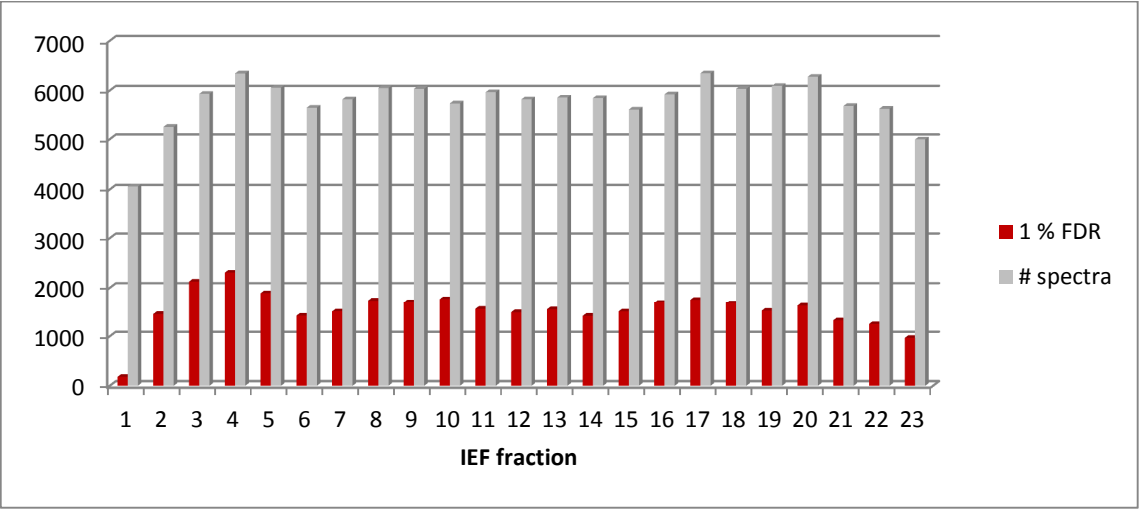
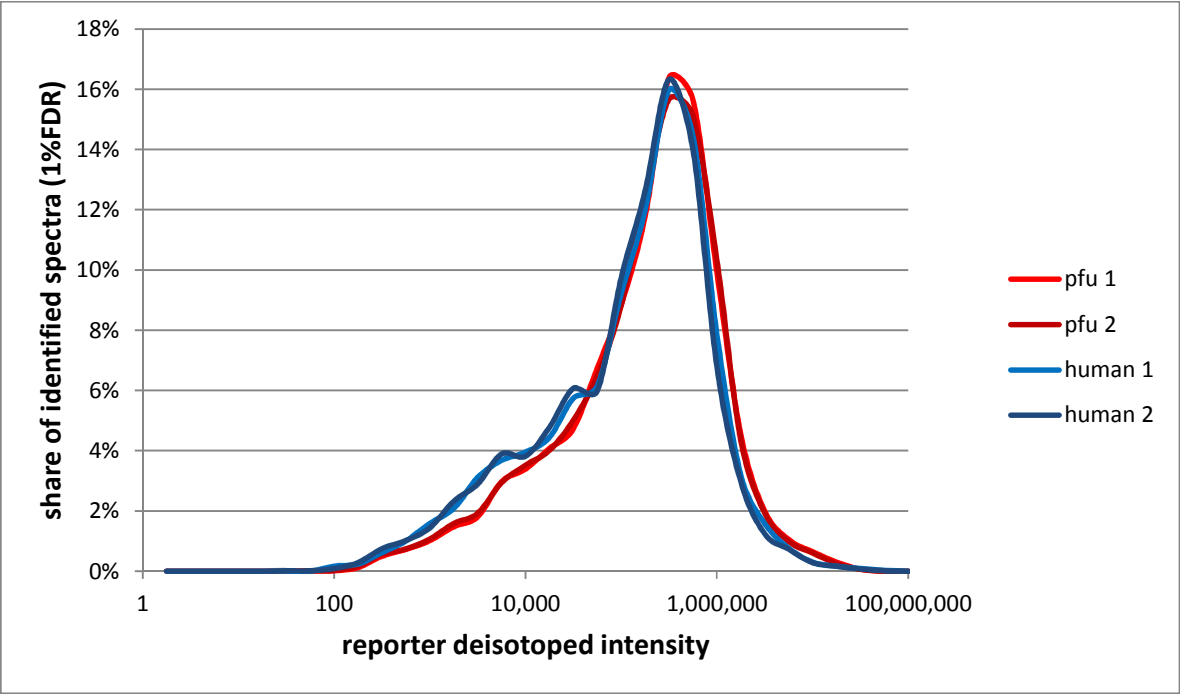


