Supporting Information

Mechanism of the human nucleocytoplasmic hexosaminidase D

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Table S1. Primers used for mutagenesis of HexD.

Mutant	**Primer**
D148A Forward	CTGCACATCGGGTGTGCTGAGGTCTATTACCTC
D148A Reverse	GAGGTAATAGACCTCAGCACACCCGATGTGCAG
D148N Forward	CTGCACATCGGGTGTAATGAGGTCTATTACC
D148N Reverse	GGTAATAGACCTCATTACACCCGATGTGCAG
E149A Forward	CACATCGGGTGTGATGCGGTCTATTACCTCGGAG
E149A Reverse	CTCCGAGGTAATAGACCGCATCACACCCGATGTG
E149Q Forward	CACATCGGGTGTGATCAGGTCTATTACCTCG
E149Q Reverse	CGAGGTAATAGACCTGATCACACCCGATGTG
H92A Forward	GTGCAGACATTTGGAGCCATGGAGTTTGTGCT
H92A Reverse	AGCACAAACTCCATGGCTCCAAATGTCTGCAC

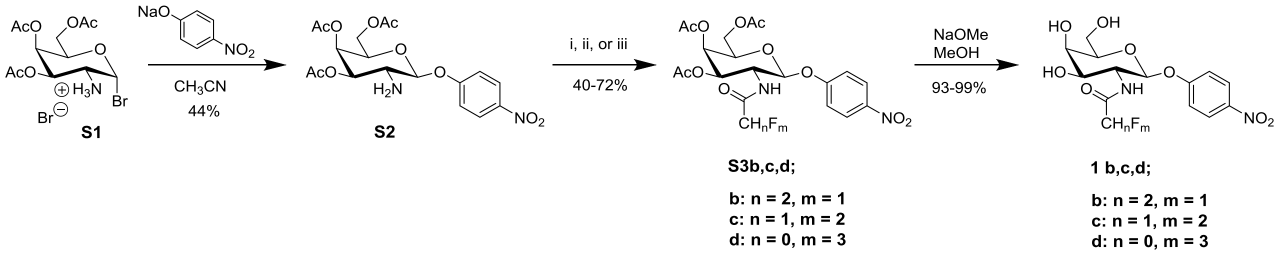
Table S2. Spectrophotometric parameters used for monitoring of Brønsted analyses.

Substrate	**pK_a**[a]	**λ (nm)**	**ε (M^{-1} cm^{-1})**[b]	**$[E]_0$ WT (μM)**	**$[E]_0$ D148A (μM)**	**$[E]_0$ E149A (μM)**
3,4-NO_2P-GalNAc (**1e**)	5.42	400	12900	0.1	1	0.1
3F4NP-GalNAc (**1f**)	6.42	388	17600	0.1	1	0.1
pNP-GalNAc (**1a**)	7.18	400	13210	0.1	2	0.1
4MU-GalNAc (**3**)	7.5	360	8600	0.2	5	0.2
mNP-GalNAc (**1g**)	8.29	330	650	1	--	1
pClP-GalNAc (**1h**)	9.47	280	720	2	--	5
P-GalNAc (**1i**)	9.99	269	969	2	--	--
pOMeP-GalNAc (**1j**)	10.2	296	1670	2	--	--

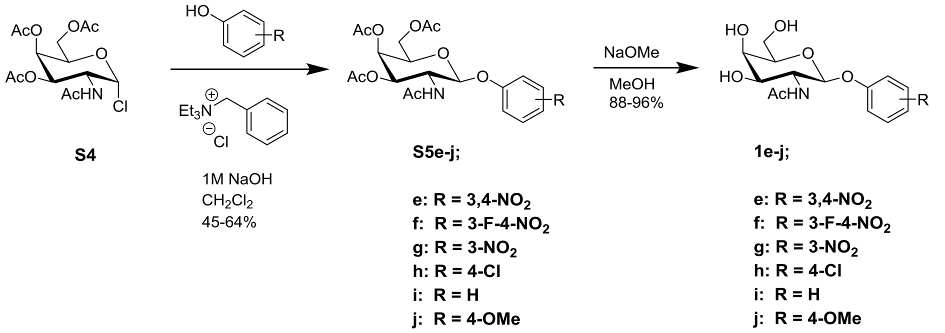
[a] pK_a values were obtained from previously reported analyses [1-3].

[b] Extinction coefficients were obtained from Macauley *et al.* [4] with the exception of 4-methoxyphenyl-*O*-GalNAc which was calculated experimentally.

Synthetic Schemes S1 and S2: Synthesis of galactosaminide substrates.

