# Supplementary Material

# Pyruvate Formate-Lyase Interacts Directly with the FocA Channel to Regulate Formate Translocation

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**Table S1.** List of identified FocA-binding partners.

| ID | Description | MW [kDa] | Peptides | Peptide spectral matches | Mascot score |
|---|---|---|---|---|---|
| P0AC25 | Probable formate transporter 1 | 31.0 | 5 | 273 | 1271 |
| P09373 | Formate acetyltransferase 1 | 85.3 | 40 | 117 | 1680 |
| A7ZSL4 | Elongation factor Tu 1 | 43.3 | 17 | 59 | 800 |
| P0A9B4 | Glyceraldehyde-3-phosphate dehydrogenase A | 35.5 | 14 | 59 | 417 |
| P0A9Q8 | Aldehyde-alcohol dehydrogenase | 96.1 | 28 | 56 | 1571 |
| P06959 | Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex | 66.1 | 21 | 51 | 647 |
| A7ZHA4 | Chaperone protein DnaK | 69.1 | 27 | 47 | 251 |
| Q8FB83 | DNA-directed RNA polymerase subunit beta | 155.1 | 33 | 42 | 1097 |
| A7ZUK2 | DNA-directed RNA polymerase subunit beta | 155.1 | 33 | 40 | 1097 |
| A7ZSK6 | 50S ribosomal protein L2 | 29.8 | 11 | 35 | 300 |
| P0A911 | Outer membrane protein | 37.2 | 9 | 34 | 151 |
| P69911 | Glutamate decarboxylase beta | 52.6 | 10 | 32 | 450 |
| A7ZUK1 | DNA-directed RNA polymerase subunit beta | 150.5 | 28 | 31 | 913 |
| P69909 | Glutamate decarboxylase alpha | 52.7 | 10 | 30 | 428 |
| Q46829 | 6-phospho-beta-glucosidase BglA | 55.3 | 11 | 28 | 138 |
| A7ZSI5 | 30S ribosomal protein S4 | 23.5 | 6 | 28 | 140 |
| B1IUY2 | Alanyl-tRNA synthetase | 96.0 | 18 | 28 | 761 |
| P62577 | Chloramphenicol acetyltransferase | 25.6 | 4 | 26 | 254 |
| P24182 | Biotin carboxylase | 49.3 | 9 | 25 | 326 |
| A7ZQM2 | Enolase | 45.6 | 12 | 25 | 322 |
| A1AJ51 | 60 kDa chaperonin 1 | 57.3 | 16 | 23 | 115 |
| P0A9P2 | Dihydrolipoyl dehydrogenase | 50.7 | 13 | 23 | 266 |
| P0A9H4 | Lysine decarboxylase, inducible | 81.2 | 14 | 23 | 412 |
| P08839 | Phosphoenolpyruvate-protein phosphotransferase | 63.5 | 13 | 22 | 471 |
| P00393 | NADH dehydrogenase | 47.3 | 8 | 21 | 465 |
| A7ZR64 | S-adenosylmethionine synthase | 41.9 | 12 | 21 | 449 |
| A7ZSJ4 | 50S ribosomal protein L6 | 18.9 | 6 | 21 | 195 |
| A7ZTR3 | Tryptophanase | 52.7 | 10 | 21 | 220 |
| P28629 | Biodegradative arginine decarboxylase | 84.4 | 14 | 20 | 467 |
| P0A797 | 6-phosphofructokinase isozyme 1 | 34.8 | 7 | 20 | 203 |
| A7ZSI4 | DNA-directed RNA polymerase subunit alpha | 36.5 | 7 | 18 | 87 |
| B7UIL4 | 30S ribosomal protein S2 | 26.7 | 6 | 18 | 177 |
| A7ZSI3 | 50S ribosomal protein L17 | 14.4 | 4 | 17 | 62 |

