Electron Transfer Dissociation of Multiply-charged Peptides: The Roles of Total Charge and Identities of Charge-bearing Sites

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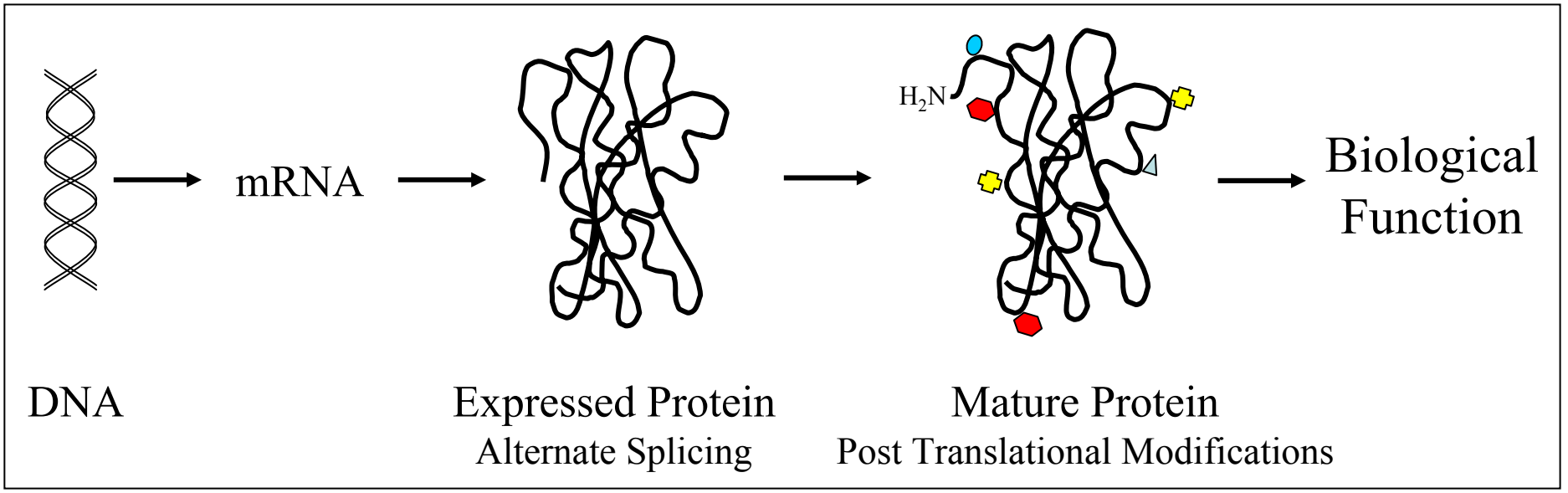
Protein Identification/Characterization:

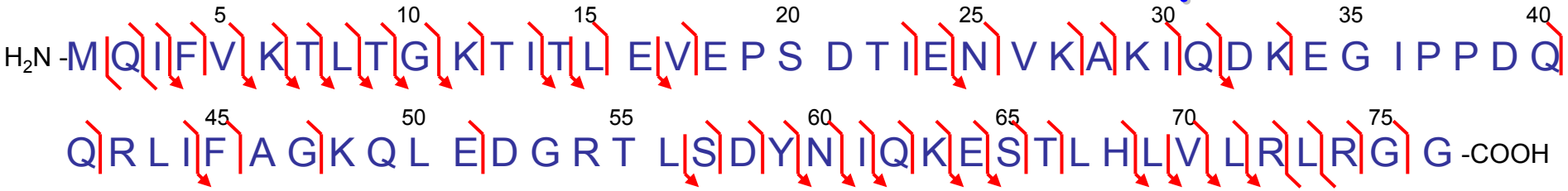
Genomics.
Static. 20,000+ Genes

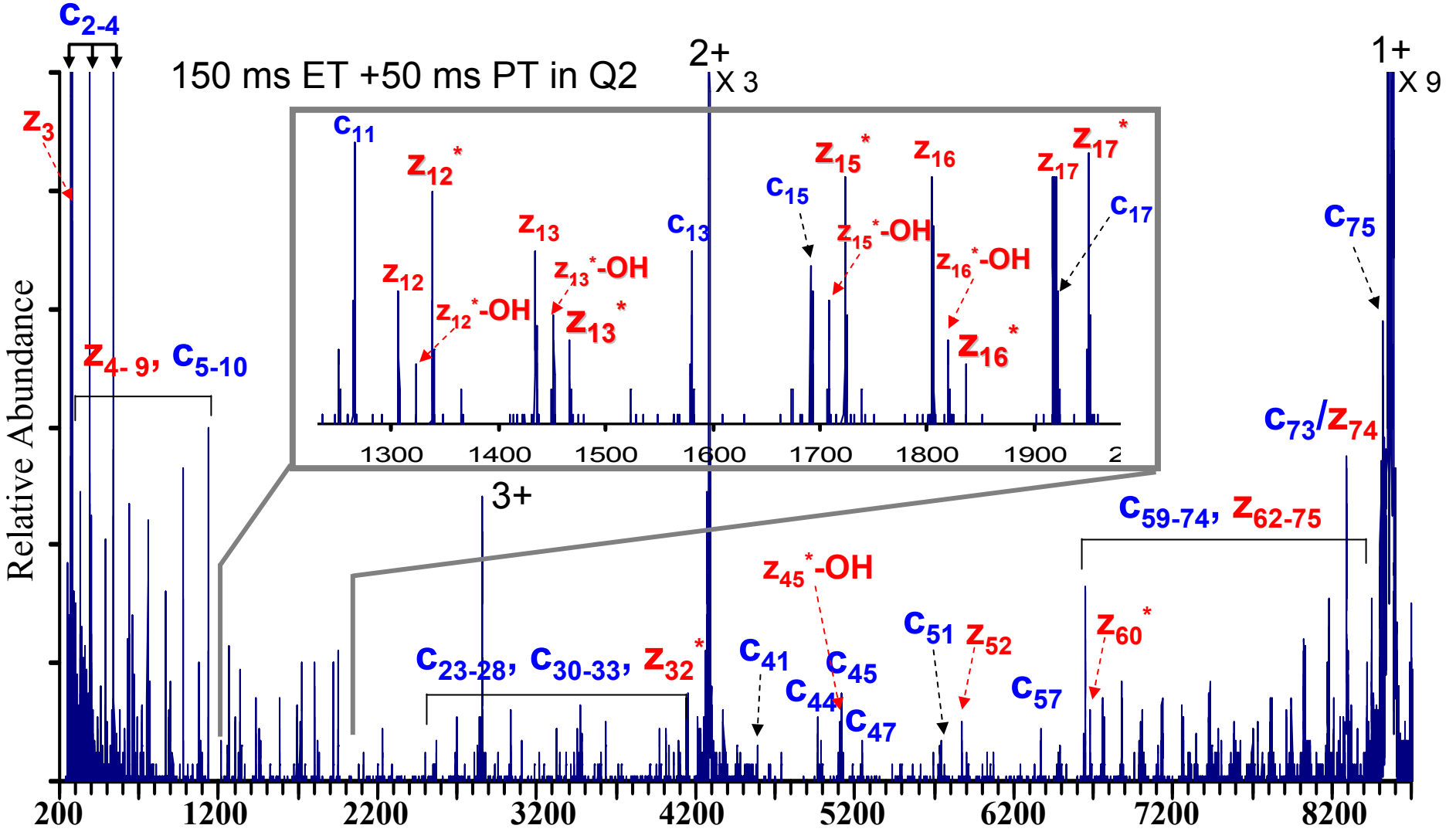
vs.

Proteomics.
Dynamic. >1,000,000 Proteins

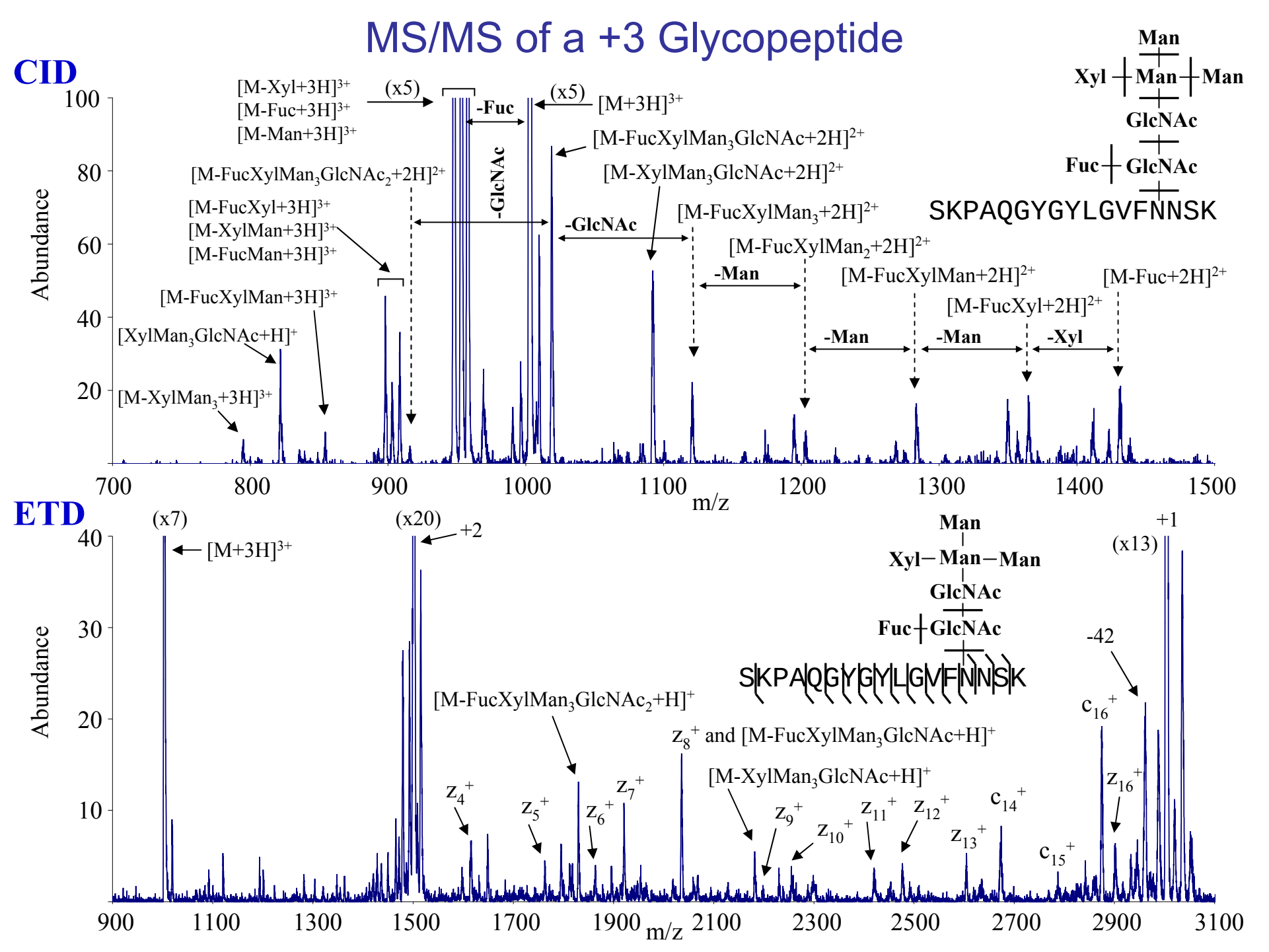


ET + PT Reactions of +12 Ubiquitin



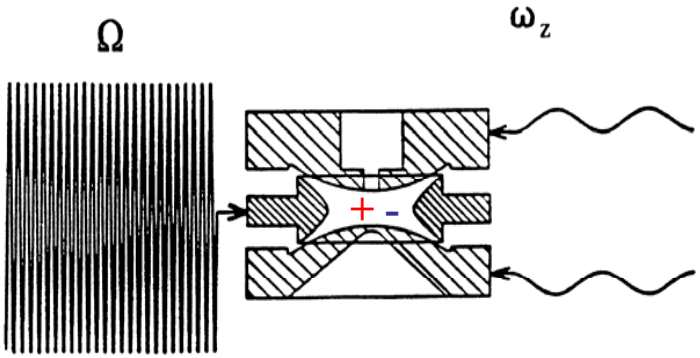


MS/MS of a +3 Glycopeptide

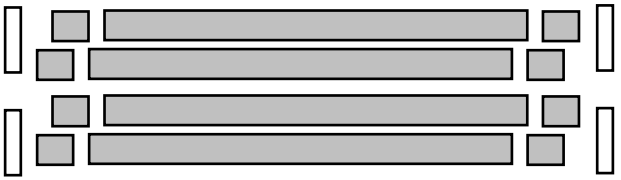


Experimental approach:

