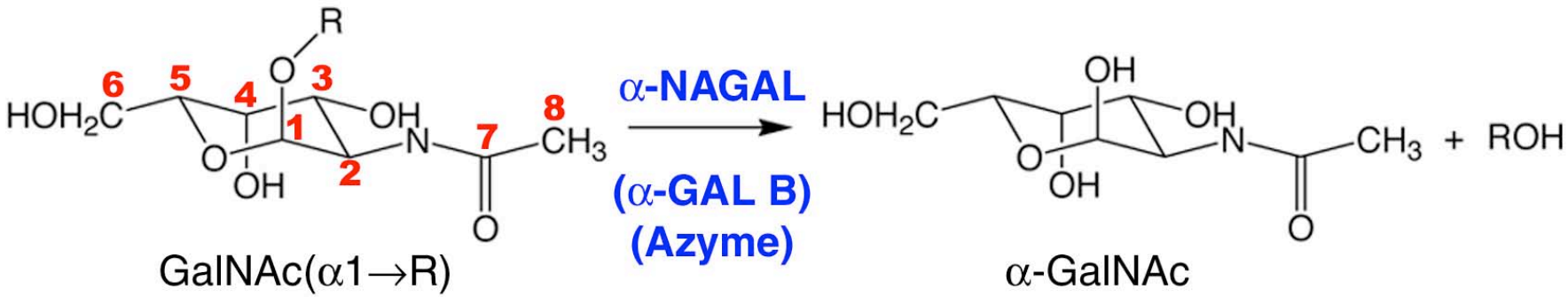
Outline

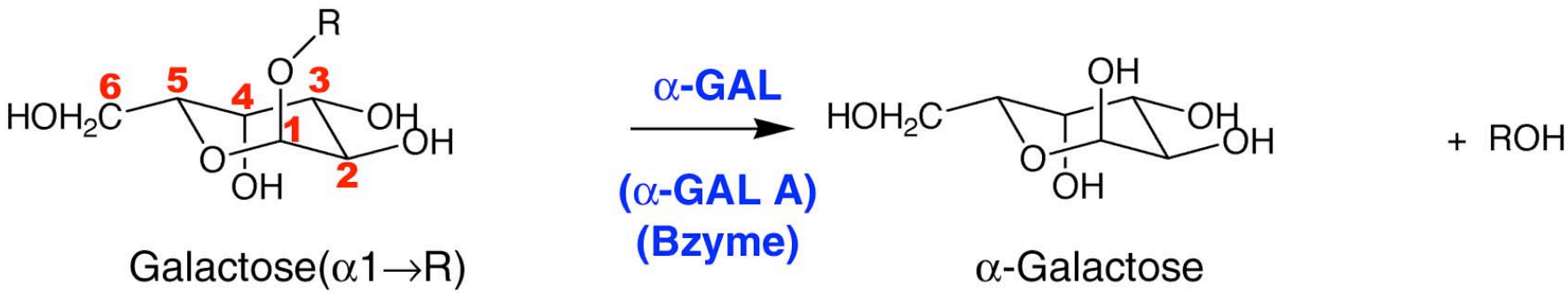
- Lysosomal storage disorders
- Structure of human α-GAL
- Mechanism of human α-GAL
- Structure human α-NAGAL
- Interconverting enzyme specificity

Lysosomal storage diseases

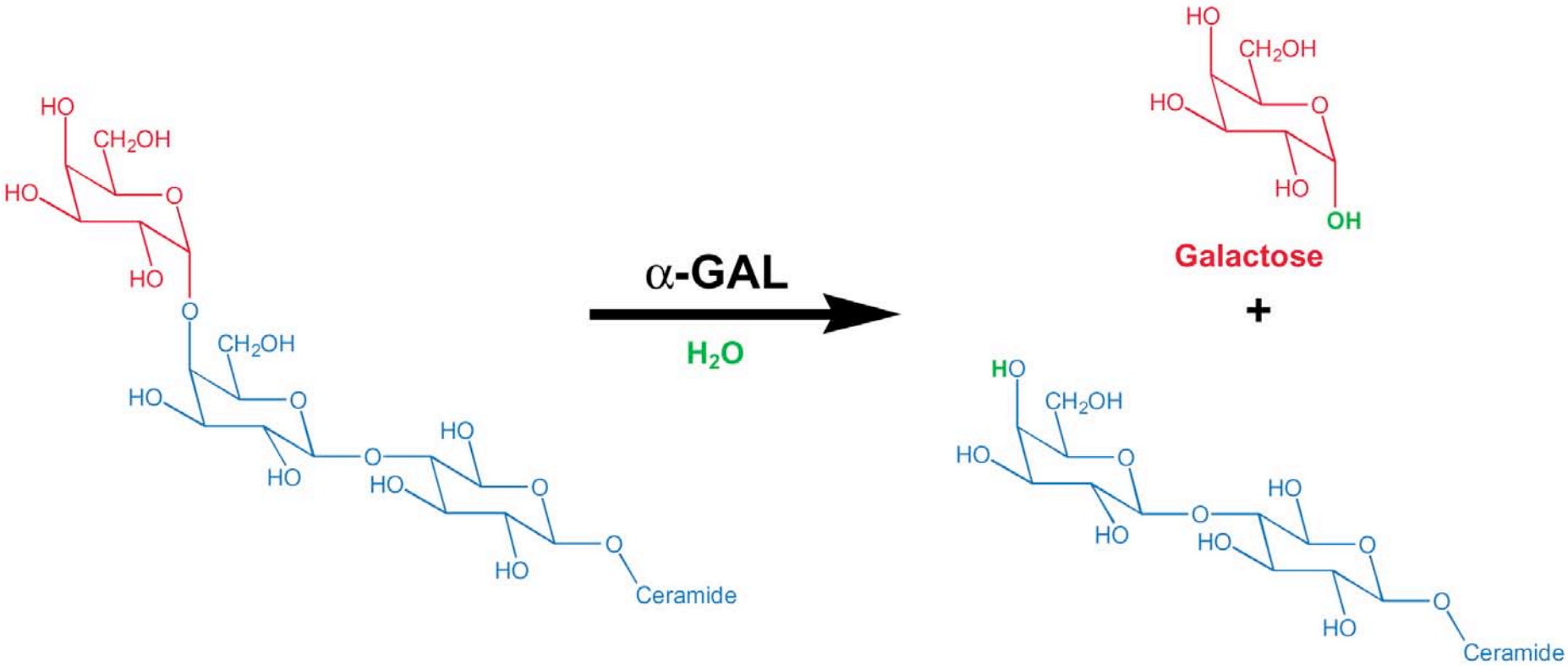
Enzyme Deficiency	Disease
α-Fucosidase	Fucosidosis
α-Mannosidase	α-Mannosidosis
β-Mannosidase	β-Mannosidosis
α-Neuraminidase	Sialidosis
α-N-Acetylgalactosaminidase	Schindler disease
β-Hexosaminidase A	Tay-Sachs disease
β-Hexosaminidase B	Sandhoff disease
β-Glucosidase	Gaucher disease
α-Galactosidase	Fabry disease
β-Galactosidase A	GM1 gangliosidosis

Enzyme specificity





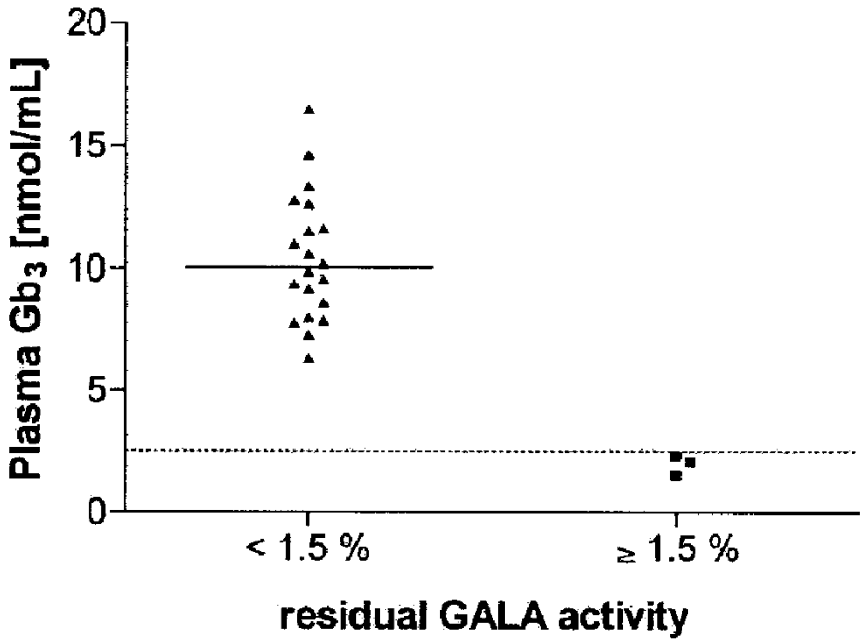
Fabry disease substrate

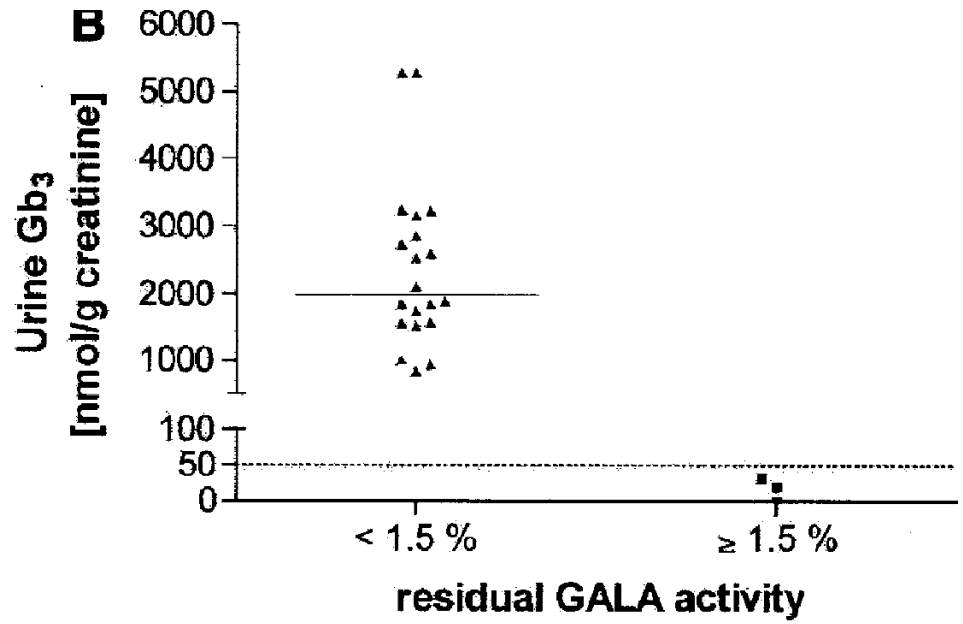


Gal(α1-4)Gal(β1-4)Glc(β1-1')Ceramide
Globotriaosylceramide **(Gb_3)**
(Fabry disease substrate)

Gal(β1-4)Glc(β1-1')Ceramide
Lactosylceramide

Substrate Accumulation in Fabry patients

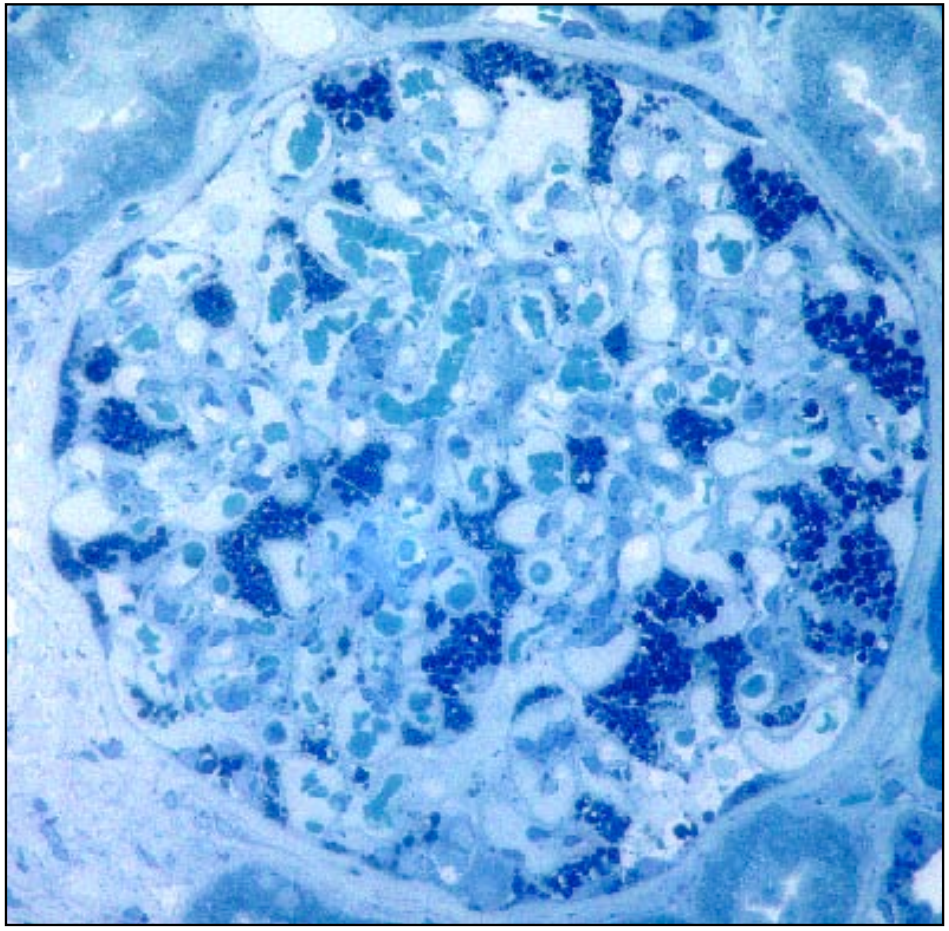




Ries *et al.*, (2005) Pediatrics **155**, e344

Fabry disease symptoms

Angiokeratomas



Gb_3 in Glomerulus

courtesy of Roscoe Brady, NIH/NINDS

Enzyme Replacement Therapy in Fabry Disease

A Randomized Controlled Trial

Raphael Schiffmann, MD
Jeffrey B. Kopp, MD
Howard A. Austin III, MD
Sharda Sabnis, MD
David F. Moore, MD, PhD
Thais Weibel, MD
James E. Balow, MD
Roscoe O. Brady, MD

Context Fabry disease is a metabolic disorder without a specific treatment, caused by a deficiency of the lysosomal enzyme α-galactosidase A (α-gal A). Most patients experience debilitating neuropathic pain and premature mortality because of renal failure, cardiovascular disease, or cerebrovascular disease.

