Proteomics Standards Research Group

Association of Biomolecular Resource Facilities Proteomics Standards Research Group (sPRG)

www.abrf.org/sprg

Phosphorylation of Proteins

- Important post-translational modification
- Major role in regulation of cellular processes.
- Analysis of phosphorylation sites is a major challenge for proteomics labs.
- Many techniques and approaches available.
- What works best?

PRG 2003 Study

- J Biomolecular Tech 14, 205-215, 2003
- 2 digested proteins (5 pmole and 0.2 fmole)
- 2 synthetic phospho-peptides (1 pmole each)
- Results:
  - 54 Data sets returned and 67 Analyses;
  - 8 Labs Identified 1 Pi-peptide;
  - 8 Labs Identified the other Pi-peptide;
  - IMAC users did not fare better.

sPRG 2007 Study

- Mixture of Proteins
  - Catalase - 10 pmol
  - Troponin T - 25 pmol
  - Osteopontin - 50 pmol
  - Ovalbumin - 50 pmol
  - Cystatin - 100 pmol
  - a-S1-casein - 250 pmol
  - a-S2-casein - 250 pmol
- Which phosphorylated residues were found?
- Which techniques were most successful?

sPRG 2007 Study

- Some known sites were detected by >50% of labs.
- Other known sites were identified by few or no labs.
- Enrichment methods did not make a big difference.
- Lack of statistical/spectral methods for identifying Pi-peptides.
- Examination of spectra underlying reported identifications indicated that identifications were not always made based on clear-cut spectral evidence.
- This study demonstrates the difficulty confronting the creation of a standard mix of phosphoproteins.

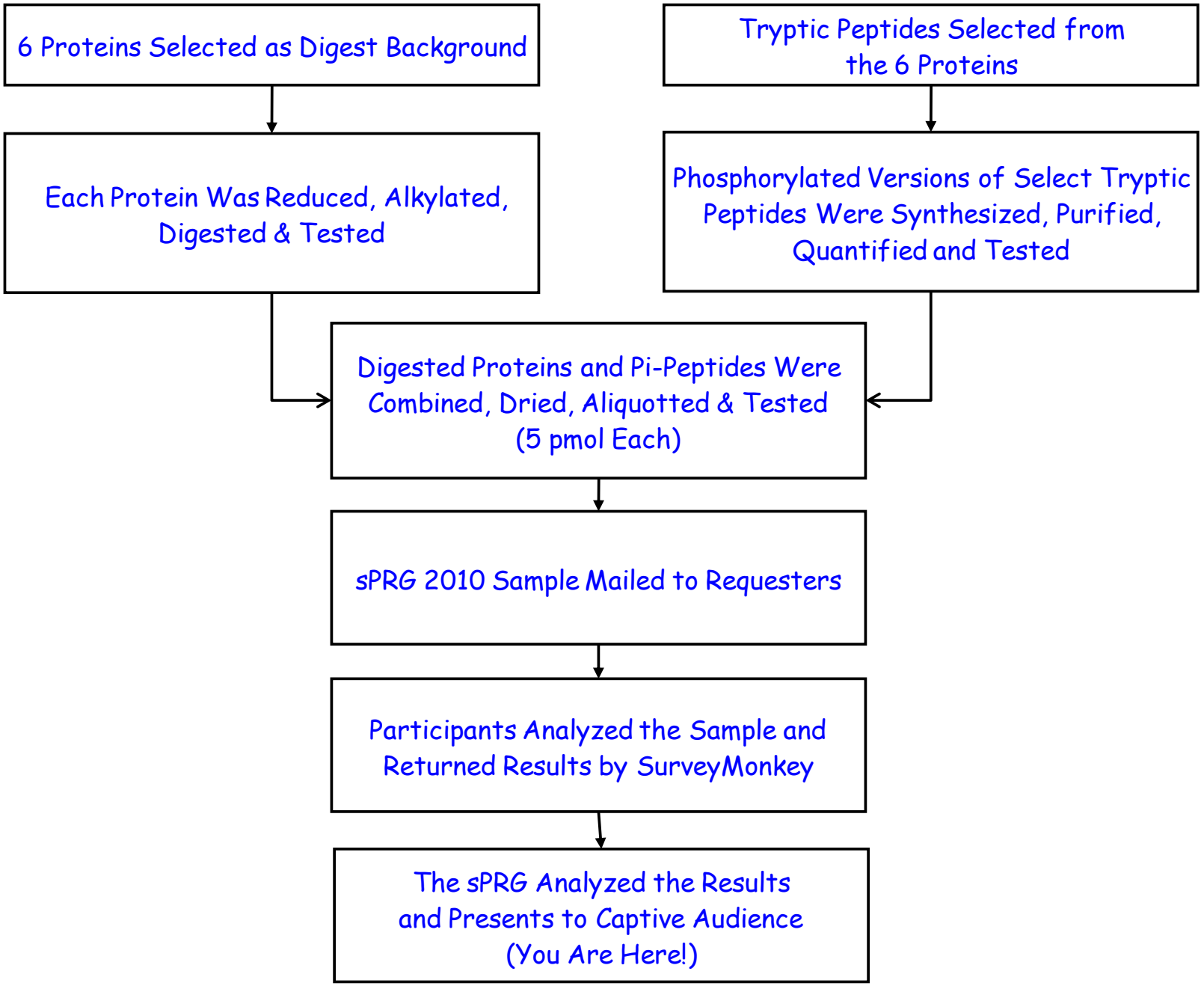
sPRG 2010 Study Goal

- Goal: A readily available phospho-peptide standard useful for:
  - Learning Pi-peptide detection before running that once in a lifetime research sample
  - Identifying Phosphorylation Sites
  - Evaluating Current and New techniques
  - Developing Your Own Techniques
  - Benchmarking
- Goal: What are the best practices being used?

sPRG 2010 Study Design

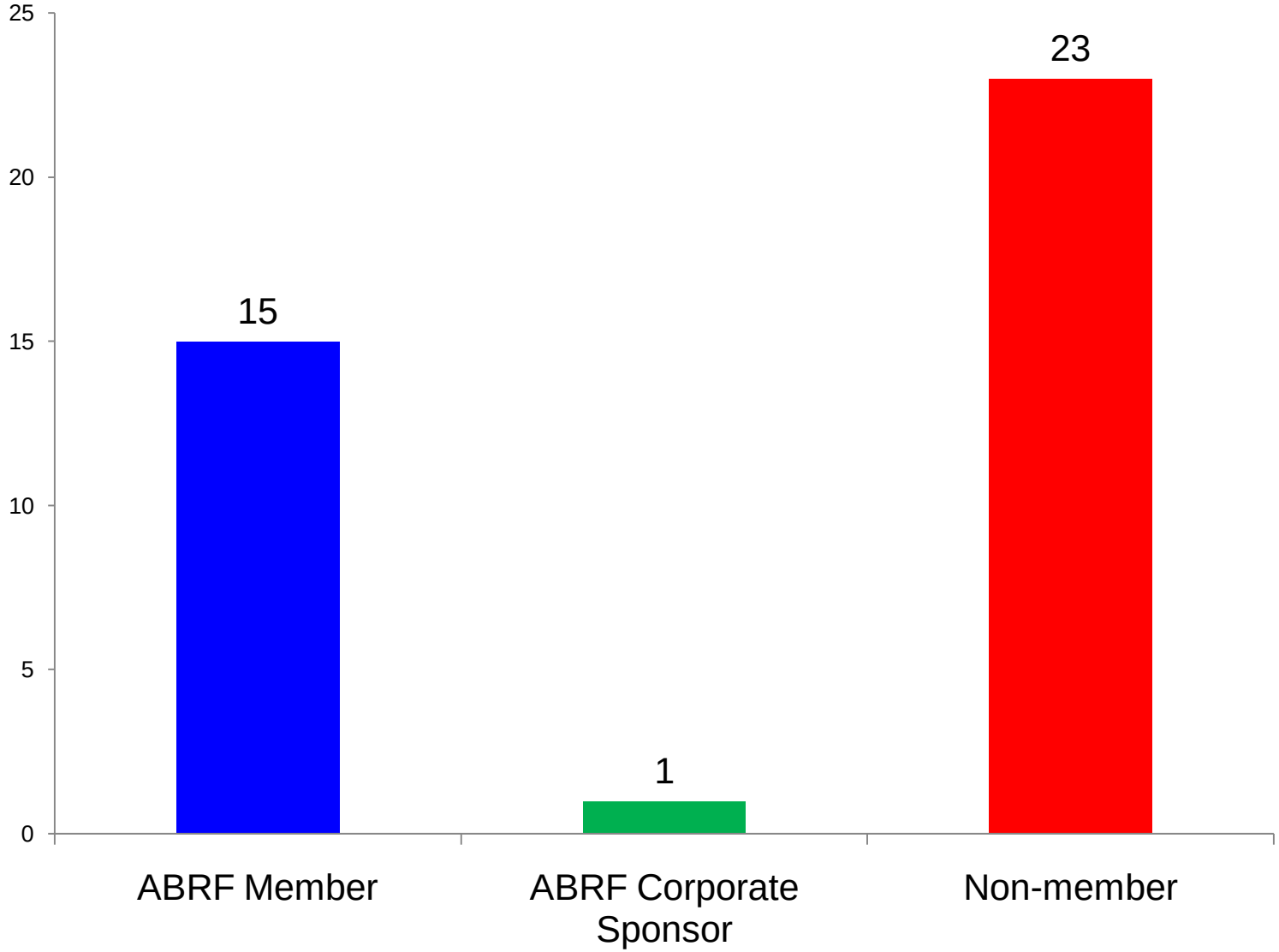
- 23 synthetic Pi-peptides
- Each present at 5 pmole
- Based on sPRG 2009 Study
- Included 2 peptides from PRG 2003
- Phosphorylations sites:
  - Ser, Thr and Tyr
  - Single Pi: 14
  - Double Pi: 5
  - Triple Pi: 3
  - Quadruple Pi: 1
  - Positional isomers.

- Digest of six proteins
- Each present at 5 pmole
- Recombinant human or bovine
- HPLC purified
- Each protein was digested separately with Trypsin
- Naturally occurring phosphopeptides present in the background digests

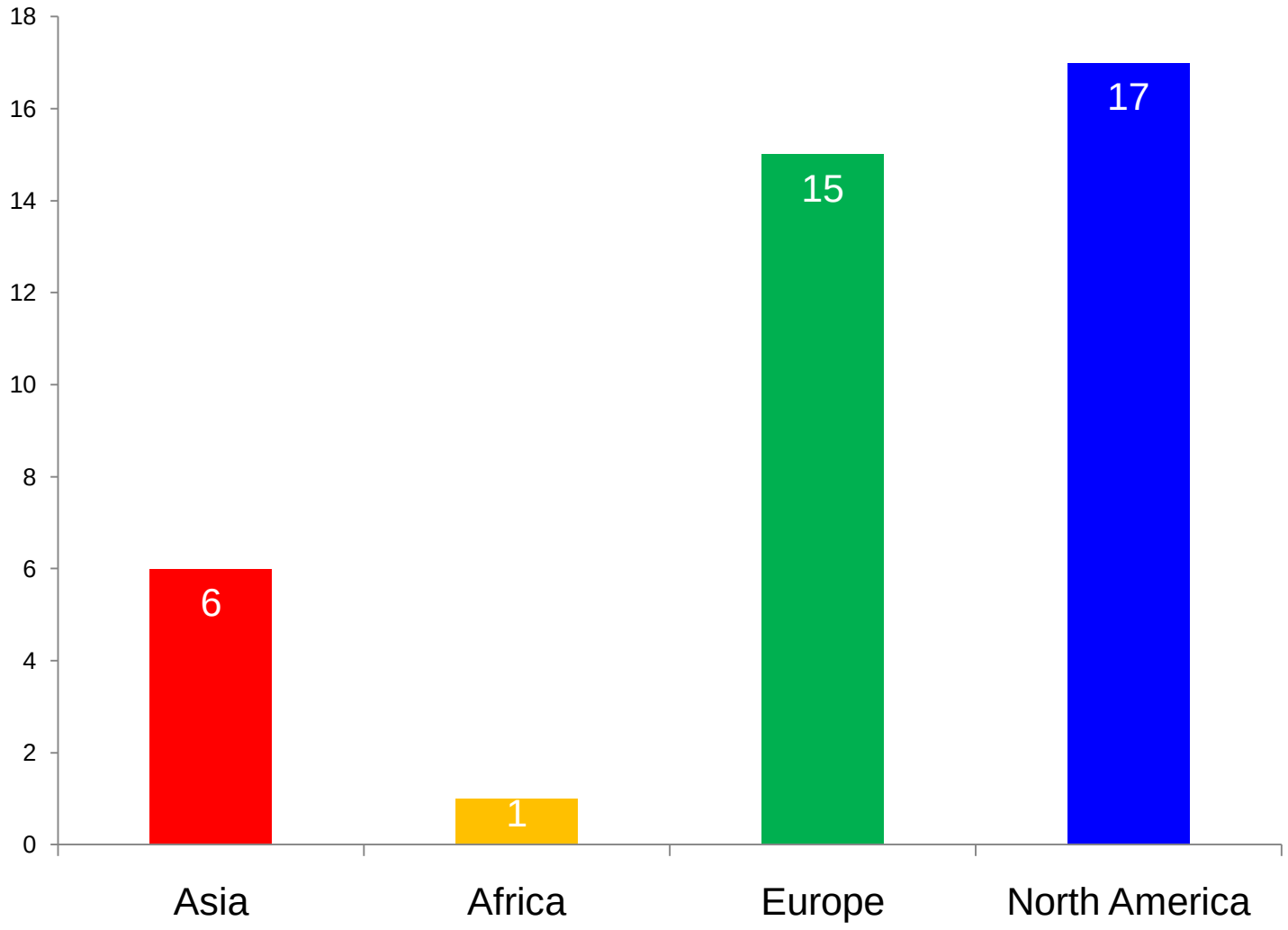

6 Proteins Selected as Digest Background
Tryptic Peptides Selected from the 6 Proteins
Each Protein Was Reduced, Alkylated, Digested & Tested
Phosphorylated Versions of Select Tryptic Peptides Were Synthesized, Purified, Quantified and Tested
Digested Proteins and Pi-Peptides Were Combined, Dried, Aliquotted & Tested (5 pmol Each)
sPRG 2010 Sample Mailed to Requesters
Participants Analyzed the Sample and Returned Results by SurveyMonkey
The sPRG Analyzed the Results and Presents to Captive Audience (You Are Here!)

Who Volunteered?

