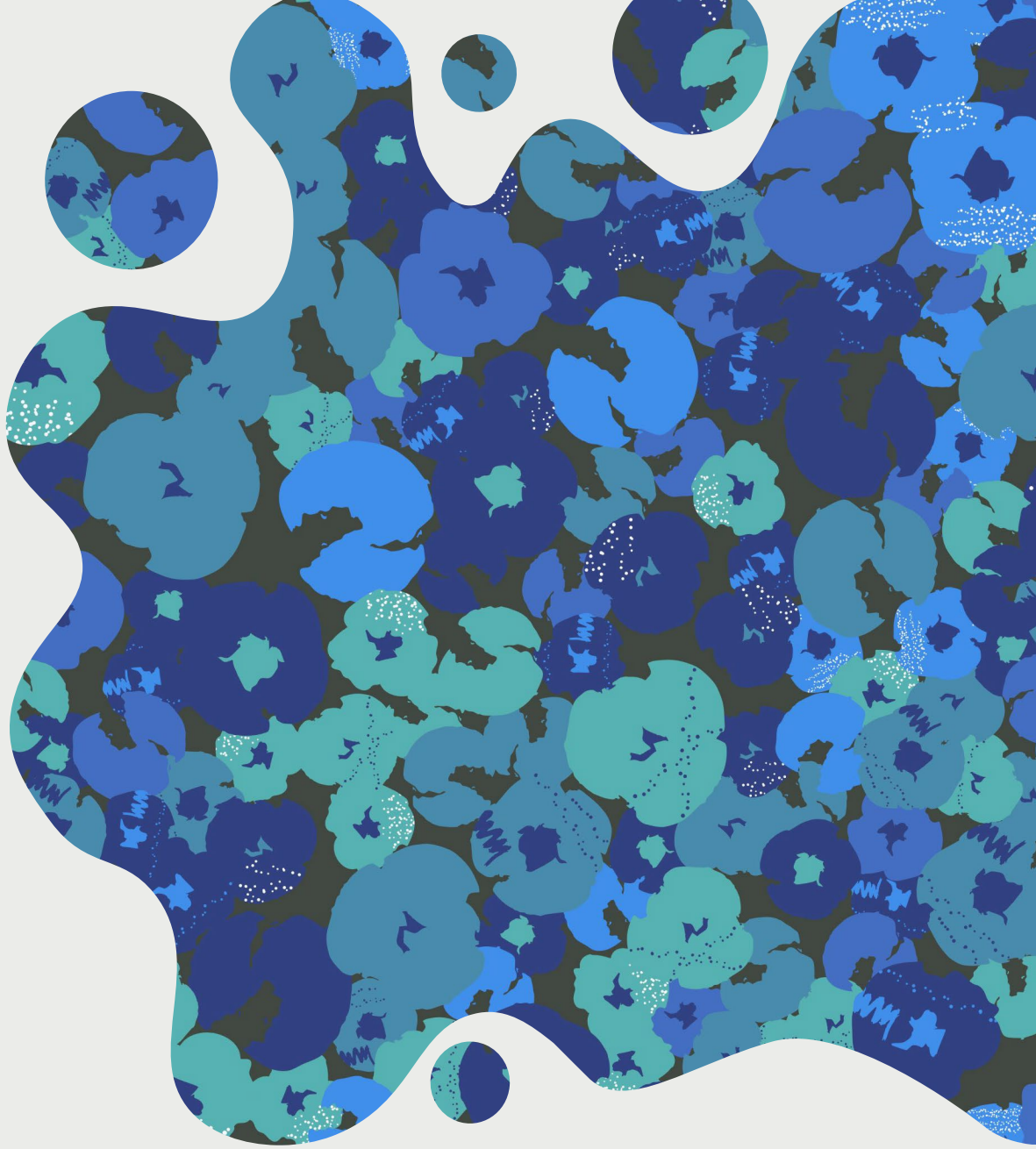
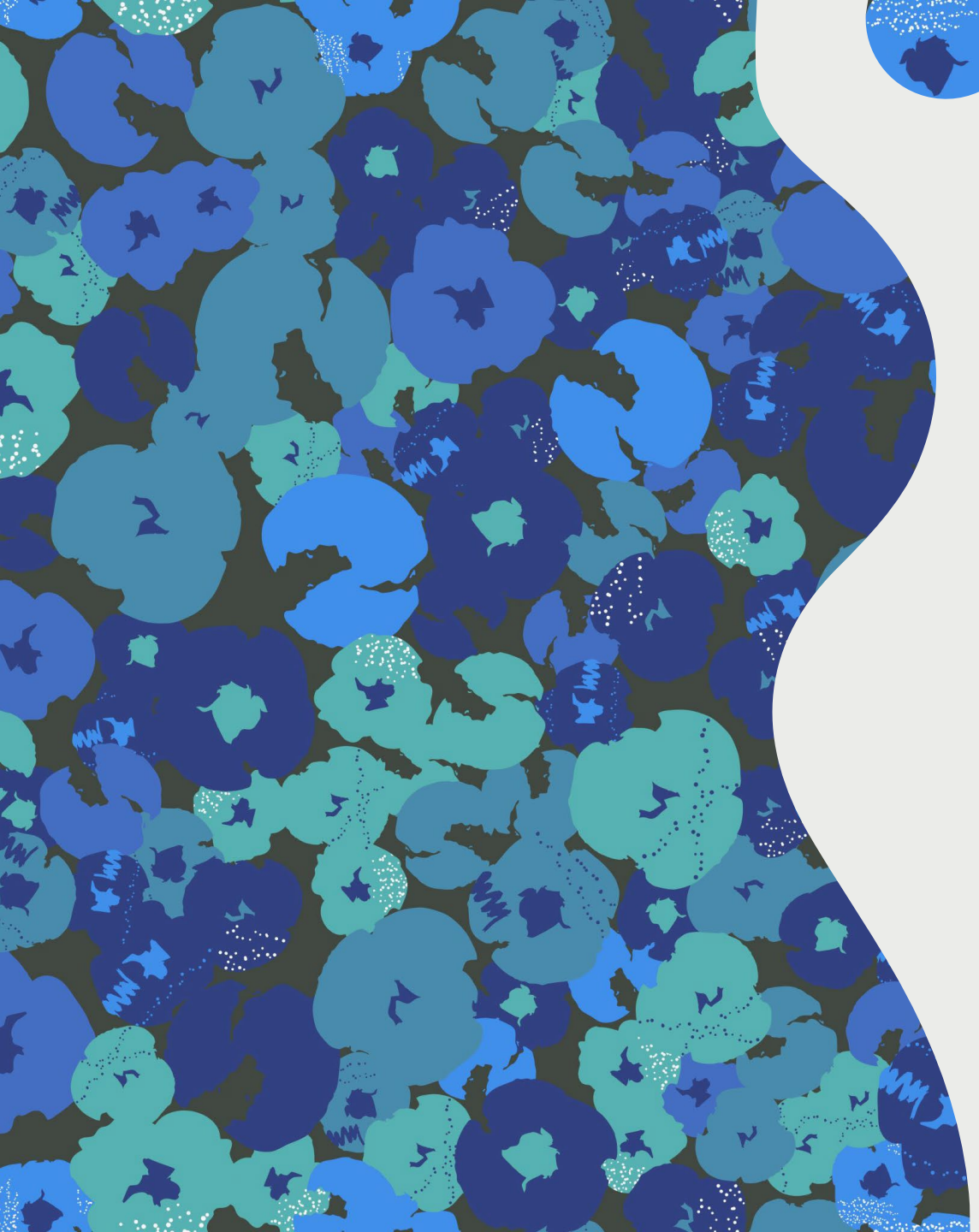
12:30-13:30	Glenn Masson: HDX-MS Theory and Practice	
13:30-14:00	Coffee	
14:00-15:00	Waters Demo	Thermo Demo
15:00-16:00	Thermo Demo	Waters Demo
16:00-16:15	Break	
16:15-17:15	Data Analysis Workshop	
17:30	The George Orwell	





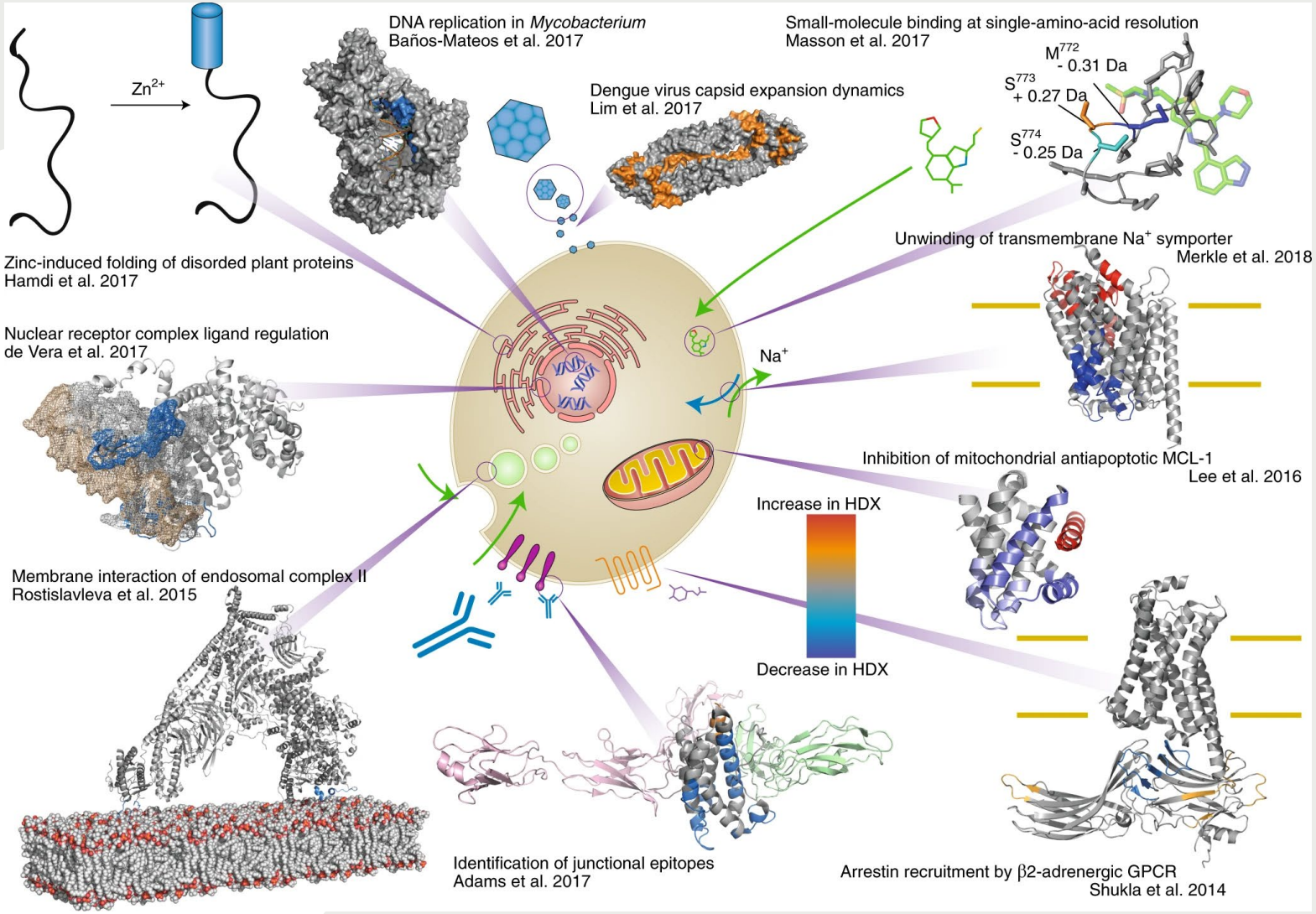
HDX-MS Theory and Practice – Overview of solvent exchange, sample considerations, limitations and controls.

BMSS Structural SIG Workshop 2024

Dr Glenn Masson

University of Dundee

HDX-MS is a powerful, widely applicable technique to monitor *in solution* protein dynamics with relatively small amount of protein



Masson, G.R., Burke, J.E., Ahn, N.G. *et al.* Recommendations for performing, interpreting and reporting hydrogen deuterium exchange mass spectrometry (HDX-MS) experiments. *Nat Methods* **16**, 595–602 (2019). https://doi.org/10.1038/s41592-019-0459-y

At its core HDX-MS simultaneously measures both secondary structure and solvent accessibility

It uses “solvent exchange” a naturally occurring phenomenon to measure both of these aspects of protein dynamics

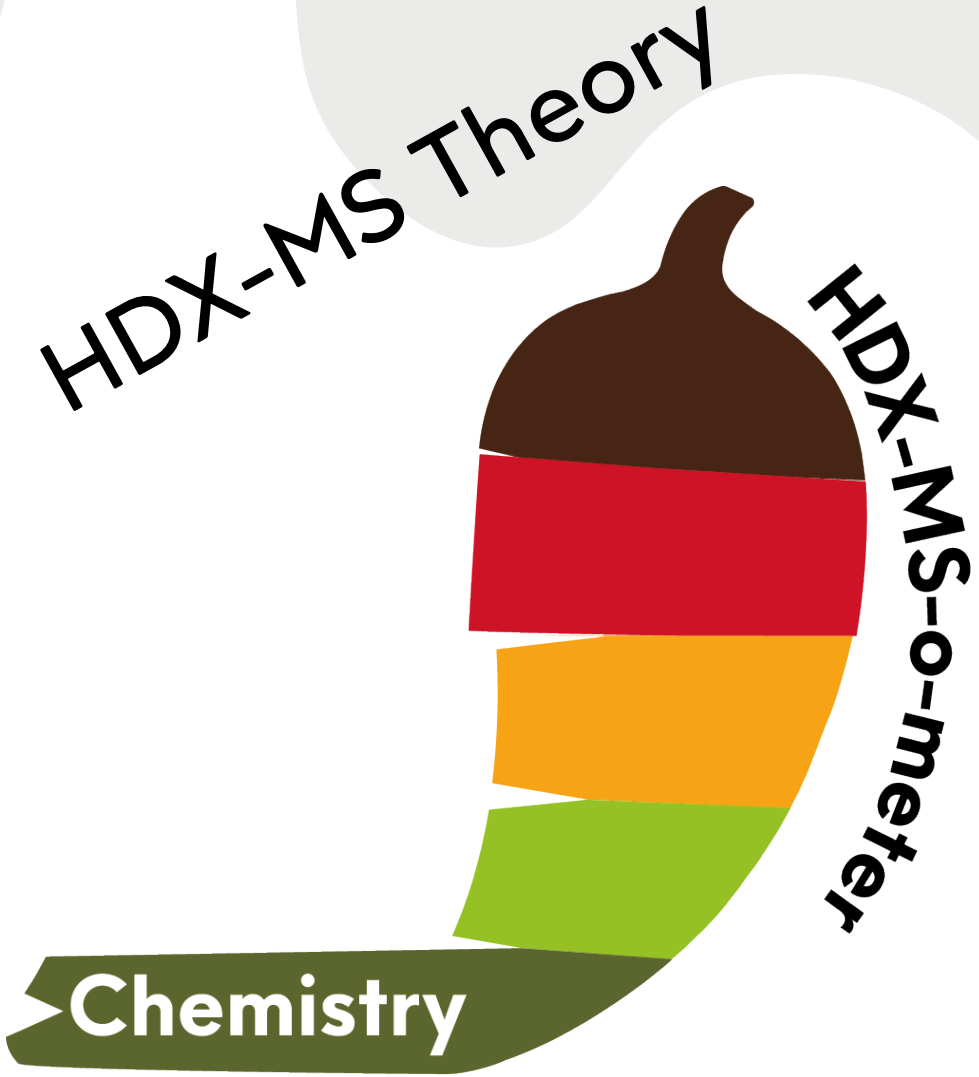
The label is one neutron, and as such, has minimal perturbations on the systems being studied.

About ~300 studies using HDX-MS are published each year.

