

THE WAY TO DEEP COVER OF HUMAN SINGLE CHROMOSOME PROTEOME IN GENE CENTRIC MODE.

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**19th CHROMOSOME- CENTRIC HPP SYMPOSIUM,
2018, 06, 16-17,SANTIAGO DE COMPOSTELA.**

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15:00

DOUBLE TARGETED STRATEGY



- **SELECTION OF SINGLE CHROMOSOME AS THE FIRST TARGET FOR PROTEOME ANALYSIS.**
- **SELECTION REACTION MONITORING MS/MS (SRM SIS) FOR TARGETED CREATION OF SELECTED CHROMOSOME PROTEOME**


PROTEIN VARIANCES FOR HUMAN EXOME (NEXTPROTE, 2015 JAN)

[Ponomarenko E. et al., OMICS JIB, 2012, 16(11), 604-11]

| # Chr. | Genes | Protein variance | | | # Chr. | Genes | Protein variance | | |
|--------|-------|------------------|-------|------|--------|-------|------------------|-------|------|
| | | AS | SAP | PTM | | | AS | SAP | PTM |
| 1 | 2056 | 2,19 | 42,48 | 7,20 | 13 | 327 | 1,99 | 43,58 | 7,07 |
| 2 | 1231 | 2,19 | 43,56 | 6,93 | 14 | 623 | 2,11 | 42,87 | 6,47 |
| 3 | 1070 | 2,21 | 44,83 | 6,63 | 15 | 602 | 1,94 | 42,43 | 6,94 |
| 4 | 761 | 2,09 | 41,81 | 6,99 | 16 | 837 | 1,90 | 40,90 | 5,81 |
| 5 | 868 | 2,00 | 42,59 | 7,08 | 17 | 1166 | 2,02 | 40,61 | 6,38 |
| 6 | 1110 | 2,10 | 41,44 | 7,24 | 18 | 276 | 1,99 | 44,26 | 5,82 |
| 7 | 934 | 2,11 | 42,16 | 6,56 | 19 | 1428 | 1,83 | 43,73 | 6,56 |
| 8 | 701 | 2,03 | 41,31 | 6,39 | 20 | 550 | 2,00 | 39,19 | 6,03 |
| 9 | 810 | 2,17 | 40,96 | 6,90 | 21 | 250 | 2,57 | 39,12 | 8,28 |
| 10 | 761 | 2,30 | 43,04 | 6,88 | 22 | 460 | 1,89 | 42,76 | 6,57 |
| 11 | 1322 | 2,09 | 44,70 | 6,25 | X | 824 | 2,03 | 42,72 | 6,52 |
| 12 | 1029 | 2,19 | 42,70 | 6,96 | Y | 47 | 1,68 | 17,33 | 3,70 |

 Within the confidence interval

 Exception

 Below the confidence interval
($p < = 0,05$)