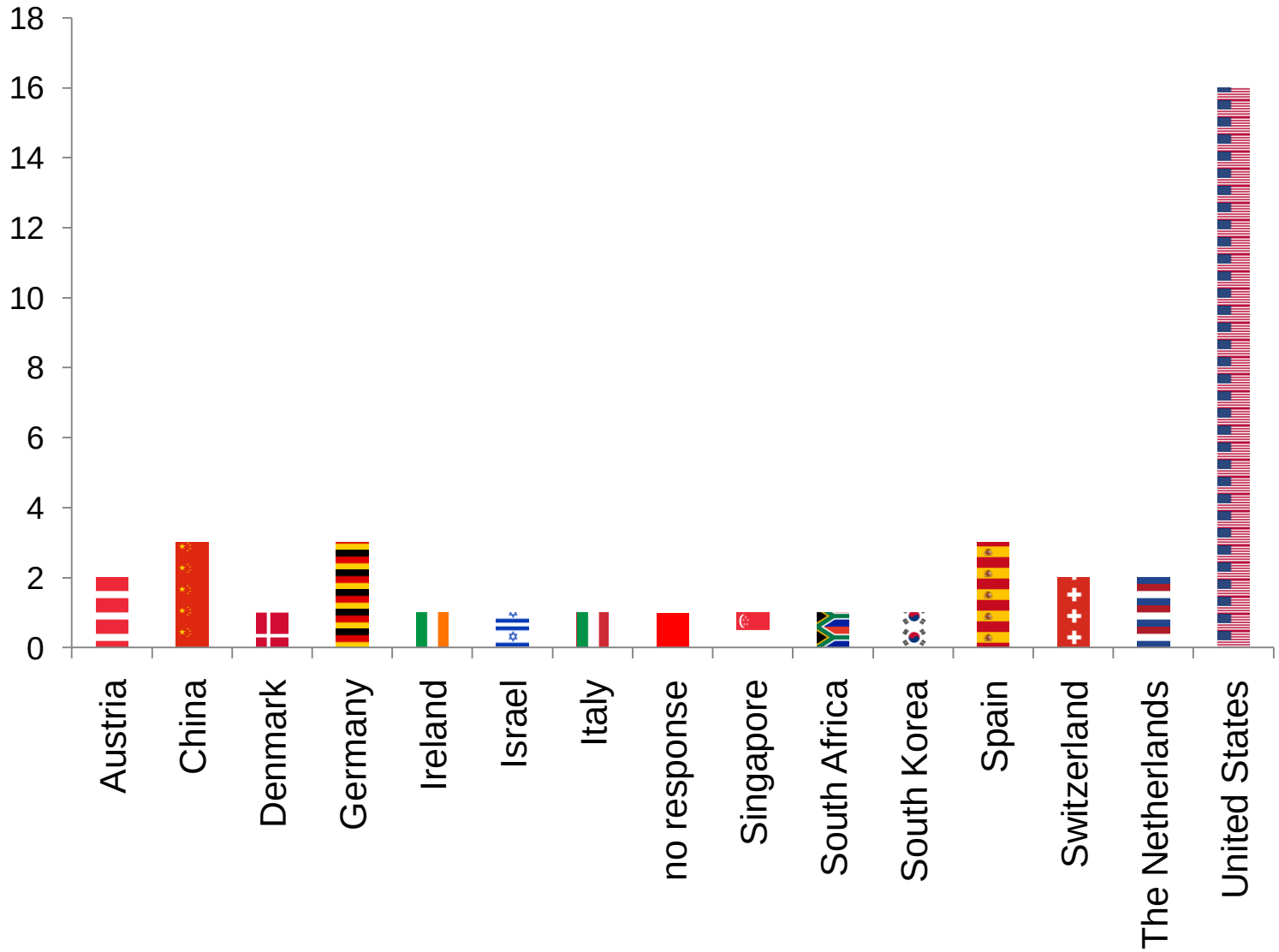
Please indicate your ABRF membership status.



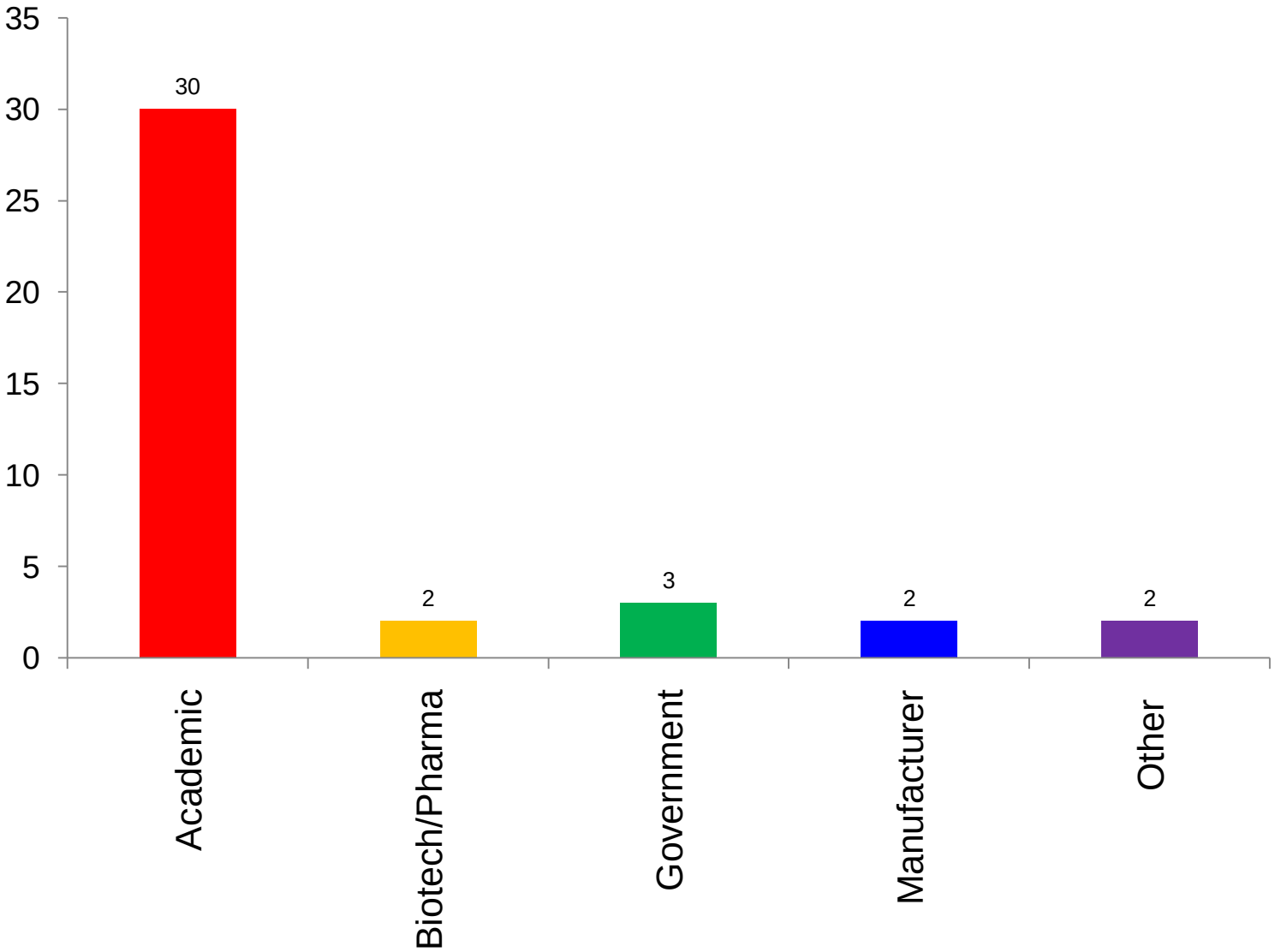
Which best describes your geographic location?



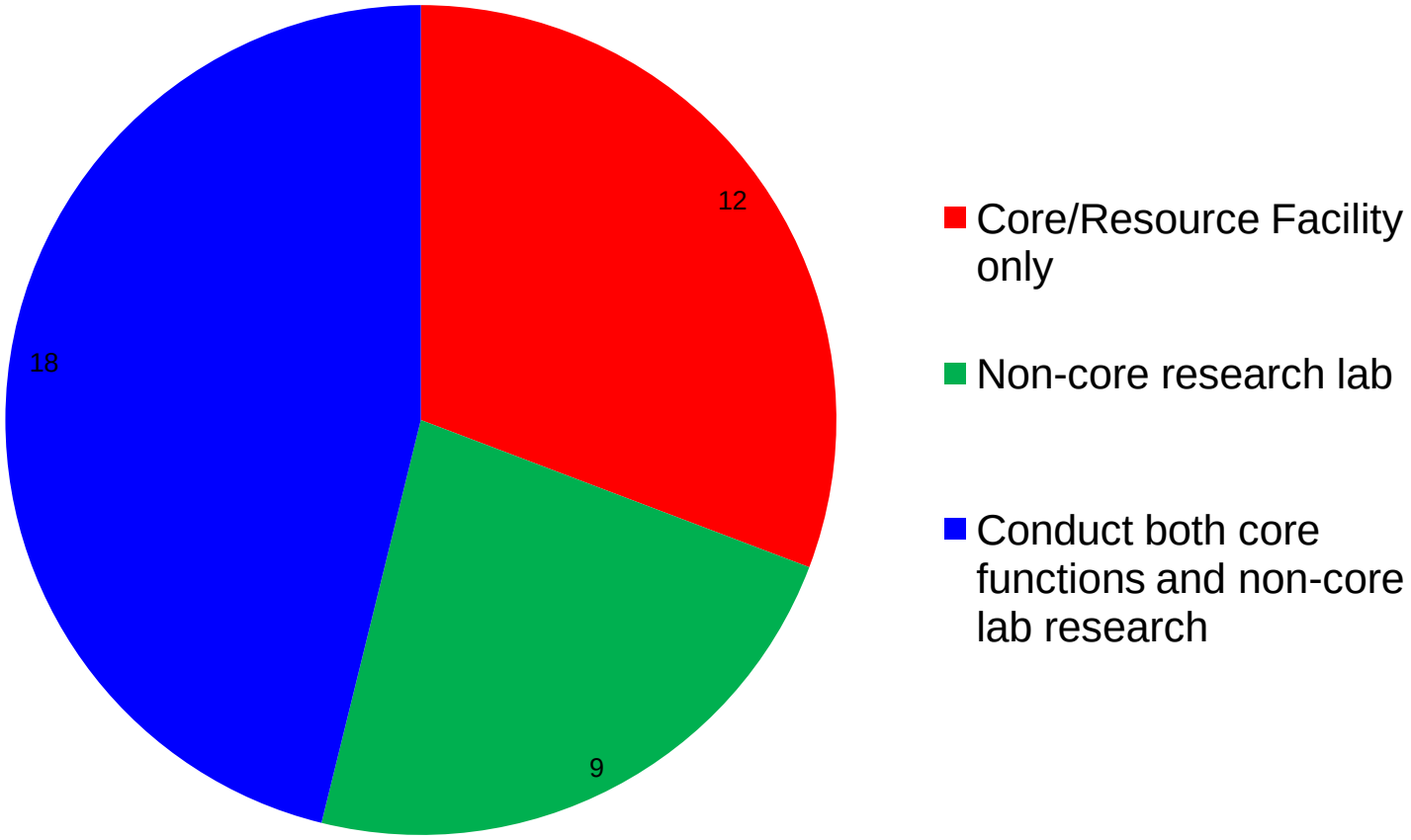
Please indicate your country



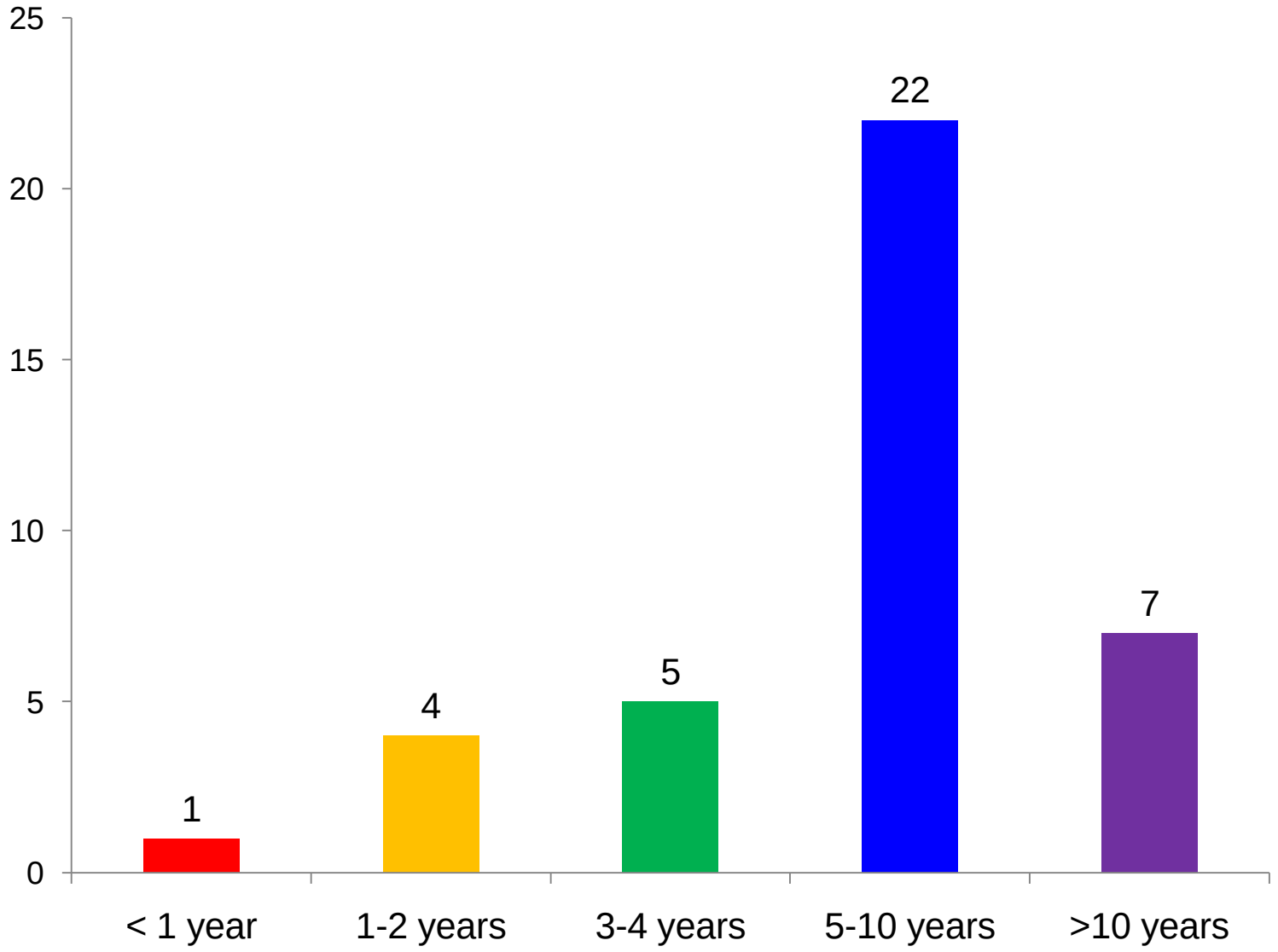
Which best describes your lab?



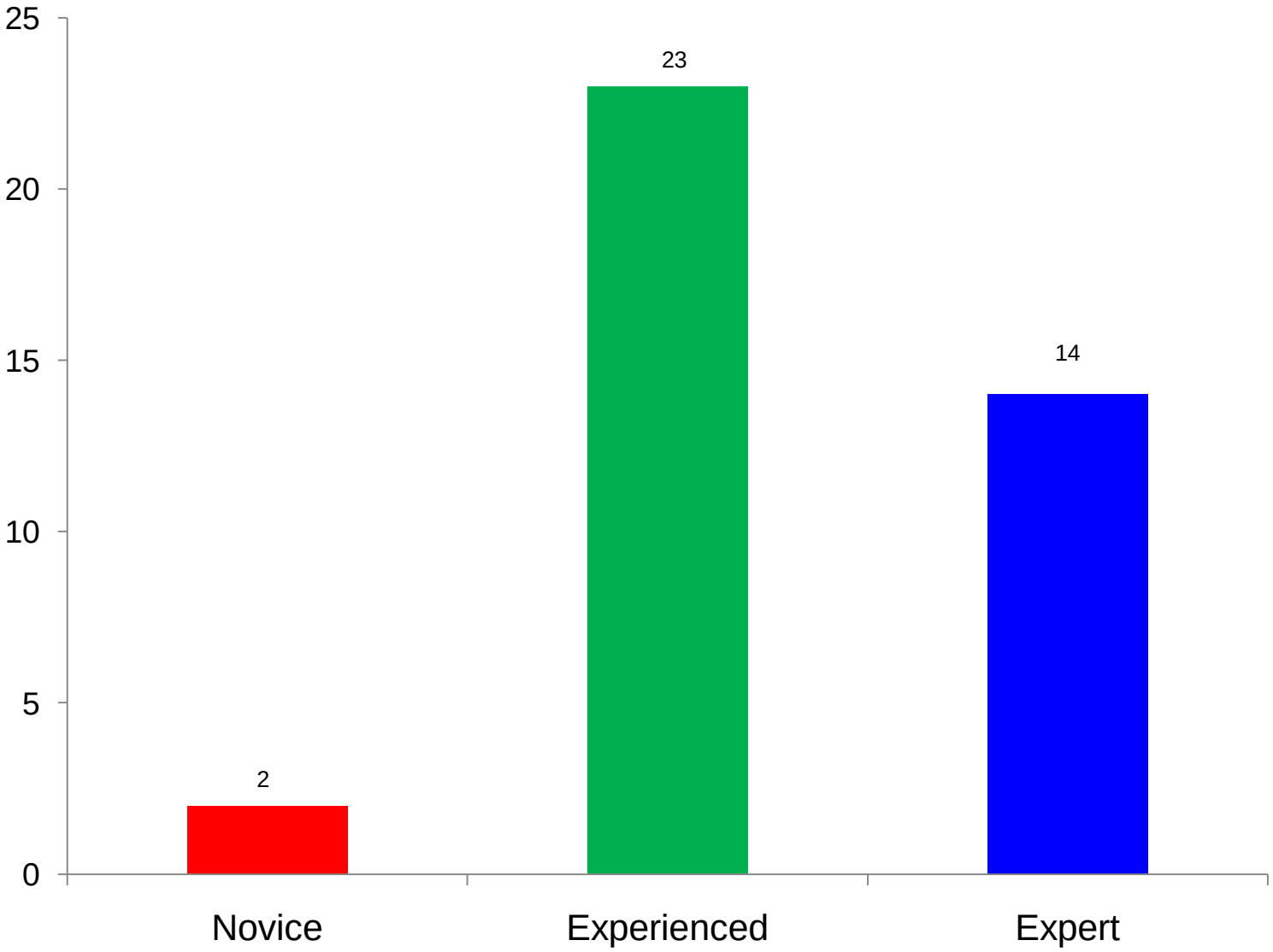
Core or resource facility status?



