

Supplementary information for:

Visualization of codon-dependent conformational rearrangements during translation termination

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Running title: Conformational rearrangements induced by RF1 codon recognition

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Supplementary Figure 1

a

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ec RF1 -----MKPSIVAKLEAL156EEHREVOALGCD156CTIAQERF156ALSR156YAO156SDVSRCTDW156VOED156ETQAMLLDDPEH156EMA156DE 81
tt RF1 -----MLDKLDRLE156EYHELEALS156SD156VL156KG156RY156SLSR156YAE156GEVIGLIREY156VLED156EQAESLLDDPEL156EMA156AE 77
tm RF1 -----MEEKKELEKLAR156DLTP156Q--M156NYGM156YAK156EEIENITNRI156EQE156FL-----L156EFG156NE 59
ec RF2 MFEFN156PVNNRIQD156ITERS156DLRGYLDYDAK156E156L156RE156VAE156PO156LV156W156N156PERA156ALGK156RSS156EAVVDLDQM156GL156ED156GLLELAVEADD156PP156EA 99
tt RF2 -----MDLERLAQRLEGLRGYLDIPQK156T156L156RE156RR156ED156SL156W156N156PEAA156KVSO156AAR156RR156TVDFRSL156SS156---D156GLLELMEELPA156RR156-SAL 87
tm RF2 MGMTSFPETK156RIE156E156LE156K156Y156K156VD156LV156SV156N156DE156IK156LE156VE156KK156LD156SV156WD156QKKA156EY156TO156QL156KR156K156NI156SE156DL156KRV156SL156FD156EV156AE156LS156DE156--D156EMA156HV 98

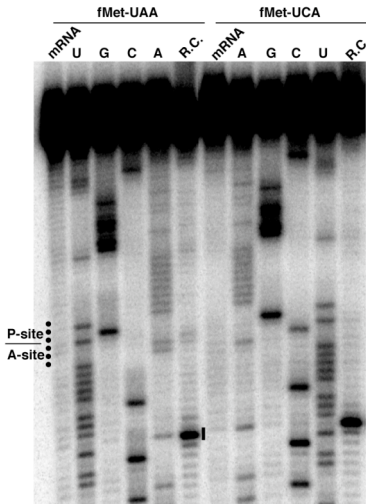
ec RF1 LREAKEKSE156Q156E156Q156LV156LL156PKDP156DDER156NA156F156EV156RAC156GG156DE156AL156FA156GD156FR156MY156SR156VA156BARR156RV156EM156ASEGE156H156GGY156K156E156I156AK156IS156ED156YGR156K156FE156GG 181
tt RF1 REALLARKEA156E156ELER156HL156PKDP156MDER156DA156VE156IRAC156GG156PE156AL156FA156RD156FR156MY156SR156VA156BEMG156ET156DL156SHPTDL156GG156FS156K156V156FE156VP156GG156YGT156K156YE156GV 177
tm RF1 LEIEKYEKE156-ID156LYQEL156LL156SPEAS156KAI156VE156IRAC156GG156PE156AL156FA156RD156FR156MY156SR156VA156BKMG156NLE156VA156I156HETDL156GG156IREV156FF156V156CK156NY156GI156K156YE156GV 158
ec RF2 VAELDALEEK156A156LE156FR156MS156-GEYDSAD156GY156DI156OAG156GG156EA156QD156VA156SM156LER156MY156SR156VA156BSRG156KTE156IE156SE156GEV156GI156K156SV156TI156K156IS156ED156Y156GW156RT156EG156V 198
tt RF2 KPELEEA156AK156D156LY156HT156LL156N156-FP156HA156E156KN156AI156TI156OAG156GG156EA156CD156VA156EM156LER156MY156SR156VA156BKMG156QVE156V156LT156PG156PE156GI156D156YA156Q156IL156V156CE156Y156GL156SP156E156GV 186
tm RF2 EEIVQELEG156AK156LE156LE156IL156N156-G156K156Y156DP156NA156Y156SV156HR156GG156ES156QD156VA156OM156LER156MY156SR156VA156BKMG156DVE156V156F156Q156PEE156GI156K156D156AT156IL156ICE156Y156GY156K156HE156GV 197

ec RF1 HR156V156RV156ATE156SO156GR156H156TS156ACT156VA156ME156EL156PA156EL156PD156IN156PA156DL156RI156ET156FR156SS156AG156GG156OV156NT156SA156RI156TH156PT156GI156V156EC156OD156RS156OH156KN156AK156AL156SV156GAR156HAA 281
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tm RF1 HR156V156RV156VE156TE156SG156GR156H156TS156ACT156VA156ME156ED156I156-E156IR156PE156DL156K156IE156TR156SG156GG156OV156NT156SA156RI156TH156PT156GI156V156SC156ON156ERS156OH156KN156AK156AL156RI156KAR156LY156Q156L 257
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tm RF2 HR156V156RV156SP156FD156ARR156H156TS156ACT156VA156ME156VD156VD156IE156IR156PE156DL156K156IE156TR156SG156GG156OV156NT156SA156RI156TH156PT156GI156V156SC156ON156ERS156OH156KN156AK156AL156KI156KAR156LY156EL 297