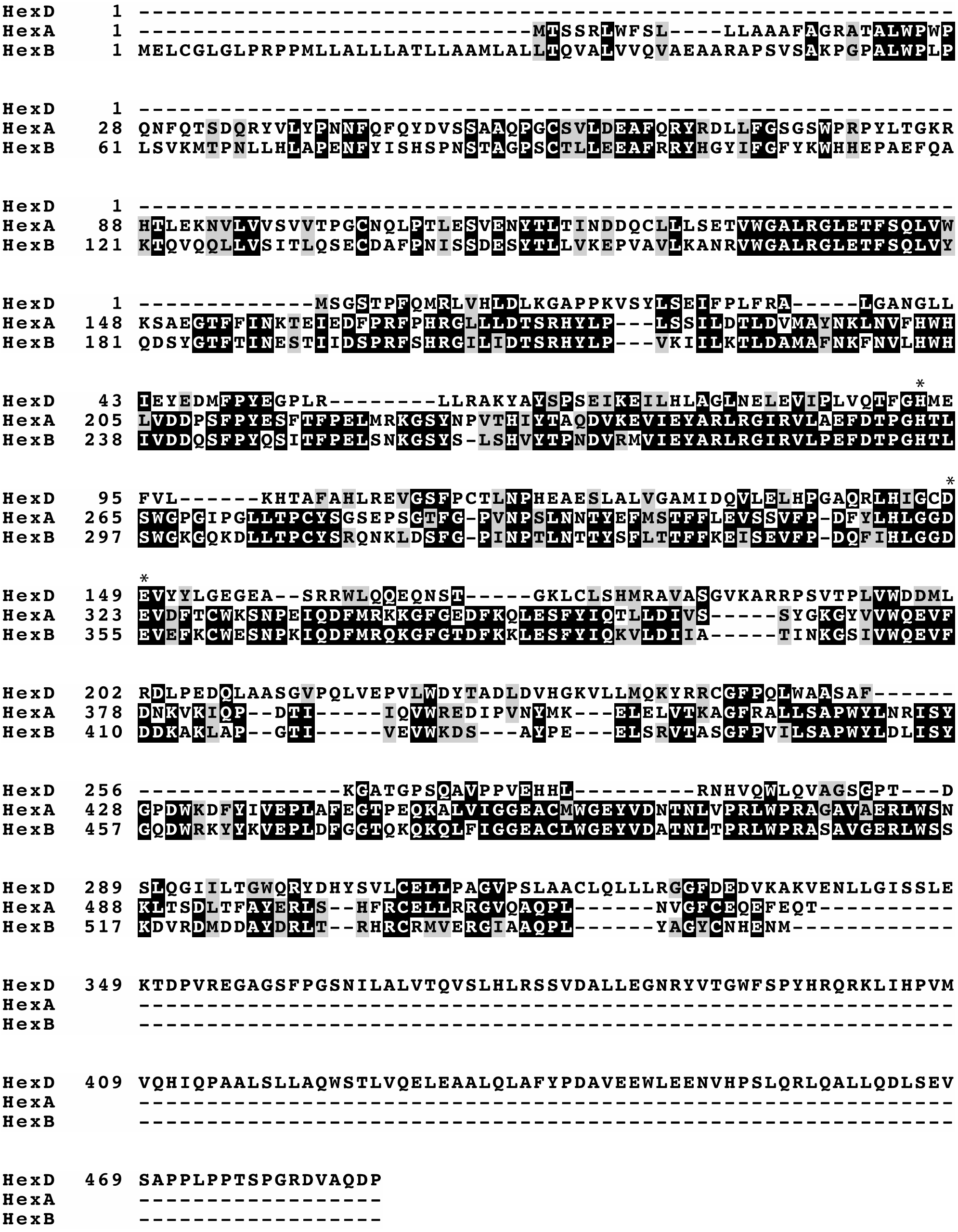


Scheme S1: Synthesis of fluorinated *N*-acyl substrates. (i) $NaOOCCH_2F$, Amberlite-120 H^+, HBTU, Et_3N, DMF, 0°C, 70%; (ii) $HOOCH_2F$, HBTU, Et_3N, DMF, 0°C, 62%; (iii) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, 0°C, 40%.



Scheme S2: Synthesis of aryl *N*-acetylgalactosamine substrate analogues.

Figure S1. Sequence alignment of HexD with the lysosomal ß-hexosaminidases HexA and HexB. Black shading shows identical residues; grey shading shows similar residues. Starred residues were mutated as part of this study. The alignment was performed in Clustal Omega and figure produced in BoxShade.



