

CIRME

November 27 2012

Milano

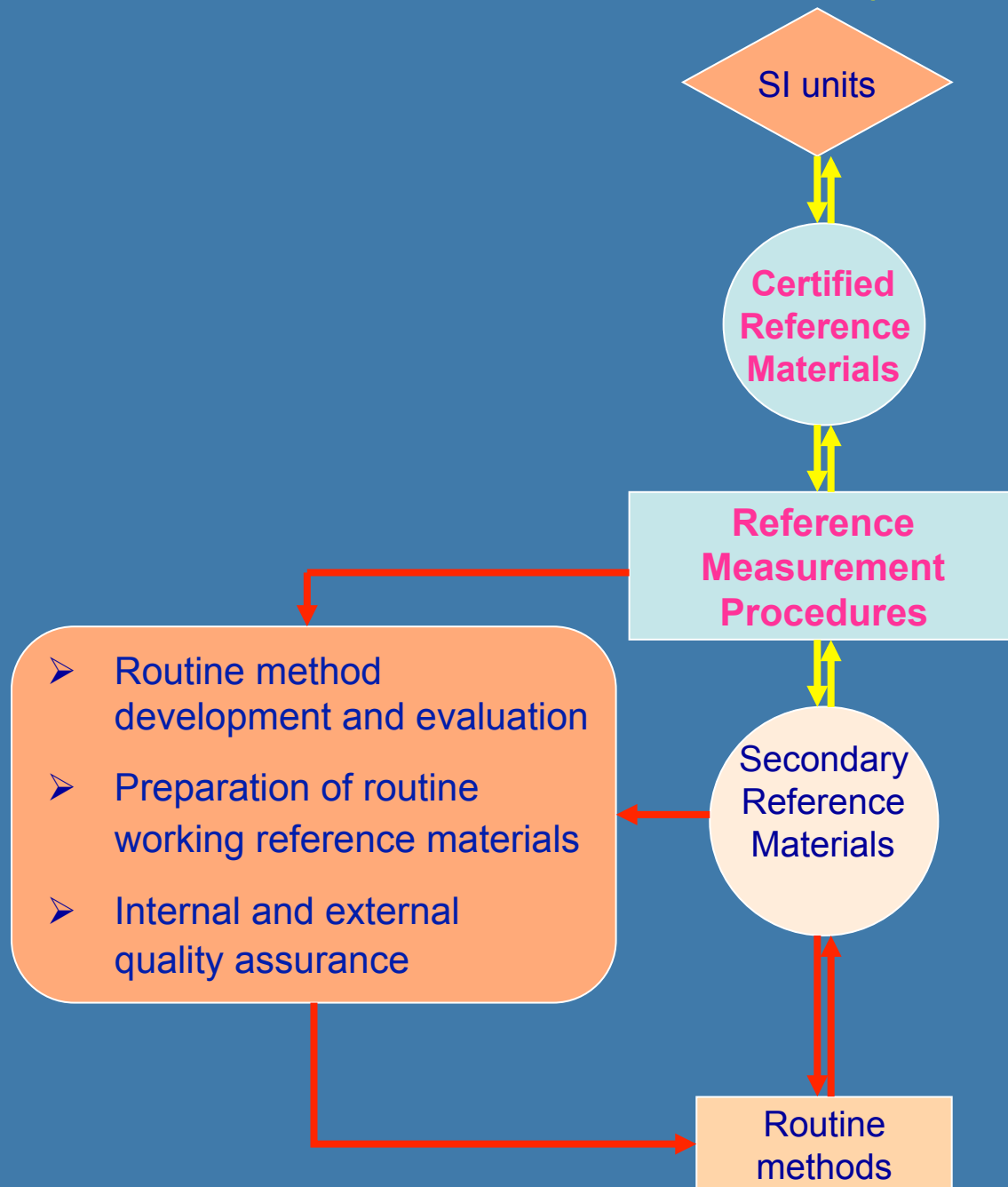
IDMS

**reference measurement