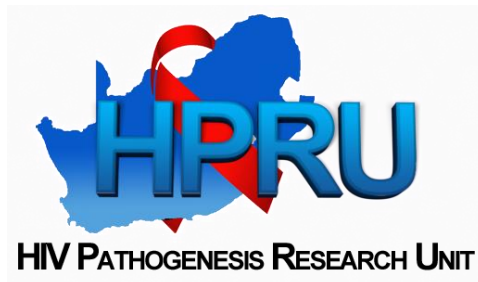

The use of
Small-Angle Neutron Scattering (SANS)
to understand dynamic changes in CD4
structure implicated in HIV-1 infection

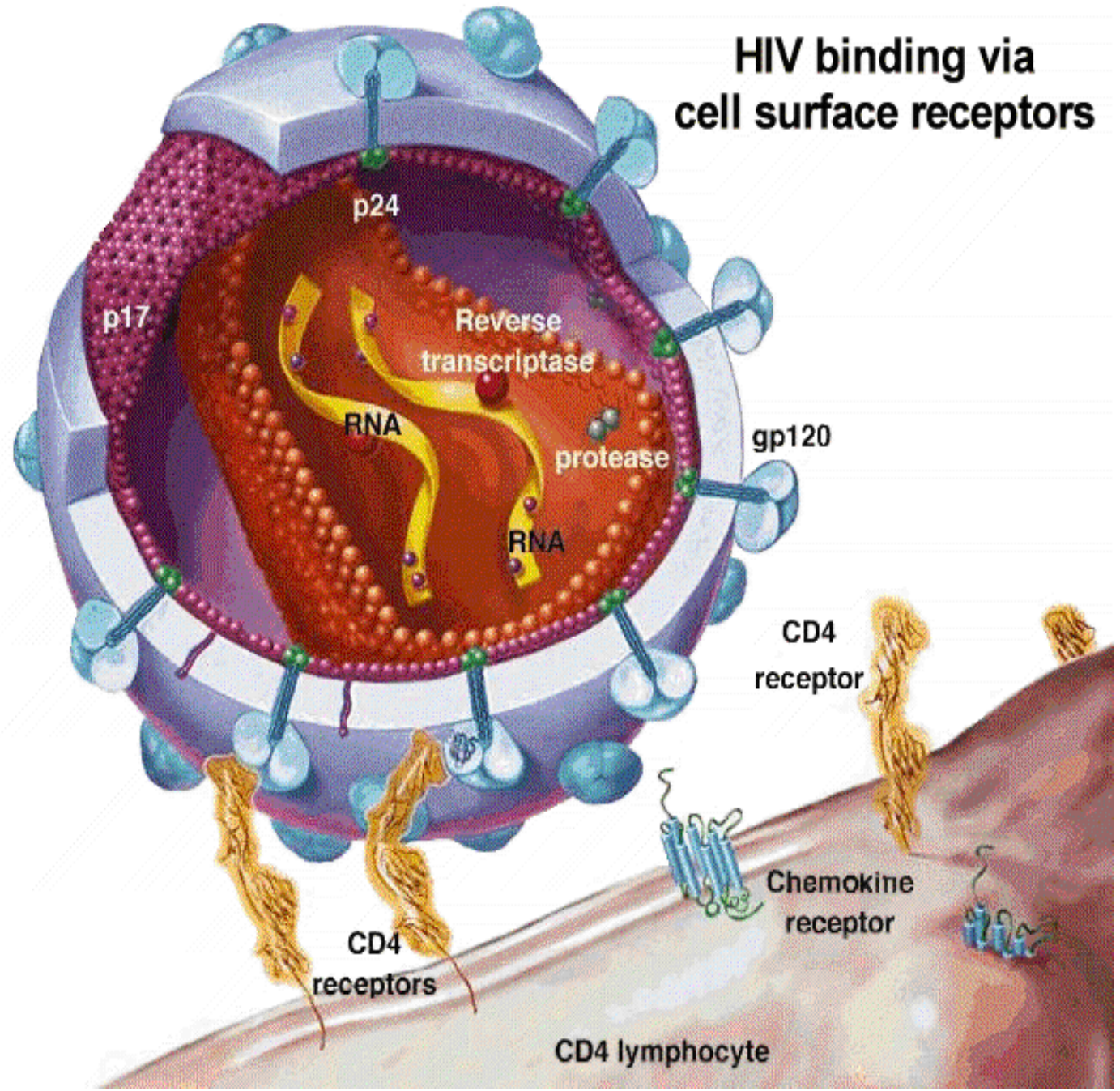
Gavin Owen, PhD

