ANAQUANT | SOLUTIONS FOR BIOANALYSIS

LC-HRMS approach to identify and quantitate potential markers for pre-clinical and clinical applications in heterogeneous samples

Quentin ENJALBERT, PhD

Context – dermo pharma

- Define potential protein markers for pre-clinical and clinical trials to evaluate the effect of skin treatment
- D-Squame® tape samples from different patients (uppermost layer of the skin)
- Main issue: huge heterogeneity in samples
- 6 samples
  - Control patients
  - Treated patients

Objectives & strategies

- Objectives:
  - Define potential protein markers for pre-clinical and clinical trials to evaluate the effect of skin treatment

- Strategies:
  - Use LC-MS for potential biomarker identification
  - Perform a consistent sample comparison that might be applied to higher sample number.

What can be done in LC-MS?

- DDA or DIA for ID and relative quantification between samples

No standard - No quantitative evaluation – Difficult to compare samples

- Targeted for absolute quantitation

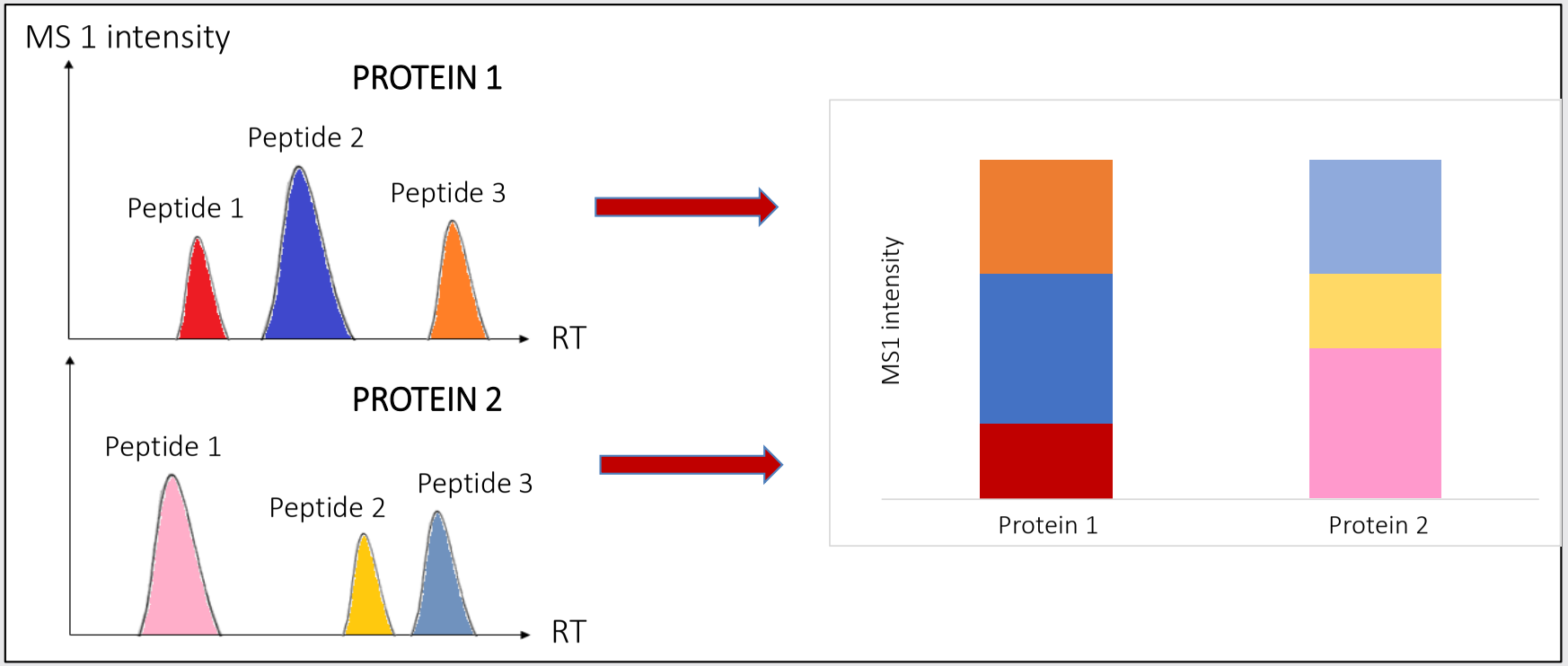
Need ID step first

Best of both world?

