Methionine Oxidation analyzed by ACE

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Overview

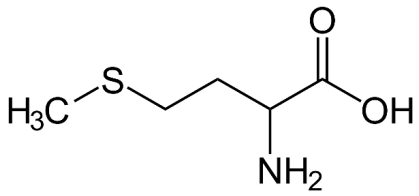
Introduction

ACE of peptides with Gold/Silver

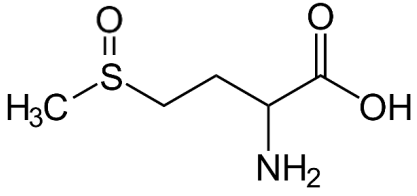
ACE of proteins with Silver

Summary

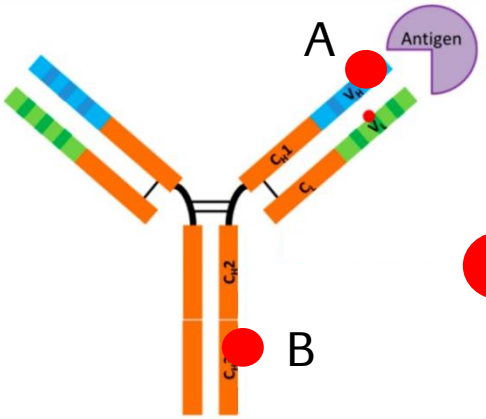
Methionine Sulfoxide



Met



Met-SO



Met oxidation site

A

Reduced efficacy

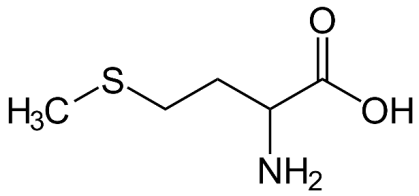