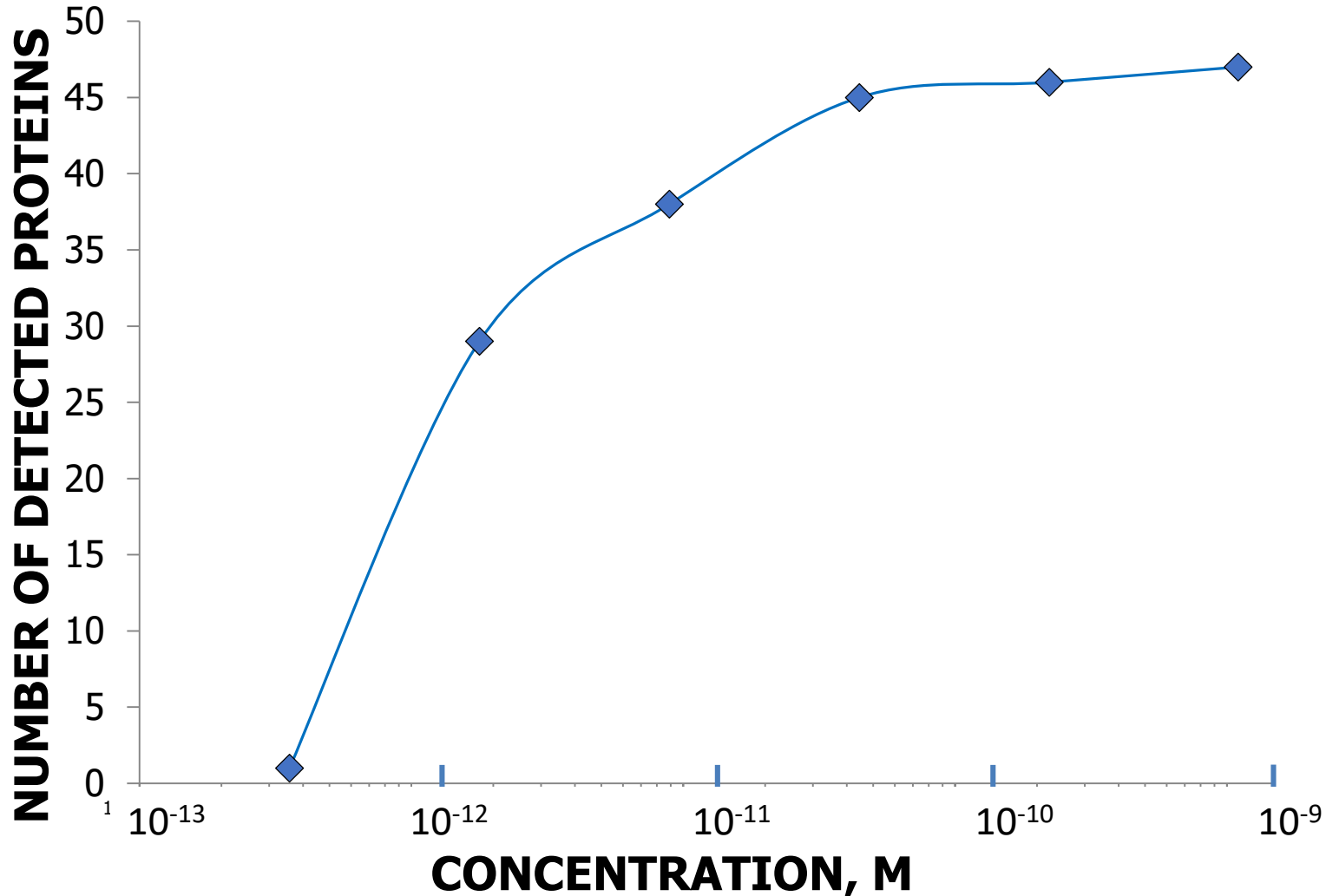
 Above the upper limit of the
confidence interval

