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Plasma Protein Binding: On RED alert!

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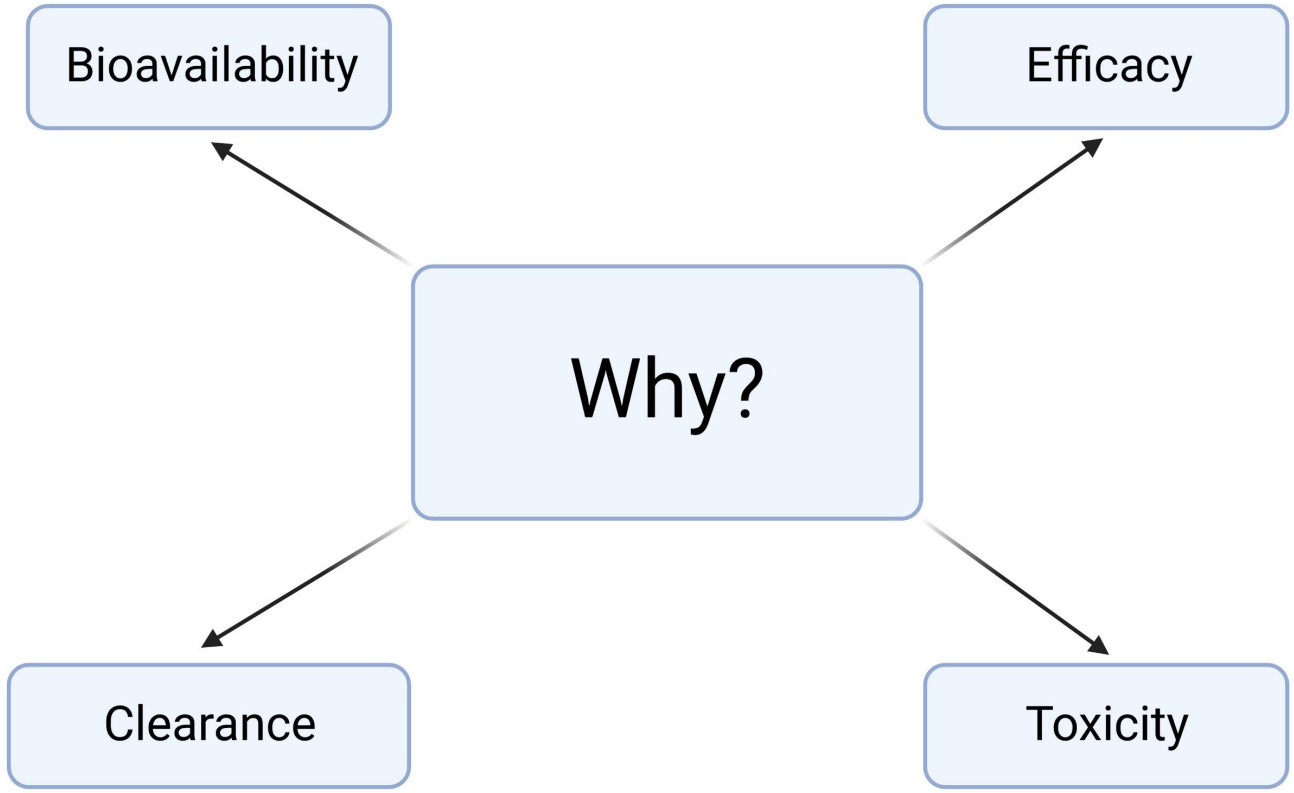
Protein binding

- Protein binding refers to the degree in which a drug is bound to proteins within the blood
- Only free drug is available for pharmacological interactions
- Unbound compound can cross cell membranes unassisted
- May release over time

Why do we care about protein binding?



What can affect protein binding?