B

Faster plasma clearance

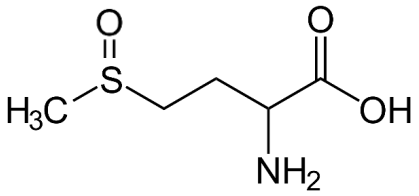
B

Immunogenicity

Methionine Sulfoxide determination

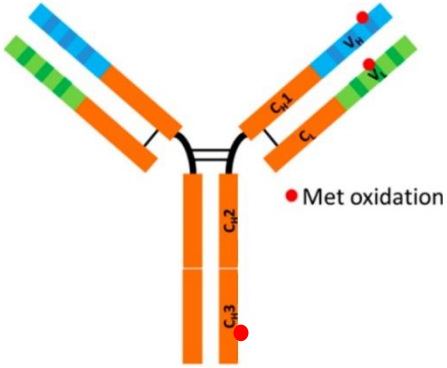


Met

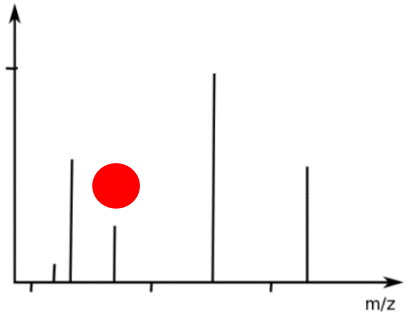


Met-SO

Mass Δ =16 u

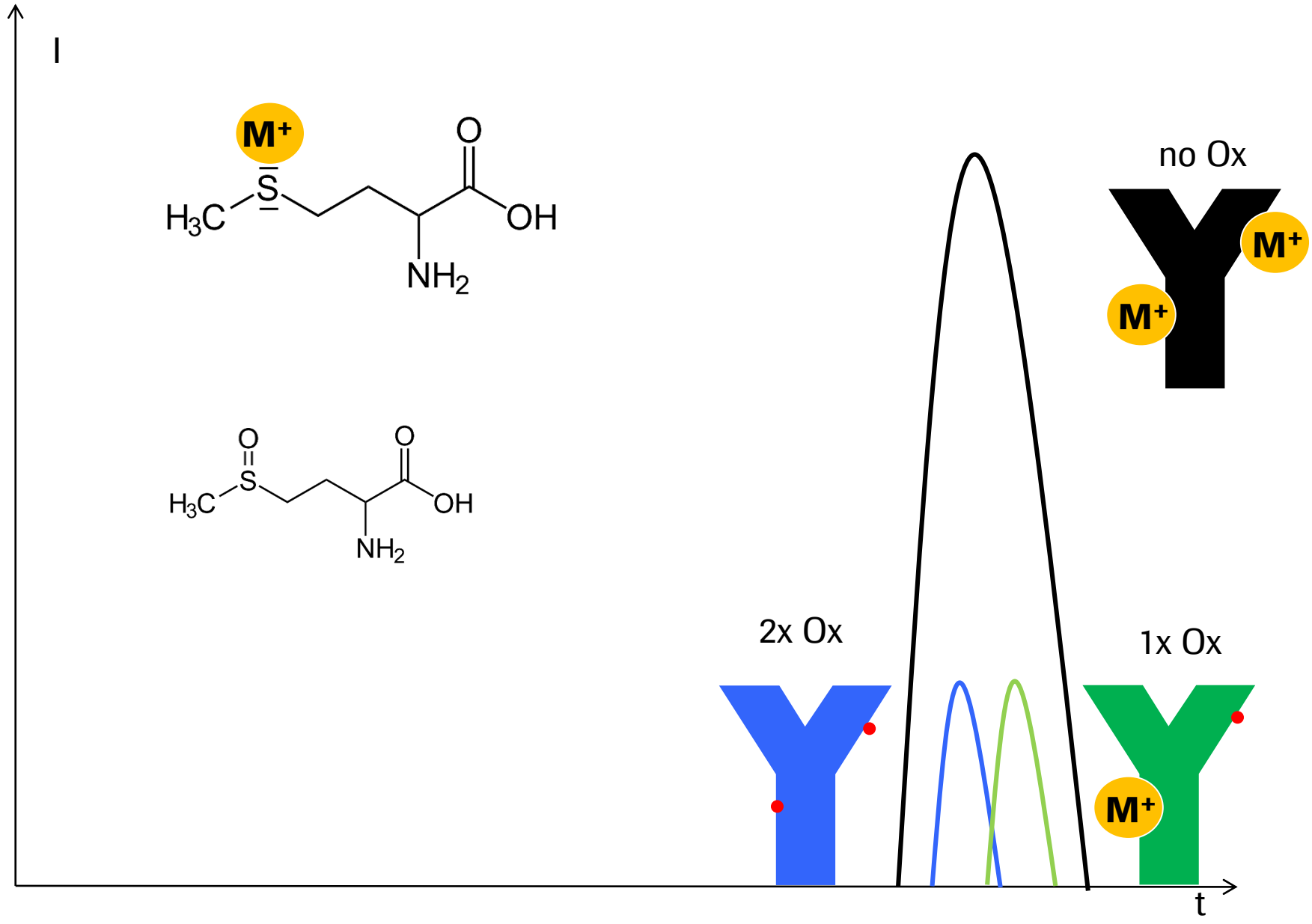


digest

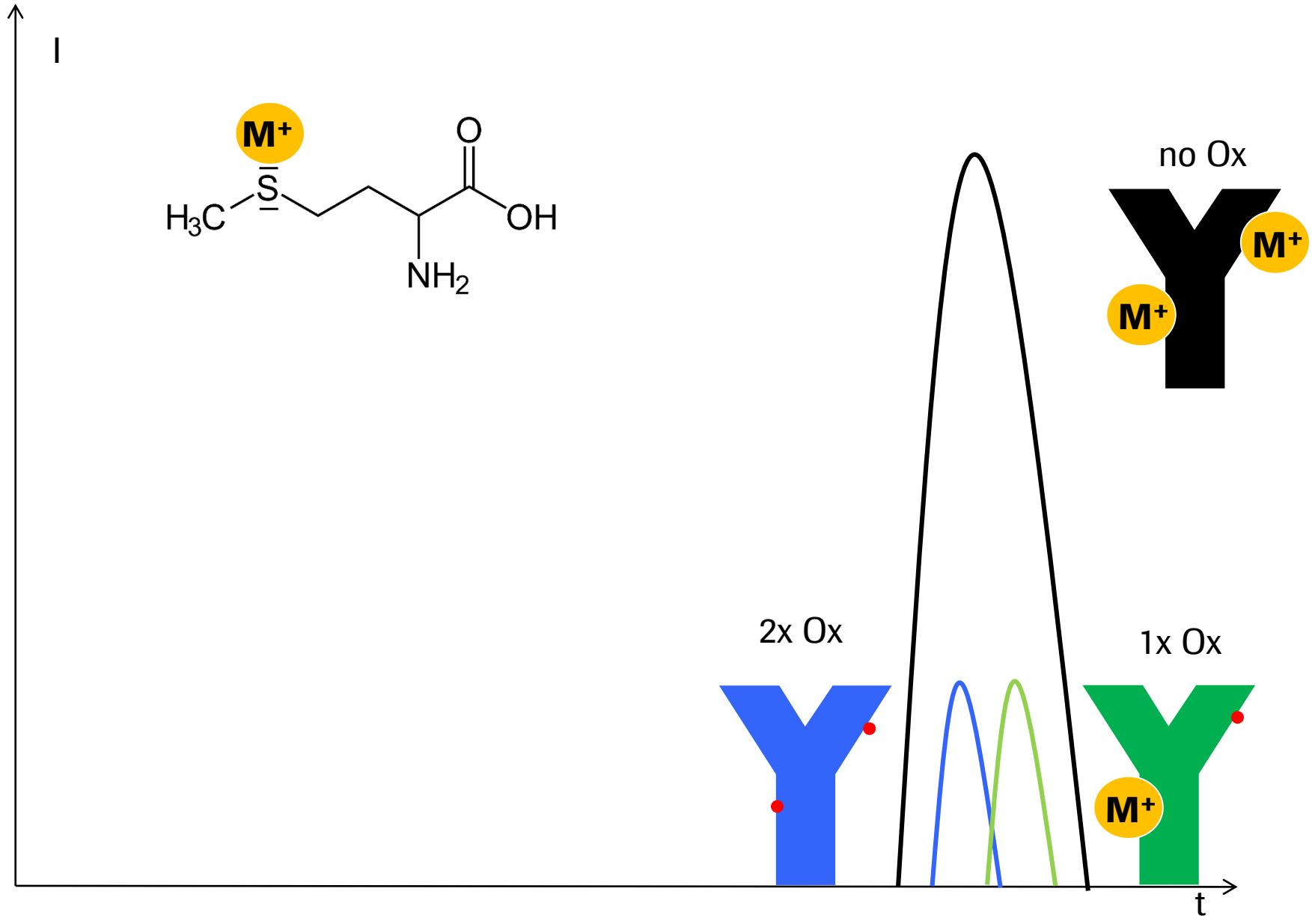


LC/MS

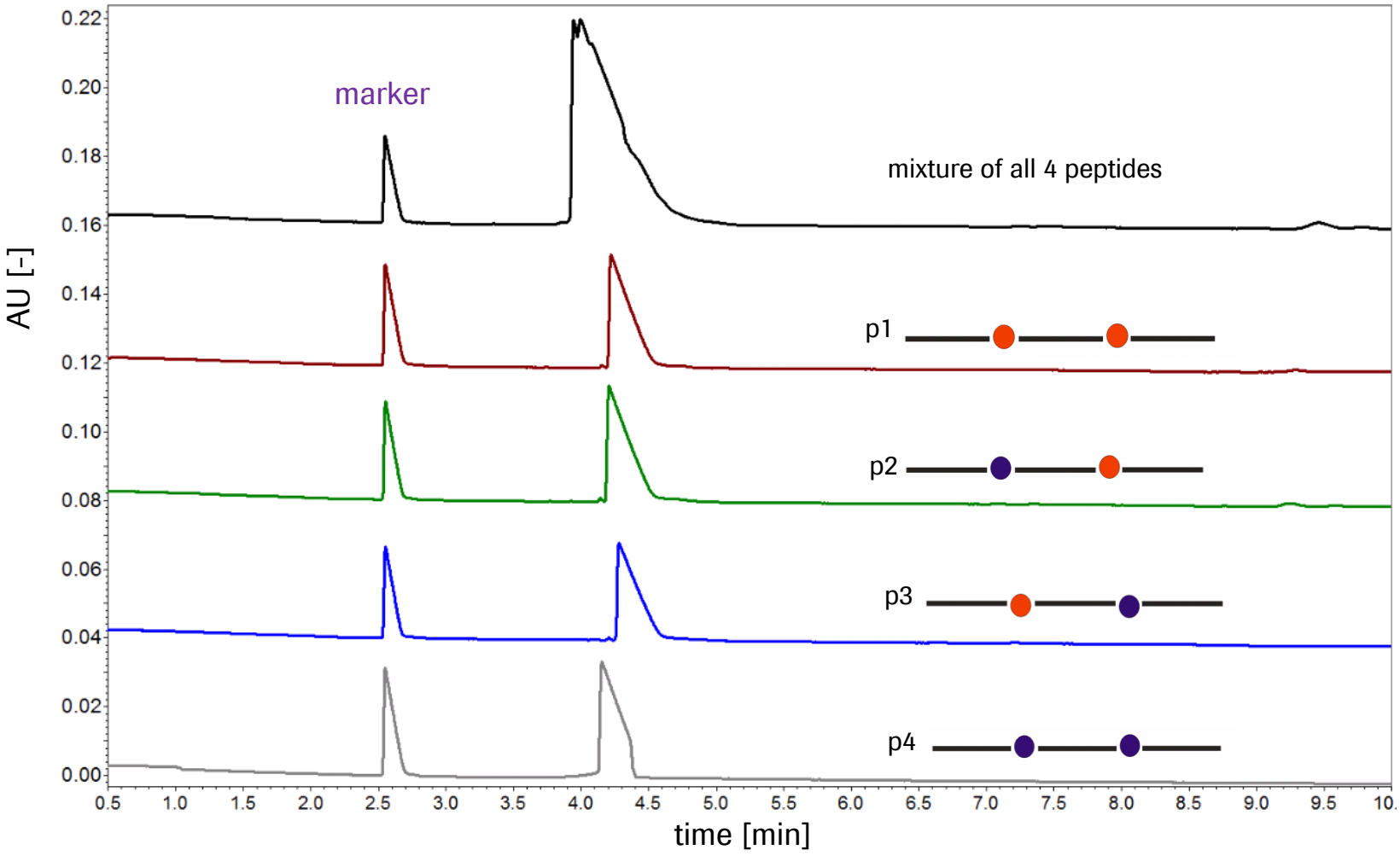
Methionine Sulfoxide determination by ACE



