Mass Spectrometric Analysis of Antibody – Epitope Peptide Complex Dissociation: Theoretical Concept and Practical Procedure of Binding Strength Characterization

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Equations
Mean charge states of each ion species, i.e. educts (e.g. holo-myoglobin ions) and of products (e.g. apo-myoglobin) as well as of ligands (e.g. heme ions) are obtained from the mass spectrum at a given collision cell voltage difference (ΔCV) instrument setting (cf. Figure 2) and can be separately determined according to equation (1).

\[ m^+ = \sum [z^+ \times \left( \frac{h^i}{\sum h^i} \right)] \]  

\[ \text{where} \]

\[ m^+ \] abundance weighted mean charge state of the multiply charged ion series
\[ z^+ \] individual charge state from the multiply charged ion series
\[ h^i \] individual ion intensity of the ion with charge state \( z^+ \) from the multiply charged ion series
\[ \sum h^i \] sum of the intensities of all the multiply charged ions from the multiply charged ion series

Heights of Gaussian fit apexes of ion signals represent relative intensities or fractions of both, products and educts, where \( h \) is the height of apex of the multiply charged complex ions (e.g. holo-myoglobin; starting material), \( i \) is the height of apex of ligand ions (e.g. heme), and \( j \) is the height of apex of the multiply charged protein ions (e.g. apo-myoglobin). Normalization of ion intensities is achieved by summation of all apex values and setting the sum to 100 %. In cases where only one or few charge states of a ligand / protein / complex appeared in the spectrum, more data points for fitting a Gaussian curve have been added by defining other charge states higher and lower than those of the appearing ion signals with respective \( m/z \) values and setting them to have “0” intensities.

\[ h + i + j \leq 100 \% \]  

\[ \text{norm (products)} = \frac{h+j}{h+i+j} \times 100\% \]  

or:

\[ \text{norm (educts)} = \frac{h}{h+i+j} \times 100\% \]  

\[ \text{norm (educts)} = 100\% - \text{norm (products)} \]

Plotting the normalized intensities of the educts of the complex dissociation reaction in the gas phase as a function of collision cell voltage difference (ΔCV) provides a sigmoidal shaped curve with Boltzmann characteristics (cf. Figure 3A). The “steep part” of the dissociation reaction dependence (interval 2dx), i.e. the “energy regime” with greatest dependence between educt ion intensity changes and ΔCV, as well as the determination of ΔCV50 from the Boltzmann fit to the data points is inferred. Also, from the Boltzmann curve, the equation of the tangent line is deduced.
\[ y = b \cdot x + c \] (6)

Slope of tangent line:
\[ b = \frac{A_2 - A_1}{4dx} \] (7)

Intercept of the tangent line with the y-axis:
\[ c = \frac{A_2 + A_1}{2} - \frac{A_2 - A_1}{4dx} \cdot \Delta CV_{50} \] (8)

Once slope and intercept are obtained, the equation of the tangent line is defined as:
\[ y = \frac{A_2 - A_1}{4dx} \cdot x + \frac{A_2 + A_1}{2} - \frac{A_2 - A_1}{4dx} \cdot \Delta CV_{50} \] (9)

With respect to determine apparent activation energies for dissociation of protein complexes, formation of an excited intermediate is assumed. The dissociation reaction follows equation (10).

\[
\text{Prot} \cdot \text{Lig} |^{m^*} \xrightleftharpoons[k_{-1}]{k_1} [\text{Prot} \cdot \text{Lig}] |^{\frac{m^*}{2}} \xrightarrow{k_2} \text{Prot}|^{p^*} + \text{Lig}|^{n^*}
\]

where
- \(\text{Prot} \cdot \text{Lig} |^{m^*}\) multiply charged protein - ligand complex ions
- \([\text{Prot} \cdot \text{Lig}] |^{\frac{m^*}{2}}\) excited intermediate of multiply charged protein - ligand complex ions
- \(\text{Lig}|^{n^*}\) singly / multiply charged ligand ions
- \(\text{Prot}|^{p^*}\) multiply charged protein ions
- \(m^*\) abundance weighted mean charge state of protein - ligand complex ions
- \(n^*\) abundance weighted mean charge state of ligand ions
- \(p^*\) abundance weighted mean charge state of protein ions
- \(k_1, k_{-1}, k_2\) rate constants of partial reactions

The apparent rate of product formation, \(k^*\), becomes independent of \(k_2\) and, hence, of an excited intermediate, when formation of a transition state (TS) is postulated, i.e. under conditions where:

\[ k_2 \gg k_1 > k_{-1} \]

Then, equation (10) can be abbreviated to:

\[
\text{Prot} \cdot \text{Lig} |^{m^*} \xrightarrow{k^*} \text{Prot}|^{p^*} + \text{Lig}|^{n^*}
\]

where
- \(k^*\) apparent rate constant of product formation.

The energy diagram of the complex dissociation reaction describes the experimental situation (cf. Figure 1A).

Since by electrospray there is always an external energy contribution \((\Delta G_{\text{ext}})\) which needs to be considered, the sum of energies affects the experimentally accessible dissociation activation energy when analyzing dissociation of electrosprayed complex ions.

\[
\Delta G_{\text{ext}} > 0 \quad (13)
\]

\[
\Delta G_{\text{ext}} = \Delta G_{\text{CR}} + \Delta G_{\text{kin}} \quad (14)
\]

where
- \(\Delta G_{\text{ext}}\) external energy of the protein - ligand complex ions due to multiple charging and acceleration in the gas phase,
- \(\Delta G_{\text{CR}}\) energy contribution from charge repulsion in multiply charged protein – ligand complex ions in the gas phase,
\( \Delta G_{\text{kin}} \) kinetic energy of protein - ligand complex ions in the gas phase.

\( \Delta G_{\text{kin}} \) includes the energy contribution arising from the applied collision cell voltage difference (\( \Delta CV \)) in the collision cell and \( \Delta G_{\text{CR}} \) arises from charge repulsion.

Similarly, because in the gas phase of a Q-ToF mass spectrometer, collision of multiply charged and accelerated complex ions takes place upon reaching elevated energies, the collision temperature (\( T_{\text{coll}} \)) which is attained by the complex during collision induced dissociation needs to be considered as well. As proposed by a model for collisional activation, [15] \( T_{\text{coll}} \) can be expressed as:

\[
T_{\text{coll}} = T_{\text{amb}} + T_{\text{ext}}
\]

(15)

where

\( T_{\text{coll}} \) protein-ligand temperature during collision in the collision cell
\( T_{\text{amb}} \) absolute ambient temperature, 298 K
\( T_{\text{ext}} \) temperature increment caused by acceleration and multiple charging of ions

and

\[
T_{\text{ext}} = \frac{x m e \Delta CV}{3N_{\text{at}} k_B}
\]

(16)

where

\( x \) overall kinetic energy converted to internal energy, equals 0.24 [15]
\( m \) abundance weighted mean charge state of multiply charged and accelerated protein - ligand complex ions
\( e \) fundamental charge, \( 1.602176634 \times 10^{-19} \) C
\( \Delta CV \) collision cell voltage difference
\( N_{\text{at}} \) number of atoms in the protein - ligand complex
\( k_B \) Boltzmann constant, \( 1.38064852 \times 10^{-23} \) J/K

Substituting equation (16) into equation (15) yields:

\[
T_{\text{coll}} = T_{\text{amb}} + \frac{x m e \Delta CV}{3N_{\text{at}} k_B} \times \Delta CV
\]

(17)

where:

\( T_{\text{coll}} \) protein-ligand temperature during collision in the collision cell
\( T_{\text{amb}} \) absolute ambient temperature, 298 K
\( x \) overall kinetic energy converted to internal energy, \( x = 0.24 \) [15]
\( m \) abundance weighted mean charge state of multiply charged and accelerated protein - ligand complex ions
\( e \) fundamental charge, \( 1.602176634 \times 10^{-19} \) C
\( N_{\text{at}} \) number of atoms in the protein - ligand complex
\( k_B \) Boltzmann constant, \( 1.38064852 \times 10^{-23} \) J/K
\( \Delta CV \) collision cell voltage difference

To determine the apparent Gibbs energy of activation of protein complex dissociation of “neutral and resting” protein - ligand complexes, the ESI-dependent external energy contributions needs to be considered. The conditions for “neutral and resting” protein - ligand complexes are met when:

\[
\Delta G_{\text{ext}} = 0
\]

(18)

It can be concluded that condition \( \Delta G_{\text{ext}} = 0 \) are met at:

\[
T_{\text{ext}} = 0
\]

(19)

and therefore equation (15) is reduced to:

\[
T_{\text{coll}} = T_{\text{amb}}
\]

(20)
which sets the temperature of the dissociation reaction of “neutral and resting” complexes to:

$$\text{T}_{\text{coll}} = \text{T}_{\text{amb}} = 298 \text{ K}$$  \hspace{1cm} (21)

Considering dissociation of “neutral and resting” protein complex ions, i.e. “$$\Delta G_{\text{ext}} = 0$$” conditions, the energy diagram of the complex dissociation reaction requires the introduction of $$\Delta G^\#_{\text{mg}}$$, which is the apparent Gibbs energy of activation that is needed for the dissociation of a protein - ligand complex in the gas phase without external energy contributions (cf. Figure 1B).

$$\Delta G^\#_{\text{mg}}$$ is the apparent Gibbs energy of activation of neutral and resting protein - ligand complexes in the gas phase derived from multiply protonated and accelerated protein - ligand complexes (m: mean of charge states, 0: no additional external energy, g: gas phase).

$$\Delta G^\#_{\text{mg}} = \Delta G^\#_{\text{mg}} + \Delta G_{\text{ext}}$$  \hspace{1cm} (22)

where

- m mean of charge states, is “0” in case of “neutral and resting” complex,
- 0 without external energy contributions,
- g gas phase.

