

## Supplementary Figures and Tables

### Figure Legends

#### **Figure A. H-D exchange time course studies of intact proenkephalin (PE) and individual peptides derived from PE.**

H-D exchange data for DXMS studies of intact PE protein (solid lines) and of peptides (dotted lines) derived from PE (by pepsin) are plotted for subdomains indicated by the amino acid numbers provided at the top of each graph. The graphs compare the number of deuterons incorporated into each peptide, y-axis (shown for +1 charge state of peptides, and for +2 charge state if it was the only charge state observed) at different times of incubation in D<sub>2</sub>O buffer for acquisition of H-D exchange data. The standard deviation of deuterium incorporation measured in replicate determinations was typically less than 5% of the mean, as reported (49, 50) These plots show similarities and differences in deuteration exchange levels for peptide amide hydrogens at specific regions in intact PE, which maybe influenced by tertiary interactions, and PE-derived peptides, which only have intrinsic interactions of the primary peptide structure.

#### **Figure B. H-D exchange of intact PE and peptides derived from PE at cleavage site domains.**

Comparison of relative H-D exchange rates at the twelve dibasic residue cleavage sites of intact PE, and peptides derived from, are illustrated at 1 second time point. More limited deuteration of intact PE is observed for the 12 cleavage site domains, compared to peptides derived from PE.

## **Table Legends**

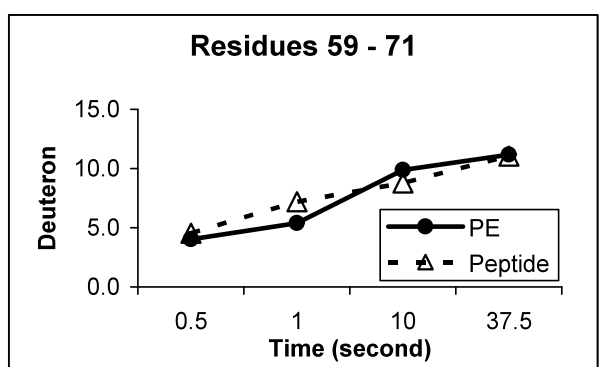
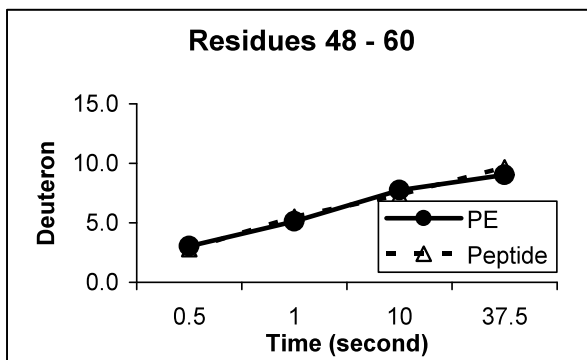
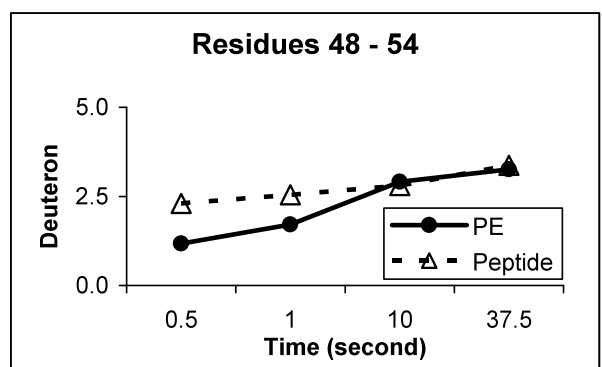
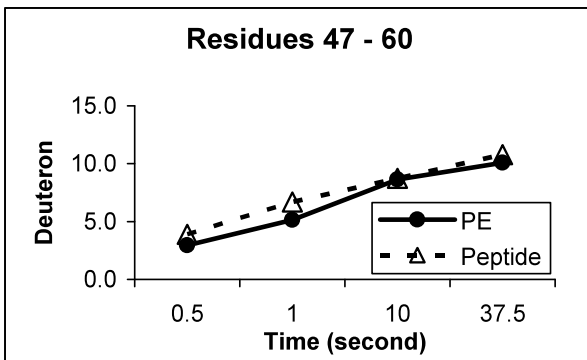
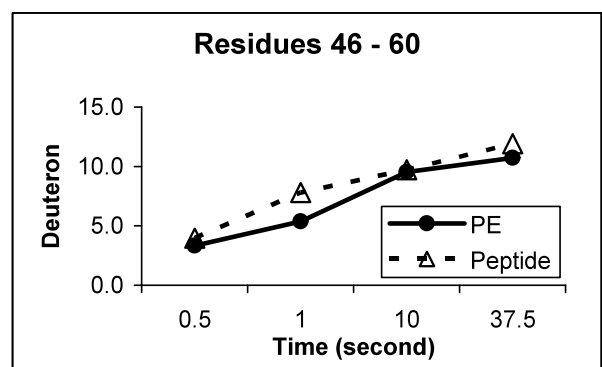
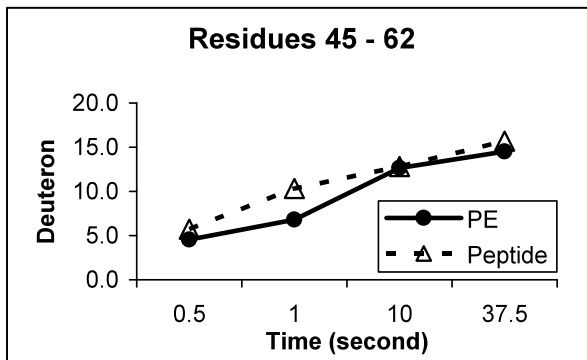
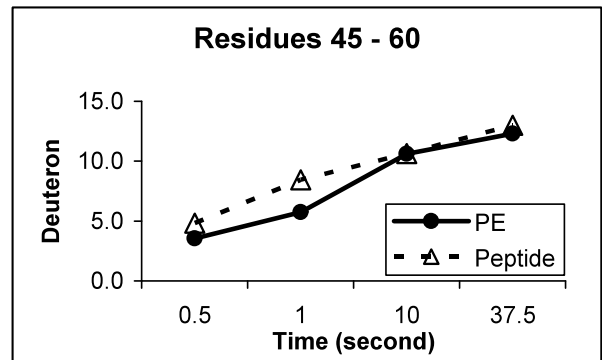
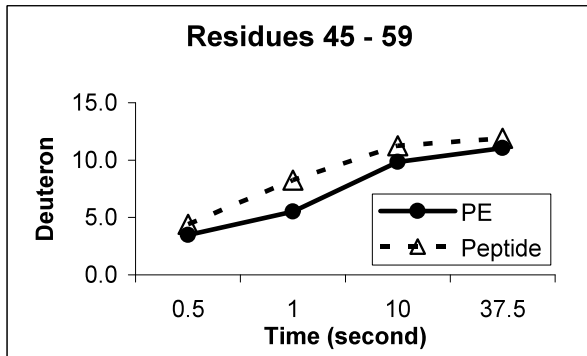
### **Table A. Charge states of peptides in the comparison of intact PE and peptides derived from PE in DXMS experiments.**

