Supporting Information

A Rapid LC-MS Method for Accurate Molecular Weight Determination of Membrane and Hydrophobic Proteins

Jennifer L. Lippens[1ǂ], Pascal F. Egea[2], Chris Spahr[1], Amit Vaish[1], James E. Keener[3], Michael T. Marty[3], Joseph A. Loo[2,4*] and Iain D. G. Campuzano[1*]

1. Amgen Discovery Research, Amgen, Thousand Oaks, California 91320, United States
2. Department of Biological Chemistry, University of California-Los Angeles, Los Angeles, California 90095, United States
3. Department of Chemistry and Biochemistry, University of Arizona, Tucson, Arizona 85721, United States
4. Department of Chemistry and Biochemistry, University of California-Los Angeles, Los Angeles, California 90095, United States

ǂ Jennifer L. Lippens is an Amgen Post-Doctoral Research Fellow

* Corresponding authors: Jennifer L. Lippens - jlippens@amgen.com; Joseph A. Loo – jloo@chem.ucla.edu ; Iain D. G. Campuzano – iainc@amgen.com

The material supplied as Supporting Information is as follows:

- Membrane protein construct preparation and purification protocols for bR mutants, AqpZ constructs, K-K, Coq10 and AmtB-MBP
- Proteolytic digestion conditions for bR mutant and Coq10
- Alternative gradient conditions
- Xevo Q-ToF tune settings and pressures
- Comparison of ion pairing agents for the polyphenol and C3 columns
- AqpZ chromatography on 7 and 15-minute gradients
- Optimization of % B on both the polyphenol and C3 columns
- Representative chromatograms from both the polyphenol and C3 columns for all protein constructs
- Coq10 chromatography at 30% B on the polyphenol and C3 columns
- Reproducibility and limit of detection for the C3 column 5-minute method
- Deconvoluted mass for Coq10
- Proteolytic digestion followed by fragmentation for bR P50A and Coq10

bR mutants preparation and purification. The L111A, T47A, P50A and P50A/T46A bacteriorhodopsin (bR) mutants were purified from *Halobacterium*. The naturally-forming crystalline sheets in the membrane, referred to as purple membrane, were separated from other components by a sucrose gradient using the density of the purple membrane. For denaturing LC-MS, the bR mutants were diluted in 200 mM ammonium acetate/ 1.1% w/v β-Octyl Glucoside (OG).

WT, W14A and GFP-tagged AqpZ preparation and purification. The WT and W14A AqpZ were prepared as briefly described here. The gene encoding *E. coli* AqpZ was cloned in a pET29b expression vector to express the protein fused to a C-terminal hexahistidine tag cleavable with thrombin. The AqpZ-GFP construct was generated by Gibson assembly of the AqpZ and superfolder GFP genes and introduced into a pRSF vector to express an AqpZ-GFP fusion protein with a C-terminal hexahistidine tag cleavable with thrombin; a Gly-Ser linker connects the AqpZ and GFP proteins. The W14A point mutation was introduced using the QuikChange mutagenesis protocol. AqpZ was expressed in C43 (DE3) cells grown in 2x LB media at 37 °C. Protein expression was induced by adding β-D thiogalactopyranoside (IPTG) at 0.8 mM final concentration when cultures reached an OD600 of 0.5; induction was carried out for 5 hours at 37 °C before cells were harvested and washed. Cells were lysed in 500 mM NaCl/ 20 mM Tris-HCl pH 8.0/ 10 % glycerol/ 0.1 mM PMSF and 2.8 mM β-mercaptoethanol (β-ME) using a C3-Emulsiflex (Avestin) pressurized at 15,000 psi. Following lysis, the extract was clarified by low-speed centrifugation at 10,000 g for 30 minutes at 4 °C prior to separating the bacterial membranes by ultracentrifugation at 120,000 g for 2 hours at 4 °C. Bacterial membrane pellets were then solubilized in lysis buffer supplemented with 200 mM OG at 4 °C overnight. Insoluble material was pelleted by ultracentrifugation at 120,000 g for 1 hour at 4 °C. Detergent-solubilized AqpZ was then purified by immobilized-nickel affinity chromatography. After loading the solubilized extract, the resin was washed with 25 column volumes (CV) of 500 mM NaCl/ 20 mM Tris-HCl pH 8.0/ 10% glycerol/ 25 mM imidazole/ 40 mM OG/ 0.1 mM PMSF and 1.4 mM β-ME; AqpZ was then eluted in 500 mM NaCl/ 20 mM Tris-HCl pH 8.0/ 250 mM imidazole/ 40 mM OG/ 0.1 mM PMSF and 1.4 mM β-ME. AqpZ was then desalted using a PD-10 desalting column equilibrated in 150 mM NaCl/ 20 mM Tris-HCl pH 8.0/ 5% glycerol/ 1.4 mM β-ME. Following desalting, AqpZ was treated with thrombin for 16 hours at ambient temperature to remove the His-tag, then purified on a Superdex 200 HR10/30 size exclusion column (GE Healthcare) equilibrated in 150 mM NaCl/ 20 mM Tris-HCl pH=8.0/ 5% glycerol and 40 mM OG. Pure *E. coli* AqpZ eluted as a single peak corresponding to the homotetramer. AqpZ fractions were pooled and passed across a small Ni-IMAC column (250 µL resin) to remove any remnants of tagged protein. The protein was then concentrated to 6.5 mg/ml for subsequent analysis. A similar process was also performed to obtain AqpZ-GFP fusion and the W14A mutant forms.

KcsA Kv1.3 preparation and purification. KcsA Kv1.3 (K-K) fused to a N-terminal hexahistidine tag was expressed in transformed *E. coli* M15 pREP4 cells. Protein expression was induced by adding IPTG at 0.5 mM final concentration; induction was carried out for 2 hours at 30 °C before cells were harvested and washed. The cells were harvested by centrifugation at 4,500 *g* for 10 min at 4 °C and lysed with 2 passes through a cell disruptor in lysis buffer consisting of 20 mM Tris-HCl pH 7.5/ 5 mM imidazole/ 150 mM KCl. The crude lysate was centrifuged at 20,000 *g* for 1 hour at 4 °C. The supernatant was removed and centrifuged at 100,000 *g* for 1 hour at 4 °C, homogenized with a Potter-Elvehjem homogenizer and then frozen. The frozen lysate was then thawed and solubilized with 3% (w/v) (final concentration) styrene maleic acid lipid polymer and incubated at 4 °C with gentle agitation overnight. The next day, after centrifugation at 100,000 *g* for 1 hour at 4 °C, the supernatant was mixed with 10 mM $MgCl_2$ and 20 mM β-Decyl Maltoside (DM), incubated for 4 hours at 4 °C, then transferred to pre-equilibrated Talon resin (Clontech) and incubated, while rocking, overnight at 4 °C. The column was then drained and washed once with lysis buffer containing 20 mM DM. This was followed by several washes with buffer containing 20 mM Tris-HCl pH 7.5/ 5 mM imidazole/ 150 mM KCl/ 5 mM DM as well as the previous buffer with 0.5 M NaCl. Each of these washes was collected for SDS PAGE analysis. The protein was then eluted from the resin with 6 CV of lysis buffer containing 100 mM imidazole and

5 mM DM. Fractions containing K-K were pooled and their concentrations determined by their A_{280} measurement. Some of this purified protein was then cleaved with caspase-3 to remove the N-terminal His-tag. To achieve cleavage, 6 µg of caspase-3 was added to 350 µg of the protein, which was then incubated at 4 °C for 6 hours. Successful His-tag cleavage was accessed by the denaturing LC-MS method described in the main text.