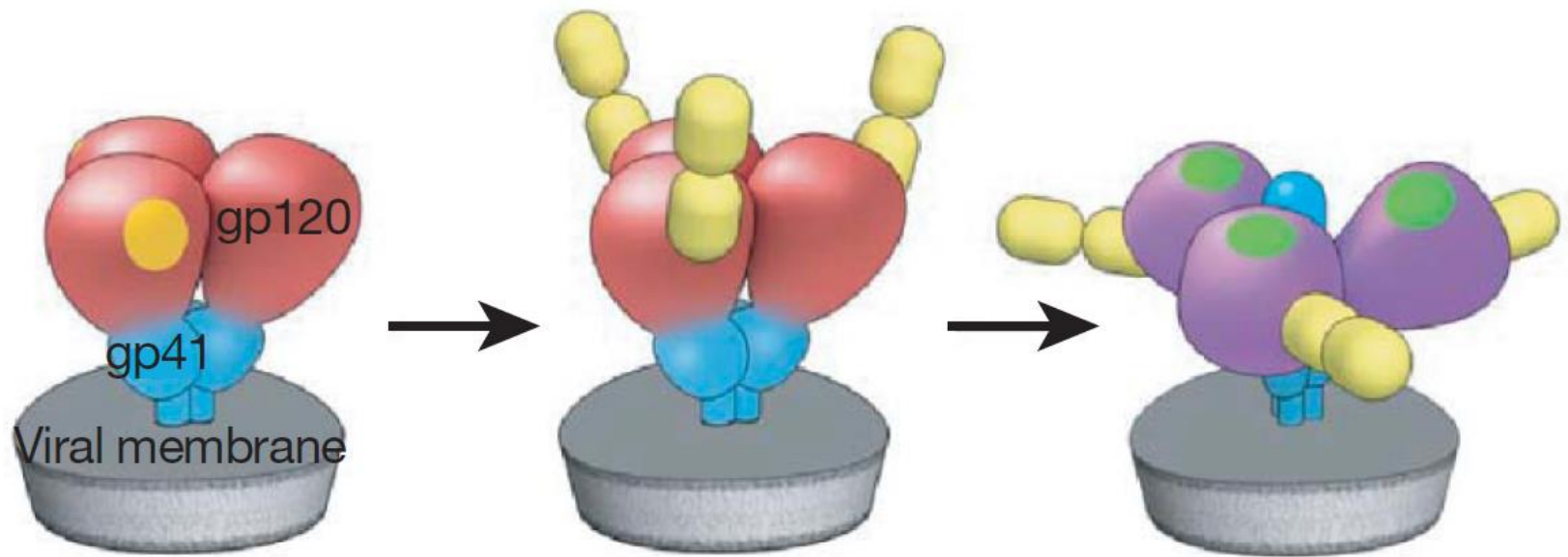
HPRU: N. Cerutti, M. Killick, M. Papathanasopoulos

ESRF-ILL: J. Channel, T. Forsyth, M. Haertlein, E. Mitchell

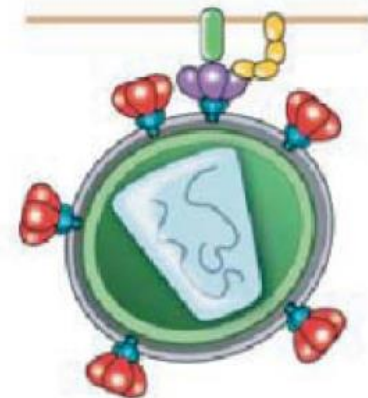
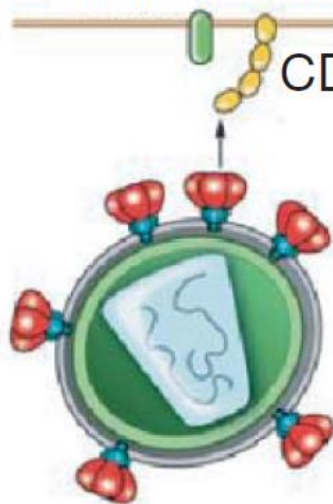