Methionine Sulfoxide determination by ACE



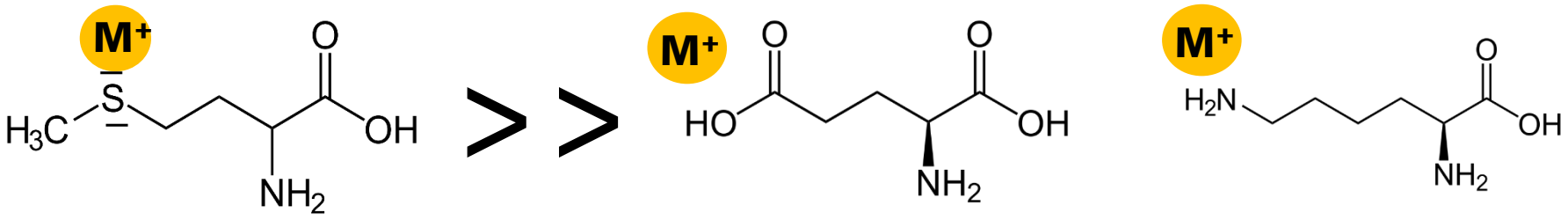
CZE of peptides differing in methionine oxidation



BGE: 50 mM phosphate; pH 3

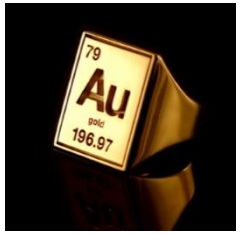
Sample: YA**M**AA**M**KA; pI ≈ 8.5 (cIEF)

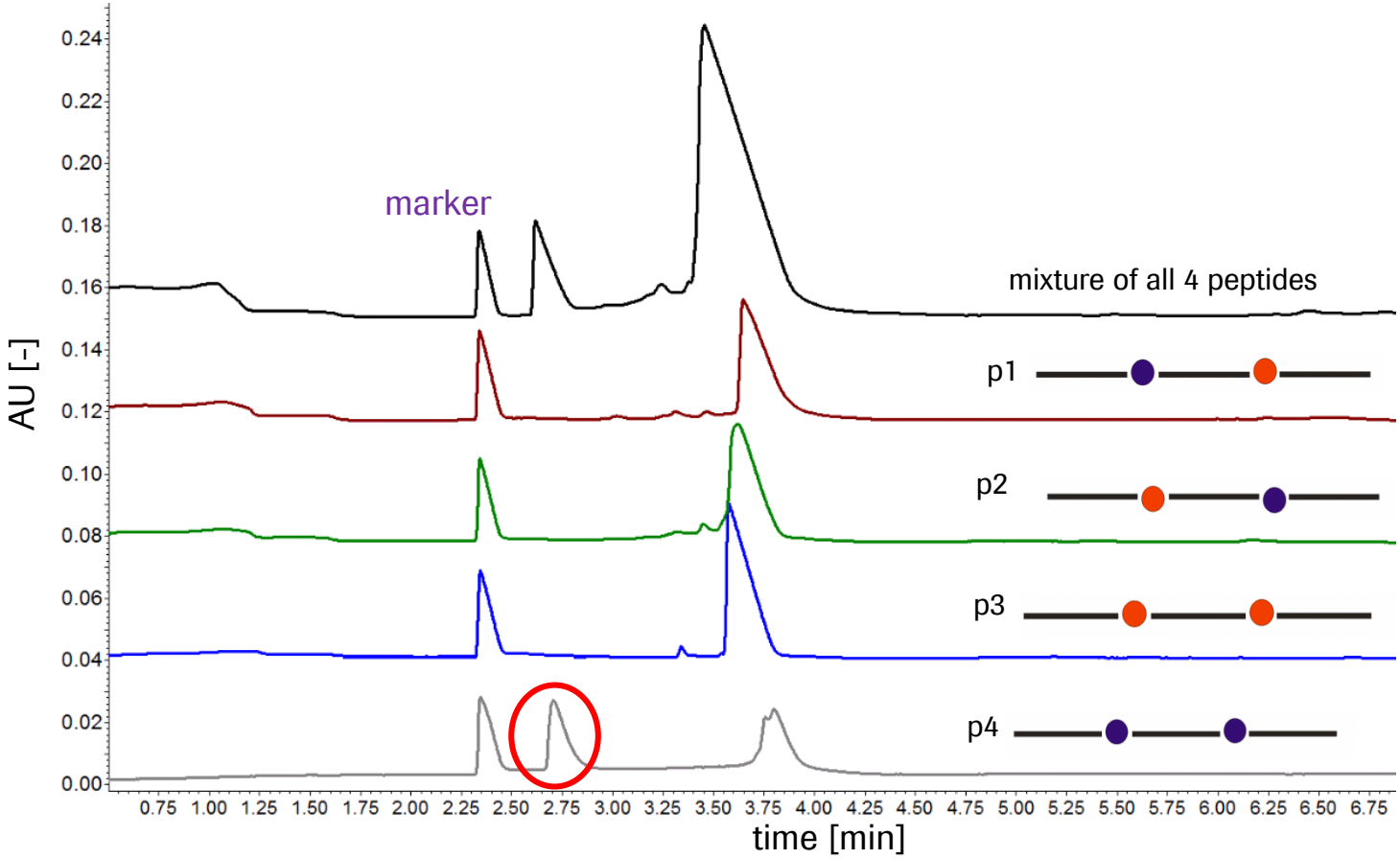
Which metal?



