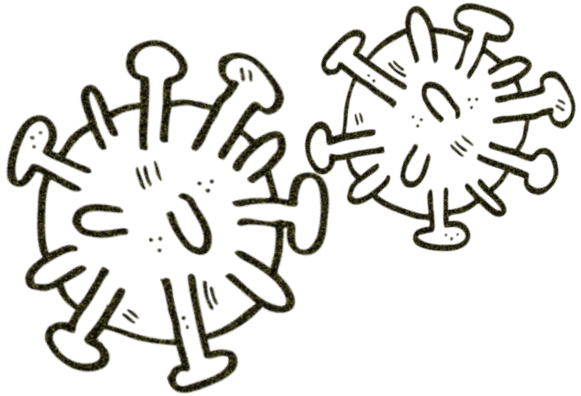
Development of an online two-dimensional SEC-UV-RPLC-MS method for the multi-attribute characterization of adeno-associated virus products

MULTIDIMENSIONAL CHROMATOGRAPHY WORKSHOP

4th February 2025

Megane Aebischer

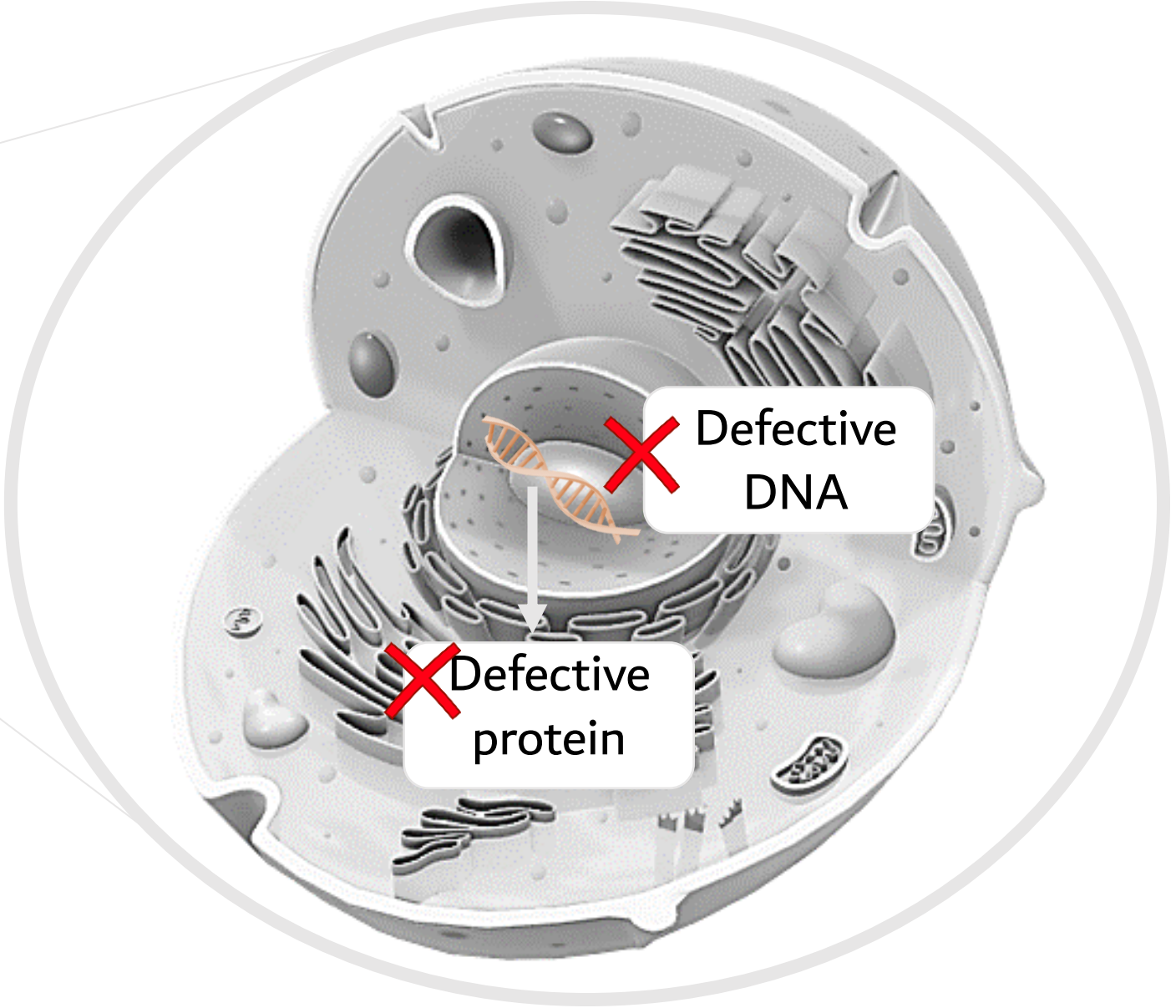
megane.aebischer@unige.ch



ISPSO
INSTITUT DES SCIENCES PHARMACEUTIQUES DE SUISSE OCCIDENTALE

UNIVERSITÉ DE GENÈVE

Genetic disease



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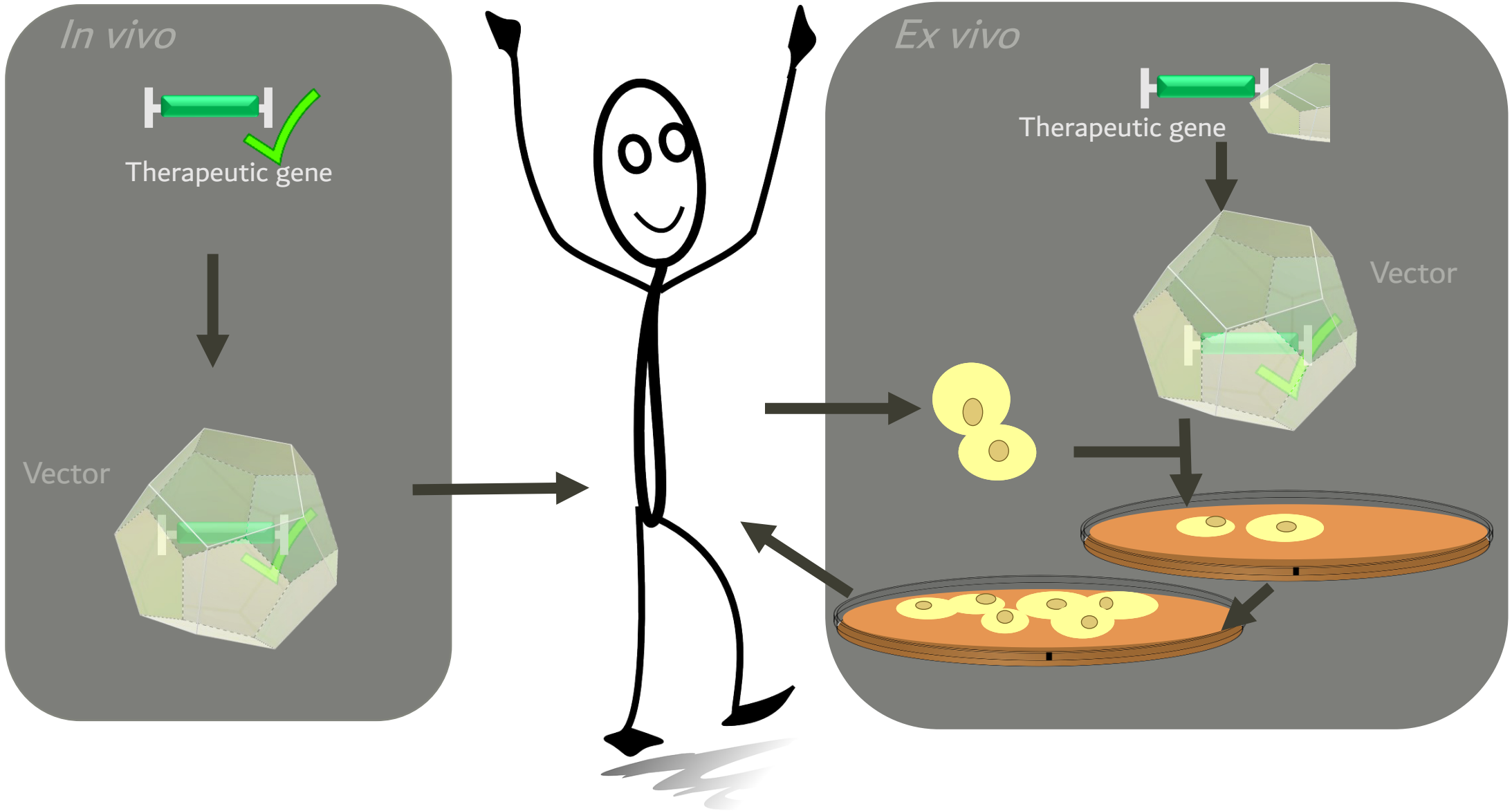
Adeno-associated virus (AAV): a vector of choice for gene therapy



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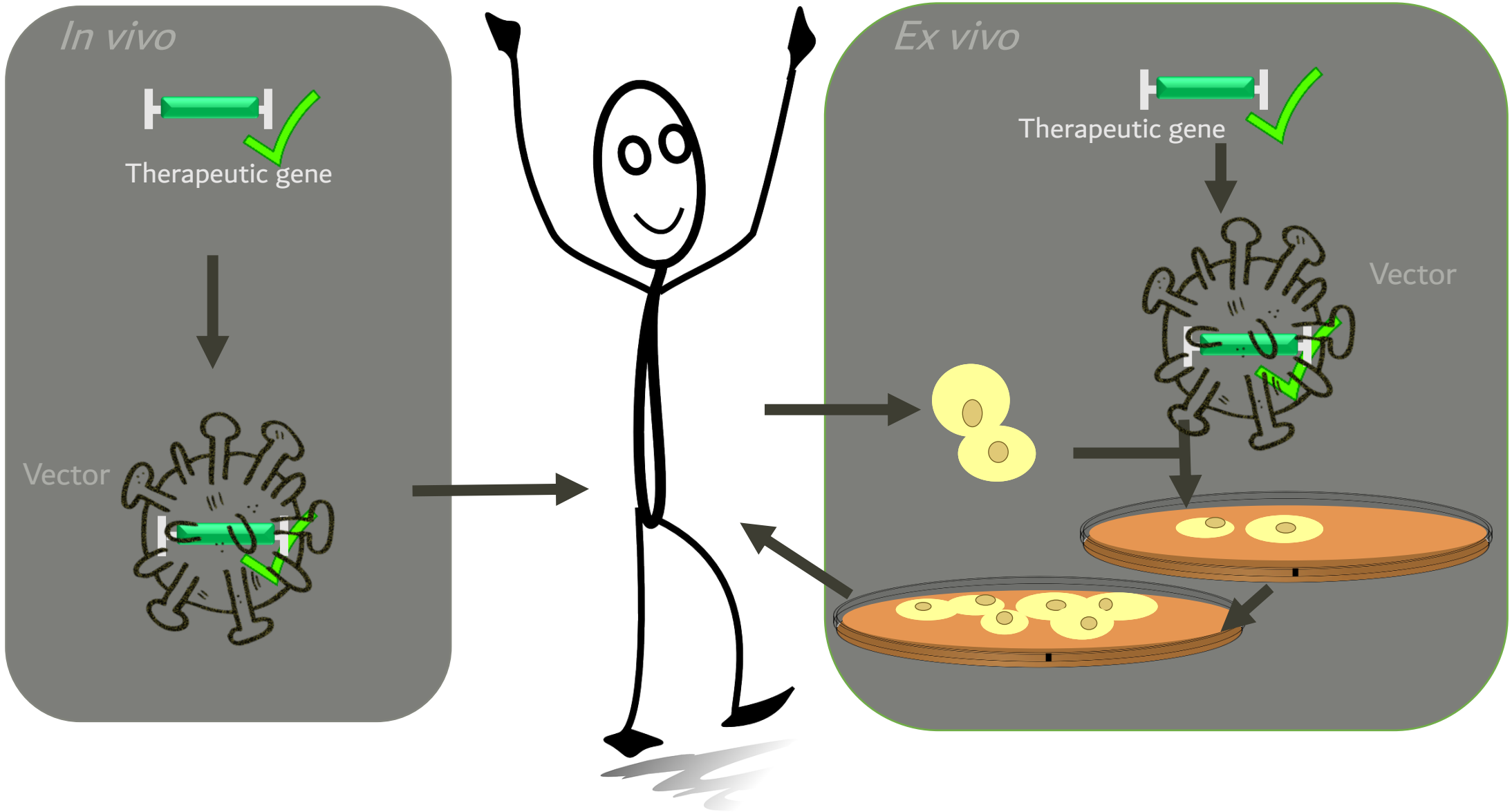

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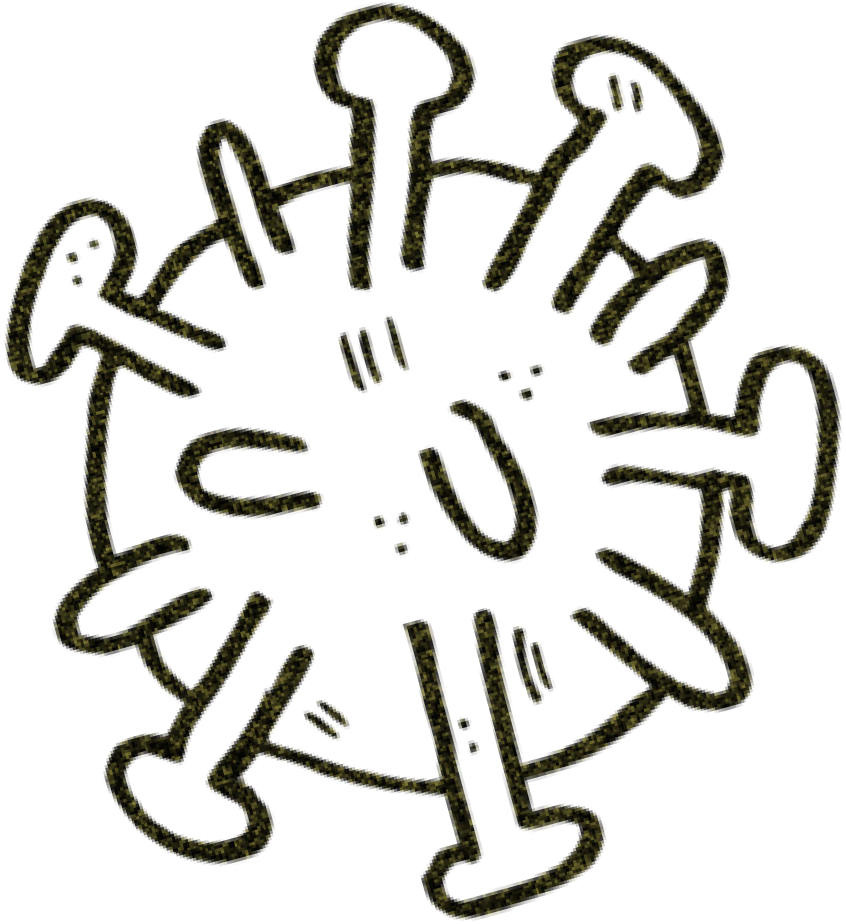