Objective To evaluate the safety and efficacy of intravenous α-gal A for Fabry disease.

Design and Setting Double-blind placebo-controlled trial conducted from December 1998 to August 1999 at the Clinical Research Center of the National Institutes of Health.

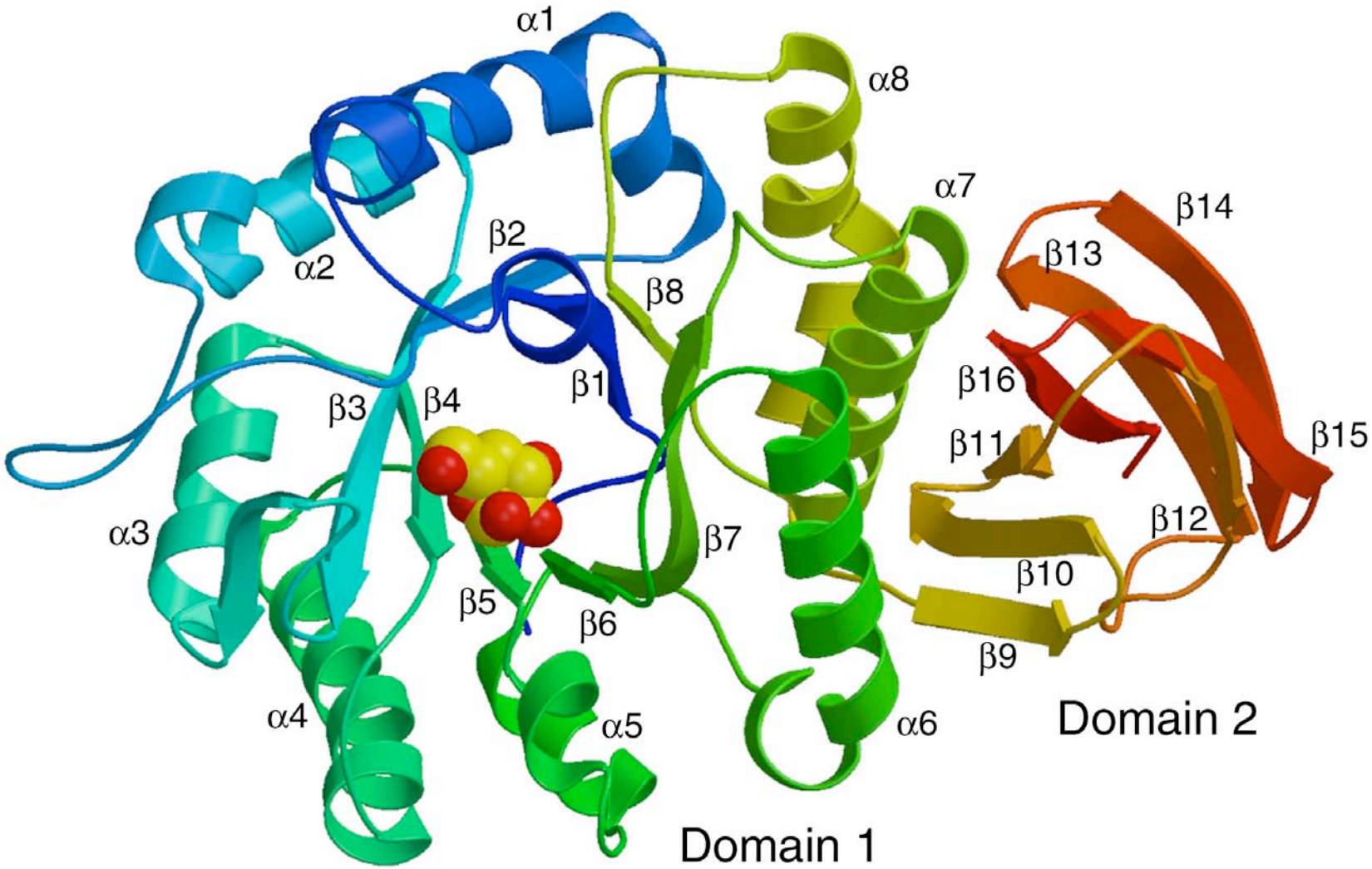
JAMA (2001) **285**:2743

SAFETY AND EFFICACY OF RECOMBINANT HUMAN α-GALACTOSIDASE A REPLACEMENT THERAPY IN FABRY'S DISEASE

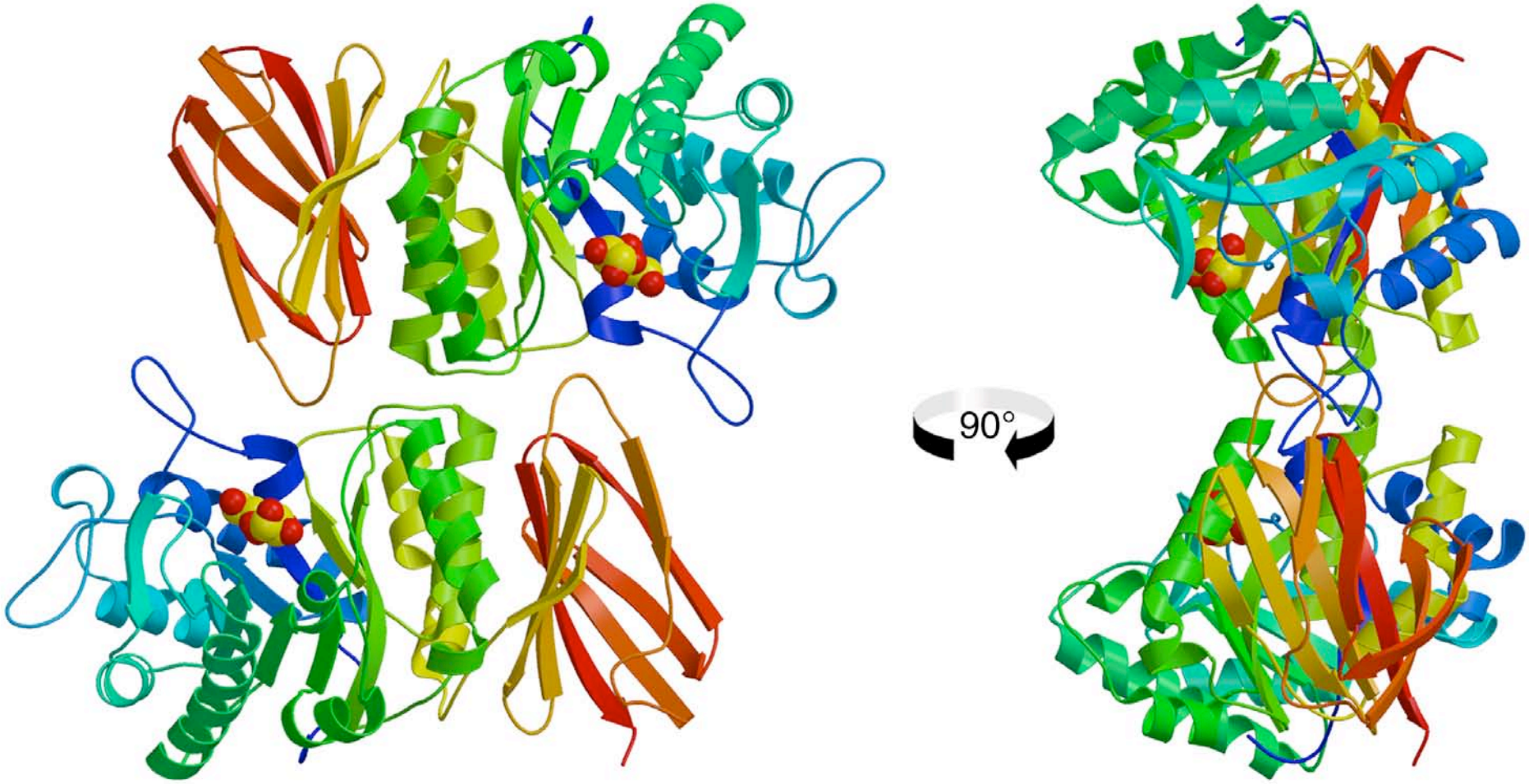
CHRISTINE M. ENG, M.D., NATHALIE GUFFON, M.D., WILLIAM R. WILCOX, M.D., PH.D.,
DOMINIQUE P. GERMAIN, M.D., PH.D., PHILIP LEE, M.R.C.P., D.M., PH.D., STEVE WALDEK, M.B., B.CH.,
LOUIS CAPLAN, M.D., GABOR E. LINTHORST, M.D., AND ROBERT J. DESNICK, PH.D., M.D.,
FOR THE INTERNATIONAL COLLABORATIVE FABRY DISEASE STUDY GROUP

