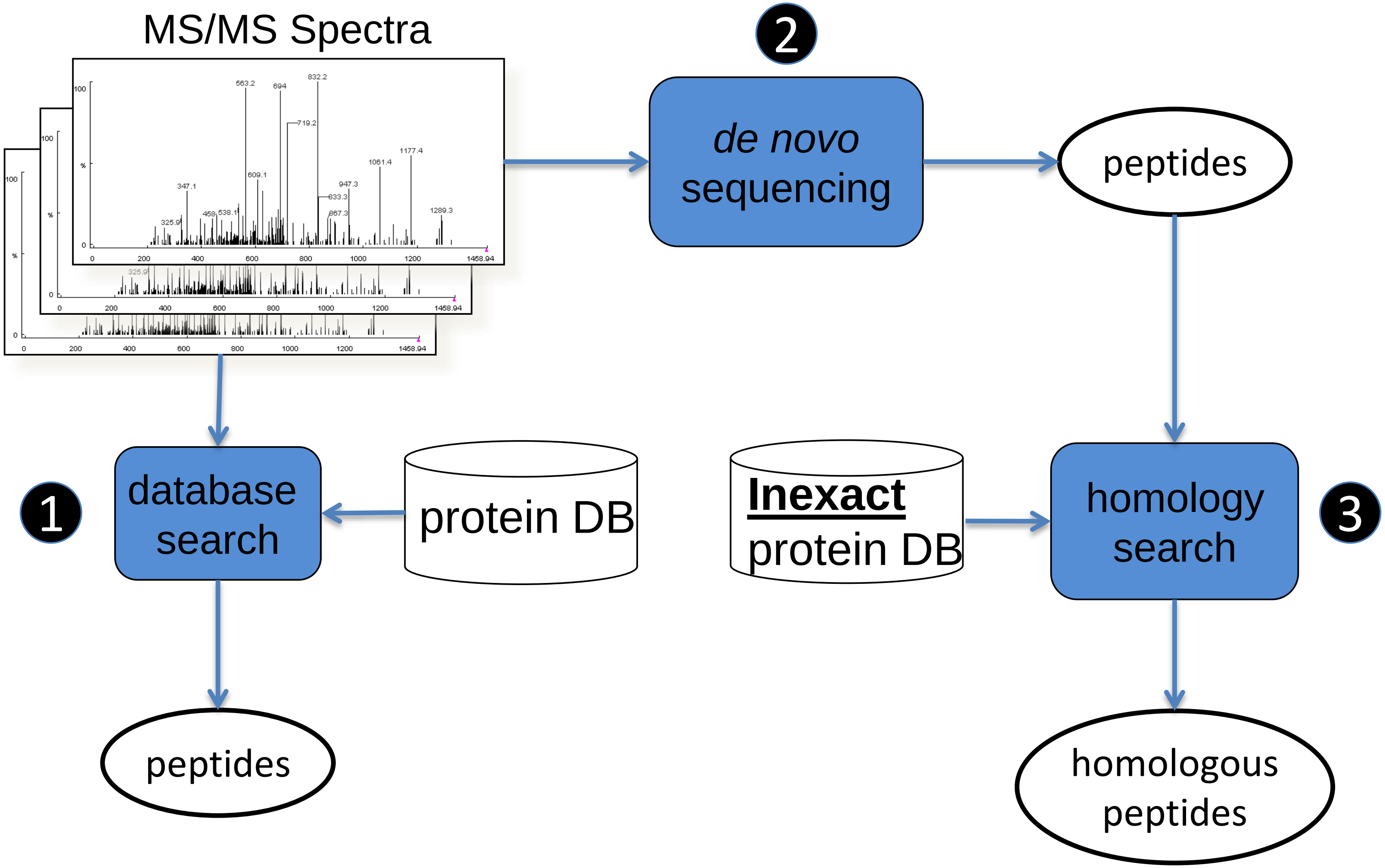
Integrating database search and de novo sequencing to improve the peptide identification

Baozhen Shan
Bioinformatics Solutions Inc.

