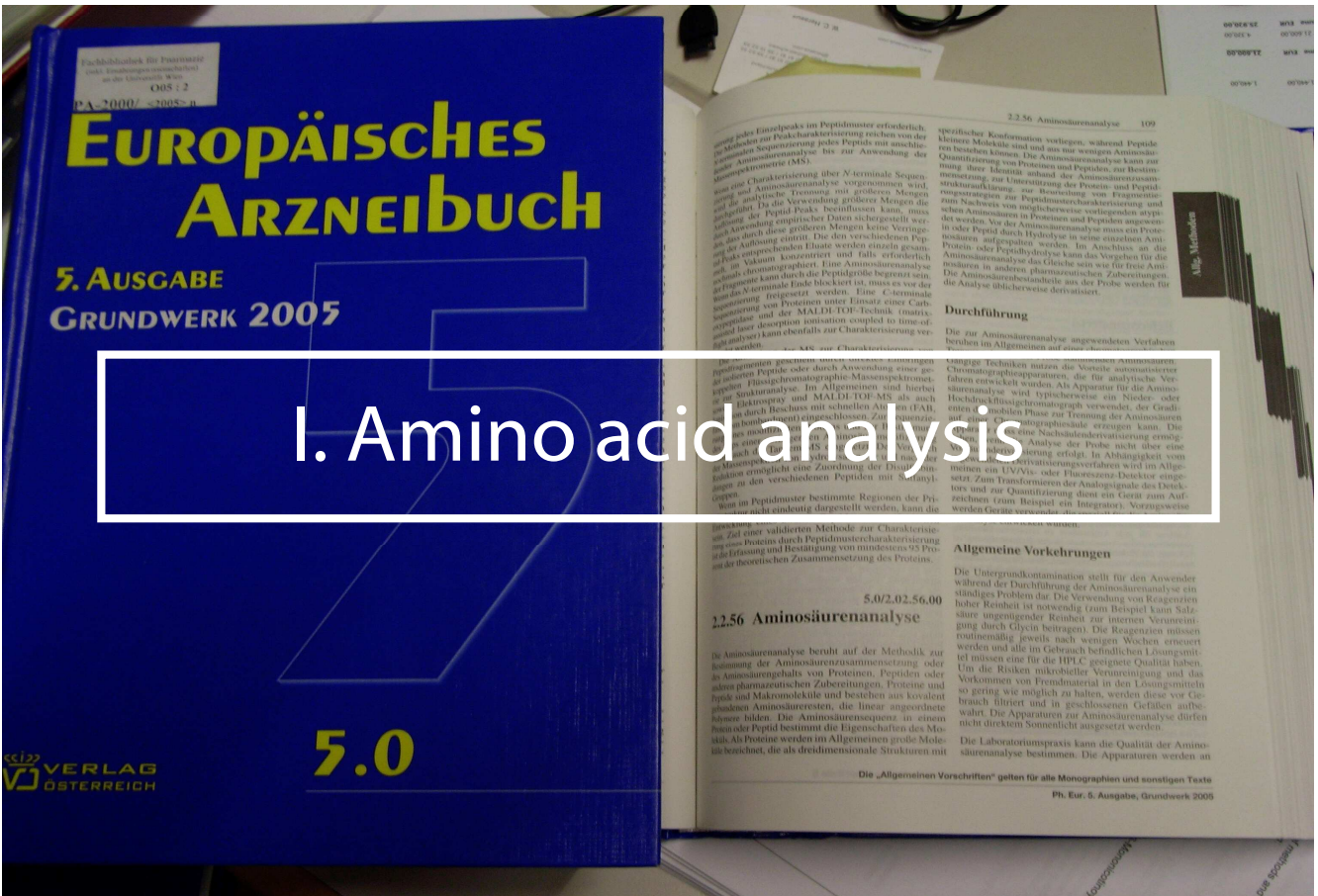


M. Kratzel

Two Examples of interactive eLearning

I. Amino acid analysis




I. Amino acid analysis

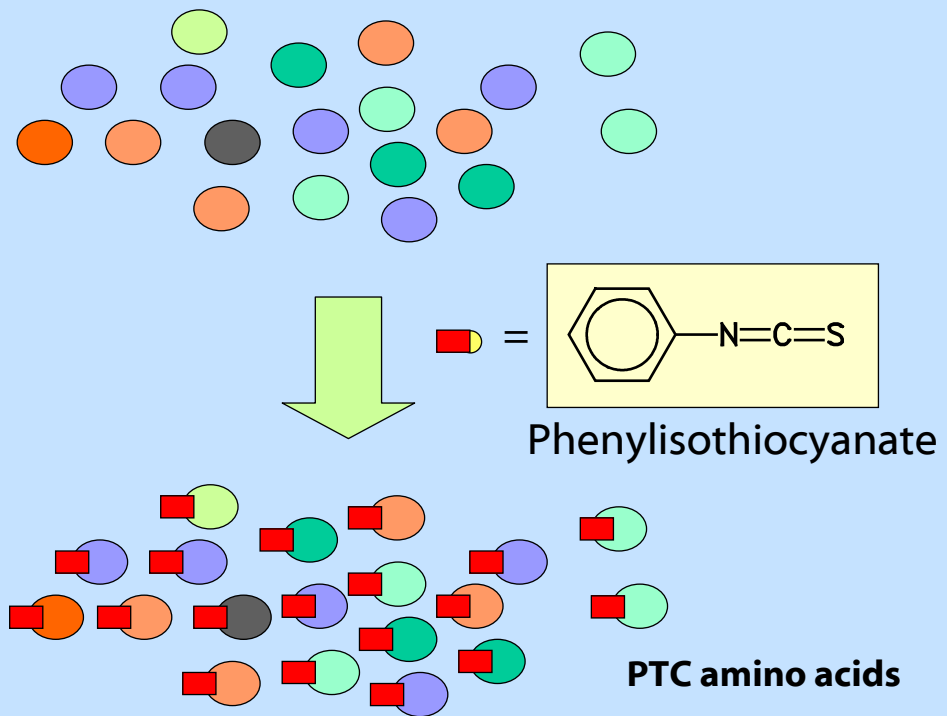
1. Hydrolysis

6 M HCl, 110 °C
24...72 h

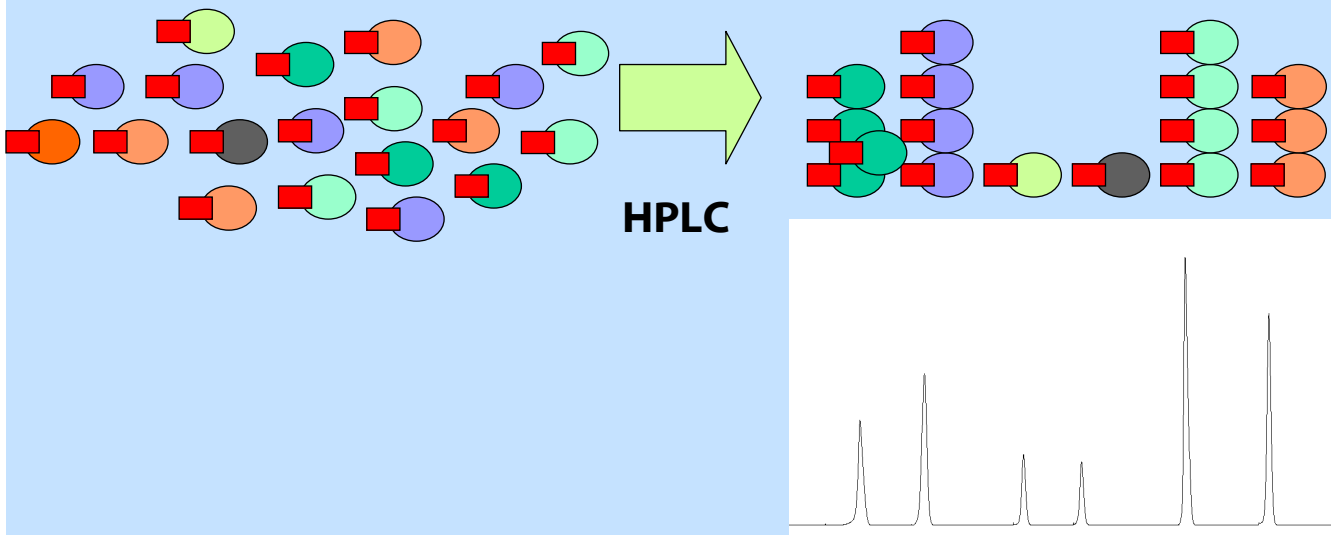
■ = amide bond(s)


universität wien
M. Kratzel: Two Examples of interactive eLearning

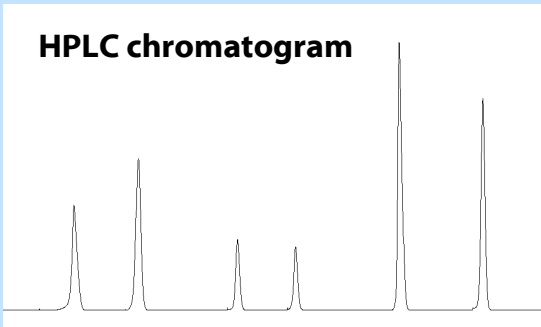
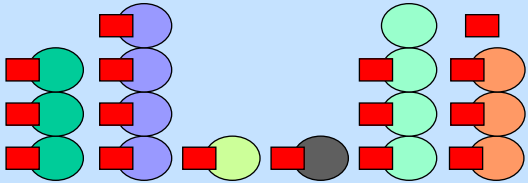
