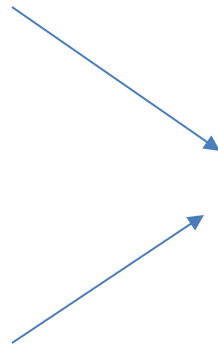
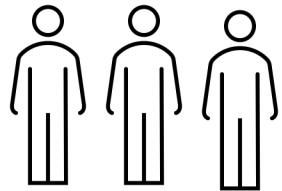
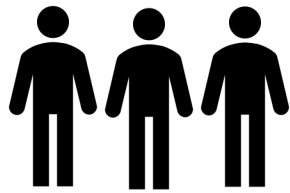


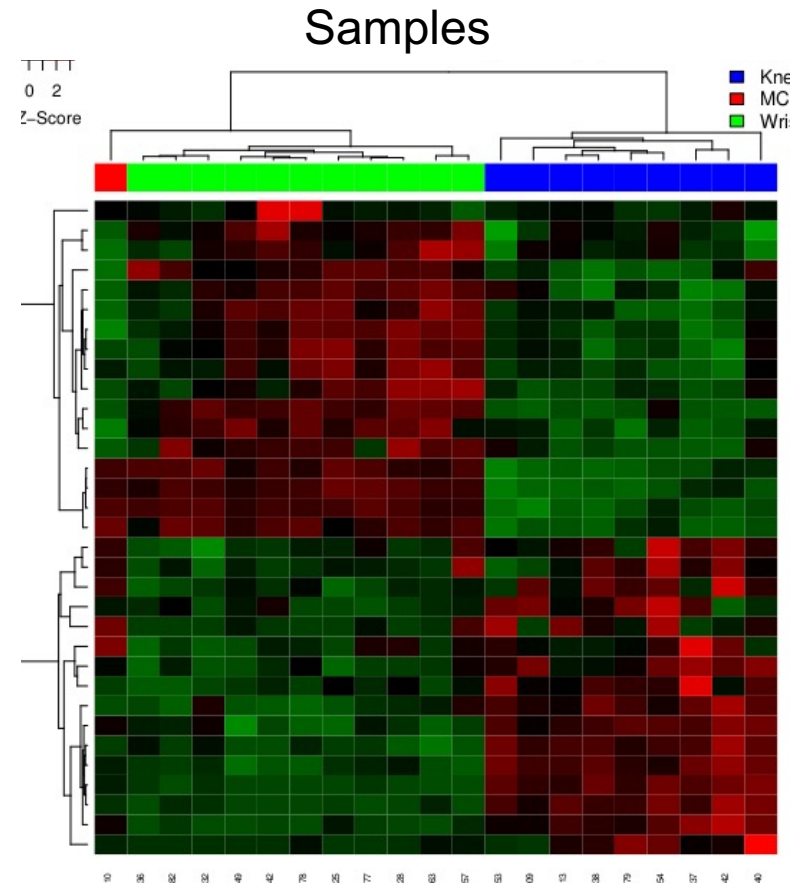
Peptide Quantification with Mass Spectrometry

Applications

- Compare protein quantity changes across two or more conditions to identify biomarkers.



Proteins

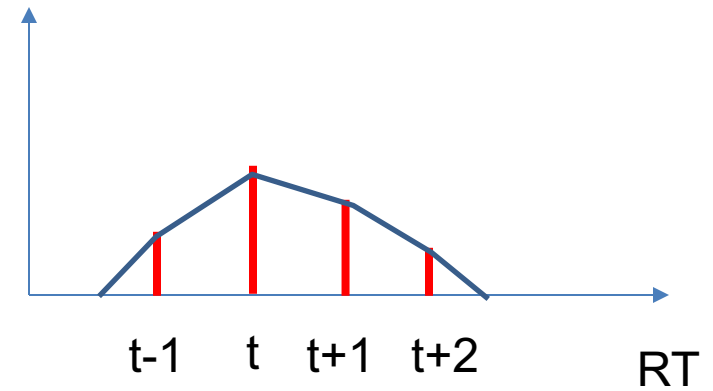
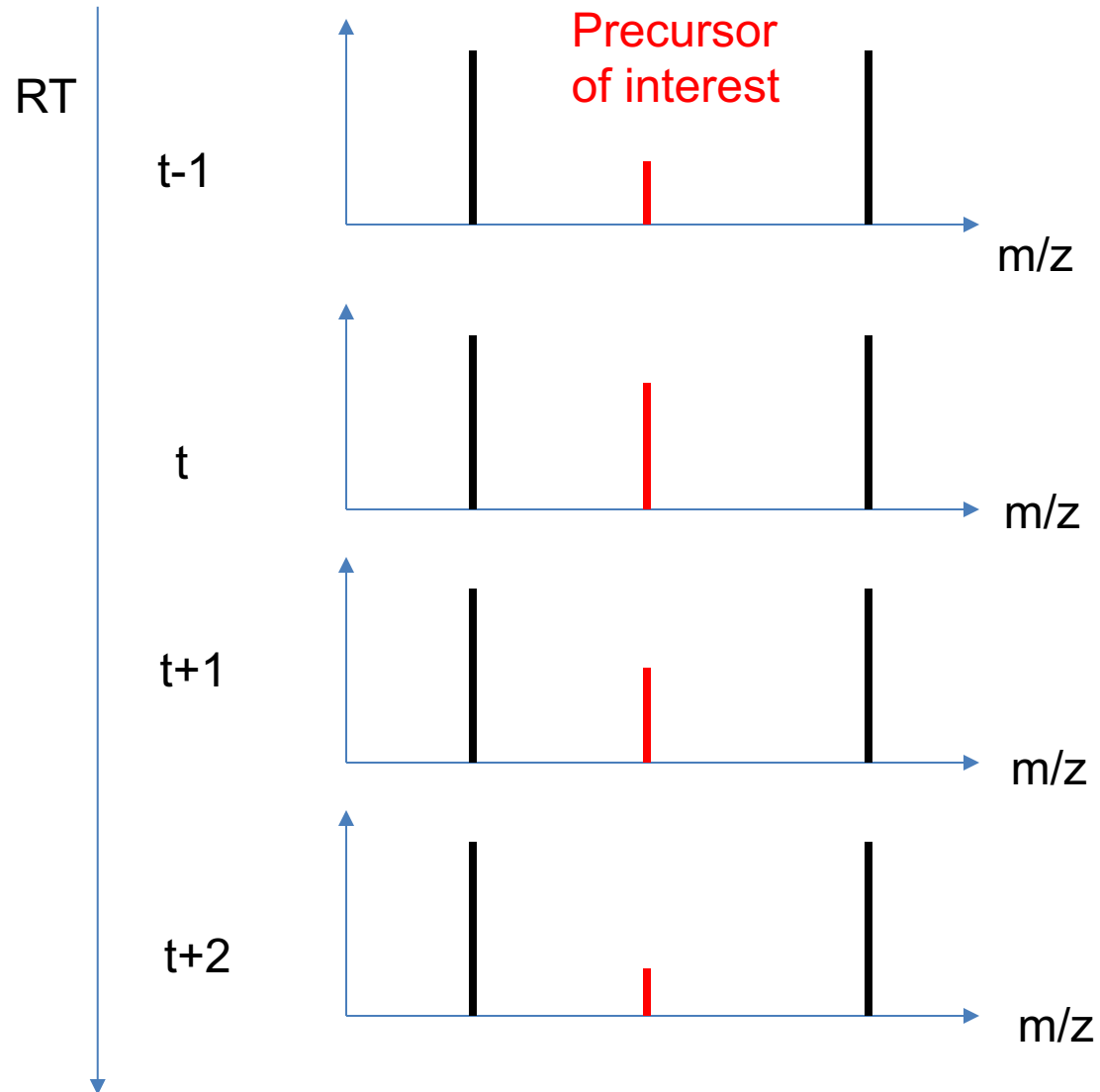


General Idea

- Mass spec is not a quantitative machine.
- Different peptides can have different efficiency for producing MS signals:
 - Peptide loss in the whole process
 - Ionization efficiency
- Signal intensity is the amount of ions arriving the detection and not the peptide quantity.
- But one can study the ratio of same peptide between two MS runs.

