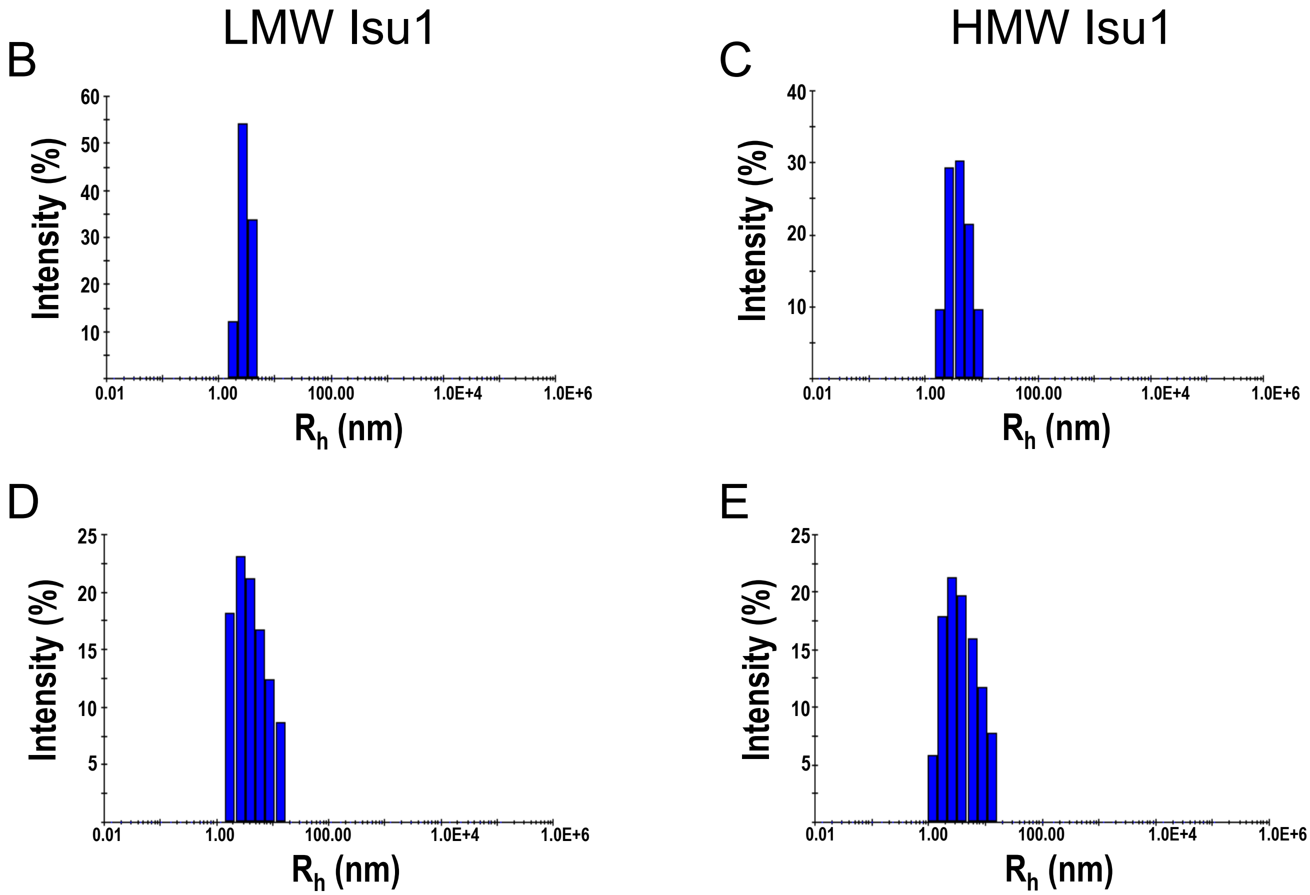
Supplementary Materials

Supplementary Figures

Supplementary Fig. S1

A

	LMW Isu1		HMW Isu1	
	No EDTA R_h (nm)	**500 mol EDTA R_h (nm)**	**No EDTA R_h (nm)**	**500 mol EDTA R_h (nm)**
Sample 1	3.5	5.6	4.6	5.0
Sample 2	3.2	5.4	5.0	/
Sample 3	2.7	4.7	3.9	/
Avg ± SD	3.1 ± 0.2	5.2 ± 0.2	4.5 ± 0.5	5.0 ± 0.4
% Mass	**100%**	**100%**	**100%**	**100%**



Supplementary Fig. S1: Chelation of endogenously bound zinc triggers oligomerization of LMW Isu1. (A) Size exclusion chromatography fractions 42 and 32 for LMW and HMW Isu1, respectively (see Fig. 1B), at a protein concentration of 1 mg/mL, were analyzed by dynamic light scattering in the presence or absence of 500 molar equivalents of EDTA, and the R_h was obtained as described in Experimental Methods. Shown are the mean ± standard deviation obtained from three independently purified sets of protein samples, each of which underwent at least 3 measurements. For HMW Isu1 in the presence of EDTA, only one purification was analyzed and we show the mean ± standard deviation obtained from 3 measurements. The *% Mass* was obtained from a distribution describing the relative proportion of each scattering species present in each sample based on its mass or volume. *100% Mass* indicates that there is only one detectable scattering species in the sample. (B-E) representative plots for LMW and HMW Isu1analyzed in the absence (B and C) or in the presence of EDTA (D and E). Each plot shows the distribution of particle sizes in the sample, which is based on the scattering intensity of each species present in the sample.

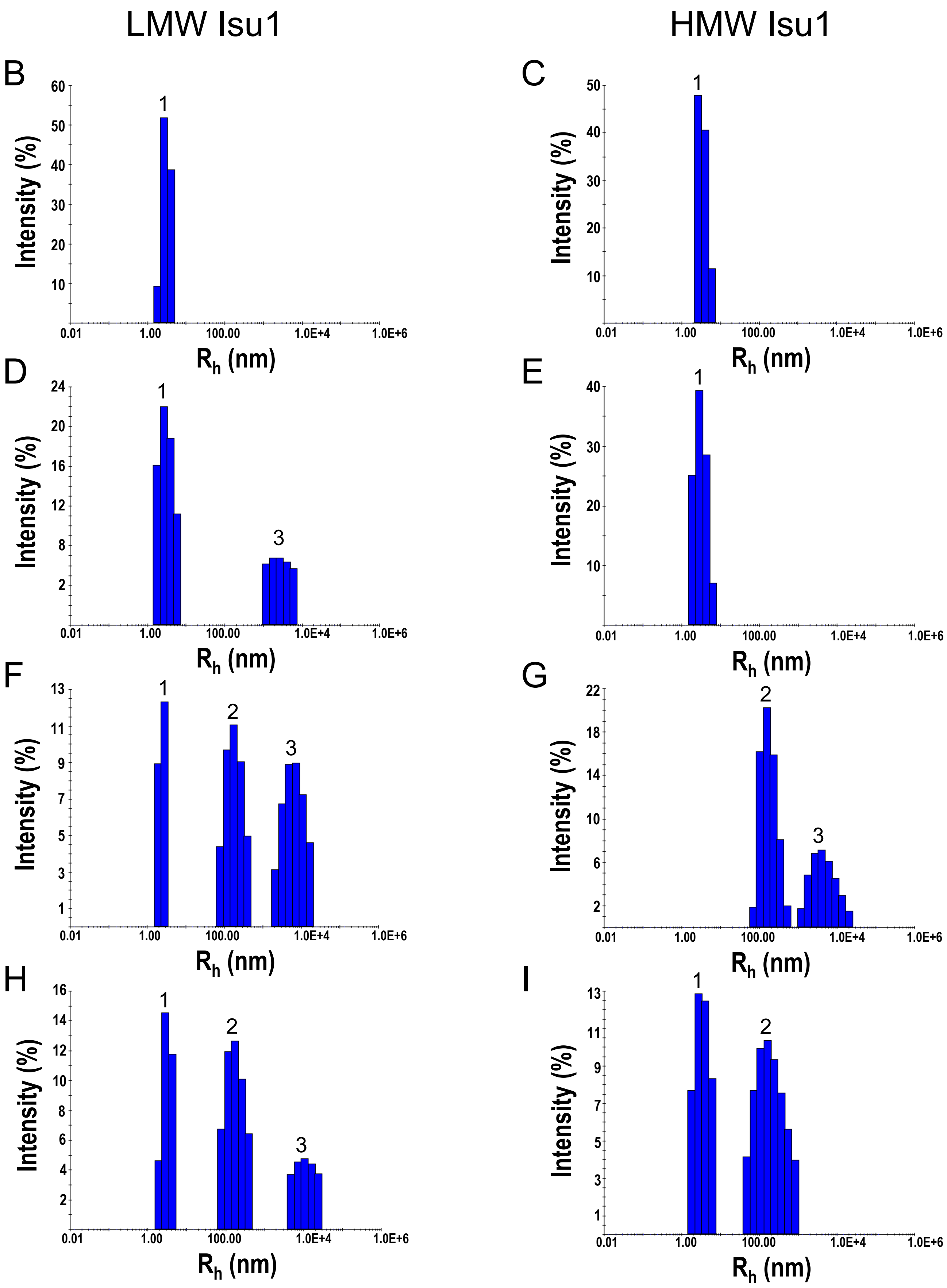
Supplementary Fig. S2

A

	LMW Isu1									
	No Zn^{2+}	**1 mol Zn^{2+}**			**10 mol Zn^{2+}**			**500 mol EDTA**		
	Peak 1 R_h (nm)	Peak 1 R_h (nm)	Peak 2 R_h (nm)	Peak 3 R_h (nm)	Peak 1 R_h (nm)	Peak 2 R_h (nm)	Peak 3 R_h (nm)	Peak 1 R_h (nm)	Peak 2 R_h (nm)	Peak 3 R_h (nm)
Measurement 1	3.2	3.4	nd	3126	2.5	201	7404	3.3	199	12826
Measurement 2	2.6	3.2	nd	4859	2.7	368	14001	3.3	210	17226
Measurement 3	3.1	2.6	nd	9223	2.9	295	17060	3.6	nd	11706
Measurement 4	2.8	2.5	nd	3196	2.9	274	nd	3.4	nd	12110
Avg	**2.9**	**2.9**	nd	**5101**	**2.8**	**284**	**12822**	**3.4**	**204.5**	**13467**
±SD	**0.3**	**0.4**	**/**	**2862**	**0.2**	**69**	**4935**	**0.1**	**7.8**	**2548**
% Mass	**100%**	**99.80%**	**/**	**0.10%**	**99.40%**	**0.00%**	**0.80%**	**99.00%**	**0.00%**	**1.00%**

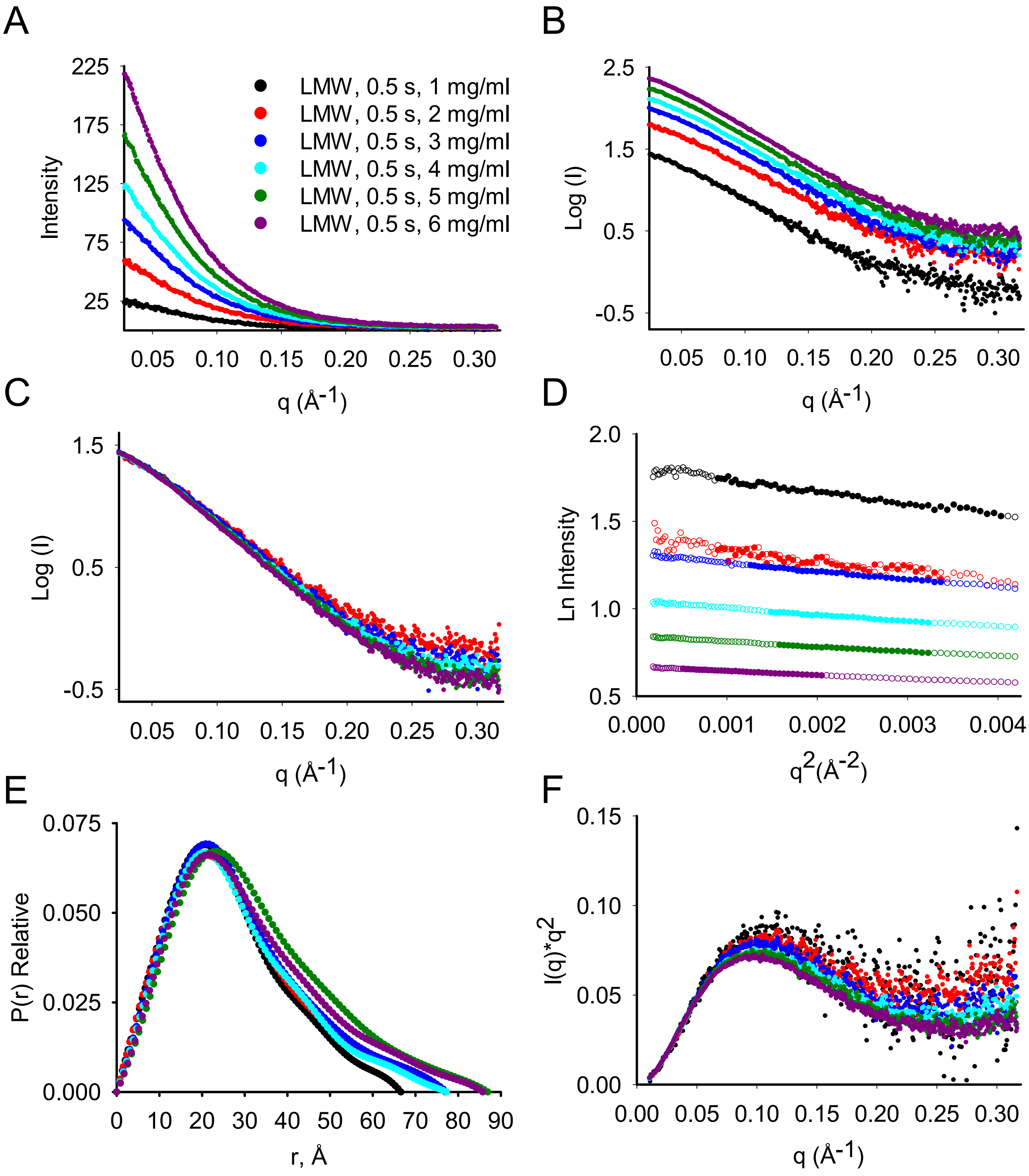
	HMW Isu1									
	No Zn^{2+}	**1 mol Zn^{2+}**			**10 mol Zn^{2+}**			**500 mol EDTA**		
	Peak 1 R_h (nm)	Peak 1 R_h (nm)	Peak 2 R_h (nm)	Peak 3 R_h (nm)	Peak 1 R_h (nm)	Peak 2 R_h (nm)	Peak 3 R_h (nm)	Peak 1 R_h (nm)	Peak 2 R_h (nm)	Peak 3 R_h (nm)
Measurement 1	3.7	3.3	nd	nd	nd	197.0	5917	3.7	258	nd
Measurement 2	3.3	4.5	nd	nd	nd	176.1	8559	3.7	508	nd
Measurement 3	3.9	4.6	nd	nd	nd	312.6	18211	3.8	170	nd
Measurement 4	3.4	3.3	nd	nd	nd	390.9	17848	3.4	285	10731
Measurement 5	3.9	3.8	nd	nd	nd	nd	2434	3.6	185	4057
Avg	**3.6**	**3.9**	**/**	**/**	**/**	**269**	**10594**	**3.6**	**281**	**7394**
±SD	**0.3**	**0.6**	**/**	**/**	**/**	**101**	**7128**	**0.2**	**136**	**4719**
% Mass	**100%**	**100%**	**/**	**/**	**/**	**4.30%**	**96.60%**	**99.80%**	**0.00%**	**0.50%**

Supplementary Fig. S2 cont.



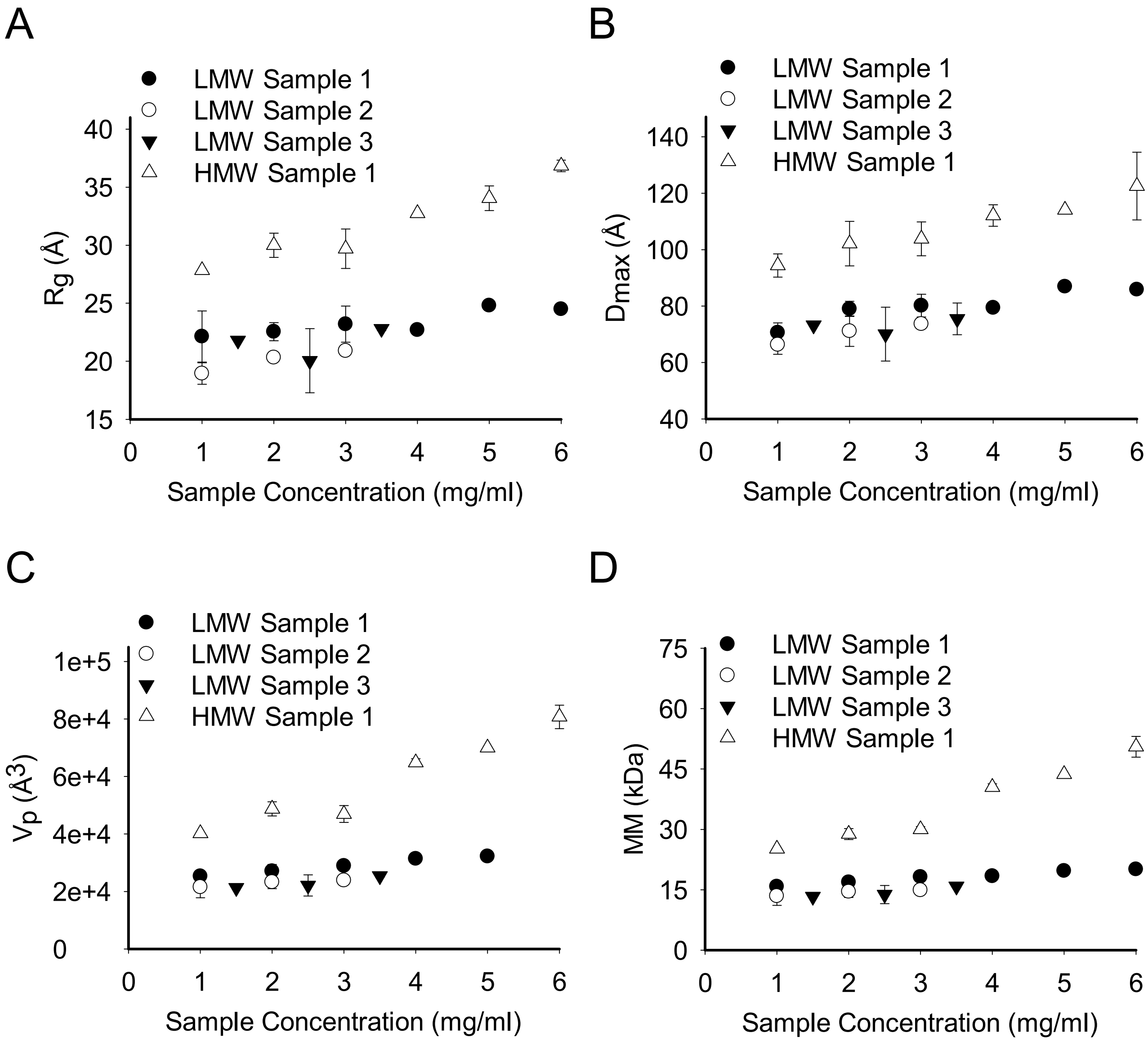
Supplementary Fig. S2: Titration of zinc into HMW Isu1 results in formation of larger species. (A) We analyzed by dynamic light scattering fractions 42 and 32 for LMW and HMW Isu1, respectively, at a protein concentration of 1 mg/ml. Each fraction was first analyzed as eluted from the gel filtration column without any additions, and was then further analyzed after each of two sequential additions of zinc (1 and 10 molar equivalents final concentration), and one more time after addition of 500 molar equivalents of EDTA. Samples were allowed to equilibrate for 1 min after each addition, after which they were subjected to 4 or 5 consecutive measurements of 50 counts each, each measurement lasting 5 min; there were no significant differences in the distribution of particle sizes between the first and the last measurement indicating the species observed were stable over the time frame of the experiment. The R_h was obtained as described in Experimental Methods. Shown are the mean ± standard deviation obtained from one purified LMW or HMW Isu1 protein sample that underwent at least 4 measurements. The *% Mass* was obtained from a distribution describing the relative proportion of each scattering species present in each sample based on the species' mass or volume; nd, not detected. (A-H) representative plots for LMW and HMW Isu1 analyzed as eluted from the column (B and C), after addition of 1 molar equivalent of zinc (D and E), after another addition of zinc to a final concentration of 10 molar equivalents (F and G), and lastly after addition of 500 molar equivalents of EDTA (H and I). Each plot shows the distribution of particle sizes in the sample, which is based on the scattering intensity of each species present in the sample.

Supplementary Fig. S3



Supplementary Figure S3: SAXS demonstrates subtle protein-concentration-dependent changes in LMW Isu1. SAXS scattering data were collected for different concentrations of LMW Isu1 at the exposure time of 0.5 sec as described in Experimental Methods and summarized in Table 2. (A) Absolute and (B) Log Intensity data vs. q after subtraction of the buffer scattering data. (C) SAXS scattering intensity profiles for increasing concentrations of LMW Isu1 with 0.5 s exposure after subtraction of the buffer scattering data and normalization of each data set to 1 mg/ml. (D) Guinier plots of the SAXS data obtained within the low angle region are indicated by hollow spheres. The associated linear regions used to extrapolate the radius of gyration (R_g) from the slope of ($R_g^{2/3}$) are indicated by solid spheres. (E) Pairwise distribution functions P(r), and (F) standard Kratky plots extracted from the data shown in (A).

Supplementary Fig. S4



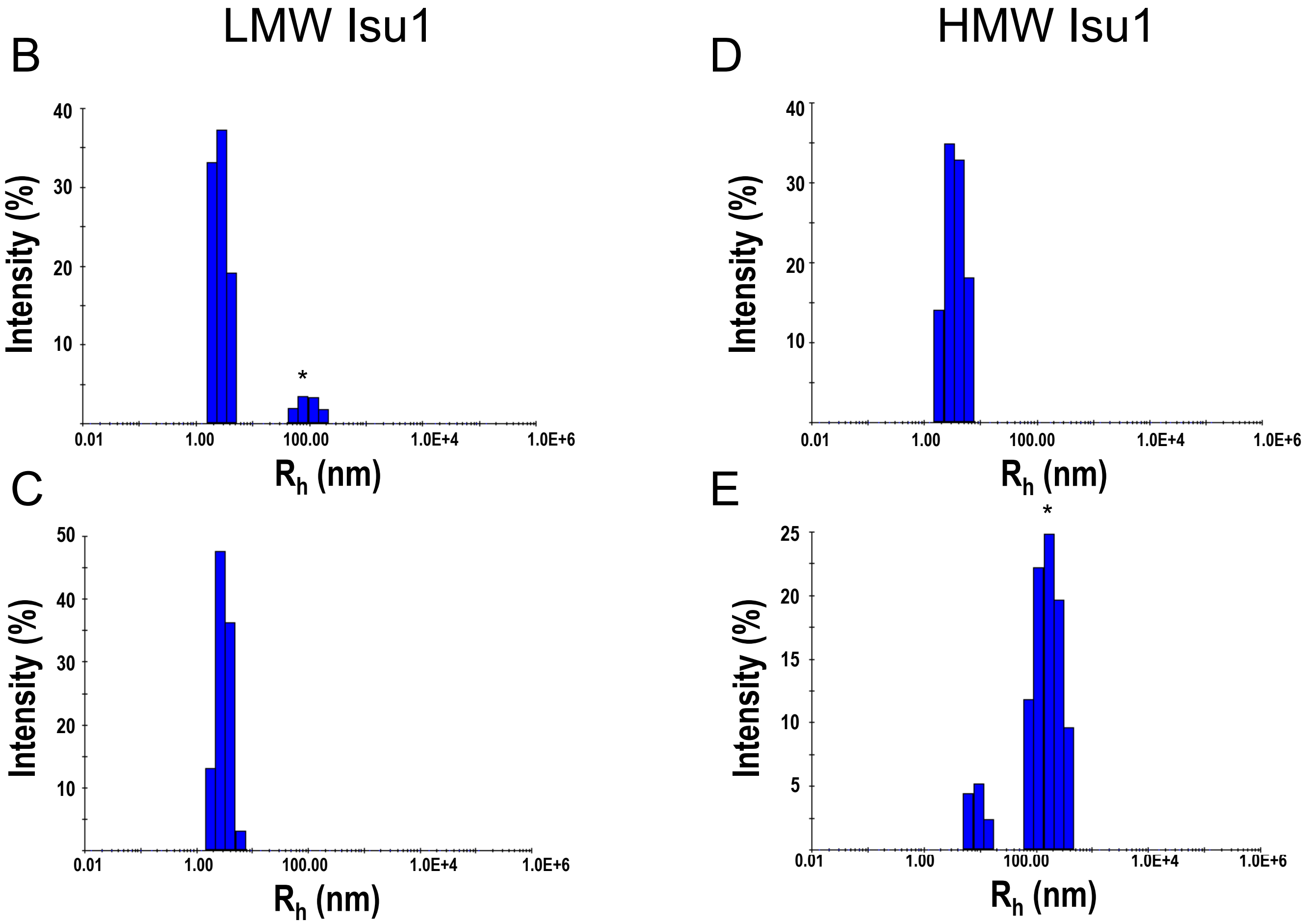
Supplementary Fig. S4. Comparison of biophysical features between LMW and HMW Isu1. (A) Radius of gyration (R_g), (B) maximum particle size (D_{max}), (C) Porod Volume (V_p), and (D) molecular mass (MM) are plotted for LMW and HMW Isu1 as a function of protein concentration. SAXS data were obtained from one preparation of HMW Isu1 (Table 2) and three independently prepared and analyzed preparations of LMW Isu1 (Table 2 and not shown). Each data point represents the mean ± standard deviation of 2-3 measurements at different exposure times for any given protein concentration.

Supplementary Fig. S5

A

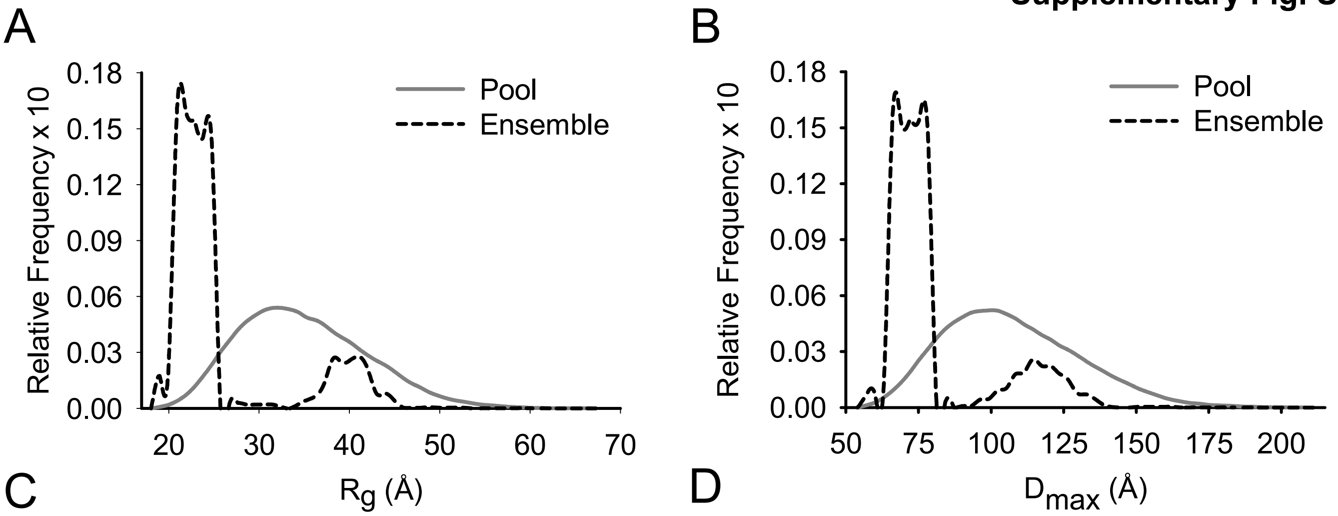
	LMW Isu1			
Protein Concentration	**2 mg/ml**	**4 mg/ml**	**6 mg /ml**	**12 mg/ml**
R_h (nm) Avg ± SD	2.9 ± 0.1	2.6 ± 0.1	3.1 ± 0.1	2.9 ± 0.2
% Mass	**99.99%**	**100%**	**100%**	**100%**

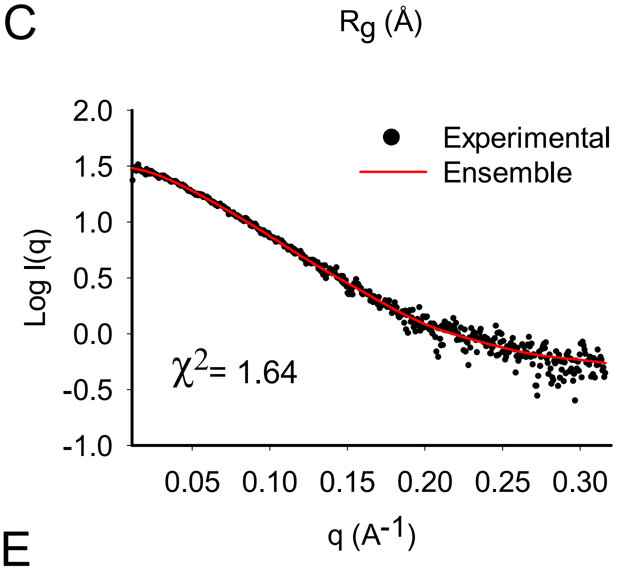
	HMW Isu1			
Protein Concentration	**2 mg/ml**	**4 mg/ml**	**6 mg /ml**	**10 mg/ml**
R_h (nm) Avg ± SD	3.8 ± 0.3	3.9 ± 0.1	5.0 ± 0.1	8.3 ± 3.4
% Mass	**100%**	**100%**	**100%**	**99.4%**



Supplementary Fig. S5: Protein concentration effects on LMW and HMW Isu1 oligomerization. (A) Size exclusion chromatography fractions 42-48 and 30-36 for LMW and HMW Isu1, respectively (see Fig. 1B), were pooled, concentrated to different protein concentrations and analyzed by dynamic light scattering. The R_h was obtained as described in Experimental Methods. Shown are the mean ± standard deviation obtained from one pool of LMW or HMW Isu1 protein sample that underwent at least 4 measurements. The *% Mass* was obtained as described in the previous figures. (B-C) representative plots for LMW Isu1at 2 mg/ml and 12 mg/ml. (D-E) representative plots for HMW Isu1at 2 mg/ml and 10 mg/ml. Each plot shows the distribution of particle sizes in the sample, which is based on the scattering intensity of each species present in the sample; *denotes minor species representing <1%.