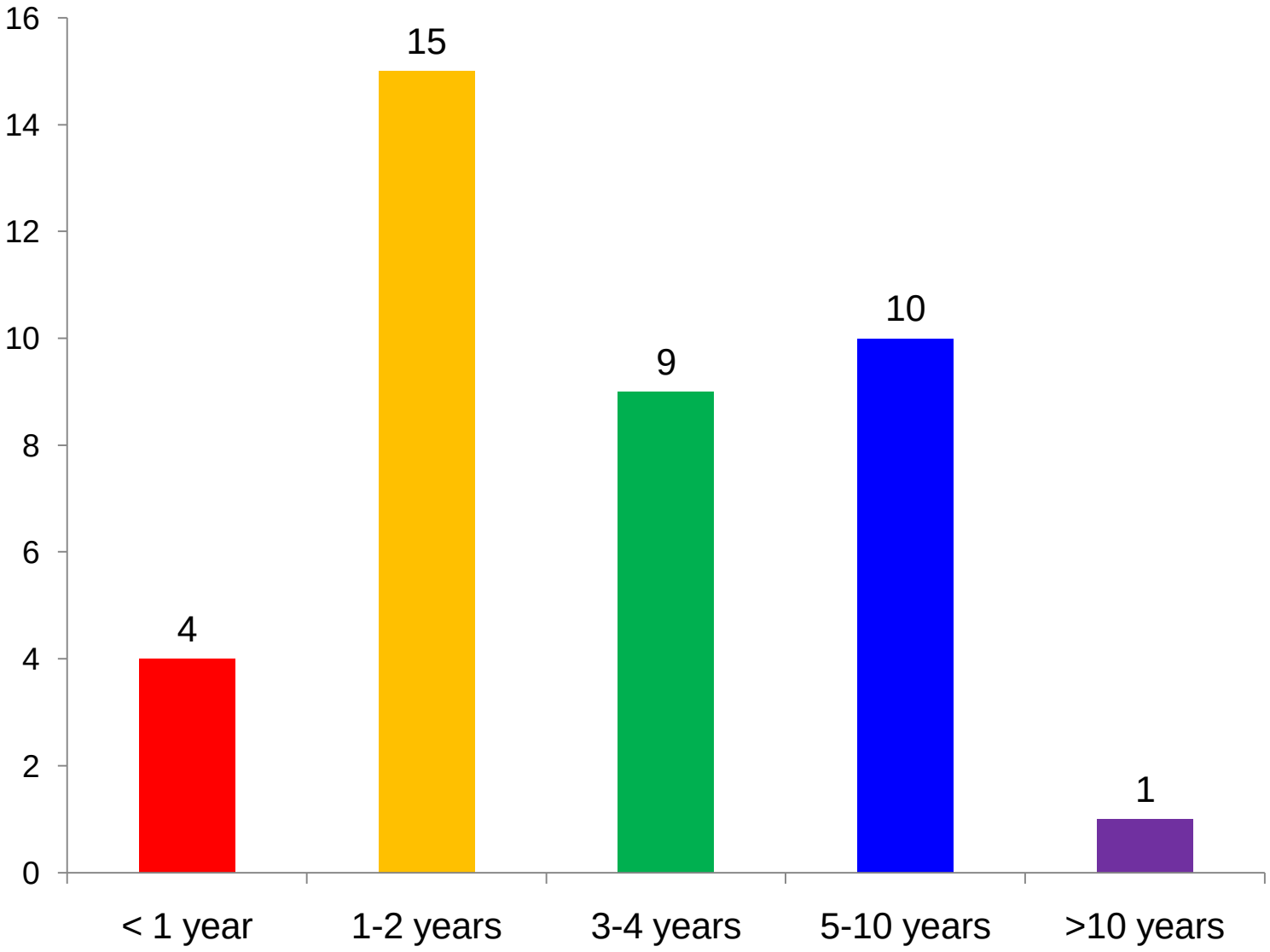
How long have you been involved in proteomics?



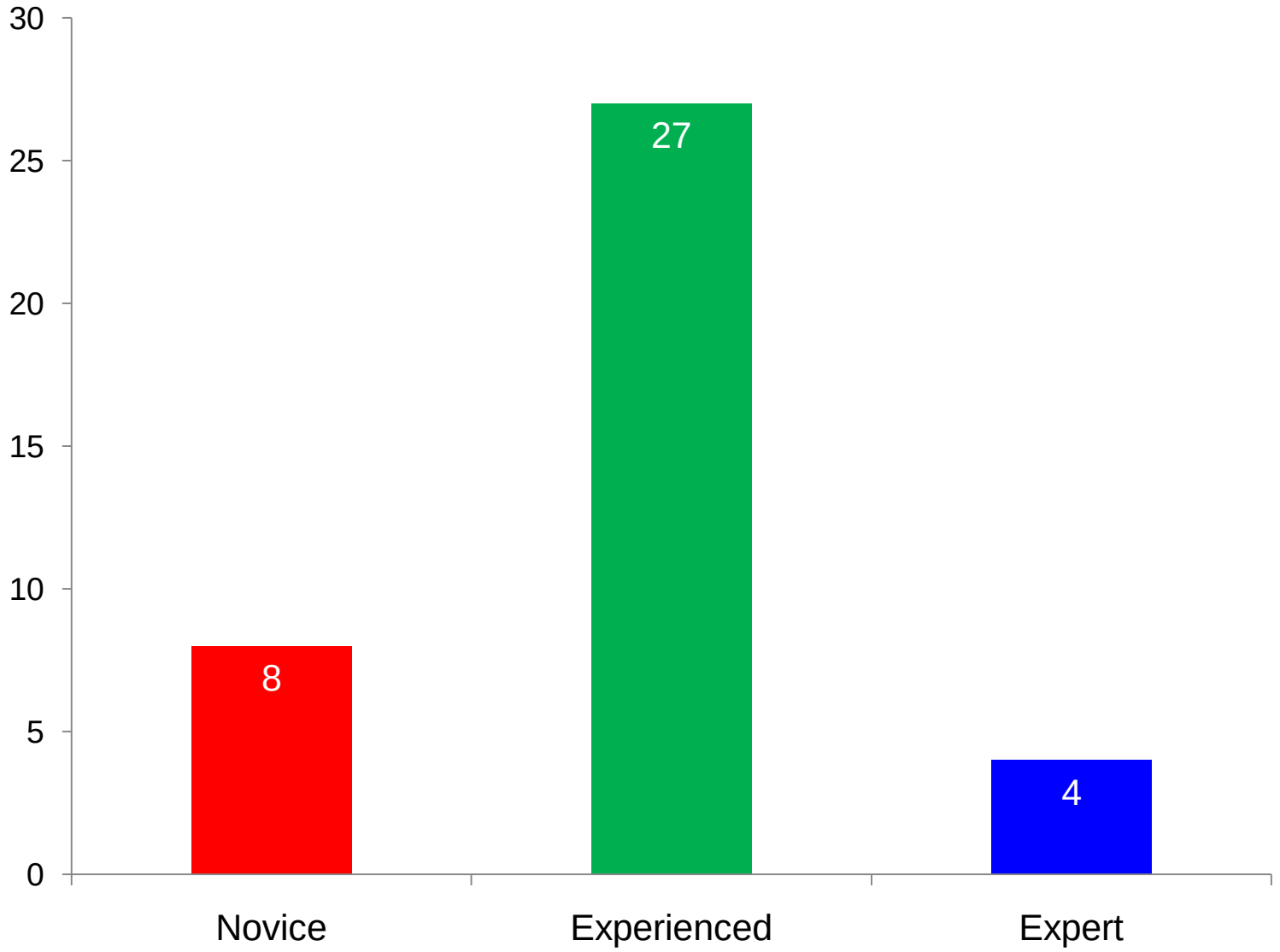
What do you consider your level of experience in proteomics?



How long have you been involved in phosphoproteomics?



What do you consider your level of experience in phosphoproteomics?



Overview of Results

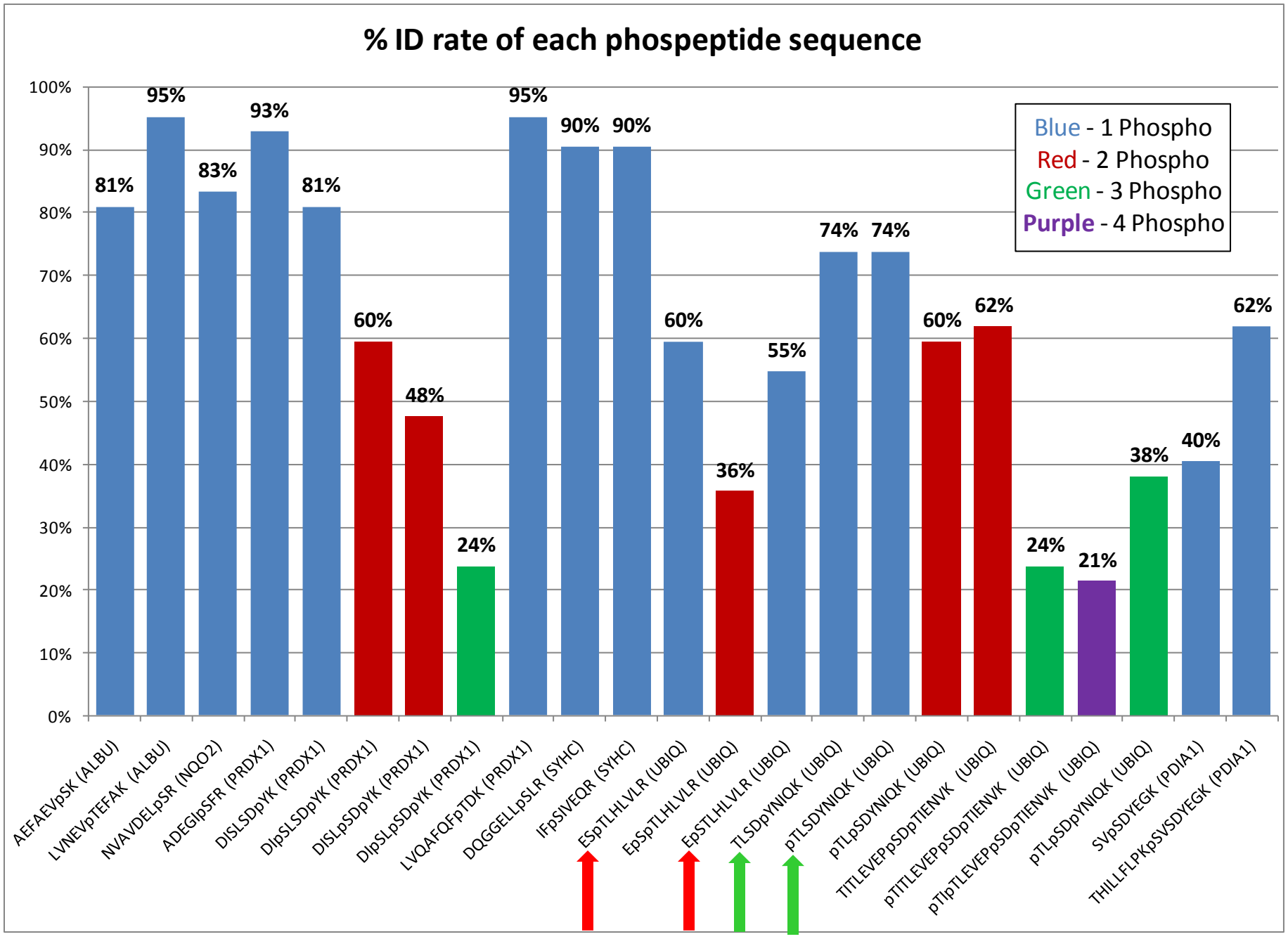
Identification Rates By Peptide

			sPRG2010		PRG 2003	
Protein	MW, Da	Sequence	# of times ID	% ID	# of times ID	% ID
ALBU_HUMAN	959.4001	AEFAEV#SK (1)	34	81%		
ALBU_HUMAN	1228.5741	LVNEV#TEFAK (1)	40	95%		
NQO2_HUMAN	1081.4805	NVAVDEL#SR (1)	35	83%		
PRDX1_HUMAN	973.3906	ADEGI#SFR (1)	39	93%		
PRDX1_HUMAN	1019.4213	DISLSD#YK (1)	34	81%		
PRDX1_HUMAN	1099.3876	DI#SLSD#YK (2)	25	60%		
PRDX1_HUMAN	1099.3876	DISL#SD#YK (2)	20	48%		
PRDX1_HUMAN	1179.3539	DI#SL#SD#YK (3)	10	24%		
PRDX1_HUMAN	1275.5901	LVQAFQF#TDK (1)	40	95%		
SYHC_HUMAN	1166.5333	DQGGELL#SLR (1)	38	90%		
SYHC_HUMAN	1070.5162	IF#SIVEQR (1)	38	90%		
UBIQ_HUMAN	1146.5798	ES#TLHLVLR (1)	25	60%		
UBIQ_HUMAN	1226.5462	E#S#TLHLVLR (2)	15	36%		
UBIQ_HUMAN	1146.5798	E#STLHLVLR (1)	23	55%		
UBIQ_HUMAN	1160.5115	TLSD#YNIQK (1)	31	74%		
UBIQ_HUMAN	1160.5115	#TLSDYNIQK (1)	31	74%		
UBIQ_HUMAN	1240.4778	#TL#SDYNIQK (2)	25	60%		
UBIQ_HUMAN	1946.8527	TITLEVEP#SD#TIENVK (2)	26	62%		
UBIQ_HUMAN	2026.8190	#TITLEVEP#SD#TIENVK (3)	10	24%		
UBIQ_HUMAN	2106.7854	#TI#TLEVEP#SD#TIENVK (4)	9	21%		
UBIQ_HUMAN	1320.4441	#TL#SD#YNIQK (3)	16	38%		
PDIA1_BOVIN	963.3587	SV#SDYEGK (1)	17	40%	8	15%
PDIA1_BOVIN	2026.0177	THILLFLPK#SVSDYEGK (1)	26	62%	8	15%

