SUPPORTING INFORMATION

Expanding the Depth and Sensitivity of Cross-Link Identification by Differential Ion Mobility Using High-Field Asymmetric Waveform Ion Mobility Spectrometry

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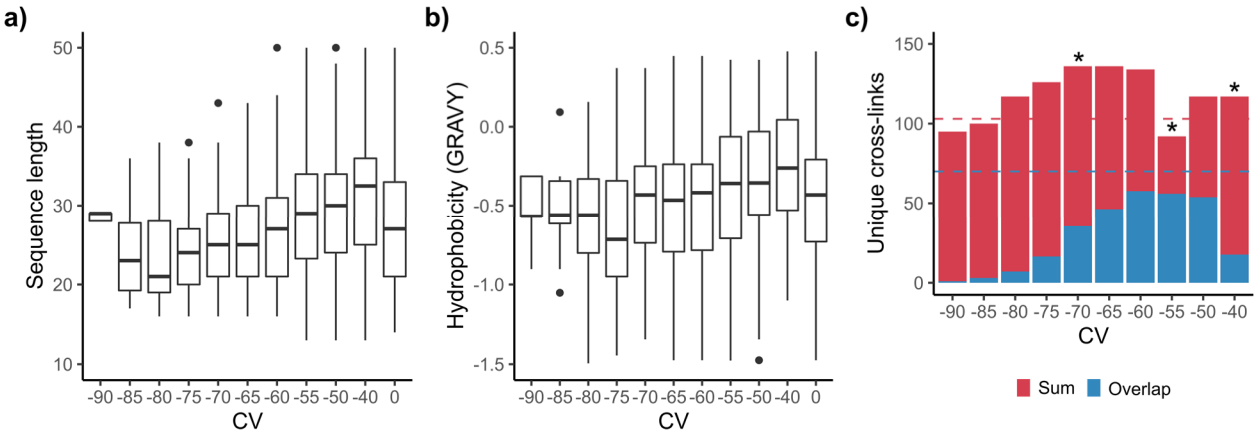
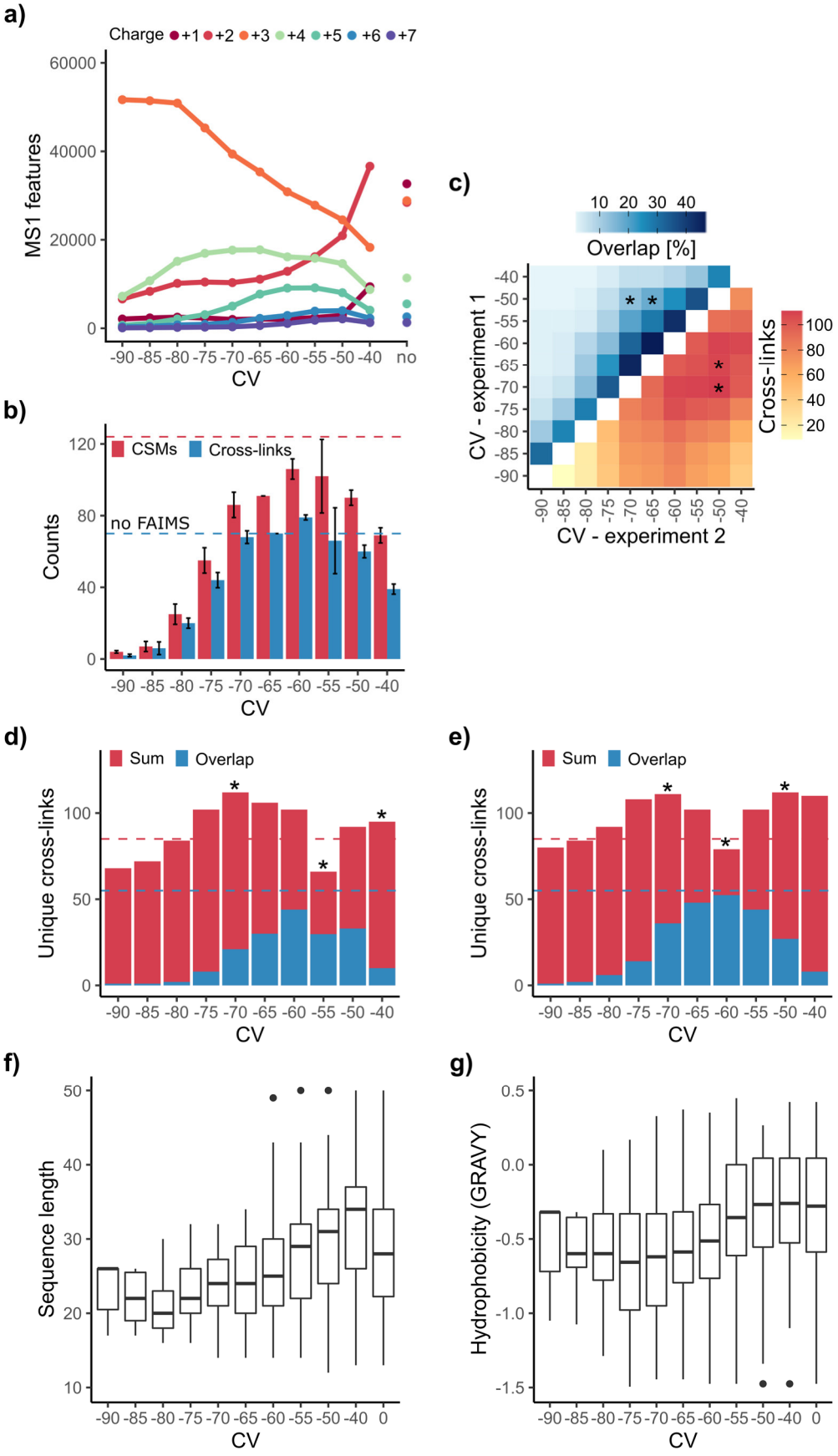
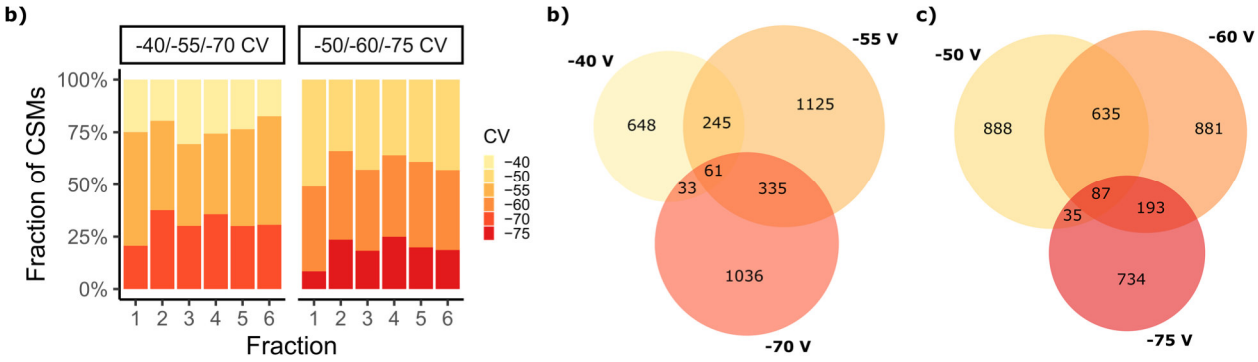