Label Free Method

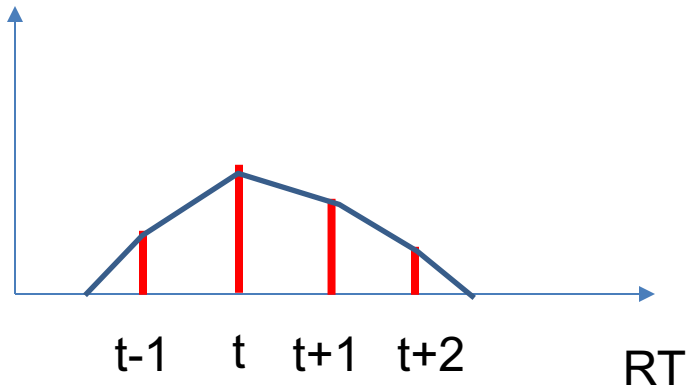
MS1 scans at different retention time



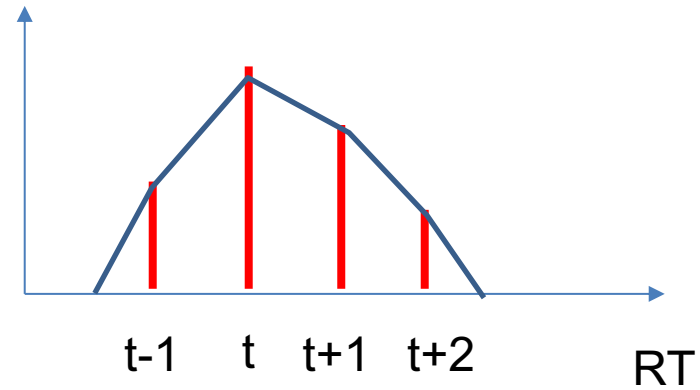
Peak area of the precursor ions

Label Free Method

Sample 1



Sample 2



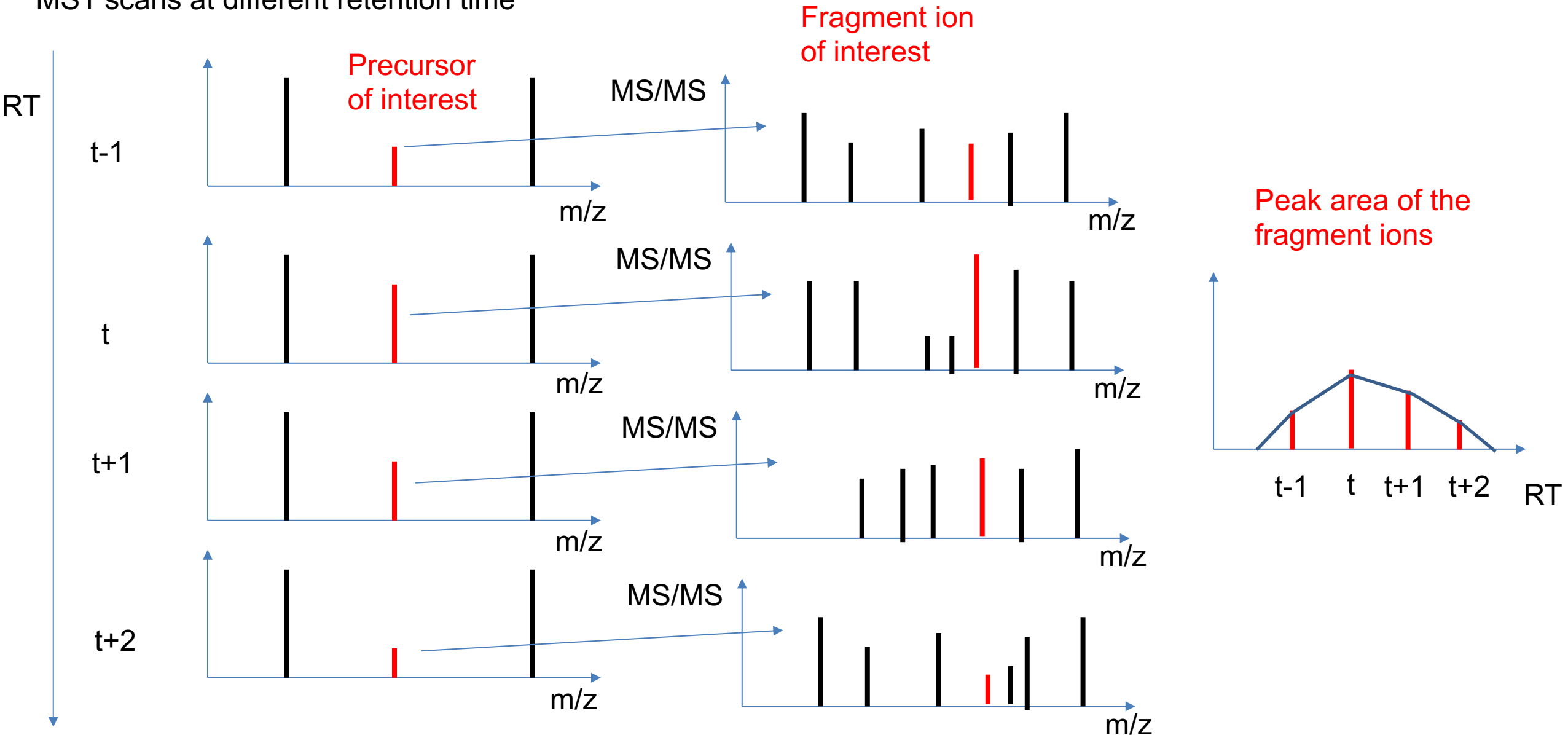
- Ratio between peak areas indicate the ratio between peptide quantity.
- Normalization may be needed to reflect the sample loading deviation.

Label Free Method

- In DDA (Data-dependent acquisition) mass spec, MS1 scans are periodically collected for precursors. MS2 scans are acquired to identify the peptides for the precursors.
- DDA on two protein samples. Identify the peptides. For each peptide identified in both samples, use their precursor peak area ratio to measure the peptide quantity change.
- Often a housekeeping protein (or a spiked-in protein) is used as the normalization factor to correct the sample loading error.