1. Approaches for LC/MSMS data analysis



MS/MS Spectra

2

de novo sequencing

peptides

1

database search

protein DB

Inexact protein DB

homology search

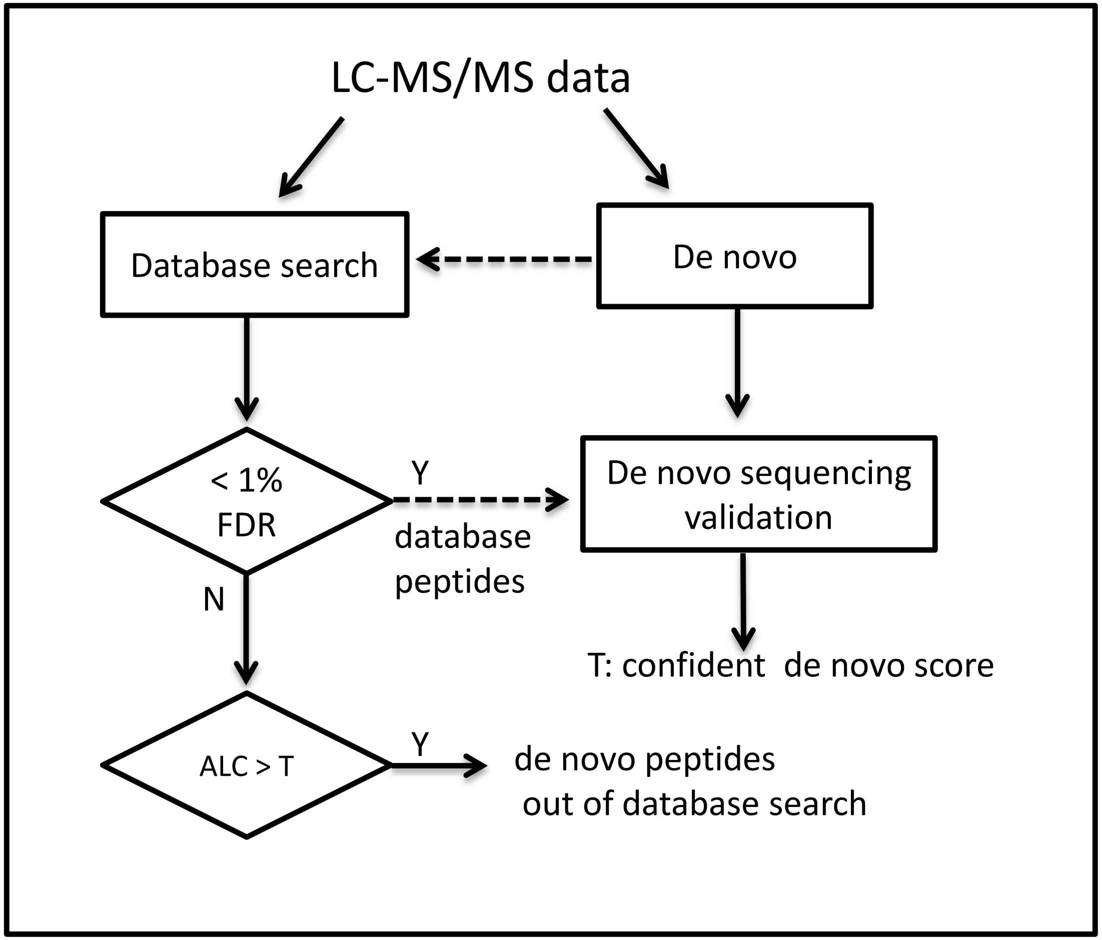
3

peptides

homologous peptides

Ma and Johnson. *MCP* (2012) 11: O111.014902

2. Integrating de novo and database search



LC-MS/MS data

Database search

De novo

< 1% FDR

Y

database peptides

De novo sequencing validation

N

T: confident de novo score

ALC > T

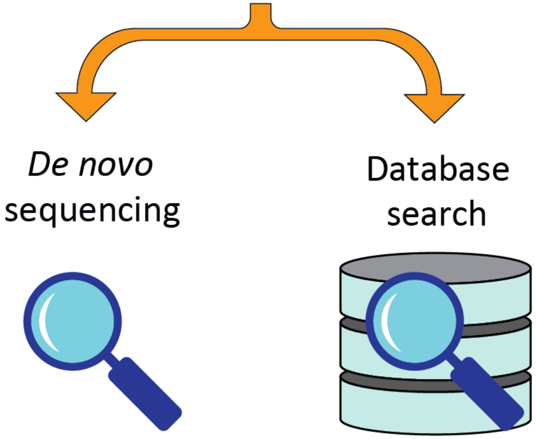
Y

de novo peptides out of database search

3. De novo sequencing improves DB search

- Problem
  - Coverage
  - Modifications
  - Incomplete database

- Solution

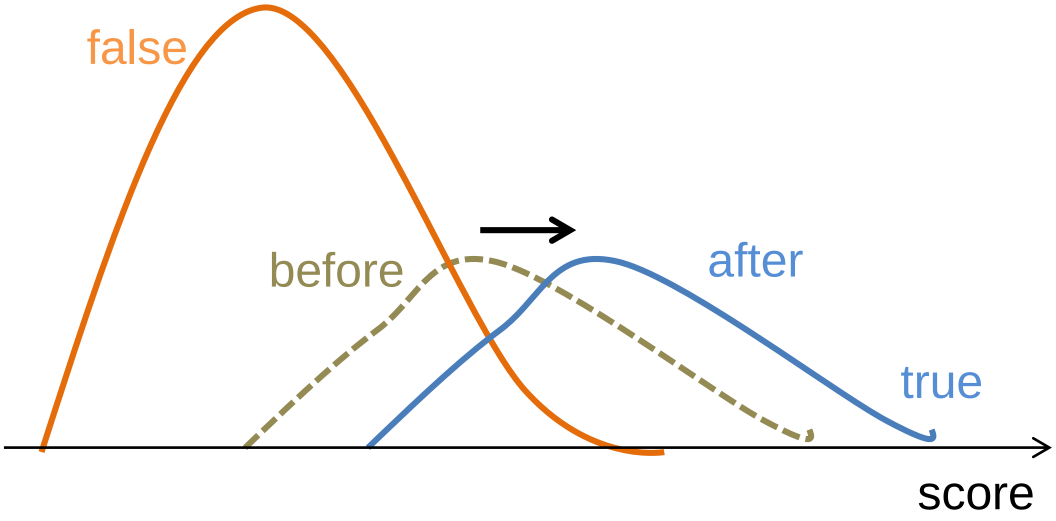


De novo sequencing

Database search

3.1. Good scoring function

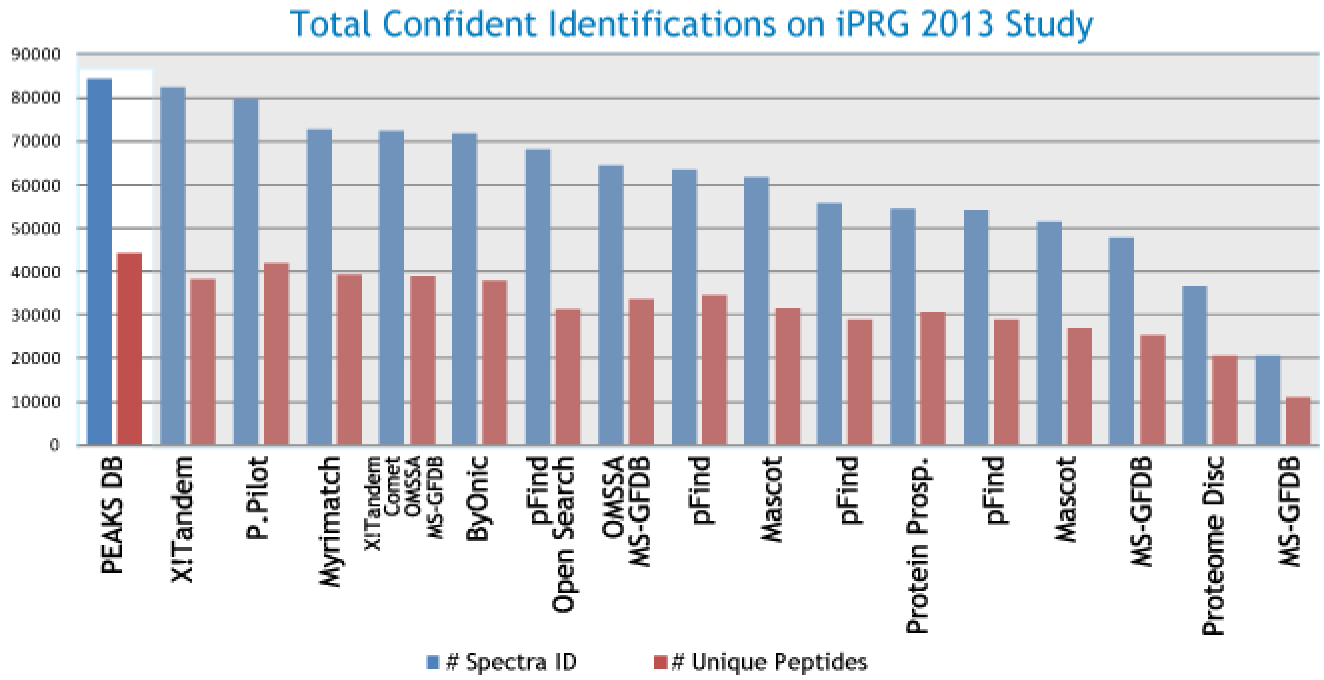
- Uses many more factors than other algorithms
  - particularly the similarity between *de novo* and DB sequence
  - many other scoring features considered
- Better separation of true and false means better accuracy and sensitivity.