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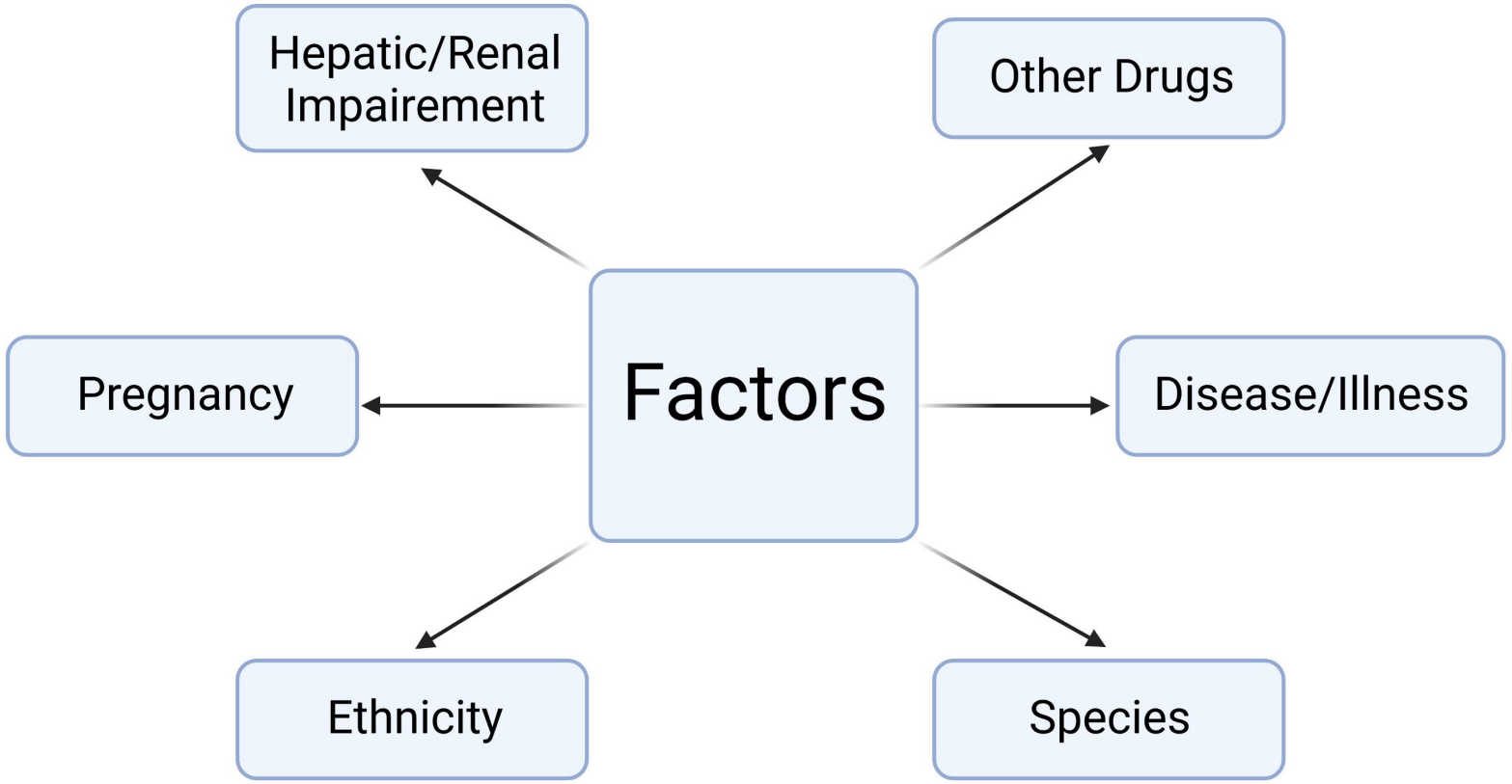
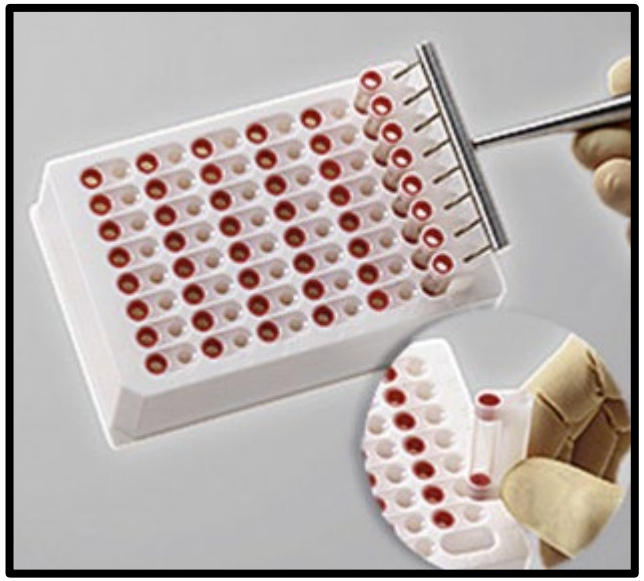


Measuring protein binding

- Ultrafiltration (UF)
- Ultracentrifugation (UC)
- Equilibrium Dialysis (ED)
- Rapid Equilibrium Dialysis (RED)



Rapid Equilibrium Dialysis (RED)

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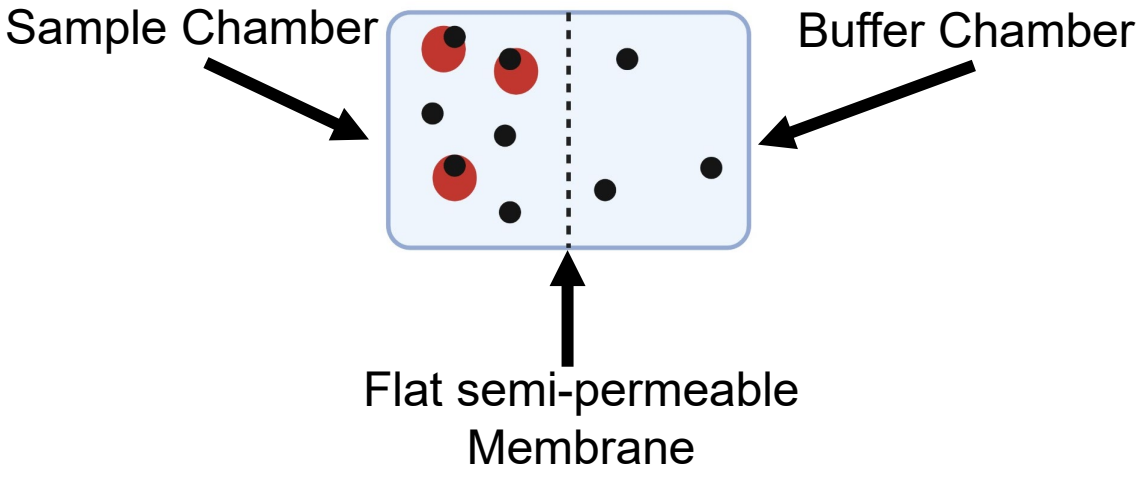