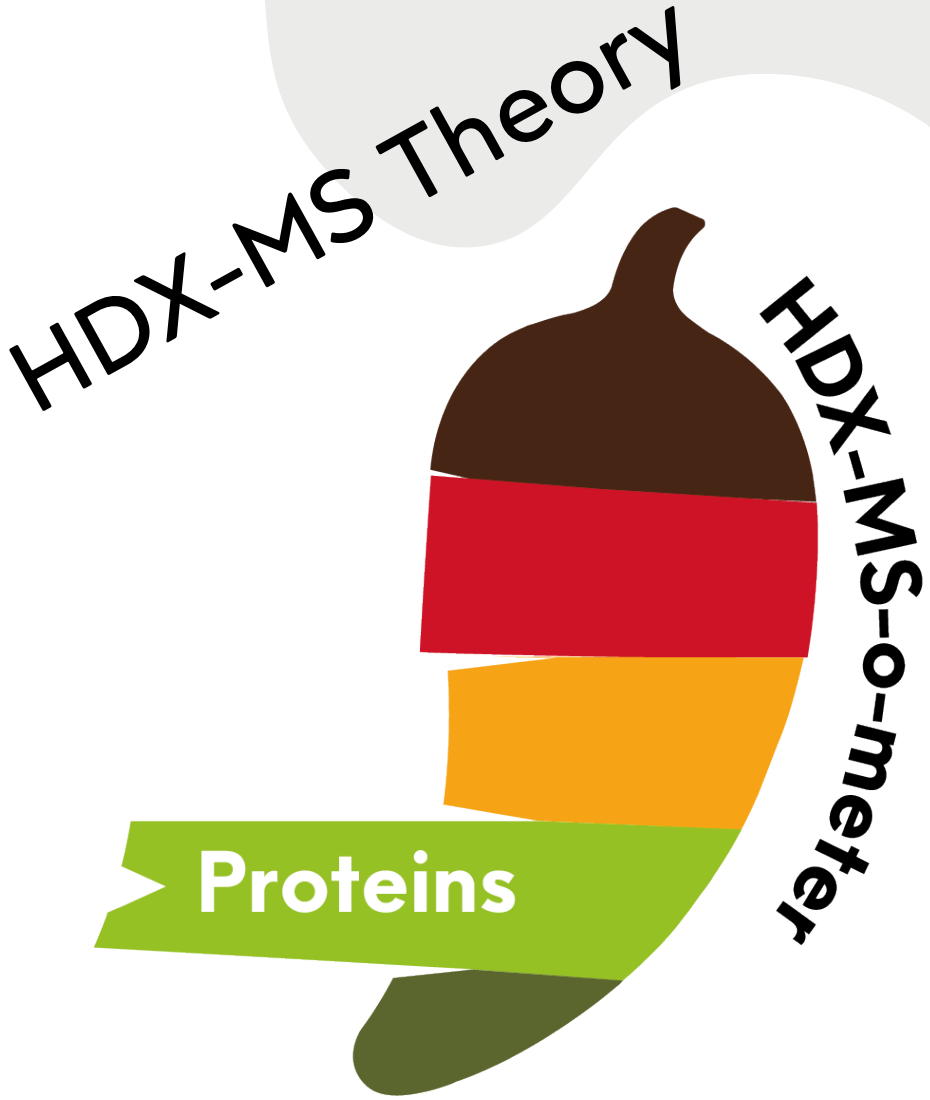
Masson, G. R., Jenkins, M. L., & Burke, J. E. (2017). An overview of hydrogen deuterium exchange mass spectrometry (HDX-MS) in drug discovery. Expert Opinion on Drug Discovery, 12(10), 981–994. https://doi.org/10.1080/17460441.2017.1363734

HDX-MS Theory

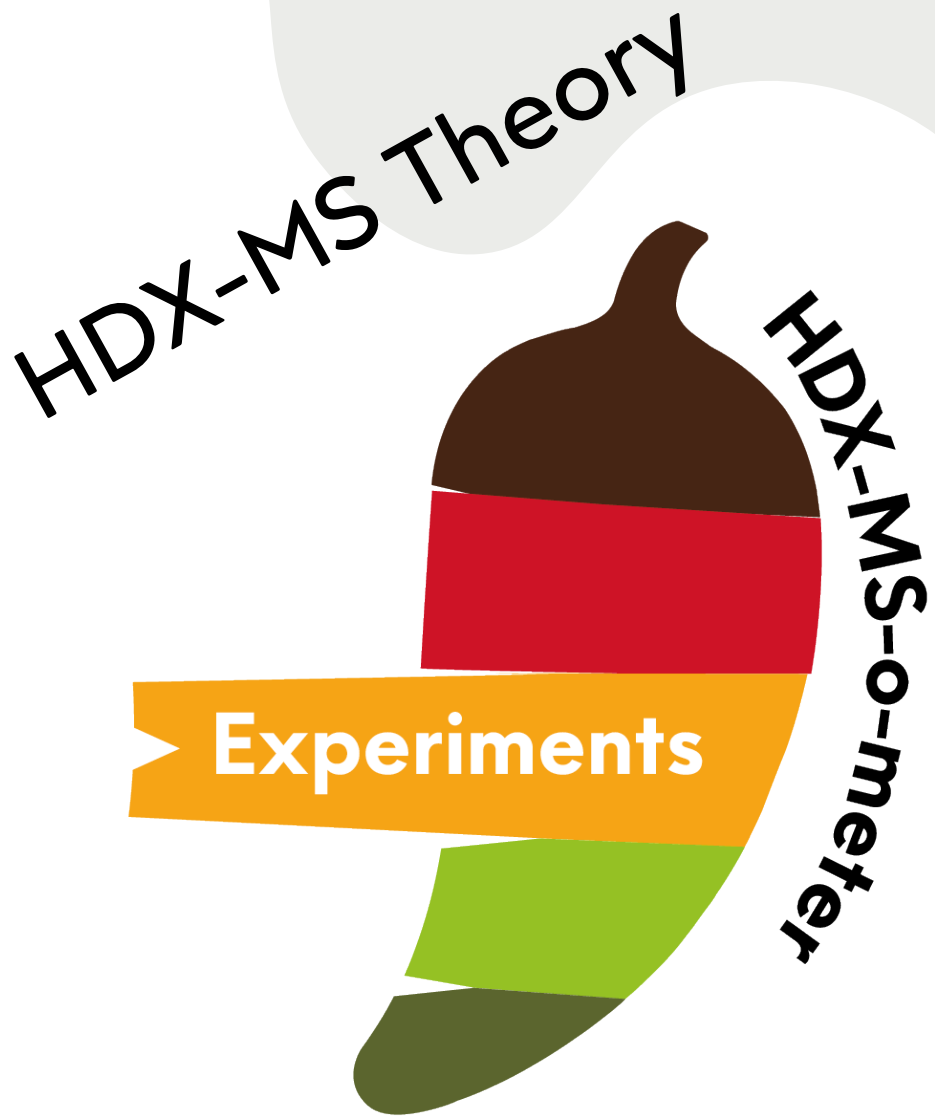
HDX-MS-o-meter



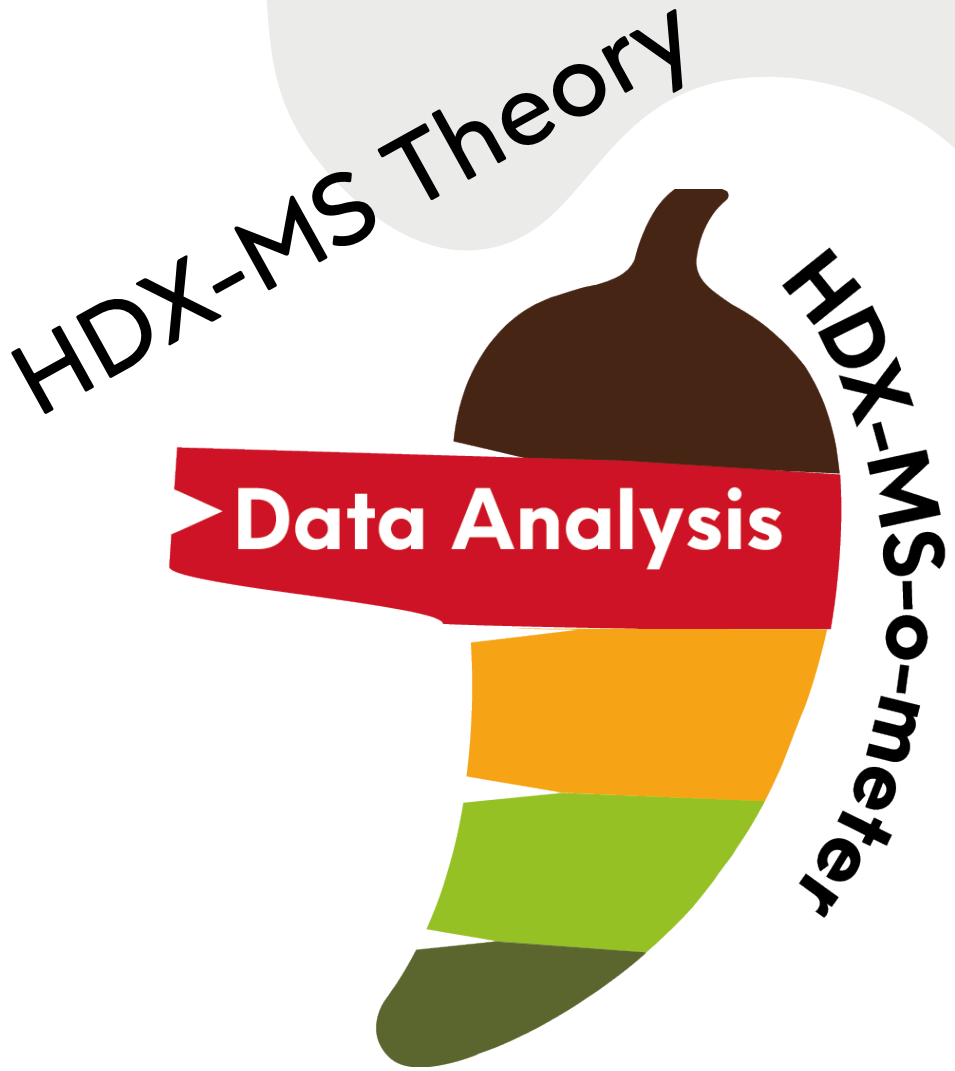
- Fundamentals of proton transfer
- How peptides exchange



- How proteins exchange
- What's a protection factor anyway

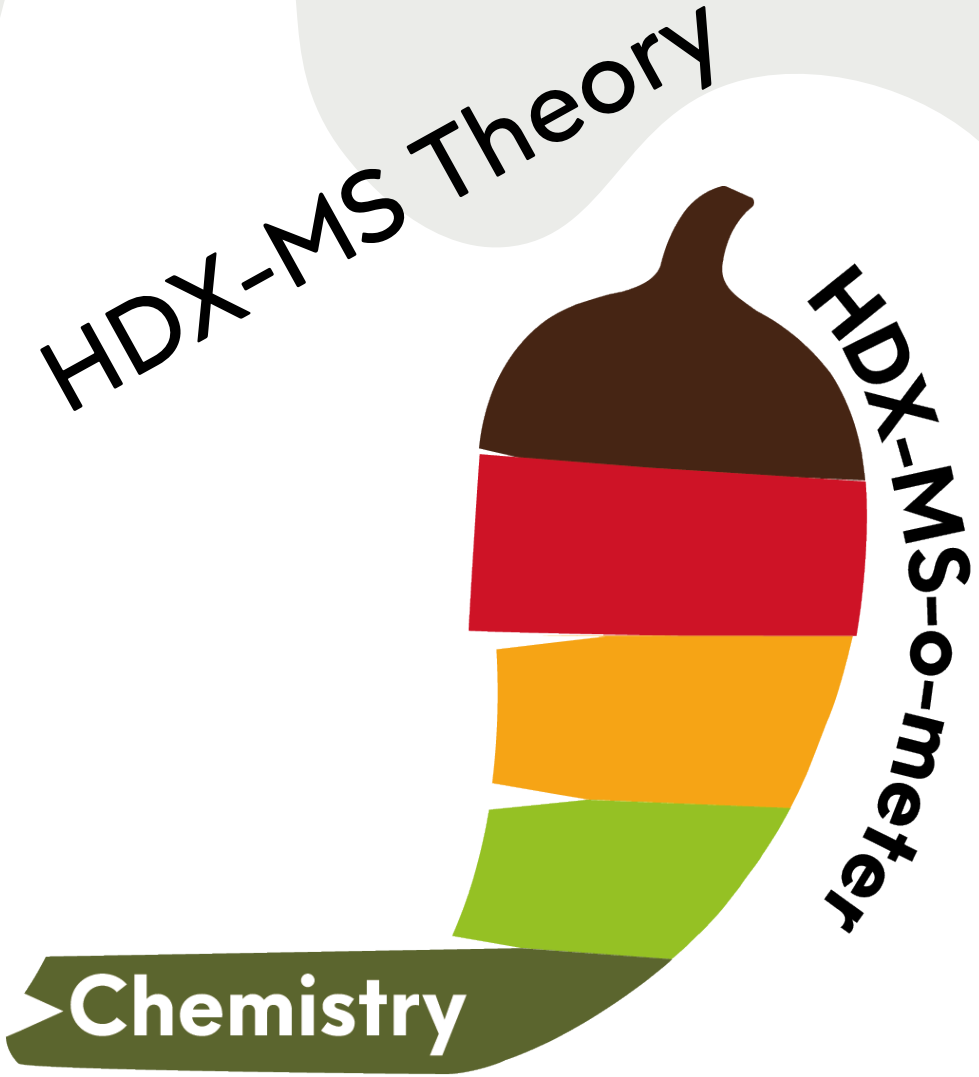


- Why mass spectrometry isn't the perfect tool to measure HDX



- Largely covered in the later session

- New & exciting tools and biology on the horizon



- Fundamentals of proton transfer
- What governs exchange rates

1. Diverse functional groups undergo exchange

2. Various factors influence solvent exchange

3. Points 1 and 2 impose limitations on experimental design.

Reference Text:
Tutorial: Chemistry of Hydrogen/Deuterium Exchange Mass Spectrometry
Yoshitomo Hamuro
Journal of the American Society for Mass Spectrometry 2021 32 (1), 133-151
DOI: *10.1021/jasms.0c00260*

Proton Exchange

$$XH^+ + Y \underset{k_{-D}}{\overset{k_D}{\rightleftharpoons}} [XH^+ \cdots Y \underset{k_{-C}}{\overset{k_C}{\rightleftharpoons}} X \cdots H^+Y] \xrightarrow{k_B} X + HY^+$$

Proton Exchange

$$XH^+ + Y \underset{k_{-D}}{\overset{k_D}{\rightleftharpoons}} [XH^+ \cdots Y \underset{k_{-C}}{\overset{k_C}{\rightleftharpoons}} X \cdots H^+Y] \xrightarrow{k_B} X + HY^+$$

Donor

Proton Exchange

Acceptor

$$XH^+ + Y \underset{k_{-D}}{\overset{k_D}{\rightleftharpoons}} [XH^+ \cdots Y \underset{k_{-C}}{\overset{k_C}{\rightleftharpoons}} X \cdots H^+Y] \xrightarrow{k_B} X + HY^+$$

Donor

Proton Exchange

Collision event

$$\mathrm{XH^+ + Y \underset{k_{-D}}{\overset{k_D}{\rightleftharpoons}} [XH^+ \cdots Y \underset{k_{-C}}{\overset{k_C}{\rightleftharpoons}} X \cdots H^+Y]}$$

Proton Exchange

$$XH^+ + Y \underset{k_{-D}}{\overset{k_D}{\rightleftharpoons}} [XH^+ \cdots Y \underset{k_{-C}}{\overset{k_C}{\rightleftharpoons}} X \cdots H^+Y]$$

Equilibrium

Proton Exchange

$$\mathrm{XH^+ + Y \underset{k_{-D}}{\overset{k_D}{\rightleftharpoons}} [XH^+ \cdots Y \underset{k_{-C}}{\overset{k_C}{\rightleftharpoons}} X \cdots H^+Y] \xrightarrow{k_B} X + HY^+}$$

Resolution

1. Concentrations of XH+···Y and X···H+Y are very low.
2. The equilibrium between XH+···Y and X···H+Y is very fast.
3. k_B is equal to k_{-D}

$$XH^+ + Y \underset{k_{-D}}{\overset{k_D}{\rightleftharpoons}} [XH^+ \cdots Y \underset{k_{-C}}{\overset{k_C}{\rightleftharpoons}} X \cdots H^+Y] \xrightarrow{k_B} X + HY^+$$

Proton Exchange

$$k_{\mathrm{T}} = k_{\mathrm{D}}/(10^{\Delta \mathrm{p}K\mathrm{a}} + 1)$$

Rate of proton transfer

$$k_{\mathrm{T}} = k_{\mathrm{D}}/(10^{\Delta \mathrm{p}K\mathrm{a}} + 1)$$

Rate of proton transfer = Rate of collision

$$k_{\mathrm{T}} = k_{\mathrm{D}} / \left(10^{\Delta \mathrm{p}Ka} + 1\right)$$

$$\mathrm{XH^+ + Y} \underset{k_{-\mathrm{D}}}{\overset{k_{\mathrm{D}}}{\rightleftharpoons}} [\mathrm{XH^+ \cdots Y} \underset{k_{-\mathrm{C}}}{\overset{k_{\mathrm{C}}}{\rightleftharpoons}} \mathrm{X \cdots H^+Y}] \overset{k_{\mathrm{B}}}{\rightarrow} \mathrm{X + HY^+}$$