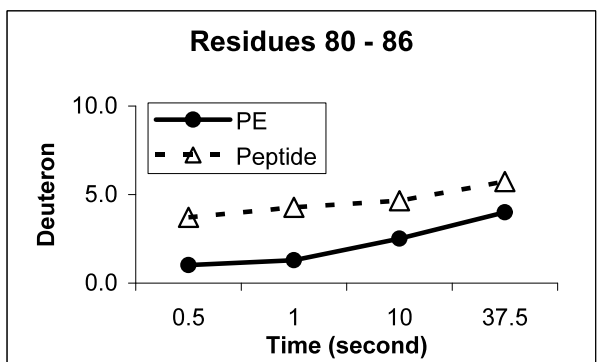
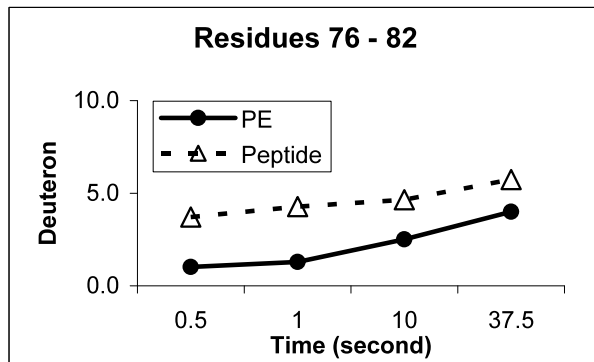
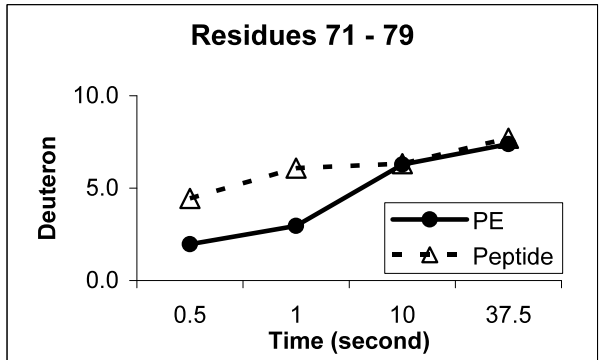
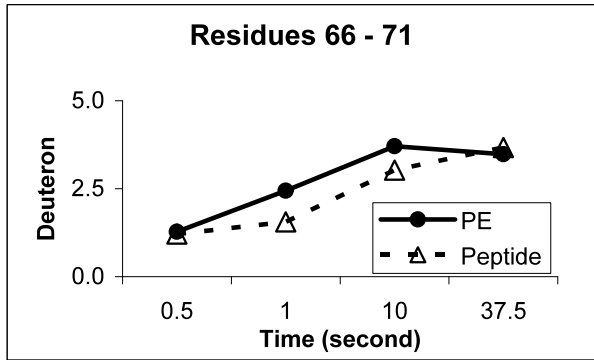
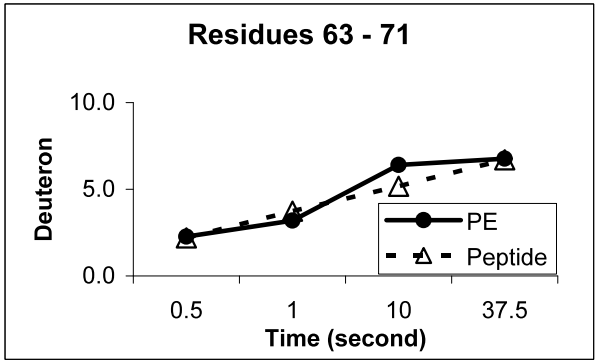
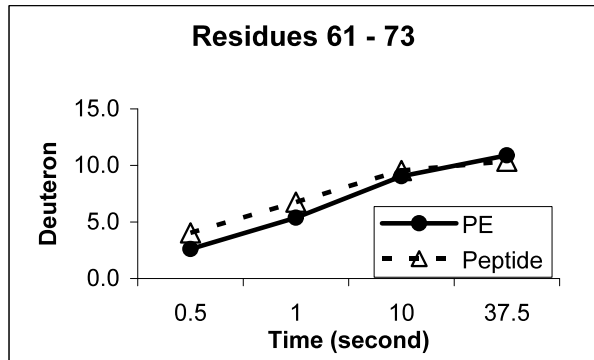
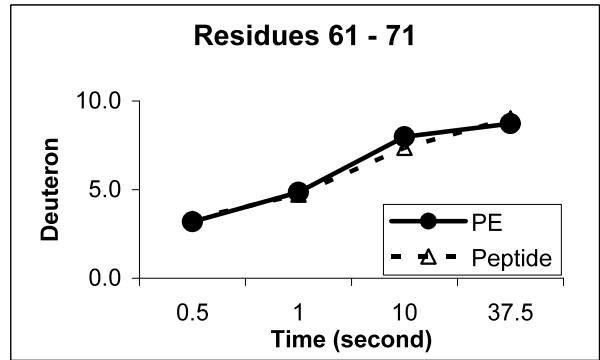
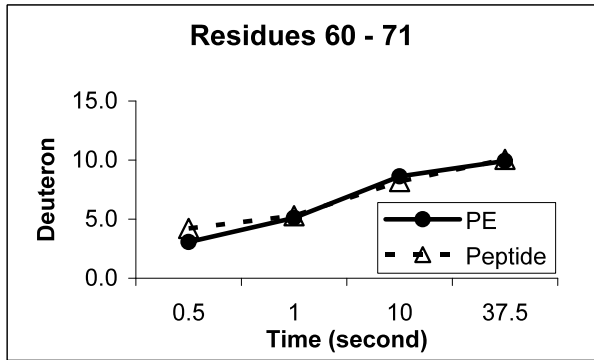
Peptides in DXMS analyses of figures 3 and 4 are listed, indicating the charge states observed in LC-MS analyses. The relative deuteration of these peptides of intact PE and for PE-derived peptides are plotted in supplemental Figure A, in time-course experiments. The relative deuteration of peptides at +1 or +2 charge states were identical. Therefore, plots of relative deuteration utilized peptides at +1 charge state, except in the case where only the +2 charge state was observed and utilized for time course plots of Figure A.