| | | | | | |
|---|---|---|---|---|---|
| A7ZSK3 | 30S ribosomal protein S3 | 26.0 | 7 | 16 | 80 |
| P00350 | 6-phosphogluconate dehydrogenase, decarboxylating | 51.4 | 7 | 14 | 87 |
| B1XBE0 | Chaperone protein DnaJ | 41.1 | 8 | 14 | 118 |
| A7ZSL5 | Elongation factor G | 77.5 | 11 | 14 | 337 |
| P0A954 | 3-oxoacyl-[acyl-carrier-protein] synthase 1 | 42.6 | 5 | 14 | 206 |
| P0A9M8 | Phosphate acetyltransferase | 77.1 | 8 | 13 | 239 |
| A7ZV47 | Adenylosuccinate synthetase | 47.3 | 7 | 13 | 256 |
| A7ZQM3 | CTP synthase | 60.3 | 8 | 13 | 227 |
| A7ZUJ8 | 50S ribosomal protein L10 | 17.7 | 4 | 12 | 81 |
| A7ZV73 | 30S ribosomal protein S18 | 9.0 | 3 | 12 | 83 |
| P0AE10 | Alkyl hydroperoxide reductase subunit C | 20.7 | 3 | 11 | 84 |
| P0AES4 | DNA gyrase subunit A | 96.9 | 4 | 11 | 103 |
| A7ZR34 | Phosphoglycerate kinase | 41.1 | 5 | 11 | 190 |
| P00562 | Bifunctional aspartokinase/homoserine dehydrogenase 2 | 88.8 | 7 | 10 | 392 |
| P0A9H8 | Cyclopropane-fatty-acyl-phospholipid synthase | 43.9 | 6 | 10 | 116 |
| P0ACG0 | DNA-binding protein H-NS | 15.5 | 4 | 10 | 102 |
| P07003 | Pyruvate dehydrogenase [ubiquinone] | 62.0 | 7 | 10 | 172 |
| A7ZSK8 | 50S ribosomal protein L4 | 22.1 | 5 | 10 | 52 |
| A7ZSJ7 | 50S ribosomal protein L5 | 20.3 | 3 | 10 | 134 |
| P0A6A5 | Acetate kinase | 43.3 | 4 | 9 | 125 |
| A7ZHC0 | Dihydrodipicolinate reductase | 28.7 | 3 | 9 | 106 |
| P21599 | Pyruvate kinase II | 51.3 | 8 | 9 | 44 |
| A7ZUK0 | 50S ribosomal protein L7/L12 | 12.3 | 3 | 9 | 40 |
| A7ZSI6 | 30S ribosomal protein S11 | 13.8 | 3 | 9 | 139 |
| A7ZNR4 | D-tagatose-1,6-bisphosphate aldolase subunit gatZ | 47.1 | 4 | 8 | 112 |
| Q8X8V9 | D-methionine-binding lipoprotein metQ | 29.4 | 6 | 8 | 44 |
| P0A7T1 | 30S ribosomal protein S13 | 13.1 | 4 | 8 | 76 |
| P07395 | Phenylalanyl-tRNA synthetase beta chain | 87.3 | 6 | 8 | 197 |
| B7L7S9 | Arginyl-tRNA synthetase | 64.7 | 6 | 8 | 138 |
| P00954 | Tryptophanyl-tRNA synthetase | 37.4 | 4 | 8 | 74 |
| P0AB72 | Fructose-bisphosphate aldolase class 2 | 39.1 | 4 | 7 | 38 |
| A7ZUH4 | Phosphoenolpyruvate carboxylase | 99.0 | 7 | 7 | 223 |
| A1AJV0 | Phosphopentomutase | 44.4 | 4 | 7 | 136 |
| A7ZVS7 | Purine nucleoside phosphorylase deoD-type | 25.9 | 5 | 7 | 116 |
| P14407 | Fumarate hydratase class I, anaerobic | 60.1 | 6 | 7 | 51 |
| P0ACD8 | Hydrogenase-1 large chain | 66.2 | 6 | 7 | 222 |
| A7ZK14 | Chromosome partition protein mukB | 170.1 | 5 | 7 | 116 |
| P0A9N6 | Pyruvate formate-lyase 1-activating enzyme | 28.2 | 4 | 7 | 101 |
| A7ZHI9 | Protein translocase subunit SecA | 102.0 | 7 | 7 | 211 |
| A7ZTA5 | Glycyl-tRNA synthetase beta subunit | 76.7 | 6 | 7 | 224 |

**Table S2**. Intermolecular cross-links between FocA and PflB; m: oxidized Met. The cross-linked amino acids are shown in bold and underlined.

| **Measured *m/z*** | **Theoretical mass** | **Charge state** | **Mass deviation [ppm]** | **AA sequences of FocA and site of cross-linking** | **AA sequences of PflB and site of cross-linking** |
|---|---|---|---|---|---|
| **1318.342** | **3953.016** | +3 | +0.9 | $^{3}$ADNPFDLLLPAAMA**K**VAE$^{20}$ | $^{302}$AQEMVDHLVm**K**LRMVR$^{317}$ |
| **1021.527** | **3062.576** | +3 | -2.8 | $^{22}$AGVY**K**ATK$^{29}$ | $^{192}$YLmKD**K**LAQFTSLQADLE$^{209}$ |
| **883.116** | **2647.332** | +3 | +0.1 | $^{3}$ADNPFDLLLPAAmA**K**VAE$^{20}$ | $^{28}$DFIQ**K**$^{32}$ |
| **838.437** | **2513.296** | +3 | +0.6 | $^{3}$ADNPFDLLLPAAmA**K**VAE$^{20}$ | $^{437}$ANLA**K**$^{441}$ |
| **830.753** | **2490.240** | +3 | +1.5 | $^{105}$ITWGQLA**K**$^{112}$ | $^{463}$**S**EPIKGDVLNYDE$^{475}$ |
| **795.067** | **2383.182** | +3 | +1.5 | $^{9}$LLLPAAmA**K**$^{17}$ | $^{118}$mIEGSBKA**Y**NR$^{128}$ |
| **750.040** | **2248.101** | +3 | +2.1 | $^{187}$SLMD**K**$^{191}$ | $^{291}$DLKAGKI**T**EQEAQE$^{304}$ |
| **744.708** | **2232.114** | +3 | -1.4 | $^{9}$LLLPAAmA**K**VAEE$^{21}$ | $^{338}$**S**IGGmGLD$^{345}$ |
| **712.704** | **2136.097** | +3 | +0.5 | $^{9}$LLLPAAMA**K**VAEE$^{21}$ | $^{192}$**Y**LmKD$^{196}$ |
| **1036.523** | **2072.037** | +2 | +0.7 | $^{3}$ADNPFDLLLPAAMA**K**$^{17}$ | $^{641}$DQ**K**$^{643}$ |
| **675.336** | **2023.997** | +3 | -0.9 | $^{187}$**S**LMD$^{190}$ | $^{129}$ELDPMIK**K**IFTE$^{140}$ |
| **642.367** | **1925.086** | +3 | +0.3 | $^{22}$AGVYKA**T**KHPLK$^{33}$ | $^{93}$QLE**K**$^{96}$ |
| **508.280** | **1522.826** | +3 | -0.4 | $^{22}$AGVY**K**$^{26}$ | $^{726}$**Y**PQLTIR$^{732}$ |
| **621.298** | **1241.590** | +2 | 0 | $^{27}$AT**K**$^{29}$ | $^{395}$**T**SSLQYE$^{401}$ |