procedures
for minor hemoglobins**

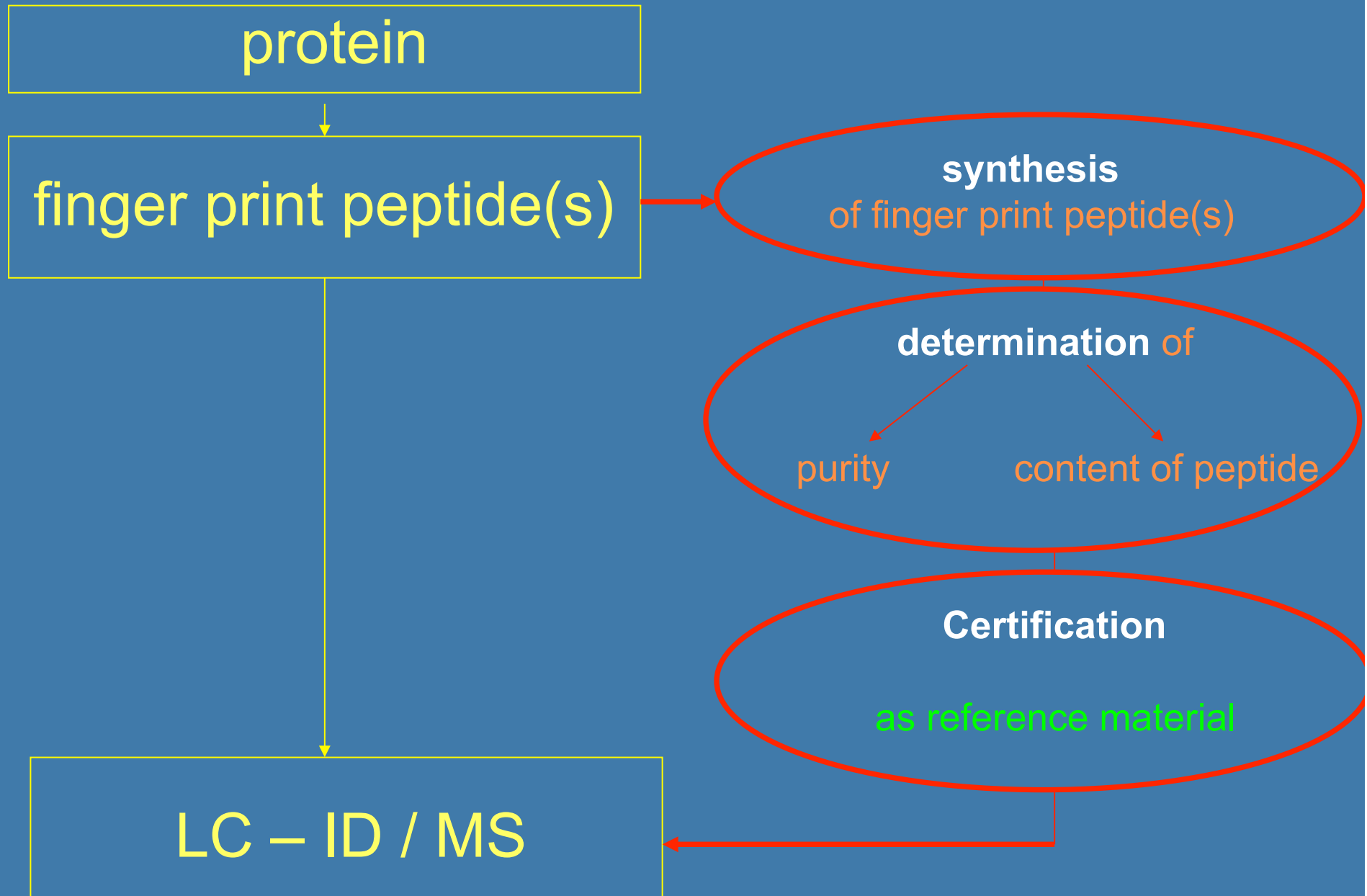


Dr. Patricia Kaiser