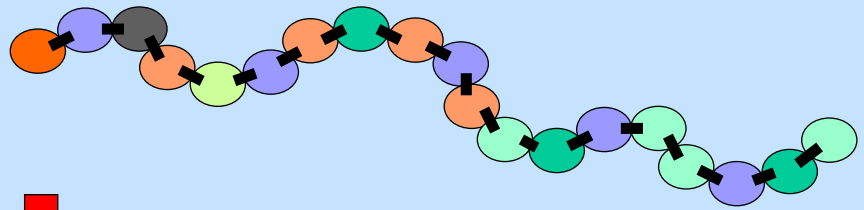
2. Derivatization



3. Separation



4. Calculation



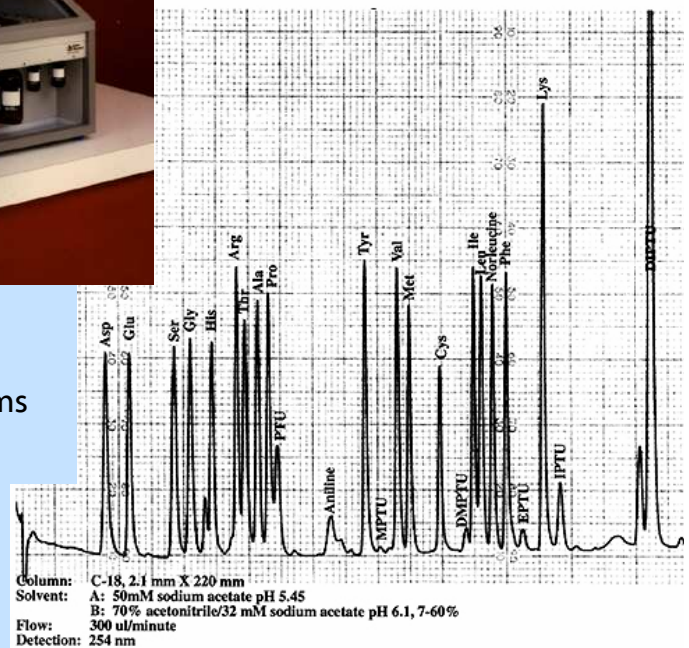
Sequence confirmation

Protein identification using databases (unique amino acid composition required)

Exact quantitation



Perkin Elmer Applied Biosystems
Model 420A PTC Derivatizer with
an on-line Perkin Elmer Applied Biosystems
Model 130A PTC Amino Acid Analyzer

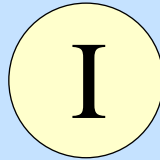


Two didactic aims...

