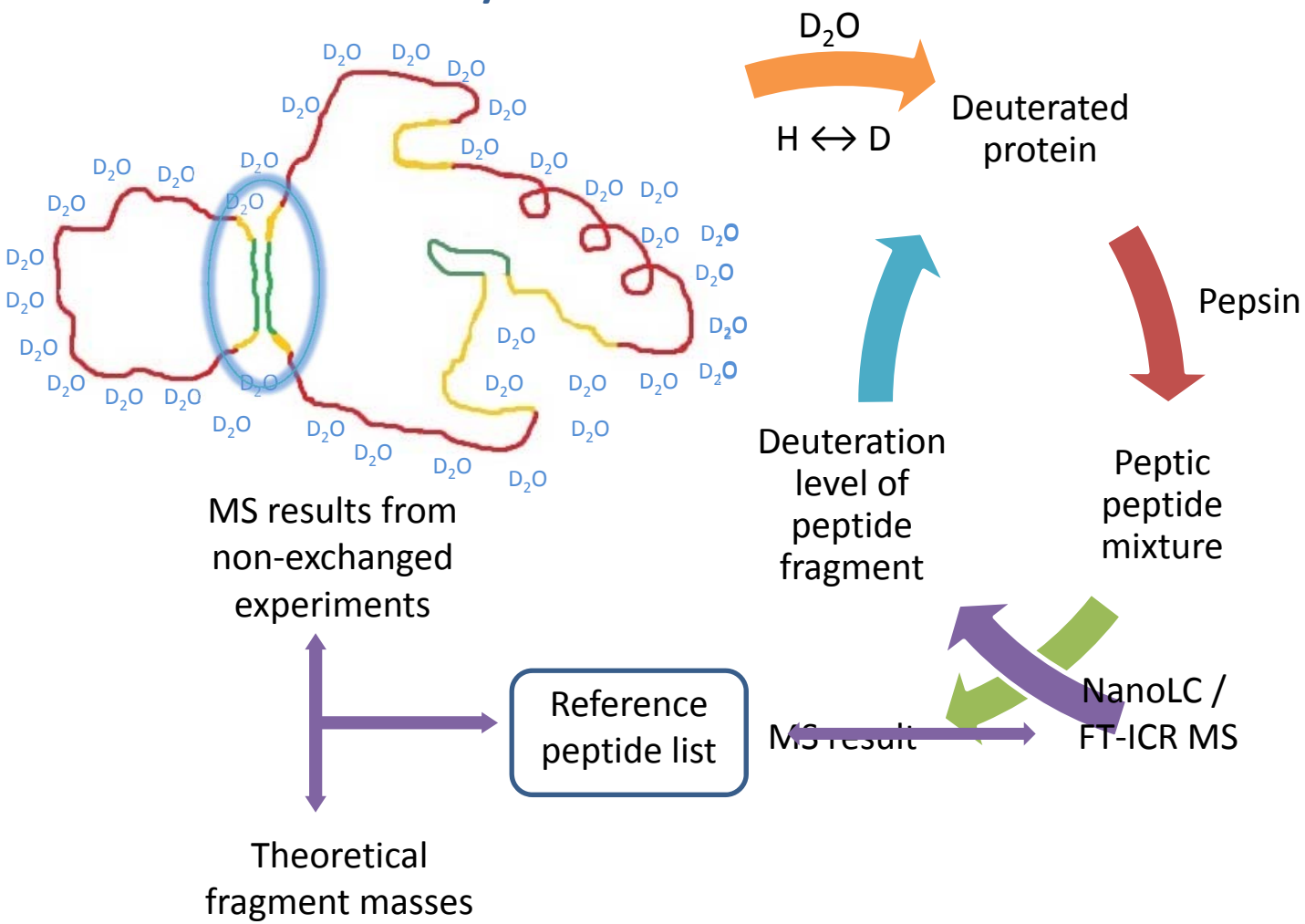
A Method Improvement in the Bottom-up H/D Exchange FT-ICR Mass Spectrometry

