In vivo incorporation of unnatural amino acids to probe structure, dynamics and ligand binding in a large protein by Nuclear Magnetic Resonance spectroscopy

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Supplemental Materials

Table S1. DNA sequences of selected OCF_3Phe-specific tRNA synthetases. Nucleotide differences are highlighted in red and underlined. Clones labeled in Bold were evaluated further.

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                1         10         20         30         40         50
All             ATGGACGAATTTGAAATGATAAAGAGAAACACATCTGAAATTATCAGCGAGGAAGAGTTA
                61         70         80         90        100        110
Library         AGAGAGGTTTTAAAAAAAGATGAAAAATCTGCTNNNATAGGTTTTGAACCAAGTGGTAAA
OCF3Phe_A6      AGAGAGGTTTTAAAAAAAGATGAAAAATCTGCTGTGATAGGTTTTGAACCAAGTGGTAAA
OCF3Phe_B6      AGAGAGGTTTTAAAAAAAGATGAAAAATCTGCTGCTATAGGTTTTGAACCAAGTGGTAAA
OCF3Phe_B7      AGAGAGGTTTTAAAAAAAGATGAAAAATCTGCTGTGATAGGTTTTGAACCAAGTGGTAAA
OCF3Phe_B10     AGAGAGGTTTTAAAAAAAGATGAAAAATCTGCTGCTATAGGTTTTGAACCAAGTGGTAAA
OCF3Phe_C2      AGAGAGGTTTTAAAAAAAGATGAAAAATCTGCTGTTATAGGTTTTGAACCAAGTGGTAAA
OCF3Phe_D5      AGAGAGGTTTTAAAAAAAGATGAAAAATCTGCTGTTATAGGTTTTGAACCAAGTGGTAAA
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OCF3Phe_E7      AGAGAGGTTTTAAAAAAAGATGAAAAATCTGCTGTGATAGGTTTTGAACCAAGTGGTAAA
OCF3Phe_F6      AGAGAGGTTTTAAAAAAAGATGAAAAATCTGCTGCTATAGGTTTTGAACCAAGTGGTAAA
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OCF3Phe_H4      AGAGAGGTTTTAAAAATAGATGAAAAATCTGCTGTGATAGGTTTTGAACCAAGTGGTAAA
                121       130        140        150        160        170
All             ATACATTTAGGGCATTATCTCCAAATAAAAAAGATGATTGATTTACAAAATGCTGGATTT
                181       190        200        210        220        230
Library         GATATAATTATANNNTTGGCTGATTTANNNGCCTATTTAAACCAGAAAGGAGAGTTGGAT
OCF3Phe_A6      GATATAATTATAGCTTTGGCTGATTTACACGCCTATTTAAACCAGAAAGGAGAGTTGGAT
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OCF3Phe_B7      GATATAATTATACTGTTGGCTGATTTACACGCCTATTTAAACCAGAAAGGAGAGTTGGAT
OCF3Phe_B10     GATATAATTATAGCTTTGGCTGATTTACATGCCTATTTAAACCAGAAAGGAGAGTTGGAT
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OCF3Phe_E7      GATATAATTATATCGTTGGCTGATTTACACGCCTATTTAAACCAGAAAGGAGAGTTGGAT
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OCF3Phe_H4      GATATAATTATAGGGTTGGCTGATTTACACGCCTATTTAAACCAGAAAGGAGAGTTGGAT
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                301       310        320        330        340        350
Library         AAATATGTTTATGGAAGTGAANNNNNNCTTGATAAGGATTATACACTGAATGTCTATAGA
OCF3Phe_A6      AAATATGTTTATGGAAGTGAACAGTGGCTTGATAAGGATTATACACTGAATGTCTATAGA
OCF3Phe_B6      AAATATGTTTATGGAAGTGAAAAGTGGCTTGATAAGGATTATACACTGAATGTCTATAGA
OCF3Phe_B7      AAATATGTTTATGGAAGTGAAGCTTGGCTTGATAAGGATTATACACTGAATGTCTATAGA
OCF3Phe_B10     AAATATGTTTATGGAAGTGAATGGATGCTTGATAAGGATTATACACTGAATGTCTATAGA
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OCF3Phe_D5      AAATATGTTTATGGAAGTGAAGAGCCTCTTGATAAGGATTATACACTGAATGTCTATAGA
OCF3Phe_D9      AAATATGTTTATGGAAGTGAATGGATGCTTGATAAGGATTATACACTGAATGTCTATAGA
OCF3Phe_E7      AAATATGTTTATGGAAGTGAAACGCAGCTTGATAAGGATTATACACTGAATGTCTATAGA
OCF3Phe_F6      AAATATGTTTATGGAAGTGAACAGGCGCTTGATAAGGATTATACACTGAATGTCTATAGA
OCF3Phe_F7      AAATATGTTTATGGAAGTGAACGTTGGCTTGATAAGGATTATACACTGAATGTCTATAGA
OCF3Phe_F8      AAATATGTTTATGGAAGTGAAAGGGAGCTTGATAAGGATTATACACTGAATGTCTATAGA
OCF3Phe_G2      AAATATGTTTATGGAAGTGAATGGATGCTTGATAAGGATTATACACTGAATGTCTATAGA
OCF3Phe_G5      AAATATGTTTATGGAAGTGAATTGGGGCTTGATAAGGATTATACACTGAATGTCTATAGA
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Table S1. Continuation.