1. Hydrolysis

2. Derivatization

3. Separation



The analytical procedure

4. Calculation

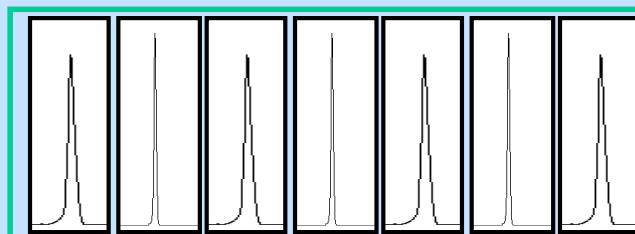
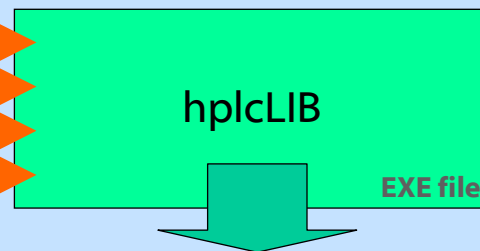
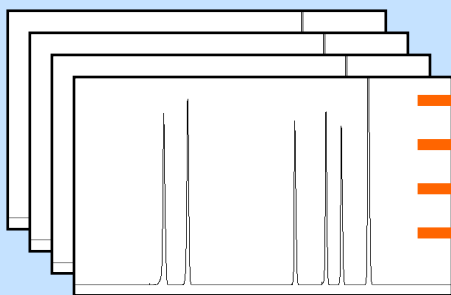


Evaluation,
Calculation,
Sequence confirmation



universität
wien

M. Kratzel: Two Examples of interactive eLearning

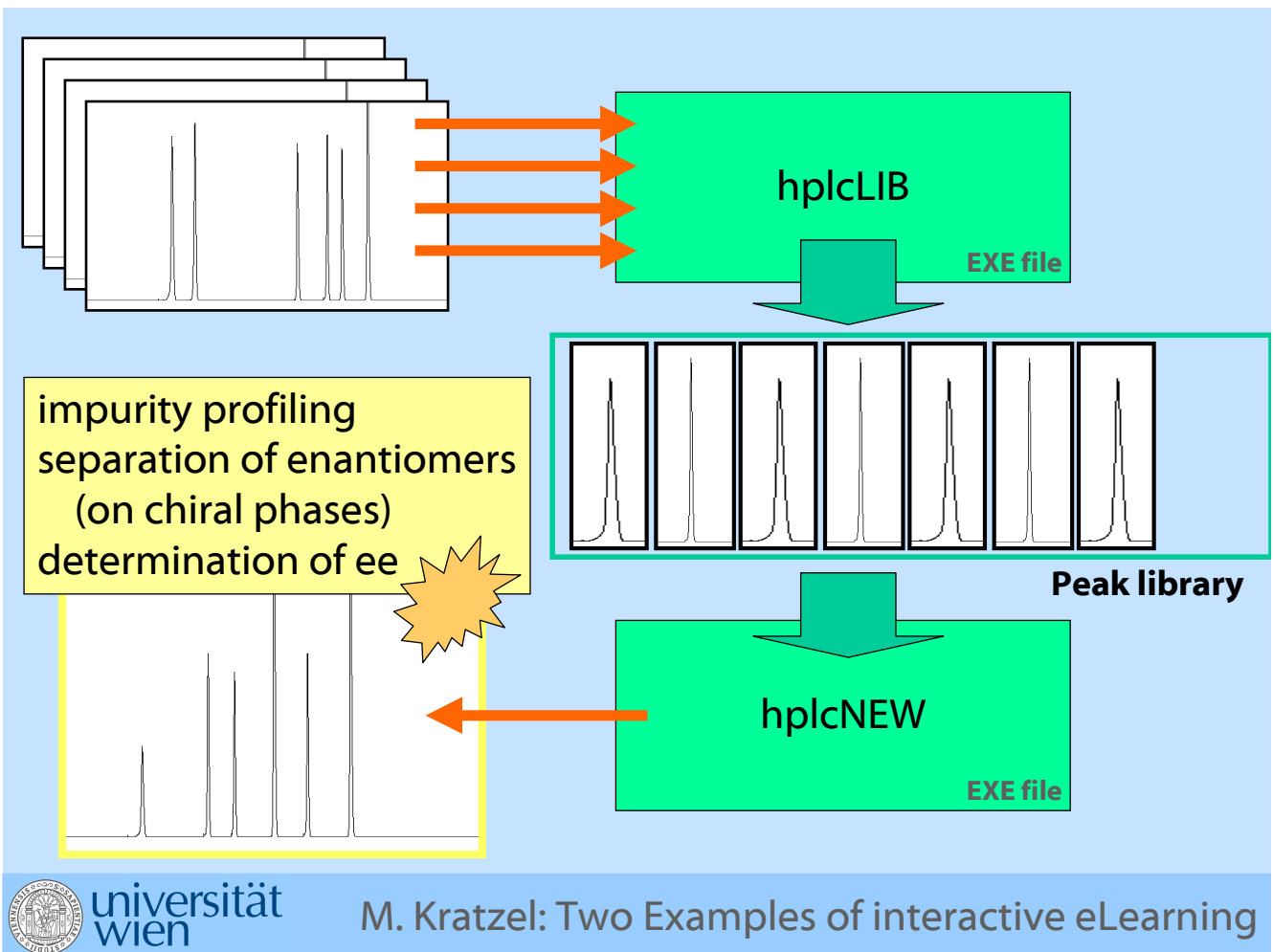
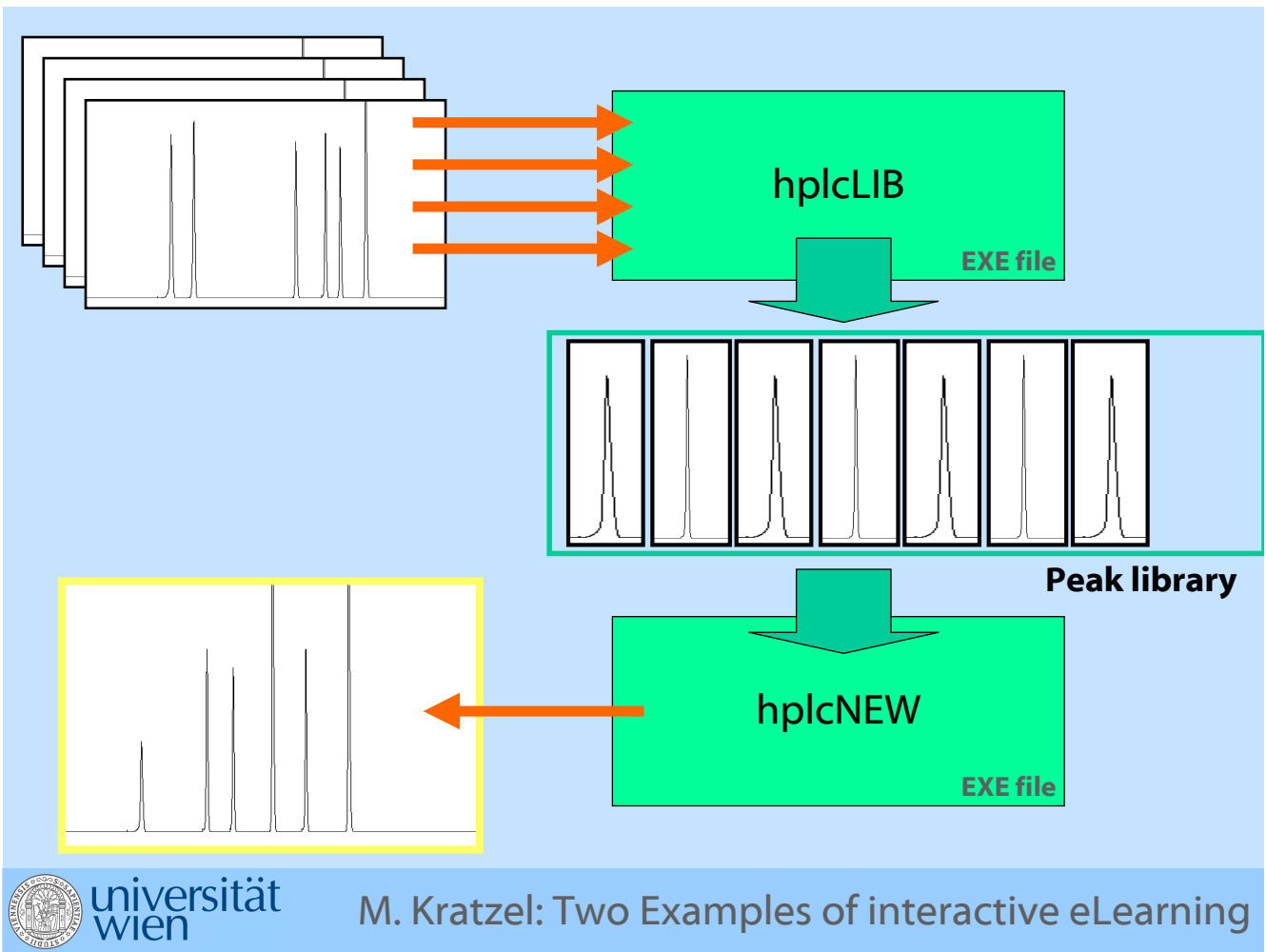