	1												13	14	15	16	17	18
1	1 H	2																2 He
2	3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
3	11 Na	12 Mg	3	4	5	6	7	8	9	10	11	12	13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
4	19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	~~28 Ni~~	~~29 Cu~~	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
5	37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	~~46 Pd~~	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
6	55 Cs	56 Ba	57	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	~~78 Pt~~	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
7	87 Fr	88 Ra	89	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Ds	111 Rg	112 Cn	113 Nh	114 Fl	115 Mc	116 Lv	117 Ts	118 Og

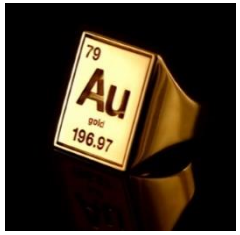
ACE of peptides differing in methionine oxidation

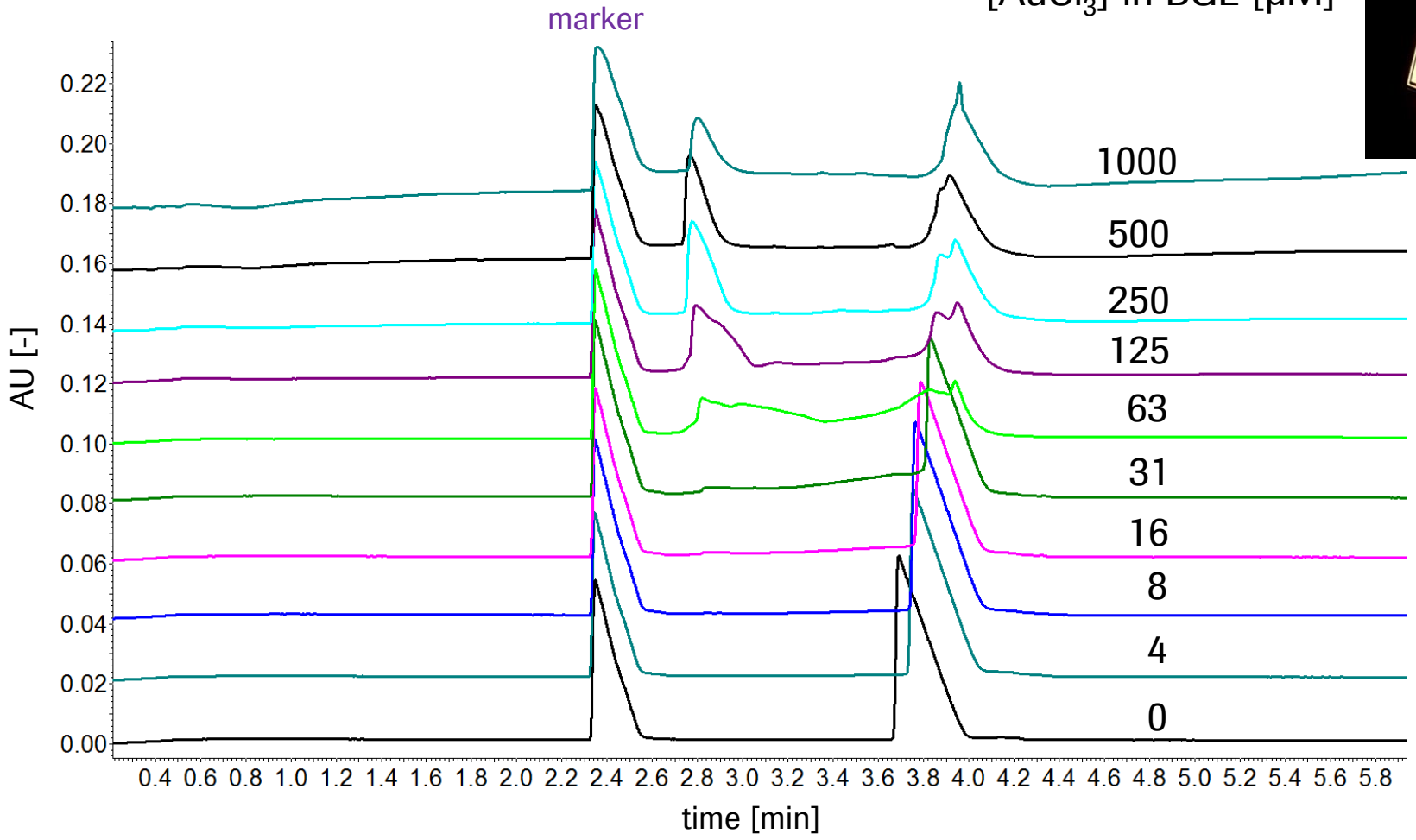




BGE: 50 mM phosphate; pH 3; **375 µM $AuCl_3$**

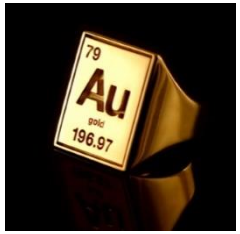
Complete peak shift cannot be forced with more Au