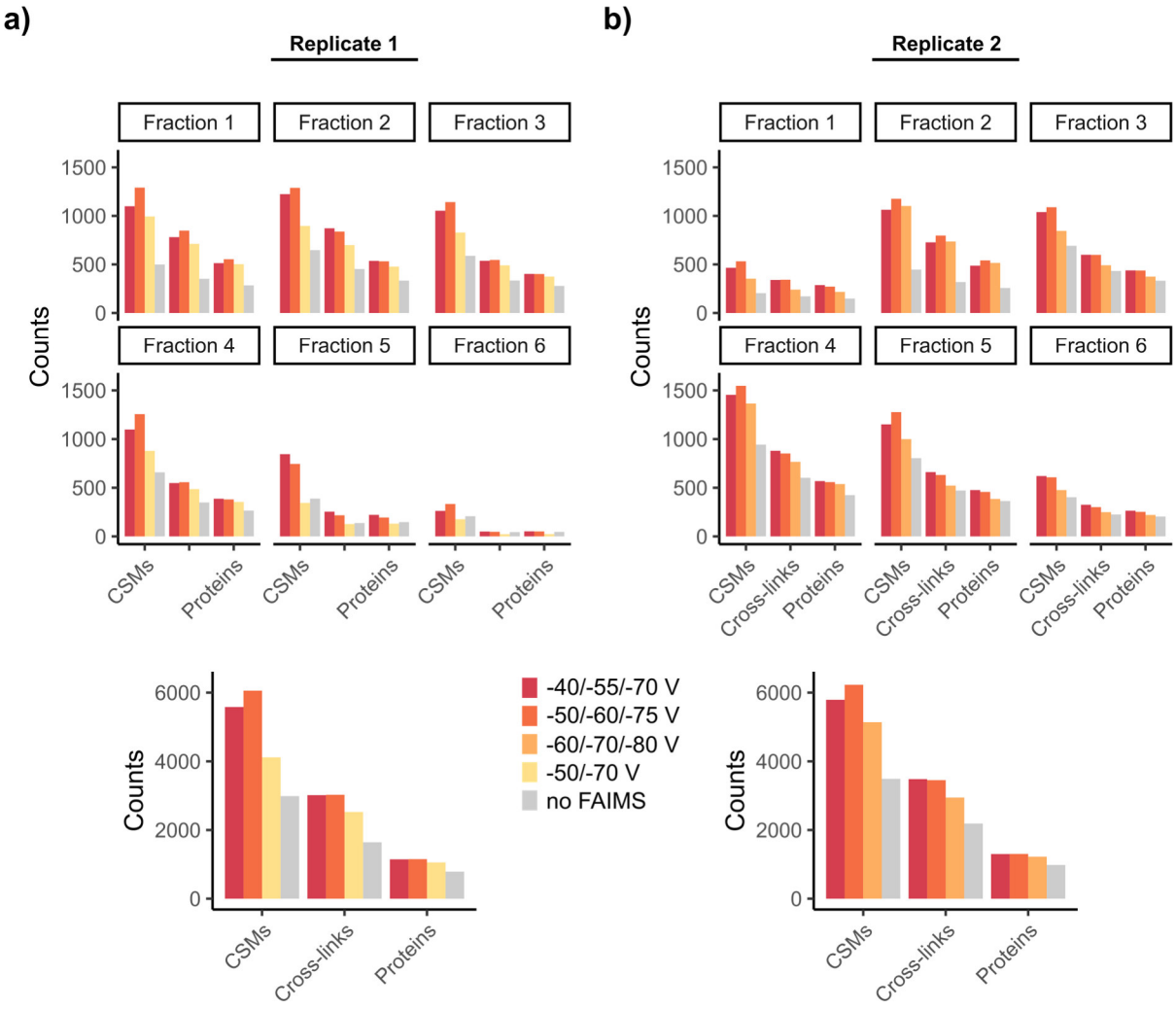
Supplementary Figure 1. Additional analysis of DSSO cross-linked eight protein mixture of the first biological replicate. (a) Distribution of the length of linearized cross-link sequence along the selected CV range. (b) Distribution of predicted hydrophobicity based on Gravy index score (> 0 hydrophobic, < 0 hydrophilic) along selected CV range. Analysis without FAIMS is indicated with “no”. (c) Stacked bar plot to determine the best 3-CV combination. A first experiment (single CV measurement at -55 V) is combined with a second experiment at different CVs. Local maxima on both sides of the initial CV suggest which three CVs to combine in order to maximize unique cross-link identification (red) and minimize overlap between CVs (blue). A 3-CV combination (-40/-55/-70, marked with *) is selected based on this analysis. Analysis without FAIMS is shown as a dotted line.