- NOT DETECTED/"MISSING"/ UPS2 PROTEINS(11) IN E. COLI EXTRACT

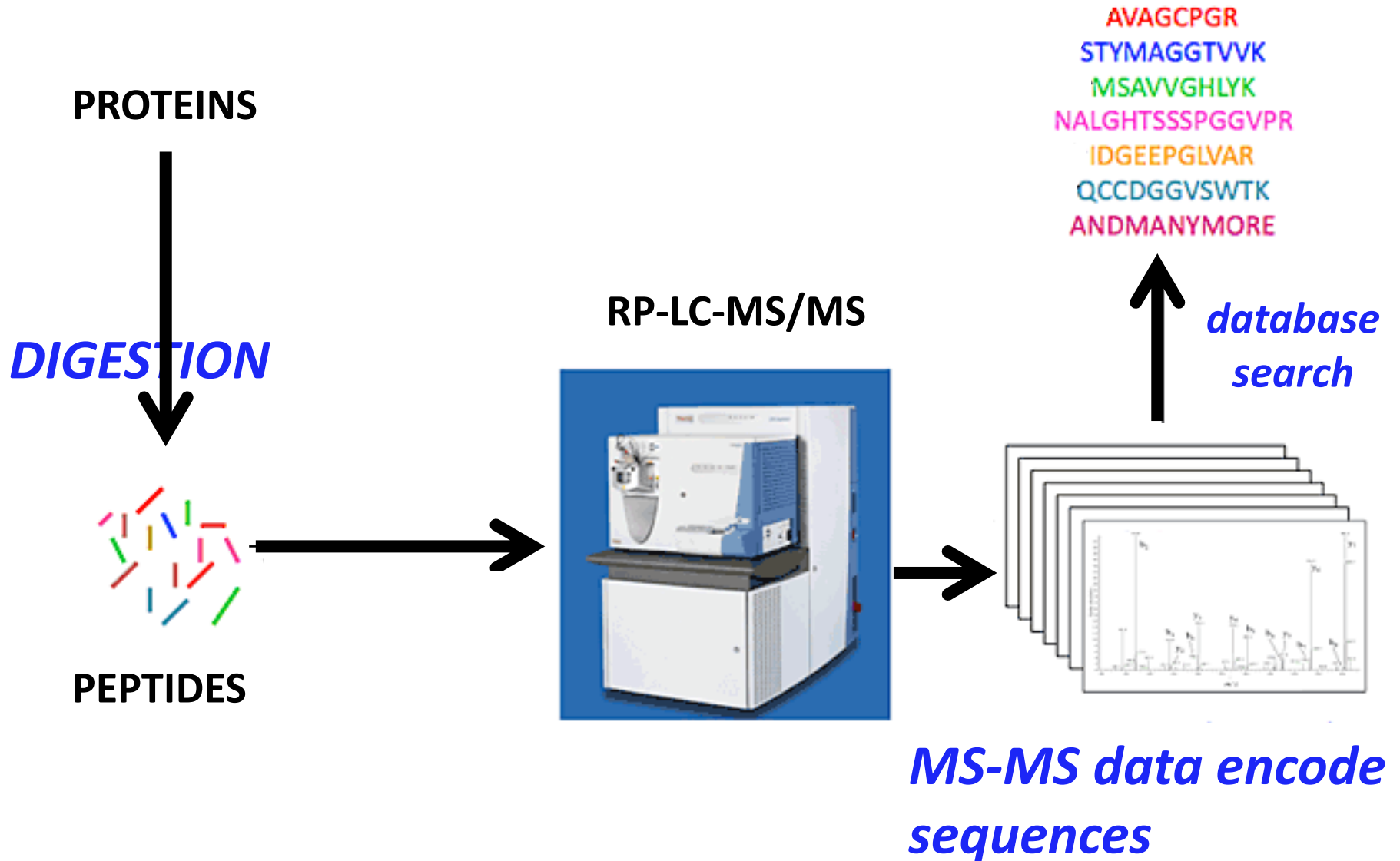
- DETECTED UPS2 PROTEINS (N=37) IN E.COLI EXTRACT

| No | UniProt, AC | Conc., M | No | UniProt AC | Conc., M | No | UniProt AC | Conc., M |
|----|-------------|----------|----|------------|----------|----------------------|------------|----------|
| 1 | P00915 | 1.00E-06 | 18 | P06732 | 1.00E-08 | 35 | P09211 | 1.00E-10 |
| 2 | P00918 | 1.00E-06 | 19 | P12081 | 1.00E-08 | 36 | P01112 | 1.00E-10 |
| 3 | P01031 | 1.00E-06 | 20 | P61626 | 1.00E-08 | 37 | P01579 | 1.00E-10 |
| 4 | P69905 | 1.00E-06 | 21 | Q15843 | 1.00E-08 | 38 | P02787 | 1.00E-10 |
| 5 | P68871 | 1.00E-06 | 22 | P02753 | 1.00E-08 | 39 | O00762 | 1.00E-10 |
| 6 | P41159 | 1.00E-06 | 23 | P16083 | 1.00E-08 | 40 | P51965 | 1.00E-10 |
| 7 | P02768 | 1.00E-06 | 24 | P63279 | 1.00E-08 | 41 | P08758 | 1.00E-11 |
| 8 | P62988 | 1.00E-06 | 25 | P01008 | 1.00E-09 | 42 | P02741 | 1.00E-11 |
| 9 | P04040 | 1.00E-07 | 26 | P61769 | 1.00E-09 | 43 | P05413 | 1.00E-11 |
| 10 | P00167 | 1.00E-07 | 27 | P55957 | 1.00E-09 | 44 | P10145 | 1.00E-11 |
| 11 | P01133 | 1.00E-07 | 28 | O76070 | 1.00E-09 | 45 | P02788 | 1.00E-11 |
| 12 | P02144 | 1.00E-07 | 29 | P08263 | 1.00E-09 | 46 | P10636 | 1.00E-11 |
| 13 | P15559 | 1.00E-07 | 30 | P01344 | 1.00E-09 | 47 | P00441 | 1.00E-11 |
| 14 | P62937 | 1.00E-07 | 31 | P01127 | 1.00E-09 | 48 | P01375 | 1.00E-11 |
| 15 | Q06830 | 1.00E-07 | 32 | P10599 | 1.00E-09 | HIGH COPIED | | |
| 16 | P63165 | 1.00E-07 | 33 | P99999 | 1.00E-10 | MEDIUM COPIED | | |
| 17 | P00709 | 1.00E-08 | 34 | P06396 | 1.00E-10 | LOW COPIED | | |