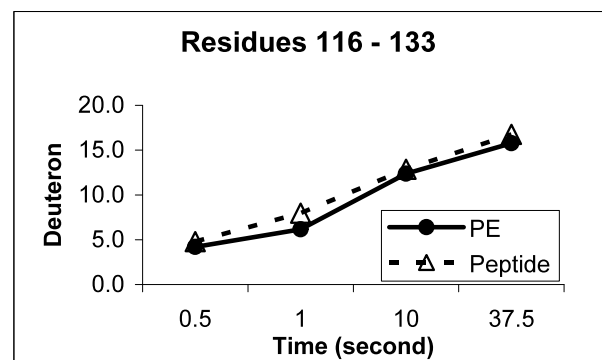
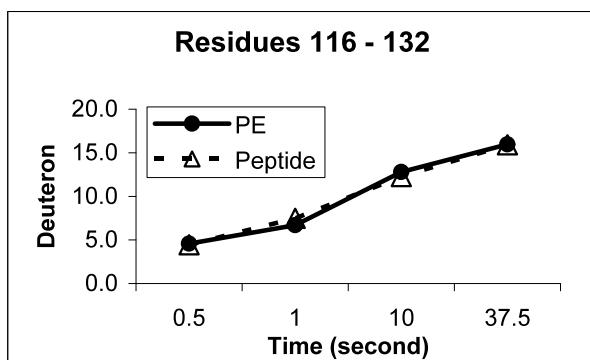
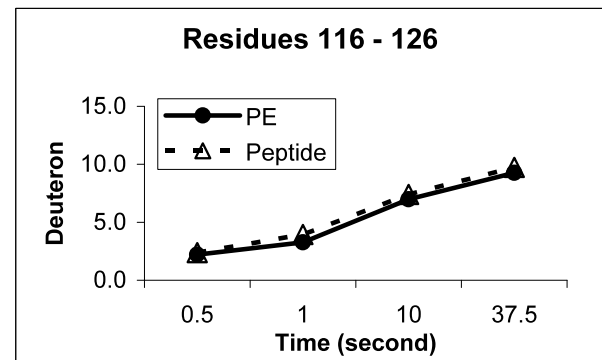
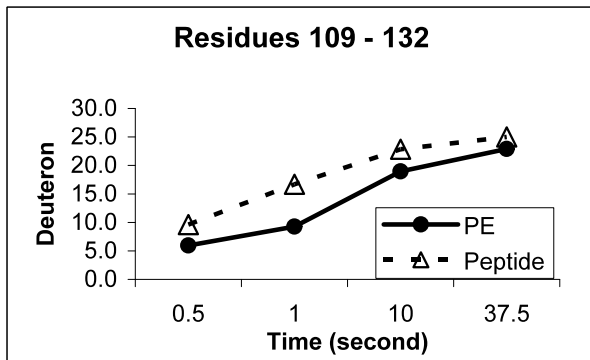
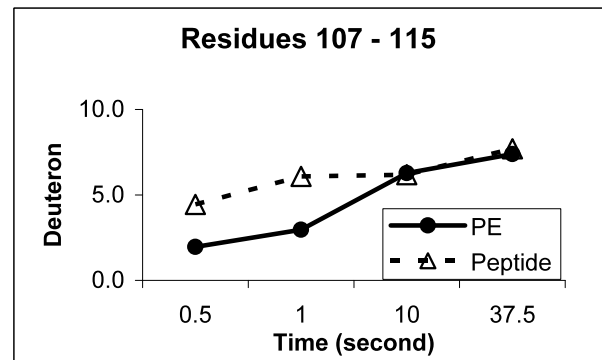
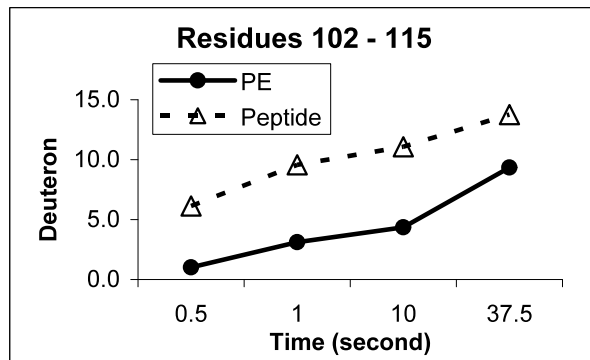
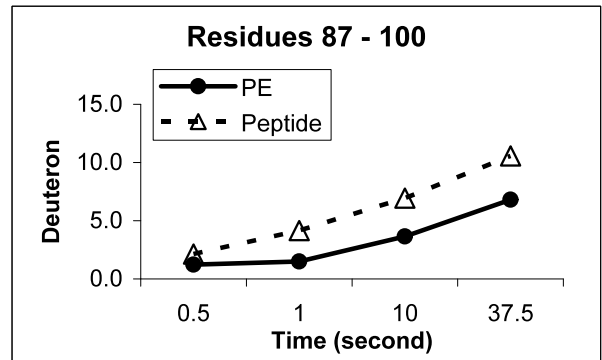
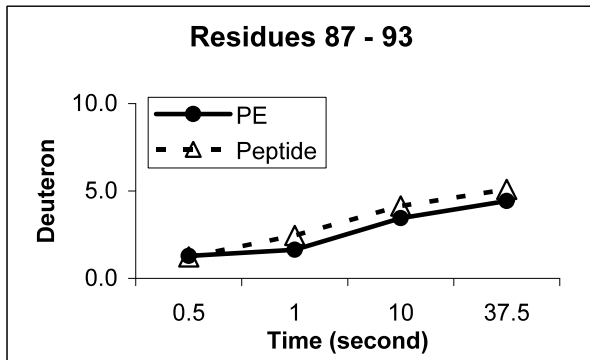
### **Table B. Peptides that span cleavage sites of PE.**