SRM (Selected Reaction Monitoring) Method

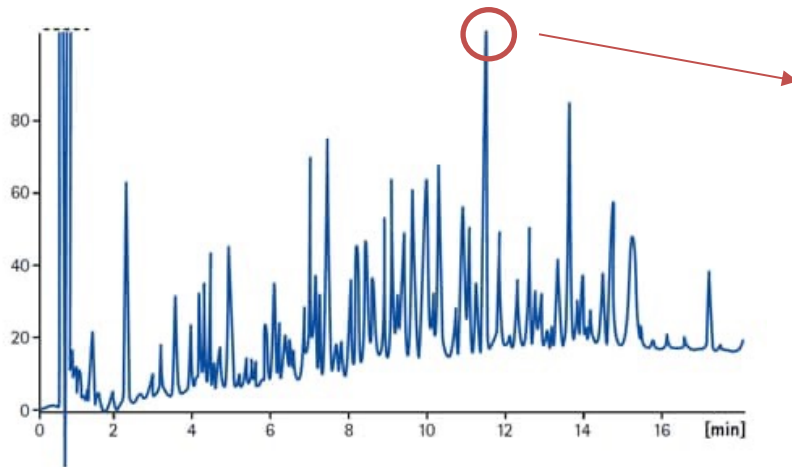
MS1 scans at different retention time



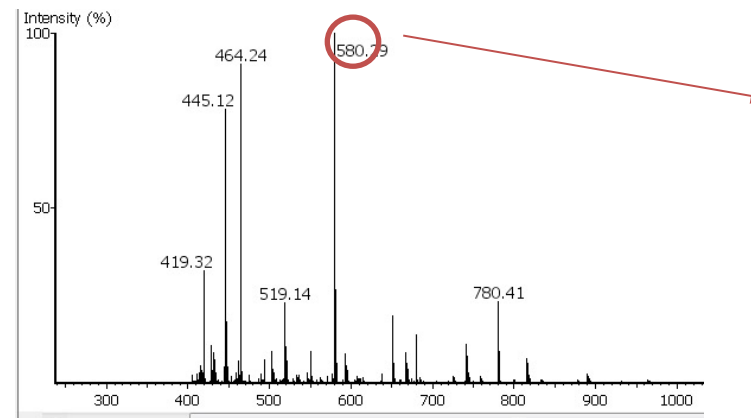
SRM and PRM

- Label free quantification with MS1 can be easily interfered by coeluting peptides with the same m/z .
- SRM is more specific. Interference signal is only from coeluting peptides with the same precursor m/z and the same fragment ion m/z .
- But it quantifies fewer peptides because it requires many MS2 scans to quantify one peptide.
- But this increases sensitivity too.
- But it requires the prior knowledge about which precursor and fragment ions to monitor.
- If multiple fragment ions are monitored simultaneously, it's called the PRM (Parallel reaction monitoring).
- SRM and PRM are sometimes called the targeted method.

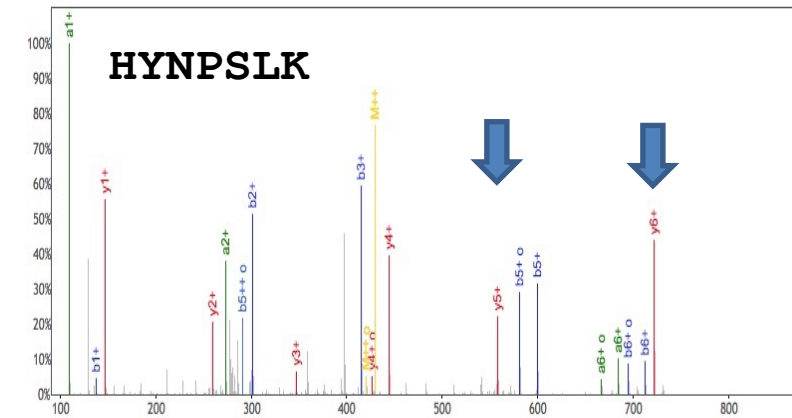
Inference to SRM/PRM Is Minimized



LC separates the peptides according to retention time (RT).



Co-eluting peptides are further separated by their mass-to-charge (m/z) in MS.



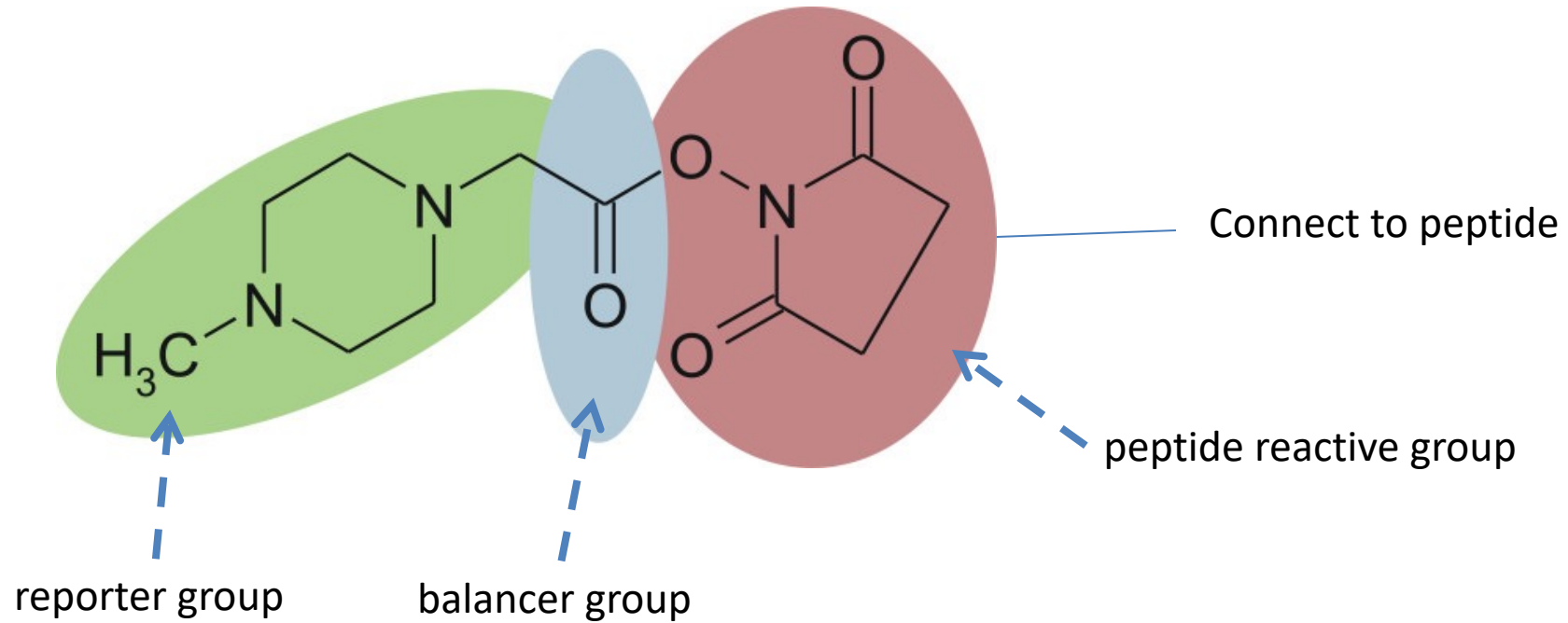
Peptides with both the same RT and m/z are further fragmented into fragment ions. Fragment ions specific to the target peptide are used to determine the quantity.

Such 3-tier separation ensures the specificity of the peptide quantification method.