Coq10$^{N\Delta30}$ preparation and purification. The His-SUMO-tagged Coq10$^{N\Delta30}$ was expressed in transformed *E. coli* BL21-(DE3) cells (Invitrogen). The *E. coli* culture was grown in LB with 50 µg/mL kanamycin at 37 °C to an OD_{600} of 0.6-0.8 before the expression was induced with 1 mM IPTG at 16 °C for 18 hours. The cells were harvested by centrifugation at 4,500 *g* for 10 min at 4 °C and lysed with a M-110P microfluidizer (Microfluidics) in lysis buffer consisting of 50 mM NaH_2PO_4, pH 8.0/ 500 mM NaCl/ 10 mM imidazole/ 10% glycerol/ 1 mM DTT/ 0.1% Triton X-100/ 1 mM phenylmethylsulfonyl fluoride (PMSF)/ Roche Complete EDTA-free protease inhibitor (Sigma-Aldrich). The crude lysate was centrifuged at 10,000 *g* for 45 min at 4 °C and the collected soluble fraction was incubated with pre-equilibrated Ni-NTA resins (Qiagen) for 2 hours at 4 °C. To remove impurities, Ni-NTA resin was step washed three times each with 2 CV of wash buffer containing 20 mM or 35 mM imidazole in 50 mM NaH_2PO_4, pH 8.0/ 300 mM NaCl/ 10% glycerol/ 1 mM DTT/ 0.1% Triton X-100, and the protein was eluted five times with 1.5 CV of wash buffer containing 200 mM imidazole. The protein eluates were pooled and concentrated using a 10 kDa Amicon (Millipore). Recombinant His-tagged SUMO protease, also known as Ulp1, was added to the concentrated eluate (approximately 400 µg of His-tagged Ulp1 was added to every 10 mL of concentrated eluate) to cleave the His-SUMO moiety during overnight dialysis against 50 mM NaH_2PO_4 pH 8.0/ 300 mM NaCl/ 10% glycerol/ 1 mM DTT/ 0.1% Triton X-100 at 4 °C using a 10,000 MWCO Slide-A-Lyzer dialysis cassette (Thermo). After overnight dialysis, the protein inside the dialysis cassette was removed and allowed to incubate with pre-equilibrated Ni-NTA resins for 2 hours at 4 °C to remove His-tagged Ulp1 protease, His-SUMO moiety (released after Ulp1 cleavage), and any uncleaved His-SUMO-tagged Coq10$^{N\Delta30}$ fusion protein, thereby leaving cleaved Coq10$^{N\Delta30}$ in the flow through. The Ni-NTA resins were washed four times each with 2 CV of wash buffer without imidazole. The combined flow through and washes were concentrated using a 10 kDa Amicon and further purified by gel filtration chromatography on a Superdex 75 Increase column (GE Healthcare) to remove any residual contaminants and to buffer exchange into 10 mM Tris, pH 7.0/ 10 mM NaCl/ 0.5 mM Tris (2-carboxyethyl)-phosphine hydrochloride (TCEP)/ 3% 2-methyl-2,4-pentanediol (MPD)/ 0.1% n-dodecyl β-D-maltoside (DDM).

AmtB preparation and purification. AmtB was purified as described in Reid. et. al. 2017. Anal. Chem.

Proteolytic digestion of bR P50A. Chymotrypsin digestion conditions were as follows: 20 µg of bR P50A was incubated at ambient temperature in 150 mM Tris, pH 7.5/ 8 M urea/ 40 mM hydroxylamine for 30 minutes before being diluted with water to a final concentration of 37.5 mM Tris pH 7.5/ 2 M urea/ 10 mM hydroxylamine. The digestion was brought to a total volume of 30 µL with deionized water after the addition of 1 µL of chymotrypsin. The digestion was then incubated overnight at 37 °C, after which 30 µL of 0.1% formic acid (FA) was added, followed by 25 mM tris (2-carboxyethyl) phosphine (TCEP) for an incubation period of 15 minutes at 37 °C prior to LC-MS/MS analysis. Following digestion with chymotrypsin and prior to acidification/ TCEP reduction, some of the material was also digested with Glu-C by adding 1 µg of Glu-C and incubating again at 37 °C overnight. This digestion reaction was also analyzed by LC-MS/MS.

For bR P50A, both chymotrypsin and chymotrypsin/ Glu-C digests were analyzed to yield N- and C-terminal information. The digests were analyzed by LC-MS/MS using an Agilent 1260 capillary HPLC linked to a Thermo Q-Exactive MS (Thermo Scientific; Waltham, MA). The HPLC utilized a ZORBAX 300SB-C18 5 µm, 250 x 0.5 mm column (Agilent Technologies; part# 5064-8266). For peptide separations, mobile phases A and B consisted of 0.1% FA in H_2O and 0.1% FA in acetonitrile, respectively. Peptides were separated at 25 µl/min at 30 °C using the following gradient: 10 minutes at 1% B, up to 55% B over 85 minutes, up to 97% B over 5 minutes, isocratic at 97% B for 5 minutes, and

then down to 1% B over 10 minutes. bR was analyzed by LC-MS/MS using a full MS scan of *m/z* [350-2000] at 70K resolution (FWHM), followed by high resolution HCD scans of the top 10 most abundant precursors at 17.5K resolution. A spray voltage of 3.5 kV, S-Lens RF level of 50, isolation width of 2.0 Da, collision energy (CE) of 27 and a 10 second dynamic exclusion were used.

Proteolytic digestion of Coq10. Glu-C digestion conditions were as follows: 5 µL of Coq10 (~10 µg) was incubated at 37 °C overnight in 10 µL of water and 5 µL of 150 mM Tris pH 7.5/ 8 M urea/ 40 mM hydroxylamine (final buffer composition of 37.5 mM Tris pH 7.5/ 2 M urea/ 10 mM hydroxylamine). To this, 0.5 µg of Glu-C was added prior to incubation. The digest was acidified by adding 20 µL of 0.1% TFA prior to LC-MS analysis. The digest was analyzed on a Thermo OrbiTrap Velos MS (Thermo Scientific; Waltham, MA) with the EASY Spray nanospray ionization source. An integrated 75 µm C18 column/emitter assembly (Thermo P/N ES 800; pepMap RSLC C18 3µm, 100 Å, 75 µm x 15 cm) was used at a spray voltage of 1.9 kV. Coq10 was analyzed using a full MS scan from *m/z* [300-2000] at 30K resolution, followed by low resolution CID of the top 10 most abundant precursor ions in the LTQ.

Alternative chromatography conditions. Both a Waters C4 and an Agilent cyano column were thoroughly investigated using an Agilent 1200 series in line with a Waters Synapt G2 ion mobility Q-ToF MS. AqpZ eluted off the C4 column during both 15 and 10-minute gradients using H_2O with 0.1% FA as mobile phase A and either acetonitrile or isopropanol (with 0.1% FA) as mobile phase B. However, after investigation of multiple 5-minute gradient configurations, no AqpZ was observed to elute. Due to these results, C4 column chemistry was abandoned, however, with further testing of various mobile phases it is possible that a 5-minute gradient could be achieved.

A cyano column was also investigated (custom Agilent column) with the following specifications: 2.1 x 50 mm, 300 Å pore size and 1.8 µm particle size. This column was investigated with various 15, 10 and 5-minute gradients employing H_2O with 1% FA as mobile phase A and acetonitrile with 1% FA as mobile phase B. Each of these gradients were investigated at both 40 and 65 °C, and for this column chemistry, less baseline fluctuation was observed at 40 °C. Each of the gradients tested succeeded in eluting AqpZ. However, this column required a higher flow rate of 750 µL/min to achieve chromatography with low background. Additionally, carry over was consistently observed, even after two post protein blank injections. For this reason, TFA at 0.1% v/v was added to both mobile phases to improve chromatography. However, even with the addition of TFA, the chromatography improved only slightly. Additionally, with the high flow rate still necessary to elute AqpZ within the 5-minute gradient described in the main text, method development was moved to a UPLC compatible C3 column for further development.

Longer 9 and 7-minute methods were both explored on the C3 column, and both eluted several of the membrane proteins investigated employing H_2O with 0.1% TFA/0.1% FA as mobile phase A and 90% *n*-propanol with 0.1% TFA/0.1% FA as mobile phase B. These methods also provided more separation of the protein and DDM as compared to the final 5-minute method. Both of the following 7-minute methods were successful in protein elution and chromatographic separation of the protein from DDM. The first gradient was held at 20% B starting from 0-2 minutes, then ramped over the next 2 minutes to 72% B, slowly ramped to 90% B at 4.5 minutes then brought to 20% B ending at 7 minutes. The other 7-minute method was also held at 20% B starting from 0-2 minutes, then ramped to 90 %B at 3 minutes and held there for 0.5 minutes. After which, the % B was ramped down to 50% B at 5 minutes then down to 20% B at 7 minutes. The 9 minute gradient was held at 20% B from 0-3 minutes then ramped as described for the first 7 minute method and held at 20% B from 7-9 minutes.