Jianqing Wu, Guillaume van der Rest

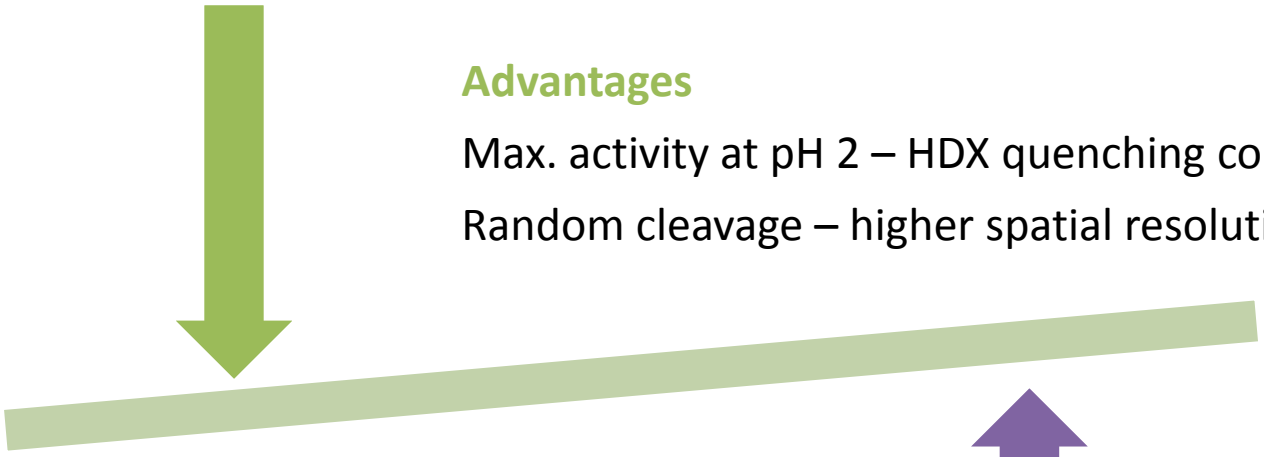
Laboratoire des Mécanismes Réactionnels, Ecole Polytechnique

29th JFSM
Orléans, 18.09.2012

Bottom-up HDX-MS: a powerful tool for protein structural study



Pepsin: the enzyme of choice in bottom-up HDX-MS



Advantages

Max. activity at pH 2 – HDX quenching condition

Random cleavage – higher spatial resolution

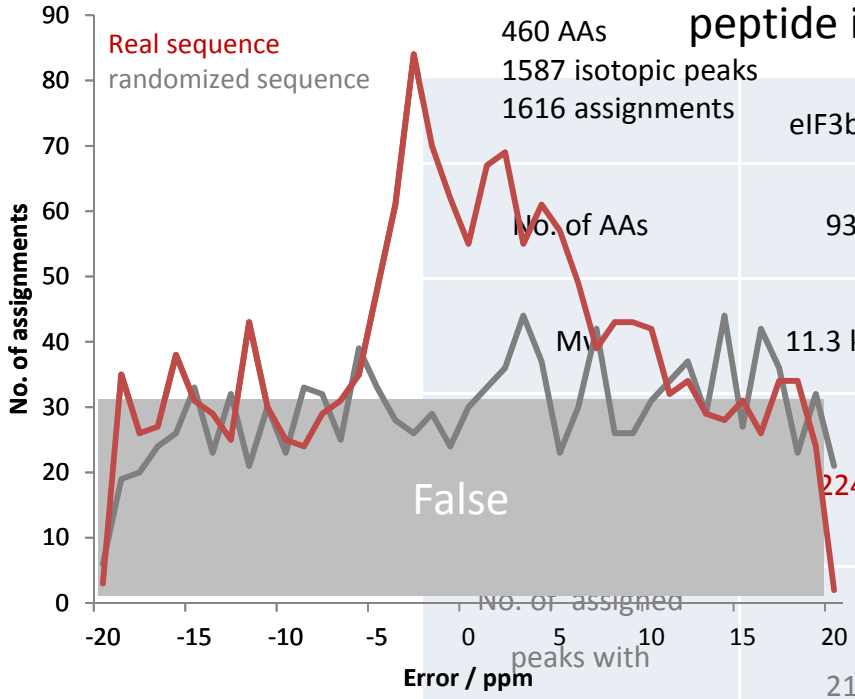
Challenges

Peptide identification using highly accurate mass

False: e.g. noises, contaminants, pepsin self-digests

Ambiguous: similar masses, different sequences

Peptide identification: is the accurate mass sufficient?



Examples of the mass-dependent peptide identification

	eIF3b-ct	eIF3i-3b-ct	aIF2Mj
No. of AAs	93	460	817
Mw	11.3 kDa	52 kDa	91.4 kDa
No. of assigned peaks with real sequence (±5 ppm)	224	664	778
No. of assigned peaks with randomized sequence (±5 ppm)	21	429	455

MS/MS: is it the only way out?