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Rate of proton transfer = Rate of collision / Difference of pKa

Difference in pKa between donor and accpetora

$$k_T = k_D/(10^{\Delta pKa} + 1)$$

$$XH^+ + Y \underset{k_{-D}}{\overset{k_D}{\rightleftharpoons}} [XH^+ \cdots Y \underset{k_{-C}}{\overset{k_C}{\rightleftharpoons}} X \cdots H^+Y] \overset{k_B}{\rightarrow} X + HY^+$$

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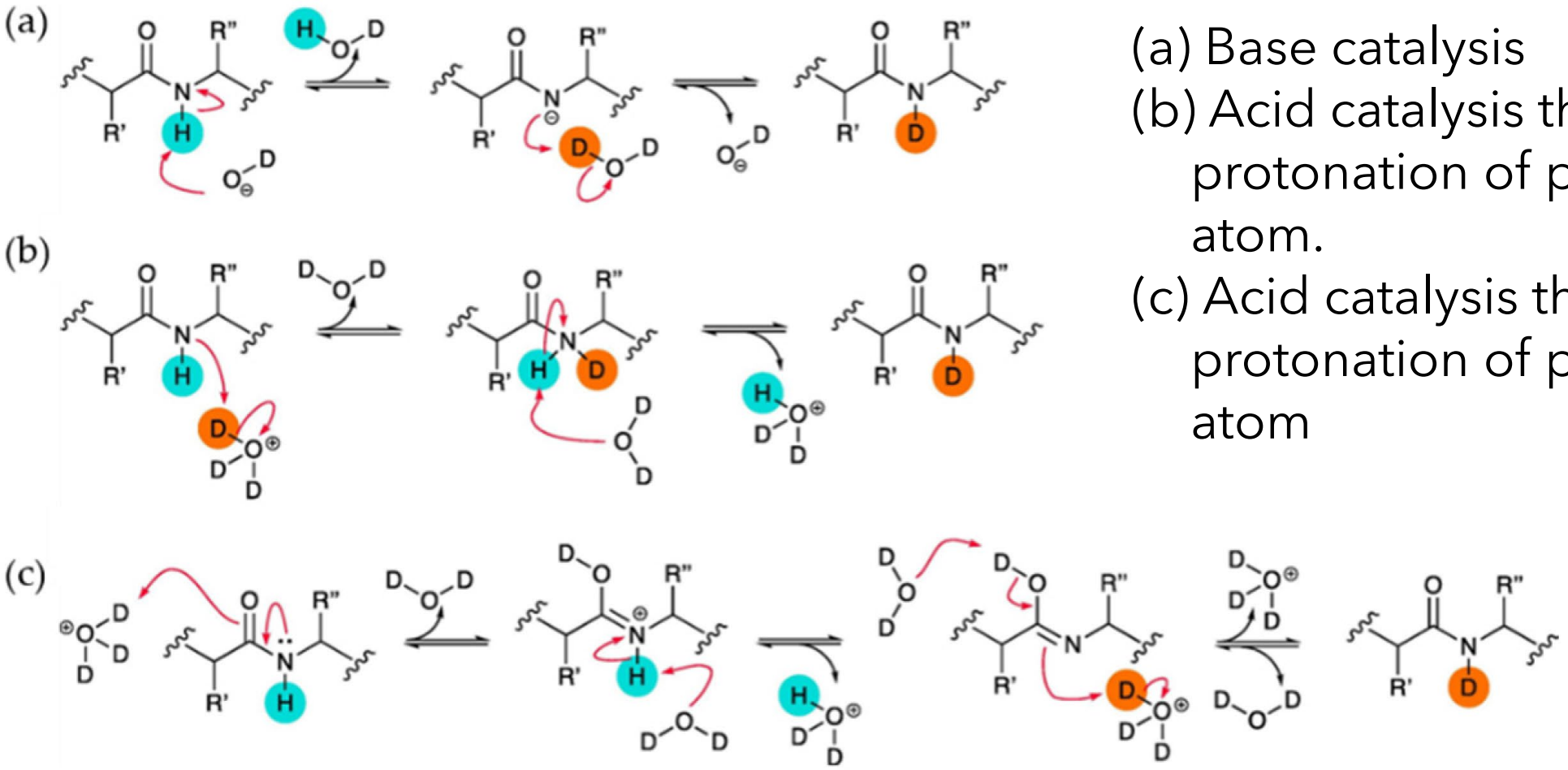
pH (or pD) and Solvent exchange

Solvent exchange can be acid, base, or D_2O catalysed



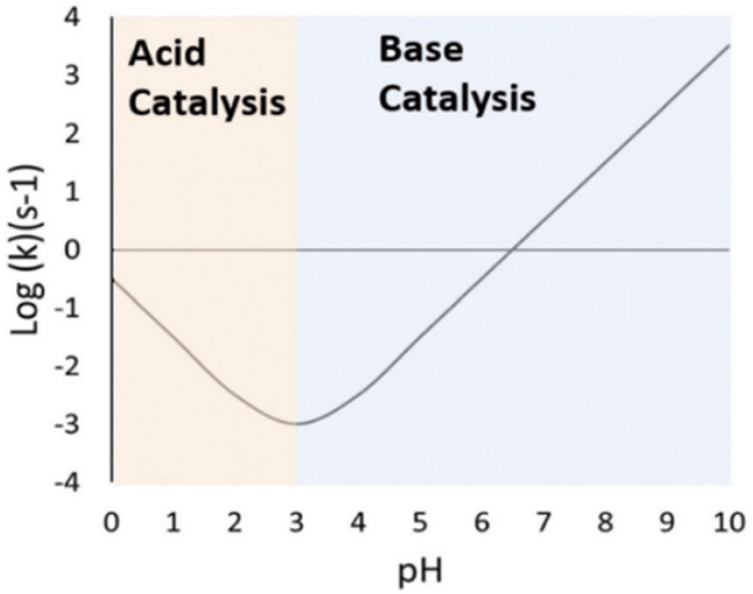
(a) Base catalysis
(b) Acid catalysis through the protonation of peptide group N atom.
(c) Acid catalysis through protonation of peptide group O atom

Narang, D.; Lento, C.; J. Wilson, D. HDX-MS: An Analytical Tool to Capture Protein Motion in Action. *Biomedicines* **2020**, *8*, 224. https://doi.org/10.3390/biomedicines8070224

pH (or pD) and Solvent exchange

Solvent exchange can be acid, base, or D_2O catalysed

The sum of those three second orders is the first order reaction rate constant: k_{ch} or "chemical rate constant"



$$k_{\mathrm{ch}} = k_{\mathrm{D}}[\mathrm{D}^+] + k_{\mathrm{OD}}[\mathrm{OD}^-] + k_{\mathrm{D2O}}[\mathrm{D_2O}]$$

$$k_{ch} = k_{ref293} * \exp\left(-\frac{E_a}{R}\left[\frac{1}{T} - \frac{1}{293}\right]\right)$$

Arrhenius Eq'n

pH (or pD) and Solvent exchange

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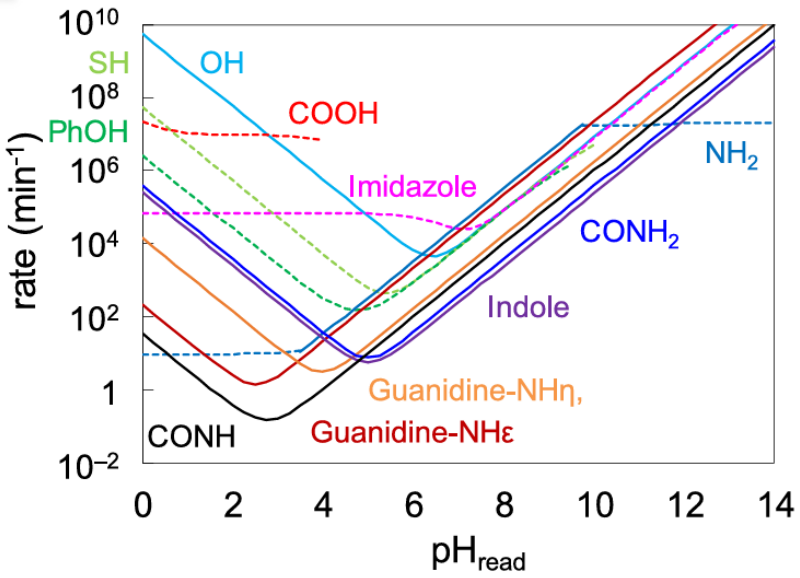


$$k_{\mathrm{ch}} = k_{\mathrm{D}}[\mathrm{D}^+] + k_{\mathrm{OD}}[\mathrm{OD}^-] + k_{\mathrm{D2O}}[\mathrm{D_2O}]$$

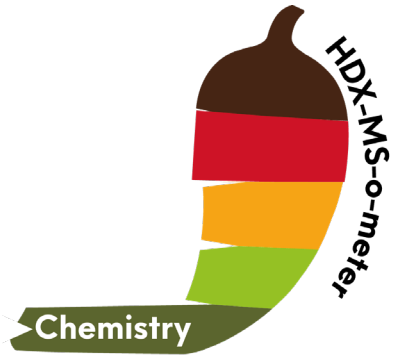
$$k_{ch} = k_{ref293} * \exp\left(-\frac{E_a}{R}\left[\frac{1}{T} - \frac{1}{293}\right]\right)$$

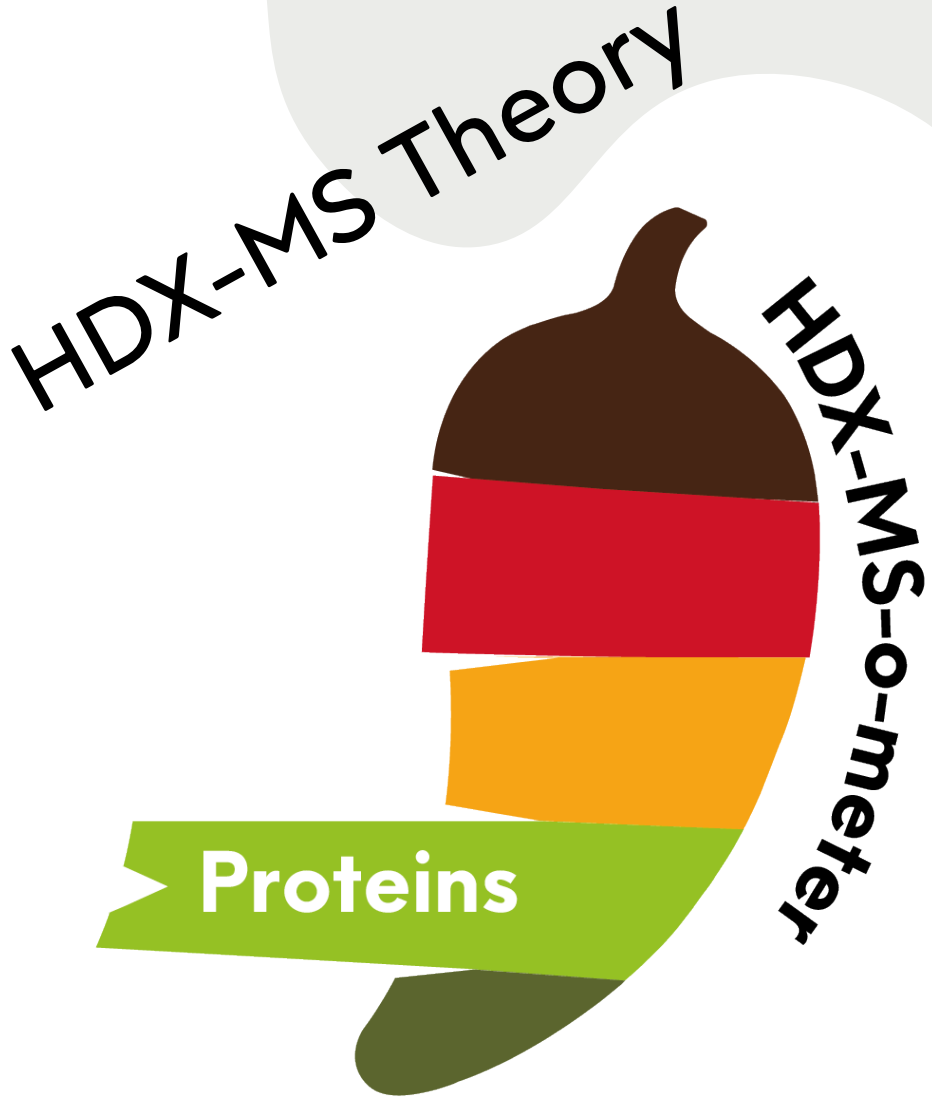
Arrhenius Eq'n

Take home messages #1

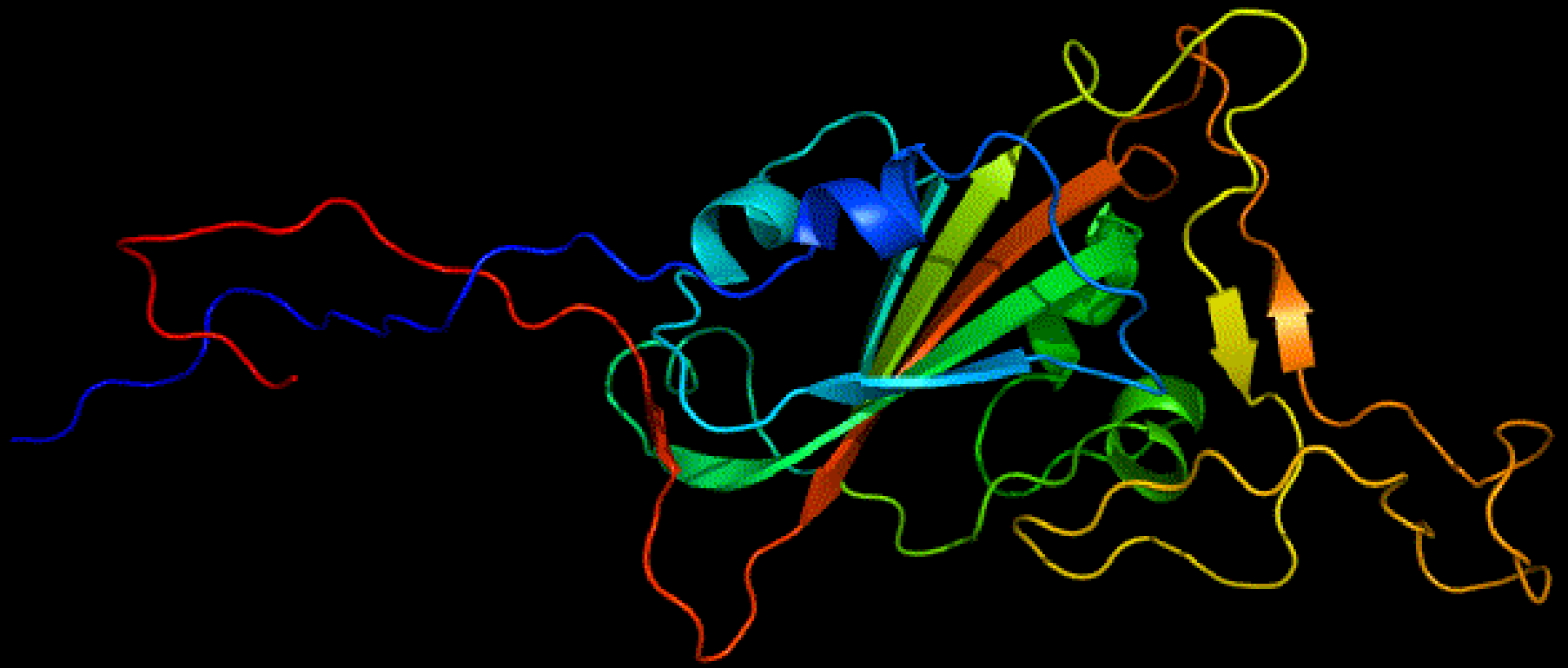
Temperature is going to be important (collision constant).

pH is going to be important (ΔpKa), [D^+], [OD^-].