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OCF3Phe_A6      GATGAAAATCCAAAGGTTGCTGAAGTTATCTATCCAATAATGCAGGTTAATGCGATTCAT
OCF3Phe_B6      GATGAAAATCCAAAGGTTGCTGAAGTTATCTATCCAATAATGCAGGTTAATGGTATTCAT
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OCF3Phe_B10     GATGAAAATCCAAAGGTTGCTGAAGTTATCTATCCAATAATGCAGGTTAATGGTAATCAT
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OCF3Phe_D5      GATGAAAATCCAAAGGTTGCTGAAGTTATCTATCCAATAATGCAGGTTAATTCTATTCAT
OCF3Phe_D9      GATGAAAATCCAAAGGTTGCTGAAGTTATCTATCCAATAATGCAGGTTAATGGTGCTCAT
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OCF3Phe_G5      GATGAAAATCCAAAGGTTGCTGAAGTTATCTATCCAATAATGCAGGTTAATTCGATTCAT
OCF3Phe_H4      GATGAAAATCCAAAGGTTGCTGAAGTTATCTATCCAATAATGCAGGTTAATGCTATTCAT
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OCF3Phe_A6      TATAAGGGCGTTGATGTTGCAGTTGGAGGGATGGAGCAGAGAAAAATACACATGTTAGCA
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OCF3Phe_B10     TATCTTGGCGTTGATGTTGCAGTTGGAGGGATGGAGCAGAGAAAAATACACATGTTAGCA
OCF3Phe_C2      TATGTGGGCGTTGATGTTGCAGTTGGAGGGATGGAGCAGAGAAAAATACACATGTTAGCA
OCF3Phe_D5      TATAGTGGCGTTGATGTTGCAGTTGGAGGGATGGAGCAGAGAAAAATACACATGTTAGCA
OCF3Phe_D9      TATCTTGGCGTTGATGTTGCAGTTGGAGGGATGGAGCAGAGAAAAATACACATGTTAGCA
OCF3Phe_E7      TATGTGGGCGTTGATGTTGCAGTTGGAGGGATGGAGCAGAGAAAAATACACATGTTAGCA
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OCF3Phe_G2      TATCTTGGGGTTGATGTTGCAGTTGGAGGGATGGAGCAGAGAAAAATACACATGTTAGCA
OCF3Phe_G5      TATAGTGGCGTTGATGTTGCAGTTGGAGGGATGGAGCAGAGAAAAATACACATGTTAGCA
OCF3Phe_H4      TATCATGGCGTTGATGTTGCAGTTGGAGGGATGGAGCAGAGAAAAATACACATGTTAGCA
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All             GGAGAAGGAAAGATGAGTTCTTCAAAAGGGAATTTTATAGCTGTTGATGACTCTCCAGAA
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All             GAGATTAGGGCTAAGATAAAGAAAGCATACTGCCCAGCTGGAGTTGTTGAAGGAAATCCA
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All             ATAATGGAGATAGCTAAATACTTCCTTGAATATCCTTTAACCATAAAAAGGCCAGAAAAA
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All             TTTGGTGGAGATTTGACAGTTAATAGCTATGAGGAGTTAGAGAGTTTATTTAAAAATAAG
                841       850       860       870       880       890
All             GAATTGCATCCAATGGATTTAAAAAATGCTGTAGCTGAAGAACTTATAAAGATTTTAGAG
                901       910       920
All             CCAATTAGAAAGAGATTATAA
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Table S2. Protein sequences of selected OCF_3Phe-specific tRNA synthetases. Amino acid differences are highlighted in red and underlined. Clones labeled in Bold were evaluated further.