ec RF1 EMAK156ROQA156FA156ST156RN156LL156GG156DR156SD156NR156RY156NP156-QGR156VD156HR156IN156LL156Y156RL156DE156VE156CK156DM156E156PI156IQ156EQ156AD156OLA156ALS156-----EQE 360
tt RF1 KRAE156BAER156IK156TL156LA156IG156T156GERSE156Q156IR156Y156NP156-QSR156VD156HR156IT156FT156HD156LG156LS156CH156TP156E156ALK156RA156D156QER156OLA156ALA156-----EG- 354
tm RF1 OK156Q156K156ERE156IS156OK156KS156IG156T156GERSE156Q156IR156Y156NP156-QNR156VD156HR156IT156YS156Y156RL156CE156LD156GD156DE156SK156LE156HD156I156ENN156LE156EV156LG156AS156VEEK 342
ec RF2 EM156Q156K156NAE156QAM156-DN156K156SD156IG156WGS156Q156IR156Y156VD156-DSR156VD156LR156GV156ET156RNT156AV156LD156GS156DD156Q156EAS156LK156AGL156----- 365
tt RF2 ER156KK156REEL156KAL156-GE156VE156PI156EWGS156Q156IR156Y156VD156-KN156Y156VD156HR156GL156MR156HD156PN156LD156GD156MD156WAG156LE156WK156AGRR156----- 356
tm RF2 EM156E156KK156RR156E156OPT156-GE156K156DIS156WGN156Q156IR156Y156HP156Y156TM156VD156HR156GV156ET156AN156V156LA156VD156GD156MD156E156AE156LV156Y156FARR156SS156----- 369

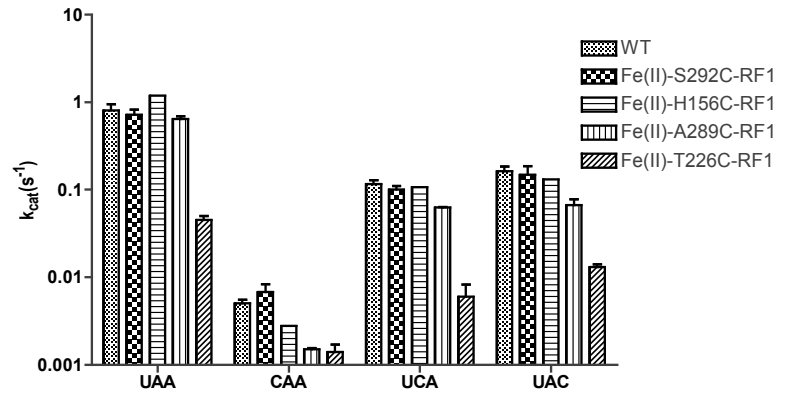
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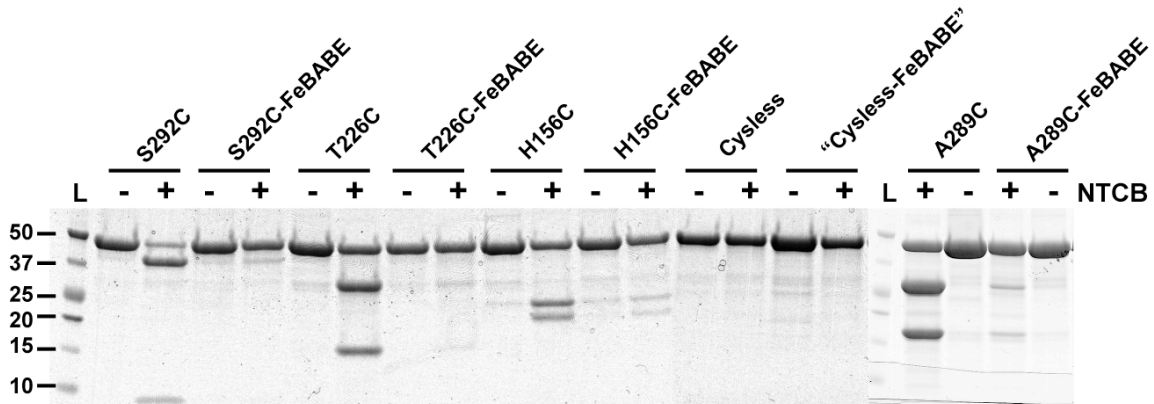