- How *proteins* exchange
- What's a protection factor anyway?

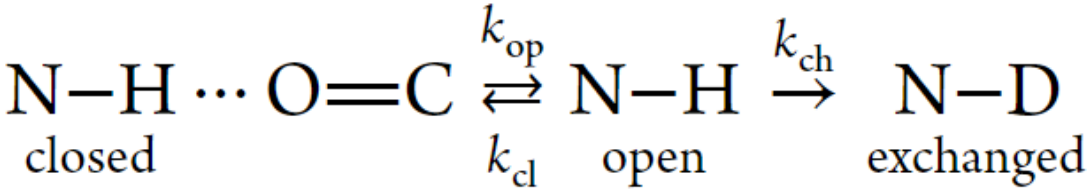


Proteins are dynamic polypeptides

Proteins “sample” solvent through opening and closing

“open” = backbone amide group *not* engaged in an h-bond

When open -> solvent exchange can occur



Linderstrøm–Lang Model

Proteins “sample” solvent through opening and closing

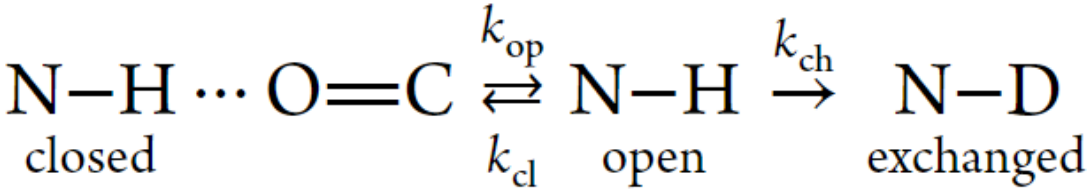
Opening rate

$$\underset{\text{closed}}{\text{N–H}\cdots\text{O=C}} \underset{k_{cl}}{\overset{k_{op}}{\rightleftarrows}} \underset{\text{open}}{\text{N–H}} \xrightarrow{k_{ch}} \underset{\text{exchanged}}{\text{N–D}}$$

Linderstrøm–Lang Model

Proteins “sample” solvent through opening and closing

Opening rate “chemical” exchange rate (will change due to pH/temp)

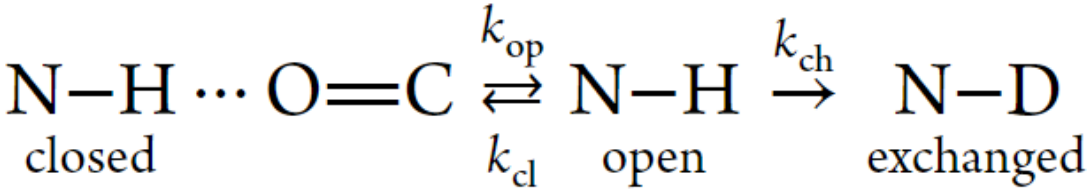


Closing rate

Linderstrøm–Lang Model

Proteins “sample” solvent through opening and closing

Opening rate “chemical” exchange rate (will change due to pH/temp)



Closing rate

Linderstrøm–Lang Model

Proteins “sample” solvent through opening and closing

Assumption: the NH is normally in a “closed” configuration

$$k_{cl} \gg k_{op}$$

$$\underset{\text{closed}}{\text{N–H}\cdots\text{O=C}} \underset{k_{cl}}{\overset{k_{op}}{\rightleftarrows}} \underset{\text{open}}{\text{N–H}} \xrightarrow{k_{ch}} \underset{\text{exchanged}}{\text{N–D}}$$

Linderstrøm–Lang Model

Proteins “sample” solvent through opening and closing

Assumption: the NH is normally in a “closed” configuration

$$k_{cl} \gg k_{op}$$

$$k_{app} = \frac{k_{ch}k_{op}}{k_{cl} + k_{ch}}$$

Linderstrøm–Lang Model

Proteins “sample” solvent through opening and closing

Assumption: the NH is normally in a “closed” configuration

$$k_{cl} \gg k_{op}$$

Steady-state approximation rate of exchange

$$k_{app} = \frac{k_{ch}k_{op}}{k_{cl} + k_{ch}}$$

Linderstrøm–Lang Model

Proteins “sample” solvent through opening and closing

Assumption: the NH is normally in a “closed” configuration

$$k_{cl} \gg k_{op}$$

Steady-state approximation rate of exchange

$$k_{app} = \frac{k_{ch}k_{op}}{k_{cl} + k_{ch}}$$

Two limiting cases depending on the relative size of k_{cl} and k_{ch}

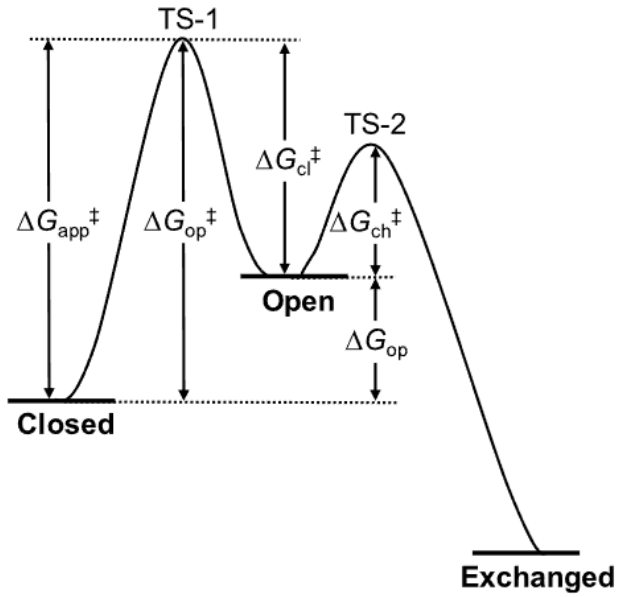
Linderstrøm–Lang Model

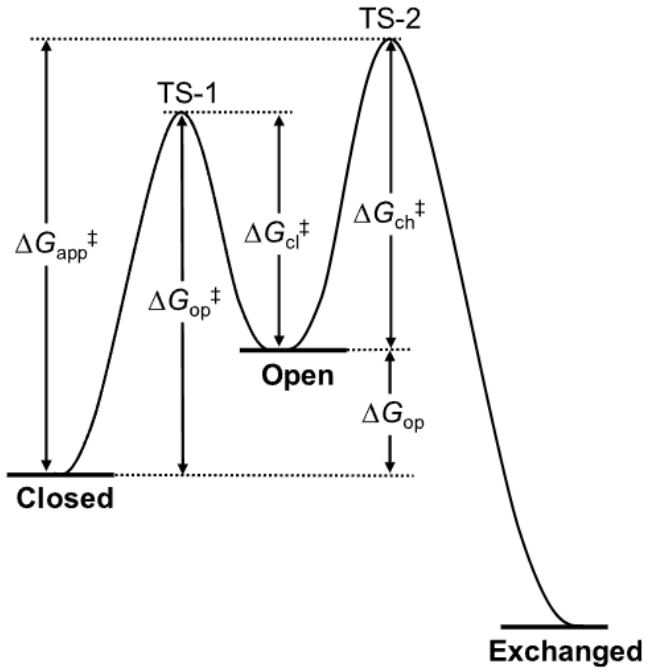
EX1 and EX2 kinetics

EX1: $k_{cl} << k_{ch}$

EX2: $k_{cl} >> k_{ch}$

Opening is slow and exchange is quick





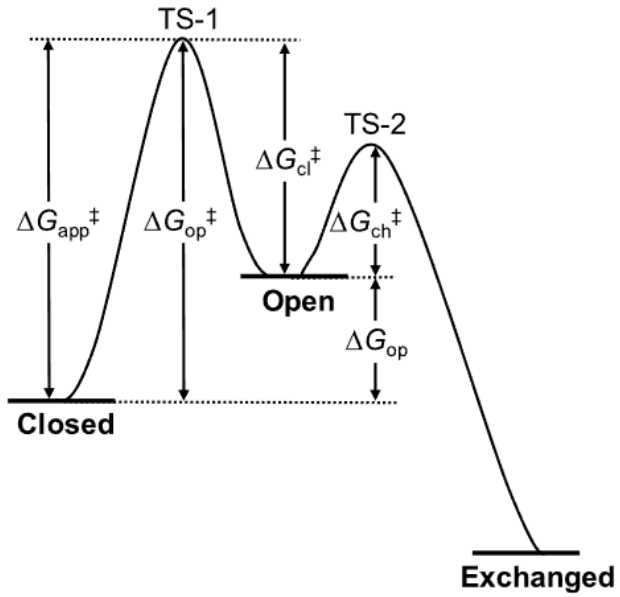
Opening is quick and exchange is slow

Linderstrøm–Lang Model

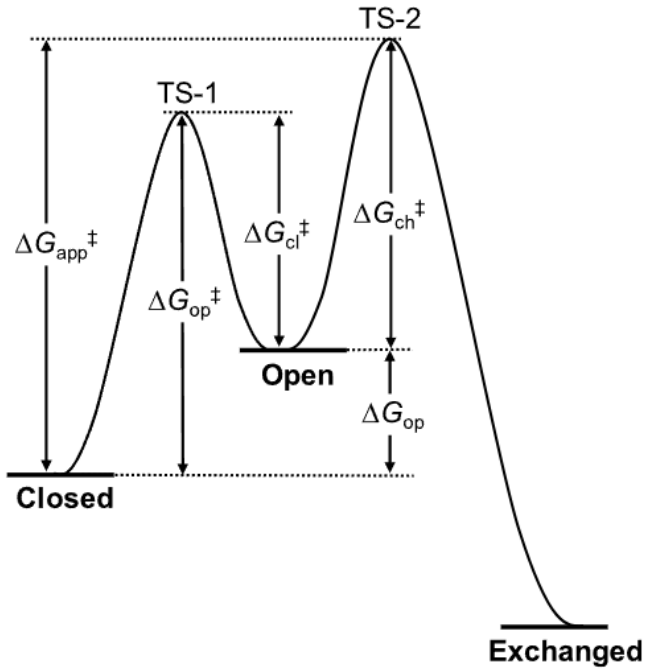
EX1 and EX2 kinetics

EX1: $k_{cl} << k_{ch}$

Measures <u>rate</u> of opening



EX2: $k_{cl} >> k_{ch}$



Measures <u>equilibrium</u> of closed and open form

Linderstrøm–Lang Model

Take home messages #2

k_{ch}

Temperature is going to be important (collision constant).
pH is going to be important (ΔpKa), $[D^+]$, $[OD^-]$.

Backbone amides only exchange when "open"
The relative speeds exchange and opening dictate what we're measuring, but <u>most</u> of the time, it's EX2, cause <u>most</u> of the time, our proteins are folded.

k_{op}

What on earth is a protection factor ln(P)?

A protection factor is an attempt to provide an numerical estimate how "protected" a residue is from engaging in solvent exchange by its non-neighbouring residues of the protein i.e. spatial neighbours (no its sequence neighbours)

Skinner SP, et al. Estimating Constraints for Protection Factors from HDX-MS Data. Biophys J. 2019 Apr 2;116(7):1194-1203. doi: 10.1016/j.bpj.2019.02.024.

What on earth is a protection factor ln(P)?

A protection factor (P) is an attempt to provide a numerical estimate how "protected" a residue. For EX2 regimes where $k_{cl} >> k_{ch}$

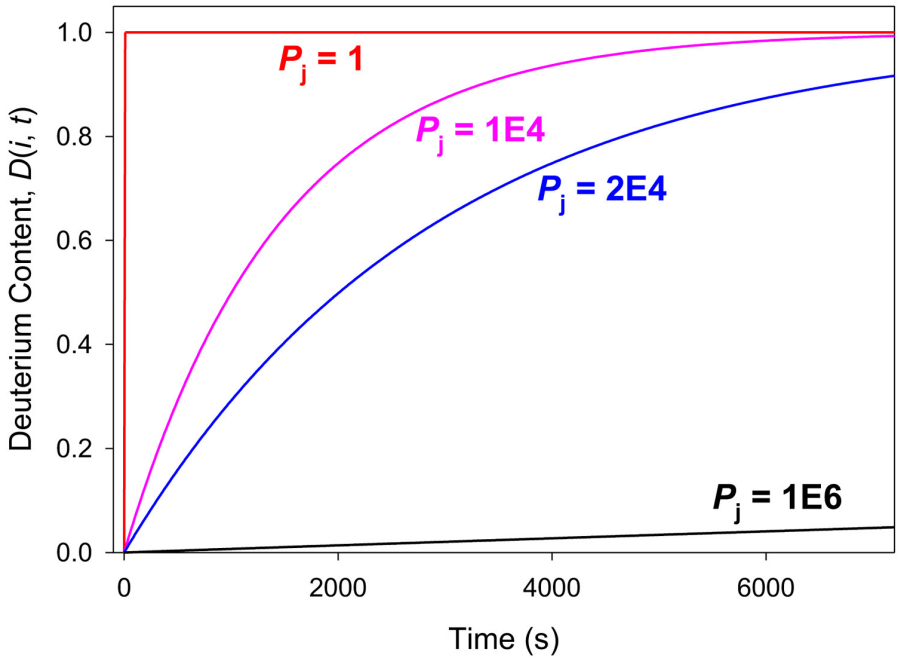
$$k_{obs} = k_{ch} / P$$

Skinner SP, et al. Estimating Constraints for Protection Factors from HDX-MS Data. Biophys J. 2019 Apr 2;116(7):1194-1203. doi: 10.1016/j.bpj.2019.02.024.

What on earth is a protection factor ln(P)?

Protection factors are thus a sum of

a) Is there already a H-bond on the protein
b) Solvent exclusion or rather, is there loads of other protein C and N atoms nearby?



Higher the protection factor, the slower the exchange.

Normally expressed as ln(P) due to the wide range involved.

Konermann L, Scrosati PM. Hydrogen/Deuterium Exchange Mass Spectrometry: Fundamentals, Limitations, and Opportunities. Mol Cell Proteomics. 2024 Oct 7:100853. doi: 10.1016/j.mcpro.2024.100853.

What on earth is a protection factor ln(P)?

Protection factors are thus a sum of

a) Is there already a H-bond on the protein (β_h)

b) Solvent exclusion or rather, is there loads of other protein C and N atoms nearby? (β_c)

EMPIRICAL measurements of seven proteins in the 2000s estimated the relative contributions of H-bonds and solvent exclusion

$$\ln(P) = (\beta_h * N_h)(X) + (\beta_c * N_c)(X)$$

What on earth is a protection factor ln(P)?

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EMPIRICAL measurements of seven proteins in the 2000s estimated the relative contributions of H-bonds and solvent exclusion

For any given state, X

$$\ln(P) = (\beta_h * N_h)(X) + (\beta_c * N_c)(X)$$

Number of C/N atoms

Number of H-bonds

What on earth is a protection factor?

Protection factors are thus a sum of

a) Is there already a H-bond on the protein (β_h)

b) Solvent exclusion or rather, is there loads of other protein C and N atoms nearby? (β_c)

EMPIRICAL measurements of <u>seven</u> proteins in the 2000s estimated the relative contributions of H-bonds and solvent exclusion

$$\ln(P) = (\beta_h * N_h)(X) + (\beta_c * N_c)(X)$$

We weight β_h with 2 and β_c with 0.35

i.e. h-bonding contributes ~ 6x more than solvent exclusion (these values are empirically determined from 7 proteins in the 2000s….)

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i.e. h-bonding contributes ~ 6x more than solvent exclusion (these values are empirically determined from 7 proteins in the 2000s….)

What on earth is a protection factor?

