Supporting Information

The Mechanisms of Human Hotdog Fold Thioesterase 2 (hTHEM2) Substrate Recognition and Catalysis Illuminated by a Structure and Function Based Analysis[#]

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Contents: Seven pages total which include Table SI1 (steady state kinetic constants measured for hTHEM2 catalysis as function of NaCl concentration), Figures SI1-5 (graphic figures depicting hTHEM2 structure) and Figure SI6 (sequence alignment of hHTEM2 homologs).

Table SI1. The steady state kinetic constants measured for hTHEM2 catalyzed hydrolysis of 3-hydroxyphenylacetyl-CoA in solutions containing 50 mM K^+HEPES (pH 7.5 and 25 ^{o}C), 1 mM DTNB and varying concentrations of NaCl. The error limits shown reflect the fit to a data set and do not represent the standard deviation calculated from repetitive determination.

[NaCl] (mM)	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m (s^{-1} μM^{-1})
0	1.44 ± 0.07	30 ± 5	4.8×10^4
1	1.48 ± 0.02	47 ± 2	3.1×10^4
10	1.50 ± 0.10	40 ± 10	3.7×10^4
50	1.43 ± 0.04	38 ± 3	3.7×10^4
100	1.20 ± 0.06	30 ± 6	4.0×10^4

Figure SI1: The existence of chloride ions as shown by the difference anomalous Fourier map constructed using anomalous difference signal and phases calculated from protein only. The densities of chloride ion (green) and the undecan-2-one-CoA sulfur atom (yellow) were observed in the map because these two atoms have similar anomalous signals at Cu K-alpha wavelength (f''-Cl=0.7e, f''-S=0.6e).
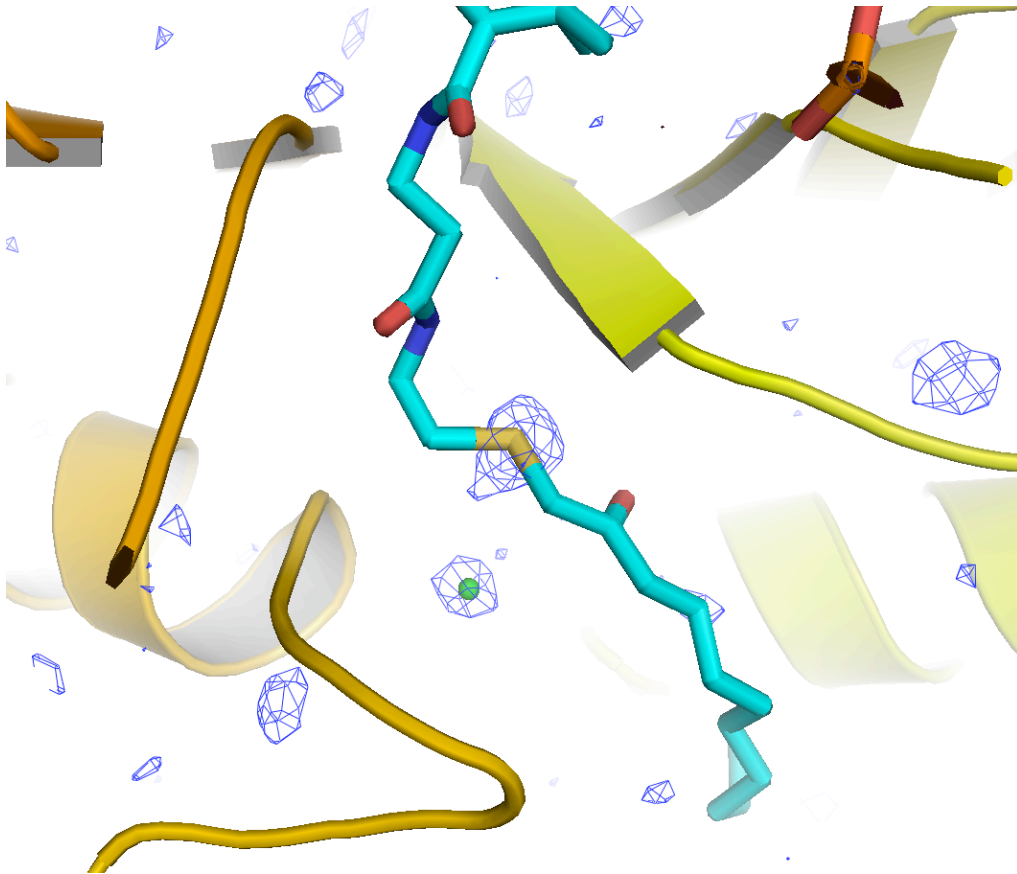
Figure SI2. Superposition of the main chain of the apo tetrameric hTHEM2 and the main chain of the undeac-2-one-CoA bound tetrameric hTHEM2
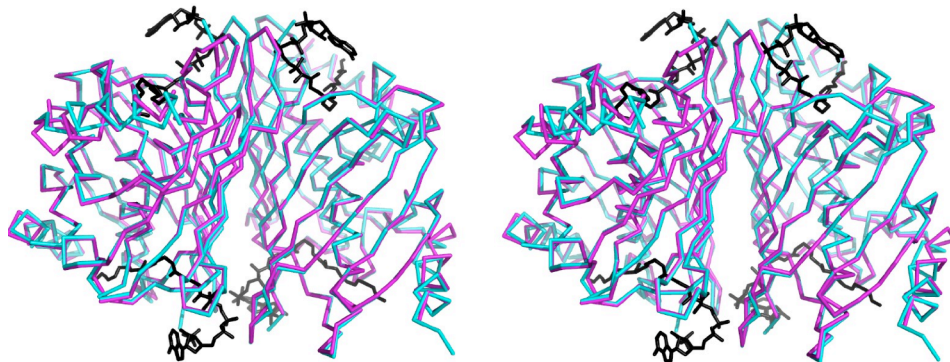

