Structural comparison of *Enterococcus faecalis* and human thymidylate synthase complexes with the substrate dUMP and its analogue FdUMP provides hints about enzyme conformational variabilities

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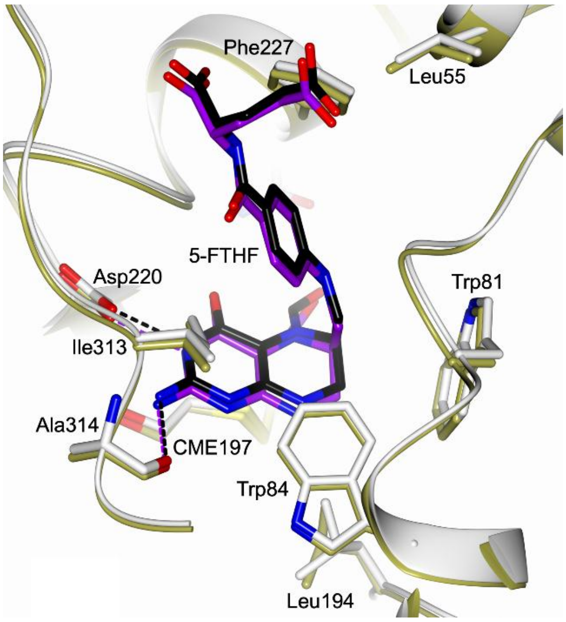
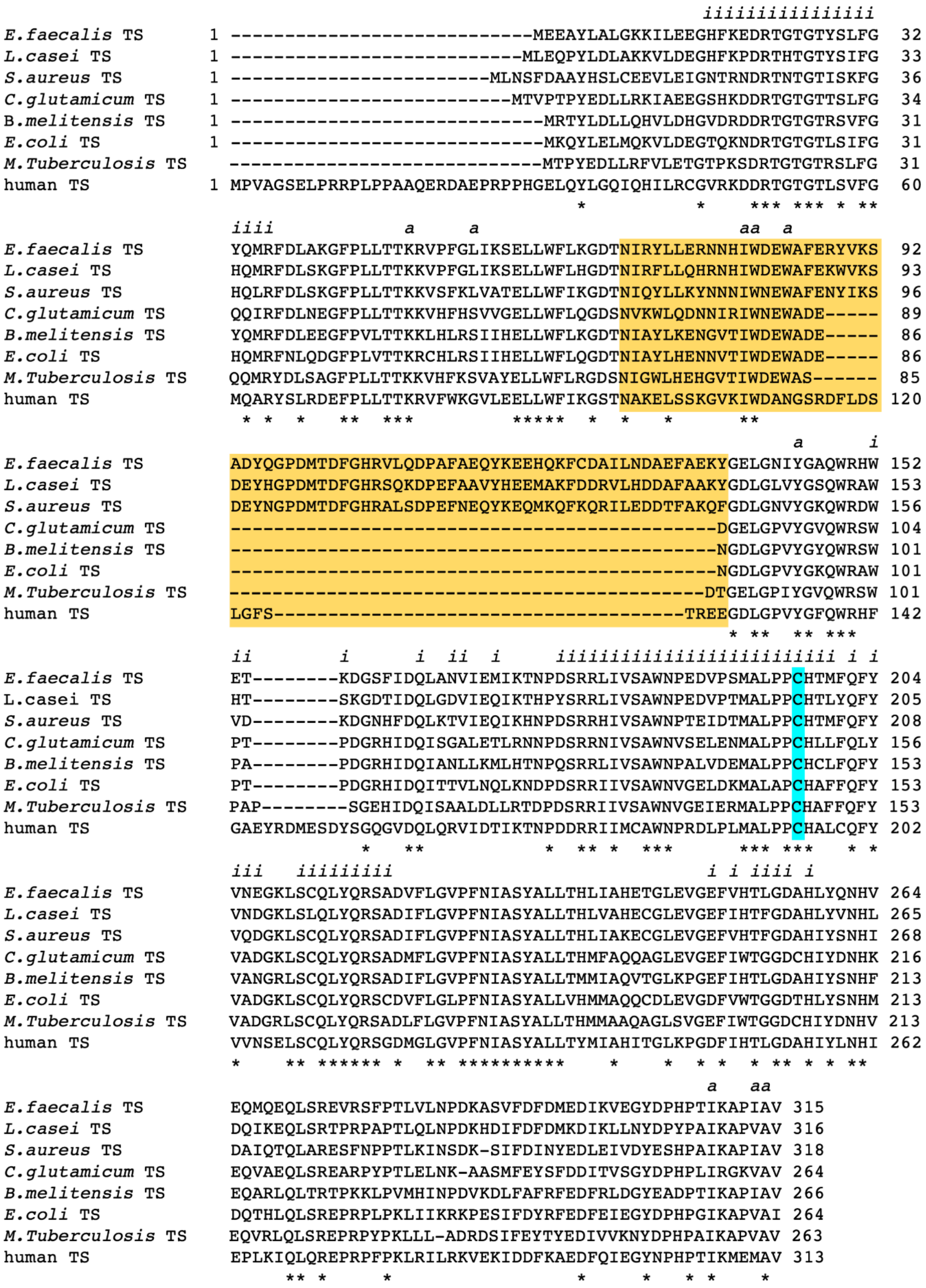