a)
Charge +1 +2 +3 +4 +5 +6 +7
MS1 features
60000
40000
20000
0
-90 -85 -80 -75 -70 -65 -60 -55 -50 -40 no
CV
b)
CSMs
Cross-links
no FAIMS
Counts
120
80
40
0
-90 -85 -80 -75 -70 -65 -60 -55 -50 -40
CV
c)
10 20 30 40
Overlap [%]
CV - experiment 1
CV - experiment 2
Cross-links
100
80
60
40
20
d)
Sum
Overlap
Unique cross-links
100
50
0
-90 -85 -80 -75 -70 -65 -60 -55 -50 -40
CV
e)
Sum
Overlap
Unique cross-links
100
50
0
-90 -85 -80 -75 -70 -65 -60 -55 -50 -40
CV
f)
Sequence length
50
40
30
20
10
-90 -85 -80 -75 -70 -65 -60 -55 -50 -40 0
CV
g)
Hydrophobicity (GRAVY)
0.5
0.0
-0.5
-1.0
-1.5
-90 -85 -80 -75 -70 -65 -60 -55 -50 -40 0
CV

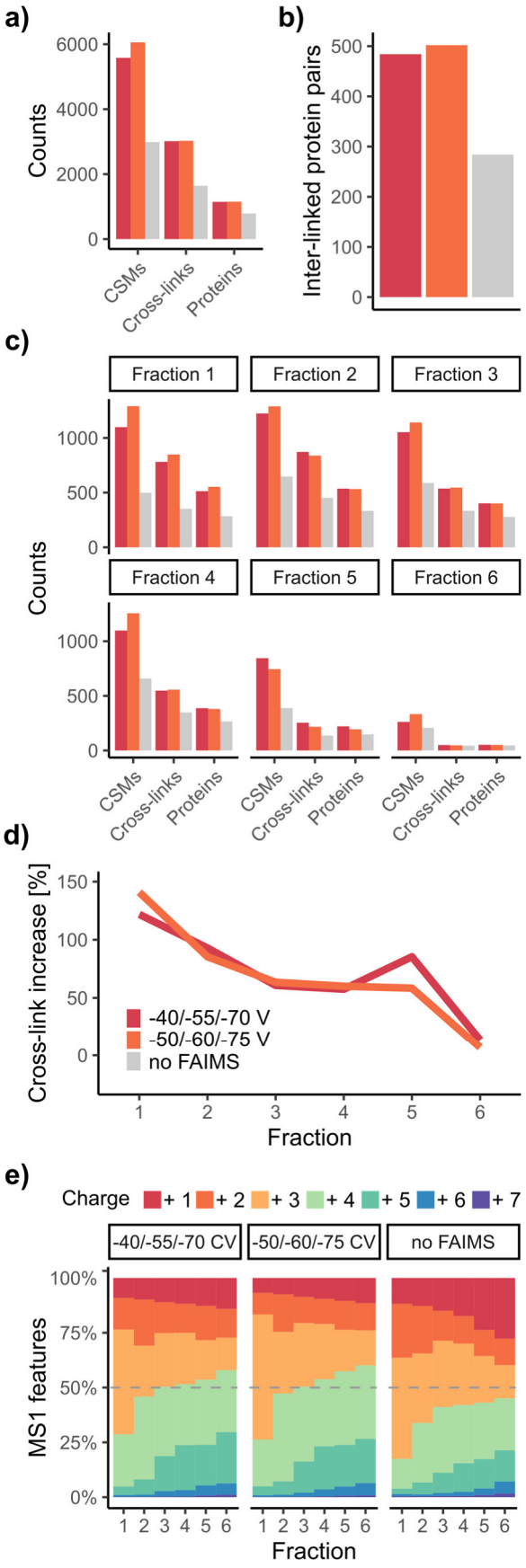
Supplementary Figure 2. Analysis of FAIMS CV settings using a second biological replicate of DSSO cross-linked eight protein mixture (analyzed in two technical duplicates). (a) Charge distribution of MS1 features along the selected FAIMS CV range and without FAIMS ("no"). (b) Number of CSMs and unique cross-links for individual CVs and without FAIMS. Analyses without FAIMS are shown as dotted lines. (c) Analysis of unique cross-links and overlap between two individual 1-CV measurements. * note the selected 2-CV combinations. (d, e) Stacked bar plot to determine the best 3-CV combination. A first experiment (single CV measurement at -55 V (d) and -60 V (e)) is combined with a second experiment at different CVs. Local maxima on both sides of the initial CV suggest which three CVs to combine in order to maximize unique cross-link identification (red) and minimize overlap between CVs (blue). A 3-CV combination (-40/-55/-70 and -50/-60/-75 V, marked with *) is selected based on this analysis. Analysis without FAIMS is shown as a dotted line. (f) Distribution of the length of linearized cross-link sequence along the selected CV range. (g) Distribution of predicted hydrophobicity based on Gravy index score (> 0 hydrophobic, < 0 hydrophilic) along selected CV range. Analysis without FAIMS is indicated with "no".