NEJM (2001) **345**:9

α-GAL architecture



α-GAL dimer structure

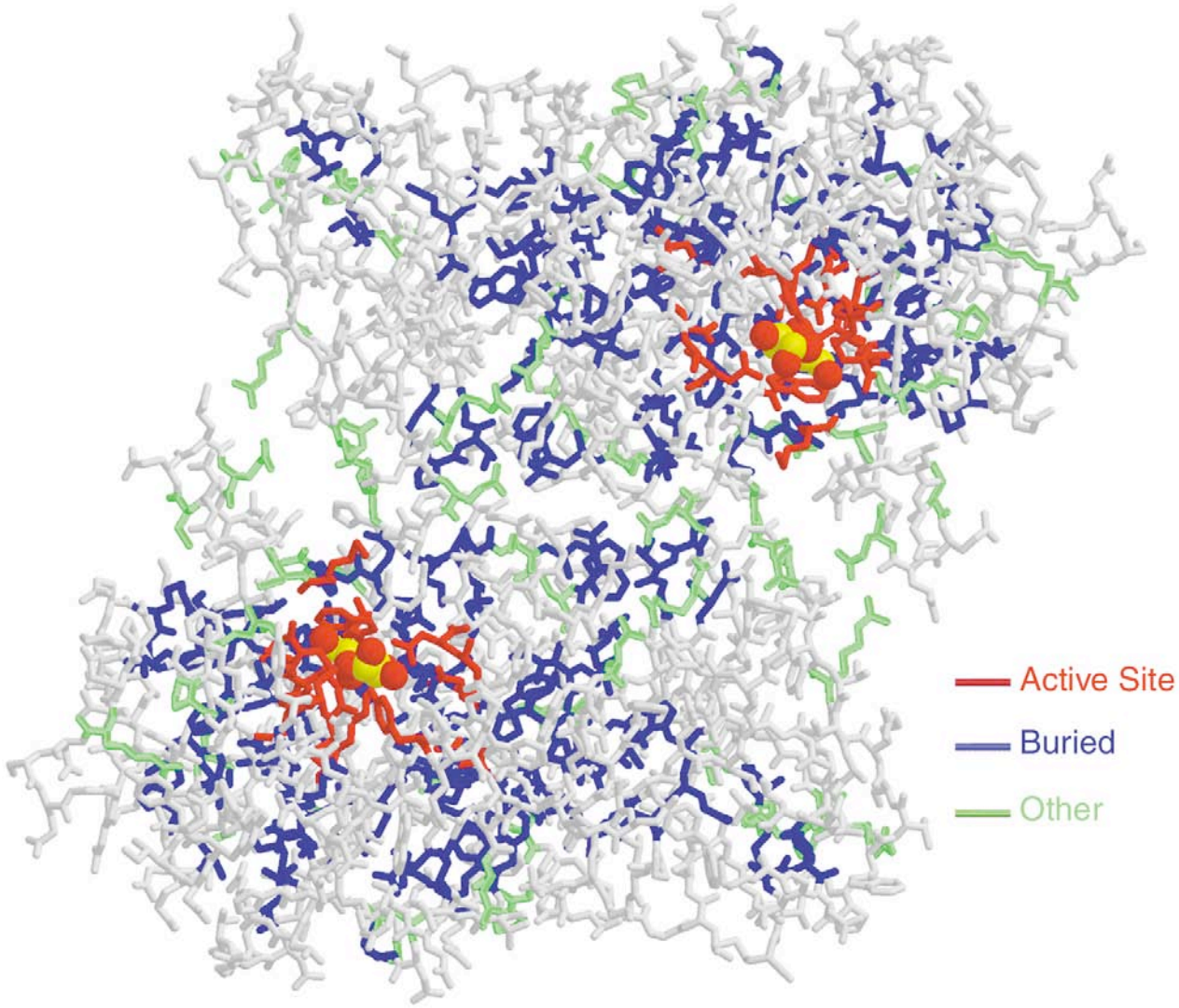


Garman *et al*. (2004) JMB **337**: 319

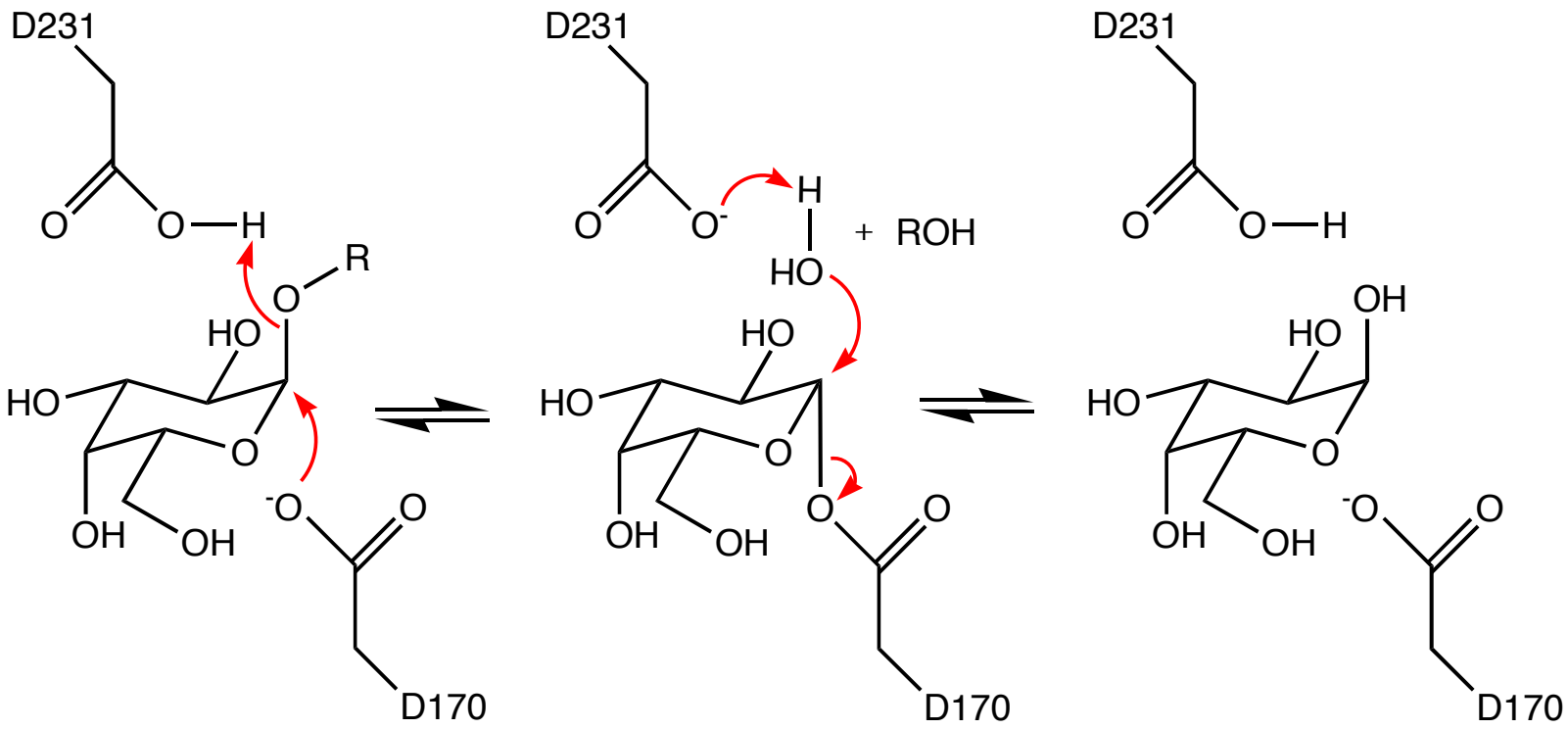
Individual mutations in Fabry disease

128	G	E	restricted phi/psi in turn	7.8	Other	Blanch (1996) Hum Mutat 8, 38
131	L	P	buried in hydrophobic pocket	0.0	Buried	Eng (1994) Hum Mol Genet 3, 1795
132	G	R	no room for side chain	0.0	Buried	Shabbeer (2002) Mol Genet Metab 76, 23
134	Y	S	active site residue	0.0	Active	Eng (1997) Mol Med 3, 174
		X	stop			Ashton-Prolla (2000) J Investig Med 48, 227
136	D	H	buried	0.1	Buried	Ashley (2001) J Hum Genet 46, 192
138	G	R	buried, no room for side chain	0.0	Buried	Eng (1997) Mol Med 3, 174
		E				Germain (2002) Mol Med 8, 306
141	T	I	buried, mutation removes N139 carbohydrate	0.1	Buried	Shabbeer (2002) Mol Genet Metab 76, 23
142	C	Y	disulfide required near active site	8.8	Active	Okumiya (1995) Hum Genet 95, 557
		R				Topaloglu (1999) Mol Med 5, 806
		X	stop			Topaloglu (1999) Mol Med 5, 806
143	A	P	larger side chain disrupts C52-C94 disulfide	3.0	Other	Eng (1994) Hum Mol Genet 3, 1795
		T				Eng (1997) Mol Med 3, 174
144	G	V	in turn; gly restricted phi/psi	5.6	Other	Eng (1994) Hum Mol Genet 3, 1795
146	P	(S)	partially hydrophobic	3.2	Other	Ploos van Amstel (1994) Hum Mol Genet 3, 503
148	S	R	buried, little room for larger side chain	0.7	Buried	Eng (1997) Mol Med 3, 174
		N				Ashton-Prolla (2000) J Investig Med 48, 227
152	Y	X	stop	1.4	-	Shabbeer (2002) Mol Genet Metab 76, 23
156	A	T	buried, no room for larger side chain	1.0	Buried	Eng (1994) Hum Mutat 3, 103
		V				Okumiya (1995) Hum Genet 95,557
		N				Azibi (2001) ASHG Abstract 2647
157	Q	X	stop	18.8	-	Eng (1994) Hum Mol Genet 3, 1795
162	W	R	mostly buried in hydrophobic pocket	2.7	Buried	Eng (1993) Am J Hum Genet 53, 1186
		C				Germain (1996) Hum Genet 98, 719
		X	stop			Rosenberg (1999) Hum Mutat Online, #292
163	G	V	in turn, restricted phi/psi	2.7	Other	Eng (1997) Mol Med 3, 174
165	D	V	buried ion pair w/H125	2.8	Buried	Davies (1994) Hum Mol Genet 3, 667
166	L	V	buried, little room for branch at beta carbon	0.4	Buried	Okumiya (1995) Hum Genet 95, 557
168	K	R	active site residue	3.3	Active	Shabbeer (2002) Mol Genet Metab 76, 23
169	F	S	buried in hydrophobic pocket	0.0	Buried	Ashley (2001) J Hum Genet 46, 192
170	D	V	catalytic residue in active site	0.0	Active	Eng (1997) Mol Med 3, 174
172	C	Y	required disulfide in active site	11.4	Active	Eng (1994) Hum Mol Genet 3, 1795

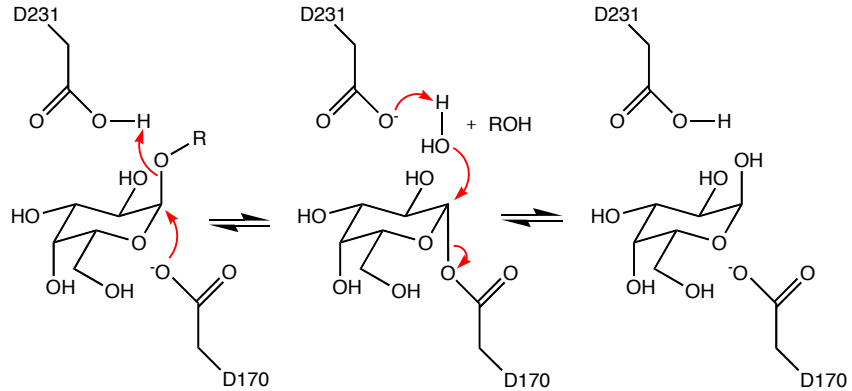
Individual mutations in Fabry disease



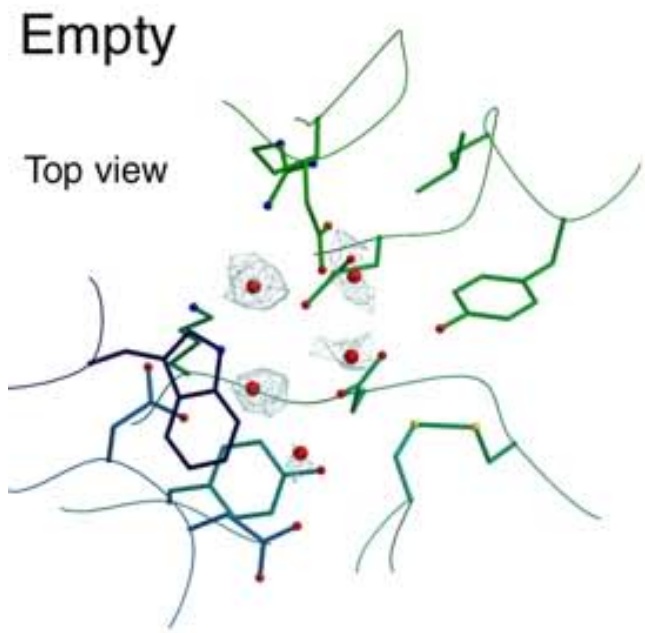
α-GAL catalytic mechanism



Steps in the catalytic cycle



Overall reaction: $E \rightleftharpoons E{\cdot}S \rightleftharpoons E{-}Int \rightleftharpoons E{\cdot}P_2 \rightleftharpoons E$ (S, P_1, H_2O, P_2)

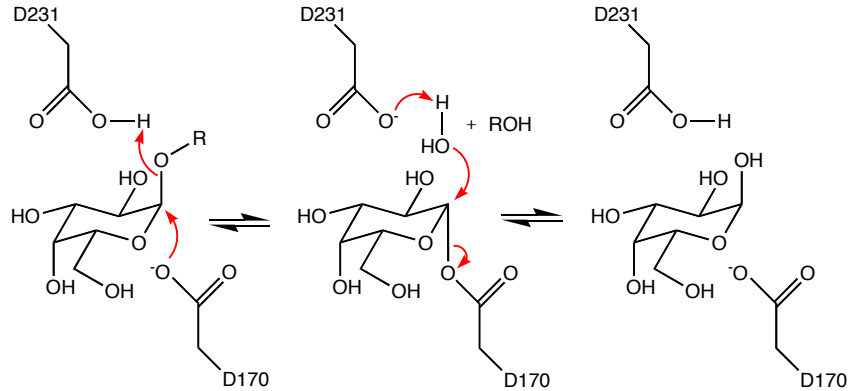


2.3Å

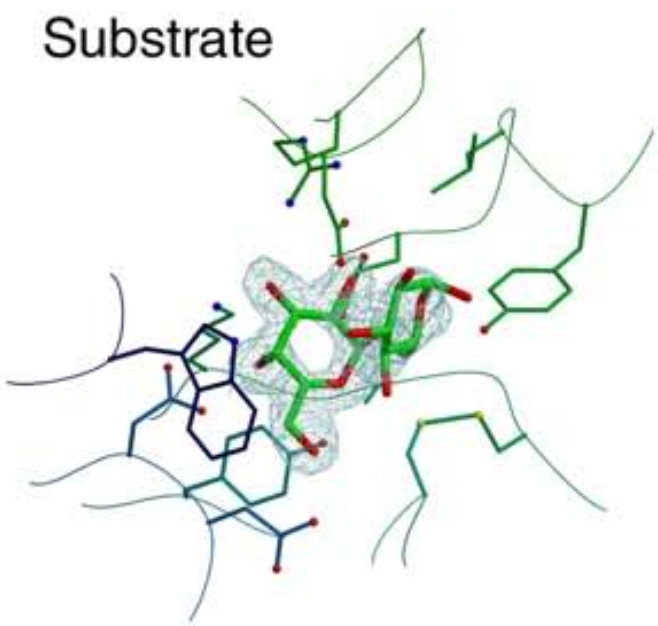
R_w/R_f 17.6% / 20.2%

Eric Salgado

Steps in the catalytic cycle



Overall reaction: $E \rightleftharpoons E{\cdot}S \rightleftharpoons E\text{–}Int \rightleftharpoons E{\cdot}P_2 \rightleftharpoons E$ (S, P_1, H_2O, P_2)