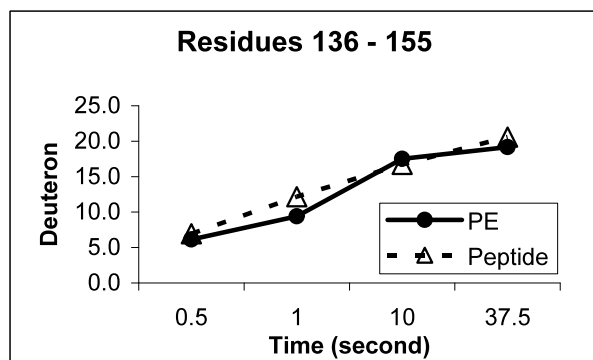
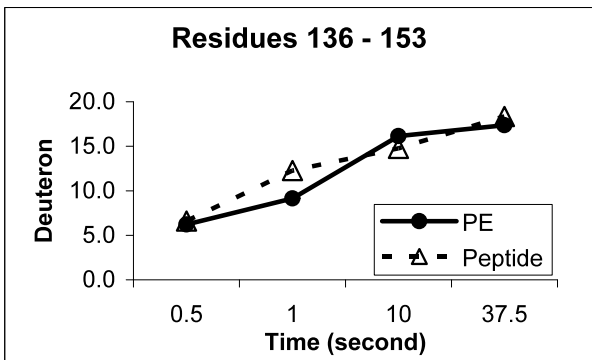
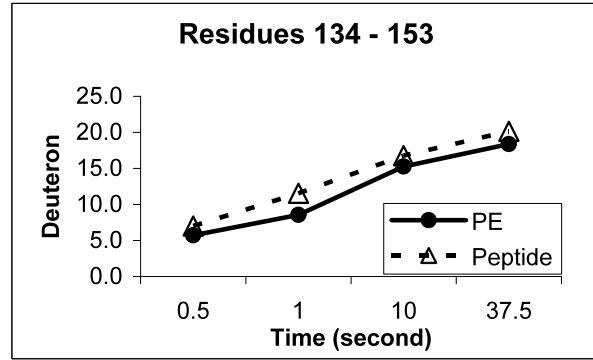
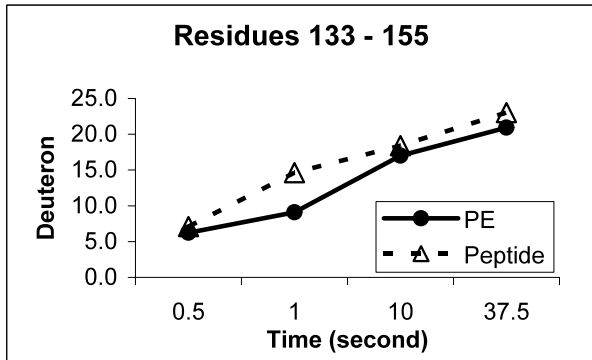
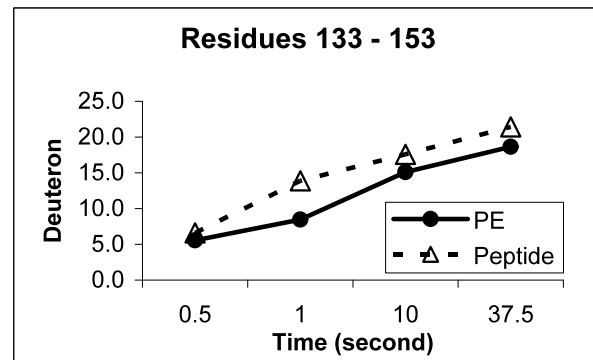
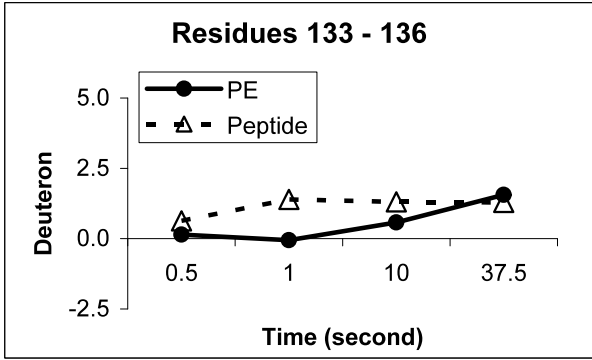
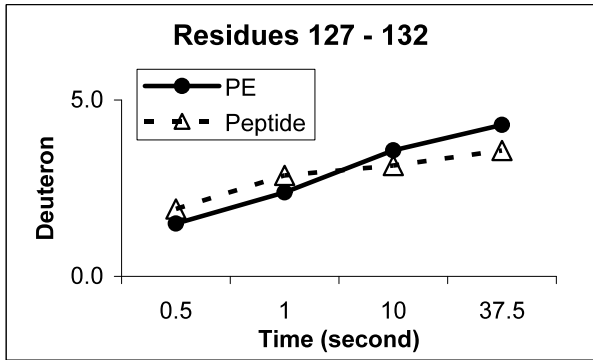
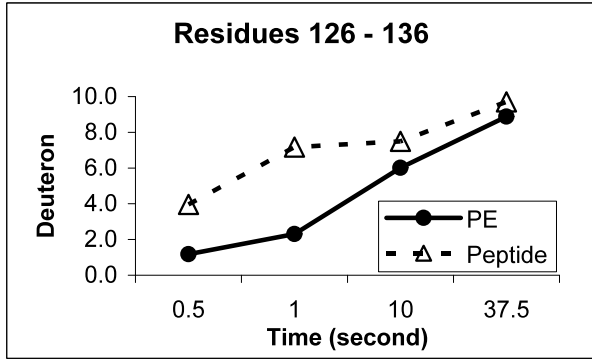
Peptides spanning the twelve cleavage sites of PE are listed. For each of the cleavages sites #1-12, the location of the dibasic cleavage sites are shown by the residues numbers. Peptides spanning each of these cleavate sites are listed, including their observed charged states. The relative deuteration of these peptides (averages) were plotted in Figure 5 for comparison of their relative accessibilities to the aqueous environment.

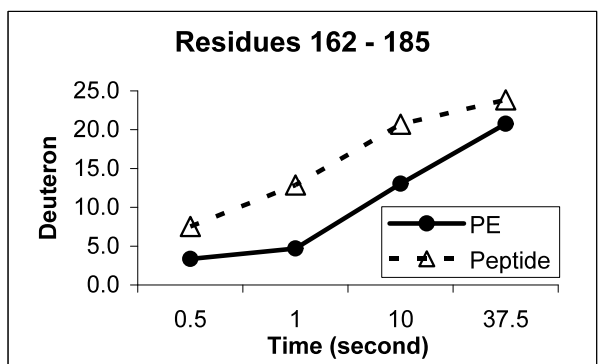
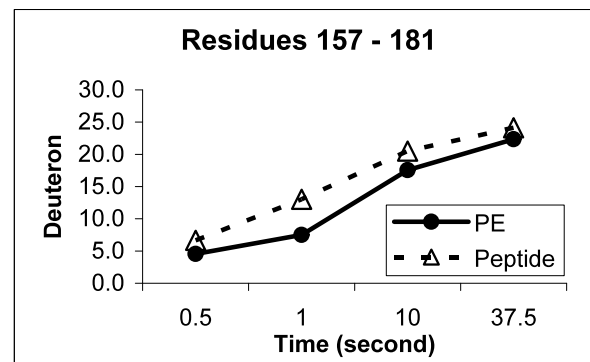
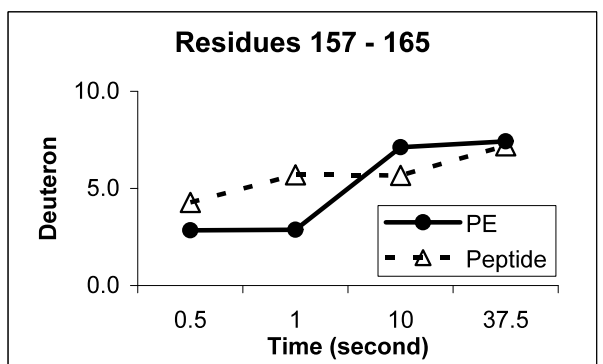
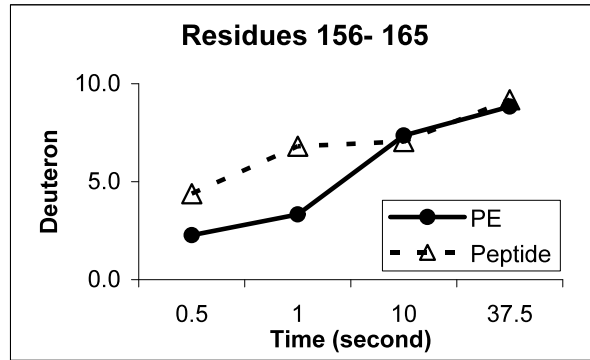
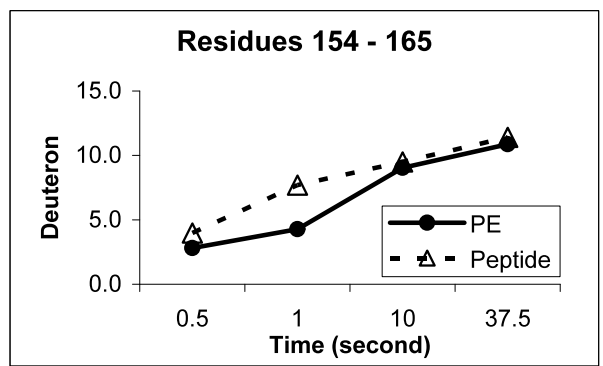
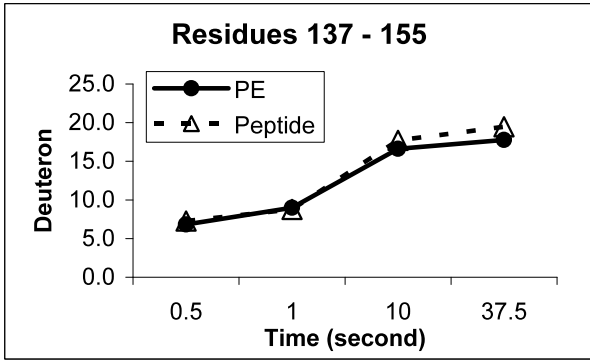
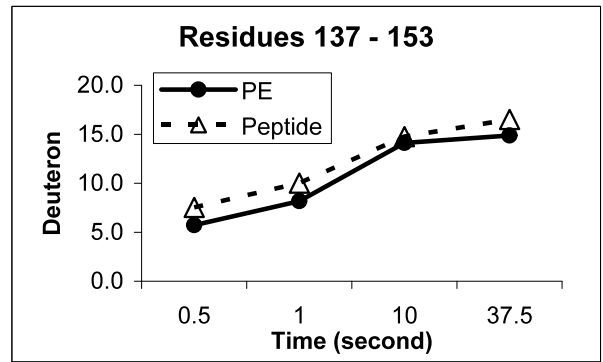
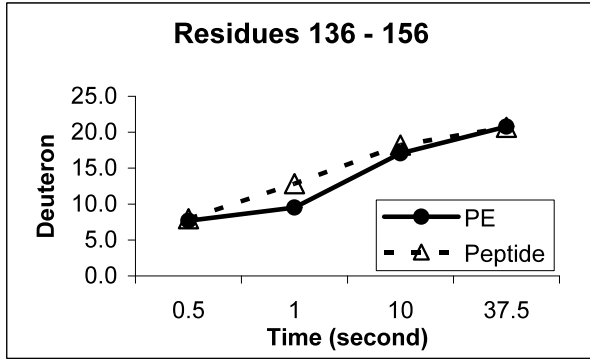
**Supplemental Figure A.**  
**H-D Exchange Time-Course of Intact PE and**  
**Peptides Derived from PE**

