

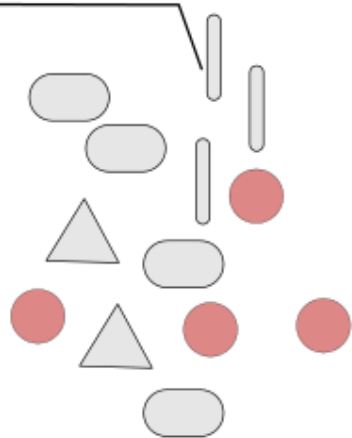
Bi8313: Praktikum z genového inženýrství (jaro 2017)

Fast protein liquid chromatography

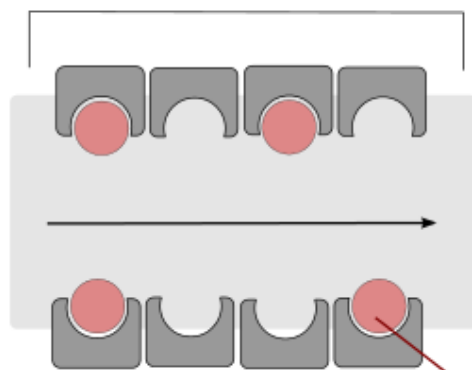


MASARYKOVA UNIVERZITA
PŘÍRODOVĚDECKÁ FAKULTA
ÚSTAV EXPERIMENTÁLNÍ BIOLOGIE
LABORATOŘ MOLEKULÁRNÍ DIAGNOSTIKY MIKROORGANISMŮ

