

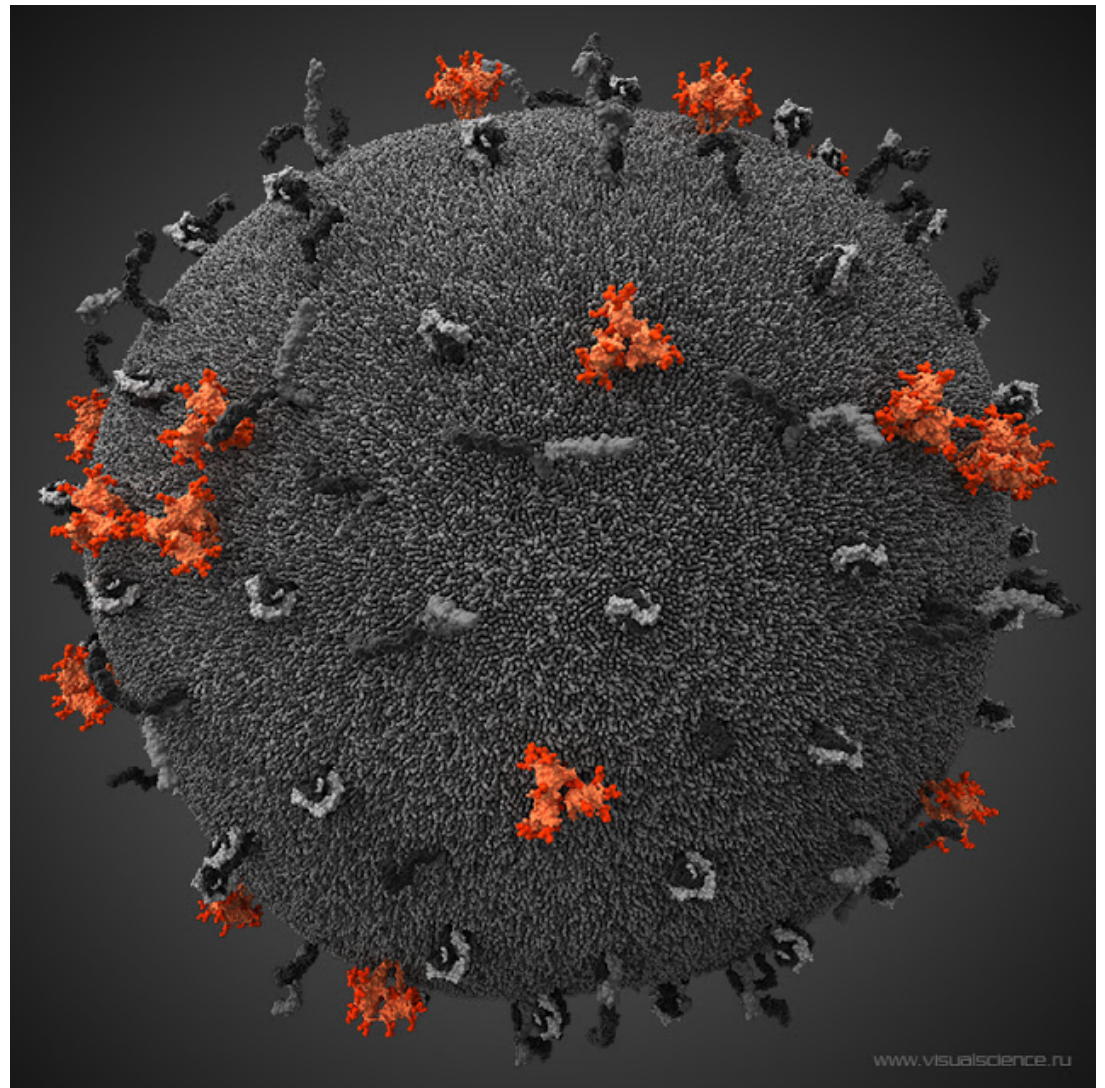
# **Unmasking the initial stages of HIV Env glycoprotein activation using H/D exchange and X-ray footprinting**

Miklos Guttman

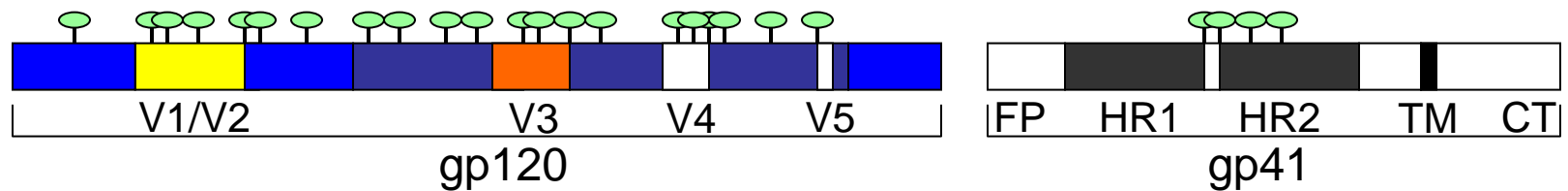
Kelly Lee's group  
Department of Medicinal Chemistry  
University of Washington, Seattle

# Env is the sole antigenic feature on HIV virions

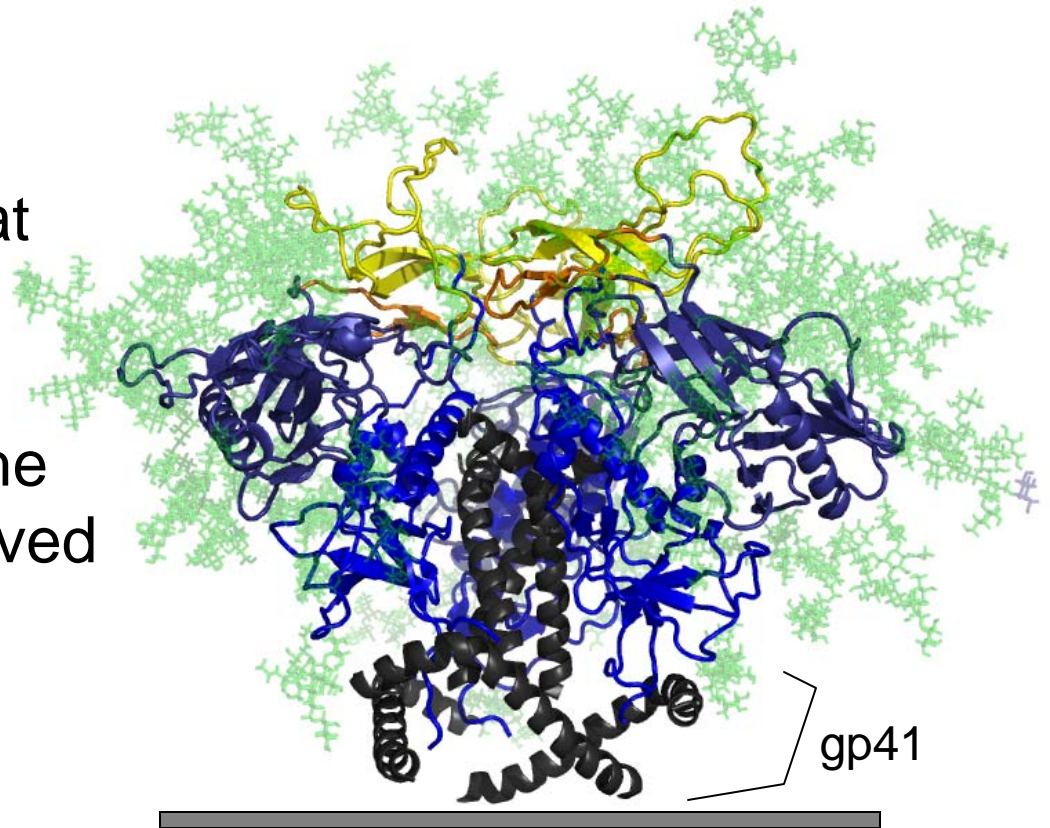
- Mediates viral entry through binding host receptors
  - Primary: CD4
  - Co-receptor: (CCR5/CXCR4)
- Evades immune system
  - High sequence variability
  - Glycan shield
  - Conserved regions only exposed upon primary receptor (CD4) binding
- Major focus of HIV vaccinology



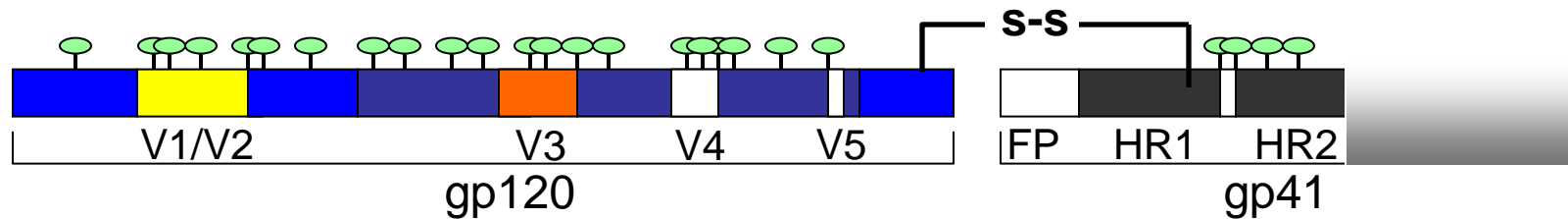
# Trimeric structure of Env



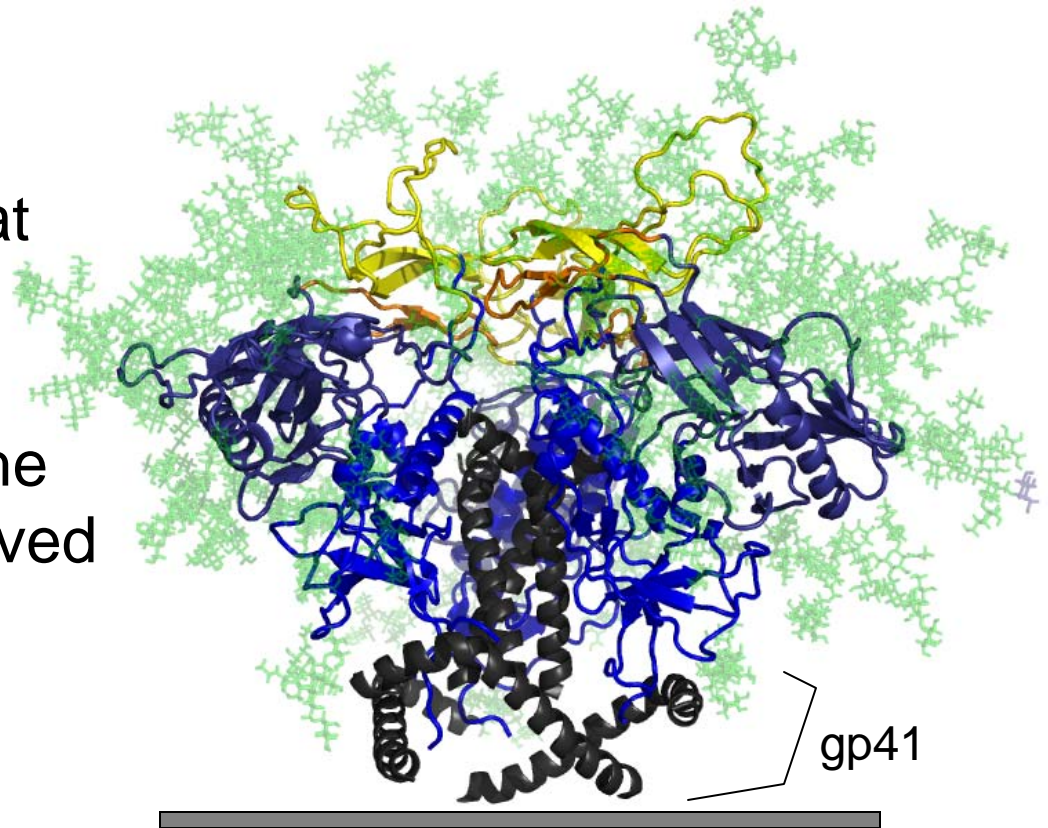
- V1/V2 and V3 form quaternary interactions at the crown of the trimer
  - Hide conserved regions
- CD4 binding opens up the trimer to unmask conserved regions (V3)