Supplementary Fig. S6



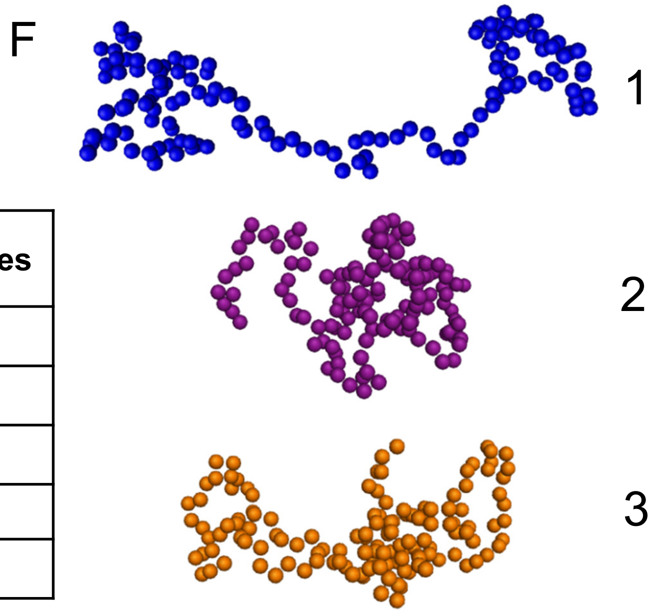


D

Histogram Program	***R$_g$* (Å)**	***D$_{max}$* (Å)**
RANCH (Pool)	34.9	107.1
GAJOE (Ensemble)	25.8	80.6

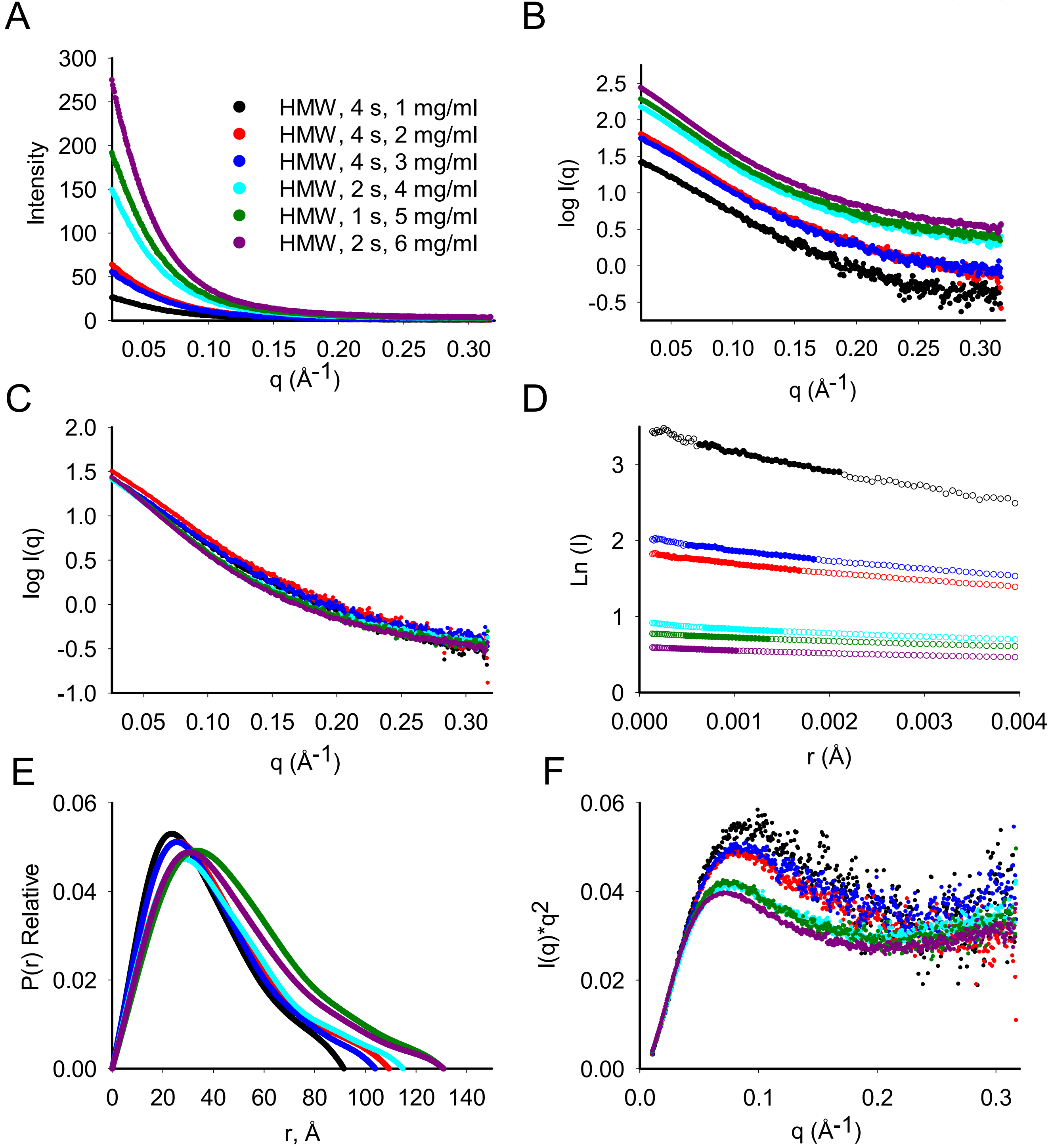
E

Model	***R$_g$*, Å**	***D$_{max}$*, Å**	**Times**
Monomer 1	39.2	109.2	6
Monomer 2	19.7	59.7	4
Monomer 3	23.3	73.6	24
Average	25.7	78.2	34
Ensemble Chi-Value: 1.64			



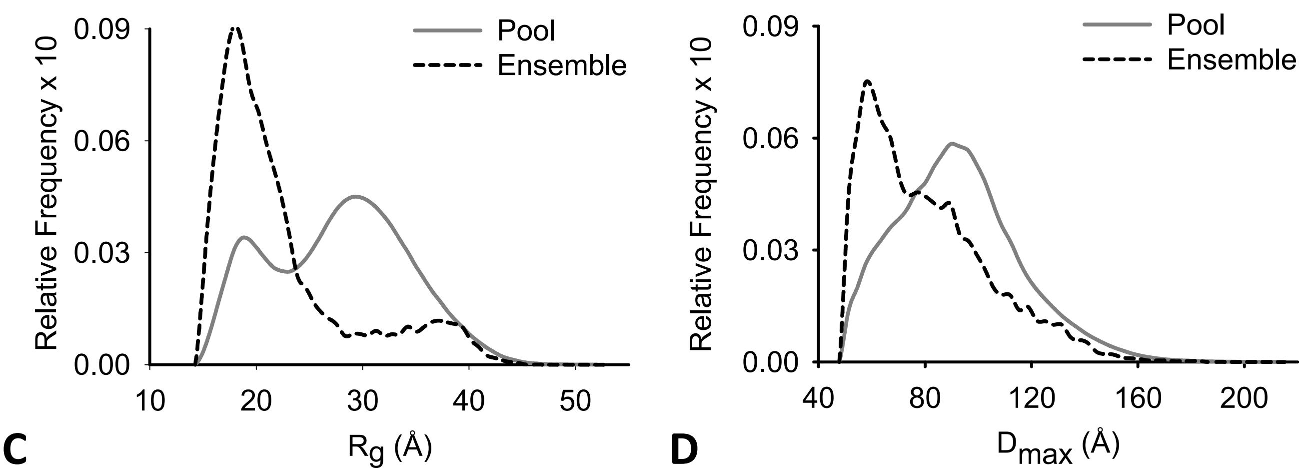
Supplementary Figure S6: EOM analysis of LMW Isu1 suggests an elongated monomer shape. SAXS scattering data for LMW Isu1 (at 1 mg protein/ml and 2 sec exposure) were subjected to EOM analysis to identify the best fit ensemble within a RANCH pool of 10,000 random Isu1 models. (A-B) Resulting distribution of frequencies of R_g and D_{max} for the best fit ensemble (dashed black) and pool (solid grey). (C) Theoretical scattering profile for the best fit ensemble (red line) fitted to experimental SAXS data (black dots). (D) Summary of the biophysical parameters determined for the pool (RANCH) and best fit ensemble (GAJOE). (E) Summary of the dimensions and frequency of each model within the best fit ensemble. (F) Bead models from the best fit ensemble oriented to display their maximum dimension. 1,2 and 3 corresponds to Monomer 1, Monomer 2, Monomer 3 in supplementary Fig. S2E.

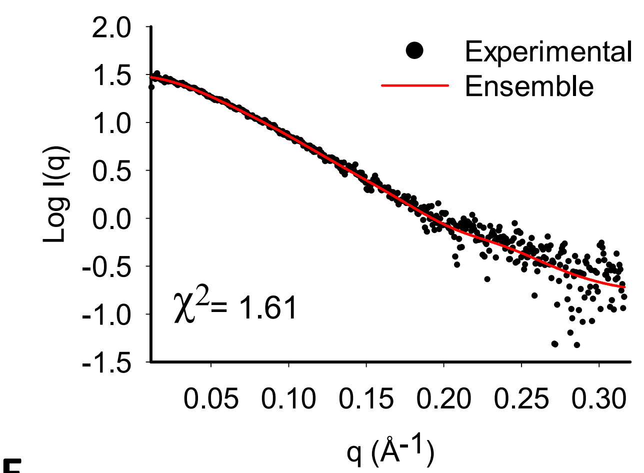
Supplementary Fig. S7



Supplementary Figure S7: SAXS demonstrates significant protein-concentration-dependent changes in HMW Isu1. SAXS scattering data were collected for different concentrations of HMW Isu1 at the indicated exposure times as described in Experimental Methods and summarized in Table 2. (A) Absolute and (B) Log Intensity data vs. q after subtraction of the buffer scattering data. (C) SAXS scattering intensity profiles for increasing concentrations of HMW Isu1 after subtraction of the buffer scattering data and normalization of each data set to 1 mg /ml. Automated scaling was carried out by identifying the minimum discrepancies between data using the program PRIMUS. (D) Guinier plots of the data at low angle region at increasing protein concentration. (E) Pairwise distribution functions, P(r), and (F) standard Kratky plots extracted from the data shown in (C).

Supplementary Fig. S8





D

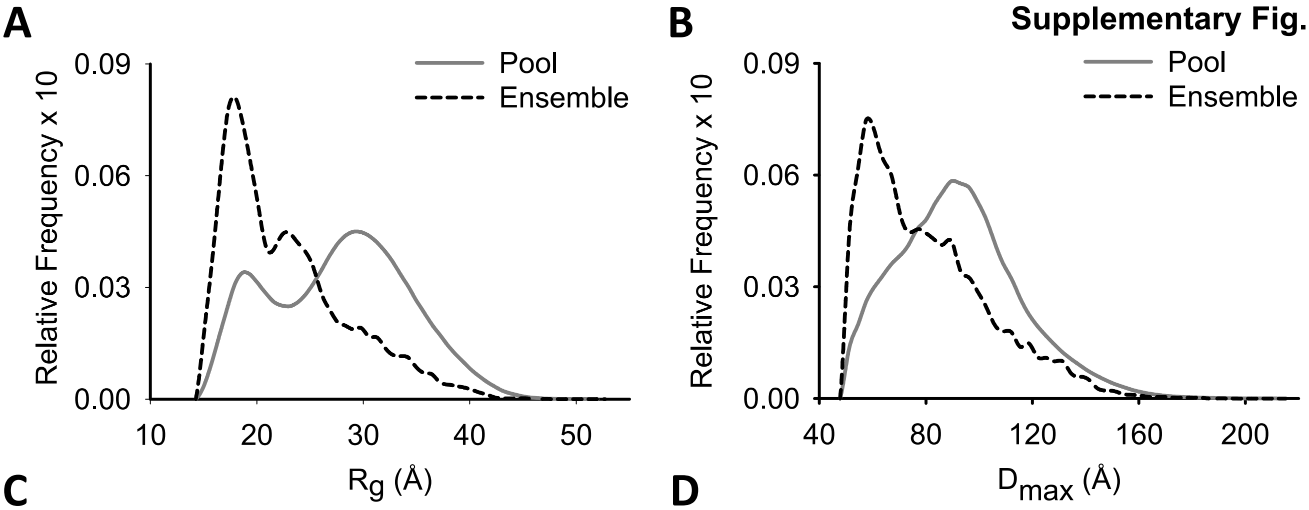
Program	R_g (Å)	D_{max} (Å)
RANCH (100,000 model pool)	27.8	92.6
GAJOE (Ensemble)	22.3	80.0

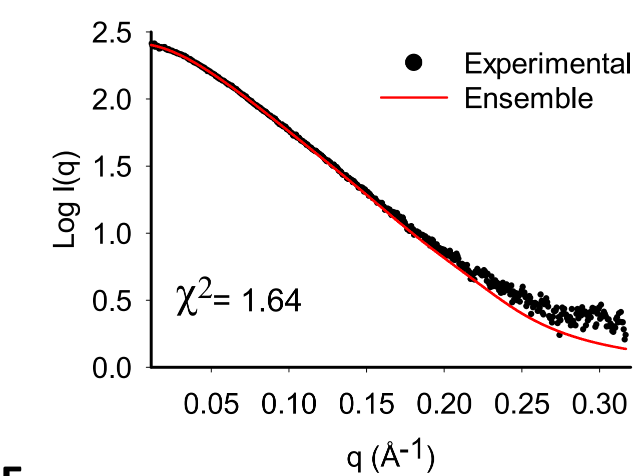
E

Oligomerization State	R_g (Å)	D_{max} (Å)	Times
monomer	17.9	60.0	2
monomer	21.6	76.2	1
monomer	17.9	58.2	1
monomer	17.6	55.9	1
dimer	33.4	123.6	2
dimer	20.5	72.8	1
Ensemble Chi-Value: 1.61			

Supplementary Figure S8: EOM analysis of LMW Isu1 suggests limited propensity to oligomerize beyond dimer. SAXS scattering data for LMW Isu1 at a protein concentration of 1 mg/ml were subjected to EOM analysis to identify the best fit ensemble within a RANCH pool of 100,000 Isu1 models with a 1:1:1:1 stoichiometry of monomer:dimer:trimer:tetramer. (A-B) Distribution of frequencies of R_g and D_{max} for the best fit ensemble (dashed black) and pool (solid grey). (C) Theoretical scattering profile for the best fit ensemble (red line) fitted to experimental SAXS data (black dots). (D) Summary of the biophysical parameters determined for the pool (RANCH) and best fit ensemble (GAJOE). (E) Summary of the dimensions and frequency of each model within the best fit ensemble.

Supplementary Fig. S9





D

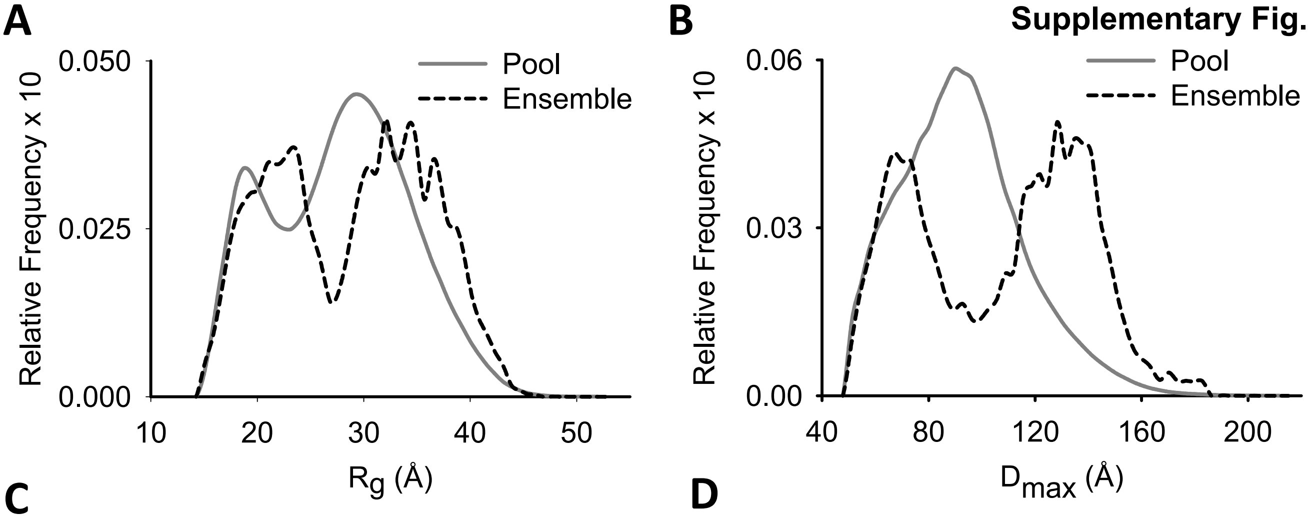
Program	R_g (Å)	D_{max} (Å)
RANCH (100,000 model pool)	27.7	92.6
GAJOE (Ensemble)	22.7	80.8

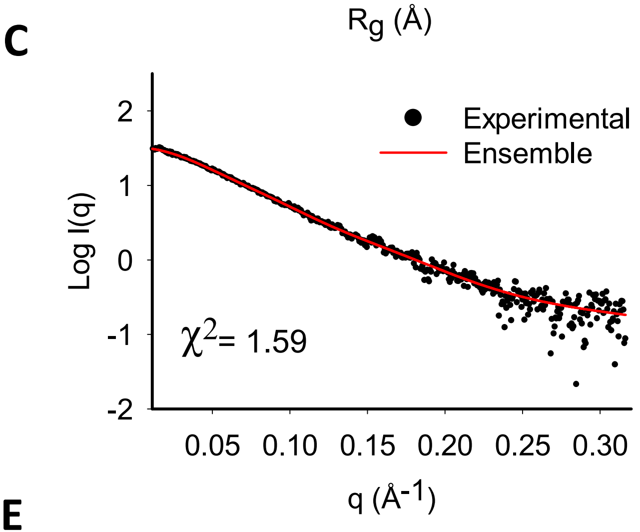
E

Oligomerization State	R_g (Å)	D_{max} (Å)	Times
monomer	22.2	82.7	1
monomer	18.2	67.8	1
monomer	16.9	62.0	2
dimer	24.0	86.3	8
dimer	39.4	138.3	2
Ensemble Chi-Value: 1.64			

Supplementary Figure S9: EOM analysis of LMW Isu1 suggests limited propensity to oligomerize beyond dimer even at high protein concentration. SAXS scattering data for LMW Isu1 at a protein concentration of 6 mg/ml were subjected to EOM analysis to identify the best fit ensemble within a RANCH pool of 100,000 Isu1 models with a 1:1:1:1 stoichiometry of monomer:dimer:trimer:tetramer. (A-B) Distribution of frequencies of R_g and D_{max} for the best fit ensemble (dashed black) and pool (solid grey). (C) Theoretical scattering profile for the best fit ensemble (red line) fitted to experimental SAXS data (black dots). (D) Summary of the biophysical parameters determined for the pool (RANCH) and best fit ensemble (GAJOE). (E) Summary of the dimensions and frequency of each model within the best fit ensemble.

Supplementary Fig. S10





D

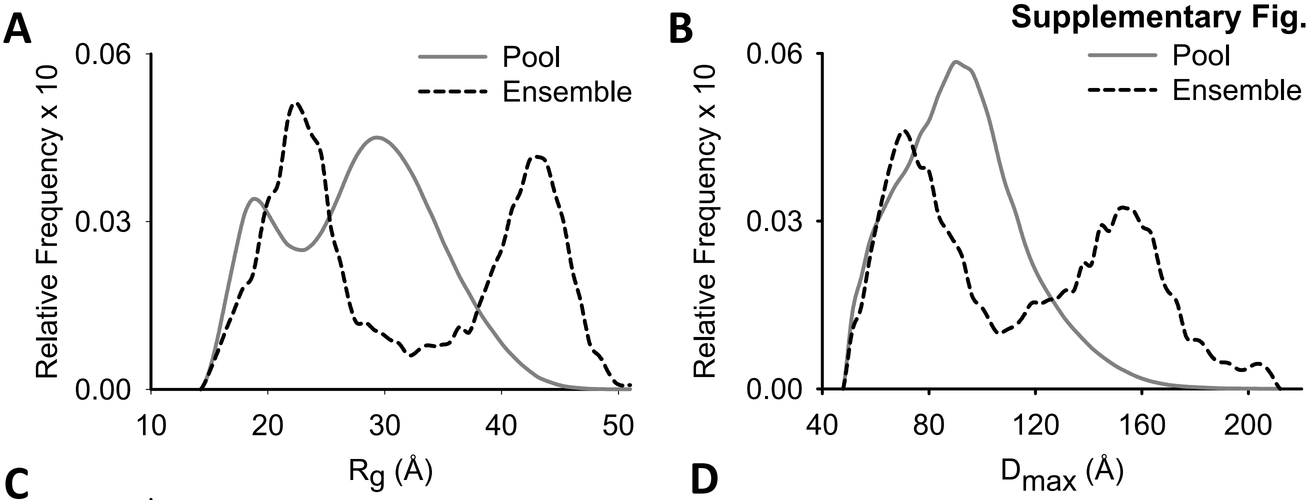
Program	R_g (Å)	D_{max} (Å)
RANCH (100,000 model pool)	27.7	107.2
GAJOE (Ensemble)	28.8	92.6

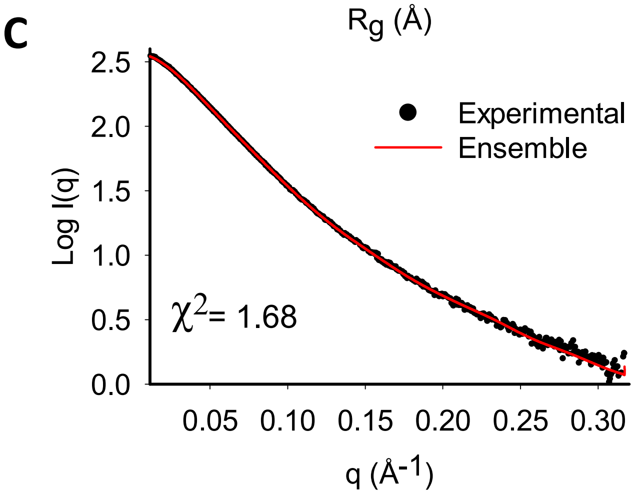
E

Oligomerization State	R_g (Å)	D_{max} (Å)	Times
monomer	19.3	67.7	3
dimer	24.7	83.1	1
dimer	37.9	116.6	4
dimer	25.0	87.2	1
trimer	22.9	71.2	1
trimer	41.3	159.8	1
Ensemble Chi-Value: 1.59			

Supplementary Figure S10: EOM analysis of HMW Isu1 suggests propensity to form dimer. SAXS scattering data for HMW Isu1 at a protein concentration of 1 mg/ml were subjected to EOM analysis to identify the best fit ensemble within a RANCH pool of 100,000 Isu1 models with a 1:1:1:1 stoichiometry of monomer:dimer:trimer:tetramer. (A-B) Distribution of frequencies of R_g and D_{max} for the best fit ensemble (dashed black) and pool (solid grey). (C) Theoretical scattering profile for the best fit ensemble (red line) fitted to experimental SAXS data (black dots). (D) Summary of the biophysical parameters determined for the pool (RANCH) and best fit ensemble (GAJOE). (E) Summary of the dimensions and frequency of each model within the best fit ensemble.

Supplementary Fig. S11





D

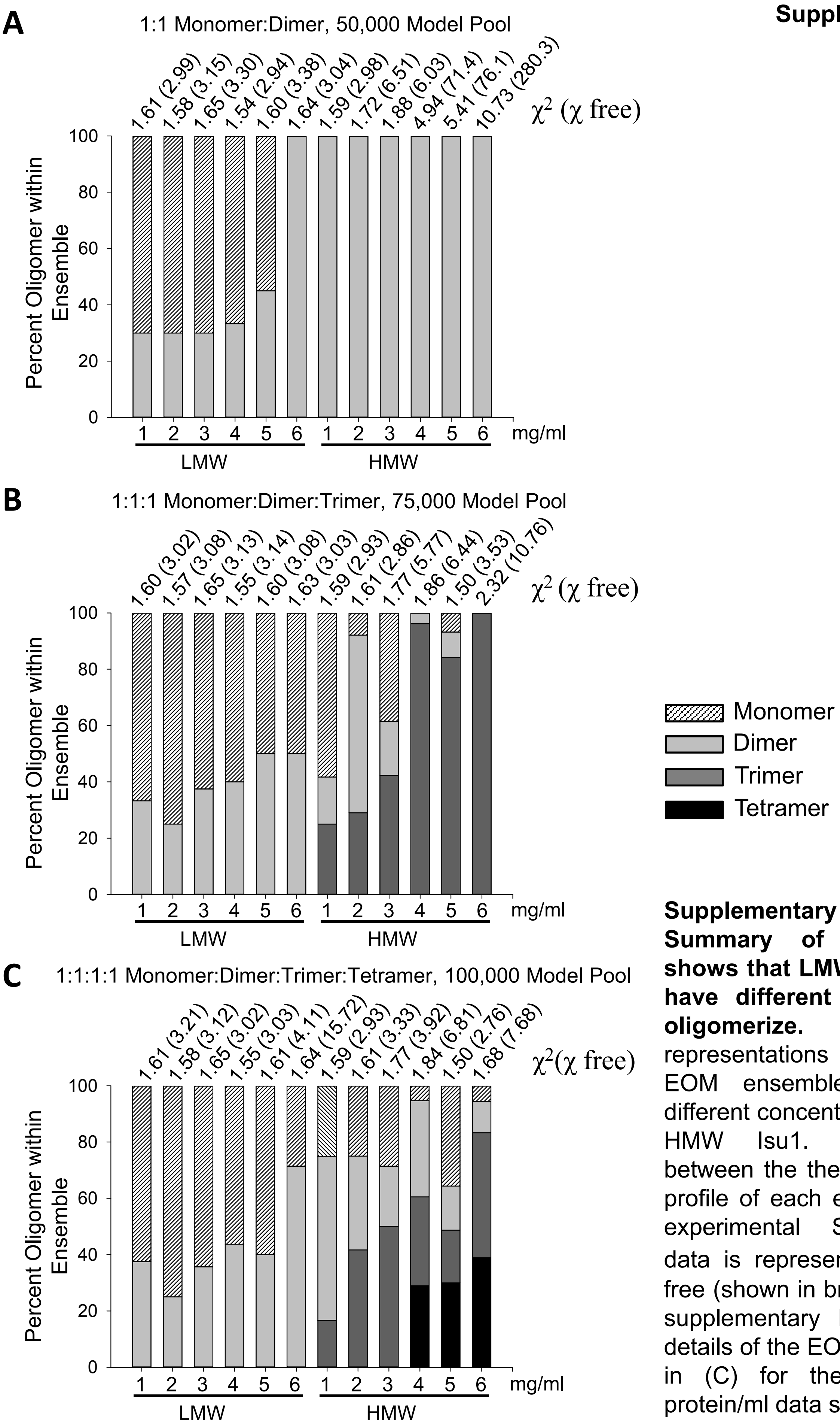
Program	R_g (Å)	D_{max} (Å)
RANCH (100,000 model pool)	27.7	92.6
GAJOE (Ensemble)	31.5	113.8

E

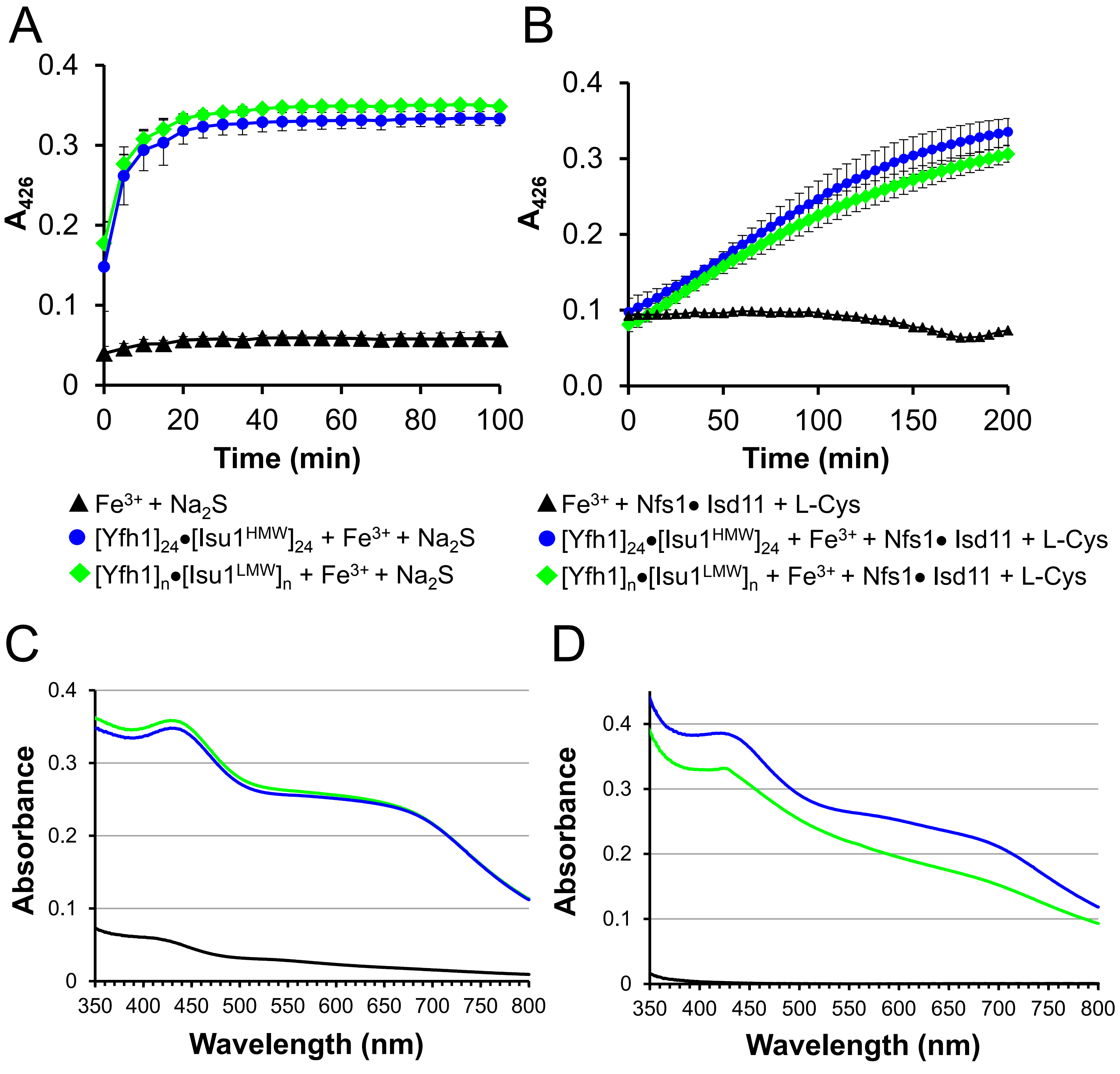
Oligomerization State	R_g (Å)	D_{max} (Å)	Times
monomer	18.1	55.7	1
dimer	27.0	73.7	1
dimer	21.6	82.8	1
trimer	23.7	76.3	6
trimer	31.8	109.1	1
trimer	41.3	159.8	1
tetramer	46.5	156.6	2
tetramer	48.6	210.6	5
Ensemble Chi-Value: 1.68			

Supplementary Figure S11: EOM analysis of HMW Isu1 suggests propensity to oligomerize beyond dimer. SAXS scattering data for HMW Isu1 at a protein concentration of 6 mg/ml were subjected to EOM analysis to identify the best fit ensemble within a RANCH pool of 100,000 Isu1 models with a 1:1:1:1 stoichiometry of monomer:dimer:trimer:tetramer. (A-B) Distribution of frequencies of R_g and D_{max} for the best fit ensemble (dashed black) and pool (solid grey). (C) Theoretical scattering profile for the best fit ensemble (red line) fitted to experimental SAXS data (black dots). (D) Summary of the biophysical parameters determined for the pool (RANCH) and best fit ensemble (GAJOE). (E) Summary of the dimensions and frequency of each model within the best fit ensemble.