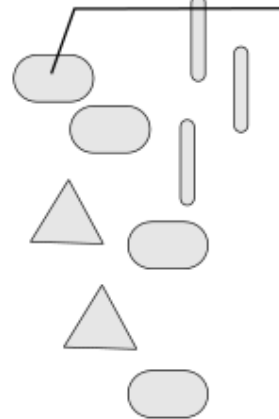
bunkový lyzát



afinitná kolóna



odpad

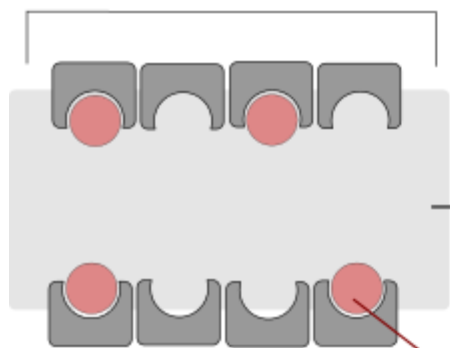


1

proteín
záujmu

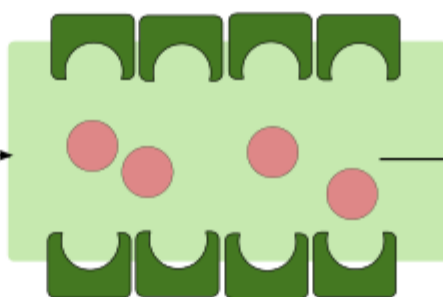
2

afinitná kolóna

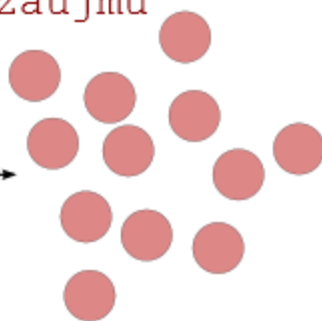


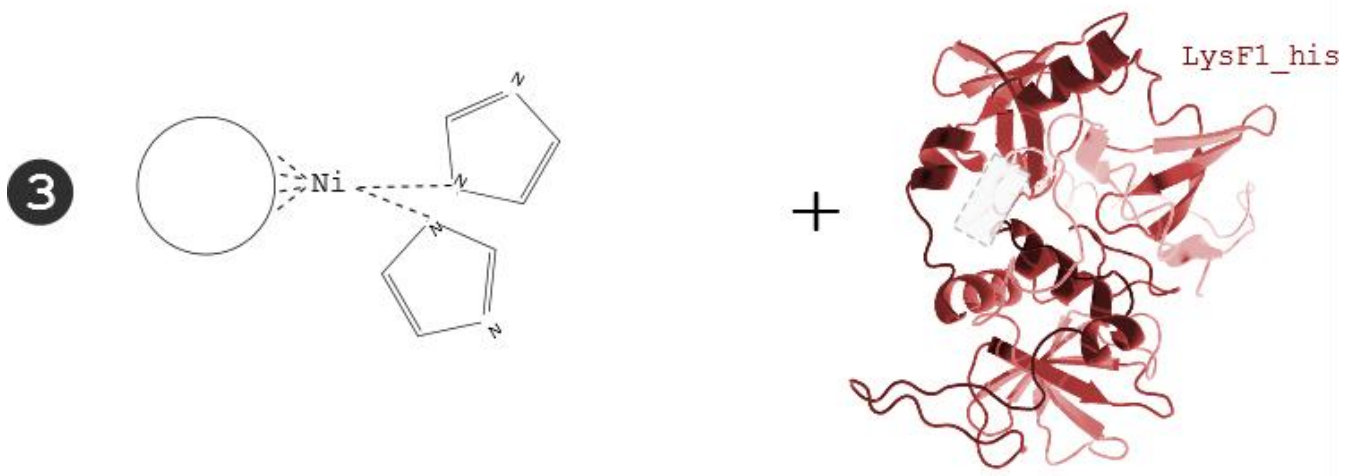
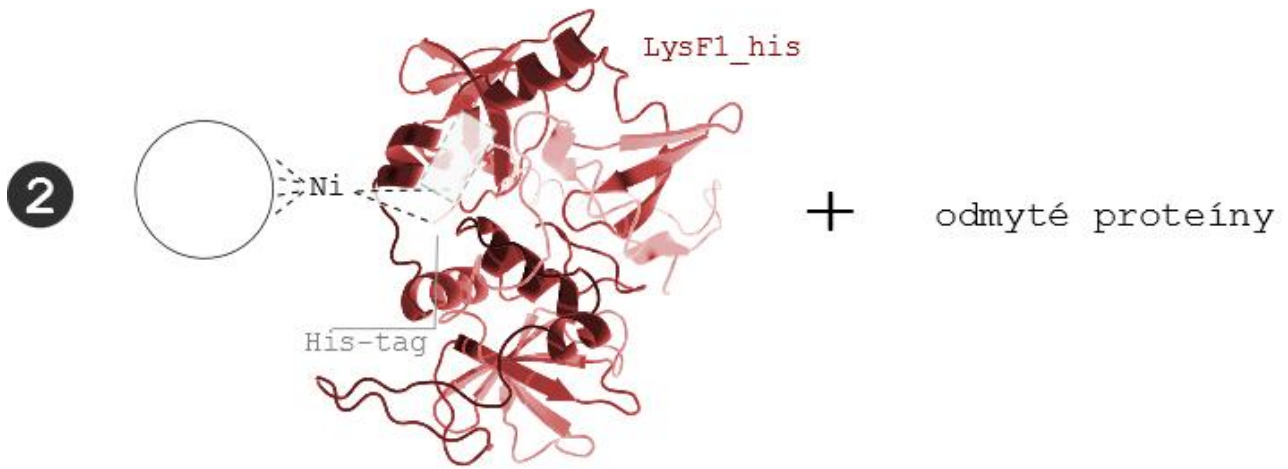
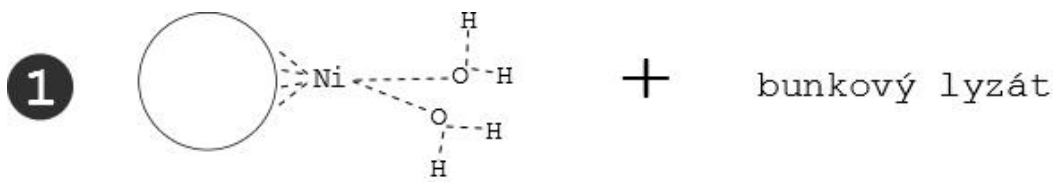
proteín
záujmu

