

SUPPORTING INFORMATION

Bacterial Expression of Human Butyrylcholinesterase as a Tool for Nerve Agent Bioscavengers Development.

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Figure S1. Primary sequence alignment of wild-type human BChE and the seven *PROSS* proposed constructs. *PROSS* conserved residues are represented in red for the maintain of BChE activity and in cyan for the dimer interface. Mutated residues are highlighted in grey. Positions are numbered from the physiologically matured protein.

hBCHE-WT	1	EDDIIITKNGKVRGMNLTVFGGTVTAFLGIPYAQPPLGRLRFKKPQSLTKWSDIWNATKYANSCC QNI DQSFPGF HGSE MWNP NTDLS EDCL YLN VWIP APKPK NATV	109
hBCHE-1	0	MEDDIIITKNGKVRGMNLTVFGGTVTAFLGIPYAQPPLGRLRFKKPQPLTKWSGIWNATKYANSCC QNI DQSFPGF HGSE MWNP NTDLS EDCL YLN VWIP APKPK NATV	109
hBCHE-2	0	MEDDIIITKNGKVRGMNLTVFGGTVTAFLGIPYAQPPLGRLRFKKPQPLTKWSGIWNATKY PNSCC QNI DQSFPGF HGSE MWNP NTDLS EDCL YLN VWIP APKPK NATV	109
hBCHE-3	0	MEDDIIITKNGKVRGMNLTVFGGTVTAFLGIPYAQPPLGRLRFKKPQPLTKWSGIWNATKYANSCC QNI DQSFPGF HGSE MWNP NTDLS EDCL YLN VWIP APKPK NATV	109
hBCHE-4	0	MEDDIIITKNGKVRGMNLTVFGGTVTAFLGIPYAQPPLGRLRFKKPQPLTKWSGIWNATKYANSCC QNI DQSFPGF HGSE MWNP NTDLS EDCL YLN VWIP APKPK NATV	109
hBCHE-5	0	MEDDIIITKNGKVRGMNLTVFGGTVTAFLGIPYAQPPLGRLRFKKPQPLTKWSGIWNATKYANSCC QNI DQSFPGF HGSE MWNP NTDLS EDCL YLN VWIP APKPK NATV	109
hBCHE-6	0	MEDDIIITKNGKVRGMNLTVFGGTVTAFLGIPYAQPPLGRLRFKKPQPLTKWSGIWNATKYANSC QNI DTSFPGF HGSE MWNP NTDLS EDCL YLN VWIP APKPK NATV	109
hBCHE-7	0	MEDDIIITKNGKVRGMNLTVFGGTVTAFLGIPYAQPPLGRLRFKKPQPLTKWSGIWNATKYANSC QNI DTSFPGF HGSE MWNP NTDLS EDCL YLN VWIP APKPK NATV	109
hBCHE-WT	110	LI WI YGGGFQ TGTSS LHV YDGKFLARVERVIVVSMN Y RVGALGFLALPGNPEAPGNMGLFDQQLALQWVQKNIAAFGGNPKSVTLF ES AGAASVSLHLLSPGSH PLFTR	219
hBCHE-1	110	LI WI YGGGFQ TGTSS LHV YDGKFLARVERVIVVSMN Y RVGALGFLALPGNPEAPGNMGLFDQQLALQWVQKNIAAFGGNPKSVTLF ES AGAASVSLHLLSPGSH PLFTR	219
hBCHE-2	110	MI WI YGGGFQ TGTSS LHV YDGKFLARVERVIVVSMN Y RVGALGFLALPGNPEAPGNMGLFDQQLALQWVQKNIAAFGGNPKSVTLF ES AGAASVSLHLLSPGSH PLFTR	219
hBCHE-3	110	MI WI YGGGFQ TGTSS LPV YDGKFLARVERVIVVSMN Y RVGALGFLALPGNPEAPGNMGLFDQQLALQWVQDNIAAFGGNPKSVTLF ES AGAASVSLHLLSPGSH PLFTR	219
hBCHE-4	110	MI WI YGGGFQ TGTSS LPV YDGKFLARVERVIVVSMN Y RVGALGFLALPGNPEAPGNMGLFDQQLALQWVQDNIAAFGGNPKSVTLF ES AGAASVSLHLLSPGSH PLFTR	219
hBCHE-5	110	MV WI YGGGFQ TGTSS LPV YDGKFLARVERVIVVSMN Y RVGALGFLALPGNPEAPGNMGLFDQQLALQWVQDNIAAFGGNPKSVTLF ES AGAASVSLHLLSPGSH PLFTR	219
hBCHE-6	110	MV WI YGGGFQ TGTSS LPV YDGKFLARVERVIVVSMN Y RVGALGFLALPGNPEAPGNMGLFDQQLALQWVQDNIAAFGGNPKSVTLF ES AGAASVSLHLLSPGSH PLFTR	219
hBCHE-7	110	MV WI YGGGFQ TGTSS LPV YDGKFLARVERVIVVSMN Y RVGALGFLALPGNPEAPGNMGLFDQQLALQWVQDNIAAFGGD PNR VT LFES AGAASVSLHLLSPGSH PLFTR	219
hBCHE-WT	220	AIL Q SGSFNAP W AVTSLYEARNRTLNLAKLTGCSREN ET IIKCLRNKDPQEILLN EAF VVPYGT PLSVN FGPTVDGDFLTMPDIL LEL GQF KKTQ ILVGV NKDEGTAF	329
hBCHE-1	220	AIL Q SGSFNAP W AVTSLYEARNRTLNLAKLTGCSREN ET IIKCLRNKDPQEILLN EAF VVPYGT PLSVN FGPTVDGDFLTMPDIL LEL GQF KKTQ ILVGV NKDEGTAF	329