Adeno-associated virus (AAV)

Non-pathogenic single-stranded DNA virus

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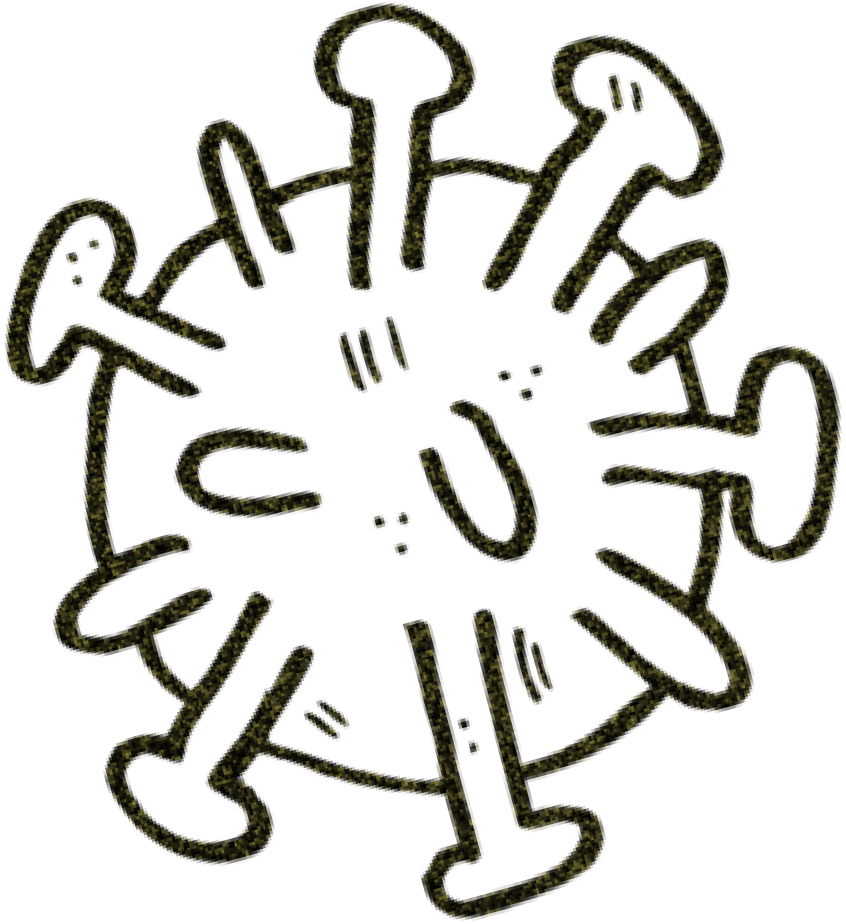
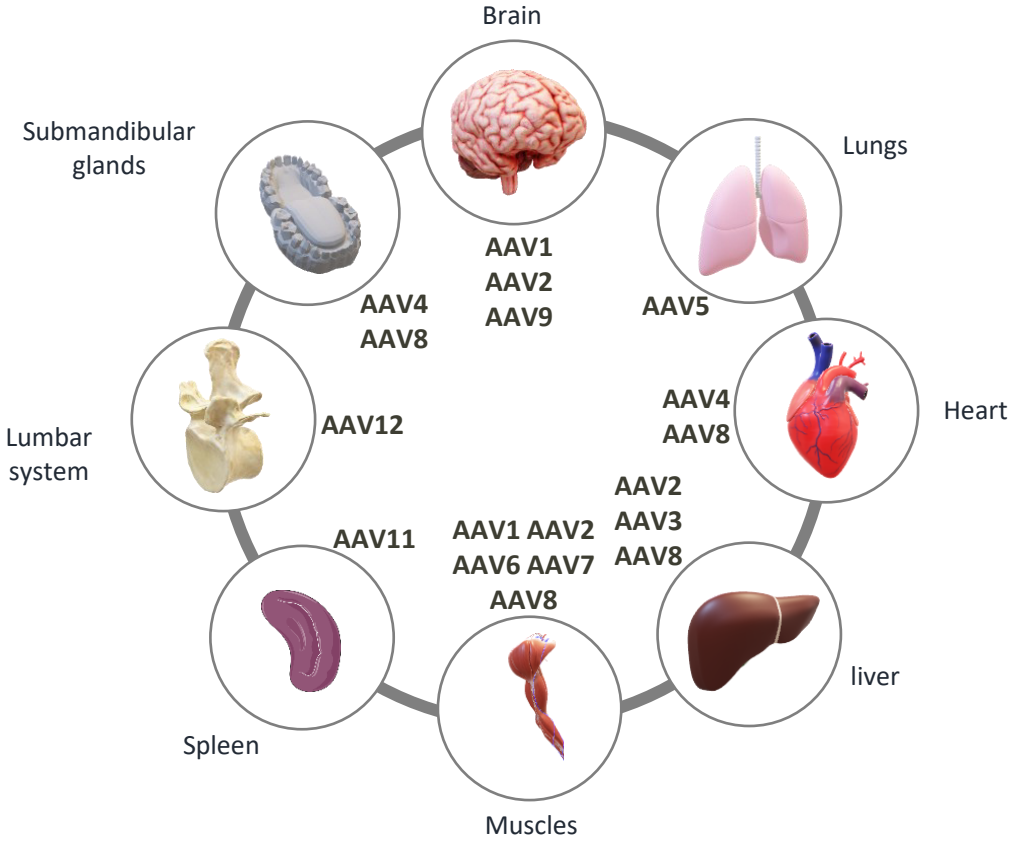


Adeno-associated virus (AAV)

Non-pathogenic single-stranded DNA virus

12 different serotypes



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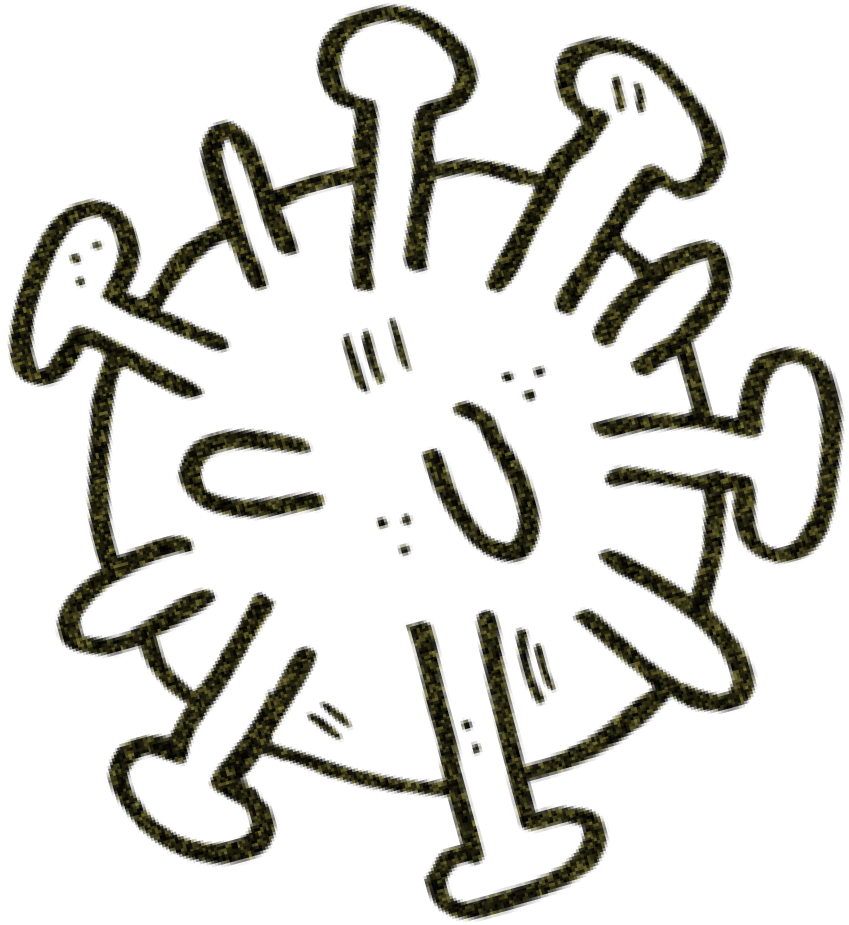
Adeno-associated virus (AAV): a vector of choice for gene therapy



Adeno-associated virus (AAV)

- Non-pathogenic single-stranded DNA virus
- 12 different serotypes
- Relatively simple biological structure

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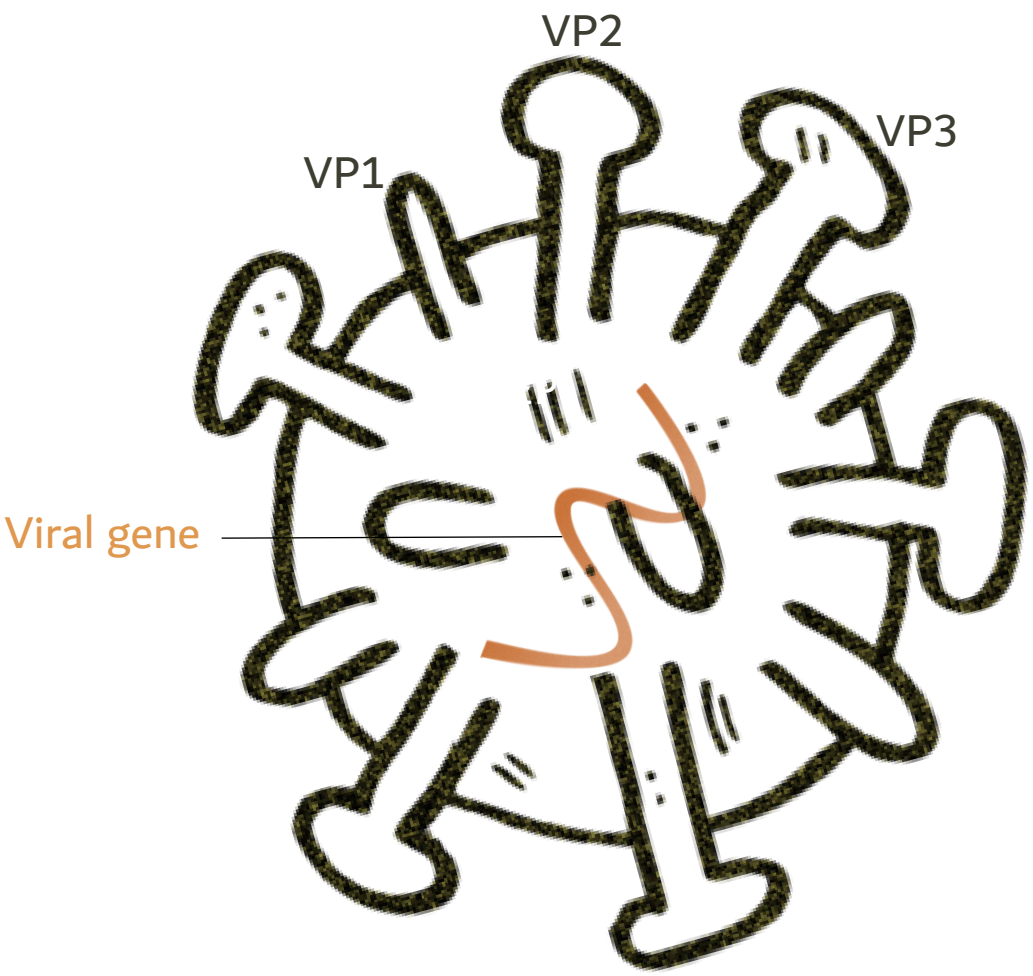
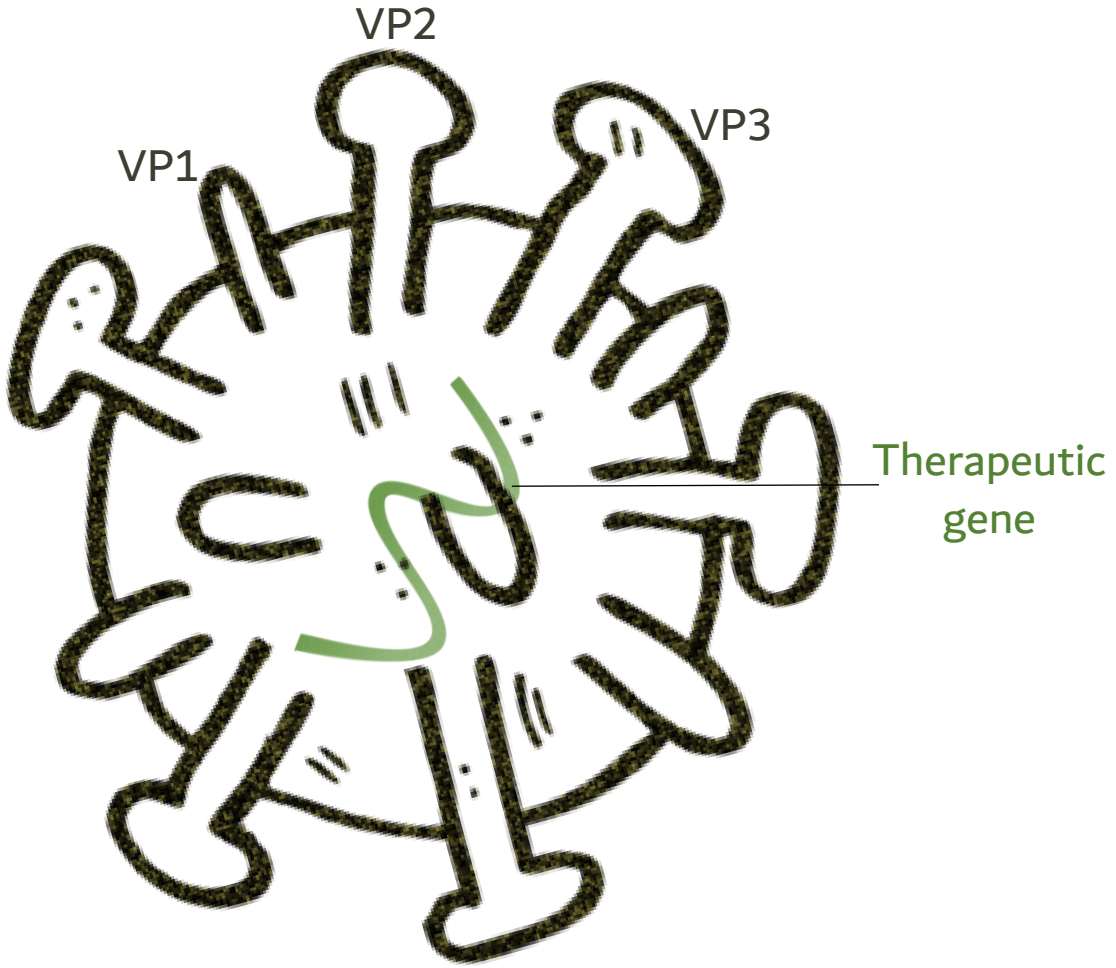
Adeno-associated virus (AAV): a vector of choice for gene therapy