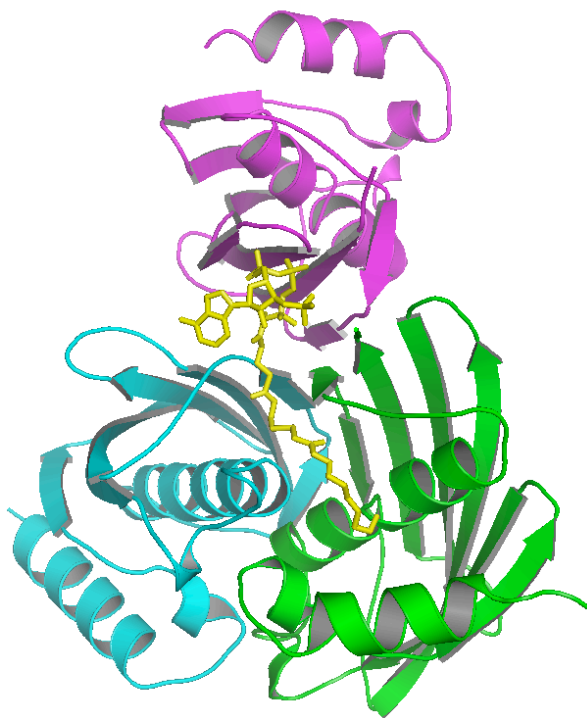Figure SI3: The undeacan-2-one-CoA (yellow) binding site formed by protomers I (cyan), II (green) and III (magenta).