# Trimeric structure of Env

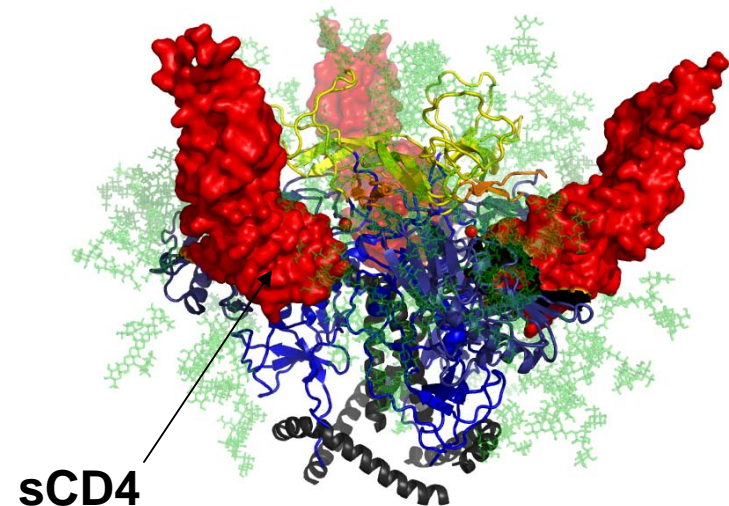
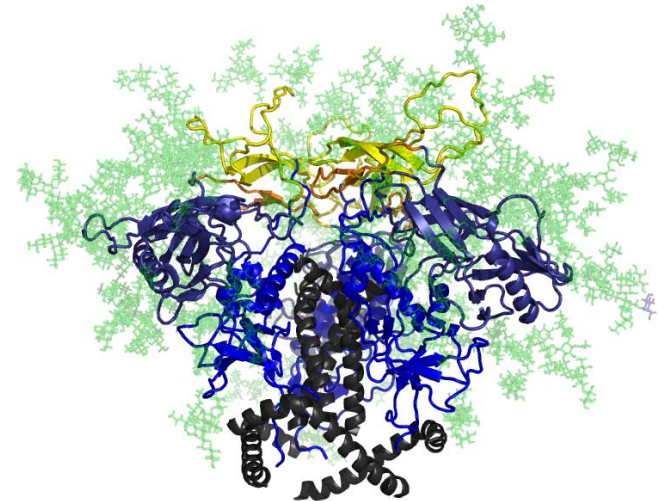


- V1/V2 and V3 form quaternary interactions at the crown of the trimer
  - Hide conserved regions
- CD4 binding opens up the trimer to unmask conserved regions (V3)
- Soluble Env trimers

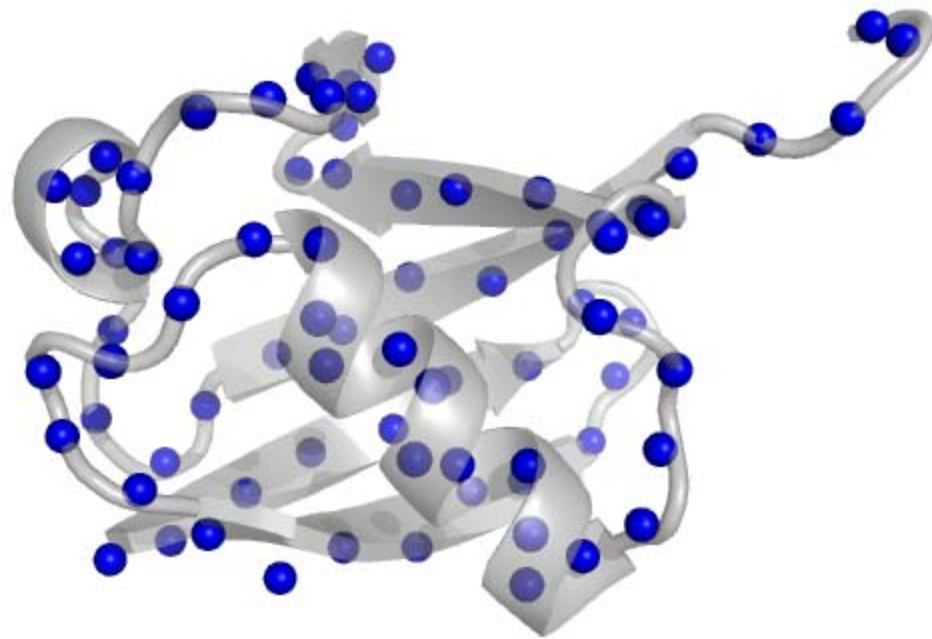
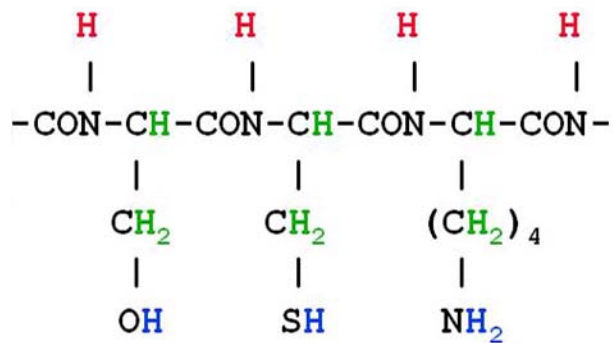


# How does CD4 binding alter Env?

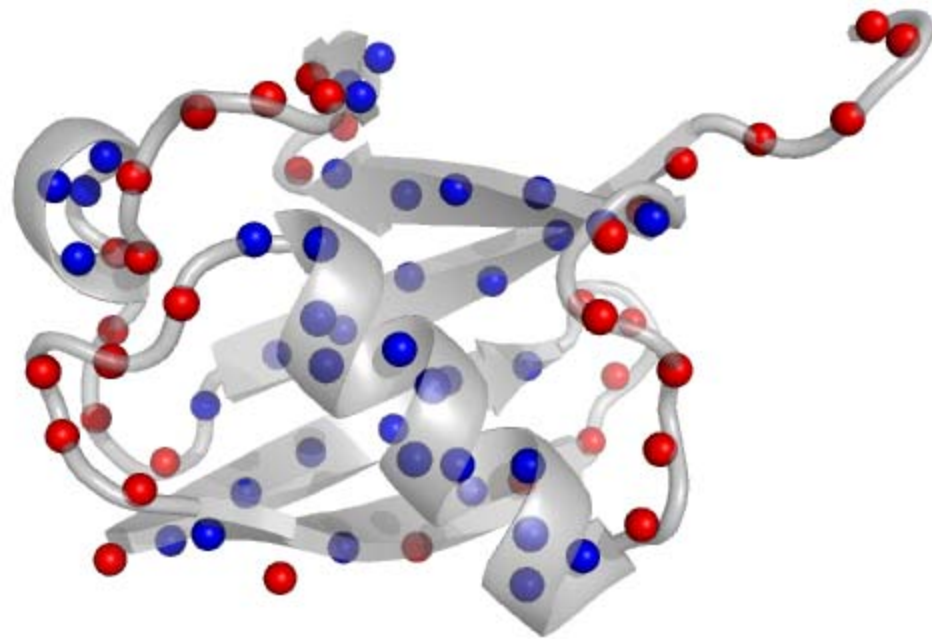
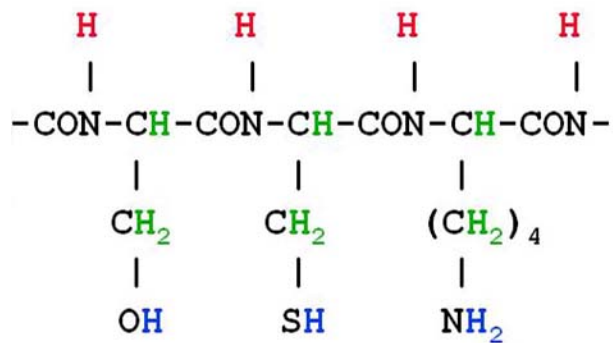
- Structures available for many ligands with isolated gp120
  - gp120 core +/- CD4 look identical
    - Kwon et al, *PNAS* 2012
  - No crystal of trimer with CD4
  - EM studies indicate large changes in the trimer upon CD4 binding
    - Liu et al, *Nature* 2008
- Compare unliganded and **sCD4** bound conformations of trimeric Env
  - H/D exchange (HDX-MS)
  - X-ray footprinting (XF-MS)



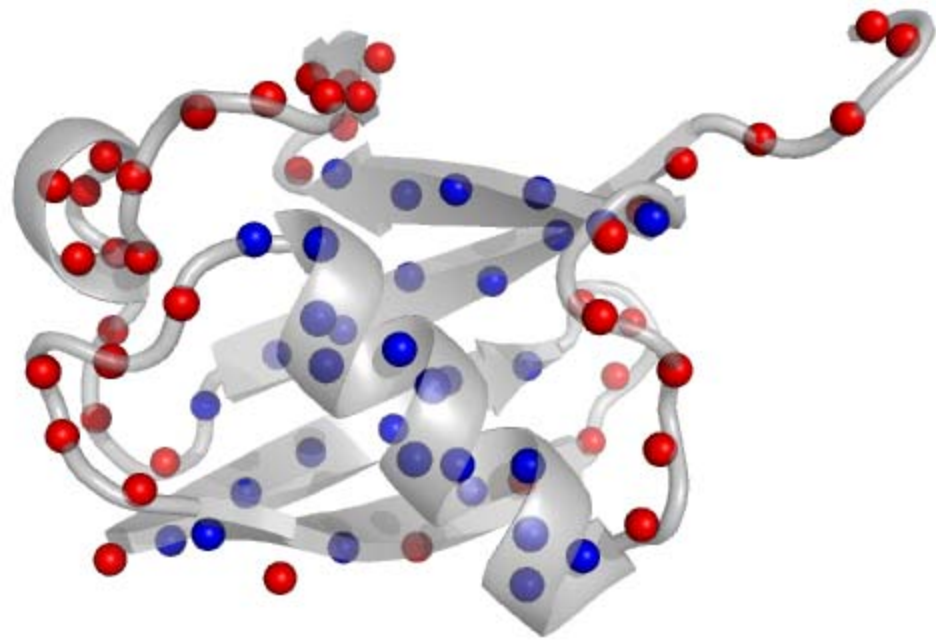
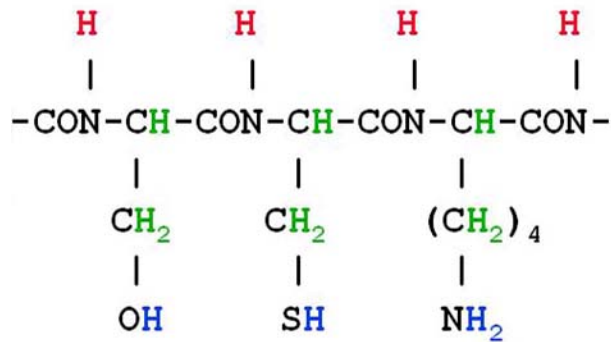
# Hydrogen/Deuterium exchange probes the accessibility of amide hydrogens



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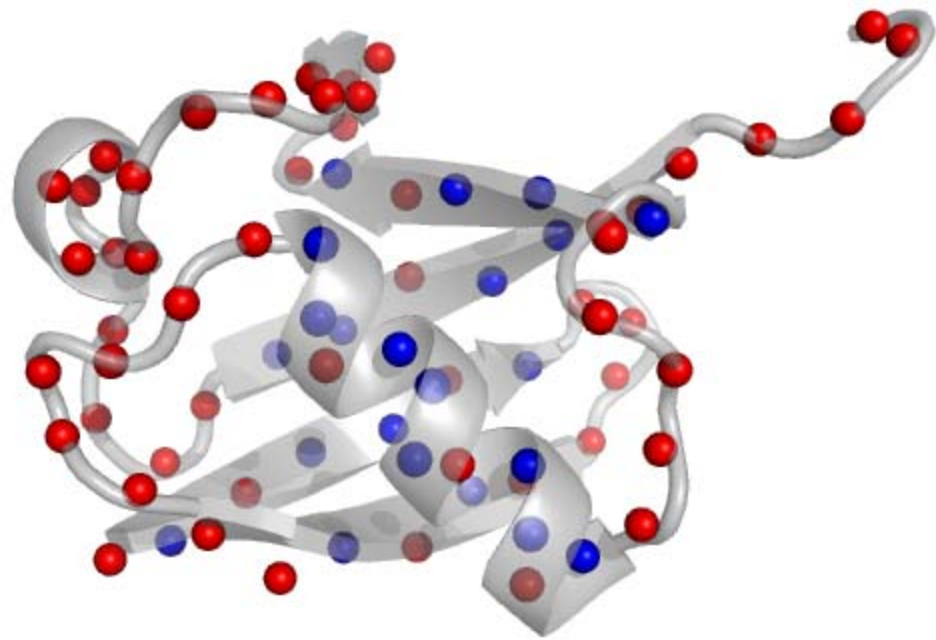
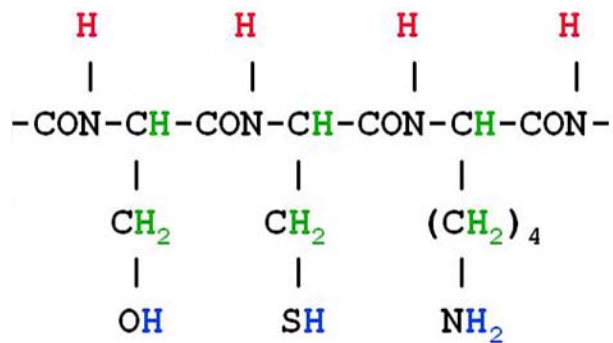