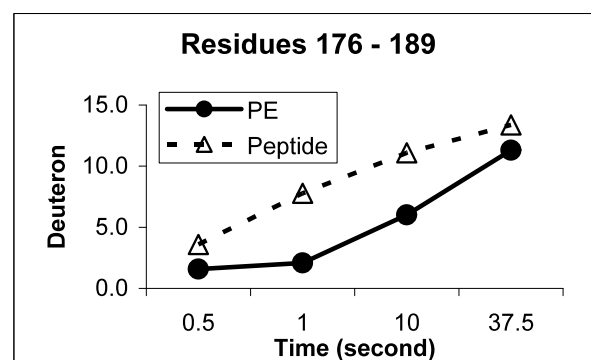
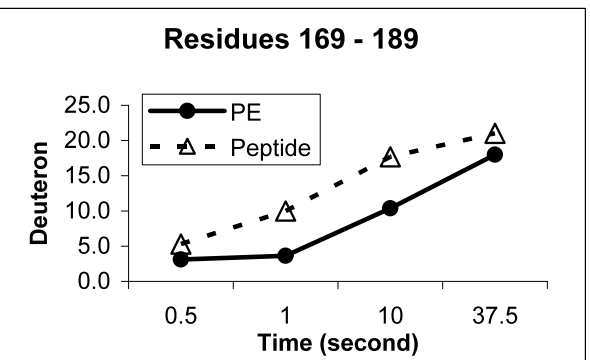
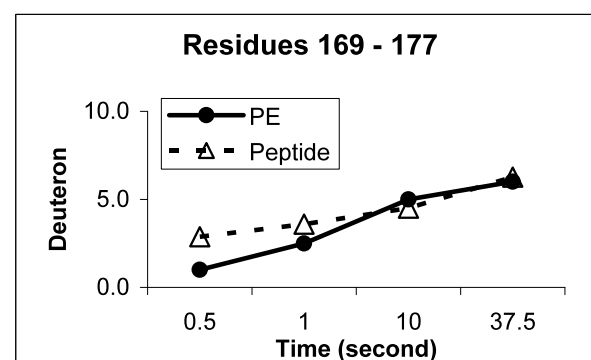
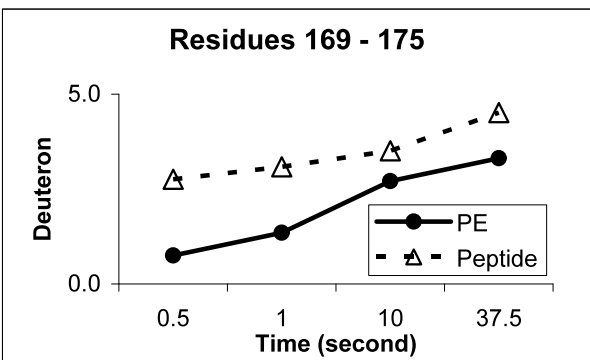
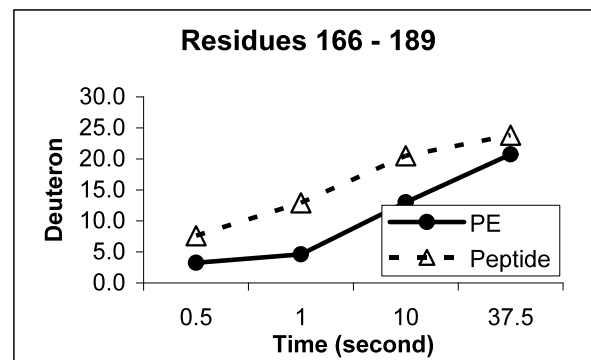
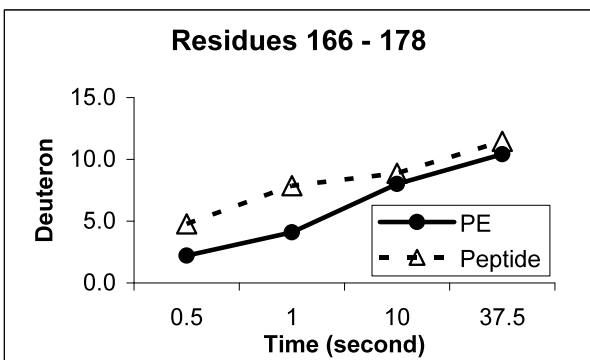
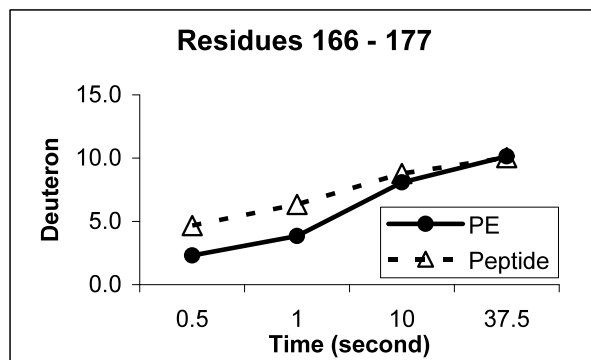
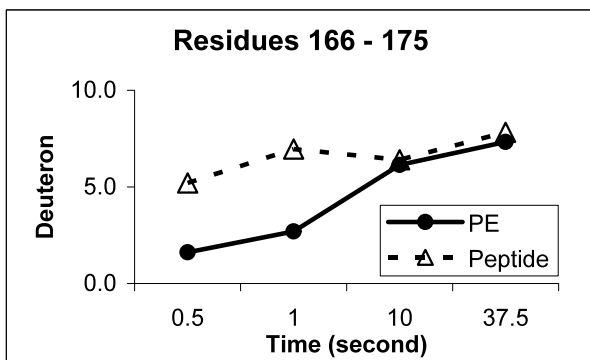