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fMet release of WT RF1 and Fe(II) tethered single cysteine RF1

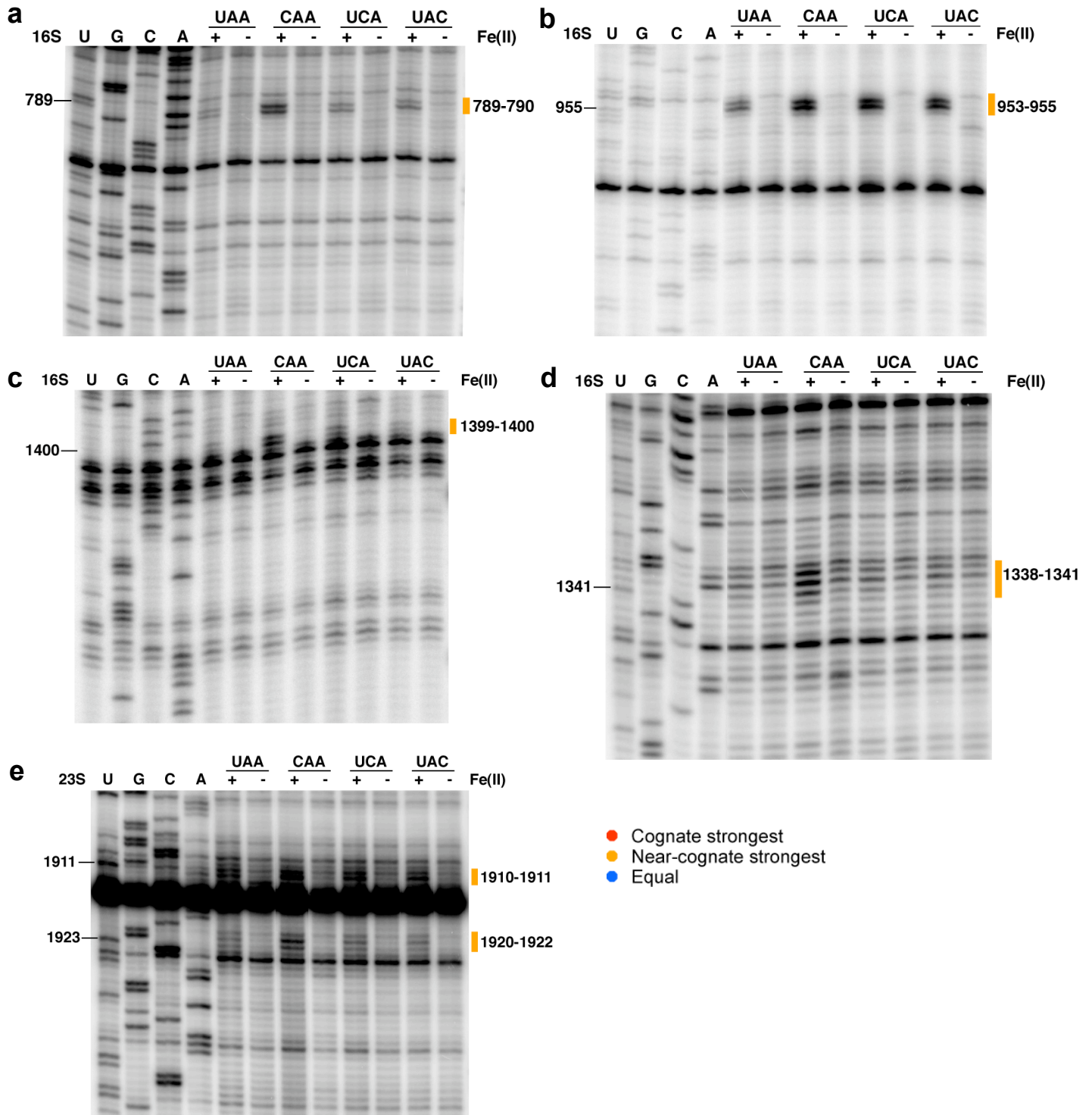


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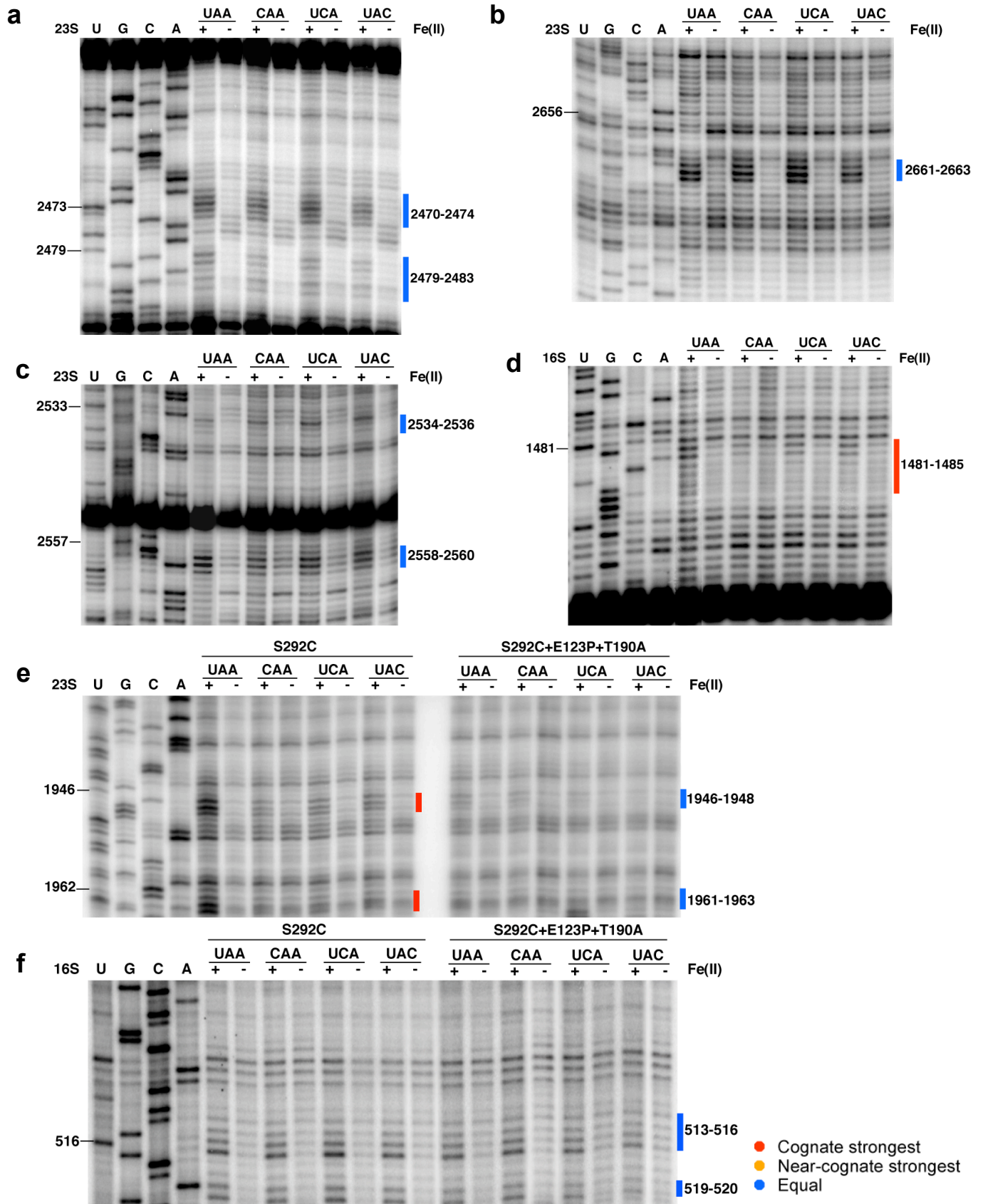
Supplementary Figure 1. Identification of candidate single cysteine RF1 variants for probing structural differences between authentic stop- and near-stop- codon complexes. (a) Alignment of several bacterial class 1 RF variants; *E. coli* (ec), *T. thermophilus* (tt), and *T. maritima* (tm). Extremely conserved residues are highlighted in black and somewhat less conserved residues in grey. Functionally important motifs including tripeptide anticodon (PV/AT or SPF), catalytic GGQ and switch loop are labeled in blue. The four single cysteine RF1 variants chosen for thorough characterization are labeled in pink while the ones that were discarded are shown in dark red. (b) Toeprinting analysis of the ribosome complexes (UAA or UCA at second codon). Lanes (U, G, C and A) indicate sequencing lane for the mRNA. (mRNA) represents a primer extension reaction on just the mRNA of interest. (R.C.) represents a primer extension reaction on the ribosome complex with mRNA positioned by tRNA^{Met} in the P site. The positions of the P-site and A-site codons are labeled on the sides of the gel and the position of the toeprint is highlighted by a black bar. (c) Functional characterization of Fe(II)-derivatized single-cysteine RF1 variants by *in vitro* peptide release assay. The bar graph shows the rate constants for catalysis at saturating release factor concentrations (k_{cat}) for wild-type RF1 and derivatized single cysteine RF1 mutants on (UAA) stop- or (CAA, UCA and UAC) near-stop-programmed ribosomes. Bars represent the mean \pm standard error from at least two experiments. (d) SDS-PAGE gel of NTCB cleavage analysis of FeBABE derivatization efficiency. (L) is a protein ladder with molecular weights indicated on the left. (+) or (-) lanes represent samples where NTCB reagent was included, or not.