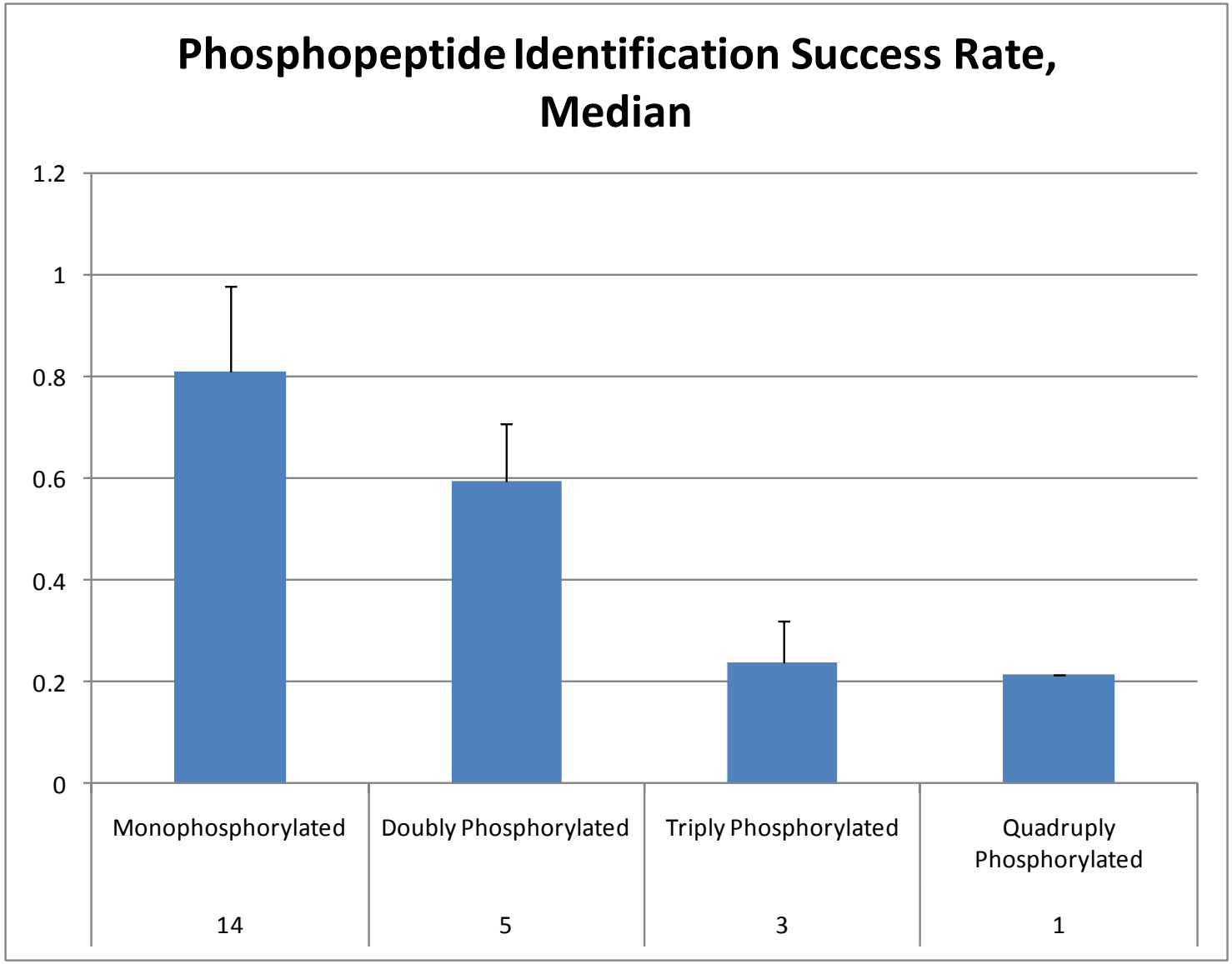
Identification Rates By Peptide

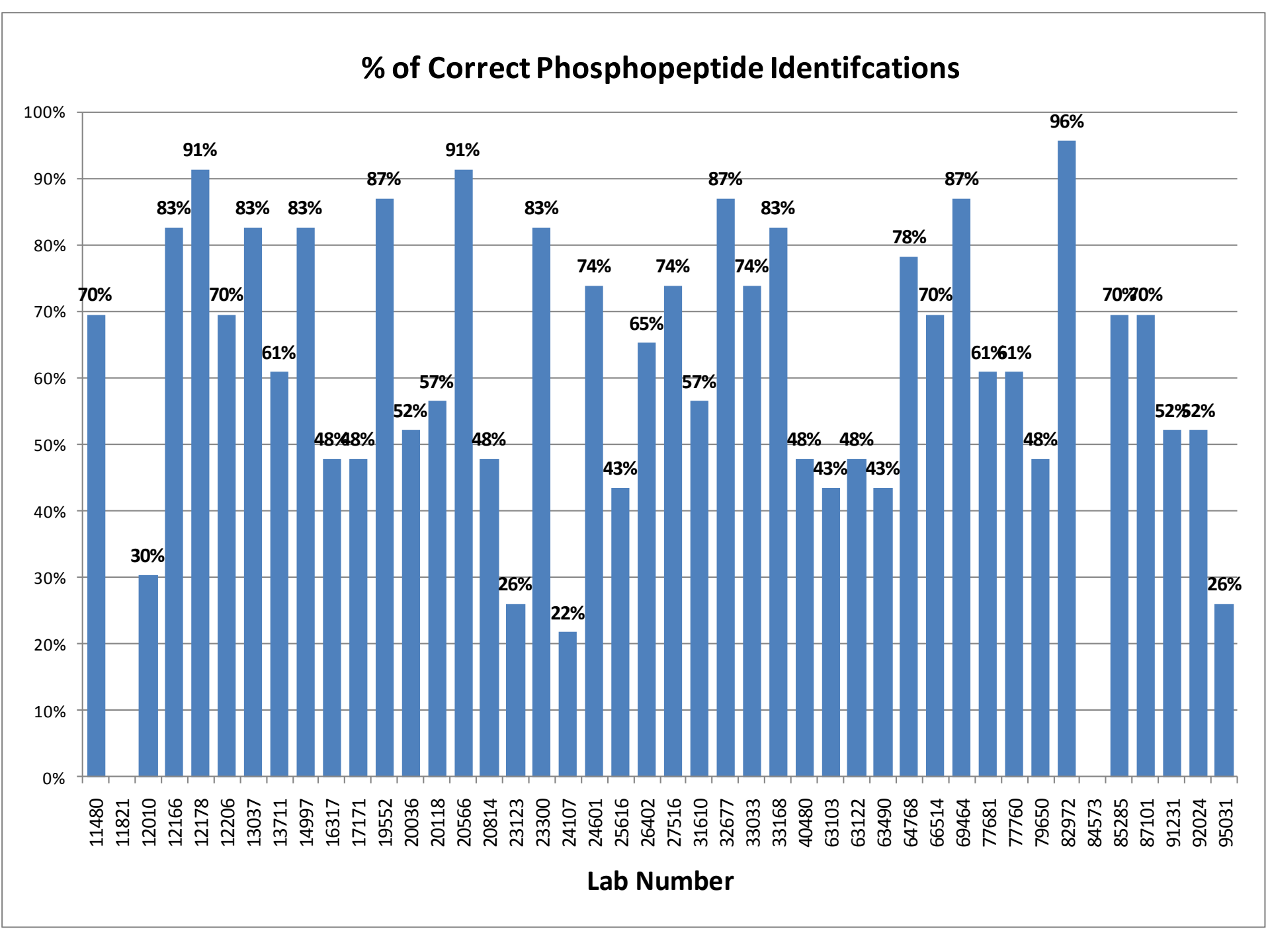
Sequence	# of p sites	# of times ID	% ID
AEFAEVpSK (ALBU)	1	34	81%
LVNEVpTEFAK (ALBU)	1	40	95%
NVAVDELpSR (NQO2)	1	35	83%
ADEGIpSFR (PRDX1)	1	39	93%
DISLSDpYK (PRDX1)	1	34	81%
DIpSLSDpYK (PRDX1)	2	25	60%
DISLpSDpYK (PRDX1)	2	20	48%
DIpSLpSDpYK (PRDX1)	3	10	24%
LVQAFQFpTDK (PRDX1)	1	40	95%
DQGGELLpSLR (SYHC)	1	38	90%
IFpSIVEQR (SYHC)	1	38	90%

Sequence	# of p sites	# of times ID	% ID
ESpTLHLVLR (UBIQ)	1	25	60%
EpSpTLHLVLR (UBIQ)	2	15	36%
EpSTLHLVLR (UBIQ)	1	23	55%
TLSDpYNIQK (UBIQ)	1	31	74%
pTLSDYNIQK (UBIQ)	1	31	74%
pTLpSDYNIQK (UBIQ)	2	25	60%
TITLEVEPpSDpTIENVK (UBIQ)	2	26	62%
pTITLEVEPpSDpTIENVK (UBIQ)	3	10	24%
pTIpTLEVEPpSDpTIENVK (UBIQ)	4	9	21%
pTLpSDpYNIQK (UBIQ)	3	16	38%
SVpSDYEGK (PDIA1)	1	17	40%
THILLFLPKpSVSDYEGK (PDIA1)	1	26	62%


% ID rate of each phospeptide sequence
Blue - 1 Phospho
Red - 2 Phospho
Green - 3 Phospho
Purple - 4 Phospho
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
81%
95%
83%
93%
81%
60%
48%
24%
95%
90%
90%
60%
36%
55%
74%
74%
60%
62%
24%
21%
38%
40%
62%
AEFAEVpSK (ALBU)
LVNEVpTEFAK (ALBU)
NVAVDELpSR (NQO2)
ADEGIpSFR (PRDX1)
DISLSDpYK (PRDX1)
DIpSLSDpYK (PRDX1)
DISLpSDpYK (PRDX1)
DIpSLpSDpYK (PRDX1)
LVQAFQFpTDK (PRDX1)
DQGGELLpSLR (SYHC)
IFpSIVEQR (SYHC)
ESpTLHLVLR (UBIQ)
EpSpTLHLVLR (UBIQ)
EpSTLHLVLR (UBIQ)
TLSDpYNIQK (UBIQ)
pTLSDYNIQK (UBIQ)
pTLpSDYNIQK (UBIQ)
TITLEVEPpSDpTIENVK (UBIQ)
pTITLEVEPpSDpTIENVK (UBIQ)
pTIpTLEVEPpSDpTIENVK (UBIQ)
pTLpSDpYNIQK (UBIQ)
SVpSDYEGK (PDIA1)
THILLFLPKpSVSDYEGK (PDIA1)

Success of Detection by # of Phosphorylations per Peptide




% of Correct Phosphopeptide Identifcations
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
70%
30%
83%
91%
70%
83%
61%
83%
48%
48%
87%
52%
57%
91%
48%
26%
83%
22%
74%
43%
65%
74%
57%
87%
74%
83%
48%
43%
48%
43%
78%
70%
87%
61%
61%
48%
96%
70%
70%
52%
52%
26%
11480
11821
12010
12166
12178
12206
13037
13711
14997
16317
17171
19552
20036
20118
20566
20814
23123
23300
24107
24601
25616
26402
27516
31610
32677
33033
33168
40480
63103
63122
63490
64768
66514
69464
77681
77760
79650
82972
84573
85285
87101
91231
92024
95031
Lab Number

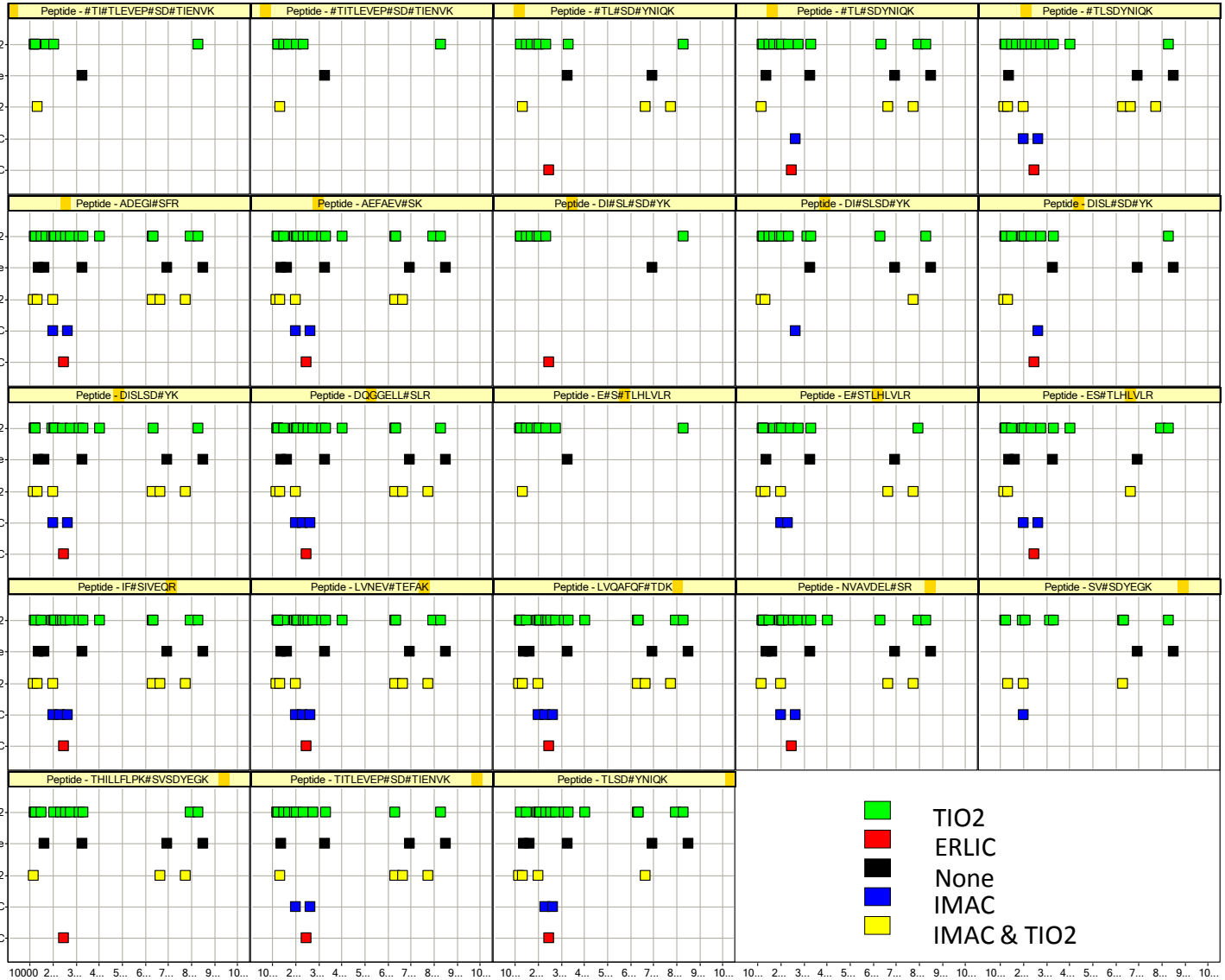
Enrichment Options

- IMAC:
  - Immobilized Metal Ion Affinity Chromatography
  - Chelated Fe^{3+} or other metal ions on a matrix retains Phosphopeptides
  - Methylation of Asp and Glu recommended
- TiO2:
  - Phosphopeptides are retained by an Ti or Zr matrix;
  - Methylation of Asp and Glu recommended
- ERLIC:
  - Electrostatic Repulsion-Hydrophilic Interaction Chromatography
  - Weak Anion Exchange Resin (PolyWax LP) run under Low pH and High Organic conditions
  - Phosphopeptides are reatined by the column