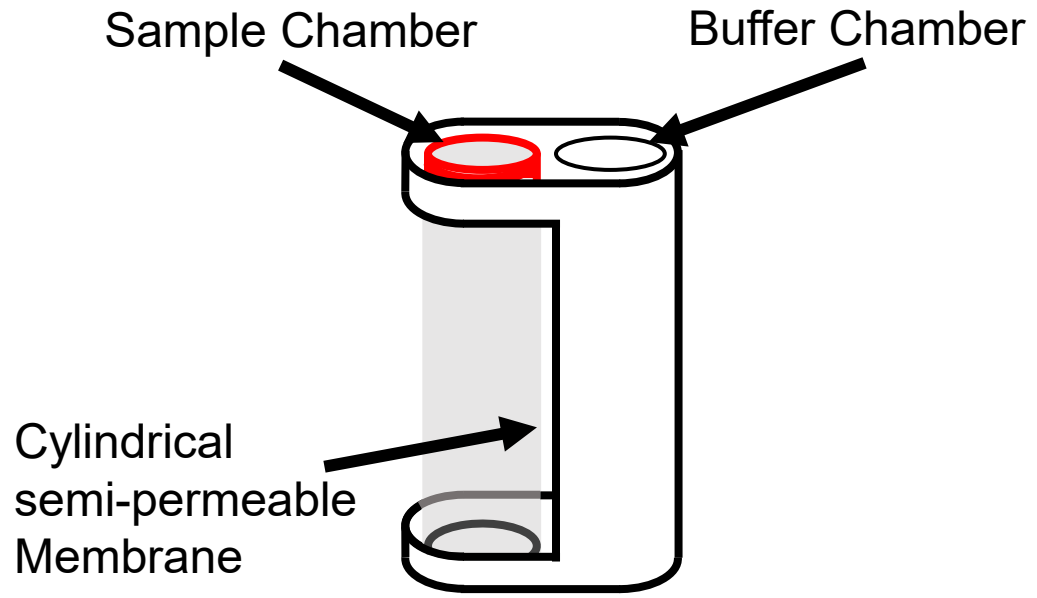

Advantage
- Larger surface area = faster equilibrium

Rapid Equilibrium Dialysis (RED)

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$$\% FU = \frac{Concentration\ white\ chamber}{Concentration\ red\ chamber} x100$$

Characterisation of RED techniques for specific compounds

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Experiment	Objectives
Binding	• Does your compound bind to the pipette tips, plate or membrane? • How can binding be overcome?
Cleaning	• Can the base plate be cleaned and reused?
Mass Balance	• Does your compound cross the membrane? • Does your compound reach equilibrium? • How long does it take for your compound to reach equilibrium? • What is the recovery of your compound?
Stability	• Is your compound stable at incubation temperature? • What does it mean if your compound isn't stable?
Time Course	• How long does it take for your compound to reach equilibrium? • What is the % fraction unbound? • Is your % fraction unbound consistent?

Tip Binding

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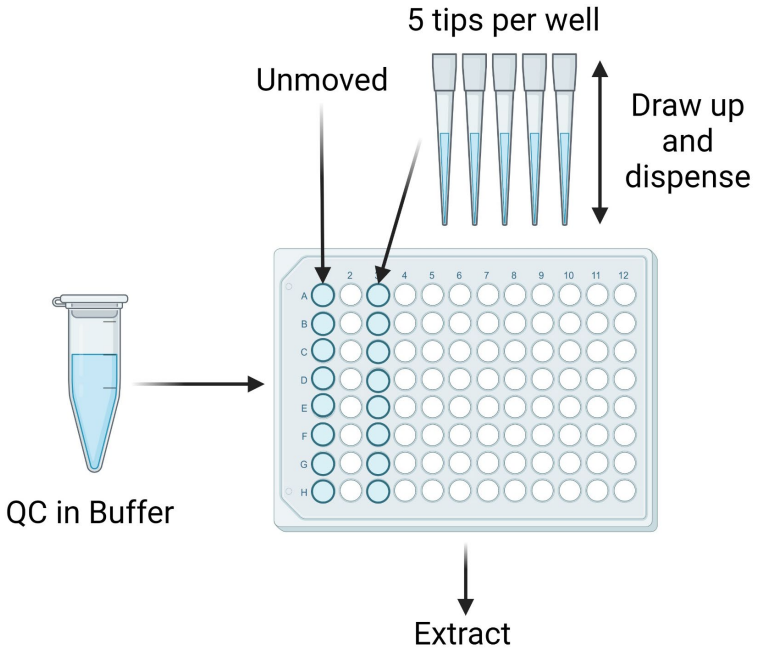

Objective	Considerations
• Does your compound bind to the **pipette tips**, plate or membrane?	• Type of pipette • Pipetting method
• How can binding be overcome?	• Pre-wet • Alternative pipette • Additives/stabilisers

Plate Binding

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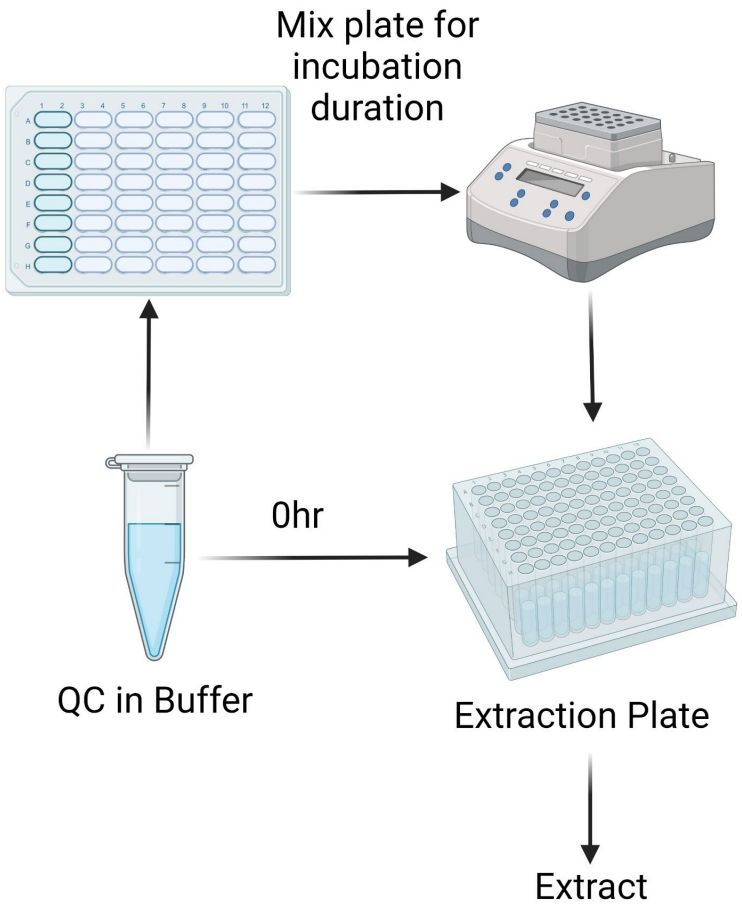