hBCHE-2	220	AIL Q SGSFNAP W AVTSP EE ARNRTLNLAKLTGCSREN ET IIKCLRNKDPQEILLN EAF VVPYGT PLSVN FGPTVDGDFLTMPDIL LEL GQF KKTQ ILVGV NKDEGTAF	329
hBCHE-3	220	AIL Q SGSFNAP W AVTSP EE ARNRTLNLAKLTGCSREN ET IIKCLRNKDPQEILDN EAF VVPYGT PLSVN FGPTVDGDFLTMPDIL LEL GQF KKTQ ILVGV NKDEGTAF	329
hBCHE-4	220	AIL Q SGSANAP W AVTSP EE ARNRTLNLAKLTGCSREN ET IIKCLRNKDPQEILDN EAF VVPYGT PLSVN FGPTVDGDFLTMPDIL LEL GQF KKTQ ILVGV NKDEGTAF	329
hBCHE-5	220	AIL Q SGSANAP W AVTSP EE ARNRTLNLAKLTGCSREN ET IIKCLRNKDPQEILDN EAF VVPYGT PLSVN FGPTVDGDFLTMPDIL LEL GQF KKTQ ILVGV NKDEGTAF	329
hBCHE-6	220	AIL Q SGSANAP W AVMS PE ARNRTLNLAKLTGCSREN ET IIKCLRNKDPQEILDN EAF VVPYGT PLSVN FGPTVDGDFLTMPDIL LEL GQF KKTQ ILVGV NKDEGTAF	329
hBCHE-7	220	AIL Q SGSANAP W AVMS PE ARNRTLNLAKLTGCSREN ET IIKCLRNKDPQEILDN EAF VVPY STPLSVN FGPTVDGDFLTMPDIL LEL GQF KKTQ ILVGV NKDEGTAF	329
hBCHE-WT	330	LV Y GAPGFSKDNNSII TR KEFQ EGLK IFFPGVSE FGKESILFH YTDWVDDQRPENYREALGDVVGDY FF ICPALE FTKKFSE WG NAFFYYFEHRSSKLPWPEWMGMHG	439
hBCHE-1	330	LV Y GAPGFSKDNNSII TR KEFQ EGLK IFFPGVSE FGKESILFH YTDWVDDQRPENYREALGDVVGDY FF ICPALE FTKKFSE WG NAFFYYFEHRSSKLPWPEWMGMHG	439
hBCHE-2	330	LV Y GAPGFSKDNNSII TR KEFQ EGLK IFFPGVSE FGKESILFH YTDWVDDQRPENYREALGDVVGDY FF ICPALE FAKKFSE HG NAFFYYFEHRSSKLPWPEWMGMHG	439
hBCHE-3	330	LV Y GAPGFSKDNNSII TR KEFQ EGLK IFFPGVSE FGKESILFH YTDWVDEQRPENYREALGDVVGDY FF ICPALE FAKKFSE HG NAFFYYFEHRSSKLPWPEWMGMHG	439
hBCHE-4	330	LV Y GAPGFSKDNNSII TR KEFQ EGLK IFFPNVSE FGKESILFH YTDWVDEQRPENYREALGDVVGDY FF ICPALE FAKKFSE HG NAFFYYFEHRSSKLPWPEWMGMHG	439
hBCHE-5	330	LV Y GAPGFSKDNNSII TR KEFQ EGLK IFFPNVSE FGKESILFH YTDWVDEQRPENYREALGDVVGDY FF ICPALE FAKKYSE HG NAFFYYFEHRSSKLPWPEWMGMHG	439
hBCHE-6	330	LV Y GAPGFSKDNNSII TR KEFQ EGLK VFFPNVSE FGKESILFH YTDWVDEQRPENYREALGVVGDY FF ICPALE FAKKYSE HG NAFFYYFEHRSSKLPWPEWMGMHG	439
hBCHE-7	330	LV Y GAPGFSKDNDSII TR KEFQ EGLK VFFPNVSE FGKESILFH YTDWVDEDRPENYRDAL AE VVGDY FF ICPALE FAKKYAE HG NAFFYYFEHRSSKLPWPEWMGMHG	439
hBCHE-WT	440	YEI EFVFG LPL ERRLN YTK AEEILSR SIV KRWAN FAK YGNP NETQ NNST SWP VFK STEQ KYLTLN TESTR IMTKLRA Q CC RF WTS FFPKV	529
hBCHE-1	440	YEI EFVFG LPL ERRLN YTK AEEILSR IK RWAN FAK YGNP NETQ NNST SWP VFK STEQ KYLTLN TESTR IMTKLRA Q CC RF WTS FFPKV	529
hBCHE-2	440	YEI EFVFG LPL ERRLN YTK AEEILSR IM KRWAN FAK YGNP NETQ NNST SWP VFK STEQ KYLTLN TESTR IMTKLRA Q CC RF WTS FFPKV	529
hBCHE-3	440	YEI EFVFG LPL ERRLN YTK AEEILSR IM KRWAN FAK YGNP NETQ NNST SWP VFK STEQ KYLTLN TESTR IMTKLRA Q CC RF WTS FFPKV	529
hBCHE-4	440	YEI EFVFG LPL ERRLN YTK AEEILSR IM RRWAN FAK YGNP NETQ NNST SWP VFK STEQ KYLTLN TESTR IMTKLRA Q CC RF WTS FFPKV	529
hBCHE-5	440	YEI EFVFG LPL ERRLN YTK AEEILSR IM RRWAN FAK YGNP NETQ NNST Q WPVFK STEQ KYLTLN TESTR IMTKLRA Q CC RF WKS FFPKV	529
hBCHE-6	440	YEI EFVFG LPL ERRLN YTK AEEILSR IM RRWAN FAK YGNP NETQ NNST Q WPVFK STEQ KYLTLN TESTR IMTKLRA Q H CRF WKS FFPKV	529
hBCHE-7	440	YEI EFVFG LPL ERRLN YTK EEILSR IM RRWAN FAK YGNP NETQ NNST Q WPVFK PT EQYLTLN TESSR IMTKLRA Q H CRF WNS FFPKV	529