Identification by Peptide vs. Method of Enrichment

#TI#TLEVER#SD#TIENVK

#TITLEVER#SD#TIENVK

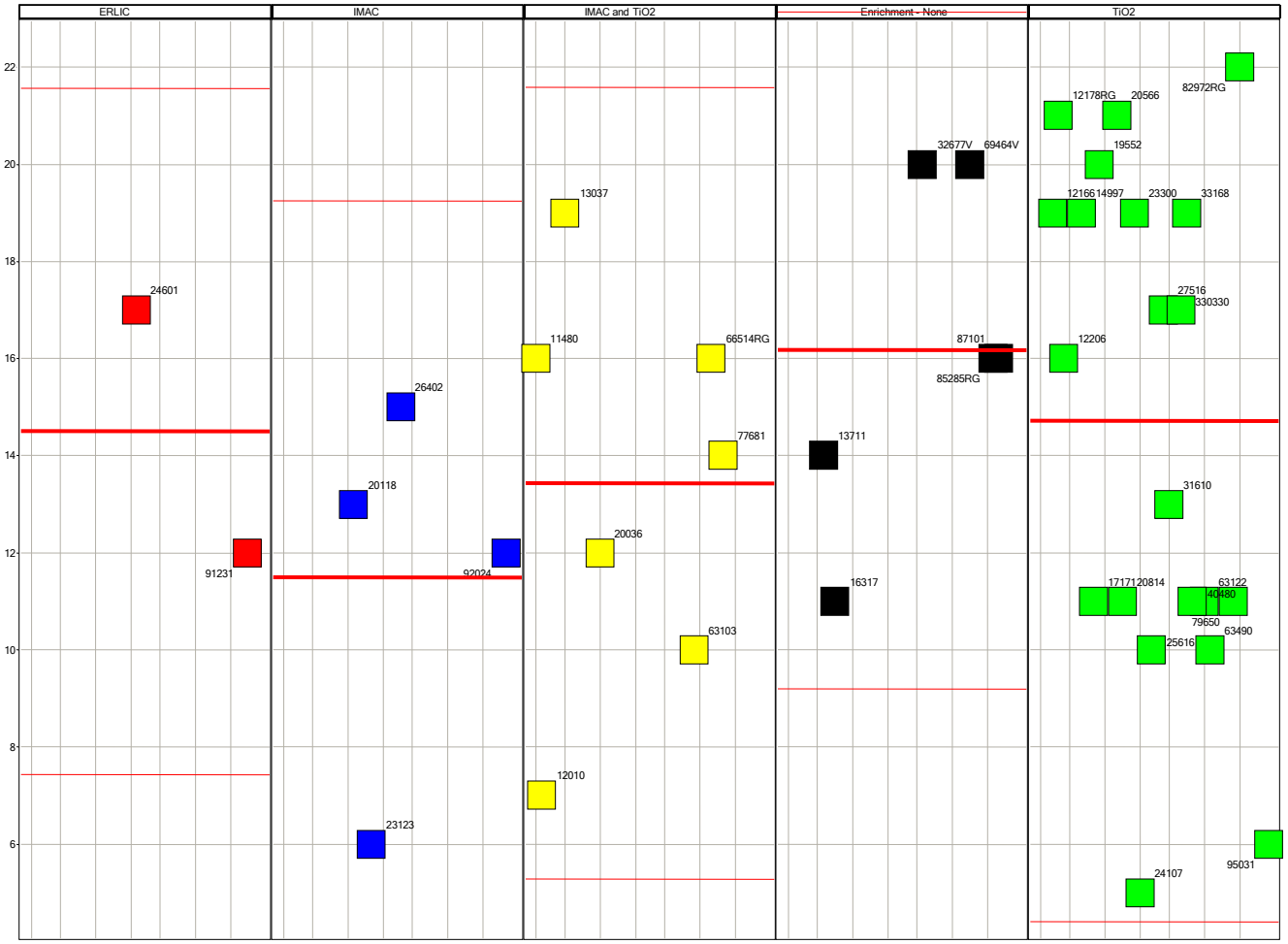


Enrichment

Entry #

Method of Enrichment vs. # of Peptides Correct

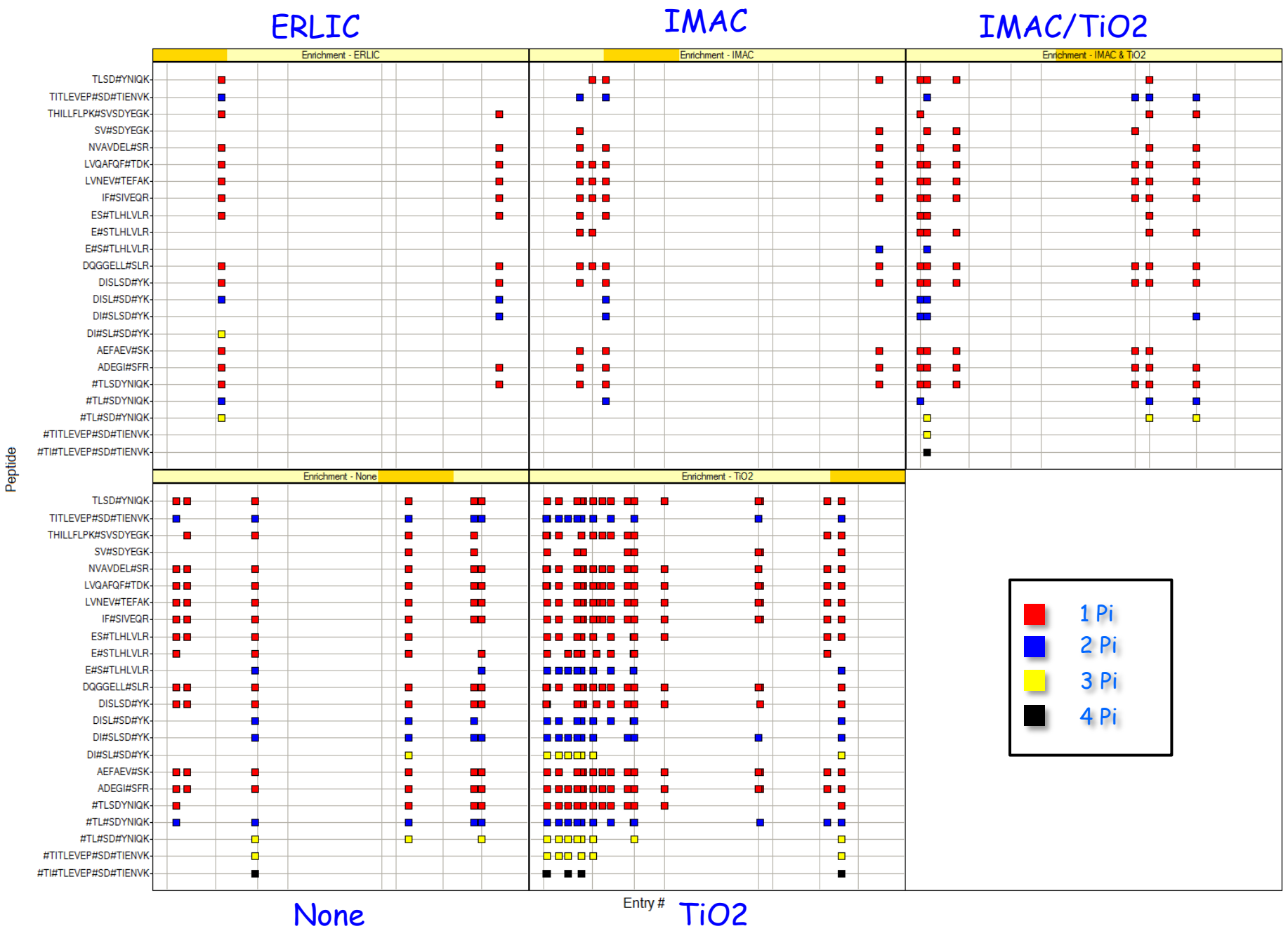
ERLIC IMAC IMAC/TiO2 None TiO2

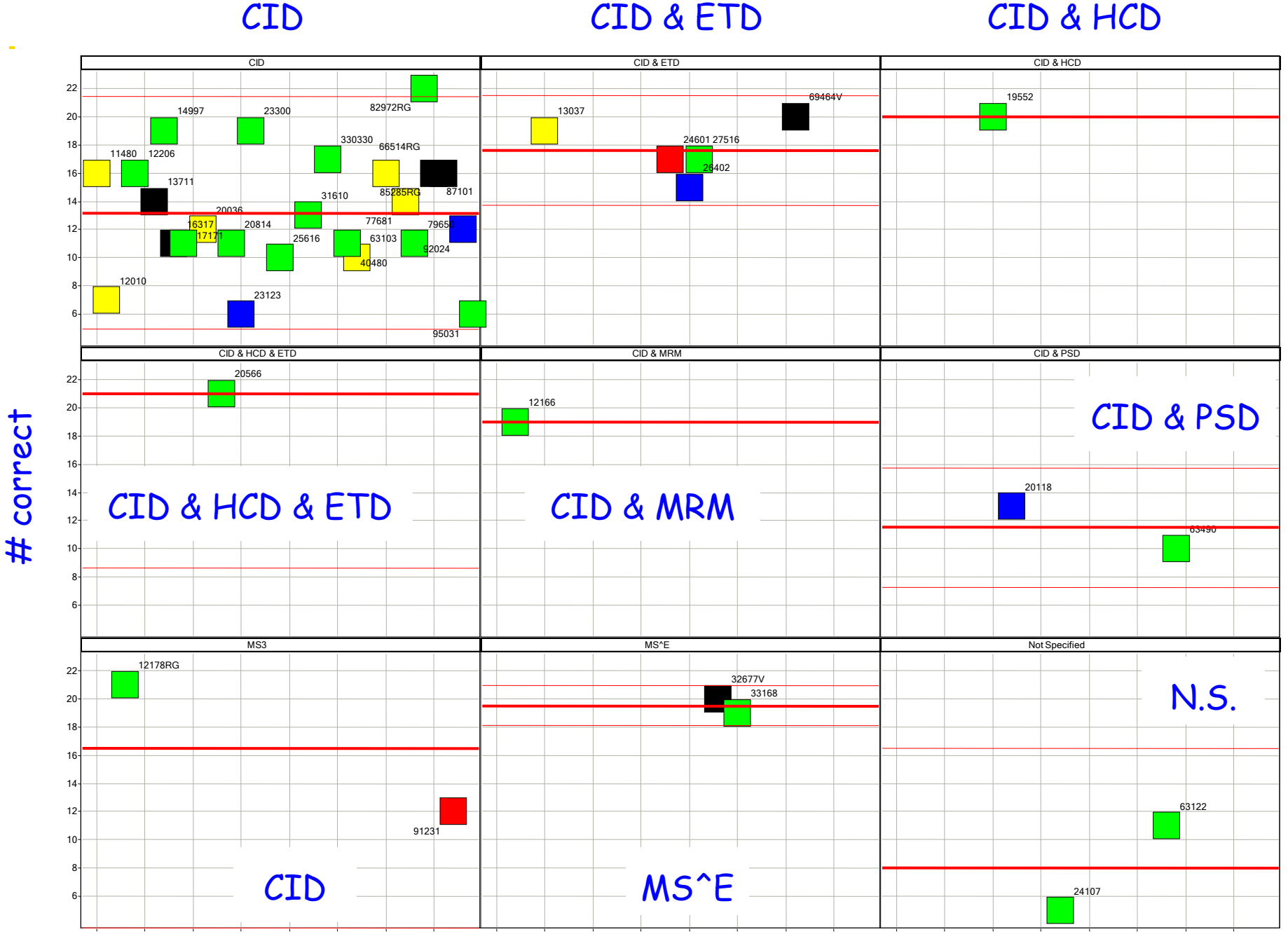


correct

- TIO2
- ERLIC
- None
- IMAC
- IMAC & TIO2

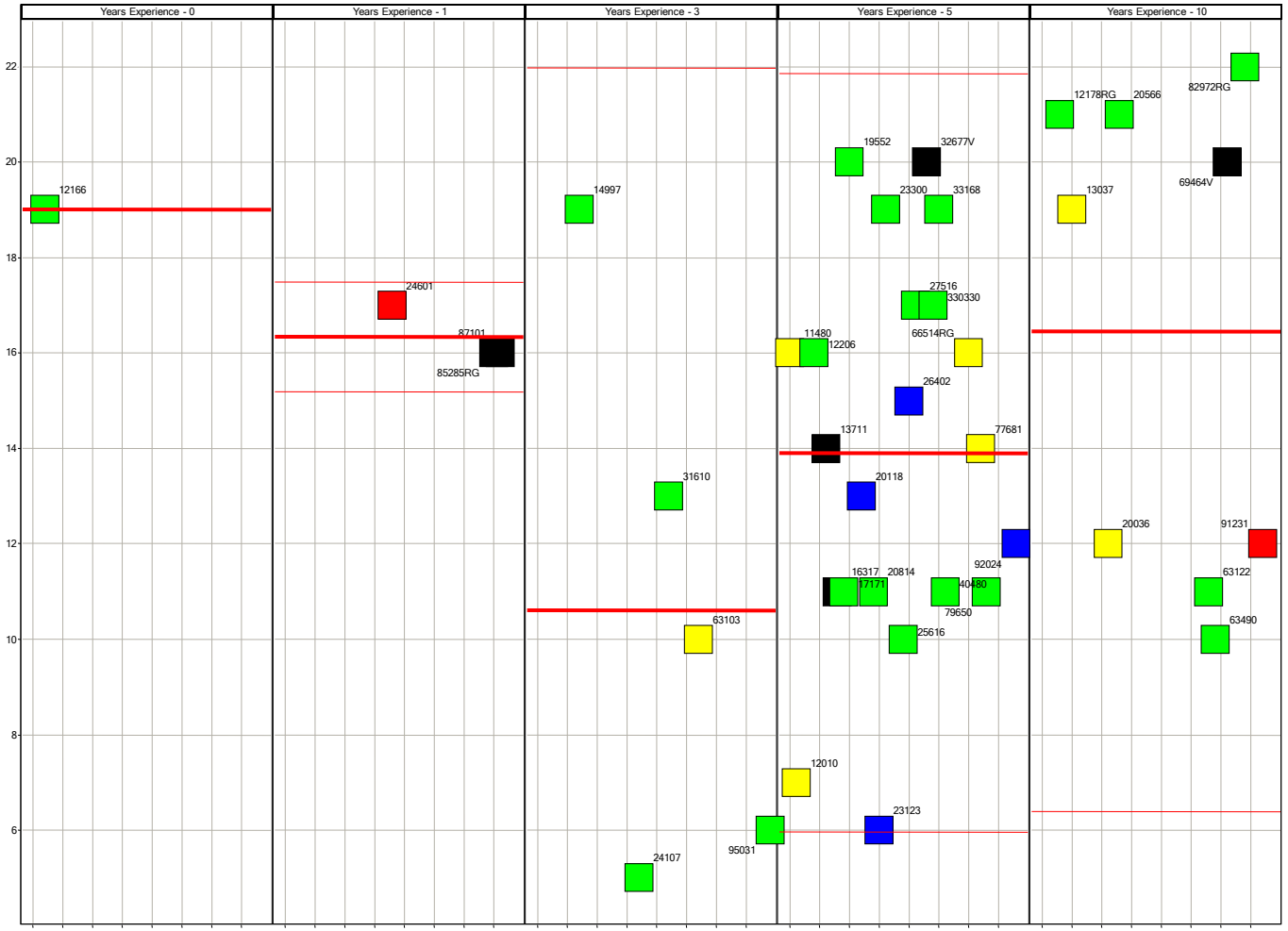
Pi-Peptides Detected by Enrichment and # of Pi's per Peptide




CID
CID & ETD
CID & HCD
correct
CID
14997
23300
82972RG
330330
66514RG
11480
12206
13711
31610
85285RG
87101
20036
16317
17171
20814
77681
79650
25616
63103
92024
40480
12010
23123
95031
CID & ETD
69464V
13037
24601 27516
26402
CID & HCD
19552
CID & HCD & ETD
20566
CID & MRM
12166
CID & PSD
20118
63490
MS3
12178RG
91231
MS^E
32677V
33168
Not Specified
N.S.
63122
24107