Objective	Considerations
• Does your compound bind to the pipette tips, **plate** or membrane?	• PTFE (Teflon) vs PP
• How can binding be overcome?	• Change plate type

Binding

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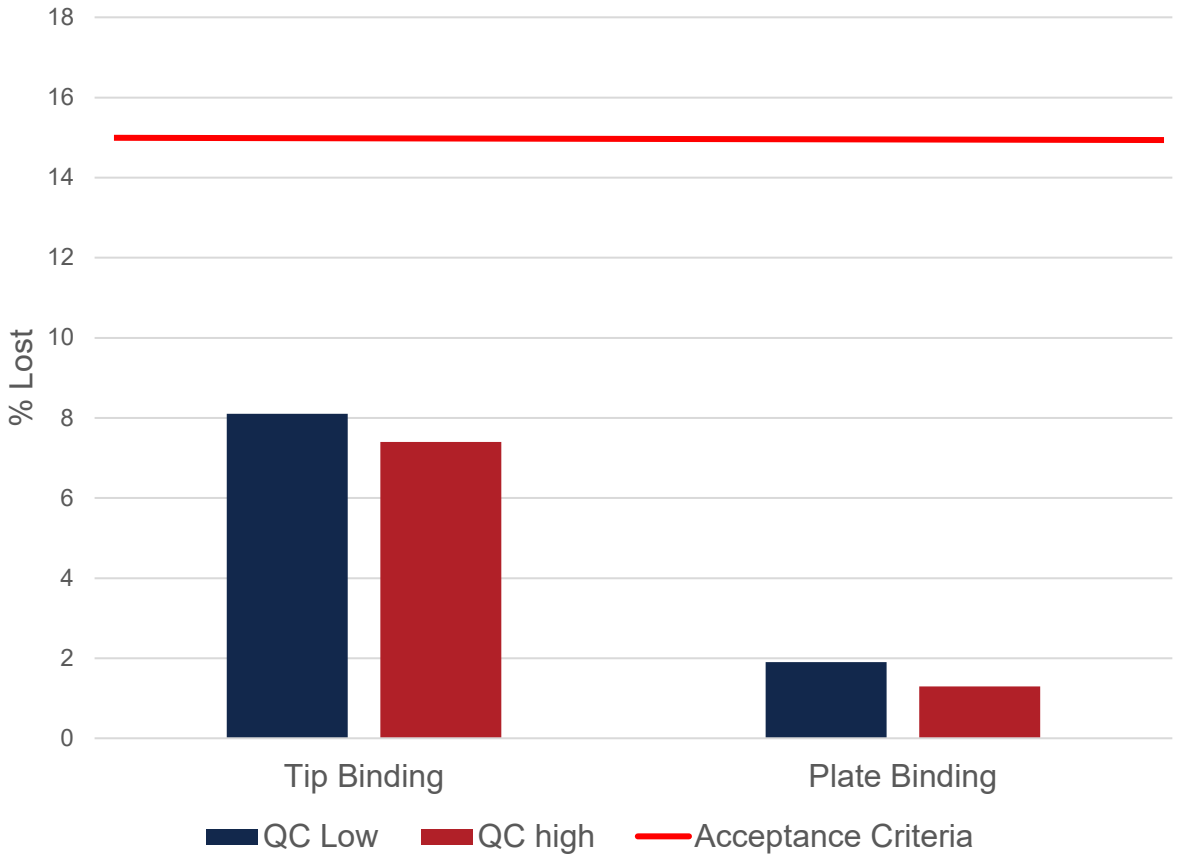
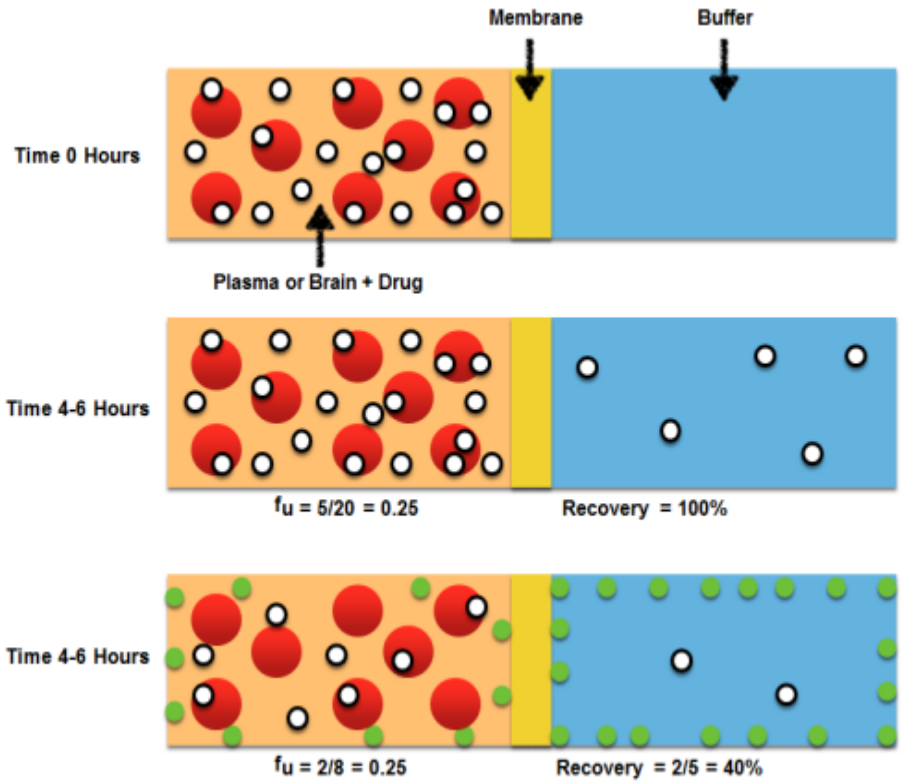
$$\% \ lost = \%RE \ Unstressed - \%RE \ Stressed$$