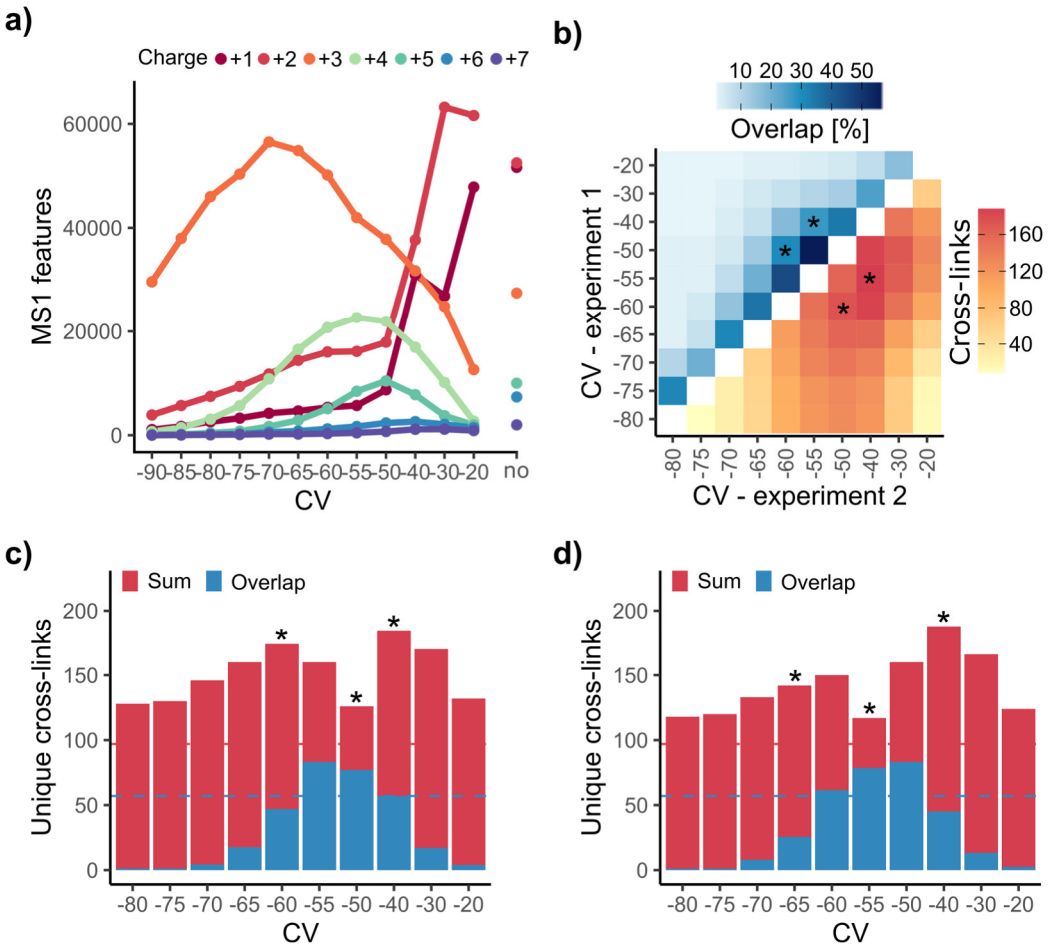
Supplementary Figure 3. Analysis of cross-link identification of individual FAIMS CVs using six SCX fractions from DSSO cross-linked HEK293T cell lysate. (a) Percentage of CSMs identified by each CV within one 3-CV combination. (b, c) Overlap of cross-links between measurements of different CVs of (b) -40/-55/-70 V and (c) -50/-60/-75 V from six SCX fractions.