Stable Isotope Labeling Method

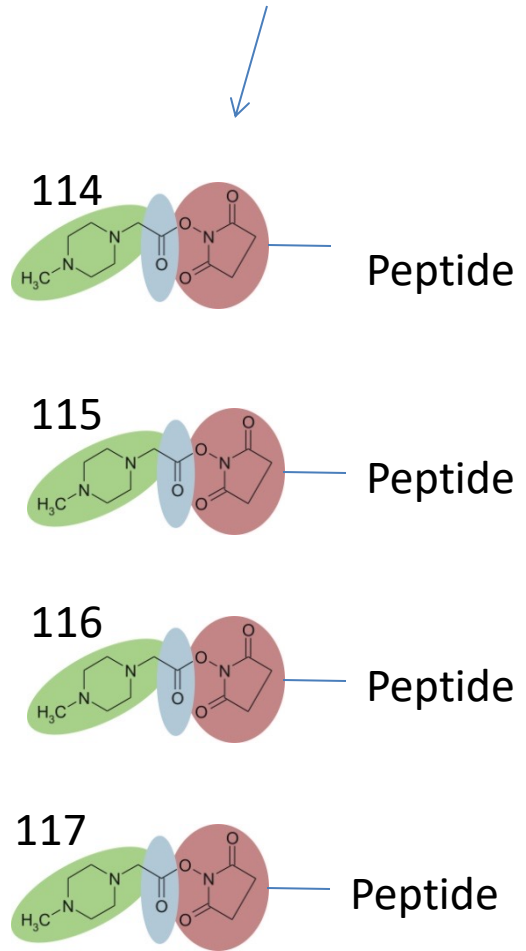
- Atoms have isotopes. E.g. C has C-12 and C-13. They differ by 1Da.
- The same peptide from two samples are labeled with different isotopic reagents, and mixed together for a single MS run.
- Use the mass difference to know which peptide is from which sample.
- Many proposals: ICAT, iTRAQ, SILAC
- We use iTRAQ as an example.

ITRAQ

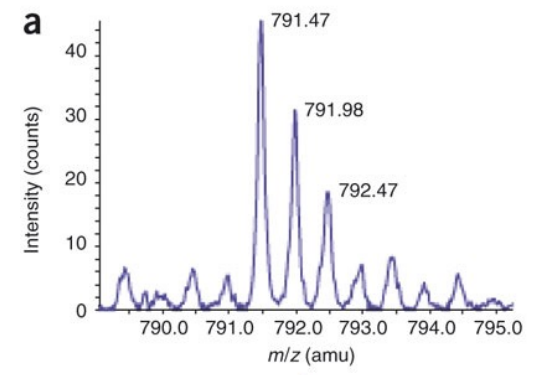


The four available tags of identical overall mass vary in their stable isotope compositions such that the reporter group has a mass of 114–117 Da and the balancer of 28–31 Da. The fragmentation site between the balancer and the reporter group is responsible for the generation of the reporter ions in the region of 114–117 m/z.

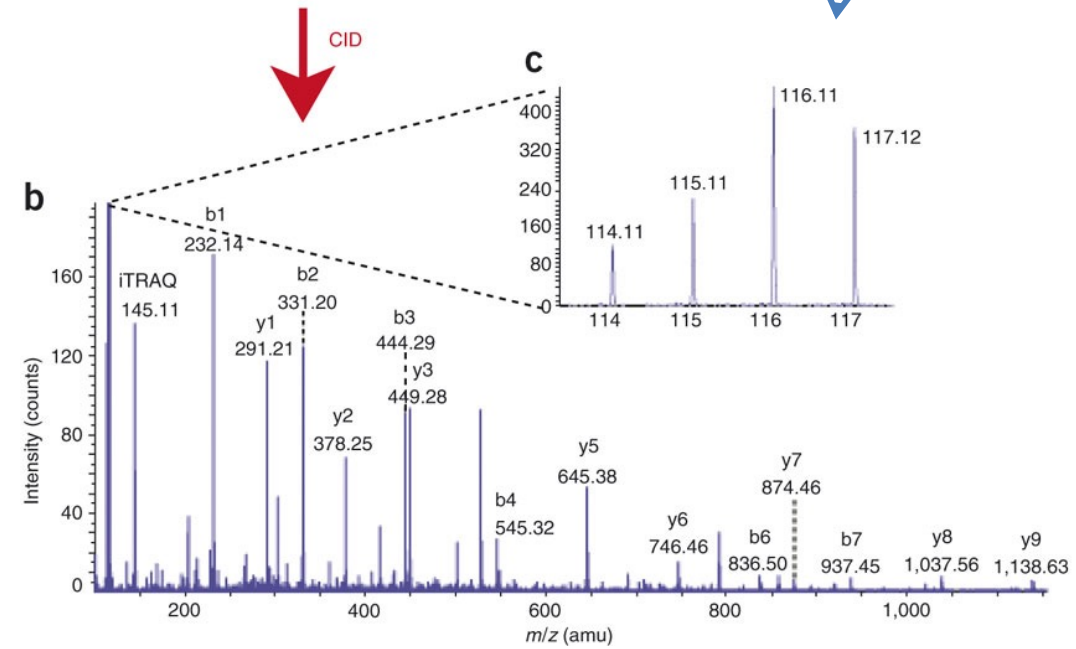
From four different samples.



Same precursor with four differently labeled peptides.



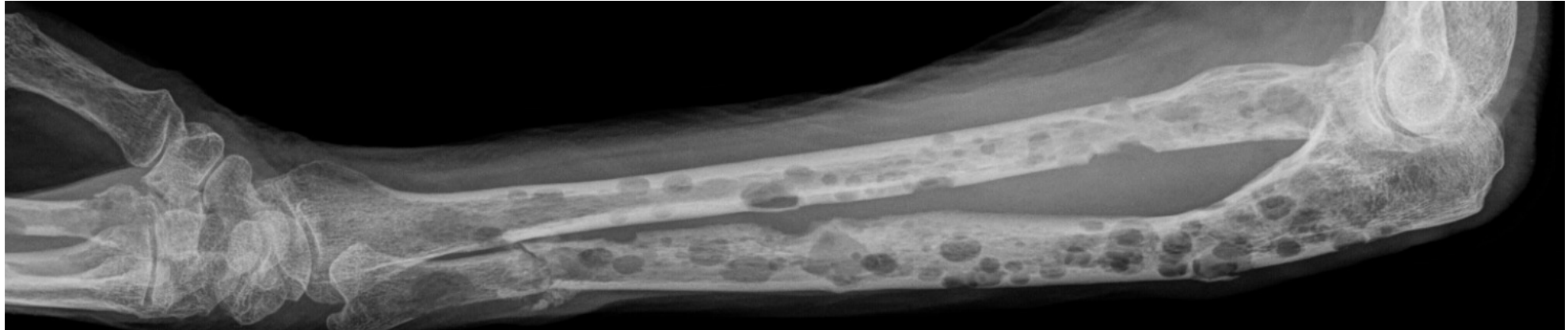
But they produce different fragment reporter ions.



Labeling based Method

- Pro: Samples are measured in the same MS run. Therefore, more accurate – no run-to-run bias.
- Cons: Labeling efficiency, error and cost.

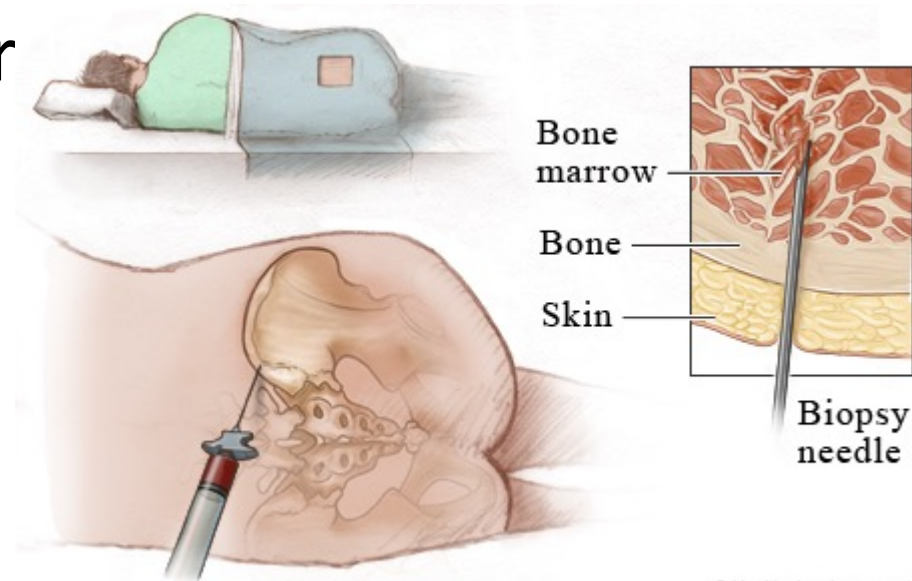
Multiple Myeloma (MM)