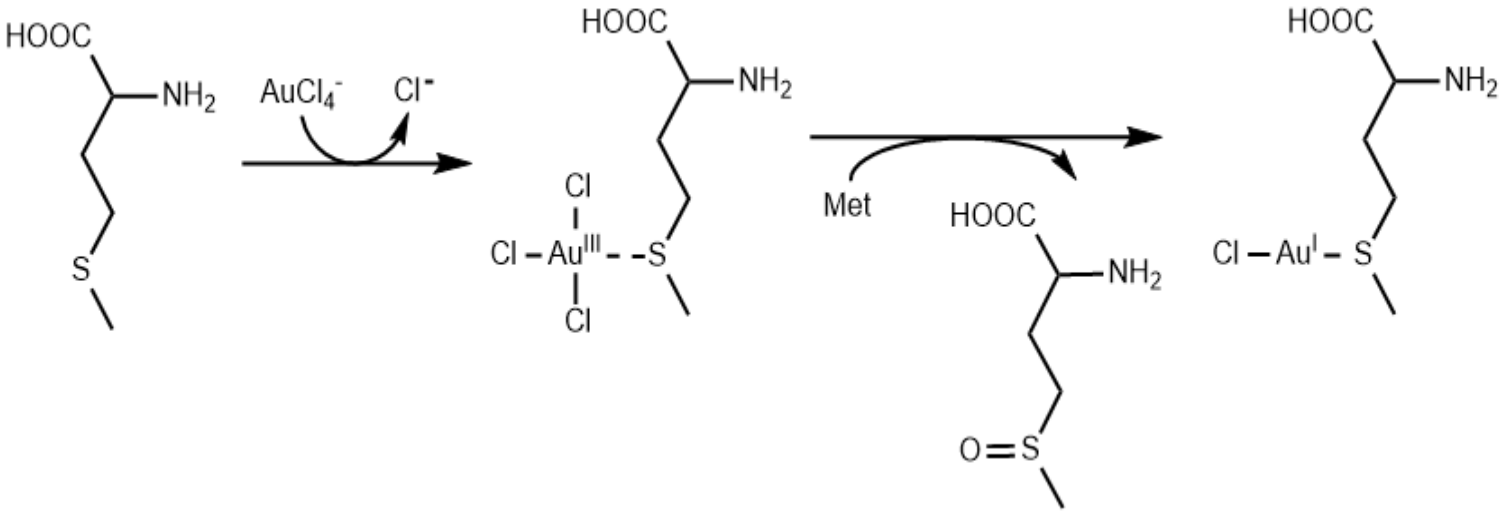


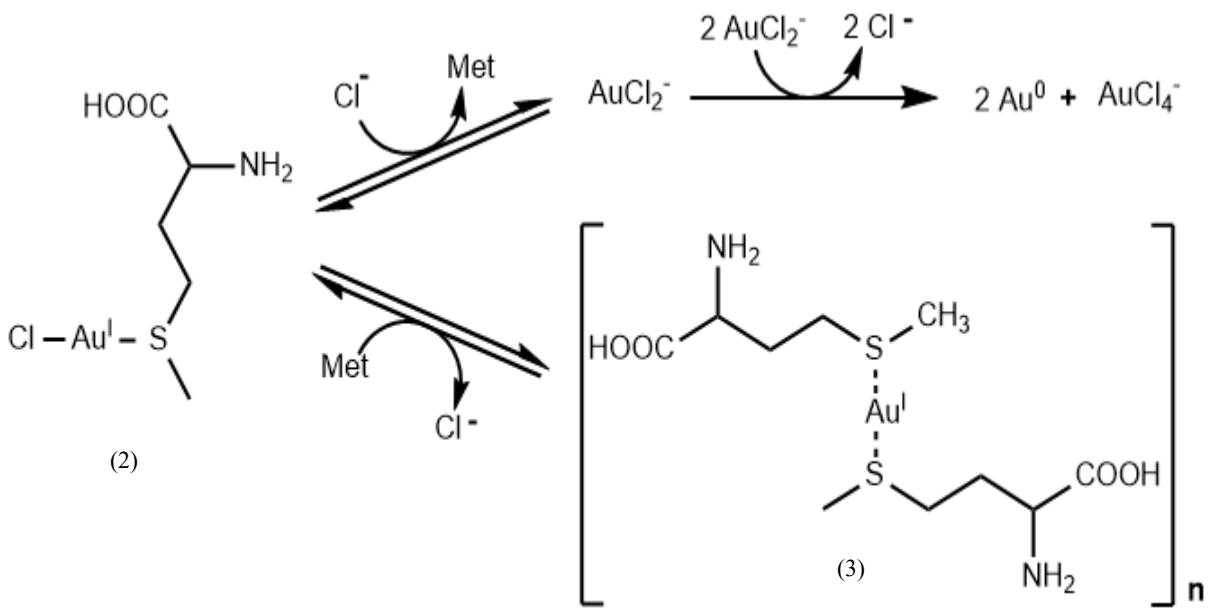


BGE: 50 mM phosphate; pH 3; **$AuCl_3$** as indicated

Why two peaks? Theory

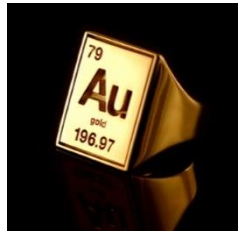


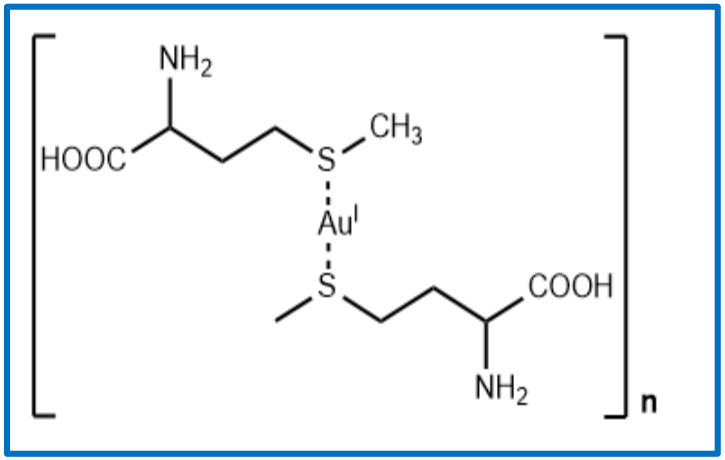


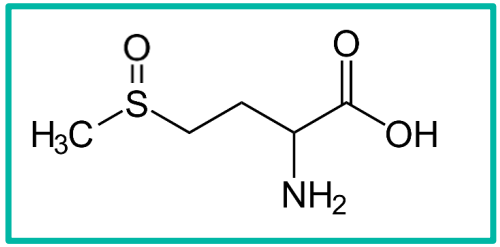


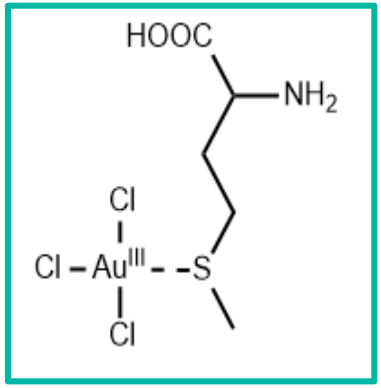
(2)

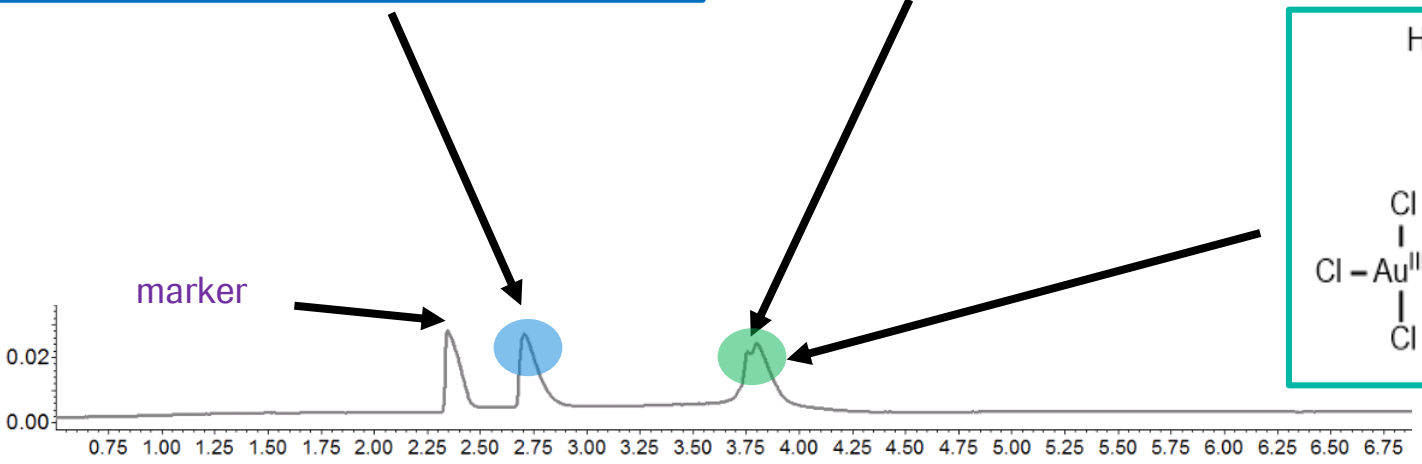
(3)


79
Au
gold
196.97

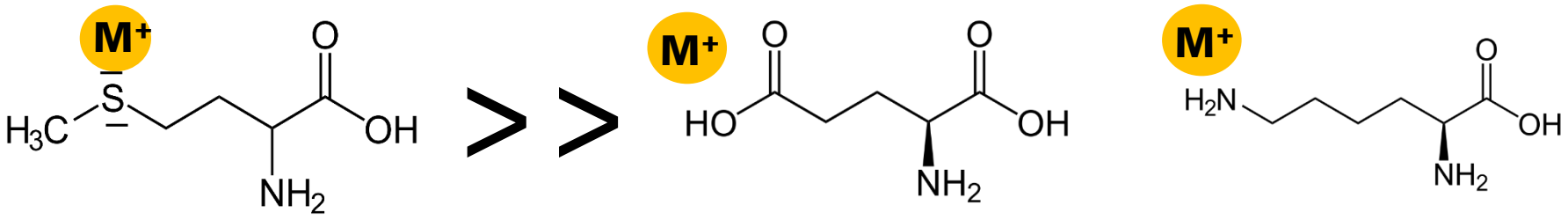

NH2
HOOC
S
CH3
Au I
S
COOH
NH2
n


O
O
S
H3C
OH
NH2


HOOC
NH2
Cl
Cl – Au III – S
Cl


marker
0.02
0.00
0.75 1.00 1.25 1.50 1.75 2.00 2.25 2.50 2.75 3.00 3.25 3.50 3.75 4.00 4.25 4.50 4.75 5.00 5.25 5.50 5.75 6.00 6.25 6.50 6.75

Which metal?