Macro-ions are studied in an electrodynamic ion trap. Important characteristics of this approach include:



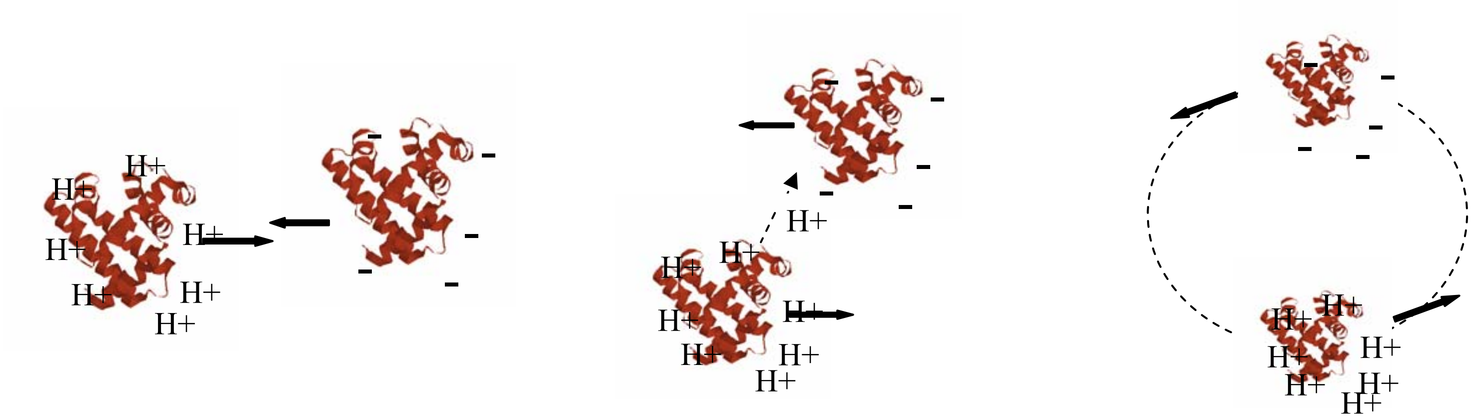
Quadrupole ion trap



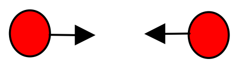
Linear Ion Trap

- High bath gas pressures (1 mtorr) and variable temperatures to provide thermal and near thermal reaction conditions.
- Ion storage and manipulation capabilities to provide for high experimental flexibility including kinetics measurements.
- Dual polarity ion storage capability to enable the study of ion/ion reactions.

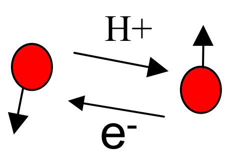
Types of Single Ion/Ion Encounters:



- Direct Hard-Sphere (Intimate) Collision

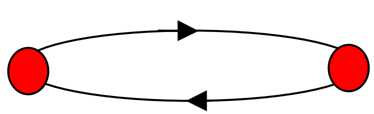


- Proton or Electron Transfer at a Crossing



  – The electrical fields are strong enough to pull protons off at distances of around 100Å

- Formation of Coulombically bound orbital complexes



  – Orbit may bring reactants into close enough proximity for reaction. Orbits can collapse due to tidal effect and/or collisions.

Cross-sections for the limiting cases:

- Direct Hard-Sphere Collision $$b_{h-s}^2 = r_{h-s}^2\left[1+\frac{2Z_1Z_2e^2}{4\pi\varepsilon_0 r_{h-s}\mu v^2}\right]$$

- Proton Hopping

$$r_{transfer}\,range = r_{hop}\,to\,(r_{hop}+r_{h-s})$$

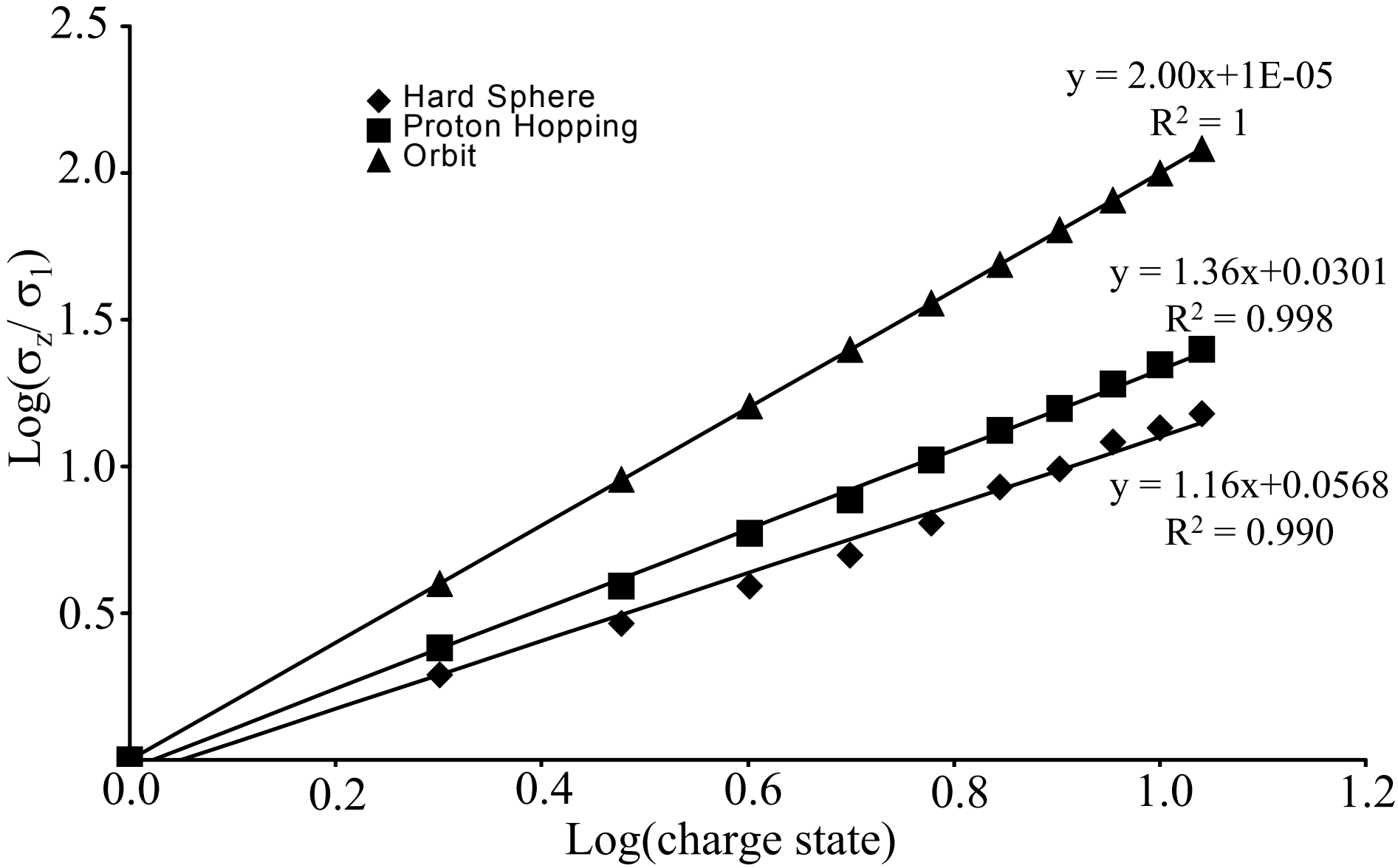
$$b_{transfer}^2 = r_{transfer}^2\left[1+\frac{2Z_1Z_2e^2}{4\pi\varepsilon_0 r_{transfer}\mu v^2}\right]$$

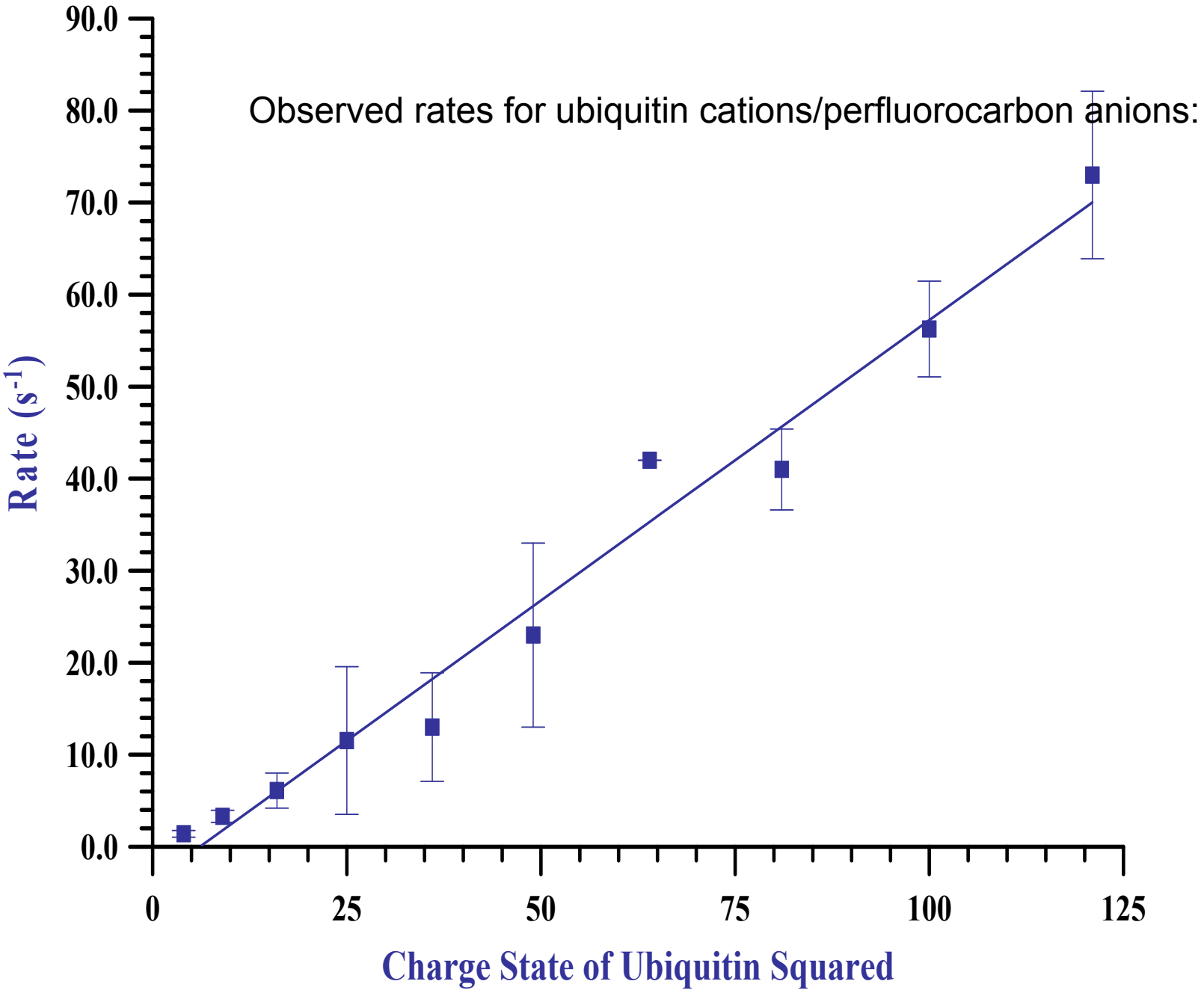
$$r_{hop} \cong \frac{Z_1Z_2e^2}{4\pi\varepsilon_0\Delta H_{transfer}}$$