If analysis of a membrane protein on the 5-minute gradient detailed in the main text does not provide a chromatographically separated protein peak the authors suggest several options as starting points for troubleshooting. The first would be to lengthen the gradient to one of the 7-minute methods described above. Both methods successfully eluted each of the membrane proteins investigated in this manuscript and provided chromatographic separation from detergents and other low MW species present in the

samples. Additionally, the same 5-minute method described in the main text can be explored at varying starting %B. As was observed for Coq10 and K-K, while good chromatography is achievable at multiple starting percentages, some membrane proteins seem to elute optimally at a slightly lower % B.

Xevo Q-ToF Tune Settings and Pressures. The Q-ToF MS was operated at the following pressures (mbar): backing 2.24, collision cell 1.17 e -2 and ToF 5.87 e -7. For all injections the capilary voltage was 3 kV, sampling cone voltage was either 25, 40, 60, or 75 V (depending on the size of the monomeric protein), source and desolvation temperatures were 80 and 300 °C, respectively. The cone and desolvation gases were maintained at 20 and 800 L/hr, respectively. The detector voltage was 1950 V and 6 V of collision energy was applied during each injection.

MaxEnt parameters were set as follows with iterations to convergence: output mass ranges (dependent on the theoretical monomeric protein mass); 1 Da/channel resolution; minimum intensity ratio left and right at 80%; width at half height for uniform Gaussian model from 0.3–1.1 Da (construct dependent). This deconvolution spectrum was then area centered for the final mass measurement.

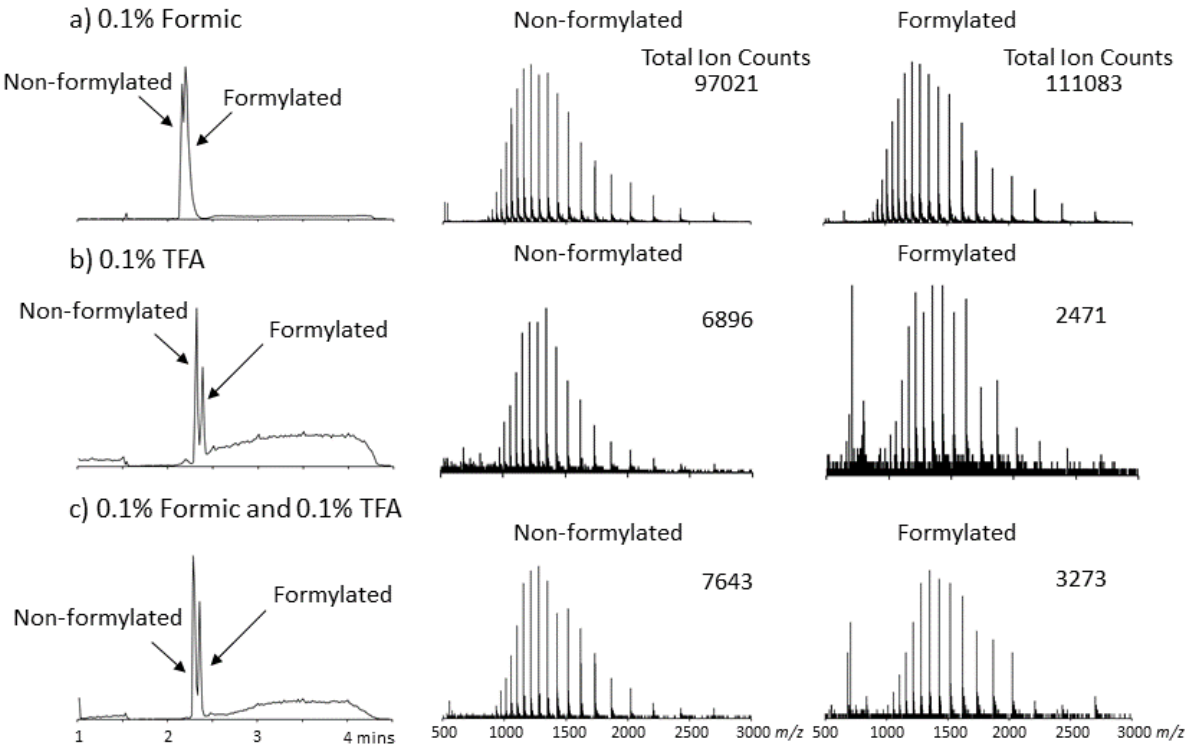

Figure S1. Comparison of WT AqpZ chromatograms and spectra obtained on the BioResolve Polyphenol column with 0.1% FA (a); 0.1% TFA (b) and 0.1% TFA/0.1% FA mix (c) as additives to both mobile phase A (H_2O) and B (90% *n*-propanol). Protein peaks are marked in each chromatogram by a black arrow. Numbers in the top right of each spectra represent the total ion counts obtained from area centered, deconvoluted data. The y-axis for the chromatograms and spectra represent intensity with arbitrary units.