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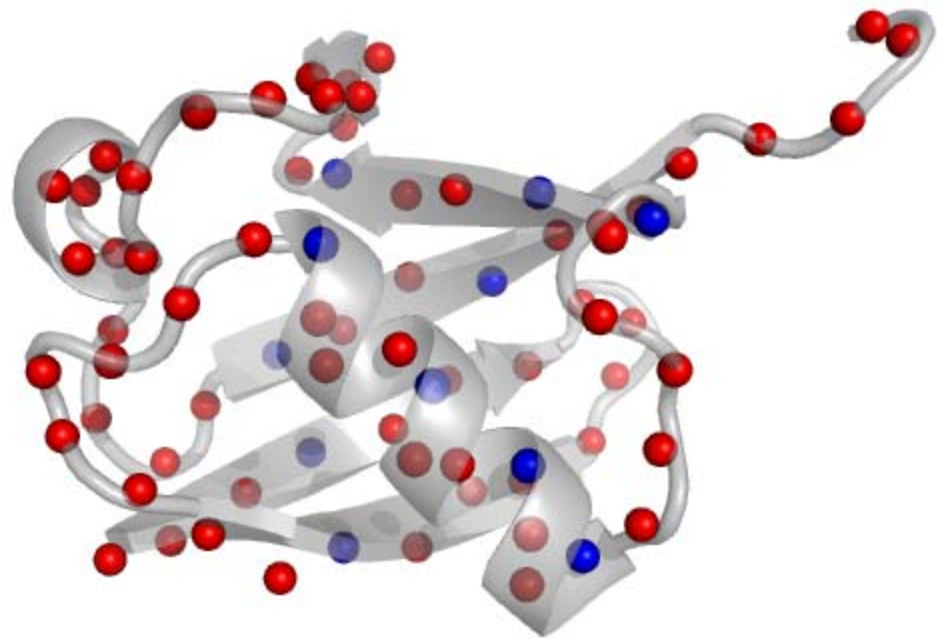
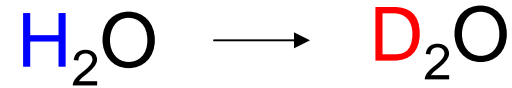
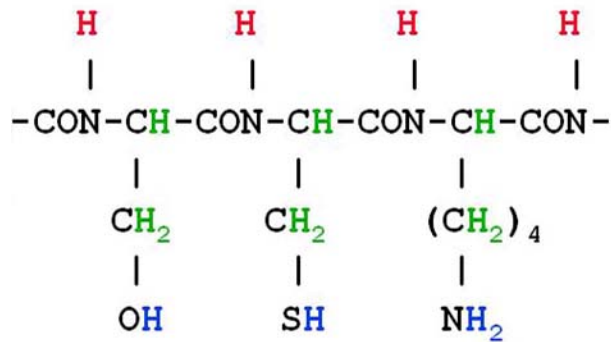




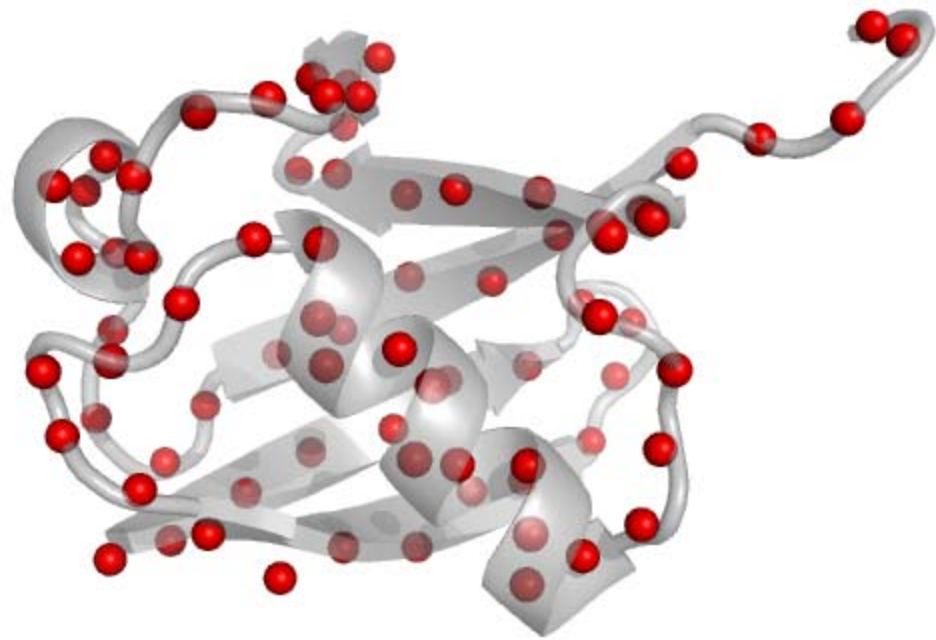
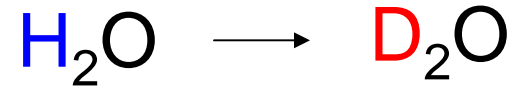
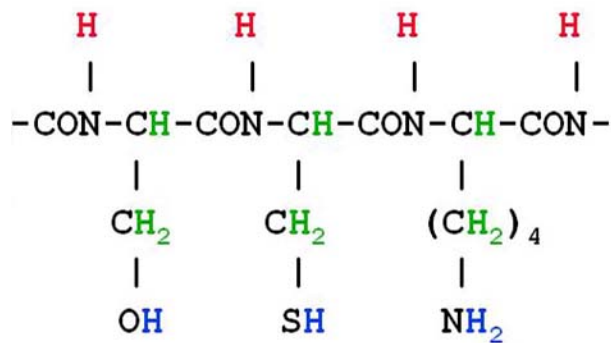
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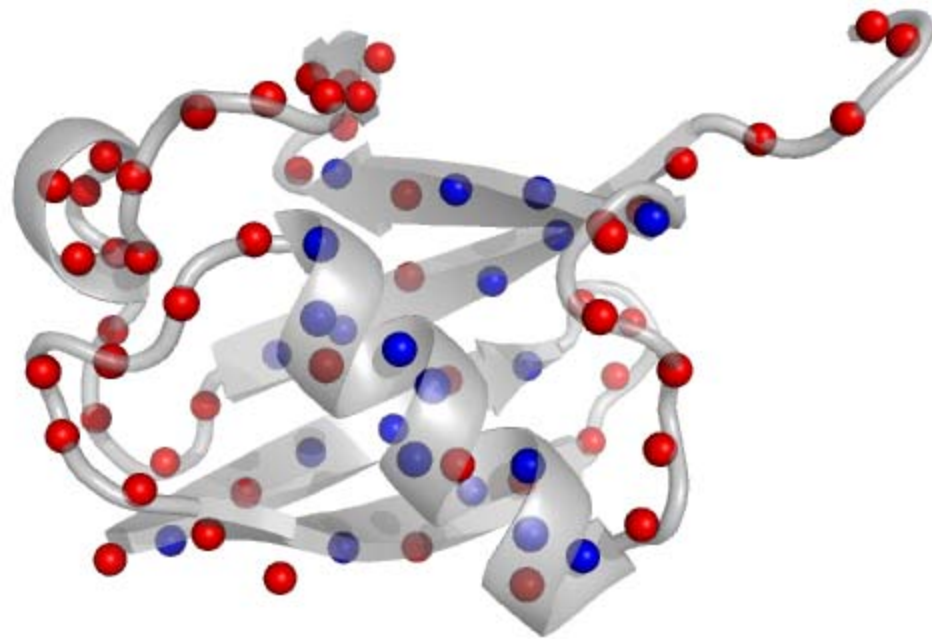
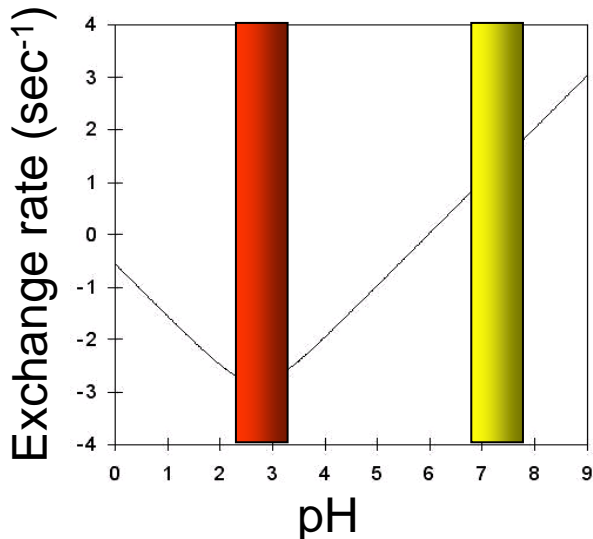
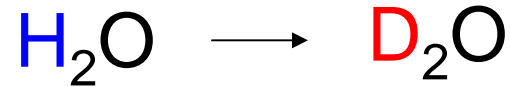
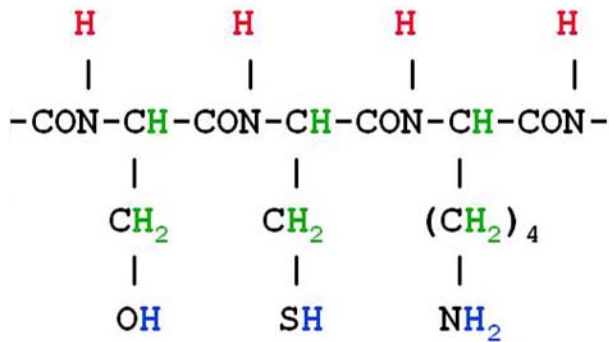


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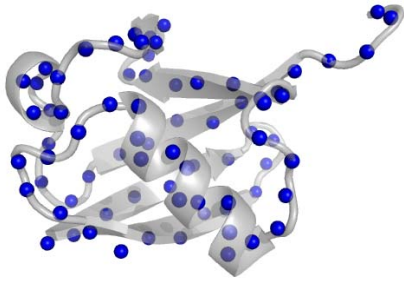
\*Labeling doesn't perturb protein structure

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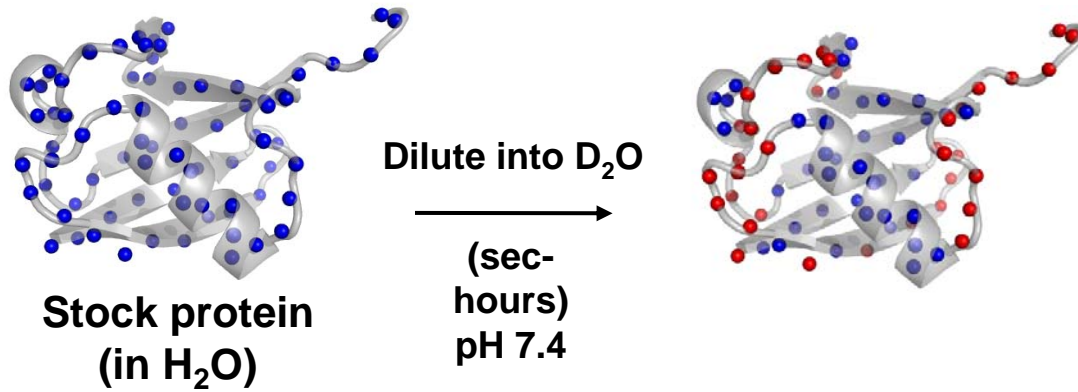
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# HDX experimental setup