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4	19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	~~28 Ni~~	~~29 Cu~~	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
5	37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	~~46 Pd~~	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
6	55 Cs	56 Ba	57	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	~~78 Pt~~	~~79 Au~~	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
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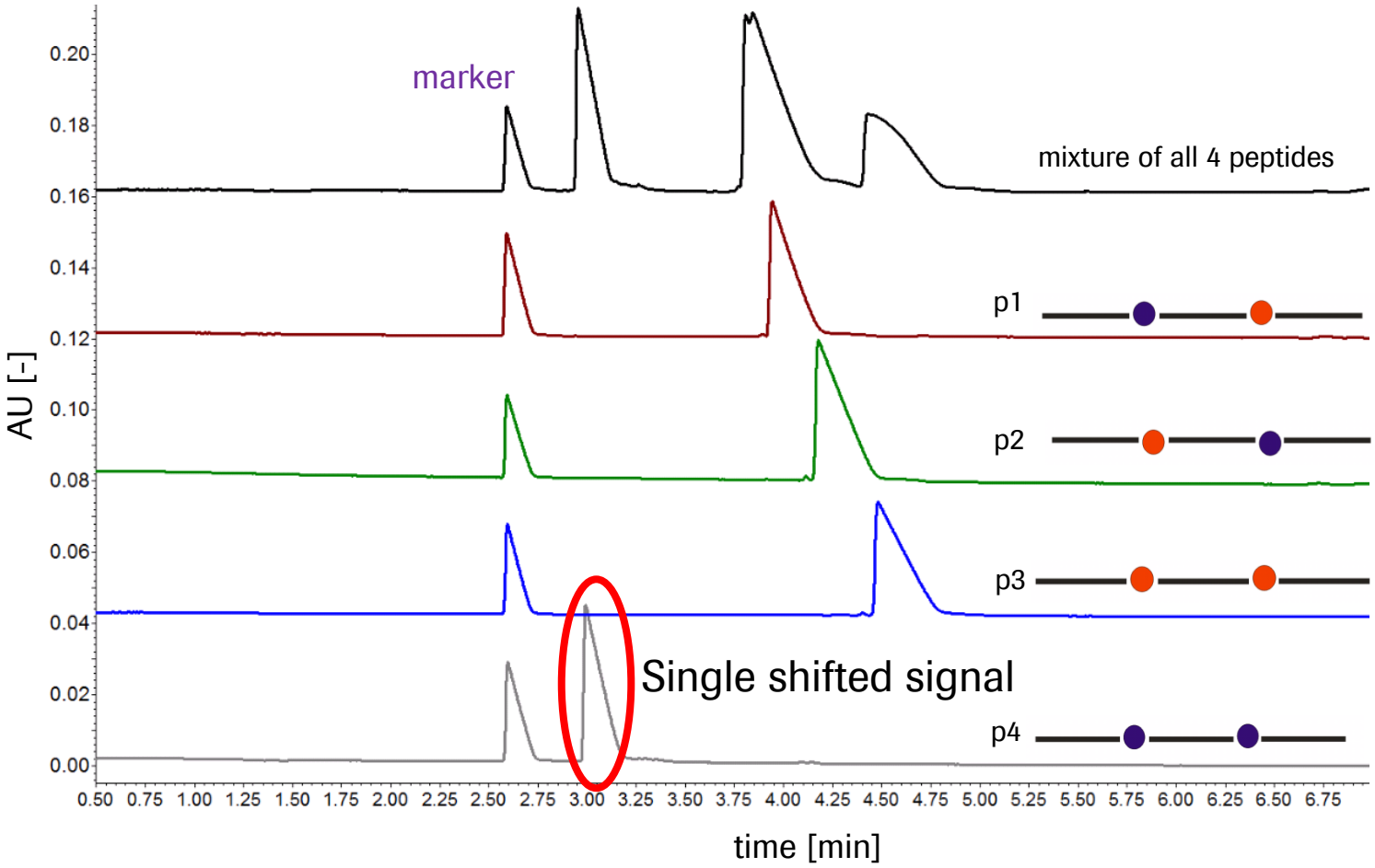
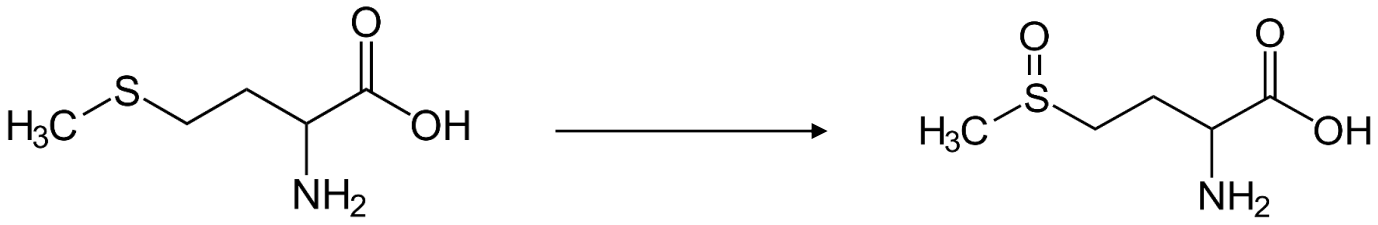
ACE of peptides differing in methionine oxidation



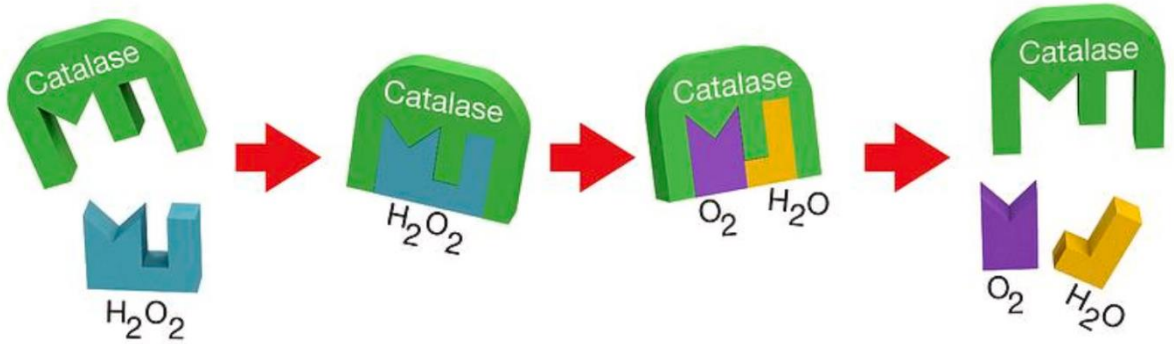
BGE: 50 mM phosphate; pH 3; **250 µM AgF**