Years of Experience vs. # of Peptides Correct

0 yrs 1 yrs 3 yrs 5 yrs 10 yrs



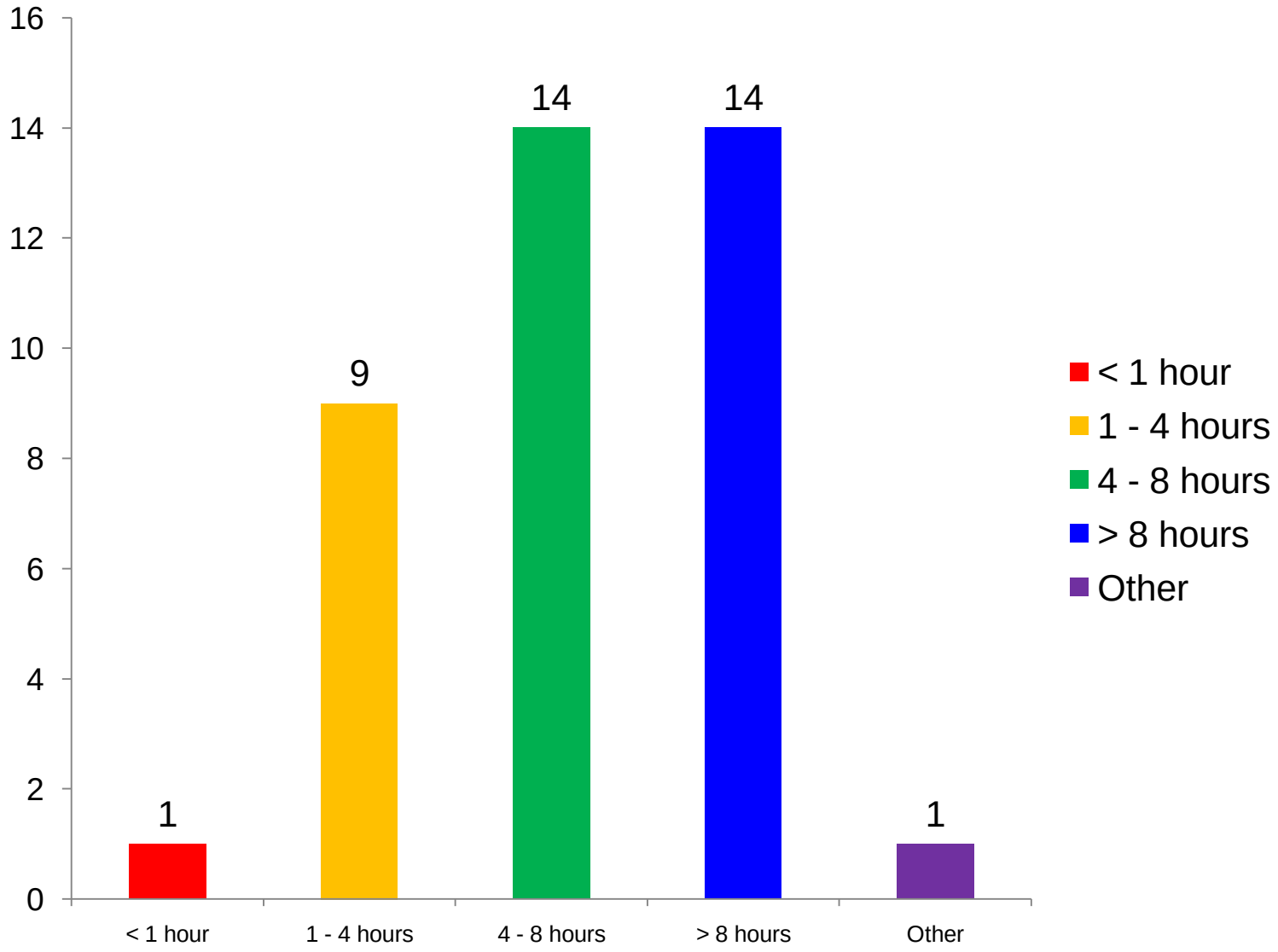
correct

TIO2
ERLIC
None
IMAC
IMAC & TIO2

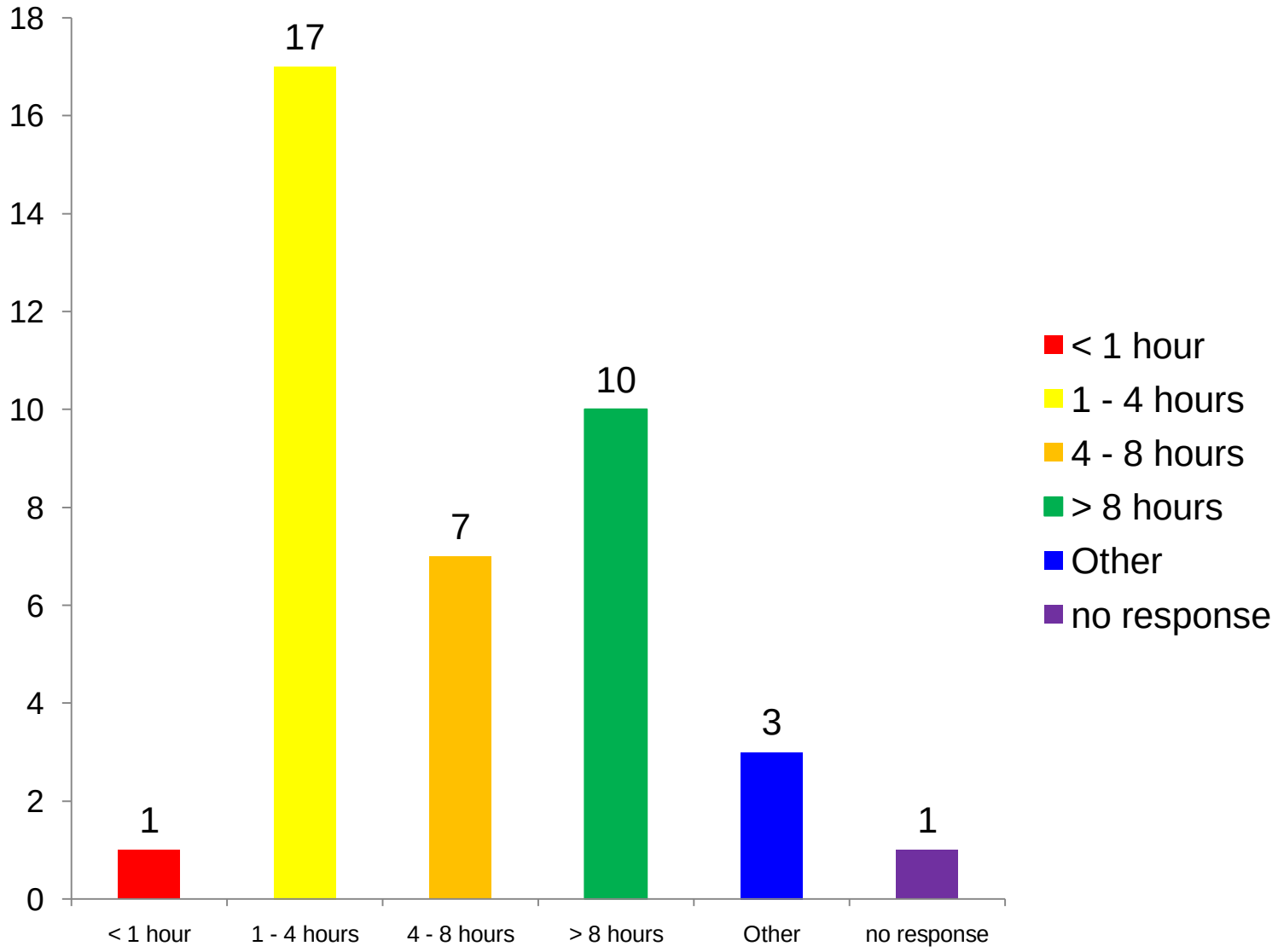
= SD
= Average

Participant Comments on the sPRG 2010 Study

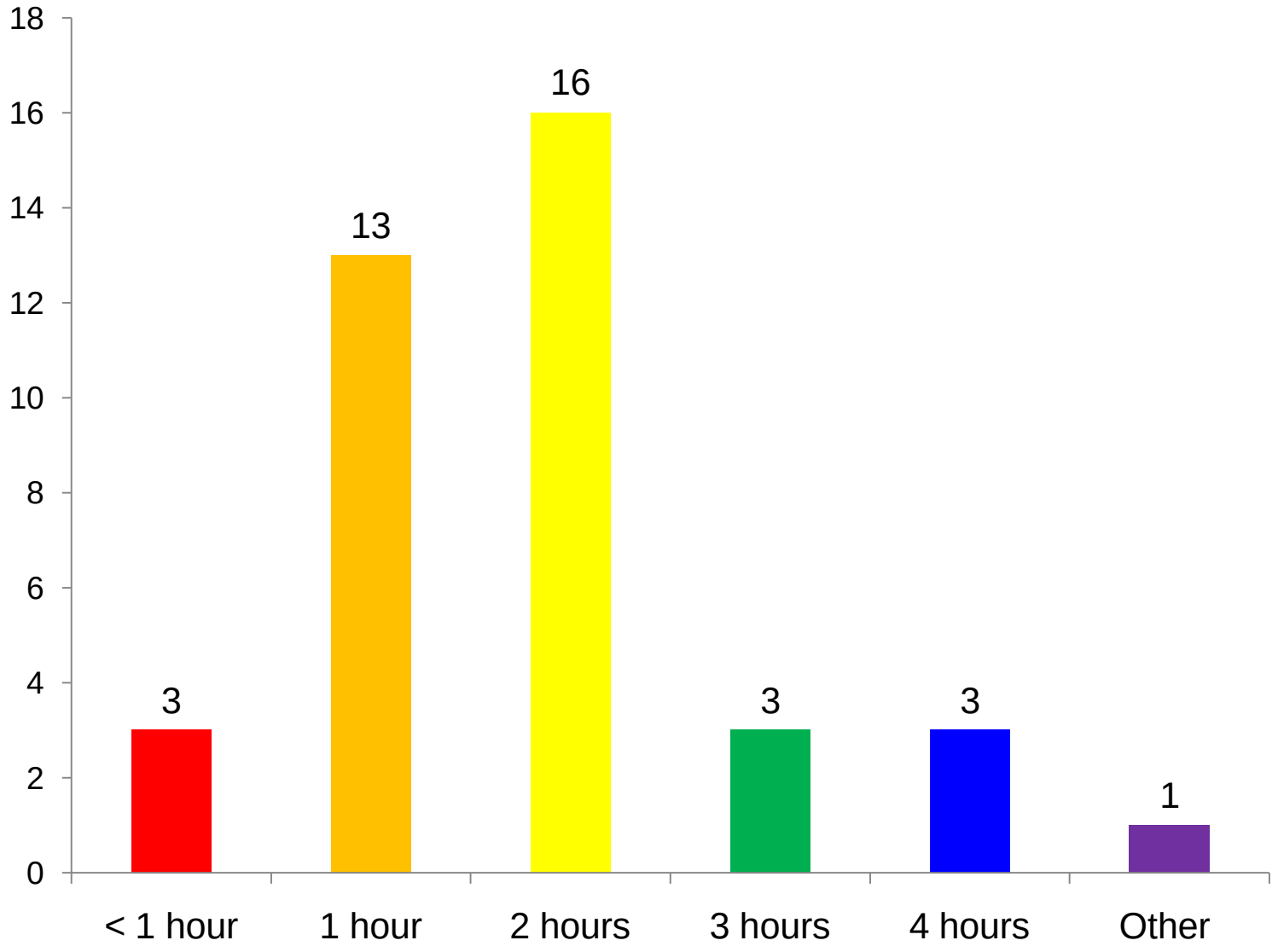
How much time did it take for you to prepare and analyze this sample?



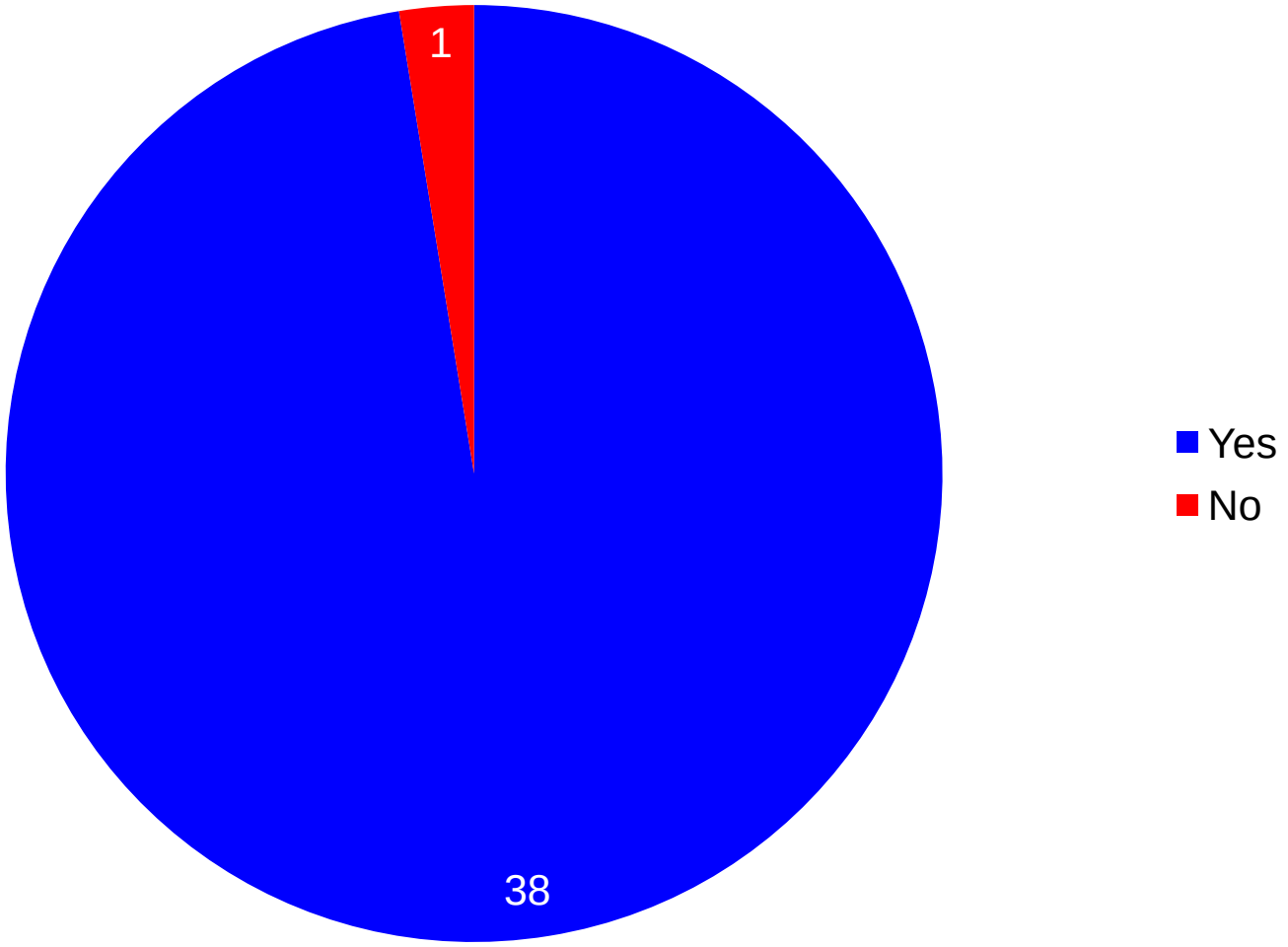
How much time did it take for you to analyze the data?