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OCF3PHE_B6          MDEFEMIKRNTSEIISEEELREVLKKDEKSAAIGFEPSGKIHLGHYLQIKKMIDLQNAGF
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OCF3PHE_C2          MDEFEMIKRNTSEIISEEELREVLKKDEKSAVIGFEPSGKIHLGHYLQIKKMIDLQNAGF
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OCF3PHE_D9          MDEFEMIKRNTSEIISEEELREVLKKDEKSALIGFEPSGKIHLGHYLQIKKMIDLQNAGF
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OCF3PHE_G5          MDEFEMIKRNTSEIISEEELREVLKKDEKSAVIGFEPSGKIHLGHYLQIKKMIDLQNAGF
OCF3PHE_H4          MDEFEMIKRNTSEIISEEELREVLKIDEKSAVIGFEPSGKIHLGHYLQIKKMIDLQNAGF
                    61        70        80        90        100       110
Library             DIIIXLADLXAYLNQKGELDEIRKIGDYNKKVFEAMGLKAKYVYGSEXXLDKDYTLNVYR
OCF3PHE_A6          DIIIALADLHAYLNQKGELDEIRKIGDYNKKVFEAMGLKAKYVYGSEQWLDKDYTLNVYR
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OCF3PHE_D5          DIIIHLADLHAYLNQKGELDEIRKIGDYNKKVFEAMGLKAKYVYGSEEPLDKDYTLNVYR
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OCF3PHE_H4          DIIIGLADLHAYLNQKGELDEIRKIGDYNKKVFEAMGLKAKYVYGSEHYLDKDYTLNVYR
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OCF3PHE_A6          LALKTTLKRARRSMELIAREDENPKVAEVIYPIMQVNAIHYKGVDVAVGGMEQRKIHMLA
OCF3PHE_B6          LALKTTLKRARRSMELIAREDENPKVAEVIYPIMQVNGIHYVGVDVAVGGMEQRKIHMLA
OCF3PHE_B7          LALKTTLKRARRSMELIAREDENPKVAEVIYPIMQVNGIHYQGVDVAVGGMEQRKIHMLA
OCF3PHE_B10         LALKTTLKRARRSMELIAREDENPKVAEVIYPIMQVNGNHYLGVDVAVGGMEQRKIHMLA
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OCF3PHE_D5          LALKTTLKRARRSMELIAREDENPKVAEVIYPIMQVNSIHYSGVDVAVGGMEQRKIHMLA
OCF3PHE_D9          LALKTTLKRARRSMELIAREDENPKVAEVIYPIMQVNGAHYLGVDVAVGGMEQRKIHMLA
OCF3PHE_E7          LALKTTLKRARRSMELIAREDENPKVAEVIYPIMQVNAIHYVGVDVAVGGMEQRKIHMLA
OCF3PHE_F6          LALKTTLKRARRSMELIAREDENPKVAEVIYPIMQVNAIHYYGVDVAVGGMEQRKIHMLA
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OCF3PHE_G5          LALKTTLKRARRSMELIAREDENPKVAEVIYPIMQVNSIHYSGVDVAVGGMEQRKIHMLA
OCF3PHE_H4          LALKTTLKRARRSMELIAREDENPKVAEVIYPIMQVNAIHYHGVDVAVGGMEQRKIHMLA
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                    241       250       260       270       280       290
All                 NFIAVDDSPEEIRAKIKKAYCPAGVVEGNPIMEIAKYFLEYPLTIKRPEKFGGDLTVNSY
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All                 EELESLFKNKELHPMDLKNAVAEELIKILEPIRKRLZ
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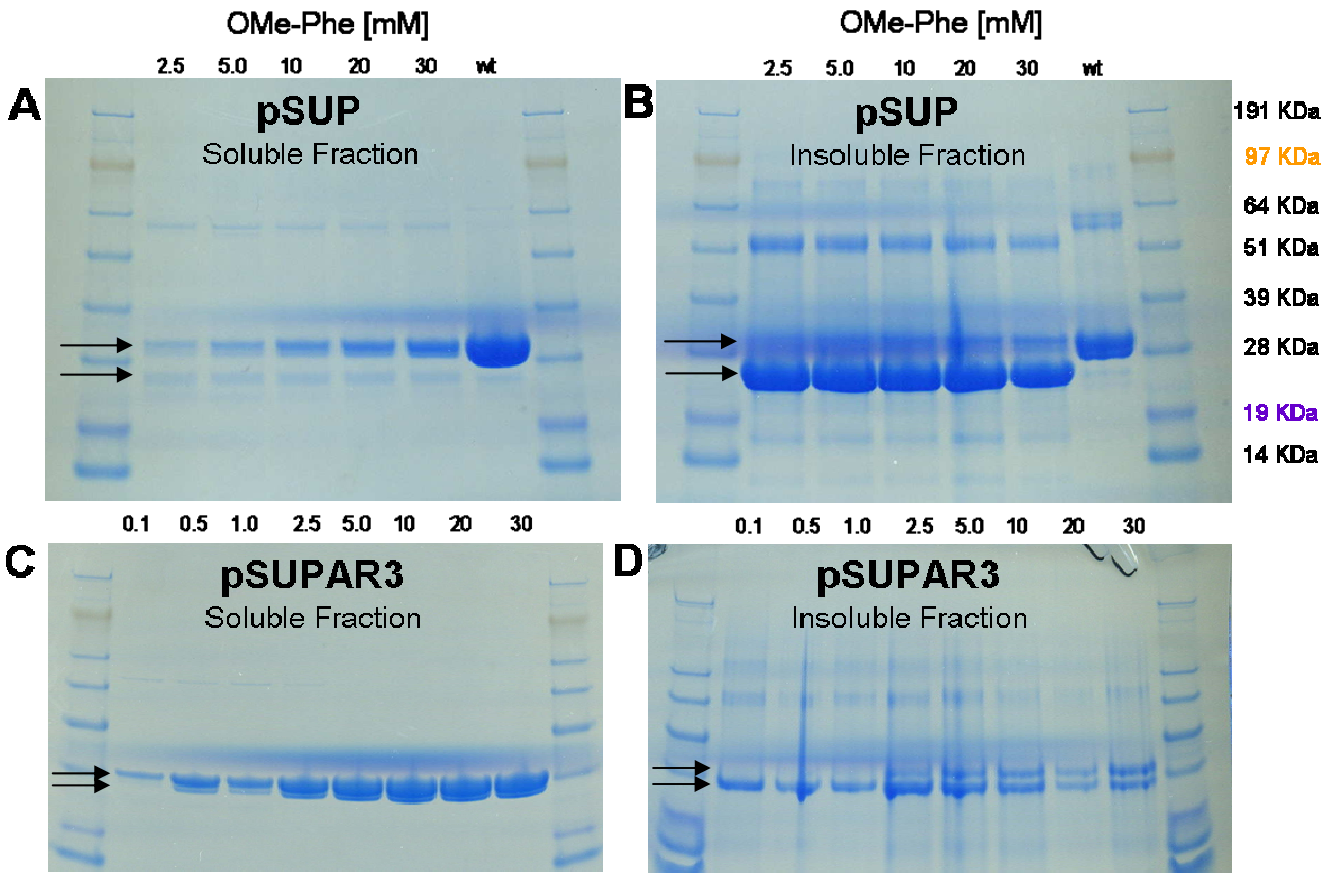
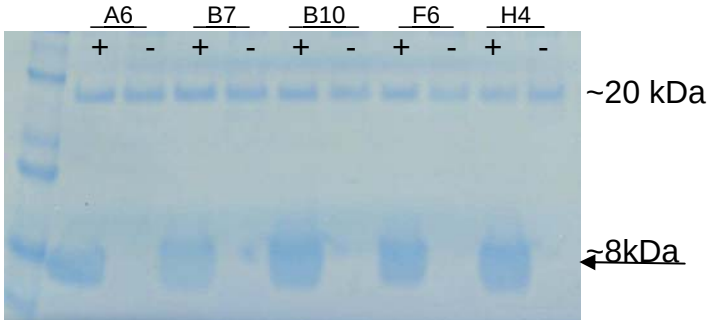


Figure S1. Incorporation of L-OMePhe into FAS-TE Tyr2454TAG using pSUP[13] (A and B) and pSUPAR3 (C and D). SDS gels of proteins purified by Nickel affinity chromatography from the soluble fraction of cell lysate (A and C) or after extraction of the insoluble pellet with 6 M guanidinium hydrochloride (B and D). 50 mL of *E. coli* culture were grown in TB media at various concentrations of OMePhe. Wild-type FAS-TE was expressed in the presence of the pSUP plasmid carrying the tRNA/tRNA synthetase pair and of 2.5 mM L-OMePhe. Truncated and full-length FAS-TE mutants are indicated by arrows and the size was confirmed by mass spectrometry. The soluble fractions generated with pSUPAR3 do not contain any detectable amounts of truncated FAS-TE as monitored by mass spectrometry.