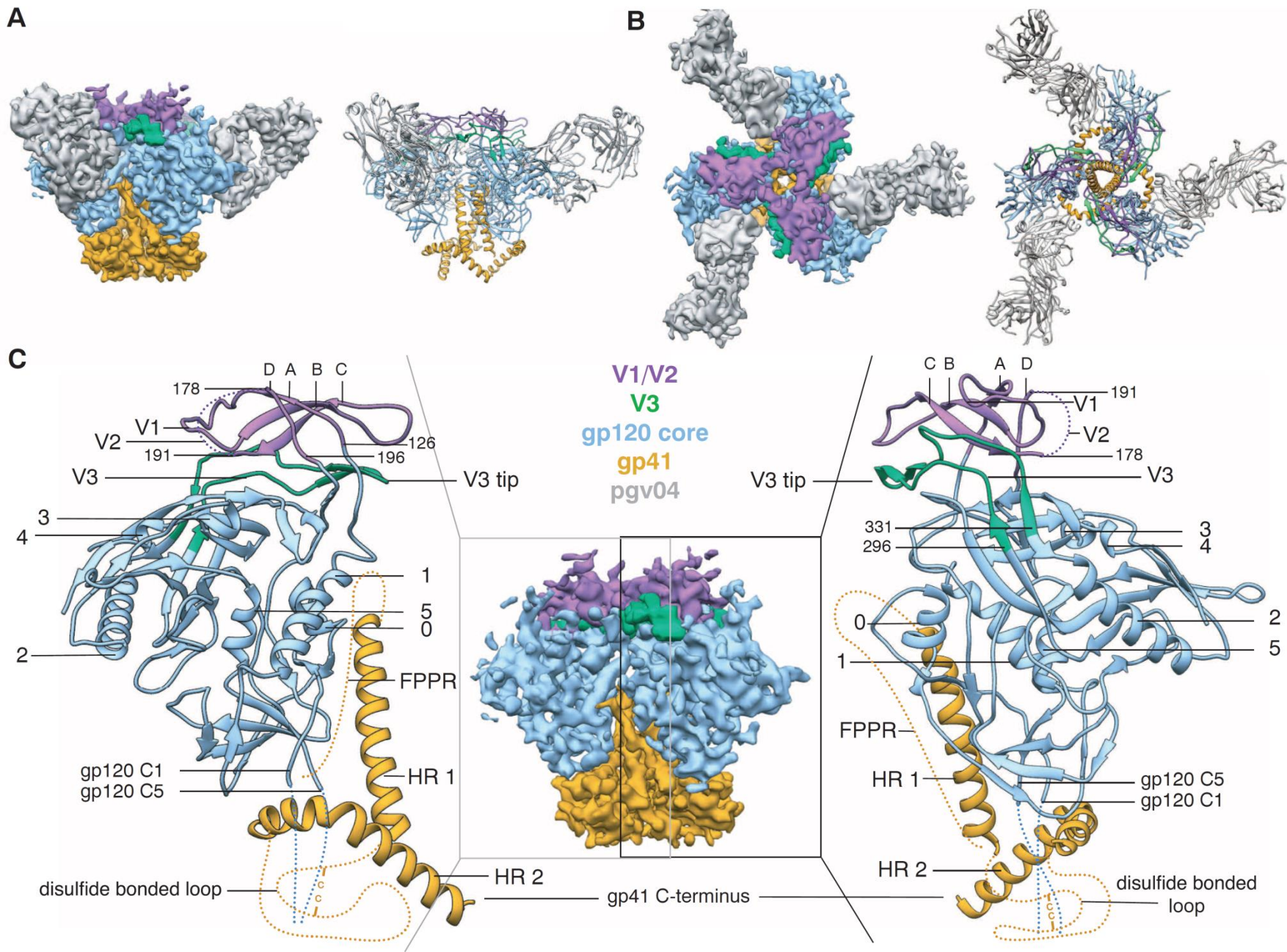
HIV binding via cell surface receptors

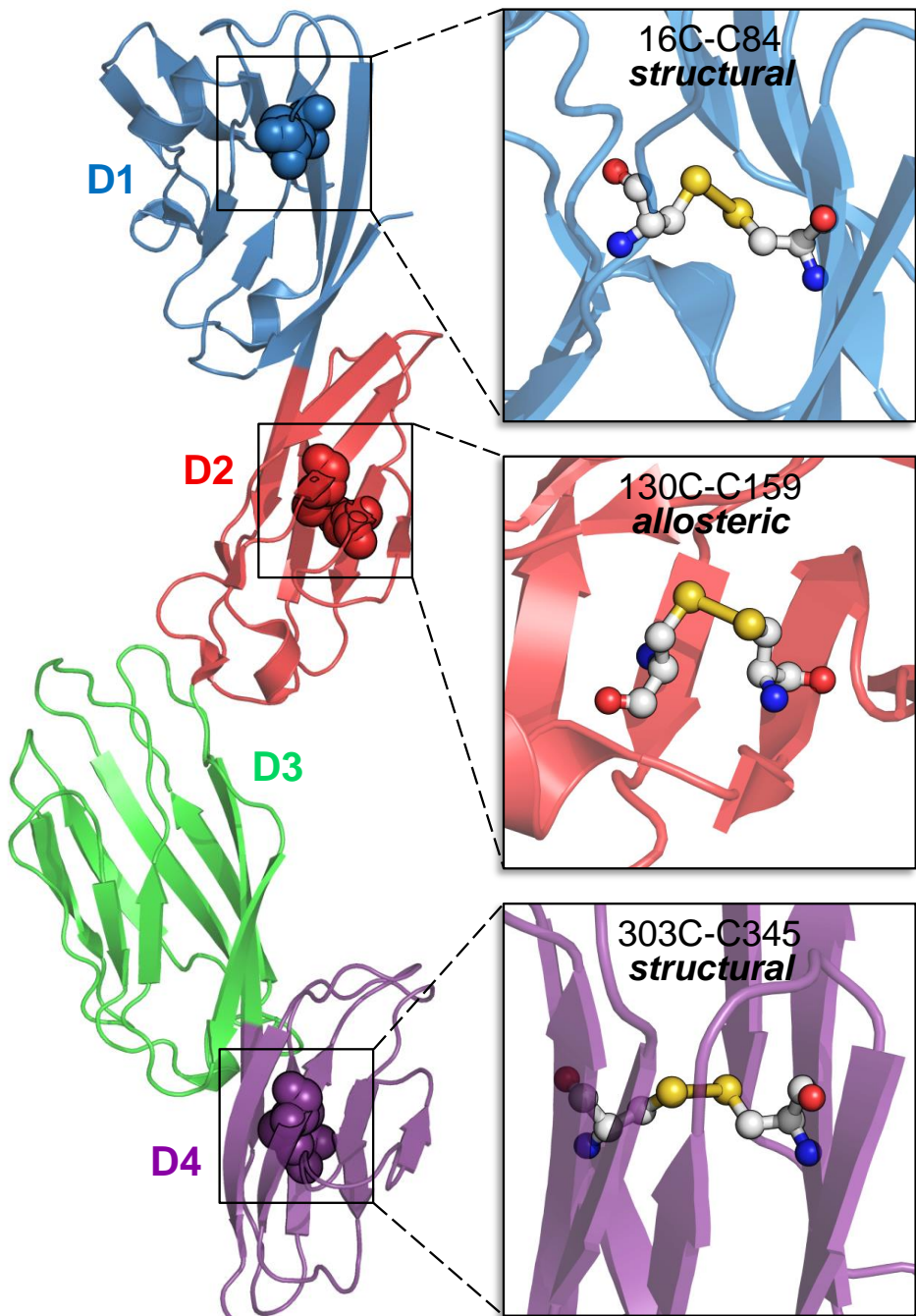




Chemokine receptor

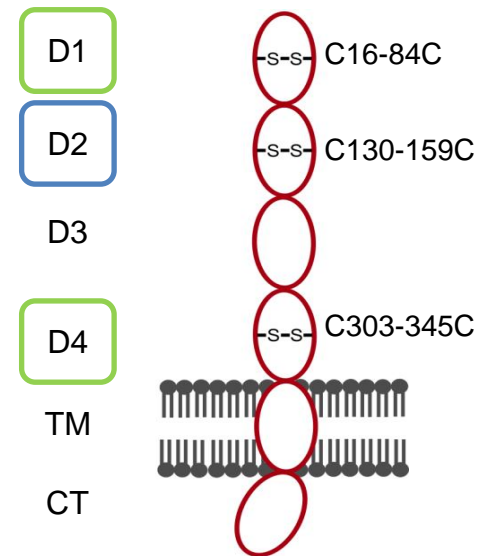




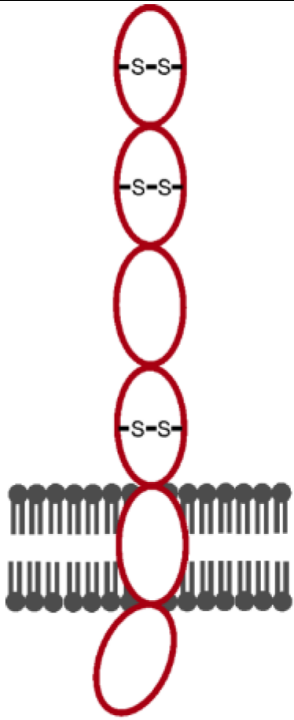


CD4