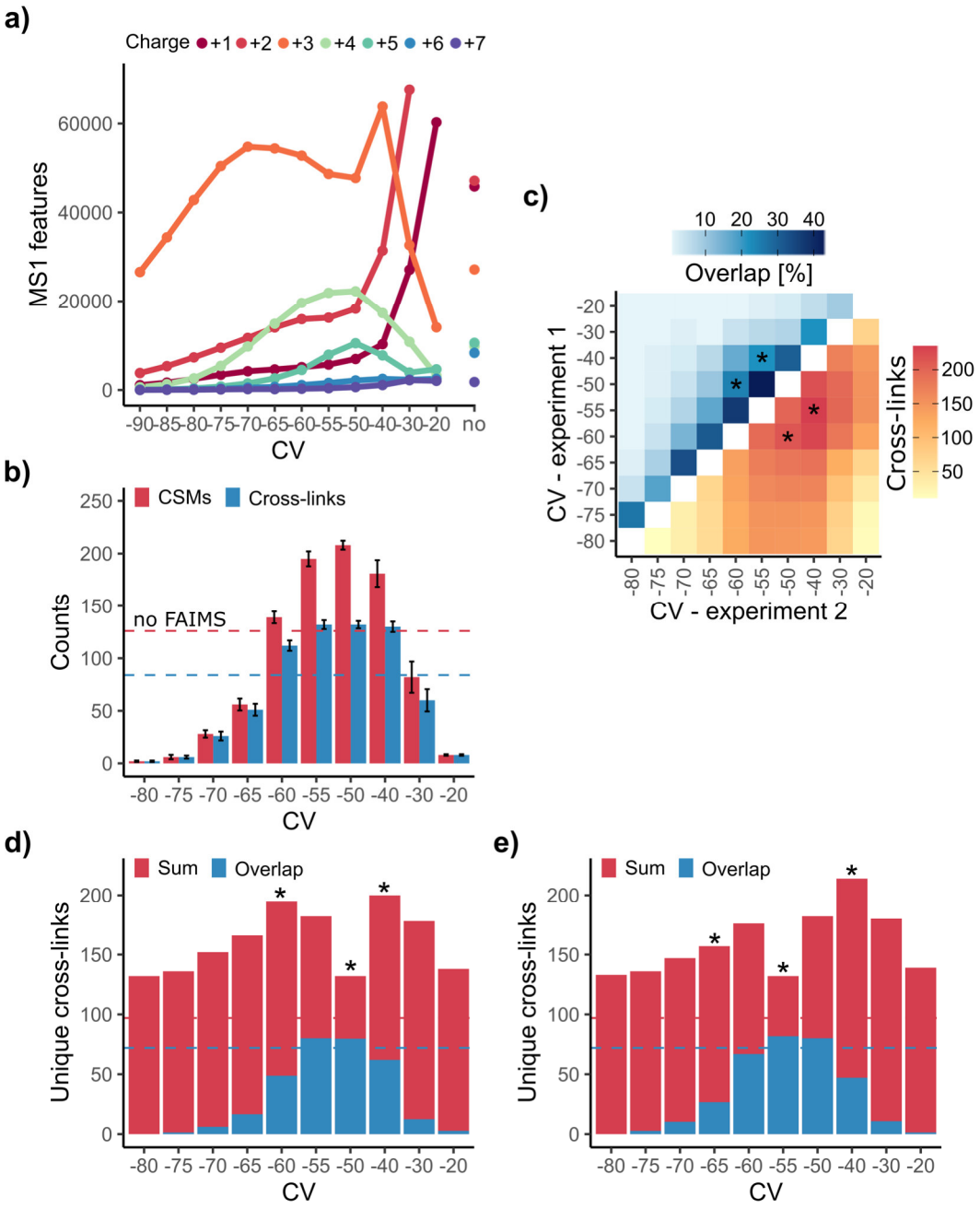
Supplementary Figure 4. Analysis of two additional CV combinations in two different biological replicates of DSSO cross-linked SCX fractionated HEK293T cell lysate. The number of CSMs, unique cross-links, and cross-linked proteins of six SCX fractions are presented separately for individual SCX fractions (top) or combined (bottom). The two best performing 3-CV combinations are compared to (a) the best performing 2-CV combination (-50/-70 V) and (b) one 3-CV combination adjusted to more hydrophilic and shorter peptides (-60/-70/-80 V).


a)
Counts
6000
4000
2000
0
CSMs
Cross-links
Proteins
b)
Inter-linked protein pairs
500
400
300
200
100
0
c)
Fraction 1
Fraction 2
Fraction 3
Fraction 4
Fraction 5
Fraction 6
Counts
1000
500
0
CSMs
Cross-links
Proteins
d)
Cross-link increase [%]
150
100
50
0
-40/-55/-70 V
-50/-60/-75 V
no FAIMS
1
2
3
4
5
6
Fraction
e)
Charge
+ 1
+ 2
+ 3
+ 4
+ 5
+ 6
+ 7
-40/-55/-70 CV
-50/-60/-75 CV
no FAIMS
MS1 features
100%
75%
50%
25%
0%
1 2 3 4 5 6
Fraction

Supplementary Figure 5. Analysis of a second biological replicate of DSSO cross-linked SCX fractionated HEK293T cell lysate. (a) Number of CSMs, unique cross-links, and cross-linked proteins from six SCX fractions using two 3-CV combinations and without FAIMS. (b) Total number of unique inter-linked protein pairs. (c) Results as in a) but are presented separately for individual SCX fractions. (d) Percentage increase of cross-link identification in different SCX fractions for two 3-CV combinations compared to analysis without FAIMS. (e) Distribution of MS1 charge states for two selected 3-CV combinations.