Figure S2. Bacterial optimized sequences of the 7 constructs proposed by the *PROSS* process. In bold and underlined are the respective KpnI and XhoI restriction sites for pThioHis vector cloning.

hBChE-1

GGTACCATGGAAGATGACATTATCATCACCACCAAAAAATGGTAAAGTGCGTGGTATGAATCTGACCGTGTTTGG
TGGCACC GTTACCGCATTTCTGGGTATTCCGTATGCACAGCCTCCGCTGGGTCGTCTGCGTTTCAAAAAACCGCA
GCCGCTGACCAAATGGTCAGGTATTTGGAATGCAACCAAATATGCAAATAGCTGCTGCCAGAATATTGATCAGAG
CTTTCCGGGTTTTTCATGGTAGCGAAATGTGGAATCCGAATACCGATCTGAGCGAAGATTGTCTGTATCTGAATGT
TTGGATTCCGGCACC GAAACCGAAAAATGCAACCGTTCTGATTTGGATTTATGGCGGTGGTTTTTCAGACCGGCAC
CAGCAGCCTGCATGTTTATGATGGTAAATTTCTGGCAGCTGTGGAACGTGTTATTGTTGTGAGCATGAATTATCG
TGTTGGTGC ACTGGGTTTTCTGGCCCTGCCTGGTAATCCGGAAGCACCGGGTAATATGGGTCTGTTTGATCAGCA
GCTGGCGCTGCAGTGGGTTTCAGAAAAACATTGCAGCATTTGGCGGTAATCCGAAAAGCGTTACCTGTTTGGTGA
AAGTGCCGGTGCAGCAAGCGTTAGCCTGCATCTGCTGAGTCCGGGTAGCCATCCGCTGTTTACCCGTGCAATTCT
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TCTGCTGAATGAAGCATTTGTTGTGCCGTATGGTACACCGCTGAGCGTTAATTTTGGTCCGACCGTTGATGGTGA
TTTTCTGACCGATATGCCGGATATTCTGCTGGA ACTGGGTCA GTTCAAAAAAACCCAGATTCTGGTTGGCGTGAA
TAAAGATGAAGGCACCGCCTTTCTGGTTTATGGCGCACCGGGTTTTAGCAAAGATAACAATAGCATTATCACCCG
CAAAGAATTTCAAGAGGGCCTGAAAATCTTTTTTCCGGGTGTTAGCGAATTTGGCAAAGAAAGCATTCTGTTCCA
TTATACCGATTGGGTTGATGATCAGCGTCCGGA AAACTATCGTGAAGCACTGGGTGATGTTGTTGGCGATTATTT
CTTTATTTGTCCGGCACTGGAATTTACCAAAAAATTCAGCGAATGGGGCAACAACGCCTTTTCTATTATTTTCGA
ACATCGGAGCAGCAA ACTGCCGTGGCCTGAATGGATGGGTGTTATGCATGGTTATGAAATCGAATTTGTTTTTGG
TCTGCCGCTGGAACGTCGTCTGAACTATACCAAAGCCGAAGAAATCCTGAGCCGTAAGATTATGAAACGTTGGGC
AAATTTTGC GAAATATGGCAACCCGAATGAAACCCAGAATAATAGCACCAGCTGGCCTGTGTTTAAAGCACC GA
ACAGAAATATCTGACCCTGAATACCGAAAGCACCCGTATTATGACCAA ACTGCGTGCCAGCAGTGTCTGTTTTT
GACCAGTTTTTTTTCCGAAAGTGAATAACTCGAG