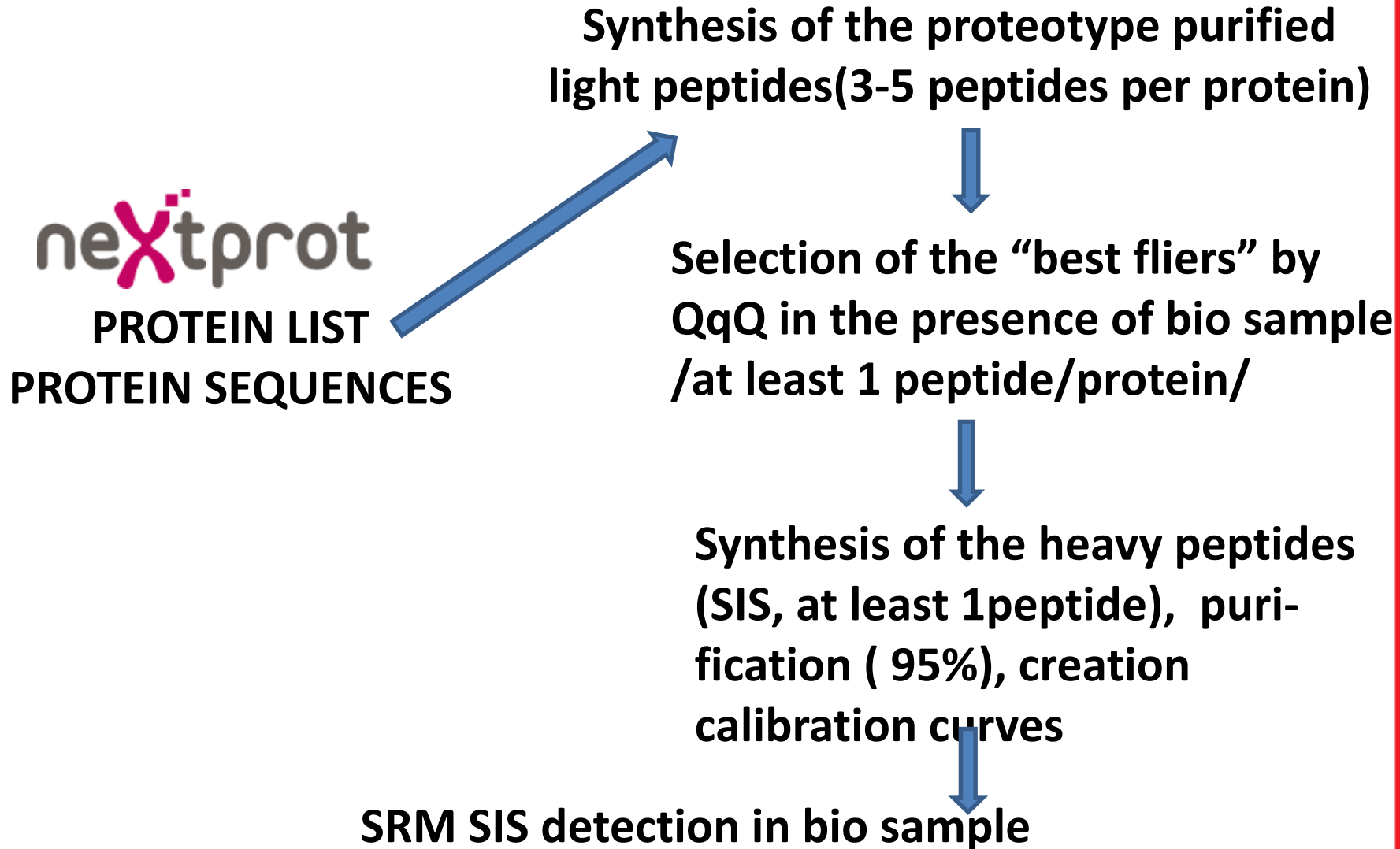
THE DEPENDENCE OF MISSING PROTEIN NUMBER ON CONCENTRATION



WORKFLOW OF SHOTGUN MS/MS TECHNOLOGY

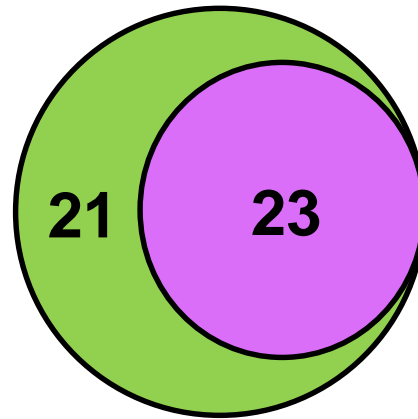


WORKFLOW OF SRM SIS



VENN DIAGRAMME OF SHORT GUN AND SRM SIS RESULTS OF PURE UPS 2