According to the Eyring-Polanyi equation [19], $$k^\#$$ is directly proportional to an apparent thermodynamic quasi equilibrium dissociation constant, $$K^\#_D$$.

$$k^\# = \frac{k_B T}{h} * K^\#_D$$  \hspace{1cm} (23)

where

- $$k^\#$$ apparent rate constant of total reaction,
- $$k_B$$ Boltzmann constant, 1.38064852 x 10^{-23} J/K,
- $$T$$ absolute temperature, K,
- $$h$$ Planck’s constant, 6.62607015 x 10^{-34} Js,
- $$K^\#_D$$ apparent thermodynamic quasi equilibrium dissociation constant.

Regarding mean charge states of complex ions and of gas phase collision temperatures, equation (23) is transformed to:

$$k^\#_{\text{mg}} = \frac{k_B T_{\text{coll}}}{h} * K^\#_{D \text{mg}}$$  \hspace{1cm} (24)

where:

- $$k^\#_{\text{mg}}$$ apparent rate constant of dissociation reaction of the mean charge state of multiply charged and accelerated protein - ligand complex ions in the gas phase
- $$T_{\text{coll}}$$ protein – ligand complex temperature during collisional dissociation in the collision cell
- m abundance weighted mean charge state of multiply charged and accelerated protein - ligand complex ions
- g gas phase
- $$K^\#_{D \text{mg}}$$ apparent gas phase thermodynamic equilibrium dissociation constants of protein - ligand complex dissociation

The apparent gas phase thermodynamic quasi equilibrium dissociation constant, $$K^\#_{D \text{mg}}$$, is also given by:

$$K^\#_{D \text{mg}} = \frac{f(\text{products})}{f(\text{educts})}$$  \hspace{1cm} (25)

combining equations (24) and (25) yields:

$$k^\#_{\text{mg}} = \frac{k_B T_{\text{coll}}}{h} * \frac{f(\text{products})}{f(\text{educts})}$$  \hspace{1cm} (26)

Upon normalization of mass spectral ion intensities, equation (26) becomes:
\[ k_{mg} = \frac{\kappa k_B T_{coll}}{h} \cdot \frac{\text{norm(products)}}{\text{norm(educts)}} \]  

(27)

or:

\[ k_{mg} = \frac{\kappa k_B T_{coll}}{h} \cdot \frac{\text{norm(products)}}{100\% \cdot \text{norm(educts)}} \]  

(28)

or:

\[ \ln k_{mg} = \ln \left( \frac{\kappa k_B T_{coll}}{h} \cdot \frac{\text{norm(products)}}{100\% \cdot \text{norm(educts)}} \right) \]  

(29)

Alternatively:

\[ \ln k_{mg} = \ln \left( \frac{\kappa k_B T_{amb} + \chi m e \cdot \Delta CV}{h} \cdot \frac{\text{norm(products)}}{100\% \cdot \text{norm(educts)}} \right) \]  

(30)

Substituting equation (17) into equation (29) yields:

\[ \ln k_{mg} = \ln \left( \frac{\kappa k_B T_{amb} + \chi m e \cdot \Delta CV}{h} \cdot \frac{\text{norm(products)}}{\text{norm(educts)}} \right) \]  

(31)

Alternatively, substituting equation (17) into equation (30) yields:

\[ \ln k_{mg} = \ln \left( \frac{\kappa k_B T_{amb} + \chi m e \cdot \Delta CV}{h} \cdot \frac{\text{100\% \cdot norm(educts)}}{\text{norm(educts)}} \right) \]  

(32)

where:

- \( k_{mg} \) apparent rate constant of dissociation reaction of the mean charge state of multiply charged and accelerated protein - ligand complex ions in the gas phase
- \( \kappa \) transmission coefficient; is equal to 1 as the products do not re-cross to the transition state
- \( k_B \) Boltzmann constant, 1.38064852 \times 10^{-23} \text{J/K}
- \( T_{amb} \) absolute ambient temperature, 298 K
- \( \chi \) overall kinetic energy converted to internal energy, \( \chi = 0.24 \ [15] \)
- \( m \) abundance weighted mean charge state of multiply charged and accelerated protein - ligand complex ions
- \( e \) fundamental charge, 1.602176634 \times 10^{-19} \text{C}
- \( N_{at} \) number of atoms in the protein - ligand complex
- \( \Delta CV \) collision cell voltage difference
- \( h \) Planck’s constant, 6.62607015 \times 10^{-34} \text{Js}
- \( \text{norm(educts)} \) normalized intensity of educts
- \( \text{norm(products)} \) normalized intensity of products

From the Arrhenius equation, the rate constant \( k_{mg} \) and the apparent energy of activation of protein – ligand complex dissociation \( \Delta G_{mg}^\# \) can be determined.

\[ k^\# = A \cdot e^{-\frac{\Delta G^\#}{RT}} \]  

(33)

where:

- \( k^\# \) apparent rate constant of total reaction
- \( A \) pre-exponential factor
- \( \Delta G^\# \) apparent Gibbs energy of activation.
- \( R \) gas constant, 8.314 J/mol•K
- \( T \) absolute temperature

or:

\[ \ln k^\# = \ln A - \frac{\Delta G^\#}{RT} \]  

(34)

or:

\[ \ln k_{mg} = \ln A - \frac{\Delta G_{mg}^\#}{R} \cdot \frac{1}{T_{coll}} \]  

(35)
where:

\( k_{mg} \) apparent rate constant of dissociation reaction of the mean charge state of multiply charged and accelerated protein - ligand complex ions in the gas phase

\( A \) pre-exponential factor

\( \Delta G_{mg}^* \) apparent Gibbs energy of activation of the abundance weighted mean charge state of multiply charged and accelerated protein - ligand complex ions in the gas phase

\( R \) gas constant, 8.314 J / mol \( \cdot \) K

\( T_{coll} \) protein-ligand temperature during collision in the collision cell

Plotting \( \ln k_{mg}^# \) as a function of \( \frac{1}{T_{coll}} \) provides the intercept with the y-axis (cf. Figure 3B), which is \( \ln A \) (pre-exponential factor) and the slope of the line, which is \(- \frac{\Delta G_{mg}^*}{R}\).

Note, at \( T_{coll} = T_{amb} = 298 \text{ K} \) it can be concluded from equation (16) that:

\[ \Delta CV = 0 \] (36)

Hence, at \( \Delta CV = 0 \) a calculated value for \( k_{m0g}^# \) is obtained i.e. the apparent rate constant of dissociation of “neutral and resting” protein - ligand complexes.

By applying the Eyring-Polanyi equation and from \( k_{m0g}^#, K_{D m0g}^# \) can be determined:

\[ \ln k_{m0g}^# = \frac{k_B T_{amb}}{h} \ln K_{D m0g}^# \] (37)

or:

\[ K_{D m0g}^# = \frac{h}{k_B T_{amb}} \ln k_{m0g}^# \] (38)

where:

\( K_{D m0g}^# \) apparent gas phase thermodynamic equilibrium dissociation constant of “neutral and resting” protein - ligand complexes

\( h \) Planck’s constant, 6.62607015 \( \times \) 10\(^{-34} \) J s

\( \kappa \) transmission coefficient; is equal to 1 as the products do not re-cross to the transition state

\( k_B \) Boltzmann constant, 1.38064852 \( \times \) 10\(^{-23} \) J / K

\( T_{amb} \) absolute ambient temperature, 298 K

\( k_{m0g}^# \) apparent rate constant of dissociation reaction of “neutral and resting” protein - ligand complexes

Thus, at \( \Delta CV = 0 \) the value for \( K_{D m0g}^# \) is calculated i.e. the apparent gas phase thermodynamic equilibrium dissociation constants of protein - ligand complex dissociation, corrected for external energy contributions; i.e. of “neutral and resting” protein - ligand complexes.

At last, by applying the van’t Hoff equation, \( \Delta G_{m0g}^# \) can be determined:

\[ \Delta G_{m0g}^# = -R T_{amb} \ln K_{D m0g}^# \] (39)

where:

\( \Delta G_{m0g}^# \) apparent Gibbs energy of activation of neutral and resting protein - ligand complexes

\( R \) gas constant, 8.314 J / mol \( \cdot \) K

\( T_{amb} \) absolute ambient temperature, 298 K

\( K_{D m0g}^# \) apparent gas phase thermodynamic equilibrium dissociation constants of neutral and resting protein - ligand complexes

Therefore, at \( \Delta CV = 0 \) the value for \( \Delta G_{m0g}^# \) is calculated i.e. the apparent Gibbs energy of activation of neutral and resting protein - ligand complexes.
Supplemental figure 1: Course of normalized ion intensities of holo-myoglobin ions (\textit{norm (educts)}) as a function of collision cell voltage differences (\(\Delta CV\)). Each data point is the mean of three independent measurements. Vertical bars give standard deviations. The curve was fitted using a Boltzmann function. The tangent line equation is taken from the Boltzmann fit. “a” describes the difference between the highest and lowest data points on the sigmoidal fit. “2dx” is the x-axis interval within which the steepest decline of educt is observed. The center of the 2dx interval is \(\Delta CV_{50}\).
Supplemental figure 2: Arrhenius plot for the course of myoglobin dissociation in the gas phase. Each data point (gray squares) is obtained from equation (32) and the value for $\ln k_{m0g}$ is taken from the point of the line at $\frac{1}{T_{amb}}$. The intercept with the y-axis is $\ln A$: 39.17.
Supplemental figure 3: Course of normalized ion intensities of RNase S ions (norm(educts)) as a function of collision cell voltage differences (ΔCV). Each data point is the mean of three independent measurements. Vertical bars give standard deviations. The curve was fitted using a Boltzmann function. The tangent line equation is taken from the Boltzmann fit. “a” describes the difference between the highest and lowest data points on the sigmoidal fit. “2dx” is the x-axis interval within which the steepest decline of educt is observed. The center of the 2dx interval is ΔCV_{50}.