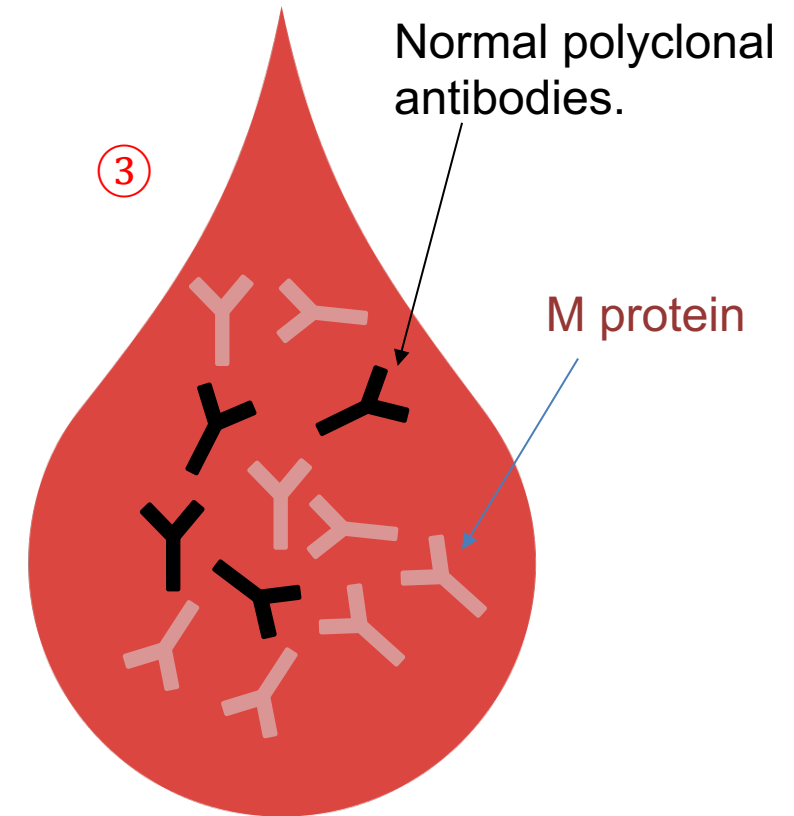
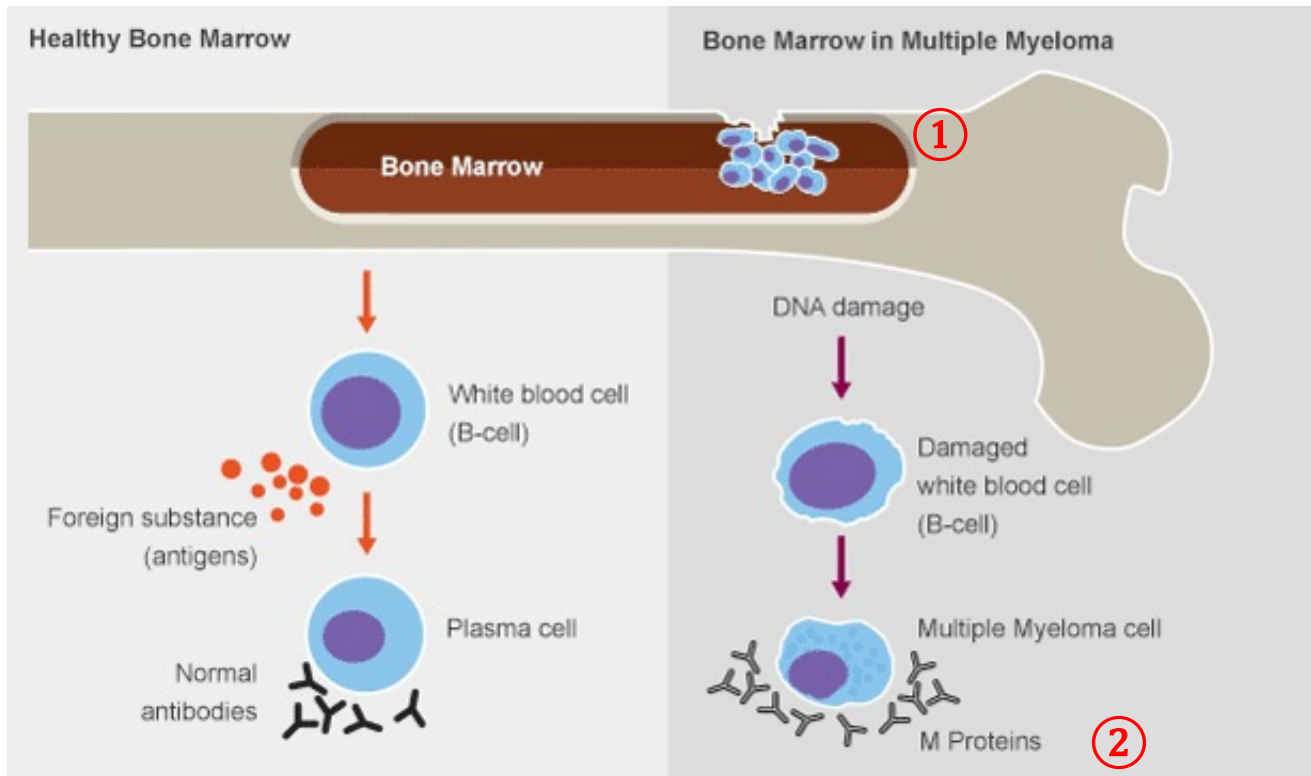
- Cancer derived from the plasma cells (therefore a blood cancer)
- Growing in bone marrow.

Multiple Myeloma Facts

- Plasma cell cancer growing in bone marrow.
- 30,000 new patients each year in US.
- Lifetime risk 1 in 143.
- Patients in remission requires periodic bone marrow aspiration to detect cancer