- Perform ID and quantitative evaluation in the same run

Top3 strategy in DDA

2 DIFFERENT PROTEINS WITH THE SAME CONCENTRATION IN MATRIX: SUM OF THE 3 BEST FLYER PEPTIDES IS CONSTANT (+/- 10% (*SILVA ET AL., 2006*))

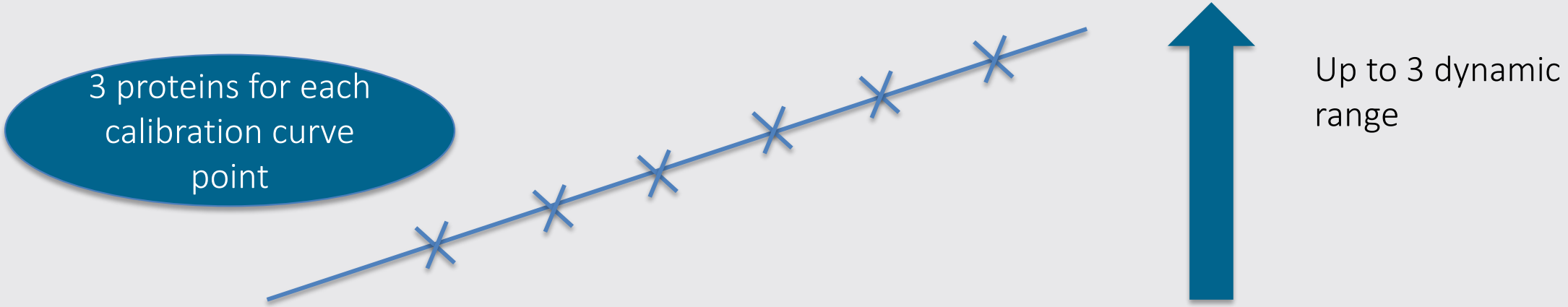


Universal relative quantification within a sample

Need of an Internal Standard for quantification evaluation

How to make a universal standard?

- Best universal standard?
  - Several proteins over a broad dynamic range



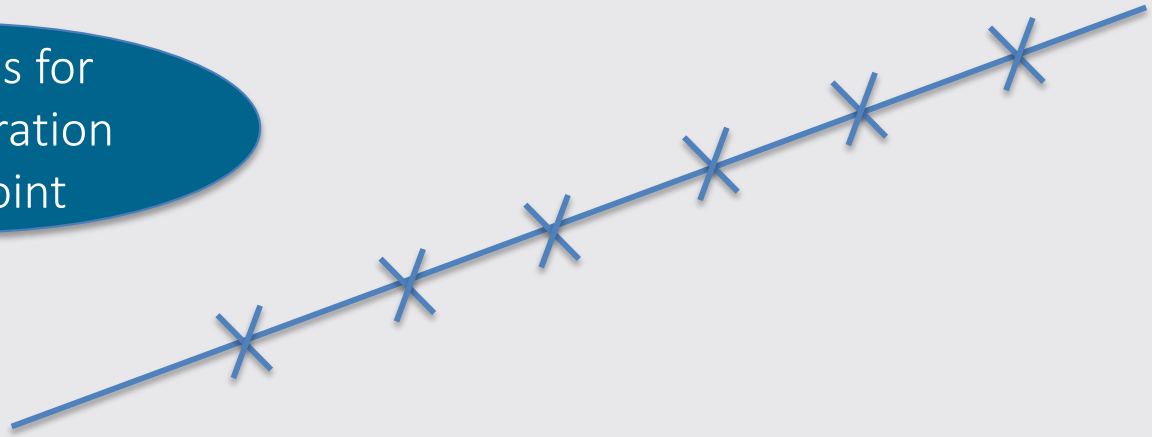
- Complicated to produce 18 calibrated proteins with different concentrations
- Batch-to-batch reproducibility
- Stability unknown

Move to peptide level?

How to make a universal standard?