Figure SI4. A zoom in on the undecan-2-one-CoA ligand I O=C-CH$_2$S unit and the surrounding residues.
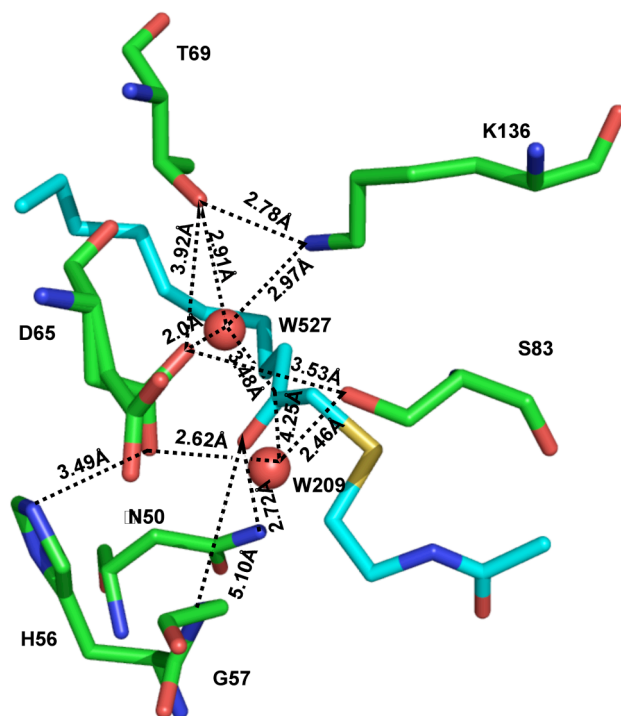
Figure SI5: Stereoview of the superposition of the hTHEM2 active site with undeca-2-one-CoA (cyan) bound and decanoyl-CoA (black) modeled.
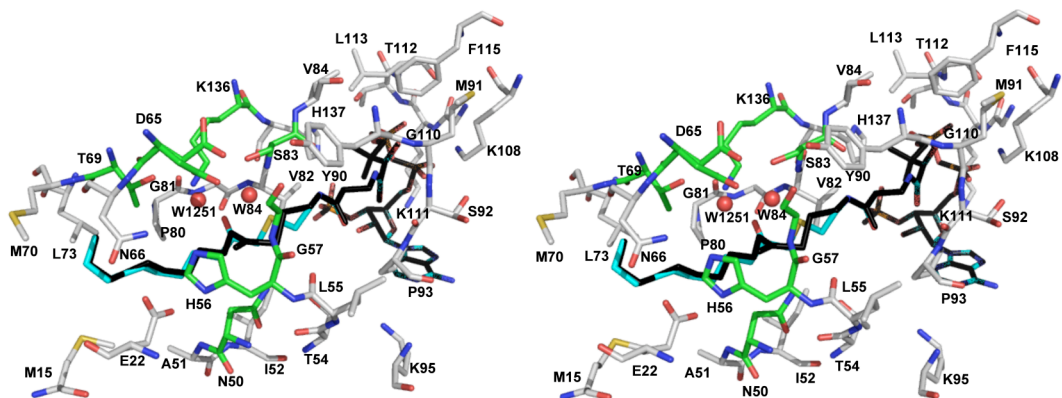
Figure SI6: Alignment of hTHEM2 eukaryotic homolog sequences having 42% or greater identity. Identical residues are highlighted in green color, similar residues are highlighted in yellow color, and the tetramerization residues are highlighted in magenta color.

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hTHEM2   ----------MTSMTQSLREVIKAMTKAR-NFERVLGKIT---LVSAAPGKVICEMKVEE 46
Q5R833   ----------MTSMTQSLREVIKAMTKAR-NFERVLGKIT---LVSAAPGKVICEMKVEE 46
Q9CQR4   ----------MSSMTQNLREVMKVMFKVP-GFDRVLEKVT---LVSAAPEKLICEMKVEE 46
A6QQ83   --------MIWVKKAKTLLEWLTHSGKFN-GFDRNLEKVT---LVSAAPGKLICEMKVEE 48
Q1LU96   ---------MASLTLNTVKQIFRAMADSP-GFDRVLSKV---EVLSAAPGKVVCEMKVEE 47
Q1LWU4   ---------MASLTLNAVKQIFRAMADSP-GFDRVLSKVSVVEVLSAAPGKVVCEMKVEE 50
Q6DER5   ---------MSSLSTSTVRAIFKALGDGP-GFDRVLSKLN---LVSAAPGKVVCELQVEE 47
Q7QJ30   --------MSGKKGLDLLRTIATVMTKTN-GYDRCLQQ---LVMVSGGDGRCMAEFKVEE 48
Q29FB2   -----------KRGVDFVKHVAEYAASKG-GFDRVLKM---IKITGGGDGRAIGEFTVEK 45
A7SG16   ----------MAALLRKGQQLWTFMTKNNPGFDRVLEK---AELAAFGGGRCIIKMTVSQ 47
Q1HPG9   ---------MGTKGIKIAELFTKTIAATK-GFDQNLRK---LKVTSCGNGSMVTEFQVGP 47
A9PCW5   MDLESVKRYLETGGYEEDKNASTIEKMPLRFFERFIMQG--LHIDLIEPGRVVCSMKVPP 58
B0FVM1   -----------------AKEPMVAKLPHRFLERFVTNG--LKVDLIEPGRIVCSMKIPP 40
A8WU80   ---------MASKYMQLAKEIVKNFGNKG-NFAGVSGAGN-CRAIHAEEGNIRVEFEVEK 49
Q18187   ---------MPSKYMQLAKEIIKNYGTKG-QFGCVSGAGN-VRAVHAEEGNLRVEFEVEK 49