M-Protein

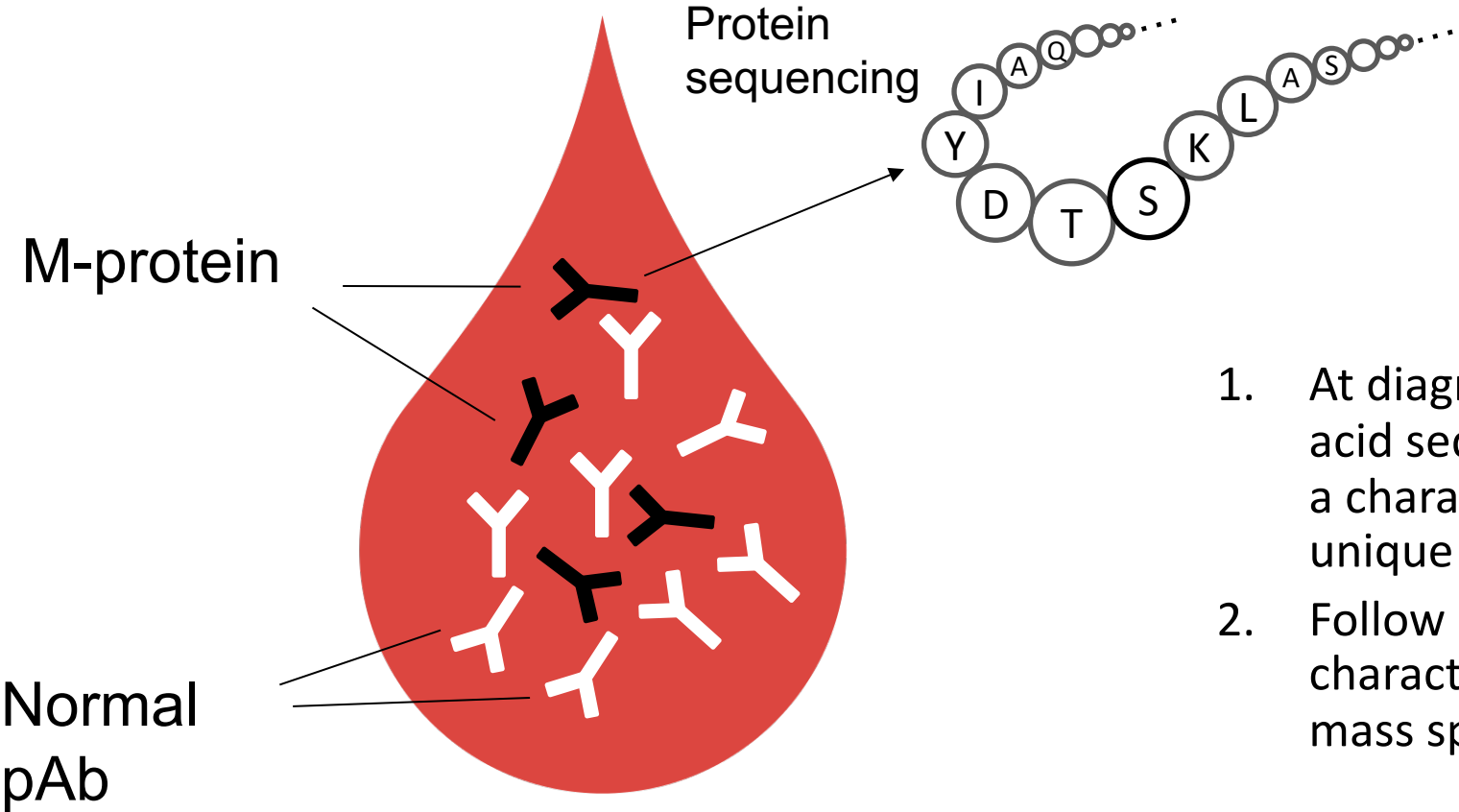


- ① Myeloma resides in bone marrow.
- ② Myeloma produces a monoclonal antibody, known as the M protein.
- ③ M protein circulates in blood, making it a perfect marker for non-invasive measurement.

M-protein hard to detect

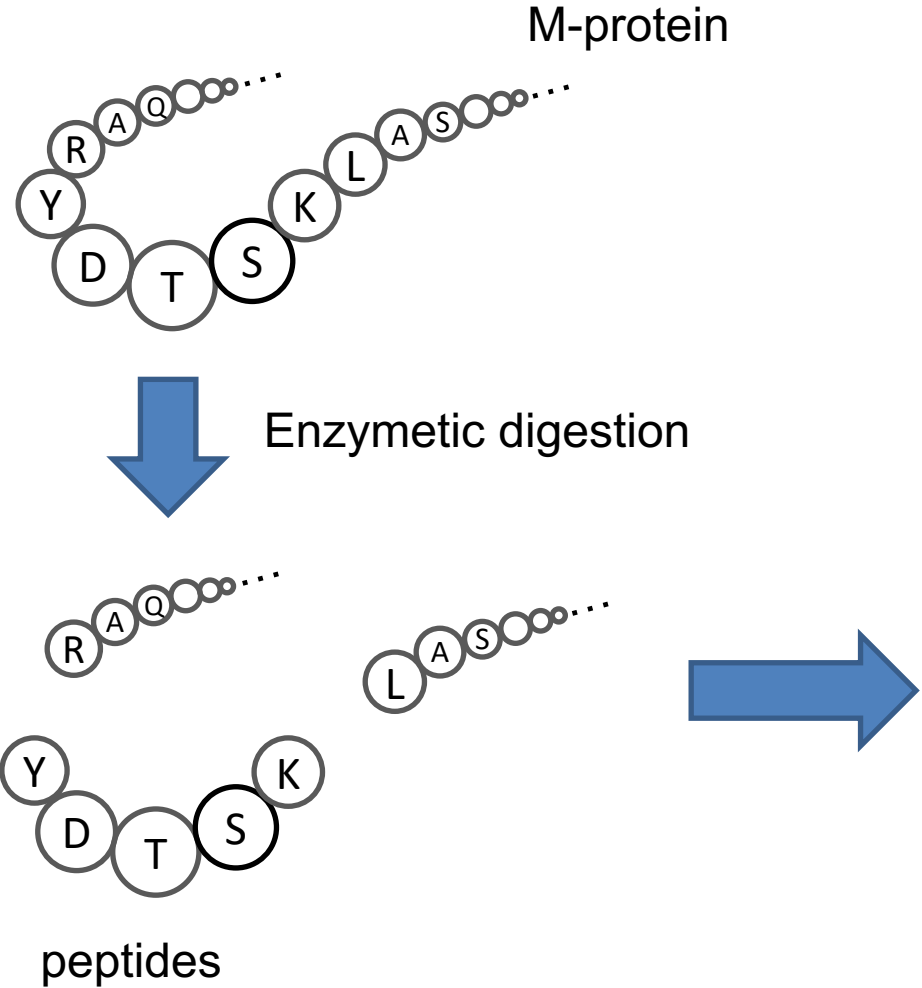
- M-protein (usually) is just a usual antibody.
- It is just one of many thousands of different clones of antibodies circulating in blood. These antibodies differ at their sequences, but are very similar in shapes.
- With some of the traditional methods, M-protein can only be realized when it is highly abundant - more than the total of all other antibodies.
- After treatment, it becomes too low to be detected.
- Such MRD (minimal residual disease) causes relapse.

EasyM's Solution



1. At diagnosis: Determine the amino acid sequence of the M-protein, pick a characteristic peptide that is unique to the M-protein.
2. Follow up: Measure the characteristic peptide with targeted mass spectrometry experiment.

LC-MS/MS



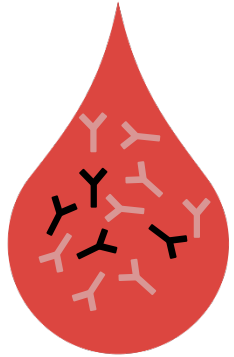
Liquid Chromatography



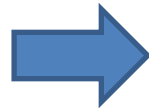
Mass Spectrometer

Rapid Novor's Platform

At diagnosis:
M protein dominates



100ul blood

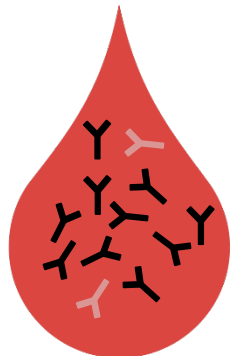


QVTLRESGPALVKPT
QTLTLCTFSGFSLST
AGMSVGWIRQPPGKA
LEWLADIWDDKKHY
NPSLKDRLTISKDTSK
NQVVLKVTNMDPADT
ATYCARDMIFNFYF
VWGQGTTVTVSSAST
M protein's
sequence

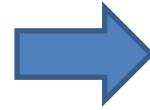


Personalized mass spec
SRM/PRM assay
targeting the M protein's
unique peptides.

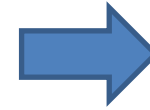
After treatment:
M protein under
other methods' detection limit.