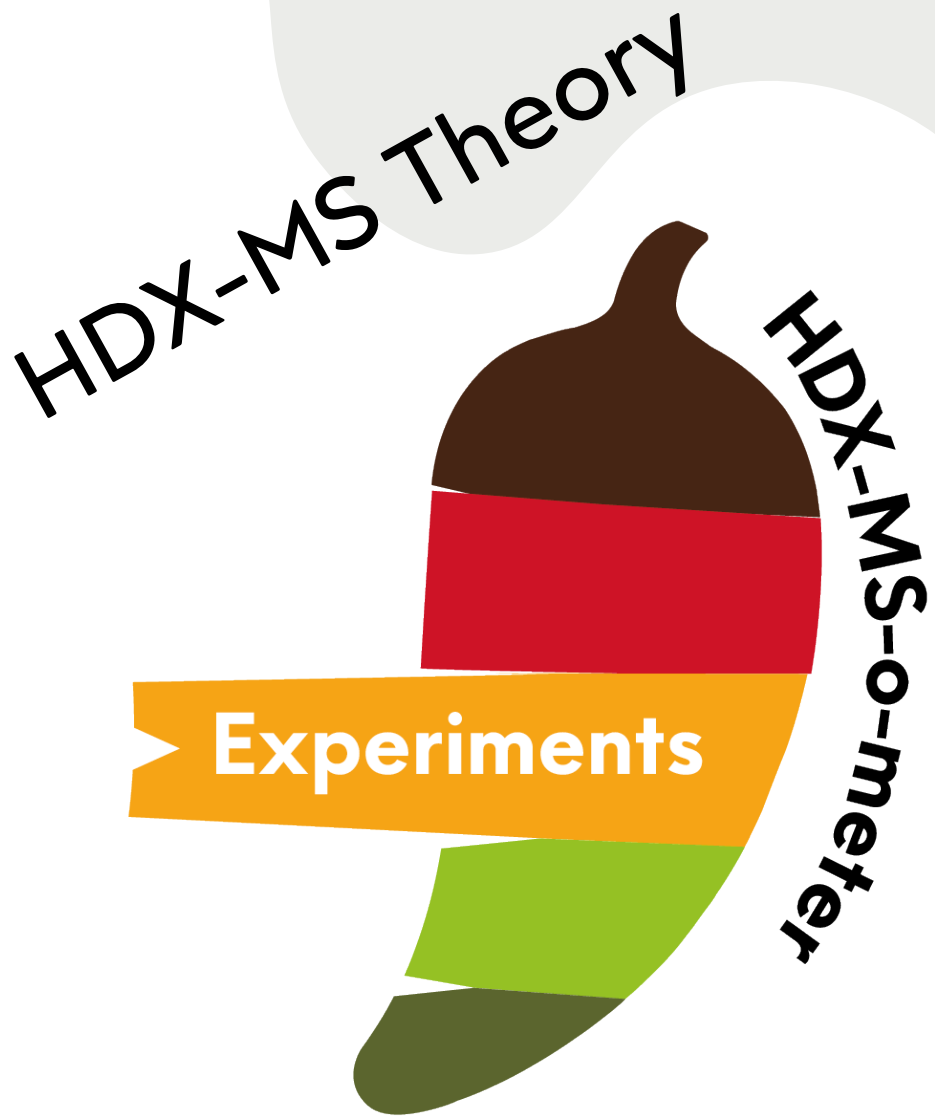
If we had a *perfect* model of protection factors, and we could *perfectly* measure HDX-MS, we would be able to essentially resolve the Linderstrom-Lang theory and infer protein structures from HDX-MS data.

There is are numerous caveats to that statement.

Take home messages #4

Protection Factors are a way of describing how “shielded” a residue from solvent. If we could accurately measure the protection factors of amides, we could model protein structures (or at least rank predicted models…).

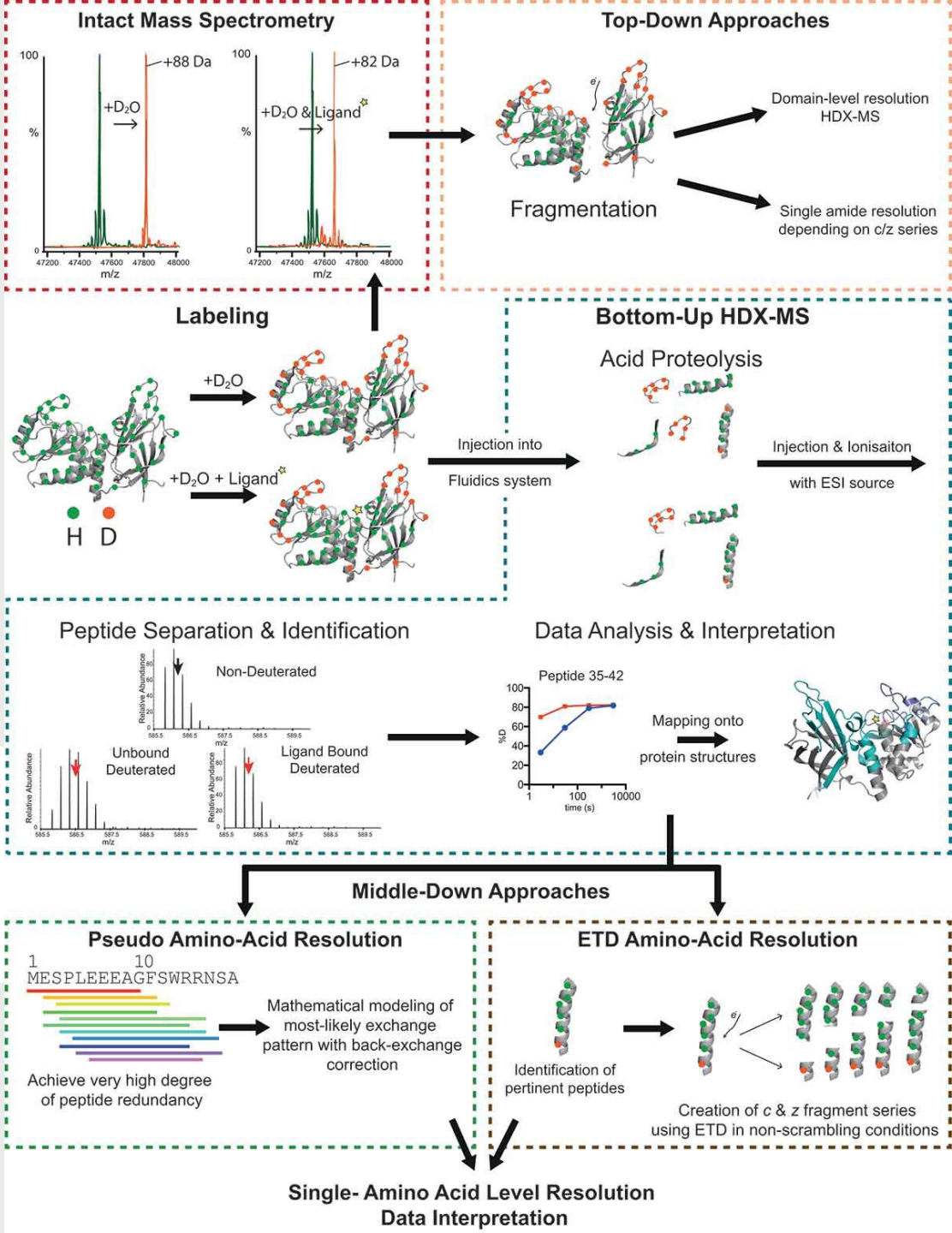
To do this though we need to very accurately measure solvent exchange.



- Why mass spectrometry is (n't) the perfect tool to measure HDX

There are multiple “routes” to measuring solvent exchange.

The most common are bottom up hdx-ms, or “peptide level” HDX-MS.



Masson, G. R., Jenkins, M. L., & Burke, J. E. (2017). An overview of hydrogen deuterium exchange mass spectrometry (HDX-MS) in drug discovery. *Expert Opinion on Drug Discovery*, *12*(10), 981–994. https://doi.org/10.1080/17460441.2017.1363734

What does an HDX experiment need to do?

We wish to measure protection factors. To do that we need to:

1) Deuterate proteins at defined timepoints

- Need to accurately start *and stop* the incorporation of deuterium*.
- Need to ensure a unidirectional incorporation of deuterium.
- Need to ensure that proteins in differing states (X) have the same k_{ch} which ensures we are observing differences in *protection factors* (and therefore changes in h-bonding and solvent exposure).

2) Measure the incorporation of that deuterium.

- Need to be able to measure BULK changes in corporation as this will give equilibrium constants, or be able to accurately separate deuterated and non-deuterated fractions, and identify peptides which strongly diverge from EX2 Need to ensure we are only measuring amide group deuterium incorporation.
- Ideally directly measure individual amides
- Minimise loss/alteration of deuterium incorporation during measurement.

*I don't have time to talk about continuous labelling HDX-MS, sorry

How good are HDX-MS experiments?

We wish to measure protection factors. To do that we need to:

1) Deuterate proteins at defined timepoints

- Need to accurately start *and stop* the incorporation of deuterium*.
- Need to ensure a unidirectional incorporation of deuterium.
- Need to ensure that proteins in differing states (X) have the same k_{ch} which ensures we are observing differences in *protection factors* (and therefore changes in h-bonding and solvent exposure).

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Konermann L, Scrosati PM. Hydrogen/Deuterium Exchange Mass Spectrometry: Fundamentals, Limitations, and Opportunities. Mol Cell Proteomics. 2024 Oct 7:100853. doi: 10.1016/j.mcpro.2024.100853. Epub ahead of print. PMID: 39383946.

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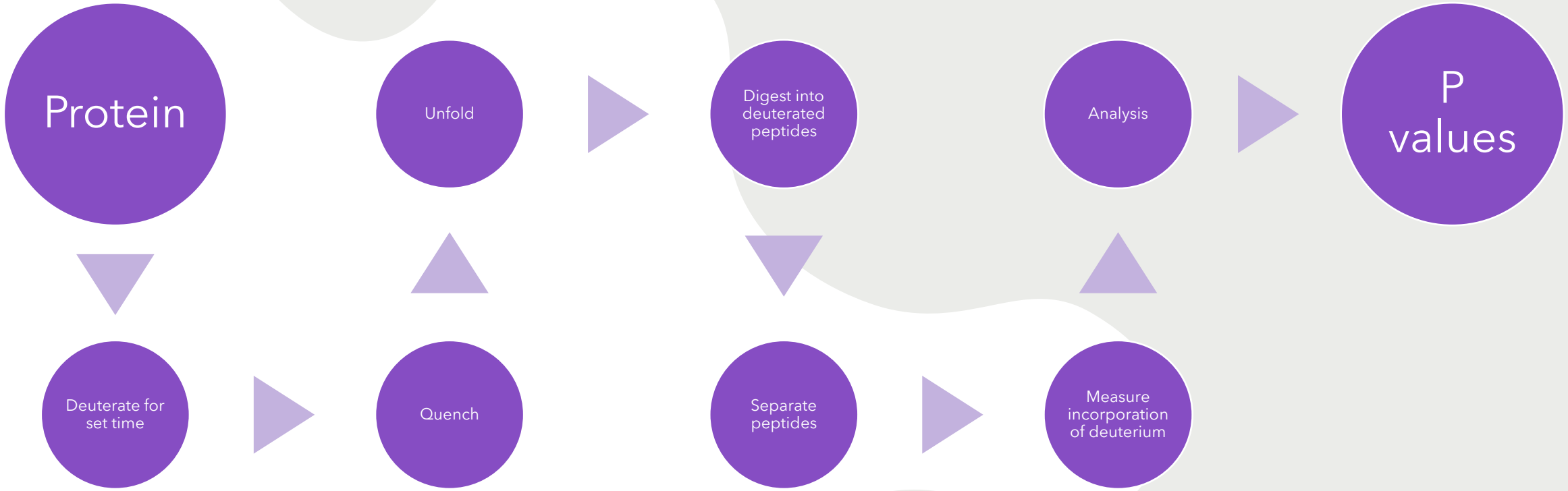
What does an HDX experiment need to do?



HDX-MS-o-meter
Experiments

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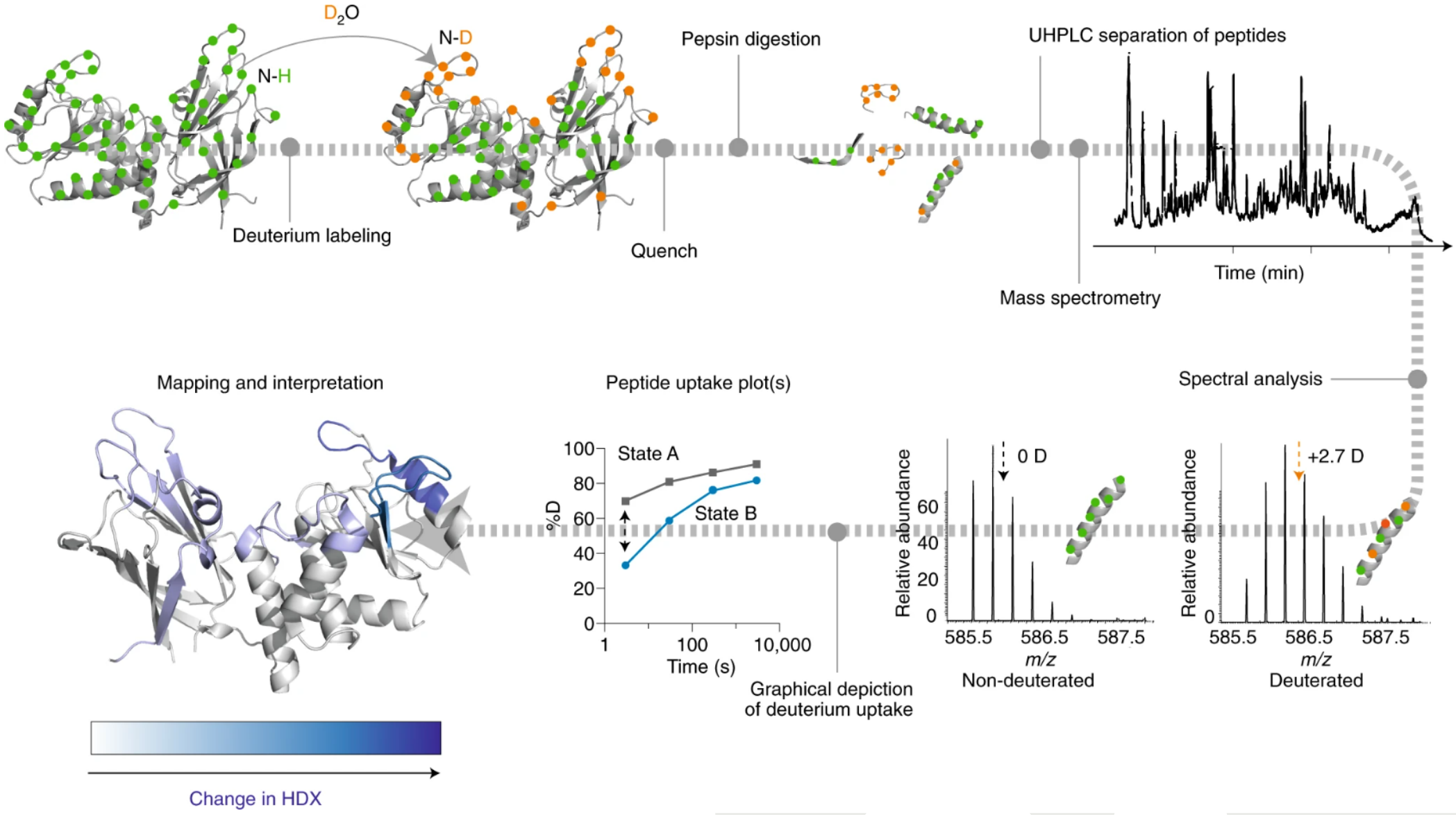