A



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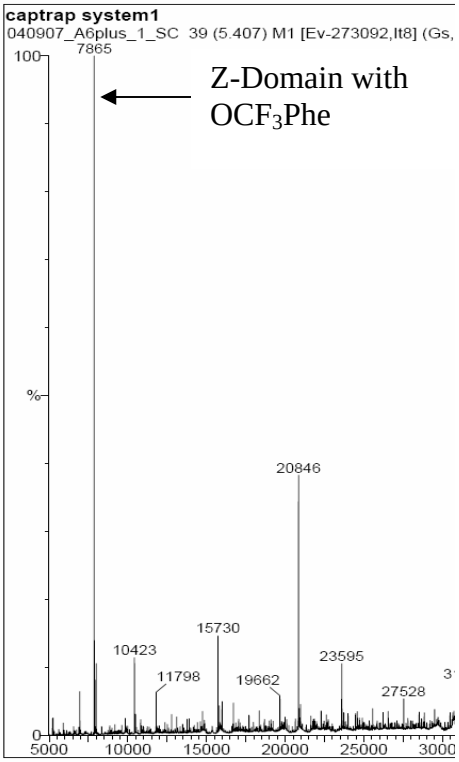

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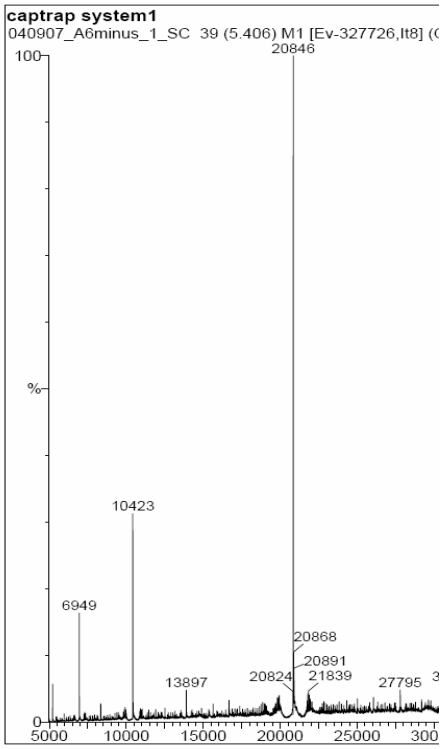
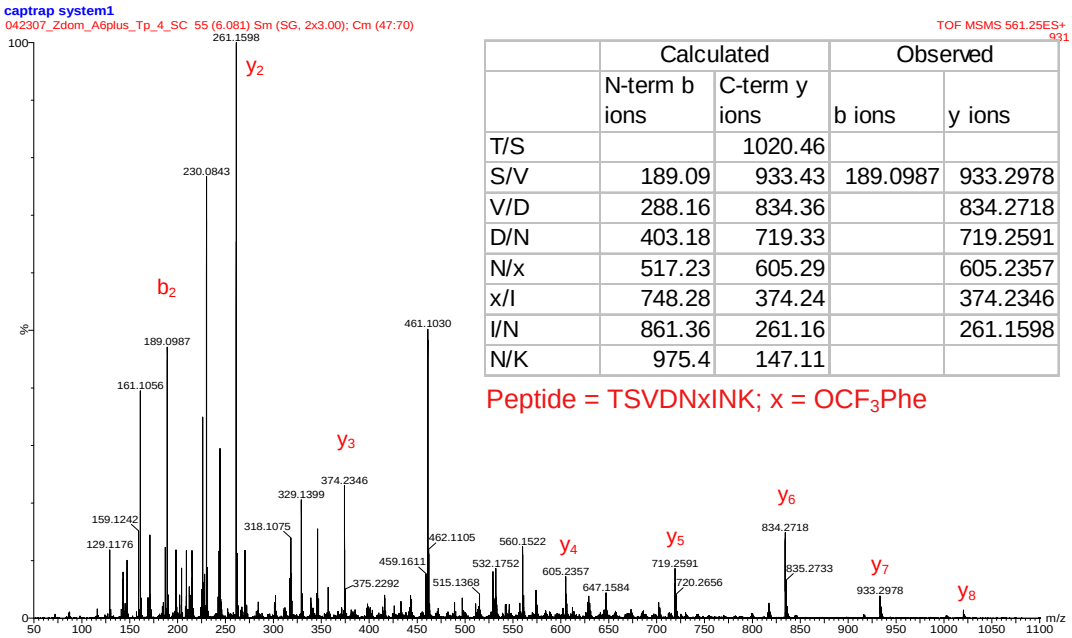


Figure S2. SDS-Gel (A) and ESI-MS spectra (B and C) of Z-domain expression with and without OCF_3Phe. (A) Incorporation of OCF_3Phe into residue 7 of Z-domain using different OCF_3Phe RS mutants (labeled by their clone number A6, B7, B10, F6 or H4) results in a band at approximately 8 kDa consistent with the expected mass of 7866 Da. The observed mass in the ESI-MS spectrum is 7865 Da (arrow, B). A contaminating *E. coli* protein of 20,846 Da (~20 kD gel band in A) is observed in all samples. In the absence of OCF_3Phe, full-length Z-domain is not observed. The resulting truncated TSVDN peptide is not observed in the SDS-gel (A) or in the ESI-MS spectra (C).

A



	Calculated		Observed	
	N-term b ions	C-term y ions	b ions	y ions
T/S		1020.46		
S/V	189.09	933.43	189.0987	933.2978
V/D	288.16	834.36		834.2718
D/N	403.18	719.33		719.2591
N/x	517.23	605.29		605.2357
x/I	748.28	374.24		374.2346
I/N	861.36	261.16		261.1598
N/K	975.4	147.11		