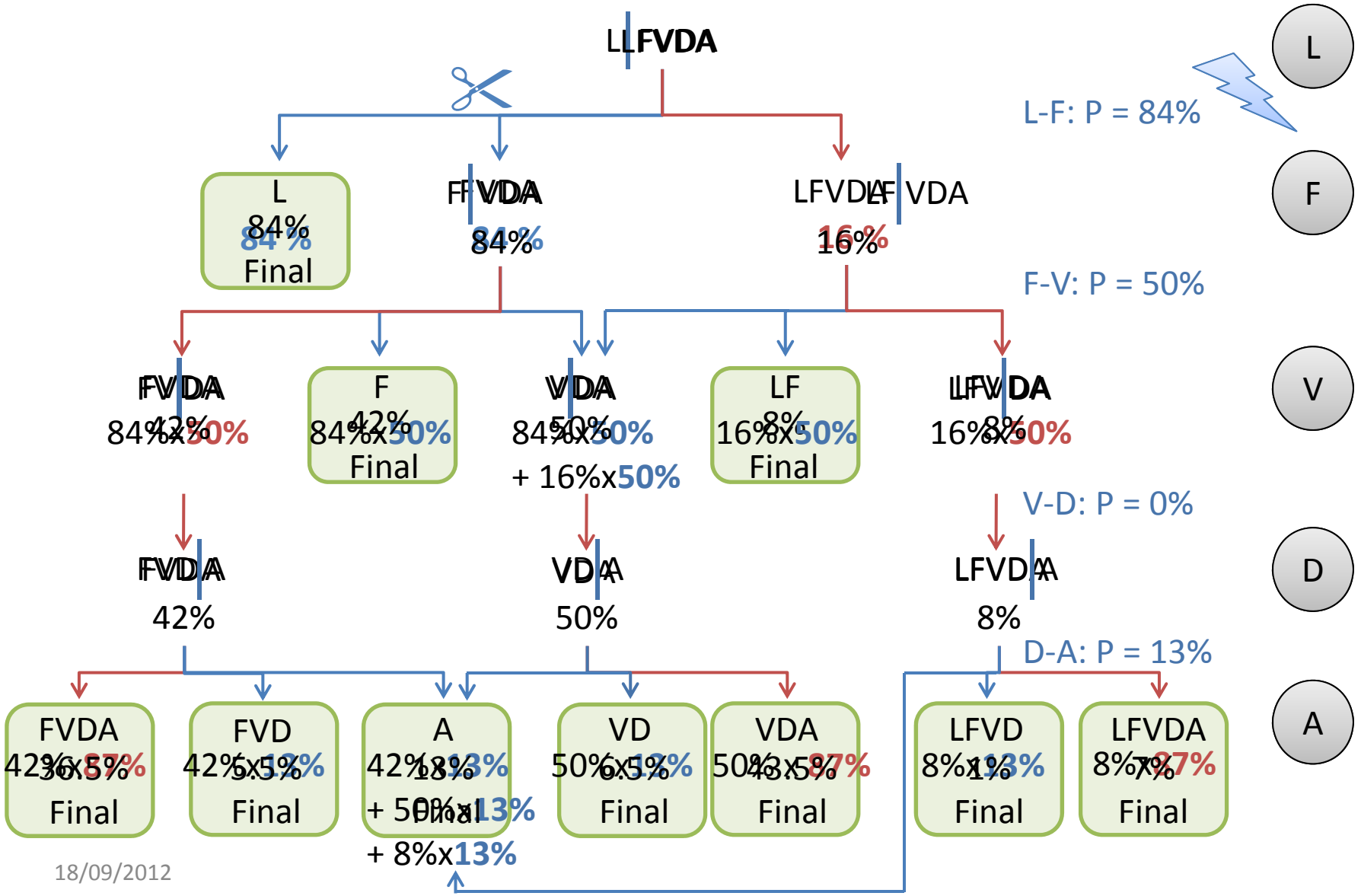
Peptide identification: pepsin specificity