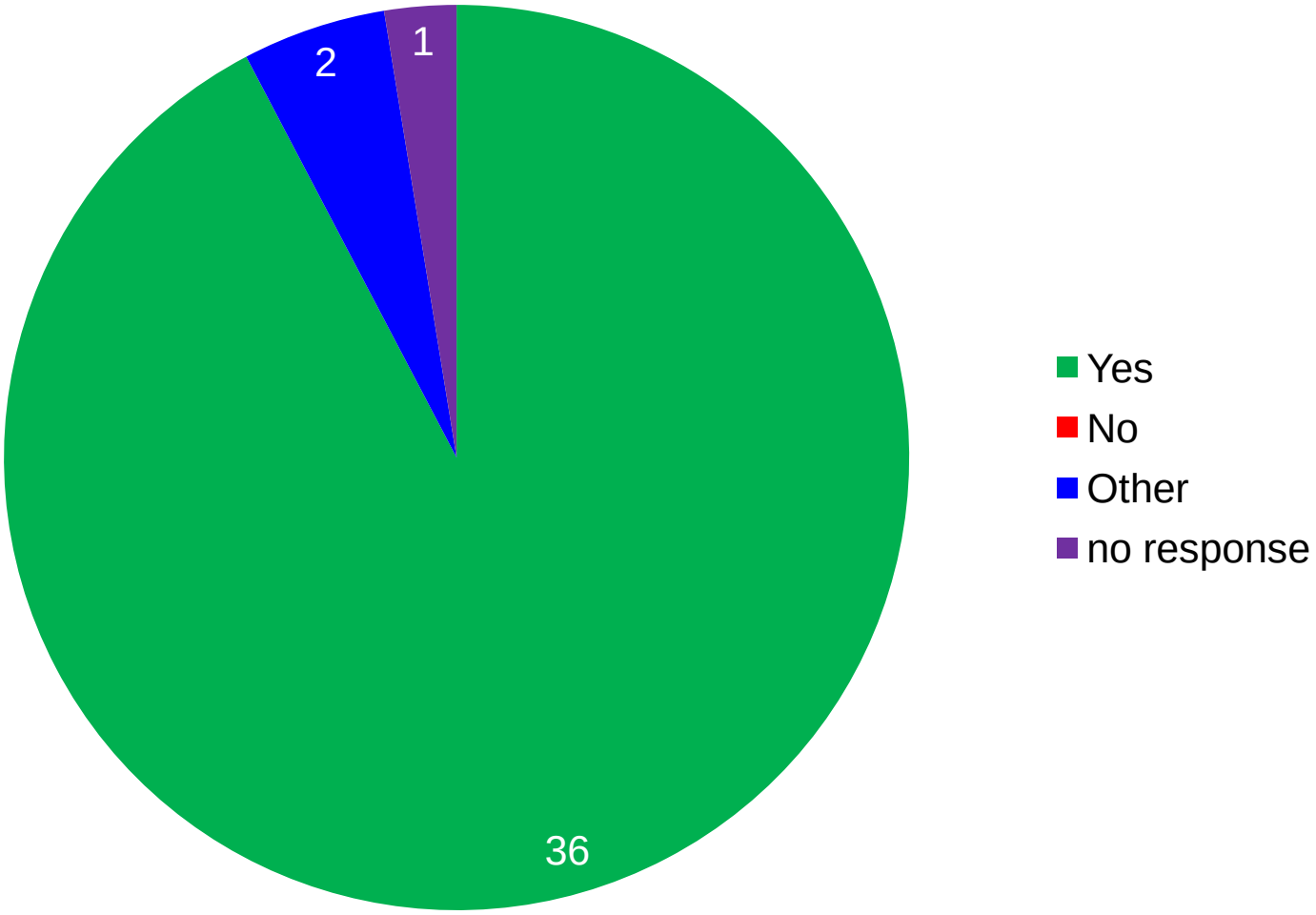
How much time did it take you to complete this survey?



Do you think this type of study has been useful?



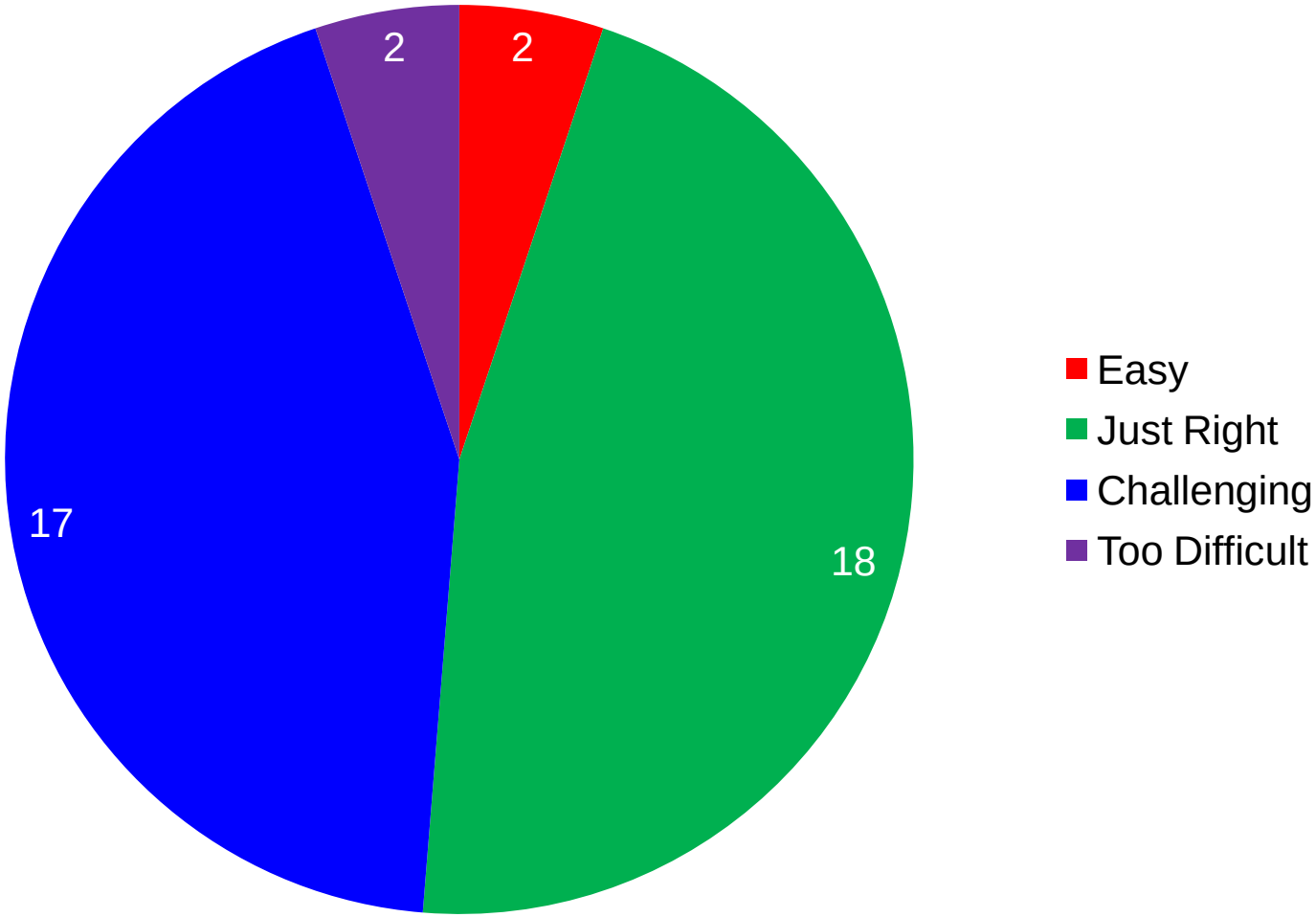
Do you think this type of standard would be useful as a positive control prior to "real world" phospho-peptide experimentation?



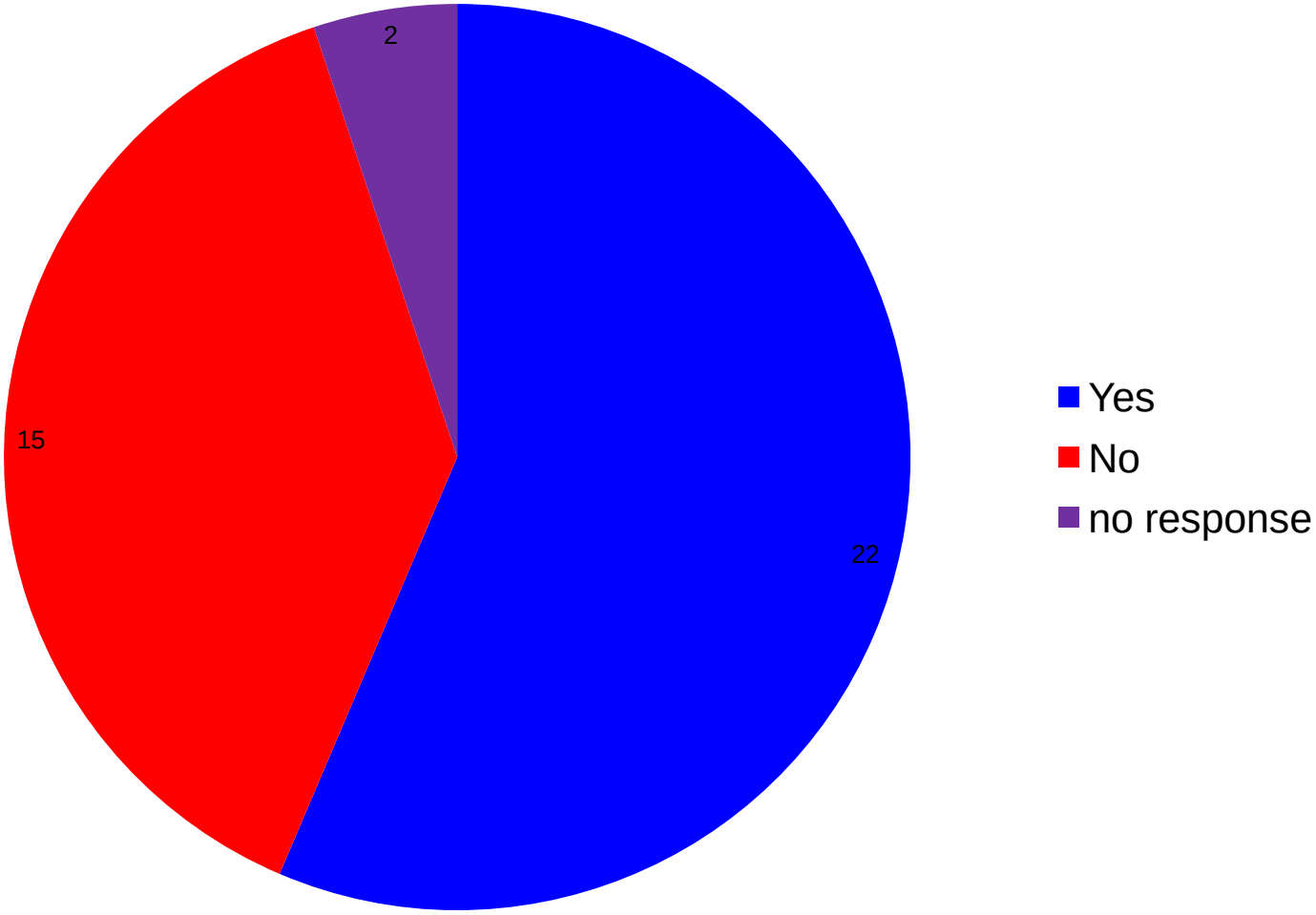
What changes would make this phosphopeptide mixture more useful?

- Make study materials available after the study
- Make clearer statements of reagent concentration and source
- Interactive study group for learning and discussion
- Include more PTMs
- Vary phosphopeptide concentrations
- Increase background complexity
- Decrease complexity

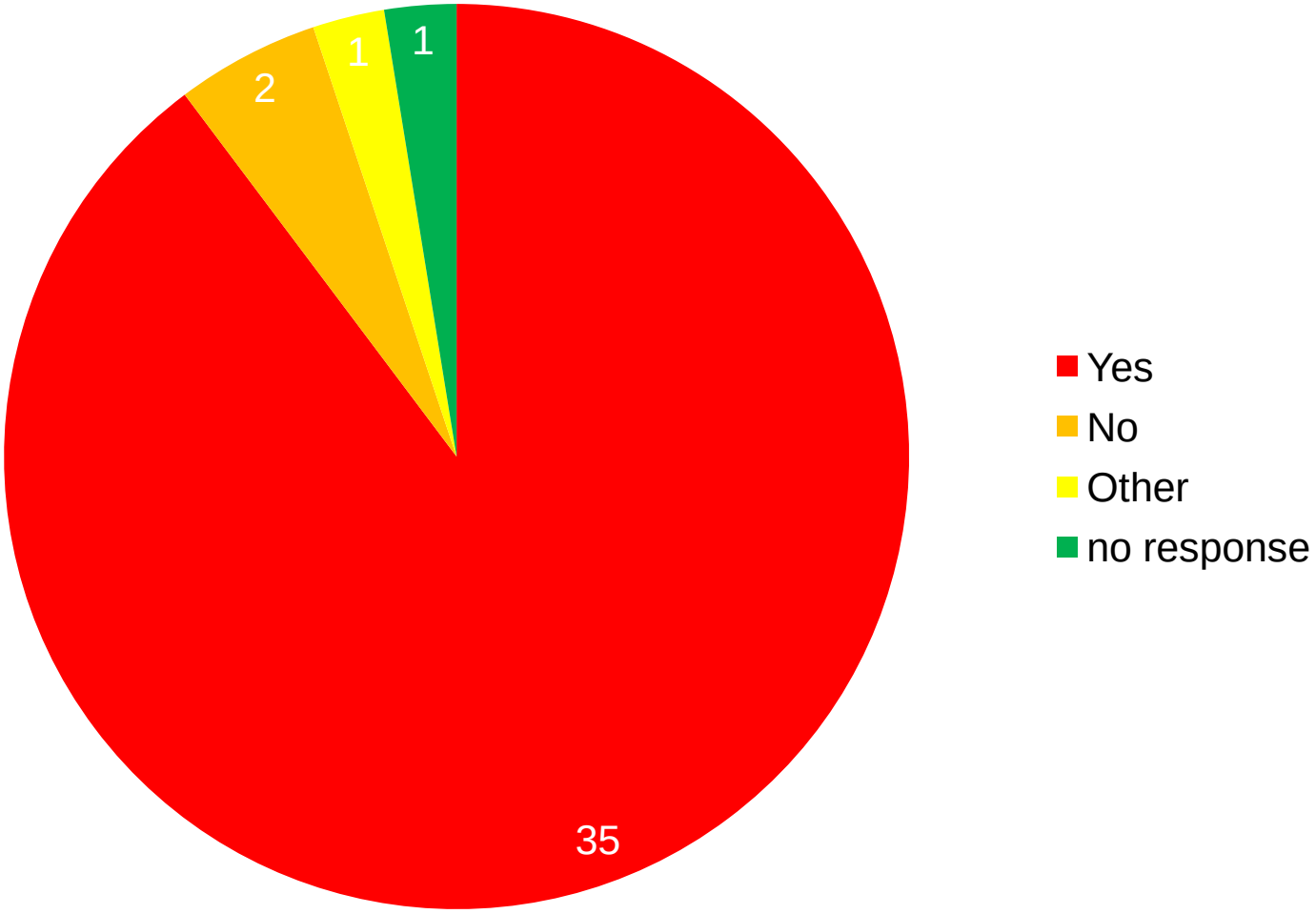
How would you rate this study's level of difficulty?