Peptide = TSVDNxINK; x = OCF_3Phe

B

Peptide = TSVDNxINK

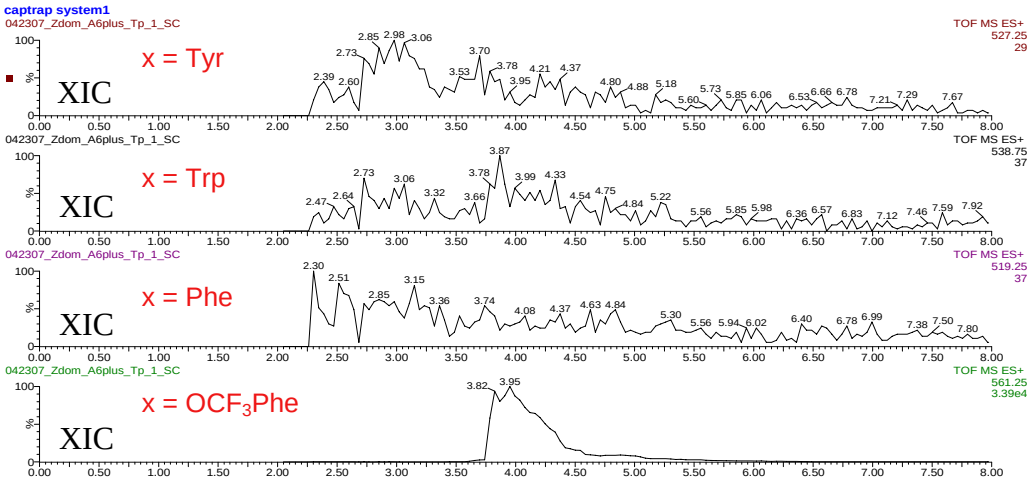


Figure S3. LC-ESI-MS analysis of tryptic digests of Z-domain with OCF_3Phe incorporated. (A) ESI-MS-MS analysis verifies incorporation of OCF_3Phe at the desired location in the tryptic peptide TSVDN(OCF_3Phe)INK. (B) Extracted single ion chromatograms (XIC) of the natural amino acid mutants Y, W, F and OCF_3Phe. Misincorporation of the natural amino acids Tyr, Trp and Phe is undetectable within the dynamic range of the experiment.

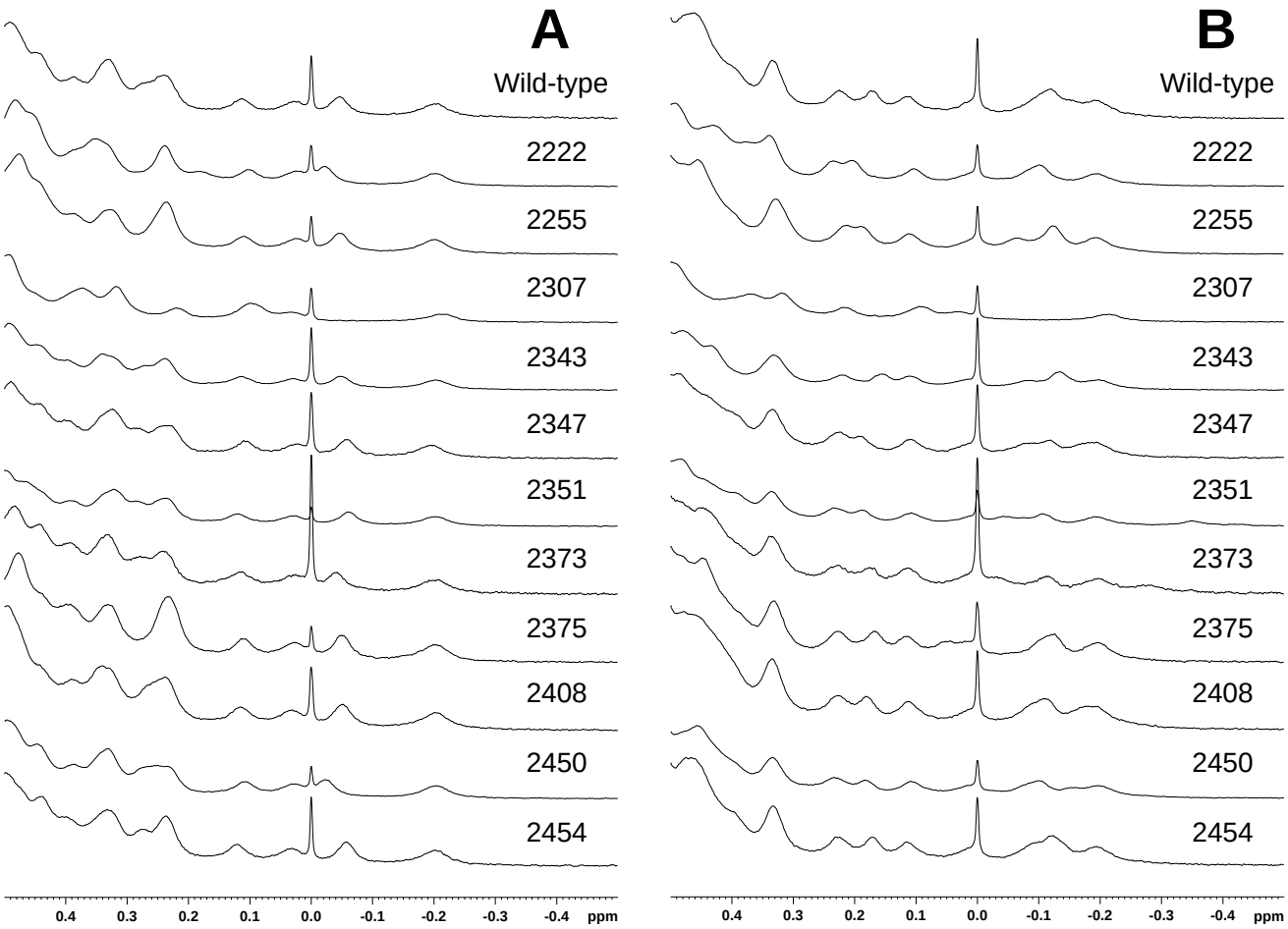


Figure S4. ^{1}H NMR of ^{13}C/^{15}N-OMePhe mutants with before (A) and after addition of tool compound (B).