Supplementary Fig. S12

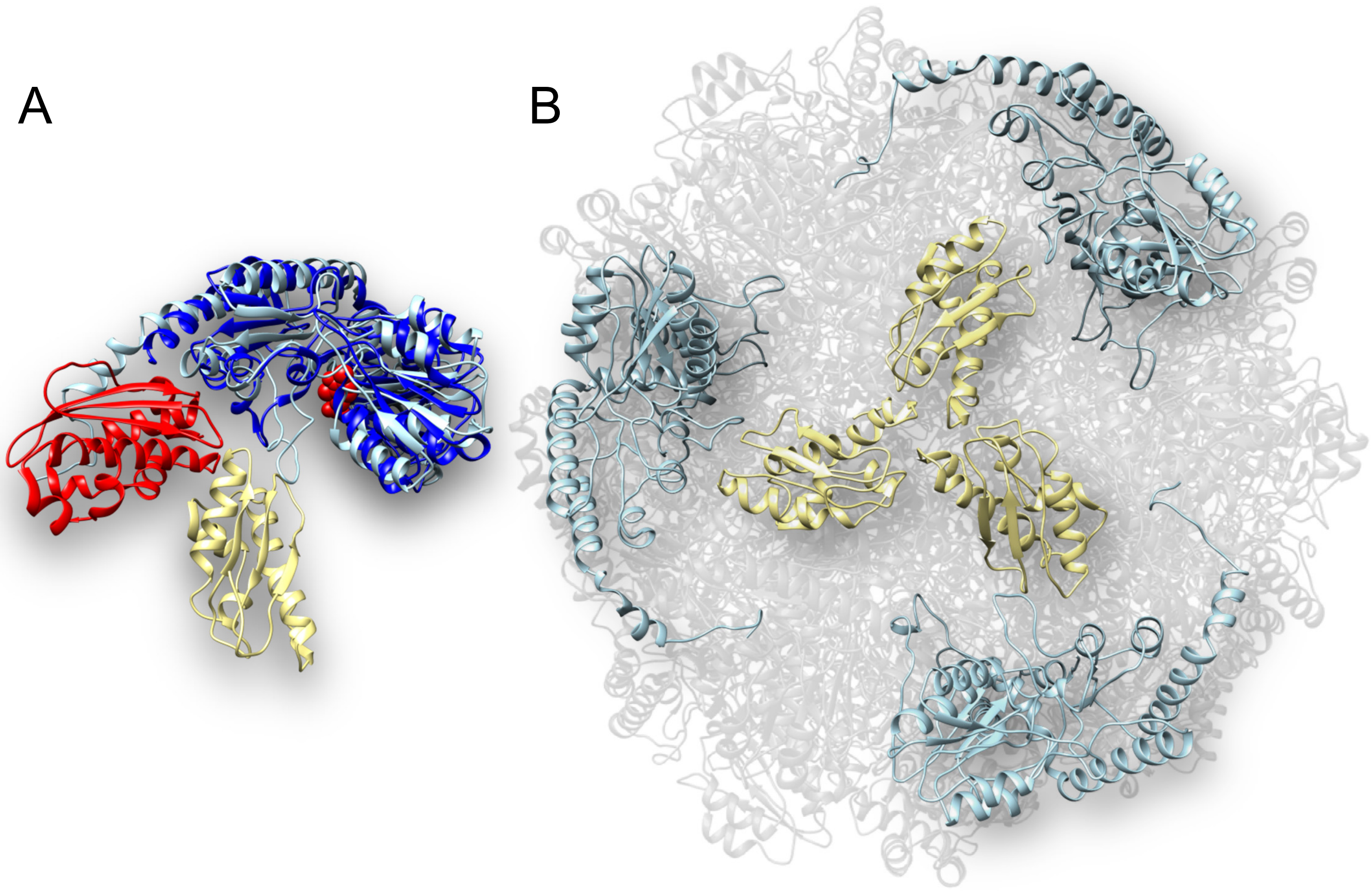


Supplementary Figure S12: Summary of EOM analysis shows that LMW and HMW Isu1 have different propensities to oligomerize. (A-C) Graphic representations of the best-fit EOM ensembles identified for different concentrations of LMW or HMW Isu1. The agreement between the theoretical scattering profile of each ensemble and the experimental SAXS scattering data is represented by χ^2 and χ free (shown in brackets). See also supplementary Figs. S8-S11 for details of the EOM analysis shown in (C) for the 1 and 6 mg protein/ml data sets.



Supplementary Fig. S13: LMW and HMW Isu1 form functional complexes with Yfh1^{Y73A} 24-mer. (A) and (B) are the same panels shown in Fig. 3 (E) and (F) and are shown again here for reference. (C) and (D) Individual UV-visible absorption spectra for the completed reactions shown in (A) and (B), respectively. Plots are color-coded as in (A) and (B).

Supplementary Fig. S14



Supplementary Fig. S14: Comparison of prokaryotic and eukaryotic Fe-S cluster assembly complexes. (A), ribbon representation of the *A. fulgidus* [IscS]•[IscU] complex (PDB code 4EB5) and the [NFS1]•[ISCU] from the human $[\mathrm{FXN}^{42\text{-}210}]_{24}$•$[\mathrm{ISCU}]_{24}$•$[\mathrm{NFS1}]_{24}$•$[\mathrm{ISD11}]_{24}$ complex (PDB code 5KZ5). Alignment of the [IscS]•[IscU] heterodimer with the [NFS1]•[ISCU] heterodimer shows the different positions of IscU (red ribbon) and ISCU (yellow ribbon) relative to the flexible loop and catalytic Cys of IscS (blue ribbon) and NFS1 (light blue ribbon). PLP cofactor is shown in red. This figure is adapted from reference 17. (B), the 3-fold axis of the human $[\mathrm{FXN}^{42\text{-}210}]_{24}$•$[\mathrm{ISCU}]_{24}$•$[\mathrm{NFS1}]_{24}$•$[\mathrm{ISD11}]_{24}$ complex shows that in the context of oligomeric frataxin, ISCU can have a trimeric configuration when bound to NFS1.

Supplementary Tables

Supplementary Table S1. Analysis of cross-linked peptides and distances between pairs of cross-linked amino acids in complex structure

Supplementary Table S1a
Single digestion (Glu-C) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
n-ME	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE					2	2				
N-Term	N-Term	5C	51.6 ± 2.6	22.5 ± 2.5	32.5 ± 4.6						
N-Term	S$_{25}$		53.0 ± 2.3	24.5 ± 2.1	33.4 ± 4.7						
N-Term	S$_{28}$		56.1 ± 2.3	26.1 ± 1.8	36.2 ± 4.9						
N-Term	S$_{29}$		57.4 ± 2.4	23.9 ± 2.2	37.6 ± 5.0						
N-Term	T$_{31}$		62.2 ± 2.8	24.8 ± 1.8	42.8 ± 4.7						
N-Term	K$_{32}$		62.1 ± 3.5	27.6 ± 1.7	42.7 ± 5.9						
N-Term	Y$_{35}$		63.7 ± 3.3	29.0 ± 1.8	44.4 ± 5.1						
N-Term	K$_{38}$	5C	61.0 ± 2.7	23.8 ± 2.1	41.0 ± 4.9						
n-MES$_{54}$S$_{55}$T$_{56}$DGQVVPQE	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE					2			1		1
S$_{54}$	K$_{32}$		61.2 ± 2.2	30.6 ± 1.4	43.3 ± 2.2						
S$_{55}$	K$_{32}$		59.4 ± 2.3	32.4 ± 1.5	43.0 ± 2.4						
T$_{56}$	K$_{32}$		58.5 ± 2.3	31.0 ± 1.7	43.7 ± 2.4						
S$_{54}$	K$_{38}$		60.0 ± 1.3	27.1 ± 1.5	37.7 ± 2.5						
S$_{55}$	K$_{38}$		58.1 ± 1.4	28.7 ± 1.7	37.7 ± 2.7						
T$_{56}$	K$_{38}$		56.8 ± 1.5	27.0 ± 2.1	38.6 ± 2.7						
n-ME	LVQGMT$_{114}$LDDAAK$_{120}$IK$_{122}$NT$_{124}$E					1	1				
N-Term	T$_{114}$		33.0 ± 2.5	34.3 ± 2.1	18.0 ± 2.9						
N-Term	K$_{120}$		41.2 ± 2.3	23.5 ± 2.2	25.6 ± 3.8						
N-Term	K$_{122}$		45.5 ± 2.1	18.7 ± 1.9	25.6 ± 2.8						
N-Term	T$_{124}$		47.1 ± 2.2	17.2 ± 1.9	27.8 ± 3.1						
K$_{72}$AHEE	IAK$_{128}$E					3	1			2	
K$_{72}$	K$_{128}$		31.4 ± 1.5	23.5 ± 1.1	28.0 ± 1.6						
K$_{72}$AHE	LVQGMT$_{114}$LDDAAK$_{120}$IK$_{122}$NT$_{124}$EIAK$_{128}$E					4	1		1	2	
K$_{72}$	T$_{114}$		15.5 ± 1.2	23.5 ± 1.0	42.0 ± 1.2						
K$_{72}$	K$_{120}$		21.6 ± 2.1	13.8 ± 1.4	30.3 ± 1.5						
K$_{72}$	K$_{122}$		26.4 ± 1.5	14.5 ± 1.5	26.5 ± 1.7						
K$_{72}$	T$_{124}$		28.8 ± 1.8	17.7 ± 1.1	24.9 ± 1.8						
LS$_{92}$E	IAK$_{128}$E					1			1		
S$_{92}$	K$_{128}$		16.4 ± 1.3	45.7 ± 1.0	12.3 ± 0.9						
LS$_{105}$HGVMT$_{110}$LE	LVQGMT$_{114}$LDDAAK$_{120}$IK$_{122}$NT$_{124}$EIAK$_{128}$E					2	1		1		
S$_{105}$	K$_{120}$		28.7 ± 1.7	37.5 ± 1.1	24.8 ± 1.7						
S$_{105}$	K$_{122}$		28.6 ± 1.4	37.7 ± 2.0	23.2 ± 1.8						
T$_{110}$	K$_{120}$		22.3 ± 1.6	37.4 ± 1.3	28.3 ± 1.1						
T$_{110}$	K$_{122}$		21.3 ± 1.4	38.5 ± 1.7	25.2 ± 1.2						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
WVS$_{151}$LRNGT$_{156}$K$_{157}$LT$_{159}$DILT$_{163}$EE	IAK$_{128}$E					1	1				
S$_{151}$	K$_{128}$		22.1 ± 0.9	37.1 ± 1.2	33.4 ± 1.3						
T$_{156}$	K$_{128}$		20.2 ± 1.5	37.3 ± 1.4	31.5 ± 1.2						
K$_{157}$	K$_{128}$		20.3 ± 1.1	35.5 ± 1.2	28.4 ± 1.2						
T$_{159}$	K$_{128}$		18.4 ± 0.7	36.5 ± 1.0	22.9 ± 1.2						
T$_{163}$	K$_{128}$		13.7 ± 0.6	41.6 ± 1.0	20.2 ± 1.0						
VEK$_{168}$AIS$_{171}$K$_{172}$SQ-c	LVQGMT$_{114}$LDDAAK$_{120}$IK$_{122}$NT$_{124}$E					2	2				
K$_{168}$	T$_{114}$		18.1 ± 0.9	44.7 ± 1.0	44.5 ± 1.3						
K$_{168}$	K$_{120}$	9C	14.1 ± 1.0	38.8 ± 1.4	35.4 ± 1.4						
K$_{168}$	K$_{122}$		11.6 ± 1.2	41.0 ± 1.3	30.1 ± 1.4						
K$_{168}$	T$_{124}$		9.8 ± 1.1	44.2 ± 1.1	25.1 ± 1.5						
K$_{172}$	T$_{114}$		24.2 ± 0.7	51.3 ± 1.1	45.3 ± 1.7						
K$_{172}$	K$_{120}$		20.2 ± 1.3	45.4 ± 1.3	37.6 ± 1.7						
K$_{172}$	K$_{122}$		16.5 ± 1.1	47.3 ± 1.4	32.4 ± 1.8						
K$_{172}$	T$_{124}$		13.6 ± 1.0	50.5 ± 1.1	27.4 ± 2.0						
S$_{171}$	K$_{120}$		19.0 ± 1.1	42.5 ± 1.3	35.3 ± 1.7						
S$_{171}$	K$_{122}$		16.3 ± 1.2	44.3 ± 1.4	29.9 ± 1.8						
K$_{168}$AIS$_{171}$K$_{172}$SQ-c	IAK$_{128}$E					8	8				
K$_{168}$	K$_{128}$		7.0 ± 0.9	50.0 ± 0.9	22.3 ± 1.0						
K$_{172}$	K$_{128}$	9C	8.0 ± 0.9	56.2 ± 1.0	23.3 ± 1.5						
S$_{171}$	K$_{128}$		9.7 ± 0.9	53.2 ± 1.0	21.1 ± 1.5						
VEK$_{168}$AIS$_{171}$K$_{172}$SQ-c	IAK$_{128}$ELS$_{131}$LPPVK$_{136}$LHCS$_{140}$MLAE					1			1		
K$_{168}$	K$_{128}$		7.0 ± 0.9	50.0 ± 0.9	22.3 ± 1.0						
K$_{168}$	S$_{131}$		10.7 ± 1.0	53.8 ± 1.5	19.4 ± 1.5						
K$_{168}$	K$_{136}$		17.4 ± 1.5	42.4 ± 1.1	21.9 ± 2.3						
K$_{168}$	S$_{140}$		17.5 ± 1.2	45.5 ± 1.2	26.4 ± 1.5						
K$_{172}$	K$_{128}$		8.0 ± 0.9	56.2 ± 1.0	23.3 ± 1.5						
K$_{172}$	S$_{131}$		7.7 ± 1.2	59.8 ± 1.6	19.7 ± 2.0						
K$_{172}$	K$_{136}$		18.9 ± 1.5	48.1 ± 1.2	25.7 ± 2.4						
K$_{172}$	S$_{140}$		17.7 ± 1.3	51.1 ± 1.2	28.7 ± 1.7						
S$_{171}$	K$_{128}$		9.7 ± 0.9	53.2 ± 1.0	21.1 ± 1.5						
S$_{171}$	K$_{136}$		20.8 ± 1.4	44.9 ± 1.2	22.7 ± 2.5						