\[ c = 152.3 \]

\[ y = -8.395 \times x + 152.31 \]

ΔCV_{50} = 12.5

2dx = 4.6
Supplemental figure 4: Arrhenius plot for the course of RNAse S dissociation in the gas phase. Each data point (gray squares) is obtained from equation (32) and the value for $\ln k_{m0g}$ is taken from the point of the line at $\frac{1}{T_{amb}}$. The intercept with the $y$-axis is $\ln A$: 128.31.
Supplemental figure 5: Course of normalized ion intensities of FLAG-peptide – antiFLAG antibody ions (norm(educts)) as a function of collision cell voltage differences (ΔCV). Each data point is the mean of three independent measurements (example: myoglobin dissociation). Vertical bars give standard deviations. The curve was fitted using a Boltzmann function. The tangent line equation is taken from the Boltzmann fit. “a” describes the difference between the highest and lowest data points on the sigmoidal fit. “dx” is the x-axis interval within which the steepest decline of educt is observed. The center of the 2dx interval is ΔCV50.

\[ y = -1.26 \times x + 162.4 \]
Supplemental figure 6: Arrhenius plot for the course of FLAG-peptide – antiFLAG antibody complex dissociation in the gas phase. Each data point (gray squares) is obtained from equation (32) and the value for \( \ln k_{mg} \) is taken from the point of the line at \( \frac{1}{T_{amb}} \). The intercept with the y-axis is lnA: 40.80.
Supplemental figure 7: Course of normalized ion intensities of Troponin I peptide – antiTroponin I antibody complex (norm (educts)) as a function of collision cell voltage differences (ΔCV). Each data point is the mean of two independent measurements. Vertical bars give standard deviations. The curve was fitted using a Boltzmann function. The tangent line equation is taken from the Boltzmann fit. “a” describes the difference between the highest and lowest data points on the sigmoidal fit. “2dx” is the x-axis interval within which the steepest decline of educt is observed. The center of the 2dx interval is ΔCV_{50}. 

\[ y = -0.6915 \times + 75.98 \]
Supplemental figure 8: Arrhenius plot for the course of Troponin I peptide – antiTroponin I antibody complex dissociation in the gas phase. Each data point (gray squares) is obtained from equation (32) and the value for $lnk_{m0g}^#$ is taken from the point of the line at $\frac{1}{T_{amb}}$. The intercept with the y-axis is $lnA$: 39.84.
Supplemental Table 1: Ion intensities, charge states, and m/z values for myoglobin at various collision cell voltage difference settings.

### 1st determination

|       | HOLO       | APO      | HEME     | HEME-Ac   | HEME-2Ac |
|-------|------------|----------|----------|-----------|----------|
| ion   | m/z        | 4V       | 20V      | 30V       | 40V      | 45V      | 50V       | 60V       | 80V       | 100V      | 120V      | 150V      | 170V      | 200V      |
| 6+    | 2928       | 0        | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
| 7+    | 2510       | 28,4     | 19,2     | 19,9      | 18,9      | 18,2       | 14,2      | 9,21      | 5,59      | 5,1       | 2,8       | 2,85      | 1,74      |           |
| 8+    | 2196       | 322      | 166      | 143       | 135       | 142        | 133       | 110       | 85,8      | 65,5      | 51        | 36        | 27,7      | 17,6      |
| 9+    | 1952       | 77,4     | 34,1     | 22,5      | 23,2      | 27,8       | 33,5      | 28,5      | 22,3      | 18,4      | 12,5      | 9,47      | 7,62      | 4,74      |
| 10+   | 1757       | 0        | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 11T        | 166      | 7        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 1+D        | 1231,4   | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 1+         | 616,2    | 0        | 2,99      | 13        | 47,4       | 101       | 172       | 241       | 317       | 379       | 393       | 345       | 312       | 262       |
|       | 2+         | 308,6    | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 3+         | 206,1    | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 1+T        | 1846,6   | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 1+D        | 1231,4   | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 1+         | 616,2    | 0        | 2,99      | 13        | 47,4       | 101       | 172       | 241       | 317       | 379       | 393       | 345       | 312       | 262       |
|       | 2+         | 308,6    | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 3+         | 206,1    | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 1+T        | 1669,6   | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 1+D        | 1113,4   | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 1+         | 557,2    | 0        | 0         | 0         | 0          | 0         | 0         | 2,69      | 21,6      | 67,6      | 140       | 168       | 182       |           |
|       | 2+         | 279,1    | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 3+         | 186,4    | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 1+T        | 1492,6   | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 1+D        | 995,4    | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 1+         | 498,2    | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 47,6      | 90        | 156       |           |           |
|       | 2+         | 249,6    | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |
|       | 3+         | 166,7    | 0        | 0         | 0         | 0          | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         |

(T=trimer; D=dimer)
## Supplemental Table 1: continued

### 2nd determination

#### HOLO

| ion  | m/z  | 4V | 20V | 30V | 40V | 45V | 50V | 55V | 60V | 80V | 100V | 120V | 150V | 170V | 200V |
|------|------|----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|
| 6+   | 2928 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 7+   | 2510 | 29 | 23,5| 17,5| 22,8| 24,4| 22,8| 17,4| 13  | 9,16 | 6,47 | 4,81 | 3,81 | 2,11 |
| 8+   | 2196 | 289| 164 | 136 | 176 | 172 | 170 | 151 | 143 | 122 | 91,6 | 73,3 | 52,8 | 38,3 | 25,7 |
| 9+   | 1952 | 62 | 26,5| 23,8| 33,8| 35,5| 39  | 34,1| 38,3| 31,8| 25  | 20   | 12,2 | 10,5 | 6,92 |
| 10+  | 1757 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |

#### APO

| ion  | m/z  | 4V | 20V | 30V | 40V | 45V | 50V | 55V | 60V | 80V | 100V | 120V | 150V | 170V | 200V |
|------|------|----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|
| 5+   | 3391 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 6+   | 2826 | 0  | 2,97| 4,01| 9,39| 15,3| 20,4| 21,4| 10,7| 6,5 | 4,95 | 3,48 | 2,8  | 0    | 0    |
| 7+   | 2422 | 0  | 4,92| 7,45| 18,9| 46,3| 87  | 123 | 146 | 103 | 79,2 | 68,4 | 56   | 49   | 33,2 |
| 8+   | 2119 | 0  | 1,67| 5,94| 18,8| 27,3| 36,2| 43,7| 47,8| 29,3| 29,6 | 30,1 | 25,5 | 22,4 | 15,7 |
| 9+   | 1884 | 0  | 1,17| 2,35| 4,03| 3,31| 4,01| 3,15| 3,22| 3,02| 3,05 | 3,02 | 2,02 | 1,27 | 1,35 |

#### HEME

| ion  | m/z  | 4V | 20V | 30V | 40V | 45V | 50V | 55V | 60V | 80V | 100V | 120V | 150V | 170V | 200V |
|------|------|----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|
| 1+T  | 1846 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 1+D  | 1231 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 1+   | 616  | 0  | 6,16| 17,8| 58,8| 127 | 211 | 290 | 353 | 488 | 558  | 560  | 518  | 466  | 366  |
| 2+   | 308  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 3+   | 206  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |

#### HEME-Ac

| ion  | m/z  | 4V | 20V | 30V | 40V | 45V | 50V | 55V | 60V | 80V | 100V | 120V | 150V | 170V | 200V |
|------|------|----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|
| 1+T  | 1669 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 1+D  | 1113 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 1+   | 498  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 89,5 | 160  | 311  | 0    | 0    |
| 2+   | 279  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 3+   | 186  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |

#### HEME-2Ac

| ion  | m/z  | 4V | 20V | 30V | 40V | 45V | 50V | 55V | 60V | 80V | 100V | 120V | 150V | 170V | 200V |
|------|------|----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|
| 1+T  | 1492 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 1+D  | 995  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 1+   | 498  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 89,5 | 160  | 311  | 0    | 0    |
| 2+   | 249  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 3+   | 166  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |

(T=trimer; D=dimer)
### Supplemental Table 1: continued

#### 3rd determination

**HOLO**

| ion | m/z  | 4V | 20V | 30V | 40V | 45V | 50V | 55V | 60V | 80V | 100V | 120V | 150V | 170V | 200V |
|-----|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|
| 6+  | 2928 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 7+  | 2510 | 24.7 | 19.8 | 21.7 | 22.2 | 20.8 | 18.5 | 14.2 | 11.7 | 9.06 | 5.71 | 5.02 | 3.92 | 6.68 | 4.28 |
| 8+  | 2196 | 255 | 141 | 155 | 159 | 150 | 131 | 110 | 103 | 81.1 | 56.4 | 45.9 | 33.6 | 27.7 | 17.2 |
| 9+  | 1952 | 55.9 | 25.1 | 32.7 | 30.2 | 31.1 | 28.1 | 26.6 | 22.6 | 20.4 | 14.4 | 12.1 | 9.37 | 27.7 | 17.2 |
| 10+ | 1757 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |

**APO**

| ion | m/z  | 4V | 20V | 30V | 40V | 45V | 50V | 55V | 60V | 80V | 100V | 120V | 150V | 170V | 200V |
|-----|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|
| 5+  | 3391 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 6+  | 2826.33 | 0 | 2.46 | 3.57 | 4.68 | 8.72 | 11.4 | 15.8 | 15.7 | 7.01 | 4.41 | 4.32 | 2.34 | 2.05 | 0    |
| 7+  | 2422.67 | 0 | 3.48 | 4.58 | 18.1 | 41   | 88.7 | 98.6 | 72.8 | 54.9 | 46.6 | 40   | 28.1 | 23.3 | 0    |
| 8+  | 2119.93 | 0 | 1.42 | 3.89 | 12.3 | 18.3 | 24   | 23.1 | 28.6 | 21.7 | 19.2 | 20   | 15.1 | 12.6 | 9.85 |
| 9+  | 1884.5 | 0 | 1   | 2.09 | 1.58 | 2.15 | 1.8  | 2.34 | 2.62 | 1.65 | 2.01 | 1.52 | 1.31 | 1    | 0    |

**HEME**

| ion | m/z  | 4V | 20V | 30V | 40V | 45V | 50V | 55V | 60V | 80V | 100V | 120V | 150V | 170V | 200V |
|-----|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|
| 1+T | 1846.6 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 1+D | 1231.4 | 0  | 5.3  | 12.3 | 50.1 | 103  | 165  | 209  | 249  | 319  | 364  | 384  | 358  | 297  | 252  |
| 1+  | 616.2 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 2+  | 308.6 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 3+  | 206.1 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |

**HEME-Ac**

| ion | m/z  | 4V | 20V | 30V | 40V | 45V | 50V | 55V | 60V | 80V | 100V | 120V | 150V | 170V | 200V |
|-----|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|
| 1+T | 1669.6 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 1+D | 1113.4 | 0  | 3.71 | 16.2 | 48.1 | 93   | 146  | 176  | 186  | 183  | 154  | 124  | 104  | 88   | 72   |
| 1+  | 557.2 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 2.73 | 19.7 | 75.9 | 143  | 173  | 190  |
| 2+  | 279.1 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 3+  | 186.4 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |

**HEME-2Ac**

| ion | m/z  | 4V | 20V | 30V | 40V | 45V | 50V | 55V | 60V | 80V | 100V | 120V | 150V | 170V | 200V |
|-----|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|
| 1+T | 1492.6 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 1+D | 995.4 | 0  | 5.3  | 8.5  | 24.1 | 46.4 | 74.7 | 97.7 | 87.8 | 73.9 | 66.3 | 58.7 | 51.1 | 44.5 | 38   |
| 1+  | 498.2 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 2+  | 249.6 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |
| 3+  | 166.7 | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |

(T=trimer; D=dimer)
Supplemental Table 2: Apex heights and mean charge states of educt and product ion signals upon gas phase dissociation of myoglobin.

| ΔCV [V] | 1<sup>st</sup> determination<sup>a)</sup> | 2<sup>nd</sup> determination<sup>a)</sup> | 3<sup>rd</sup> determination<sup>a)</sup> |
|---------|-------------------------------------|-------------------------------------|-------------------------------------|
|         | m+ h  n+ i  p+ j                      | m+ h  n+ i  p+ j                      | m+ h  n+ i  p+ j                      |
| 4       | 8,1 322 1 0 - 0                       | 8,1 289,4 1 0 - 0                    | 8,1 255,2 1 0 - 0                    |
| 20      | 8,1 166,7 1 3 7,3 3,3                 | 8 167,8 1 6,2 7,3 5,4                | 8 143,3 1 5,4 7,3 3,8,0              |
| 30      | 8 146,4 1 13,2 7,3 4,8                | 8 137,9 1 18,1 7,3 7,8               | 8,1 156,3 1 12,5 7,3 4,8             |
| 40      | 8 137,5 1 48,1 7,4 16,5               | 8 177,7 1 59,7 7,4 18,7              | 8 161,1 1 50,9 7,4 18,9              |
| 45      | 8 143,6 1 102,6 7,3 38,2              | 8 173,7 1 129 7,4 48,4               | 8,1 151,3 1 104,6 7,3 41,6           |
| 50      | 8,1 133,3 1 174,7 7,2 81,2            | 8,1 170,7 1 214,3 7,2 87,9           | 8,1 132 1 167,5 7,2 71               |
| 55      | n.d. n.d. n.d. n.d. n.d. n.d.        | 8,1 151,1 1 294,5 7,2 123,2          | 8,1 110,3 1 212,2 7 88,9             |
| 60      | 8,1 110,1 1 244,6 7,1 99,7           | 8,1 143,1 1 358,4 7,2 146            | 8,1 103,3 1 252,8 7,1 98,9           |
| 80      | 8,1 85,8 1 324,6 7,1 79,5            | 8,1 122 1 499,9 7,1 104,1            | 8,1 81,1 1 326,7 7,1 73,5            |
| 100     | 8,1 65,7 1 406,5 7,2 58,3            | 8,1 91,7 1 611,8 7,2 79,3            | 8,1 56,4 1 389,6 7,2 54,9            |
| 120     | 8,1 51 1 466,9 7,2 52,8              | 8,1 73,4 1 674,2 7,3 69,7            | 8,1 45,9 1 467 7,3 47,4              |
| 150     | 8,1 36,1 1 539 7,3 41                | 8,1 52,8 1 852,5 7,3 57,4            | 8,1 33,6 1 552,3 7,2 40,2            |
| 170     | 8,1 27,7 1 576,7 7,2 34              | 8,1 38,3 1 903,8 7,3 50,7            | 8,3 27,7 1 576,6 7,3 28,8            |
| 200     | 8,1 17,6 1 607 7,3 23,9              | 8,1 25,7 1 1028 7,3 34,2             | 8,3 17,2 1 611,3 7,3 23,7            |

<sup>a)</sup> arbitrary units; cf. Figure 4; n.d.: not determined
Supplemental Table 3: Ion intensities, charge states, and m/z values for RNAse S at various collision cell voltage difference settings.

1st determination

**RNAse S Complex 1-19...21-124**

| ion | m/z   | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|-----|-------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 5+  | 2727,90 | 1,83 | 1,01 | 2,84 | 3,63 | 4,82 | 3,14 | 3,89 | 3,44 | 1,46 |
| 6+  | 2272,97 | 51,10 | 36,30 | 67,40 | 109,00 | 124,00 | 99,20 | 98,20 | 49,80 | 19,10 |
| 7+  | 1948,36 | 150,00 | 116,00 | 166,00 | 135,00 | 98,60 | 46,30 | 32,10 | 20,50 | 9,56 |
| 8+  | 1704,93 | 0,90 | 0,58 | 0,67 | 0,00 | 0,29 | 0,00 | 0,28 | 0,16 | 0,28 |
| 9+  | 1515,49 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,12 |

**RNAse S Complex 1-20...22-124**

| ion | m/z   | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|-----|-------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 5+  | 2709,68 | 0,00 | 0,00 | 0,00 | 0,93 | 0,80 | 1,41 | 2,31 | 1,84 | 0,86 |
| 6+  | 2258,30 | 31,20 | 20,80 | 0,00 | 67,30 | 71,80 | 55,60 | 57,00 | 22,90 | 4,22 |
| 7+  | 1935,95 | 78,10 | 64,70 | 48,30 | 70,50 | 49,90 | 23,20 | 20,30 | 12,70 | 5,69 |
| 8+  | 1693,60 | 0,56 | 0,46 | 0,18 | 0,00 | 0,11 | 0,30 | 0,00 | 0,00 | 0,00 |
| 9+  | 1505,78 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |

**S Protein 21-124**

| ion | m/z   | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|-----|-------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 3+  | 3845,75 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| 4+  | 2884,76 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,36 | 1,24 | 42,80 | 44,80 |
| 5+  | 2308,44 | 0,00 | 5,77 | 53,80 | 178,00 | 294,00 | 270,00 | 303,00 | 355,00 | 392,00 |
| 6+  | 1923,71 | 6,00 | 5,26 | 8,56 | 7,47 | 8,53 | 5,36 | 6,54 | 6,41 | 11,30 |
| 7+  | 1648,71 | 0,00 | 0,00 | 1,03 | 0,00 | 0,20 | 0,13 | 0,21 | 0,16 | 0,16 |

**S Protein 22-124**

| ion | m/z   | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|-----|-------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 3+  | 3816,46 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| 4+  | 2862,45 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,40 | 1,10 | 29,20 | 28,40 |
| 5+  | 2289,00 | 0,00 | 12,80 | 42,80 | 120,00 | 190,00 | 175,00 | 194,00 | 217,00 | 241,00 |
| 6+  | 1908,50 | 1,06 | 1,00 | 2,43 | 3,15 | 4,09 | 3,04 | 2,62 | 1,01 | 5,71 |
| 7+  | 1636,20 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,17 | 0,06 | 0,16 | 0,16 |

**S Peptide 1-19**

| ion | m/z   | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|-----|-------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 1+  | 4189,30 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| 2+  | 2095,35 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 5,61 | 0,00 | 0,00 |
| 3+  | 699,15  | 0,00 | 1,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| 4+  | 542,30  | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |

**S Peptide 1-20**

| ion | m/z   | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|-----|-------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 1+  | 4330,36 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| 2+  | 2166,18 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| 3+  | 1083,57 | 0,06 | 0,34 | 9,50 | 42,40 | 78,90 | 83,00 | 95,90 | 141,00 | 157,00 |
| 4+  | 542,30  | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |

D=Dimer
Supplemental Table 3: continued

2nd determination

| RNAse S Complex 1-19...21-124 | Ion | m/z   | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|-------------------------------|-----|-------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 5+                            |     | 2727,2| 0.46| 0.70| 1.06| 0.75| 0.94| 1.44| 2.11| 1.31| 1.12|
| 6+                            |     | 2272,7| 108.00| 134.00| 153.00| 114.00| 122.00| 115.00| 177.00| 54.10| 22.00|
| 7+                            |     | 1948,2| 177.00| 213.00| 161.00| 71.30| 41.90| 28.20| 35.80| 13.30| 7.51|
| 8+                            |     | 1705 | 1.29| 1.05| 0.57| 0.59| 0.42| 0.00| 0.25| 0.09| 0.17|
| 9+                            |     | 1515,5| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00|

| RNAse S Complex 1-20...22-124 | Ion | m/z   | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|-------------------------------|-----|-------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 5+                            |     | 2709,6| 0.26| 0.36| 0.87| 0.46| 1.22| 1.40| 1.51| 1.50| 0.70|
| 6+                            |     | 2258 | 57.80| 75.20| 87.50| 64.80| 68.30| 64.00| 97.50| 26.40| 8.64|
| 7+                            |     | 1935,7| 96.20| 111.00| 86.70| 39.70| 39.70| 15.70| 20.40| 7.22| 4.65|
| 8+                            |     | 1693,9| 0.68| 0.68| 0.50| 0.13| 0.35| 0.19| 0.27| 0.22| 0.20|
| 9+                            |     | 1505,8| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00|

| S Protein 21-124 | Ion | m/z   | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|-----------------|-----|-------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 3+              |     | 3845,8| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00|
| 4+              |     | 2884,7| 0.00| 0.00| 0.00| 0.00| 0.00| 1.01| 2.20| 39.40| 46.00|
| 5+              |     | 2308,6| 24.80| 32.00| 78.90| 121.00| 151.00| 151.00| 250.00| 188.00| 185.00|
| 6+              |     | 1923,3| 8.19| 9.08| 8.22| 4.29| 3.32| 2.57| 3.49| 4.23| 3.74|
| 7+              |     | 1648,8| 0.00| 0.00| 0.00| 0.00| 0.23| 0.00| 0.00| 0.00| 0.22|

| S Protein 22-124 | Ion | m/z   | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|-----------------|-----|-------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 3+              |     | 3816,46| 0.00| 0.00| 0.00| 0.00| 0.00| 1.01| 2.20| 39.40| 46.00|
| 4+              |     | 2862,59| 0.00| 0.25| 0.00| 0.00| 0.00| 0.71| 2.11| 24.60| 27.90|
| 5+              |     | 2288,33| 51.00| 64.20| 76.60| 95.80| 104.00| 108.00| 171.00| 125.00| 115.00|
| 6+              |     | 1908,23| 1.70| 2.37| 1.93| 1.26| 1.30| 1.36| 1.53| 2.23| 2.57|
| 7+              |     | 1636,20| 0.00| 0.18| 0.00| 0.00| 0.32| 0.00| 0.00| 0.00| 0.18|

| S Peptide 1-19 | Ion | m/z   | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|----------------|-----|-------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 1+D            |     | 4189,30| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00|
| 1+             |     | 2095,15| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00|
| 2+             |     | 1048,59| 7.58| 80.00| 142.00| 189.00| 195.00| 281.00| 379.00| 405.00|
| 3+             |     | 699,05| 0.06| 0.00| 0.00| 0.00| 0.11| 0.17| 0.19| 0.19| 0.00|
| 4+             |     | 524,53| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00|

| S Peptide 1-20 | Ion | m/z   | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|----------------|-----|-------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 1+D            |     | 4330,36| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00|
| 1+             |     | 2166,18| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00|
| 2+             |     | 1083,59| 0.18| 1.67| 21.40| 39.40| 64.90| 71.40| 107.00| 145.00| 157.00|
| 3+             |     | 722,72| 0.00| 0.00| 0.12| 0.00| 0.00| 0.17| 0.00| 0.00| 0.00|
| 4+             |     | 542,30| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00|

D=Dimer
Supplemental Table 3: continued

3rd determination

### RNAse S Complex 1-19...21-124

| m/z      | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|----------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 5+       | 2727,6 | 1,59 | 1,57 | 2,88 | 1,07 | 3,15 | 2,84 | 3,68 | 1,96 | 0,59 |
| 6+       | 2272,9 | 130,00 | 183,00 | 234,00 | 195,00 | 229,00 | 178,00 | 269,00 | 52,70 | 14,60 |
| 7+       | 1948,4 | 215,00 | 281,00 | 278,00 | 127,00 | 84,00 | 50,40 | 60,30 | 15,70 | 5,57 |
| 8+       | 1705,2 | 1,03 | 0,39 | 0,31 | 0,30 | 0,33 | 0,38 | 0,75 | 0,37 | 0,29 |
| 9+       | 1515,5 | 0,00 | 0,00 | 0,06 | 0,00 | 0,13 | 0,15 | 0,00 | 0,12 | 0,18 |

### RNAse S Complex 1-20...22-124

| m/z      | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|----------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 5+       | 2709,68 | 0,62 | 0,58 | 0,65 | 1,56 | 1,54 | 1,70 | 2,75 | 1,64 | 0,34 |
| 6+       | 2258,39 | 79,60 | 109,00 | 137,00 | 116,00 | 135,00 | 108,00 | 153,00 | 25,80 | 6,34 |
| 7+       | 1935,92 | 119,00 | 161,00 | 155,00 | 69,9 | 44,50 | 27,10 | 33,30 | 8,68 | 4,14 |
| 8+       | 1694,24 | 0,77 | 0,69 | 0,28 | 0,38 | 0,28 | 0,13 | 0,39 | 0,41 | 0,26 |
| 9+       | 1505,76 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,07 | 0,00 | 0,11 | 0,18 |

### S Protein 21-124

| m/z      | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|----------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 3+       | 3845,75 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| 4+       | 2884,90 | 0,22 | 0,12 | 0,00 | 0,38 | 0,71 | 0,94 | 3,58 | 40,50 | 33,60 |
| 5+       | 2308,05 | 10,40 | 15,50 | 92,40 | 184,00 | 288,00 | 259,00 | 429,00 | 228,00 | 156,00 |
| 6+       | 1923,58 | 8,76 | 12,60 | 11,20 | 6,18 | 5,21 | 2,71 | 3,30 | 8,68 | 4,14 |
| 7+       | 1648,87 | 0,00 | 0,00 | 0,24 | 0,12 | 0,24 | 0,57 | 0,33 | 0,41 | 0,26 |

### S Protein 22-124

| m/z      | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|----------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 3+       | 3816,46 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| 4+       | 2862,33 | 0,21 | 0,30 | 0,00 | 0,35 | 0,73 | 0,87 | 2,65 | 26,80 | 22,50 |
| 5+       | 2288,35 | 34,90 | 39,80 | 75,80 | 125,00 | 189,00 | 174,00 | 278,00 | 140,00 | 98,90 |
| 6+       | 1908,24 | 0,00 | 0,00 | 0,24 | 0,00 | 0,24 | 0,57 | 0,33 | 0,41 | 0,26 |
| 7+       | 1636,20 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |

### S Peptide 1-19

| m/z      | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|----------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 1+D      | 4189,30 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| 1+       | 2095,15 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| 2+       | 1048,07 | 0,21 | 6,41 | 71,10 | 160,00 | 253,00 | 270,00 | 493,00 | 372,00 | 362,00 |
| 3+       | 699,05 | 0,00 | 0,00 | 0,06 | 0,00 | 0,00 | 0,31 | 0,48 | 0,00 | 0,00 |
| 4+       | 524,53 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,21 | 0,20 | 0,00 | 0,00 |

### S Peptide 1-20

| m/z      | 3V | 8V | 11V | 13V | 15V | 17V | 20V | 30V | 50V |
|----------|----|----|-----|-----|-----|-----|-----|-----|-----|
| 1+D      | 4330,36 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| 1+       | 2166,18 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| 2+       | 1083,60 | 0,00 | 1,04 | 18,40 | 47,20 | 90,50 | 100,00 | 190,00 | 144,00 | 141,00 |
| 3+       | 722,72 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,24 | 0,00 | 0,23 | 0,00 |
| 4+       | 542,30 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |

D=Dimer
**Supplemental Table 4:** Apex heights and mean charge states of educt and product ion signals upon gas phase dissociation of RNAse S.

1st determination

| ΔCV [V] | RNAse S 1-19..21-124 | RNAse S 1-20..22-124 | S-Protein 21-124 | S-Protein 22-124 | S-Peptide 1-19 | S-Peptide 1-20 |
|---------|-----------------------|-----------------------|-------------------|-------------------|----------------|----------------|
|         | m_{(1)}^+  h_{(1)} | m_{(1)}^+  h_{(2)} | p_{(1)}^+  j_{(1)} | p_{(2)}^+  j_{(2)} | n_{(1)}^+  i_{(1)} | n_{(1)}^+  i_{(2)} |
| 3       | 6,7 150,63 6,7 79,04 6,0 6,0 6,0 1,06 | 2,0 0,18 2,0 0,07 |
| 8       | 6,7 117,42 6,8 65,32 5,5 5,92 5,1 12,84 | 2,4 1,51 2,0 0,35 |
| 11      | 6,7 169,46 7,0 48,58 5,2 53,28 5,1 42,85 | 2,0 40,45 2,0 9,78 |
| 13      | 6,5 141,61 6,5 73,14 5,0 179,10 5,0 120,54 | 2,0 157,44 2,0 43,64 |
| 15      | 6,5 125,54 6,4 73,50 5,0 295,43 5,0 190,09 | 2,0 265,43 2,0 81,14 |
| 17      | 6,3 101,91 6,3 56,47 5,0 271,48 5,0 176,16 | 2,0 268,56 2,0 85,37 |
| 20      | 6,2 98,79 6,2 56,98 5,0 305,32 5,0 195,58 | 2,0 264,41 2,0 98,68 |
| 30      | 6,2 51,54 6,3 23,88 4,9 358,12 4,9 224,21 | 2,0 386,44 2,0 144,48 |
| 50      | 6,3 19,91 6,5 5,74 4,9 397,52 4,9 244,36 | 2,0 439,48 2,0 161,00 |