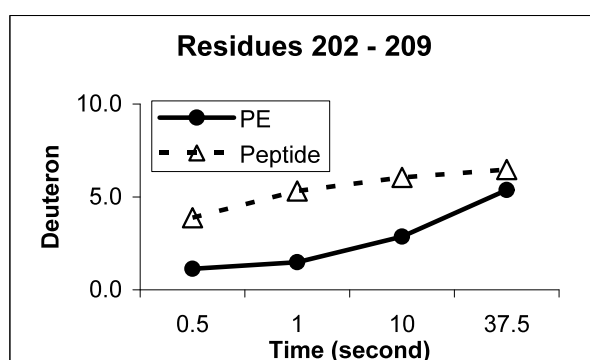
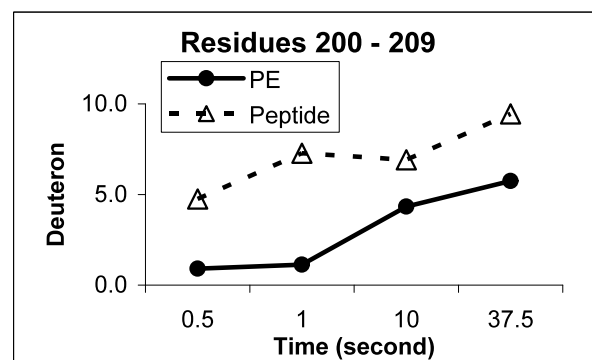
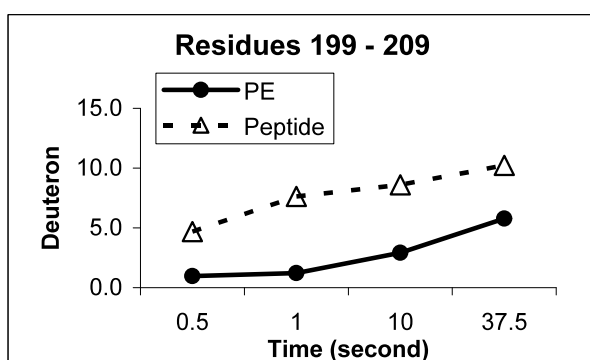
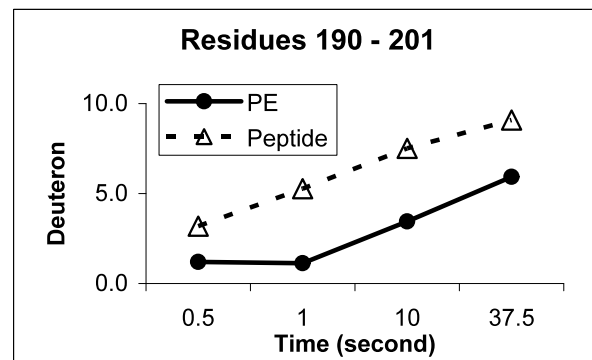
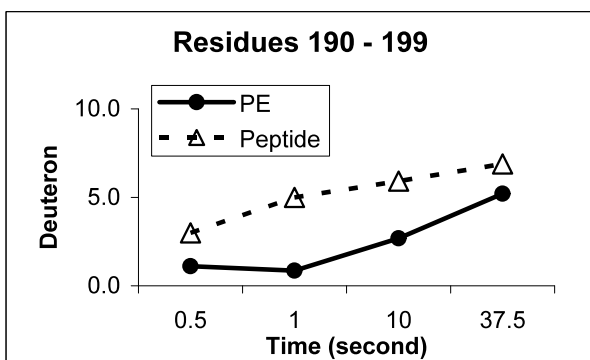
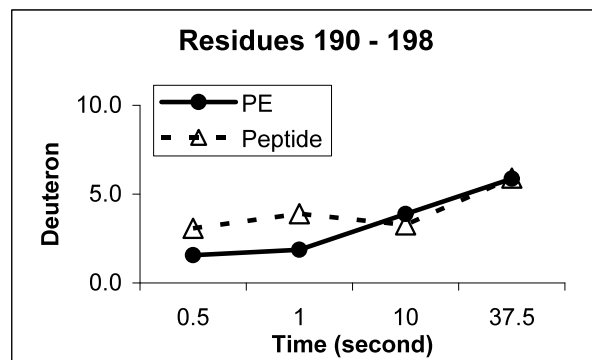
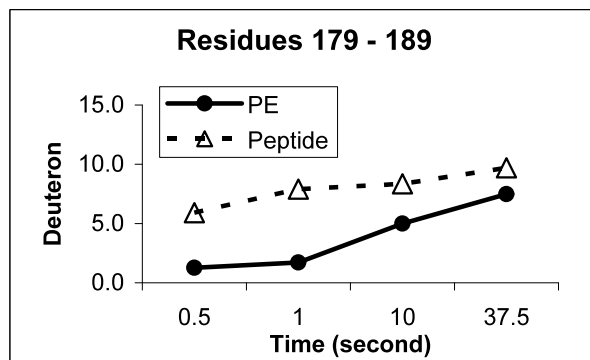
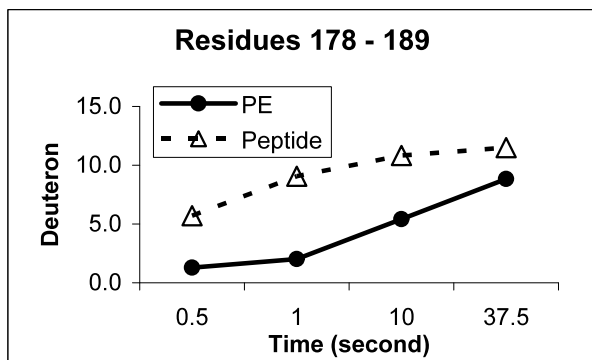