- Move to the peptide level
  - Easier to produce
  - 3 peptides for each protein: 18 proteins → 54 peptides

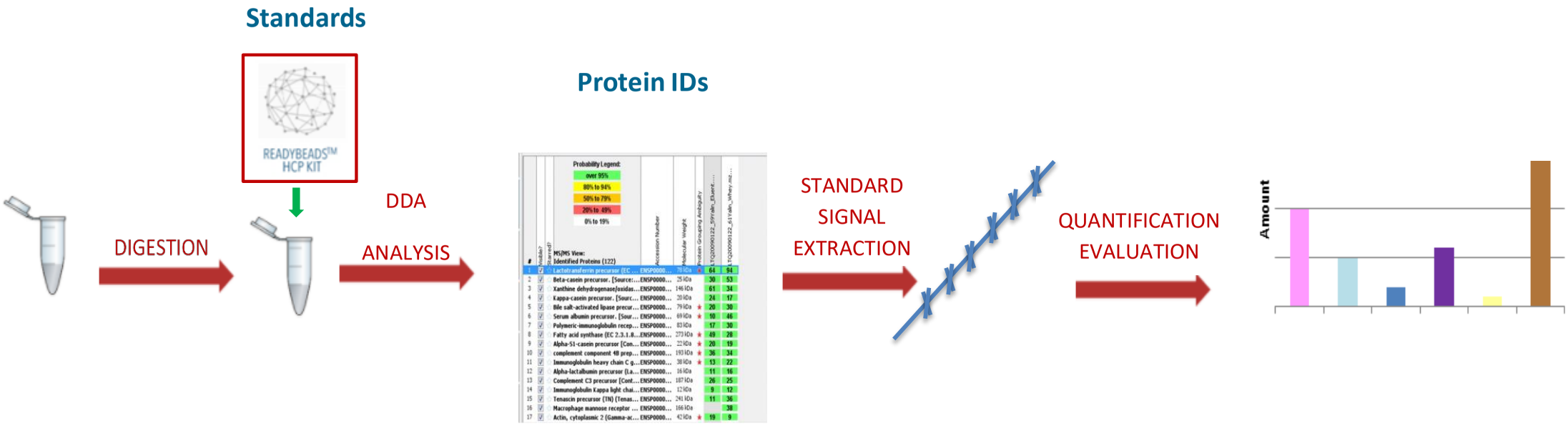
9 peptides for each calibration curve point



Up to 3 dynamic range

How to deal with 54 calibrated peptides over a dynamic range?
USE OF READYBEADS® TECHNOLOGY

Workflow with the Universal Quantification READYBEADS



Identification and quantification evaluation in a single analysis
Based on classic shotgun workflow

Identification results

- Sample prep done on 1.5 µg total protein amount
- Injection on nano-LC-Qexactive HF system (Thermo). Column acclaim pepmap, 0.75µm*50cm.
- 1 hour gradient.

	Number of identified proteins in 6 D-Squame® samples					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number of identified proteins with 2 peptide	161	133	324	365	267	71

Large heterogeneity between samples, even in a same group.
Difficult to perform classic normalization.

Quantification evaluation

- Independent and individual protein quantification evaluation for each sample

Proteins ID (Confidential)	Sample 1 (µg/mL)	Sample 2 (µg/mL)	Sample 3 (µg/mL)	Sample 4 (µg/mL)	Sample 5 (µg/mL)	Sample 6 (µg/mL)
A	0,002	ND	0,003	0,005	0,002	ND
B	0,008	0,001	0,015	ND	0,002	0,001
C	0,008	ND	0,006	0,005	0,001	ND
D	0,150	0,002	0,029	0,019	0,002	0,002
E	0,010	0,004	0,032	0,019	0,014	ND
F	0,025	0,008	0,045	0,044	0,007	ND
G	ND	0,001	0,016	0,025	0,003	ND
H	0,062	0,009	0,042	0,041	0,002	ND

Direct comparison possible thanks to the quantification evaluation assessment

Take-home messages

1. Protein ID and Quantification evaluation within the same LC-MS run.
   - In this case up to 361 proteins ID and with an estimated concentration within the sample
2. Intra and Inter sample quantification evaluation
3. Universal: can be applied to any taxonomy, compatible with DDA and DIA
4. Easy to use workflow
5. Sample comparison time independent