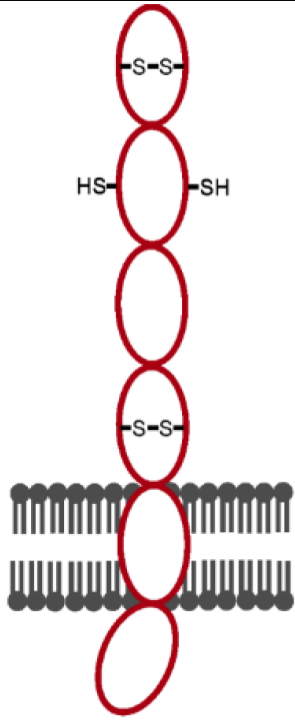
- Physiological function = adaptive immune system
- Primary receptor for HIV-1 surface antigen env
 - **binds gp120**
- CD4 exists in different redox isoforms
 - **different functions**



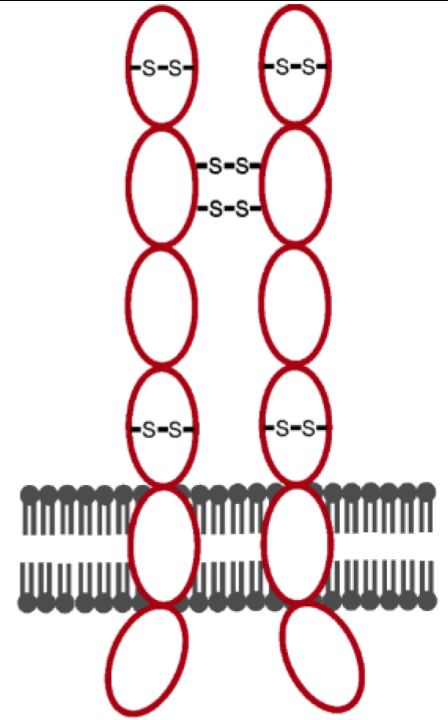
Resting T-cell	Binds gp120	Activated T-cell
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Monomer
(Oxidised)

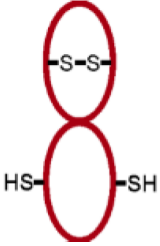


Monomer
(Reduced)

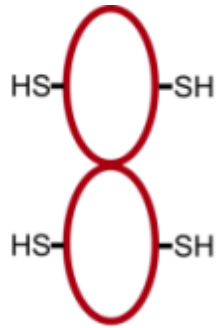


Domain-swapped Dimer
(Oxidised)