**Table S3.** Strains used in this study.

| Strain or plasmid | Relevant genotype or characteristic(s) | Reference of source |
|---|---|---|
| **Strains** | | |
| BL21 (DE3) | F$^-$ *ompT hsdS*($r_B^- m_B^-$) *gal dcm lacY1* (DE3) | Novagen |
| MC4100 | F$^-$ *araD139* Δ(*argF-lac*)*U169 ptsF25 deoC1 relA1 flbB5301 rspL150* $^-$ | 1 |
| RM201 | Like MC4100 but Δ*pfl-25* Ω(*pfl::cat* pACYC184) (Δ*focApflB*) | 2 |
| RM220 | Like MC4100 but Δ*pflB-pflA* | 3 |
| REK701 | Like MC4100 but *focA* (*focA* codons 114 and 115 changed to UAG and UAA, respectively) | 4 |
| REK702 | Like MC4100 but GUG codon of *focA* converted to AUG | 4 |
| RM223 | MC4100 but *pflB pflA tdcE* | 3 |
| PM08 | MC4100, *pflA*::Tn*5* | C. Pinske |
| 234M1 | MC4100 but *pflA*, Cm$^R$ | 5 |
| BL21Δ*pflA* | BL21 but *pflA,* Cm$^R$, Tet$^R$ | This study |
| DHM1 | F-, *cya-854*, *recA1, endA1, gyrA96 (Nal r)*, *thi1*, *hsdR17*, *spoT1*, *rfbD1, glnV44(AS).* | 6 |
| **Plasmids** | | |
| pKNT25 | Kan$^R$, expression vector | Euromedex |
| pKT25 | Kan$^R$, expression vector | Euromedex |
| pUT18 | Amp$^R$, expression vector | Euromedex |
| pUT18C | Amp$^R$, expression vector | Euromedex |
| pASK-IBA5focA | Amp$^R$, expression vector with the gene *focA*, encoding *N*-terminally Strep-tagged FocA | 7 |

| | | |
|---|---|---|
| pASK-IBA3focA | Amp$^{R}$, expression vector with the gene *focA*, encoding *C*-terminally Strep-tagged FocA | 7 |
| pCA24NpflB | Cm$^{R}$, *pflB* encoding His-tagged PflB in pCA24N | National BioResources Project (NIG, Japan); 8 |
| pJW0886 | Cm$^{R}$, ASKA plasmid with *pflB* | National BioResources Project (NIG, Japan): *E. coli* |
| pKT25-zip | Control vector, BACTH-system | Euromedex |
| pUT18-zip | Control vector, BACTH-system | Euromedex |
| pKT25-focA | Kan$^{R}$, pKT25 with the gene *focA* | This study |
| pKNT25-focA | Kan$^{R}$, pKNT25 with the gene *focA* | This study |
| pUT18-pflB 1-424 | Amp$^{R}$, pUT18 with portion of the *pflB* gene encoding aa 1-424 | This study |
| pUT18C-pflB 414-603 | Amp$^{R}$, pUT18C with portion of the *pflB* gene encoding aa 414-603 | This study |
| pUT18C-pflB 603-752 | Amp$^{R}$, pUT18C with portion of the *pflB* gene encoding aa 603-752 | This study |
| p29 | Cm$^{R}$, *focA-pflB-act* in pACYC184 | 9 |

**Table S4. Oligonucleotide primers used in this study.**

| Primer name | Sequence |
|---|---|
| pKNT25-*focA* PstI for | 5' – TAC TGC TGC AGG AAA GCT GAC AAC CCT TTT GAT-3' |
| pKNT25-*focA* EcoRI rev | 5'- AGA TGG AAT TCC ATG GTG GTC GTT TTC ACG CAG-3' |
| pKT25-*focA* PstI for | 5'- ACT TCT GCA GGA AAA GCT GAC AAC CCT TTT GAT-3' |
| pKT25-*focA* EcoRI rev | 5'- ACG GAA TTC TTA ATG GTG GTC GTT TTC ACG CAG-3' |
| pUT18-*pflB* 2-424 PstI for | 5'- AAC TGC AGG TCC GAG CTT AAT GAA AAG TTA GCC ACA-3' |
| pUT18-*pflB* 2-424 EcoRI rev | 5'- ATC GAA TTC TTA TTT ACC AAC GAT CAT CGG GCT-3' |
| pUT18C-*pflB* 414-603 PstI for | 5'- ATC TGC AGG AGC CCG ATG ATC GTT GGT AAA-3' |
| pUT18C-*pflB* 414-603 EcoRI rev | 5'- ACG AAT TCA TTA AGA AGT GAT GGT CAG AAC AGA-3' |
| pUT18C-*pflB* 603-752 PstI for | 5'- AAC TGC AGG TCT GTT CTG ACC ATC ACT TCT-3' |
| pUT18C-*pflB* 603-752 EcoRI rev | 5'- ATC GAA TTC TTA CAT AGA TTG AGT GAA GGT ACG AGT AAT-3' |