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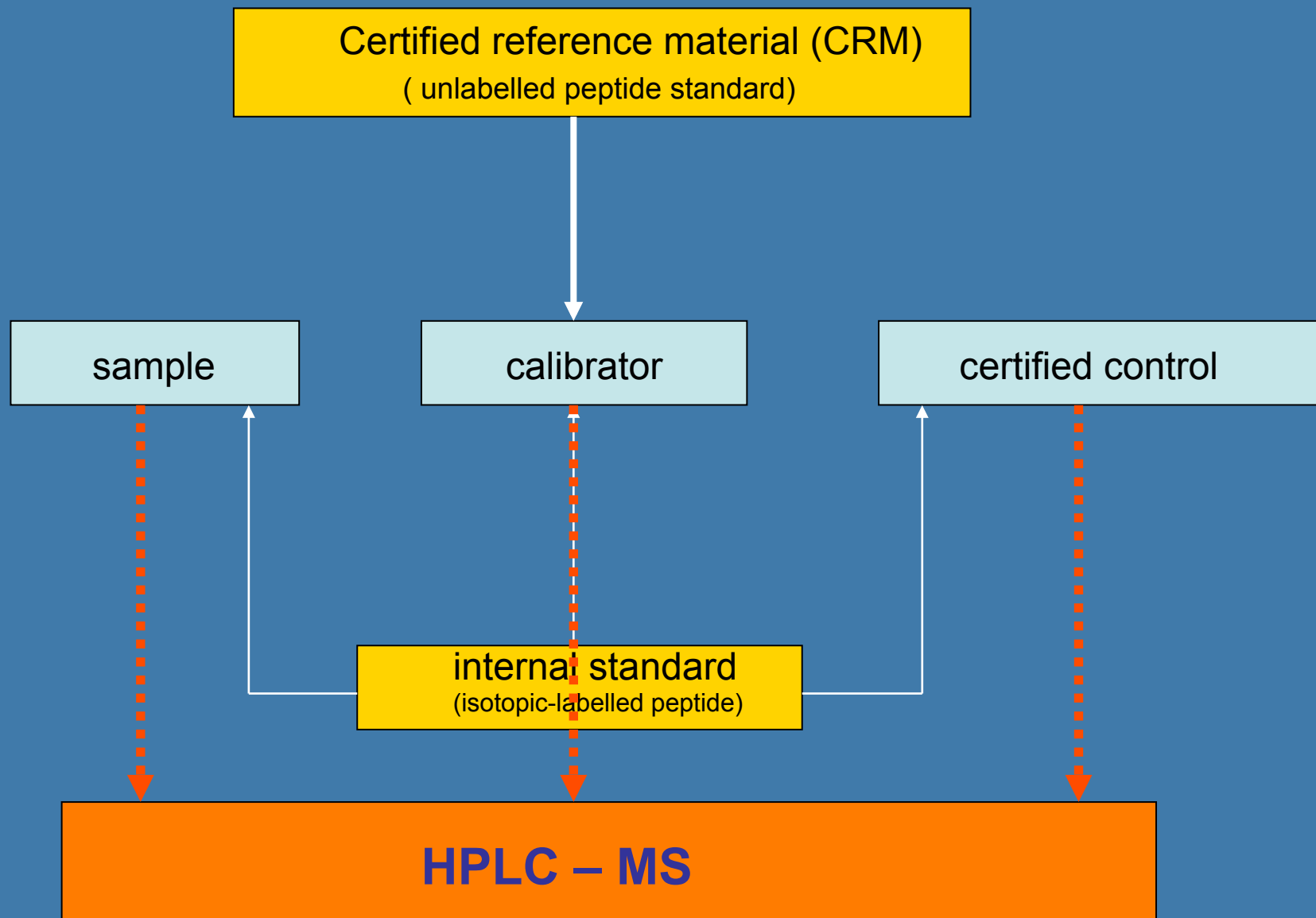
Traceability chain