hBChE-2

GGTACCATGGAAGATGACATTATCATCACCACCAAAAAATGGTAAAGTGCGTGGTATGAATCTGACCGTGTTTGG
TGGCACC GTTACCGCATTTCTGGGTATTCCGTATGCACAGCCTCCGCTGGGTCGTCTGCGTTTCAAAAAACCGCA
GCCGCTGACCAAATGGTCAGGTATTTGGAATGCAACCAAATATCCGAATAGCTGCTGCCAGAATATTGATCAGAG
CTTTCCGGGTTTTTCATGGTAGCGAAATGTGGAATCCGAATACCGATCTGAGCGAAGATTGTCTGTATCTGAATGT
TTGGATTCCGGCACC GAAACCGAAAAATGCAACCGTTATGATTTGGATTTATGGCGGTGGTTTTTCAGACCGGCAC
CAGCAGCCTGCATGTTTATGATGGTAAATTTCTGGCAGCTGTGGAACGTGTTATTGTTGTGAGCATGAATTATCG
TGTTGGTGC ACTGGGTTTTCTGGCCCTGCCTGGTAATCCGGAAGCACCGGGTAATATGGGTCTGTTTGATCAGCA
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AAGTGCCGGTGCAGCAAGCGTTAGCCTGCATCTGCTGAGTCCGGGTAGCCATCCGCTGTTTACCCGTGCAATTCT
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AAA ACTGCTGGGTTGTAGCCGTGAAAATGAAACCGAGATTATCAAATGCCTGCGCAATAAAGATCCGCAAGAAAT
TCTGCTGAATGAAGCATTTGTTGTGCCGTATGGTACACCGCTGAGCGTTAATTTTGGTCCGACCGTTGATGGTGA
TTTTCTGACCGATATGCCGGATATTCTGCTGGA ACTGGGTCA GTTCAAAAAAACCCAGATTCTGGTTGGCGTGAA
TAAAGATGAAGGCACCGCCTTTCTGGTTTATGGCGCACCGGGTTTTAGCAAAGATAACAATAGCATTATCACCCG
CAAAGAATTTCAAGAGGGCCTGAAAATCTTTTTTCCGGGTGTTAGCGAATTTGGCAAAGAAAGCATTCTGTTCCA
TTATACCGATTGGGTTGATGATCAGCGTCCGGA AAACTATCGTGAAGCACTGGGTGATGTTGTTGGCGATTATTT
CTTTATTTGTCCGGCACTGGAATTTGCCAAAAAATTCAGCGAATATGGCAACAACGCCTTTTCTATTATTTTCGA
ACATCGGAGCAGCAA ACTGCCGTGGCCTGAATGGATGGGTGTTATGCATGGTTATGAAATCGAATTTGTTTTTGG
TCTGCCGCTGGAACGTCGTAAAAA CTATACCAAAGCCGAAGAAATCCTGAGCCGTGAGATTATGAAACGTTGGGC
AAATTTTGC GAAATATGGCAACCCGAATGAAACCCAGAATAATAGCACCAGCTGGCCTGTGTTTAAAGCACC GA
ACAGAAATATCTGACCCTGAATACCGAAAGCACCCGTATTATGACCAA ACTGCGTGCCAGCAGTGTCTGTTTTT
GACCAGTTTTTTTTCCGAAAGTGAATAACTCGAG

hBChE-3

GGTACCATGGAAGATGACATTATCATCACCACCAAAAAATGGTAAAGTGCGTGGTATGAATCTGACCGTGTTTGG
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GCCGCTGACCAAATGGTCAGGTATTTGGAATGCAACCAAATATGCCAATAGCTGCTGCCAGAATATTGATCAGAG
CTTTCCGGGTTTTTCATGGTAGCGAAATGTGGAATCCGAATACCGATCTGAGCGAAGATTGTCTGTATCTGAATGT
TTGGATTCCGGCACC GAAACCGAAAAATGCAACCGTTATGATTTGGATTTATGGCGGTGGTTTTTCAGACCGGCAC
CAGCAGCCTGCCGGTTTATGATGGTAAATTTCTGGCAGCTGTGGAACGTGTTATTGTTGGT GAGCATGAATTATCG