Double digestion (Glu-C and Asp-N) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
nME	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE					8	1	1	5		1
N-Term	N-Term	5C	51.6 ± 2.6	22.5 ± 2.5	32.5 ± 4.6						
N-Term	S$_{25}$		53.0 ± 2.3	24.5 ± 2.1	33.4 ± 4.7						
N-Term	S$_{28}$		56.1 ± 2.3	26.1 ± 1.8	36.2 ± 4.9						
N-Term	S$_{29}$		57.4 ± 2.4	23.9 ± 2.2	37.6 ± 5.0						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
N-Term	T_{31}		62.2 ± 2.8	24.8 ± 1.8	42.8 ± 4.7						
N-Term	K_{32}		62.1 ± 3.5	27.6 ± 1.7	42.7 ± 5.9						
N-Term	Y_{35}		63.7 ± 3.3	29.0 ± 1.8	44.4 ± 5.1						
N-Term	K_{38}	5C	61.0 ± 2.7	23.8 ± 2.1	41.0 ± 4.9						
nME	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIEHY$_{43}$T$_{44}$HPRNVGS$_{51}$LDK$_{54}$K$_{55}$LPNVGTGLVGAPACG					2			1		1
N-Term	N-Term		51.6 ± 2.6	22.5 ± 2.5	38.7 ± 3.6						
N-Term	S_{25}		53.0 ± 2.3	24.5 ± 2.1	40.9 ± 3.6						
N-Term	S_{28}		56.1 ± 2.3	26.1 ± 1.8	41.4 ± 3.6						
N-Term	S_{29}		57.4 ± 2.4	23.9 ± 2.2	38.8 ± 3.7						
N-Term	T_{31}		62.2 ± 2.8	24.8 ± 1.8	39.6 ± 4.2						
N-Term	K_{32}		62.1 ± 3.5	27.6 ± 1.7	42.5 ± 4.1						
N-Term	Y_{35}		63.7 ± 3.3	29.0 ± 1.8	41.6 ± 4.1						
N-Term	K_{38}		61.0 ± 2.7	23.8 ± 2.1	36.4 ± 4.1						
N-Term	Y_{43}		61.0 ± 3.0	29.8 ± 2.7	38.3 ± 4.0						
N-Term	T_{44}		63.9 ± 3.0	29.6 ± 2.7	37.1 ± 3.8						
N-Term	S_{51}		72.3 ± 2.7	29.7 ± 2.8	34.2 ± 4.2						
N-Term	K_{54}		62.7 ± 3.0	28.5 ± 2.4	31.5 ± 3.4						
N-Term	K_{55}		62.5 ± 3.2	32.7 ± 3.2	33.6 ± 3.1						
N-Term	T_{61}		50.1 ± 2.7	27.2 ± 2.3	26.3 ± 2.7						
nMES$_{54}$S$_{55}$T$_{56}$	DDAAK$_{120}$IK$_{122}$NT$_{124}$EIAK$_{128}$E					12	3	1	6		2
N-Term	K_{120}		41.2 ± 2.3	23.5 ± 2.2	26.5 ± 2.7						
N-Term	K_{122}		45.5 ± 2.1	18.7 ± 1.9	25.6 ± 2.8						
N-Term	T_{124}		47.1 ± 2.2	17.2 ± 1.9	27.8 ± 3.1						
N-Term	K_{128}		48.2 ± 2.1	18.9 ± 2.0	32.5 ± 3.3						
S_{54}	K_{120}		38.8 ± 1.6	21.5 ± 1.8	24.4 ± 2.2						
S_{55}	K_{120}		36.4 ± 1.7	20.1 ± 1.9	23.8 ± 2.2						
T_{56}	K_{120}		34.7 ± 1.7	16.9 ± 2.0	26.4 ± 2.0						
S_{54}	K_{122}		42.9 ± 1.4	16.7 ± 1.9	23.5 ± 2.5						
S_{55}	K_{122}		40.5 ± 1.5	15.8 ± 2.3	22.7 ± 2.7						
T_{56}	K_{122}		39.0 ± 1.8	12.7 ± 2.4	24.8 ± 2.6						
S_{54}	K_{128}		45.8 ± 1.2	17.6 ± 1.1	30.6 ± 2.4						
S_{55}	K_{128}		43.5 ± 1.1	17.8 ± 1.3	29.4 ± 2.6						
T_{56}	K_{128}		42.7 ± 1.1	16.0 ± 1.5	30.6 ± 2.3						
nMES$_{54}$S$_{55}$T$_{56}$	DVMRLQIK$_{78}$VN					2			2		
N-Term	K_{78}		42.8 ± 2.7	33.8 ± 2.4	27.5 ± 2.8						
S_{54}	K_{78}		42.4 ± 1.2	34.3 ± 1.4	28.6 ± 1.7						
S_{55}	K_{78}		40.8 ± 1.5	33.7 ± 1.7	30.7 ± 2.0						
T_{56}	K_{78}		38.5 ± 1.3	30.4 ± 1.9	33.6 ± 1.4						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
nMES$_{54}$S$_{55}$T$_{56}$DGQVVPQE	HY$_{43}$T$_{44}$HPRNVGS$_{51}$LDK$_{54}$K$_{55}$LPNVGTGLVGAPACG					1			1		
N-Term	Y$_{43}$		61.0 ± 3.0	29.8 ± 2.7	38.3 ± 4.0						
N-Term	T$_{44}$		63.9 ± 3.0	29.6 ± 2.7	37.1 ± 3.8						
N-Term	S$_{51}$		72.3 ± 2.7	29.7 ± 2.8	34.2 ± 4.2						
N-Term	K$_{54}$		62.7 ± 3.0	28.5 ± 2.4	31.5 ± 3.4						
N-Term	K$_{55}$		62.5 ± 3.2	32.7 ± 3.2	33.6 ± 3.1						
N-Term	T$_{61}$		50.1 ± 2.7	27.2 ± 2.3	26.3 ± 2.7						
S$_{54}$	K$_{54}$		62.1 ± 1.0	32.7 ± 1.7	34.4 ± 2.4						
S$_{55}$	K$_{54}$		60.3 ± 1.2	33.5 ± 1.9	35.7 ± 2.6						
T$_{56}$	K$_{54}$		58.2 ± 1.2	31.2 ± 2.0	37.2 ± 2.3						
S$_{54}$	K$_{55}$		62.2 ± 1.3	35.9 ± 1.6	36.5 ± 2.1						
S$_{55}$	K$_{55}$		60.5 ± 1.5	36.6 ± 1.7	38.0 ± 2.3						
T$_{56}$	K$_{55}$		58.3 ± 1.3	34.1 ± 1.8	39.7 ± 2.0						
nMES$_{54}$S$_{55}$T$_{56}$	LS$_{131}$LPPVK$_{136}$LHCS$_{140}$MLAEDAIK$_{148}$AAIK$_{152}$					4			2		2
N-Term	S$_{131}$		52.6 ± 2.2	18.5 ± 2.4	33.3 ± 2.4						
N-Term	K$_{136}$		55.0 ± 2.2	9.9 ± 2.1	22.1 ± 2.2						
N-Term	S$_{140}$		57.1 ± 2.6	11.3 ± 2.4	23.3 ± 2.1						
N-Term	K$_{148}$		46.7 ± 2.1	22.1 ± 2.4	21.1 ± 2.1						
N-Term	K$_{152}$		45.9 ± 2.3	27.3 ± 2.5	22.9 ± 2.4						
S$_{54}$	K$_{136}$		52.0 ± 1.9	7.0 ± 1.6	20.7 ± 2.5						
S$_{55}$	K$_{136}$		49.4 ± 1.9	7.1 ± 1.5	19.2 ± 2.6						
T$_{56}$	K$_{136}$		48.3 ± 1.8	6.1 ± 0.8	19.9 ± 2.5						
S$_{54}$	K$_{148}$		44.7 ± 1.2	21.5 ± 1.4	21.1 ± 2.0						
S$_{55}$	K$_{148}$		42.5 ± 1.4	20.4 ± 1.8	21.4 ± 2.0						
T$_{56}$	K$_{148}$		40.4 ± 1.3	17.1 ± 1.9	24.2 ± 1.9						
S$_{54}$	K$_{152}$		44.1 ± 1.1	26.7 ± 1.3	22.4 ± 1.6						
S$_{55}$	K$_{152}$		42.0 ± 1.4	25.3 ± 1.6	23.3 ± 1.8						
T$_{56}$	K$_{152}$		39.5 ± 1.3	22.2 ± 1.7	26.4 ± 1.7						
nMES$_{54}$S$_{55}$T$_{56}$	IAK$_{128}$ELS$_{131}$LPPVK$_{136}$LHCS$_{140}$MLAE					3			1		2
N-Term	K$_{128}$		48.2 ± 2.1	18.9 ± 2.0	32.5 ± 3.3						
N-Term	S$_{131}$		52.6 ± 2.2	18.5 ± 2.4	33.3 ± 2.4						
N-Term	K$_{136}$		55.0 ± 2.2	9.9 ± 2.1	22.1 ± 2.2						
N-Term	S$_{140}$		57.1 ± 2.6	11.3 ± 2.4	23.3 ± 2.1						
S$_{54}$	K$_{128}$		45.8 ± 1.2	17.6 ± 1.1	30.6 ± 2.4						
S$_{55}$	K$_{128}$		43.5 ± 1.1	17.8 ± 1.3	29.4 ± 2.6						
T$_{56}$	K$_{128}$		42.7 ± 1.1	16.0 ± 1.5	30.6 ± 2.3						
S$_{54}$	K$_{136}$		52.0 ± 1.9	7.0 ± 1.6	20.7 ± 2.5						
S$_{55}$	K$_{136}$		49.4 ± 1.9	7.1 ± 1.5	19.2 ± 2.6						
T$_{56}$	K$_{136}$		48.3 ± 1.8	6.1 ± 0.8	19.9 ± 2.5						
nME	LS$_{131}$LPPVK$_{136}$LHCS$_{140}$MLAE					10	1				9
N-Term	S$_{131}$		52.6 ± 2.2	18.5 ± 2.4	33.3 ± 2.4						
N-Term	K$_{136}$		55.0 ± 2.2	9.9 ± 2.1	22.1 ± 2.2						
N-Term	S$_{140}$		57.1 ± 2.6	11.3 ± 2.4	23.3 ± 2.1						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
nME	DAIK$_{148}$AAIK$_{152}$DY$_{154}$K$_{155}$S$_{156}$K$_{157}$RNT$_{160}$PT$_{162}$MLS$_{165}$-c					6			3	1	2
N-Term	K$_{148}$		46.7 ± 2.1	22.1 ± 2.4	21.1 ± 2.1						
N-Term	K$_{152}$		45.9 ± 2.3	27.3 ± 2.5	22.9 ± 2.4						
N-Term	Y$_{154}$		44.2 ± 2.4	31.5 ± 2.5	27.6 ± 2.3						
N-Term	K$_{155}$		43.1 ± 2.3	32.3 ± 2.4	26.9 ± 2.3						
N-Term	S$_{156}$		46.4 ± 2.3	32.9 ± 2.5	25.6 ± 2.2						
N-Term	K$_{157}$		46.0 ± 2.5	35.3 ± 2.6	28.8 ± 2.1						
N-Term	T$_{160}$		42.6 ± 2.4	36.2 ± 2.4	28.1 ± 2.4						
N-Term	T$_{162}$		43.8 ± 2.2	34.5 ± 2.2	25.2 ± 2.5						
N-Term	S$_{165}$		46.4 ± 2.0	29.3 ± 2.2	20.5 ± 2.4						
S$_{54}$S$_{55}$T$_{56}$DGQVVPQE	IAK$_{128}$E					5	2		2		1
S$_{54}$	K$_{128}$		45.8 ± 1.2	17.6 ± 1.1	29.1 ± 2.7						
S$_{55}$	K$_{128}$		43.5 ± 1.1	17.8 ± 1.3	30.3 ± 2.9						
T$_{56}$	K$_{128}$		42.7 ± 1.1	16.0 ± 1.5	31.9 ± 2.7						
K$_{72}$AHEE	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE					4	1		3		
K$_{72}$	N-Term		36.8 ± 1.6	31.0 ± 1.7	32.6 ± 1.8						
K$_{72}$	S$_{25}$		38.9 ± 1.5	33.7 ± 1.4	35.3 ± 1.6						
K$_{72}$	S$_{28}$		42.0 ± 1.4	35.9 ± 1.3	38.9 ± 1.6						
K$_{72}$	S$_{29}$		42.4 ± 1.5	34.1 ± 1.6	37.2 ± 1.8						
K$_{72}$	T$_{31}$		47.1 ± 1.8	36.8 ± 2.1	38.5 ± 1.9						
K$_{72}$	K$_{32}$		47.7 ± 2.0	39.1 ± 1.8	41.3 ± 1.7						
K$_{72}$	Y$_{35}$		49.0 ± 1.7	39.5 ± 1.4	43.3 ± 2.1						
K$_{72}$	K$_{38}$		45.2 ± 1.8	33.9 ± 1.5	38.0 ± 2.0						
K$_{72}$AHEE	LS$_{131}$LPPVK$_{136}$LHCS$_{140}$MLAE					2	1				1
K$_{72}$	S$_{131}$		36.6 ± 1.4	27.5 ± 1.8	28.7 ± 2.1						
K$_{72}$	K$_{136}$		36.2 ± 1.8	17.3 ± 1.7	17.4 ± 2.1						
K$_{72}$	S$_{140}$		38.7 ± 1.7	21.0 ± 1.7	24.4 ± 1.7						
K$_{72}$AHEE	DAAK$_{120}$IK$_{122}$NT$_{124}$E					5	2	1			2
K$_{72}$	K$_{120}$		21.6 ± 2.1	13.8 ± 1.4	30.3 ± 1.5						
K$_{72}$	K$_{122}$		26.4 ± 1.5	14.5 ± 1.5	26.5 ± 1.7						
K$_{72}$	T$_{124}$		28.8 ± 1.8	17.7 ± 1.1	24.9 ± 1.8						
K$_{72}$AHEE	DAIK$_{148}$AAIK$_{152}$DY$_{154}$K$_{155}$S$_{156}$K$_{157}$RNT$_{160}$PT$_{162}$MLS$_{165}$-c					5	1	1	1		2
K$_{72}$	K$_{148}$		27.1 ± 1.6	12.3 ± 1.1	30.3 ± 1.5						
K$_{72}$	K$_{152}$		26.5 ± 1.5	14.0 ± 1.2	34.5 ± 1.6						
K$_{72}$	Y$_{154}$		25.9 ± 1.5	19.2 ± 1.2	39.6 ± 1.5						
K$_{72}$	K$_{155}$		24.5 ± 1.5	17.6 ± 1.3	39.1 ± 1.6						
K$_{72}$	S$_{156}$		28.0 ± 1.5	18.3 ± 1.1	39.6 ± 1.7						
K$_{72}$	K$_{157}$		28.5 ± 1.5	21.9 ± 1.1	42.8 ± 1.6						
K$_{72}$	T$_{160}$		24.3 ± 1.7	19.0 ± 1.4	41.2 ± 1.8						
K$_{72}$	T$_{162}$		24.8 ± 1.7	15.4 ± 1.5	37.7 ± 2.1						
K$_{72}$	S$_{165}$		26.5 ± 1.6	10.1 ± 1.7	32.4 ± 2.1						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Yfh1	Isu1	Figure	Yfh1-Isu1 intra-dimer	Yfh1-Isu1 intra-trimer	Yfh1-Isu1 inter-trimers	# of Peptides	≤5%	≤10%	≤20%	≤25%	Below 25%
K$_{72}$AHEE	DVMRLQIK$_{78}$VNDS$_{82}$T$_{83}$GVIE					2			1		1
K$_{72}$	K$_{78}$		26.9 ± 1.4	26.2 ± 1.0	44.2 ± 1.4						
K$_{72}$	S$_{82}$		19.5 ± 1.4	28.8 ± 1.2	49.8 ± 1.5						
K$_{72}$	T$_{83}$		16.5 ± 1.7	27.5 ± 1.4	47.9 ± 1.6						
K$_{72}$AHEE	DVK$_{90}$FK$_{92}$T$_{93}$FGCGS$_{98}$AIAS$_{102}$S$_{103}$S$_{104}$Y$_{105}$MT$_{107}$E					1			1		
K$_{72}$	K$_{90}$		31.2 ± 1.4	26.9 ± 1.2	40.9 ± 1.4						
K$_{72}$	K$_{92}$		36.6 ± 1.4	26.4 ± 1.0	37.2 ± 1.3						
K$_{72}$	T$_{93}$		37.9 ± 1.4	26.0 ± 1.0	34.6 ± 1.3						
K$_{72}$	S$_{98}$		39.0 ± 1.5	28.6 ± 1.2	32.1 ± 1.5						
K$_{72}$	S$_{102}$		31.8 ± 1.5	24.7 ± 1.1	33.0 ± 1.2						
K$_{72}$	S$_{103}$		31.4 ± 1.5	24.2 ± 0.8	34.5 ± 1.2						
K$_{72}$	S$_{104}$		31.2 ± 1.3	27.5 ± 0.8	38.0 ± 1.2						
K$_{72}$	Y$_{105}$		28.5 ± 1.2	26.9 ± 0.9	37.4 ± 1.1						
K$_{72}$	T$_{107}$		26.6 ± 1.4	25.0 ± 0.8	38.6 ± 1.1						
K$_{72}$AHEE	IAK$_{128}$E					4			1		3
K$_{72}$	K$_{128}$		31.4 ± 1.5	23.5 ± 1.1	28.0 ± 1.6						
K$_{72}$AHEE	DK$_{54}$K$_{55}$LPNVGT$_{61}$GLVGAPACGDVMRLQIK$_{78}$VN					1			1		
K$_{72}$	K$_{54}$		45.8 ± 1.5	31.9 ± 1.4	42.3 ± 1.3						
K$_{72}$	K$_{55}$		46.2 ± 1.6	33.7 ± 1.1	45.4 ± 1.3						
K$_{72}$	T$_{61}$		32.1 ± 1.5	21.0 ± 1.1	38.1 ± 1.4						
K$_{72}$	K$_{78}$		26.9 ± 1.4	26.2 ± 1.0	44.2 ± 1.4						
DS$_{87}$LE	DAAK$_{120}$IK$_{122}$NT$_{124}$EIAK$_{128}$E					8	1	2	2		3
S$_{87}$	K$_{120}$		17.9 ± 1.2	28.8 ± 1.3	24.0 ± 1.1						
S$_{87}$	K$_{122}$		19.5 ± 1.2	29.5 ± 1.4	19.2 ± 1.1						
S$_{87}$	K$_{128}$		19.8 ± 0.9	37.5 ± 1.0	16.0 ± 1.3						
DS$_{87}$LE	DK$_{54}$K$_{55}$LPNVGT$_{61}$GLVGAPACGDVMRLQIK$_{78}$VN					1	1				
S$_{87}$	K$_{54}$		43.0 ± 1.2	42.9 ± 1.4	40.3 ± 1.5						
S$_{87}$	K$_{55}$		44.4 ± 1.4	44.6 ± 1.1	43.4 ± 1.4						
S$_{87}$	K$_{78}$	5C	27.9 ± 1.1	39.9 ± 0.6	38.8 ± 0.8						
DS$_{87}$LEE	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE					3			2		1
S$_{87}$	N-Term		23.7 ± 1.1	44.8 ± 1.3	19.3 ± 2.2						
S$_{87}$	K$_{32}$		37.5 ± 2.0	52.6 ± 1.4	32.2 ± 2.1						
S$_{87}$	K$_{38}$		36.7 ± 1.4	47.1 ± 1.3	30.9 ± 2.4						
DS$_{87}$LE	DAIK$_{148}$AAIK$_{152}$					1			1		
S$_{87}$	K$_{148}$		25.5 ± 0.8	26.8 ± 0.8	27.2 ± 0.8						
S$_{87}$	K$_{152}$		28.3 ± 0.8	26.5 ± 0.8	32.7 ± 1.0						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
LS$_{105}$HGVMT$_{110}$LE	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE					2		2			
S$_{105}$	N-Term		30.9 ± 1.2	50.3 ± 1.4	26.4 ± 2.2						
S$_{105}$	K$_{32}$		47.9 ± 1.6	62.1 ± 1.2	43.5 ± 2.5						
S$_{105}$	K$_{38}$		47.7 ± 1.3	57.9 ± 1.5	42.5 ± 2.2						
T$_{110}$	N-Term	5C	23.2 ± 1.2	53.0 ± 1.2	23.0 ± 1.8						
T$_{110}$	K$_{32}$		40.3 ± 1.6	63.6 ± 1.6	40.6 ± 2.2						
T$_{110}$	K$_{38}$		39.9 ± 1.5	58.7 ± 1.5	40.6 ± 2.0						
LS$_{105}$HGVMT$_{110}$LEIPAFGT$_{118}$Y$_{119}$VINK$_{123}$QP PNK$_{128}$QIWLAS$_{134}$PLS$_{137}$GPNRF	DAAK$_{120}$IK$_{122}$NT$_{124}$E					2			1		1
K$_{123}$	K$_{120}$		21.1 ± 1.2	29.6 ± 1.3	32.7 ± 1.1						
K$_{123}$	K$_{122}$	9C	22.4 ± 1.4	31.4 ± 1.7	30.0 ± 1.8						
K$_{123}$	T$_{124}$		21.9 ± 1.3	32.8 ± 1.5	26.8 ± 1.7						
K$_{128}$	K$_{120}$	9C	19.3 ± 1.3	25.3 ± 1.3	39.9 ± 1.4						
K$_{128}$	K$_{122}$		22.0 ± 1.5	28.5 ± 1.8	36.8 ± 2.0						
K$_{128}$	T$_{124}$		23.3 ± 1.3	30.6 ± 1.8	33.8 ± 1.9						
S$_{105}$	K$_{120}$		28.7 ± 1.7	37.5 ± 1.1	24.8 ± 1.7						
S$_{105}$	K$_{122}$		28.6 ± 1.4	37.7 ± 2.0	23.2 ± 1.8						
T$_{110}$	K$_{120}$		22.3 ± 1.6	37.4 ± 1.3	28.3 ± 1.1						
T$_{110}$	K$_{122}$		21.3 ± 1.4	38.5 ± 1.7	25.2 ± 1.2						
T$_{118}$	K$_{120}$		17.7 ± 1.1	40.5 ± 1.3	35.3 ± 1.3						
T$_{118}$	K$_{122}$		14.5 ± 1.2	42.7 ± 1.6	31.2 ± 1.4						
Y$_{119}$	K$_{120}$		16.5 ± 1.1	37.7 ± 1.4	35.0 ± 1.4						
Y$_{119}$	K$_{122}$		14.3 ± 1.6	40.0 ± 1.5	31.1 ± 1.6						
S$_{134}$	K$_{120}$		12.7 ± 1.3	36.2 ± 1.3	39.3 ± 1.6						
S$_{134}$	K$_{122}$		10.5 ± 1.2	39.2 ± 1.7	35.1 ± 1.7						
S$_{137}$	K$_{120}$		10.8 ± 0.9	36.9 ± 1.2	44.2 ± 1.6						
S$_{137}$	K$_{122}$		8.2 ± 1.5	40.6 ± 1.4	39.7 ± 1.8						
DVELS$_{105}$HGVMT$_{110}$LE	DAAK$_{120}$IK$_{122}$NT$_{124}$EIAK$_{128}$E					7	1		4		2
S$_{105}$	K$_{120}$		28.7 ± 1.7	37.5 ± 1.1	24.8 ± 1.7						
S$_{105}$	K$_{122}$		28.6 ± 1.4	37.7 ± 2.0	23.2 ± 1.8						
S$_{105}$	K$_{128}$		27.3 ± 1.1	43.4 ± 1.4	22.3 ± 2.2						
T$_{110}$	K$_{120}$		22.3 ± 1.6	37.4 ± 1.3	28.3 ± 1.1						
T$_{110}$	K$_{122}$		21.3 ± 1.4	38.5 ± 1.7	25.2 ± 1.2						
T$_{110}$	K$_{128}$		19.4 ± 1.2	45.7 ± 1.1	21.3 ± 1.6						
LS$_{105}$HGVMT$_{110}$LE	DAIK$_{148}$AAIK$_{152}$					2		1			1
S$_{105}$	K$_{148}$		36.3 ± 1.1	38.8 ± 1.3	31.2 ± 1.7						
S$_{105}$	K$_{152}$		39.7 ± 1.1	39.7 ± 1.2	35.3 ± 1.7						
T$_{110}$	K$_{148}$		29.6 ± 0.9	37.6 ± 1.3	34.2 ± 1.0						
T$_{110}$	K$_{152}$		33.8 ± 1.1	37.3 ± 1.2	39.2 ± 1.0						
DILT$_{163}$EE	IAK$_{128}$ELS$_{131}$LPPVK$_{136}$LHCS$_{140}$MLAE					2		1			1
T$_{163}$	K$_{128}$		13.7 ± 0.6	41.6 ± 1.0	20.2 ± 1.0						
T$_{163}$	K$_{136}$		21.3 ± 1.6	34.1 ± 1.1	16.0 ± 2.2						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Yfh1	Isu1	Figure	Yfh1-Isu1 intra-dimer	Yfh1-Isu1 intra-trimer	Yfh1-Isu1 inter-trimers	# of Peptides	≤5%	≤10%	≤20%	≤25%	Below 25%
$WVS_{151}LRNGT_{156}K_{157}LT_{159}DILT_{163}E$	$n\text{-}GS_{25}HMS_{28}S_{29}IT_{31}K_{32}RLY_{35}HPK_{38}VIE$					1			1		
K_{157}	N-Term		27.1 ± 1.1	43.2 ± 1.4	30.7 ± 1.9						
K_{157}	S_{25}		29.6 ± 1.0	46.0 ± 1.1	33.4 ± 1.6						
K_{157}	S_{28}		33.0 ± 0.9	48.2 ± 1.1	38.8 ± 1.3						
K_{157}	S_{29}		32.7 ± 1.0	46.5 ± 1.2	38.0 ± 1.8						
K_{157}	T_{31}		37.2 ± 1.3	49.4 ± 1.8	39.7 ± 1.7						
K_{157}	K_{32}		38.4 ± 1.7	51.6 ± 1.8	42.0 ± 1.6						
K_{157}	Y_{35}		39.9 ± 1.3	51.4 ± 1.3	45.7 ± 1.6						
K_{157}	K_{38}		35.2 ± 1.1	46.0 ± 1.4	40.9 ± 1.6						
S_{151}	N-Term		29.6 ± 0.9	44.8 ± 1.1	35.7 ± 2.0						
T_{156}	N-Term		27.3 ± 1.4	45.0 ± 1.6	33.2 ± 1.9						
T_{159}	N-Term		24.3 ± 0.9	44.1 ± 1.1	25.5 ± 1.9						
T_{163}	N-Term		18.6 ± 0.9	49.1 ± 1.1	21.0 ± 1.6						
S_{151}	K_{32}		40.8 ± 1.6	54.2 ± 1.6	47.7 ± 1.7						
T_{156}	K_{32}		37.4 ± 1.8	52.9 ± 2.0	44.0 ± 1.8						
T_{159}	K_{32}		37.0 ± 1.8	52.4 ± 1.5	37.6 ± 1.7						
T_{163}	K_{32}		31.2 ± 1.9	56.1 ± 1.5	32.9 ± 1.6						
S_{151}	K_{38}		36.9 ± 1.1	49.1 ± 1.4	46.8 ± 1.7						
T_{156}	K_{38}		33.6 ± 1.1	47.3 ± 1.6	43.2 ± 1.6						
T_{159}	K_{38}		34.8 ± 1.2	46.8 ± 1.4	36.5 ± 1.7						
T_{163}	K_{38}		29.7 ± 1.0	50.1 ± 1.4	32.8 ± 1.6						
$WVS_{151}LRNGT_{156}K_{157}LT_{159}DILT_{163}E$	$IAK_{128}ELS_{131}LPPVK_{136}LHCS_{140}MLAE$					6			1		5
K_{157}	K_{128}	9C	20.3 ± 1.1	35.5 ± 1.2	28.4 ± 1.2						
K_{157}	S_{131}		25.7 ± 1.3	40.0 ± 1.6	28.0 ± 1.6						
K_{157}	K_{136}	9C	23.2 ± 1.8	30.2 ± 1.4	21.0 ± 1.8						
K_{157}	S_{140}		26.3 ± 1.7	34.1 ± 1.6	29.1 ± 1.4						
S_{151}	K_{128}		22.1 ± 0.9	37.1 ± 1.2	33.4 ± 1.3						
T_{156}	K_{128}		20.2 ± 1.5	37.3 ± 1.4	31.5 ± 1.2						
T_{159}	K_{128}		18.4 ± 0.7	36.5 ± 1.0	22.9 ± 1.2						
T_{163}	K_{128}		13.7 ± 0.6	41.6 ± 1.0	20.2 ± 1.0						
S_{151}	K_{136}		22.0 ± 1.6	33.2 ± 1.5	26.4 ± 1.9						
T_{156}	K_{136}		21.9 ± 2.1	32.9 ± 1.4	24.4 ± 1.8						
T_{159}	K_{136}		23.4 ± 1.6	29.7 ± 1.2	16.4 ± 1.8						
T_{163}	K_{136}		21.3 ± 1.6	34.1 ± 1.1	16.0 ± 2.2						
$DLLNGEWVS_{151}LRNGT_{156}K_{157}LT_{159}DILT_{163}$	$LS_{131}LPPVK_{136}LHCS_{140}MLAE$					4			1		3
K_{157}	S_{131}		25.7 ± 1.3	40.0 ± 1.6	28.0 ± 1.6						
K_{157}	K_{136}		23.2 ± 1.8	30.2 ± 1.4	21.0 ± 1.8						
K_{157}	S_{140}		26.3 ± 1.7	34.1 ± 1.6	29.1 ± 1.4						
S_{151}	K_{136}		22.0 ± 1.6	33.2 ± 1.5	26.4 ± 1.9						
T_{156}	K_{136}		21.9 ± 2.1	32.9 ± 1.4	24.4 ± 1.8						
T_{159}	K_{136}		23.4 ± 1.6	29.7 ± 1.2	16.4 ± 1.8						
T_{163}	K_{136}		21.3 ± 1.6	34.1 ± 1.1	16.0 ± 2.2						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
K$_{168}$AIS$_{171}$K$_{172}$SQ	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE					6	1	2	2	1	
K$_{168}$	N-Term		10.4 ± 0.9	57.5 ± 1.1	20.0 ± 1.6						
K$_{168}$	S$_{25}$		12.4 ± 0.8	60.2 ± 0.8	21.6 ± 1.5						
K$_{168}$	S$_{28}$		16.7 ± 0.9	61.5 ± 0.7	27.6 ± 1.6						
K$_{168}$	S$_{29}$		17.5 ± 1.0	59.3 ± 0.8	28.1 ± 2.0						
K$_{168}$	T$_{31}$		22.5 ± 1.2	61.1 ± 1.8	30.0 ± 1.8						
K$_{168}$	K$_{32}$		23.2 ± 1.7	63.6 ± 1.5	31.4 ± 1.8						
K$_{168}$	Y$_{35}$		26.4 ± 1.4	62.5 ± 1.6	36.6 ± 1.8						
K$_{168}$	K$_{38}$	9C	22.4 ± 0.9	57.3 ± 1.4	33.1 ± 1.6						
K$_{172}$	N-Term	9C	5.0 ± 0.6	63.6 ± 1.2	19.0 ± 1.9						
K$_{172}$	S$_{25}$		6.0 ± 0.8	66.3 ± 0.9	19.7 ± 2.0						
K$_{172}$	S$_{28}$		11.7 ± 0.7	67.4 ± 0.8	25.6 ± 2.0						
K$_{172}$	S$_{29}$		13.4 ± 1.0	65.1 ± 0.9	26.9 ± 2.3						
K$_{172}$	T$_{31}$		18.4 ± 1.1	66.6 ± 1.8	28.8 ± 2.0						
K$_{172}$	K$_{32}$	9C	18.8 ± 1.6	69.2 ± 1.6	29.6 ± 2.1						
K$_{172}$	Y$_{35}$		23.1 ± 1.5	67.9 ± 1.8	35.4 ± 2.1						
K$_{172}$	K$_{38}$		19.8 ± 1.0	62.8 ± 1.5	32.7 ± 1.8						
S$_{171}$	N-Term		8.6 ± 0.5	60.5 ± 1.2	17.3 ± 1.7						
S$_{171}$	K$_{32}$		20.5 ± 1.8	65.6 ± 1.6	27.4 ± 1.9						
S$_{171}$	K$_{38}$		21.1 ± 1.1	59.3 ± 1.5	30.0 ± 1.7						
K$_{168}$AIS$_{171}$K$_{172}$SQ	DVMRLQIK$_{78}$VN					1				1	
K$_{168}$	K$_{78}$		23.0 ± 0.6	45.7 ± 0.7	47.8 ± 1.0						
K$_{172}$	K$_{78}$		27.2 ± 0.5	51.8 ± 0.8	48.6 ± 1.5						
S$_{171}$	K$_{78}$		25.0 ± 0.5	48.8 ± 0.8	46.2 ± 1.4						
VEK$_{168}$AIS$_{171}$K$_{172}$SQ	DK$_{54}$K$_{55}$LPNVGT$_{61}$GLVGAPACG					1			1		
K$_{168}$	K$_{54}$	5C	31.9 ± 1.1	49.0 ± 1.1	47.3 ± 1.3						
K$_{168}$	K$_{55}$		33.9 ± 1.2	49.8 ± 0.9	50.6 ± 1.4						
K$_{168}$	T$_{61}$		25.0 ± 0.6	39.5 ± 0.5	45.7 ± 1.1						
K$_{172}$	K$_{54}$		32.7 ± 1.3	53.9 ± 1.1	48.4 ± 1.8						
K$_{172}$	K$_{55}$		35.1 ± 1.6	54.6 ± 1.0	51.6 ± 1.8						
K$_{172}$	T$_{61}$		28.8 ± 0.7	45.3 ± 0.6	47.5 ± 1.4						
S$_{171}$	K$_{54}$		32.7 ± 1.3	50.3 ± 1.1	45.3 ± 1.6						
S$_{171}$	K$_{55}$		34.8 ± 1.5	51.1 ± 1.0	48.5 ± 1.7						
VEK$_{168}$AIS$_{171}$K$_{172}$SQ	DAAK$_{120}$IK$_{122}$NT$_{124}$E					8	7			1	
K$_{168}$	K$_{120}$	9C	14.1 ± 1.0	38.8 ± 1.4	35.4 ± 1.4						
K$_{168}$	K$_{122}$		11.6 ± 1.2	41.0 ± 1.3	30.1 ± 1.4						
K$_{168}$	T$_{124}$		9.8 ± 1.1	44.2 ± 1.1	25.1 ± 1.5						
K$_{172}$	K$_{120}$		20.2 ± 1.3	45.4 ± 1.3	37.6 ± 1.7						
K$_{172}$	K$_{122}$		16.5 ± 1.1	47.3 ± 1.4	32.4 ± 1.8						
K$_{172}$	T$_{124}$		13.6 ± 1.0	50.5 ± 1.1	27.4 ± 2.0						
S$_{171}$	K$_{120}$		19.0 ± 1.1	42.5 ± 1.3	35.3 ± 1.7						
S$_{171}$	K$_{122}$		16.3 ± 1.2	44.3 ± 1.4	29.9 ± 1.8						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
VEK$_{168}$AIS$_{171}$K$_{172}$SQ	DAIK$_{148}$AAIK$_{152}$DY$_{154}$K$_{155}$S$_{156}$K$_{157}$RNT$_{160}$PT$_{162}$MLS$_{165}$-c					3		1	1	1	
K_{168}	K_{148}		19.4 ± 0.7	35.5 ± 0.7	38.8 ± 1.1						
K_{168}	K_{152}		24.4 ± 0.6	32.8 ± 0.6	44.8 ± 1.1						
K_{168}	Y_{154}		25.8 ± 0.6	36.3 ± 0.6	48.3 ± 1.0						
K_{168}	K_{155}		27.1 ± 0.5	33.4 ± 0.6	49.2 ± 1.0						
K_{168}	S_{156}		30.1 ± 0.5	31.6 ± 0.7	51.2 ± 1.1						
K_{168}	K_{157}		31.0 ± 0.6	34.7 ± 0.8	53.2 ± 1.1						
K_{168}	T_{160}		31.6 ± 0.7	30.2 ± 0.8	53.1 ± 1.2						
K_{168}	T_{162}		32.0 ± 0.8	25.8 ± 0.7	51.5 ± 1.5						
K_{168}	S_{165}		29.3 ± 1.0	24.8 ± 0.8	46.8 ± 1.8						
K_{172}	K_{148}		24.3 ± 0.9	41.8 ± 0.8	41.4 ± 1.4						
K_{172}	K_{152}		29.7 ± 0.8	39.1 ± 0.7	47.4 ± 1.4						
K_{172}	Y_{154}		31.0 ± 0.6	42.4 ± 0.7	50.3 ± 1.4						
K_{172}	K_{155}		32.7 ± 0.7	39.7 ± 0.8	51.5 ± 1.4						
K_{172}	S_{156}		35.5 ± 0.7	37.6 ± 0.7	53.7 ± 1.4						
K_{172}	K_{157}		36.2 ± 0.6	40.6 ± 0.7	55.4 ± 1.4						
K_{172}	T_{160}		37.6 ± 0.9	36.4 ± 1.0	55.8 ± 1.4						
K_{172}	T_{162}		38.1 ± 1.1	32.3 ± 0.9	54.7 ± 1.7						
K_{172}	S_{165}		35.1 ± 1.2	31.4 ± 1.0	50.3 ± 2.0						
S_{171}	K_{148}		23.5 ± 0.8	38.6 ± 0.7	38.6 ± 1.4						
S_{171}	K_{152}		28.5 ± 0.7	36.1 ± 0.6	44.7 ± 1.4						
S_{171}	K_{155}		31.0 ± 0.6	36.9 ± 0.7	48.9 ± 1.3						
S_{171}	K_{157}		34.5 ± 0.7	37.8 ± 0.7	52.7 ± 1.4						
K$_{168}$AIS$_{171}$K$_{172}$SQ	IAK$_{128}$E					12	7		5		
K_{168}	K_{128}		7.0 ± 0.9	50.0 ± 0.9	22.3 ± 1.0						
K_{172}	K_{128}	9C	8.0 ± 0.9	56.2 ± 1.0	23.3 ± 1.5						
S_{171}	K_{128}		9.7 ± 0.9	53.2 ± 1.0	21.1 ± 1.5						
K$_{168}$AIS$_{171}$K$_{172}$SQ	DS$_{82}$T$_{83}$GVIE					2		1	1		
K_{168}	S_{82}		28.8 ± 0.7	44.3 ± 0.8	54.9 ± 1.2						
K_{168}	T_{83}		26.0 ± 0.7	44.6 ± 1.6	52.2 ± 1.1						
K_{172}	S_{82}		34.5 ± 0.7	50.5 ± 0.9	56.0 ± 1.5						
K_{172}	T_{83}		31.8 ± 0.8	51.0 ± 1.5	53.2 ± 1.4						

Supplementary Table S1b
Single digestion (Glu-C) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Yfh1	**Yfh1**	**Figure**	**Yfh1 intra-monomer**	**Yfh1-Yfh1 intra-trimer**	**Yfh1-Yfh1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
n-ME	$S_{54}S_{55}T_{56}$DGQVVPQE					2	2				
N-Term	S_{54}		6.4 ± 0.6	60.6 ± 2.4	20.5 ± 4.5						
N-Term	S_{55}		8.7 ± 0.6	58.9 ± 2.6	19.5 ± 4.4						
N-Term	T_{56}		9.5 ± 1.0	56.3 ± 2.7	19.3 ± 3.9						
n-ME	K_{72}AHEE					8	7			1	
N-Term	K_{72}	8E	20.7 ± 2.6	44.2 ± 2.2	15.5 ± 2.5						
n-ME	WVS_{151}LRNG$T_{156}K_{157}$LT_{159}DILT_{163}EE					1	1				
N-Term	S_{151}		37.5 ± 1.9	29.2 ± 1.7	26.4 ± 3.5						
N-Term	T_{156}		36.2 ± 2.2	29.1 ± 2.3	23.3 ± 3.7						
N-Term	K_{157}		33.4 ± 2.0	31.1 ± 2.0	20.9 ± 3.5						
N-Term	T_{159}		31.7 ± 2.1	32.0 ± 1.9	17.7 ± 3.9						
N-Term	T_{163}		34.7 ± 2.3	29.8 ± 1.9	17.4 ± 4.8						
n-ME	VEK_{168}AI$S_{171}K_{172}$SQ-c					7	6			1	
N-Term	K_{168}		42.3 ± 2.5	25.1 ± 2.0	23.3 ± 4.7						
N-Term	K_{172}		47.3 ± 2.5	25.2 ± 2.2	27.9 ± 4.5						
N-Term	S_{171}		43.9 ± 2.6	27.6 ± 2.2	24.3 ± 4.5						
K_{72}AHEE	ADDY_{80}LDHLLDS_{87}LEE					14	9	1	3		1
K_{72}	Y_{80}	8E	10.2 ± 1.0	27.9 ± 0.8	21.5 ± 1.7						
K_{72}	S_{87}		15.5 ± 0.8	29.2 ± 0.8	23.6 ± 2.3						
K_{72}AHEE	$S_{54}S_{55}T_{56}$DGQVVPQE					2	2				
K_{72}	S_{54}	8E	17.9 ± 1.9	41.3 ± 1.7	11.8 ± 1.7						
K_{72}	S_{55}		15.7 ± 1.9	39.4 ± 2.0	11.8 ± 1.7						
K_{72}	T_{56}		13.4 ± 1.9	36.7 ± 2.1	14.8 ± 1.7						
K_{72}AHEE	K_{72}AHEE					2	1	1			
K_{72}	K_{72}	8E	NA	30.3 ± 1.5	22.5 ± 2.0						
K_{72}AHEE	K_{168}AI$S_{171}K_{172}$SQ-c					9	9				
K_{72}	K_{168}		26.7 ± 1.1	25.8 ± 1.0	34.0 ± 1.3						
K_{72}	K_{172}		33.0 ± 1.1	30.7 ± 1.2	35.3 ± 1.7						
K_{72}	S_{171}		30.0 ± 1.1	30.6 ± 1.0	37.5 ± 1.5						
LS_{92}E	K_{72}AHEE					2	1				1
S_{92}	K_{72}		24.0 ± 0.9	32.5 ± 1.4	28.8 ± 2.9						
LS_{92}E	K_{168}AI$S_{171}K_{172}$SQ-c					1	1				
S_{92}	K_{168}		12.0 ± 1.5	49.1 ± 0.7	17.2 ± 1.2						
S_{92}	K_{172}		14.8 ± 1.5	53.4 ± 1.0	15.9 ± 1.8						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Yfh1	**Yfh1**	**Figure**	**Yfh1 intra-monomer**	**Yfh1-Yfh1 intra-trimer**	**Yfh1-Yfh1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
K$_{168}$AIS$_{171}$K$_{172}$SQ-c	IPAFGTY$_{119}$VINK$_{123}$QPPNK$_{128}$QIWLAS$_{134}$PLS$_{137}$GPN RFDLLNGE					2	2				
K$_{168}$	Y$_{119}$		14.8 ± 1.0	46.3 ± 0.5	24.7 ± 1.5						
K$_{168}$	K$_{123}$		24.8 ± 0.6	43.1 ± 0.5	36.5 ± 1.8						
K$_{168}$	K$_{128}$		27.9 ± 0.7	34.5 ± 0.8	41.6 ± 1.7						
K$_{168}$	S$_{134}$		15.4 ± 1.0	41.3 ± 0.9	26.7 ± 1.3						
K$_{168}$	S$_{137}$		16.9 ± 1.1	37.4 ± 1.2	28.2 ± 1.4						
K$_{172}$	Y$_{119}$		18.6 ± 1.1	52.6 ± 0.6	27.2 ± 1.8						
K$_{172}$	K$_{123}$		30.0 ± 0.8	49.4 ± 0.7	39.3 ± 2.0						
K$_{172}$	K$_{128}$		33.9 ± 1.0	40.9 ± 0.9	45.1 ± 1.9						
K$_{172}$	S$_{134}$		19.9 ± 1.2	47.6 ± 0.8	29.9 ± 1.7						
K$_{172}$	S$_{137}$		21.2 ± 1.4	43.6 ± 1.2	31.9 ± 1.7						
S$_{171}$	K$_{123}$		29.1 ± 0.7	47.6 ± 0.6	38.4 ± 2.0						
S$_{171}$	K$_{128}$		32.9 ± 0.7	39.2 ± 0.9	43.5 ± 1.9						
VEK$_{168}$AIS$_{171}$K$_{172}$SQ-c	WVS$_{151}$LRNGT$_{156}$K$_{157}$LT$_{159}$DILT$_{163}$EE					4	4				
K$_{168}$	S$_{151}$		21.1 ± 0.7	37.1 ± 1.0	39.3 ± 1.2						
K$_{168}$	T$_{156}$		18.1 ± 0.8	37.6 ± 1.3	37.7 ± 1.2						
K$_{168}$	K$_{157}$		17.5 ± 0.4	36.4 ± 1.1	34.5 ± 1.2						
K$_{168}$	T$_{159}$		14.6 ± 0.2	38.4 ± 0.8	29.0 ± 1.2						
K$_{168}$	T$_{163}$		8.6 ± 0.2	43.8 ± 0.7	26.3 ± 1.0						
K$_{172}$	S$_{151}$		27.5 ± 0.9	43.3 ± 0.9	39.0 ± 1.7						
K$_{172}$	T$_{156}$		24.7 ± 1.1	43.6 ± 1.3	36.9 ± 1.6						
K$_{172}$	K$_{157}$		24.2 ± 0.8	42.2 ± 1.1	34.1 ± 1.6						
K$_{172}$	T$_{159}$		21.1 ± 0.5	43.7 ± 1.0	28.8 ± 1.6						
K$_{172}$	T$_{163}$		15.0 ± 0.5	48.9 ± 1.0	24.8 ± 1.4						
S$_{171}$	K$_{157}$		22.2 ± 0.5	41.4 ± 1.1	36.9 ± 1.4						
K$_{168}$AIS$_{171}$K$_{172}$SQ-c	S$_{54}$S$_{55}$T$_{56}$DGQVVPQE					1	1				
K$_{168}$	S$_{54}$		40.3 ± 1.3	23.8 ± 1.3	22.7 ± 2.4						
K$_{168}$	S$_{55}$		38.1 ± 1.3	23.6 ± 1.4	24.2 ± 2.7						
K$_{168}$	T$_{56}$		37.4 ± 1.1	21.3 ± 1.3	25.6 ± 2.3						
K$_{172}$	S$_{54}$		45.5 ± 1.4	24.8 ± 1.4	27.5 ± 2.7						
K$_{172}$	S$_{55}$		43.5 ± 1.4	25.4 ± 1.5	29.0 ± 2.9						
K$_{172}$	T$_{56}$		43.0 ± 1.3	23.6 ± 1.5	29.9 ± 2.4						
K$_{168}$AIS$_{171}$K$_{172}$SQ-c	VEK$_{168}$AIS$_{171}$K$_{172}$SQ-c					6	6				
K$_{168}$	K$_{168}$		NA	52.1 ± 0.7	27.6 ± 0.8						
K$_{168}$	K$_{172}$		6.8 ± 0.5	57.2 ± 0.9	24.2 ± 1.2						
K$_{168}$	S$_{171}$		5.2 ± 0.2	57.0 ± 0.7	26.0 ± 1.1						
K$_{172}$	K$_{168}$		6.8 ± 0.5	57.2 ± 0.9	24.2 ± 1.2						
K$_{172}$	K$_{172}$		NA	63.5 ± 1.0	22.9 ± 1.4						
K$_{172}$	S$_{171}$		3.8 ± 0.0	63.5 ± 0.8	21.6 ± 1.3						