- Orbital Complex $$b_{orb.}^2 \approx \frac{4Z_1^2Z_2^2e^4}{\left(4\pi\varepsilon_0\mu v^2\right)^2}$$

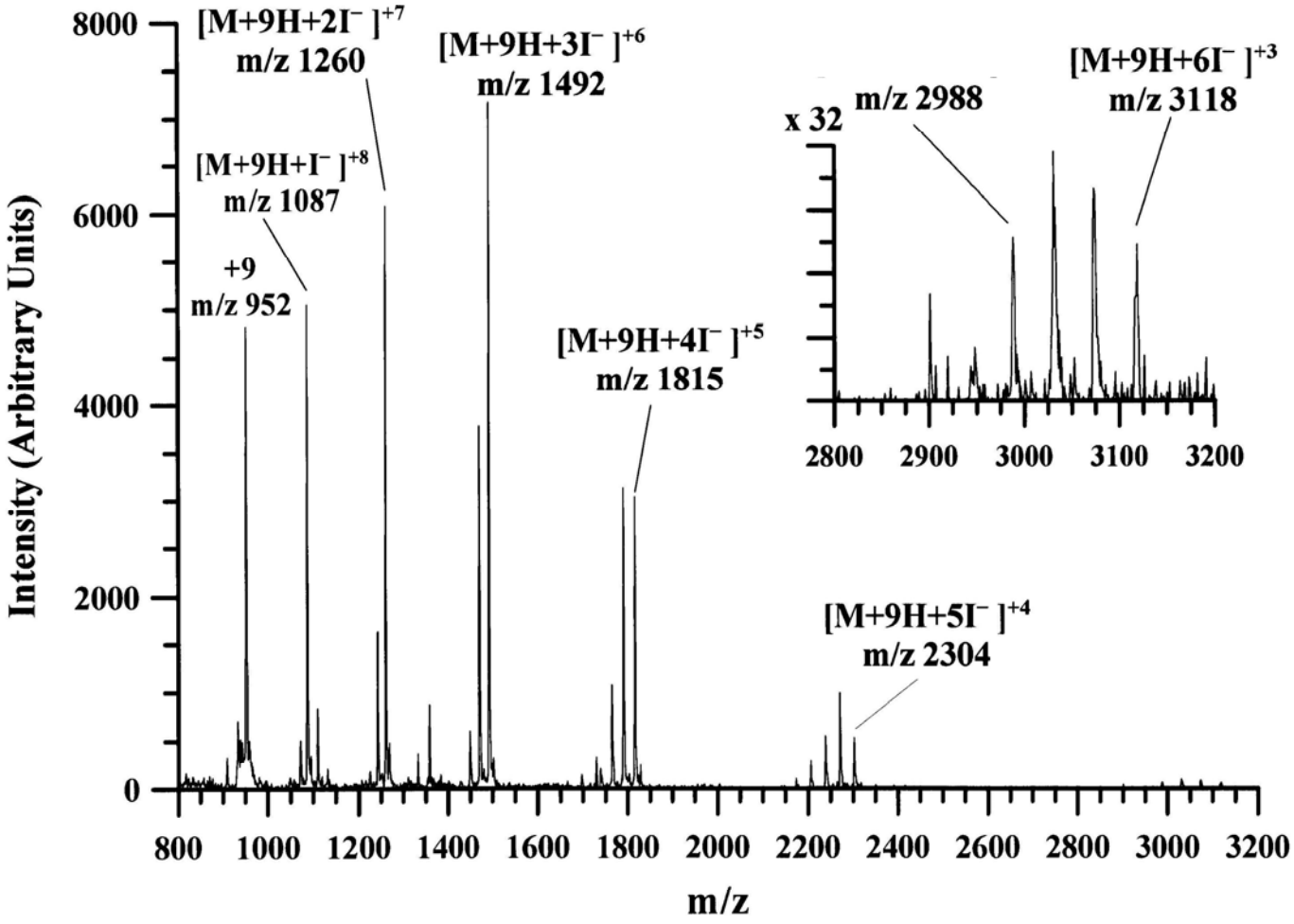
(Thomson 3-body model)

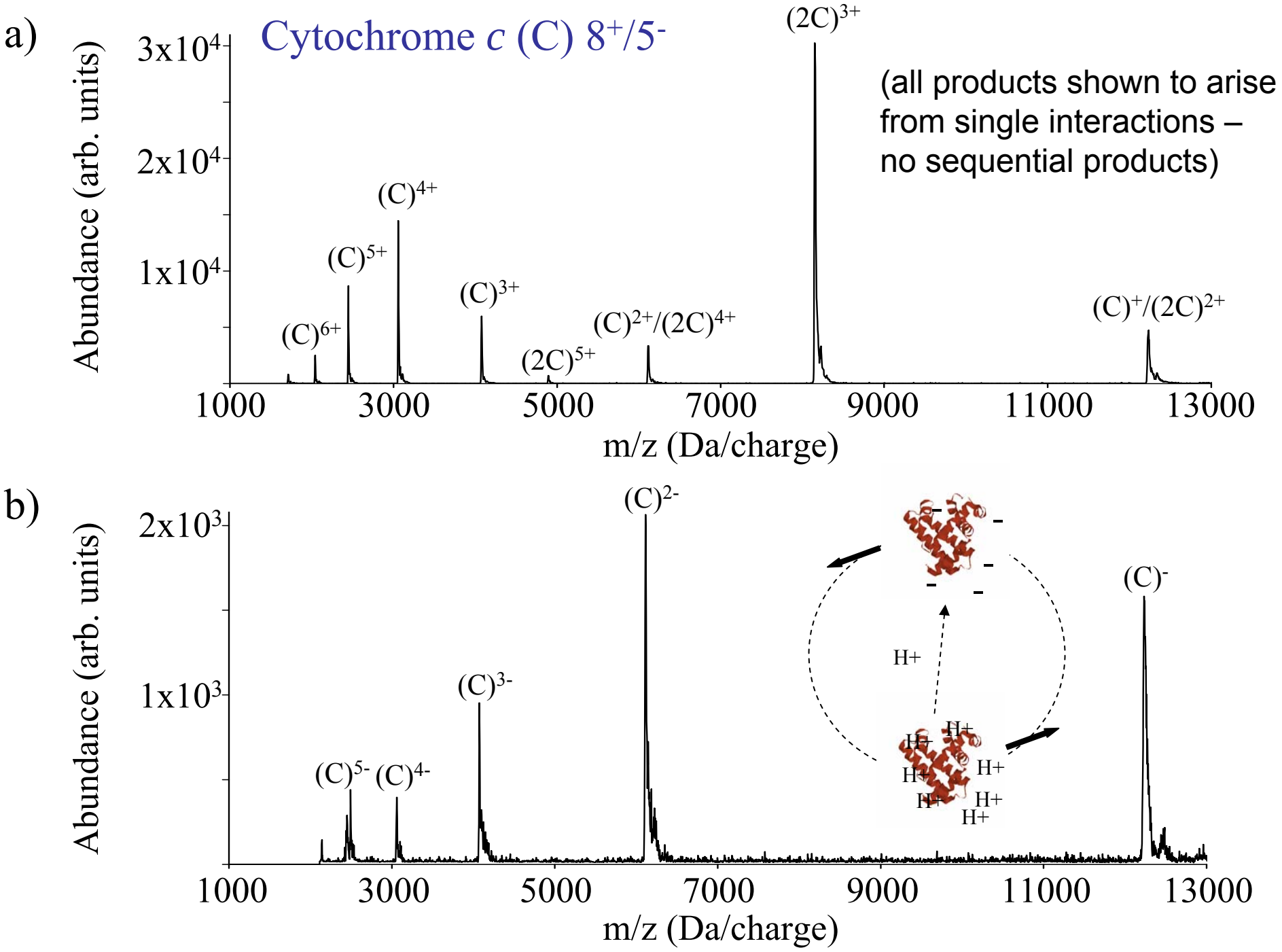
Predicted rate dependencies of different models on z




Observed rates for ubiquitin cations/perfluorocarbon anions:
Rate (s^{-1})
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
25
50
75
100
125
Charge State of Ubiquitin Squared

Ubiquitin $(M+9H)^{9+}/I^-$ (25 ms)




a)
Cytochrome c (C) 8+/5-
(all products shown to arise from single interactions – no sequential products)
Abundance (arb. units)
3x10^4
2x10^4
1x10^4
(C)6+
(C)5+
(C)4+
(C)3+
(2C)5+
(C)2+/(2C)4+
(2C)3+
(C)+/(2C)2+
1000
3000
5000
7000
9000
11000
13000
m/z (Da/charge)
b)
Abundance (arb. units)
2x10^3
1x10^3
(C)5-
(C)4-
(C)3-
(C)2-
(C)-
H+
m/z (Da/charge)

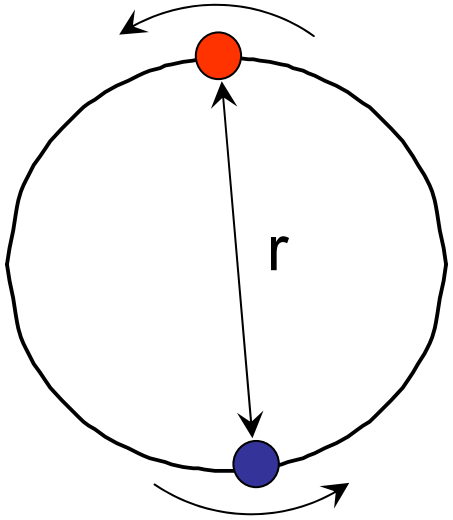
Dynamics of Protein/Protein Reactions

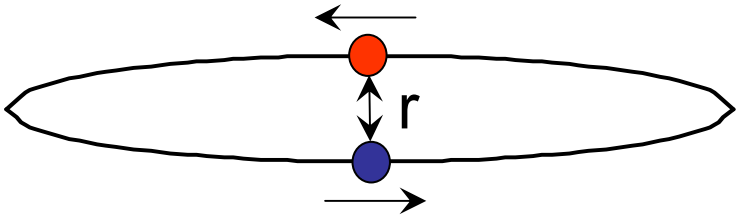
Low eccentricity orbit

- r slowly decreases as KE is removed by tidal effects, He collisions
- ions do not interact until KE is low
- acid/base and non-covalent interactions bind chains together