Zhang et al, *MCP* (2012) 11, M111.010587.2011.

High sensitivity and accuracy

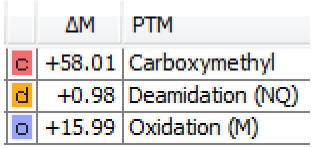
- ABRF iPRG 2013 study



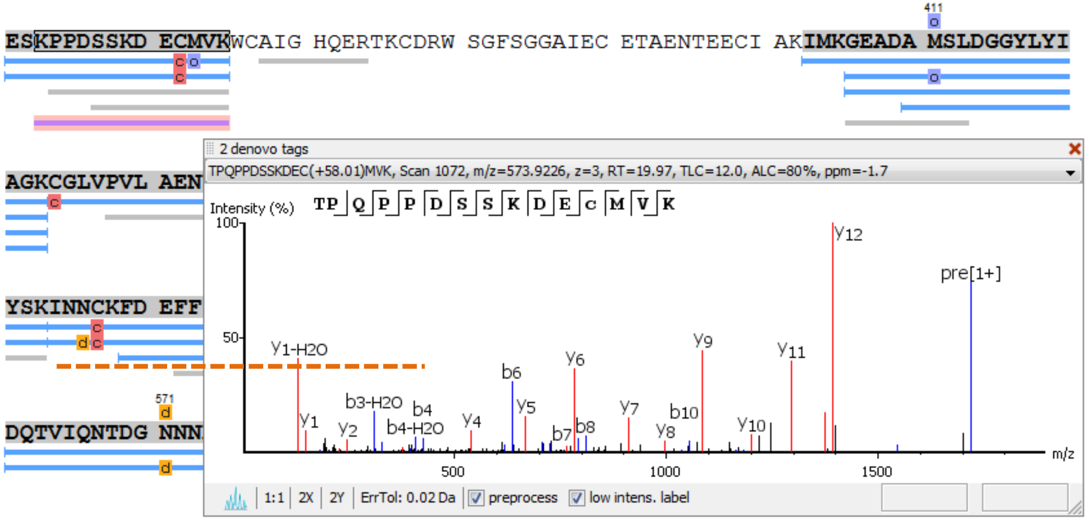
3.2. De novo assisted PTM “Blind Search”

- Search for PTM when there is a tag match.

PEAKS DB



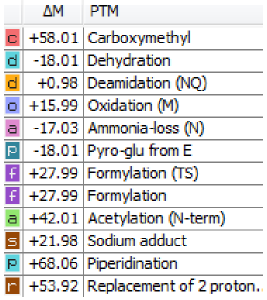
	ΔM	PTM
c	+58.01	Carboxymethyl
d	+0.98	Deamidation (NQ)
o	+15.99	Oxidation (M)



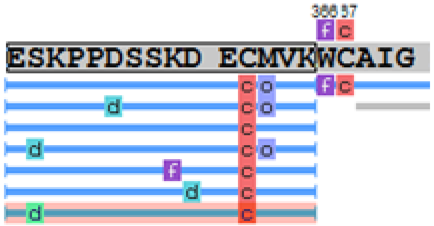
2 denovo tags

TPQPPDSSKDEC(+58.01)MVK, Scan 1072, m/z=573.9226, z=3, RT=19.97, TLC=12.0, ALC=80%, ppm=-1.7

PEAKS PTM



	ΔM	PTM
c	+58.01	Carboxymethyl
d	-18.01	Dehydration
d	+0.98	Deamidation (NQ)
o	+15.99	Oxidation (M)
a	-17.03	Ammonia-loss (N)
p	-18.01	Pyro-glu from E
f	+27.99	Formylation (TS)
f	+27.99	Formylation
a	+42.01	Acetylation (N-term)
s	+21.98	Sodium adduct
p	+68.06	Piperidination
r	+53.92	Replacement of 2 proton..

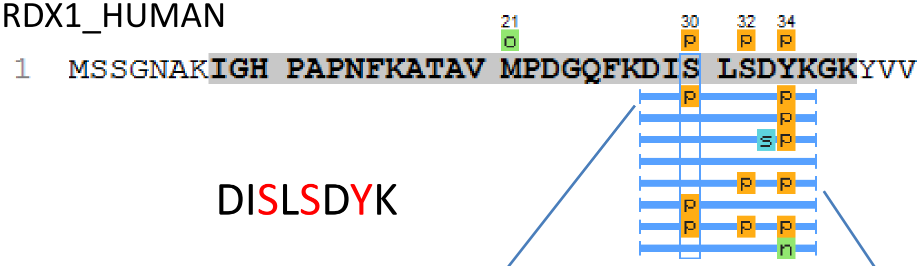


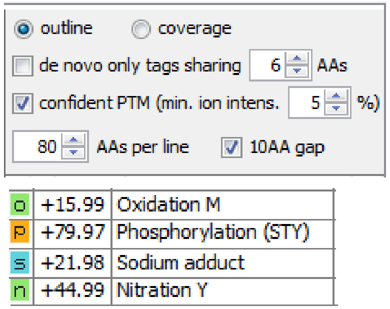
X. Han et al., *JPR* (2011), 10, 2930-2936.