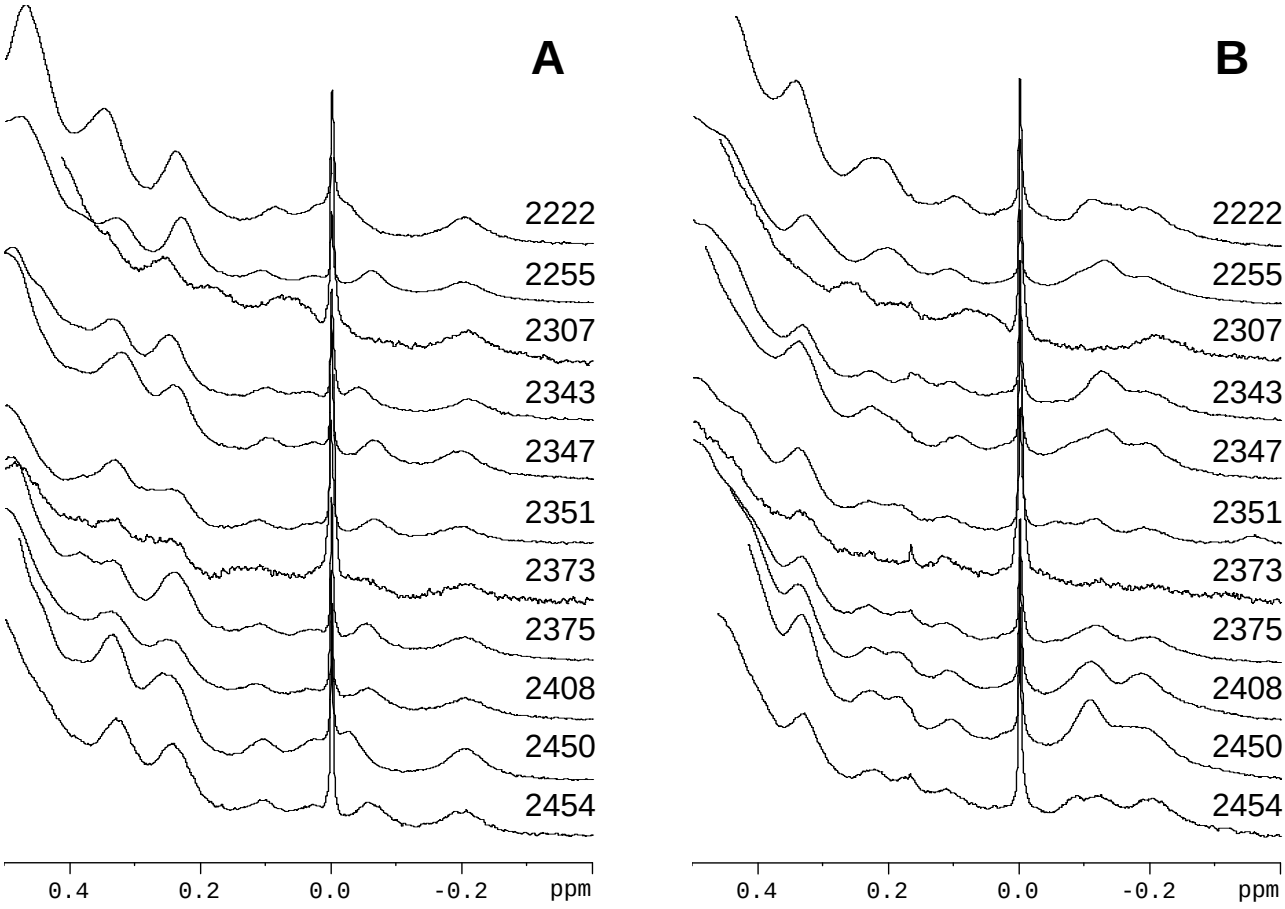


Figure S5. ^{1}H NMR of OCF_3Phe mutants before (A) and after addition of tool compound (B).

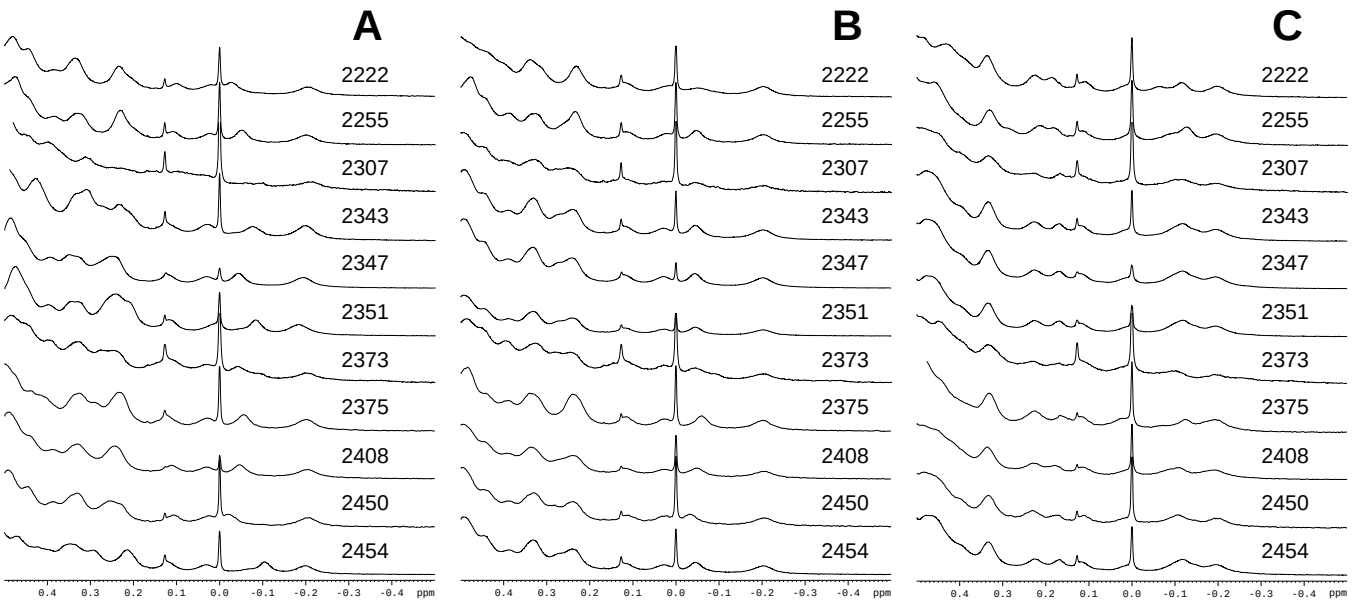


Figure S6. ^{1}H NMR of ^{15}N-oNBTyr mutants before (A) and after UV cleavage (B) and after addition of tool compound (C).