Supplementary Figure 2



Supplementary Figure 2. Primer extension analysis of cleavage pattern of 16S rRNA (a-d) and 23S rRNA (e) from saturating levels of Fe(II)-H156C-RF1 in ribosome complexes with UAA, CAA, UCA or UAC codons in the A site. (U, G, C and A) are sequencing lanes. (+) represents the Fe(II)-H156C-RF1 sample while (–) represents the sample treated with mock labeled Cysless-RF1. Primers used begin at position 906 (a), 1046 (b), 1490 (c) and 1391 (d) of 16S rRNA and 2042 (e) of 23S rRNA. Bars and numbers at right indicate cleavages corresponding to nucleotides within 16S rRNA or 23S rRNA.

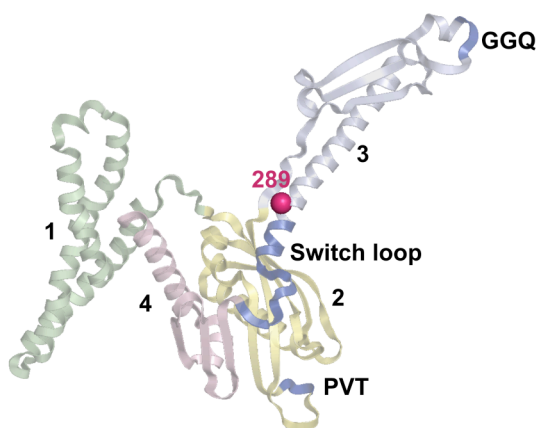
Supplementary Figure 3



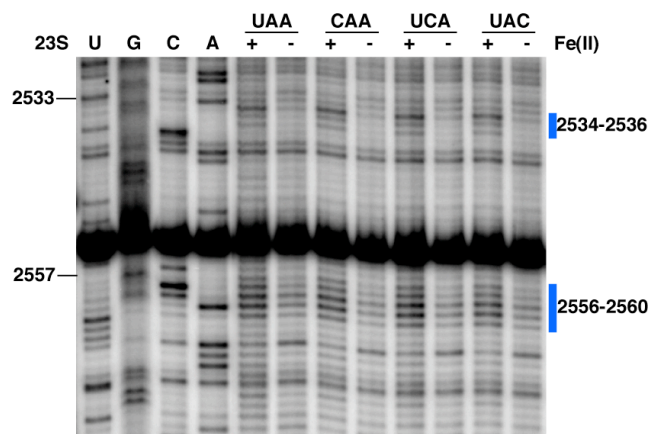
Supplementary Figure 3. Primer extension analysis of cleavage pattern of 23S rRNA (a-c and e) and 16S rRNA (d, f) from saturating levels of Fe(II)-S292C-RF1 with or without the indicated mutations in ribosome complexes with UAA, CAA, UCA or UAC codons in the A site. (U, G, C and A) are sequencing lanes. (+) represents the Fe(II)-S292C-RF1 (with or without the indicated mutations) sample while (–) represents the sample treated with mock labeled Cysless-RF1. Primers used begin at position 2542 (a), 2741 (b), 2639 (c) and 2042 (e) of 23S rRNA as well as 1508 (d) and 565 (f) of 16S rRNA. Bars and numbers at right indicate cleavages corresponding to nucleotides within 16S and 23S rRNA.