ABRF iPRG 2012 study

One spiked peptide

PRDX1_HUMAN





● outline ○ coverage

☐ de novo only tags sharing 6 AAs

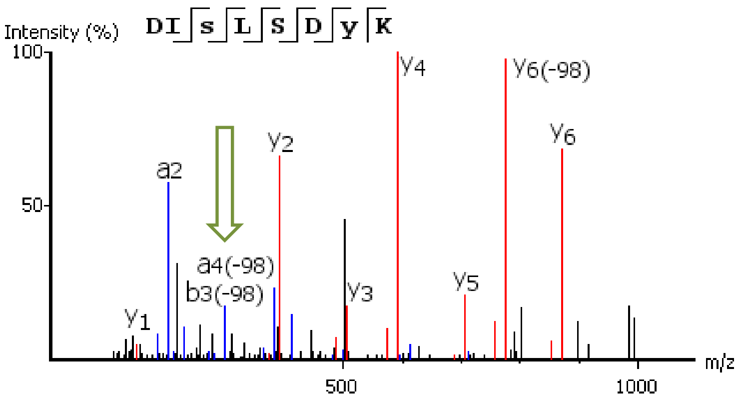
☑ confident PTM (min. ion intens. 5 %)

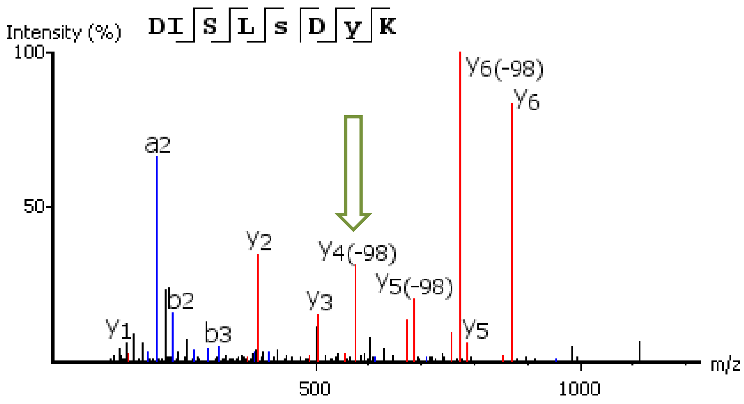
80 AAs per line ☑ 10AA gap

o	+15.99	Oxidation M
p	+79.97	Phosphorylation (STY)
s	+21.98	Sodium adduct
n	+44.99	Nitration Y

DISLSDYK

3,7-phospho *vs* 5,7-phospho

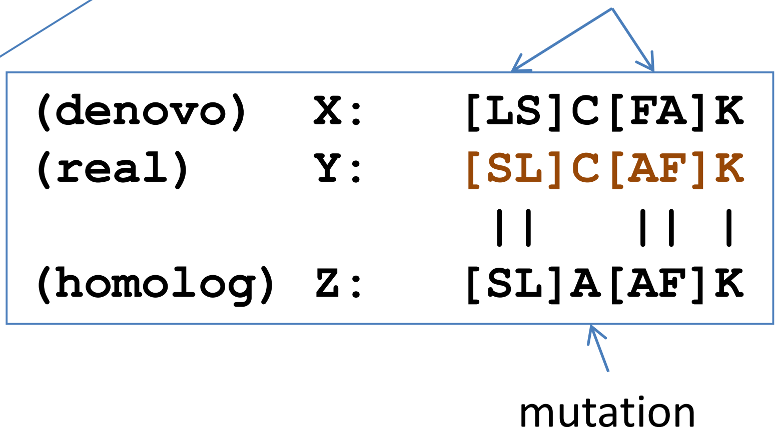




3.3. SPIDER homology search

Problem： de novo errors、database mutations

```
(denovo)   X:    LSCFAK
                      |
(homolog)  Z:    SLAAFK
```