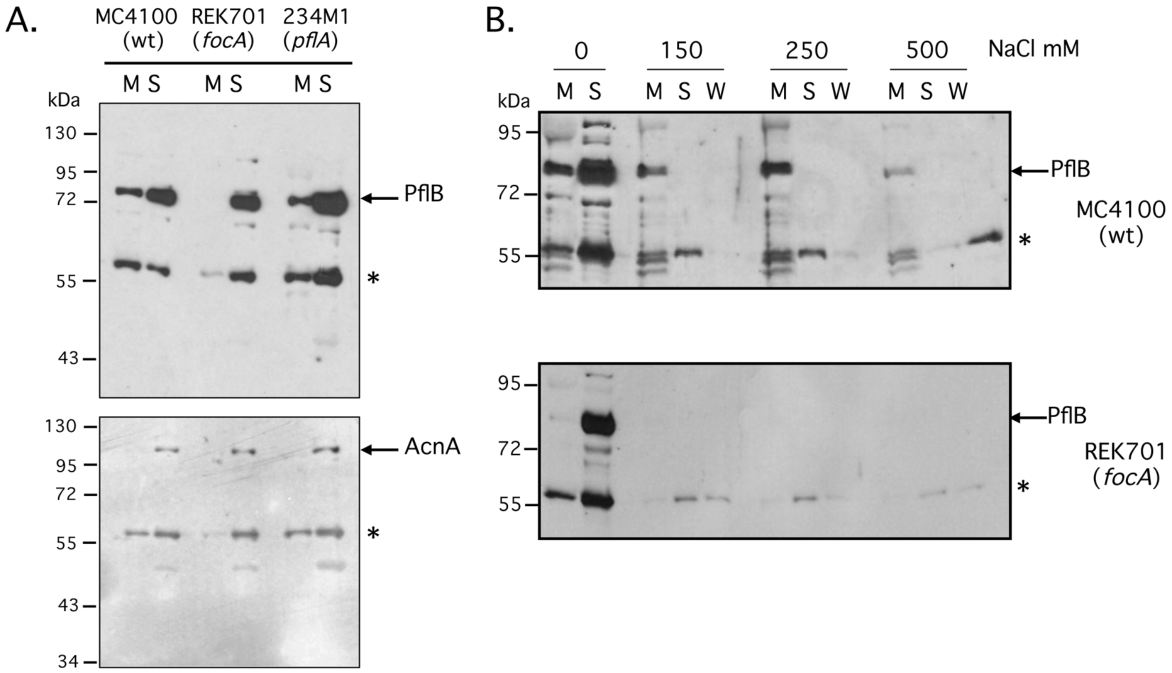


**Figure S1.** FocA-dependent association of PflB with the cytoplasmic membrane. A. Crude extracts derived from the strains indicated were separated into soluble (S) and membrane (M) fractions in a buffer containing 150 mM NaCl as indicated in the Experimental procedures. The membrane fraction was resuspended in the same buffer and centrifuged again at 105 000 *g* for 45 min. Aliquots (5 µg of protein) of the sub-cellular fractions were separated by 10% (w/v) SDS-PAGE and after transfer to nitrocellulose membranes were challenged with either anti-PflB (upper panel) or anti-AcnA (lower panel) antibodies. The locations of PflB and aconitase A (AcnA) are indicated by the arrow on the right of the panels. The locations of the molecular mass size markers (Fermentas) are indicated on the left of the panels in kDa. The asterisk indicates an unidentified cross-reacting polypeptide. B. Sub-cellular fractions from strains MC4100 (upper panel) and the *focA* mutant REK701 (lower panel) were prepared in buffer that including the indicated concentrations of NaCl. The membranes were washed (W) a second time in the same buffer with the indicated NaCl concentration. Aliquots (10 µg of protein in upper panel and 5 µg in lower panel) of the indicated sub-cellular fractions were separated by SDS-PAGE as above. The migration position of PflB is indicated. The asterisk denotes the unknown 57 kDa cross-reacting polypeptide