TGTTGGTGCACCTGGGTTTTCTGGCCCTGCCTGGTAATCCGGAAGCACCGGGTAATATGGGTCTGTTTGATCAGCA
GCTGGCGCTGCAGTGGGTTCAAGATAACATTGCAGCATTGGCGGTAATCCGAAAAGCGTTACCCTGTTTGGTGA
AAGTGCCGGTGCAGCAAGCGTTAGCCTGCATCTGCTGAGTCCGGGTAGCCATCCGCTGTTTACCCTGCAATTCT
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TTTTCTGACCGATATGCCGGATATTCTGCTGGAACCTGGGTGAGTTCAAAAAAACCCAGATTCTGGTTGGCGTGAA
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AAATTTTGCAGAAATATGGTAACCCGAATGAAACCCAGAATAATAGCACCAGCTGGCCTGTGTTTAAAGCACC
ACAGAAATATCTGACCCTGAATACCGAAAGCACCCGTATTATGACCAAACCTGCGTGCCAGCAGTGTCTGTTTTG
GACCAGCTTTTTTCCCAAAGTGTGATAACTCGAG

hBChE-4

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CTTTCCGGGTTTTTCATGGTAGCGAAATGTGGAATCCGAATACCGATCTGAGCGAAGATTGTCTGTATCTGAATGT
TTGGATTCCGGCACCGAAACCGAAAAATGCAACCGTTATGATTTGGATTTATGGCGGTGGTTTTTCAGACCGGCAC
CAGCAGCCTGCCGGTTTATGATGGTAAATTTCTGGCACGTGTTGAACGTGTTATTGTGGTGAGCATGAATTATCG
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ACATCGGAGCAGCAAACCTGCCGTGGCCTGAATGGATGGGTGTTATGCATGGTTATGAAATCGAATTTGTTTTTGG
TCTGCCGCTGGAACGTCGTCTGAATTATACCAAAGCCGAAGAAATCCTGAGTCGCGAAATTATGCGTCGTTGGGC
AAATTTTGCAGAAATATGGTAACCCGAATGAAACCCAGAATAATAGCACCAGCTGGCCTGTGTTTAAAGCACC
ACAGAAATATCTGACCCTGAATACCGAAAGCACCCGTATTATGACCAAACCTGCGTGCCAGCAGTGTCTGTTTTG
GACCAGCTTTTTTCCCAAAGTGTGATAACTCGAG

hBChE-5

GGTACCATGGAAGATGACATTATCATCACCACCAAAAAATGGTAAAGTGCGTGGTATGAATCTGACCGTGTTTGG
TGGCACCGTTACCGCATTTCTGGGTATTCCGTATGCACAGCCTCCGCTGGGTGCTCTGCGTTTCAAAAAACCGCA
GCCGCTGACCAAATGGTCAGGTATTTGGAATGCAACCAAATATGCCAATAGCTGCTGCCAGAATATTGATCAGAG
CTTTCCGGGTTTTTCATGGTAGCGAAATGTGGAATCCGAATACCGATCTGAGCGAAGATTGTCTGTATCTGAATGT
TTGGATTCCGGCACCGAAACCGAAAAATGCAACCGTTATGGTTTGGATTTATGGCGGTGGTTTTTCAGACCGGCAC
CAGCAGCCTGCCGGTTTATGATGGTAAATTTCTGGCACGTGTTGAACGTGTTATTGTGGTGAGCATGAATTATCG
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TCTGCCGCTGGAACGTCGTCTGAATTATACCAAAGCCGAAGAAATCCTGAGTCGCGAAATTATGCGTCGTTGGGC
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ACAGAAATATCTGACCCTGAATACCGAAAGCACCCGTATTATGACCAAACCTGCGTGCCAGCAGTGTCTGTTTTTG
GAAAAGCTTTTTTCCCAAAGTGTGATAACTCGAG