Probability of cleavage at P1-P1’ positions

	F	L	M	C	E	W	Y	D	A	Q	N	T	S	G	V	I	K	H	R	P	Ave.
Y	65%	68%	30%	25%	70%	71%	36%	48%	48%	47%	39%	23%	31%	17%	11%	7%	0%	0%	0%	0%	33%
F	85%	84%	64%	75%	53%	40%	33%	37%	38%	24%	28%	21%	5%	9%	8%	8%	0%	0%	0%	0%	28%
W	60%	60%	50%	57%	63%	-	50%	17%	17%	33%	38%	17%	23%	17%	24%	30%	0%	0%	0%	0%	28%
I	65%	63%	62%	40%	36%	15%	20%	42%	30%	40%	25%	18%	13%	9%	9%	0%	2%	0%	0%	0%	24%
M	83%	58%	42%	0%	29%	33%	25%	20%	30%	20%	0%	11%	0%	11%	6%	0%	0%	0%	0%	0%	21%
V	50%	53%	61%	21%	31%	10%	16%	25%	28%	23%	16%	11%	12%	0%	4%	2%	0%	0%	0%	0%	19%
L	64%	56%	66%	36%	21%	29%	25%	12%	16%	7%	4%	8%	4%	13%	7%	1%	0%	0%	0%	0%	18%
C	20%	54%	0%	50%	36%	22%	0%	33%	6%	33%	25%	0%	20%	12%	0%	18%	0%	0%	0%	0%	17%
A	55%	54%	38%	18%	35%	8%	9%	13%	14%	7%	9%	7%	6%	4%	3%	2%	0%	0%	0%	0%	16%
E	42%	45%	29%	20%	9%	24%	19%	6%	6%	2%	0%	0%	4%	0%	4%	6%	0%	0%	0%	0%	10%
D	44%	46%	38%	0%	11%	21%	17%	5%	5%	0%	5%	2%	2%	4%	0%	0%	0%	0%	0%	0%	10%
R	42%	34%	26%	29%	9%	13%	16%	9%	8%	6%	4%	0%	5%	3%	0%	0%	0%	0%	0%	0%	10%
N	42%	45%	7%	0%	13%	0%	11%	0%	4%	5%	0%	0%	4%	0%	0%	0%	0%	0%	0%	0%	9%
S	52%	42%	22%	0%	4%	6%	14%	2%	5%	0%	0%	5%	2%	0%	3%	0%	0%	0%	0%	0%	9%
T	31%	27%	25%	35%	3%	29%	4%	12%	5%	3%	6%	9%	3%	2%	0%	0%	0%	0%	0%	0%	9%
H	43%	33%	29%	17%	6%	0%	10%	15%	22%	15%	0%	11%	3%	0%	0%	0%	0%	0%	0%	0%	9%
K	47%	33%	32%	0%	12%	13%	0%	2%	3%	3%	0%	4%	0%	2%	0%	2%	0%	0%	0%	0%	8%
Q	33%	26%	17%	0%	11%	0%	20%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	6%
P	17%	24%	18%	20%	8%	0%	6%	0%	5%	0%	0%	0%	2%	0%	11%	2%	0%	5%	0%	0%	6%
G	28%	7%	8%	6%	1%	0%	10%	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	3%
Ave.	46%	44%	35%	23%	20%	17%	16%	13%	13%	10%	9%	7%	5%	4%	4%	2%	0%	0%	0%	0%	14%

Hamoru et al., *Rapid Commun. Mass Spectrom*. 2008; **22**: 1041-1046

Peptide identification: probability of occurrence (POc)