Adeno-associated virus (AAV)



Recombinant adeno-associated virus (rAAV)

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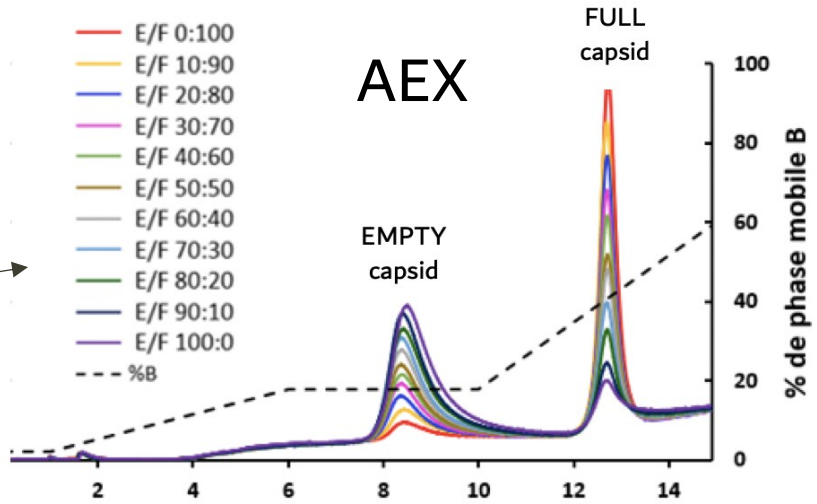
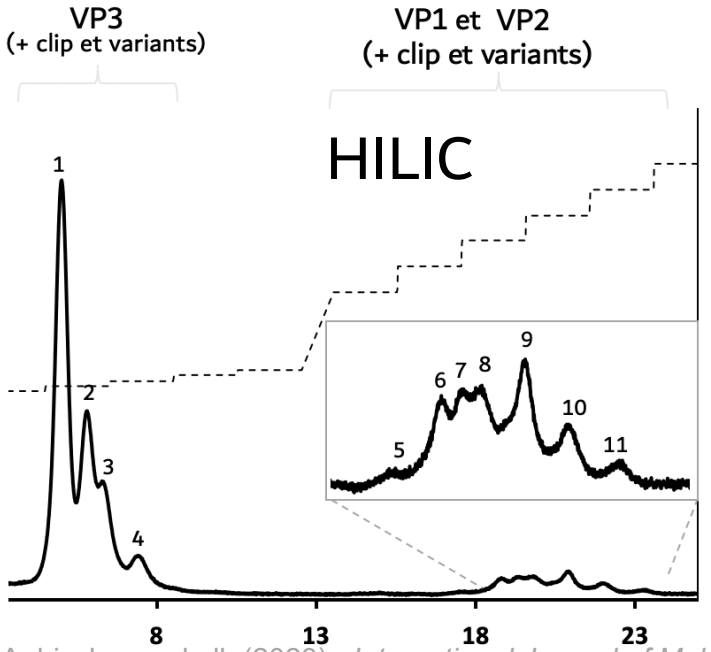
Critical quality attributes (CQA)

CQA	Analytical methods
Full/Empty Capsid	AUC, TEM, IEX, SEC-MALS, CE-SDS/cIEF
Aggregation and Fragmentation	SEC, SEC-MALS, DLS
Capsid Integrity (VP1-3 Ratio)	RPLC, CE-SDS/cIEF
Capsid Identity (VP1-3), post-translational modifications	LC-MS
Particle Concentration (viral titer)	SEC-MALS, ELISA
Genomic titer, identity and integrity	qPCR/ddPCR IP-RPLC
Infectivity, potency, mode of action	Cell based assays

AUC = Analytical Ultra Centrifugation; TEM = Transmission Electron Microscopy; IEX = Ion Exchange Chromatography, SEC = Size Exclusion Chromatography; MALS = Multi-angle Light Scattering; DLS = Dynamic Light Scattering; ELISA = Enzyme-Linked Immunosorbent Assay; RPLC = Reversed-Phase Liquid Chromatography; CE-SDS = Capillary electrophoresis sodium dodecyl sulfate; cIEF = capillary IsoElectric Focusing; q/ddPCR = quantitative/droplet digital Polymerase Chain Reaction



Aebischer and all. (2022). *International Journal of Molecular Sciences*, *23*(20), 12332.



Aebischer and all. (2023). *International Journal of Molecular Sciences*, *24*(10), 8503.

1D-LC methods are well established for characterizing CQAs, but the industry needs fast methods that can provide as much information as possible in a single run.

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2D-LC multi-attributes method

CQA#1 Aggregation	CQA#2 Capsid integrity	CQA#3 Capsid identity	CQA#4 Post-translational modifications
	 1 : 1 : 10	AAV2, AAV5, AAV6, AAV8, AAV9 … ?	Acetylation, phosphorylation …

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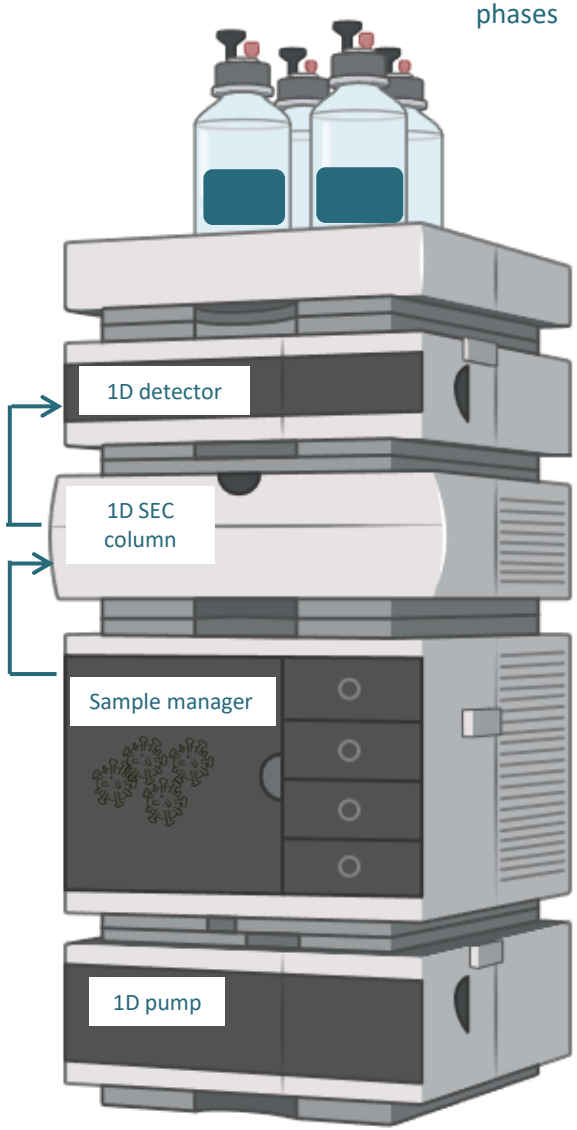
2D-LC workflow



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Agilent 1290 2D-LC

(10 ports with active solvent modulation)

Agilent Q-TOF 6530

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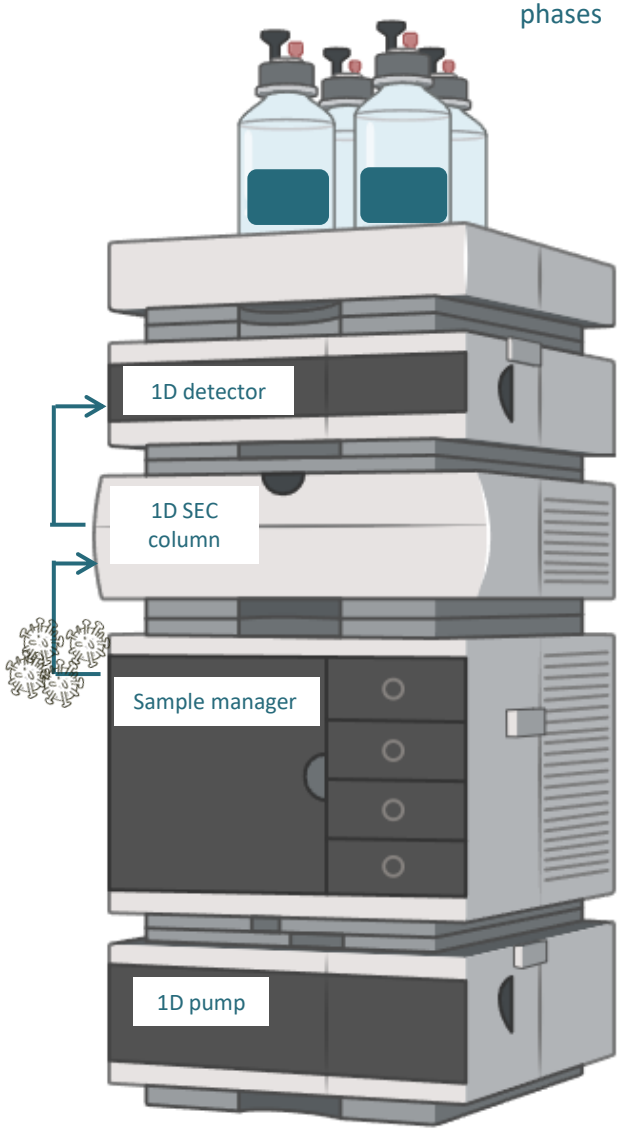
2D-LC workflow



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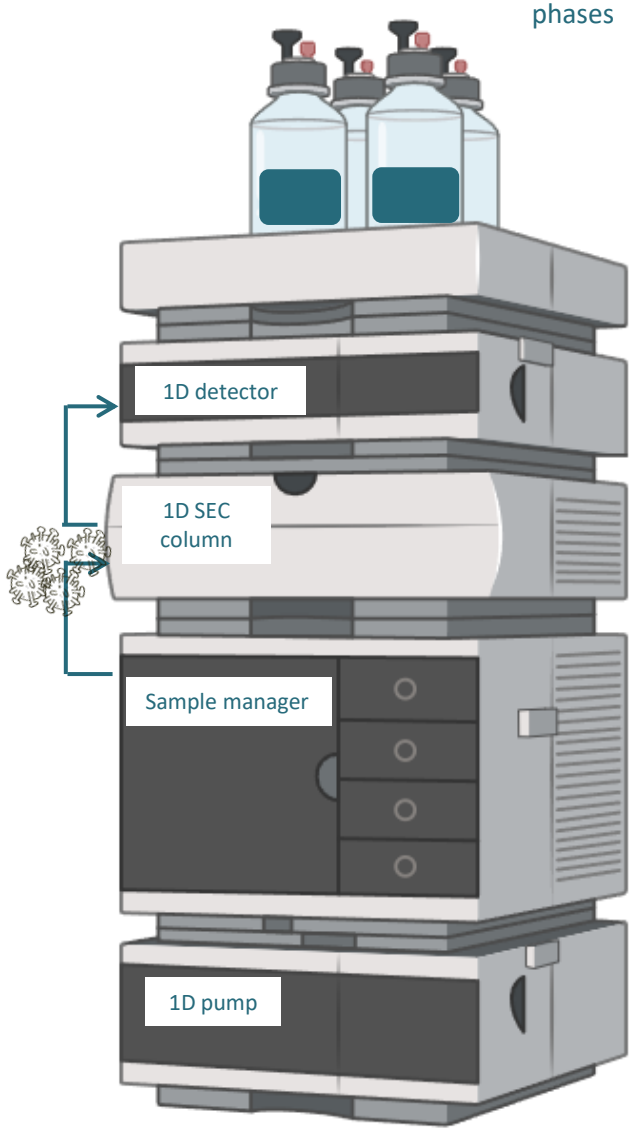

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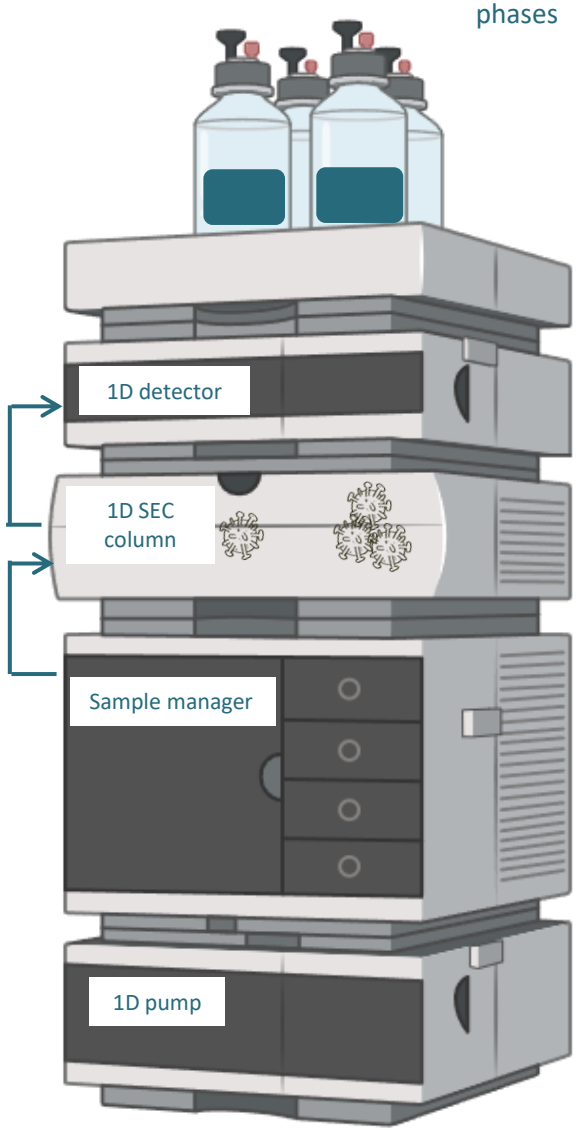