a) arbitrary units; cf. Figure 5

2nd determination

| ΔCV [V] | RNAse S 1-19..21-124 | RNAse S 1-20..22-124 | S-Protein 21-124 | S-Protein 22-124 | S-Peptide 1-19 | S-Peptide 1-20 |
|---------|-----------------------|-----------------------|-------------------|-------------------|----------------|----------------|
|         | m_{(1)}^+  h_{(1)} | m_{(1)}^+  h_{(2)} | p_{(1)}^+  j_{(1)} | p_{(2)}^+  j_{(2)} | n_{(1)}^+  i_{(1)} | n_{(1)}^+  i_{(2)} |
| 3       | 6,6 178,50 6,6 96,00 5,3 96,00 5,0 51,30 | 2,1 0,30 2,0 0,18 |
| 8       | 6,6 217,22 6,6 111,64 5,2 111,64 5,0 64,62 | 2,0 7,81 2,0 1,72 |
| 11      | 6,5 165,55 6,5 89,17 5,1 89,17 5,0 78,49 | 2,0 82,33 2,0 21,99 |
| 13      | 6,4 116,12 6,4 65,55 5,0 65,55 5,0 96,26 | 2,0 146,14 2,0 96,26 |
| 15      | 6,3 123,01 6,2 69,31 5,0 69,31 5,0 104,49 | 2,0 194,47 2,0 66,80 |
| 17      | 6,2 119,16 6,2 64,79 5,0 64,79 5,0 108,77 | 2,0 200,63 2,0 73,43 |
| 20      | 6,2 177,28 6,2 99,01 5,0 99,01 5,0 173,30 | 2,0 289,13 2,0 110,13 |
| 30      | 6,2 54,50 6,2 26,62 4,9 26,62 4,9 126,13 | 2,0 387,73 2,0 149,24 |
| 50      | 6,2 22,23 6,3 8,96 4,8 8,96 4,8 116,52 | 2,0 412,82 2,0 160,37 |

a) arbitrary units; cf. Figure 5

3rd determination

| ΔCV [V] | RNAse S 1-19..21-124 | RNAse S 1-20..22-124 | S-Protein 21-124 | S-Protein 22-124 | S-Peptide 1-19 | S-Peptide 1-20 |
|---------|-----------------------|-----------------------|-------------------|-------------------|----------------|----------------|
|         | m_{(1)}^+  h_{(1)} | m_{(1)}^+  h_{(2)} | p_{(1)}^+  j_{(1)} | p_{(2)}^+  j_{(2)} | n_{(1)}^+  i_{(1)} | n_{(1)}^+  i_{(2)} |
| 3       | 6,6 216,06 6,6 121,18 5,5 35,24 5,0 35,24 | 2,0 0,22 2,0 0,22 |
| 8       | 6,6 286,31 6,6 161,90 5,4 15,84 5,0 40,20 | 2,0 6,60 2,0 1,07 |
| 11      | 6,5 277,28 6,5 158,20 5,1 92,77 5,0 76,20 | 2,0 7,31 2,0 18,94 |
| 13      | 6,4 200,68 6,4 120,32 5,0 185,14 5,0 125,68 | 2,0 164,68 2,0 48,58 |
| 15      | 6,3 232,78 6,2 138,67 5,0 289,83 5,0 190,07 | 2,0 93,15 2,0 93,15 |
| 17      | 6,2 181,59 6,2 108,78 5,0 260,73 5,0 175,44 | 2,0 277,80 2,0 102,92 |
| 20      | 6,2 270,91 6,2 162,23 5,0 433,17 5,0 281,03 | 2,0 504,90 2,0 195,48 |
| 30      | 6,2 53,61 6,2 26,36 4,9 229,49 4,9 141,04 | 2,0 380,94 2,0 148,21 |
| 50      | 6,3 14,93 6,4 6,55 4,9 157,36 4,8 100,07 | 2,0 369,42 2,0 143,90 |

a) arbitrary units; cf. Figure 5
**Supplemental Table 5:** Ion intensities, charge states, and m/z values for FLAG-peptide - antiFLAG antibody complex at various collision cell voltage difference settings.

### 1st determination

#### Antibody + 2 peptides

| ion | m/z   | 20  | 40  | 60  | 80  | 100 | 120 | 150 | 170 | 200 |
|-----|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 21+ | 7183  | 0.3 | 0.19| 0.2 | 0   | 0   | 0   | 0   | 0   | 0   |
| 22+ | 6859  | 1   | 1   | 0.9 | 0.18| 0.13| 0.13| 0   | 0   | 0   |
| 23+ | 6562  | 1.76| 1.24| 1.17| 0.29| 0.31| 0.14| 0   | 0   | 0   |
| 24+ | 6283  | 2.1 | 2.01| 1.76| 0.52| 0.51| 0.23| 0   | 0   | 0   |
| 25+ | 6032  | 2.94| 3.15| 2.52| 0.65| 0.51| 0.34| 0   | 0   | 0   |
| 26+ | 5797  | 1.89| 1.84| 1.43| 0.36| 0.42| 0.2 | 0   | 0   | 0   |
| 27+ | 5586  | 0.6 | 0.52| 0.3 | 0   | 0   | 0   | 0   | 0   | 0   |

#### Antibody + 1 peptide

| ion | m/z   | 20  | 40  | 60  | 80  | 100 | 120 | 150 | 170 | 200 |
|-----|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 21+ | 7149  | 0.14| 0.1 | 0.36| 0   | 0   | 0   | 0   | 0   | 0   |
| 22+ | 6814  | 0.54| 1.12| 0.78| 0.34| 0.29| 0   | 0   | 0   | 0   |
| 23+ | 6516  | 0.74| 1.73| 1.31| 0.56| 0.4 | 0.21| 0   | 0   | 0   |
| 24+ | 6239  | 0.78| 2.44| 1.75| 0.6 | 0.78| 0.26| 0   | 0   | 0   |
| 25+ | 5993  | 0.65| 2.58| 2.26| 0.76| 0.61| 0.44| 0   | 0   | 0   |
| 26+ | 5761  | 0.3 | 1.74| 1.47| 0.56| 0.57| 0   | 0   | 0   | 0   |
| 27+ | 5542  | 0.12| 0.2 | 0.5 | 0.2 | 0.16| 0   | 0   | 0   | 0   |

#### Antibody

| ion | m/z   | 20  | 40  | 60  | 80  | 100 | 120 | 150 | 170 | 200 |
|-----|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 21+ | 7094  | 0   | 0   | 0.2 | 0   | 0   | 0   | 0   | 0   | 0   |
| 22+ | 6767  | 0   | 0.5 | 0.49| 0.17| 0.18| 0   | 0   | 0   | 0   |
| 23+ | 6472  | 0   | 0.67| 0.7 | 0.26| 0.18| 0.15| 0   | 0   | 0   |
| 24+ | 6199  | 0   | 0.77| 0.68| 0.23| 0.27| 0.23| 0   | 0   | 0   |
| 25+ | 5949  | 0   | 0.86| 0.68| 0.32| 0.26| 0.16| 0   | 0   | 0   |
| 26+ | 5721  | 0   | 0.55| 0.53| 0.19| 0.16| 0.15| 0   | 0   | 0   |
| 27+ | 5513  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |

#### FLAG peptide

| ion | m/z   | 20  | 40  | 60  | 80  | 100 | 120 | 150 | 170 | 200 |
|-----|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1+T | 3037  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 1+D | 2025  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 1+  | 1013  | 0   | 0   | 0   | 0.91| 3.86| 6.15| 5.73| 4.22| 3.61|
| 2+  | 507   | 0   | 0   | 0   | 1.14| 2.96| 2.12| 2.35| 1.82| 1.14|
| 3+  | 338   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |

(T=trimer; D=dimer)
Supplemental Table 5: continued

2nd determination

### Antibody + 2 peptides

| ion | m/z   | 20 | 40 | 60 | 70 | 80 | 100 | 150 | 170 | 200 |
|-----|-------|----|----|----|----|----|-----|-----|-----|-----|
| 21+ | 7183  | 0.44| 0.62| 0.88| 0.53| 0.58| 0   | 0   | 0   | 0   |
| 22+ | 6859  | 1   | 1,98| 2,37| 2,53| 1,69| 1   | 0.3 | 0   | 0   |
| 23+ | 6562  | 1.27| 3.4 | 5.07| 4.49| 3.4 | 1.76| 0.81| 0   | 0   |
| 24+ | 6283  | 1.86| 5.86| 10.5| 7.35| 5.23| 3.17| 1.29| 0   | 0   |
| 25+ | 6032  | 2.17| 7.8 | 12  | 10.7| 6.92| 4.25| 1.97| 0   | 0   |
| 26+ | 5797  | 1   | 4.08| 5.47| 4.35| 3.35| 2.44| 1.67| 0   | 0   |
| 27+ | 5586  | 0.25| 0.85| 0.76| 0.76| 0.61| 0.5 | 0.42| 0   | 0   |

### Antibody + 1 peptide

| ion | m/z   | 20 | 40 | 60 | 70 | 80 | 100 | 150 | 170 | 200 |
|-----|-------|----|----|----|----|----|-----|-----|-----|-----|
| 21+ | 7149  | 0.44| 0.78| 1.19| 1.13| 0.88| 0   | 0   | 0   | 0   |
| 22+ | 6814  | 1.25| 2.69| 3.62| 3.41| 2.96| 1.55| 0.59| 0   | 0   |
| 23+ | 6561  | 1.58| 4.22| 6.81| 6.39| 5.18| 3.14| 1.14| 0   | 0   |
| 24+ | 6239  | 1.86| 5.97| 10.4| 8.72| 7.58| 4.45| 2.33| 0   | 0   |
| 25+ | 5993  | 1.89| 7.39| 10.9| 9.56| 8.03| 5.73| 2.83| 0   | 0   |
| 26+ | 5761  | 1.03| 3.98| 5.83| 4.95| 3.4 | 2.74| 1.82| 0   | 0   |
| 27+ | 5542  | 0.2 | 0.94| 1.06| 0.92| 0.85| 0.55| 0.45| 0   | 0   |