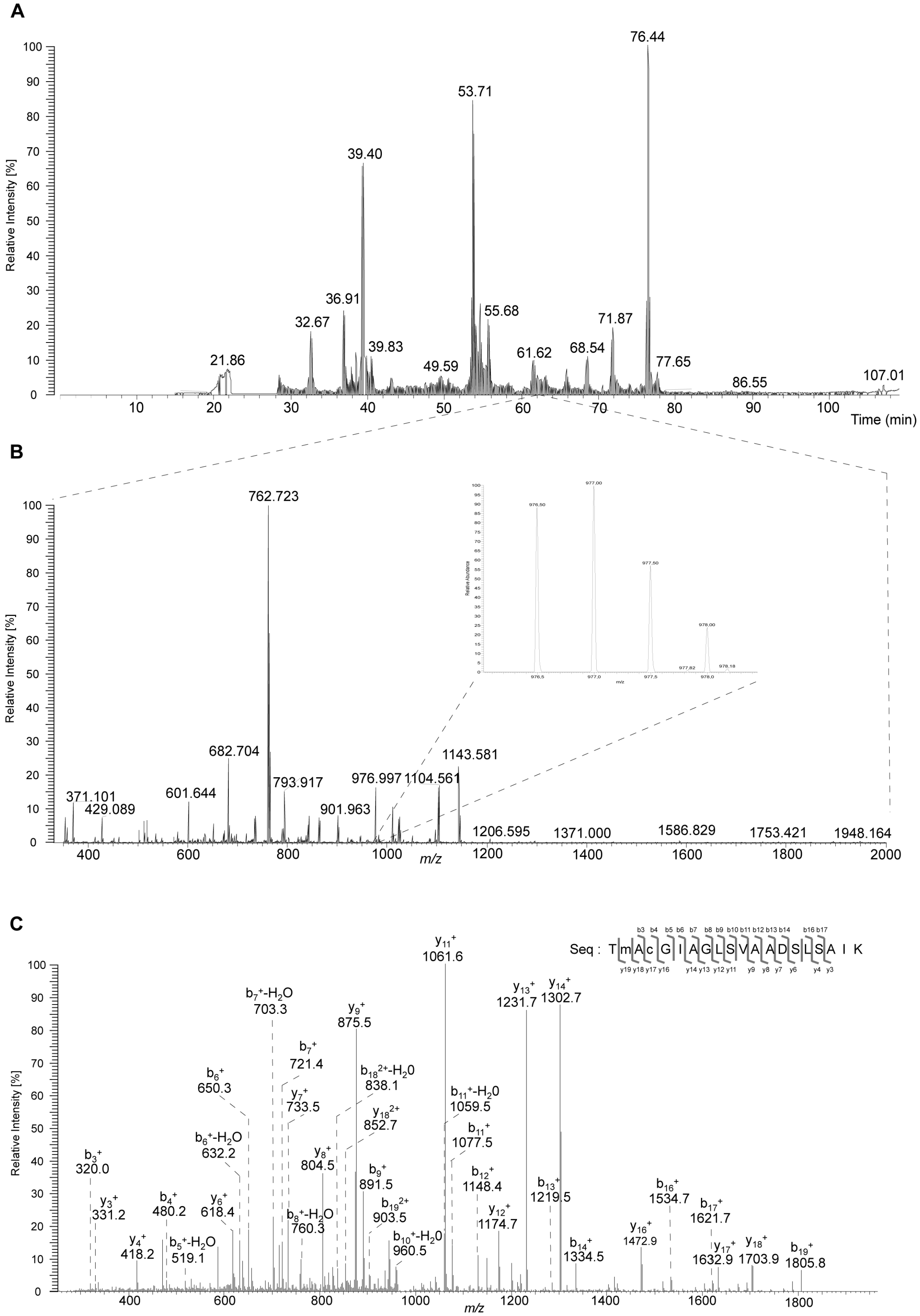

A
Relative Intensity [%]
Time (min)
21.86
32.67
36.91
39.40
39.83
49.59
53.71
55.68
61.62
68.54
71.87
76.44
77.65
86.55
107.01
B
Relative Intensity [%]
m/z
371.101
429.089
601.644
682.704
762.723
793.917
901.963
976.997
1104.561
1143.581
1206.595
1371.000
1586.829
1753.421
1948.164
976.50
977.00
977.50
978.00
977.82
978.18
Relative Abundance
C
Relative Intensity [%]
m/z
Seq : T m A c G I A G L S V A A D S L S A I K
b3 b4 b5 b6 b7 b8 b9 b10 b11 b12 b13 b14 b16 b17
y19 y18 y17 y16 y14 y13 y12 y11 y9 y8 y7 y6 y4 y3
b3+ 320.0
y3+ 331.2
y4+ 418.2
b4+ 480.2
b5+-H2O 519.1
y6+ 618.4
b6+-H2O 632.2
b6+ 650.3
b7+-H2O 703.3
b7+ 721.4
y7+ 733.5
b8+-H2O 760.3
y8+ 804.5
b18 2+-H20 838.1
y18 2+ 852.7
y9+ 875.5
b9+ 891.5
b19 2+ 903.5
b10+-H20 960.5
b11+-H20 1059.5
y11+ 1061.6
b11+ 1077.5
b12+ 1148.4
y12+ 1174.7
b13+ 1219.5
y13+ 1231.7
y14+ 1302.7
b14+ 1334.5
y16+ 1472.9
b16+ 1534.7
b17+ 1621.7
y17+ 1632.9
y18+ 1703.9
b19+ 1805.8

**Figure S2.** MS analysis of PflB peptide TmAcGIAGLSVAADSLSAIK. (A) Chromatogram of the total ion current (TIC), (B) mass spectrum recorded at 74.08 minutes, (C) Fragment ion mass spectrum (MS/MS) of the doubly charged precursor ion at *m/z* 976.495. m: oxidized methionine, c: carbamidomethylated cysteine.