hBChE-6

GGTACCCATGGAAGATGACATTATCATCACCACCAAAAAATGGTAAAGTGCGTGGTATGAATCTGACCGTGTTTGG
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CTTTCCGGGTTTTTCATGGTAGCGAAATGTGGAATCCGAATACCGATCTGAGCGAAGATTGTCTGTATCTGAATGT
TTGGATTCCGGCACCGAAACCGAAAAATGCAACCGTTATGGTTTGGATTATGGTGGTGGTTTTTCAGACCGGCAC
CAGCAGCCTGCCGGTTTATGATGGTAAATTTCTGGCAGTGTTGAACGTGTTATTGTGGTGAGCATGAATTATCG
TGTTGGTGCACTGGGTTTTCTGGCCCTGCCTGGTAATCCGGAAGCACCGGGTAATATGGGTCTGTTTGATCAGCA
GCTGGCACTGAAATGGGTTCAAGATAACATTGCAGCATTTGGCGGTAATCCGAAAAGCGTTACCCTGTTTGGTGA
AAGTGCCGGTGCAGCAAGCGTTAGCCTGCATCTGCTGAGTCCGGGTAGCCATCCGCTGTTTACCCTGCAATTCT
GCAGAGCGGTAGCGCAAATGCACCGTGGGCAGTTATGAGTCCGGAAGAAGCACGTAATCGTACCCTGAATCTGGC
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TCTGGATAATGAAGCCTTTGTTGTGCCGTATGGTACACCGCTGAGCGTTAATTTTGGTCCGACCGTTGATGGTGA
TTTTCTGACCGATATGCCGGATACACTGCTGGAACCTGGGTGAGTTCAAAAAAACCCAGATTCTGGTTGGCGTGAA
TAAAGATGAAGGCACCGCCTTTCTGGTTTATGGCGCACCGGGTTTTAGCAAAGATAACAATAGCATTATCACCCG
CAAAGAATTTCAAGAGGGTCTGAAAGTGTTTTTCCGAACGTTAGCGAATTTGGCAAAGAAAGCATCCTGTTTCA
TTATACCGATTGGGAAGATGAAGATCGTCCGGAACCTATCGTGAAGCACTGGGTGAAGTTGTTGGCGATTATTT
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AAATTTTGCAGAAATATGGTAACCCGAATGAAACCCAGAATAATAGCACCCAGTGGCCTGTGTTTAAAAGCACCGA
ACAGAAATATCTGACCCTGAATACCGAAAGCACCCGTATTATGACCAAACCTGCGTGACAGCATTTGTCGTTTTTG
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hBChE-7

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Figure S3. Determination of kinetic parameters of hBChE-7 for BTC hydrolysis. Upper panel, enzymatic activity titration by increasing concentration of substrate. Lower panel, titration of residual activity by increasing concentration of echothiophate.