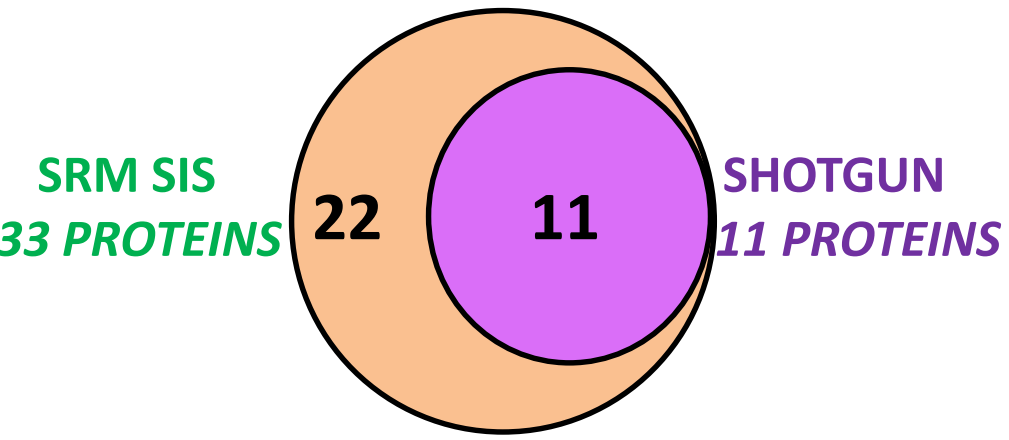
SRM SIS
44 proteins



Shotgun
23 proteins

TOTALLY 44 PROTEINS

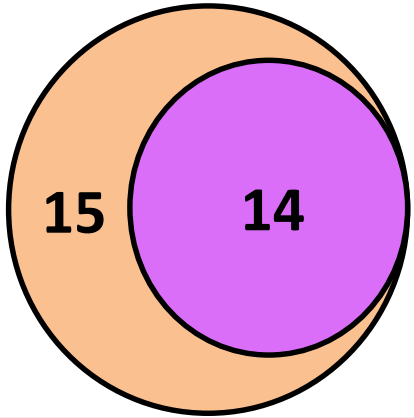
VENN DIAGRAMM OF SHORT GUN AND SRM SIS INVESTIGATION OF UPS2 WITH 1D FRACTIONATION