No non-specific binding

Non-specific binding present

Cleaning

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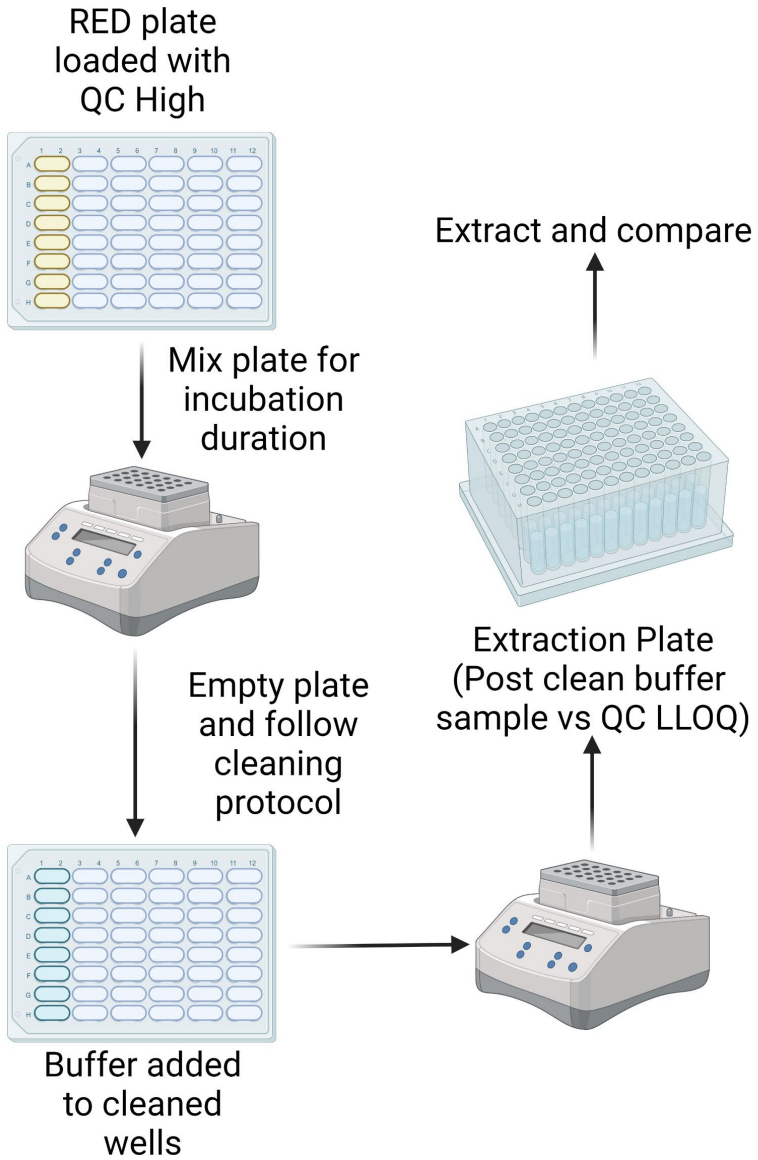
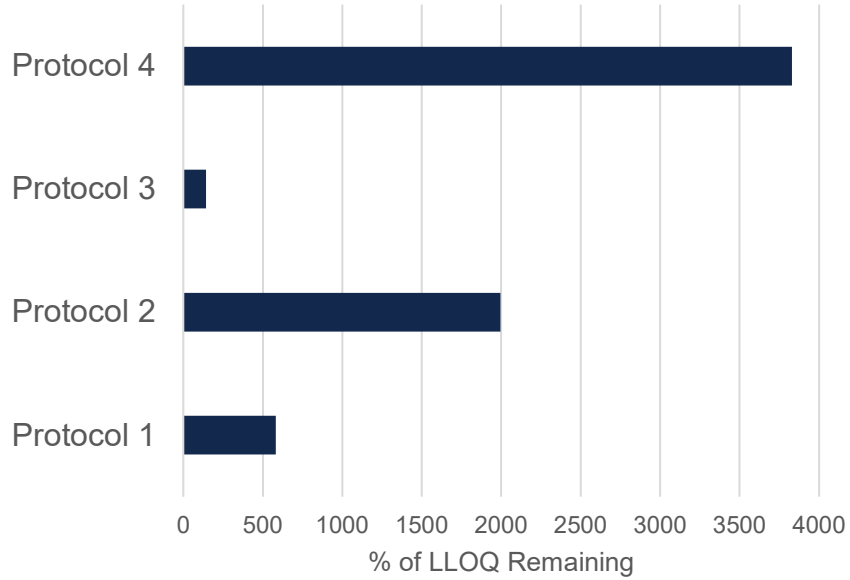


Objective	Considerations
• Can a reusable Teflon plate be used?	• Binding • Solvents • Temperature • Mix Speed • Mix Duration
• Why wouldn't you just use single use?	• Binding