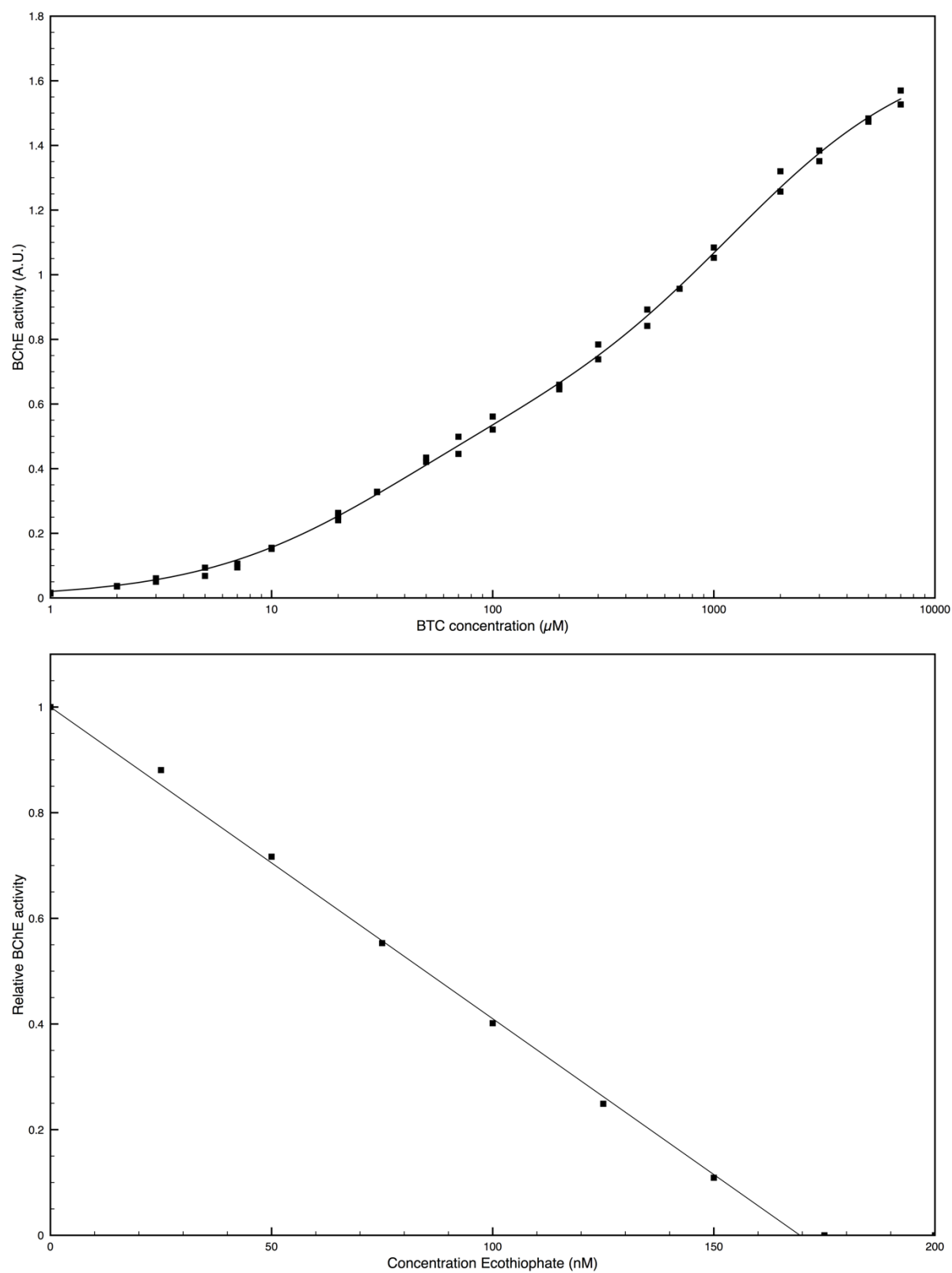


Figure S4. Effects of different buffers on hBChE-7 thermal stability. Examples of normalized DSF scans of hBChE-7 in different buffer conditions, without (plain trace) or with 0.25M NaCl (dashed trace). 0.1M KH_2PO_4 pH 6.0, red trace; 0.1M Cacodylate pH 6.5, blue trace; 0.1M NaH_2PO_4 pH 7.5, green trace; 0.1M HEPES pH 7.5, magenta trace. For HEPES, increasing concentrations of NaCl, show the stabilizing effect of the buffer ionic strength.