High eccentricity orbit

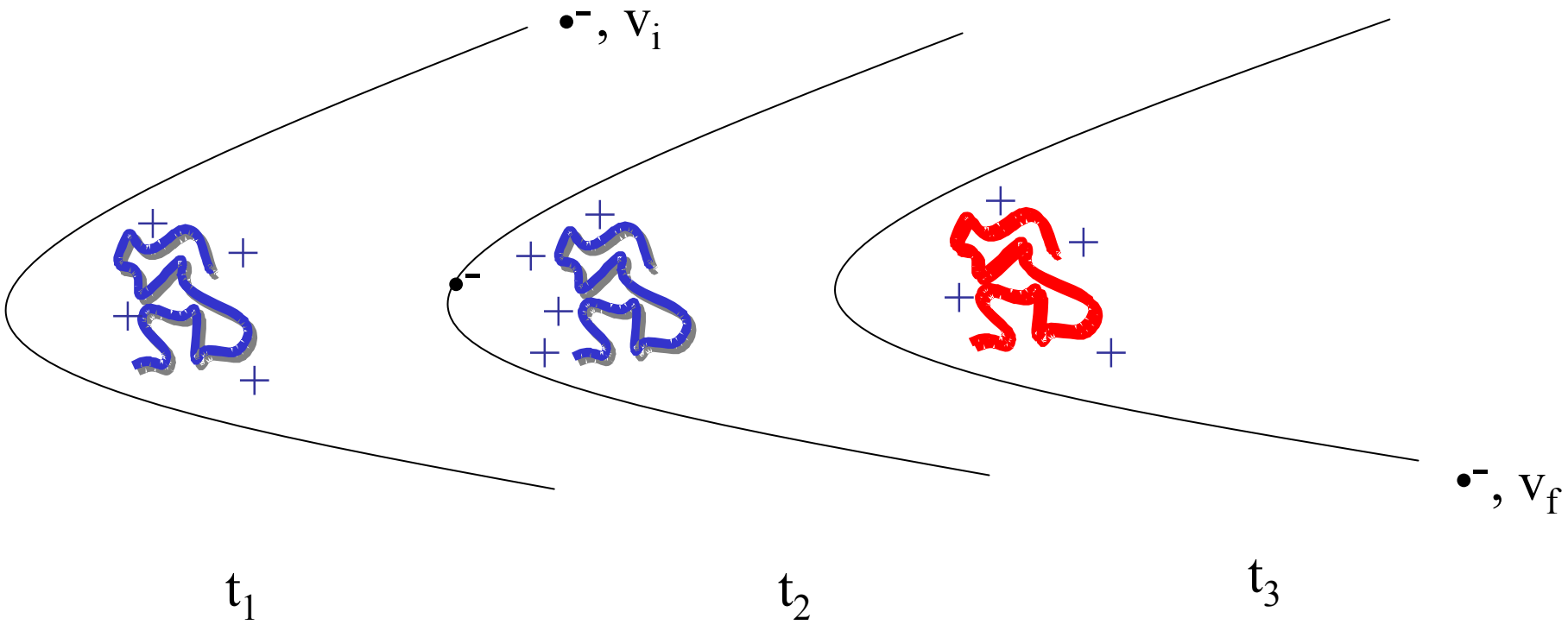
- KE is highest when r is low
- when protons transfer, Coulombic attraction is reduced and ions separate





r ≡ distance of closest approach

A “tidal” mechanism for ion/ion capture, Xslational → ε transfer



Factors in ETD:

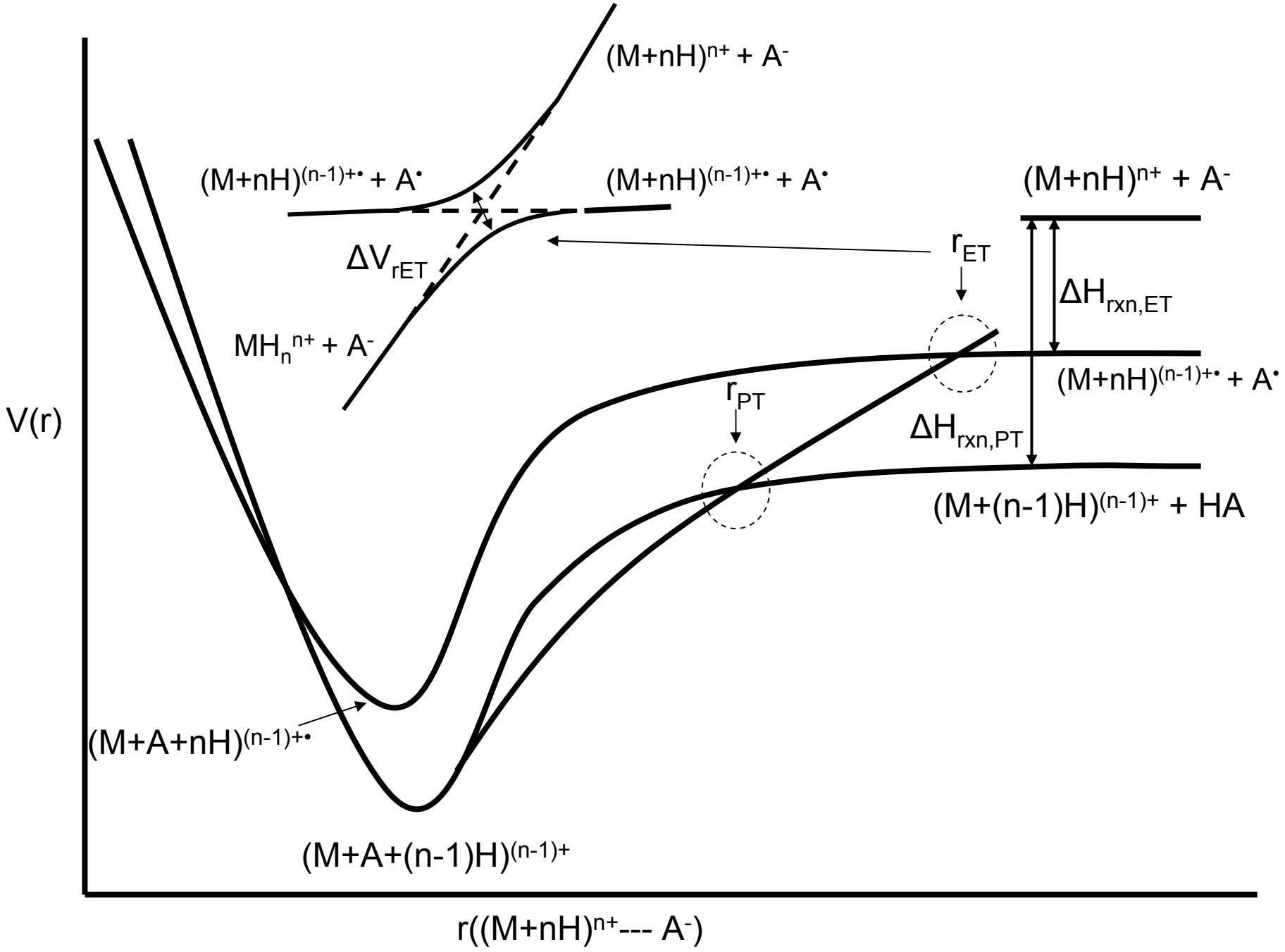
- Nature of the anion: EA, FCFs
- Nature of the cation:
  - Identities of charge bearing sites
  - Total charge
  - High order structure
  - PTMs
    - -SS-
    - Phosphate
    - Sugars
    - Etc.

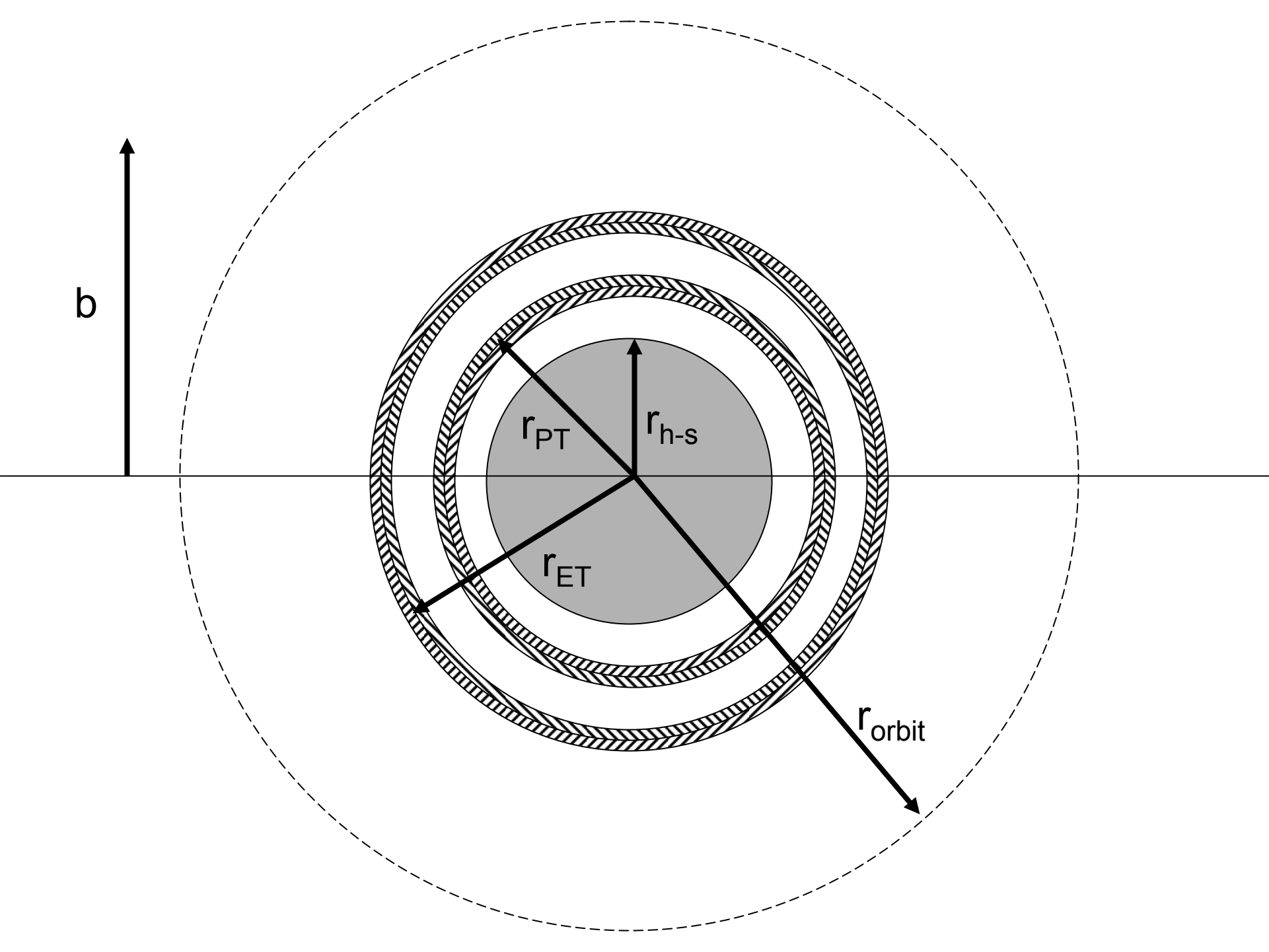
Reaction enthalpies for PT and ET reactions with model cations:

	PA (kcal/mol)	EA (kcal/mol)	ΔHrxn by PT_1/PT_2[b] (kcal/mol)	ΔHrxn by ET_1/ET_2[b] (kcal/mol)	relative exothermicity[b] $\Delta H_{PT} - \Delta H_{ET}$ (kcal/mol)
Guanidine(+)	237.7 (neutral)	92.8 (cation)			
Glycine (+)	204.3 (neutral)	122.6 (cation)			
$SO_2^{-\bullet}$	327.2 (R^-)	27.2 (R)	-89.5/-122.9	-65.6/-95.4	-23.9/-27.5
$O_3^{-\bullet}$	342.4 (R^-)	49.7 (R)	-104.7/-138.1	-43.1/-72.9	-61.6/-65.2
$S_3^{-\bullet}$	315.9 (R^-)	55.1 (R)	-78.2/-111.6	-37.7/-67.5	-40.5/-44.1
$phNNph^{-\bullet}$ [a]	348.8 (R^-)	13.1 (R)	-111.1/-144.5	-79.7/-109.5	-31.4/-35
$SF_6^{-\bullet}$	345.1 (R^-)	29.2 (R)	-107.4/-140.8	-63.6/-93.4	-44/-47.4
CH_3COO^-	345.9 (R^-)	77.4 (R)	-108.2/-141.6	-15.5/-45.2	-92.8/-96.4
I^- [c]	314.3 (R^-)	70.6 (R)	-76.6/-110.0	-22.2/-52.0	-54.4/-58.0
$CS_2^{-\bullet}$	317.6 (R^-)	11.8 [c] (R)	-79.9/-113.3	-81.0/-110.8	+1.1/-2.5

a. experimental values from FT-ICR bracketing method[i]
b, c and d: the first number is calculated against guanidine and the 2nd number is calculated against glycine.
e. experimental values from NIST webbook[ii]

[i] Ingemann, S.; Fokkens, R.H.; Nibbering, N.M.M. *J. Org. Chem.* **1991**, *56*, 607-612.
[ii] NIST Chemistry WebBook, NIST Standard Database 69, January, 2005, http://webbook.nist.gov/chemistry/


(M+nH)n+ + A-
(M+nH)(n-1)+• + A•
(M+nH)(n-1)+• + A•
(M+nH)n+ + A-
rET
ΔVrET
ΔHrxn,ET
MHn n+ + A-
(M+nH)(n-1)+• + A•
V(r)
rPT
ΔHrxn,PT
(M+(n-1)H)(n-1)+ + HA
(M+A+nH)(n-1)+•
(M+A+(n-1)H)(n-1)+
r((M+nH)n+--- A-)


b
r_{PT}
$r_{h\text{-}s}$
r_{ET}
r_{orbit}

$$\sigma_{ET} = P_{ET}\pi b_{ET}^2$$

$$P_{ET} \approx 2P_{LZ}(1-P_{LZ})$$

P_{LZ} is the Landau-Zener probability for transition between adiabatic states

$$b_{ET}^2 \approx r_{ET}^2\left[1 + \frac{2Z_1Z_2e^2}{4\pi\varepsilon_0 r_{ET}\mu v^2}\right]$$

r_{ET} is the crossing point. We treat the two ions as point charges.

$P_{LZ} = e$

$v =$ $\left[\frac{2|Z$ $\frac{Z_3Z_4}{r_{et}^2})|$

ix element

Fundamental factors in the model:

m_{anion}, m_{cation}

RE

EA

Z_1, Z_2, Z_3, Z_4

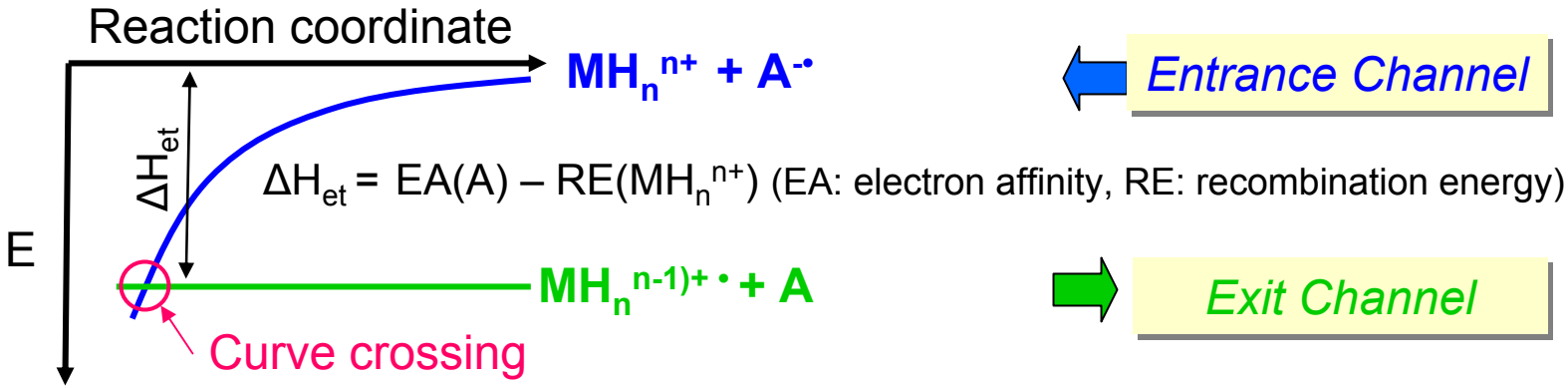
(Frank-Condon Factors)

Expt'l. variables

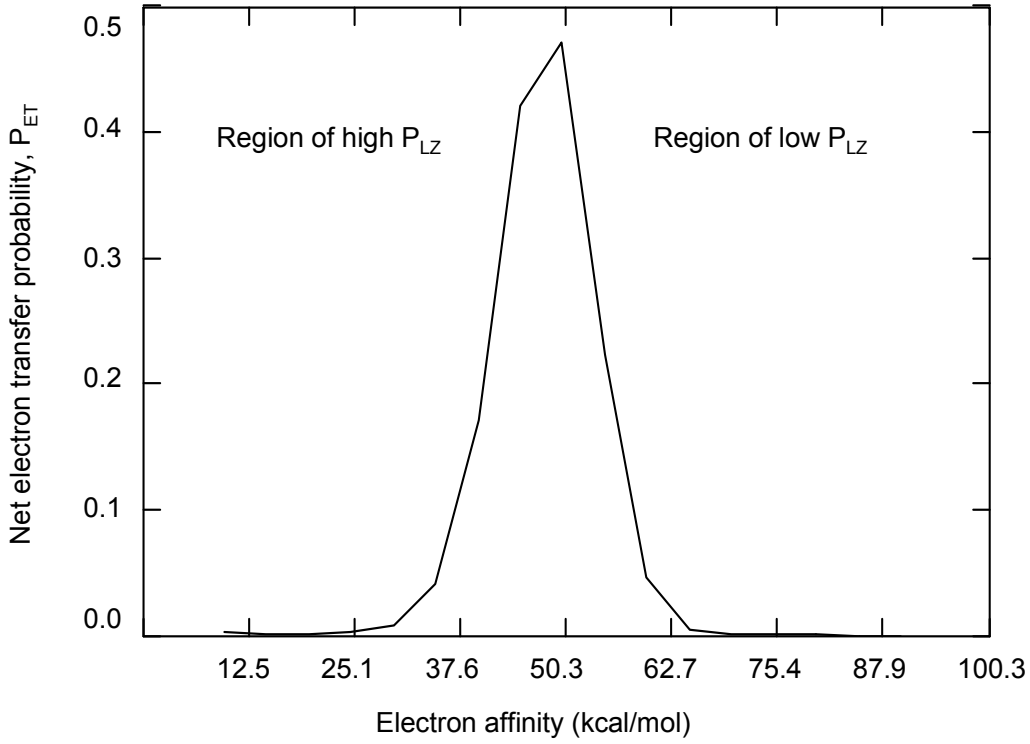
parameterized model

$$H_{12}=1.044\left[\frac{(2EA)^{1/2} + (2RE)^{1/2}}{2}\right](EA)^{1/2}(RE)^{1/2}r_{ET}\exp\left[-0.857\left[\frac{(2EA)^{1/2} + (2RE)^{1/2}}{2}\right]r_{ET}\right.$$

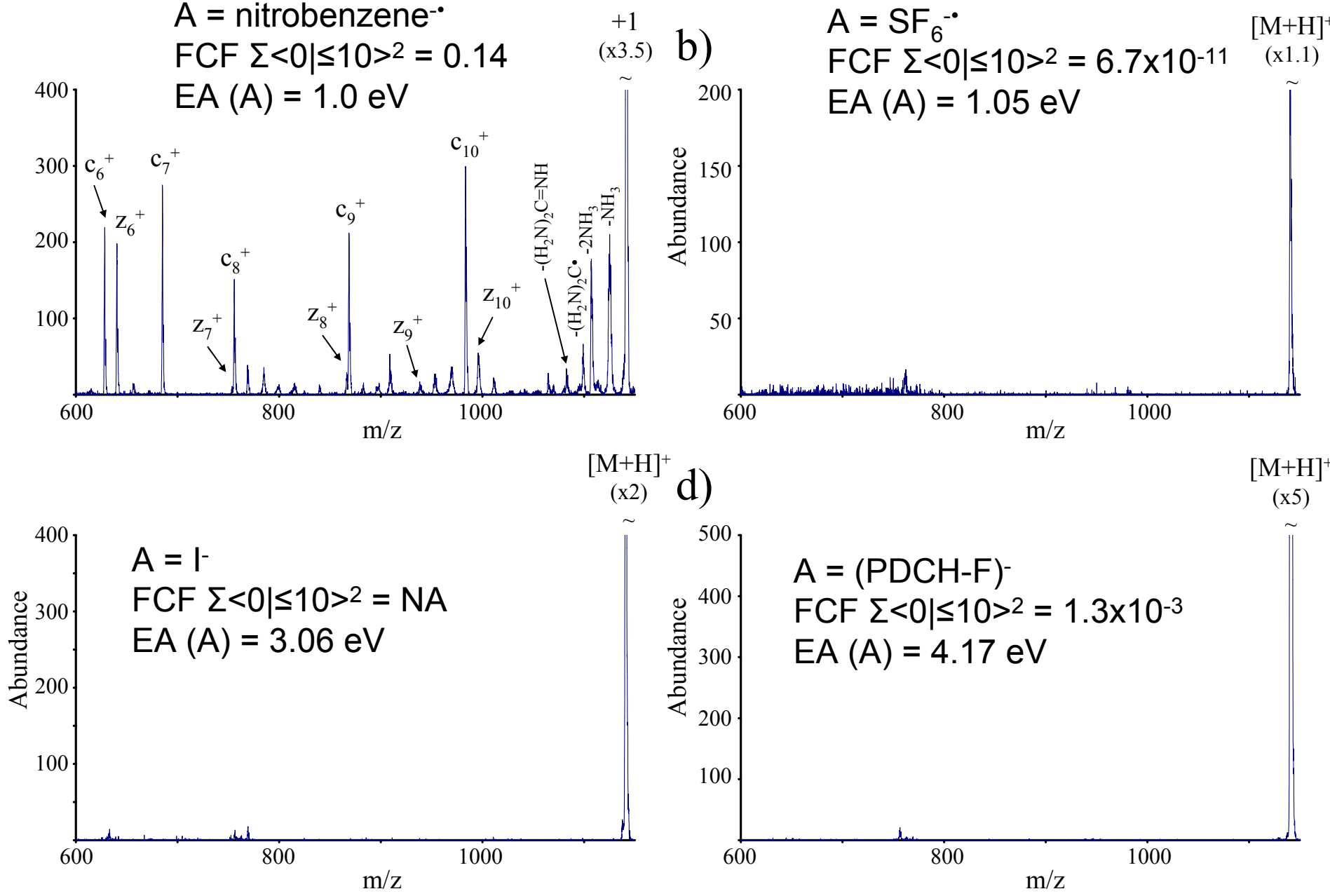
Olson RE. *J. Chem. Phys.* 1972; **56:** 2979.