Experiments

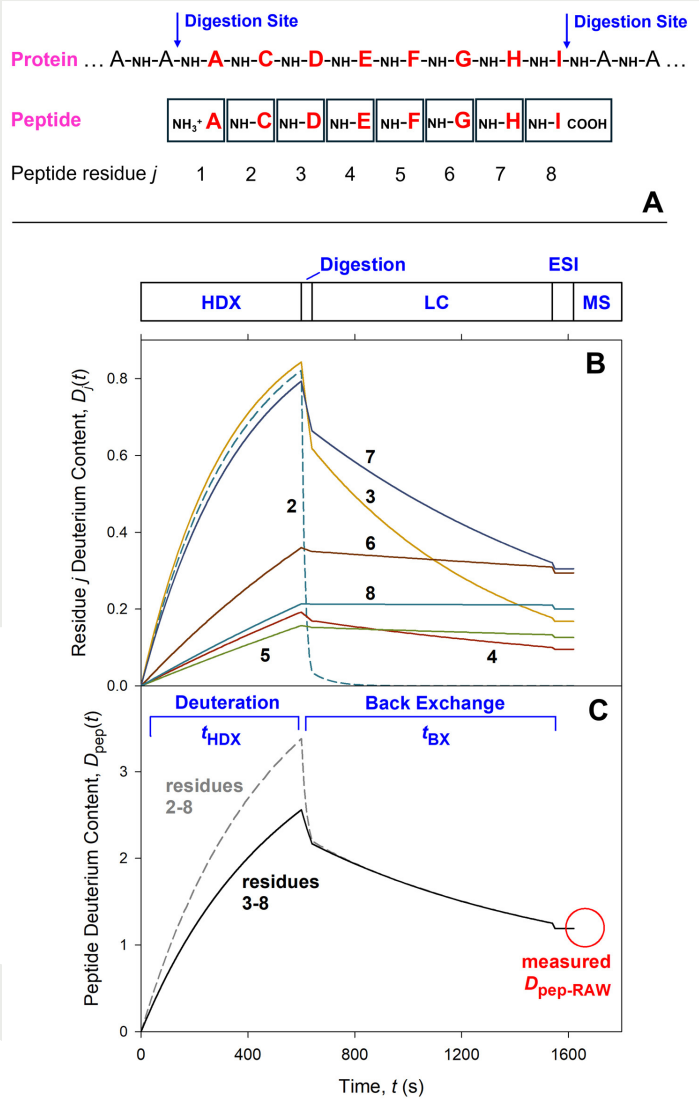
HDX-MS-o-meter

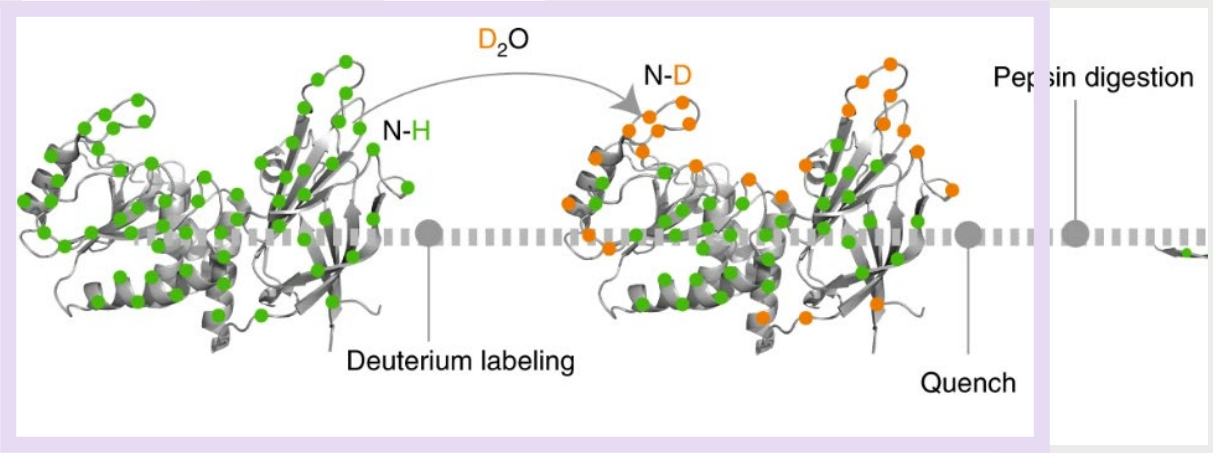
Back exchange, or why HDX-MS is imperfect.

Back exchange is the loss of deuterium from the backbone amides as the deuterated protein is exposed to H_2O typically during digestion, peptide separation and ionisation (it may also occur in the gas phase, perhaps).

Typically this results in 30%-50% loss of deuterium signal… no big deal?

- It's instrument dependent
- It's method dependent
- It's peptide dependent





Deuteration:

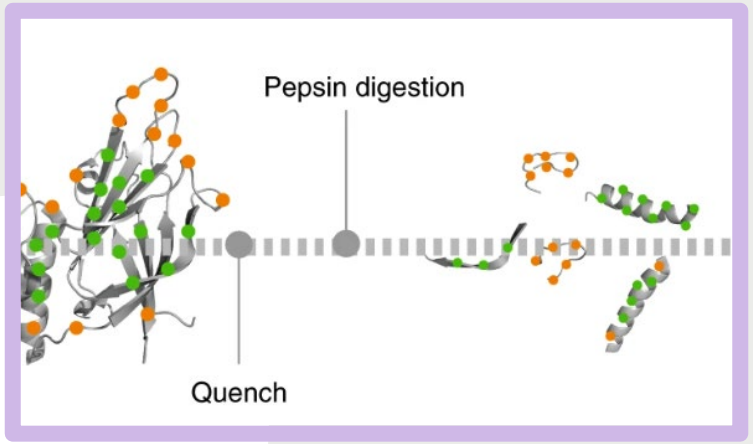
- Typically conducted on recombinantly expressed highly purified proteins, in solution, at a pH range of 6-8.
- Unidirectional deuteration is conducted by dilution of concentrated protein solution into a deuterated buffer solution
- Deuteration needs to be conducted for defined lengths of time, can be done manually or by robot.

Quenching:

- Achieved by a drop in pH (to ~2.5), freezing in liquid nitrogen or (temporarily) dropping temperature to ~0°C

Experiments

HDX-MS-o-meter

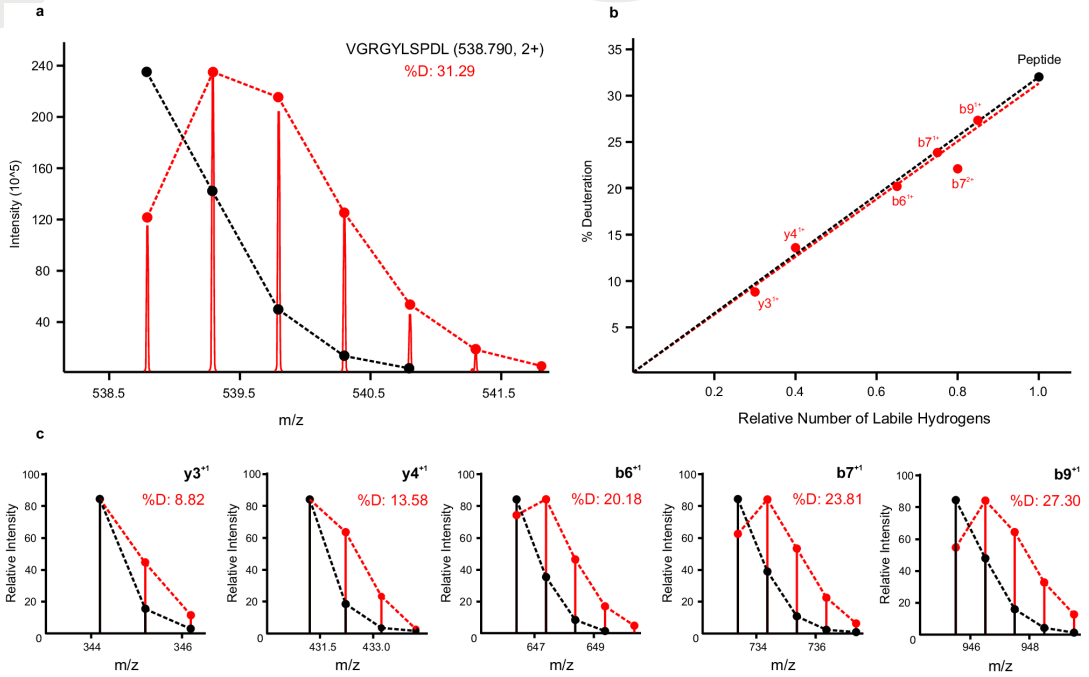


Digestion:

- Needs an acidic function, non-sequence specific protease (pepsin, Nepenthesin, Fungal Protease etc.).
- Can be done on-line (i.e. using a fluidics system) or offline using beads with proteases on.
- Typically 30s-2min of ~15°C in pH 2.5 buffer

Why can’t we do HDX-MS/MS with CID?

High energy fragmentation of peptides (such as CID) causes the deuterium signal to be randomised i.e. the number of labile Hydorgens directly correlates with the % deuteration

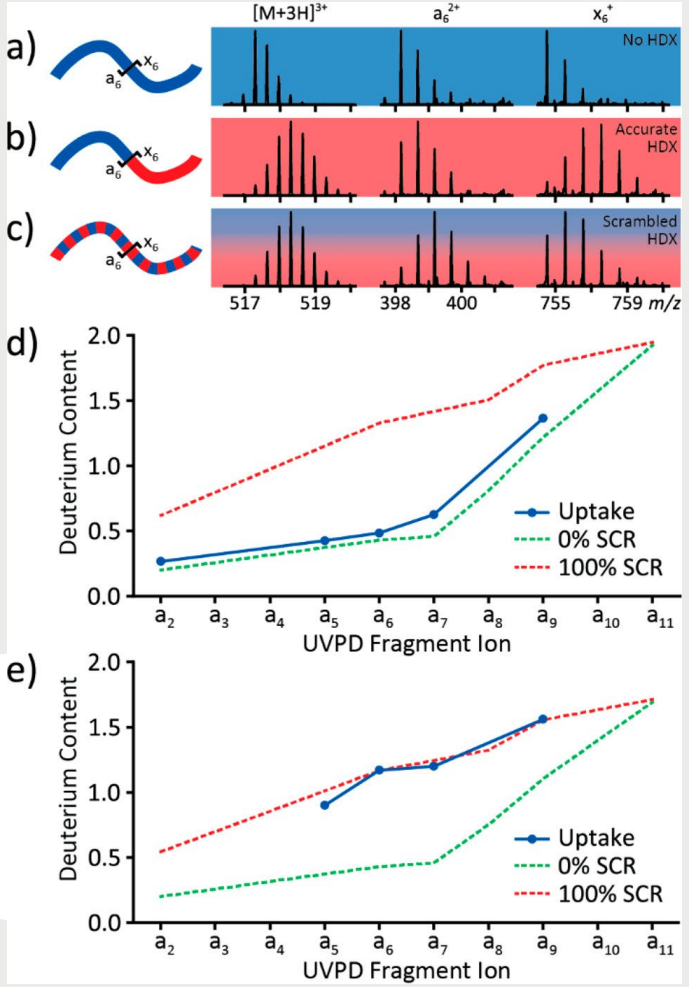


Filandr, F., Sarpe, V., Raval, S. *et al.* Automating data analysis for hydrogen/deuterium exchange mass spectrometry using data-independent acquisition methodology. *Nat Commun* **15**, 2200 (2024). https://doi.org/10.1038/s41467-024-46610-3

Why can't we do HDX-MS/MS with CID?

- However, we can now use softer fragmentation methods to minimize and/or eliminate scrambling:
  - electron transfer dissociation (ETD)
  - electron capture dissociation (ECD)
  - UV Photodissociation (UVPD)

- Typically, we use the "P1" peptide to monitor scrambling "HHHHHHIIKIIK"



Mistarz, Ulrik H et al. "UV Photodissociation Mass Spectrometry Accurately Localize Sites of Backbone Deuteration in Peptides." *Analytical chemistry* vol. 90,2 (2018): 1077-1080. doi:10.1021/acs.analchem.7b04683

Glenn R. Masson, Sarah L. Maslen, Roger L. Williams; Analysis of phosphoinositide 3-kinase inhibitors by bottom-up electron-transfer dissociation hydrogen/deuterium exchange mass spectrometry. Biochem J 1 June 2017; 474 (11): 1867–1877. doi:https://doi.org/10.1042/BCJ20170127

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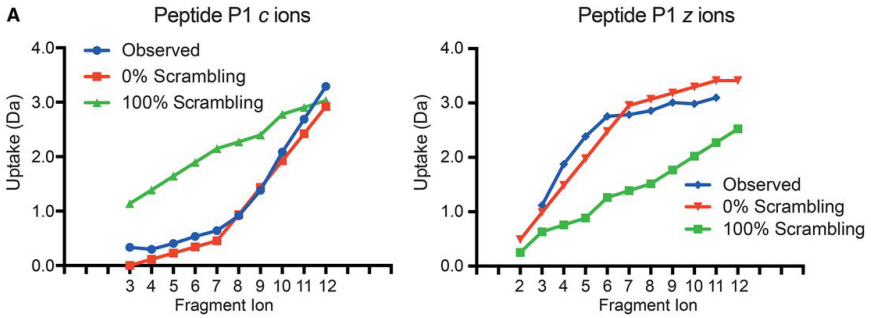

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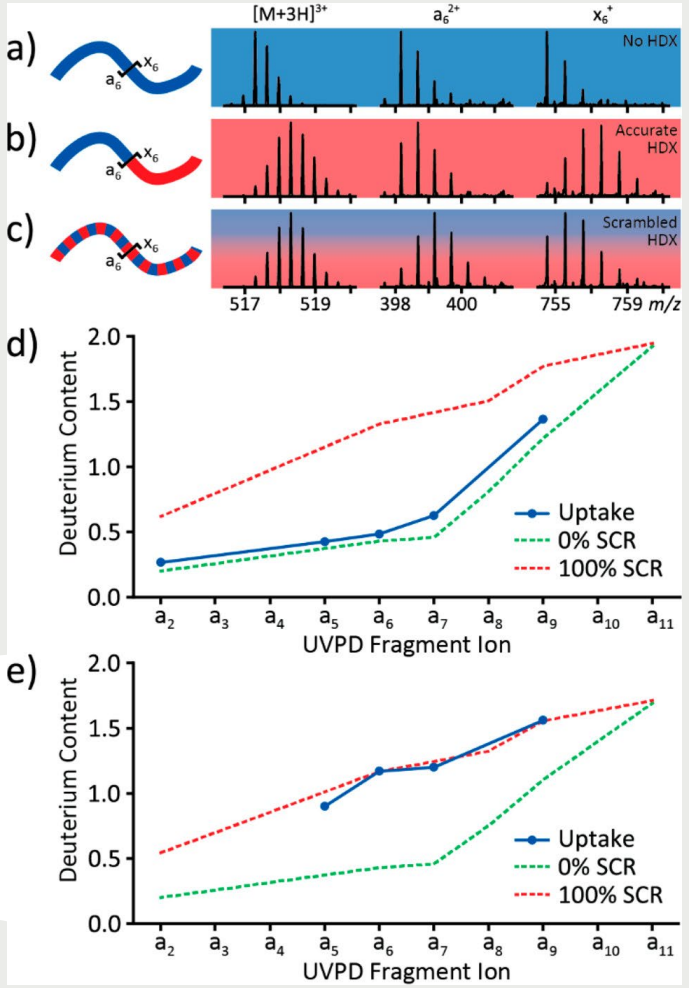

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HDX-MS-o-meter
Experiments

Why can't we do HDX-MS/MS with CID?