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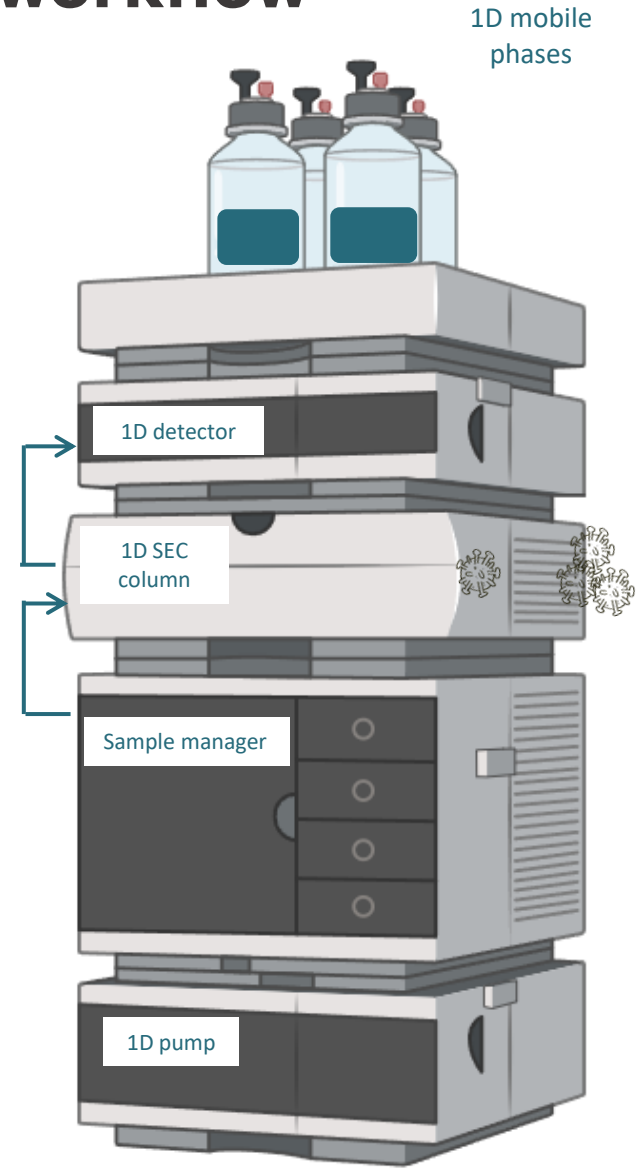

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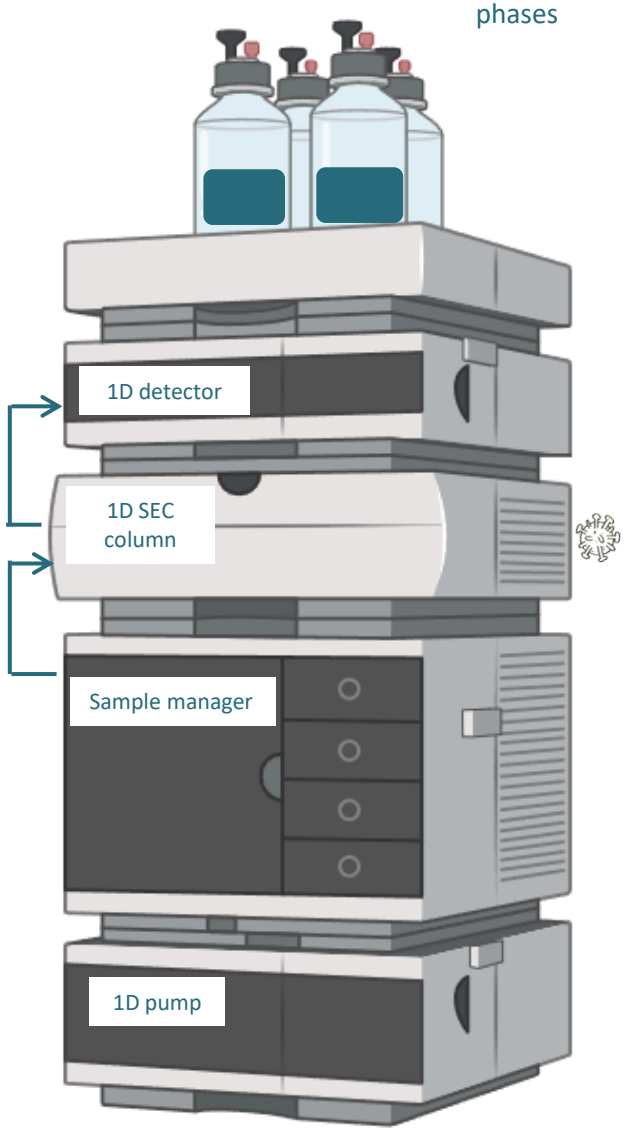

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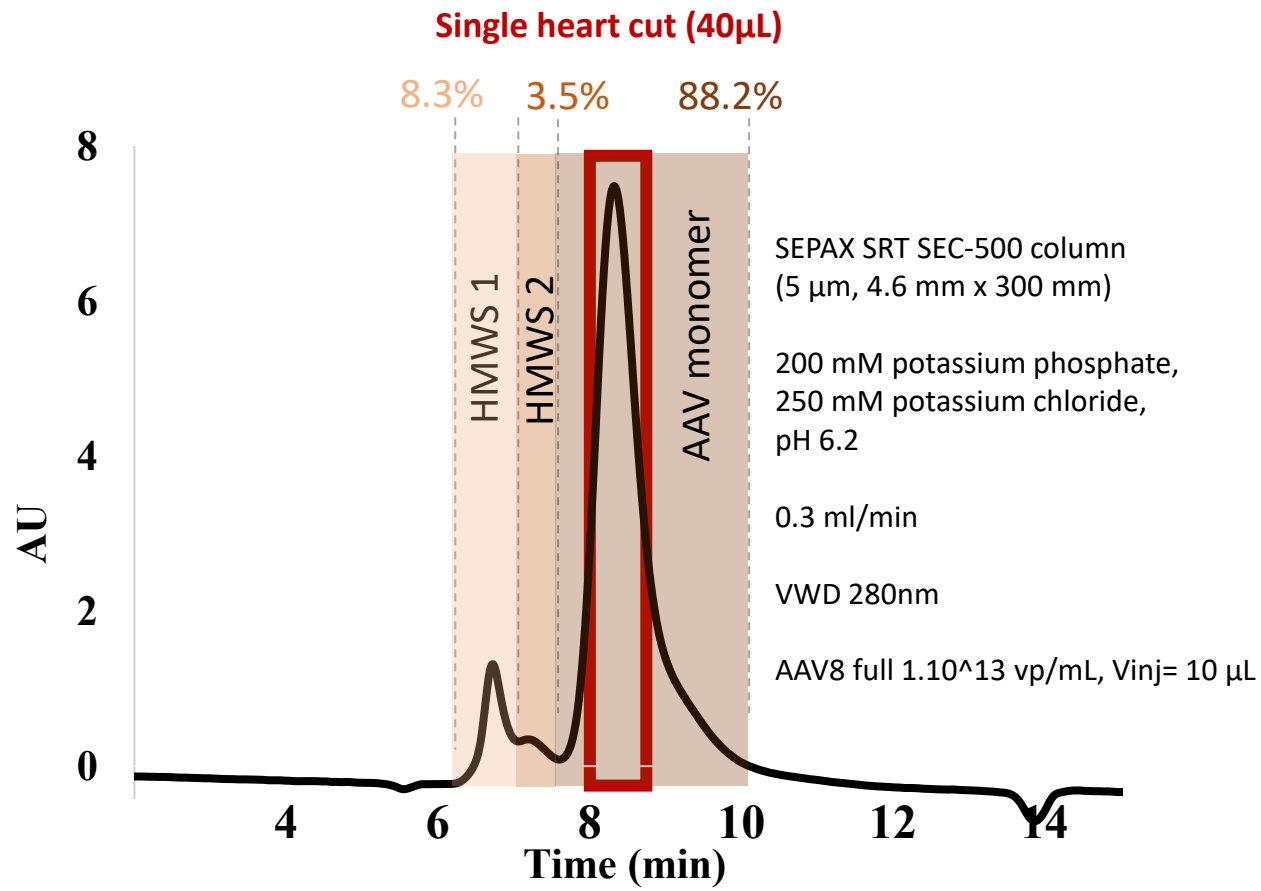
CQA#1 Aggregation



SEPAX SRT SEC-500 column
(5 µm, 4.6 mm x 300 mm)

200 mM potassium phosphate,
250 mM potassium chloride,
pH 6.2

0.3 ml/min

VWD 280nm

AAV8 full 1.10^13 vp/mL, Vinj= 10 µL

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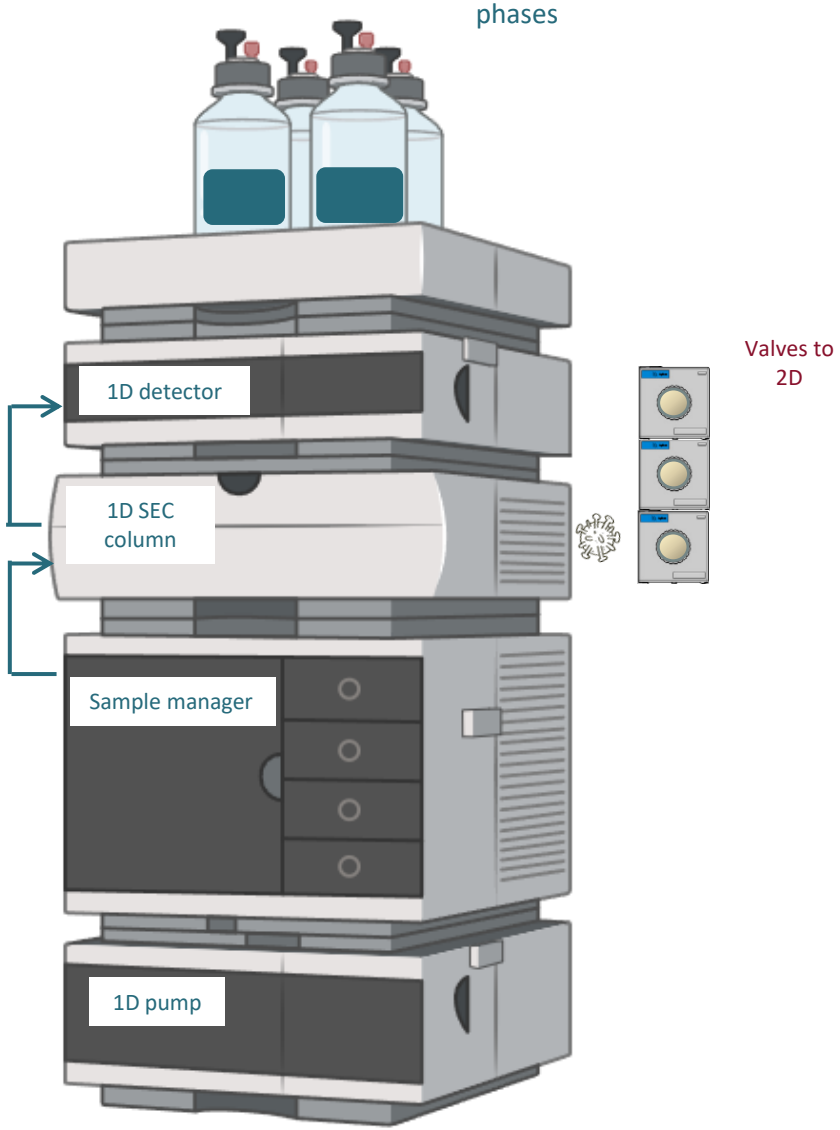
2D-LC workflow



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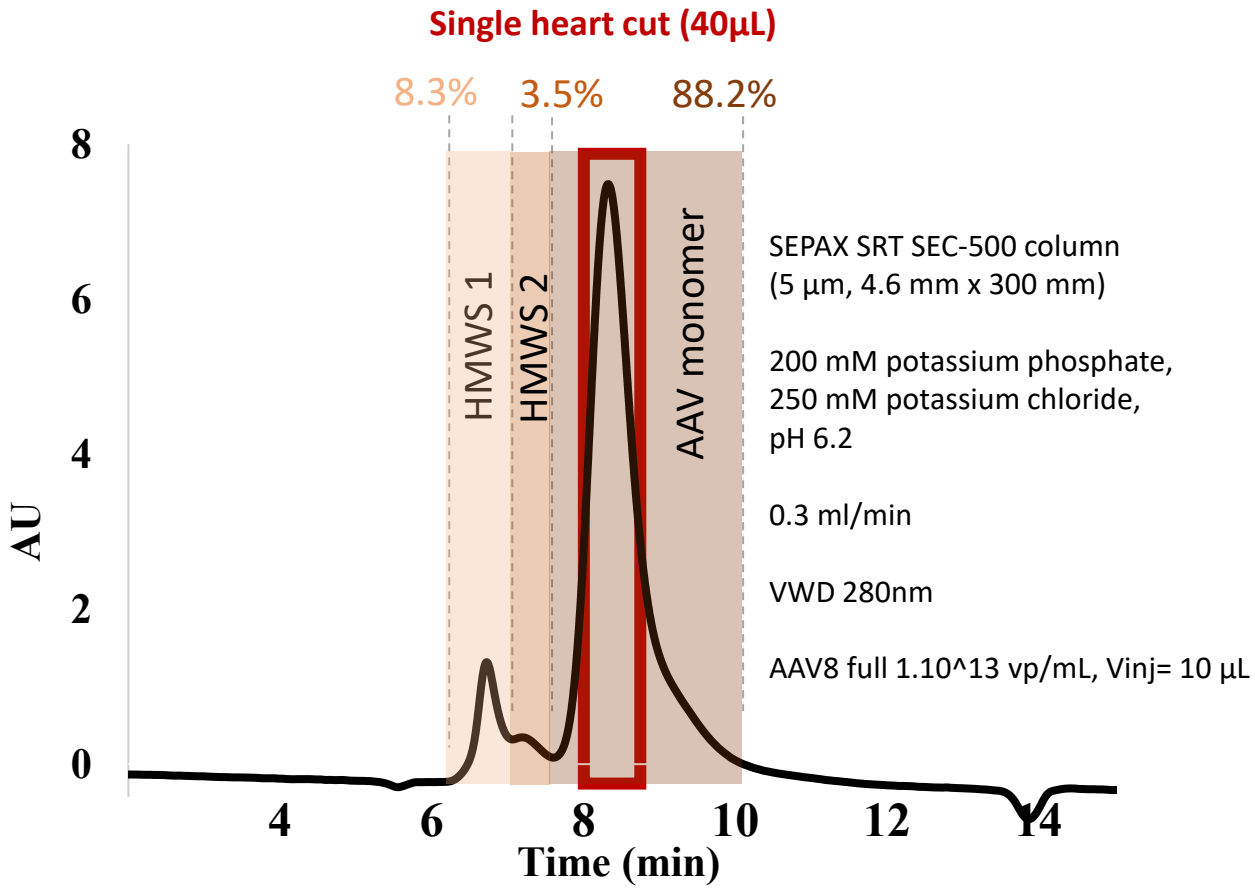