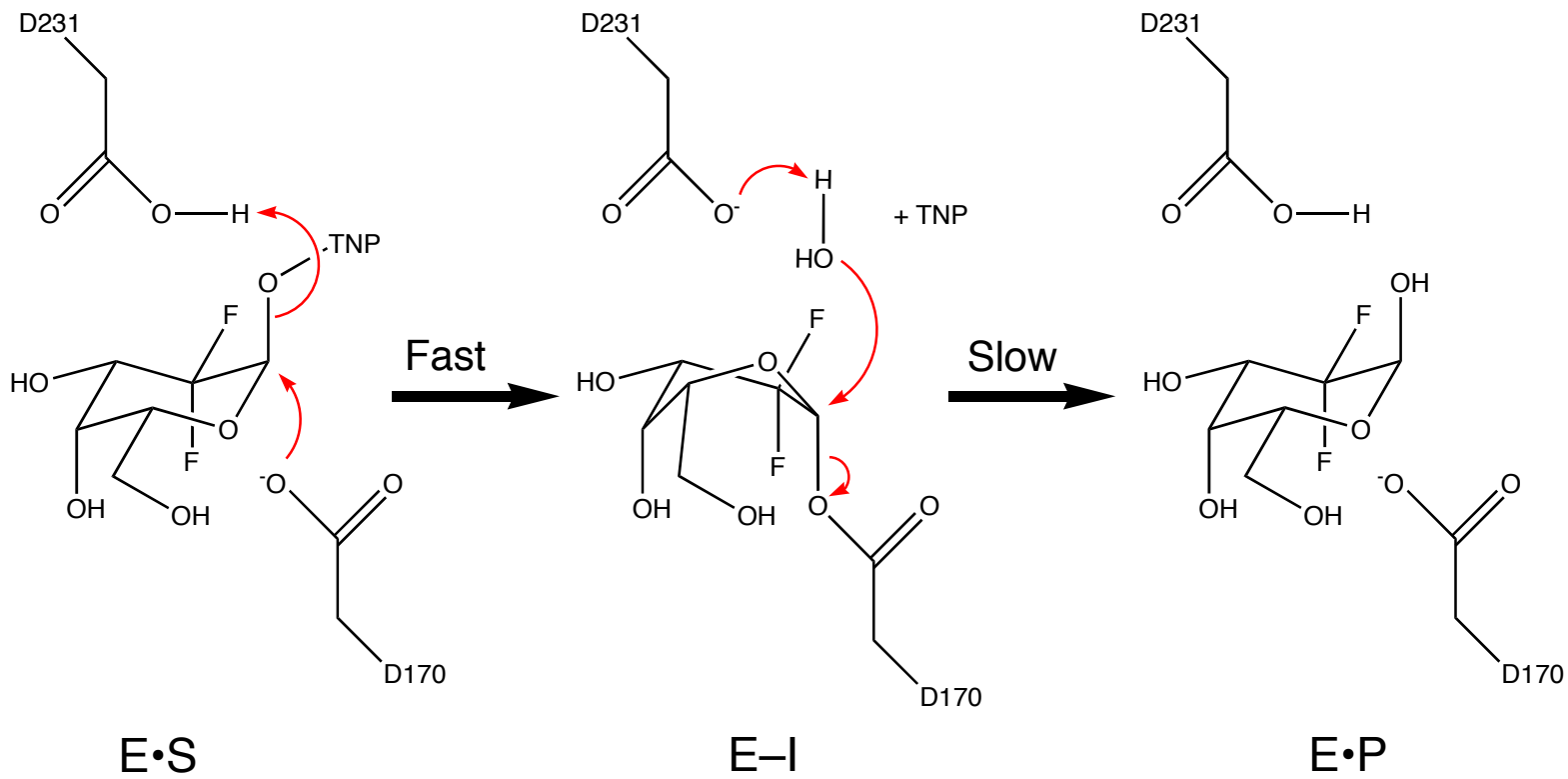


1.9Å

R_w/R_f 16.5% / 19.7%

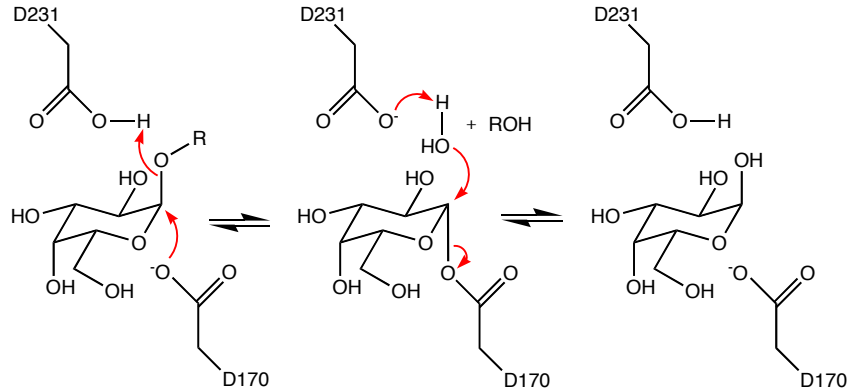
Abby Guce

Trapping a covalent intermediate

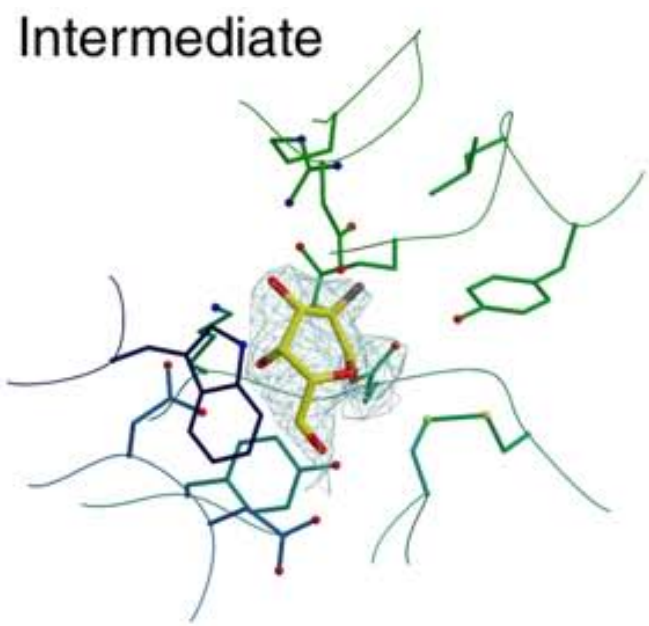


Harry Brumer, KTH Stockholm

Steps in the catalytic cycle



Overall reaction: $E \rightleftharpoons E{\cdot}S \rightleftharpoons E{-}Int \rightleftharpoons E{\cdot}P_2 \rightleftharpoons E$ (S, P_1, H_2O, P_2)

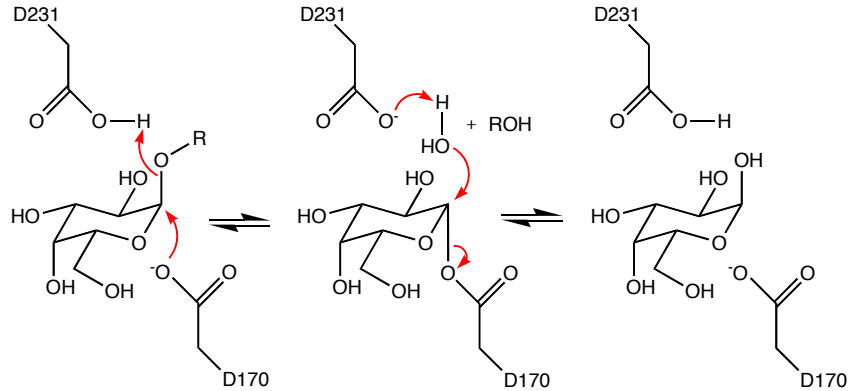


2.3Å

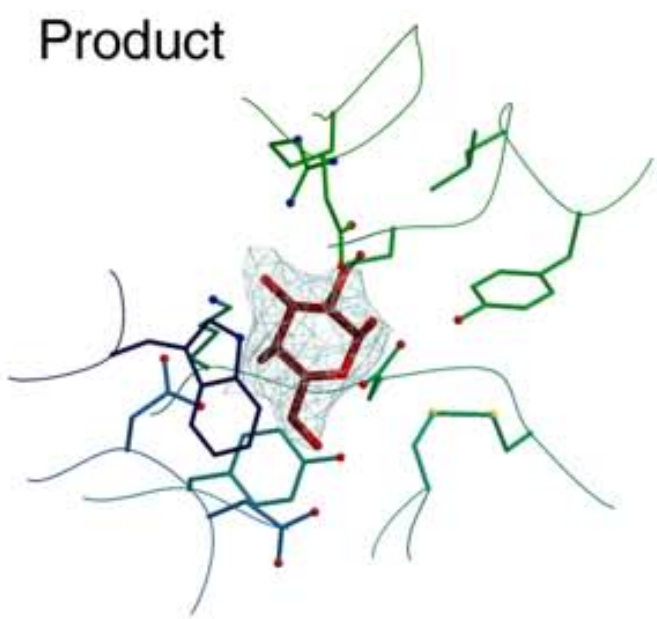
R_w/R_f 16.5% / 22.1%

Nat Clark

Steps in the catalytic cycle



Overall reaction: $E \rightleftharpoons E{\cdot}S \rightleftharpoons E\text{–}Int \rightleftharpoons E{\cdot}P_2 \rightleftharpoons E$ (S; P_1; H_2O; P_2)



2.3Å

R_w/R_f 19.1% / 22.7%

Eric Salgado

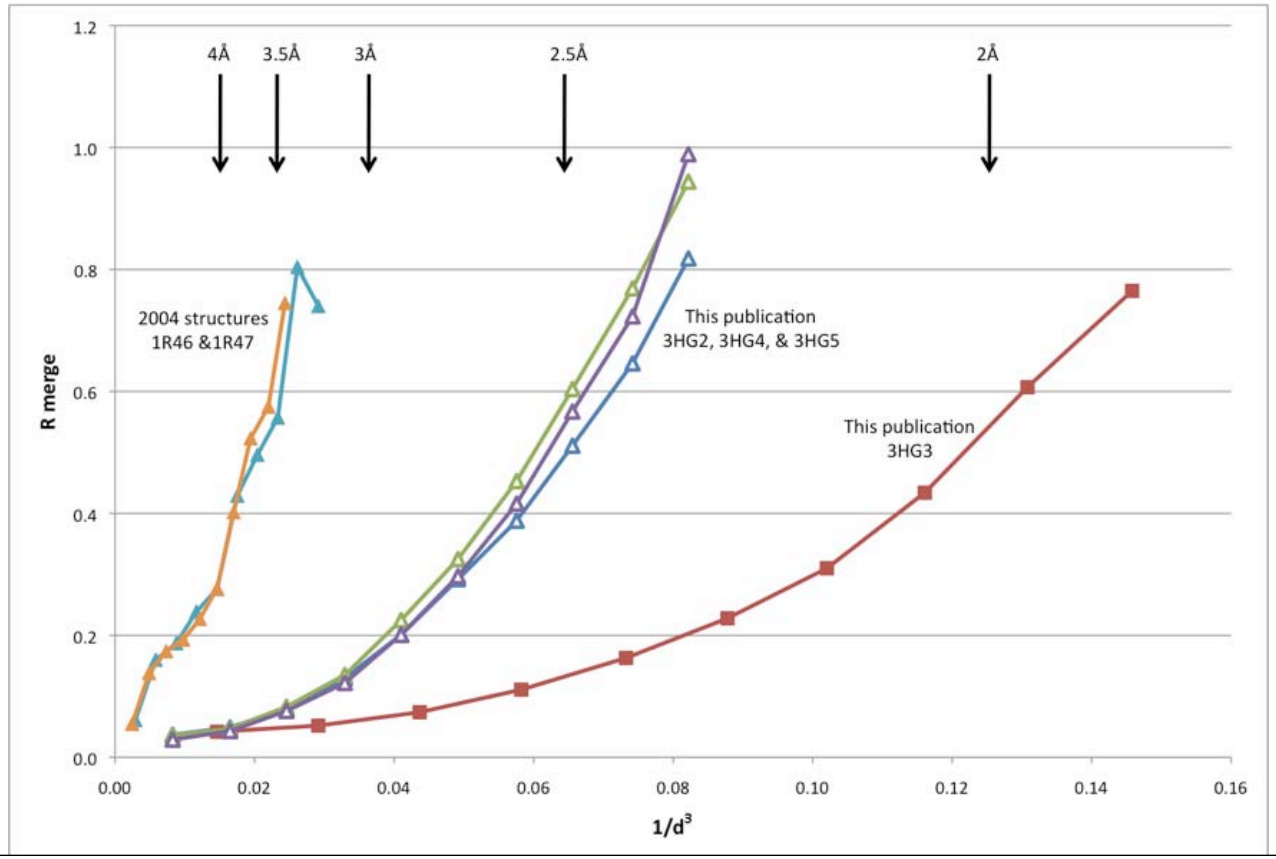
Steps in the catalytic cycle

Active site:	Empty	Substrate	Intermediate	Product
PDB code:	**3HG2**	**3HG3**	**3HG4**	**3HG5**
Protein				
Protein sequence	Wild type	D170A	Wild type	Wild type
Space group	$P3_221$	$P2_12_12_1$	$P3_221$	$P3_221$
Cell lengths, Å	90.8, 90.8, 217.2	59.5, 106.1, 181.7	90.2, 90.2, 216.6	90.8, 90.8, 217.2
Cell angles, °	90, 90, 120	90, 90, 90	90, 90, 120	90, 90, 120
X-ray data				
X-ray source	Cu anode	BNL X25	Cu anode	Cu anode
Wavelength, Å	1.54	1.0085	1.54	1.54
Resolution (last shell)	50-2.3 (2.38-2.3)	50-1.90 (1.97-1.9)	50-2.3 (2.38-2.3)	50-2.3 (2.38-2.3)
Observations	637,404	1,248,391	739,029	398,547
Unique observations	45,612	90,308	46,337	47,076
Completeness, % (last shell)	97.2 (95.4)	98.6 (88.4)	99.7 (100.0)	99.9 (100.0)
Multiplicity (last shell)	14.0 (14.2)	13.8 (9.9)	15.9 (16.0)	8.5 (8.4)
R_{sym} (last shell)[a]	0.115 (0.818)	0.088 (0.755)	0.112 (0.944)	0.099 (0.989)
I/σ_I (last shell)	25.5 (3.5)	30.2 (3.1)	28.3 (3.4)	21.4 (2.3)
Refinement				
R_{work}/R_{free},[b] %	17.6/20.2	16.5/19.7	16.5/22.1	19.1/22.7
No. of atoms	6767	7539	7251	6694
Protein	6255	6377	6262	6255
Carbohydrate	156	216	276	167
Water	329	893	682	236
Other	27	53	31	36
Average B, Å^2	36.4	23.1	49.8	43.4

Guce, Clark *et al.*, (2010) JBC **285**, 3625

Referee comments

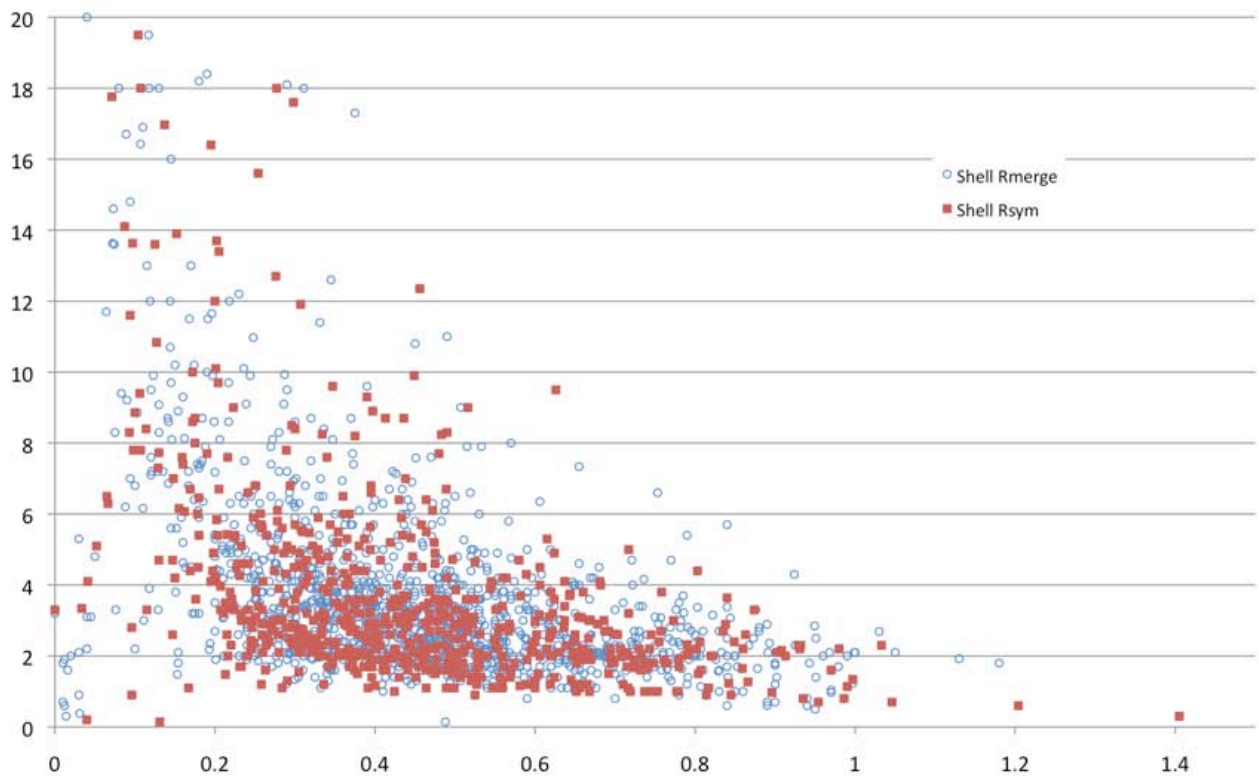
"B. It is a mistake of the authors to choose not to collect the best (synchrotron) data for their complexes. Why report these at "2.3A" resolution when they could have been at 1.9, or better? It looks very unprofessional. In the US synchrotron access is trivial, often with remote data collection and couriered crystals. There is no reason why this hasn't been done correctly (especially given the next point)."