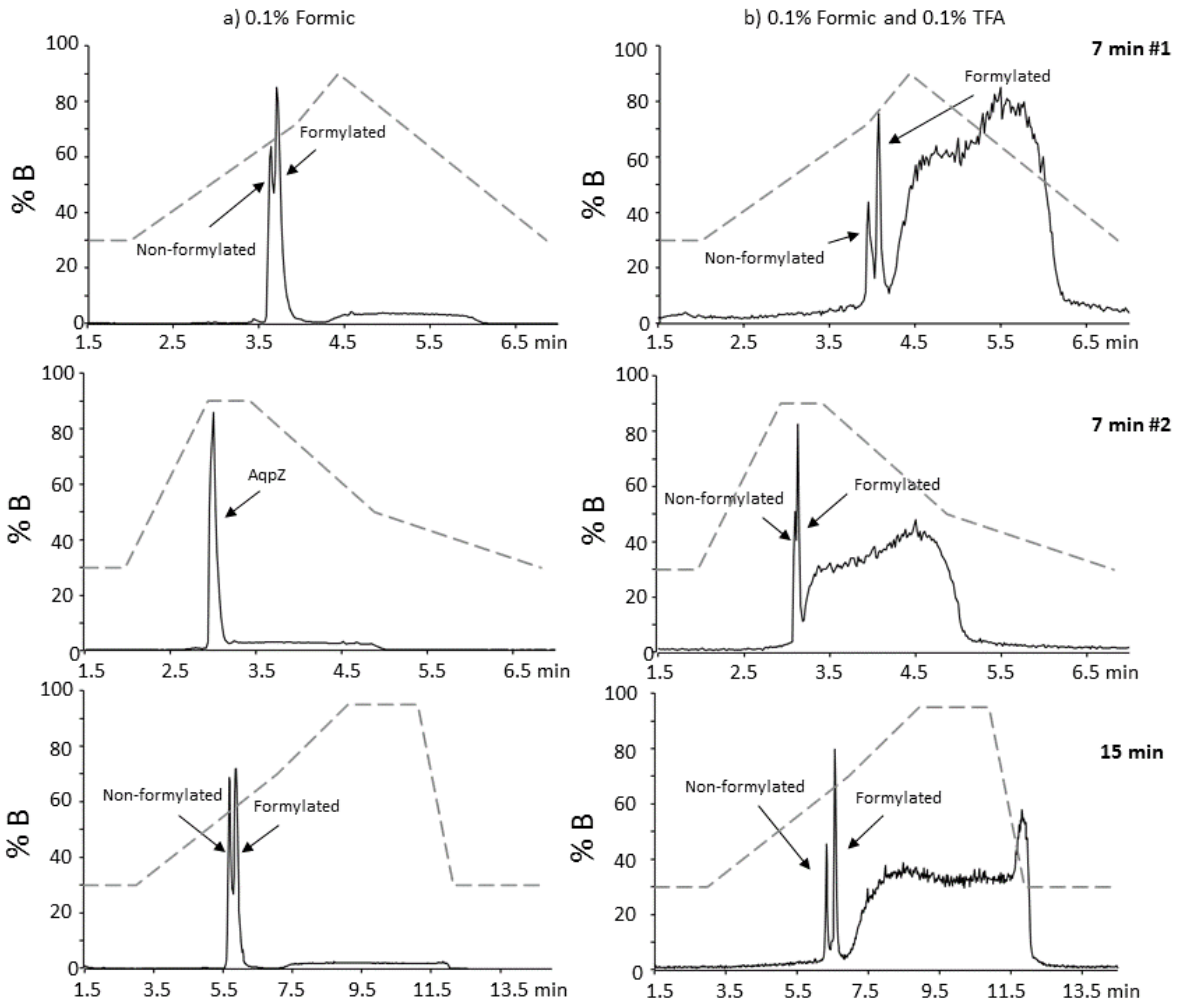

Figure S2. Comparison of three gradients for the separation of non-formylated and formylated WT AqpZ. Profiles of % B (dashed lines) are overlaid on each reconstructed ion chromatogram (RIC). Both 7-minute methods are detailed in the *Supporting Information*. As shown, improved separation is consistently observed with the mix of 0.1% formic/ 0.1% TFA, regardless of gradient length, as compared to 0.1% formic acid.

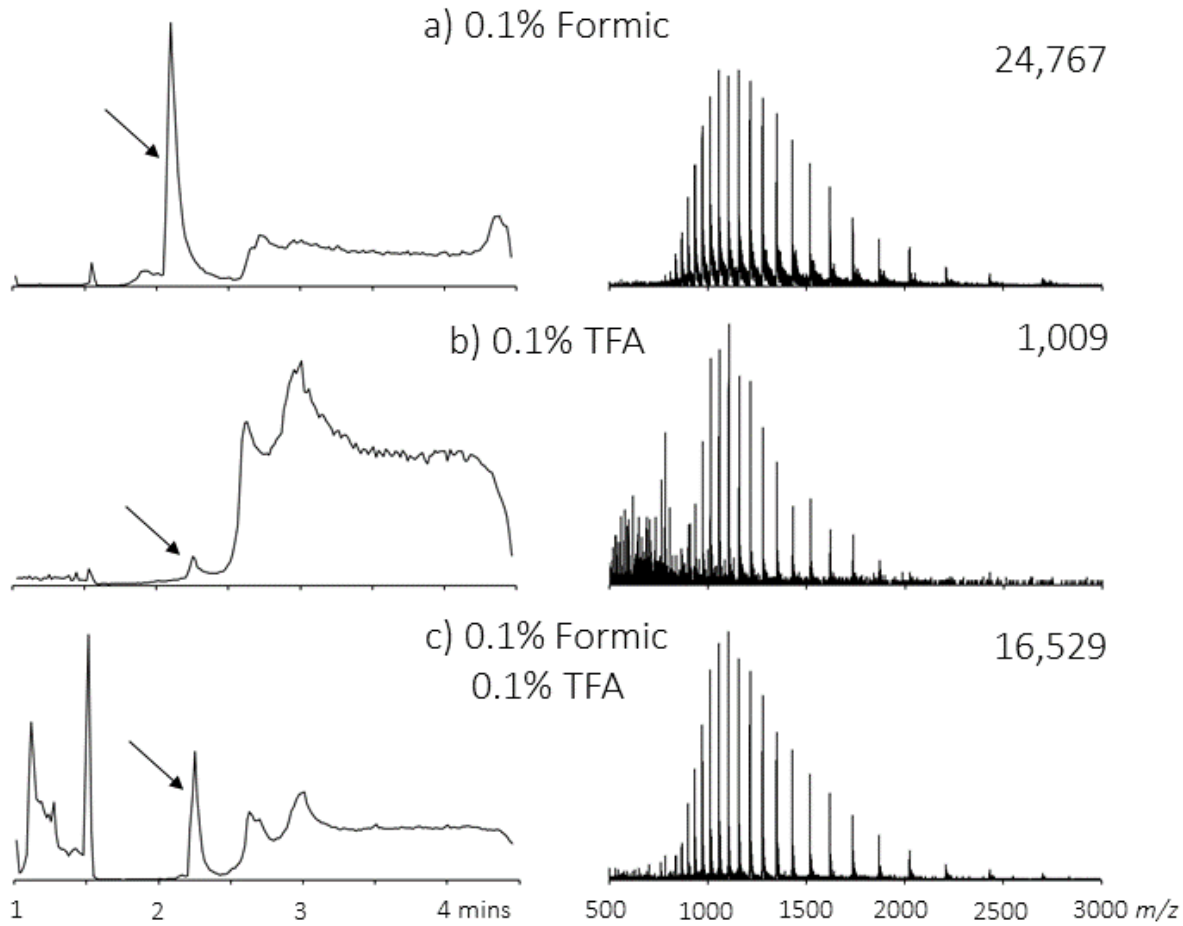

Figure S3. Comparison of WT AqpZ chromatograms and spectra obtained on the ZORBAX 300SB C3 column with 0.1% FA (a); 0.1% TFA (b) and 0.1% TFA/0.1% FA mix (c) as additives to both mobile phase A (H_2O) and B (90% n-propanol). Protein peaks are marked in each chromatogram by a black arrow. Numbers in the top right of each spectra represent the total ion counts obtained from area centered, deconvoluted data. The y-axis for the chromatograms and spectra represent intensity with arbitrary units.