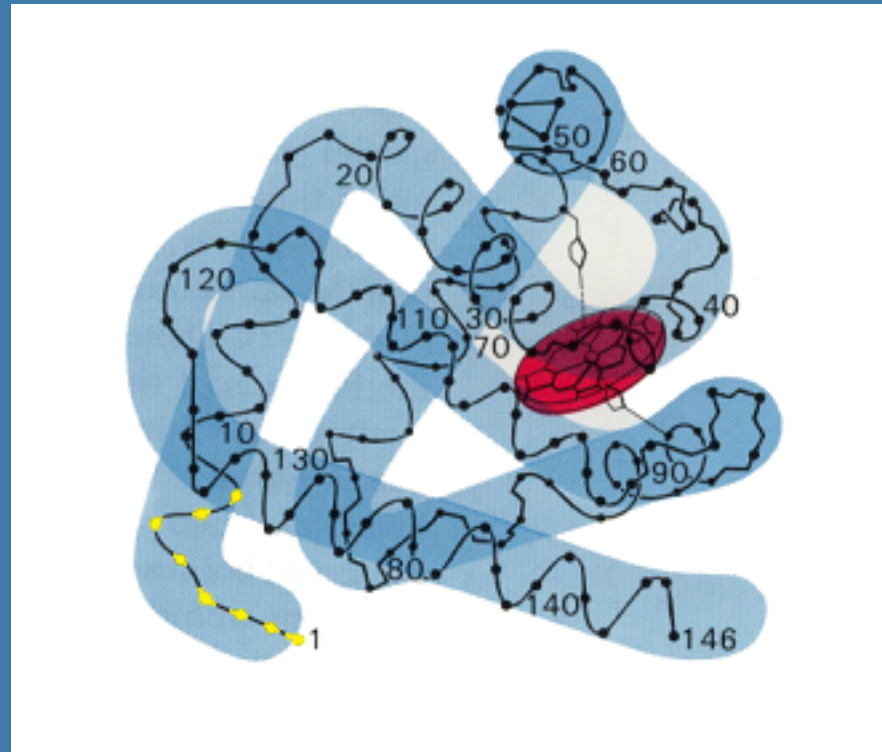
Quantification of proteins by peptide mapping



Isotope-dilution mass spectrometry



β -chain of haemoglobin



HbA_{1c}
 $\alpha 2\beta 2$

HbA0	Val – His – Leu – Thr – Pro – Glu – Glu – Lys –
HbA1c	Gluc – Val – His – Leu – Thr – Pro – Glu – Glu – Lys –

1. Preparation of synthetic peptides

HD0

(VHLTPE = non-glycated β -N-term. hexapeptide)

GD0

(1-Deoxyfructosyl-VHLTPE = glycated β -N-term. hexapeptide)

HD7

(VHXTPE with X = Leu (Isopropyl-D7))

GD7

(1-Deoxyfructosyl-VHLTPE with X = Leu (Isopropyl-D7))

2. Purification of the synthetic peptides

by preparative HPLC

3. Determination of the purity of the non- labelled peptides HD0 and GD0

by HPLC - MS

4. Determination of the content of peptide in HD0 and GD0 stock solution

by HPLC – ID / MS

hydrolysis of peptide:

with 6 M HCl, 65 h at 120 °C

HPLC:

HILIC - column 3,5 μm , 150 x 2.1 mm
5 mM NH_4Ac / ACN

MS:

MRM m/z 132 / 138 (leucine)
 116 / 122 (proline)
 120 / 125 (threonine)

and ^{13}C and ^{15}N -labeled int. standards

[Arsene et al. Anal.Chem.2008,804154-4160]

using aminoacid Standard Reference Material from NIST with
certified target values and defined uncertainty of measurement

IFCC reference measurement procedure HbA1c in blood by HPLC-ESI/MS

Principle of measurement:

Determination of the ratio of glycated and non-glycated
 β -N-terminal hexapeptides of haemoglobin

1. Haemolysis of whole blood sample

2. Proteolytic cleavage of the haemolysate

3. HPLC-ESI-MS analysis

Proteolytic cleavage of the haemolysate



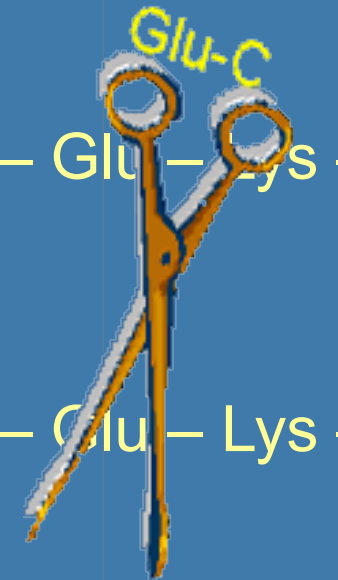
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HbA0

Val – His – Leu – Thr – Pro – Glu – Glu – Lys –

HbA1c

Glc – Val – His – Leu – Thr – Pro – Glu – Glu – Lys –



Glu – Lys –
Glu – Lys –

IFCC reference measurement procedure for HbA1c in blood by HPLC-ESI/MS

Principle of measurement:

Determination of the ratio of glycated and non-glycated
 β -N-terminal hexapeptides of haemoglobin