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  - electron transfer dissociation (ETD)
  - electron capture dissociation (ECD)
  - UV Photodissociation (UVPD)

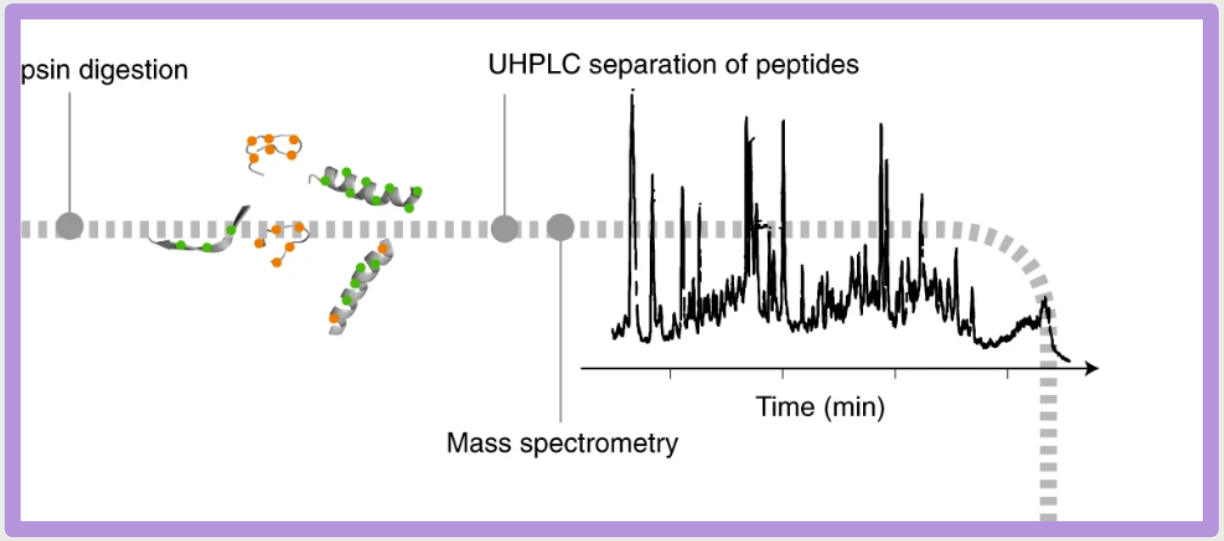
- Typically, we use the "P1" peptide to monitor scrambling "HHHHHHIIKIIK"

But…

- Relatively low throughput (10-12 peptides a run).
- Need fairly high abundance peptides
- For ETD/ECD z > ~3
- Scrambling is often never 0%
- Analysis is still burdensome…

Peptide Separation:

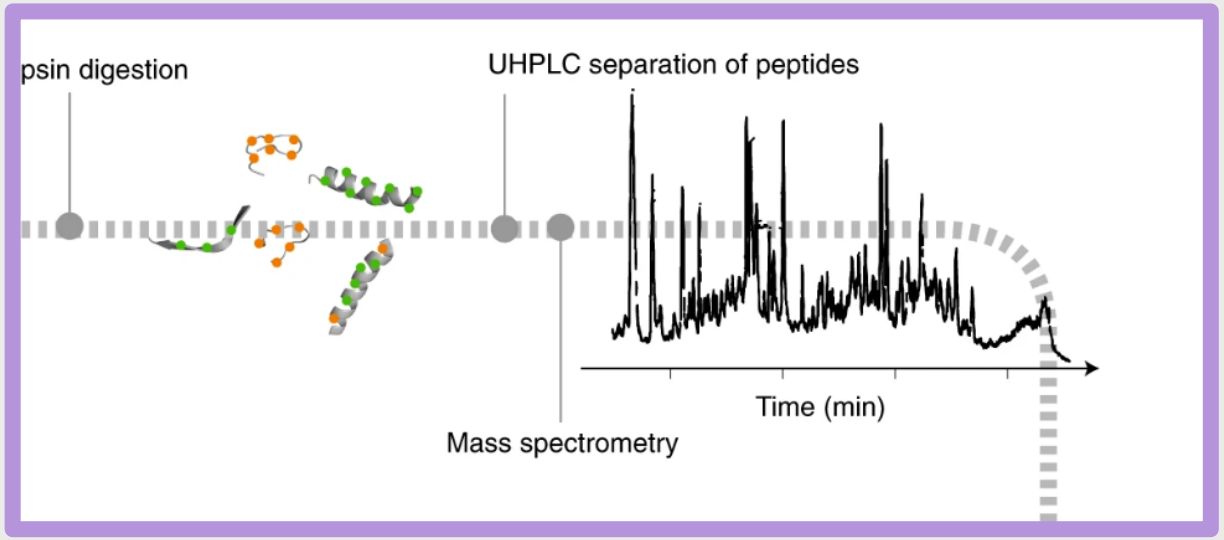
- Typically needs done at 0.1 °C reverse-phase UPLC system.
- ESI typically involves high temperatures

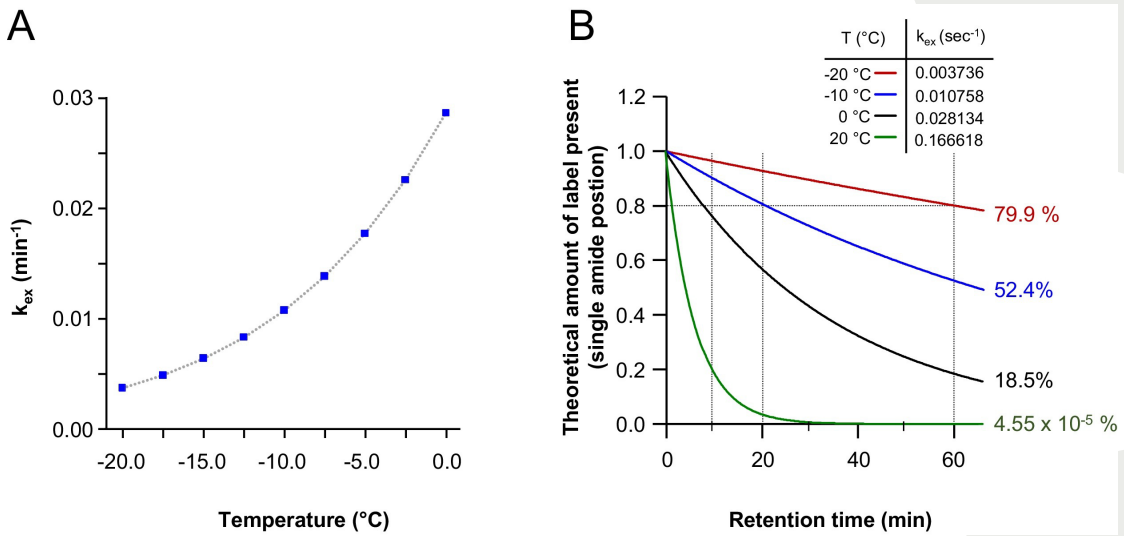


Peptide Separation:

- Typically needs done at 0.1 °C reverse-phase UPLC system.
- ESI typically involves high temperatures

- Can be ameliorated somewhat by subzero chromatography (at the expense of longer chromatograms



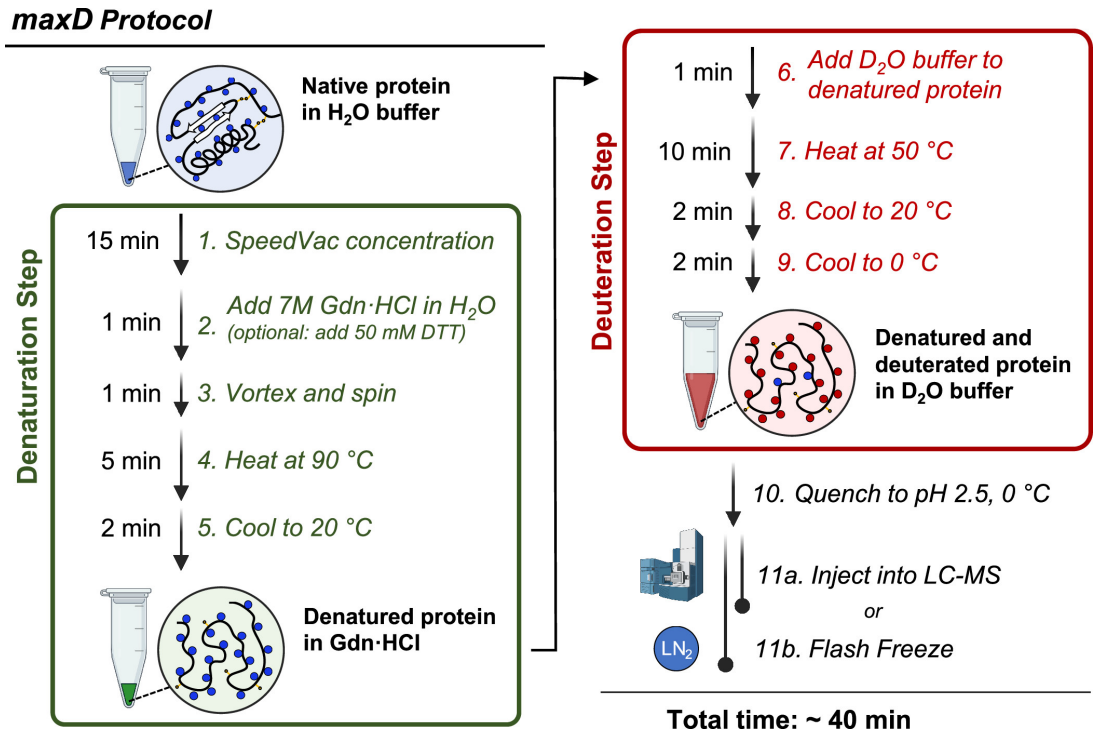


Wales, Thomas E et al. "Subzero Celsius separations in three-zone temperature controlled hydrogen deuterium exchange mass spectrometry." *Journal of chromatography. A* vol. 1523 (2017): 275-282. doi:10.1016/j.chroma.2017.05.067

How to (partially) fix back-exchange

We can control for peptide-level backexchange by creating a “maxD” control. We create a completely deuterated protein and then we measure how much deuterium is lost on each peptide.

Doesn’t solve the “residue by residue” issue, without ECD/ETD.



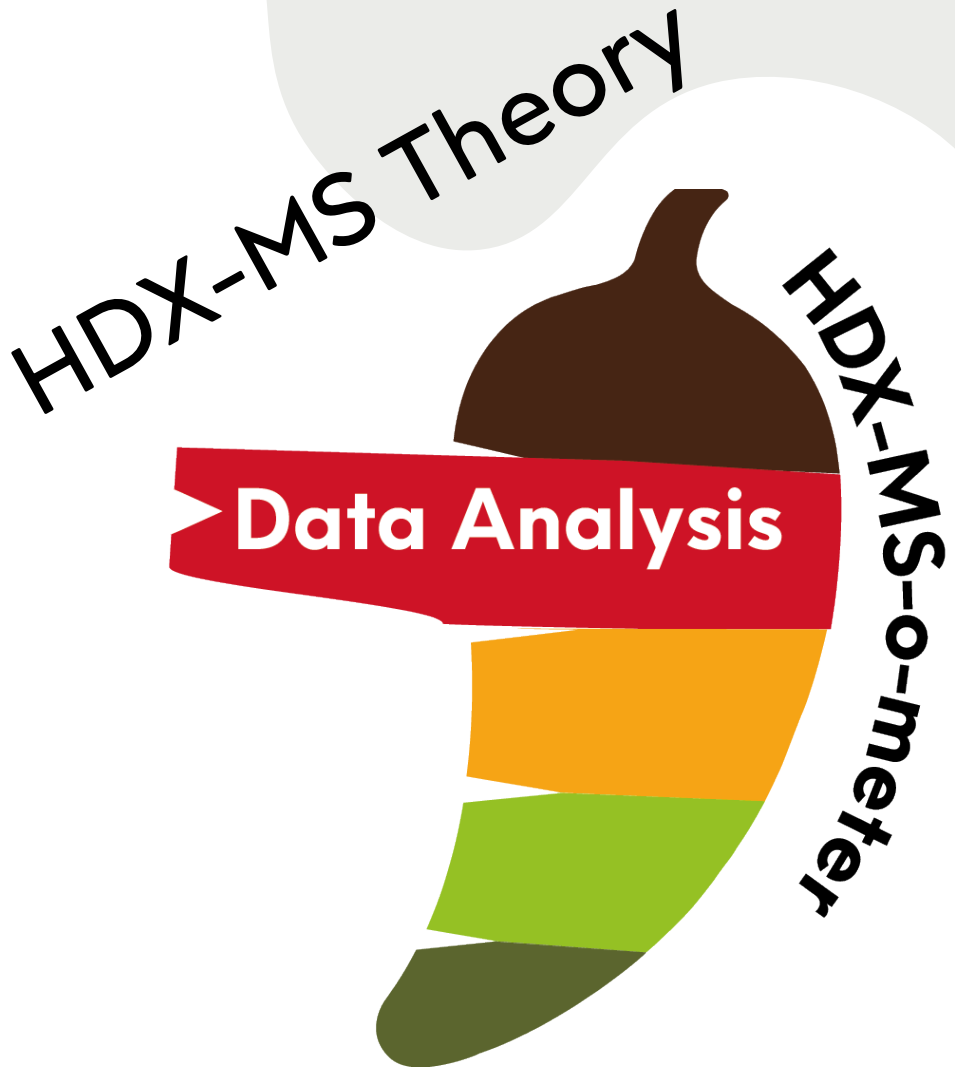
Most HDX Software allows you to load a maximally deuterated control somewhere to make the correction for you.

Peterle, Daniele et al. “Simple and Fast Maximally Deuterated Control (maxD) Preparation for Hydrogen-Deuterium Exchange Mass Spectrometry Experiments.” *Analytical chemistry* vol. 94,28 (2022): 10142-10150. doi:10.1021/acs.analchem.2c01446

How to (partially) fix back-exchange

Back-exchange essentially restricts us to *qualitative experiments i.e.* we compare state A and B (e.g. ligand bound and un-bound) and we largely ignore protection factors.

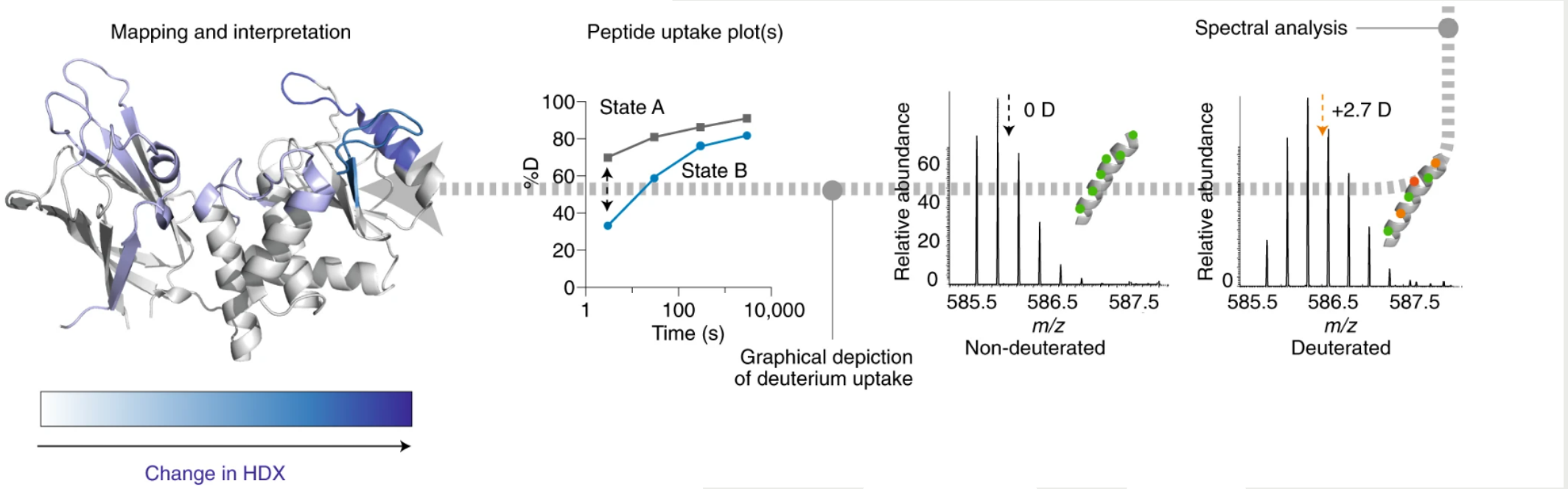
There's a whole ocean of structural biology untapped due to this…



- Largely covered in the later session

1) Identify your non-deuterated peptides from your non-deuterated runs (PLGS/Mascot)
2) Determine the level of deuteration from subsequent deuterated samples (HDExaminer, MSStudio, DynamX, Newmarket…)

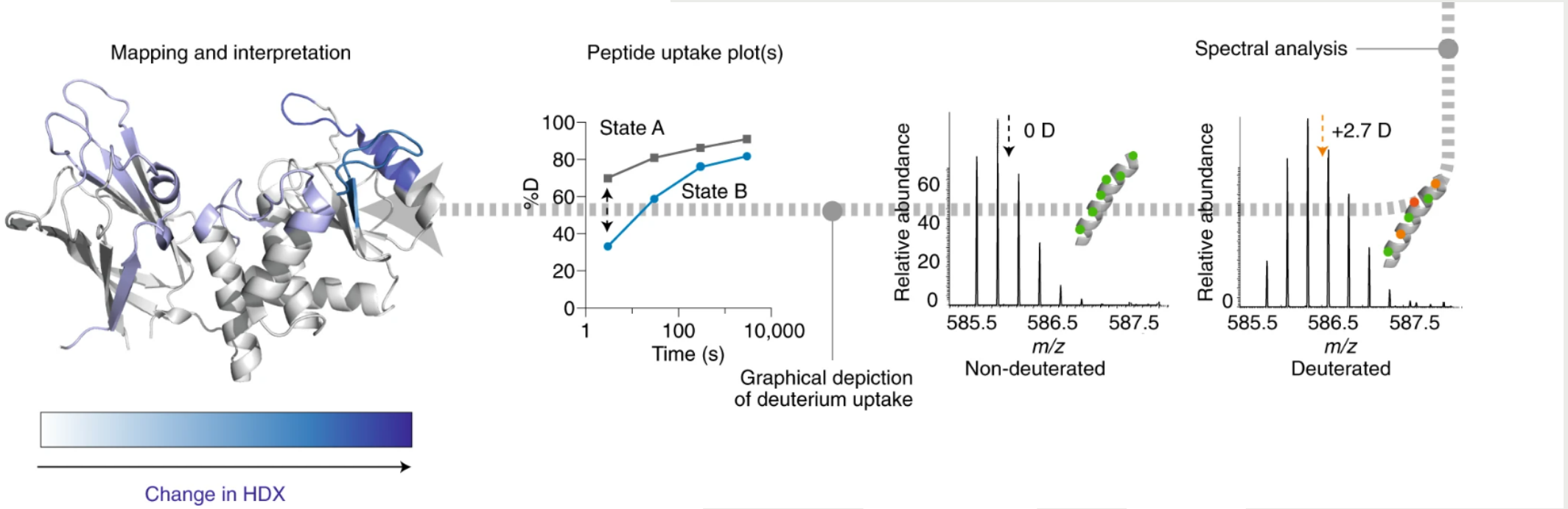
Your data <u>never</u> gets better when you add deuterium, so always throw away poor non-deuterated peptides!