$\Delta H_{et} = EA(A) - RE(MH_n^{n+})$ (EA: electron affinity, RE: recombination energy)



(KGAILKGAILR + 3H$^+$)/A$^-$



Reagent	Franck-Condon factor $\Sigma \langle 0 \vert \leq 10 \rangle^2$	EA(R) (kcal/mol)	% ETD	
norbornodiene	1.1×10^{-2}	5.6	7.2	
cis-stilbene	5.2×10^{-3}	10.4	9.8	
O_2	9.7×10^{-1}	10.4	4.9	
CS_2	4.9×10^{-5}	11.8	<0.01	←
azobenzene	1.8×10^{-1}	13.1	48.8	
fluoranthene	3.6×10^{-1}	14.5	37.4	
perylene	4.1×10^{-1}	22.4	20.9	
nitrobenzene	1.4×10^{-1}	23.0	14.7	
SF_6	6.7×10^{-11}	24.2	<0.01	←
SO_2	4.6×10^{-1}	25.5	30.1	
m-dinitrobenzene	2.7×10^{-1}	38.3	26.6	
o-dinitrobenzene	1.2×10^{-4}	38.3	17.2	
S_2O	3.5×10^{-1}	43.3	7.3	
SO_3	6.9×10^{-8}	43.8	<0.01	←
p-dinitrobenzene	1.8×10^{-1}	46.1	16.4	
S_3	5.2×10^{-1}	48.3	7.0	
O_3	3.8×10^{-1}	48.5	4.8	
$NO_2^{\bullet}$	2.3×10^{-1}	52.4	8.5	
1,3,5-trinitrobenzene	6.5×10^{-1}	60.6	7.9	
CO_3	9.0×10^{-1}	62.0	<0.01	←
$I^{\bullet}$	N/A	70.6	<0.01	←
$CH_3COO^{\bullet}$	5.8×10^{-3}	77.4	<0.01	←
$NO_3^{\bullet}$	7.6×10^{-1}	90.8	<0.01	←
$[PDCH-F]^{\bullet}$	1.3×10^{-3}	96.2	<0.01	←
H_2PO_4	1.3×10^{-8}	105.4	<0.01	←
HSO_4	4.9×10^{-2}	109.5	<0.01	←

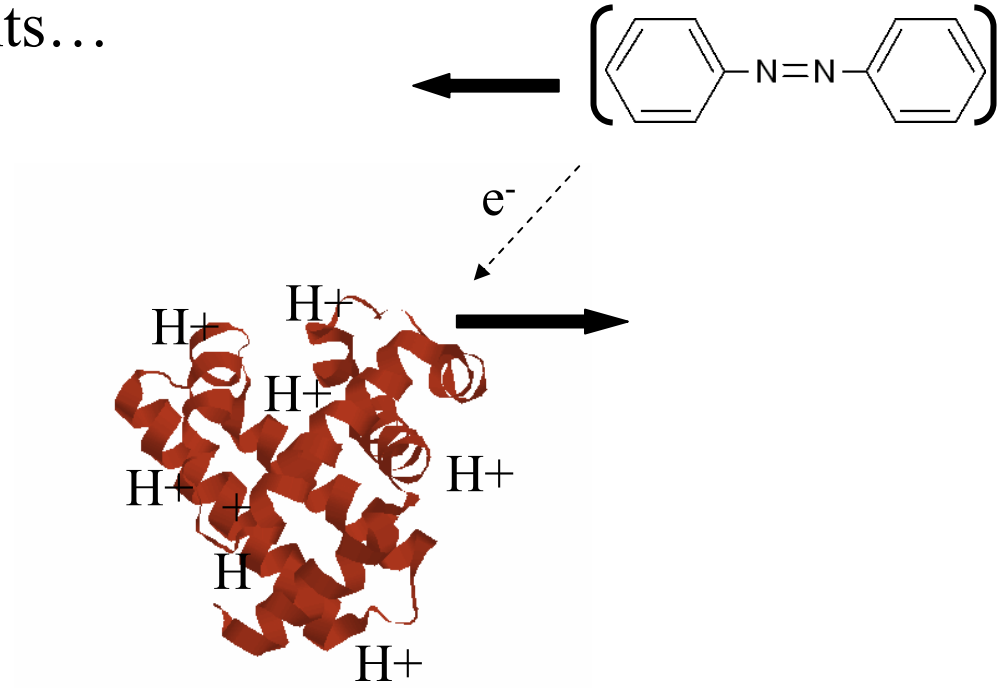
Anion characteristics:

Favorable F-C factors
EA of neutral not too high (<3 eV)

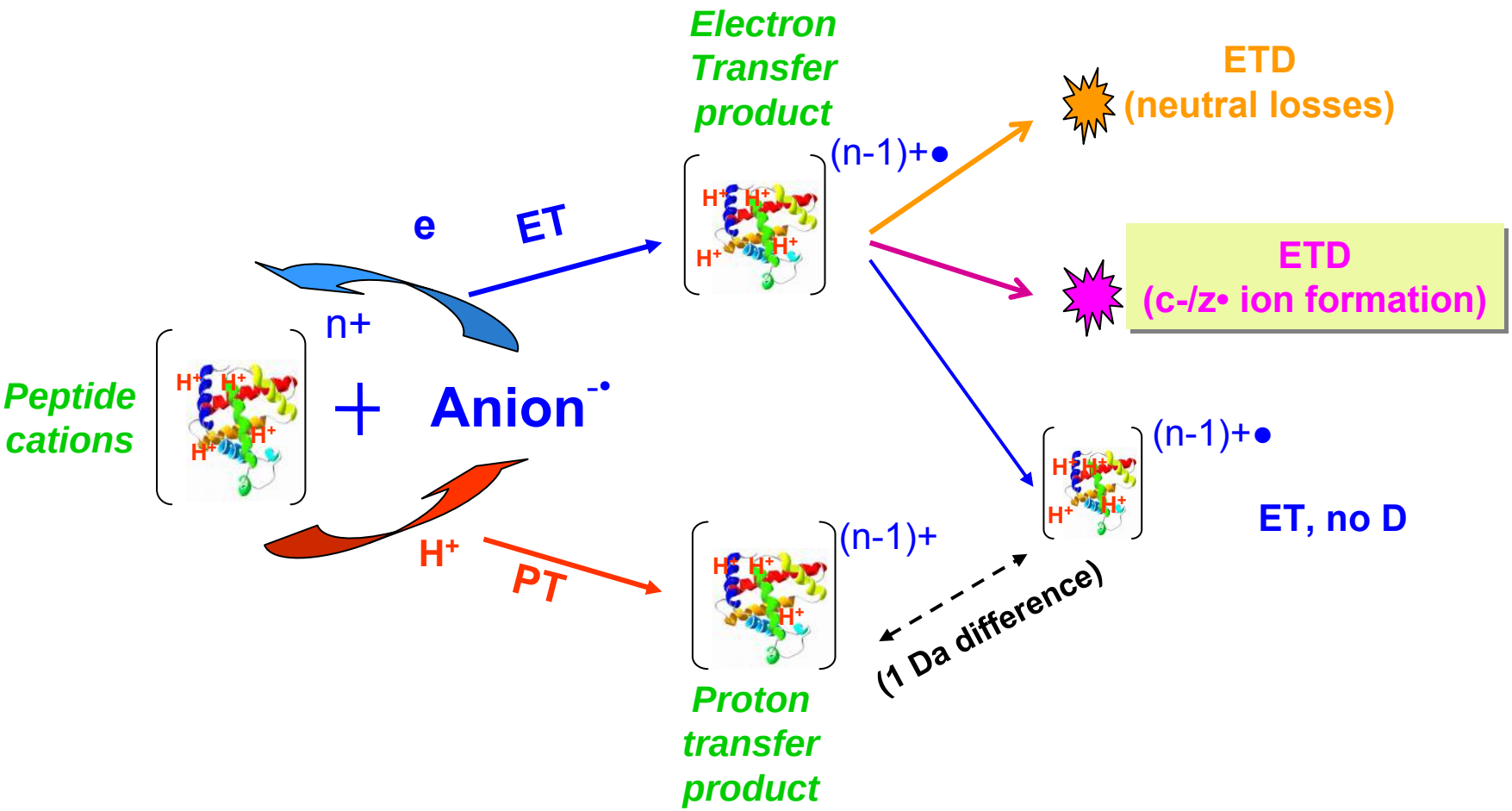
Thanks to Jamie Stearns and Tim Zwier for teaching us how to do the FCF calculations

Conclusions about nature of anion:

- ET becomes competitive with PT when EA reagent is not "too high" (< 3 eV) and when FCF are not "too low" ($<10^{-2}$)
- Dipole-bound anions, Rydberg atoms should make good ET reagents…



Major Competing Channels in an ETD Expt.