Double digestion (Glu-C and Asp-N) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Yfh1	**Yfh1**	**Figure**	**Yfh1 intra-monomer**	**Yfh1-Yfh1 intra-trimer**	**Yfh1-Yfh1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
nME	nMS$_{54}$S$_{55}$T$_{56}$DGQVVPQE					5	3	1	1		
N-Term	N-Term	8E	NA	63.4 ± 2.9	20.2 ± 5.7						
N-Term	S$_{54}$		6.4 ± 0.6	60.6 ± 2.4	20.5 ± 4.5						
N-Term	S$_{55}$		8.7 ± 0.6	58.9 ± 2.6	19.5 ± 4.4						
N-Term	T$_{56}$		9.5 ± 1.0	56.3 ± 2.7	19.3 ± 3.9						
nME	LS$_{105}$HGVMT$_{110}$LE					1	1				
N-Term	S$_{105}$		37.5 ± 1.4	63.0 ± 1.6	29.4 ± 2.4						
N-Term	T$_{110}$		39.7 ± 1.6	62.3 ± 1.7	27.6 ± 3.3						
K$_{72}$AHEE	nMS$_{54}$S$_{55}$T$_{56}$DGQVVPQE					23	14	4	2	1	2
K$_{72}$	N-Term		20.7 ± 2.6	44.2 ± 2.2	15.0 ± 2.2						
K$_{72}$	S$_{54}$	8E	17.9 ± 1.9	41.3 ± 1.7	11.8 ± 1.7						
K$_{72}$	S$_{55}$		15.7 ± 1.9	39.4 ± 2.0	11.8 ± 1.7						
K$_{72}$	T$_{56}$		13.4 ± 1.9	36.7 ± 2.1	14.8 ± 1.7						
K$_{72}$AHEE	LS$_{105}$HGVMT$_{110}$LE					2	2				
K$_{72}$	S$_{105}$		26.8 ± 1.3	34.3 ± 1.5	26.3 ± 2.1						
K$_{72}$	T$_{110}$		25.8 ± 1.3	28.6 ± 1.2	30.3 ± 2.2						
K$_{72}$AHEE	VEK$_{168}$AIS$_{171}$K$_{172}$SQ-c					20	7	3	4	1	5
K$_{72}$	K$_{168}$		26.7 ± 1.1	25.8 ± 1.0	34.0 ± 1.3						
K$_{72}$	K$_{172}$		33.0 ± 1.1	30.7 ± 1.2	35.3 ± 1.7						
K$_{72}$	S$_{171}$		30.0 ± 1.1	30.6 ± 1.0	37.5 ± 1.5						
DDY$_{80}$L	WVS$_{151}$LRNGT$_{156}$K$_{157}$LT$_{159}$DILT$_{163}$E					2			1		1
Y$_{80}$	K$_{157}$		11.7 ± 0.9	22.5 ± 0.7	29.8 ± 1.1						
DS$_{87}$LE	K$_{168}$AIS$_{171}$K$_{172}$SQ					2	1				1
S$_{87}$	K$_{168}$		14.8 ± 0.7	40.5 ± 0.7	22.0 ± 1.1						
S$_{87}$	K$_{172}$		20.1 ± 0.8	45.1 ± 1.0	22.1 ± 1.8						
LS$_{92}$E	K$_{72}$AHEE					1			1		
S$_{92}$	K$_{72}$		24.0 ± 0.9	32.5 ± 1.4	28.8 ± 2.9						
ELS$_{92}$E	WVS$_{151}$LRNGT$_{156}$K$_{157}$LT$_{159}$					2			1		1
S$_{92}$	K$_{157}$		19.3 ± 0.7	39.7 ± 0.9	27.7 ± 2.3						
LS$_{92}$E	K$_{168}$AIS$_{171}$K$_{172}$SQ					5	3	1	1		
S$_{92}$	K$_{168}$		12.0 ± 1.5	49.1 ± 0.7	17.2 ± 1.2						
S$_{92}$	K$_{172}$		14.8 ± 1.5	53.4 ± 1.0	15.9 ± 1.8						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Yfh1	**Yfh1**	**Figure**	**Yfh1 intra-monomer**	**Yfh1-Yfh1 intra-trimer**	**Yfh1-Yfh1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
DLLNGEWVS$_{151}$LRNGT$_{156}$K$_{157}$LT$_{159}$	IPAFGT$_{118}$Y$_{119}$VINK$_{123}$QPPNK$_{128}$QIWLAS$_{134}$PLS$_{137}$GPNRF					1			1		
K_{157}	T_{118}		18.8 ± 1.1	33.7 ± 0.8	24.6 ± 1.2						
K_{157}	Y_{119}		16.4 ± 0.9	30.9 ± 0.7	25.6 ± 1.6						
K_{157}	K_{123}		15.5 ± 0.9	27.1 ± 0.7	33.8 ± 1.7						
K_{157}	K_{128}		14.1 ± 0.9	18.4 ± 1.0	36.4 ± 1.6						
K_{157}	S_{134}		14.4 ± 1.3	26.1 ± 0.9	25.3 ± 1.3						
K_{157}	S_{137}		15.3 ± 1.1	23.0 ± 1.2	25.4 ± 1.0						
S_{151}	K_{123}		14.7 ± 0.8	25.1 ± 0.7	35.6 ± 1.5						
S_{151}	K_{128}		10.3 ± 1.0	15.9 ± 0.7	38.5 ± 1.4						
T_{156}	K_{123}		17.9 ± 0.9	28.6 ± 0.9	37.2 ± 1.8						
T_{156}	K_{128}		14.9 ± 0.9	19.7 ± 1.0	40.0 ± 1.5						
T_{159}	K_{123}		14.6 ± 0.9	29.1 ± 0.6	30.0 ± 1.9						
T_{159}	K_{128}		16.5 ± 0.7	20.9 ± 0.9	32.9 ± 1.8						
DILT$_{163}$EE	K$_{168}$AIS$_{171}$K$_{172}$SQ-c					2			1		1
T_{163}	K_{168}		8.6 ± 0.2	43.8 ± 0.7	26.3 ± 1.0						
T_{163}	K_{172}		15.0 ± 0.5	48.9 ± 1.0	24.8 ± 1.4						
VEK$_{168}$AIS$_{171}$K$_{172}$SQ-c	nMS$_{54}$S$_{55}$T$_{56}$DGQVVPQE					23	7	2	11		3
K_{168}	N-Term		42.3 ± 2.5	25.1 ± 2.0	24.5 ± 3.3						
K_{168}	S_{54}		40.3 ± 1.3	23.8 ± 1.3	22.7 ± 2.4						
K_{168}	S_{55}		38.1 ± 1.3	23.6 ± 1.4	24.2 ± 2.7						
K_{168}	T_{56}		37.4 ± 1.1	21.3 ± 1.3	25.6 ± 2.3						
K_{172}	N-Term		47.3 ± 2.5	25.2 ± 2.2	28.9 ± 3.6						
K_{172}	S_{54}		45.5 ± 1.4	24.8 ± 1.4	27.5 ± 2.7						
K_{172}	S_{55}		43.5 ± 1.4	25.4 ± 1.5	29.0 ± 2.9						
K_{172}	T_{56}		43.0 ± 1.3	23.6 ± 1.5	29.9 ± 2.4						
S_{171}	N-Term		43.9 ± 2.6	27.6 ± 2.2	25.3 ± 3.5						
K$_{168}$AIS$_{171}$K$_{172}$SQ-c	LS$_{105}$HGVMT$_{110}$LE					3	1	1	1		
K_{168}	S_{105}		26.2 ± 0.9	46.6 ± 1.2	24.2 ± 1.8						
K_{168}	T_{110}		18.9 ± 1.2	48.4 ± 1.0	24.4 ± 1.4						
K_{172}	T_{110}		29.6 ± 0.9	50.9 ± 1.5	27.2 ± 2.2						
K_{172}	S_{105}		22.3 ± 1.1	53.2 ± 1.2	25.4 ± 2.1						
K$_{168}$AIS$_{171}$K$_{172}$SQ-c	VEK$_{168}$AIS$_{171}$K$_{172}$SQ-c					15	14	1			
K_{168}	K_{168}		NA	52.1 ± 0.7	27.6 ± 0.8						
K_{168}	K_{172}		6.8 ± 0.5	57.2 ± 0.9	24.2 ± 1.2						
K_{168}	S_{171}		5.2 ± 0.2	57.0 ± 0.7	27.5 ± 1.1						
K_{172}	K_{168}		6.8 ± 0.5	57.2 ± 0.9	24.2 ± 1.2						
K_{172}	K_{172}		NA	63.5 ± 1.0	22.9 ± 1.4						
K_{172}	S_{171}		3.8 ± 0.0	63.5 ± 0.8	26.3 ± 1.4						

Supplementary Table S1c

Single digestion (Glu-C) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Isu1	**Isu1**	**Figure**	**Isu1 intra-monomer**	**Isu1-Isu1 intra-trimer**	**Isu1-Isu1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
IAK$_{128}$E	LVQGMT$_{114}$LDDAAK$_{120}$IK$_{122}$NT$_{124}$E					5	5				
K$_{128}$	T$_{114}$		22.7 ± 0.9	36.8 ± 0.8	30.0 ± 1.1						
K$_{128}$	K$_{120}$		15.1 ± 1.2	43.6 ± 1.6	31.8 ± 1.5						
K$_{128}$	K$_{122}$		10.2 ± 0.6	48.9 ± 1.2	31.7 ± 1.0						
K$_{128}$	T$_{124}$		6.2 ± 0.4	51.6 ± 1.4	29.8 ± 1.1						
IAK$_{128}$E	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE					2	2				
K$_{128}$	N-Term	5C, 7G	8.0 ± 1.2	62.3 ± 1.0	24.6 ± 2.2						
K$_{128}$	S$_{25}$		11.0 ± 1.0	65.1 ± 0.9	26.1 ± 2.1						
K$_{128}$	S$_{28}$		16.0 ± 1.2	66.8 ± 1.2	32.7 ± 2.1						
K$_{128}$	S$_{29}$		16.0 ± 1.4	64.7 ± 1.0	33.7 ± 2.5						
K$_{128}$	T$_{31}$		20.5 ± 1.4	66.8 ± 1.9	35.7 ± 2.0						
K$_{128}$	K$_{32}$	5C	21.9 ± 1.5	69.3 ± 1.5	36.8 ± 2.2						
K$_{128}$	Y$_{35}$		25.4 ± 1.0	68.4 ± 1.5	42.3 ± 2.3						
K$_{128}$	K$_{38}$	5C	20.7 ± 0.9	63.2 ± 1.5	39.2 ± 2.0						
IAK$_{128}$E	IAK$_{128}$E					1		1			
K$_{128}$	K$_{128}$	7G	NA	54.7 ± 1.1	27.2 ± 1.3						
LS$_{131}$LPPVK$_{136}$LHCS$_{140}$MLAE	LVQGMT$_{114}$LDDAAK$_{120}$IK$_{122}$NT$_{124}$E					2			2		
K$_{136}$	T$_{114}$		27.7 ± 1.9	31.7 ± 1.4	22.3 ± 1.9						
K$_{136}$	K$_{120}$		16.6 ± 1.8	38.7 ± 1.9	26.6 ± 2.2						
K$_{136}$	K$_{122}$		11.4 ± 1.8	43.3 ± 1.7	28.5 ± 2.3						
K$_{136}$	T$_{124}$		8.7 ± 1.6	45.3 ± 2.0	28.3 ± 2.1						
S$_{131}$	K$_{120}$		20.0 ± 1.3	48.2 ± 1.9	30.1 ± 1.9						
S$_{131}$	K$_{122}$		14.9 ± 1.1	53.4 ± 1.6	29.7 ± 1.5						
S$_{140}$	K$_{120}$		17.9 ± 1.9	41.6 ± 1.5	33.3 ± 1.4						
S$_{140}$	K$_{122}$		12.9 ± 1.7	46.6 ± 1.6	34.9 ± 1.8						

Double digestion (Glu-C and Asp-N) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Isu1	**Isu1**	**Figure**	**Isu1 intra-monomer**	**Isu1-Isu1 intra-trimer**	**Isu1-Isu1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
DVMRLQIK$_{78}$VN	DVMRLQIK$_{78}$VNDS$_{82}$T$_{83}$GVIE					1			1		
K$_{78}$	K$_{78}$		NA	30.1 ± 0.5	59.9 ± 0.9						
K$_{78}$	S$_{82}$	7H	12.4 ± 0.2	18.4 ± 0.4	57.2 ± 0.7						
K$_{78}$	T$_{83}$		12.6 ± 0.7	20.4 ± 0.5	54.2 ± 1.0						
IAK$_{128}$E	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE					6			2		4
K$_{128}$	N-Term	5C, 7G	8.0 ± 1.2	62.3 ± 1.0	24.6 ± 2.2						
K$_{128}$	S$_{25}$		11.0 ± 1.0	65.1 ± 0.9	26.1 ± 2.1						
K$_{128}$	S$_{28}$		16.0 ± 1.2	66.8 ± 1.2	32.7 ± 2.1						
K$_{128}$	S$_{29}$		16.0 ± 1.4	64.7 ± 1.0	33.7 ± 2.5						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Isu1	Isu1	Figure	Isu1 intra-monomer	Isu1-Isu1 intra-trimer	Isu1-Isu1 inter-trimers	# of Peptides	≤5%	≤10%	≤20%	≤25%	Below 25%
K_{128}	T_{31}		20.5 ± 1.4	66.8 ± 1.9	35.7 ± 2.0						
K_{128}	K_{32}	5C	21.9 ± 1.5	69.3 ± 1.5	36.8 ± 2.2						
K_{128}	Y_{35}		25.4 ± 1.0	68.4 ± 1.5	42.3 ± 2.3						
K_{128}	K_{38}	5C	20.7 ± 0.9	63.2 ± 1.5	39.2 ± 2.0						
DAIK$_{148}$AAIK$_{152}$	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIEHY$_{43}$T$_{44}$HPRNVGS$_{51}$L					2				1	1
K_{148}	N-Term		25.8 ± 1.3	45.8 ± 1.1	42.7 ± 1.6						
K_{148}	S_{25}		27.6 ± 0.9	47.5 ± 0.9	44.8 ± 1.4						
K_{148}	S_{28}		27.8 ± 0.9	49.0 ± 1.0	46.8 ± 1.1						
K_{148}	S_{29}		26.0 ± 0.8	48.9 ± 1.1	44.4 ± 1.4						
K_{148}	T_{31}		28.9 ± 1.5	53.1 ± 1.5	45.5 ± 1.9						
K_{148}	K_{32}		30.8 ± 1.4	53.6 ± 2.1	48.4 ± 1.6						
K_{148}	Y_{35}		30.3 ± 1.1	53.7 ± 1.6	48.7 ± 1.8						
K_{148}	K_{38}		25.2 ± 1.3	50.2 ± 1.4	43.8 ± 2.2						
K_{148}	Y_{43}		25.8 ± 1.8	48.6 ± 1.2	48.5 ± 2.2						
K_{148}	T_{44}		26.6 ± 1.3	50.7 ± 1.0	47.4 ± 1.8						
K_{148}	S_{51}		30.5 ± 1.2	56.7 ± 0.9	45.5 ± 2.2						
K_{152}	N-Term		31.6 ± 1.2	42.9 ± 1.1	42.6 ± 1.6						
K_{152}	S_{25}		33.3 ± 0.9	44.5 ± 0.9	44.5 ± 1.3						
K_{152}	S_{28}		33.0 ± 0.8	45.3 ± 0.9	46.7 ± 1.0						
K_{152}	S_{29}		31.2 ± 0.7	44.9 ± 1.0	44.4 ± 1.4						
K_{152}	T_{31}		33.9 ± 1.4	48.8 ± 1.4	45.3 ± 1.9						
K_{152}	K_{32}		35.6 ± 1.4	49.5 ± 2.0	48.1 ± 1.6						
K_{152}	Y_{35}		34.4 ± 1.1	49.1 ± 1.4	48.9 ± 1.8						
K_{152}	K_{38}		29.7 ± 1.3	45.5 ± 1.4	44.2 ± 2.2						
K_{152}	Y_{43}		28.7 ± 1.7	43.5 ± 1.2	49.9 ± 2.0						
K_{152}	T_{44}		29.3 ± 1.2	45.4 ± 0.9	48.8 ± 1.6						
K_{152}	S_{51}		32.2 ± 1.0	50.9 ± 0.9	47.3 ± 2.1						
LVQGMT$_{114}$LD	DAAK$_{120}$IK$_{122}$NT$_{124}$EIAK$_{128}$E					67			11	5	51
T_{114}	K_{120}		12.0 ± 0.6	23.9 ± 1.4	38.6 ± 1.4						
T_{114}	K_{122}		16.6 ± 1.0	27.0 ± 1.0	33.3 ± 1.5						
T_{114}	K_{128}		22.7 ± 0.9	36.8 ± 0.8	30.0 ± 1.1						
DAAK$_{120}$IK$_{122}$NT$_{124}$E	DAAK$_{120}$IK$_{122}$NT$_{124}$E					1		1			
K_{120}	K_{120}		NA	30.1 ± 1.5	41.7 ± 1.5						
K_{120}	K_{122}		6.3 ± 0.4	33.9 ± 1.9	36.7 ± 1.4						
K_{120}	T_{124}		10.6 ± 1.2	37.8 ± 1.7	32.4 ± 1.5						
K_{122}	K_{120}		6.3 ± 0.4	33.9 ± 1.9	36.7 ± 1.4						
K_{122}	K_{122}		NA	39.3 ± 1.8	37.9 ± 1.4						
K_{122}	T_{124}		5.3 ± 0.3	43.0 ± 1.4	33.2 ± 1.6						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Isu1	**Isu1**	**Figure**	**Isu1 intra-monomer**	**Isu1-Isu1 intra-trimer**	**Isu1-Isu1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
DAAK$_{120}$IK$_{122}$NT$_{124}$E	IAK$_{128}$ELS$_{131}$LPPVK$_{136}$LHCS$_{140}$MLAE					2			1		1
K_{120}	K_{128}		15.1 ± 1.2	43.6 ± 1.6	31.8 ± 1.5						
K_{120}	S_{131}		20.0 ± 1.3	48.2 ± 1.9	30.1 ± 1.9						
K_{120}	K_{136}	7G	16.6 ± 1.8	38.7 ± 1.9	26.6 ± 2.2						
K_{120}	S_{140}		17.9 ± 1.9	41.6 ± 1.5	33.3 ± 1.4						
K_{122}	K_{128}		10.2 ± 0.6	48.9 ± 1.2	31.7 ± 1.0						
K_{122}	S_{131}		14.9 ± 1.1	53.4 ± 1.6	29.7 ± 1.5						
K_{122}	K_{136}	7G	11.4 ± 1.8	43.3 ± 1.7	28.5 ± 2.3						
K_{122}	S_{140}		12.9 ± 1.7	46.6 ± 1.6	34.9 ± 1.8						
T_{124}	K_{128}		6.2 ± 0.4	51.6 ± 1.4	29.8 ± 1.1						
T_{124}	K_{136}		8.7 ± 1.6	45.3 ± 2.0	28.3 ± 2.1						
DAAK$_{120}$IK$_{122}$NT$_{124}$EIAK$_{128}$E	LS$_{131}$LPPVK$_{136}$LHCS$_{140}$MLAE					15				3	12
K_{120}	S_{131}		20.0 ± 1.3	48.2 ± 1.9	30.1 ± 1.9						
K_{120}	K_{136}	7G	16.6 ± 1.8	38.7 ± 1.9	26.6 ± 2.2						
K_{120}	S_{140}		17.9 ± 1.9	41.6 ± 1.5	33.3 ± 1.4						
K_{122}	S_{131}		14.9 ± 1.1	53.4 ± 1.6	29.7 ± 1.5						
K_{122}	K_{136}	7G	11.4 ± 1.8	43.3 ± 1.7	28.5 ± 2.3						
K_{122}	S_{140}		12.9 ± 1.7	46.6 ± 1.6	34.9 ± 1.8						
K_{128}	S_{131}		5.7 ± 0.7	58.8 ± 1.5	24.7 ± 1.9						
K_{128}	K_{136}		11.4 ± 1.5	47.5 ± 1.3	27.7 ± 2.4						
K_{128}	S_{140}		12.8 ± 1.1	51.2 ± 1.4	32.9 ± 1.8						
DAAK$_{120}$IK$_{122}$NT$_{124}$EIAK$_{128}$E	DK$_{54}$K$_{55}$LPNVGT$_{61}$GLVGAPACG					1			1		
K_{120}	K_{54}	5C	28.7 ± 1.3	44.7 ± 1.1	54.3 ± 1.2						
K_{120}	K_{55}		30.3 ± 1.2	45.1 ± 1.0	57.7 ± 1.3						
K_{120}	T_{61}		16.9 ± 1.1	32.2 ± 0.8	52.1 ± 1.1						
K_{122}	K_{54}		27.8 ± 1.4	49.7 ± 1.3	56.2 ± 1.8						
K_{122}	K_{55}		30.0 ± 1.6	50.1 ± 1.2	59.6 ± 1.9						
K_{122}	T_{61}		18.6 ± 0.8	37.7 ± 1.1	54.0 ± 1.4						
K_{128}	K_{54}		31.1 ± 1.1	54.9 ± 1.2	53.8 ± 1.7						
K_{128}	K_{55}		33.8 ± 1.4	55.5 ± 0.9	57.1 ± 1.7						
K_{128}	T_{61}		25.7 ± 0.6	44.4 ± 0.8	51.7 ± 1.4						
T_{124}	K_{54}		30.6 ± 1.2	53.4 ± 1.3	56.2 ± 1.7						
T_{124}	K_{55}		33.1 ± 1.4	53.9 ± 1.2	59.5 ± 1.8						
DDAAK$_{120}$IK$_{122}$NT$_{124}$EIAK$_{128}$E	HY$_{43}$T$_{44}$HPRNVGS$_{51}$LDK$_{54}$K$_{55}$LPNVGT$_{61}$GLVGAPAC					1				1	
K_{120}	Y_{43}		29.5 ± 2.2	52.1 ± 1.8	51.4 ± 1.7						
K_{120}	T_{44}		31.0 ± 1.7	52.1 ± 1.4	51.8 ± 1.6						
K_{120}	S_{51}		36.7 ± 1.8	51.7 ± 1.4	54.7 ± 1.9						
K_{120}	K_{54}	5C	28.7 ± 1.3	44.7 ± 1.1	54.3 ± 1.2						
K_{120}	K_{55}		30.3 ± 1.2	45.1 ± 1.0	57.7 ± 1.3						
K_{120}	T_{61}		16.9 ± 1.1	32.2 ± 0.8	52.1 ± 1.1						
K_{122}	Y_{43}		27.4 ± 2.5	57.5 ± 1.8	52.1 ± 2.3						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Isu1	**Isu1**	**Figure**	**Isu1 intra-monomer**	**Isu1-Isu1 intra-trimer**	**Isu1-Isu1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
K_{122}	T_{44}		28.9 ± 2.0	57.3 ± 1.4	52.9 ± 2.1						
K_{122}	S_{51}		34.6 ± 1.5	56.3 ± 1.7	56.9 ± 2.4						
K_{122}	K_{54}		27.8 ± 1.4	49.7 ± 1.3	56.2 ± 1.8						
K_{122}	K_{55}		30.0 ± 1.6	50.1 ± 1.2	59.6 ± 1.9						
K_{122}	T_{61}		18.6 ± 0.8	37.7 ± 1.1	54.0 ± 1.4						
K_{128}	Y_{43}		26.9 ± 1.5	63.3 ± 1.9	48.0 ± 2.2						
K_{128}	T_{44}		29.0 ± 1.2	62.6 ± 1.5	49.5 ± 2.0						
K_{128}	S_{51}		36.4 ± 1.3	60.1 ± 1.3	55.3 ± 2.5						
K_{128}	K_{54}		31.1 ± 1.1	54.9 ± 1.2	53.8 ± 1.7						
K_{128}	K_{55}		33.8 ± 1.4	55.5 ± 0.9	57.1 ± 1.7						
K_{128}	T_{61}		25.7 ± 0.6	44.4 ± 0.8	51.7 ± 1.4						
DAAK$_{120}$IK$_{122}$NT$_{124}$E	DAIK$_{148}$AAIK$_{152}$DY$_{154}$K$_{155}$S$_{156}$K$_{157}$RNT$_{160}$PT$_{162}$MLS$_{165}$-c					17	6	2	5		4
K_{120}	K_{148}		8.1 ± 1.2	28.0 ± 1.2	40.0 ± 1.1						
K_{120}	K_{152}		12.0 ± 1.1	24.2 ± 1.1	42.8 ± 1.0						
K_{120}	Y_{154}		14.9 ± 1.0	26.8 ± 1.0	48.1 ± 1.0						
K_{120}	K_{155}	7H	14.8 ± 1.0	23.2 ± 1.1	47.0 ± 1.0						
K_{120}	S_{156}		17.8 ± 1.1	22.2 ± 0.9	46.7 ± 1.2						
K_{120}	K_{157}		19.7 ± 1.0	25.2 ± 0.9	50.2 ± 1.2						
K_{120}	T_{160}		18.6 ± 1.2	18.8 ± 1.1	47.8 ± 1.3						
K_{120}	T_{162}		18.2 ± 1.3	14.3 ± 1.0	43.6 ± 1.4						
K_{120}	S_{165}		15.4 ± 1.6	15.6 ± 1.3	38.5 ± 1.1						
K_{122}	K_{148}		10.1 ± 0.7	33.6 ± 1.2	36.8 ± 1.7						
K_{122}	K_{152}		15.6 ± 0.7	29.9 ± 1.1	38.9 ± 1.6						
K_{122}	Y_{154}		18.9 ± 0.9	32.5 ± 1.1	44.2 ± 1.6						
K_{122}	K_{155}		19.4 ± 0.8	29.0 ± 1.1	42.9 ± 1.6						
K_{122}	S_{156}		21.9 ± 0.7	27.7 ± 1.1	42.3 ± 1.6						
K_{122}	K_{157}		23.8 ± 0.8	30.6 ± 1.0	45.8 ± 1.6						
K_{122}	T_{160}		23.6 ± 0.9	24.4 ± 1.2	43.4 ± 1.9						
K_{122}	T_{162}		22.9 ± 1.1	20.1 ± 1.0	39.2 ± 2.1						
K_{122}	S_{165}		19.2 ± 1.4	21.3 ± 1.3	34.3 ± 1.8						
T_{124}	K_{148}		14.9 ± 0.8	37.2 ± 1.5	35.7 ± 2.0						
T_{124}	K_{152}		20.7 ± 0.8	34.0 ± 1.4	37.1 ± 1.8						
T_{124}	K_{155}		24.5 ± 0.9	33.5 ± 1.4	40.6 ± 1.8						
T_{124}	K_{157}		28.8 ± 0.9	35.2 ± 1.3	43.5 ± 1.6						
IAK$_{128}$E	IAK$_{128}$E					1			1		
K_{128}	K_{128}		NA	54.7 ± 1.1	27.2 ± 1.3						
IAK$_{128}$E	DAAK$_{120}$IK$_{122}$NT$_{124}$E					8	4		2		1
K_{128}	K_{120}		15.1 ± 1.2	43.6 ± 1.6	31.8 ± 1.5						
K_{128}	K_{122}		10.2 ± 0.6	48.9 ± 1.2	31.7 ± 1.0						
K_{128}	T_{124}		6.2 ± 0.4	51.6 ± 1.4	29.8 ± 1.1						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Isu1	Isu1	Figure	Isu1 intra-monomer	Isu1-Isu1 intra-trimer	Isu1-Isu1 inter-trimers	# of Peptides	≤5%	≤10%	≤20%	≤25%	Below 25%
$\mathrm{IAK_{128}E}$	$\mathrm{DAIK_{148}AAIK_{152}DY_{154}K_{155}S_{156}K_{157}RNT_{160}PT_{162}MLS_{165}\text{-}c}$					9			3		6
K_{128}	K_{148}		19.2 ± 0.8	40.3 ± 0.9	38.3 ± 1.6						
K_{128}	K_{152}		25.0 ± 0.7	37.2 ± 0.9	38.9 ± 1.3						
K_{128}	Y_{154}		27.5 ± 0.7	40.3 ± 0.9	43.9 ± 1.1						
K_{128}	K_{155}		28.7 ± 0.7	37.1 ± 1.0	42.1 ± 1.2						
K_{128}	S_{156}		31.2 ± 0.7	35.5 ± 0.9	40.9 ± 1.0						
K_{128}	K_{157}		32.6 ± 0.7	38.5 ± 0.9	44.4 ± 0.9						
K_{128}	T_{160}		33.2 ± 0.9	33.1 ± 1.2	41.5 ± 1.2						
K_{128}	T_{162}		32.8 ± 1.0	28.7 ± 1.1	37.1 ± 1.0						
K_{128}	S_{165}		29.2 ± 1.1	28.6 ± 1.0	33.1 ± 1.4						
$\mathrm{LS_{131}LPPVK_{136}LHCS_{140}MLAE}$	$\mathrm{DAIK_{148}AAIK_{152}DY_{154}K_{155}S_{156}K_{157}RNT_{160}PT_{162}MLS_{165}\text{-}c}$					10					10
K_{136}	K_{148}	7G	18.1 ± 1.8	44.2 ± 1.8	27.4 ± 2.7						
K_{136}	K_{152}		23.9 ± 1.8	40.6 ± 1.7	28.6 ± 2.6						
K_{136}	Y_{154}		28.0 ± 1.8	43.0 ± 1.8	33.7 ± 2.5						
K_{136}	K_{155}		28.5 ± 1.8	39.4 ± 1.8	32.2 ± 2.7						
K_{136}	S_{156}		30.1 ± 1.7	38.4 ± 1.9	31.4 ± 2.4						
K_{136}	K_{157}		32.5 ± 1.8	41.1 ± 2.0	35.0 ± 2.3						
K_{136}	T_{160}		32.3 ± 1.6	34.5 ± 1.9	32.5 ± 2.7						
K_{136}	T_{162}		30.6 ± 1.4	30.2 ± 1.7	28.4 ± 2.7						
K_{136}	S_{165}		25.7 ± 1.5	31.7 ± 1.7	23.7 ± 2.7						
S_{131}	K_{148}		23.0 ± 1.4	45.3 ± 1.1	38.0 ± 2.0						
S_{131}	K_{152}		29.0 ± 1.4	42.1 ± 1.1	37.9 ± 1.7						
S_{131}	K_{155}		32.9 ± 1.3	41.9 ± 1.3	40.8 ± 1.6						
S_{131}	K_{157}		36.4 ± 1.3	42.9 ± 1.3	42.5 ± 1.4						
S_{140}	K_{148}		16.4 ± 0.8	45.0 ± 1.6	32.0 ± 1.4						
S_{140}	K_{152}		22.1 ± 0.9	40.6 ± 1.4	33.1 ± 1.1						
S_{140}	K_{155}		26.8 ± 0.9	38.9 ± 1.3	37.0 ± 1.1						
S_{140}	K_{157}		29.5 ± 0.7	39.2 ± 1.0	38.6 ± 1.0						
$\mathrm{DY_{154}K_{155}S_{156}K_{157}RNT_{160}PT_{162}MLS_{165}\text{-}c}$	$\mathrm{LS_{131}LPPVK_{136}LHCS_{140}MLAEDAIK_{148}AAIK_{152}}$					1			1		
K_{155}	S_{131}		32.9 ± 1.3	41.9 ± 1.3	42.0 ± 1.5						
K_{155}	K_{136}		28.5 ± 1.8	39.4 ± 1.8	32.5 ± 1.4						
K_{155}	S_{140}		26.8 ± 0.9	38.9 ± 1.3	37.0 ± 1.1						
K_{155}	K_{148}		10.8 ± 0.2	23.3 ± 0.6	46.2 ± 0.8						
K_{155}	K_{152}	7H	5.1 ± 0.1	19.0 ± 0.5	50.3 ± 0.7						
K_{157}	S_{131}		36.4 ± 1.3	42.9 ± 1.3	43.7 ± 1.1						
K_{157}	K_{136}		32.5 ± 1.8	41.1 ± 2.0	35.3 ± 1.4						
K_{157}	S_{140}		29.5 ± 0.7	39.2 ± 1.0	38.6 ± 1.1						
K_{157}	K_{148}		14.5 ± 0.2	24.0 ± 0.5	49.0 ± 0.7						
K_{157}	K_{152}		8.9 ± 0.1	19.3 ± 0.5	53.1 ± 0.7						
Y_{154}	K_{136}		28.0 ± 1.8	43.0 ± 1.8	34.0 ± 1.4						
S_{156}	K_{136}		30.1 ± 1.7	38.4 ± 1.9	31.7 ± 1.4						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Isu1	Isu1	Figure	Isu1 intra-monomer	Isu1-Isu1 intra-trimer	Isu1-Isu1 inter-trimers	# of Peptides	≤5%	≤10%	≤20%	≤25%	Below 25%
T_{160}	K_{136}		32.3 ± 1.6	34.5 ± 1.9	32.9 ± 1.4						
T_{162}	K_{136}		30.6 ± 1.4	30.2 ± 1.7	28.7 ± 1.6						
S_{165}	K_{136}		25.7 ± 1.5	31.7 ± 1.7	24.1 ± 1.4						
Y_{154}	K_{148}		10.0 ± 0.1	26.8 ± 0.5	46.6 ± 0.9						
S_{156}	K_{148}		12.4 ± 0.2	22.0 ± 0.5	45.9 ± 0.7						
T_{160}	K_{148}		15.5 ± 0.4	18.0 ± 0.6	48.2 ± 0.8						
T_{162}	K_{148}		15.9 ± 0.5	15.4 ± 0.8	44.7 ± 0.9						
S_{165}	K_{148}		12.6 ± 0.7	18.9 ± 1.1	39.5 ± 1.0						
Y_{154}	K_{152}		5.4 ± 0.1	22.4 ± 0.5	50.4 ± 0.7						
S_{156}	K_{152}		6.4 ± 0.2	17.9 ± 0.5	50.1 ± 0.8						
T_{160}	K_{152}		9.8 ± 0.3	13.4 ± 0.6	52.6 ± 0.8						
T_{162}	K_{152}	7H	11.3 ± 0.4	12.4 ± 0.8	49.4 ± 1.0						
S_{165}	K_{152}		9.5 ± 0.6	17.0 ± 1.0	44.0 ± 1.0						
$\text{DAIK}_{148}\text{AAIK}_{152}$	$\text{DAAK}_{120}\text{IK}_{122}\text{NT}_{124}\text{E}$					17	8		1		8
K_{148}	K_{120}		8.1 ± 1.2	28.0 ± 1.2	39.9 ± 1.3						
K_{148}	K_{122}		10.1 ± 0.7	33.6 ± 1.2	36.0 ± 1.3						
K_{148}	T_{124}		14.9 ± 0.8	37.2 ± 1.5	35.2 ± 1.2						
K_{152}	K_{120}		12.0 ± 1.1	24.2 ± 1.1	42.8 ± 1.2						
K_{152}	K_{122}		15.6 ± 0.7	29.9 ± 1.1	38.4 ± 1.1						
K_{152}	T_{124}		20.7 ± 0.8	34.0 ± 1.4	36.8 ± 1.0						
$\text{DAIK}_{148}\text{AAIK}_{152}$	$\text{DK}_{54}\text{K}_{55}\text{LPNVGT}_{61}\text{GLVGAPACG}$					1			1		
K_{148}	K_{54}		22.4 ± 1.2	46.7 ± 0.9	45.9 ± 1.2						
K_{148}	K_{55}		23.9 ± 1.1	46.2 ± 0.8	48.9 ± 1.3						
K_{148}	T_{61}		10.2 ± 0.6	32.7 ± 0.6	44.2 ± 1.3						
K_{152}	K_{54}		23.5 ± 1.1	41.0 ± 0.9	48.3 ± 1.0						
K_{152}	K_{55}		24.2 ± 0.9	40.3 ± 0.8	51.6 ± 1.0						
K_{152}	T_{61}		9.2 ± 0.5	26.9 ± 0.6	47.7 ± 1.0						
$\text{DAIK}_{148}\text{AAIK}_{152}$	$\text{DVK}_{90}\text{FK}_{92}\text{T}_{93}\text{FGCGS}_{98}\text{AIAS}_{102}\text{S}_{103}\text{S}_{104}\text{Y}_{105}\text{MT}_{107}\text{E}$					3			1		2
K_{148}	K_{90}		14.9 ± 0.7	33.9 ± 0.7	48.7 ± 1.0						
K_{148}	K_{92}		15.6 ± 0.6	40.0 ± 0.6	43.9 ± 1.0						
K_{148}	T_{93}		16.3 ± 0.4	42.2 ± 0.6	41.4 ± 1.0						
K_{148}	S_{98}		21.3 ± 0.8	45.7 ± 0.9	40.1 ± 0.9						
K_{148}	S_{102}		16.1 ± 1.1	38.5 ± 0.9	42.2 ± 0.9						
K_{148}	S_{103}		14.2 ± 0.9	36.9 ± 0.8	43.1 ± 0.7						
K_{148}	S_{104}		17.1 ± 1.0	36.7 ± 0.8	46.7 ± 0.7						
K_{148}	Y_{105}		17.3 ± 1.1	35.0 ± 0.8	46.9 ± 0.8						
K_{148}	T_{107}		14.0 ± 1.1	31.4 ± 0.8	47.5 ± 0.8						
K_{152}	K_{90}		17.2 ± 0.5	28.9 ± 0.5	51.1 ± 0.7						
K_{152}	K_{92}		19.0 ± 0.4	34.9 ± 0.4	45.9 ± 0.7						
K_{152}	T_{93}		20.6 ± 0.3	37.2 ± 0.5	43.1 ± 0.7						
K_{152}	S_{98}		26.8 ± 0.7	41.7 ± 0.8	40.6 ± 0.8						