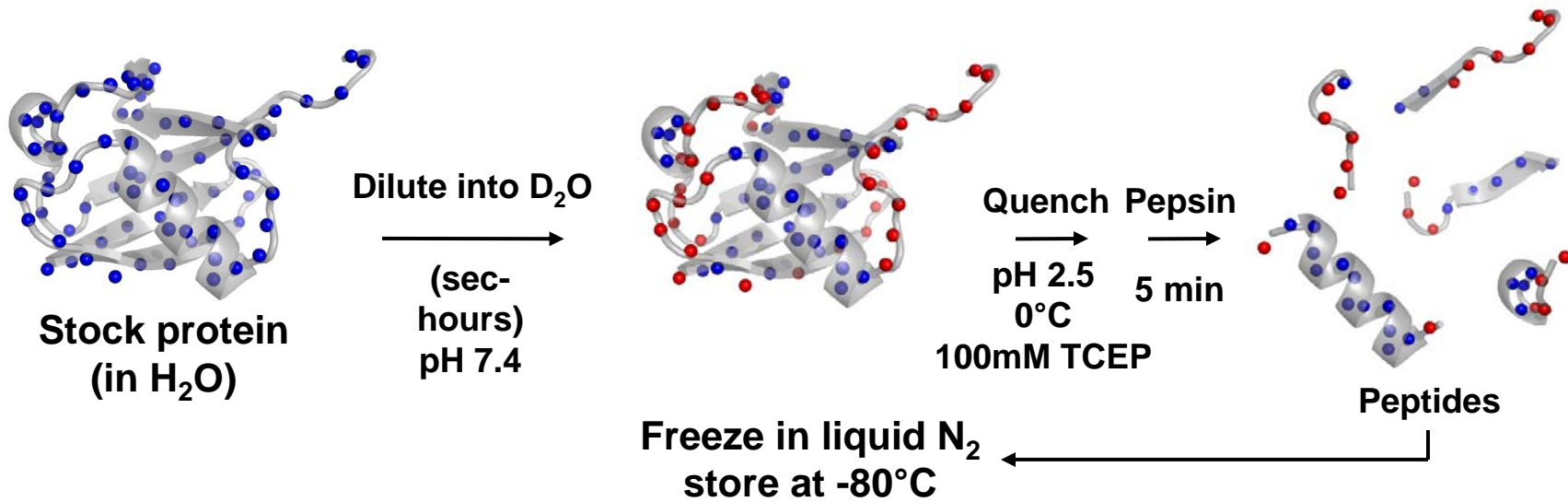


**Stock protein  
(in H<sub>2</sub>O)**

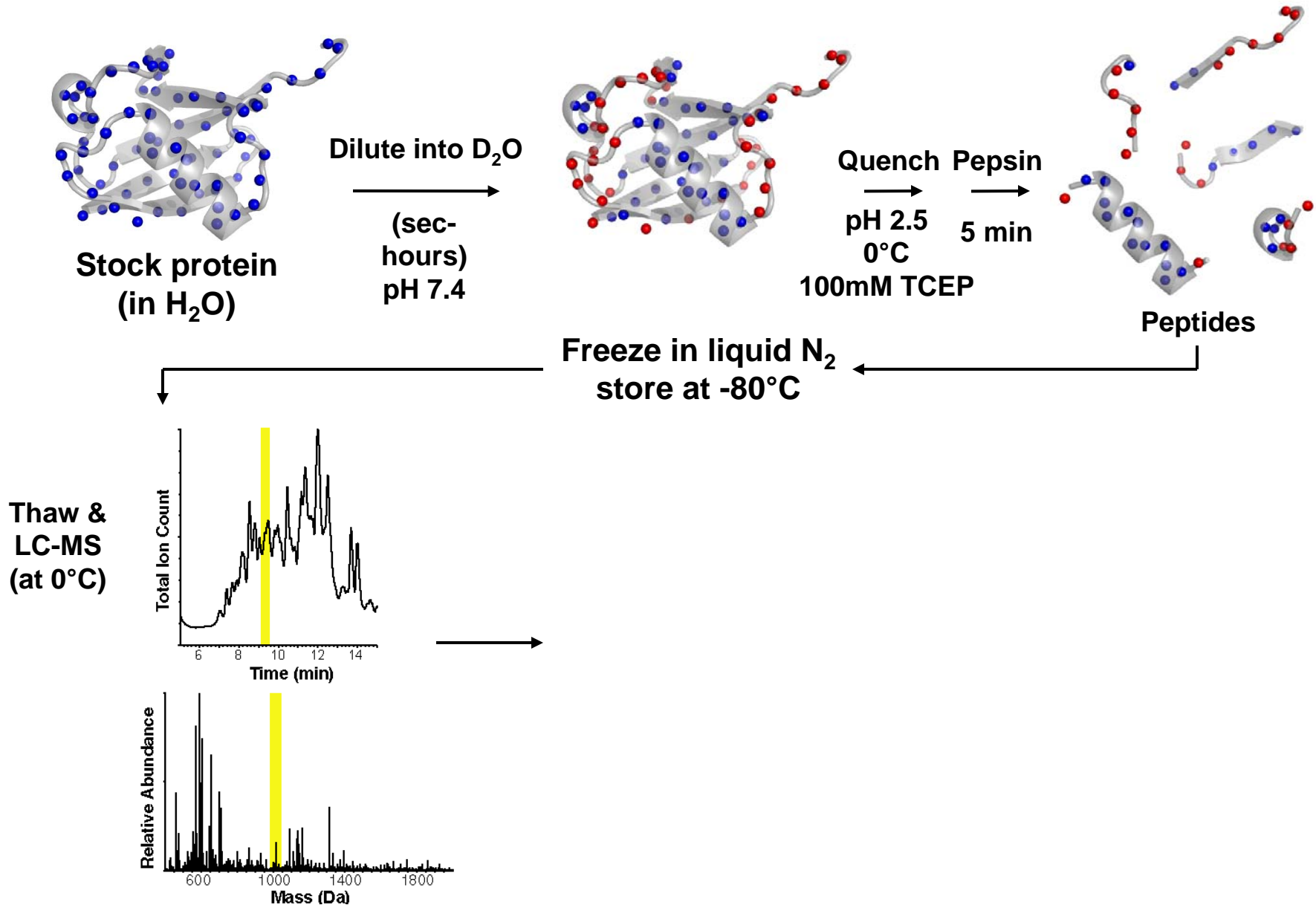
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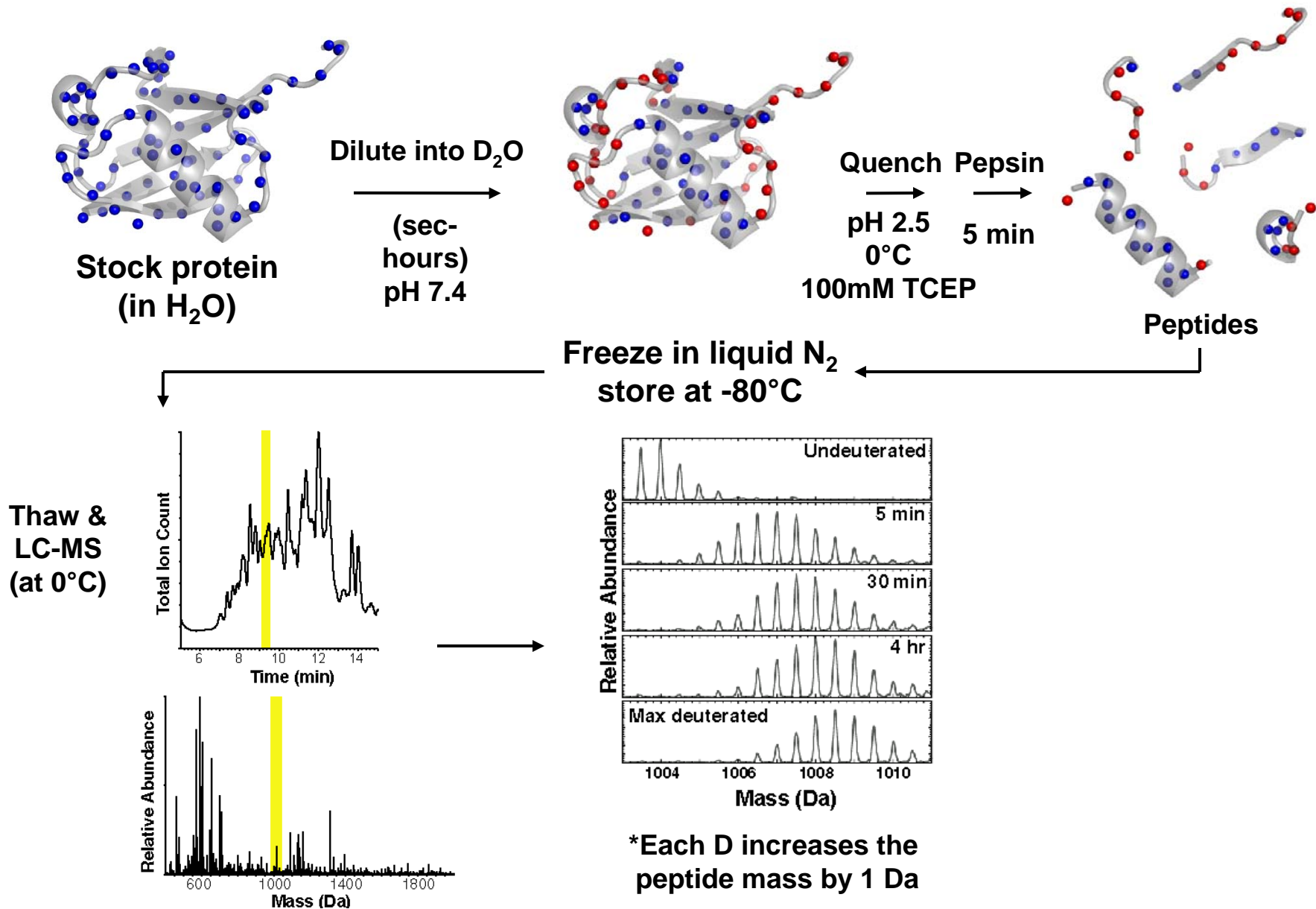


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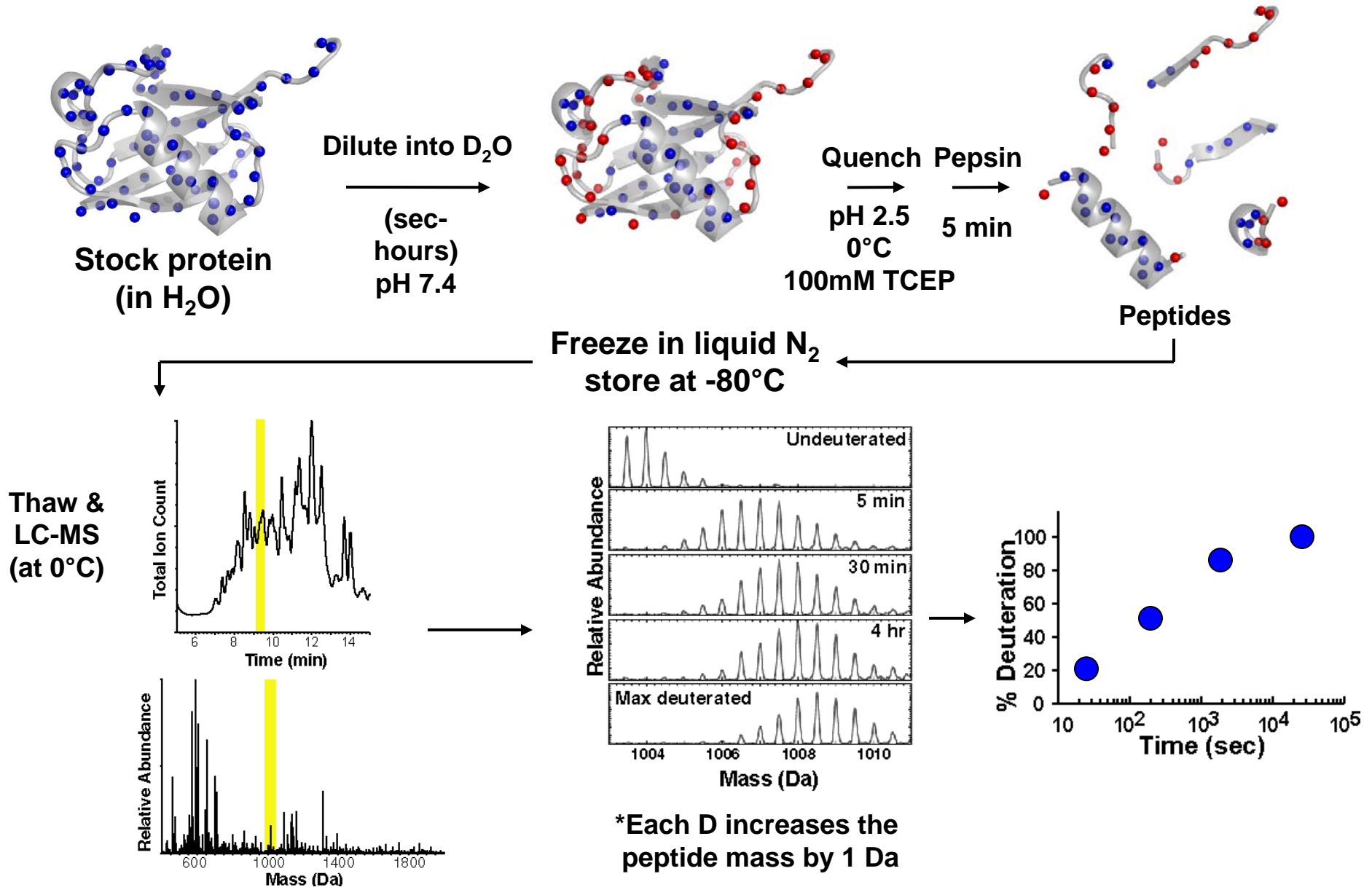