Referee comments

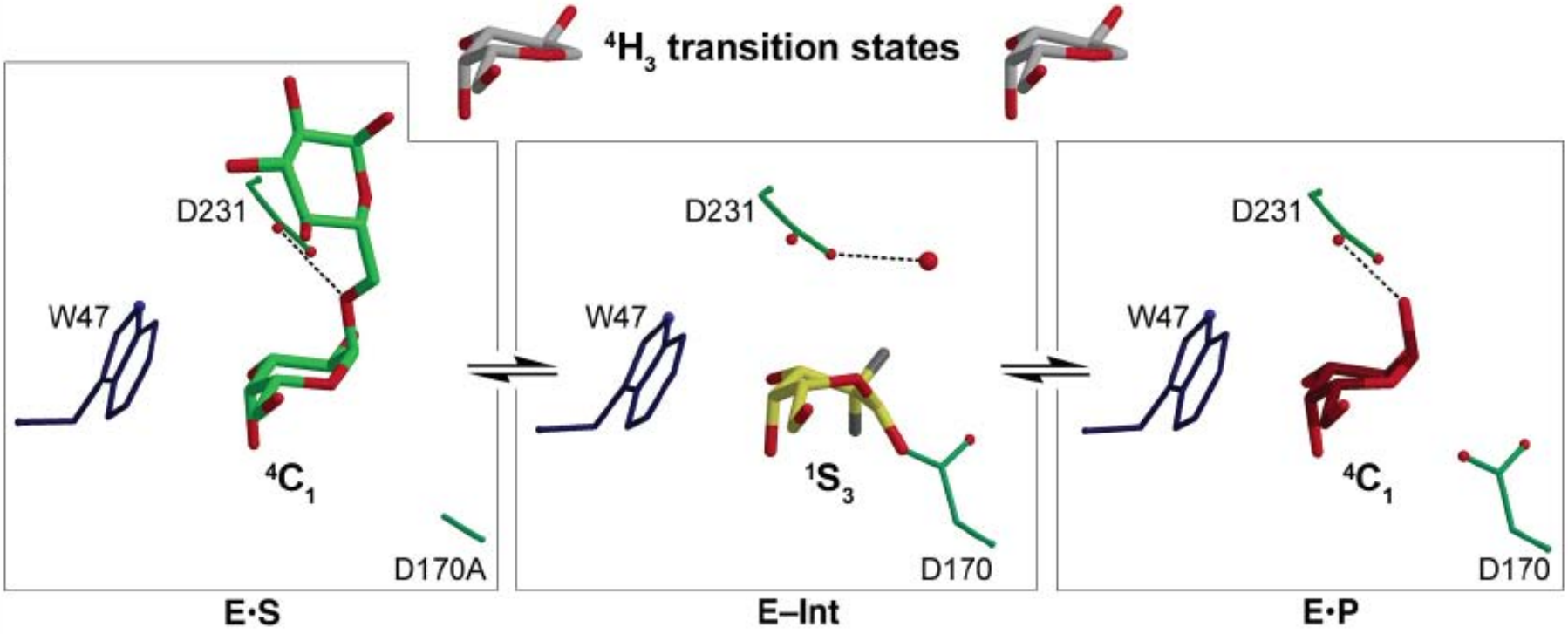
"C. The authors' outer resolution bin Rmerges are from 75 to 100%. (a) They must put up a good argument why these values are acceptable (in terms of map quality, Rcryst stats in these outer bins, likelihood weighting approaches) and (b) even if they choose to use these data, they should stick with current norms and report the structure – in the text – at a more or less community acceptable Rmerge of 50% so we can compare these structures with those from other groups. This is especially important as the authors are claiming that these structures are improvements over past "3.25A" structures. It may well be that those in this paper are equally well 3.2A by any other groups' criteria? ..."

I/σ
in the
last shell



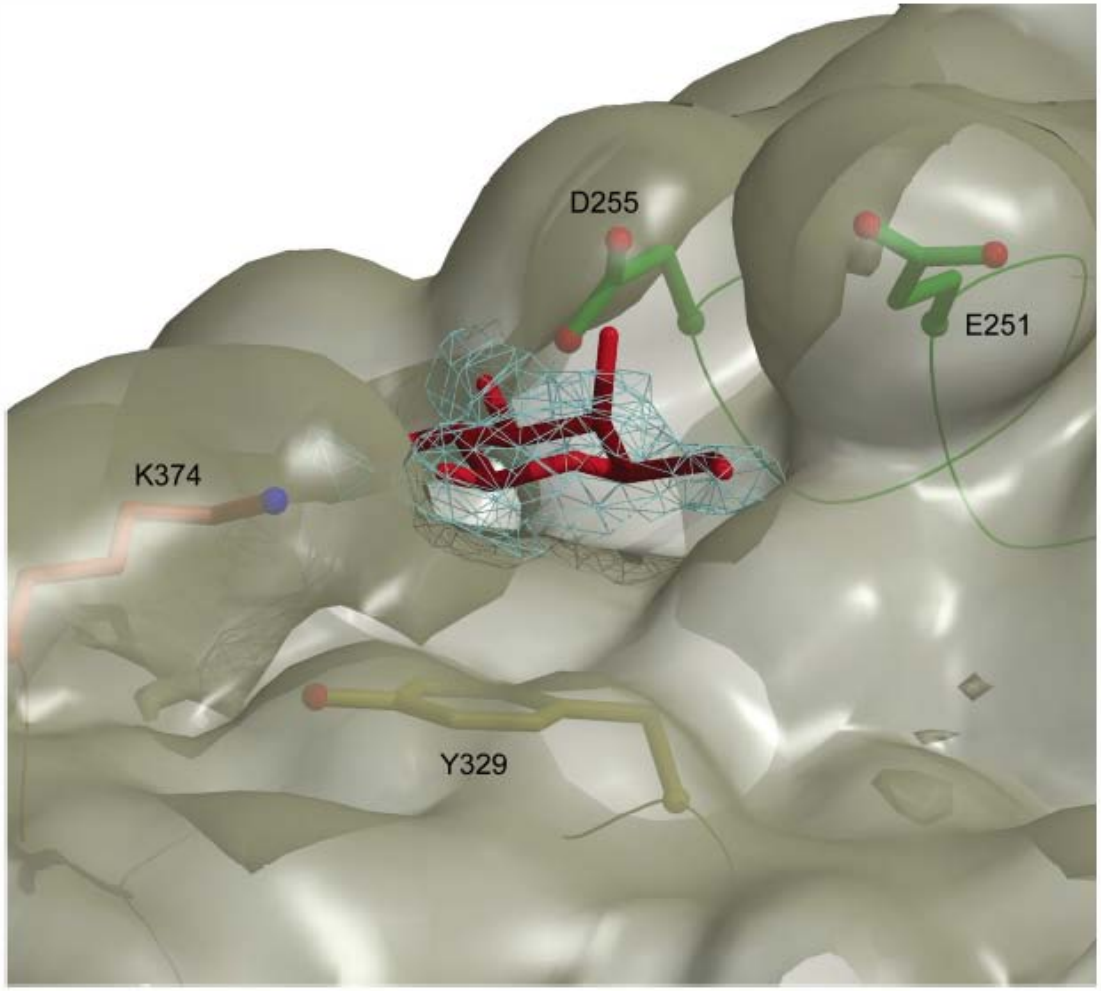
R_{merge} or R_{sym} in the last shell

Modeling the high energy transition states



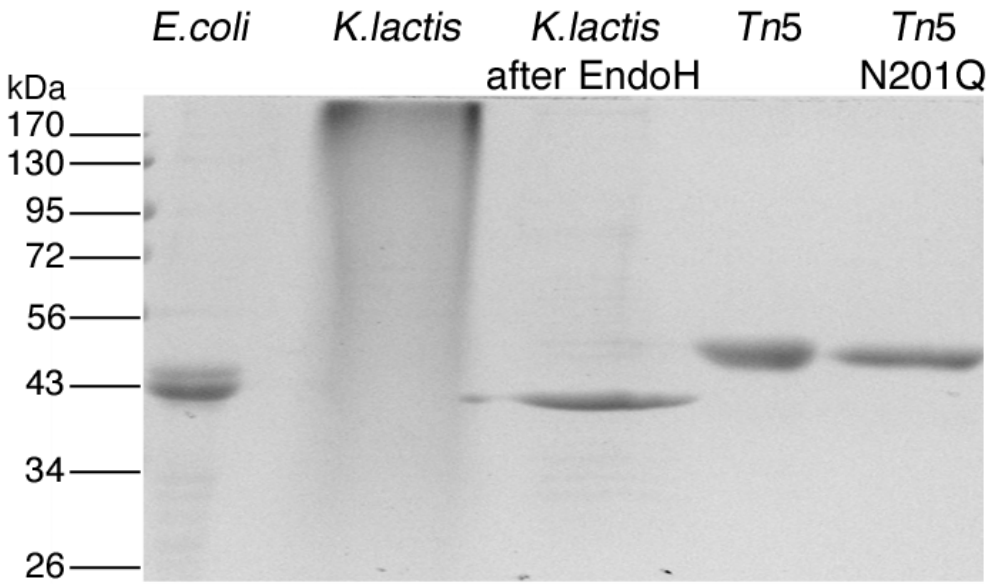
Guce, Clark *et al.*, (2010) JBC **285**, 3625