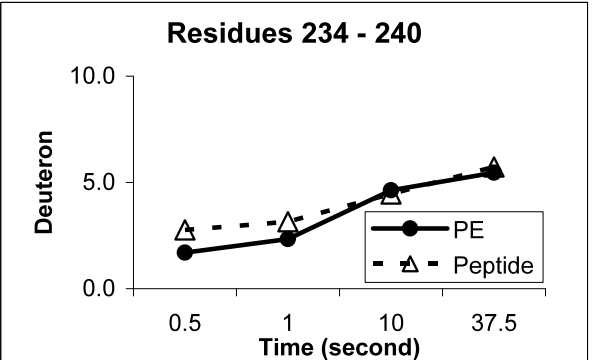
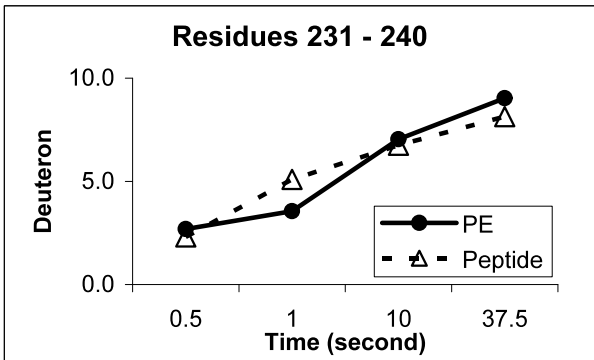
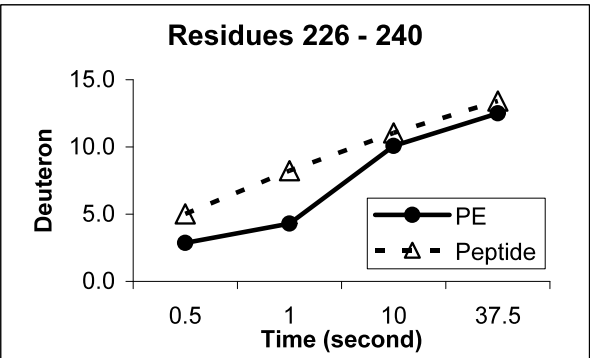
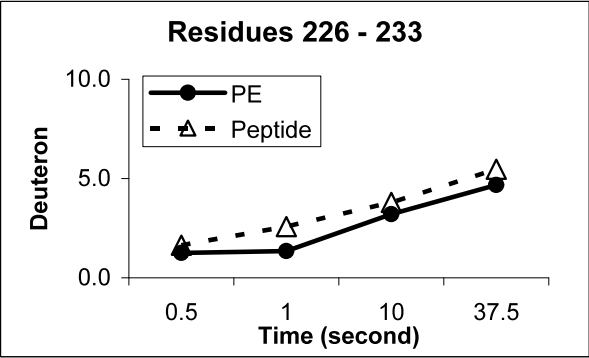
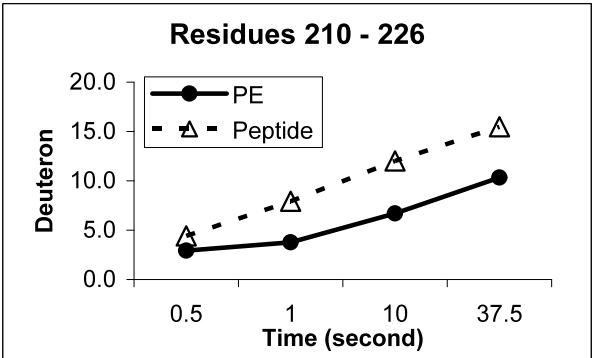
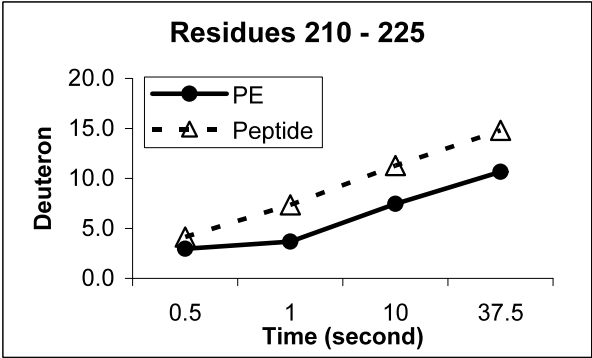
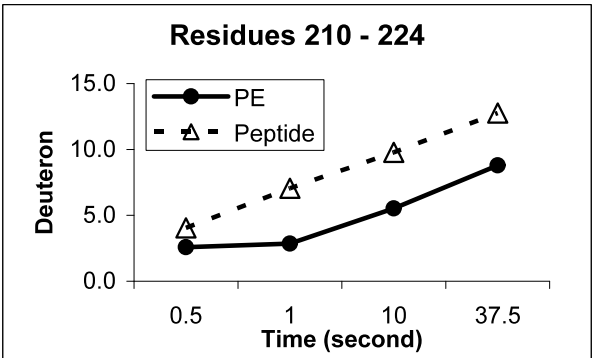
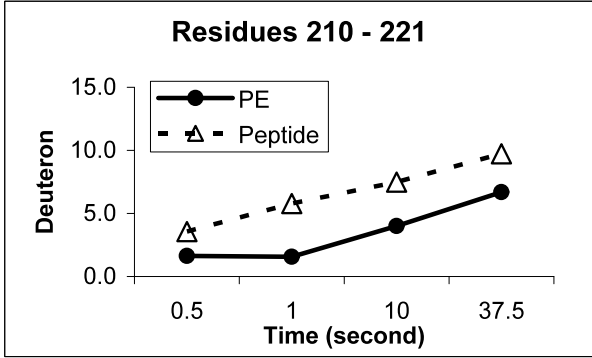




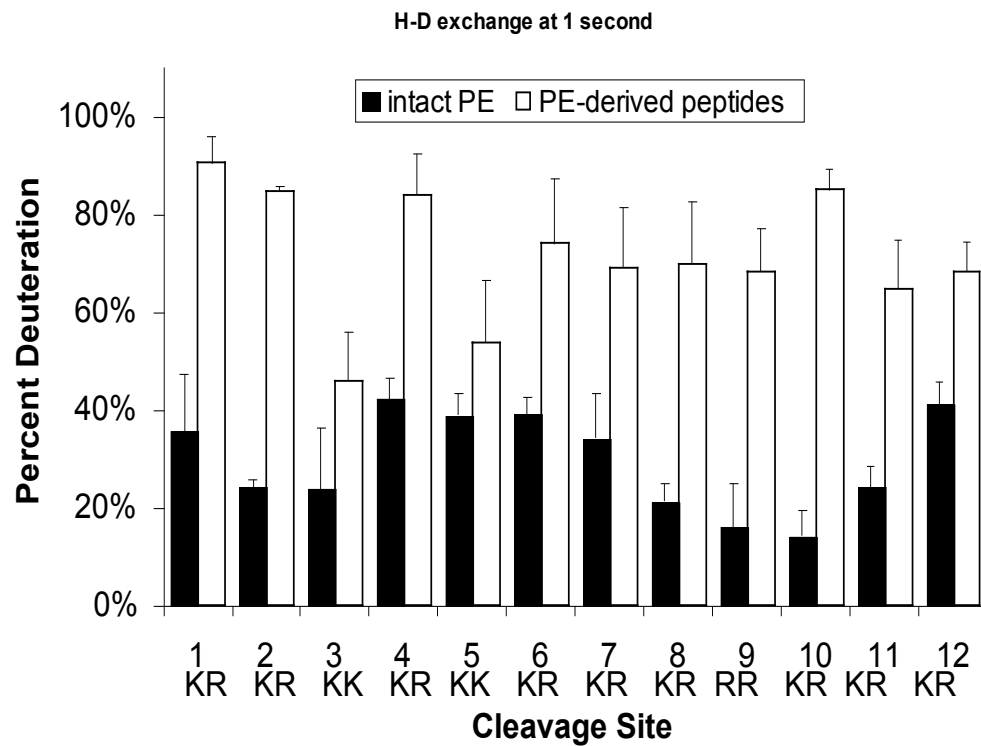








**Figure B**  
**H-D Exchange of Intact PE and Peptides Derived from PE**  
**at Cleavage Site Domains**



**Supplemental Table A**  
**Charge States of Peptides in the Comparison of intact PE**  
**and Peptides Derived from PE in DXMS Experiments**