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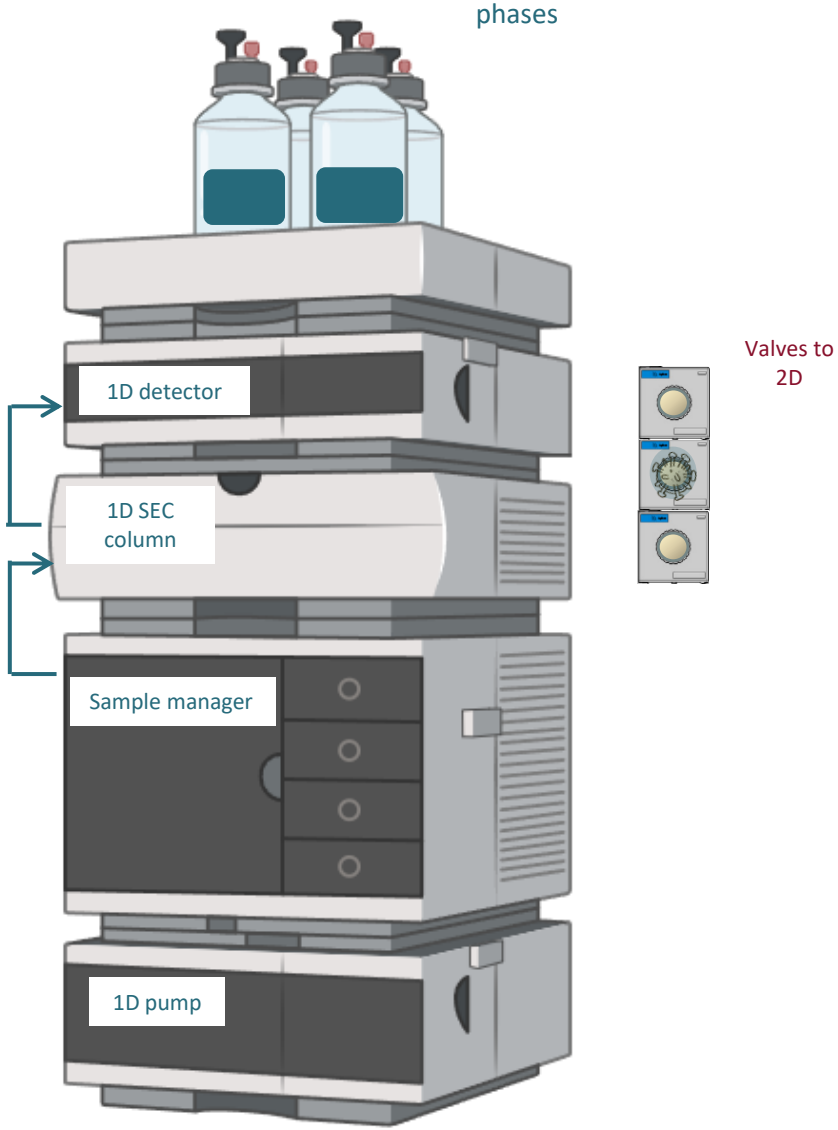

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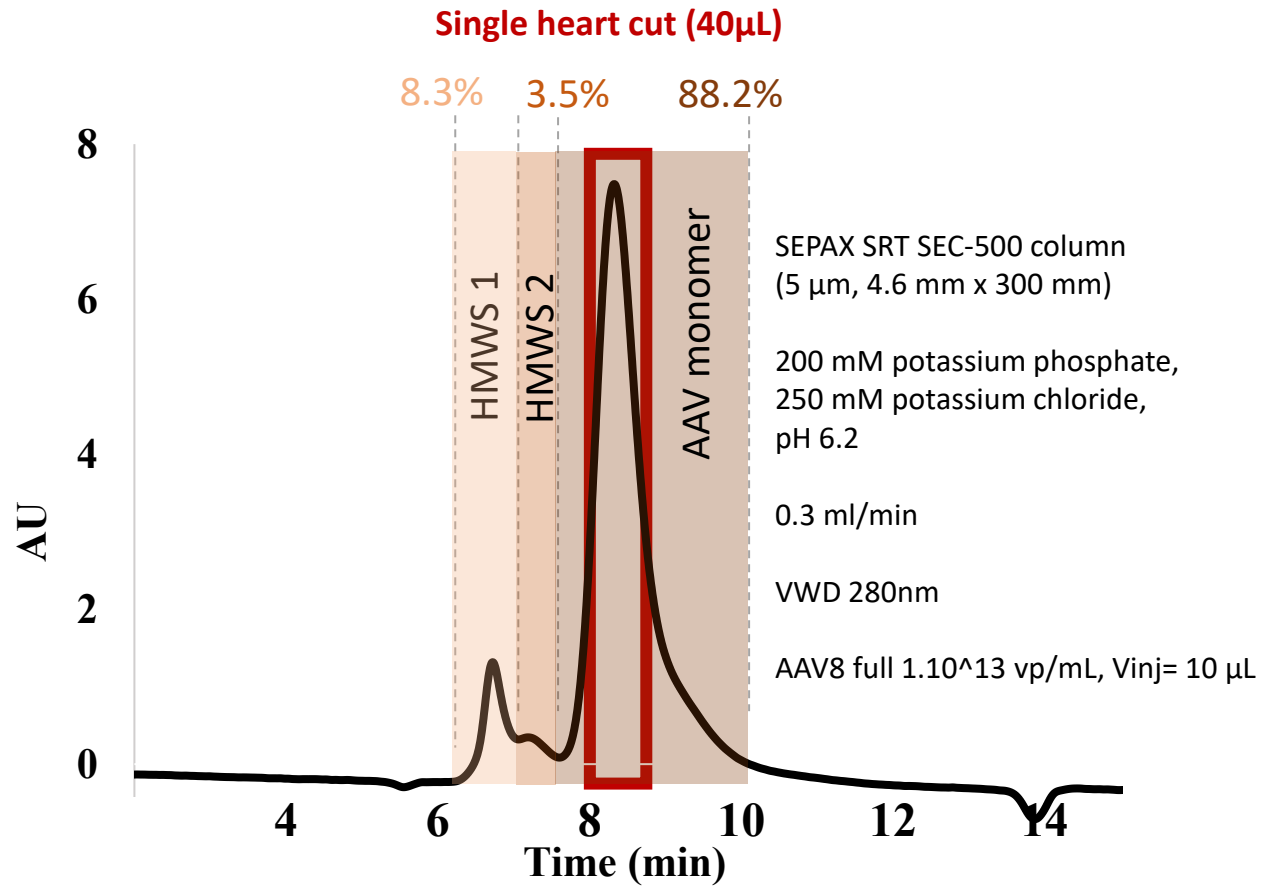

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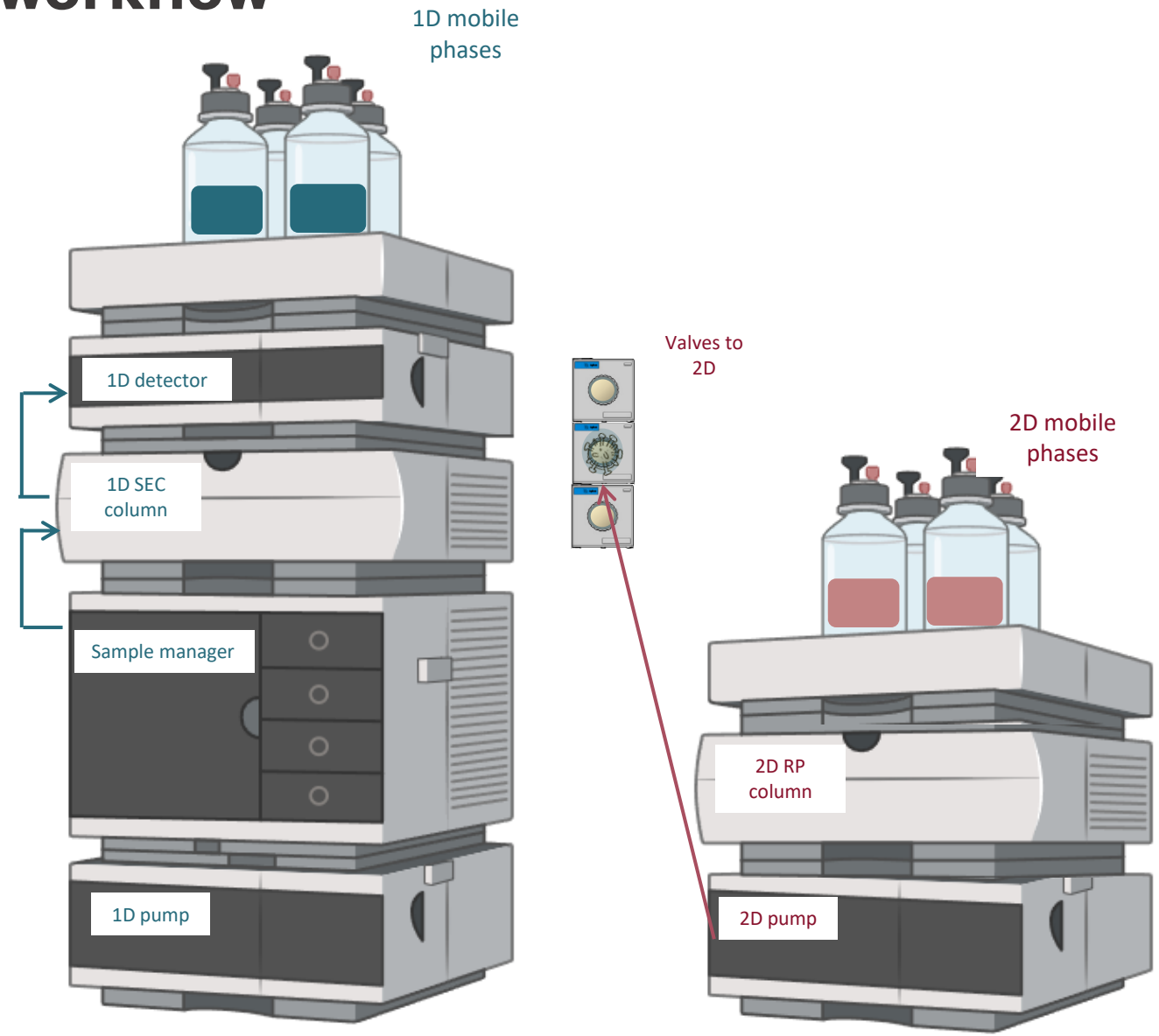

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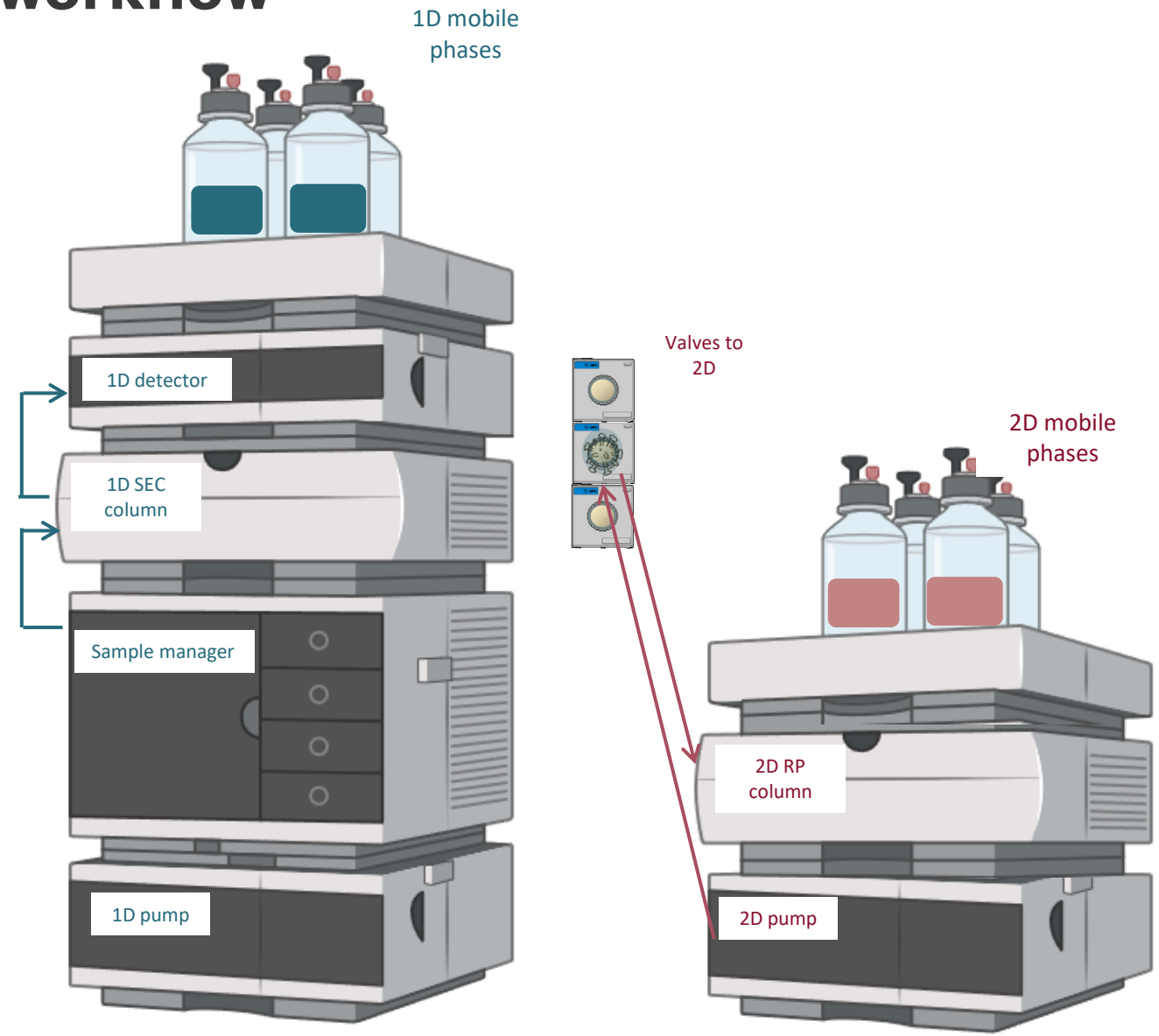