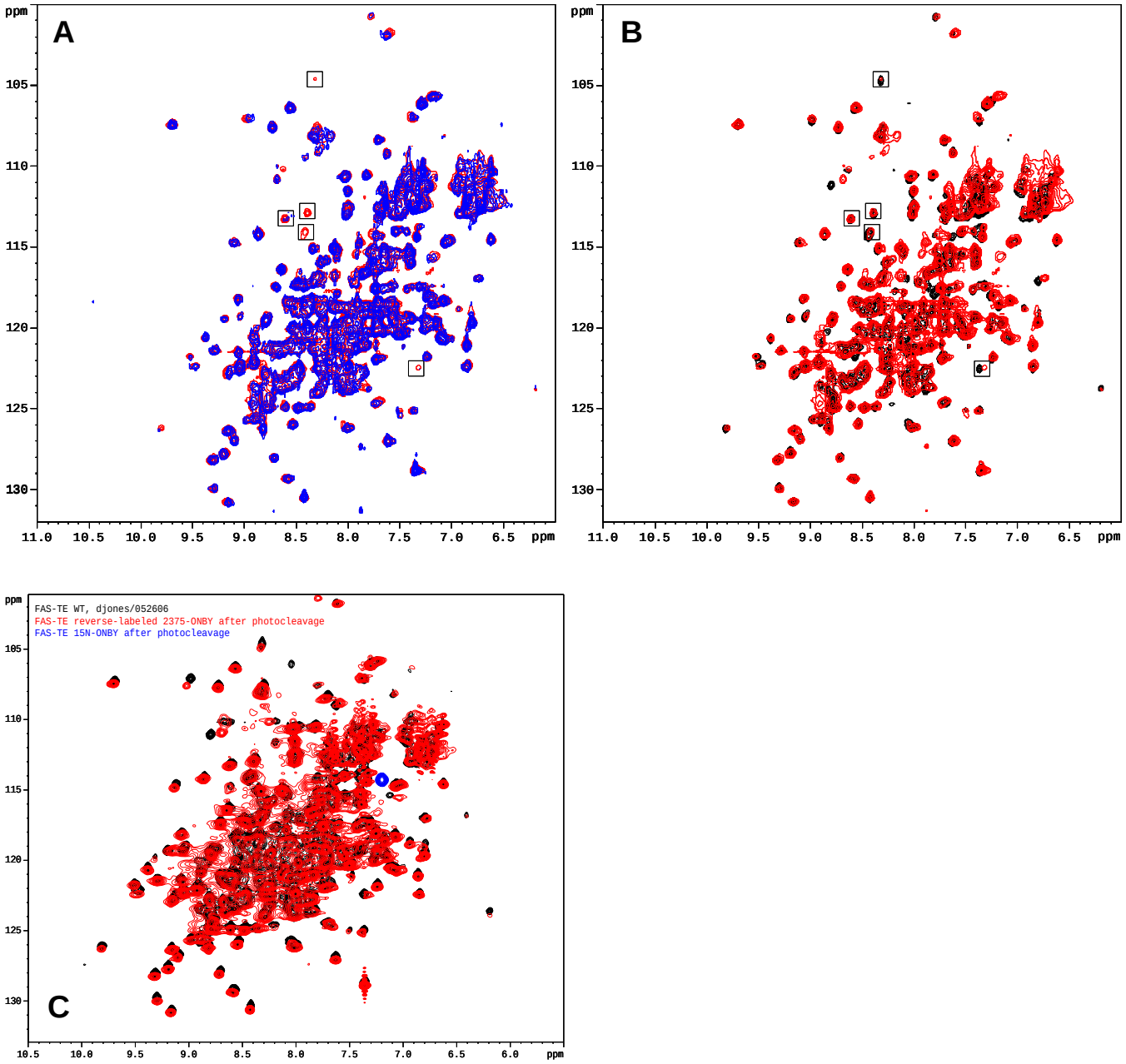


Figure S7. Reverse labeled samples indicating structural integrity of FAS-TE mutants. A) Overlay of ^{1}H-^{15}N HSQC spectra of ^{15}N-labeled FAS-TE with unlabeled oNBTyr incorporated in place of Tyr-2343 before (blue) and after (red) UV-illumination; B) Overlay of UV-cleaved Tyr-2343-oNBTyr FAS-TE (red) with wild-type FAS-TE (black); C) Overlay of UV-cleaved Tyr-2375-oNBTyr mutant (blue) with the spectrum of uniformly ^{15}N-labeled wild-type FAS-TE (black) and UV-cleaved ^{15}N-labeled Phe-2375-oNBTyr produced with unlabeled oNBTyr. As expected, the resonance peak of Tyr-2375 is missing from the wild-type spectrum (Phe-2375) and from the reverse labeled Phe-2375-oNBTyr sample but the rest of the spectrum is almost identical.