Peptide		
Start	End	Charge
45	59	+1
45	59	+2
45	60	+2
45	62	+2
46	60	+1
46	60	+2
47	60	+1
47	60	+2
48	54	+1
48	54	+2
48	60	+1
48	60	+2
59	71	+2
60	71	+1
60	71	+2
61	71	+1
61	71	+2
61	73	+2
63	71	+1
63	71	+2
66	71	+1
71	79	+1
71	79	+2
76	82	+1
76	82	+2
80	86	+1
80	86	+2
87	93	+1
87	100	+2
102	115	+2
107	115	+1
107	115	+2

Peptide		
Start	End	Charge
109	132	+2
116	126	+2
116	132	+2
116	133	+2
126	136	+1
126	136	+2
127	132	+1
133	136	+1
133	153	+2
133	155	+2
134	153	+2
136	153	+2
136	155	+2
136	156	+2
137	153	+2
137	155	+2
154	165	+1
154	165	+2
156	165	+1
156	165	+2
157	165	+2
157	181	+2
162	185	+2
162	185	+3
166	175	+1
166	175	+2
166	177	+2
166	178	+2
166	189	+2
166	189	+3
169	175	+1
169	175	+2

Peptide		
Start	End	Charge
169	177	+2
169	189	+2
176	189	+1
176	189	+2
178	189	+2
179	189	+1
179	189	+2
190	198	+1
190	198	+2
190	199	+2
190	201	+2
199	209	+1
199	209	+2
200	209	+1
200	209	+2
202	209	+1
202	209	+2
210	221	+1
210	221	+2
210	224	+2
210	225	+1
210	225	+2
210	226	+2
226	233	+2
226	240	+1
226	240	+2
231	240	+1
234	240	+1
234	240	+2

**Supplemental Table B.  
Peptides Spanning PE Cleavage Sites**

Cleavage site	Intact Protein	PE-derived Peptide	Cleavage site	Intact Protein	PE-derived Peptide	Cleavage site	Intact Protein	PE-derived Peptide		
#1 74-75	61-79 (+2)	71-79 (+1)	#7 170-171	156-175 (+2)	157-181 (+2)	#9 191-192	190-198 (+1)	190-198 (+1)		
	71-79 (+1)	71-79 (+2)		156-175 (+3)	162-185 (+2)		190-198 (+2)	190-198 (+2)		
	71-79 (+2)			157-165 (+2)	162-185 (+3)		190-199 (+2)	190-199 (+2)		
	72-86 (+2)			157-181 (+2)	166-175 (+1)		190-201 (+2)	190-201 (+2)		
#2 81-82	72-86 (+2)	76-82 (+1)		157-181 (+3)	166-175 (+2)	190-209 (+2)	190-209 (+2)	#10 204-205	190-209 (+2)	199-209 (+1)
	76-82 (+1)	76-82 (+2)		162-185 (+2)	166-177 (+2)	190-209 (+3)	199-209 (+3)		199-209 (+2)	199-209 (+2)
	76-82 (+2)	80-86 (+1)		162-185 (+3)	166-178 (+2)	199-209 (+1)	199-209 (+1)		199-210 (+2)	199-210 (+2)
	80-86 (+1)	80-86 (+2)		166-175 (+2)	166-189 (+2)	199-209 (+2)	199-209 (+2)		200-209 (+1)	200-209 (+1)
	80-86 (+2)			166-177 (+2)	166-189 (+3)	200-209 (+1)	200-209 (+1)		200-209 (+2)	200-209 (+2)
#3 88-89	87-93 (+1)	87-93		166-178 (+2)	169-175 (+1)	200-209 (+2)	200-209 (+2)	202-209 (+1)	202-209 (+1)	
	87-100 (+2)	87-97 (+1)		166-189 (+2)	169-175 (+2)	202-209 (+1)	202-209 (+1)	202-209 (+2)	202-209 (+2)	
		87-97 (+2)		166-189 (+3)	169-177 (+2)	202-209 (+2)	202-209 (+2)	202-209 (+2)	202-209 (+2)	
		87-100 (+1)		169-175 (+1)	169-189 (+2)	202-209 (+1)	202-209 (+1)	202-209 (+2)	202-209 (+2)	
		87-100 (+2)		169-175 (+2)	169-189 (+3)					
#4 110-111	97-120 (+2)	102-115 (+2)	169-177 (+2)	169-189 (+3)	#11 211-212	210-221 (+1)	210-217 (+1)			
	102-115 (+2)	107-115 (+1)	169-189 (+2)	210-221 (+2)		210-217 (+2)	210-217 (+2)			
	107-115 (+1)	107-115 (+2)	#8 184-185	162-185 (+2)		162-185 (+2)	210-224 (+2)	210-221 (+1)		
	107-115 (+2)	109-132 (+2)		162-185 (+3)		162-185 (+3)	210-225 (+1)	210-221 (+2)		
	109-132 (+2)			166-189 (+2)		166-189 (+2)	210-225 (+2)	210-224 (+2)		
		166-189 (+3)		166-189 (+3)	210-226 (+2)	210-225 (+1)				
		169-189 (+2)		169-189 (+2)		210-225 (+2)				
#5 117-118	109-132 (+2)	109-132 (+2)	176-189 (+1)	169-189 (+3)	#12 235-236	218-242 (+2)	226-240 (+1)			
	116-126 (+2)	116-126 (+2)	176-189 (+2)	176-189 (+1)		226-240 (+1)	226-240 (+2)			
	116-132 (+2)	116-132 (+1)	178-189 (+2)	176-189 (+2)		226-240 (+2)	226-240 (+3)			
	116-133 (+2)	116-132 (+2)	179-189 (+1)	176-189 (+3)		231-240 (+1)	231-240 (+1)			
	116-153 (+3)	116-133 (+2)	179-189 (+2)	178-189 (+2)		234-240 (+1)	234-240 (+1)			
#6 160-161	133-165 (+3)	154-165 (+1)	182-189 (+1)	179-189 (+1)	234-240 (+2)	234-240 (+2)				
	154-165 (+1)	154-165 (+2)								
	154-165 (+2)	156-165 (+1)								
	156-165 (+1)	156-165 (+2)								
	156-165 (+2)	157-165 (+1)								
	156-175 (+2)	157-165 (+2)								
	156-175 (+3)	157-181 (+2)								
	157-165 (+2)	158-165 (+1)								
	157-181 (+2)	158-165 (+2)								
	157-181 (+3)									