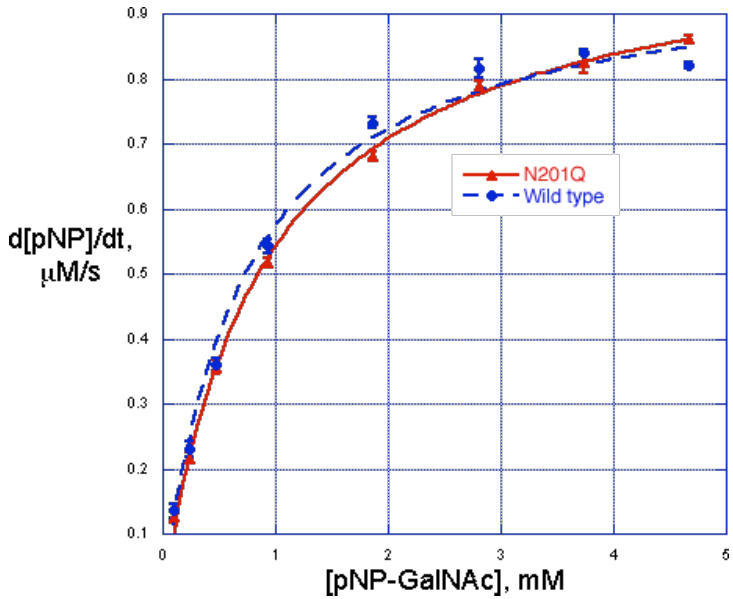
Second binding site on α-Gal



Guce, Clark *et al.*, (2010) JBC **285**, 3625

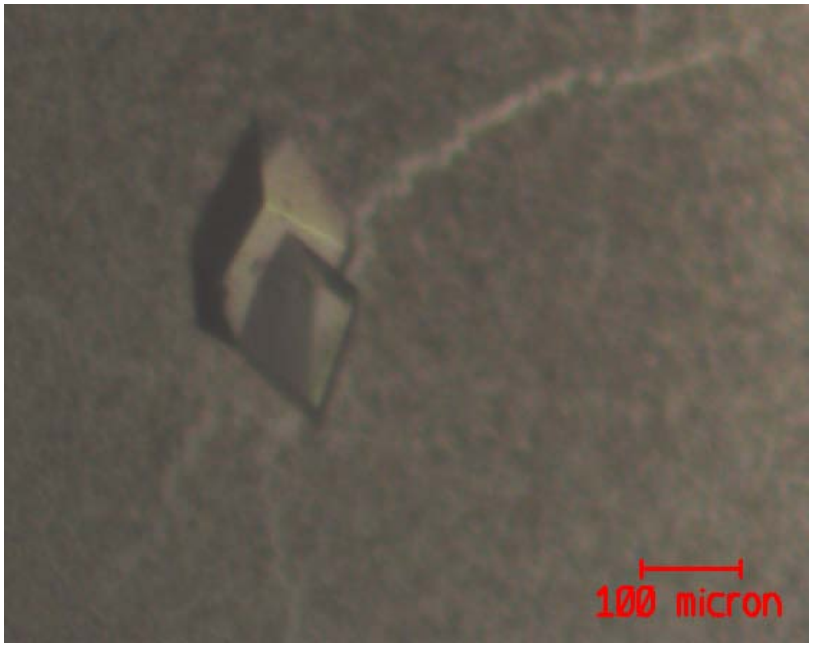
Human α-NAGAL expression



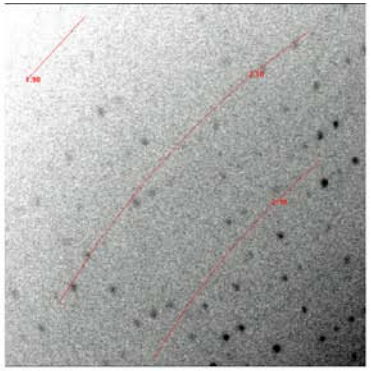


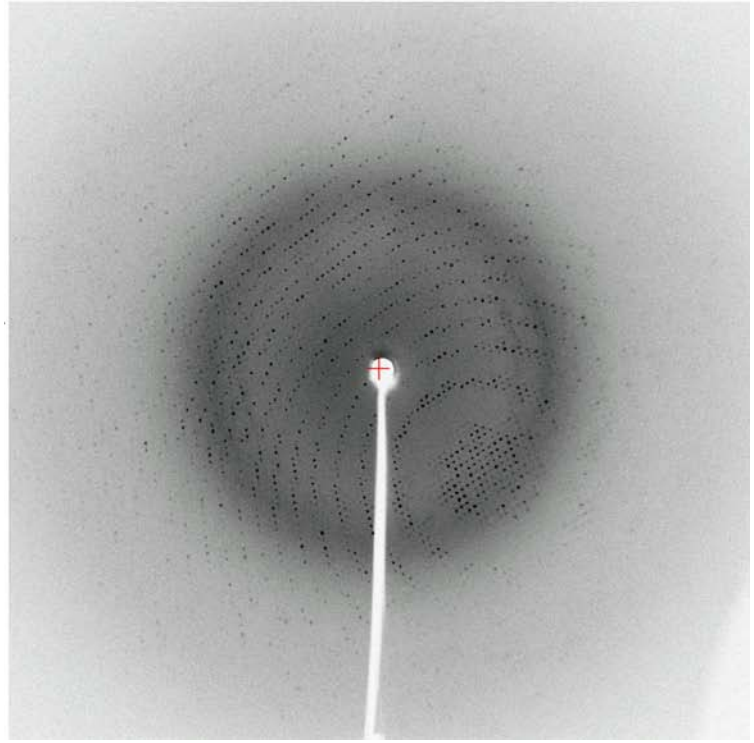
Clark & Garman (2009) JMB **393**, 435

Human α-NAGAL crystallization



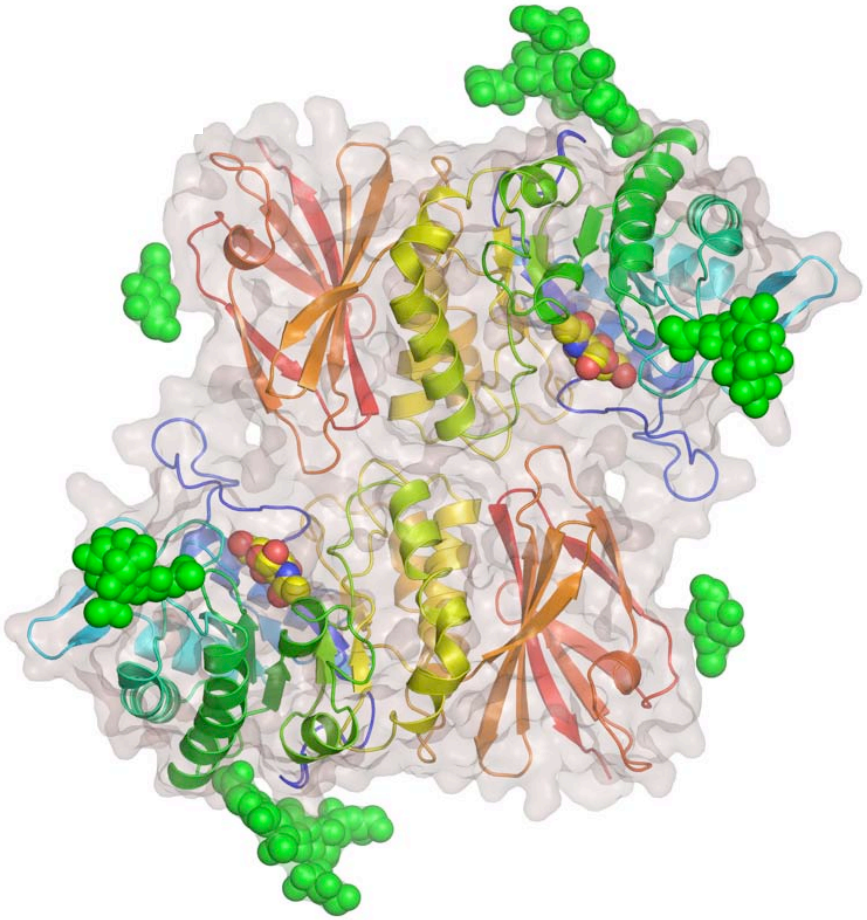
Four structures from this!

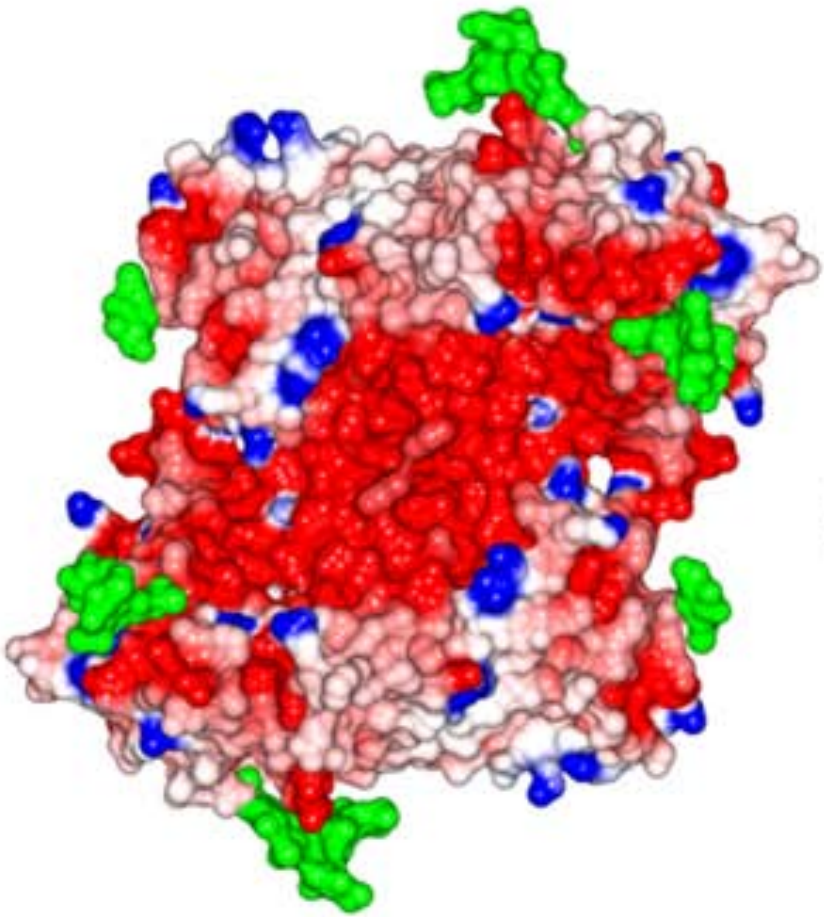




Nat Clark

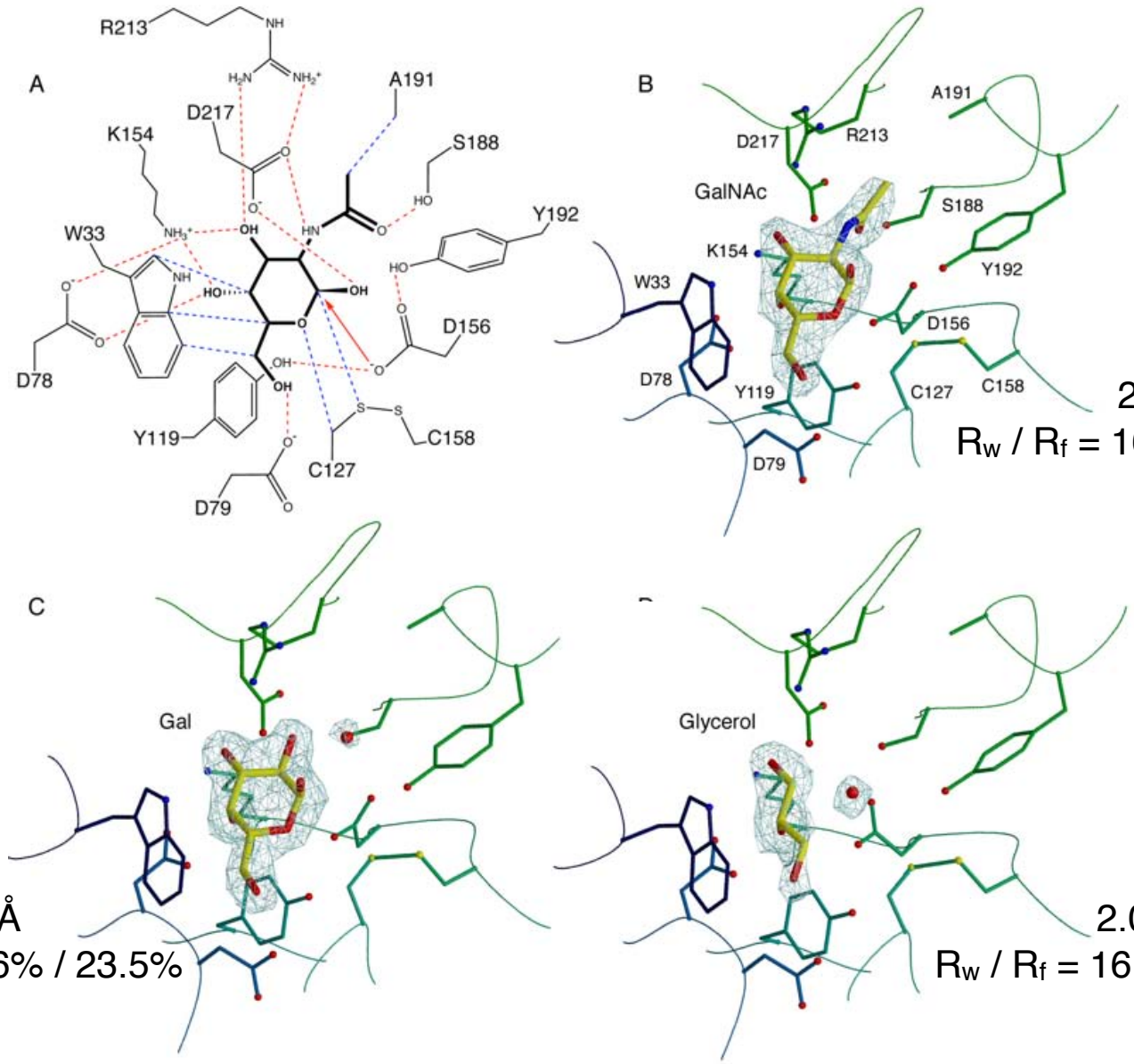
Human α-NAGAL structure





Nat Clark

Human α-NAGAL ligand binding



2.2Å
R_w / R_f = 16.5% / 20.5%

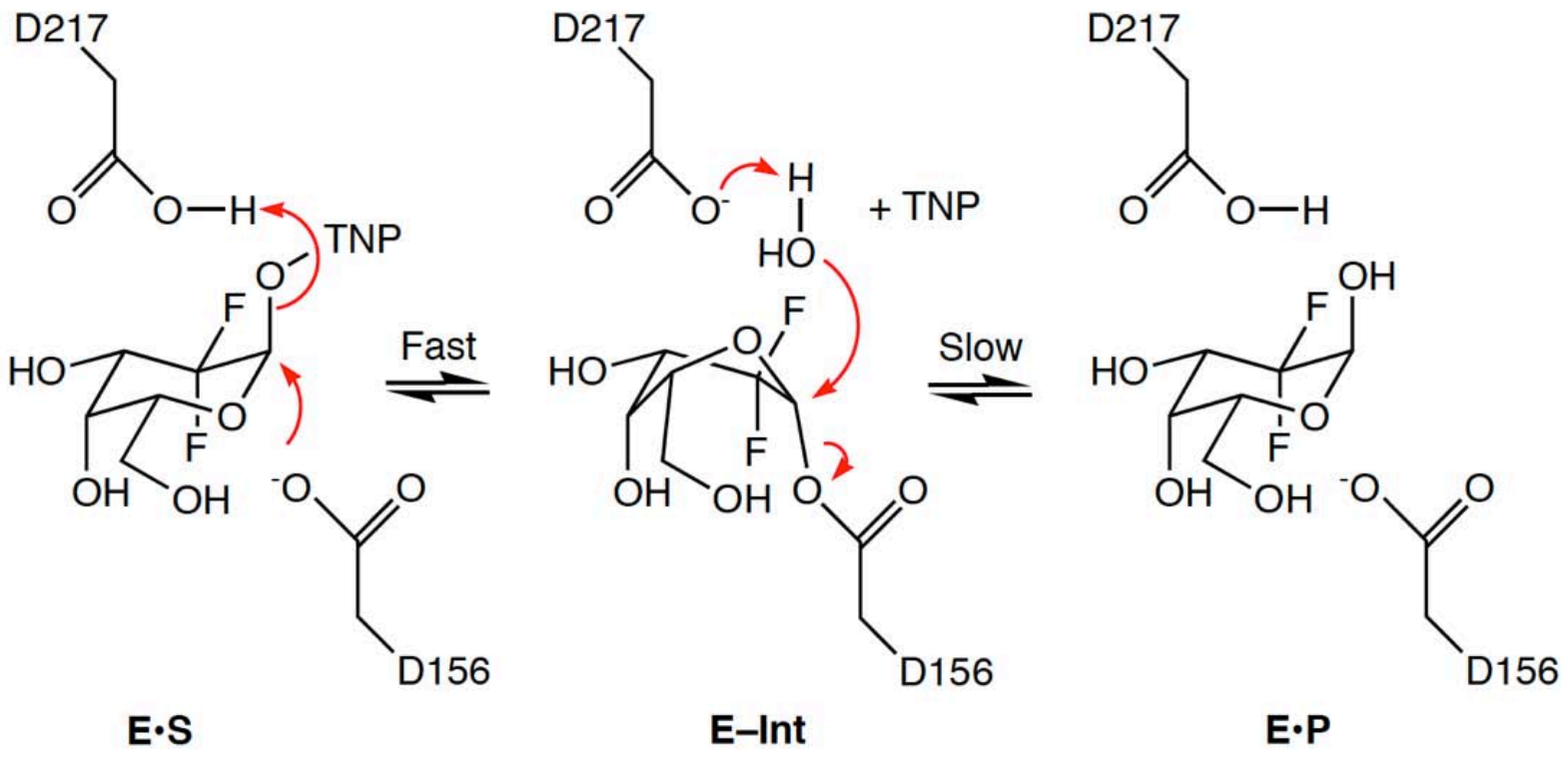
1.9Å
R_w / R_f = 19.6% / 23.5%

2.0Å
R_w / R_f = 16.1% / 19.4%

Clark & Garman (2009) JMB **393**, 435

Referee's comments

"2. The mechanism would be more convincing if the authors would isolate, crystallize and determine the structure of the covalent intermediate at high resolution. Co-crystallizing the enzyme with 2-fluoro-α-gal-fluoride or related compound would allow the authors to produce the structure of the intermediate. Contacting Steve Withers would be very helpful and he might even have the appropriate compound for them to try..."