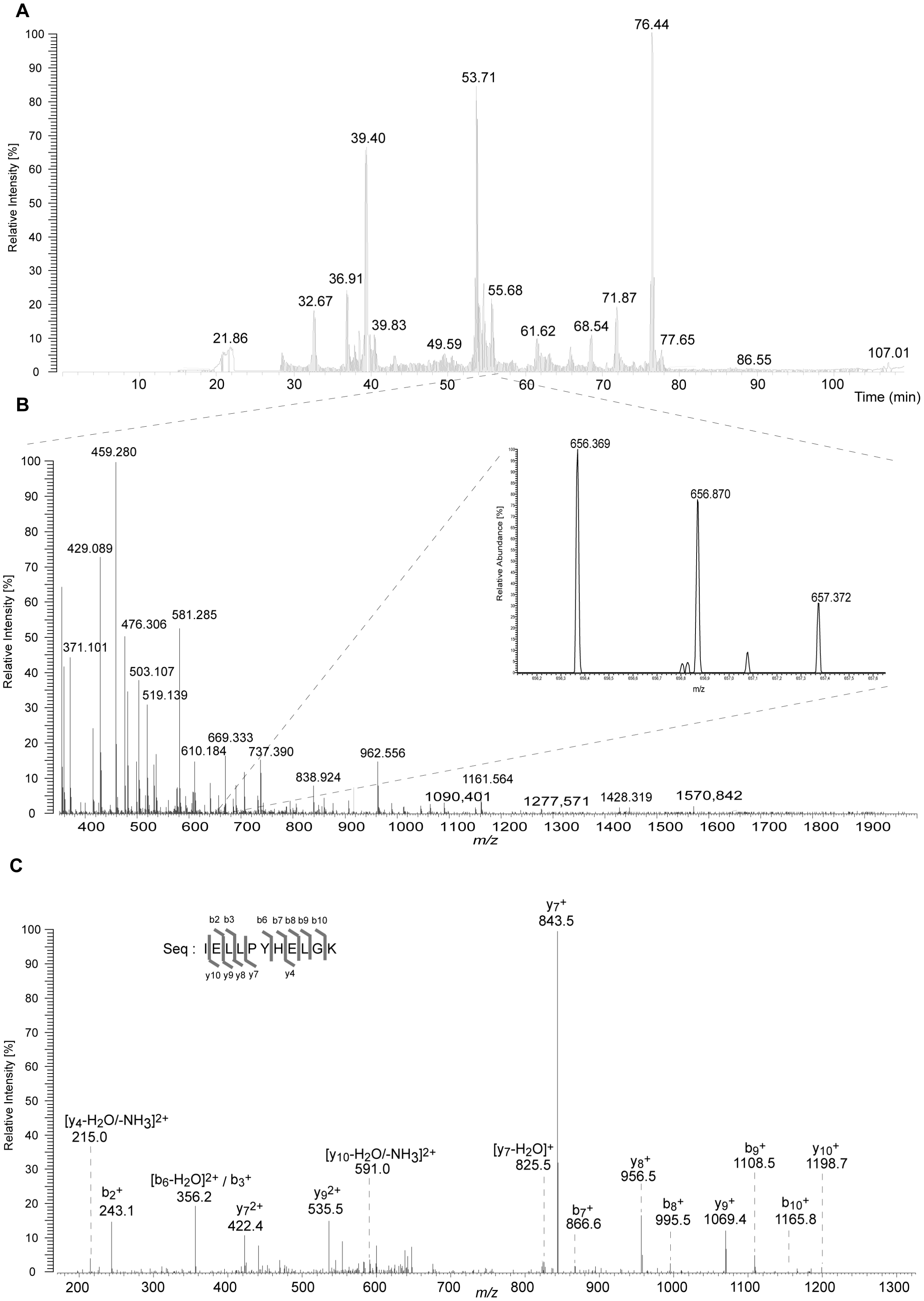
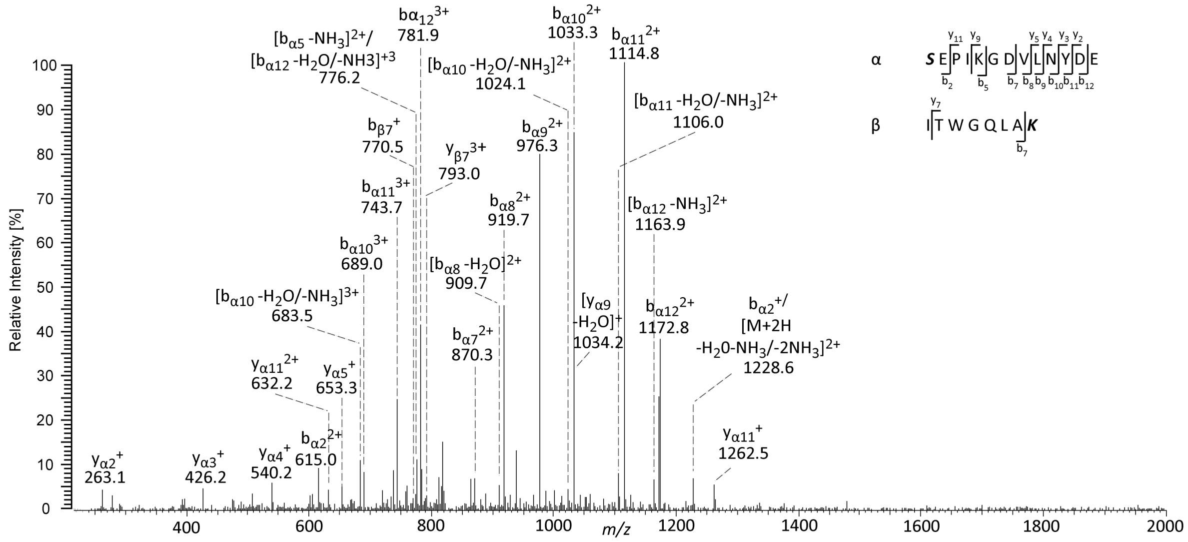

A
Relative Intensity [%]
76.44
53.71
39.40
36.91
32.67
21.86
39.83
49.59
55.68
61.62
68.54
71.87
77.65
86.55
107.01
Time (min)
B
Relative Intensity [%]
459.280
429.089
476.306
581.285
371.101
503.107
519.139
610.184
669.333
737.390
838.924
962.556
1161.564
1090,401
1277,571
1428.319
1570,842
m/z
656.369
656.870
657.372
Relative Abundance [%]
m/z
C
Relative Intensity [%]
Seq : I E L L P Y H E L G K
b2 b3 b6 b7 b8 b9 b10
y10 y9 y8 y7 y4
y7+ 843.5
[y4-H2O/-NH3]2+ 215.0
b2+ 243.1
[b6-H2O]2+ / b3+ 356.2
y72+ 422.4
y92+ 535.5
[y10-H2O/-NH3]2+ 591.0
[y7-H2O]+ 825.5
b7+ 866.6
y8+ 956.5
b8+ 995.5
y9+ 1069.4
b9+ 1108.5
b10+ 1165.8
y10+ 1198.7
m/z