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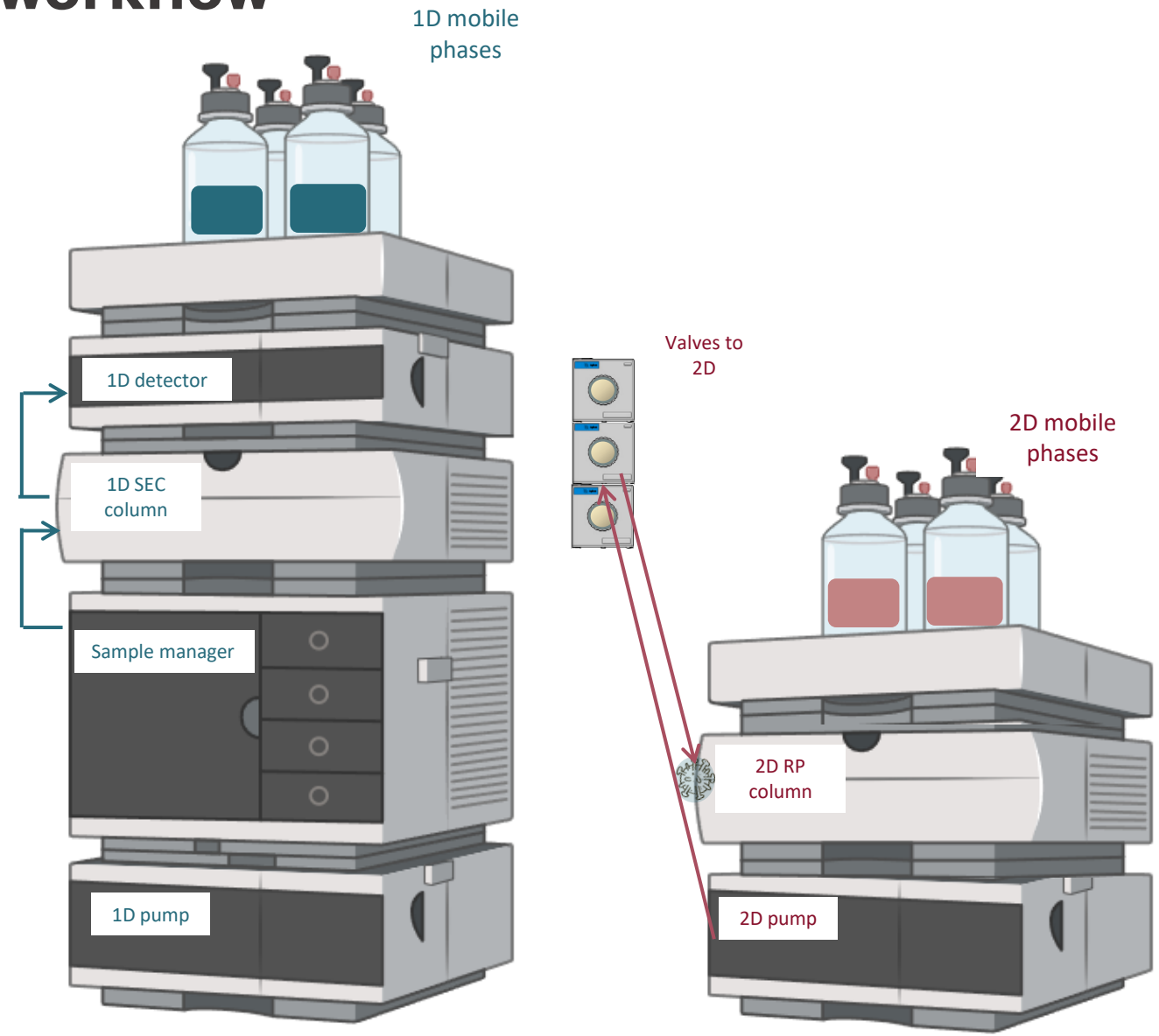

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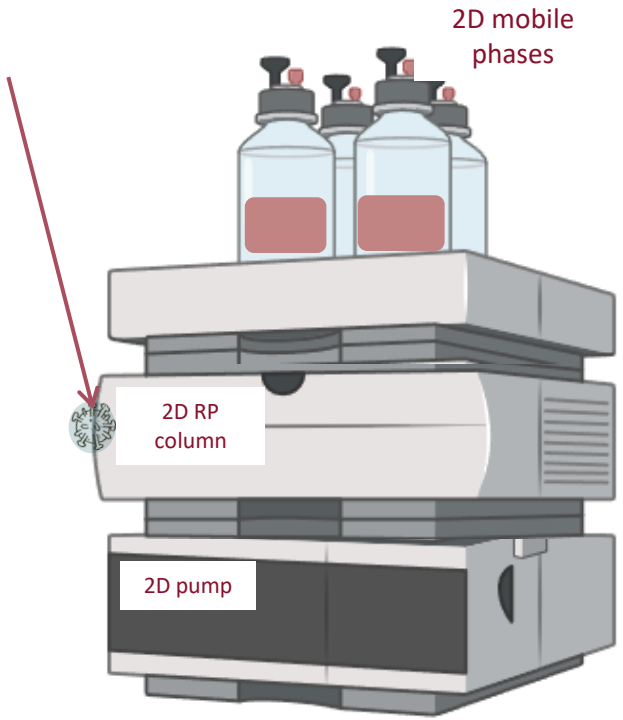

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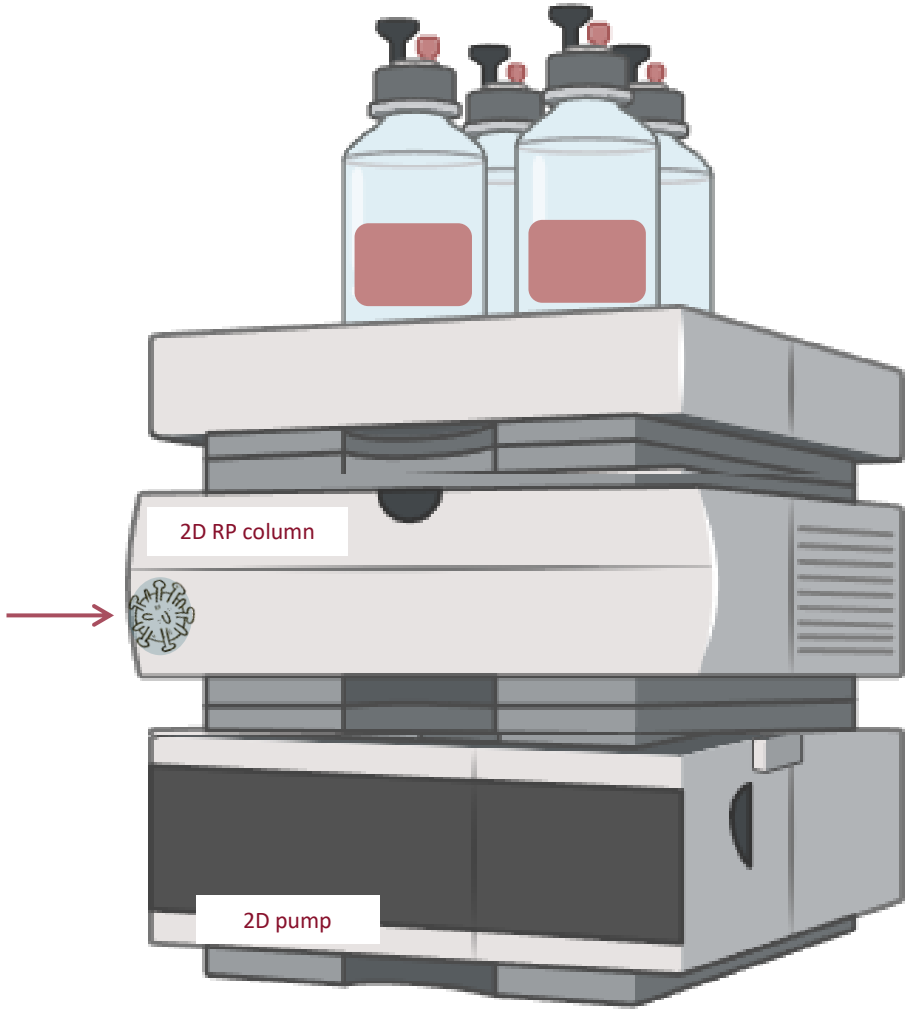

ACQUITY UPLC Protein BEH C4 column (1.7 µm, 300 Å, 2.1 mm × 150 mm)

MPA H2O 0.1% **DFA**, MPB ACN 0.1% **DFA**, Flow 0.2 mL/min, column temperature 60 °C

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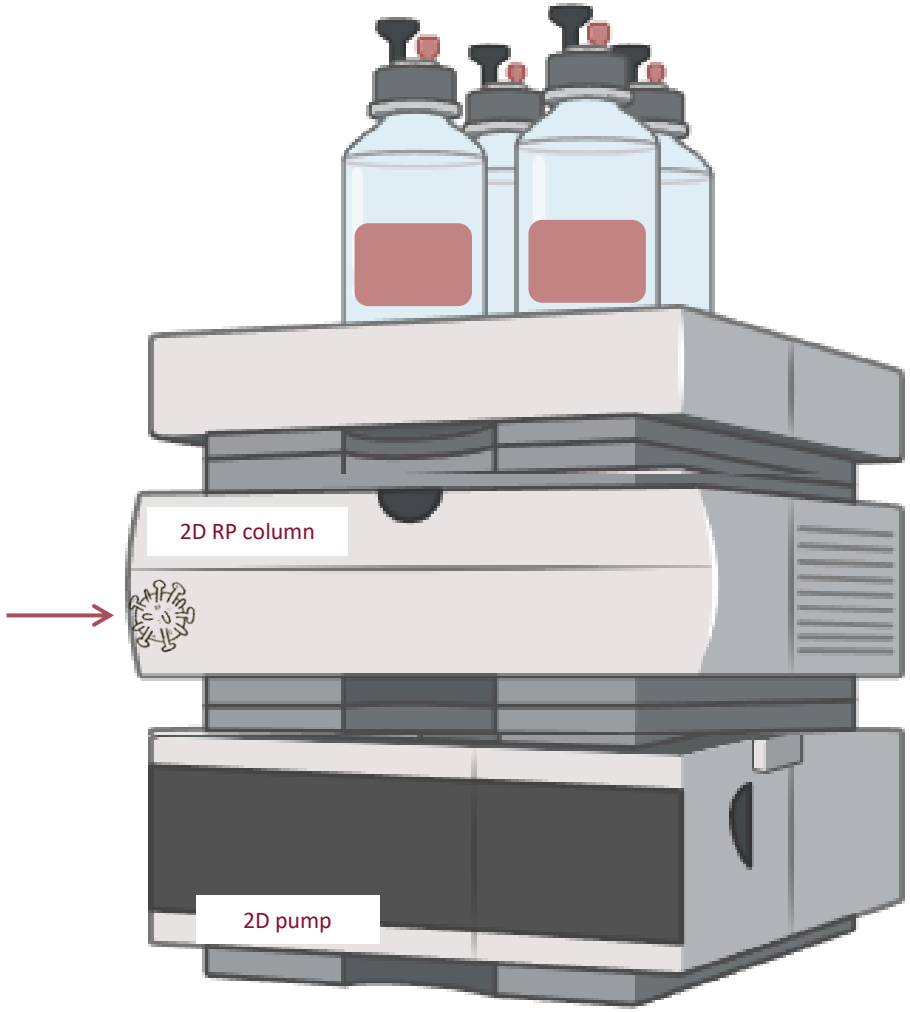
2D-LC workflow



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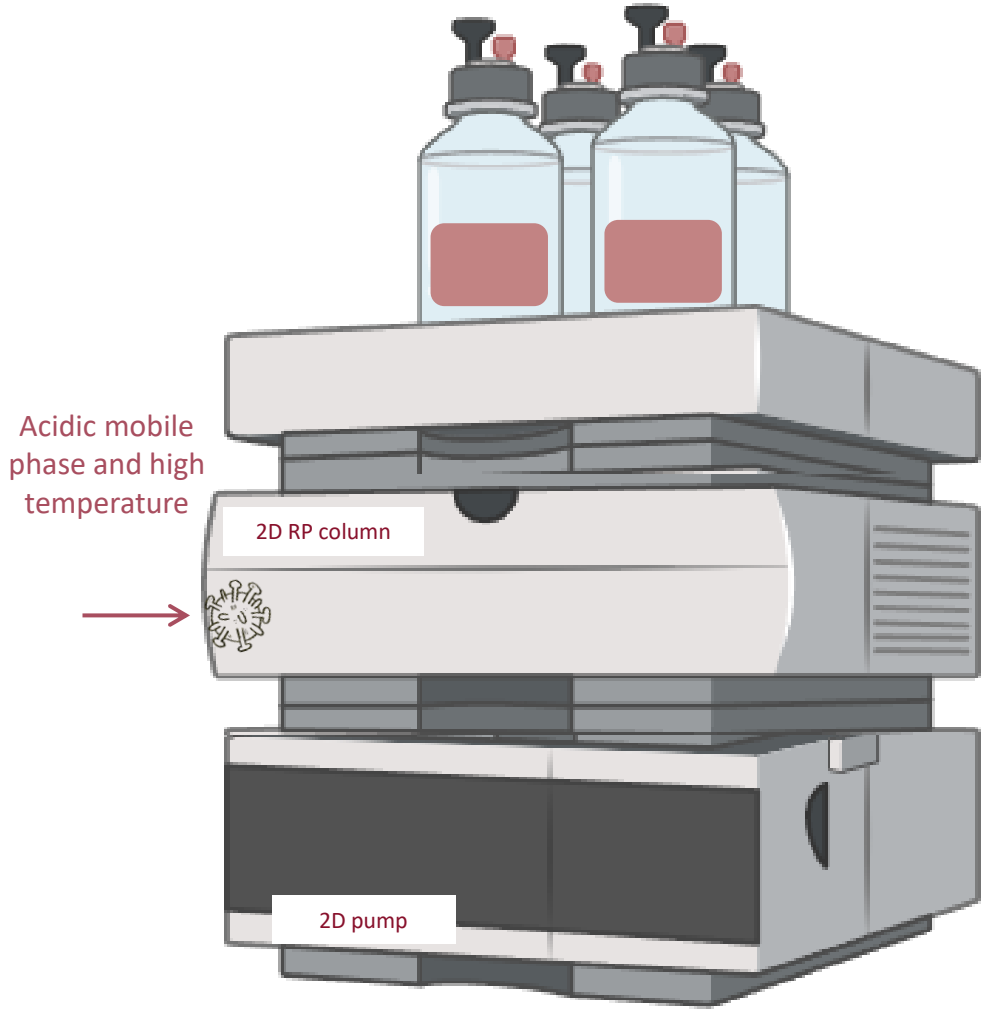