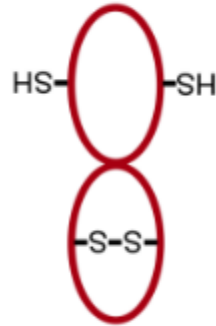
Binds gp120



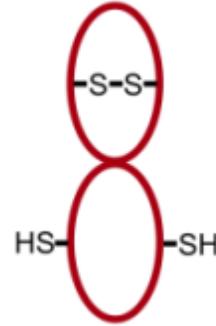
Reduced WT



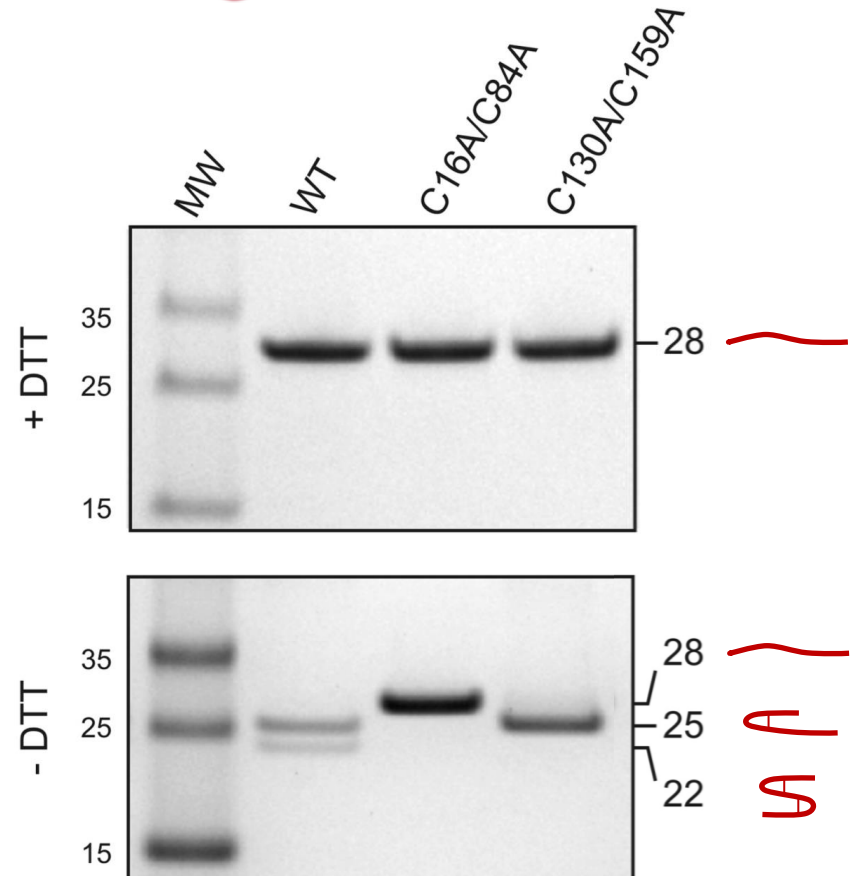
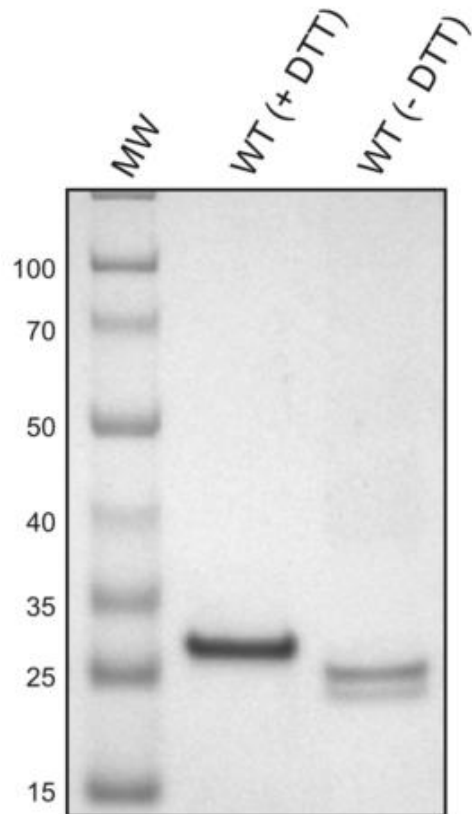
**Partially reduced WT/
C16A-C84A**