Supplementary Figure 4

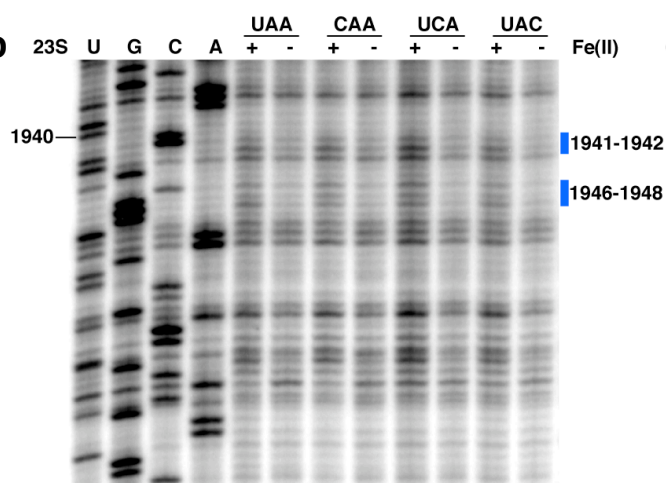
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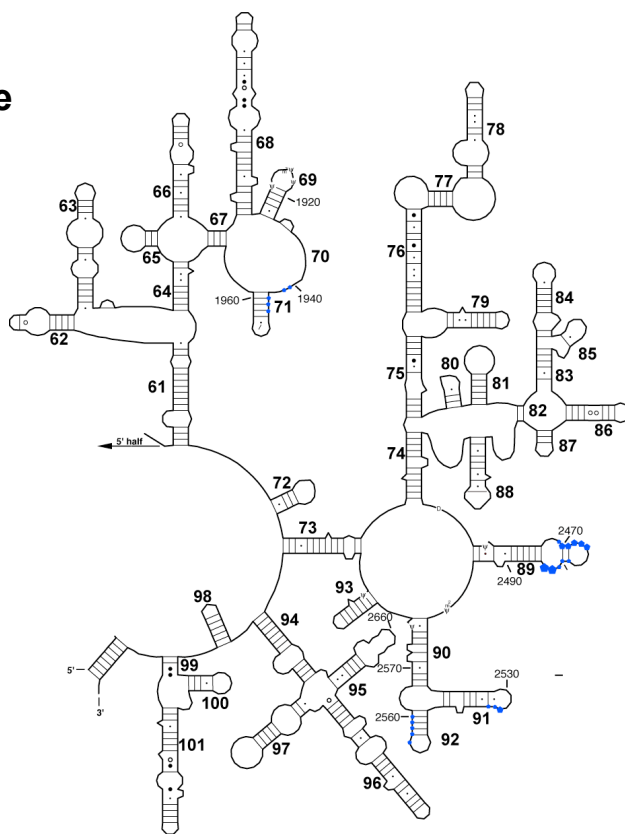
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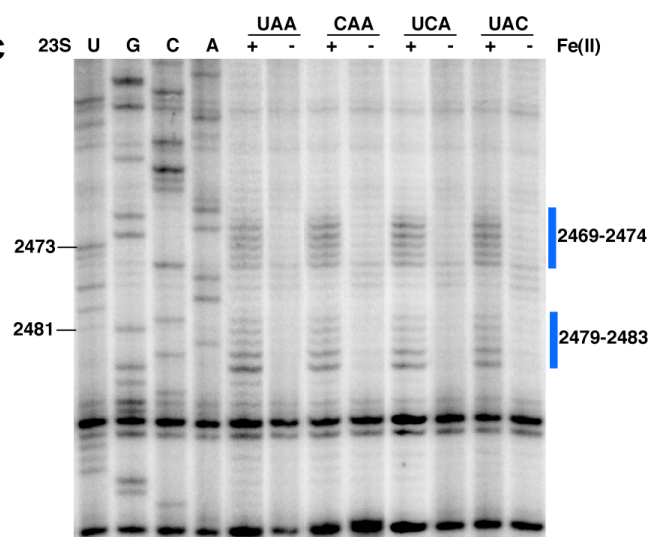
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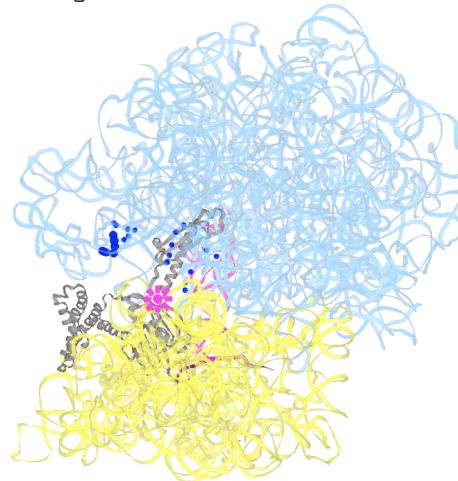