### Antibody

| ion | m/z   | 20 | 40 | 60 | 70 | 80 | 100 | 150 | 170 | 200 |
|-----|-------|----|----|----|----|----|-----|-----|-----|-----|
| 21+ | 7094  | 0   | 0.49| 0.57| 0.45| 0.51| 0   | 0   | 0   | 0   |
| 22+ | 6767  | 0   | 1.14| 1.91| 1.66| 1.28| 0.75| 0.3 | 0   | 0   |
| 23+ | 6472  | 0   | 1.63| 2.65| 2.63| 2.05| 1.16| 0.63| 0   | 0   |
| 24+ | 6199  | 0.62| 1.71| 3.11| 2.48| 2.43| 1.69| 0.85| 0   | 0   |
| 25+ | 5949  | 0.53| 2.02| 2.81| 2.33| 1.81| 1.53| 1.01| 0   | 0   |
| 26+ | 5721  | 0.38| 1.17| 1.49| 1.58| 0.97| 0.93| 0.58| 0   | 0   |
| 27+ | 5513  | 0   | 0.37| 0.37| 0.39| 0.33| 0   | 0   | 0   | 0   |

### FLAG peptide

| ion | m/z   | 20 | 40 | 60 | 70 | 80 | 100 | 150 | 170 | 200 |
|-----|-------|----|----|----|----|----|-----|-----|-----|-----|
| 1+T | 3037  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 1+D | 2025  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 1+  | 1013  | 0   | 0   | 1.18| 7.03| 8.75| 12.5| 3.15| 3.15| 3.15|
| 2+  | 507   | 0   | 0   | 3.32| 4.77| 8   | 4.68| 0   | 0   | 0   |
| 3+  | 338   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |

(T=trimer; D=dimer)
Supplemental Table 5: continued

3rd determination

### Antibody + 2 peptides

| ion  | m/z   | 40 | 60 | 70 | 80 | 100 | 120 | 150 | 170 |
|------|-------|----|----|----|----|-----|-----|-----|-----|
| 21+  | 7183  | 0  | 0  | 0  | 0  | 0   | 0   | 0   | 0   |
| 22+  | 6859  | 0,79 | 0,63 | 0,61 | 0,39 | 0,51 | 0,1 | 0   | 0   |
| 23+  | 6562  | 1,63 | 1,39 | 1,24 | 1,31 | 1,02 | 0,3 | 0   | 0   |
| 24+  | 6283  | 3,45 | 3,19 | 3,33 | 2,62 | 2,05 | 0,84 | 0   | 0   |
| 25+  | 6032  | 5,98 | 5,36 | 5,94 | 4,77 | 3,35 | 1,81 | 0,47 | 0   |
| 26+  | 5797  | 4,44 | 4,36 | 4   | 3,57 | 2,39 | 1,33 | 0,39 | 0   |
| 27+  | 5586  | 1,35 | 1,17 | 1,12 | 0,89 | 0,7  | 0,61 | 0   | 0   |

### Antibody + 1 peptide

| ion  | m/z   | 40 | 60 | 70 | 80 | 100 | 120 | 150 | 170 |
|------|-------|----|----|----|----|-----|-----|-----|-----|
| 21+  | 7149  | 0  | 0  | 0  | 0  | 0   | 0   | 0   | 0   |
| 22+  | 6814  | 0  | 0,63 | 0,64 | 0,65 | 0,52 | 0,32 | 0   | 0   |
| 23+  | 6516  | 0  | 1,53 | 1,97 | 1,82 | 1,45 | 0,67 | 0,2 | 0   |
| 24+  | 6239  | 0  | 2,44 | 3,24 | 3,2  | 2,43 | 1,77 | 0,34 | 0   |
| 25+  | 5993  | 0  | 4,3  | 5,43 | 4,84 | 3,53 | 1,97 | 0,5 | 0   |
| 26+  | 5761  | 0  | 3,41 | 3,73 | 3,87 | 3   | 1,59 | 0,39 | 0   |
| 27+  | 5542  | 0  | 0,1  | 1,3  | 1,09 | 0,99 | 0,5  | 0   | 0   |

### Antibody

| ion  | m/z   | 40 | 60 | 70 | 80 | 100 | 120 | 150 | 170 |
|------|-------|----|----|----|----|-----|-----|-----|-----|
| 21+  | 7094  | 0  | 0  | 0  | 0  | 0   | 0   | 0   | 0   |
| 22+  | 6767  | 0  | 0,29 | 0,32 | 0,39 | 0,38 | 0   | 0   | 0   |
| 23+  | 6472  | 0  | 0,51 | 0,51 | 0,87 | 0,64 | 0,29 | 0   | 0   |
| 24+  | 6199  | 0  | 0,86 | 0,99 | 1,23 | 0,83 | 0,54 | 0,21 | 0   |
| 25+  | 5949  | 0  | 1,09 | 1,58 | 1,49 | 1,13 | 0,75 | 0,33 | 0   |
| 26+  | 5721  | 0  | 1,13 | 1,27 | 0,88 | 0,76 | 0,44 | 0,24 | 0   |
| 27+  | 5513  | 0  | 0,35 | 0,52 | 0,45 | 0   | 0   | 0   | 0   |

### FLAG peptide

| ion  | m/z   | 40 | 60 | 70 | 80 | 100 | 120 | 150 | 170 |
|------|-------|----|----|----|----|-----|-----|-----|-----|
| 1+T  | 3037  | 0  | 0  | 0  | 0  | 0   | 0   | 0   | 0   |
| 1+D  | 2025  | 0  | 0  | 0  | 0  | 0   | 0   | 0   | 0   |
| 1+   | 1013  | 0  | 0  | 1,71 | 4,97 | 7,44 | 5,97 | 1,36 | 1,36 |
| 2+   | 507   | 0  | 0  | 2,28 | 3,75 | 4,51 | 1,9  | 0   | 0   |
| 3+   | 338   | 0  | 0  | 0   | 0   | 0   | 0   | 0   | 0   |

(T=trimer; D=dimer)
### Supplemental Table 6: Apex heights and mean charge states of educt and product ion signals upon gas phase dissociation of FLAG-peptide – antiFLAG antibody complex.

| ΔCV [V] | 1<sup>st</sup> determination<sup>a)</sup> | 2<sup>nd</sup> determination<sup>a)</sup> | 3<sup>rd</sup> determination<sup>a)</sup> |
|---------|---------------------------------|---------------------------------|---------------------------------|
|         | m<sub>(1)+</sub> h<sub>(1)</sub> m<sub>(2)+</sub> h<sub>(2)</sub> n+ i p+ j | m<sub>(1)+</sub> h<sub>(1)</sub> m<sub>(2)+</sub> h<sub>(2)</sub> n+ i p+ j | m<sub>(1)+</sub> h<sub>(1)</sub> m<sub>(2)+</sub> h<sub>(2)</sub> n+ i p+ j |
| 4       | 23,8 0,804 24,4 2,913 0 0 0 | - 0 24 1,868 0 0 | - 0 - 5,981 0 0 |
| 20      | 23,8 0,804 24,4 2,913 0 0 | 23,9 1,871 24 2,137 | 0 0 | 0 0 - 5,981 0 0 |
| 40      | 24,2 2,611 24,5 3,134 0 24,1 0,834 | 24,2 7,032 24,4 7,668 0 | 24, 1,199 | 0 0 24,9 5,981 0 0 |
| 60      | 24,3 2,145 24,3 2,455 0 23,8 0,748 | 24,2 11,364 24,3 12,551 1,7 3,534 23,9 2,421 | 24,7 4,454 24,9 5,492 | 0 24,8 1,142 |
| 70      | n.d. n.d. n.d. n.d. n.d. n.d. n.d. | 24,2 9,666 24,3 10,529 1,4 7,248 23,9 1,979 | 24,8 5,142 24,9 5,933 | 1,6 2,172 24,9 1,612 |
| 80      | 24,4 0,704 24,4 0,645 1,6 1,217 24,1 0,337 | 24,1 8,254 24,3 6,657 1,5 9,165 23,9 1,765 | 24,8 4,776 24,9 4,798 1,4 5,147 24,6 1,413 |
| 100     | 24,5 0,726 24,4 0,56 1,4 4,003 24 0,293 | 24,4 5,345 24,5 4,129 1,3 12,28 24,1 1,016 | 24,8 3,49 24,8 3,314 1,4 7,644 24,3 1,044 |
| 120     | 24,2 0,401 24,7 0,328 1,3 6,059 24,4 0,226 | 24,6 2,843 24,8 1,958 1,2 9,043 24,3 0,324 | 24,8 2,087 25,2 1,81 1,3 6,019 24,7 0,743 |
| 150     | - 0 - 0 1,3 5,609 - | - 0 - 0 1 3,199 - | 24,8 0,503 25,5 0,482 1 1,381 25 0,352 |
| 170     | - 0 - 0 1,3 4,308 - | - 0 - 0 1 3,199 - | - 0 - 0 1 1,381 - |
| 200     | - 0 - 0 1,2 3,64 - | - 0 - 0 1 3,199 - | - 0 - 0 1 1,381 - |

<sup>a)</sup> arbitrary units; cf. Figure 6; n.d.: not determined
Supplemental Table 7: Ion intensities, charge states, and m/z values for Troponin I peptide - antiTroponinI at various collision cell voltage difference settings.