Peak library



universität
wien

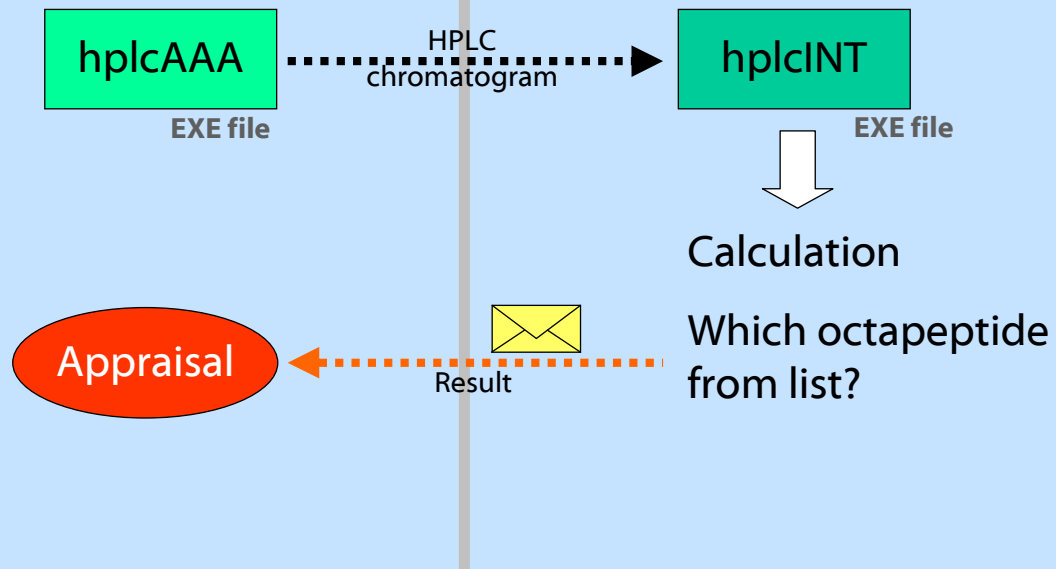
M. Kratzel: Two Examples of interactive eLearning