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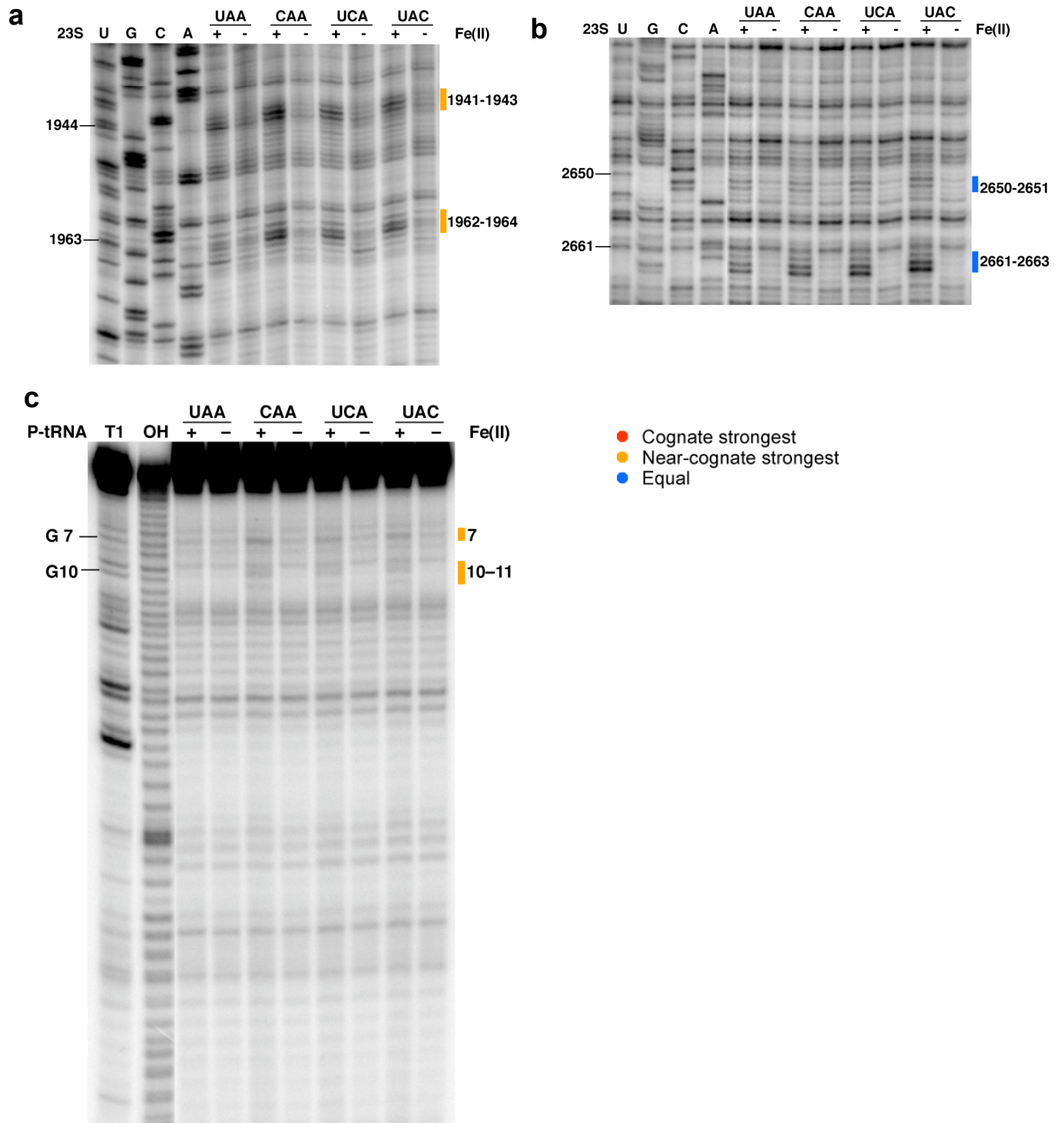
- Cognate strongest
- Near-cognate strongest
- Equal