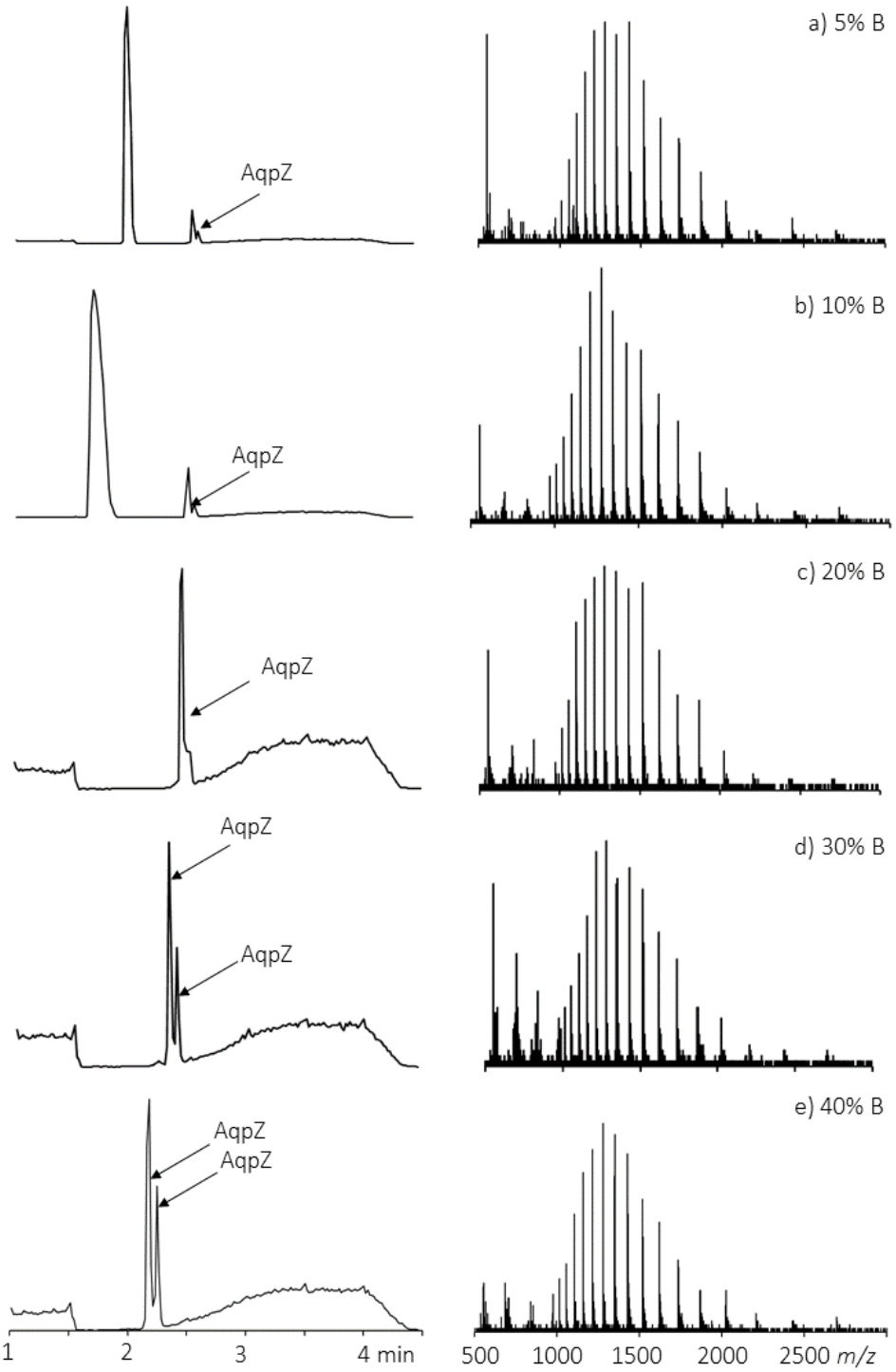

Figure S4. Determination of optimal starting %B with WT AqpZ at (a) 5%, (b) 10%, (c) 20%, (d) 30% and (e) 40% starting %B. Optimal starting concentration was determined from chromatographic separation of non-formylated and formylated protein. Spectra shown are a combination of both the non-formylated and formylated AqpZ as for 5, 10 and 20% runs the chromatographic separation between the two species was difficult to discern. The y-axis for the chromatograms and spectra represent intensity with arbitrary units.

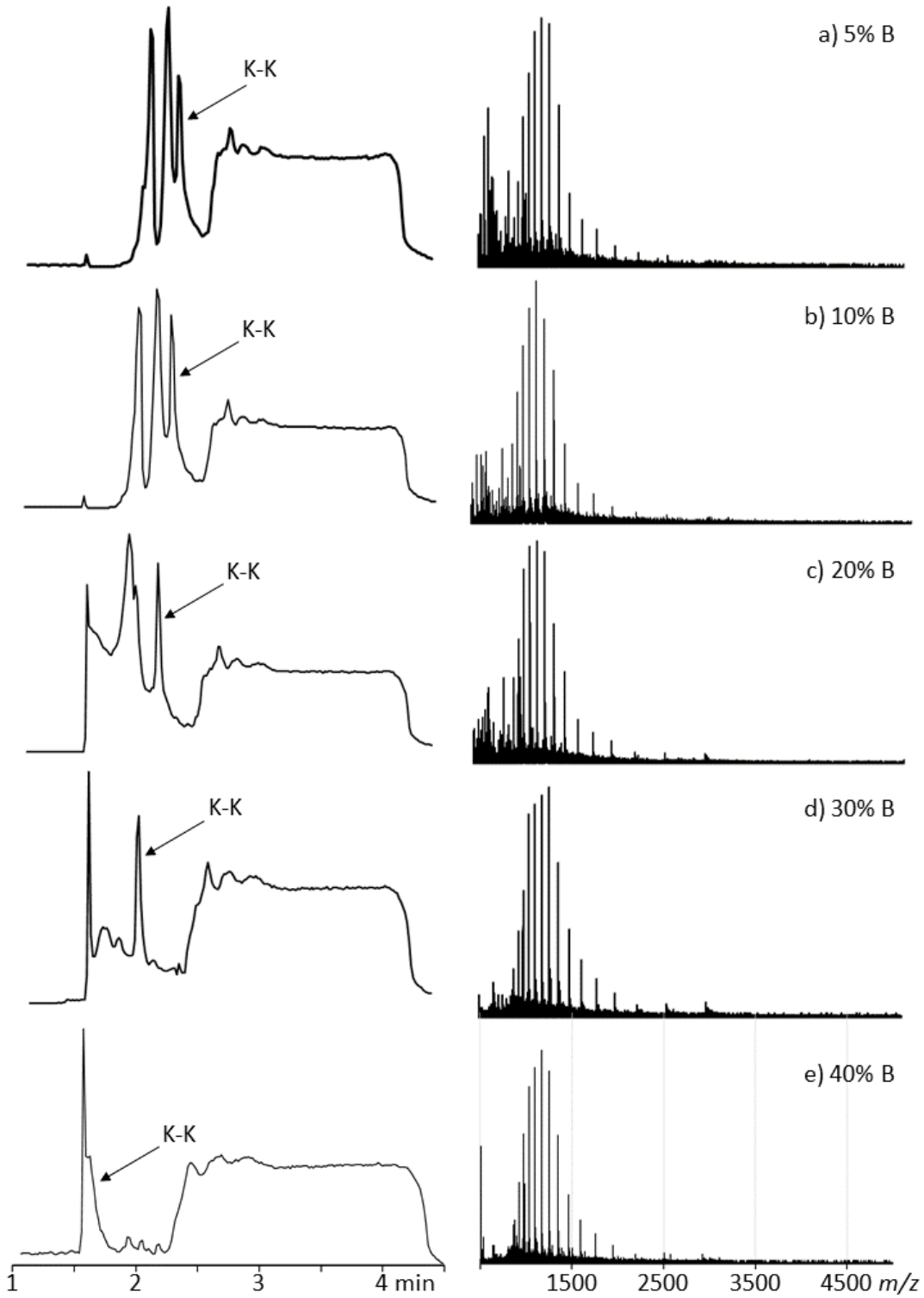

Figure S5. Determination of optimal starting %B with K-K on ZORBAX 300SB C3 column at (a) 5%, (b) 10%, (c) 20%, (d) 30% and (e) 40% starting %B. Optimal starting concentration was determined from both chromatographic separation and lowest signal for low *m/z* species. The y-axis for the chromatograms and spectra represent intensity with arbitrary units.