Flowchart - Amino acid analysis

Teacher's side

Student's side



universität
wien

M. Kratzel: Two Examples of interactive eLearning

Web pages - Amino acid analysis

<http://synthon.pch.univie.ac.at/px-te/simul/as001.htm>

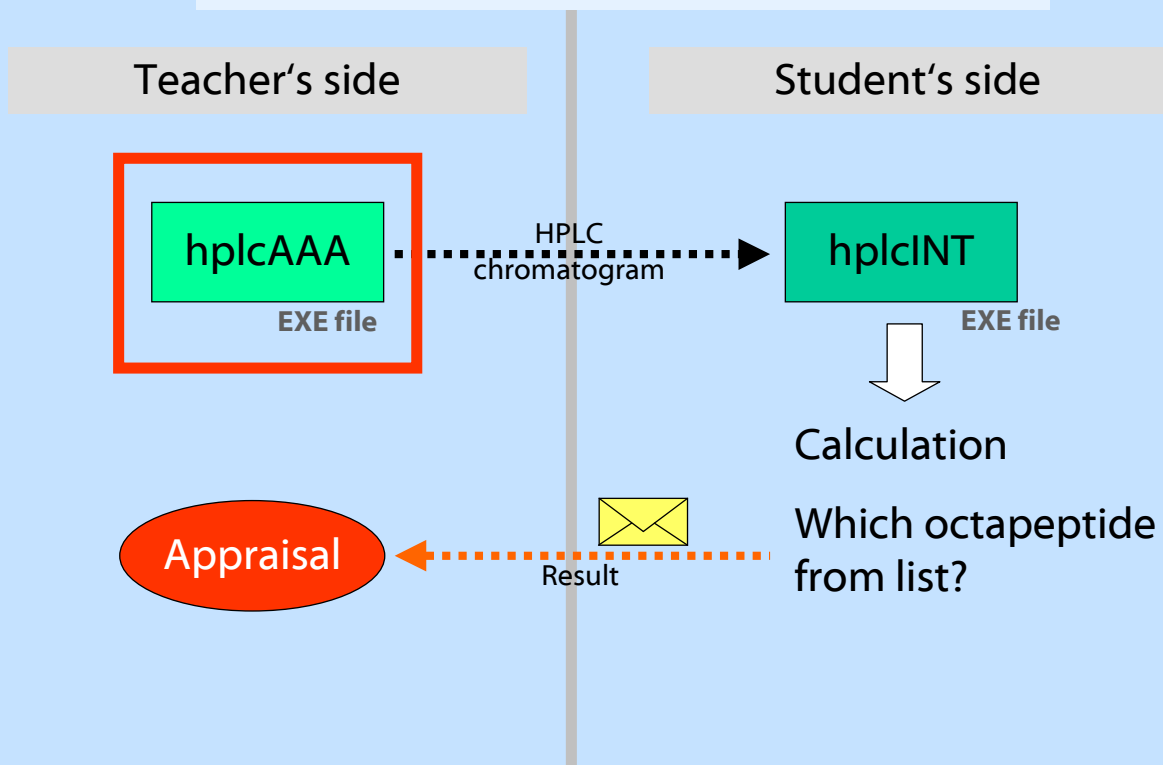
http://www.univie.ac.at/ulg-pqm/aawe/programm_aawe_ws07.html