**Figure S3.** MS analysis of PflA peptide IELLPYHELGK. (A) Chromatogram of the total ion current (TIC), (B) mass spectrum recorded at 58.12 minutes, and (C) fragment ion mass spectrum (MS/MS) of the doubly charged precursor ion at *m/z* 656.368.

**A.**



**B.**

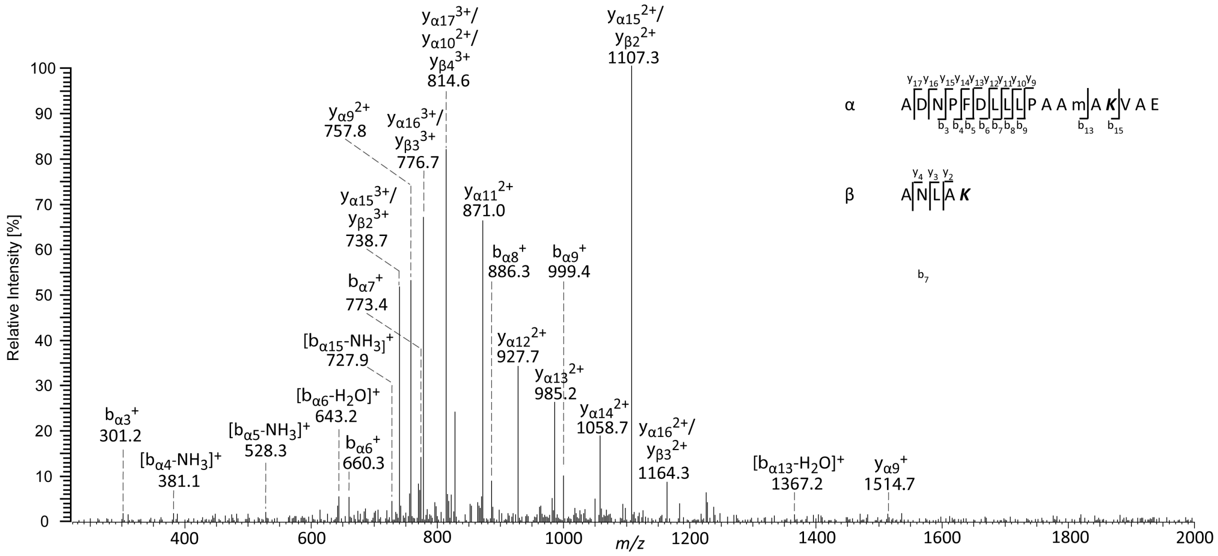


**Figure S4.** Identification of FocA-peptides cross-linked to PflB. A. Fragment ion mass spectrum (MS/MS) of a triply charged cross-linked product at *m/z* 830.753. The cross-linked product was unambiguously assigned to amino acids 105-112 (ITWGQLAK) of FocA connected to amino acids 463-475 (SEPIKGDVLNYDE) of PflB. The fragment ions point to Lys112 (FocA) cross-linked with Ser463 (PflB). B. Fragment ion mass spectrum (MS/MS) of a triply charged cross-linked product at *m/z* 838.437. The cross-linked product was unambiguously assigned to amino acids 3-20 (ADNPFDLLLPAAmAKVAE) of FocA connected to amino acids 437-441 (ANLAK) of PflB. The fragment ions point to Lys17 (FocA) cross-linked with Lys441 (PflB); m indicates an oxidized Met.