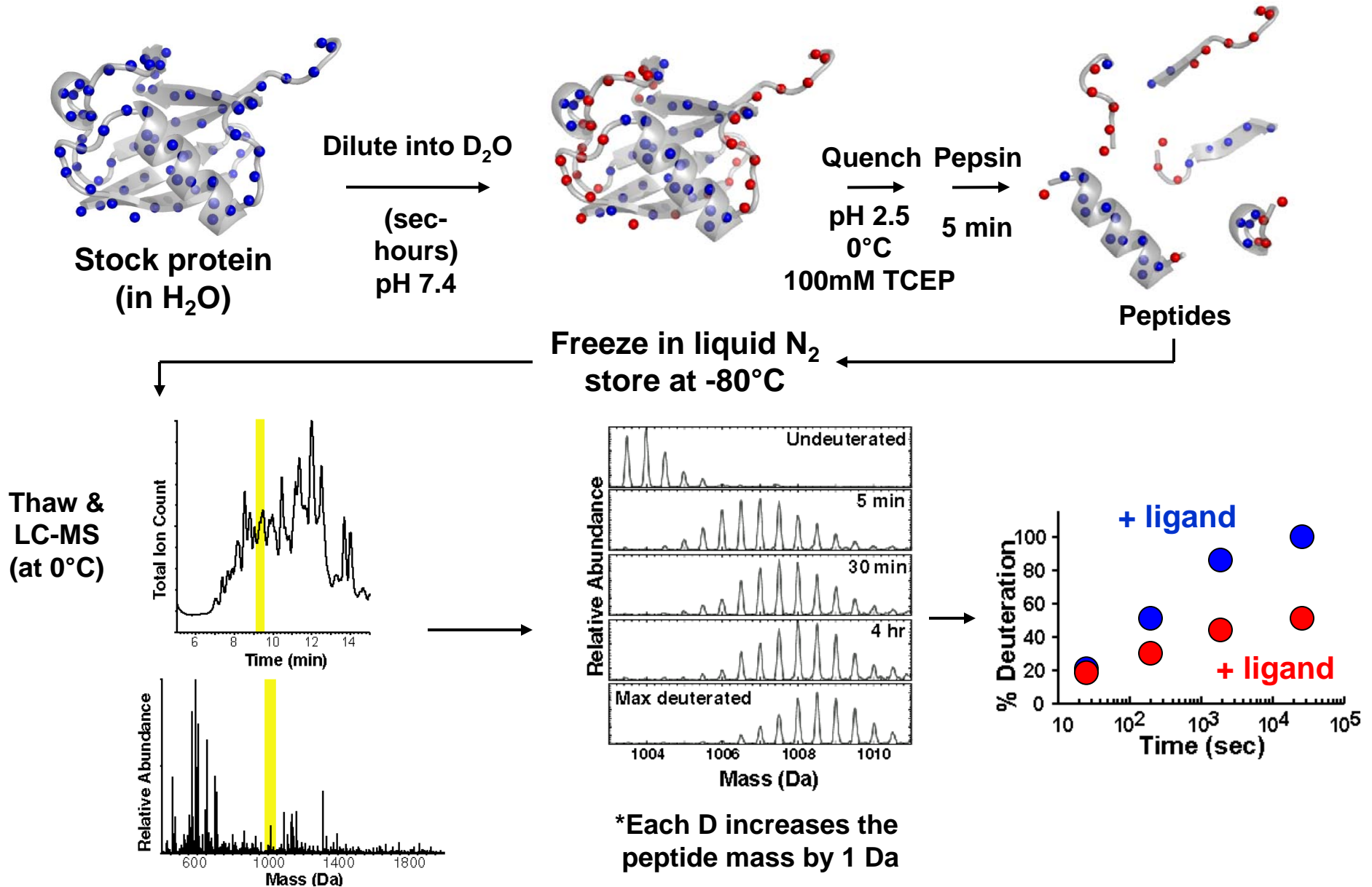
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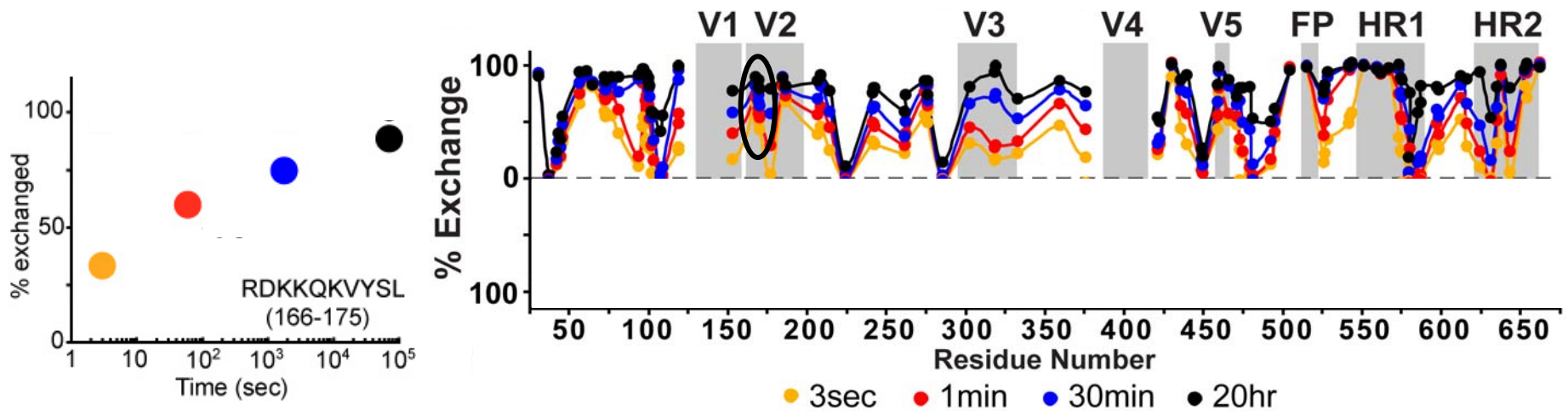
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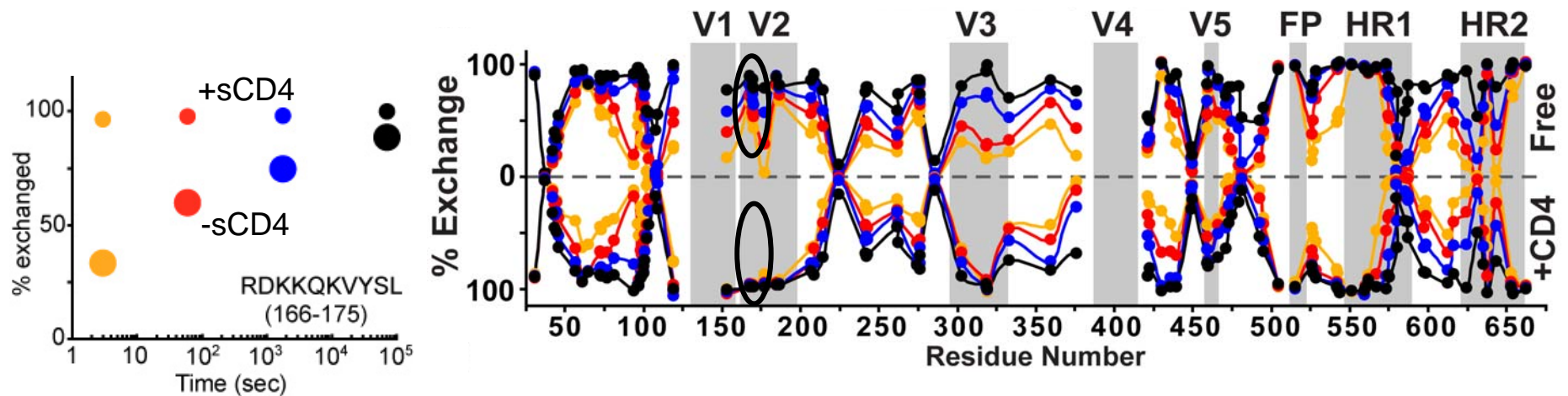


# Visualizing the H/DX data



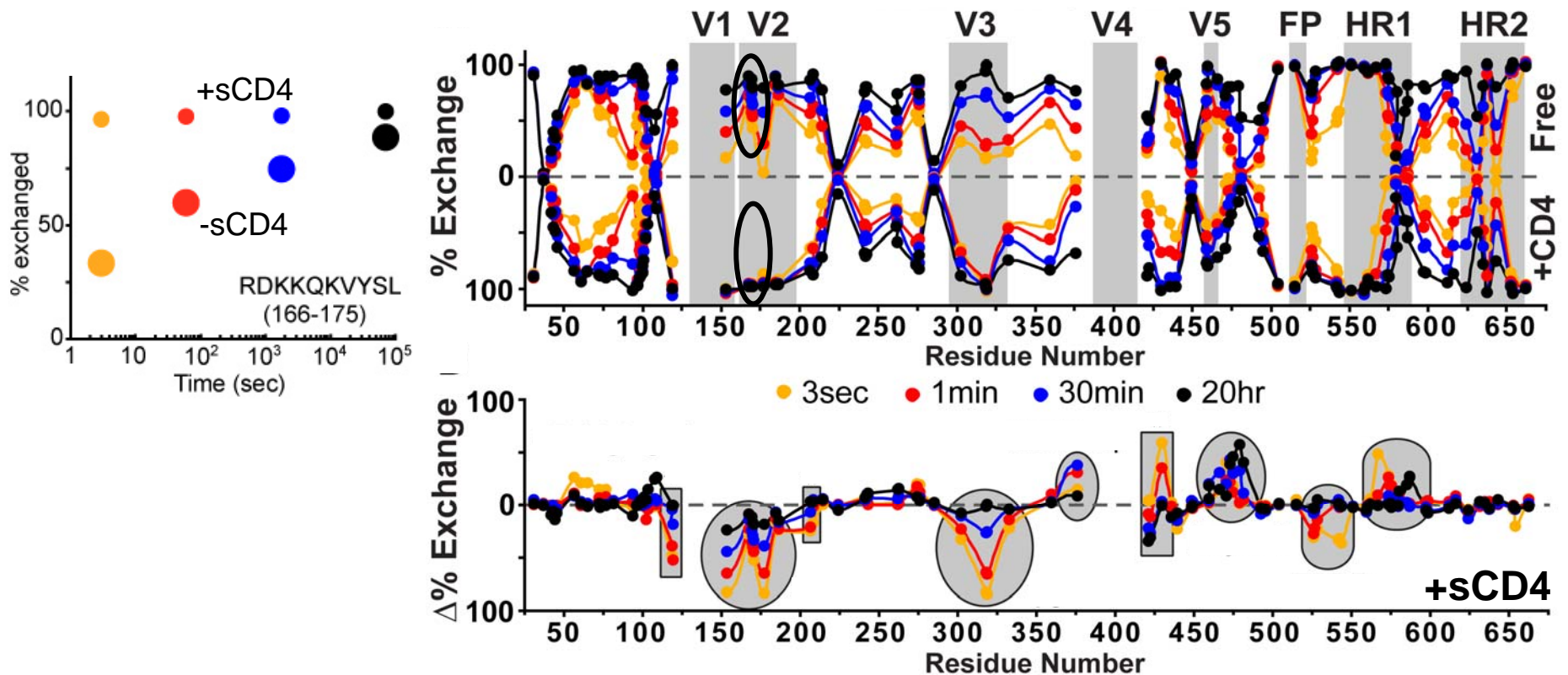
- Data for each peptide is plotted on the primary sequence

# Visualizing the H/DX data



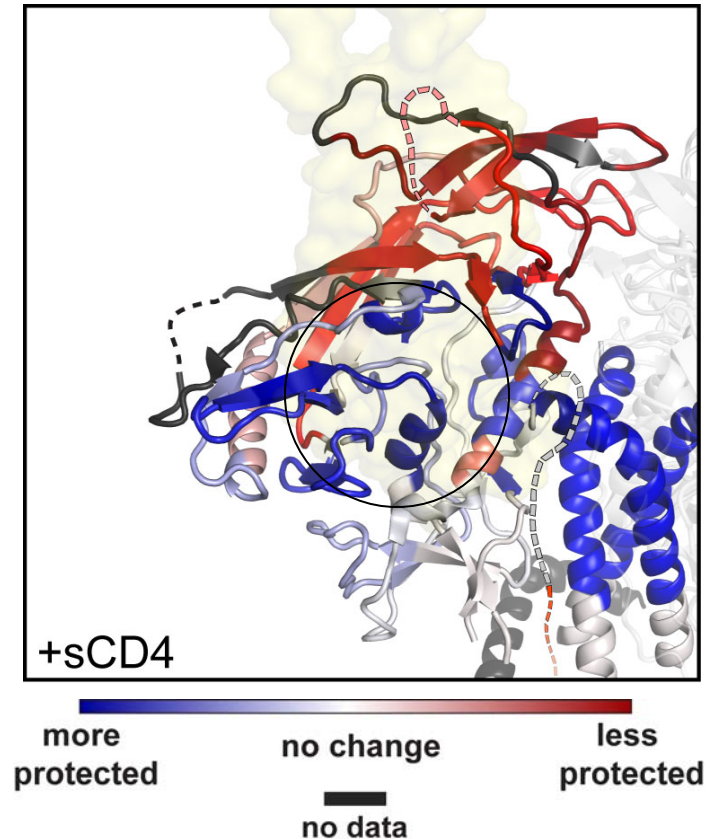
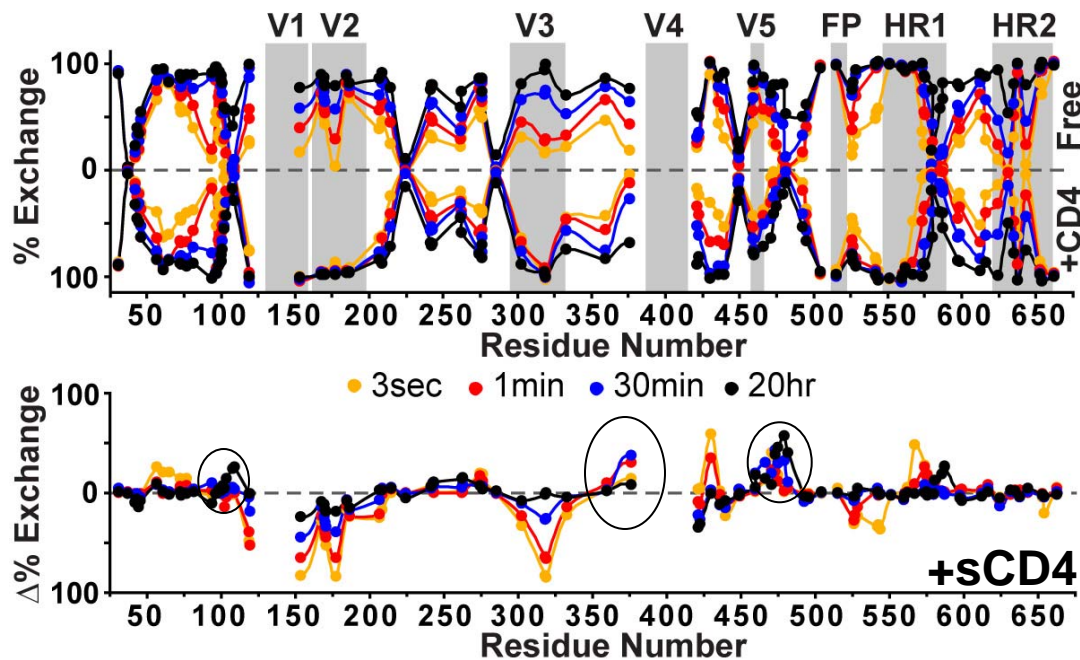
- Data for each peptide is plotted on the primary sequence
- Butterfly/mirror plots are useful for comparing HDX for two protein states (+/- ligand)