Cross-linked peptides			Distance (Å)			False Discovery Rate (FDR)					
Isu1	**Isu1**	**Figure**	**Isu1 intra-monomer**	**Isu1-Isu1 intra-trimer**	**Isu1-Isu1 inter-trimers**	**# of Peptides**	**≤5%**	**≤10%**	**≤20%**	**≤25%**	**Below 25%**
K_{152}	S_{102}		21.4 ± 0.9	34.6 ± 0.8	43.5 ± 0.7						
K_{152}	S_{103}		19.0 ± 0.7	32.6 ± 0.7	44.8 ± 0.5						
K_{152}	S_{104}		21.4 ± 0.7	32.6 ± 0.7	48.3 ± 0.5						
K_{152}	Y_{105}		21.8 ± 0.8	31.3 ± 0.7	48.4 ± 0.6						
K_{152}	T_{107}		17.4 ± 0.8	27.2 ± 0.6	49.6 ± 0.6						
$DAIK_{148}AAIK_{152}$	$DY_{154}K_{155}S_{156}K_{157}RNT_{160}PT_{162}MLS_{165}$-c					16			2		14
K_{148}	Y_{154}		10.0 ± 0.1	26.8 ± 0.5	46.5 ± 0.9						
K_{148}	K_{155}		10.8 ± 0.2	23.3 ± 0.6	46.0 ± 0.9						
K_{148}	S_{156}		12.4 ± 0.2	22.0 ± 0.5	45.6 ± 1.0						
K_{148}	K_{157}		14.5 ± 0.2	24.0 ± 0.5	48.8 ± 1.0						
K_{148}	T_{160}		15.5 ± 0.4	18.0 ± 0.6	47.7 ± 1.0						
K_{148}	T_{162}		15.9 ± 0.5	15.4 ± 0.8	44.2 ± 1.1						
K_{148}	S_{165}		12.6 ± 0.7	18.9 ± 1.1	38.9 ± 1.0						
K_{152}	Y_{154}		5.4 ± 0.1	22.4 ± 0.5	50.4 ± 0.7						
K_{152}	K_{155}		5.1 ± 0.1	19.0 ± 0.5	50.2 ± 0.7						
K_{152}	S_{156}		6.4 ± 0.2	17.9 ± 0.5	50.0 ± 0.8						
K_{152}	K_{157}		8.9 ± 0.1	19.3 ± 0.5	53.2 ± 0.8						
K_{152}	T_{160}		9.8 ± 0.3	13.4 ± 0.6	52.3 ± 0.9						
K_{152}	T_{162}		11.3 ± 0.4	12.4 ± 0.8	49.0 ± 1.0						
K_{152}	S_{165}		9.5 ± 0.6	17.0 ± 1.0	43.5 ± 0.9						

Supplementary Table S1d. Alternative N-terminus #1

Single digestion (Glu-C) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)		
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**
n-ME	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
N-Term	N-Term		55.0 ± 0.0	26.9 ± 0.6	18.8 ± 0.9
N-Term	S$_{25}$		58.5 ± 0.0	27.9 ± 0.8	16.1 ± 1.0
N-Term	S$_{28}$		64.9 ± 0.0	34.3 ± 0.8	16.2 ± 1.2
N-Term	S$_{29}$		63.6 ± 0.0	36.8 ± 0.7	17.0 ± 1.2
N-Term	T$_{31}$		66.5 ± 0.0	40.0 ± 0.6	21.0 ± 1.2
N-Term	K$_{32}$		68.2 ± 0.0	38.8 ± 0.5	23.0 ± 1.3
N-Term	Y$_{35}$		71.8 ± 0.0	43.9 ± 0.6	23.6 ± 1.3
N-Term	K$_{38}$		68.9 ± 0.0	43.6 ± 0.7	19.4 ± 1.3
n-MES$_{54}$S$_{55}$T$_{56}$DGQVVPQE	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
S$_{54}$	K$_{32}$		69.8 ± 0.0	42.5 ± 0.7	24.8 ± 1.3
S$_{55}$	K$_{32}$		67.7 ± 0.0	41.3 ± 0.7	27.2 ± 1.3
T$_{56}$	K$_{32}$		64.6 ± 0.0	38.1 ± 0.7	28.2 ± 1.3
S$_{54}$	K$_{38}$		70.2 ± 0.0	36.7 ± 0.8	22.4 ± 1.4
S$_{55}$	K$_{38}$		68.0 ± 0.0	35.8 ± 0.7	24.4 ± 1.3
T$_{56}$	K$_{38}$		64.9 ± 0.0	32.8 ± 0.7	24.4 ± 1.3

Double digestion (Glu-C and Asp-N) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)		
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**
nME	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
N-Term	N-Term		55.0 ± 0.0	26.9 ± 0.6	18.8 ± 0.9
N-Term	S$_{25}$		58.5 ± 0.0	27.9 ± 0.8	16.1 ± 1.0
N-Term	S$_{28}$		64.9 ± 0.0	34.3 ± 0.8	16.2 ± 1.2
N-Term	S$_{29}$		63.6 ± 0.0	36.8 ± 0.7	17.0 ± 1.2
N-Term	T$_{31}$		66.5 ± 0.0	40.0 ± 0.6	21.0 ± 1.2
N-Term	K$_{32}$		68.2 ± 0.0	38.8 ± 0.5	23.0 ± 1.3
N-Term	Y$_{35}$		71.8 ± 0.0	43.9 ± 0.6	23.6 ± 1.3
N-Term	K$_{38}$		68.9 ± 0.0	43.6 ± 0.7	19.4 ± 1.3
nME	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIEHY$_{43}$T$_{44}$HPRNVGS$_{51}$LDK$_{54}$K$_{55}$LPNVGTGLVGAPACG				
N-Term	N-Term		55.0 ± 0.0	34.1 ± 0.4	18.8 ± 0.9
N-Term	S$_{25}$		58.5 ± 0.0	34.3 ± 0.4	16.1 ± 1.0
N-Term	S$_{28}$		64.9 ± 0.0	35.1 ± 0.6	16.2 ± 1.2
N-Term	S$_{29}$		63.6 ± 0.0	32.6 ± 0.6	17.0 ± 1.2
N-Term	T$_{31}$		66.5 ± 0.0	35.2 ± 0.7	21.0 ± 1.2
N-Term	K$_{32}$		68.2 ± 0.0	39.0 ± 0.7	23.0 ± 1.3
N-Term	Y$_{35}$		71.8 ± 0.0	38.4 ± 0.8	23.6 ± 1.3
N-Term	K$_{38}$		68.9 ± 0.0	32.9 ± 0.8	19.4 ± 1.3
N-Term	Y$_{43}$		75.6 ± 0.0	33.9 ± 0.9	22.9 ± 1.3
N-Term	T$_{44}$		74.5 ± 0.0	32.6 ± 0.9	25.0 ± 1.3
N-Term	S$_{51}$		75.2 ± 0.0	32.9 ± 0.9	38.6 ± 1.3
N-Term	K$_{54}$		65.7 ± 0.0	30.1 ± 0.7	36.8 ± 1.2
N-Term	K$_{55}$		63.6 ± 0.0	31.6 ± 0.6	39.1 ± 1.2
N-Term	T$_{61}$		47.9 ± 0.0	23.3 ± 0.3	32.7 ± 0.9
nMES$_{54}$S$_{55}$T$_{56}$	DVMRLQIK$_{78}$VN				
N-Term	K$_{78}$		40.4 ± 0.0	29.1 ± 0.1	30.4 ± 0.5
S$_{54}$	K$_{78}$		43.2 ± 0.0	32.5 ± 0.1	28.7 ± 0.6
S$_{55}$	K$_{78}$		41.4 ± 0.0	31.9 ± 0.1	30.3 ± 0.7
T$_{56}$	K$_{78}$		37.8 ± 0.0	28.6 ± 0.1	32.9 ± 0.6
nMES$_{54}$S$_{55}$T$_{56}$DGQVVPQE	HY$_{43}$T$_{44}$HPRNVGS$_{51}$LDK$_{54}$K$_{55}$LPNVGTGLVGAPACG				
N-Term	Y$_{43}$		75.6 ± 0.0	33.9 ± 0.9	22.9 ± 1.3
N-Term	T$_{44}$		74.5 ± 0.0	32.6 ± 0.9	25.0 ± 1.3
N-Term	S$_{51}$		75.2 ± 0.0	32.9 ± 0.9	38.6 ± 1.3
N-Term	K$_{54}$		65.7 ± 0.0	30.1 ± 0.7	36.8 ± 1.2
N-Term	K$_{55}$		63.6 ± 0.0	31.6 ± 0.6	39.1 ± 1.2
N-Term	T$_{61}$		47.9 ± 0.0	23.3 ± 0.3	32.7 ± 0.9
S$_{54}$	K$_{54}$		68.2 ± 0.0	35.6 ± 0.7	39.3 ± 1.3
S$_{55}$	K$_{54}$		66.3 ± 0.0	36.1 ± 0.7	40.0 ± 1.3
T$_{56}$	K$_{54}$		62.8 ± 0.0	33.0 ± 0.7	39.3 ± 1.2
S$_{54}$	K$_{55}$		66.3 ± 0.0	37.1 ± 0.6	41.2 ± 1.2
S$_{55}$	K$_{55}$		64.5 ± 0.0	37.5 ± 0.7	41.9 ± 1.2
T$_{56}$	K$_{55}$		60.9 ± 0.0	34.3 ± 0.7	41.5 ± 1.1

Cross-linked peptides		Figure	Distance (Å)		
Yfh1	**Isu1**		**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**
$\mathrm{K_{72}AHEE}$	$\mathrm{n\text{-}GS_{25}HMS_{28}S_{29}IT_{31}K_{32}RLY_{35}HPK_{38}VIE}$				
K_{72}	N-Term	5D	36.9 ± 0.0	14.2 ± 0.4	37.0 ± 1.1
K_{72}	S_{25}		40.1 ± 0.0	15.8 ± 0.3	35.7 ± 1.2
K_{72}	S_{28}		45.8 ± 0.0	21.0 ± 0.4	36.8 ± 1.2
K_{72}	S_{29}		44.0 ± 0.0	19.7 ± 0.5	36.0 ± 1.1
K_{72}	T_{31}		47.1 ± 0.0	24.1 ± 0.5	39.6 ± 1.1
K_{72}	K_{32}	5D	49.4 ± 0.0	26.8 ± 0.5	42.7 ± 1.2
K_{72}	Y_{35}		52.3 ± 0.0	29.2 ± 0.5	42.1 ± 1.2
K_{72}	K_{38}	5D	48.6 ± 0.0	24.8 ± 0.5	36.8 ± 1.1
$\mathrm{K_{72}AHEE}$	$\mathrm{DVMRLQIK_{78}VNDS_{82}T_{83}GVIE}$				
K_{72}	K_{78}		21.6 ± 0.0	25.0 ± 0.3	47.3 ± 0.8
K_{72}	S_{82}		15.4 ± 0.0	26.6 ± 0.3	41.2 ± 0.7
K_{72}	T_{83}		12.1 ± 0.0	26.3 ± 0.3	38.1 ± 0.7
$\mathrm{K_{72}AHEE}$	$\mathrm{DK_{54}K_{55}LPNVGT_{61}GLVGAPACGDVMRLQIK_{78}VN}$				
K_{72}	K_{54}		45.6 ± 0.0	32.8 ± 0.4	43.8 ± 0.9
K_{72}	K_{55}		44.1 ± 0.0	33.5 ± 0.4	46.2 ± 0.9
K_{72}	T_{61}		27.2 ± 0.0	19.7 ± 0.3	38.6 ± 0.7
K_{72}	K_{78}		21.6 ± 0.0	25.0 ± 0.3	45.3 ± 0.6
$\mathrm{DS_{87}LE}$	$\mathrm{DK_{54}K_{55}LPNVGT_{61}GLVGAPACGDVMRLQIK_{78}VN}$				
S_{87}	K_{54}		45.0 ± 0.0	43.6 ± 0.1	43.7 ± 1.0
S_{87}	K_{55}		44.2 ± 0.0	45.0 ± 0.2	45.2 ± 1.0
S_{87}	K_{78}		25.7 ± 0.0	40.2 ± 0.2	39.5 ± 0.8
$\mathrm{DS_{87}LEE}$	$\mathrm{nGS_{25}HMS_{28}S_{29}IT_{31}K_{32}RLY_{35}HPK_{38}VIE}$				
S_{87}	N-Term		42.8 ± 0.0	14.1 ± 0.4	42.6 ± 1.2
S_{87}	K_{32}		53.8 ± 0.0	26.8 ± 0.3	50.0 ± 1.1
S_{87}	K_{38}		50.6 ± 0.0	26.6 ± 0.3	44.2 ± 1.1
$\mathrm{LS_{105}HGVMT_{110}LE}$	$\mathrm{nGS_{25}HMS_{28}S_{29}IT_{31}K_{32}RLY_{35}HPK_{38}VIE}$				
S_{105}	N-Term		47.4 ± 0.0	29.7 ± 0.4	40.3 ± 1.0
S_{105}	K_{32}		60.7 ± 0.0	43.4 ± 0.3	52.3 ± 1.1
S_{105}	K_{38}		57.5 ± 0.0	42.5 ± 0.3	51.0 ± 1.1
T_{110}	N-Term		43.4 ± 0.0	24.8 ± 0.3	33.4 ± 1.0
T_{110}	K_{32}		55.2 ± 0.0	38.8 ± 0.3	44.3 ± 1.1
T_{110}	K_{38}		51.4 ± 0.0	39.2 ± 0.2	42.9 ± 1.1
$\mathrm{WVS_{151}LRNGT_{156}K_{157}LT_{159}DILT_{163}E}$	$\mathrm{n\text{-}GS_{25}HMS_{28}S_{29}IT_{31}K_{32}RLY_{35}HPK_{38}VIE}$				
K_{157}	N-Term	5D	31.2 ± 0.0	12.4 ± 0.3	40.2 ± 0.9
K_{157}	S_{25}		33.7 ± 0.0	14.6 ± 0.3	38.1 ± 1.0
K_{157}	S_{28}		38.7 ± 0.0	22.6 ± 0.3	40.0 ± 1.0
K_{157}	S_{29}		36.8 ± 0.0	23.2 ± 0.3	40.9 ± 1.0
K_{157}	T_{31}		40.3 ± 0.0	27.8 ± 0.3	45.0 ± 1.0
K_{157}	K_{32}		43.1 ± 0.0	29.0 ± 0.3	46.8 ± 1.0
K_{157}	Y_{35}		45.3 ± 0.0	33.0 ± 0.3	47.0 ± 1.0
K_{157}	K_{38}		40.9 ± 0.0	29.9 ± 0.3	42.9 ± 1.0
S_{151}	N-Term		25.6 ± 0.0	17.9 ± 0.3	39.3 ± 1.0
T_{156}	N-Term		27.5 ± 0.0	13.4 ± 0.3	39.3 ± 0.9
T_{159}	N-Term		36.5 ± 0.0	11.9 ± 0.4	40.4 ± 0.9
T_{163}	N-Term		39.2 ± 0.0	10.9 ± 0.3	37.3 ± 0.8
S_{151}	K_{32}		38.7 ± 0.0	34.5 ± 0.3	46.7 ± 1.0
T_{156}	K_{32}		39.3 ± 0.0	30.0 ± 0.3	45.5 ± 1.0
T_{159}	K_{32}		48.0 ± 0.0	27.5 ± 0.3	47.3 ± 1.0
T_{163}	K_{32}		49.0 ± 0.0	24.8 ± 0.2	43.0 ± 1.0
S_{151}	K_{38}		36.8 ± 0.0	35.8 ± 0.3	42.1 ± 1.0
T_{156}	K_{38}		37.2 ± 0.0	31.6 ± 0.3	41.0 ± 1.0
T_{159}	K_{38}		45.4 ± 0.0	27.8 ± 0.3	44.0 ± 1.0
T_{163}	K_{38}		45.6 ± 0.0	26.3 ± 0.3	40.2 ± 0.9
$\mathrm{K_{168}AIS_{171}K_{172}SQ}$	$\mathrm{n\text{-}GS_{25}HMS_{28}S_{29}IT_{31}K_{32}RLY_{35}HPK_{38}VIE}$				
K_{168}	N-Term		41.3 ± 0.0	15.5 ± 0.1	31.5 ± 0.7
K_{168}	S_{25}		42.9 ± 0.0	14.6 ± 0.1	28.5 ± 0.7
K_{168}	S_{28}		45.5 ± 0.0	20.3 ± 0.3	29.3 ± 0.9
K_{168}	S_{29}		42.8 ± 0.0	23.3 ± 0.2	31.4 ± 0.8
K_{168}	T_{31}		45.4 ± 0.0	27.1 ± 0.2	35.0 ± 0.9
K_{168}	K_{32}		48.9 ± 0.0	26.4 ± 0.2	35.8 ± 0.9
K_{168}	Y_{35}		49.4 ± 0.0	30.7 ± 0.3	36.4 ± 1.0
K_{168}	K_{38}		44.4 ± 0.0	29.6 ± 0.3	33.6 ± 0.9
K_{172}	N-Term		47.7 ± 0.0	21.9 ± 0.1	29.7 ± 0.6

Cross-linked peptides			Distance (Å)		
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**
K$_{172}$	S$_{25}$		48.9 ± 0.0	20.3 ± 0.1	26.5 ± 0.7
K$_{172}$	S$_{28}$	5D	50.8 ± 0.0	23.9 ± 0.3	26.9 ± 0.9
K$_{172}$	S$_{29}$		48.0 ± 0.0	27.4 ± 0.3	29.8 ± 0.8
K$_{172}$	T$_{31}$		50.3 ± 0.0	30.6 ± 0.3	33.0 ± 0.9
K$_{172}$	K$_{32}$	5D	54.0 ± 0.0	29.3 ± 0.2	33.0 ± 1.0
K$_{172}$	Y$_{35}$		53.8 ± 0.0	33.2 ± 0.3	34.1 ± 1.0
K$_{172}$	K$_{38}$		48.8 ± 0.0	32.8 ± 0.3	32.4 ± 1.0
S$_{171}$	N-Term		46.7 ± 0.0	18.5 ± 0.1	32.8 ± 0.6
S$_{171}$	K$_{32}$		53.3 ± 0.0	25.7 ± 0.2	35.7 ± 0.9
S$_{171}$	K$_{38}$		48.4 ± 0.0	29.1 ± 0.4	34.5 ± 0.9
K$_{168}$AIS$_{171}$K$_{172}$SQ	DVMRLQIK$_{78}$VN				
K$_{168}$	K$_{78}$		23.7 ± 0.0	45.9 ± 0.2	43.0 ± 0.8
K$_{172}$	K$_{78}$		29.6 ± 0.0	53.0 ± 0.1	40.5 ± 0.8
S$_{171}$	K$_{78}$		26.6 ± 0.0	49.8 ± 0.1	43.8 ± 0.7
VEK$_{168}$AIS$_{171}$K$_{172}$SQ	DK$_{54}$K$_{55}$LPNVGT$_{61}$GLVGAPACG				
K$_{168}$	K$_{54}$		36.2 ± 0.0	49.0 ± 0.1	50.7 ± 1.1
K$_{168}$	K$_{55}$		36.0 ± 0.0	50.0 ± 0.1	52.2 ± 1.0
K$_{168}$	T$_{61}$		23.9 ± 0.0	40.7 ± 0.0	44.6 ± 1.0
K$_{172}$	K$_{54}$		38.8 ± 0.0	54.0 ± 0.2	51.9 ± 1.1
K$_{172}$	K$_{55}$		38.8 ± 0.0	55.3 ± 0.2	53.0 ± 1.1
K$_{172}$	T$_{61}$		29.4 ± 0.0	47.4 ± 0.0	45.7 ± 1.1
S$_{171}$	K$_{54}$		37.9 ± 0.0	50.2 ± 0.2	48.9 ± 1.1
S$_{171}$	K$_{55}$		37.5 ± 0.0	51.6 ± 0.1	50.2 ± 1.1

Single digestion (Glu-C) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)		
Isu1	**Isu1**	**Figure**	**Isu1 intra-monomer**	**Isu1-Isu1 intra-trimer**	**Isu1-Isu1 inter-trimers**
IAK$_{128}$E	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
K$_{128}$	N-Term		39.5 ± 0.0	20.0 ± 0.1	25.8 ± 0.7
K$_{128}$	S$_{25}$	5D	40.6 ± 0.0	19.9 ± 0.2	23.0 ± 0.8
K$_{128}$	S$_{28}$		43.1 ± 0.0	26.2 ± 0.3	24.7 ± 1.0
K$_{128}$	S$_{29}$		40.6 ± 0.0	29.0 ± 0.3	26.8 ± 0.9
K$_{128}$	T$_{31}$		43.5 ± 0.0	32.8 ± 0.2	30.8 ± 1.0
K$_{128}$	K$_{32}$		47.1 ± 0.0	32.1 ± 0.2	31.8 ± 1.0
K$_{128}$	Y$_{35}$		47.3 ± 0.0	36.6 ± 0.3	32.9 ± 1.1
K$_{128}$	K$_{38}$		42.1 ± 0.0	35.5 ± 0.3	29.8 ± 1.0

Double digestion (Glu-C and Asp-N) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)		
Isu1	**Isu1**	**Figure**	**Isu1 intra-monomer**	**Isu1-Isu1 intra-trimer**	**Isu1-Isu1 inter-trimers**
IAK$_{128}$E	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
K$_{128}$	N-Term		39.5 ± 0.0	20.0 ± 0.1	25.8 ± 0.7
K$_{128}$	S$_{25}$		40.6 ± 0.0	19.9 ± 0.2	23.0 ± 0.8
K$_{128}$	S$_{28}$		43.1 ± 0.0	26.2 ± 0.3	24.7 ± 1.0
K$_{128}$	S$_{29}$		40.6 ± 0.0	29.0 ± 0.3	26.8 ± 0.9
K$_{128}$	T$_{31}$		43.5 ± 0.0	32.8 ± 0.2	30.8 ± 1.0
K$_{128}$	K$_{32}$		47.1 ± 0.0	32.1 ± 0.2	31.8 ± 1.0
K$_{128}$	Y$_{35}$		47.3 ± 0.0	36.6 ± 0.3	32.9 ± 1.1
K$_{128}$	K$_{38}$		42.1 ± 0.0	35.5 ± 0.3	29.8 ± 1.0
DAIK$_{148}$AAIK$_{152}$	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIEHY$_{43}$T$_{44}$HPRNVGS$_{51}$L				
K$_{148}$	N-Term		24.2 ± 0.0	14.1 ± 0.4	37.3 ± 0.9
K$_{148}$	S$_{25}$		26.3 ± 0.0	17.3 ± 0.5	34.8 ± 1.0
K$_{148}$	S$_{28}$		29.4 ± 0.0	24.4 ± 0.4	34.5 ± 1.0
K$_{148}$	S$_{29}$		26.8 ± 0.0	25.1 ± 0.3	34.8 ± 0.9
K$_{148}$	T$_{31}$		29.5 ± 0.0	28.4 ± 0.3	38.0 ± 1.0
K$_{148}$	K$_{32}$		32.7 ± 0.0	28.4 ± 0.3	40.3 ± 1.0
K$_{148}$	Y$_{35}$		34.3 ± 0.0	33.4 ± 0.3	39.3 ± 1.0
K$_{148}$	K$_{38}$		29.9 ± 0.0	32.1 ± 0.3	34.9 ± 1.0
K$_{148}$	Y$_{43}$		34.7 ± 0.0	40.5 ± 0.3	34.5 ± 1.0
K$_{148}$	T$_{44}$		33.3 ± 0.0	41.2 ± 0.3	36.2 ± 0.9
K$_{148}$	S$_{51}$		35.0 ± 0.0	51.5 ± 0.2	46.6 ± 0.9
K$_{152}$	N-Term		20.0 ± 0.0	17.1 ± 0.5	41.8 ± 1.0
K$_{152}$	S$_{25}$		22.5 ± 0.0	20.7 ± 0.4	39.5 ± 1.0
K$_{152}$	S$_{28}$		26.0 ± 0.0	27.1 ± 0.4	39.0 ± 1.1
K$_{152}$	S$_{29}$		23.5 ± 0.0	27.1 ± 0.3	38.8 ± 1.0
K$_{152}$	T$_{31}$		26.2 ± 0.0	29.9 ± 0.3	41.9 ± 1.0

Cross-linked peptides			Distance (Å)		
Isu1	Isu1	Figure	Isu1 intra-monomer	Isu1-Isu1 intra-trimer	Isu1-Isu1 inter-trimers
K_{152}	K_{32}		28.9 ± 0.0	30.3 ± 0.3	44.4 ± 1.0
K_{152}	Y_{35}		31.2 ± 0.0	35.1 ± 0.3	43.0 ± 1.0
K_{152}	K_{38}		27.4 ± 0.0	33.6 ± 0.3	38.4 ± 1.0
K_{152}	Y_{43}		33.3 ± 0.0	42.0 ± 0.3	37.1 ± 1.0
K_{152}	T_{44}		32.1 ± 0.0	42.2 ± 0.3	38.4 ± 0.9
K_{152}	S_{51}		35.3 ± 0.0	50.6 ± 0.2	46.9 ± 0.8
$\mathrm{DAAK_{120}IK_{122}NT_{124}EIAK_{128}E}$	$\mathrm{DK_{54}K_{55}LPNVGT_{61}GLVGAPACG}$				
K_{120}	K_{54}		33.9 ± 0.0	46.6 ± 0.2	49.9 ± 0.8
K_{120}	K_{55}		33.8 ± 0.0	46.7 ± 0.3	52.5 ± 0.8
K_{120}	T_{61}	5D	17.6 ± 0.0	33.3 ± 0.2	44.9 ± 0.6
K_{122}	K_{54}		32.3 ± 0.0	51.3 ± 0.1	45.8 ± 0.9
K_{122}	K_{55}		32.7 ± 0.0	51.6 ± 0.1	48.3 ± 0.8
K_{122}	T_{61}		19.0 ± 0.0	39.5 ± 0.2	41.0 ± 0.6
K_{128}	K_{54}		36.4 ± 0.0	54.1 ± 0.1	48.8 ± 0.9
K_{128}	K_{55}		36.8 ± 0.0	54.9 ± 0.1	51.1 ± 0.9
K_{128}	T_{61}		25.1 ± 0.0	44.4 ± 0.1	43.8 ± 0.7
T_{124}	K_{54}		34.7 ± 0.0	52.8 ± 0.1	46.9 ± 0.9
T_{124}	K_{55}		35.1 ± 0.0	53.3 ± 0.1	49.2 ± 0.9
$\mathrm{DDAAK_{120}IK_{122}NT_{124}EIAK_{128}E}$	$\mathrm{HY_{43}T_{44}HPRNVGS_{51}LDK_{54}K_{55}LPNVGT_{61}GLVGAPACG}$				
K_{120}	Y_{43}		39.5 ± 0.0	41.7 ± 0.3	37.5 ± 1.0
K_{120}	T_{44}		38.5 ± 0.0	42.7 ± 0.3	39.3 ± 1.0
K_{120}	S_{51}		40.8 ± 0.0	53.6 ± 0.2	50.3 ± 0.9
K_{120}	K_{54}		33.9 ± 0.0	46.6 ± 0.2	49.9 ± 0.8
K_{120}	K_{55}		33.8 ± 0.0	46.7 ± 0.3	52.5 ± 0.8
K_{120}	T_{61}	5D	17.6 ± 0.0	33.3 ± 0.2	44.9 ± 0.6
K_{122}	Y_{43}		38.2 ± 0.0	43.8 ± 0.4	32.2 ± 1.0
K_{122}	T_{44}		37.0 ± 0.0	45.3 ± 0.3	34.3 ± 1.0
K_{122}	S_{51}		38.0 ± 0.0	57.8 ± 0.2	46.8 ± 1.0
K_{122}	K_{54}		32.3 ± 0.0	51.3 ± 0.1	45.8 ± 0.9
K_{122}	K_{55}		32.7 ± 0.0	51.6 ± 0.1	48.3 ± 0.8
K_{122}	T_{61}		19.0 ± 0.0	39.5 ± 0.2	41.0 ± 0.6
K_{128}	Y_{43}		44.3 ± 0.0	43.0 ± 0.4	33.1 ± 1.1
K_{128}	T_{44}		42.7 ± 0.0	45.1 ± 0.4	35.7 ± 1.1
K_{128}	S_{51}		41.1 ± 0.0	59.3 ± 0.2	50.3 ± 1.1
K_{128}	K_{54}		25.1 ± 0.0	44.4 ± 0.1	43.8 ± 0.7
K_{128}	K_{55}		34.7 ± 0.0	52.8 ± 0.1	46.9 ± 0.9
K_{128}	T_{61}		35.1 ± 0.0	53.3 ± 0.1	49.2 ± 0.9
$\mathrm{DAIK_{148}AAIK_{152}}$	$\mathrm{DK_{54}K_{55}LPNVGT_{61}GLVGAPACG}$				
K_{148}	K_{54}		27.2 ± 0.0	50.1 ± 0.2	47.5 ± 0.7
K_{148}	K_{55}		26.9 ± 0.0	48.1 ± 0.2	50.5 ± 0.7
K_{148}	T_{61}		10.2 ± 0.0	32.4 ± 0.1	45.3 ± 0.5
K_{152}	K_{54}	5D	26.9 ± 0.0	44.1 ± 0.2	48.6 ± 0.7
K_{152}	K_{55}		26.3 ± 0.0	42.1 ± 0.2	51.6 ± 0.7
K_{152}	T_{61}		9.6 ± 0.0	26.6 ± 0.1	46.9 ± 0.6