Experiments
HDX-MS-o-meter

Covered later today

1) Need to identify non-deuterated peptides.
2) Need to measure the mass on the deuterated peptides.



- New & exciting tools and biology on the horizon

1. Single-amino acid HDX-MS by either ECD/ETD/UVPD
2. Subzero chromatography
3. Single-amino acid HDX-MS by data analysis
4. (cyclic) Ion Mobility for better resolution and bigger datasets
5. DIA-based peptide measurement and identification

   > Cellular HDX??

1. Single-amino acid HDX-MS by either ECD/ETD/UVPD ✓
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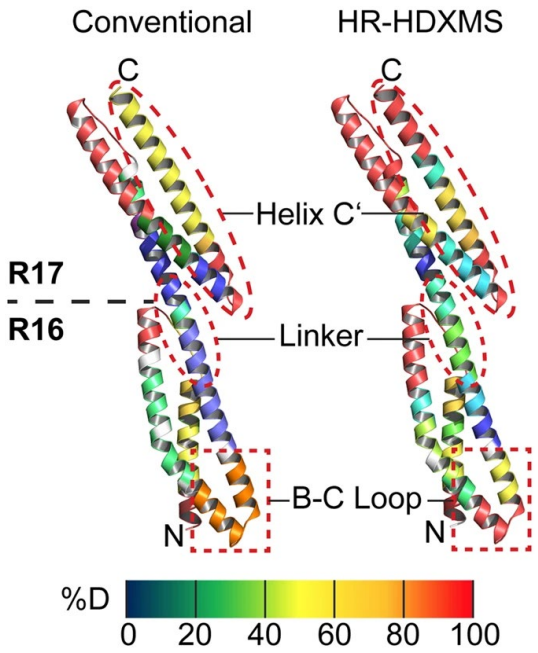
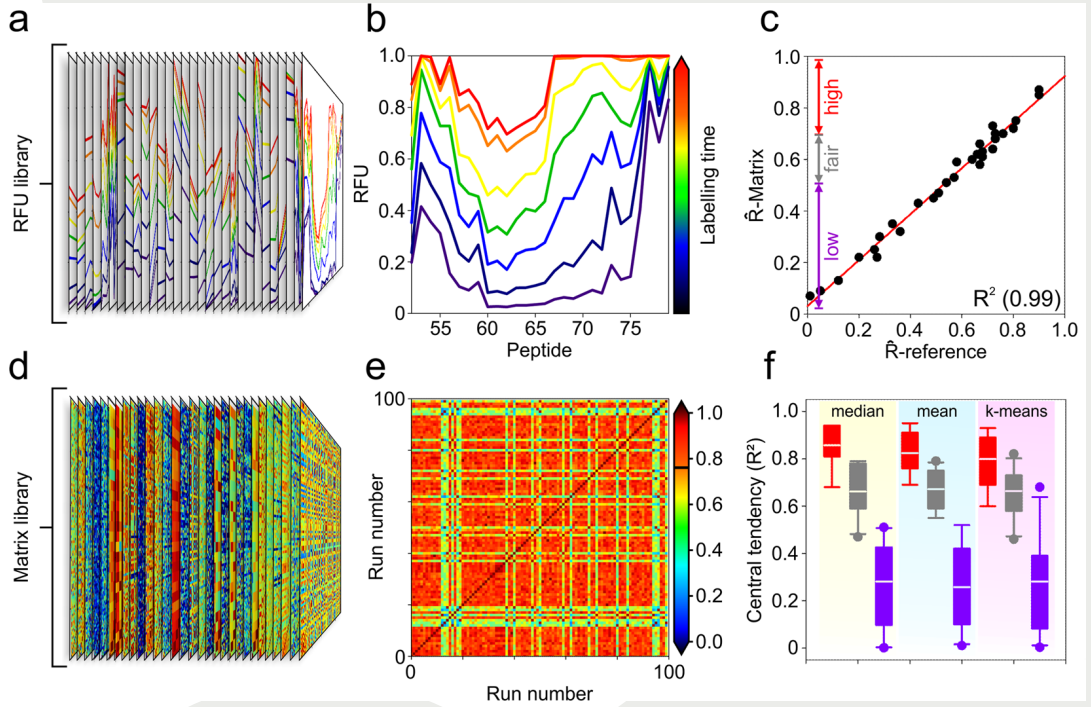
   > Cellular HDX??

Single-amino acid HDX-MS by data analysis

Aim: Take peptide-level data collected in the standard protocol and determine single amino acid level of deuteration.





Salmas, R.E., Borysik, A.J. HDXmodeller: an online webserver for high-resolution HDX-MS with auto-validation. *Commun Biol* **4**, 199 (2021). https://doi.org/10.1038/s42003-021-01709-x

Gessner, C., Steinchen, W., Bédard, S. *et al.* Computational method allowing Hydrogen-Deuterium Exchange Mass Spectrometry at single amide Resolution. *Sci Rep* **7**, 3789 (2017). https://doi.org/10.1038/s41598-017-03922-3

Single-amino acid HDX-MS by data analysis

Aim: Take peptide-level data collected in the standard protocol and determine single amino acid level of deuteration.

Why isn't this widely adopted?

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Single-amino acid HDX-MS by data analysis

Aim: Take peptide-level data collected in the standard protocol and determine single amino acid level of deuteration.

Why isn't this widely adopted?

1. You typically need high levels of peptide redundancy, with good back exchange controls
2. Need to a high degree of computational/technical insight to get it going
3. The field hasn't settled yet on "one" solution

Salmas, R.E., Borysik, A.J. HDXmodeller: an online webserver for high-resolution HDX-MS with auto-validation. *Commun Biol* **4**, 199 (2021). https://doi.org/10.1038/s42003-021-01709-x

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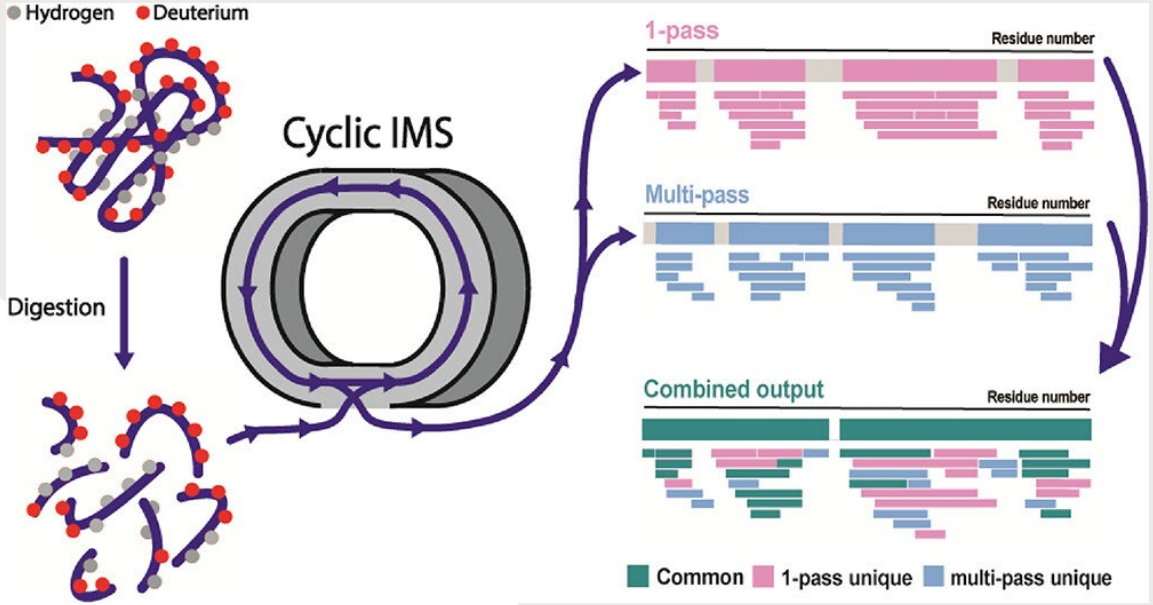
(cyclic) Ion Mobility for better resolution and bigger datasets

Aim: Allows better resolution of peptides on compressed chromatograms

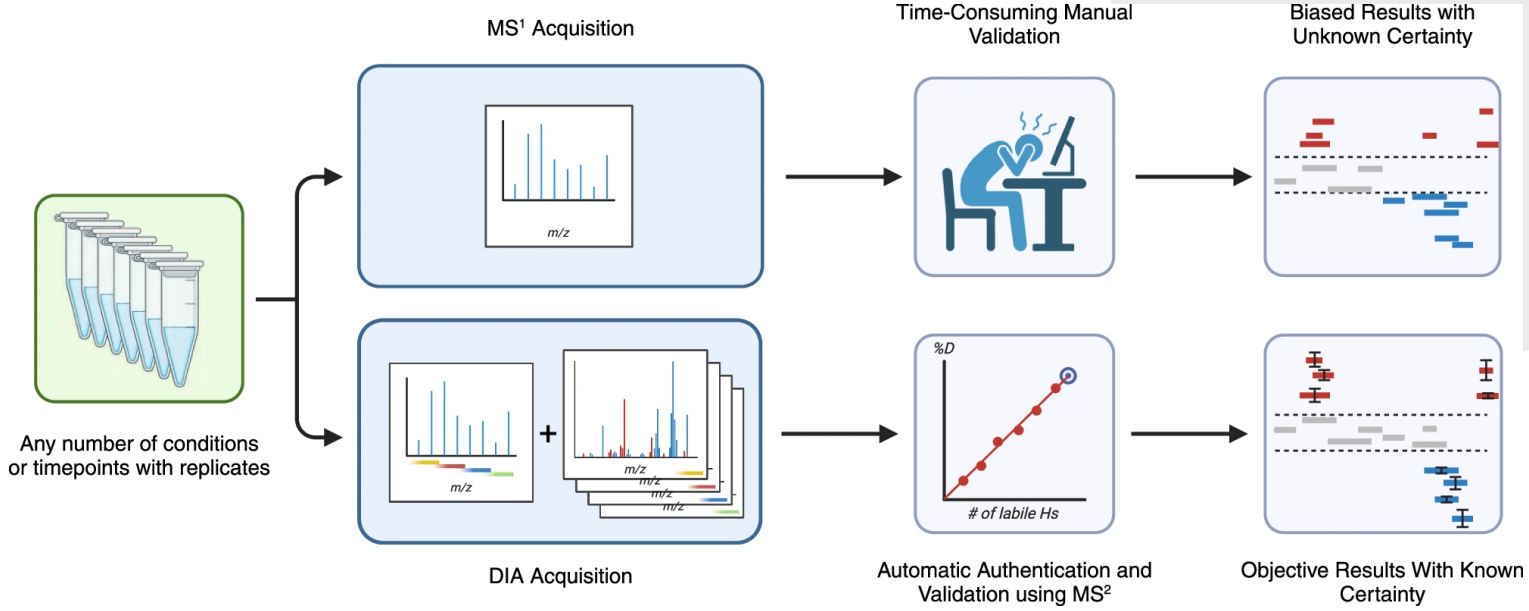
Promising, but I think that the main thing we could do is essentially eliminate or greatly reduce chromatographic separation and thus vastly reduce back-exchange…



Griffiths, Damon et al. "Cyclic Ion Mobility for Hydrogen/Deuterium Exchange-Mass Spectrometry Applications." *Analytical chemistry* vol. 96,15 (2024): 5869-5877. doi:10.1021/acs.analchem.3c05753

DIA-based peptide measurement and identification & Cellular HDX??

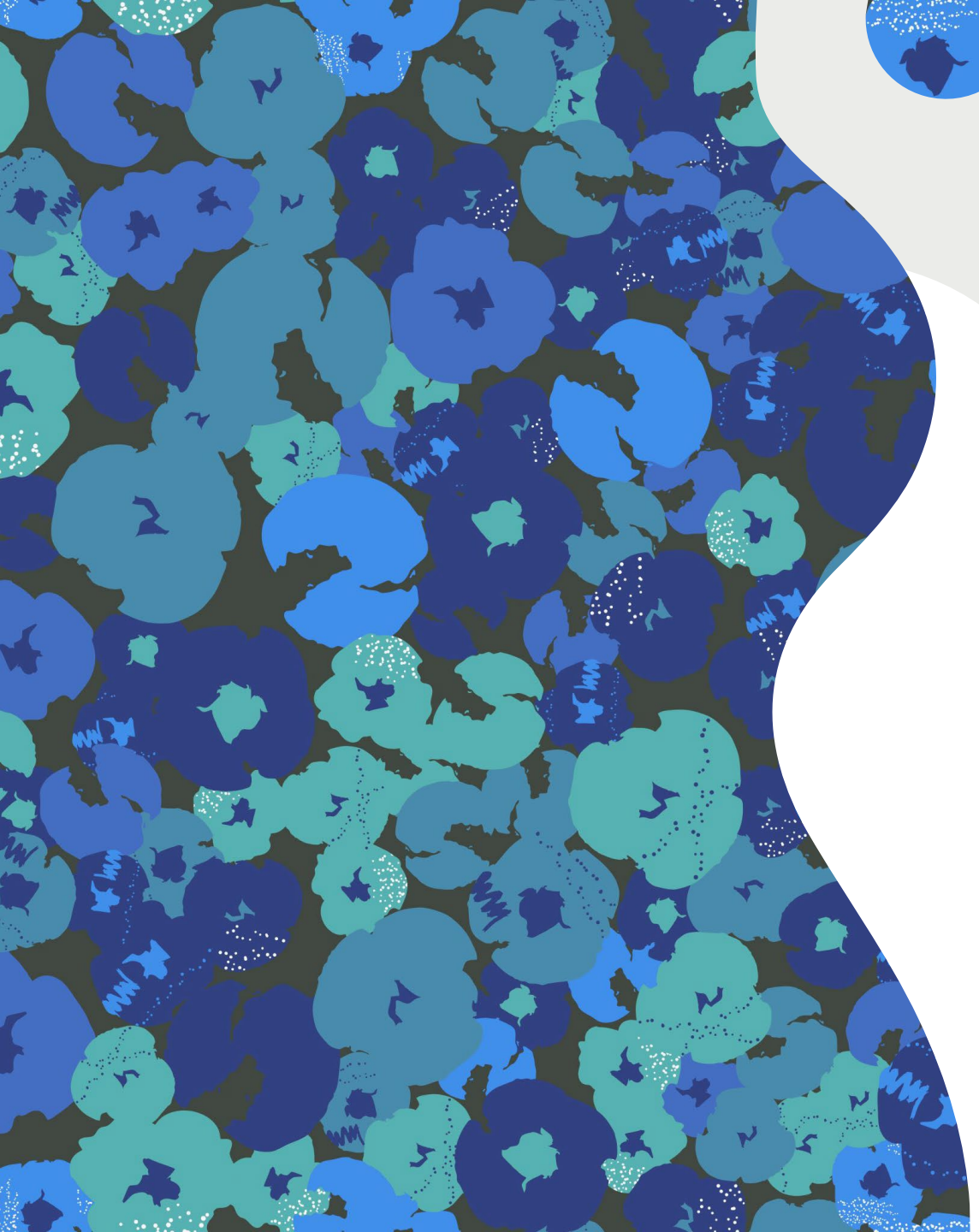
Aim: Use DIA/machine learning to do peptide identification + HDX-MS in one step, and confirms the MS data with MS/MS



Could be a game changer in both time and accuracy of data analysis!

Will allow for the analysis of *very* large datasets

Filandr, F., Sarpe, V., Raval, S. *et al.* Automating data analysis for hydrogen/deuterium exchange mass spectrometry using data-independent acquisition methodology. *Nat Commun* **15**, 2200 (2024). https://doi.org/10.1038/s41467-024-46610-3



Conclusions

- Currently the vast majority of HDX-MS studies are qualitative studies comparing e.g. drug binding to proteins. This is largely due to back-exchange being an unresolved issue.
- Better theory, systems, controls, or data analysis systems are required to develop quantitative HDX, which would allow structure determination from protection factors.
- Recent advances in HDX-MS are to push the number of peptides identified, and the speed at which we can process those peptides