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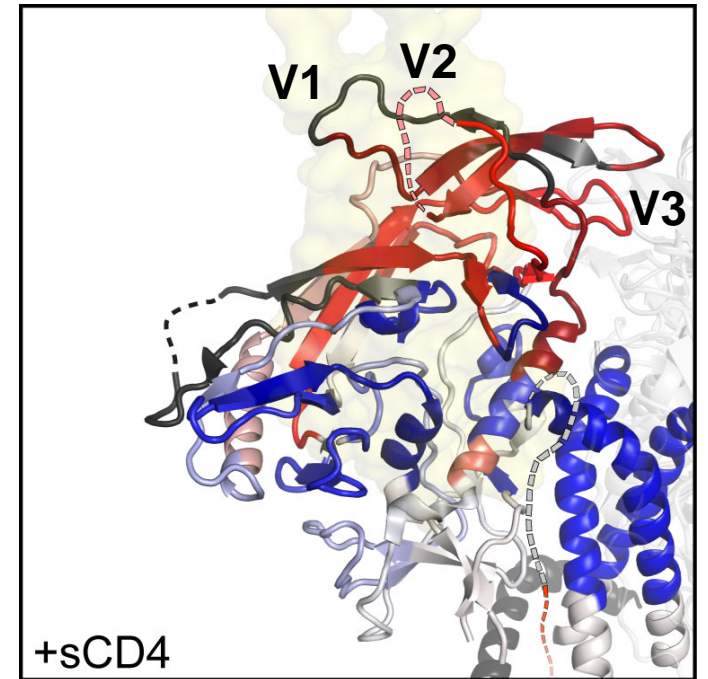
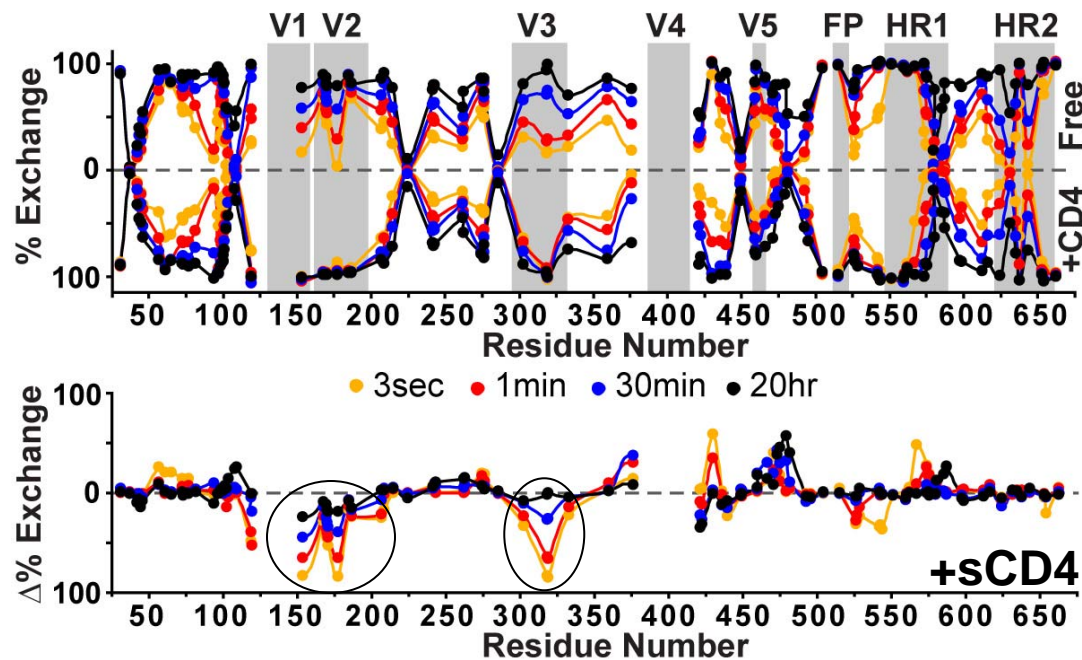
- Data for each peptide is plotted on the primary sequence
- Butterfly/mirror plots are useful for comparing HDX for two protein states (+/- ligand)
- Difference plots show raw difference at each time point

# CD4 binding induces elaborate changes



- CD4 binding surface becomes protected

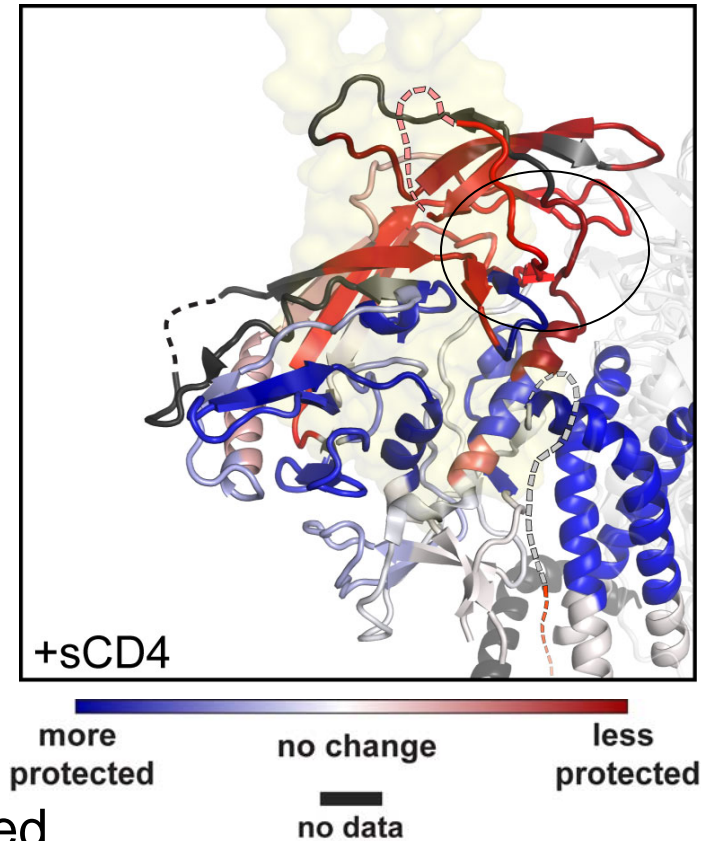
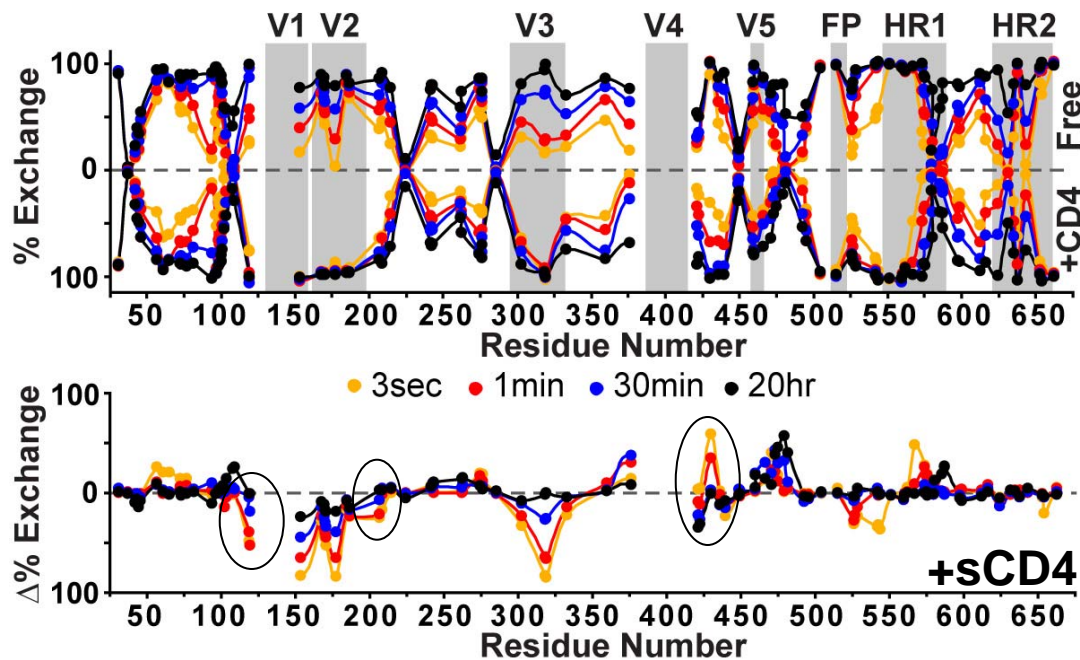
# CD4 binding induces elaborate changes



- CD4 binding surface becomes protected
- Variable loops V1/V2 & V3 become disordered

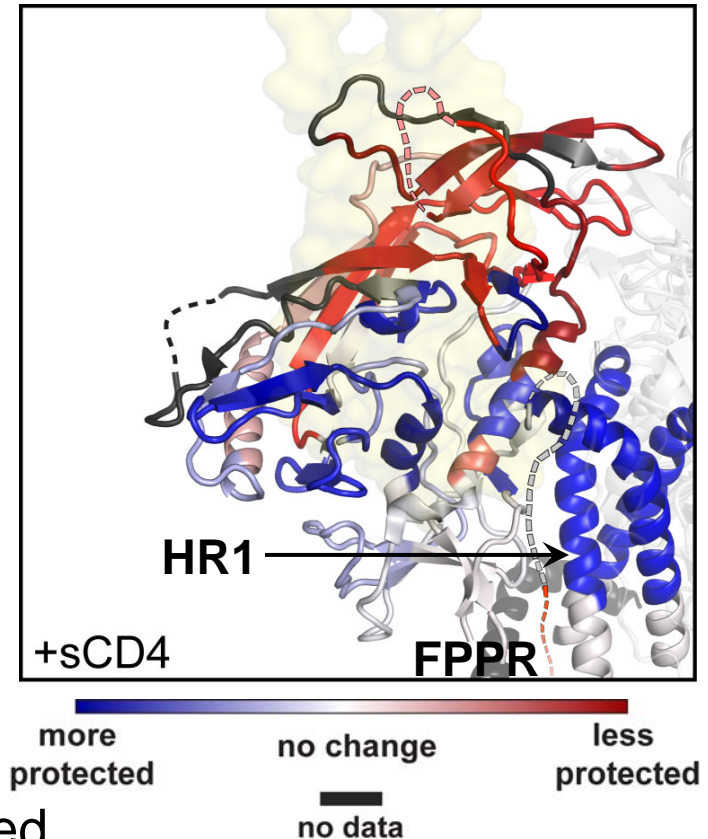
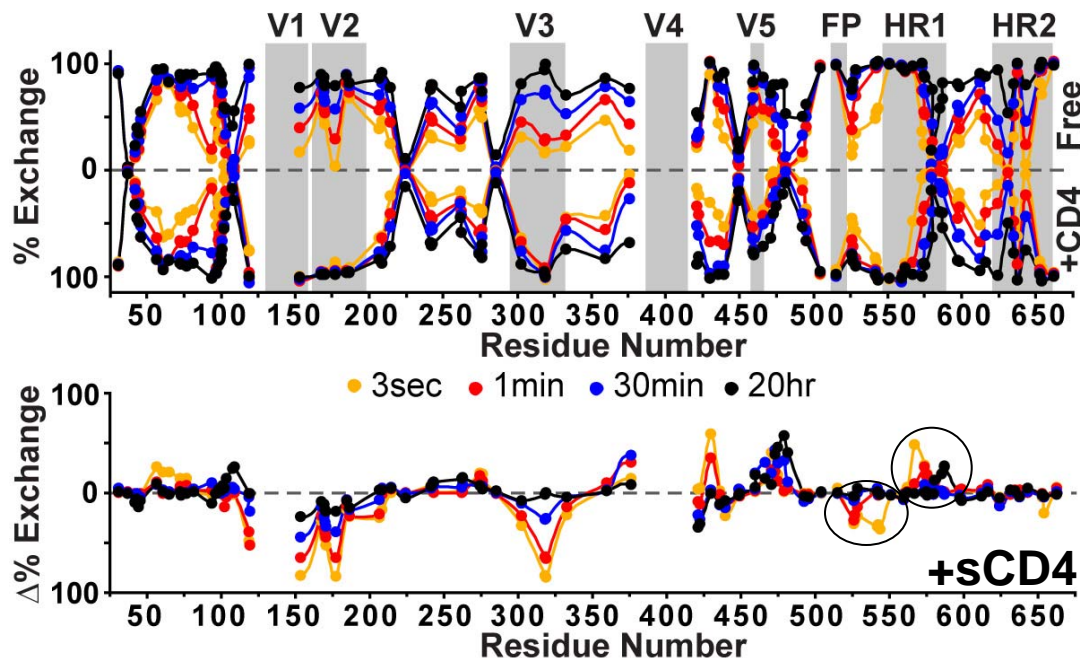


# CD4 binding induces elaborate changes



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- Variable loops V1/V2 & V3 become disordered
- Crown region of gp120 reorganized