universität
wien

M. Kratzel: Two Examples of interactive eLearning

Flowchart - Amino acid analysis



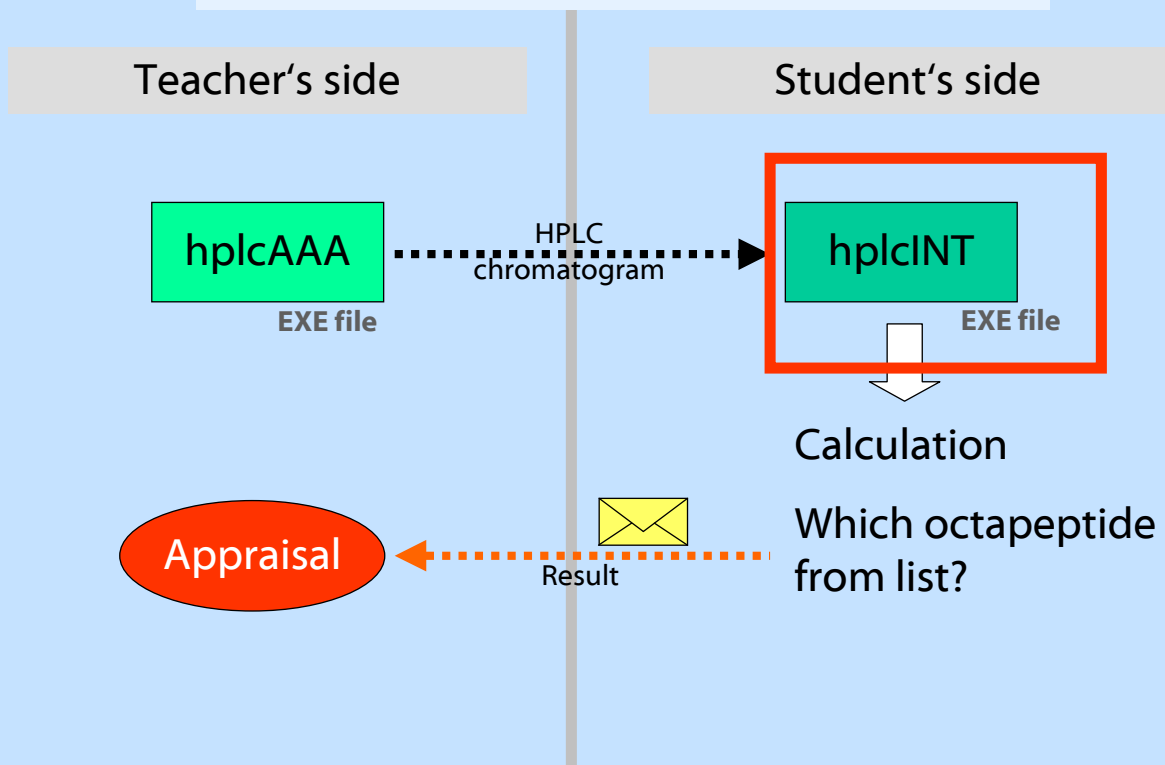
Web pages - Sample download

<http://www.univie.ac.at/ulg-pqm/aawe/downloads.php>

http://www.univie.ac.at/ulg-pqm/aawe/programm_aawe_ws07.html

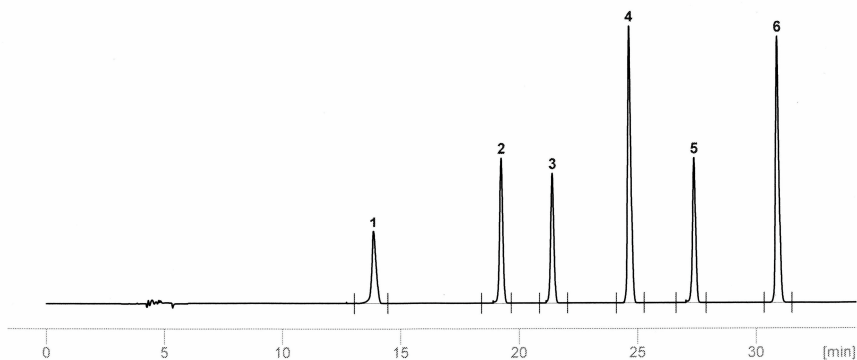


Flowchart - Amino acid analysis



D:\eLearning\MEIER_M_3.HPD

hplcINT
(C) M.Kratzel



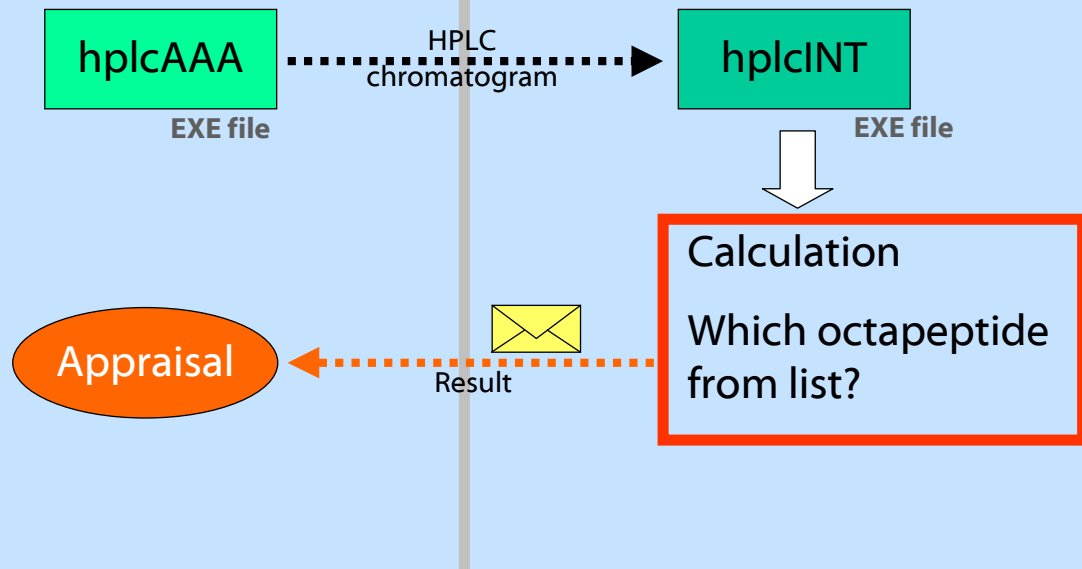
#	r. t.	PA	rel. %	
1	13.85	1001.19	9.09	Asp
2	19.24	1478.38	13.42	Arg
3	21.40	1319.32	11.98	Ala
4	24.62	2903.01	26.35	Val
5	27.37	1481.18	13.44	Ile
6	30.88	2833.77	25.72	Phe



Flowchart - Amino acid analysis

Teacher's side

Student's side



Some (but no more) comments on evaluation:

1. The reference samples contain amino acids in equimolar amounts. Different peak areas refer to different UV absorption of PTC derivatives.
2. The result comprises the relative (molar) amounts of present amino acids.
3. And which octapeptide relates to your result?

Microsoft Excel - CalcAAA.xls

Datei Bearbeiten Ansicht Einfügen Format Extras Daten Fenster ?

Arial 10 F X U

B23 = 1001,19

	A	B	C	D	E	F	G	H	I	J	K	L
1	AA	PA	Corr. Factor									
2												
3	Standard 1											
4												
5	Asp	1193,17	1,2562									
6	Glu	1215,98	1,2326									
7	Ala	1498,86	1,0000									
8	Ile	1667,46	0,8989									
9												
10												
11	Standard 2											
12												
13	Arg	1672,6	0,8966									
14	Ala	1499,64	1,0000									
15	Val	1700,34	0,8820									
16	Leu	1638,29	0,9154									
17	Phe	1651,64	0,9080									
18	Lys	2648,54	0,5662									
19												
20												
21	Sample		corr. PA									
22												
23	Asp	1001,19	1257,69									
24	Arg	1478,38	1325,50									
25	Ala	1319,32	1319,32									
26	Val	2903,01	2560,35									
27	Ile	1481,18	1331,42									
28	Phe	2833,77	2572,98									
29												
30												

Tabelle1 | Tabelle2 | Tabelle3 | **Tabelle4** | Tabelle5 | Tabelle6 | Tabelle7

Bereit



Microsoft Excel - CalcAAA.xls

Datei Bearbeiten Ansicht Einfügen Format Extras Daten Fenster ?

Arial 10 F X U

F14 =

	A	B	C	D	E	F	G	H	I	J	K	L
1	AA	PA	Corr. Factor									
2												
3	Standard 1											
4												
5	Asp	1193,17	1,2562									
6	Glu	1215,98	1,2326									
7	Ala	1498,86	1,0000									
8	Ile	1667,46	0,8989									
9												
10												
11	Standard 2											
12												
13	Arg	1672,6	0,8966									
14	Ala	1499,64	1,0000									
15	Val	1700,34	0,8820									
16	Leu	1638,29	0,9154									
17	Phe	1651,64	0,9080									
18	Lys	2648,54	0,5662									
19												
20												
21	Sample		corr. PA	rel. %								
22												
23	Asp	1001,19	1257,69	12,13	1 x Asp (D)							
24	Arg	1478,38	1325,50	12,79	1 x Arg (R)							
25	Ala	1319,32	1319,32	12,73	1 x Ala (A)							
26	Val	2903,01	2560,35	24,70	2 x Val (V)							
27	Ile	1481,18	1331,42	12,84	1 x Ile (I)							
28	Phe	2833,77	2572,98	24,82	2 x Phe (F)	"=> No. 23: DVFAVIFR						
29												
30			10367,27									

Tabelle1 | Tabelle2 | Tabelle3 | **Tabelle4** | Tabelle5 | Tabelle6 | Tabelle7

Bereit



II. Assay Validation

ICH parameters versus type of validation

Type of Analytical Procedure	Identification	Impurity Testing		Assay
		Quantitative	Limit Tests	
Accuracy	No	Yes	No	Yes
Precision				
Repeatability	No	Yes	No	Yes
Interm. Prec.	No	Yes	No	Yes
Specificity	Yes	Yes	Yes	Yes
LOD	No	No	Yes	No
LOQ	No	Yes	No	No
Linearity	No	Yes	No	Yes
Range	No	Yes	No	Yes