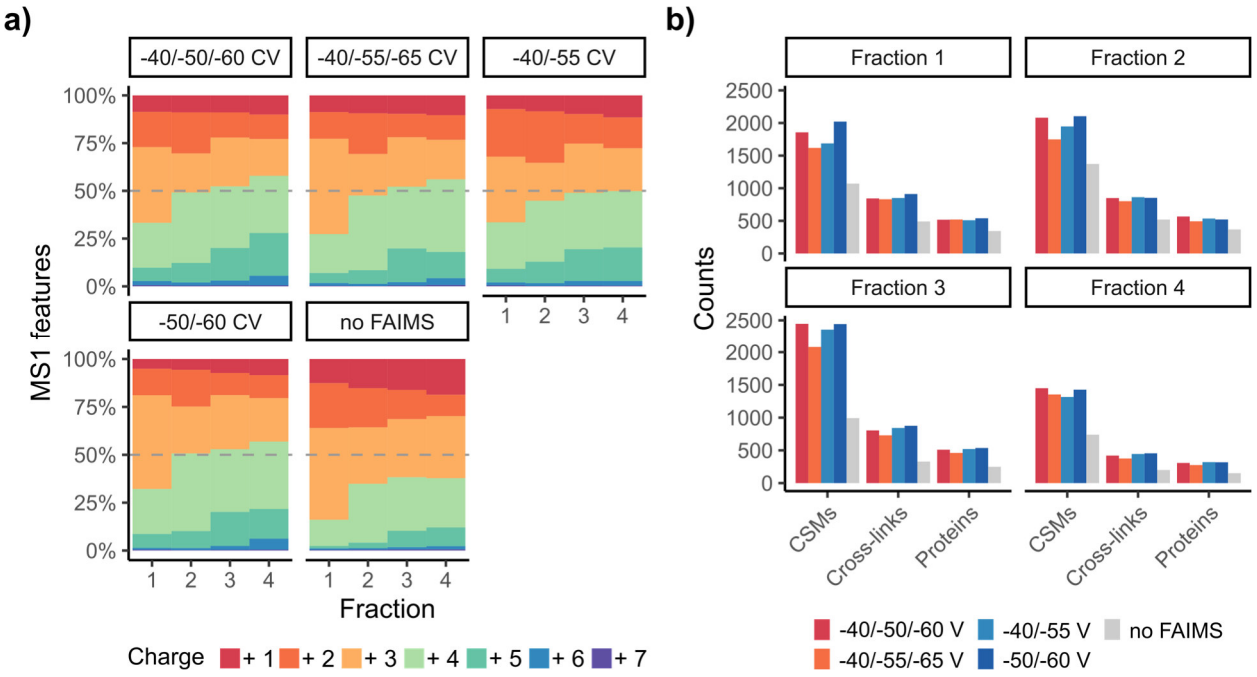


Supplementary Figure 6. Analysis of FAIMS CV settings for a DSS cross-linked eight protein mixture (analyzed in two technical duplicates). (a) Charge distribution of MS1 features along the selected FAIMS CV range and without FAIMS ("no"). (b) Analysis of unique cross-links and overlap between two individual 1-CV measurements. * note the selected 2-CV combinations. (c, d) Stacked bar plot to determine the best 3-CV combination. A first experiment (single CV measurement at -50 V (c) and -55 V (d)) is combined with a second experiment at different CVs. Local maxima on both sides of the initial CV suggest which three CVs to combine in order to maximize unique cross-link identification (red) and minimize overlap between CVs (blue). A 3-CV combination (-40/-50/-60 and -40/-55/-65 V, marked with *) is selected based on this analysis. Analysis without FAIMS is shown as a dotted line.