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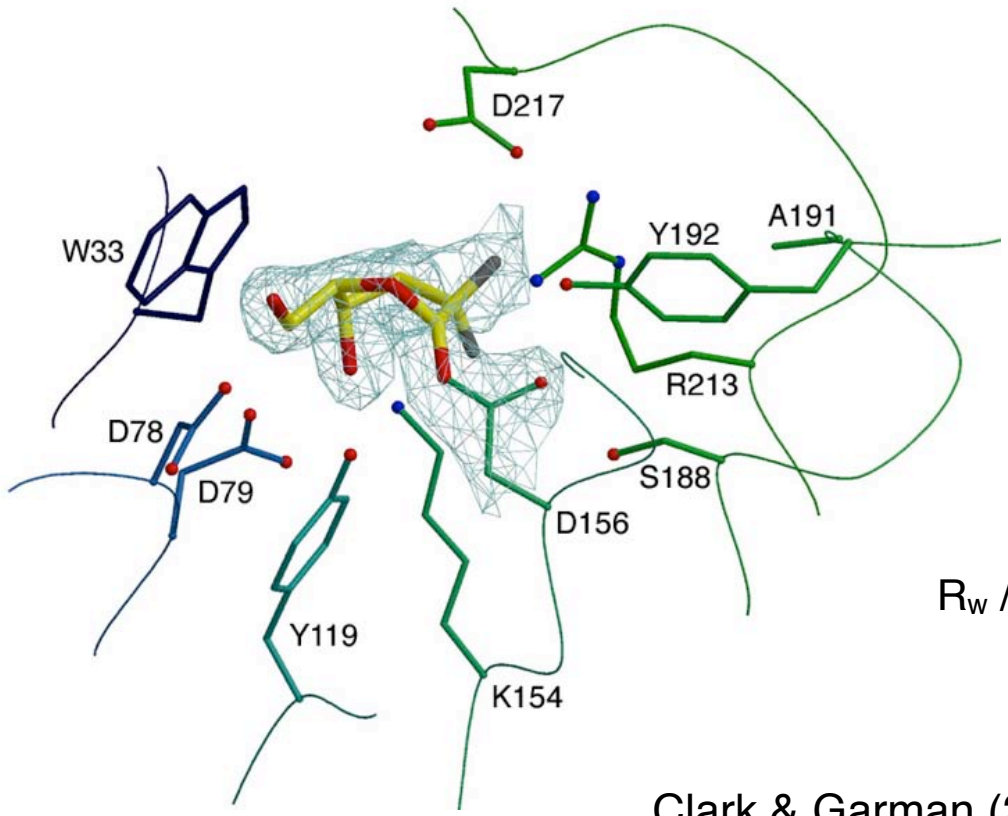


2.15Å

R_w / R_f = 16.4% / 19.9%

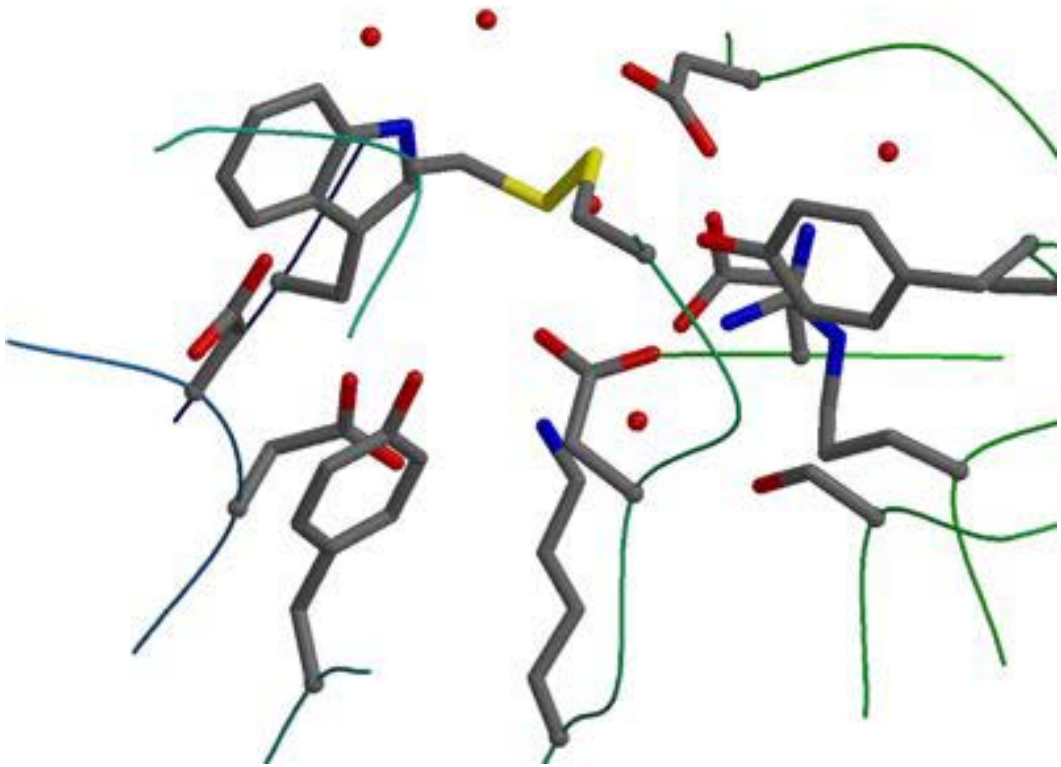
Clark & Garman (2009) JMB **393**, 435

Human α-NAGAL structures

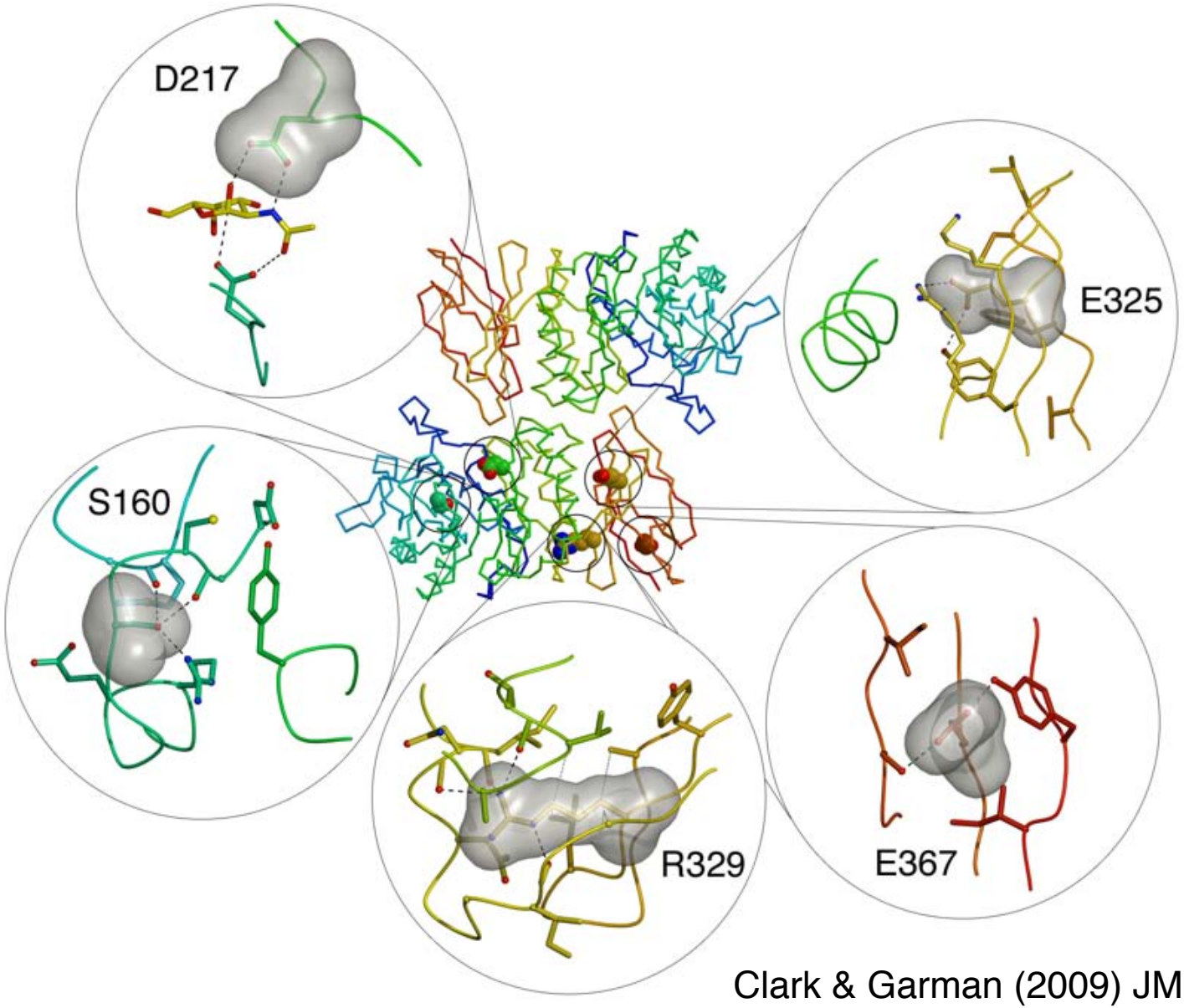
	Protein Data Bank accession code			
	3H53	3H54	3H55	3IGU
Data collection				
Ligand soak	None	GalNAc	Gal	F_2-α-Gal-TNP
X-ray source	Rotating anode	Rotating anode	BNL X6A	BNL X6A
Wavelength (Å)	1.54	1.54	0.979	0.980
Space group	*C*2	*C*2	*C*2	*C*2
Cell parameters				
a, *b*, *c* (Å)	153.5, 114.3, 68.4	153.4, 114.2, 68.3	153.9, 114.5, 68.4	151.6, 113.6, 68.4
α, β, γ (°)	90, 96.1, 90	90, 95.6, 90	90, 96.2, 90	90, 96.1, 90
Resolution (last shell) (Å)	50–2.01 (2.08–2.01)	50–2.2 (2.28–2.2)	50–1.9 (1.97–1.9)	50–2.15 (2.23–2.15)
No. of observations (last shell)	772,346 (60,051)	356,155 (24,762)	319,671 (32,173)	265,173 (21,625)
Unique observations (last shell)	77,785 (7283)	59,205 (5733)	87,369 (8937)	62,455 (6056)
Completeness (last shell) (%)	99.3 (93.0)	99.1 (95.9)	95.8 (98.2)	99.7 (97.4)
Multiplicity (last shell)	9.9 (8.2)	6.0 (4.2)	3.7 (3.6)	4.2 (3.5)
R_{sym} (last shell)[a]	0.085 (0.610)	0.106 (0.674)	0.117 (0.698)	0.078 (0.634)
$\langle I/\sigma_I \rangle$ (last shell)	29.0 (3.2)	15.9 (2.0)	10.1 (1.7)	17.6 (1.8)
Refinement				
R_{work}/R_{free}[b]	0.161/0.194	0.165/0.205	0.196/0.235	0.164/0.199