Figure S1. Active site view of the structural comparison between subunit B in the substrate-free *Ef*TS (PDB id 3UWL [5], gold cartoon and carbon atoms) and in the complex with dUMP (white cartoon and carbon atoms). In subunits B and D of both structures, the catalytic cysteine is modified as S,S-(2-hydroxyethyl)thiocysteine (CME197) and the cofactor-like molecule 5-formyl-6-tetrahydrofolate (5-FTHF, in sticks, carbon atoms are colored black and purple in the native *Ef*TS and in the complex with dUMP, respectively) occupies the active site displaying an highly conserved binding mode. H-bonds are displayed as dashed lines (colored black and purple in the native *Ef*TS and in the complex with dUMP, respectively). Oxygen atoms are colored red, nitrogen blue, sulfur yellow, and phosphorous magenta.



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                                                                  iiiiiiiiiiiiiiii
E.faecalis TS       1 ----------------------------MEEAYLALGKKILEEGHFKEDRTGTGTYSLFG  32
L.casei TS          1 ---------------------------MLEQPYLDLAKKVLDEGHFKPDRTHTGTYSIFG  33
S.aureus TS         1 ------------------------MLNSFDAAYHSLCEEVLEIGNTRNDRTNTGTISKFG  36
C.glutamicum TS     1 --------------------------MTVPTPYEDLLRKIAEEGSHKDDRTGTGTTSLFG  34
B.melitensis TS     1 -----------------------------MRTYLDLLQHVLDHGVDRDDRTGTGTRSVFG  31
E.coli TS           1 -----------------------------MKQYLELMQKVLDEGTQKNDRTGTGTLSIFG  31
M.Tuberculosis TS     -----------------------------MTPYEDLLRFVLETGTPKSDRTGTGTRSLFG  31
human TS            1 MPVAGSELPRRPLPPAAQERDAEPRPPHGELQYLGQIQHILRCGVRKDDRTGTGTLSVFG  60
                                                      *          *    *** *** * **

                      iiii            a     a                        aa  a
E.faecalis TS         YQMRFDLAKGFPLLTTKRVPFGLIKSELLWFLKGDTNIRYLLERNNHIWDEWAFERYVKS  92
L.casei TS            HQMRFDLSKGFPLLTTKKVPFGLIKSELLWFLHGDTNIRFLLQHRNHIWDEWAFEKWVKS  93
S.aureus TS           HQLRFDLSKGFPLLTTKKVSFKLVATELLWFIKGDTNIQYLLKYNNNIWNEWAFENYIKS  96
C.glutamicum TS       QQIRFDLNEGFPLLTTKKVHFHSVVGELLWFLQGDSNVKWLQDNNIRIWNEWADE-----  89
B.melitensis TS       YQMRFDLEEGFPVLTTKKLHLRSIIHELLWFLKGDTNIAYLKENGVTIWDEWADE-----  86
E.coli TS            HQMRFNLQDGFPLVTTKRCHLRSIIHELLWFLQGDTNIAYLHENNVTIWDEWADE-----  86
M.Tuberculosis TS     QQMRYDLSAGFPLLTTKKVHFKSVAYELLWFLRGDSNIGWLHEHGVTIWDEWAS------  85
human TS              MQARYSLRDEFPLLTTKRVFWKGVLEELLWFIKGSTNAKELSSKGVKIWDANGSRDFLDS 120
                       * *  *   **  ***         *****  *  *   *      **