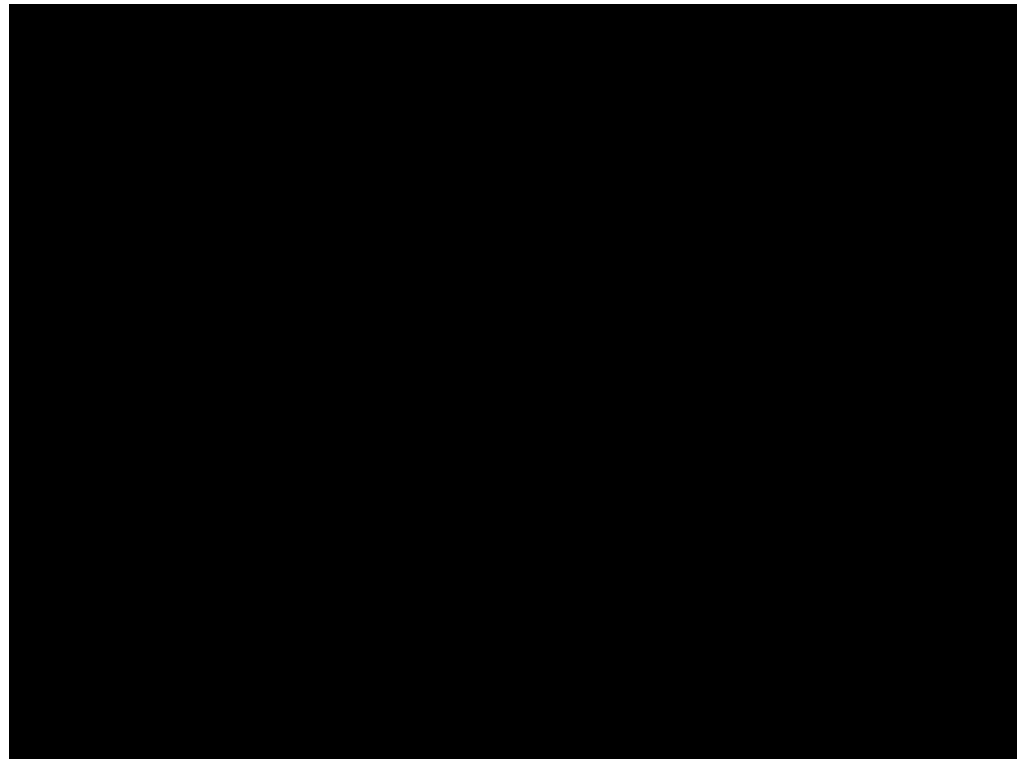
# CD4 binding induces elaborate changes



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- Crown region of gp120 reorganized
- In gp41: HR1 is more protected; while FPFR is less

# XF-MS at SSRL BL4-2

- Establish dosage
  - (Alexa 488 dye)
- Irradiate in quartz capillary at various flow rates for different exposure times
  - ~8 to 200 msec
  - Eject sample into collection tubes with methionine
    - (20mM final)
  - Run wash cycle
- Compare unliganded and CD4-bound Env trimers



# XF-MS data processing

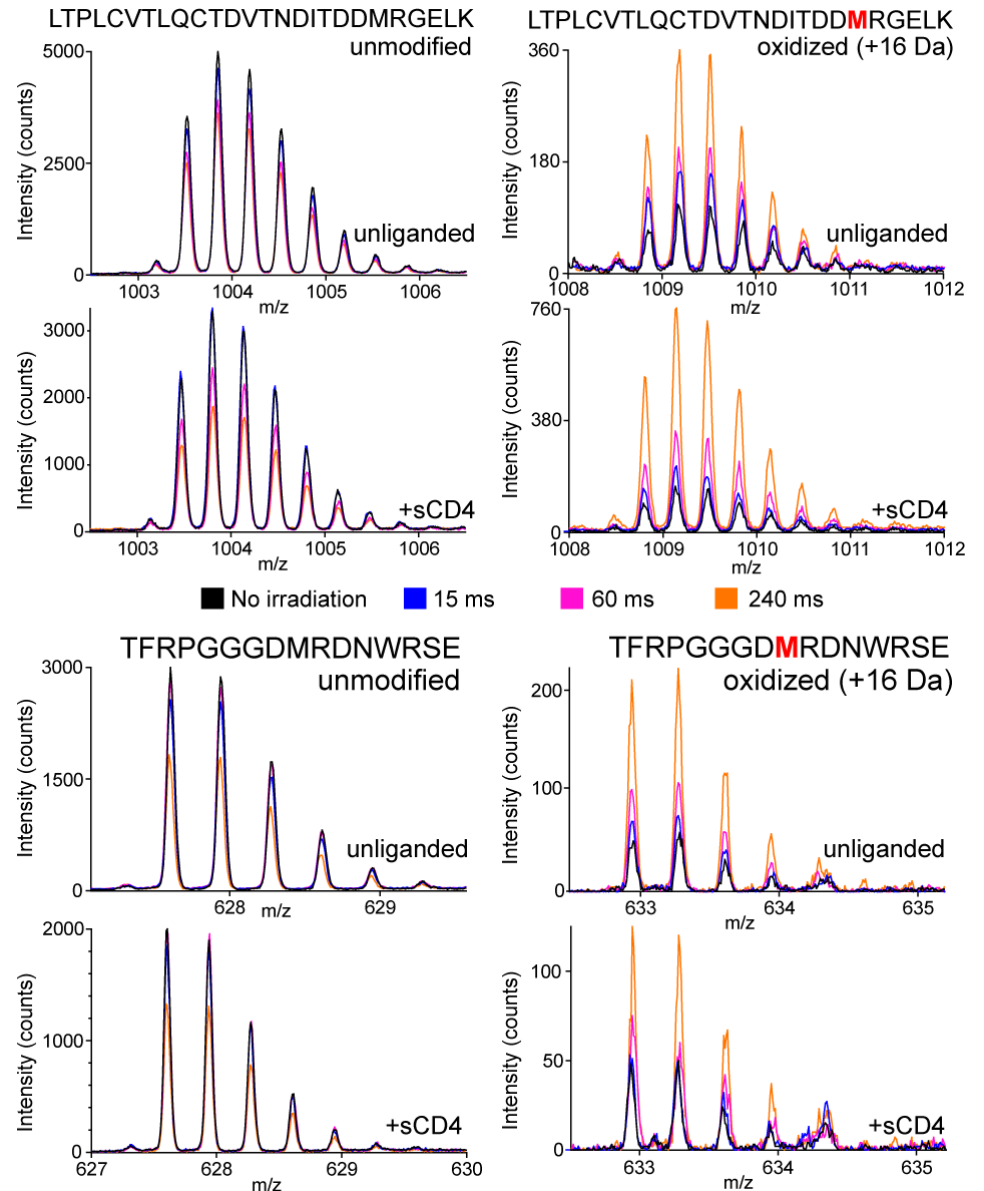
## Sample processing:

- Denaturation
  - GndHCl & DTT
- Cysteine alkylation
  - IAA
- Deglycosylation (PNGaseF)
  - Dilute sample to 0.5M GndHCl
- Digestion
  - Split sample: LysC & GluC

## LC-MS:

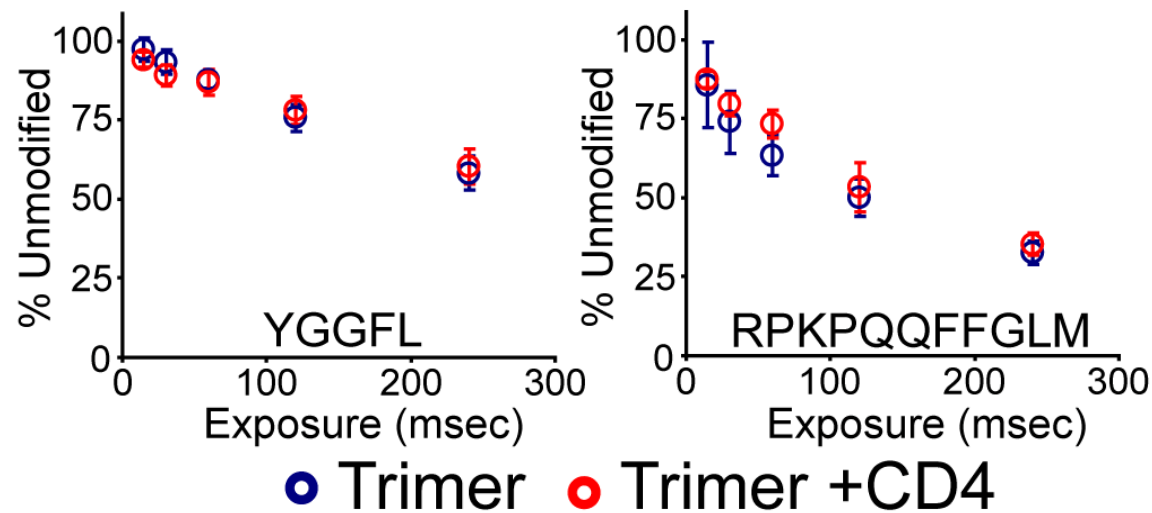
- 30 minute gradient over C18 column
- ESI-QTOF (Waters Synapt)
- Integrate and measure the intensity of each unmodified and oxidized peptide.
- Calculate and plot % modified:

$$\frac{\text{Intensity modified}}{\text{Intensity of unmodified + modified (all)}}$$

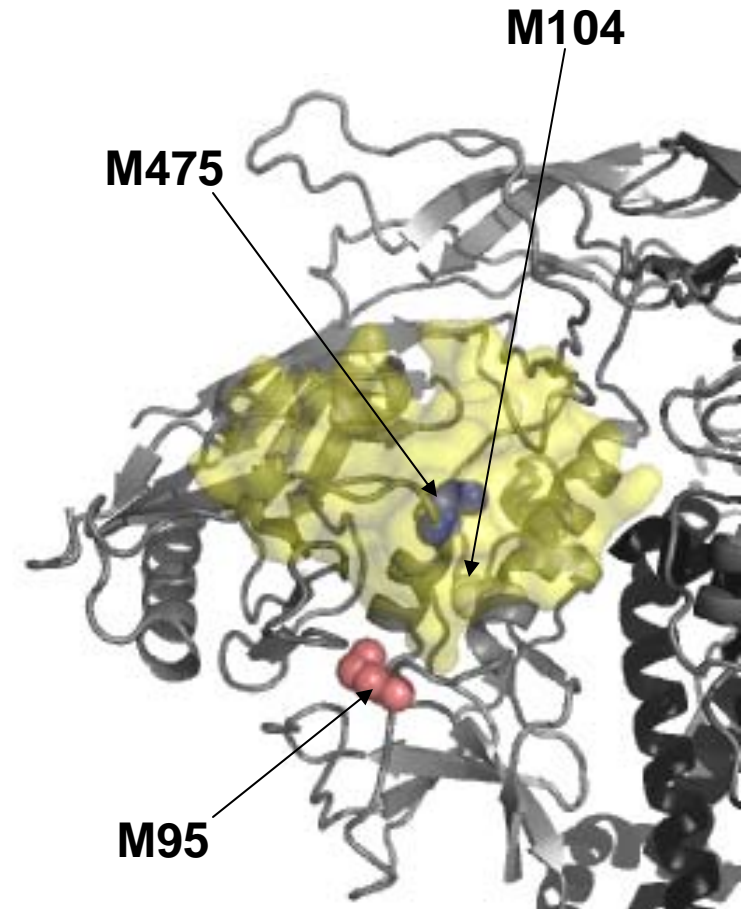
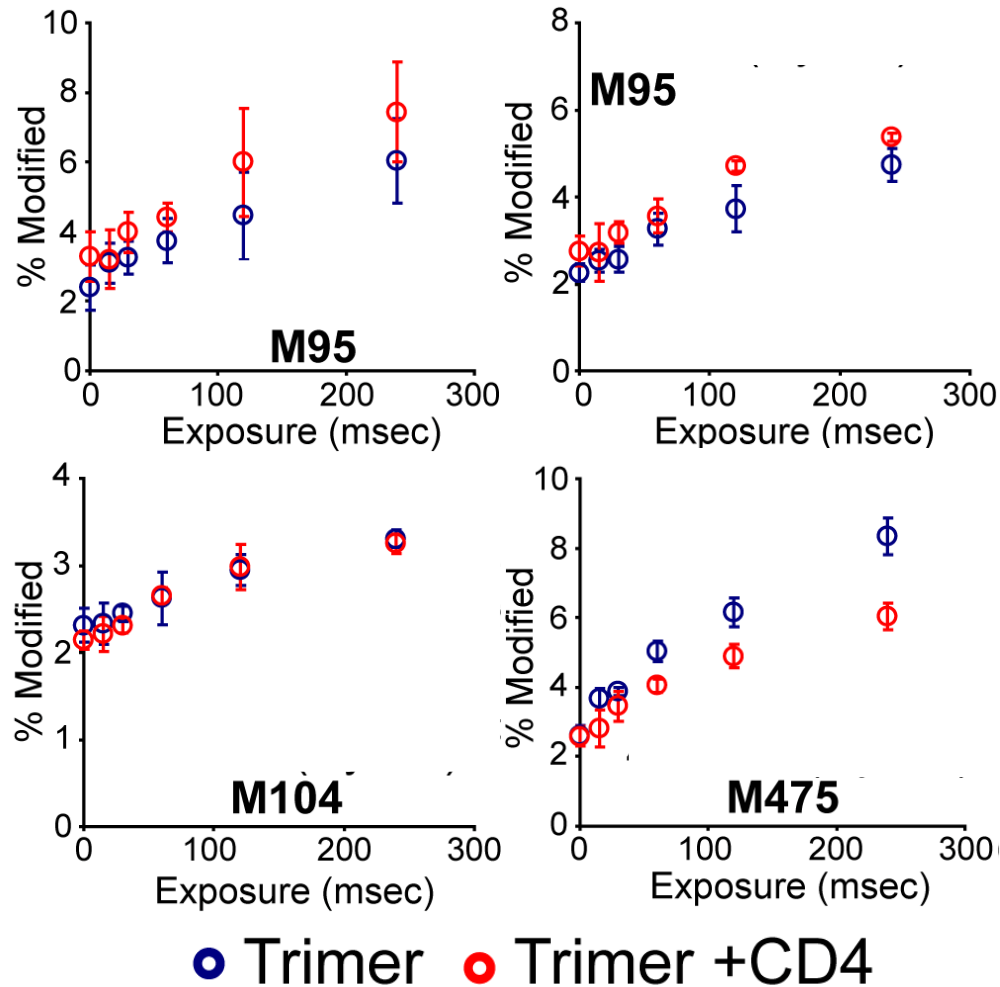