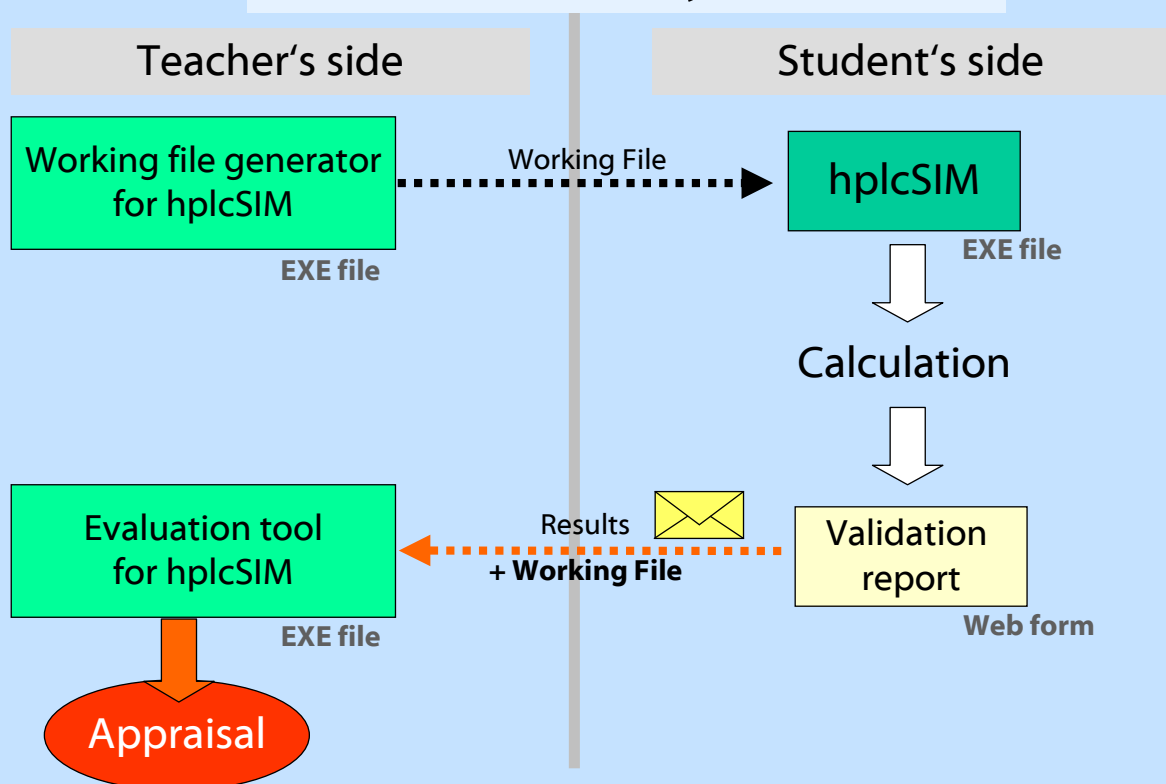
Validation plan

Assay of Nitrendipine by HPLC

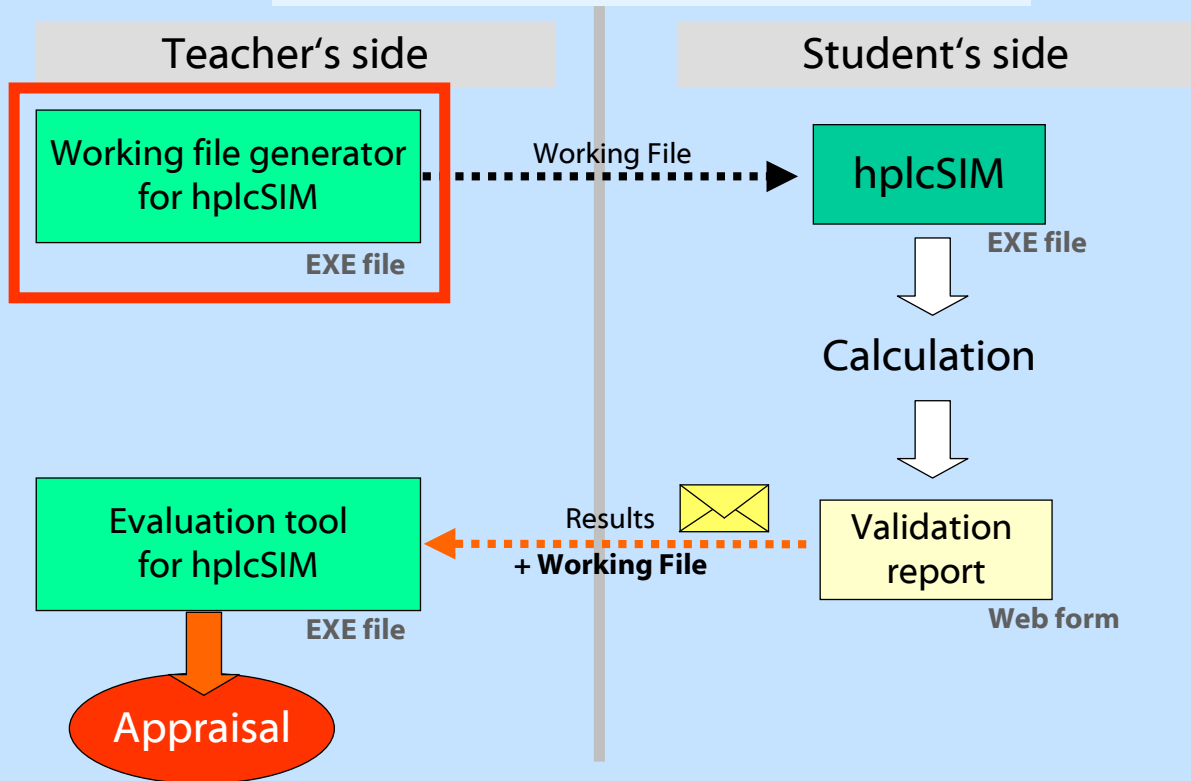
- I. System Suitability
- II. Specificity
- III. Precision
- IV. Linearity
- ~~V. LOD + LOQ~~
- (VI. Range) \Rightarrow included in IV. Linearity
- VII. Accuracy
- VIII. Precision - Repeatability
- IX: Precision - Intermediate precision