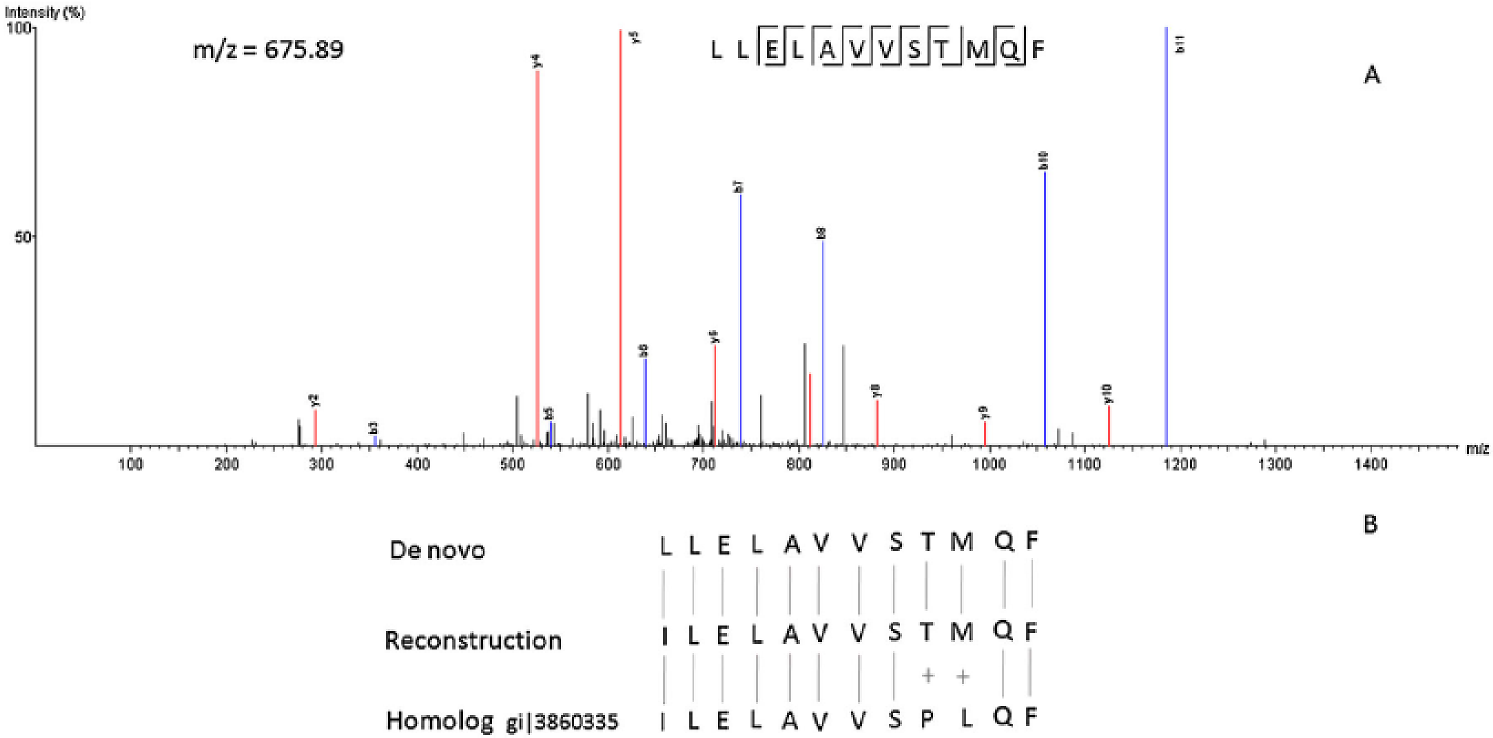


```
(denovo)   X:    [LS]C[FA]K
(real)     Y:    [SL]C[AF]K
                  ||   || |
(homolog)  Z:    [SL]A[AF]K
```

Solution： minimize de novo errors and mutations

Y. Han *et al*, *JBCB* (2005) 3, 697-716.

Peptides in camel milk



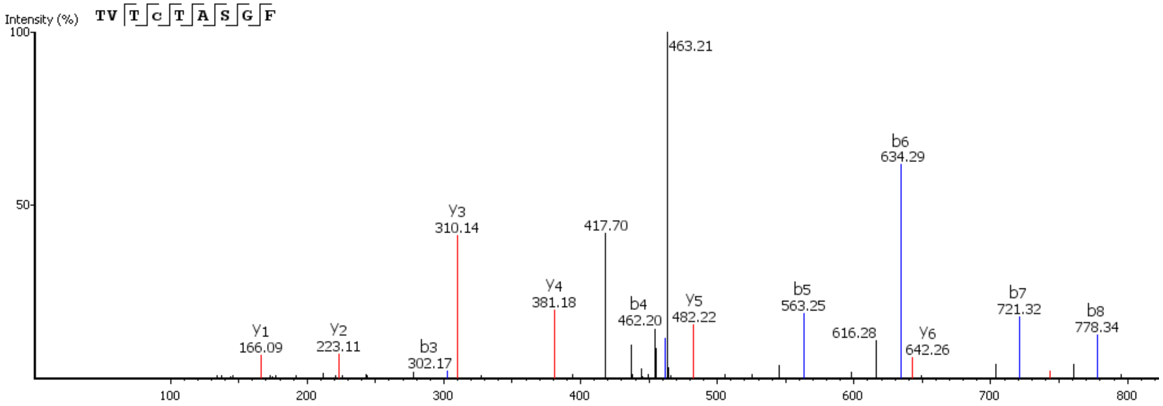
J. Mass Spectrom. **2013**, *48*, 779–794