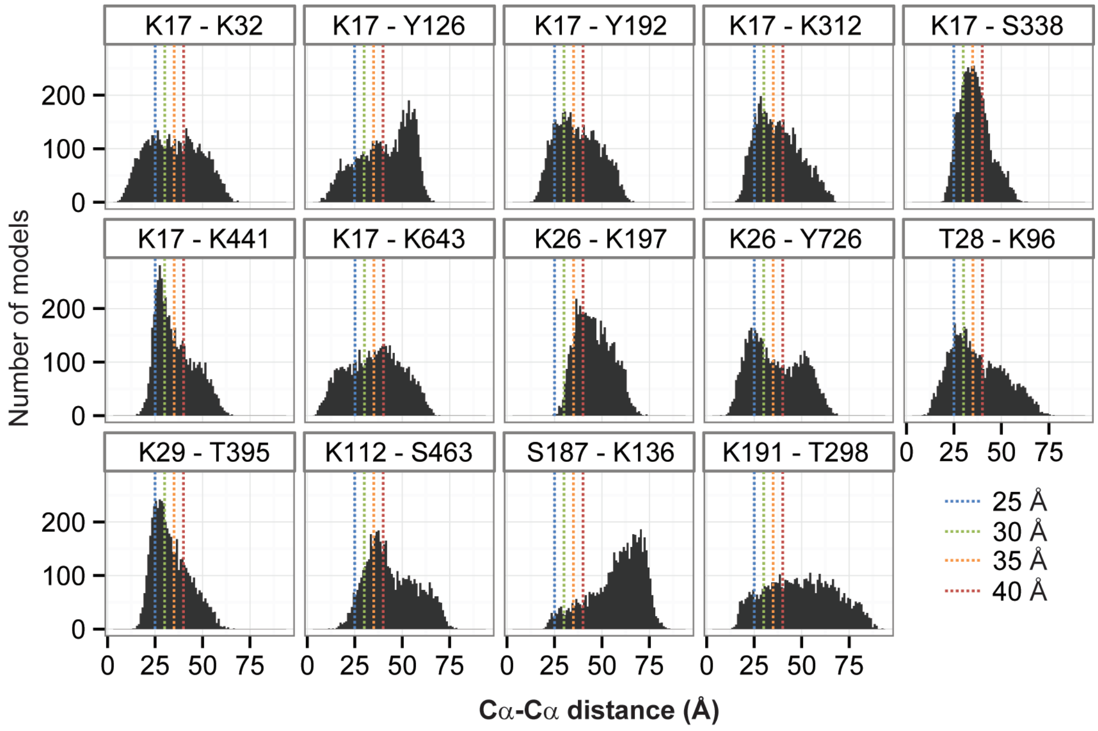


**Figure S5.** Histogram of Cα-Cα cross-link distances for each residue pair across the model population. Evaluated cutoff distances are represented by vertical dashed lines.

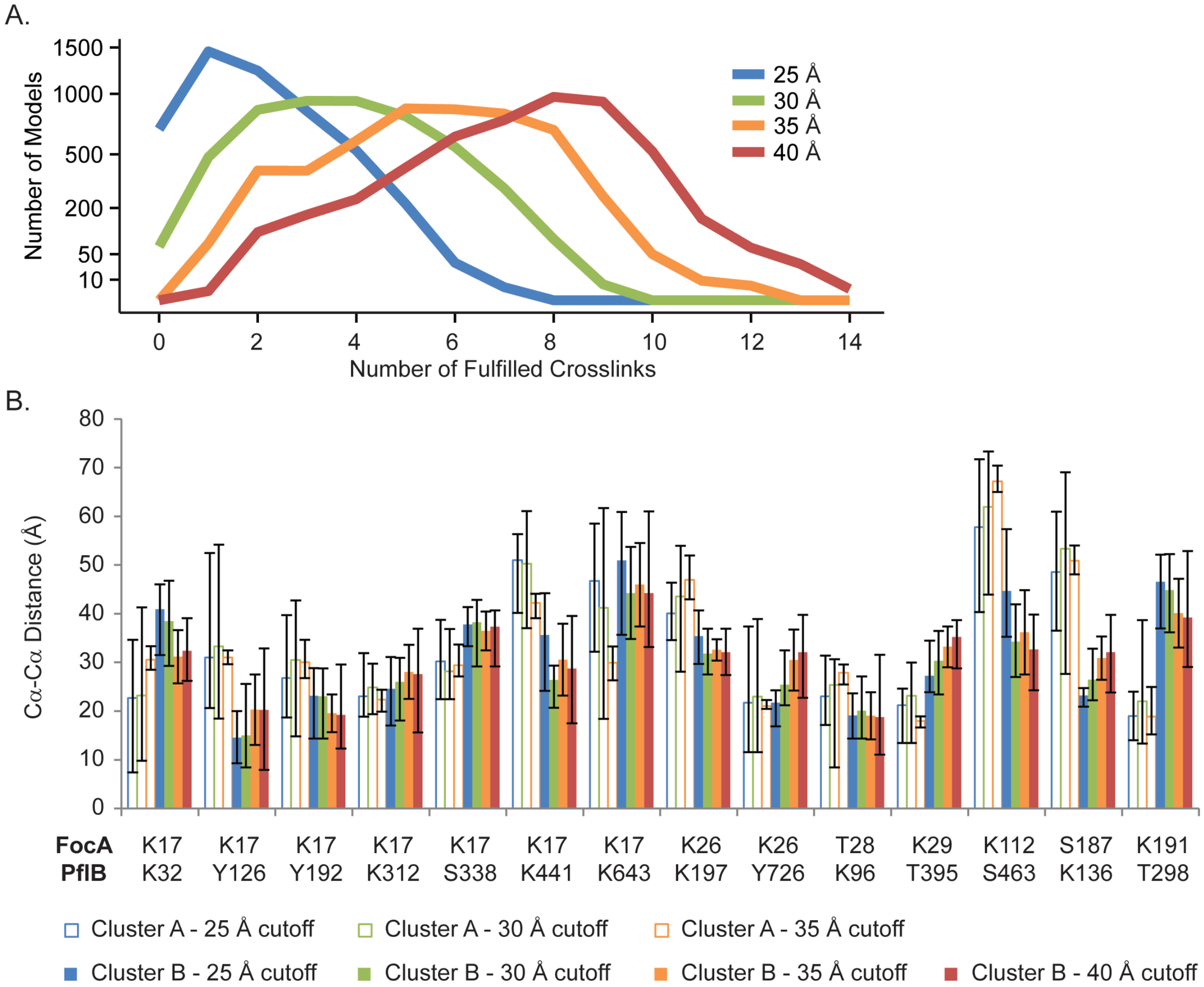


**Figure S6.** A. Number of models fulfilling cross-links at each cutoff distance. B. Average Cα- Cα distance for each cross-link residue pair in each cluster set. Cluster A and Cluster B correspond to Conformation A and B discussed in the main text. Error bars show the maximum and minimum Cα- Cα distance.

## References

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