Supplementary Figures

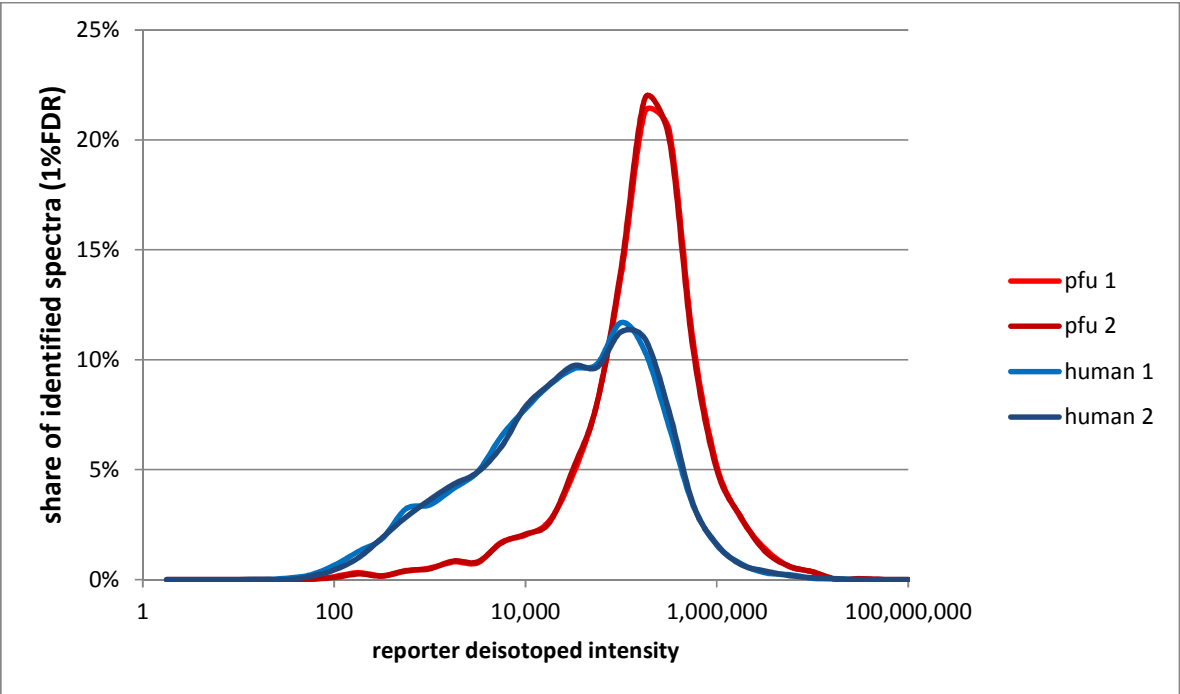
Supplementary figure 1:



Supplementary figure 2A:



Supplementary figure 2B:



Supplementary Figure Legends

Supplementary figure 1: **Identification rates in human platelet IEF fractions. Every IEF fraction was measured in a single run.** Fraction 24 is not shown, it was abandoned due to a lack of spectra.

Fractions 2 to 22 are potentially good samples, with more than 1,000 MS/MS spectra identified at 1% FDR among more than 5,000. Fractions 3, 9 and 20 were retained for their IEF distance and high number of identified spectra.

Supplementary figure 2: (A) The human (target) and pfu (decoy) samples cover the same range of reporter intensities as illustrated here by the distribution of human and pfu reporter deisotoped intensities measured in human and pfu spectra, respectively. The similarity of both distributions is mandatory for the quality of interference rate estimation. (B) presents a counter-example of an unsuccessful experiment (similar design to experiment 1, data not shown) where the human reporter deisotoped intensities in human spectra cannot be compared with the pfu reporter deisotoped intensities in pfu spectra. This deviation is most likely due to a pipetting imprecision. Both distributions were estimated in bin size of $0.25 \log_{10}$ of the intensity.

Supplementary Table

Supplementary table 1:

| Experiment | # MS/MS spectra | Species searched | Pride Accession | # matches at 1% FDR |
|----------------|-----------------|----------------------------|-----------------|---------------------|
| 1 | 24,641 | <i>Homo sapiens</i> | 27082 | 6,681 |
| | | <i>Pyrococcus furiosus</i> | 27083 | 7,583 |
| 2 | 17,804 | <i>Homo sapiens</i> | 27084 | 3,536 |
| | | <i>Pyrococcus furiosus</i> | 27085 | 7,269 |
| 3a replicate 1 | 11,615 | <i>Homo sapiens</i> | 27094 | 1,915 |
| | | <i>Pyrococcus furiosus</i> | 27096 | 2,433 |
| 3a replicate 2 | 11,399 | <i>Homo sapiens</i> | 27095 | 2,033 |
| | | <i>Pyrococcus furiosus</i> | 27097 | 2,266 |
| 3b replicate 1 | 22,078 | <i>Homo sapiens</i> | 27090 | 3,534 |
| | | <i>Pyrococcus furiosus</i> | 27092 | 4,257 |
| 3b replicate 2 | 22,299 | <i>Homo sapiens</i> | 27091 | 3,570 |
| | | <i>Pyrococcus furiosus</i> | 27093 | 4,458 |
| 3c replicate 1 | 21,593 | <i>Homo sapiens</i> | 27086 | 2,968 |
| | | <i>Pyrococcus furiosus</i> | 27088 | 4,243 |
| 3c replicate 2 | 21,625 | <i>Homo sapiens</i> | 27087 | 2,846 |
| | | <i>Pyrococcus furiosus</i> | 27089 | 4,198 |

Supplementary Table legend

Supplementary table 1:

This table presents a summary of the various datasets obtained in experiments 1 to 3. Every dataset was searched against a human human database and against a pfu database and validated at 1% FDR. The result of every search was uploaded in PRIDE and can be accessed using the corresponding accession number. The number of retained peptide to spectrum matches at 1% FDR is given in the last column for each identification set.