### 1st determination

| Ion     | m/z  | 2V  | 4V  | 6V  | 8V  | 12V | 16V | 20V | 30V | 40V | 50V | 60V | 70V | 80V |
|---------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Troponin I peptide |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| D+1     | 3629 | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  |
| 1+      | 1815 | 6   | 8   | 7   | 7   | 12  | 11  | 12  | 26  | 45  | 63  | 111 | 133 | 152 |
| 2+      | 908  | 118 | 187 | 256 | 352 | 692 | 1634| 2155| 4871| 6635| 9454| 11135| 10995| 11326|
| 3+      | 606  | 59  | 76  | 126 | 134 | 238 | 513 | 692 | 1254| 1788| 2324| 2294| 2234| 2118|
| 4+      | 454  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  |

| Antibody |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 28+       | 5231| 506 | 582 | 542 | 400 | 330 | 773 | 652 | 643 | 588 | 587 | 581 | 513 | 507 |
| 27+       | 5424| 1963| 2302| 2188| 1773| 1616| 2834| 2646| 2631| 2519| 2622| 2564| 2315| 2218|
| 26+       | 5632| 3689| 4083| 4114| 3696| 3484| 5073| 4974| 5110| 5317| 5485| 5376| 5124| 5021|
| 25+       | 5860| 2851| 3293| 3291| 3274| 3274| 3893| 3959| 4544| 513 | 507 | 507 | 507 | 507 |
| 24+       | 6102| 1315| 1404| 1517| 1659| 1685| 1712| 1835| 2330| 2667| 2971| 3142| 3031| 3131|
| 23+       | 6365| 381 | 363 | 404 | 494 | 551 | 496 | 582 | 811 | 1124| 1175| 1339| 1303| 1359|

| Antibody with 1 peptide |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 28+                    | 5296| 1185| 1512| 1400| 1025| 872 | 1888| 1667| 1564| 1413| 1381| 1295| 1102| 1024|
| 27+                    | 5489| 4192| 4916| 4812| 4034| 3773| 6061| 5581| 5683| 5456| 5356| 5065| 4633| 4530|
| 26+                    | 5701| 6474| 7368| 7391| 7017| 6850| 8697| 8623| 9133| 9295| 9384| 9198| 8567| 8360|
| 25+                    | 5930| 4458| 4767| 5074| 5132| 5196| 5764| 5828| 6548| 7029| 7181| 7353| 6989| 7002|
| 24+                    | 6179| 1770| 1816| 2020| 2231| 2283| 2206| 2417| 3059| 3366| 3591| 3768| 3550| 3697|
| 23+                    | 6446| 388 | 419 | 445 | 524 | 597 | 533 | 608 | 859 | 1062| 1168| 1314| 1270| 1312|

| Antibody with 2 peptides |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 28+                    | 5359| 726 | 901 | 842 | 632 | 528 | 1128| 1022| 968 | 789 | 741 | 669 | 603 | 533 |
| 27+                    | 5558| 2275| 2588| 2607| 2238| 2072| 3214| 2949| 2894| 2716| 2540| 2413| 2204| 2107|
| 26+                    | 5773| 3056| 3320| 3392| 3349| 3322| 3993| 3846| 3911| 4042| 3822| 3681| 3425| 3421|
| 25+                    | 6003| 1805| 1916| 2045| 2150| 2104| 2302| 2259| 2484| 2581| 2505| 2486| 2306| 2307|
| 24+                    | 6250| 618 | 638 | 688 | 753 | 822 | 748 | 800 | 892 | 1021| 957 | 1020| 905 | 967 |
| 23+                    | 6523| 121 | 129 | 150 | 156 | 167 | 156 | 171 | 195 | 239 | 254 | 271 | 262 | 286 |

D = dimer; ion intensity values were imputed
4+ ion intensity values were imputed
# Supplemental Table 7: continued

## 2nd determination

### Troponin I peptide

| ion | m/z | 2V | 4V | 6V | 8V | 12V | 16V | 20V | 30V | 40V | 50V | 60V | 70V | 80V |
|-----|-----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| D1+ | 3629 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 1+  | 1816 | 5  | 5  | 8  | 8  | 7  | 16 | 8  | 18 | 29 | 58 | 94 | 135| 156|
| 2+  | 908  | 75 | 111| 193| 328| 465| 1052| 1709| 3463| 6093| 7789| 10328| 10711| 10635|
| 3+  | 606  | 31 | 52 | 91 | 121| 153| 289 | 448 | 799 | 1373| 1513| 1621| 1573| 1376|
| 4+  | 455  | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

### Antibody

| ion | m/z | 2V | 4V | 6V | 8V | 12V | 16V | 20V | 30V | 40V | 50V | 60V | 70V | 80V |
|-----|-----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 28+ | 5231| 278| 252| 294| 367| 276 | 377 | 469 | 542 | 636 | 434 | 530 | 92 | 484 |
| 27+ | 5424| 1222|991 |1211|1536|897 |1356|1608|1870|2164|1790|2002|1957|1785|
| 26+ | 5632| 2507|2038|2481|3083|1928|2504|3042|3327|3720|3465|3906|3782|3400|
| 25+ | 5860| 1846|2289|2727|2017|2299|2567|2826|3119|3222|3550|3472|3149| |
| 24+ | 6102| 342 |308 |262 |349 |422 |418 |432 |434 |526 |590 |732 |812 |759 |
| 23+ | 6365| 308 |262 |349 |422 |418 |432 |434 |526 |590 |732 |812 |759 | |

### Antibody with 1 peptide

| ion | m/z | 2V | 4V | 6V | 8V | 12V | 16V | 20V | 30V | 40V | 50V | 60V | 70V | 80V |
|-----|-----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 28+ | 5296| 674 |604 |738 |894 |627 |928 |1165|1327|1550|1082|1212|1146|1018|
| 27+ | 5489| 2872|2334|2804|3500|1953|2980|3499|4040|4434|3693|3900|3787|3376|
| 26+ | 5701| 5092|3991|4896|6006|3268|4375|5216|5795|6203|5923|6374|6107|5356|
| 25+ | 5930| 3733|2981|3640|4547|2533|3097|3505|3807|4196|4523|4931|4606|4209|
| 24+ | 6179| 1559|1242|1547|1936|1205|1331|1496|1640|1857|2175|2405|2299|2129|
| 23+ | 6446| 364 |310 |400 |471 |341 |382 |410 |492 |566 |710 |807 |790 |754 |

### Antibody with 2 peptides

| ion | m/z | 2V | 4V | 6V | 8V | 12V | 16V | 20V | 30V | 40V | 50V | 60V | 70V | 80V |
|-----|-----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 28+ | 5359| 422 |445 |518 |578 |559 |736 |878 |934 |956 |689 |671|707 |581 |
| 27+ | 5558| 1615|1565|1749|2001|1904|2238|2529|2630|2578|2074|2017|1906|1759|
| 26+ | 5773| 2469|2316|2578|3025|2951|3135|3353|3329|3259|2959|2859|2693|2570|
| 25+ | 6003| 1531|1501|1647|2011|2124|2114|2171|2136|2102|2029|1990|1825|1828|
| 24+ | 6250| 545 |508 |615 |729 |857 |810 |853 |808 |807 |903 |867 |789 |848 |
| 23+ | 6523| 112 |114 |128 |143 |191 |189 |193 |196 |235 |256 |263 |268 |304 |

D = dimer; ion intensity values were imputed
4+ ion intensity values were imputed
Supplemental Table 8: Apex heights and mean charge states of educt and product ion signals upon gas phase dissociation of TroponinI immune complex.

ΔCV [V] | 1st determination a) | 2nd determination a)
|----------------|-----------------|-----------------|
| \( m_{(1)^+} \) \( h_{(1)} \) \( m_{(2)^+} \) \( h_{(2)} \) \( n^+ \) \( i \) \( p^+ \) \( j \) | \( m_{(1)^+} \) \( h_{(1)} \) \( m_{(2)^+} \) \( h_{(2)} \) \( n^+ \) \( i \) \( p^+ \) \( j \) |
| 2 | 25,9 | 6370 | 26,1 | 3029 | 2,2 | 108 | 25,7 | 3611 |
| 4 | 25,9 | 7203 | 26,1 | 3322 | 2,3 | 175 | 25,7 | 4107 |
| 6 | 25,9 | 7271 | 26,1 | 3395 | 2,2 | 239 | 25,6 | 4094 |
| 8 | 25,8 | 6881 | 26,0 | 3287 | 2,1 | 361 | 25,5 | 3794 |
| 12 | 25,7 | 6756 | 25,9 | 3210 | 2,3 | 1186 | 25,4 | 3667 |
| 16 | 26,0 | 8592 | 26,1 | 4041 | 2,2 | 2082 | 25,7 | 4990 |
| 20 | 25,9 | 8402 | 26,1 | 3825 | 2,2 | 2872 | 25,7 | 4917 |
| 30 | 25,8 | 8892 | 26,0 | 3886 | 2,1 | 5105 | 25,5 | 5237 |
| 40 | 25,7 | 9092 | 26,0 | 3931 | 2,2 | 7176 | 25,4 | 5469 |
| 50 | 25,7 | 9160 | 25,9 | 3721 | 2,1 | 9712 | 25,4 | 5774 |
| 60 | 25,6 | 9057 | 25,9 | 3580 | 2,0 | 11192 | 25,3 | 5816 |
| 70 | 25,6 | 8495 | 25,9 | 3323 | 2,0 | 11050 | 25,3 | 5485 |
| 80 | 25,6 | 8389 | 25,9 | 3291 | 1,9 | 11501 | 25,3 | 5482 |

a) arbitrary units; cf. Figure 7