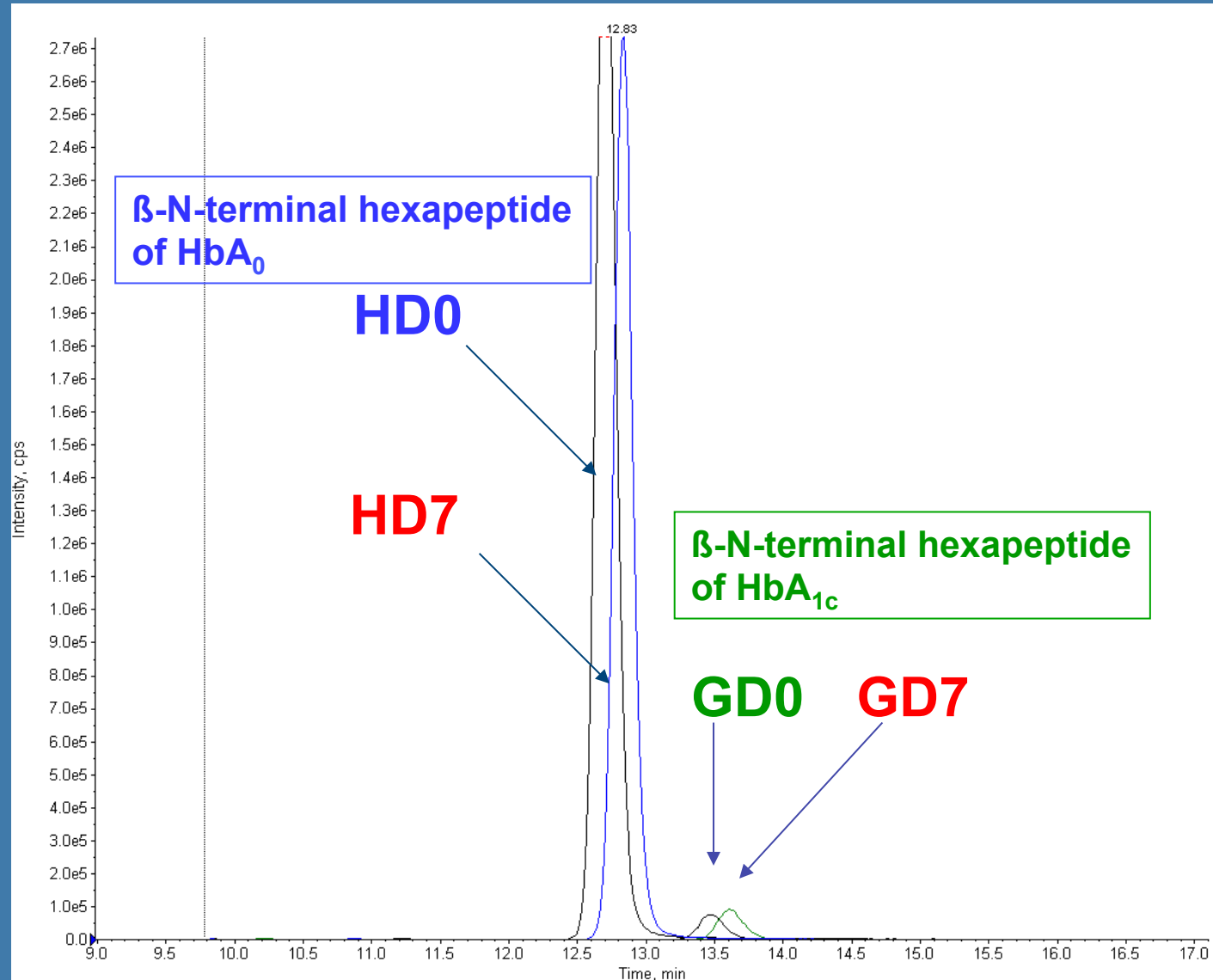
1. Haemolysis of whole blood sample

2. Proteolytic cleavage of the haemolysate

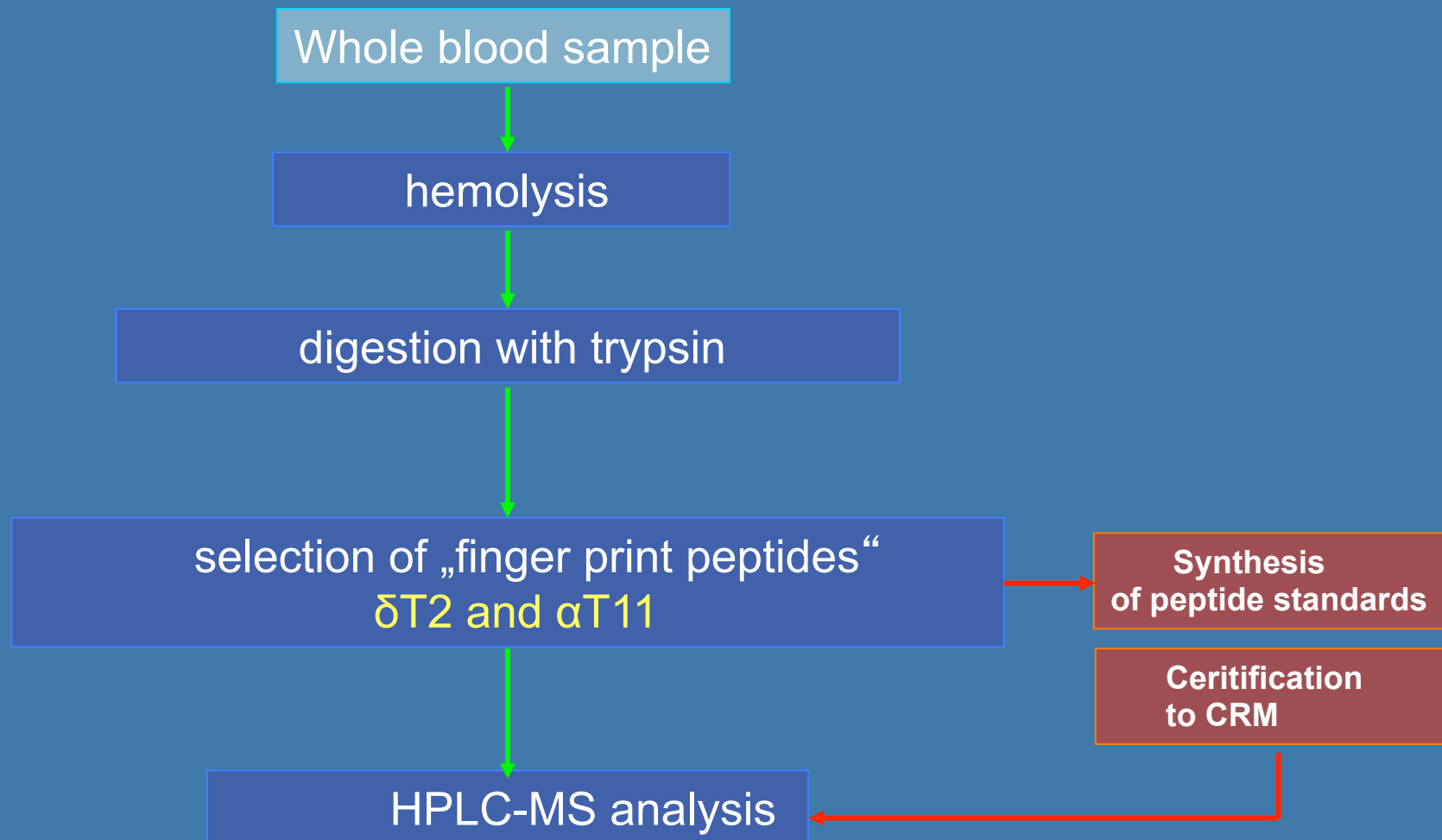
3. HPLC-ESI-MS analysis

LC-ID/MS chromatogram

HbA_{1c} determination

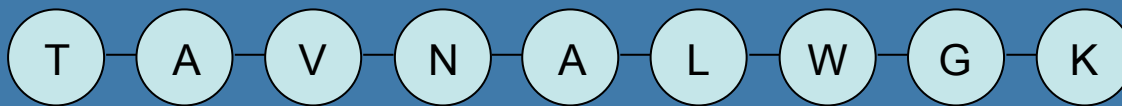


Isotope dilution mass spectrometry reference measurement procedure for HbA₂ ($\alpha 2\delta 2$)