Product Partitioning Definitions:

$$\%\ PT = \frac{\sum PT}{\sum PT + (ET, no\ D) + ETD} \times 100\%$$

$$\%\ ET = \%\ ET, no\ D + \%\ ETD$$

$$\%\ ET, no\ D = \frac{\sum ET, no\ D}{\sum PT + (ET, no\ D) + ETD} \times 100\%$$

$$\%\ ETD = \frac{\sum ETD}{\sum PT + (ET, no\ D) + ETD} \times 100\%$$

$$\%\ c, z/ETD = \frac{\sum c, z}{\sum ETD} \times 100\%$$

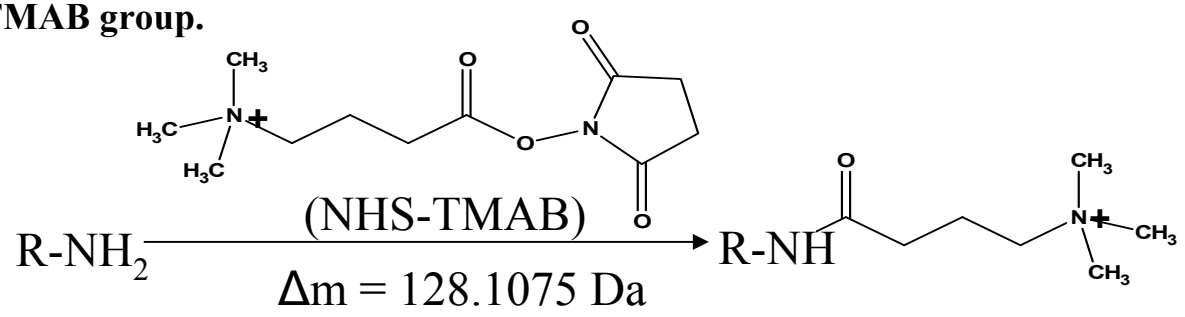
$$\%\ side\text{-}chain/ETD = \frac{\sum side\text{-}chain}{\sum ETD} \times 100\%$$

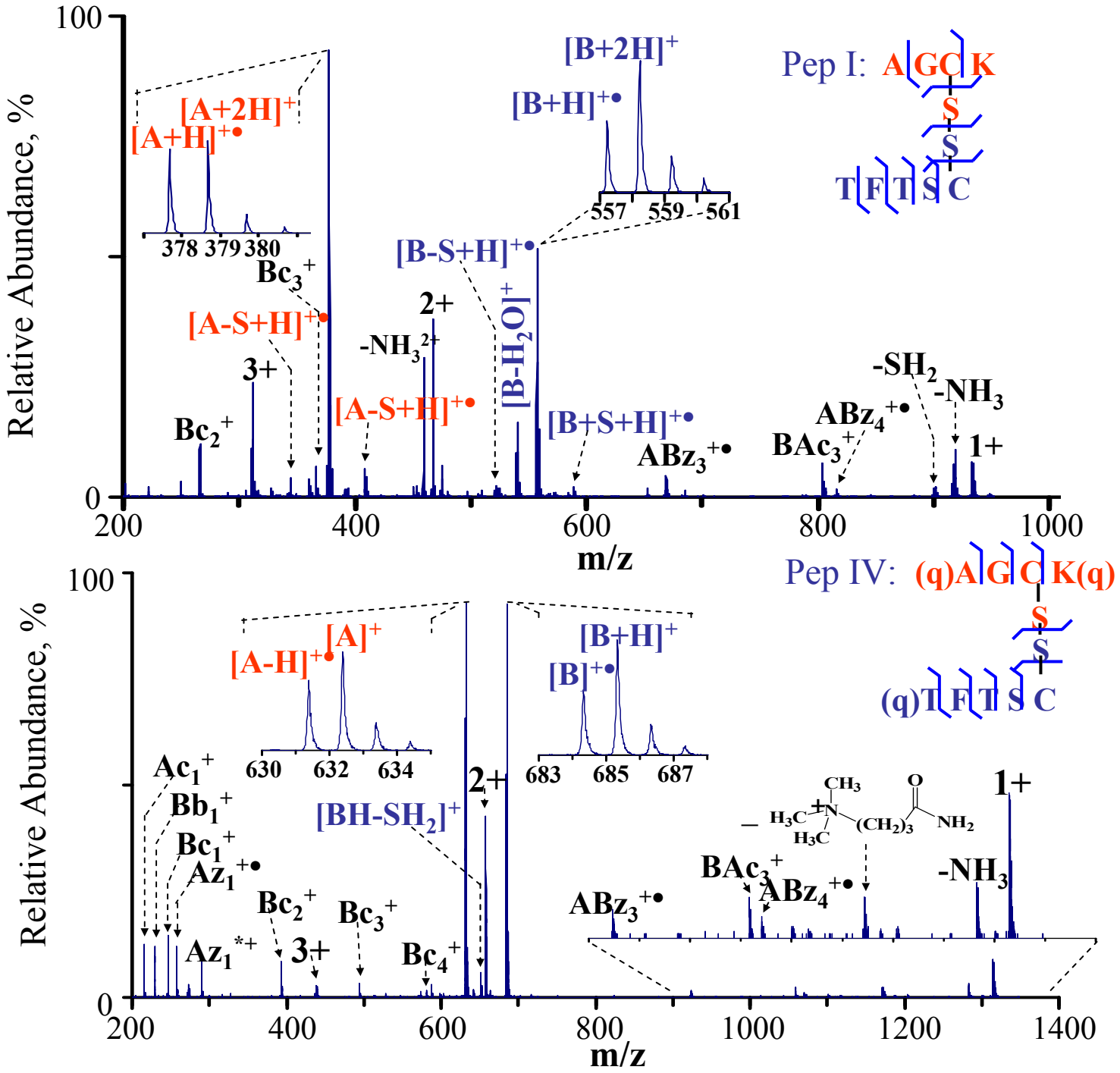
Factors in ETD:

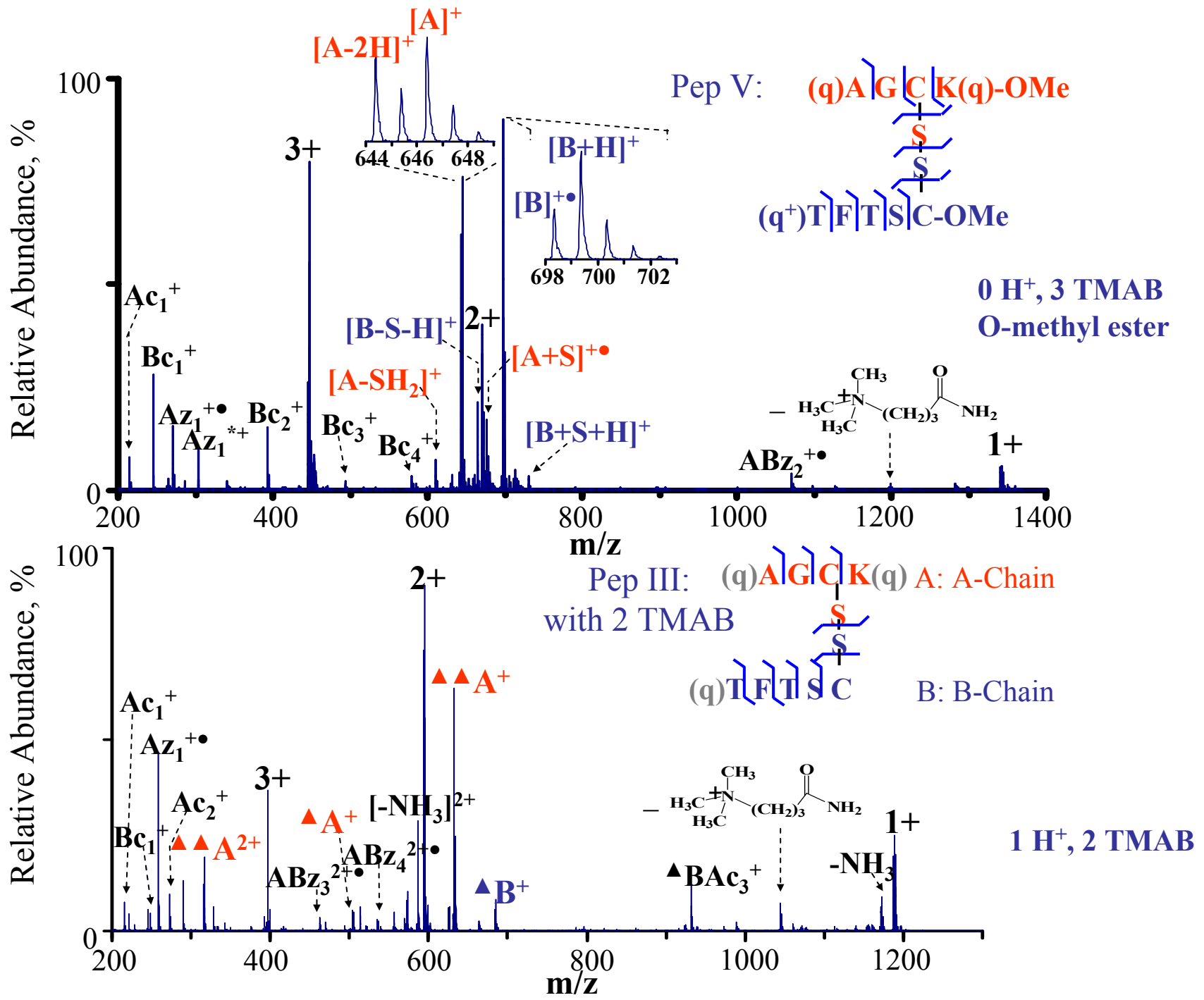
- Nature of the anion: EA, FCFs
- Nature of the cation:
  - Identities of charge bearing sites
  - Total charge
  - High order structure
  - PTMs
    - -SS-
    - Phosphate
    - Sugars
    - Etc.

Peptide Label	Peptide Sequence	Peptide Label	Peptide Sequence
Peptide I	AGCK | TFTSC	**Peptide VI**	AGCKNFFWK | TFTSC
Peptide II*	(q)AGCK | TFTSC	**Peptide VII***	(q)AGCKNFFWK | TFTSC
Peptide III*	(q)AGCK(q) | TFTSC	**Peptide VIII***	(q)AGCKNFFWK(q) | TFTSC
Peptide IV	(q)AGCK(q) | (q)TFTSC	**Peptide IX***	(q)AGCKNFFWK(q) | (q)TFTSC
Peptide V	(q)AGCK(q)-OMe | (q)TFTSC-OMe	**Peptide X**	(q)AGCK(q)NFFWK(q) | (q)TFTSC