4. DB search validates *De novo* sequencing

- Problem

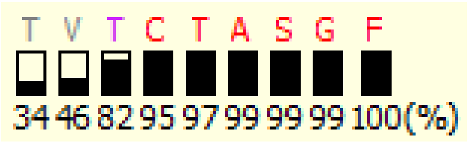
  De novo sequencing

  - Ambiguity of de novo sequence
  - Partially correct tags

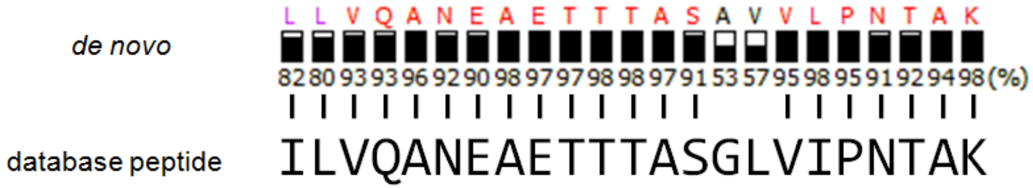


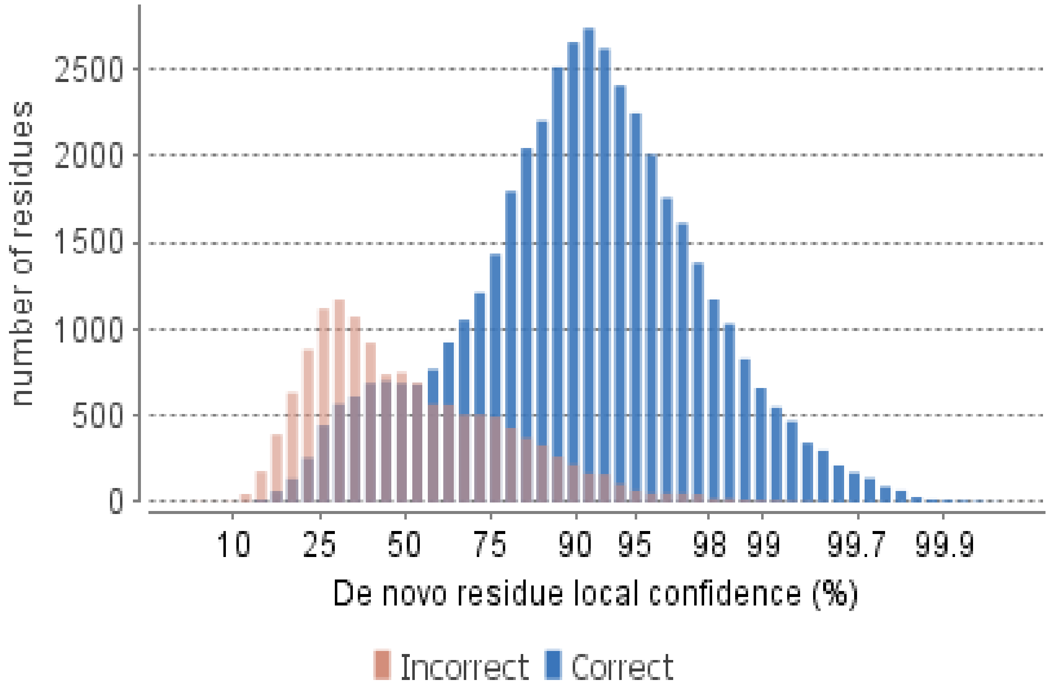
- Solution

  Local confidence score

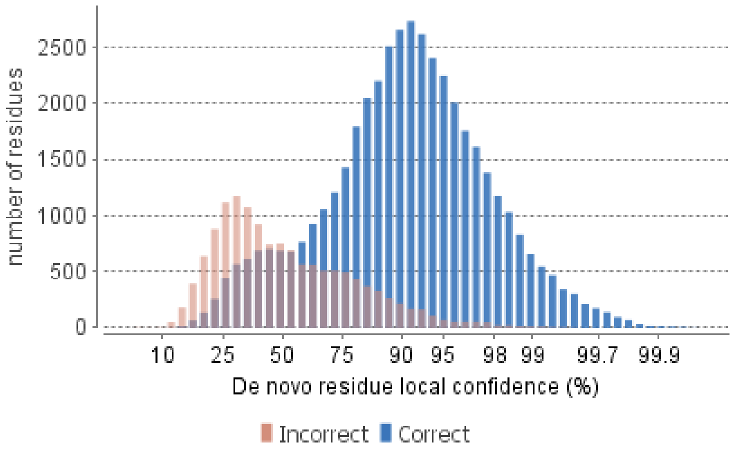

T V T C T A S G F
34 46 82 95 97 99 99 99 100(%)

Validation with DB peptides

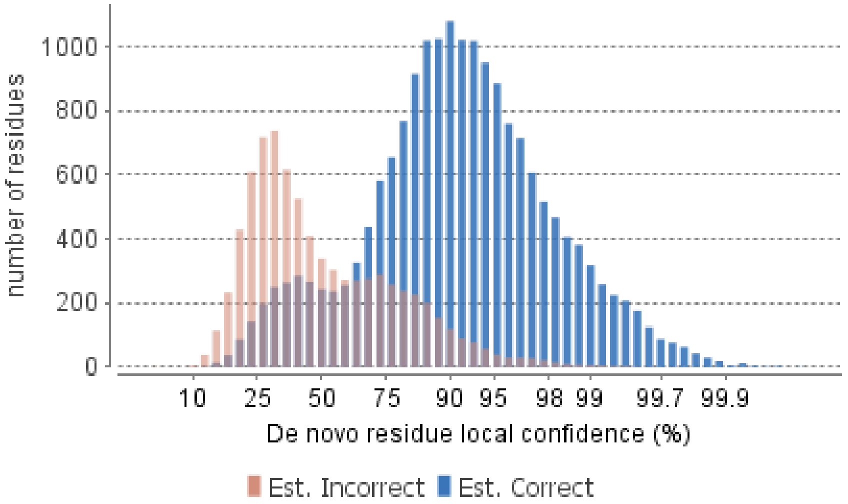




De novo - only peptides



de novo peptides validated by DB



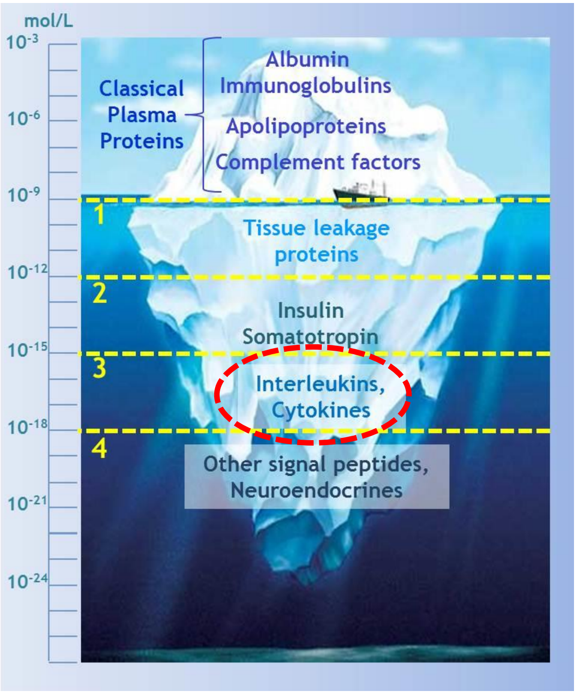
score distribution
of *de novo* “only” peptides
with estimated correctness