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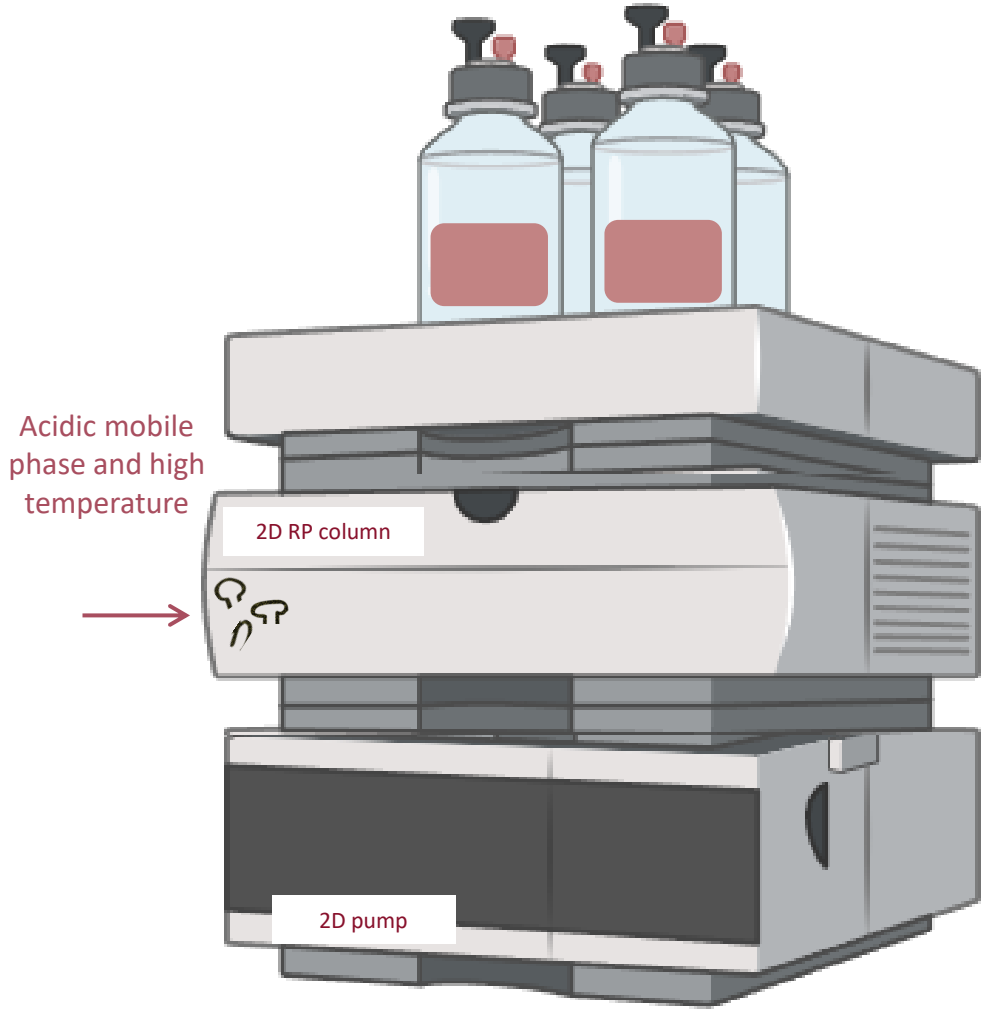

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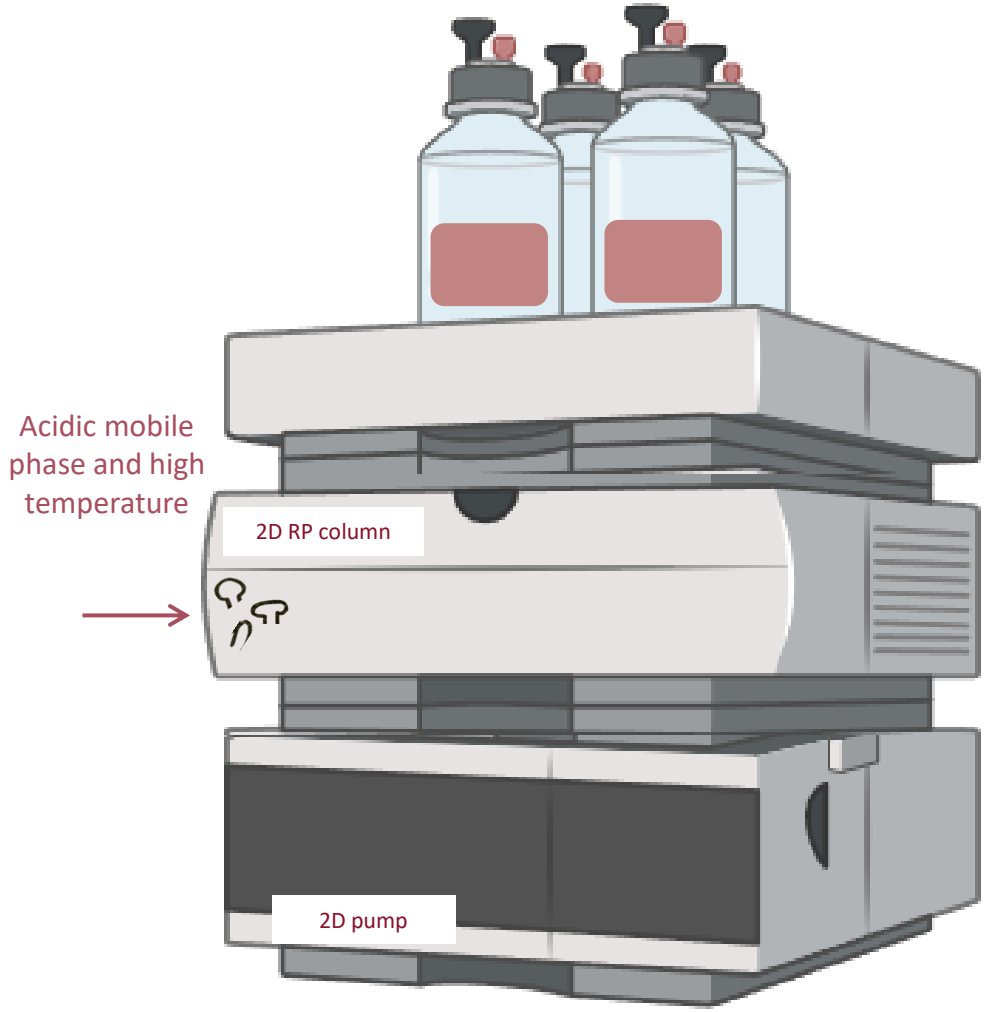

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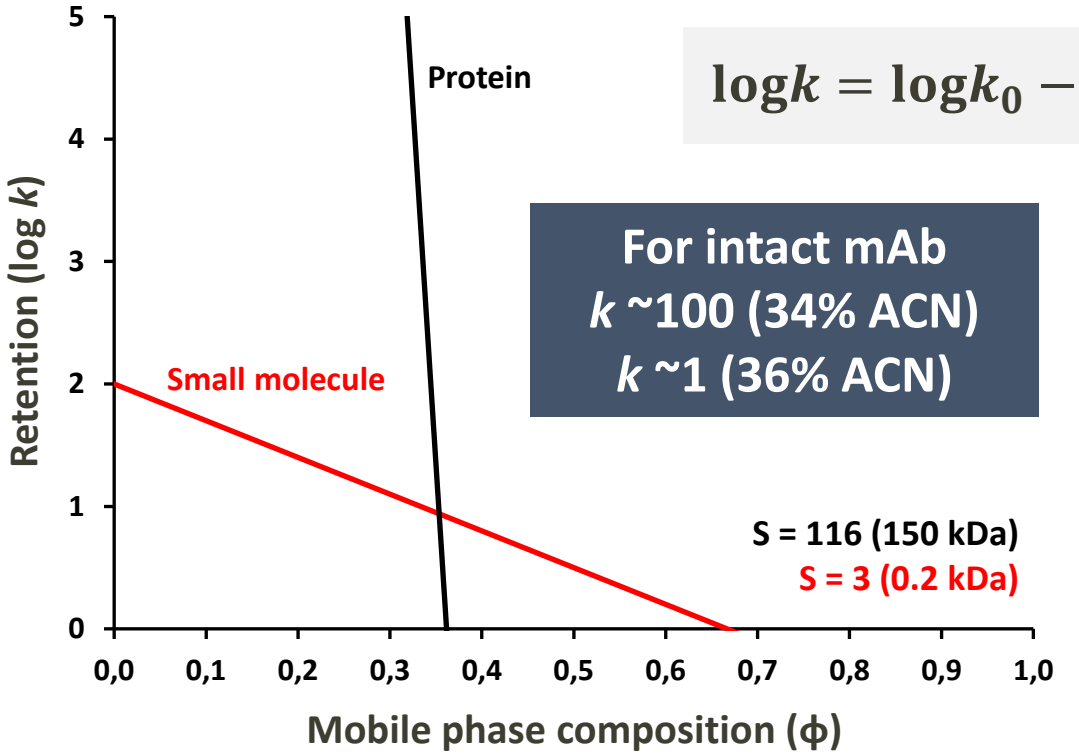
On-off mechanism of proteins:

Linear Solvent Strength (LSS)

$$\log k = \log k_0 - S \times \varphi$$



For intact mAb
k **~100 (34% ACN)**
k **~1 (36% ACN)**

S = 116 (150 kDa)
S = 3 (0.2 kDa)

Fekete et al. *Analytical Chemistry* 2019, 91, 12954-12961

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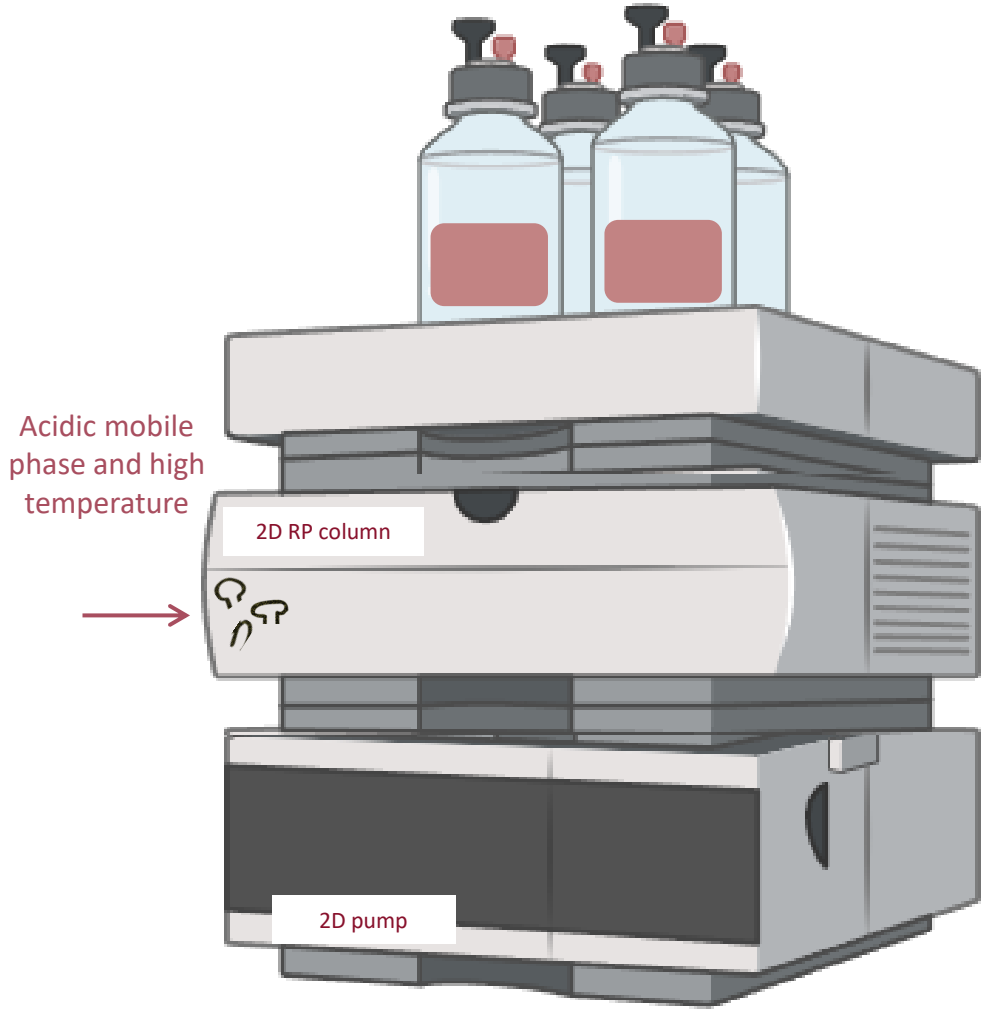
2D-LC workflow



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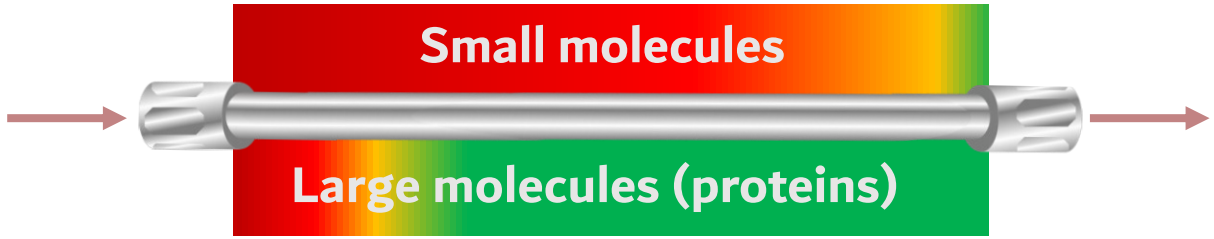
On-off mechanism of proteins:

Small molecules:

- Consecutive adsorption-desorption steps: continuous interactions with the stationary phase
- The whole lenght of the column is used for the separation



Large molecules (proteins) :

- Binding at the column inlet, desorption at a precise composition, elution without further interaction
- Only the column inlet is used for the separation