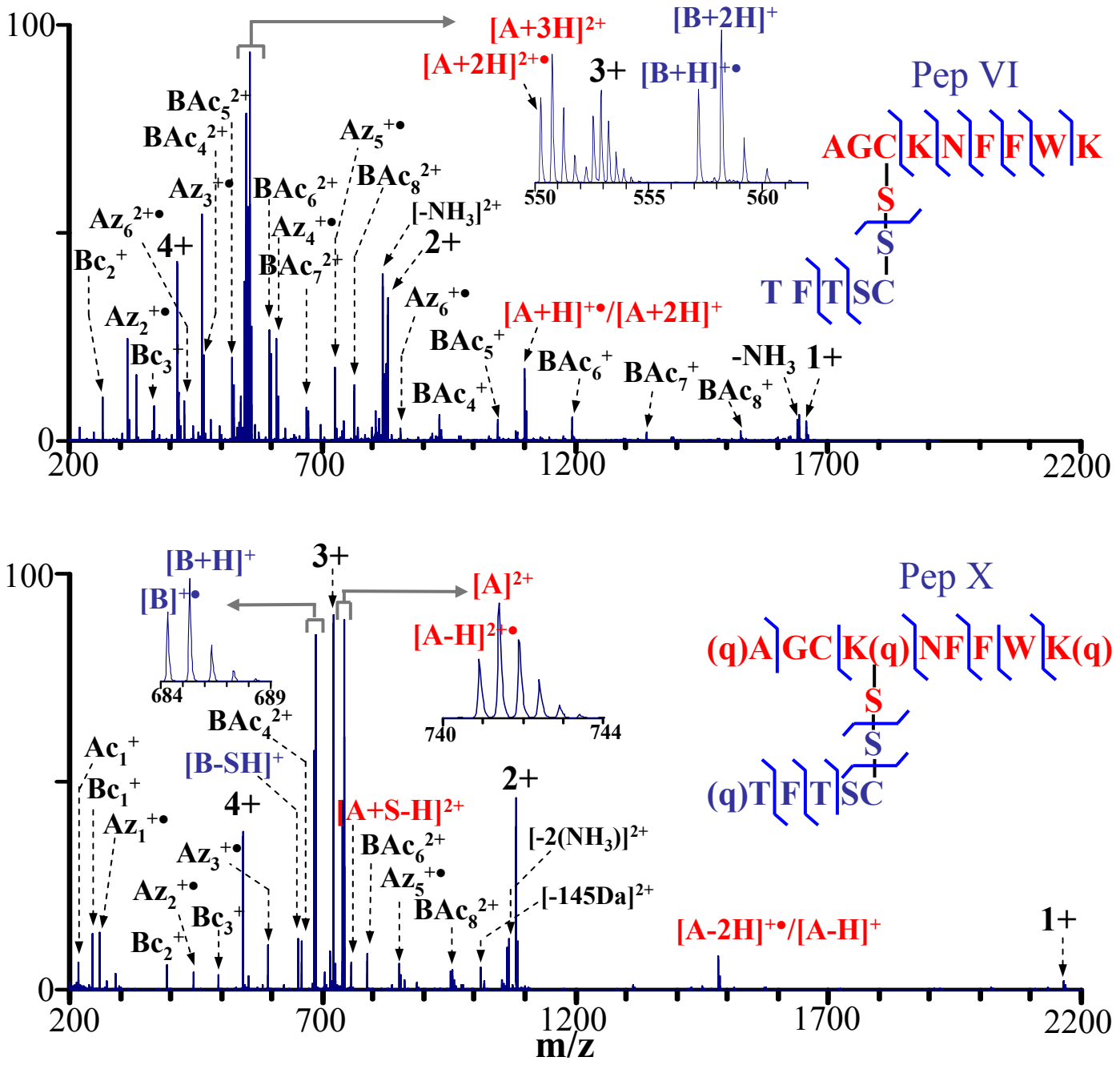
*** represents the peptides have a mixture of structures with combinations of TMAB on N-termini or lysine side chains. The corresponding peptide sequences stand for only one of the possible structures. q represents the TMAB group.**



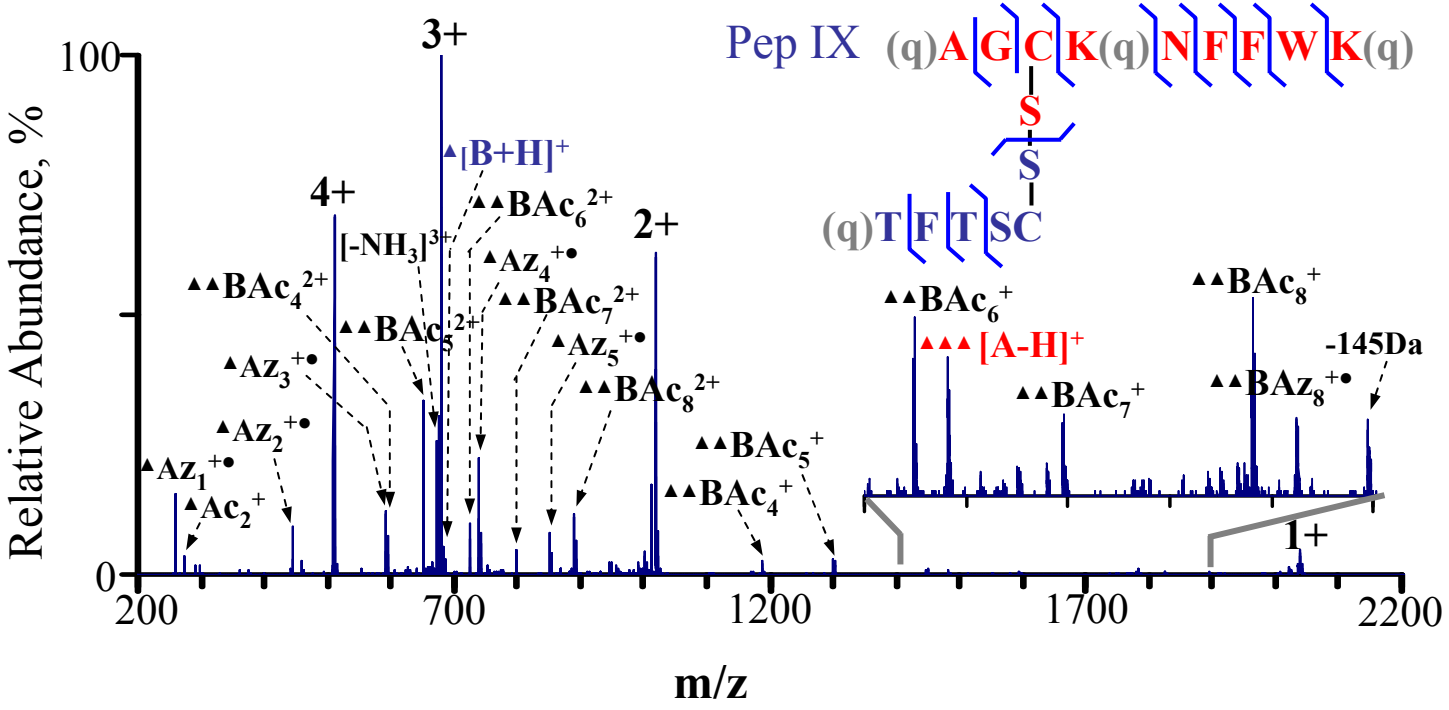

Relative Abundance, %
100
0
200
400
600
800
1000
m/z
[A+2H]+
[A+H]+•
378 379 380
[B+2H]+
[B+H]+•
557 559 561
Pep I: AGCK
S
S
TFTSC
Bc3+
[A-S+H]+•
2+
-NH32+
3+
Bc2+
[A-S+H]+•
[B-S+H]+•
[B-H2O]+
[B+S+H]+•
ABz3+•
ABz4+•
BAc3+
-SH2
-NH3
1+
Relative Abundance, %
100
0
200
400
600
800
1000
1200
1400
m/z
[A]+
[A-H]+•
630 632 634
[B+H]+
[B]+•
683 685 687
Pep IV: (q)AGCK(q)
S
S
(q)TFTSC
Ac1+
Bb1+
Bc1+
Az1+•
Az1*+
Bc2+
3+
Bc3+
Bc4+
[BH-SH2]+
2+
CH3
H3C
N+
H3C
(CH2)3
O
NH2
1+
BAc3+
ABz4+•
ABz3+•
-NH3


[A]+
[A-2H]+
644 646 648
[B+H]+
[B]+•
698 700 702
Pep V: (q)A G C K(q)-OMe
S
S
(q+)T F T S C-OMe
0 H+, 3 TMAB
O-methyl ester
Relative Abundance, %
100
0
200 400 600 800 1000 1200 1400
m/z
3+
2+
1+
Ac1+
Bc1+
Az1+•
Az1*+
Bc2+
Bc3+
Bc4+
[B-S-H]+
[A-SH2]+
[A+S]+•
[B+S+H]+
ABz2+•
CH3
H3C N+ H3C
(CH2)3
O
NH2
Pep III: (q)A G C K(q) A: A-Chain
with 2 TMAB
S
S
(q)T F T S C B: B-Chain
1 H+, 2 TMAB
Relative Abundance, %
100
0
200 400 600 800 1000 1200
m/z
2+
3+
1+
▲▲A+
Ac1+
Az1+•
Ac2+
Bc1+
▲▲A2+
▲A+
[-NH3]2+
ABz32+•
ABz42+•
▲B+
▲BAc3+
-NH3
CH3
H3C N+ H3C
(CH2)3
O
NH2

3+ Peptide Ions React with Azobenzene Anions					
Peptide	**#H^+,# TMAB**	**ETD%**	**ET, no D%**	**PT%**	**SS, ETD%**
Peptide I	**3H^+,0 TMAB**	84	< 1	15	73
Peptide II	**2H^+,1 TMAB**	86	< 1	13	64
Peptide III	**1H^+,2 TMAB**	64	5	31	33
Peptide IV	**0H^+,3 TMAB**	81	< 1	18	78
Peptide V	**0H^+,3 TMAB**	83	< 1	16	81


100
0
200
700
1200
1700
2200
[A+3H]2+
[A+2H]2+•
3+
[B+2H]+
[B+H]+•
550
555
560
Pep VI
AGCKNFFWK
S
S
T F T SC
BAc5 2+
BAc4 2+
Az5 +•
Az3 +•
BAc6 2+
BAc8 2+
[-NH3]2+
Az6 2+•
Az4 +•
4+
2+
Bc2 +
BAc7 2+
Az6 +•
Az2 +•
[A+H]+•/[A+2H]+
BAc5 +
Bc3 +
BAc6 +
BAc7 +
-NH3
1+
BAc4 +
BAc8 +
[B+H]+
3+
[A]2+
[B]+•
[A-H]2+•
684
689
740
744
Pep X
(q)AGCK(q)NFFWK(q)
S
S
(q)T F T SC
BAc4 2+
Ac1 +
[B-SH]+
Bc1 +
4+
2+
[A+S-H]2+
Az1 +•
BAc6 2+
[-2(NH3)]2+
Az3 +•
Az5 +•
Az2 +•
Bc3 +
BAc8 2+
[-145Da]2+
Bc2 +
[A-2H]+•/[A-H]+
1+
m/z

1 H^+, 3 TMAB


Pep IX (q)AGCK(q)NFFWK(q)
S
S
(q)TFTSC
Relative Abundance, %
100
0
200
700
1200
1700
2200
m/z
3+
4+
2+
1+
[B+H]+
BAc6 2+
Az4 +•
BAc4 2+
BAc5 2+
BAc7 2+
Az5 +•
Az3 +•
BAc8 2+
Az2 +•
BAc5 +
Az1 +•
BAc4 +
Ac2 +
[-NH3]3+
BAc6 +
[A-H]+
BAc7 +
BAc8 +
BAz8 +•
-145Da

	4+ Peptide Ions React with Azobenzene Anions				
Peptide	**#H^+,# TMAB**	**ETD%**	**ET, no D%**	**PT%**	**SS, ETD%**
Peptide VI	**$4H^+$,0 TMAB**	84	3	13	30
Peptide VII	**$3H^+$,1 TMAB**	89	4	7	40
Peptide VIII	**$2H^+$,2 TMAB**	78	6	16	23
Peptide IX	**$1H^+$,3 TMAB**	53	11	36	6
Peptide X	**$0H^+$,4 TMAB**	63	11	26	57

Mechanisms for -SS- cleavage in ETD/ECD:

- Capture of e^- at protonated site, transfer of H• to -SS-
- Capture of e^- in Coulomb stabilized -SS- antibonding orbital
- Capture of e^- in Rydberg orbital of charge site with subsequent transfer to -SS- anti-bonding orbital ("through bond electron transfer")

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DOE OBES

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