f



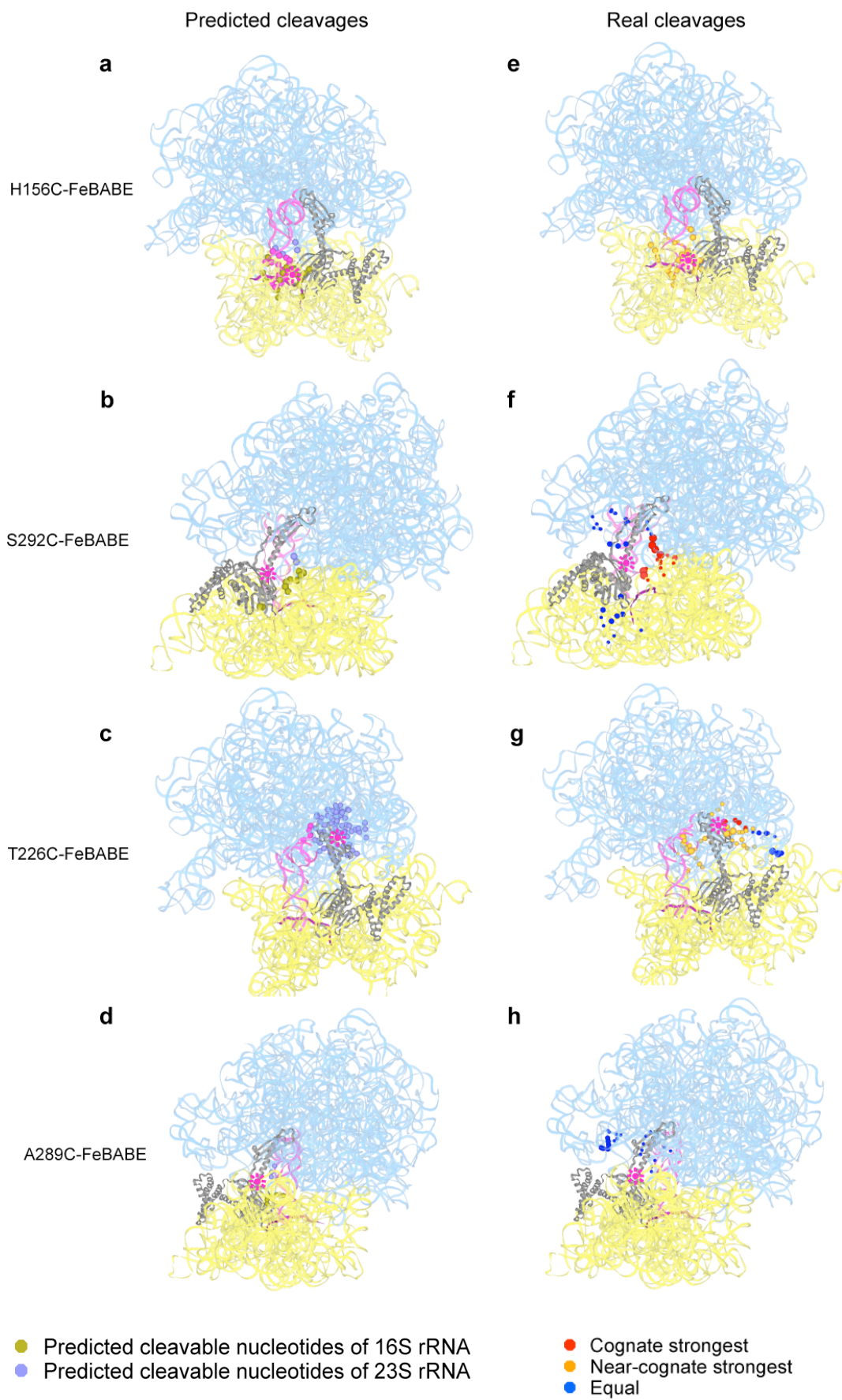
Supplementary Figure 4. Directed hydroxyl radical probing of the ribosome environment near position 289 (next to the switch loop of RF1) in ribosome complexes programmed with various codons in the A site. (a) Ribbon diagram of RF1. Sphere indicates C α of residue 289 where Fe(II) is tethered. (b-d) Primer extension analysis of cleavage of 23S rRNA from saturating levels of Fe(II)-A289C-RF1 incubated with the indicated ribosome complexes with primers beginning at position (b) 2042, (c) 2542 and (d) 2639. (U, G, C and A) are sequencing lanes. (+) represents the Fe(II)-A289C-RF1 sample while (-) represents the sample treated with mock labeled Cysless-RF1. Bars and numbers at right indicate cleavages corresponding to nucleotides within 23S rRNA. (e) Directed hydroxyl radical cleavage sites from Fe(II)-A289C-RF1 displayed on the secondary structure of the 3' half of 23S rRNA. (g) All cleavage sites modeled on tertiary structure of RF1 bound ribosome complex (PDB entry: 3D5A and 3D5B), as in Figure 1.

Supplementary Figure 5



Supplementary Figure 5. Primer extension analysis and denaturing sequencing gel analysis of cleavage pattern of 23S rRNA (a-b) and P-site tRNA (c) from saturating levels of Fe(II)-T226C-RF1 in ribosome complexes with UAA, CAA, UCA or UAC codons in the A site. (U, G, C and A) are sequencing lanes. (+) represents the Fe(II)-T226C-RF1 sample while (–) represents the sample treated with mock labeled Cysless-RF1. Primers used are 2042 (a) and 2741 (b). (c) Denaturing sequencing gel analysis of cleavage of P-site tRNA from Fe(II)-T226C-RF1. (T1) cleavage by RNaseT1; (OH) alkaline hydrolysis ladder. Bars and numbers at right indicate cleavages corresponding to nucleotides within 23S rRNA or P-site tRNA.

Supplementary Figure 6



Supplementary Figure 6. Comparison of the predicted cleavable nucleotides (a-d) and the observed cleaved nucleotides (e-h) by hydroxyl radicals from position 156 (a, e), 292 (b, f), 226 (c, g) and 289 (d, h). Cleavable nucleotides were predicted by locating all the nucleotides within probing range (24 Å) of the positions where Fe(II) is tethered based on reported crystal structures (PDB entry: 3D5A and 3D5B). Predicted nucleotides of 16S rRNA are shown in olive green while those of 23S rRNA are shown in light blue. Observed cleavages are colored as throughout the paper; cleavages stronger on cognate (red), stronger on near-cognate (orange), and equivalent on all (blue).