Cross-linked peptides			Distance (Å)		
Yfh1	Yfh1	Figure	Yfh1 intra-monomer	Yfh1-Yfh1 intra-trimer	Yfh1-Yfh1 inter-trimers
nME	$\mathrm{LS_{105}HGVMT_{110}LE}$				
N-Term	S_{105}		38.8 ± 0.0	32.5 ± 0.9	25.4 ± 1.4
N-Term	T_{110}		41.8 ± 0.0	24.4 ± 0.9	24.4 ± 1.4

Supplementary Table S1e. Alternative N-terminus #2

Single digestion (Glu-C) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)		
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**
n-ME	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
N-Term	N-Term		82.2 ± 0.0	52.9 ± 0.7	33.2 ± 1.4
N-Term	S$_{25}$		85.3 ± 0.0	53.7 ± 0.8	33.9 ± 1.4
N-Term	S$_{28}$		86.2 ± 0.0	50.8 ± 0.9	34.0 ± 1.4
N-Term	S$_{29}$		83.6 ± 0.0	47.3 ± 0.9	30.3 ± 1.4
N-Term	T$_{31}$		82.3 ± 0.0	44.6 ± 0.9	30.5 ± 1.4
N-Term	K$_{32}$		85.1 ± 0.0	47.7 ± 0.9	34.3 ± 1.4
N-Term	Y$_{35}$		85.2 ± 0.0	47.5 ± 0.9	39.0 ± 1.4
N-Term	K$_{38}$		88.5 ± 0.0	48.4 ± 0.9	41.8 ± 1.5
n-MES$_{54}$S$_{55}$T$_{56}$DGQVVPQE	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
S$_{54}$	K$_{32}$		87.0 ± 0.0	51.9 ± 0.9	38.3 ± 1.5
S$_{55}$	K$_{32}$		84.9 ± 0.0	51.6 ± 0.8	40.5 ± 1.4
T$_{56}$	K$_{32}$		81.6 ± 0.0	48.9 ± 0.8	40.0 ± 1.4
S$_{54}$	K$_{38}$		90.7 ± 0.0	53.2 ± 0.9	46.2 ± 1.5
S$_{55}$	K$_{38}$		88.7 ± 0.0	53.6 ± 0.9	48.0 ± 1.4
T$_{56}$	K$_{38}$		85.3 ± 0.0	50.9 ± 0.8	46.9 ± 1.4

Double digestion (Glu-C and Asp-N) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)		
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**
nME	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
N-Term	N-Term		82.2 ± 0.0	52.9 ± 0.7	33.2 ± 1.4
N-Term	S$_{25}$		85.3 ± 0.0	53.7 ± 0.8	33.9 ± 1.4
N-Term	S$_{28}$		86.2 ± 0.0	50.8 ± 0.9	34.0 ± 1.4
N-Term	S$_{29}$		83.6 ± 0.0	47.3 ± 0.9	30.3 ± 1.4
N-Term	T$_{31}$		82.3 ± 0.0	44.6 ± 0.9	30.5 ± 1.4
N-Term	K$_{32}$		85.1 ± 0.0	47.7 ± 0.9	34.3 ± 1.4
N-Term	Y$_{35}$		85.2 ± 0.0	47.5 ± 0.9	39.0 ± 1.4
N-Term	K$_{38}$		88.5 ± 0.0	48.4 ± 0.9	41.8 ± 1.5
nME	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIEHY$_{43}$T$_{44}$HPRNVGS$_{51}$LDK$_{54}$K$_{55}$LPNVGTGLVGAPACG				
N-Term	N-Term		82.2 ± 0.0	52.9 ± 0.7	33.2 ± 1.4
N-Term	S$_{25}$		85.3 ± 0.0	53.7 ± 0.8	33.9 ± 1.4
N-Term	S$_{28}$		86.2 ± 0.0	50.8 ± 0.9	34.0 ± 1.4
N-Term	S$_{29}$		83.6 ± 0.0	47.3 ± 0.9	30.3 ± 1.4
N-Term	T$_{31}$		82.3 ± 0.0	44.6 ± 0.9	30.5 ± 1.4
N-Term	K$_{32}$		85.1 ± 0.0	47.7 ± 0.9	34.3 ± 1.4
N-Term	Y$_{35}$		85.2 ± 0.0	47.5 ± 0.9	39.0 ± 1.4
N-Term	K$_{38}$		88.5 ± 0.0	48.4 ± 0.9	41.8 ± 1.5
N-Term	Y$_{43}$		88.7 ± 0.0	49.0 ± 0.9	46.9 ± 1.5
N-Term	T$_{44}$		85.0 ± 0.0	45.6 ± 0.9	44.1 ± 1.4
N-Term	S$_{51}$		71.3 ± 0.0	35.1 ± 0.8	32.4 ± 1.3
N-Term	K$_{54}$		63.4 ± 0.0	32.6 ± 0.7	30.5 ± 1.1
N-Term	K$_{55}$		62.6 ± 0.0	34.1 ± 0.6	34.0 ± 1.0
N-Term	T$_{61}$	5E	47.5 ± 0.0	23.5 ± 0.2	32.3 ± 0.6
nMES$_{54}$S$_{55}$T$_{56}$	DVMRLQIK$_{78}$VN				
N-Term	K$_{78}$		40.4 ± 0.0	29.1 ± 0.1	30.4 ± 0.7
S$_{54}$	K$_{78}$		43.2 ± 0.0	32.5 ± 0.1	28.7 ± 0.8
S$_{55}$	K$_{78}$		41.4 ± 0.0	31.9 ± 0.2	30.3 ± 0.8
T$_{56}$	K$_{78}$		37.8 ± 0.0	28.6 ± 0.1	32.9 ± 0.8
nMES$_{54}$S$_{55}$T$_{56}$DGQVVPQE	HY$_{43}$T$_{44}$HPRNVGS$_{51}$LDK$_{54}$K$_{55}$LPNVGTGLVGAPACG				
N-Term	Y$_{43}$		88.7 ± 0.0	49.0 ± 0.9	46.9 ± 1.5
N-Term	T$_{44}$		85.0 ± 0.0	45.6 ± 0.9	44.1 ± 1.4
N-Term	S$_{51}$		71.3 ± 0.0	35.1 ± 0.8	32.4 ± 1.3
N-Term	K$_{54}$		63.4 ± 0.0	32.6 ± 0.7	30.5 ± 1.1
N-Term	K$_{55}$		62.6 ± 0.0	34.1 ± 0.6	34.0 ± 1.0
N-Term	T$_{61}$	5E	47.5 ± 0.0	23.5 ± 0.2	32.3 ± 0.6
S$_{54}$	K$_{54}$		65.8 ± 0.0	37.5 ± 0.7	32.5 ± 1.2
S$_{55}$	K$_{54}$		63.8 ± 0.0	37.1 ± 0.7	33.8 ± 1.2
T$_{56}$	K$_{54}$		60.3 ± 0.0	33.6 ± 0.7	34.1 ± 1.1
S$_{54}$	K$_{55}$		65.2 ± 0.0	39.1 ± 0.6	35.7 ± 1.1
S$_{55}$	K$_{55}$		63.3 ± 0.0	38.8 ± 0.7	37.0 ± 1.1
T$_{56}$	K$_{55}$		59.7 ± 0.0	35.3 ± 0.6	37.3 ± 1.1

Cross-linked peptides			**Distance (Å)**		
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**
K$_{72}$AHEE	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
K$_{72}$	N-Term		64.4 ± 0.0	41.3 ± 0.5	54.0 ± 1.3
K$_{72}$	S$_{25}$		67.1 ± 0.0	43.4 ± 0.4	54.2 ± 1.3
K$_{72}$	S$_{28}$		67.1 ± 0.0	43.5 ± 0.5	52.4 ± 1.3
K$_{72}$	S$_{29}$		64.1 ± 0.0	40.1 ± 0.5	48.7 ± 1.3
K$_{72}$	T$_{31}$		62.4 ± 0.0	39.2 ± 0.5	47.5 ± 1.3
K$_{72}$	K$_{32}$		65.5 ± 0.0	42.8 ± 0.5	50.9 ± 1.3
K$_{72}$	Y$_{35}$		65.5 ± 0.0	45.1 ± 0.5	53.3 ± 1.2
K$_{72}$	K$_{38}$		68.4 ± 0.0	48.4 ± 0.5	54.4 ± 1.2
K$_{72}$AHEE	DVMRLQIK$_{78}$VNDS$_{82}$T$_{83}$GVIE				
K$_{72}$	K$_{78}$		21.6 ± 0.0	25.0 ± 0.3	47.5 ± 0.9
K$_{72}$	S$_{82}$		15.4 ± 0.0	26.6 ± 0.3	41.4 ± 0.7
K$_{72}$	T$_{83}$		12.1 ± 0.0	26.3 ± 0.3	38.3 ± 0.8
K$_{72}$AHEE	DK$_{54}$K$_{55}$LPNVGT$_{61}$GLVGAPACGDVMRLQIK$_{78}$VN				
K$_{72}$	K$_{54}$	5E	43.8 ± 0.0	27.3 ± 0.5	43.4 ± 1.0
K$_{72}$	K$_{55}$		43.4 ± 0.0	29.3 ± 0.5	46.1 ± 0.9
K$_{72}$	T$_{61}$	5E	26.9 ± 0.0	19.2 ± 0.3	38.7 ± 0.6
K$_{72}$	K$_{78}$		21.6 ± 0.0	25.0 ± 0.3	45.4 ± 0.5
DS$_{87}$LE	DK$_{54}$K$_{55}$LPNVGT$_{61}$GLVGAPACGDVMRLQIK$_{78}$VN				
S$_{87}$	K$_{54}$		46.4 ± 0.0	35.7 ± 0.2	46.1 ± 1.1
S$_{87}$	K$_{55}$		46.3 ± 0.0	38.6 ± 0.2	47.8 ± 1.0
S$_{87}$	K$_{78}$	5E	25.7 ± 0.0	40.2 ± 0.2	39.8 ± 0.8
DS$_{87}$LEE	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
S$_{87}$	N-Term		69.2 ± 0.0	37.1 ± 0.3	52.8 ± 1.1
S$_{87}$	K$_{32}$		67.3 ± 0.0	42.7 ± 0.3	56.7 ± 1.2
S$_{87}$	K$_{38}$		68.7 ± 0.0	51.5 ± 0.3	65.4 ± 1.3
LS$_{105}$HGVMT$_{110}$LE	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
S$_{105}$	N-Term		75.3 ± 0.0	53.1 ± 0.3	59.9 ± 1.2
S$_{105}$	K$_{32}$		75.1 ± 0.0	58.5 ± 0.3	65.5 ± 1.3
S$_{105}$	K$_{38}$		78.2 ± 0.0	66.9 ± 0.3	74.2 ± 1.3
T$_{110}$	N-Term		70.0 ± 0.0	47.6 ± 0.2	51.8 ± 1.2
T$_{110}$	K$_{32}$		68.5 ± 0.0	54.8 ± 0.3	57.0 ± 1.3
T$_{110}$	K$_{38}$		70.8 ± 0.0	64.1 ± 0.3	65.8 ± 1.3
WVS$_{151}$LRNGT$_{156}$K$_{157}$LT$_{159}$DILT$_{163}$E	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
K$_{157}$	N-Term		58.4 ± 0.0	41.4 ± 0.3	54.5 ± 1.0
K$_{157}$	S$_{25}$		60.6 ± 0.0	44.0 ± 0.3	54.5 ± 1.1
K$_{157}$	S$_{28}$		60.0 ± 0.0	46.2 ± 0.3	54.8 ± 1.1
K$_{157}$	S$_{29}$		56.8 ± 0.0	43.1 ± 0.3	51.3 ± 1.1
K$_{157}$	T$_{31}$		54.9 ± 0.0	43.6 ± 0.3	51.9 ± 1.1
K$_{157}$	K$_{32}$		58.2 ± 0.0	47.1 ± 0.3	55.4 ± 1.1
K$_{157}$	Y$_{35}$		58.6 ± 0.0	50.8 ± 0.3	60.2 ± 1.1
K$_{157}$	K$_{38}$		61.1 ± 0.0	54.9 ± 0.3	62.1 ± 1.2
S$_{151}$	N-Term		53.7 ± 0.0	47.0 ± 0.2	56.1 ± 1.1
T$_{156}$	N-Term		54.6 ± 0.0	42.4 ± 0.3	53.8 ± 1.0
T$_{159}$	N-Term		63.4 ± 0.0	39.3 ± 0.3	54.0 ± 1.0
T$_{163}$	N-Term		64.5 ± 0.0	34.8 ± 0.2	48.5 ± 1.0
S$_{151}$	K$_{32}$		54.6 ± 0.0	52.9 ± 0.3	55.8 ± 1.1
T$_{156}$	K$_{32}$		54.6 ± 0.1	48.5 ± 0.3	53.7 ± 1.1
T$_{159}$	K$_{32}$		62.5 ± 0.0	44.8 ± 0.3	56.2 ± 1.1
T$_{163}$	K$_{32}$		62.0 ± 0.0	42.0 ± 0.3	51.4 ± 1.2
S$_{151}$	K$_{38}$		58.7 ± 0.0	60.3 ± 0.3	61.6 ± 1.1
T$_{156}$	K$_{38}$		57.8 ± 0.0	56.2 ± 0.3	59.8 ± 1.2
T$_{159}$	K$_{38}$		64.7 ± 0.0	53.0 ± 0.3	63.8 ± 1.2
T$_{163}$	K$_{38}$		63.0 ± 0.0	51.3 ± 0.3	59.7 ± 1.3
K$_{168}$AIS$_{171}$K$_{172}$SQ	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
K$_{168}$	N-Term		64.2 ± 0.0	33.0 ± 0.3	40.4 ± 1.0
K$_{168}$	S$_{25}$		65.6 ± 0.0	35.4 ± 0.3	40.4 ± 1.1
K$_{168}$	S$_{28}$		63.1 ± 0.0	39.8 ± 0.4	42.1 ± 1.2
K$_{168}$	S$_{29}$		59.8 ± 0.0	37.2 ± 0.4	38.9 ± 1.2
K$_{168}$	T$_{31}$		56.9 ± 0.0	39.6 ± 0.4	40.9 ± 1.2
K$_{168}$	K$_{32}$		59.8 ± 0.0	42.9 ± 0.4	44.3 ± 1.2
K$_{168}$	Y$_{35}$		58.6 ± 0.0	48.9 ± 0.3	50.6 ± 1.2
K$_{168}$	K$_{38}$		59.7 ± 0.0	53.6 ± 0.3	53.3 ± 1.3
K$_{172}$	N-Term		68.9 ± 0.0	32.3 ± 0.3	35.5 ± 1.1

Cross-linked peptides			Distance (Å)		
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**
K$_{172}$	S$_{25}$		70.0 ± 0.0	34.5 ± 0.4	35.7 ± 1.2
K$_{172}$	S$_{28}$		67.0 ± 0.0	39.8 ± 0.5	38.5 ± 1.3
K$_{172}$	S$_{29}$		63.7 ± 0.0	37.5 ± 0.5	35.7 ± 1.3
K$_{172}$	T$_{31}$		60.4 ± 0.0	40.7 ± 0.5	38.4 ± 1.3
K$_{172}$	K$_{32}$		63.2 ± 0.0	43.8 ± 0.4	41.7 ± 1.3
K$_{172}$	Y$_{35}$		61.5 ± 0.0	50.6 ± 0.4	48.7 ± 1.3
K$_{172}$	K$_{38}$		61.9 ± 0.0	55.3 ± 0.4	52.0 ± 1.4
S$_{171}$	N-Term		68.4 ± 0.0	29.6 ± 0.3	38.0 ± 1.0
S$_{171}$	K$_{32}$		63.0 ± 0.0	40.5 ± 0.4	43.2 ± 1.3
S$_{171}$	K$_{38}$		61.8 ± 0.1	51.8 ± 0.4	53.2 ± 1.4
K$_{168}$AIS$_{171}$K$_{172}$SQ	DVMRLQIK$_{78}$VN				
K$_{168}$	K$_{78}$		23.7 ± 0.0	45.9 ± 0.2	43.0 ± 0.8
K$_{172}$	K$_{78}$		29.6 ± 0.0	53.0 ± 0.1	40.4 ± 0.8
S$_{171}$	K$_{78}$		26.6 ± 0.0	49.9 ± 0.2	43.8 ± 0.8
VEK$_{168}$AIS$_{171}$K$_{172}$SQ	DK$_{54}$K$_{55}$LPNVGT$_{61}$GLVGAPACG				
K$_{168}$	K$_{54}$		39.8 ± 0.0	38.9 ± 0.1	46.5 ± 0.8
K$_{168}$	K$_{55}$		39.9 ± 0.0	41.7 ± 0.0	50.1 ± 0.8
K$_{168}$	T$_{61}$	5E	24.1 ± 0.0	40.2 ± 0.1	48.9 ± 0.6
K$_{172}$	K$_{54}$		44.1 ± 0.0	43.7 ± 0.1	45.7 ± 0.9
K$_{172}$	K$_{55}$		44.1 ± 0.0	46.7 ± 0.1	49.4 ± 0.9
K$_{172}$	T$_{61}$		29.8 ± 0.0	46.9 ± 0.0	49.9 ± 0.7
S$_{171}$	K$_{54}$		42.9 ± 0.0	39.9 ± 0.1	47.9 ± 0.8
S$_{171}$	K$_{55}$		42.7 ± 0.0	42.9 ± 0.1	51.6 ± 0.8

Single digestion (Glu-C) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)		
Isu1	**Isu1**	**Figure**	**Isu1 intra-monomer**	**Isu1-Isu1 intra-trimer**	**Isu1-Isu1 inter-trimers**
IAK$_{128}$E	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
K$_{128}$	N-Term		62.0 ± 0.0	38.4 ± 0.3	38.4 ± 1.1
K$_{128}$	S$_{25}$		63.3 ± 0.0	40.9 ± 0.4	38.8 ± 1.1
K$_{128}$	S$_{28}$		60.8 ± 0.0	45.7 ± 0.4	40.5 ± 1.2
K$_{128}$	S$_{29}$		57.4 ± 0.0	43.2 ± 0.4	37.1 ± 1.2
K$_{128}$	T$_{31}$		54.6 ± 0.0	45.7 ± 0.4	38.8 ± 1.3
K$_{128}$	K$_{32}$		57.7 ± 0.0	48.9 ± 0.4	42.4 ± 1.3
K$_{128}$	Y$_{35}$		57.1 ± 0.0	54.8 ± 0.3	48.3 ± 1.3
K$_{128}$	K$_{38}$		58.1 ± 0.0	59.6 ± 0.3	51.3 ± 1.3

Double digestion (Glu-C and Asp-N) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)		
Isu1	**Isu1**	**Figure**	**Isu1 intra-monomer**	**Isu1-Isu1 intra-trimer**	**Isu1-Isu1 inter-trimers**
IAK$_{128}$E	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
K$_{128}$	N-Term		62.0 ± 0.0	38.4 ± 0.3	38.4 ± 1.1
K$_{128}$	S$_{25}$		63.3 ± 0.0	40.9 ± 0.4	38.8 ± 1.1
K$_{128}$	S$_{28}$		60.8 ± 0.0	45.7 ± 0.4	40.5 ± 1.2
K$_{128}$	S$_{29}$		57.4 ± 0.0	43.2 ± 0.4	37.1 ± 1.2
K$_{128}$	T$_{31}$		54.6 ± 0.0	45.7 ± 0.4	38.8 ± 1.3
K$_{128}$	K$_{32}$		57.7 ± 0.0	48.9 ± 0.4	42.4 ± 1.3
K$_{128}$	Y$_{35}$		57.1 ± 0.0	54.8 ± 0.3	48.3 ± 1.3
K$_{128}$	K$_{38}$		58.1 ± 0.0	59.6 ± 0.3	51.3 ± 1.3
DAIK$_{148}$AAIK$_{152}$	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIEHY$_{43}$T$_{44}$HPRNVGS$_{51}$L				
K$_{148}$	N-Term		48.2 ± 0.0	39.4 ± 0.3	48.4 ± 1.1
K$_{148}$	S$_{25}$		50.2 ± 0.0	42.6 ± 0.3	47.9 ± 1.1
K$_{148}$	S$_{28}$		48.9 ± 0.0	45.8 ± 0.3	46.7 ± 1.1
K$_{148}$	S$_{29}$		45.7 ± 0.0	43.4 ± 0.3	43.2 ± 1.1
K$_{148}$	T$_{31}$		43.4 ± 0.0	44.1 ± 0.3	43.0 ± 1.1
K$_{148}$	K$_{32}$		46.6 ± 0.0	47.2 ± 0.3	46.3 ± 1.1
K$_{148}$	Y$_{35}$		46.5 ± 0.0	50.7 ± 0.2	50.3 ± 1.0
K$_{148}$	K$_{38}$		48.9 ± 0.0	55.4 ± 0.3	51.5 ± 1.1
K$_{148}$	Y$_{43}$		49.7 ± 0.0	59.4 ± 0.2	55.9 ± 1.1
K$_{148}$	T$_{44}$		45.9 ± 0.0	56.1 ± 0.2	53.5 ± 1.1
K$_{148}$	S$_{51}$		32.2 ± 0.0	41.5 ± 0.2	45.1 ± 0.9
K$_{152}$	N-Term		44.4 ± 0.0	42.1 ± 0.3	52.8 ± 1.1
K$_{152}$	S$_{25}$		46.7 ± 0.0	45.4 ± 0.3	52.2 ± 1.1
K$_{152}$	S$_{28}$		46.0 ± 0.0	48.0 ± 0.3	50.3 ± 1.1
K$_{152}$	S$_{29}$		42.9 ± 0.0	45.7 ± 0.3	46.8 ± 1.1

Cross-linked peptides			Distance (Å)		
Isu1	**Isu1**	**Figure**	**Isu1 intra-monomer**	**Isu1-Isu1 intra-trimer**	**Isu1-Isu1 inter-trimers**
K_{152}	T_{31}		41.0 ± 0.0	45.9 ± 0.3	46.1 ± 1.0
K_{152}	K_{32}		44.1 ± 0.0	48.8 ± 0.3	49.3 ± 1.1
K_{152}	Y_{35}		44.3 ± 0.0	51.4 ± 0.2	52.6 ± 1.0
K_{152}	K_{38}		47.3 ± 0.0	55.9 ± 0.2	53.3 ± 1.1
K_{152}	Y_{43}		48.6 ± 0.0	59.1 ± 0.2	57.0 ± 1.1
K_{152}	T_{44}		44.8 ± 0.0	55.7 ± 0.2	54.7 ± 1.0
K_{152}	S_{51}		30.5 ± 0.0	41.1 ± 0.2	47.1 ± 0.8
$DAAK_{120}IK_{122}NT_{124}EIAK_{128}E$	$DK_{54}K_{55}LPNVGT_{61}GLVGAPACG$				
K_{120}	K_{54}		32.6 ± 0.0	37.8 ± 0.2	46.4 ± 0.8
K_{120}	K_{55}		33.4 ± 0.0	39.6 ± 0.3	49.7 ± 0.8
K_{120}	T_{61}		17.4 ± 0.0	32.7 ± 0.3	44.7 ± 0.5
K_{122}	K_{54}		33.0 ± 0.0	41.6 ± 0.1	41.0 ± 0.8
K_{122}	K_{55}		34.0 ± 0.0	43.7 ± 0.1	44.4 ± 0.8
K_{122}	T_{61}		19.0 ± 0.0	38.9 ± 0.2	40.7 ± 0.5
K_{128}	K_{54}		39.4 ± 0.0	44.1 ± 0.1	41.9 ± 0.9
K_{128}	K_{55}		40.1 ± 0.0	46.7 ± 0.1	45.5 ± 0.9
K_{128}	T_{61}		25.3 ± 0.0	43.8 ± 0.1	43.3 ± 0.7
T_{124}	K_{54}		35.9 ± 0.0	43.1 ± 0.1	41.3 ± 0.9
T_{124}	K_{55}		36.8 ± 0.0	45.4 ± 0.2	44.7 ± 0.8
$DDAAK_{120}IK_{122}NT_{124}EIAK_{128}E$	$HY_{43}T_{44}HPRNVGS_{51}LDK_{54}K_{55}LPNVGT_{61}GLVGAPACG$				
K_{120}	Y_{43}		56.0 ± 0.0	62.5 ± 0.2	60.2 ± 1.2
K_{120}	T_{44}		52.2 ± 0.0	59.2 ± 0.2	57.6 ± 1.2
K_{120}	S_{51}		38.7 ± 0.0	44.5 ± 0.2	47.6 ± 1.0
K_{120}	K_{54}		32.6 ± 0.0	37.8 ± 0.2	46.4 ± 0.8
K_{120}	K_{55}		33.4 ± 0.0	39.6 ± 0.3	49.7 ± 0.8
K_{120}	T_{61}		17.4 ± 0.0	32.7 ± 0.3	44.7 ± 0.5
K_{122}	Y_{43}		54.0 ± 0.0	65.1 ± 0.3	55.7 ± 1.2
K_{122}	T_{44}		50.3 ± 0.0	62.0 ± 0.2	53.0 ± 1.2
K_{122}	S_{51}		37.8 ± 0.0	47.8 ± 0.2	42.4 ± 1.0
K_{122}	K_{54}		33.0 ± 0.0	41.6 ± 0.1	41.0 ± 0.8
K_{122}	K_{55}		34.0 ± 0.0	43.7 ± 0.1	44.4 ± 0.8
K_{122}	T_{61}		19.0 ± 0.0	38.9 ± 0.2	40.7 ± 0.5
K_{128}	Y_{43}		58.0 ± 0.0	65.4 ± 0.3	57.2 ± 1.3
K_{128}	T_{44}		54.4 ± 0.0	62.5 ± 0.2	54.7 ± 1.3
K_{128}	S_{51}		43.4 ± 0.0	49.1 ± 0.2	43.7 ± 1.1
K_{128}	K_{54}		39.4 ± 0.0	44.1 ± 0.1	41.9 ± 0.9
K_{128}	K_{55}		40.1 ± 0.0	46.7 ± 0.1	45.5 ± 0.9
K_{128}	T_{61}		25.3 ± 0.0	43.8 ± 0.1	43.3 ± 0.7
$DAIK_{148}AAIK_{152}$	$DK_{54}K_{55}LPNVGT_{61}GLVGAPACG$				
K_{148}	K_{54}		25.8 ± 0.0	34.4 ± 0.2	45.1 ± 0.7
K_{148}	K_{55}		26.3 ± 0.0	35.8 ± 0.2	48.5 ± 0.7
K_{148}	T_{61}		9.9 ± 0.0	30.5 ± 0.2	45.2 ± 0.4
K_{152}	K_{54}	5E	23.2 ± 0.0	33.4 ± 0.2	47.6 ± 0.7
K_{152}	K_{55}		23.7 ± 0.0	34.2 ± 0.3	50.7 ± 0.7
K_{152}	T_{61}		9.0 ± 0.0	27.8 ± 0.2	47.0 ± 0.4

Cross-linked peptides			Distance (Å)		
Yfh1	**Yfh1**	**Figure**	**Yfh1 intra-monomer**	**Yfh1-Yfh1 intra-trimer**	**Yfh1-Yfh1 inter-trimers**
nME	$LS_{105}HGVMT_{110}LE$				
N-Term	S_{105}		38.8 ± 0.0	32.5 ± 0.9	25.9 ± 1.5
N-Term	T_{110}		41.8 ± 0.0	24.4 ± 0.9	25.0 ± 1.5

Supplementary Table S1f. Alternative N-terminus #3

Single digestion (Glu-C) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)		
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**
n-ME	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
N-Term	N-Term		59.8 ± 0.0	53.0 ± 0.4	52.9 ± 0.9
N-Term	S$_{25}$		62.6 ± 0.0	52.4 ± 0.5	51.4 ± 1.0
N-Term	S$_{28}$		67.8 ± 0.0	50.4 ± 0.6	50.7 ± 1.1
N-Term	S$_{29}$		67.8 ± 0.0	48.8 ± 0.6	50.8 ± 1.1
N-Term	T$_{31}$		73.5 ± 0.0	49.8 ± 0.7	51.7 ± 1.2
N-Term	K$_{32}$		74.7 ± 0.0	51.9 ± 0.7	54.5 ± 1.2
N-Term	Y$_{35}$		68.8 ± 0.0	46.5 ± 0.6	53.0 ± 1.1
N-Term	K$_{38}$		70.6 ± 0.0	44.1 ± 0.7	49.0 ± 1.1
n-MES$_{54}$S$_{55}$T$_{56}$DGQVVPQE	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
S$_{54}$	K$_{32}$		78.4 ± 0.0	57.3 ± 0.6	56.7 ± 1.2
S$_{55}$	K$_{32}$		77.0 ± 0.0	57.6 ± 0.6	58.1 ± 1.2
T$_{56}$	K$_{32}$		73.3 ± 0.0	54.3 ± 0.6	58.2 ± 1.1
S$_{54}$	K$_{38}$		74.0 ± 0.0	49.6 ± 0.7	51.3 ± 1.2
S$_{55}$	K$_{38}$		72.5 ± 0.0	50.0 ± 0.6	52.4 ± 1.2
T$_{56}$	K$_{38}$		68.8 ± 0.0	46.8 ± 0.7	52.2 ± 1.1