Mass Balance

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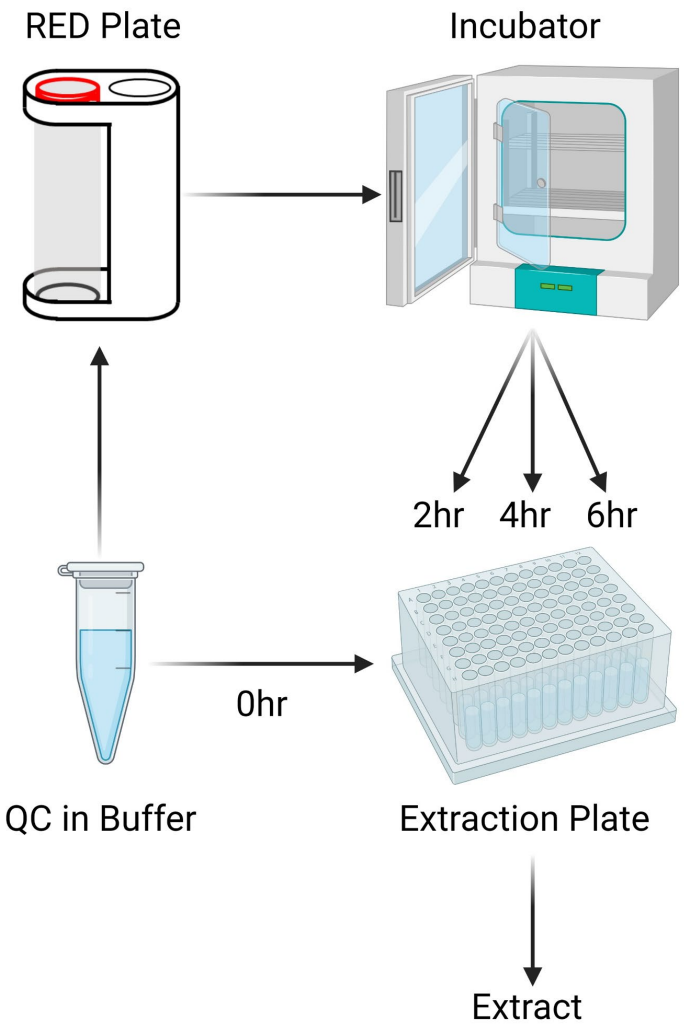

Objective	Considerations
Does your compound cross the membrane?	• Size 8000Da & 12000Da • Stability • Binding
Does your compound reach equilibrium?	• Can RED be used to determine %FU • In buffer = no protein binding
How long does it take for your compound to reach equilibrium?	• Extraction time • F/T cycles
What is the recovery of your compound?	• Stability • Binding