                                                                          a      i
E.faecalis TS         ADYQGPDMTDFGHRVLQDPAFAEQYKEEHQKFCDAILNDAEFAEKYGELGNIYGAQWRHW 152
L.casei TS            DEYHGPDMTDFGHRSQKDPEFAAVYHEEMAKFDDRVLHDDAFAAKYGDLGLVYGSQWRAW 153
S.aureus TS          DEYNGPDMTDFGHRALSDPEFNEQYKEQMKQFKQRILEDDTFAKQFGDLGNVYGKQWRDW 156
C.glutamicum TS       ---------------------------------------------DGELGPVYGVQWRSW 104
B.melitensis TS       ---------------------------------------------NGDLGPVYGYQWRSW 101
E.coli TS            ---------------------------------------------NGDLGPVYGKQWRAW 101
M.Tuberculosis TS     --------------------------------------------DTGELGPIYGVQWRSW 101
human TS              LGFS--------------------------------------TREEGDLGPVYGFQWRHF 142
                                                                    * **  ** ***

                      ii        i      i  ii  i     iiiiiiiiiiiiiiiiiiiiiiiiii i i
E.faecalis TS         ET--------KDGSFIDQLANVIEMIKTNPDSRRLIVSAWNPEDVPSMALPPCHTMFQFY 204
L.casei TS            HT--------SKGDTIDQLGDVIEQIKTHPYSRRLIVSAWNPEDVPTMALPPCHTLYQFY 205
S.aureus TS           VD--------KDGNHFDQLKTVIEQIKHNPDSRRHIVSAWNPTEIDTMALPPCHTMFQFY 208
C.glutamicum TS       PT--------PDGRHIDQISGALETLRNNPDSRRNIVSAWNVSELENMALPPCHLLFQLY 156
B.melitensis TS       PA--------PDGRHIDQIANLLKMLHTNPQSRRLIVSAWNPALVDEMALPPCHCLFQFY 153
E.coli TS            PT--------PDGRHIDQITTVLNQLKNDPDSRRIIVSAWNVGELDKMALAPCHAFFQFY 153
M.Tuberculosis TS     PAP--------SGEHIDQISAALDLLRTDPDSRRIIVSAWNVGEIERMALPPCHAFFQFY 153
human TS              GAEYRDMESDYSGQGVDQLQRVIDTIKTNPDDRRIIMCAWNPRDLPLMALPPCHALCQFY 202
                                  *   **           *  ** *  ***      *** ***   * *

                      iii   iiiiiiiii                             i i iiii i
E.faecalis TS         VNEGKLSCQLYQRSADVFLGVPFNIASYALLTHLIAHETGLEVGEFVHTLGDAHLYQNHV 264
L.casei TS            VNDGKLSLQLYQRSADIFLGVPFNIASYALLTHLVAHECGLEVGEFIHTFGDAHLYVNHL 265
S.aureus TS           VQDGKLSCQLYQRSADIFLGVPFNIASYALLTHLIAKECGLEVGEFVHTFGDAHIYSNHI 268
C.glutamicum TS       VADGKLSCQLYQRSADMFLGVPFNIASYALLTHMFAQQAGLEVGEFIWTGGDCHIYDNHK 216
B.melitensis TS       VANGRLSCQLYQRSADIFLGVPFNIASYALLTMMIAQVTGLKPGEFIHTLGDAHIYSNHF 213
E.coli TS            VADGKLSCQLYQRSCDVFLGLPFNIASYALLVHMMAQQCDLEVGDFVWTGGDTHLYSNHM 213
M.Tuberculosis TS     VADGRLSCQLYQRSADLFLGVPFNIASYALLTHMMAAQAGLSVGEFIWTGGDCHIYDNHV 213
human TS              VVNSELSCQLYQRSGDMGLGVPFNIASYALLTYMIAHITGLKPGDFIHTLGDAHIYLNHI 262
                      *    ** ****** *  ** **********    *    *  * *  * ** * * **

                                                                  a   aa
E.faecalis TS         EQMQEQLSREVRSFPTLVLNPDKASVFDFDMEDIKVEGYDPHPTIKAPIAV 315
L.casei TS            DQIKEQLSRTPRPAPTLQLNPDKHDIFDFDMKDIKLLNYDPYPAIKAPVAV 316
S.aureus TS           DAIQTQLARESFNPPTLKINSDK-SIFDINYEDLEIVDYESHPAIKAPIAV 318
C.glutamicum TS       EQVAEQLSREARPYPTLELNK-AASMFEYSFDDITVSGYDPHPLIRGKVAV 264
B.melitensis TS       EQARLQLTRTPKKLPVMHINPDVKDLFAFRFEDFRLDGYEADPTIKAPIAV 266
E.coli TS            DQTHLQLSREPRPLPKLIIKRKPESIFDYRFEDFEIEGYDPHPGIKAPVAI 264
M.Tuberculosis TS     EQVRLQLSREPRPYPKLLL-ADRDSIFEYTYEDIVVKNYDPHPAIKAPVAV 263
human TS              EPLKIQLQREPRPFPKLRILRKVEKIDDFKAEDFQIEGYNPHPTIKMEMAV 313
                           ** *     *                 *     *   * *    *
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Figure S2. Sequence alignment among TS from different sources. Conserved residues are indicated by asterisks. The catalytic cysteine and the small domain of each TS enzyme are highlighted in cyan and orange, respectively. *Ef*TS residues belonging to the interface area and to the active site are indicated by "*i*" and "*a*", respectively.