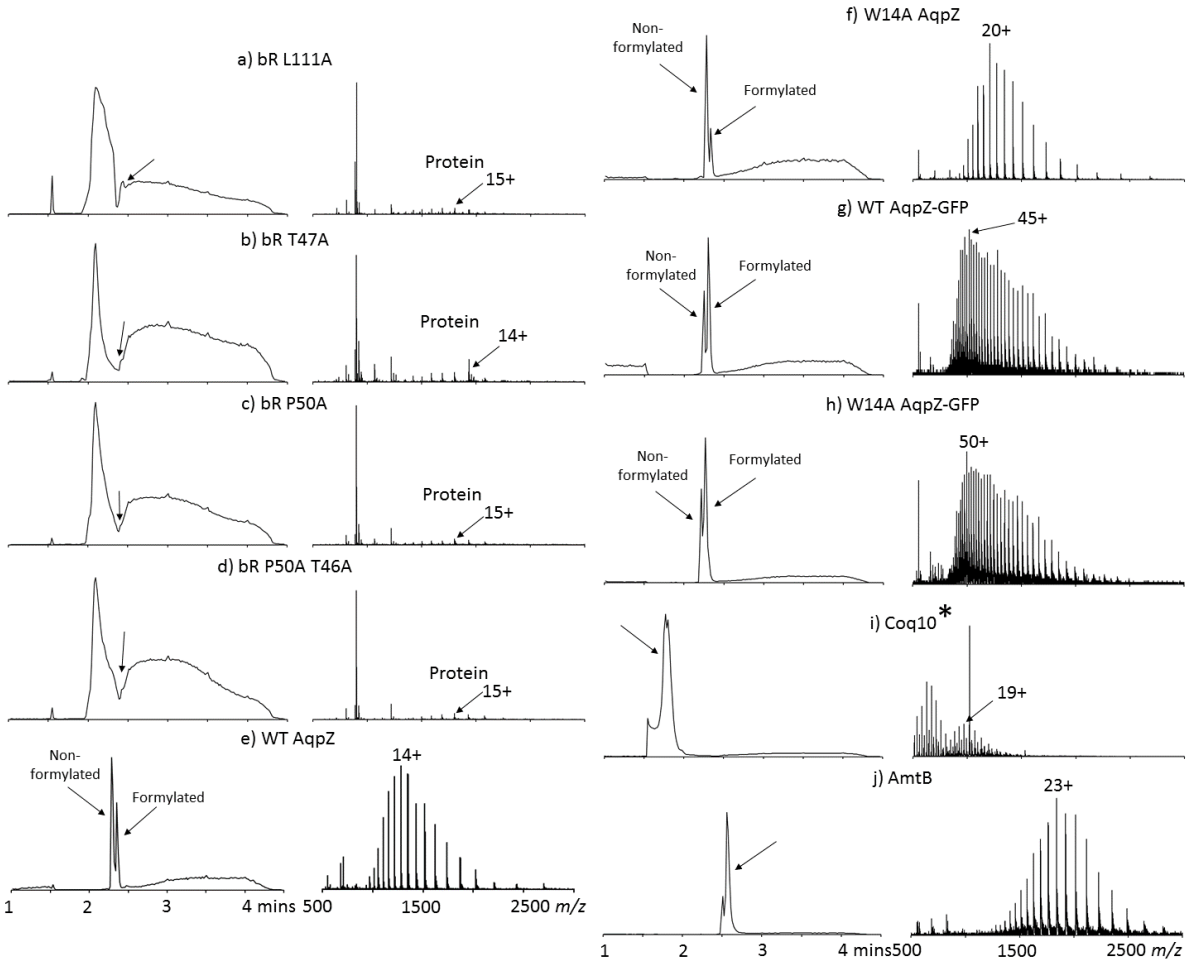

Figure S6. Representative chromatograms and spectra obtained with the BioResolve Polyphenol column from (a) bR L111A; (b) bR T47A; (c) bR P50A; (d) bR P50A T46A; (e) WT AqpZ; (f) W14A AqpZ; (g) WT AqpZ-GFP; (h) W14A AqpZ-GFP; (i) Coq10; (j) AmtB-MBP and (l) AmtB. Protein signal in the chromatograms are marked with a black arrow. The * denotes that the Coq10 chromatographic and MS spectrum data were obtained at 25% B starting as opposed to 30 % B. The most intense charge state is labeled in each MS spectrum. For bR mutant samples, the protein peak is chromatographically unresolved. Monitoring the protein by UV absorbance shows the retention time at about 2.4 minutes for all mutants. The y-axis for the chromatograms and spectra represent intensity with arbitrary units.

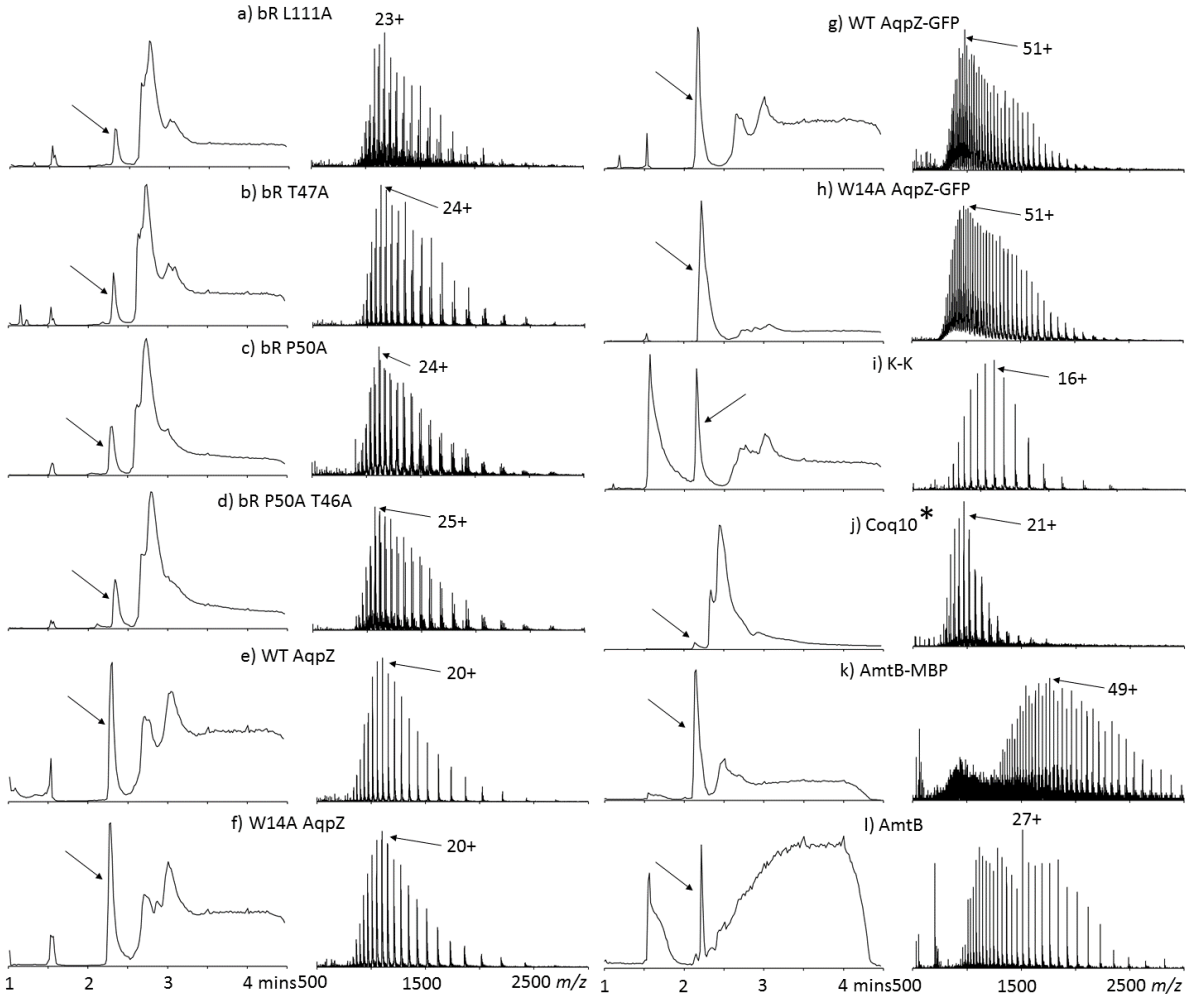

Figure S7. Representative chromatograms and spectra obtained with the ZORBAX 300SB C3 from (a) bR L111A; (b) bR T47A; (c) bR P50A; (d) bR P50A T46A; (e) WT AqpZ; (f) W14A AqpZ; (g) WT AqpZ-GFP; (h) W14A AqpZ-GFP; (i) K-K; (j) Coq10; (k) AmtB-MBP and (l) AmtB. Protein signal in the chromatograms are marked with an arrow. The * denotes that the Coq10 chromatographic and MS spectrum data were obtained at 20% B starting as opposed to 30% B. The most intense charge state is labeled in each MS spectrum. The y-axis for the chromatograms and spectra represent intensity with arbitrary units.