Clark & Garman (2009) JMB **393**, 435

α-NAGAL reaction cycle

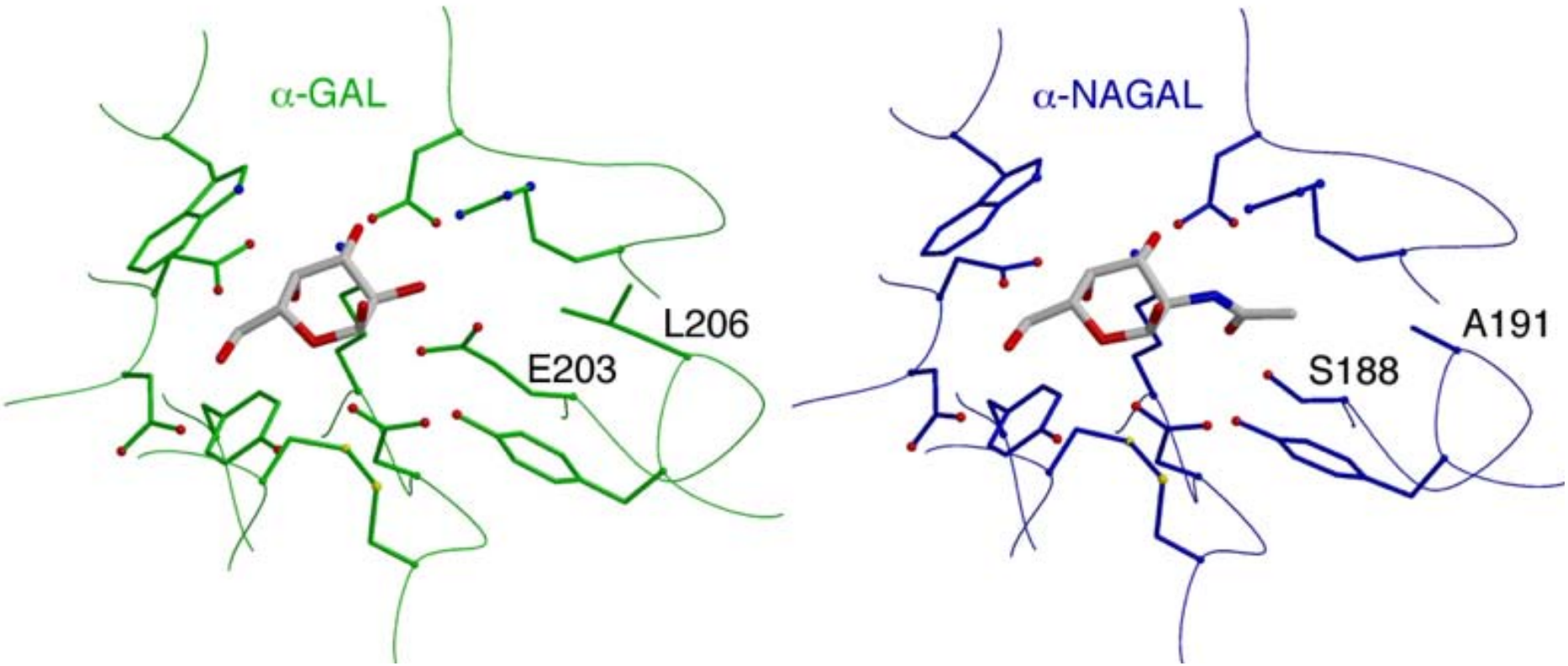


Schindler disease mutations



Clark & Garman (2009) JMB **393**, 435

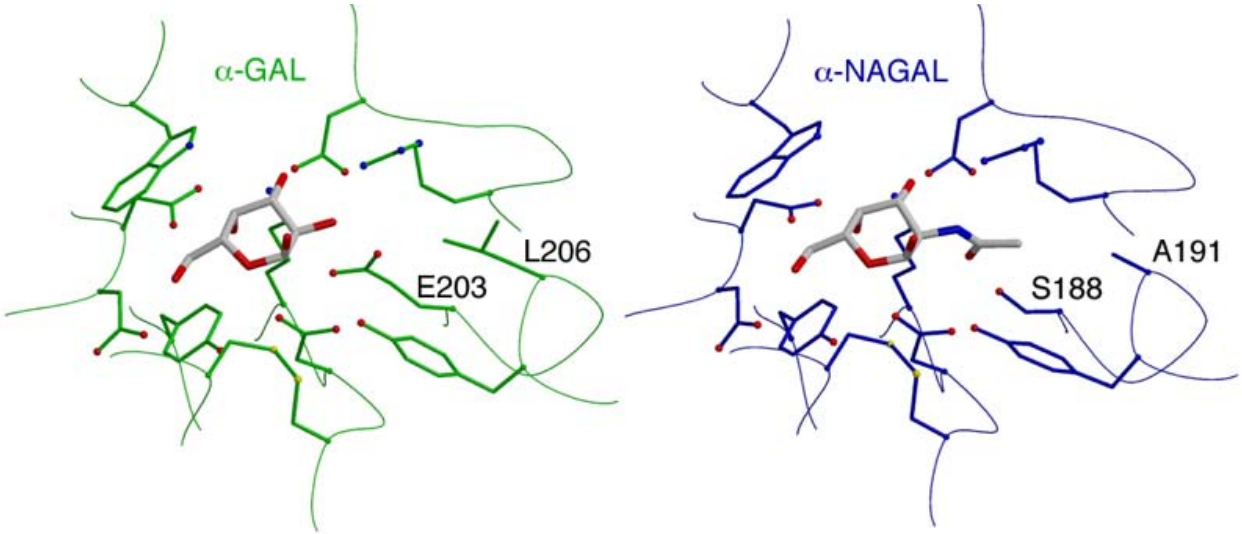
Active site similarities

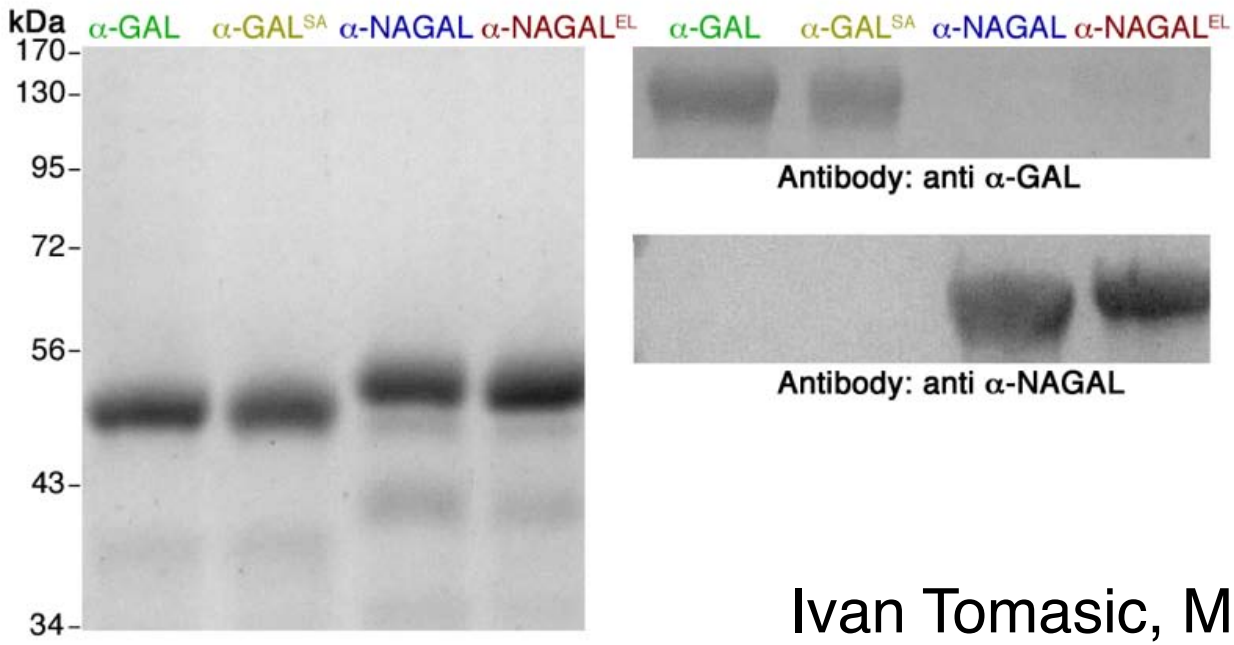


Garman *et al.*, (2004) JMB **337**: 319

Clark & Garman (2009) JMB **393**, 435

Active site similarities





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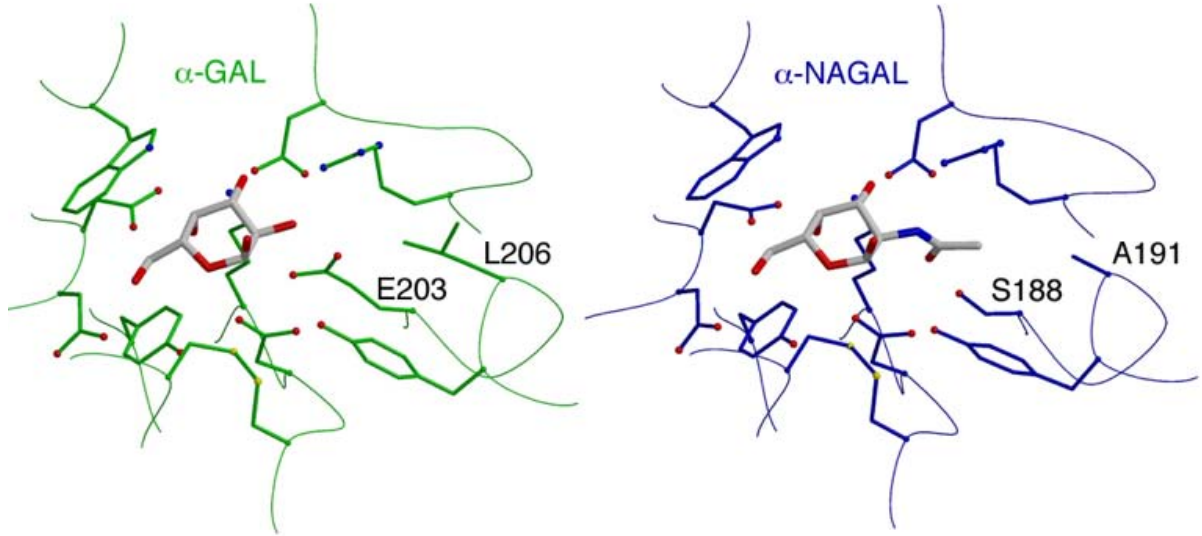
Active site similarities



Substrate:	pNP-α-Gal			pNP-α-GalNAc		
Enzyme	K_M (mM)	k_{cat} (sec^{-1})	k_{cat} / K_M (mM^{-1} sec^{-1})	K_M (mM)	k_{cat} (sec^{-1})	k_{cat} / K_M (mM^{-1} sec^{-1})
α-GAL	6.88 ± 0.07	37.8 ± 0.2	5.49 ± 0.06	No activity detected *		
α-GAL^{SA}	49.1 ± 7.2	1.20 ± 0.14	0.024 ± 0.005	21.0 ± 0.8	21.5 ± 0.7	1.03 ± 0.03
α-NAGAL	27.5 ± 4.7	10.7 ± 0.9	0.39 ± 0.07	0.68 ± 0.01	15.1 ± 0.1	22.4 ± 0.1
α-NAGAL^{EL}	7.58 ± 0.07	13.7 ± 0.1	1.81 ± 0.02	No activity detected *		

* $k_{cat} < 0.01$ sec^{-1}

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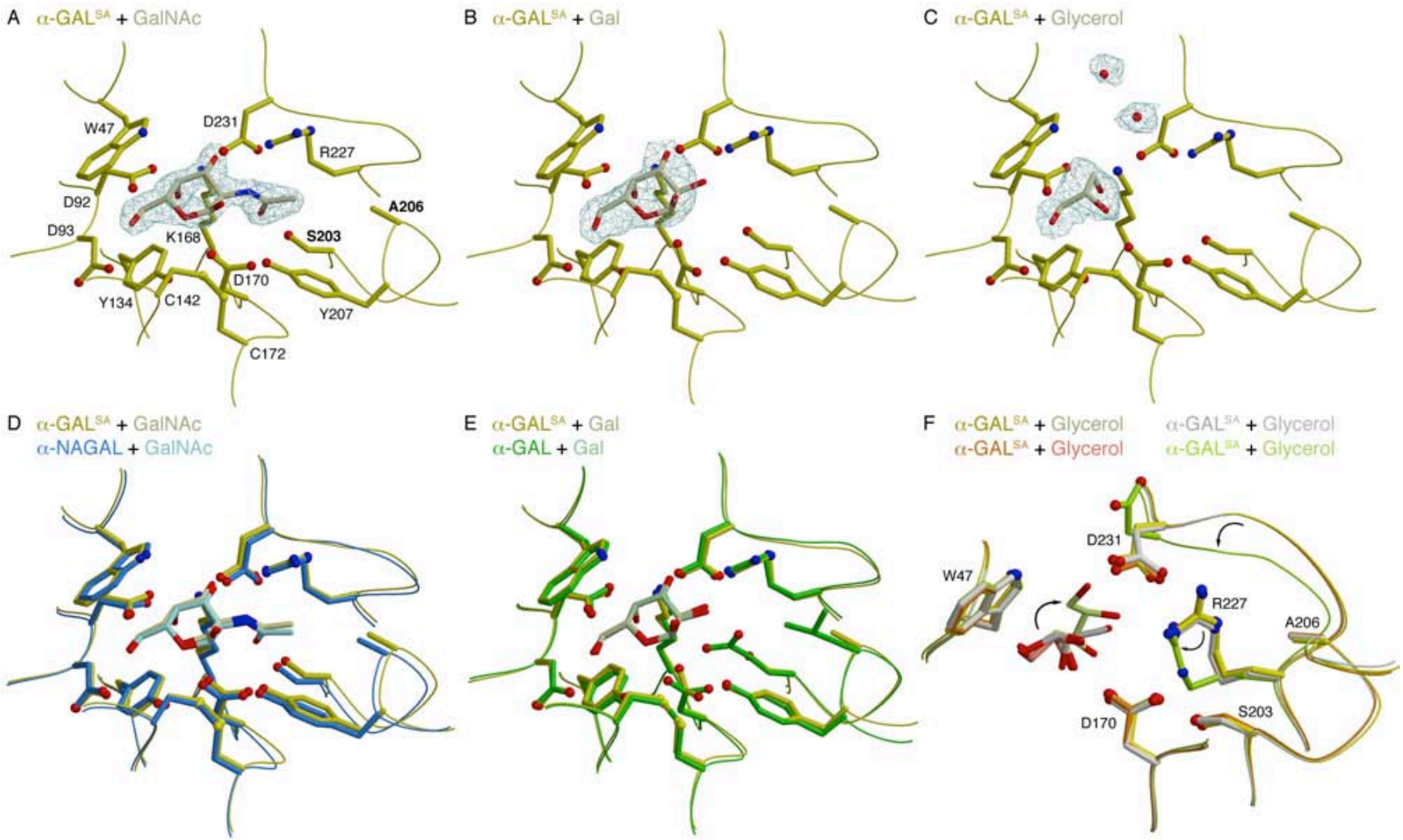
Interconverting substrate specificities



Tomasic, Metcalf, et al., submitted

Interconverting substrate specificities

Ligand:	GalNAc	Galactose	Glycerol	Glycerol
PDB ID:	3LX9	3LXA	3LXB	3LXC
Data Collection:				
Beamline	APS 24-ID-C	NSLS X6A	NSLS X6A	APS 24-ID-C
Wavelength (Å)	1.07188	0.98010	0.98010	1.07188
Space group	$C222_1$	$P2_12_12_1$	$P2_12_12_1$	$C222_1$
Resolution (Å) (last shell)	50-2.05 (2.09-2.05)	50-3.0 (3.11-3.0)	50-2.85 (2.90-2.85)	50-2.35 (2.39-2.35)
Cell parameters *a*, *b*, *c* (Å)	89.95, 139.49, 182.58	59.50, 105.85, 181.85	59.57, 106.92, 181.51	89.75, 139.77, 182.45
No. of observations	481,042	172,299	136,330	193,566
No. of unique observations (last shell)	72,009 (3,354)	23,638 (2,309)	27,897 (1,334)	47,535 (2,335)
Multiplicity (last shell)	6.7 (3.6)	7.3 (7.2)	4.9 (4.9)	4.1 (4.0)
Completeness (%) (last shell)	99.4 (93.3)	100.0 (99.9)	99.3 (99.9)	98.6 (98.7)
R_{sym} (last shell)	0.120 (0.701)	0.271 (0.851)	0.177 (0.616)	0.153 (0.862)
I/σ_I (last shell)	18.6 (1.6)	7.6 (1.8)	9.1 (2.2)	12.3 (2.0)
Refinement:				
R_{work}/R_{free} , %	17.62/21.82	21.45/24.39	22.50/26.38	18.35/23.68

Tomasic, Metcalf, et al., submitted

Acknowledgements

UMass Amherst
Eric Salgado
Abby Guce
Rishi Arora
Nat Clark
Cassidy Dobson
Nilima Kolli
Yadilette Rivera-Colón
Matt Metcalf
Ivan Tomasic
Michelle Dyke
Jia Lin
Yurie Kim
Jerome Rogich

NIH/NIAID
Dave Garboczi
Linda Hannick

NY Blood Center
Alex Zhu

NIH/NINDS
Roscoe Brady
Markus Ries
Raphael Schiffmann

KTH Stockholm
Harry Brumer

Transkaryotic Therapies

Genzyme

UMass Amherst Macromolecular X-ray Facility
NSLS X6A & X25, APS SBC, SER-CAT, & NE-CAT

Charles H. Hood Foundation
Institute for Cellular Engineering (NSF)
Chemistry-Biology Interface program (NIH)
NIH R01-DK76877