elúcia

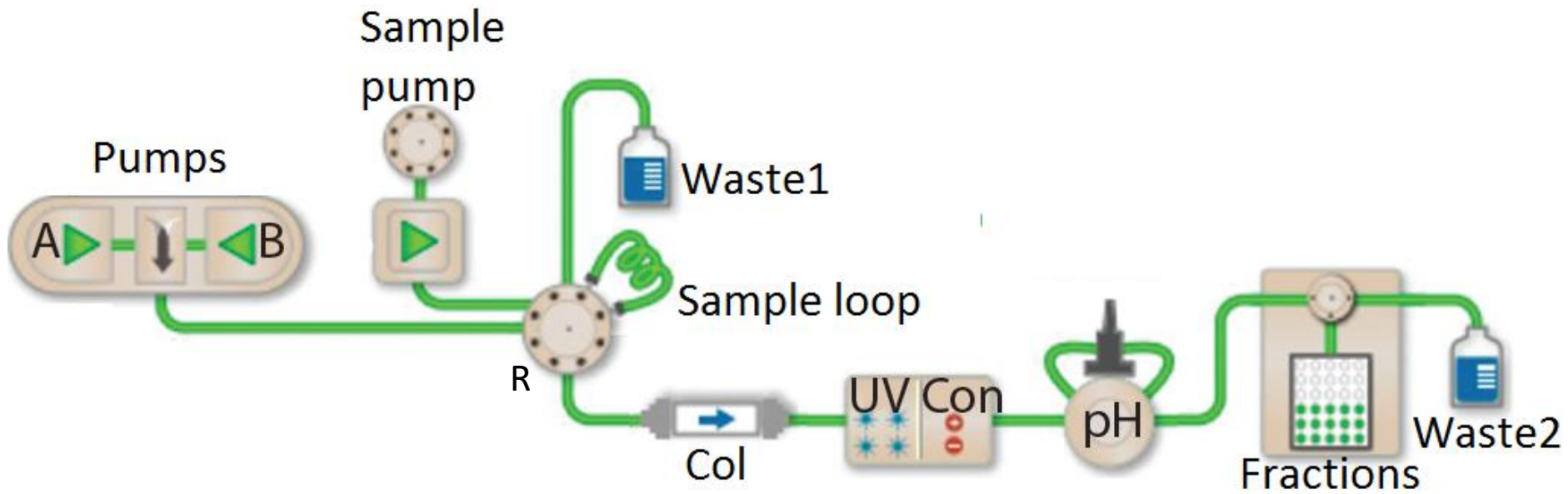


čistý
proteín
záujmu

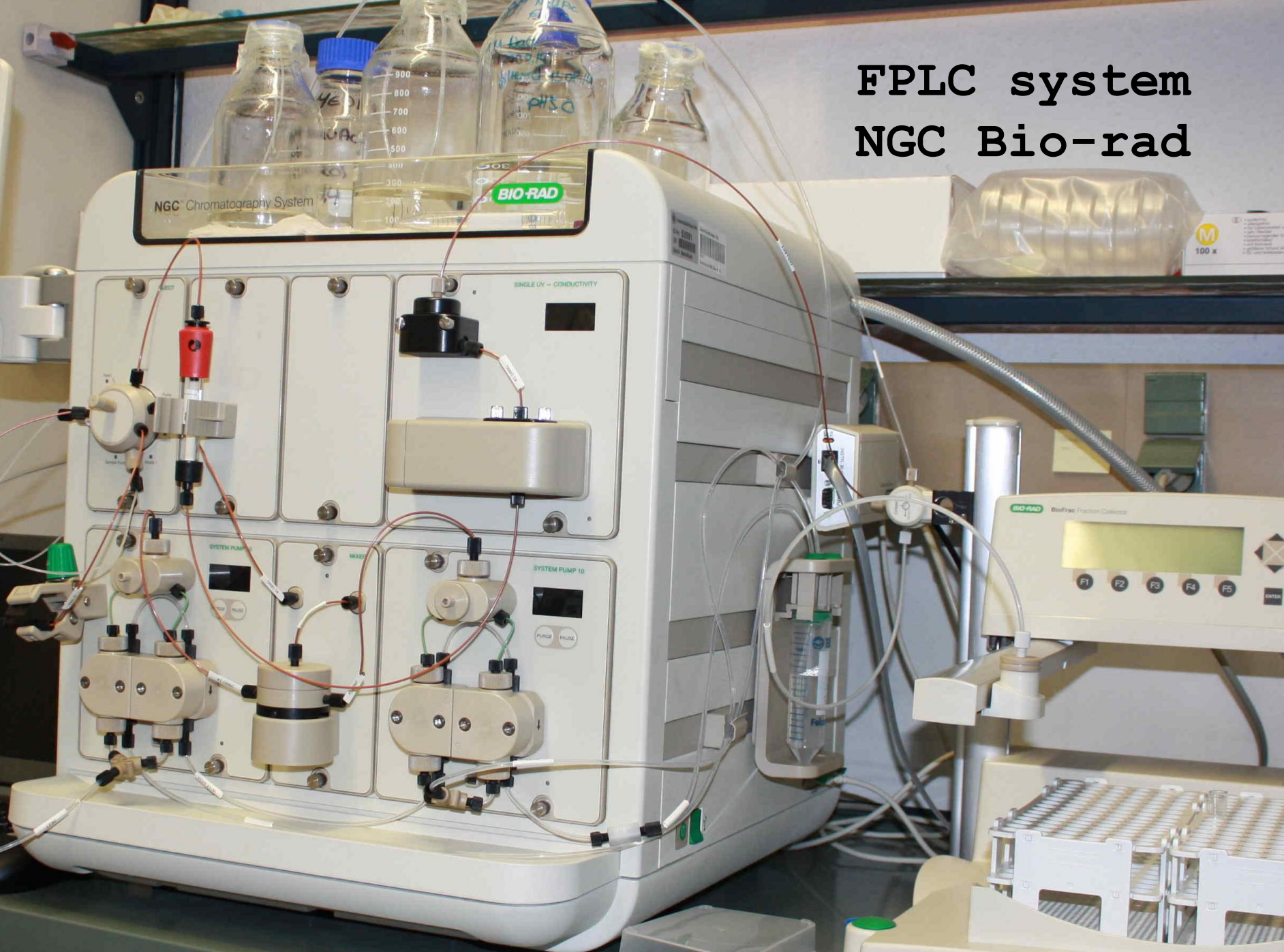


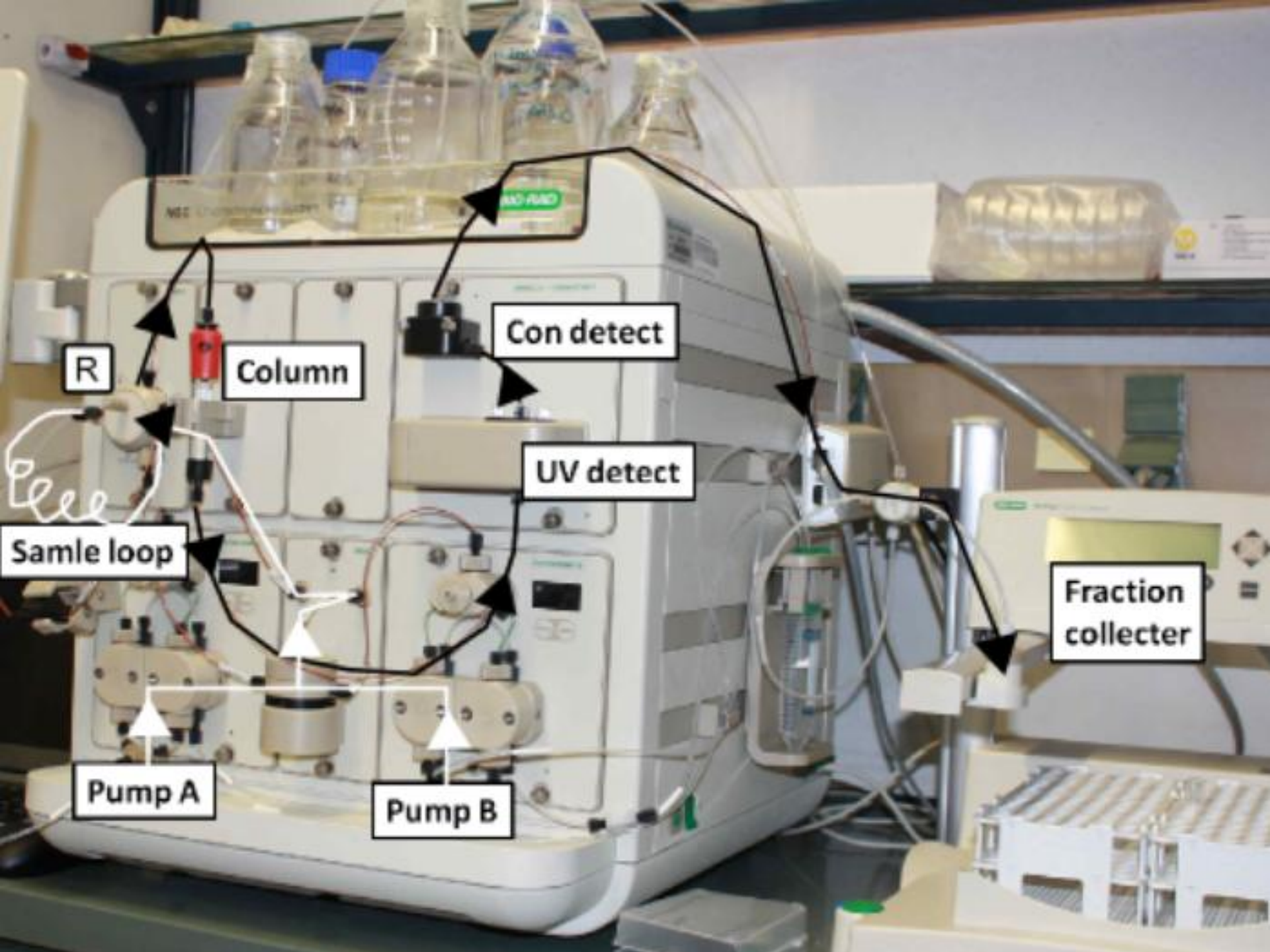


FPLC system - schéma