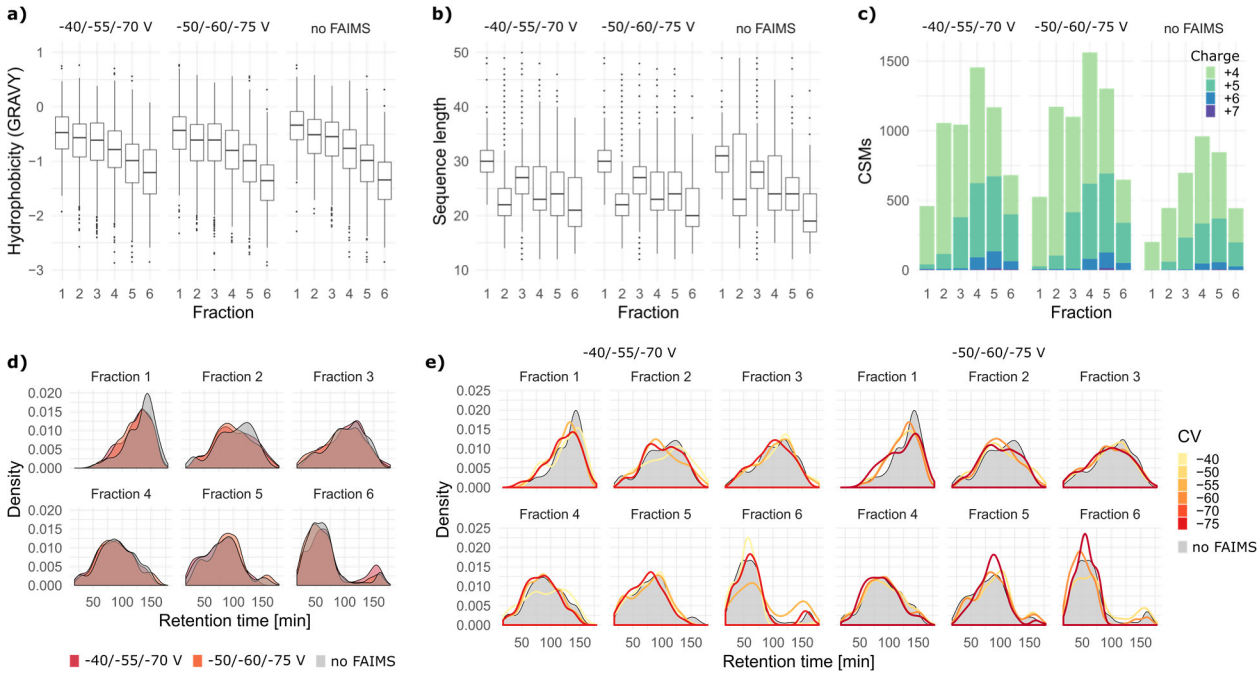


Supplementary Figure 7. Analysis of FAIMS CV settings for a second biological replicate of DSS cross-linked eight protein mixture (analyzed in two technical duplicates). (a) Charge distribution of MS1 features along the selected FAIMS CV range and without FAIMS ("no"). (b) Number of CSMs and unique cross-links for individual CVs and without FAIMS. Analyses without FAIMS are shown as dotted lines. (c) Analysis of unique cross-links and overlap between two individual 1-CV measurements. * note the selected 2-CV combinations. (d, e) Stacked bar plot to determine the best 3-CV combination. A first experiment (single CV measurement at -50 V (d) and -55 V (e)) is combined with a second experiment at different CVs. Local maxima on both sides of the initial CV suggest which three CVs to combine in order to maximize unique cross-link identification (red) and minimize overlap between CVs (blue). A 3-CV

combination (-40/-50/-60 and -40/-55/-65 V, marked with *) is selected based on this analysis. Analysis without FAIMS is shown as a dotted line.



Supplementary Figure 8. Analysis of four SCX fractions of DSS cross-linked HEK293T cell lysate using two 2-CV and two 3-CV combinations and without FAIMS. (a) Distribution of MS1 charge states. (b) Number of CSMs, unique cross-links, and cross-linked proteins presented separately for individual SCX fractions.



Supplementary Figure 9. Analysis of physiochemical properties of cross-links along SCX fractions and LC/MS retention time using six SCX fractions from DSSO cross-linked HEK293T cell lysate. (a, b) Distribution of (a) predicted hydrophobicity based on Gravy index score (> 0 hydrophobic, < 0 hydrophilic) and (b) distribution of the length of linearized cross-link sequence along SCX fractions in different FAIMS setup. (c) Number of CSMs in different charge states along SCX fractions in different FAIMS setup. (d, e) Density distribution of CSMs along LC/MS gradient. Results of 3-CV combinations are shown in (d) and individual CVs within one 3-CV combination are shown in (e).