TOTALLY 23 PROTEINS DETECTED IN E.COLI

TOTALLY 23 PROTEINS DETECTED IN HBP

SRM SIS 29 PROTEINS



SHOTGUN 14 PROTEINS

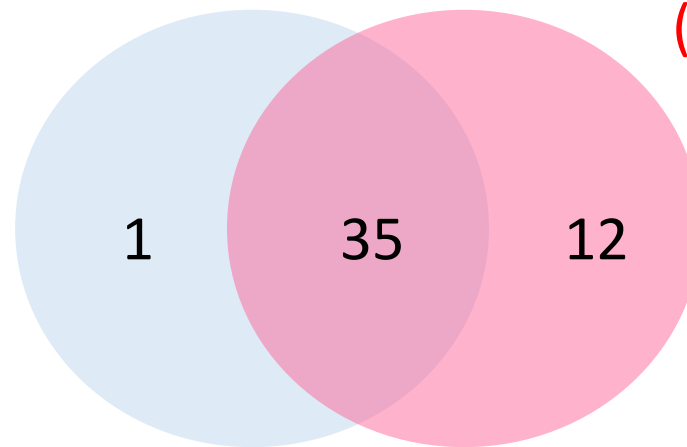
SUMMARISING TABLE

| Sample | Method | Number of detected proteins | Sensitivity, Sn |
|----------------------------|------------------------------|-----------------------------|-----------------|
| UPS2-set in purified state | SRM SIS | 44 | 92% |
| | Shotgun (database search) | 23 | 48% |
| UPS2-set + E.Coli | SRM SIS | 33 | 69% |
| | Shotgun (database search) | 11 | 23% |
| UPS2-set + HBP | SRM SIS | 29 | 63% |
| | Shotgun (database search) | 14 | 48% |