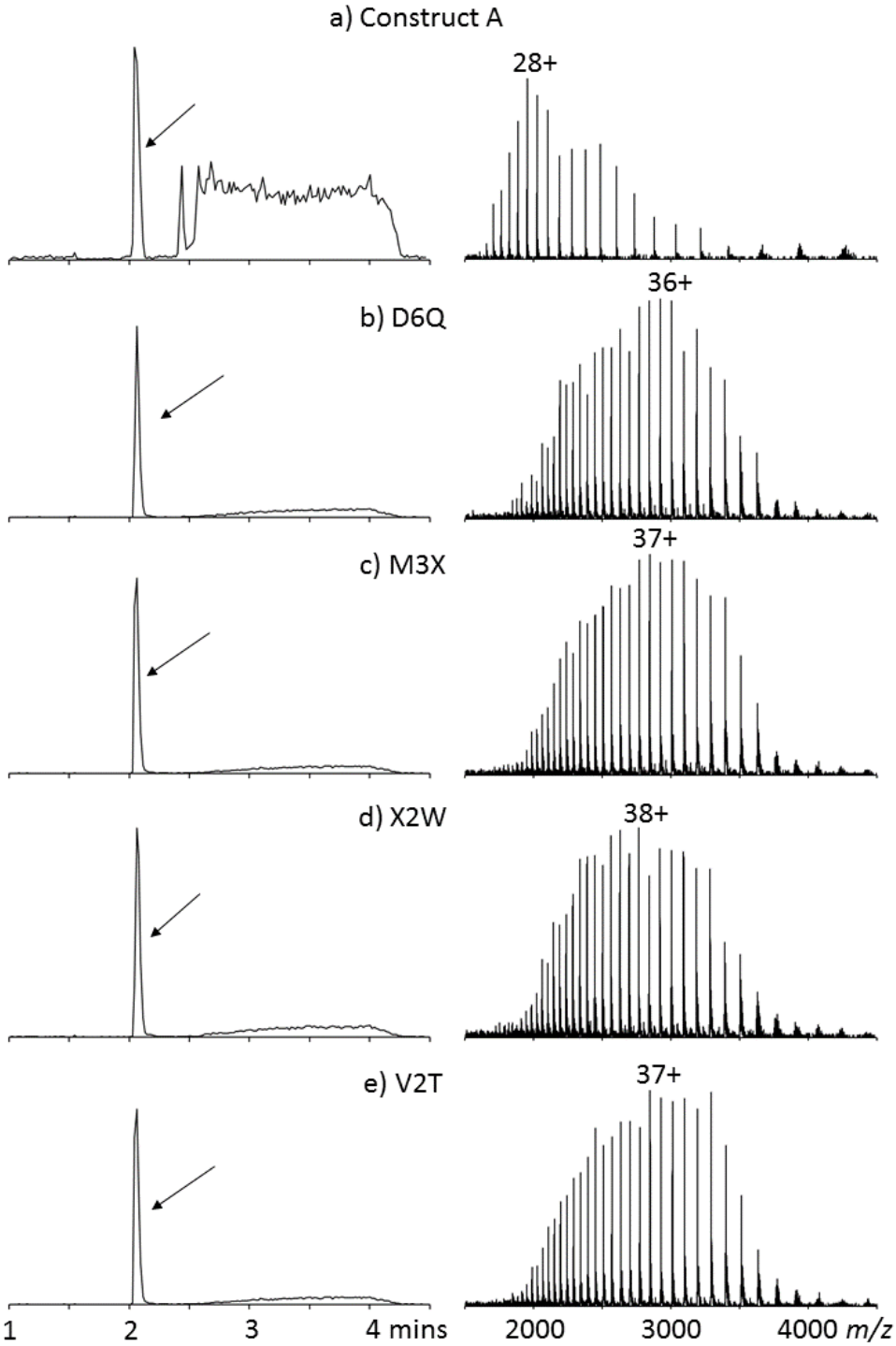

Figure S8. Representative RICs and spectra for five of the bispecific antibody constructs analyzed (a) construct A; (b) D6Q; (c) M3X; (d) X2W; and (e) V2T. The most intense charge state is labeled in each MS spectrum. The y-axis for the chromatograms and spectra represent intensity with arbitrary units.

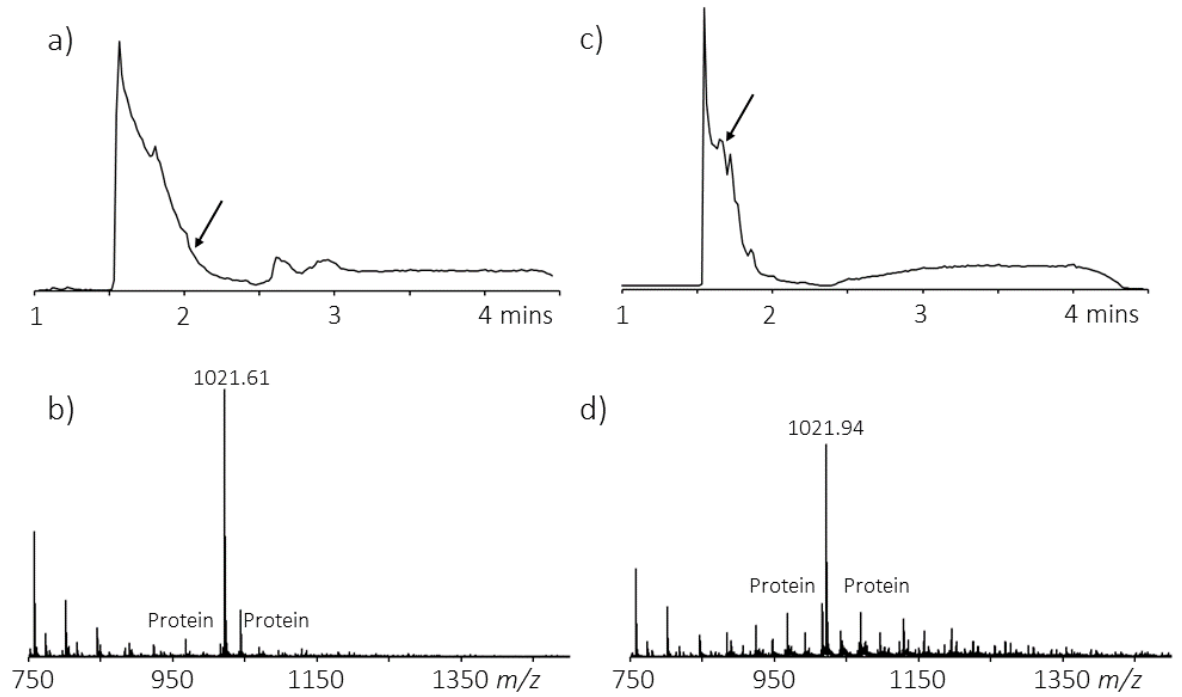

Figure S9. C3 chromatography (a) and spectrum (b) and polyphenol chromatography (c) and spectrum (d) obtained for Coq10 at 30 %B. Coq10 elutes as a shoulder of the first peak (denoted by an arrow) and the protein signal is heavily suppressed by the signal at 1021.61 *m/z*. Both the clipped and unclipped species were observed in the deconvoluted spectrum and provided average masses of 18,306.77 ± 0.22 Da and 20,302.38 ± 0.05 Da, respectively. The mass accuracy obtained from triplicate injections at 30 %B was Δ 0.66 and 0.23 Da for the clipped and unclipped species, respectively. Protein signal suppression was less on the polyphenol column and again, both the clipped and unclipped species were observed in the deconvoluted spectrum. The species provided average masses of 18,313.67 ± 0.31 Da and 20,310.11 ± 0.10 Da with mass accuracy of Δ +6.2 and +7.5 Da for the clipped and unclipped species, respectively. The y-axis for the chromatograms and spectra represent intensity with arbitrary units.

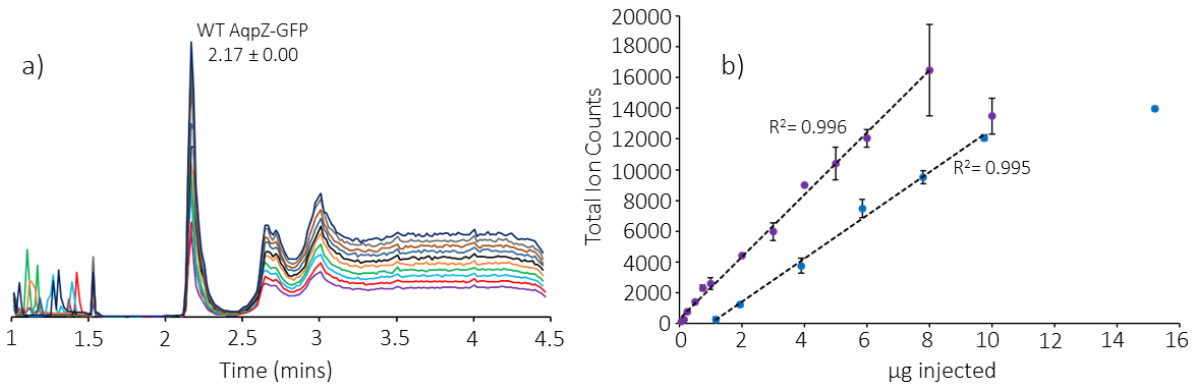

Figure S10. Reproducibility and limit of detection experiments on the ZORBAX 300SB C3 column. Reproducibility tests (a) with WT AqpZ-GFP at 1.6 µg/injection. Each run is represented with a different color and shows an overall reproducibility of retention time of 2.17 ± 0.00 minutes. Limit of detection studies (b) with bR P50A T46A (blue) and K-K (purple), injection amounts based on A_{280} readings ranged from 0.2 to 15 µg and 0.075 to 10 µg for bR P50A T46A and K-K, respectively. Outliers for bR P50A T46A existed at 0.2 and 15 µg and for K-K at 10 µg's. Total ion counts on the y-axis were obtained from MaxEnt deconvolution of the LC integrated peak area. The y-axis for the chromatograms represent intensity with arbitrary units.