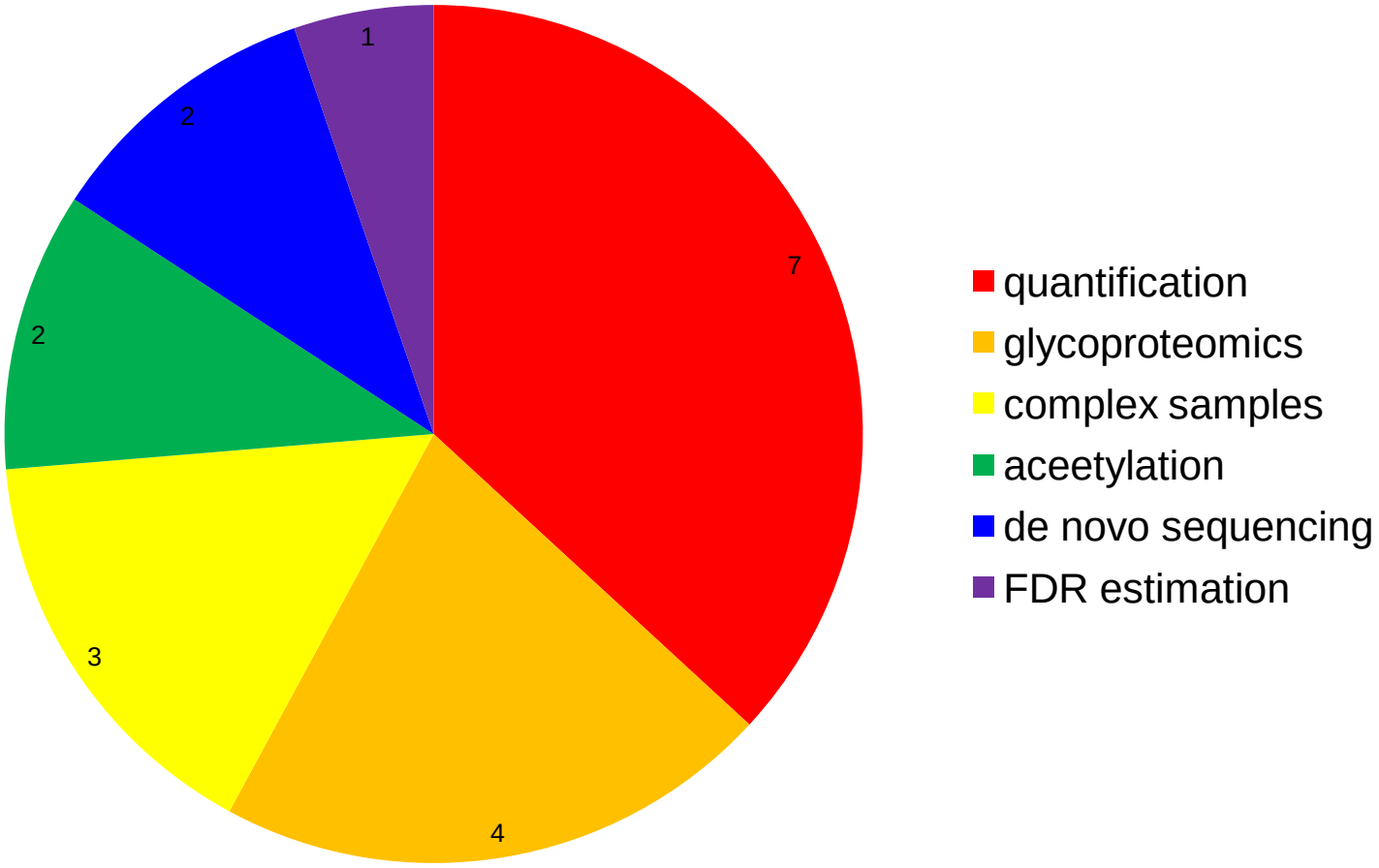
Have you participated in previous ABRF studies?



Based on this study, would you consider participating in future ABRF studies?



What type for study would you like to see the ABRF sPRG conduct in the future?



sPRG 2010 Study: Acknowledgements

- Mathias Madalinski of the Mechtler Lab (ICP Austria) for peptide synthesis, purification and analysis.
- Jim Makusky (NIH) as The Anonymizer.
- Sigma for the purified proteins.

To the Fearless 43 Participants:

Thank You!

Please Visit Our Poster

RG-8

ABRF-sPRG2010 Study: Development of a Phosphopeptide Standard for Proteomics

Please Visit Our Website

www.abrf.org ,

Click on Research Groups then sPRG

Questions, Ideas or Interested in Joining Us?

Contact

Alexander Ivanov (New Chair)

aivanov@hsph.harvard.edu

or

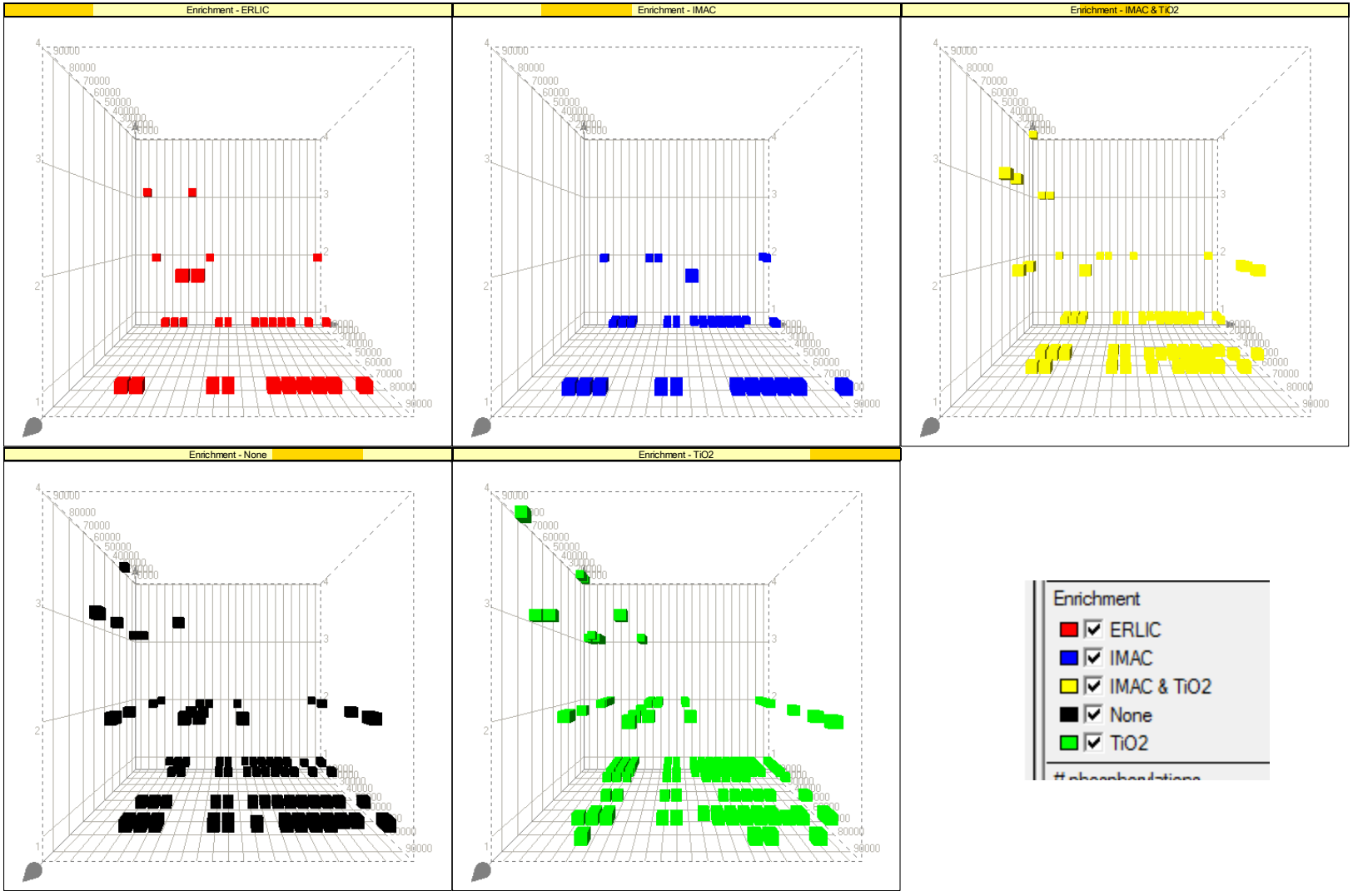
Jim Farmar (Old Chair)

jgf7k@virginia.edu

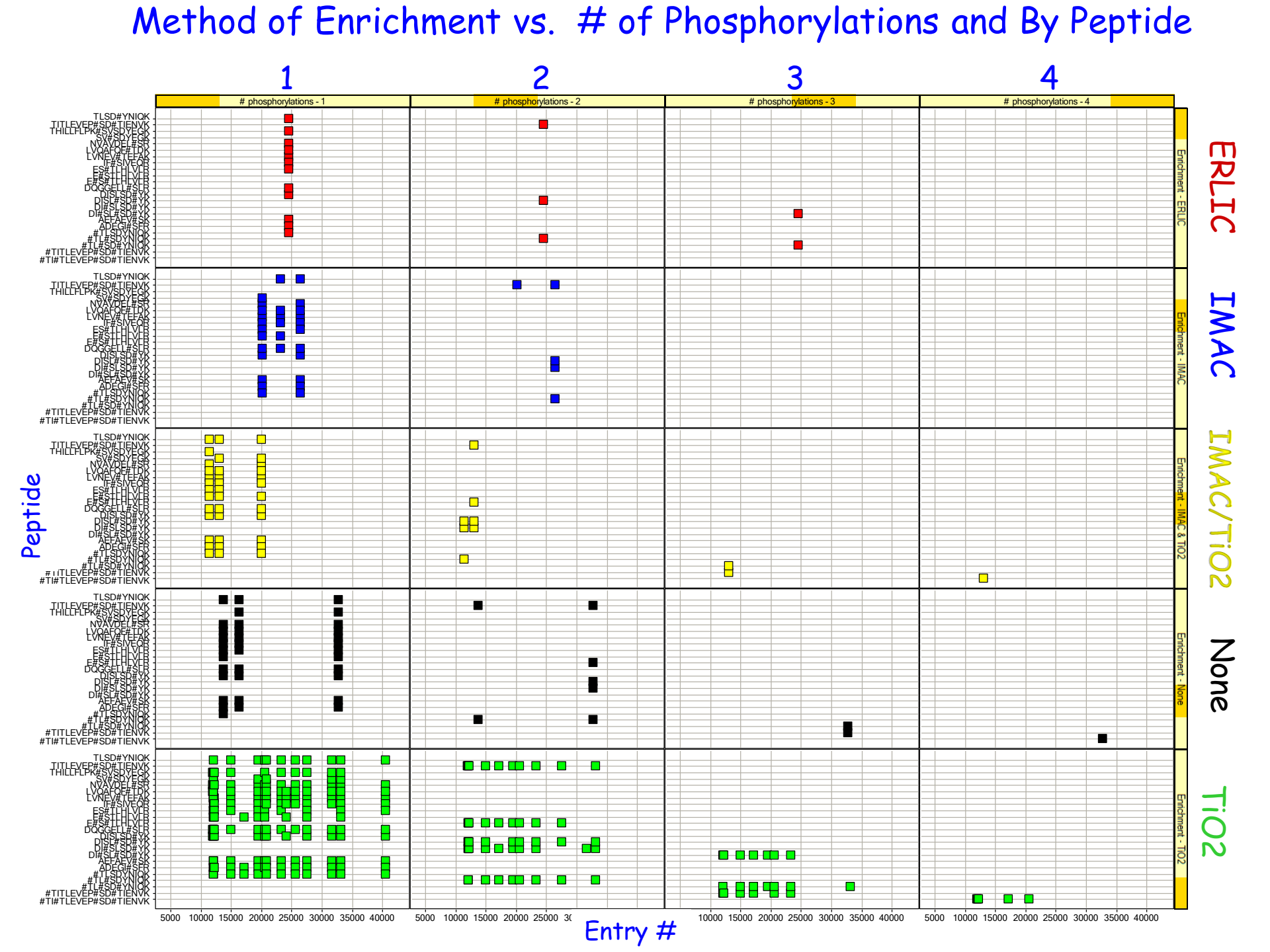
sPRG Members 2010

- Christopher Colangelo - Yale University
- Jim Farmar - Univ. of Virginia
- Alexander Ivanov (Chair) - Harvard School of Public Health
- Christopher Kinsinger - National Cancer Institute
- Jeffrey Kowalak (EB Liaison) - NIMH
- Karl Mechtler – Research Institute of Molecular Pathology, Austria
- Brett Phinney - UC Davis Genome Center
- Manfred Raida - Experimental Therapeutic Center
- Susan Weintraub - Univ. of Texas Health Science Center at San Antonio

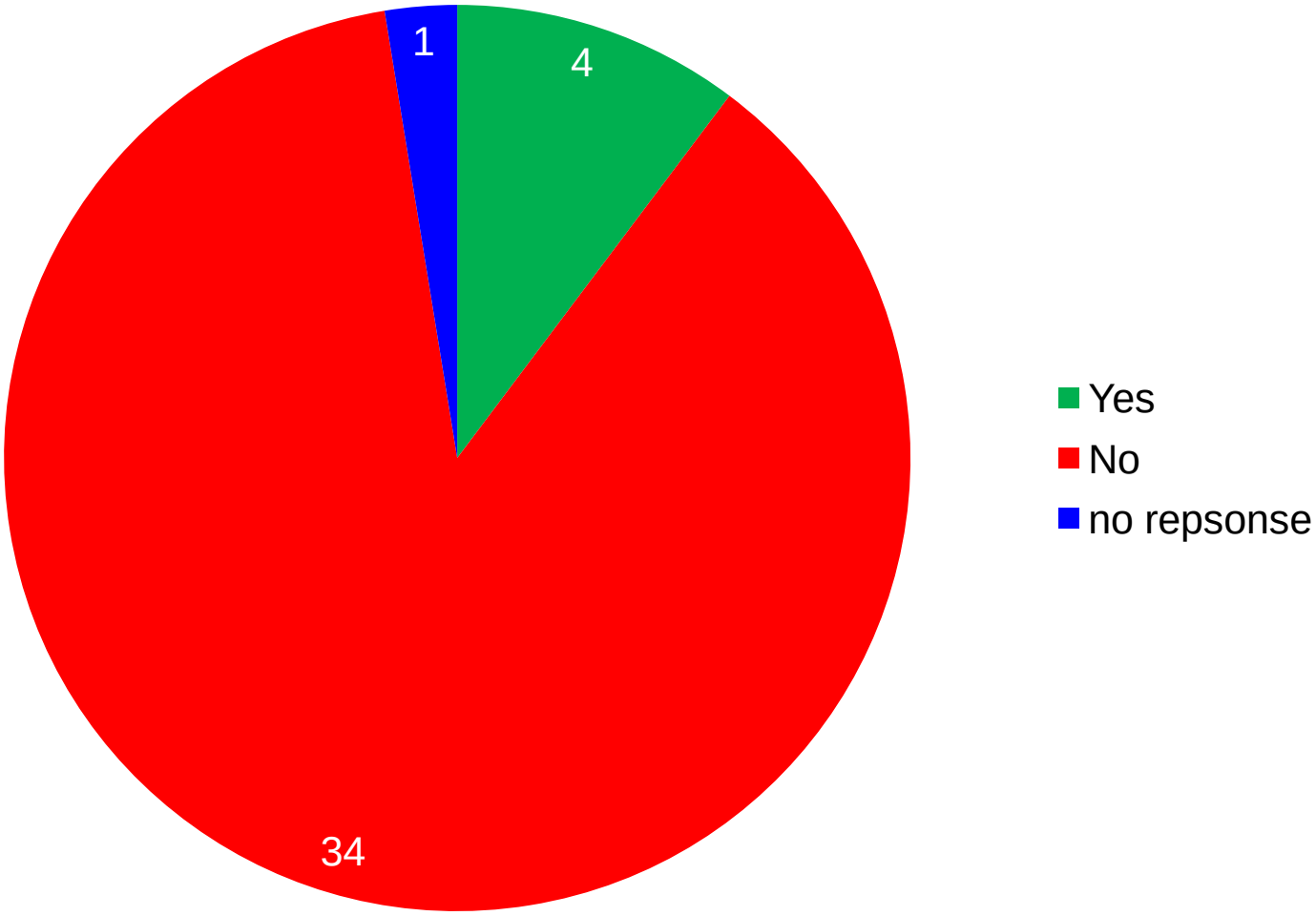
Pi-Peptides Detected by Enrichment and # of Pi's per Peptide



Y axis = # phosphorylations
X axis = peptide
Z = entry number


Method of Enrichment vs. # of Phosphorylations and By Peptide
1
2
3
4
phosphorylations - 1
phosphorylations - 2
phosphorylations - 3
phosphorylations - 4
Peptide
TLSD#YNIQK
TITLEVEP#SD#TIENVK
THILLFLPK#SVSDYEGK
SV#SDYEGR
NVAVDEL#SR
LVQAFQE#TDK
LVNEV#TEFAK
IF#SIVEQR
ES#TLHLVLR
EA#SLHLVLR
DQGGELL#SLR
DISL#SD#VK
DI#SL#SD#VK
DI#SLE#SD#VK
AELEALV#SK
ADEGH#SFR
#TLSDYNIQK
#TL#SDYNIQK
#TL#SD#YNIQK
#TITLEVEP#SD#TIENVK
#TI#TLEVEP#SD#TIENVK
Enrichment - ERLIC
Enrichment - IMAC
Enrichment - IMAC & TiO2
Enrichment - None
Enrichment - TiO2
ERLIC
IMAC
IMAC/TiO2
None
TiO2
5000
10000
15000
20000
25000
30000
35000
40000
Entry #

Did you or your lab receive more than one study sample?



Did you have knowledge of the results from another lab at anytime during this study?