Flowchart - Assay validation



Flowchart - Assay validation



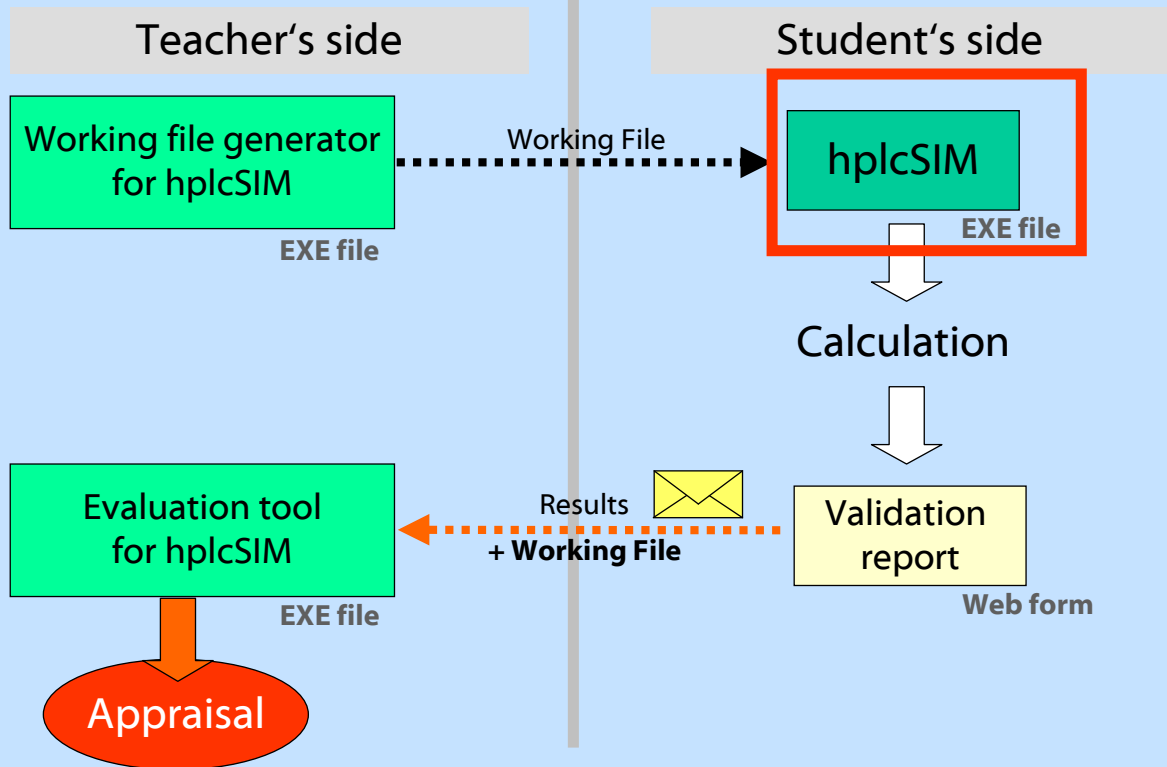
Web pages - Assay validation

http://synthon.pch.univie.ac.at/px-te/simul/valid_hplc_01.htm

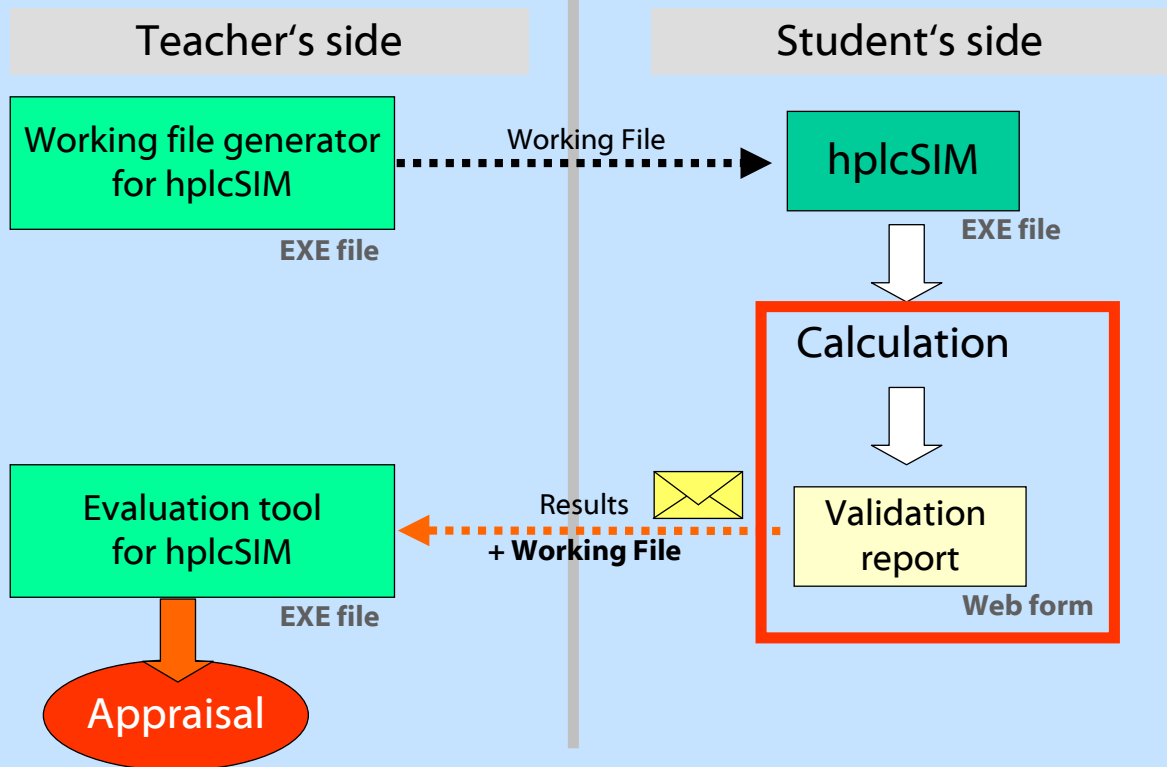
http://www.univie.ac.at/ulg-pqm/aawe/programm_aawe_ws07.html



Flowchart - Assay validation



Flowchart - Assay validation



Web form - Validation report

<http://synthon.pch.univie.ac.at/px-te/simul/abschlussbericht3.htm>

http://www.univie.ac.at/ulg-pqm/aawe/programm_aawe_ws07.html

Flowchart - Assay validation