50ul blood

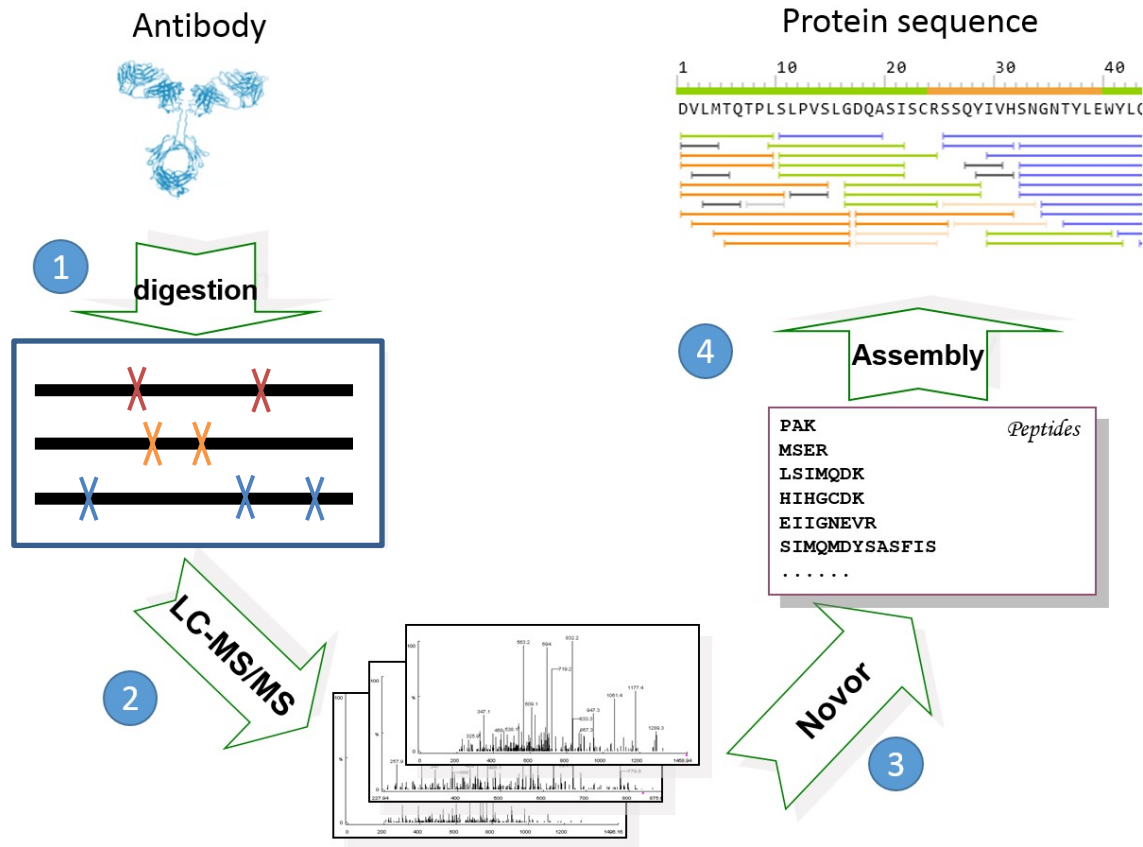


Mass spectrometer



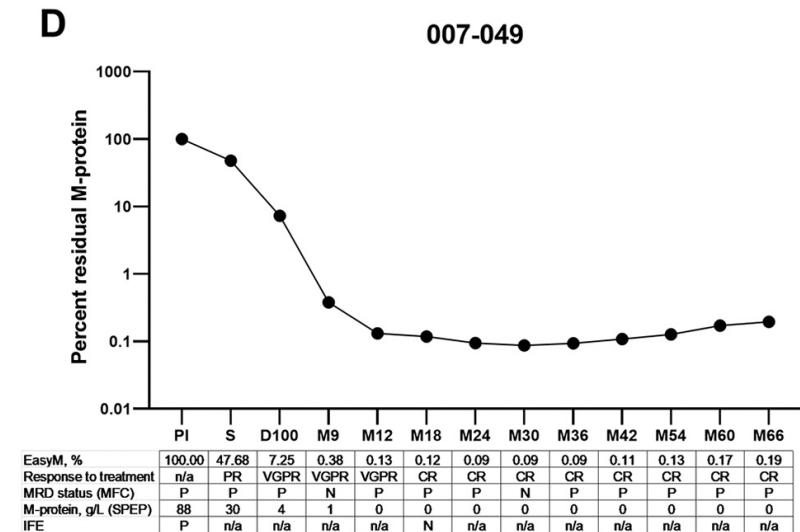
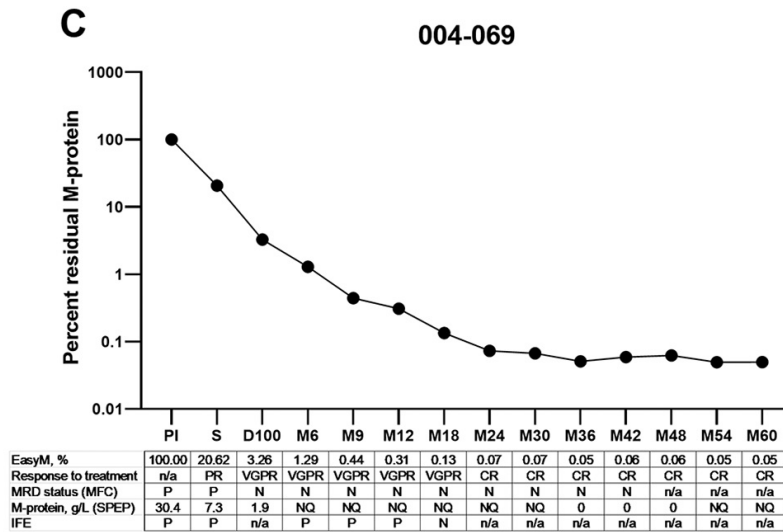
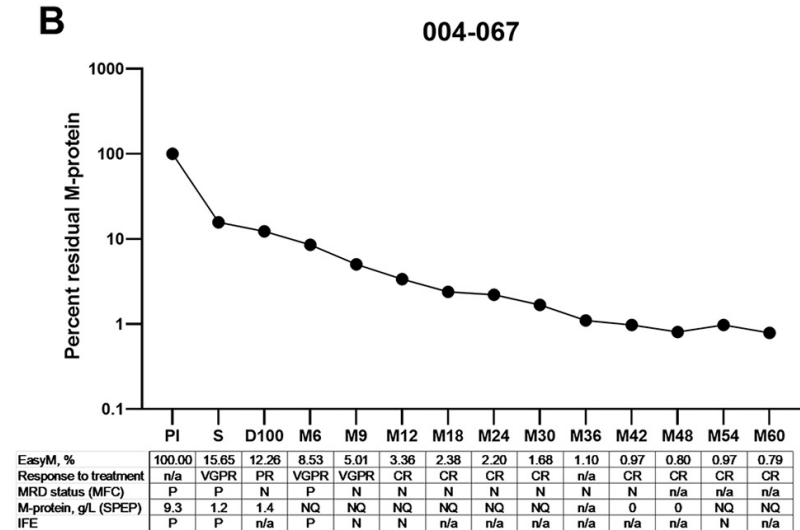
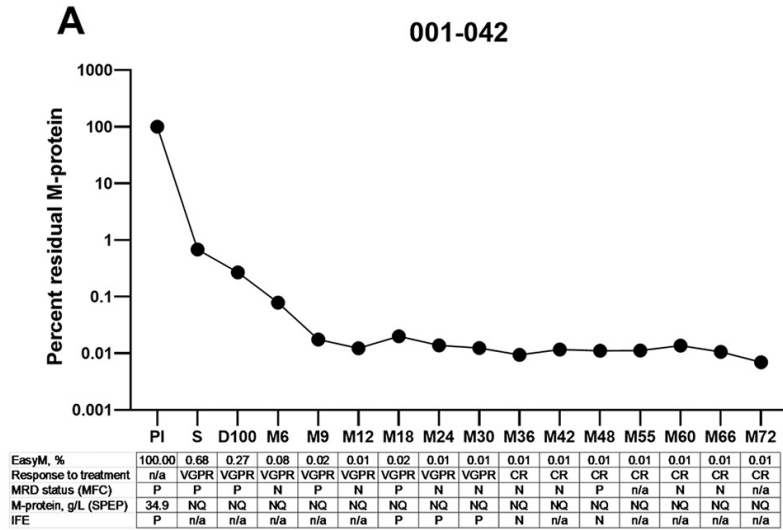
Quantity of the M protein as an
indicator of tumor burden.