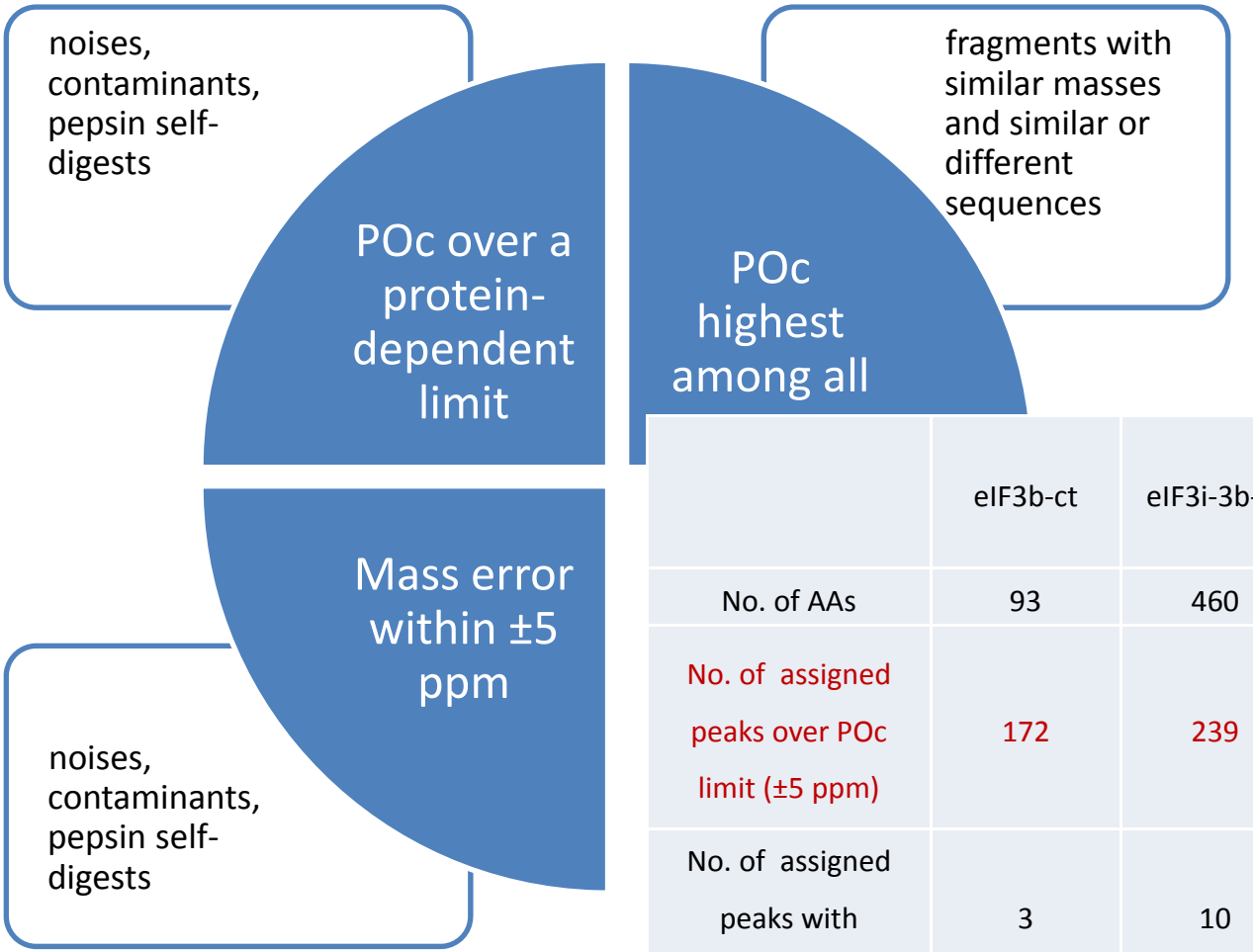
Peptide identification: POc filter

noises, contaminants, pepsin self-digests

POc over a protein-dependent limit

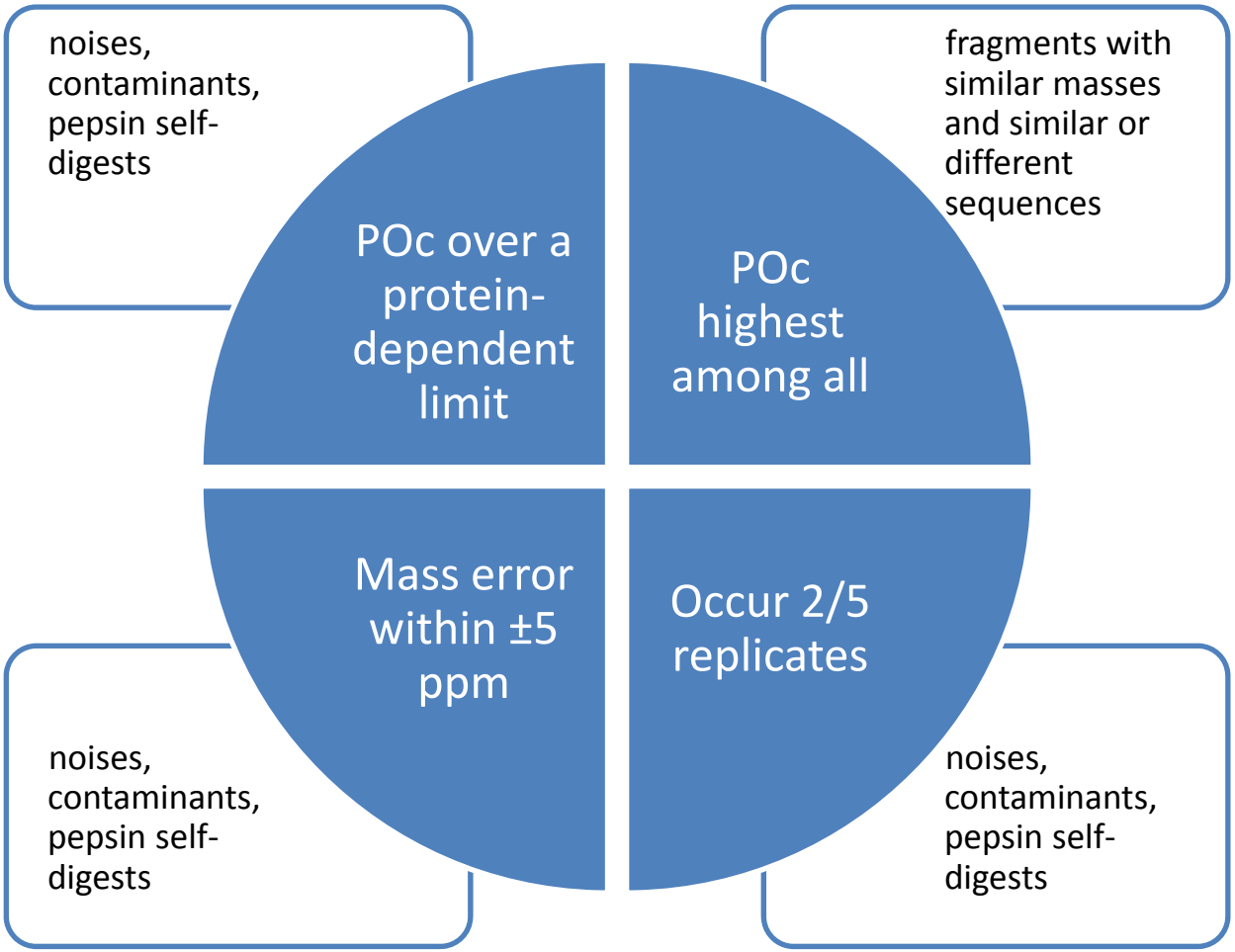
#fragments(POc<limit) = #fragments(randomized sequence)

Peptide identification: mass filter & POc selection



	eIF3b-ct	eIF3i-3b-ct	aIF2Mj
No. of AAs	93	460	820
No. of assigned peaks over POc limit (±5 ppm)	172	239	305
No. of assigned peaks with ambiguities	3	10	22

Peptide identification: reproducibility



Discussions: MS/MS verification

	eIF3b-ct	eIF3i-3b-ct	aIF2Mj
No. of AAs	93	367	820
No. of MS/MS	11	18	15

ALL the MS/MS results show the peptides are correctly identified.

Experimental peak mass	m/z of possible fragment	Mass error /ppm	ion	Fragment position	sequence
813.3714	813.3701	1.82	2.38%	eIF3b-ct_53-66	KIFDVQPEDASDDF
	813.3750	-4.45	0.034%	eIF3b-ct_63-76	SDDFTTIEEIVEEV
1002.5292	1002.5271	2.12	1.05%	eIF3i_129-146	LAILDNVMKNPGSINIYE
	1002.5271	2.12	0.205%	eIF3i_130-147	AILDNVMKNPGSINIYEI
698.3788	698.3787	0.16	1.43%	eIF3i_306-318	IGRVQGHFGPLNT
	698.3782	0.94	0.289%	eIF3i_308-320	RVQGHFGPLNTVA
	698.3802	-1.94	0.0084%	eIF3i_291-301	GKFEARFYHKI

Discussions: sequence coverage

3b-ct: 93 AAs
90 fragment

3i: 367 AAs
216 fragments

Conclusions

- Peptide identification can be achieved with the accurate mass and POc.
- MS/MS is no longer required.
- High spatial resolution becomes possible due to the high sequence coverage.
- Assessment of the false positives requires more MS/MS verifications.

Acknowledgment

- Edith Nicol, Christian Malosse, Laboratoire des Mécanismes Réactionnels, Ecole Polytechnique

- Pierre-Damien Coureux, Emmanuelle Schmitt, Yves Méchulam, Laboratoire de Biochimie, Ecole Polytechnique

Thank you!