Mass Balance

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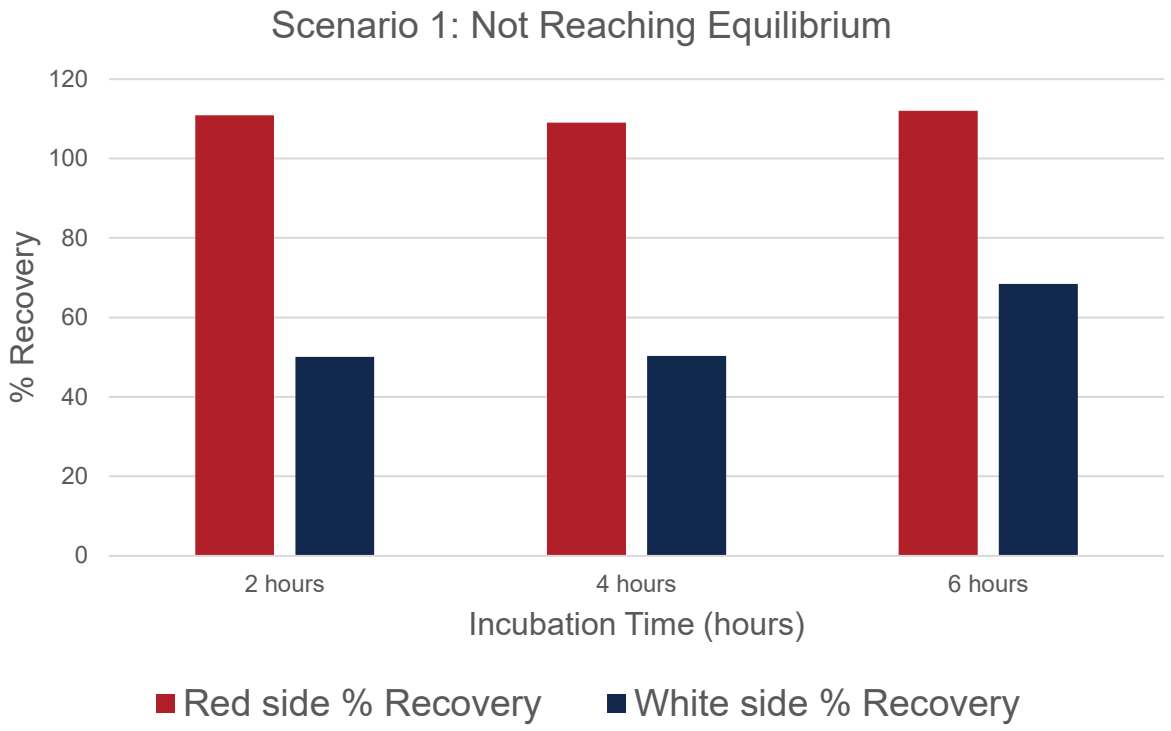
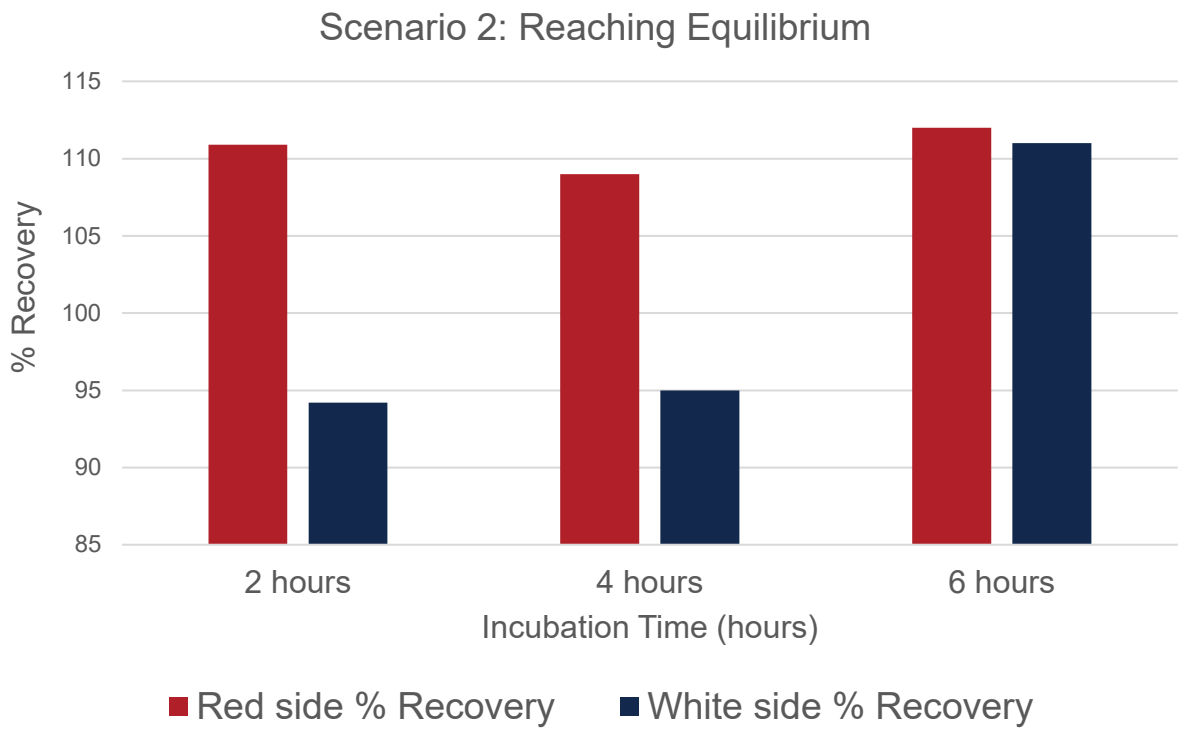
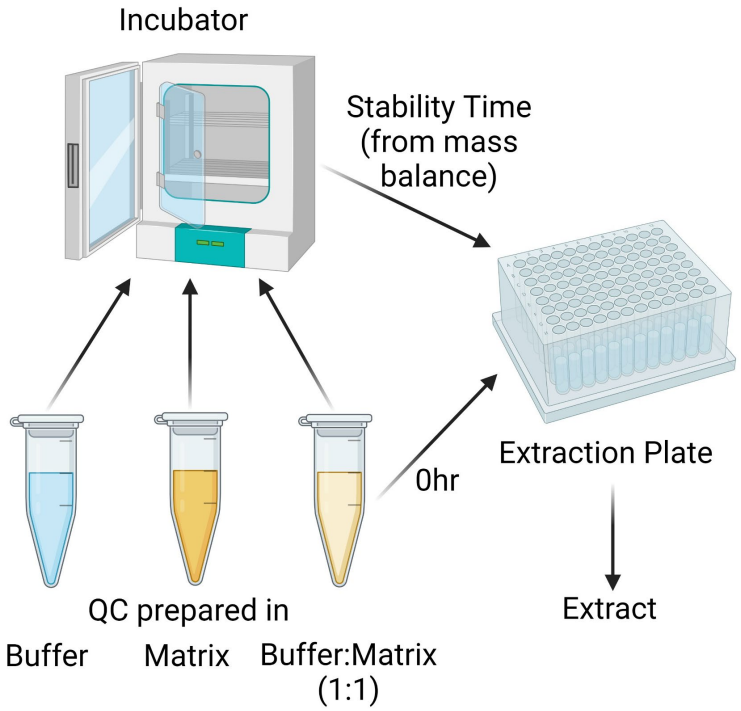
$$Expected\ Concentration(ng/mL) = \frac{(Total\ volume\ of\ red\ side\ (mL) x\ QC\ concentration(ng/mL)}{(Total\ volume\ of\ red + white\ side\ (mL))}$$

$$Recovery\ (\%) = \frac{Mean\ concentration\ (ng/mL)}{Overall\ expected\ concentration\ (ng/mL)} x100$$





RED Stability



Objective	Considerations
Is your compound stable at incubation temperature?	• Time
What does it mean if your compound isn't stable?	• Buffer • Matrix • Generated sample