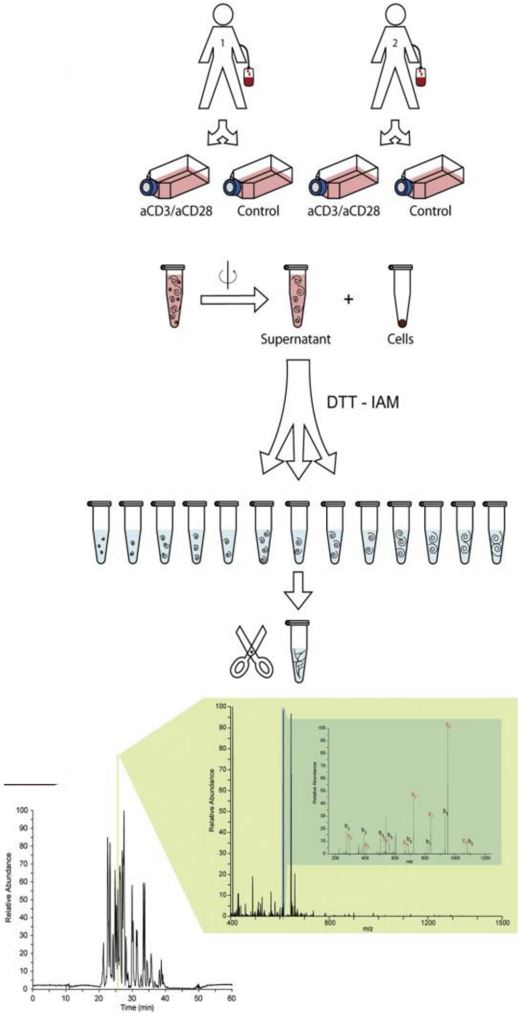
5. Finding endogenous peptides

- Extracellular proteome

  low abundance, esp. signaling peptides

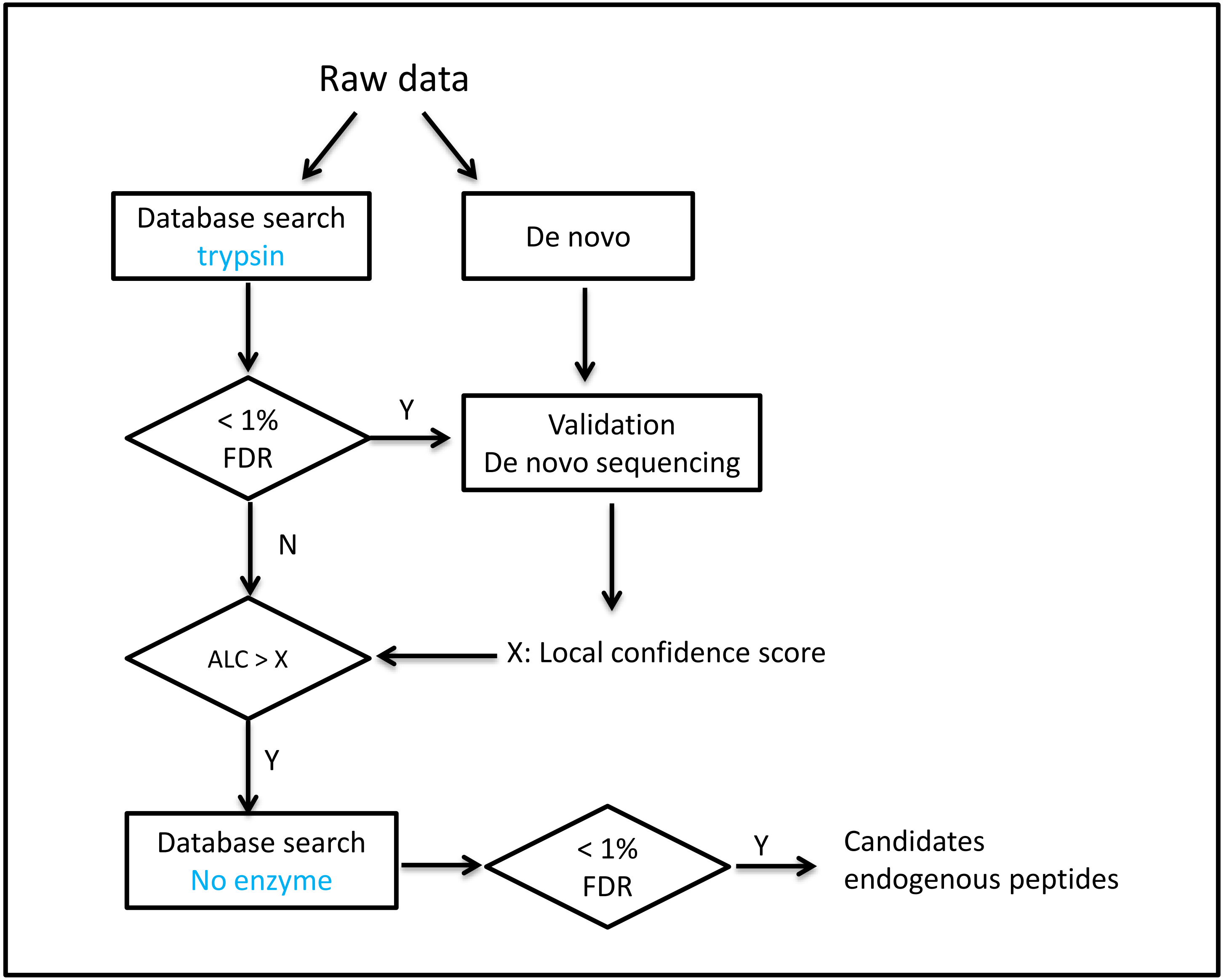
  contaminated by intracellular proteins





Finoulst et al. J. Proteomics (2012)