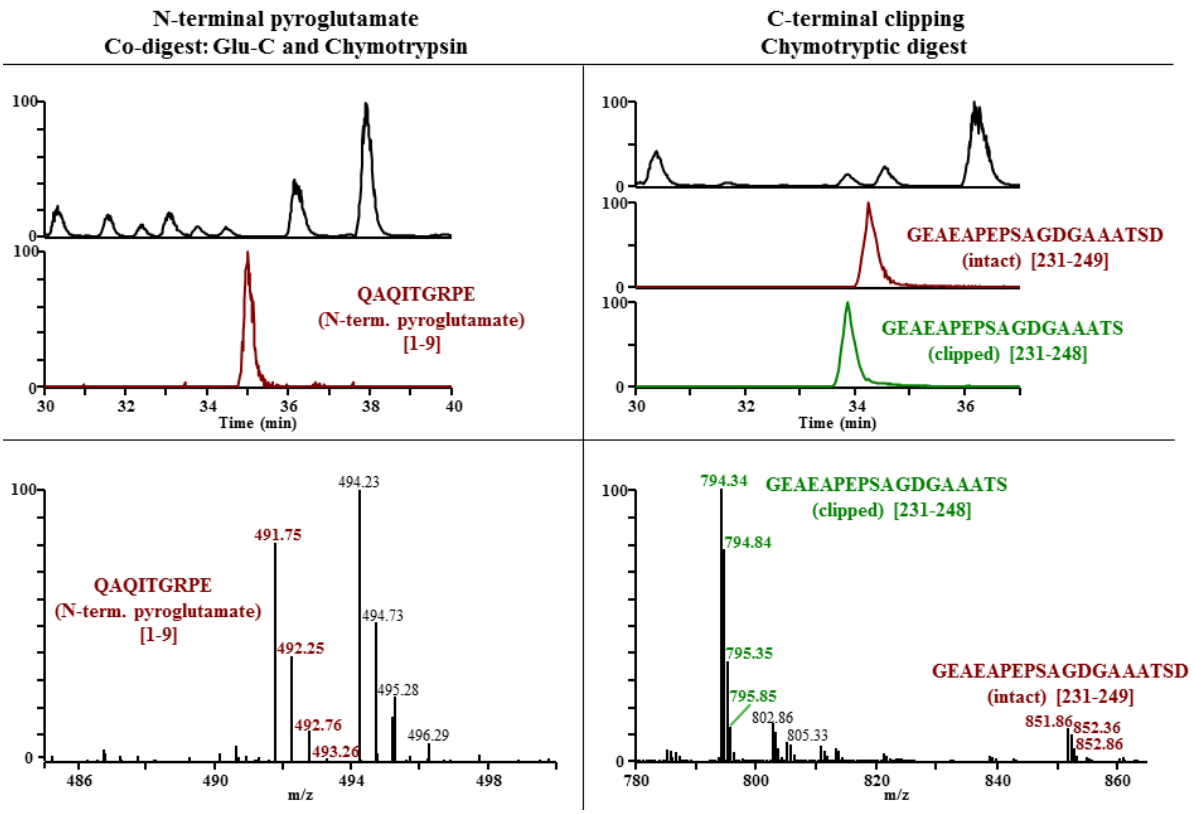

Figure S11. Chymotrypsin and chymotrypsin/ Glu-C digestion of bR P50A revealed both N- and C-terminal modification. LC-MS/MS of a chymotrypsin/ Glu-C digest (*left panel*) shows the presence of pyroglutamate on the N-terminus. XIC signal of N-terminal peptide [top], full MS signal of same peptide [bottom]. LC-MS/MS of a chymotrypsin digest (*right panel*) shows two C-terminal peptide species: intact and one clipped missing the C-terminal aspartate. XIC signal of both C-terminal species [top]. The relative intensities of the two species show that loss of the aspartate had occurred in roughly 85% of this construct [bottom].

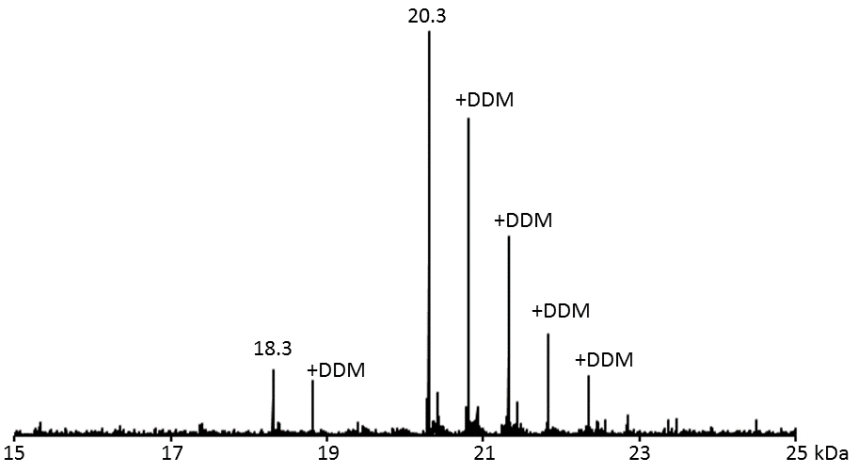

Figure S12. Deconvoluted spectrum of Coq10 showing the presence of a clipped sequence and the intact sequence at 18,307.04 Da and 20,302.13 Da, respectively. Both species display DDM adduction, more pronounced on the intact construct. Each DDM adduct corresponds to a mass addition of +510 Da. Coq10 is the only protein construct for which detergent adducts were observed upon spectral deconvolution. The y-axis for the spectrum represent intensity with arbitrary units.

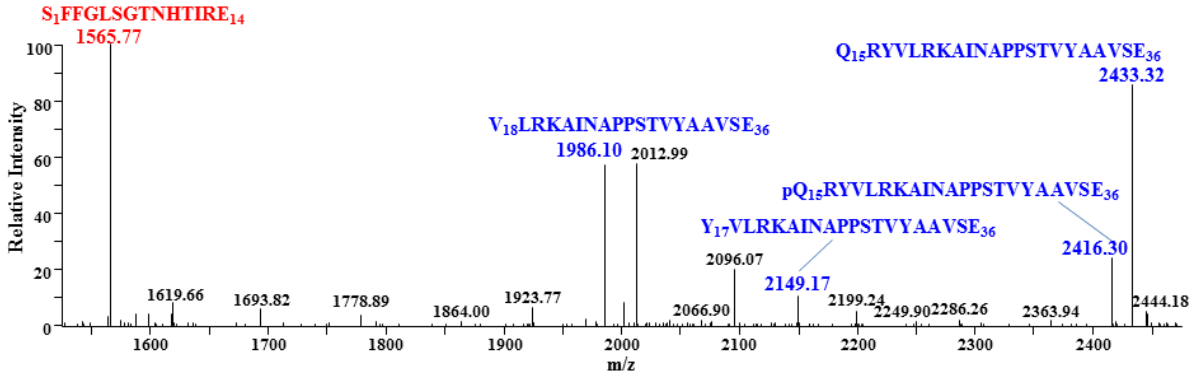

Figure S13. Deconvoluted full MS data from the Glu-C digest of Coq10 revealed two major N-terminal peptides (1565.77; S_1 – E_{14} and 1986.10; V_{18}- E_{36}). The confirmation of N-terminal cleavage was provided by the appearance of several truncated forms of the expected Glu-C derived Q_{15}- E_{36} peptide that could only be observed if N-terminal clipping was present. These peptides are V_{18}-E_{36} (1986.10) and Y_{17}- E_{36} (2149.17 *m/z*).