VENN DIAGRAMM OF 2D alkaline LC FRACTIONATION WITH SHORT GUN AND SRM SIS TECHNOLOGIES OF PURE UPS2

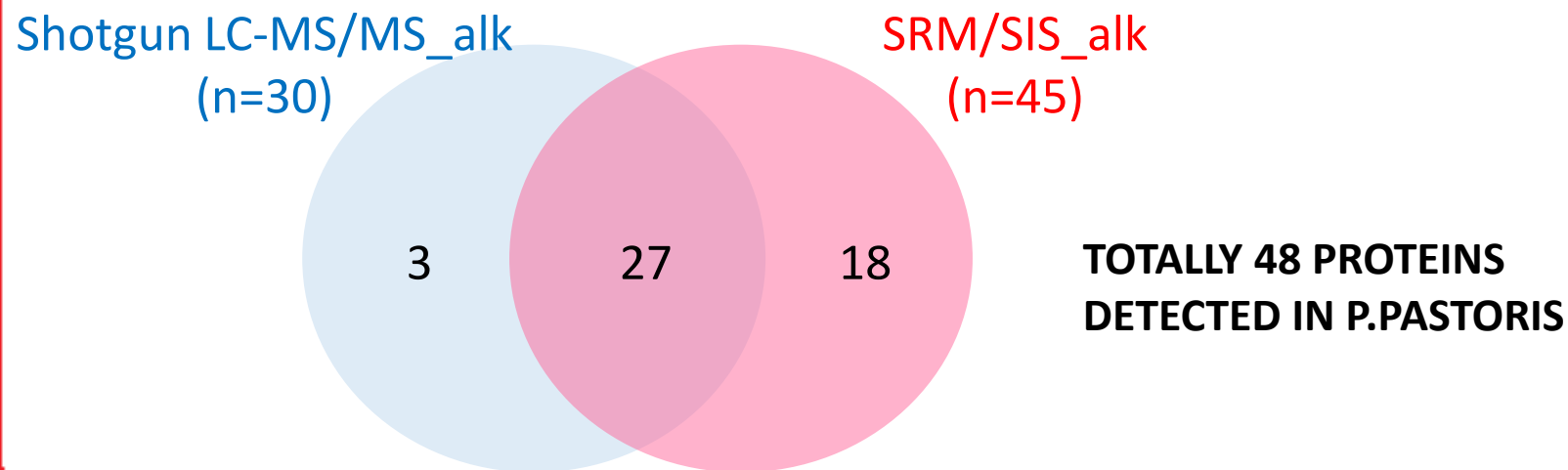
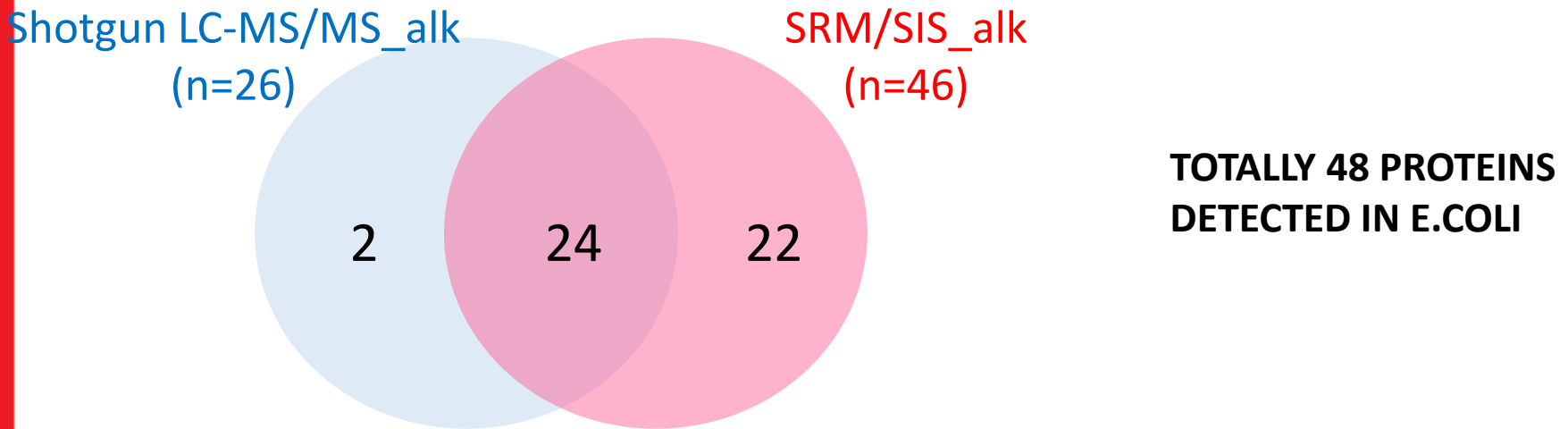
Shotgun LC-MS/MS_alk
(n=36)

SRM/SIS_alk
(n=47)