Workflow for endogenous peptides identification



Raw data

Database search
trypsin

De novo

< 1%
FDR

Y

Validation
De novo sequencing

N

ALC > X

X: Local confidence score

Y

Database search
No enzyme

< 1%
FDR

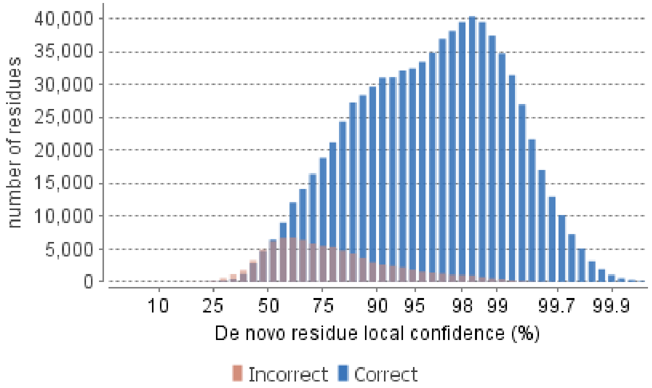
Y

Candidates
endogenous peptides

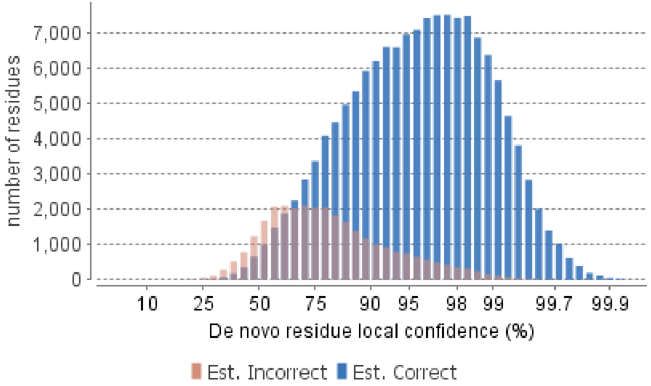
Identification of peptides

	# MS/MS	# peptides
LC-MS/MS	1954303	
Database search	584614	18625
De novo sequencing	15597	987

validated

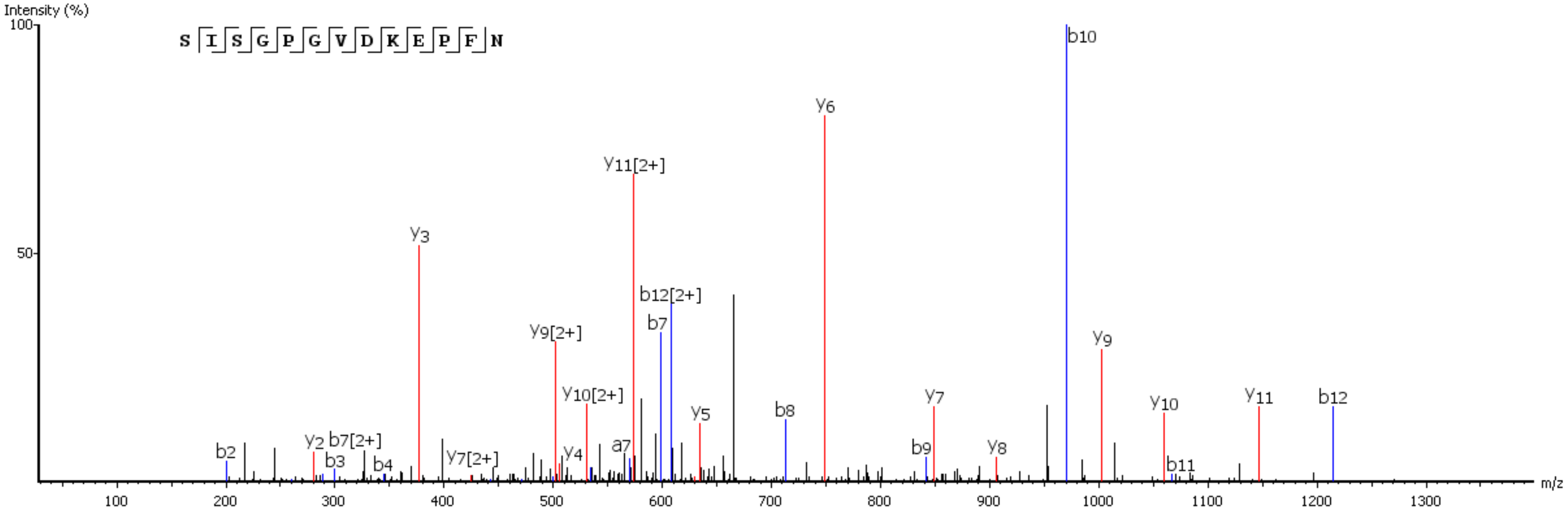


estimated



70 Human non-tryptic peptides

Example of an endogenous peptide

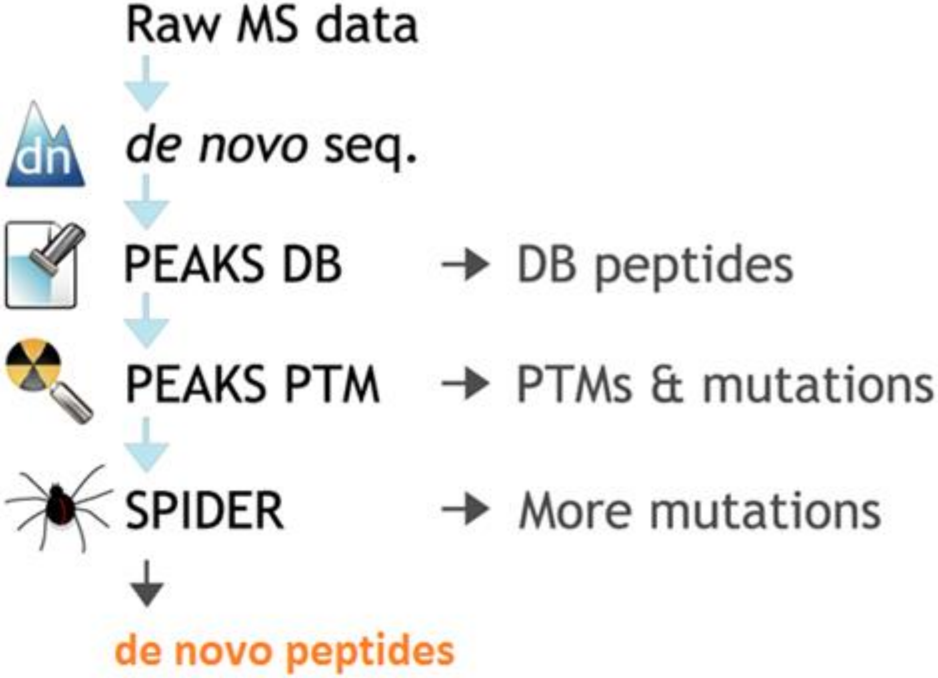


Q08554|DSC1_HUMAN

a member of the desmocollin subfamily

extracellular region

Implement in PEAKS



Raw MS data

↓

de novo seq.

↓

PEAKS DB → DB peptides

↓

PEAKS PTM → PTMs & mutations

↓

SPIDER → More mutations

↓

de novo peptides

Acknowledgement

- PEAKS R&D team at BSI
- Collaborations
  - Prof. Bin Ma at UW
  - Prof. Gilles A. Lajoie at UWO
  - Prof. Kaizhong Zhang at UWO
  - Prof. Peter Verhaert at Delft University of Technology
  - Cuijie Zhang at Samuel Lunenfeld Research Institute