FPLC system
NGC Bio-rad





R

Column

Con detect

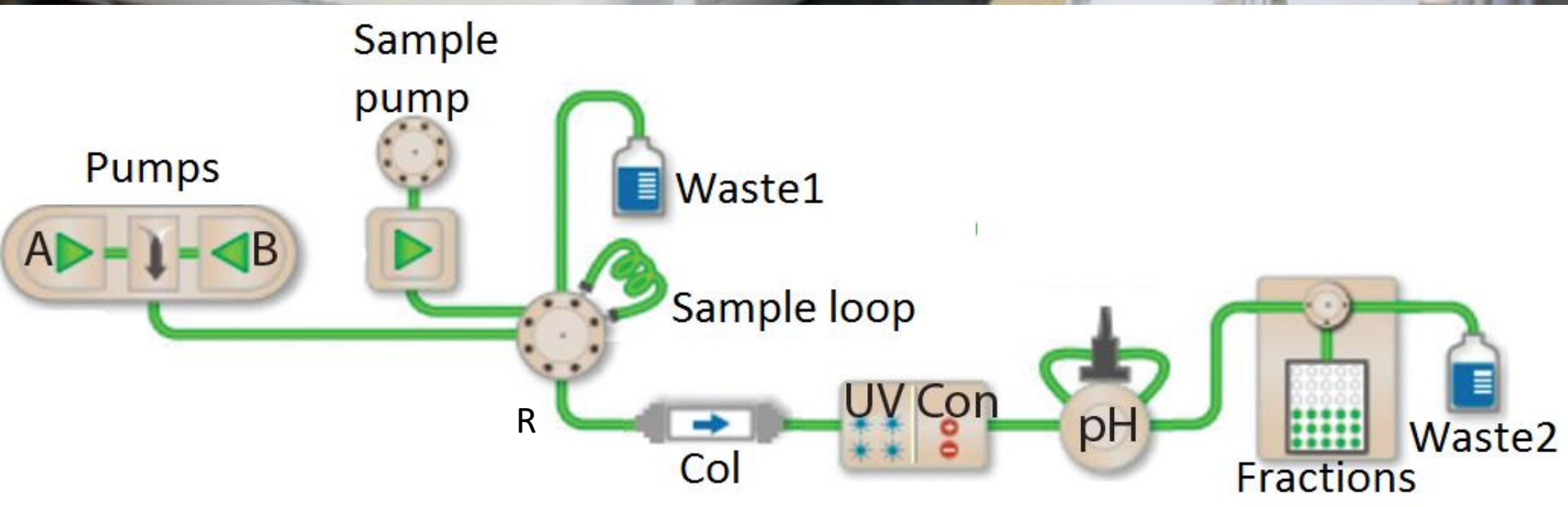
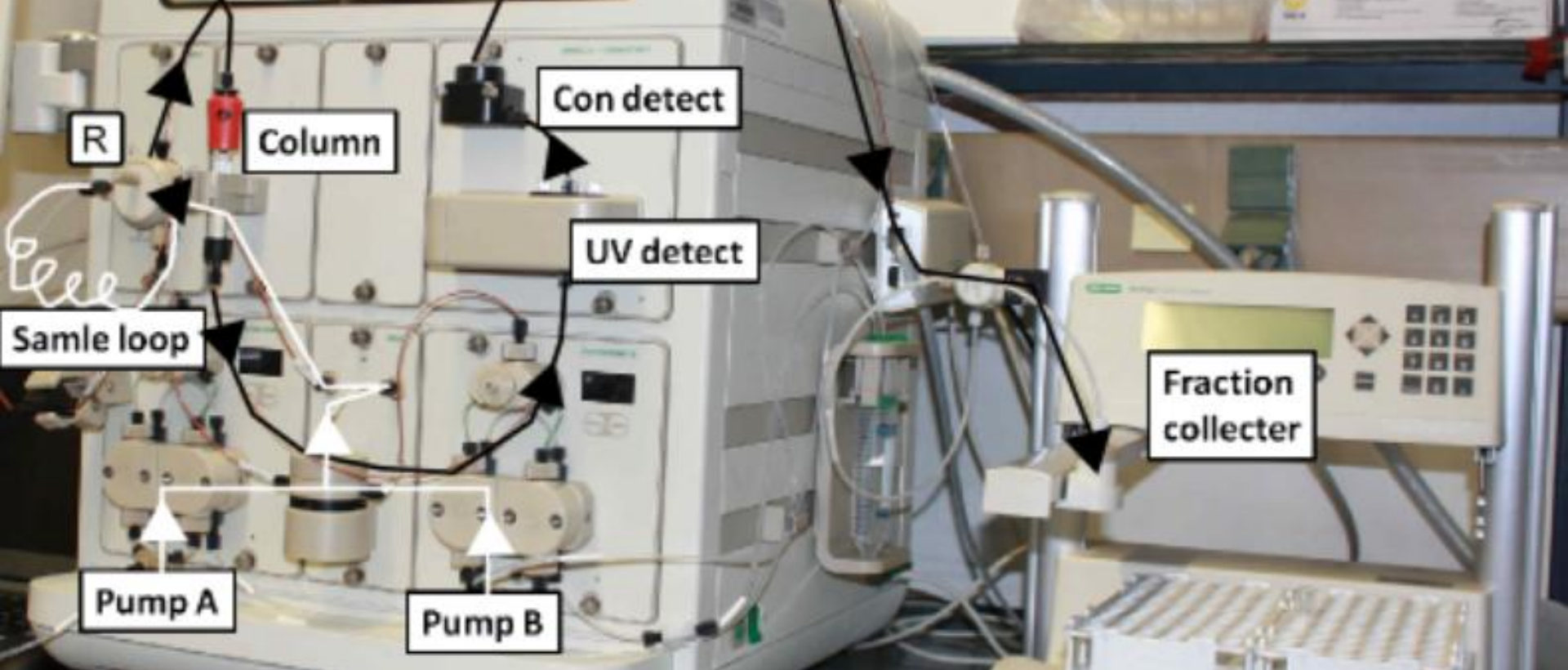
UV detect

Sample loop

Fraction collector

Pump A

Pump B



Buffer A

HEPES 100mM, pH = 6.8

NaCl 200mM

Imidazole 20 mM

PMSF 0.2mM (optional)

Buffer B

HEPES 100mM, pH = 6.8

NaCl 200mM

Imidazole 500 mM

PMSF 0.2mM (optional)

Purification Buffer

stable pH

optimal ionic strength

elution substance

minimal proteolysis

<https://www.youtube.com/watch?v=DfzB6BtSjBY>