TOTAL 48

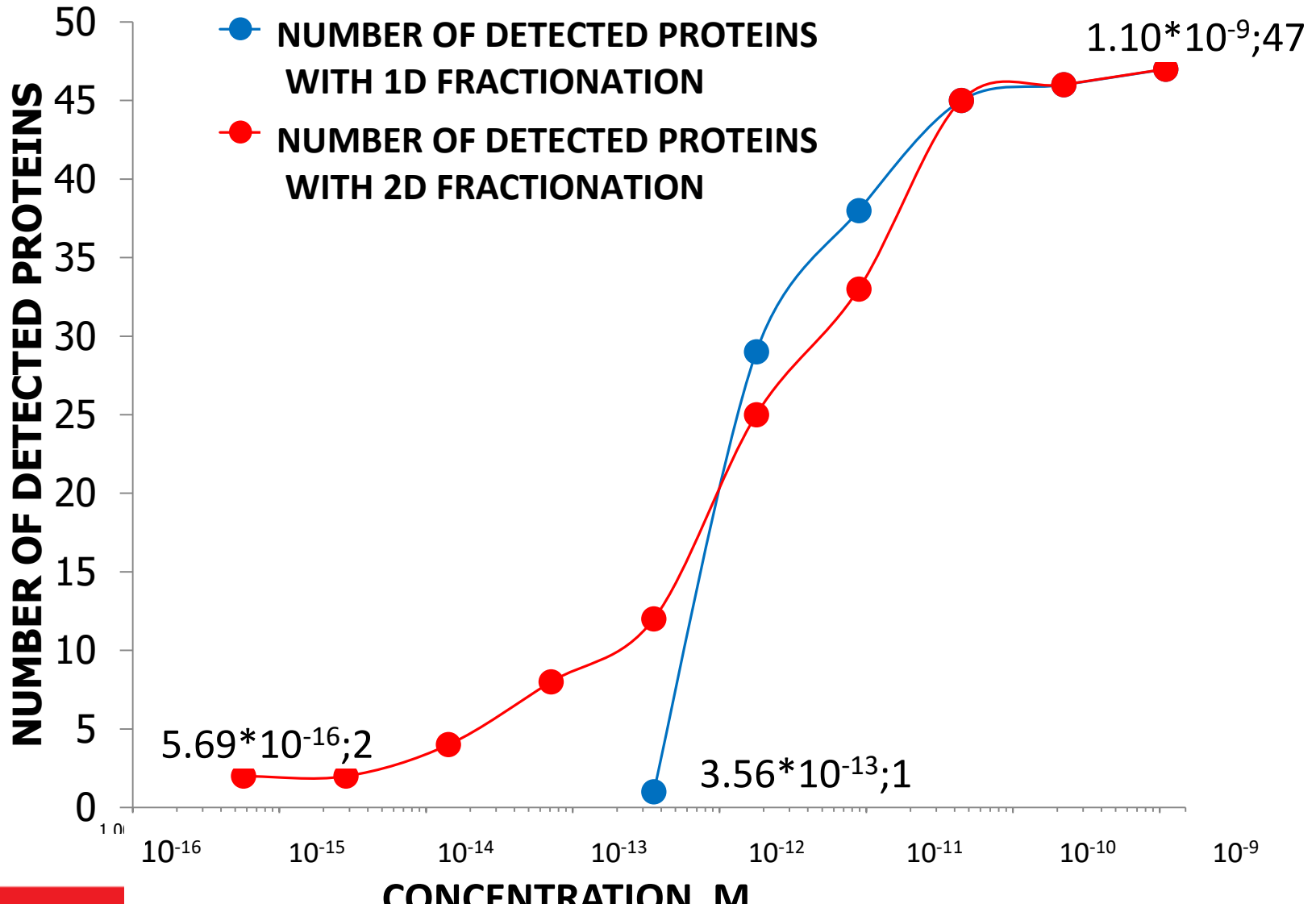
VENN DIAGRAMM OF SHORT GUN AND SRM SIS INVESTIGATION OF UPS2 IN BIOLOGICAL MATRICES.



WAY SHORT GUN MS/MS NECESSARY FOR SRM SIS ?

- Additional proteins can be identified (qualitative analysis)
- Better choice of proteotypic peptides selection/better protein coverage/

DEPENDENCE OF DETECTED NUMBER PROTEINS ON CONCENTRATION.



THE SENSITIVITY OF SHORT GUN AND SRM SIS WITH 2D ALKENE FRACTIONATION TECHNOLOGIES .

| Biological matrix | Technology | The number of proteins detected /undetected | Sensitivity (Sn), % |
|-------------------|----------------------------|--|---------------------|
| E. coli | shotgun LC-MS/MS | 26 /22 | 54.1 |
| | SRM SIS | 46 /2 | 95.8 |
| | shotgun LC-MS/MS + SRM SIS | 48 /0 | 100 |
| P. pastoris | shotgun LC-MS/MS | 30 /18 | 62.5 |
| | SRM SIS | 45 /3 | 93.7 |
| | shotgun LC-MS/MS + SRM SIS | 48 /0 | 100 |

FINAL WORKFLOW

PROTEINS

DIGESTION



ADDITION OF PROTEOTYPING SIS PEPTIDES

ALKALINE FRACTIONATION

26 FRACTIONS

RP-LC-SRM SIS

RP-LC-MS/MS



List of detected proteins



List of identified proteins

TOTAL NUMBER OF REVEALED PROTEINS

ACKNOWLEDGEMENTS

Institute of Biomedical Chemistry

- *V.Zgoda*
- *A.Kopylov*
- *T. Farafonova*
- *S. Novikova*
- *A.Lisitsa*
- *E.Ponomarenko*
- *K.Poverennaya*
- *E. Ilgisonis*