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HexD     1 ------------------------------------------------------------
HexA     1 -----------------------------MTSSRLWFSL----LLAAAFAGRATALWPWP
HexB     1 MELCGLGLPRPPMLLALLLATLLAAMLALLTQVALVVQVAEAARAPSVSAKPGPALWPLP

HexD     1 ------------------------------------------------------------
HexA    28 QNFQTSDQRYVLYPNNFQFQYDVSSAAQPGCSVLDEAFQRYRDLLFGSGSWPRPYLTGKR
HexB    61 LSVKMTPNLLHLAPENFYISHSPNSTAGPSCTLLEEAFRRYHGYIFGFYKWHHEPAEFQA

HexD     1 ------------------------------------------------------------
HexA    88 HTLEKNVLVVSVVTPGCNQLPTLESVENYTLTINDDQCLLLSETVWGALRGLETFSQLVW
HexB   121 KTQVQQLLVSITLQSECDAFPNISSDESYTLLVKEPVAVLKANRVWGALRGLETFSQLVY

HexD     1 -------------MSGSTPFQMRLVHLDLKGAPPKVSYLSEIFPLFRA-----LGANGLL
HexA   148 KSAEGTFFINKTEIEDFPRFPHRGLLLDTSRHYLP---LSSILDTLDVMAYNKLNVFHWH
HexB   181 QDSYGTFTINESTIIDSPRFSHRGILIDTSRHYLP---VKIILKTLDAMAFNKFNVLHWH

                                                                    *
HexD    43 IEYEDMFPYEGPLR--------LLRAKYAYSPSEIKEILHLAGLNELEVIPLVQTFGHME
HexA   205 LVDDPSFPYESFTFPELMRKGSYNPVTHIYTAQDVKEVIEYARLRGIRVLAEFDTPGHTL
HexB   238 IVDDQSFPYQSITFPELSNKGSYS-LSHVYTPNDVRMVIEYARLRGIRVLPEFDTPGHTL

                                                                      *
HexD    95 FVL------KHTAFAHLREVGSFPCTLNPHEAESLALVGAMIDQVLELHPGAQRLHIGCD
HexA   265 SWGPGIPGLLTPCYSGSEPSGTFG-PVNPSLNNTYEFMSTFFLEVSSVFP-DFYLHLGGD
HexB   297 SWGKGQKDLLTPCYSRQNKLDSFG-PINPTLNTTYSFLTTFFKEISEVFP-DQFIHLGGD

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HexD   149 EVYYLGEGEA--SRRWLQQEQNST-----GKLCLSHMRAVASGVKARRPSVTPLVWDDML
HexA   323 EVDFTCWKSNPEIQDFMRKKGFGEDFKQLESFYIQTLLDIVS-----SYGKGYVVWQEVF
HexB   355 EVEFKCWESNPKIQDFMRQKGFGTDFKKLESFYIQKVLDIIA-----TINKGSIVWQEVF

HexD   202 RDLPEDQLAASGVPQLVEPVLWDYTADLDVHGKVLLMQKYRRCGFPQLWAASAF------
HexA   378 DNKVKIQP--DTI-----IQVWREDIPVNYMK---ELELVTKAGFRALLSAPWYLNRISY
HexB   410 DDKAKLAP--GTI-----VEVWKDS---AYPE---ELSRVTASGFPVILSAPWYLDLISY

HexD   256 ---------------KGATGPSQAVPPVEHHL---------RNHVQWLQVAGSGPT---D
HexA   428 GPDWKDFYIVEPLAFEGTPEQKALVIGGEACMWGEYVDNTNLVPRLWPRAGAVAERLWSN
HexB   457 GQDWRKYYKVEPLDFGGTQKQKQLFIGGEACLWGEYVDATNLTPRLWPRASAVGERLWSS

HexD   289 SLQGIILTGWQRYDHYSVLCELLPAGVPSLAACLQLLLRGGFDEDVKAKVENLLGISSLE
HexA   488 KLTSDLTFAYERLS--HFRCELLRRGVQAQPL------NVGFCEQEFEQT----------
HexB   517 KDVRDMDDAYDRLT--RHRCRMVERGIAAQPL------YAGYCNHENM------------

HexD   349 KTDPVREGAGSFPGSNILALVTQVSLHLRSSVDALLEGNRYVTGWFSPYHRQRKLIHPVM
HexA       ------------------------------------------------------------
HexB       ------------------------------------------------------------

HexD   409 VQHIQPAALSLLAQWSTLVQELEAALQLAFYPDAVEEWLEENVHPSLQRLQALLQDLSEV
HexA       ------------------------------------------------------------
HexB       ------------------------------------------------------------

HexD   469 SAPPLPPTSPGRDVAQDP
HexA       ------------------
HexB       ------------------
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