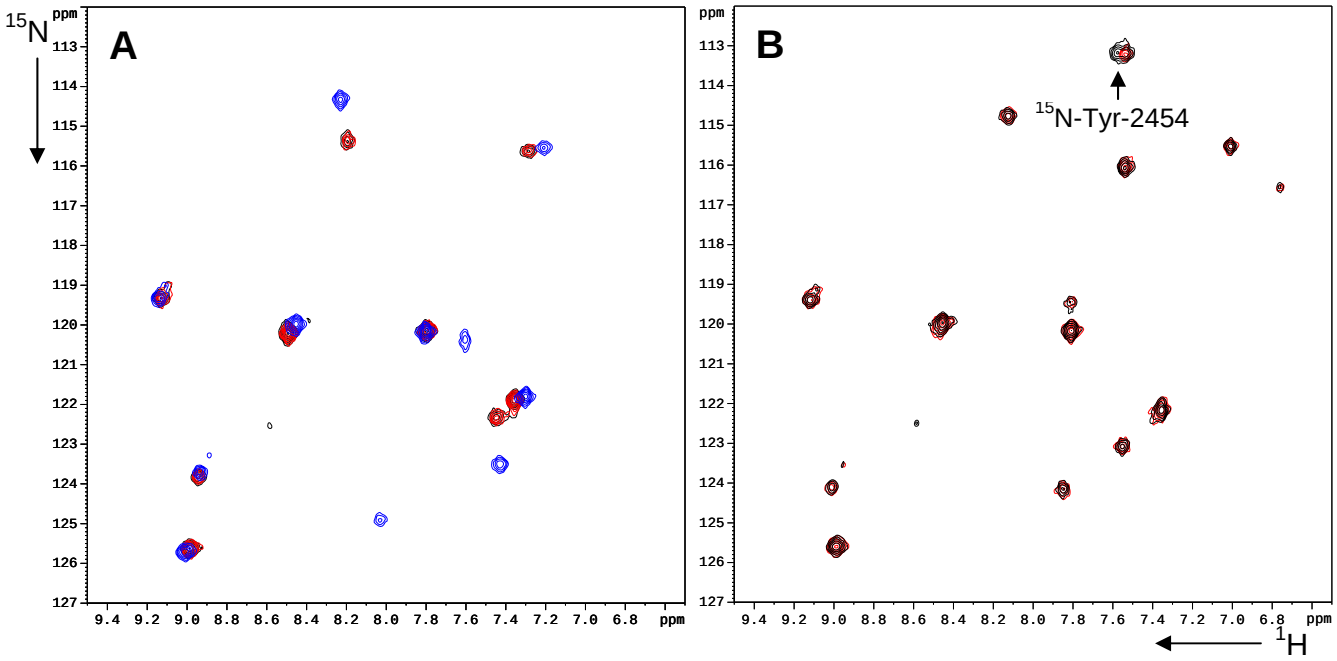


Figure S8. Wild-type and Tyr-2454-oNBTyr mutant FAS-TE are structurally similar. A) Overlay of ^{1}H-^{15}N HSQC data for wild-type protein selectively labeled with ^{15}N-tyrosine (black) and Tyr-2454-oNBTyr selectively labeled with ^{15}N-tyrosine and unlabeled oNBTyr at position 2454 before (blue) and after UV-cleavage (red). B) Overlay of wild-type protein (black) and of Tyr-2454-oNBTyr (red) after the addition of tool compound. The wild-type protein has one extra peak for Tyr-2454 that is unlabeled in the Tyr-2454-oNBTyr sample.

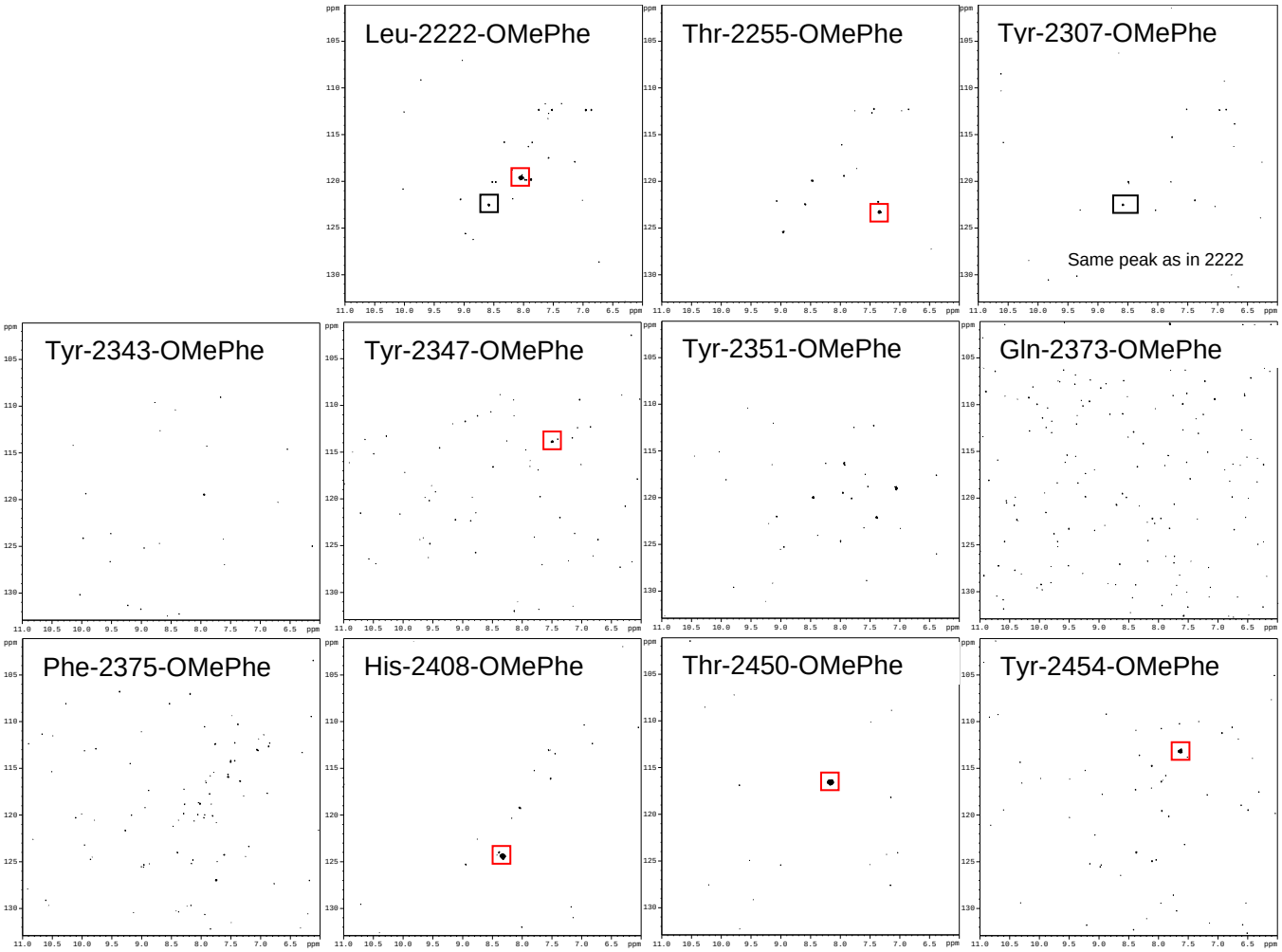


Figure S9. ^{1}H-^{15}N HSQC spectra of FAS-TE mutants with $^{13}C/^{15}N$-labeled OMePhe incorporated at 11 different positions after the addition of four molar equivalent of tool compound. Only six of the 11 mutants show a clear single amide resonance peak (Leu-2222-OMePhe, Thr-2255-OMePhe, Tyr-2347-OMePhe, His-2408-OMePhe, Thr-2450-OMePhe and Tyr-2454-OMePhe) after addition of tool compound.