Protein Sequencing with REmAb™

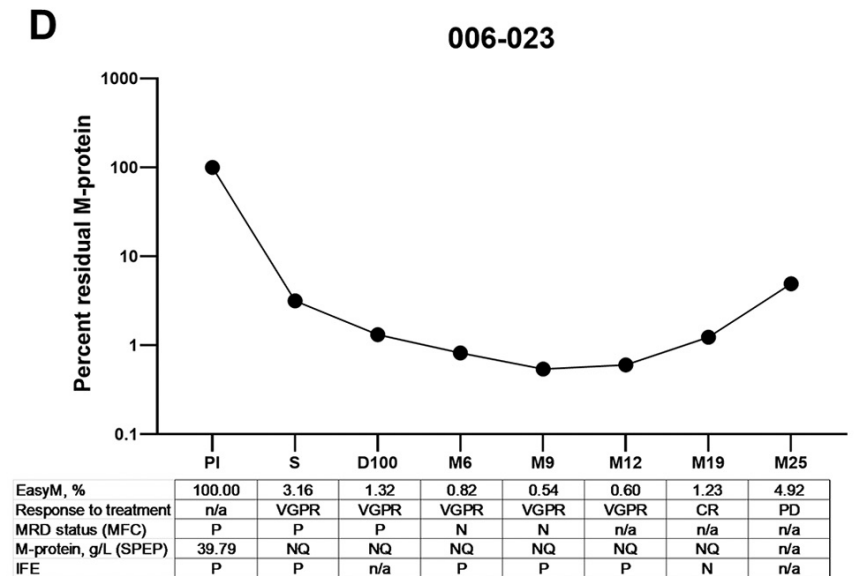
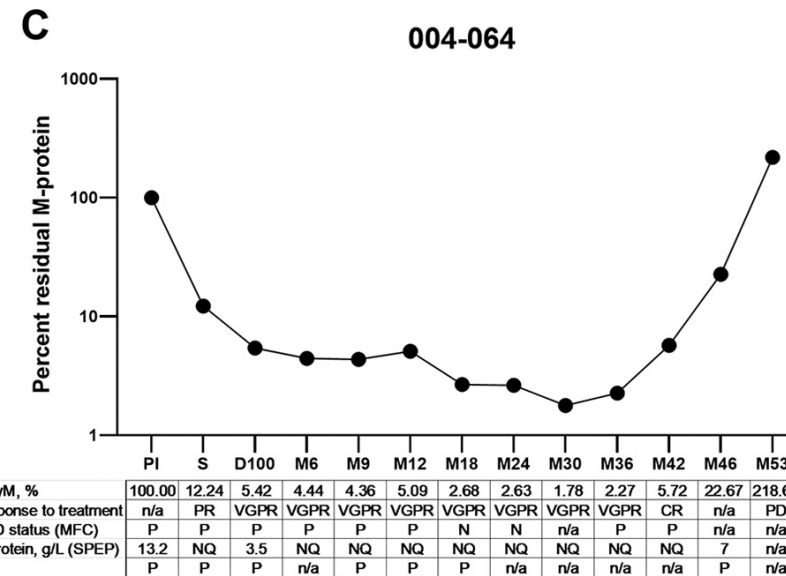
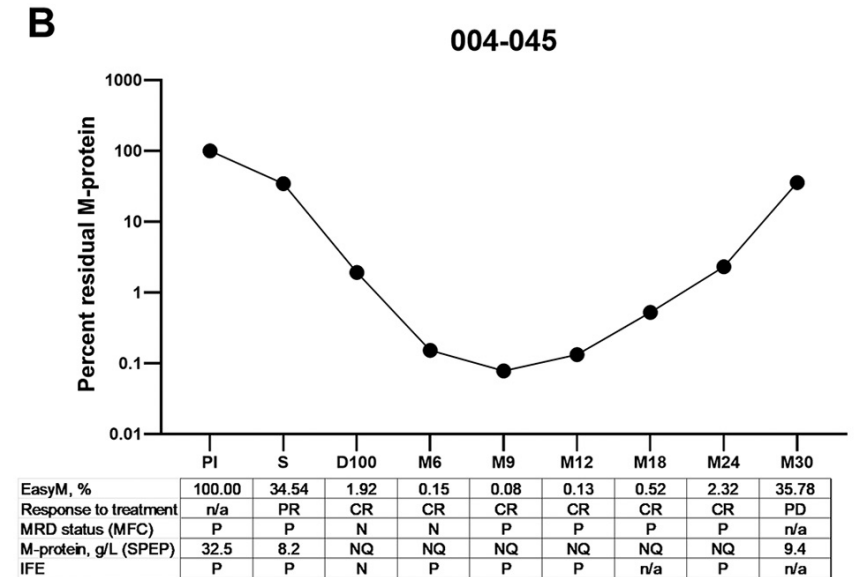
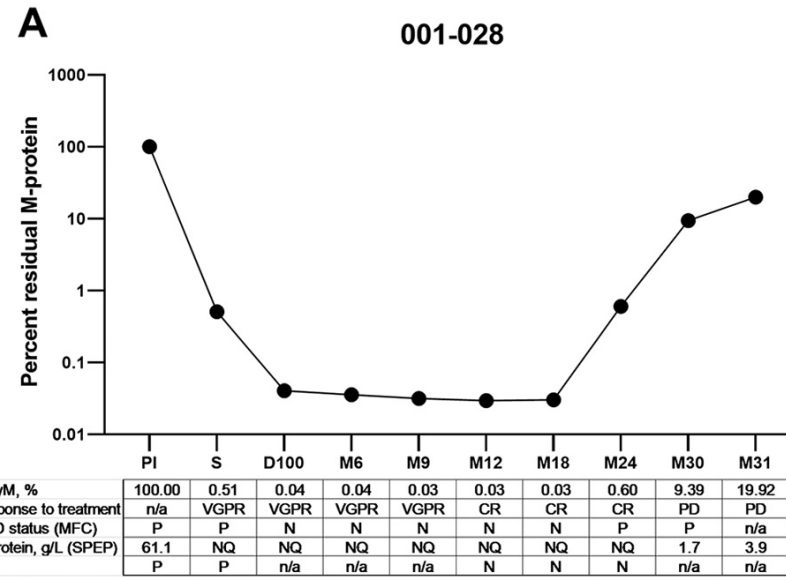


1. Digest with multiple enzymes
2. LC-MS/MS
3. Peptide de novo sequencing
4. Sequence assembly

Patients with no relapse



Patients relapsed



EasyM - Mission Accomplished

- Blood based. No need for bone marrow aspiration.
- 1000 times more sensitive than other blood based method.
- Detect relapse about half year earlier than conventional methods.