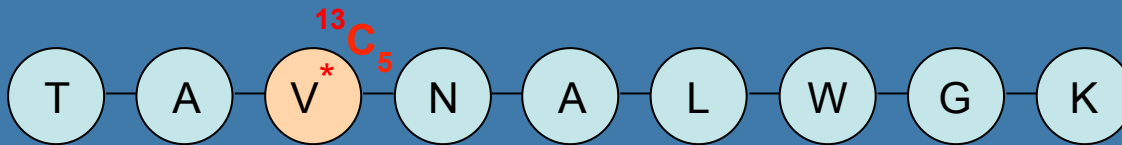


Calibrators for HbA₂ determination by HPLC-IDMS

α T11

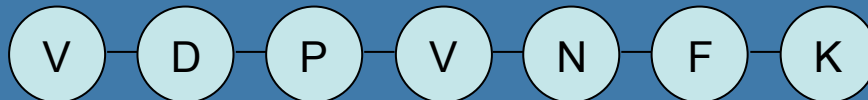


CRM

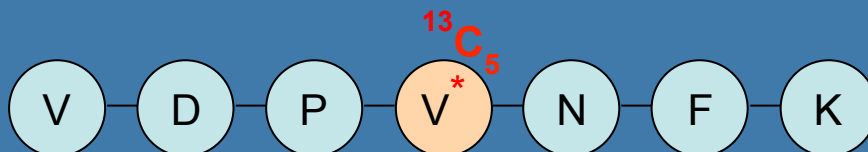


internal standard

δ T2



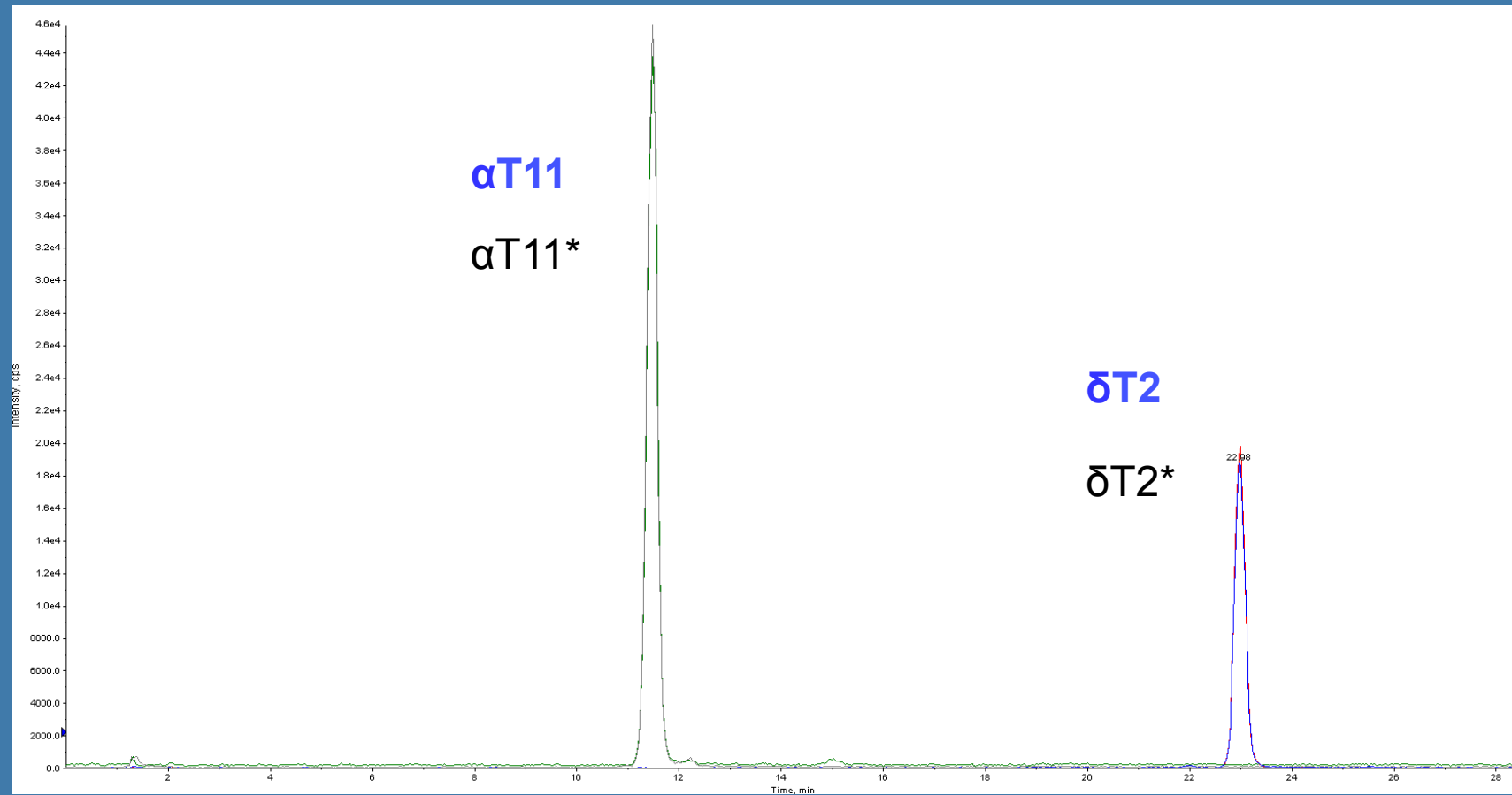
CRM



internal standard

LC-ID/MS chromatogram

HbA₂ determination



Kaiser P, Akerboom T, Ohlendorf R, and Reinauer H

Liquid chromatography-isotope dilution-mass spectrometry
as a new basis for the reference measurement procedure
for hemoglobin A1c determination.

Clin Chem 2010; 56: 750-4

Thank you for your attention