Double digestion (Glu-C and Asp-N) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)		
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**
nME	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
N-Term	N-Term		59.8 ± 0.0	53.0 ± 0.4	52.9 ± 0.9
N-Term	S$_{25}$		62.6 ± 0.0	52.4 ± 0.5	51.4 ± 1.0
N-Term	S$_{28}$		67.8 ± 0.0	50.4 ± 0.6	50.7 ± 1.1
N-Term	S$_{29}$		67.8 ± 0.0	48.8 ± 0.6	50.8 ± 1.1
N-Term	T$_{31}$		73.5 ± 0.0	49.8 ± 0.7	51.7 ± 1.2
N-Term	K$_{32}$		74.7 ± 0.0	51.9 ± 0.7	54.5 ± 1.2
N-Term	Y$_{35}$		68.8 ± 0.0	46.5 ± 0.6	53.0 ± 1.1
N-Term	K$_{38}$		70.6 ± 0.0	44.1 ± 0.7	49.0 ± 1.1
nME	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIEHY$_{43}$T$_{44}$HPRNVGS$_{51}$LDK$_{54}$K$_{55}$LPNVGTGLVGAPACG				
N-Term	N-Term		59.8 ± 0.0	53.0 ± 0.4	52.9 ± 0.9
N-Term	S$_{25}$		62.6 ± 0.0	52.4 ± 0.5	51.4 ± 1.0
N-Term	S$_{28}$		67.8 ± 0.0	50.4 ± 0.6	50.7 ± 1.1
N-Term	S$_{29}$		67.8 ± 0.0	48.8 ± 0.6	50.8 ± 1.1
N-Term	T$_{31}$		73.5 ± 0.0	49.8 ± 0.7	51.7 ± 1.2
N-Term	K$_{32}$		74.7 ± 0.0	51.9 ± 0.7	54.5 ± 1.2
N-Term	Y$_{35}$		68.8 ± 0.0	46.5 ± 0.6	53.0 ± 1.1
N-Term	K$_{38}$		70.6 ± 0.0	44.1 ± 0.7	49.0 ± 1.1
N-Term	Y$_{43}$		73.5 ± 0.0	40.8 ± 0.7	44.5 ± 1.2
N-Term	T$_{44}$		71.1 ± 0.0	38.5 ± 0.7	43.4 ± 1.2
N-Term	S$_{51}$		58.9 ± 0.0	31.0 ± 0.4	44.4 ± 0.9
N-Term	K$_{54}$		55.2 ± 0.0	28.0 ± 0.4	39.3 ± 0.8
N-Term	K$_{55}$		52.7 ± 0.0	30.3 ± 0.3	40.5 ± 0.8
N-Term	T$_{61}$	5F	45.2 ± 0.0	23.4 ± 0.1	32.5 ± 0.5
nMES$_{54}$S$_{55}$T$_{56}$	DVMRLQIK$_{78}$VN				
N-Term	K$_{78}$		40.4 ± 0.0	29.1 ± 0.1	30.4 ± 0.7
S$_{54}$	K$_{78}$		43.2 ± 0.0	32.5 ± 0.1	28.7 ± 0.8
S$_{55}$	K$_{78}$		41.4 ± 0.0	31.9 ± 0.1	30.3 ± 0.8
T$_{56}$	K$_{78}$		37.8 ± 0.0	28.6 ± 0.1	32.9 ± 0.8
nMES$_{54}$S$_{55}$T$_{56}$DGQVVPQE	HY$_{43}$T$_{44}$HPRNVGS$_{51}$LDK$_{54}$K$_{55}$LPNVGTGLVGAPACG				
N-Term	Y$_{43}$		73.5 ± 0.0	40.8 ± 0.7	44.5 ± 1.2
N-Term	T$_{44}$		71.1 ± 0.0	38.5 ± 0.7	43.4 ± 1.2
N-Term	S$_{51}$		58.9 ± 0.0	31.0 ± 0.4	44.4 ± 0.9
N-Term	K$_{54}$		55.2 ± 0.0	28.0 ± 0.4	39.3 ± 0.8
N-Term	K$_{55}$		52.7 ± 0.0	30.3 ± 0.3	40.5 ± 0.8
N-Term	T$_{61}$		45.2 ± 0.0	23.4 ± 0.1	32.5 ± 0.5
S$_{54}$	K$_{54}$		58.0 ± 0.0	33.1 ± 0.4	40.4 ± 1.0
S$_{55}$	K$_{54}$		56.2 ± 0.0	33.4 ± 0.5	40.6 ± 0.9
T$_{56}$	K$_{54}$		52.6 ± 0.0	30.1 ± 0.4	40.6 ± 0.9
S$_{54}$	K$_{55}$		55.7 ± 0.0	35.1 ± 0.4	41.2 ± 0.9
S$_{55}$	K$_{55}$		54.0 ± 0.0	35.2 ± 0.4	41.6 ± 0.9
T$_{56}$	K$_{55}$		50.4 ± 0.0	31.8 ± 0.4	41.9 ± 0.8

Cross-linked peptides			Distance (Å)		
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**
K$_{72}$AHEE	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
K$_{72}$	N-Term		47.3 ± 0.0	43.7 ± 0.4	66.5 ± 0.8
K$_{72}$	S$_{25}$		49.2 ± 0.0	43.5 ± 0.5	65.5 ± 0.9
K$_{72}$	S$_{28}$		52.6 ± 0.0	44.7 ± 0.5	63.7 ± 0.9
K$_{72}$	S$_{29}$		52.2 ± 0.0	44.7 ± 0.5	62.6 ± 0.9
K$_{72}$	T$_{31}$		57.0 ± 0.0	47.7 ± 0.5	63.1 ± 1.0
K$_{72}$	K$_{32}$		58.6 ± 0.0	50.2 ± 0.5	65.5 ± 1.0
K$_{72}$	Y$_{35}$		52.5 ± 0.0	46.6 ± 0.4	61.7 ± 0.9
K$_{72}$	K$_{38}$		53.2 ± 0.0	44.3 ± 0.5	58.3 ± 0.9
K$_{72}$AHEE	DVMRLQIK$_{78}$VNDS$_{82}$T$_{83}$GVIE				
K$_{72}$	K$_{78}$		21.6 ± 0.0	25.0 ± 0.3	47.5 ± 0.9
K$_{72}$	S$_{82}$		15.4 ± 0.0	26.6 ± 0.3	41.3 ± 0.7
K$_{72}$	T$_{83}$		12.1 ± 0.0	26.3 ± 0.3	38.3 ± 0.8
K$_{72}$AHEE	DK$_{54}$K$_{55}$LPNVGT$_{61}$GLVGAPACGDVMRLQIK$_{78}$VN				
K$_{72}$	K$_{54}$	5F	35.9 ± 0.0	29.8 ± 0.3	44.6 ± 0.7
K$_{72}$	K$_{55}$		34.1 ± 0.0	29.8 ± 0.3	46.8 ± 0.7
K$_{72}$	T$_{61}$		24.4 ± 0.0	18.1 ± 0.3	38.5 ± 0.6
K$_{72}$	K$_{78}$		21.6 ± 0.0	25.0 ± 0.3	45.4 ± 0.5
DS$_{87}$LE	DK$_{54}$K$_{55}$LPNVGT$_{61}$GLVGAPACGDVMRLQIK$_{78}$VN				
S$_{87}$	K$_{54}$		36.0 ± 0.0	43.5 ± 0.1	41.2 ± 0.9
S$_{87}$	K$_{55}$		35.8 ± 0.0	43.6 ± 0.2	43.3 ± 0.8
S$_{87}$	K$_{78}$	5F	25.7 ± 0.0	40.2 ± 0.2	39.8 ± 0.8
DS$_{87}$LEE	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
S$_{87}$	N-Term		55.6 ± 0.0	52.9 ± 0.4	65.9 ± 0.9
S$_{87}$	K$_{32}$		61.9 ± 0.0	59.6 ± 0.3	65.7 ± 1.0
S$_{87}$	K$_{38}$		55.0 ± 0.0	54.7 ± 0.2	57.9 ± 1.0
LS$_{105}$HGVMT$_{110}$LE	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
S$_{105}$	N-Term		69.0 ± 0.0	67.4 ± 0.4	69.6 ± 1.0
S$_{105}$	K$_{32}$		75.8 ± 0.0	74.7 ± 0.4	74.0 ± 1.0
S$_{105}$	K$_{38}$		68.8 ± 0.0	69.2 ± 0.3	66.7 ± 1.1
T$_{110}$	N-Term		63.9 ± 0.0	62.9 ± 0.4	73.1 ± 1.0
T$_{110}$	K$_{32}$		69.0 ± 0.0	71.5 ± 0.3	75.4 ± 1.0
T$_{110}$	K$_{38}$		61.7 ± 0.0	66.7 ± 0.3	67.7 ± 1.0
WVS$_{151}$LRNGT$_{156}$K$_{157}$LT$_{159}$DILT$_{163}$E	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
K$_{157}$	N-Term		47.9 ± 0.0	46.8 ± 0.5	76.1 ± 0.7
K$_{157}$	S$_{25}$		49.0 ± 0.0	46.9 ± 0.4	74.9 ± 0.7
K$_{157}$	S$_{28}$		50.7 ± 0.0	49.8 ± 0.4	74.0 ± 0.8
K$_{157}$	S$_{29}$		50.0 ± 0.0	50.6 ± 0.4	73.8 ± 0.8
K$_{157}$	T$_{31}$		53.8 ± 0.0	54.2 ± 0.3	74.4 ± 0.9
K$_{157}$	K$_{32}$		55.8 ± 0.0	56.7 ± 0.4	77.1 ± 0.9
K$_{157}$	Y$_{35}$		49.8 ± 0.0	54.2 ± 0.3	74.6 ± 0.8
K$_{157}$	K$_{38}$		49.4 ± 0.0	52.1 ± 0.3	70.8 ± 0.9
S$_{151}$	N-Term		47.6 ± 0.0	47.9 ± 0.5	73.8 ± 0.7
T$_{156}$	N-Term		45.5 ± 0.0	45.2 ± 0.5	74.2 ± 0.7
T$_{159}$	N-Term		51.2 ± 0.0	49.3 ± 0.4	77.5 ± 0.6
T$_{163}$	N-Term		51.5 ± 0.0	49.7 ± 0.4	74.5 ± 0.6
S$_{151}$	K$_{32}$		55.8 ± 0.0	59.6 ± 0.4	74.7 ± 0.9
T$_{156}$	K$_{32}$		53.2 ± 0.0	56.4 ± 0.4	74.6 ± 0.9
T$_{159}$	K$_{32}$		58.5 ± 0.0	57.6 ± 0.3	79.3 ± 0.9
T$_{163}$	K$_{32}$		56.7 ± 0.0	57.8 ± 0.2	76.2 ± 0.9
S$_{151}$	K$_{38}$		49.6 ± 0.0	55.1 ± 0.4	68.0 ± 0.9
T$_{156}$	K$_{38}$		46.9 ± 0.0	52.1 ± 0.3	68.1 ± 0.9
T$_{159}$	K$_{38}$		51.9 ± 0.0	52.8 ± 0.3	73.3 ± 0.9
T$_{163}$	K$_{38}$		49.7 ± 0.0	53.6 ± 0.2	70.7 ± 0.9
K$_{168}$AIS$_{171}$K$_{172}$SQ	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
K$_{168}$	N-Term		53.0 ± 0.0	52.0 ± 0.3	68.3 ± 0.6
K$_{168}$	S$_{25}$		53.6 ± 0.0	51.2 ± 0.3	66.6 ± 0.7
K$_{168}$	S$_{28}$		52.9 ± 0.0	53.9 ± 0.2	66.2 ± 0.8
K$_{168}$	S$_{29}$		51.1 ± 0.0	55.5 ± 0.2	66.6 ± 0.8
K$_{168}$	T$_{31}$		53.5 ± 0.0	58.3 ± 0.1	67.2 ± 0.9
K$_{168}$	K$_{32}$		55.1 ± 0.0	61.0 ± 0.1	70.1 ± 0.9
K$_{168}$	Y$_{35}$		48.0 ± 0.0	60.7 ± 0.0	69.2 ± 0.9
K$_{168}$	K$_{38}$		47.5 ± 0.0	57.6 ± 0.0	65.0 ± 0.9
K$_{172}$	N-Term		58.0 ± 0.0	57.0 ± 0.3	66.1 ± 0.7

Cross-linked peptides			Distance (Å)		
Yfh1	**Isu1**	**Figure**	**Yfh1-Isu1 intra-dimer**	**Yfh1-Isu1 intra-trimer**	**Yfh1-Isu1 inter-trimers**
K$_{172}$	S$_{25}$		58.5 ± 0.0	55.9 ± 0.3	64.4 ± 0.7
K$_{172}$	S$_{28}$		57.0 ± 0.0	58.4 ± 0.1	64.4 ± 0.8
K$_{172}$	S$_{29}$		54.7 ± 0.0	60.2 ± 0.1	65.2 ± 0.9
K$_{172}$	T$_{31}$		56.5 ± 0.0	62.5 ± 0.0	65.9 ± 1.0
K$_{172}$	K$_{32}$		58.0 ± 0.1	65.3 ± 0.0	68.8 ± 1.0
K$_{172}$	Y$_{35}$		50.6 ± 0.0	65.7 ± 0.0	68.8 ± 0.9
K$_{172}$	K$_{38}$		50.1 ± 0.0	62.3 ± 0.1	64.5 ± 1.0
S$_{171}$	N-Term		55.4 ± 0.0	53.9 ± 0.3	69.1 ± 0.6
S$_{171}$	K$_{32}$		56.2 ± 0.0	61.7 ± 0.0	71.0 ± 0.9
S$_{171}$	K$_{38}$		48.5 ± 0.0	58.6 ± 0.1	66.5 ± 0.9
K$_{168}$AIS$_{171}$K$_{172}$SQ	DVMRLQIK$_{78}$VN				
K$_{168}$	K$_{78}$		23.7 ± 0.0	44.4 ± 0.2	43.0 ± 0.8
K$_{172}$	K$_{78}$		29.6 ± 0.0	51.0 ± 0.3	40.4 ± 0.8
S$_{171}$	K$_{78}$		26.6 ± 0.0	49.3 ± 0.2	43.8 ± 0.8
VEK$_{168}$AIS$_{171}$K$_{172}$SQ	DK$_{54}$K$_{55}$LPNVGT$_{61}$GLVGAPACG				
K$_{168}$	K$_{54}$		28.8 ± 0.0	49.4 ± 0.0	51.8 ± 1.0
K$_{168}$	K$_{55}$		29.8 ± 0.0	48.8 ± 0.1	51.9 ± 0.9
K$_{168}$	T$_{61}$		22.9 ± 0.0	39.7 ± 0.1	45.7 ± 0.7
K$_{172}$	K$_{54}$		32.2 ± 0.0	55.4 ± 0.0	47.4 ± 1.0
K$_{172}$	K$_{55}$		33.8 ± 0.0	55.1 ± 0.0	47.9 ± 1.0
K$_{172}$	T$_{61}$		29.0 ± 0.0	46.5 ± 0.0	42.8 ± 0.8
S$_{171}$	K$_{54}$		30.3 ± 0.0	51.8 ± 0.0	51.1 ± 1.0
S$_{171}$	K$_{55}$		31.6 ± 0.0	51.6 ± 0.0	51.5 ± 1.0
LS$_{105}$HGVMT$_{110}$LE	DAIK$_{148}$AAIK$_{152}$				
S$_{105}$	K$_{148}$		31.8 ± 0.0	37.5 ± 0.3	30.7 ± 1.0
S$_{105}$	K$_{152}$		35.7 ± 0.0	37.2 ± 0.4	35.0 ± 1.0
T$_{110}$	K$_{148}$		25.7 ± 0.0	37.5 ± 0.3	34.2 ± 1.0
T$_{110}$	K$_{152}$		30.5 ± 0.0	36.0 ± 0.3	39.2 ± 1.0

Single digestion (Glu-C) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)		
Isu1	**Isu1**	**Figure**	**Isu1 intra-monomer**	**Isu1-Isu1 intra-trimer**	**Isu1-Isu1 inter-trimers**
IAK$_{128}$E	n-GS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
K$_{128}$	N-Term		55.2 ± 0.0	55.4 ± 0.4	63.1 ± 0.6
K$_{128}$	S$_{25}$		55.5 ± 0.0	54.9 ± 0.3	61.7 ± 0.7
K$_{128}$	S$_{28}$		54.5 ± 0.0	58.3 ± 0.2	61.6 ± 0.8
K$_{128}$	S$_{29}$		52.8 ± 0.0	60.0 ± 0.2	62.0 ± 0.8
K$_{128}$	T$_{31}$		54.8 ± 0.0	63.1 ± 0.1	63.0 ± 0.9
K$_{128}$	K$_{32}$		56.6 ± 0.0	65.8 ± 0.1	65.9 ± 0.9
K$_{128}$	Y$_{35}$		50.0 ± 0.0	65.3 ± 0.1	64.7 ± 0.9
K$_{128}$	K$_{38}$		48.9 ± 0.0	62.5 ± 0.1	60.7 ± 1.0

Double digestion (Glu-C and Asp-N) and analysis of cross-linked peptides and distances

Cross-linked peptides			Distance (Å)		
Isu1	**Isu1**	**Figure**	**Isu1 intra-monomer**	**Isu1-Isu1 intra-trimer**	**Isu1-Isu1 inter-trimers**
IAK$_{128}$E	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIE				
K$_{128}$	N-Term		55.2 ± 0.0	55.4 ± 0.4	63.1 ± 0.6
K$_{128}$	S$_{25}$		55.5 ± 0.0	54.9 ± 0.3	61.7 ± 0.7
K$_{128}$	S$_{28}$		54.5 ± 0.0	58.3 ± 0.2	61.6 ± 0.8
K$_{128}$	S$_{29}$		52.8 ± 0.0	60.0 ± 0.2	62.0 ± 0.8
K$_{128}$	T$_{31}$		54.8 ± 0.0	63.1 ± 0.1	63.0 ± 0.9
K$_{128}$	K$_{32}$		56.6 ± 0.0	65.8 ± 0.1	65.9 ± 0.9
K$_{128}$	Y$_{35}$		50.0 ± 0.0	65.3 ± 0.1	64.7 ± 0.9
K$_{128}$	K$_{38}$		48.9 ± 0.0	62.5 ± 0.1	60.7 ± 1.0
DAIK$_{148}$AAIK$_{152}$	nGS$_{25}$HMS$_{28}$S$_{29}$IT$_{31}$K$_{32}$RLY$_{35}$HPK$_{38}$VIEHY$_{43}$T$_{44}$HPRNVGS$_{51}$L				
K$_{148}$	N-Term		39.1 ± 0.0	40.3 ± 0.5	69.5 ± 0.6
K$_{148}$	S$_{25}$		39.5 ± 0.0	40.6 ± 0.4	67.9 ± 0.7
K$_{148}$	S$_{28}$		40.1 ± 0.0	45.2 ± 0.3	65.9 ± 0.7
K$_{148}$	S$_{29}$		39.3 ± 0.0	46.8 ± 0.3	65.3 ± 0.7
K$_{148}$	T$_{31}$		42.6 ± 0.0	51.2 ± 0.2	65.2 ± 0.8
K$_{148}$	K$_{32}$		44.7 ± 0.0	53.7 ± 0.3	67.8 ± 0.8
K$_{148}$	Y$_{35}$		39.2 ± 0.0	52.0 ± 0.3	65.5 ± 0.8

Cross-linked peptides			Distance (Å)		
Isu1	**Isu1**	**Figure**	**Isu1 intra-monomer**	**Isu1-Isu1 intra-trimer**	**Isu1-Isu1 inter-trimers**
K_{148}	K_{38}		38.1 ± 0.0	50.5 ± 0.2	61.4 ± 0.8
K_{148}	Y_{43}		37.4 ± 0.0	50.3 ± 0.2	55.9 ± 0.9
K_{148}	T_{44}		34.9 ± 0.0	49.1 ± 0.2	54.7 ± 0.9
K_{148}	S_{51}		25.8 ± 0.0	46.8 ± 0.2	54.6 ± 0.7
K_{152}	N-Term		34.9 ± 0.0	36.6 ± 0.5	72.3 ± 0.6
K_{152}	S_{25}		35.3 ± 0.0	37.4 ± 0.4	70.8 ± 0.7
K_{152}	S_{28}		36.8 ± 0.0	42.5 ± 0.4	68.3 ± 0.7
K_{152}	S_{29}		36.5 ± 0.0	44.0 ± 0.4	67.4 ± 0.7
K_{152}	T_{31}		40.2 ± 0.0	48.9 ± 0.3	67.0 ± 0.8
K_{152}	K_{32}		42.4 ± 0.0	51.1 ± 0.3	69.5 ± 0.8
K_{152}	Y_{35}		37.7 ± 0.0	49.0 ± 0.4	66.8 ± 0.8
K_{152}	K_{38}		36.5 ± 0.0	48.0 ± 0.3	62.7 ± 0.8
K_{152}	Y_{43}		36.2 ± 0.0	48.6 ± 0.3	57.0 ± 0.9
K_{152}	T_{44}		34.0 ± 0.0	47.2 ± 0.3	55.8 ± 0.8
K_{152}	S_{51}		26.5 ± 0.0	43.7 ± 0.3	55.1 ± 0.7
DAAK_{120}IK_{122}NT_{124}EIAK_{128}E	D$K_{54}K_{55}$LPNVGT_{61}GLVGAPACG				
K_{120}	K_{54}		27.2 ± 0.0	43.4 ± 0.3	51.7 ± 0.7
K_{120}	K_{55}		27.3 ± 0.0	42.0 ± 0.3	53.5 ± 0.6
K_{120}	T_{61}		15.4 ± 0.0	31.4 ± 0.3	44.5 ± 0.4
K_{122}	K_{54}		26.7 ± 0.0	49.1 ± 0.2	47.9 ± 0.7
K_{122}	K_{55}		27.7 ± 0.0	47.9 ± 0.3	49.4 ± 0.6
K_{122}	T_{61}	5F	17.8 ± 0.0	37.8 ± 0.2	40.6 ± 0.4
K_{128}	K_{54}		30.5 ± 0.0	53.5 ± 0.1	51.1 ± 0.9
K_{128}	K_{55}		32.0 ± 0.0	52.8 ± 0.1	52.2 ± 0.8
K_{128}	T_{61}		24.3 ± 0.0	43.0 ± 0.2	43.2 ± 0.6
T_{124}	K_{54}		29.1 ± 0.0	51.0 ± 0.2	48.7 ± 0.8
T_{124}	K_{55}		30.2 ± 0.0	49.9 ± 0.2	50.0 ± 0.7
DDAAK_{120}IK_{122}NT_{124}EIAK_{128}E	H$Y_{43}T_{44}$HPRNVGS_{51}LD$K_{54}K_{55}$LPNVGT_{61}GLVGAPACG				
K_{120}	Y_{43}		44.3 ± 0.0	54.0 ± 0.3	58.7 ± 1.0
K_{120}	T_{44}		41.9 ± 0.0	52.6 ± 0.3	57.4 ± 1.0
K_{120}	S_{51}		32.1 ± 0.0	49.7 ± 0.3	56.1 ± 0.8
K_{120}	K_{54}		27.2 ± 0.0	43.4 ± 0.3	51.7 ± 0.7
K_{120}	K_{55}		27.3 ± 0.0	42.0 ± 0.3	53.5 ± 0.6
K_{120}	T_{61}		15.4 ± 0.0	31.4 ± 0.3	44.5 ± 0.4
K_{122}	Y_{43}		43.1 ± 0.0	58.0 ± 0.1	54.1 ± 1.0
K_{122}	T_{44}		40.6 ± 0.0	56.9 ± 0.1	53.0 ± 1.0
K_{122}	S_{51}		30.8 ± 0.0	55.5 ± 0.2	52.7 ± 0.8
K_{122}	K_{54}		26.7 ± 0.0	49.1 ± 0.2	47.9 ± 0.7
K_{122}	K_{55}		27.7 ± 0.0	47.9 ± 0.3	49.4 ± 0.6
K_{122}	T_{61}		17.8 ± 0.0	37.8 ± 0.2	40.6 ± 0.4
K_{128}	Y_{43}		47.2 ± 0.0	60.5 ± 0.1	56.2 ± 1.1
K_{128}	T_{44}		44.5 ± 0.0	59.7 ± 0.1	55.3 ± 1.1
K_{128}	S_{51}		33.2 ± 0.0	59.8 ± 0.1	56.4 ± 0.9
K_{128}	K_{54}		30.5 ± 0.0	53.5 ± 0.1	51.1 ± 0.9
K_{128}	K_{55}		32.0 ± 0.0	52.8 ± 0.1	52.2 ± 0.8
K_{128}	T_{61}		24.3 ± 0.0	43.0 ± 0.2	43.2 ± 0.6
DAIK_{148}AAIK_{152}	D$K_{54}K_{55}$LPNVGT_{61}GLVGAPACG				
K_{148}	K_{54}		20.3 ± 0.0	40.7 ± 0.2	50.8 ± 0.6
K_{148}	K_{55}	5F	20.2 ± 0.0	37.9 ± 0.3	52.9 ± 0.5
K_{148}	T_{61}		8.1 ± 0.0	29.7 ± 0.1	45.4 ± 0.3
K_{152}	K_{54}	5F	20.4 ± 0.0	34.8 ± 0.2	51.8 ± 0.6
K_{152}	K_{55}		19.5 ± 0.0	32.0 ± 0.3	54.3 ± 0.5
K_{152}	T_{61}	5F	7.1 ± 0.0	23.9 ± 0.1	47.2 ± 0.4

Cross-linked peptides			Distance (Å)		
Yfh1	**Yfh1**	**Figure**	**Yfh1 intra-monomer**	**Yfh1-Yfh1 intra-trimer**	**Yfh1-Yfh1 inter-trimers**
nME	LS_{105}HGVMT_{110}LE				
N-Term	S_{105}		38.8 ± 0.0	32.5 ± 0.9	25.9 ± 1.5
N-Term	T_{110}		41.8 ± 0.0	24.4 ± 0.9	25.0 ± 1.5

Shown are all of the Yfh1-Isu1 (Supplementary Table S1a), Yfh1-Yfh1 (Supplementary Table S1b), and Isu1-Isu1 (Supplementary Table S1c) cross-linked peptides with the corresponding FDR (False Discovery Rate) identified as described in Experimental Procedures. Supplementary Tables S1d - S1f correspond to the alternative orientations of the N-terminal portion of Isu1. Some of the cross-links are shown in Fig. 4-8, as indicated in the table (column labeled Figure). The number of times any given peptide was identified by MS/MS is also shown (column labeled # of peptides). The distance constraints and maximum allowable distance constraints between K-K, K-N-term, N-term-N-term, K-Y, K-S, K-T, N-term-S, N-term-T, and N-term-Y were calculated as described in Experimental Procedures and are shown below. Distances were measured between all possible pairs of cross-linked residues within each of the cross-linked peptides in the entire complex structure, and are expressed as mean ± standard deviation. For each distance, we show the mean ± standard deviation of 24 measurements except for certain *inter-trimers* distances that are only present 12 times in the $[\text{Yfh1}]_{24}\bullet[\text{Isu1}]_{24}$ structure. The small standard deviations demonstrate that in the structure there are small differences in the orientation of the side chain of any given cross-linked residue among the 24 Yfh1 or Isu1 subunits. Distances measured in the $[\text{Yfh1}]_{24}\bullet[\text{Isu1}]_{24}$ structure that are equal to or lower than the distance constraints are highlighted in light gray; distances that are equal to or lower than the maximum allowable distance constraints are highlighted in dark gray, and distances greater than the maximum allowable distance constraints are highlighted in yellow. In Supplementary Table S1a, S1d-S1f, Yfh1-Isu1 *intra-dimer* distances are measured between Yfh1 and Isu1 subunits of the same $[\text{Yfh1}]\bullet[\text{Isu1}]$ heterodimer; Yfh1-Isu1 *intra-trimer* distances are measured between Yfh1 and Isu1 subunits of the same $[\text{Yfh1}]_3\bullet[\text{Isu1}]_3$ sub-complex, and Yfh1-Isu1 *inter-trimers* distances are measured between Yfh1 and Isu1 subunits of two adjacent $[\text{Yfh1}]_3\bullet[\text{Isu1}]_3$ sub-complexes. In Supplementary Table S1b, S1c or S1d-S1f, Yfh1 or Isu1 *intra-monomer* distances are measured within individual Yfh1 or Isu1 subunits, Yfh1-Yfh1 or Isu1-Isu1 *intra-trimer* distances are measured between Yfh1 or Isu1 subunits of the same $[\text{Yfh1}]_3\bullet[\text{Isu1}]_3$ sub-complex, and Yfh1-Yfh1 or Isu1-Isu1 *inter-trimers* distances are measured between Yfh1 or Isu1 subunits of two adjacent $[\text{Yfh1}]_3\bullet[\text{Isu1}]_3$ sub-complexes. Peptides beginning with the letter n are N-terminal peptides. NA, not applicable; NM, not measured.

	Distance Constraints (Å)
K-K	24
K-N-term	19.2
N-term-N-term	14.4
K-Y	24.1
K-S	20.1
K-T	20.1
N-term-S	15.3
N-term-T	15.3
N-term-Y	19.3

Maximum Allowable Distance Constraints (Å)			
	Yfh1-Isu1	Yfh1-Yfh1	Isu1-Isu1
K-K	24-31.8	24-34.2	24-29.4
K-N-term	19.2-27	19.2-29.4	19.2-24.6
N-term-N-term	14.4-22.2	14.4-24.6	14.4-19.8
K-Y	24-31.8	24-34.3	24-29.5
K-S	20.1-27.9	20.1-30.3	20.1-25.5
K-T	20.1-27.9	20.1-30.3	20.1-25.5
N-term-S	15.3-23.1	15.3-25.5	15.3-20.7
N-term-T	15.3-23.1	15.3-25.5	15.3-20.7
N-term-Y	19.3-27.1	19.3-29.5	19.3-24.7

Supplementary Table S2. Zinc binding properties of Yfh1^{Y73A} 24-mer, LMW Isu1 and HMW Isu1, and their complexes

Before Assembly				After Assembly and SEC		
Protein	Yfh1^{Y73A} Oligomer	LMW Isu1	HMW Isu1	Complex	[Yfh1]$_{24}$•[Isu1^{LMW}]$_{24}$	[Yfh1]$_{24}$•[Isu1^{HMW}]$_{24}$
Zn^{2+}/ subunit	0.395 ± 0.004	0.677 ± 0.003	0.025 ± 0.000	Zn^{2+}/ [Yfh1]•[Isu1]	1.508 ± 0.182	1.604 ± 0.090

Before Assembly: Yfh1^{Y73A} 24-mer, LMW Isu1, and HMW Isu1 were isolated and their zinc content was measured as described in Experimental Procedures. After Assembly and SEC: The same protein preparations were used to assemble [Yfh1]$_{24}$•[Isu1^{LMW}]$_{24}$ and [Yfh1]$_{24}$•[Isu1^{HMW}]$_{24}$ complexes that were isolated by size exclusion chromatography (SEC). Fractions 52-56 of each complex were independently used for zinc and protein concentration measurements and the average Zn^{2+}/ [Yfh1]•[Isu1] heterodimer molar ratios were calculated. For each complex we show the mean ± standard deviation obtained from one set of fractions 52, 53, 55 and 56 from one complex preparation, and two sets of fraction 54 from two different complex preparations; each fraction was measured in duplicate. The zinc concentration was 0.011 µg/ml in the assembly reaction buffer and 0.024 µg/ml in the SEC buffer. The total amount of zinc present in the elution volume of each complex was 1.26 µg, sufficient to account for the increase in zinc content exhibited by the [Yfh1]$_{24}$•[Isu1^{LMW}]$_{24}$ and the [Yfh1]$_{24}$•[Isu1^{HMW}]$_{24}$ complex after SEC.