IgG1 Analysis

- *1% H_2O_2* for 1-6 hours

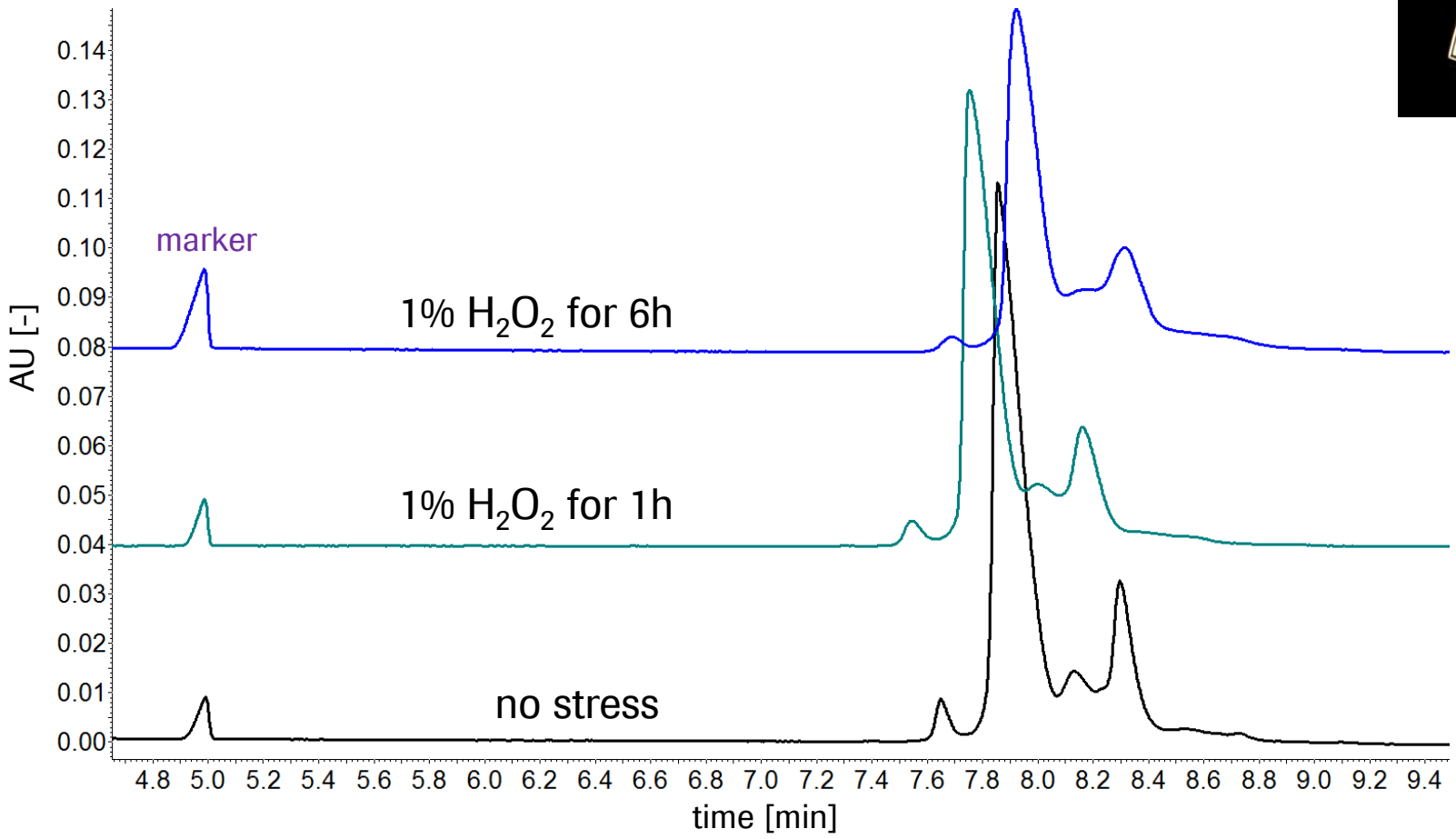


- Stopping with 20 U of catalase



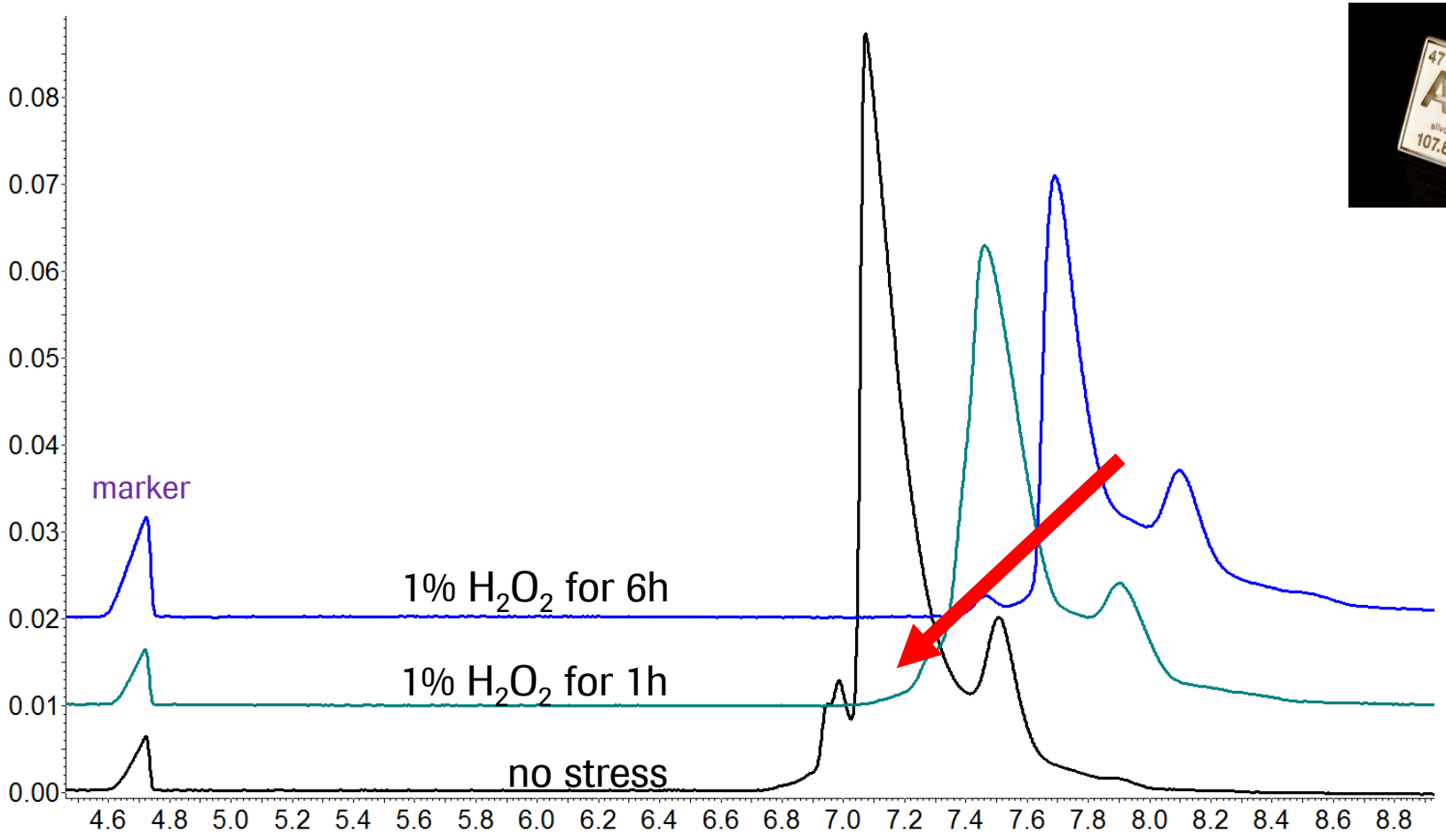
- IgG1; approx. 150 kDa; pI 8.5; **12x Met**; 32x Cys