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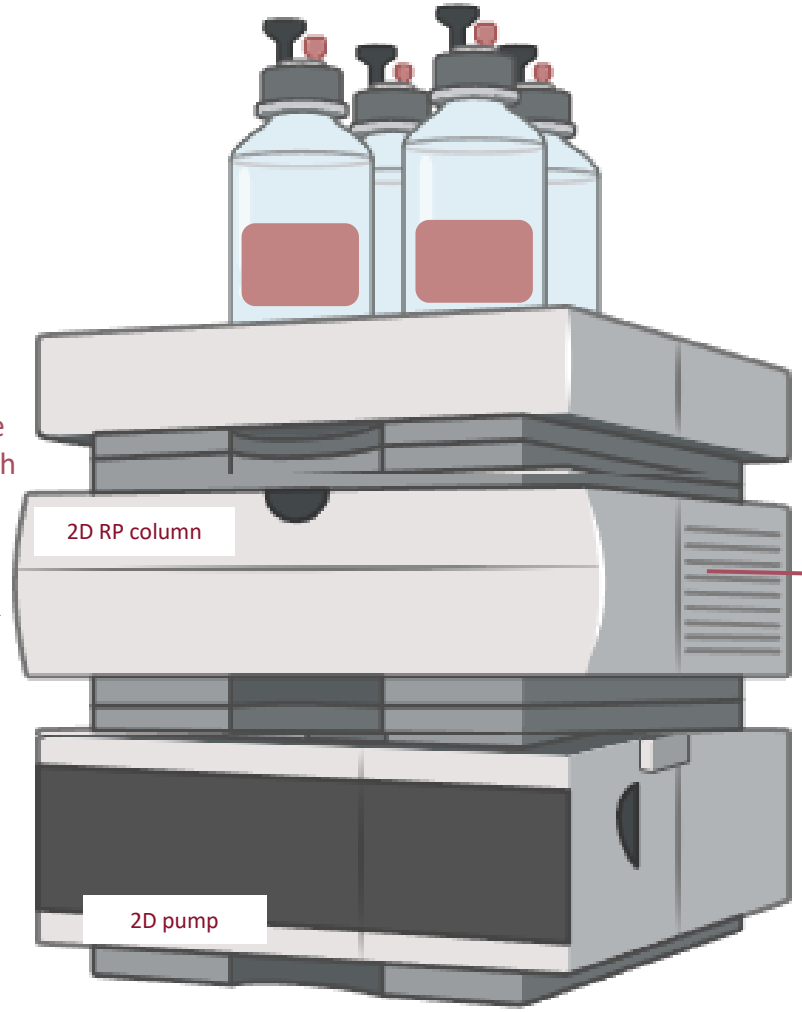
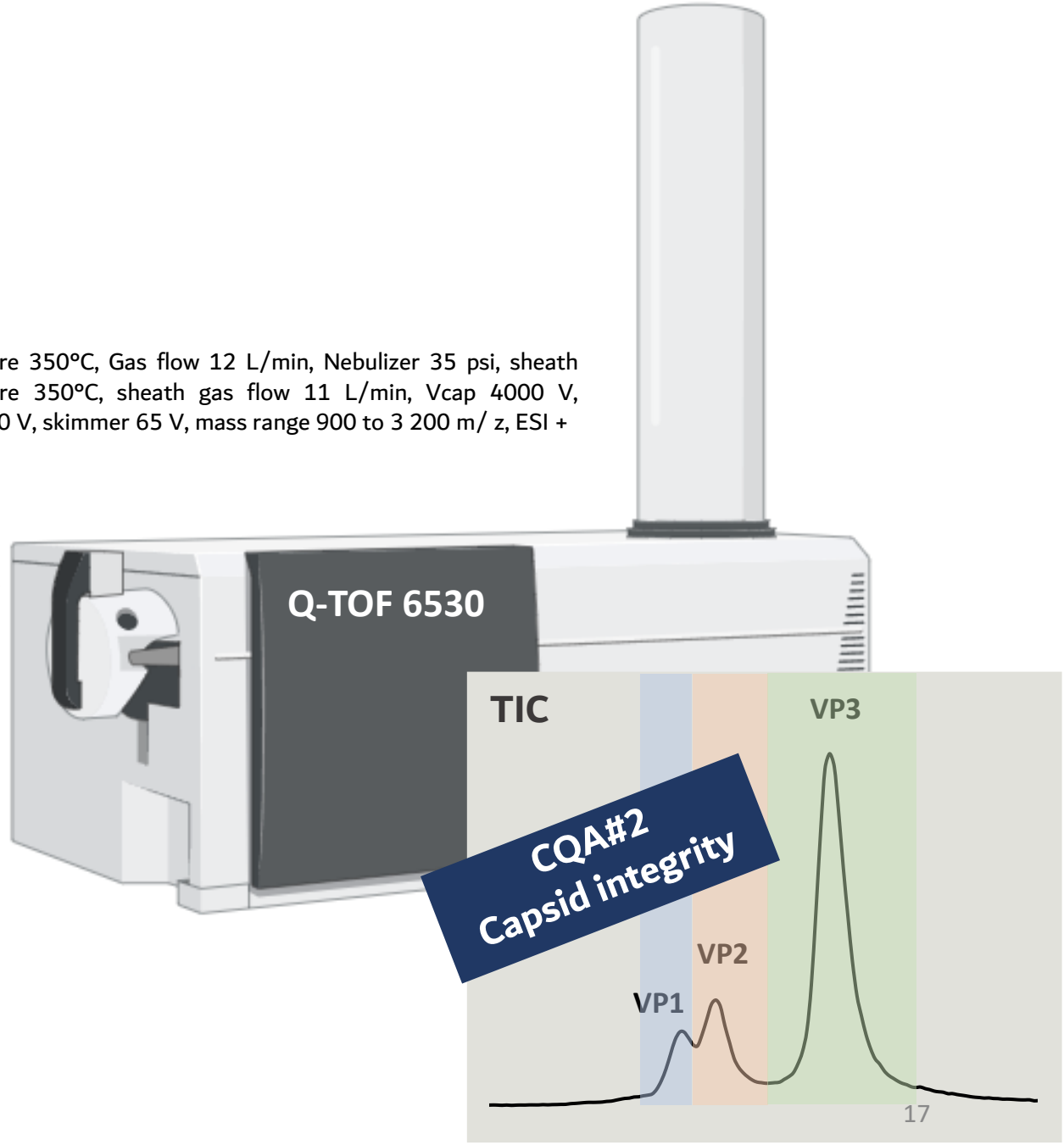
2D-LC workflow



MS:

Gas temperature 350°C, Gas flow 12 L/min, Nebulizer 35 psi, sheath gas temperature 350°C, sheath gas flow 11 L/min, Vcap 4000 V, Fragmentor 180 V, skimmer 65 V, mass range 900 to 3 200 m/ z, ESI +



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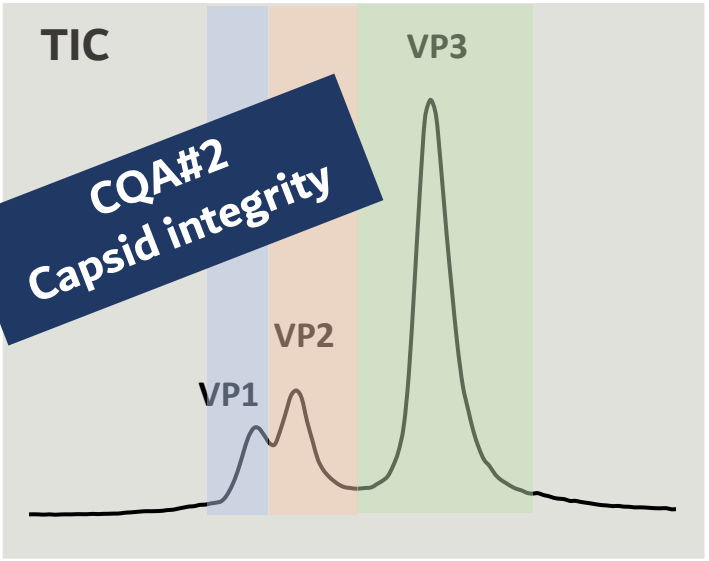
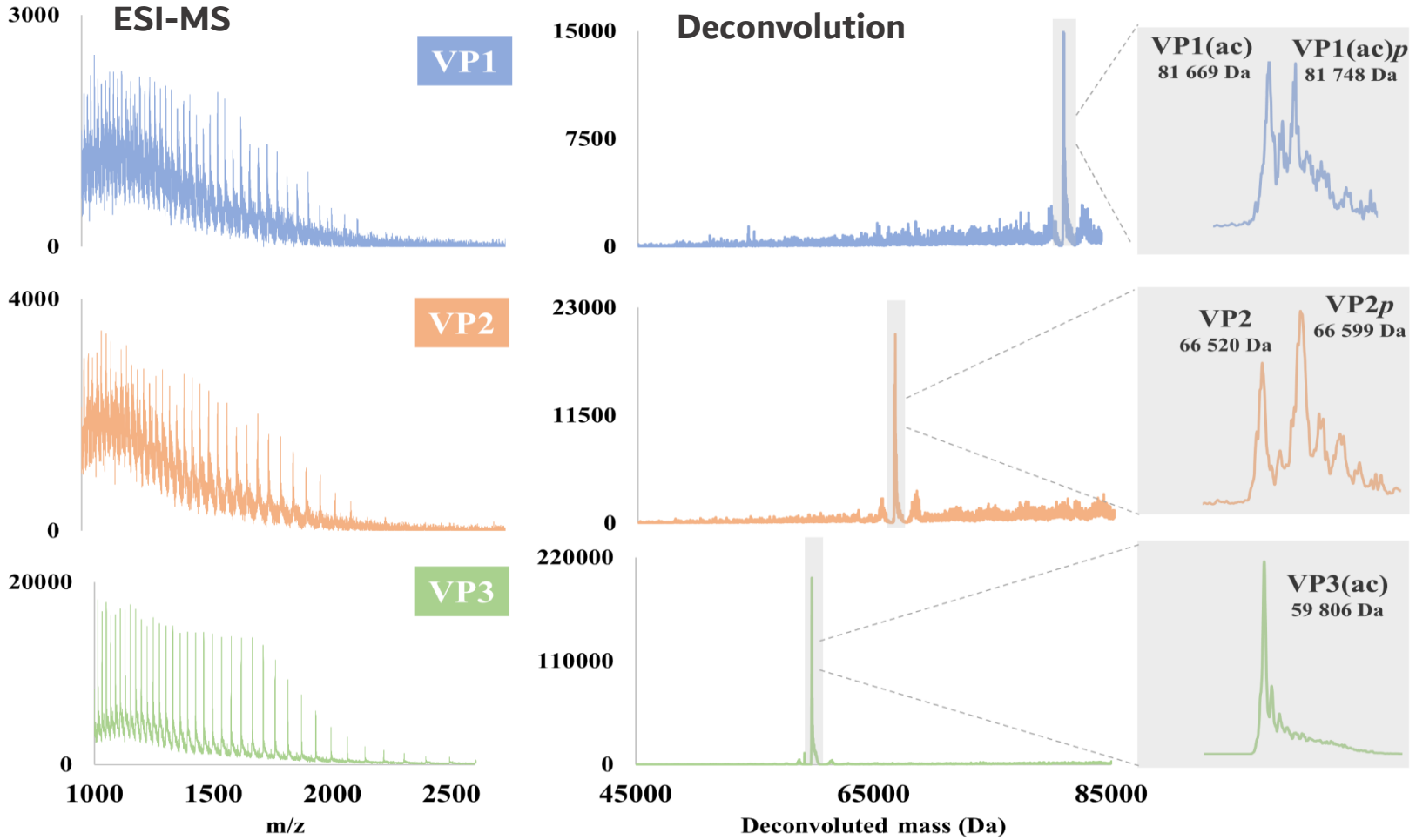
2D-LC workflow

CQA#3
Capsid identity

CQA#4
Post-translational modifications





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2D-LC multi-attributes method

CQA#1 Aggregation	CQA#2 Capsid integrity	CQA#3 Capsid identity	CQA#4 Post-translational modifications
	1 : 1 : 10	AAV2, AAV5, AAV6, AAV8, AAV9 … ?	Acetylation, phosphorylation …

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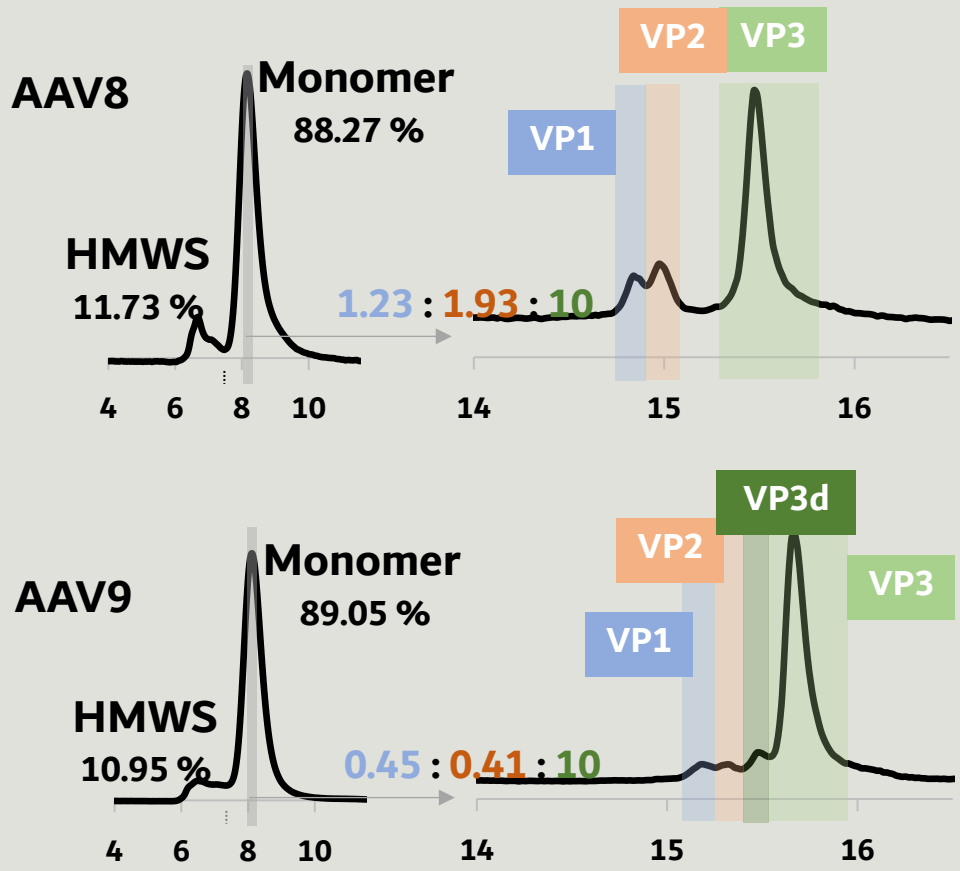
Application : *generic method applied to other serotypes*



For all the serotypes, the **monomer** has approximatively **the same size**:

→The cut in 1D does not have to be readjusted because all the monomers will have a close elution time.

For all the serotypes, **VPs** have approximatively **the same structure**:

→Elution composition range in RPLC does not have to be readjusted because all VPs will have a close retention time.

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Conclusion

- Development SEC-UV-RP-MS method able to characterize **4 CQAs in a single injection.**
- Confirmation of the **applicability of 2D-LC to characterize AAV** based products.
- The method's ability to deliver detailed characterization without requiring extensive adjustments for **different serotypes** confirms its broad applicability and versatility.

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