Table S1. Data collection and processing (Values for the outer shell are given in parentheses).

	***Ef*TS – dUMP**	***Ef*TS – FdUMP**	**hTS – dUMP**	**hTS – FdUMP**
PDB code	**6QYA**	**6QXS**	**6QXH**	**6QXG**
Diffraction source	I04-1 (DLS)	I24 (DLS)	P14 (PetraIII)	I03 (DLS)
Wavelength (Å)	0.92819	0.9861	0.97625	0.97625
Temperature (K)	100	100	100	100
Detector	Dectris Pilatus 6M	Dectris Pilatus 6M	Dectris Pilatus 6M	Dectris Pilatus 6M
Crystal-detector distance (mm)	293.4	437.0	381.9	363.5
Rotation range per image (°)	0.25	0.25	0.20	0.25
Total rotation range (°)	200	160	360	300
Exposure time per image (s)	0.40	0.08	0.10	0.30
Space group	$P2_1$	$P2_1$	C2	I2
a, b, c (Å)	73.16; 95.10; 97.41	72.04; 94.84; 97.00	182.11; 61.59; 101.10	103.22; 61.89; 169.36
β (°)	93.63	94.01	115.38	98.96
Mosaicity (°)	0.51	0.75	0.36	0.55
Resolution range (Å)	73.01-1.76 (1.86-1.76)	96.76-2.88 (3.04-2.88)	34.19-2.04 (2.15-2.04)	33.37-2.08 (2.19-2.08)
Total No. of reflections	389827 (58951)	61694 (8929)	393536 (57948)	281895 (40808)
No. of unique reflections	125005 (18480)	28081 (4058)	62630 (9114)	63324 (9136)
Completeness (%)	95.1 (96.5)	95.1 (94.7)	97.0 (97.2)	99.4 (98.8)
Redundancy	3.1 (3.2)	2.2 (2.2)	6.3 (6.4)	4.5 (4.5)
$\langle I/\sigma(I) \rangle$	6.9 (2.9)	4.2 (2.0)	12.8 (3.3)	7.5 (2.0)
R_{meas}	0.131 (0.425)	0.198 (0.466)	0.072 (0.528)	0.103 (0.617)
Overall B factor from Wilson plot (Å^2)	10.40	30.71	43.18	37.05

Table S2. Structure solution and refinement (Values for the outer shell are given in parentheses).

	*Ef*TS – dUMP	*Ef*TS – FdUMP	hTS – dUMP	hTS – FdUMP
PDB code	**6QYA**	**6QXS**	**6QXH**	**6QXG**
Resolution range (Å)	68.07-1.76 (1.81-1.76)	96.76-2.88 (2.96-2.88)	34.22-2.04 (2.09-2.04)	32.36-2.08 (2.13-2.08)
Completeness (%)	94.88 (96.44)	94.42 (95.20)	96.69 (96.57)	97.86 (98.68)
No. of reflections, working set	118686 (8891)	26635 (1942)	58183 (4281)	59309 (4378)
No. of reflections, test set	6295 (471)	1350 (119)	4434 (310)	3181 (243)
Final R_{cryst}	0.184 (0.230)	0.200 (0.291)	0.187 (0.357)	0.181 (0.277)
Final R_{free}	0.220 (0.258)	0.252 (0.296)	0.237 (0.339)	0.225 (0.303)
Estimated error on coordinated based on R value (Å)	0.13	0.45	0.19	0.19
No. of non-H atoms				
Protein	9894	9670	6804	6786
Ion	16	10	6	5
Ligand	140	110	67	63
Water	1523	327	205	413
Total	11573	10117	7082	7267
R.m.s. deviations				
Bonds (Å)	0.022	0.007	0.010	0.012
Angles (°)	2.271	1.613	1.940	2.010
Average *B* factors (Å^2)	24.67	38.77	69.4	82.93
Ramachandran plot				
Most favoured (%)	97.9	95.2	96.9	96.5
Allowed (%)	2.1	4.8	3.1	3.5