CZE of IgG1 differing in H_2O_2 stress



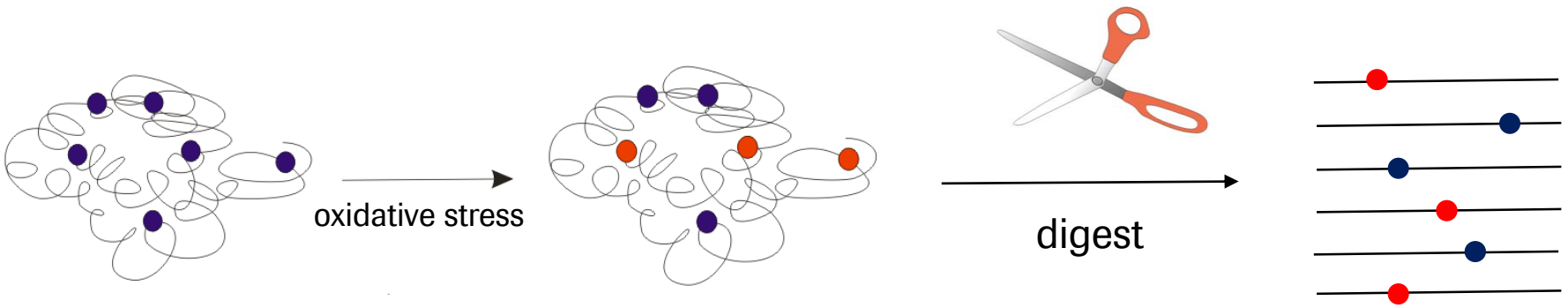
BGE: 400 mM EACA; 2 mM TETA; 0.05% HPMC; pH 5.7

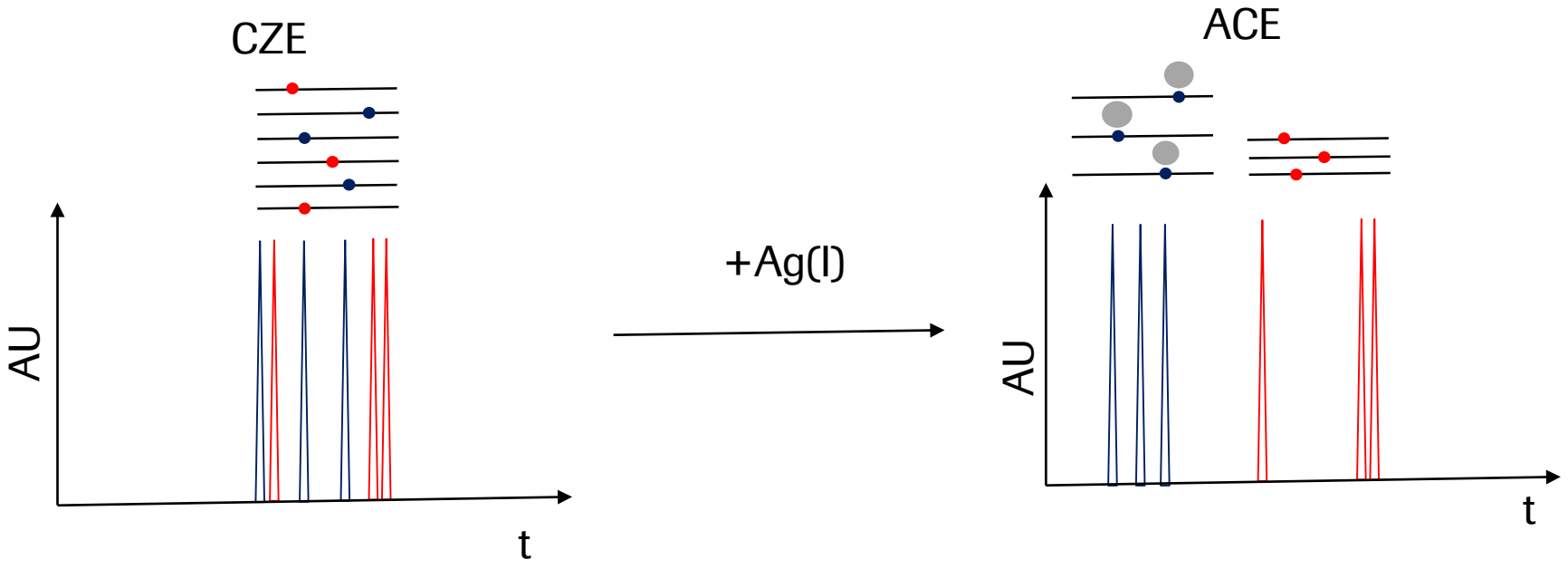
ACE of IgG1 differing in H_2O_2 stress



BGE: 400 mM EACA; 2 mM TETA; 0.05% HPMC; pH 5.7 +**500 µM AgF**

Outlook: Silver Peptide Map of Proteins

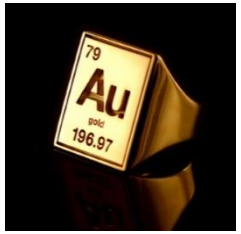




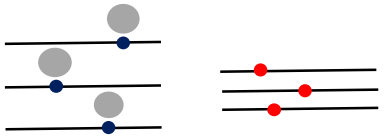
Summary

- ACE-CZE allows to monitor **methionine oxidation in peptides** applying Ag(I) ions in the BGE

- Au(III) ions seem unsuitable because of oxidation of Methionine



- Ongoing: Silver Peptide Map with different Proteins



Acknowledgements

- PD Dr. Maria Schwarz
- Dr. Bernd Moritz
- Dr. Steffen Kiessig
- Andrea Bathke
- Anja Bathke

Doing now what patients need next