# Internal dosimeters for XF-MS

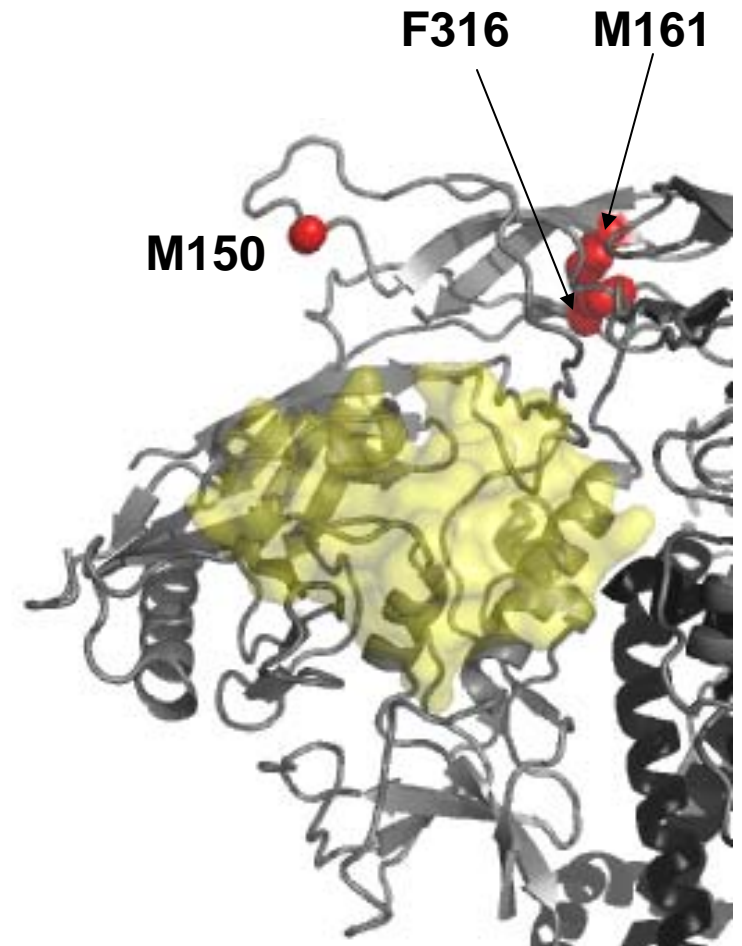
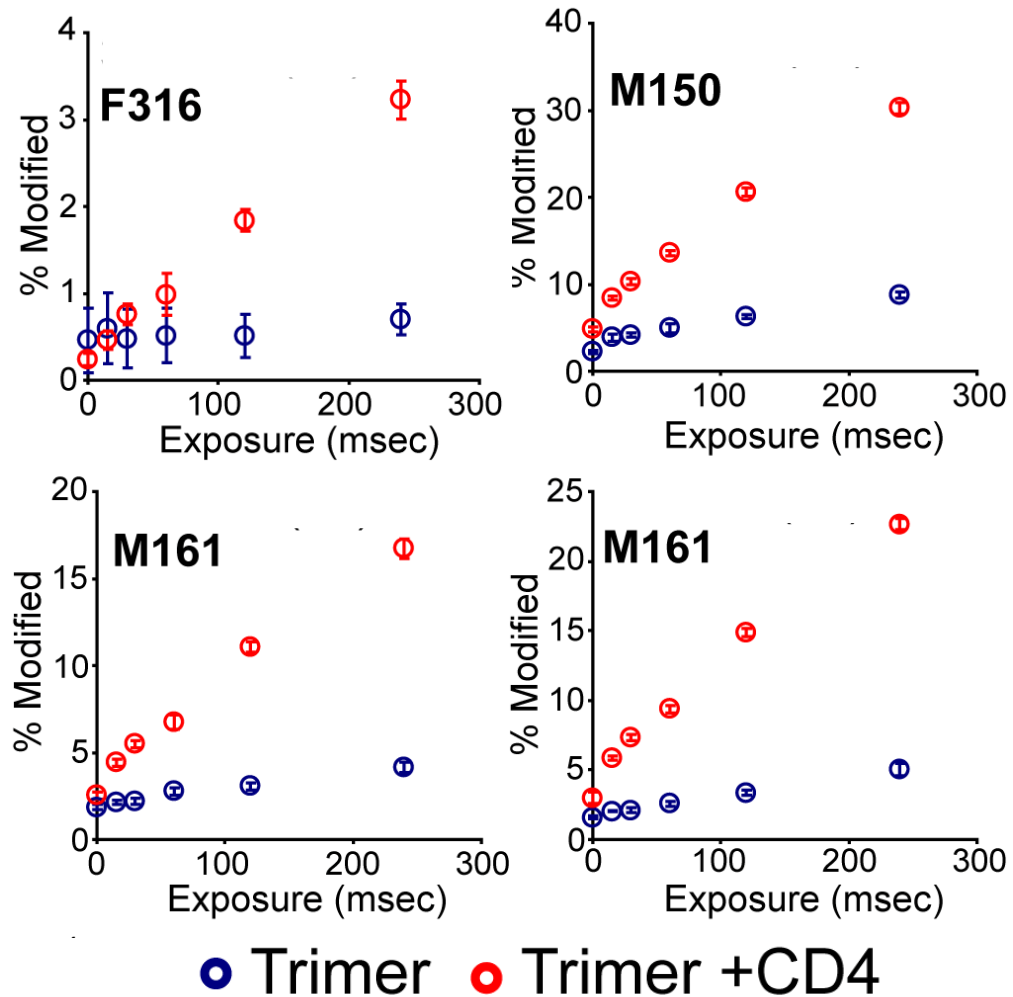
- Protein concentrations and buffers kept consistent, experiments performed side by side (in duplicate).
- Internal standard to ensure datasets received identical dosage:
  - Leucine enkephalin & Substance P
  - Look at the decrease in percent unmodified



# Mixed changes near CD4 binding surface on gp120

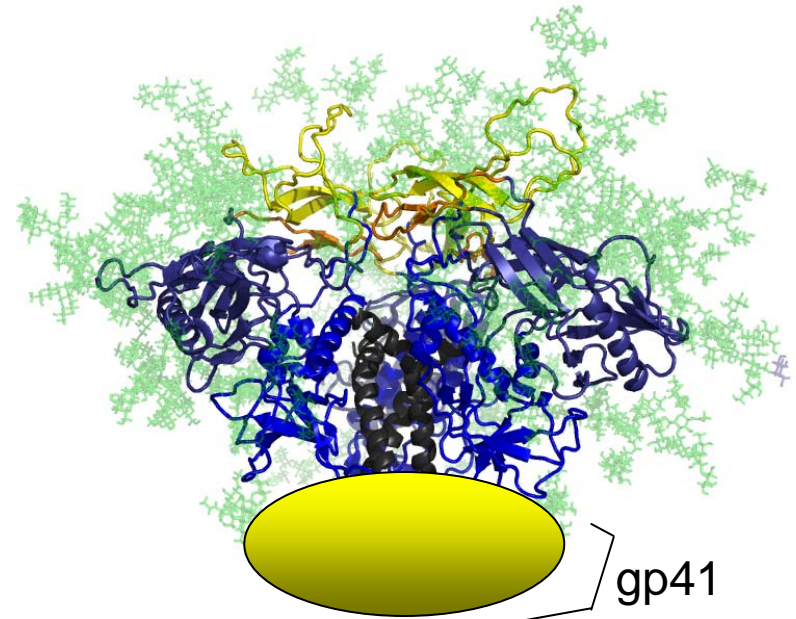
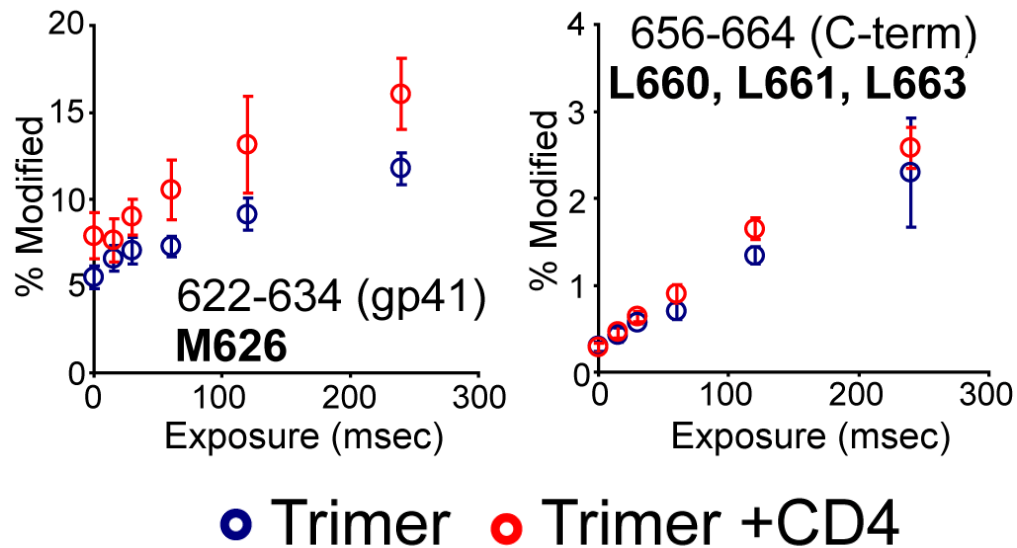


# Increased accessibility at V1/V2 and V3



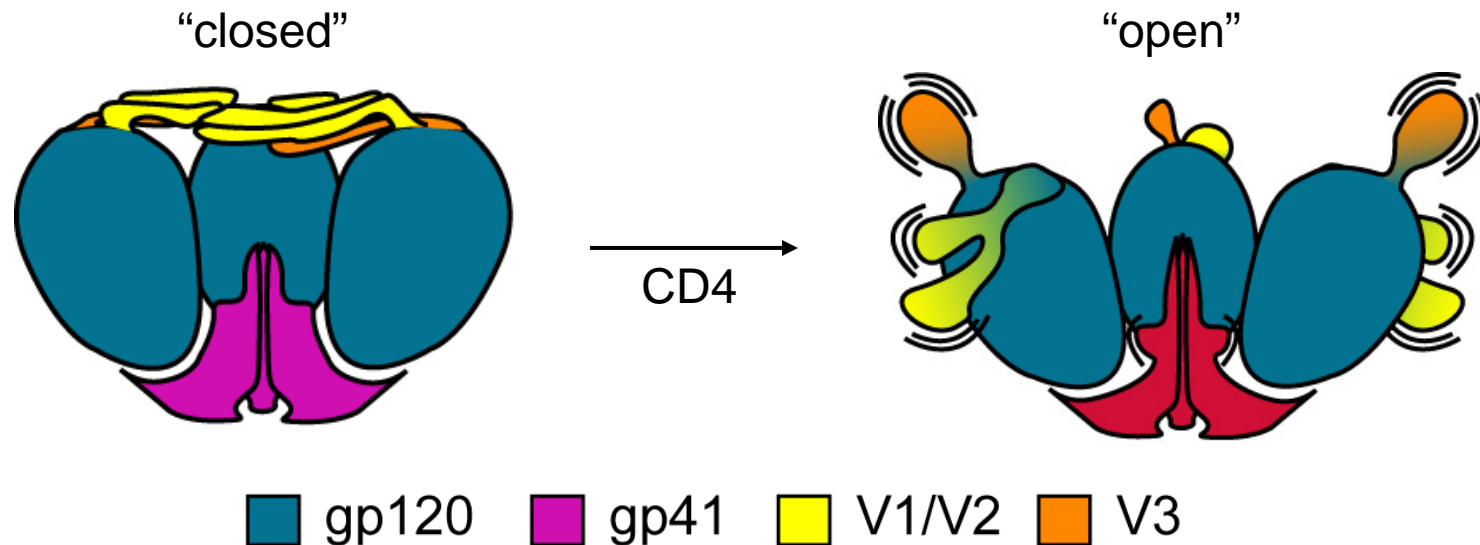
# Additional changes within gp41

- Additional changes are seen at M626 and the C-terminus of gp41
  - (not well resolved in the current crystal structures)





# H/DX & XF-MS reveal the extensive changes upon CD4 binding



- Opening of V1/V2 and V3 loops
- Reorganization of the gp120 subunits
- Changes to gp41 may act to “prime” gp41 for subsequent activation (by co-receptor)

# Acknowledgements

## University of Washington

- Kelly K. Lee (PI)
- Natalie K. Garcia
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- John P. Moore

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- Ian A. Wilson

## University of Amsterdam

- Rogier Sanders

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- Tsutomu Matsui

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- F32 award
  - F32-GM097805

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