Time Course

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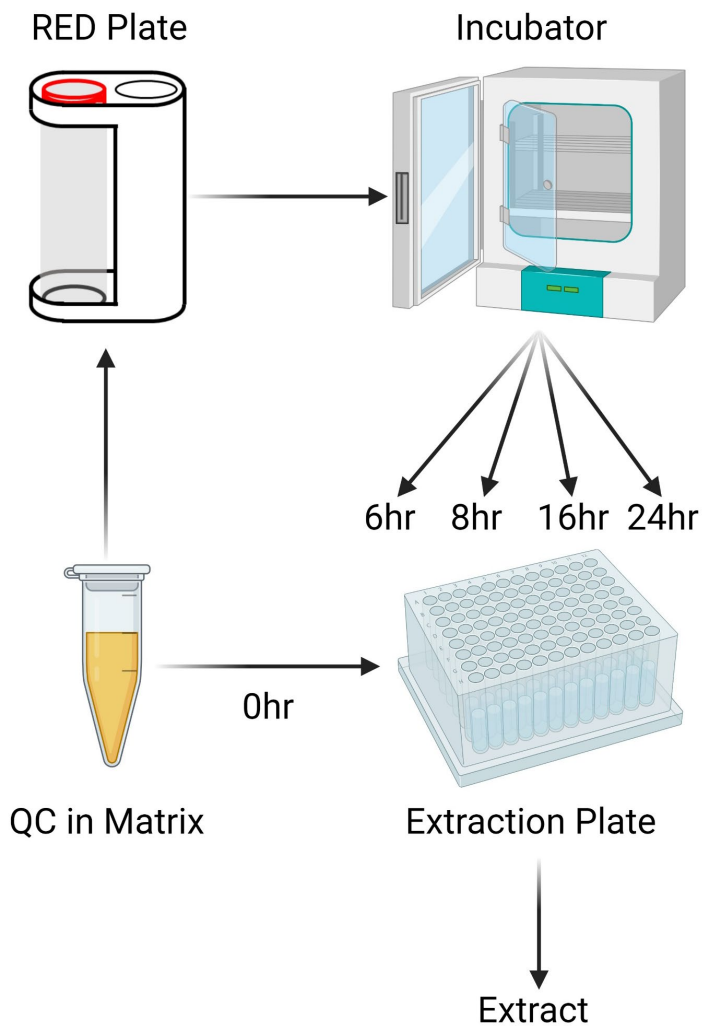

Objective	Considerations
How long does it take for your compound to reach equilibrium?	• Taken from MB experiment • Addition of proteins
What is the % fraction unbound?	
Is your % fraction unbound consistent?	• Multiple QC levels

Time Course

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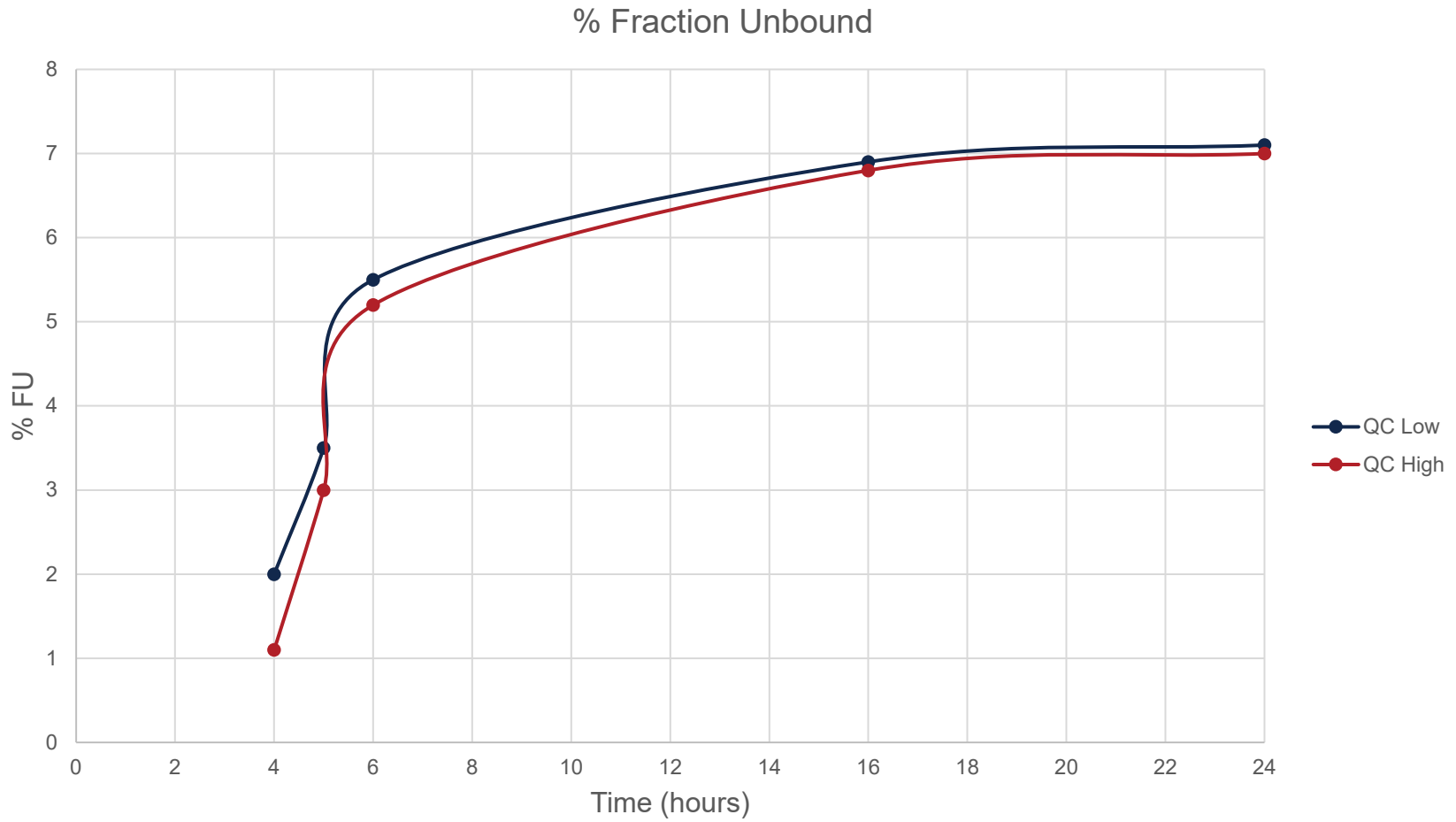

Best Practices and Conclusions

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- Consider time taken to transfer samples from RED plate to extraction plate
- Not all compounds are feasible for RED
- There are points in RED that are ‘STOP’ points
- RED takes time and should be planned carefully

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Thank you for your attention

Any questions?

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