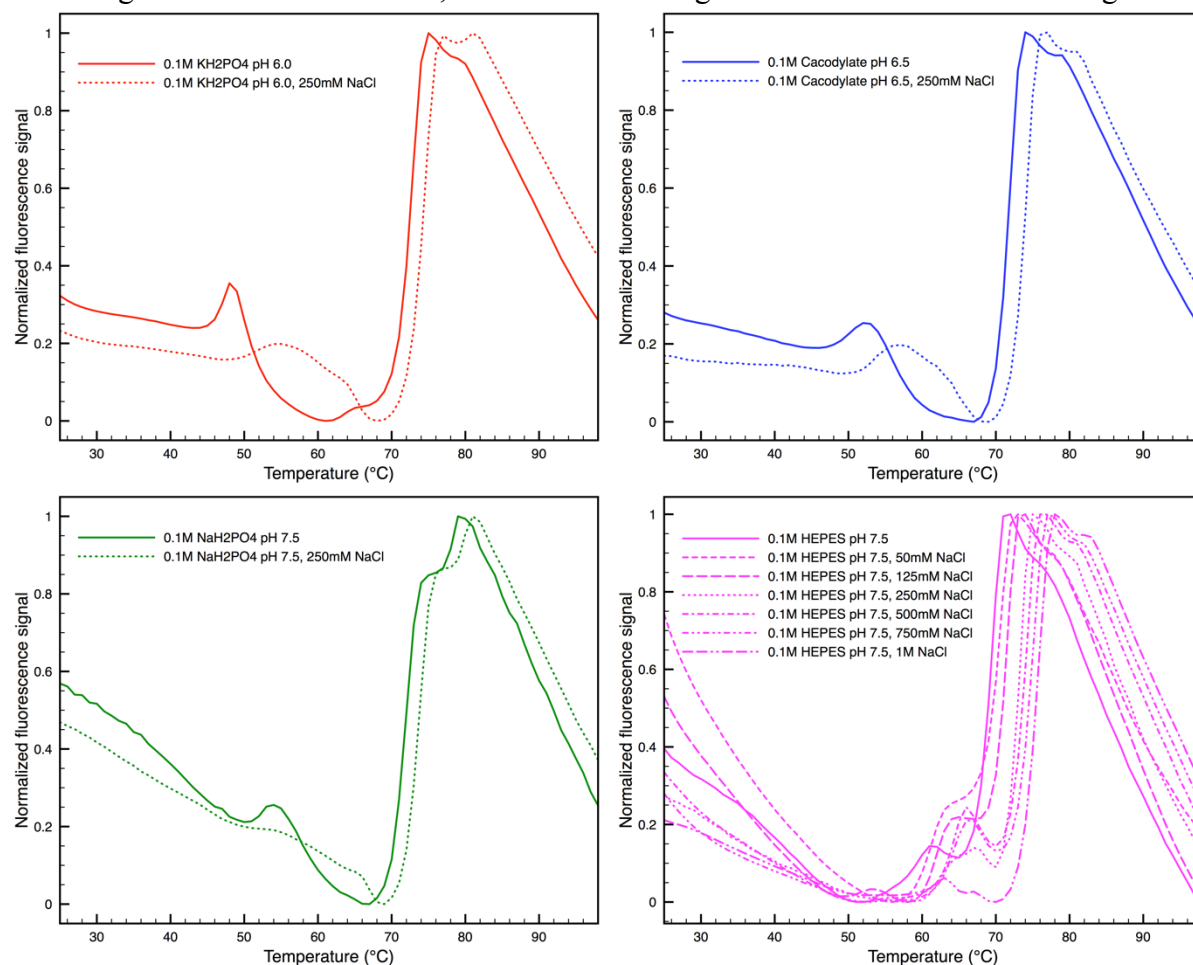


Figure S5. Composition of the buffer screen used in the DSF assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	Citrate pH 4.0	Na Acetate pH 4.5	Citrate pH 5.0	MES pH 6.0	KH ₂ PO ₄ pH 6.0	Citrate pH 6.0	Bis-Tris pH 6.5	Cacodylate pH 6.5	NaH ₂ PO ₄ pH 7.0	KH ₂ PO ₄ pH 7.0	HEPES pH 7.0
B	MOPS pH 7.0	Am. Acetate pH 7.3	Tris-HCl pH 7.5	NaH ₂ PO ₄ pH 7.5	HEPES pH 7.5	Imidazole pH 8.0	Tris-HCl pH 8.0	Tricine pH 8.0	Bicine pH 8.0	Bicine pH 8.5	Tris-HCl pH 8.5	Bicine pH 9.0
C	Water 250mM NaCl	Citrate pH 4.0 250mM NaCl	Na Acetate pH 4.5 250mM NaCl	Citrate pH 5.0 250mM NaCl	MES pH 6.0 250mM NaCl	KH ₂ PO ₄ pH 6.0 250mM NaCl	Citrate pH 6.0 250mM NaCl	Bis-Tris pH 6.5 250mM NaCl	Cacodylate pH 6.5 250mM NaCl	NaH ₂ PO ₄ pH 7.0 250mM NaCl	KH ₂ PO ₄ pH 7.0 250mM NaCl	HEPES pH 7.0 250mM NaCl
D	MOPS pH 7.0 250mM NaCl	Am. Acetate pH 7.3 250mM NaCl	Tris-HCl pH 7.5 250mM NaCl	NaH ₂ PO ₄ pH 7.5 250mM NaCl	HEPES pH 7.5 250mM NaCl	Imidazole pH 8.0 250mM NaCl	Tris-HCl pH 8.0 250mM NaCl	Tricine pH 8.0 250mM NaCl	Bicine pH 8.0 250mM NaCl	Bicine pH 8.5 250mM NaCl	Tris-HCl pH 8.5 250mM NaCl	Bicine pH 9.0 250mM NaCl
E	10mM MES pH 6.0	50mM MES pH 6.0	100mM MES pH 6.0	250mM MES pH 6.0	10mM KH ₂ PO ₄ pH 6.0	50 mM KH ₂ PO ₄ pH 6.0	100m KH ₂ PO ₄ pH 6.0	250mM KH ₂ PO ₄ pH 6.0	10mM Citrate pH 6.0	50 mM Citrate pH 6.0	100mM Citrate pH 6.0	250mM Citrate pH 6.0
F	10mM NaH ₂ PO ₄ pH 7.5	50mM NaH ₂ PO ₄ pH 7.5	100mM NaH ₂ PO ₄ pH 7.5	250mM NaH ₂ PO ₄ pH 7.5	10mM HEPES pH 7.5	50mM HEPES pH 7.5	100mM HEPES pH 7.5	250mM HEPES pH 7.5	10mM Tris- HCl pH 7.5	50mM Tris- HCl pH 7.5	100mM Tris- HCl pH 7.5	250mM Tris- HCl pH 7.5
G	50mM MES pH 6.0 50mM NaCl	50mM MES pH 6.0 125mM NaCl	50mM MES pH 6.0 250mM NaCl	50mM MES pH 6.0 500mM NaCl	50mM MES pH 6.0 750mM NaCl	50mM MES pH 6.0 1M NaCl	50mM MOPS pH 7.0 50mM NaCl	50mM MOPS pH 7.0 125mM NaCl	50mM MOPS pH 7.0 250mM NaCl	50mM MOPS pH 7.0 500mM NaCl	50mM MOPS pH 7.0 750mM NaCl	50mM MOPS pH 7.0 1M NaCl
H	50mM HEPES pH 7.5 50mM NaCl	50mM HEPES pH 7.5 125mM NaCl	50mM HEPES pH 7.5 250mM NaCl	50mM HEPES pH 7.5 500mM NaCl	50mM HEPES pH 7.5 750mM NaCl	50mM HEPES pH 7.5 1M NaCl	50mM Tris- HCl pH 8.0 50mM NaCl	50mM Tris- HCl pH 8.0 125mM NaCl	50mM Tris- HCl pH 8.0 250mM NaCl	50mM Tris- HCl pH 8.0 500mM NaCl	50mM Tris- HCl pH 8.0 750mM NaCl	50mM Tris- HCl pH 8.0 1M NaCl