hTHEM2   EHTNAIGTLHGGLTATLVDNISTMALLCTE-RGAPGVSVDMNITYMSPAKLGEDIVITAH 105
Q5R833   EHTNAIGTLHGGLTATLVDNISTMALLCTE-RGAPGVSVDMNITYMSPAKLGEDIVITAH 105
Q9CQR4   QHTNKLGTLHGGLTATLVDSISTMALMCTE-RGAPGVSVDMNITYMSPAKIGEEIVITAH 105
A6QQ83   QHANKMGTLHGGMIATLVDVISSLALLCTE-RGISGVSVDMNITYMSPAKVGEDILITAH 107
Q1LU96   QHTNRGGTLHGGMTATLVDMISTMAIMYSE-RGAPGVSVDMNITYMNAAKIGEDILITAQ 106
Q1LWU4   QHTNRGGTLHGGMTATLVDMISTMAIMYSE-RGAPGVSVDMNITYMNAAKIGEDILITAQ 109
Q6DER5   EHTNRGGTLHGGLTATLVDTVSTVALLHTE-RGAPGVSVDMNITYMAAKIGDSVLITAQ 106
Q7QJ30   EHLNRAGGLHGGYTATIVDVTTYALMTKEN-ATPGVSVDIHVSYLKGARLGDEVIIDAN 107
Q29FB2   EHLNRQGTLHGGLTATIVDNVTTYALMSTG--SHPGVTATLNVSYMSAAKPGELVEIDAR 103
A7SG16   EHENRMGTLHGGLTATMVDDVTTMAIISQT--GQAGVSVDMNISYLKAACRGDEVIFEGI 105
Q1HPG9   EHLNQRGTLHGGFIAHLVDAISTYALTTNENVDTRGVSIDLSLSFYSAAKEGDNIEVEAK 107
A9PCW5   RLLNGGNFLHGGATATLVDLVGSAAIFTVG-APATGVSVEINVSYLDAAFADEEIEIEAR 117
B0FVM1   HLLNAGKFLHGGATATLVDLIGSAVIYTAG-ASHSGVSVEINVSYLDAAFLDEDIEIESK 99
A8WU80   DQTNHFETLHGGCTATLIDNYTTAALLLTK-QARPGVSVDLHVTYLSAAKIGETLVLDST 108
Q18187   DQSNHFNTLHGGCTSTLIDIFTTGALLLTK-PARPGVSVDLHVTYLTAAKIGETLVLDST 108
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hTHEM2   VLKQGKTLAFTSVDLTNKATGKLIAQGRHTKHLGN---------------- 140
Q5R833   VLKQGKTLAFTSVDLTNKATGKLIAQGRHTKHLGN---------------- 140
Q9CQR4   ILKQGKTLAFASVDLTNKTTGKLIAQGRHTKHLGN---------------- 140
A6QQ83   VLKEGRSLSFASVDLMNKATGKLIAQGRHTKHMGNRPPEGSVKKSNNE--- 155
Q1LU96   VLKQGRTLAFATVDLTNKANGKLIAQGRHTKHLGS---------------- 141
Q1LWU4   VLKQGRTLAFATVDLTNKANGKLIAQGRHTKHLGS---------------- 144
Q6DER5   VLKQGRTLAFATVDVTNKVSGKLIAQGRHTKHLGN---------------- 141
Q7QJ30   TVRAGRNLAFLECELRHKKDNSIIAKASHTKYIGSS-------------- 143
Q29FB2   TLRAGRKMAYLDCVLKRKSDGRILASGGQVKYVDFDQE------------- 141
A7SG16   CNKAGKNLAFSTAEIKLK-DGTVLAMGKHTKYIGNSPKVIDFDGNCNQTLY 155
Q1HPG9   TRKTGKKIAFLEVEVRNKDKNQVLASGRHTKYIGI--------------- 142
A9PCW5   VLRVGKAVGVVSVELKKKTGKIIAQGRHTKYLAVPSKL------------ 156
B0FVM1   ALRVGKAVAVVSVELRKKTTGKIIAQGRHTKYFAP--------------- 134
A8WU80   VTKQGRTLAFTKAELYRKRDNVMIATGVHTKAFPAMKKSE---------- 148
Q18187   VIKQGKTLAFTKAELYRKSDNVMIATGVHTKAFPTVKKAN---------- 148
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