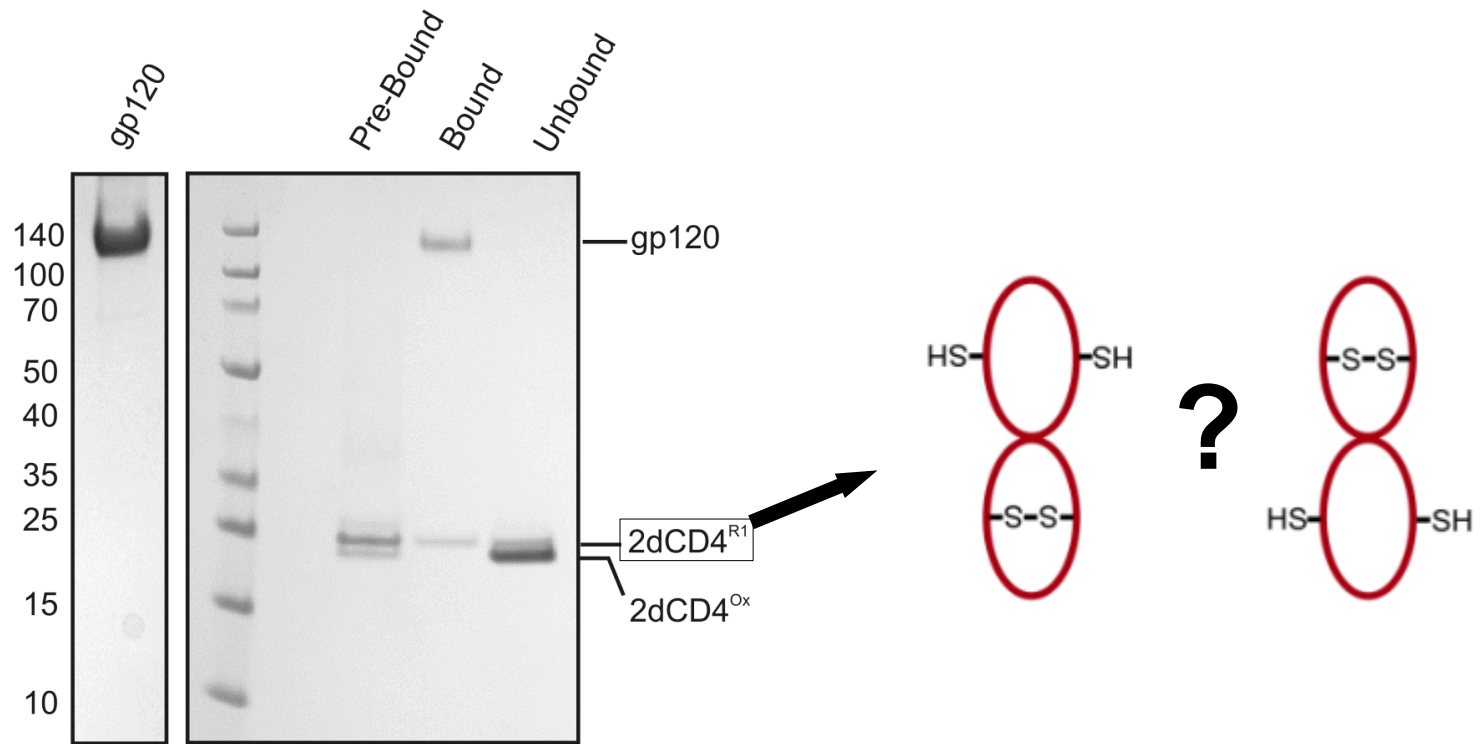
**Partially Reduced WT/
C130A-C159A**



Oxidised WT



Binding to Env?



Human CD4 Metastability Is a Function of the Allosteric Disulfide Bond in Domain 2

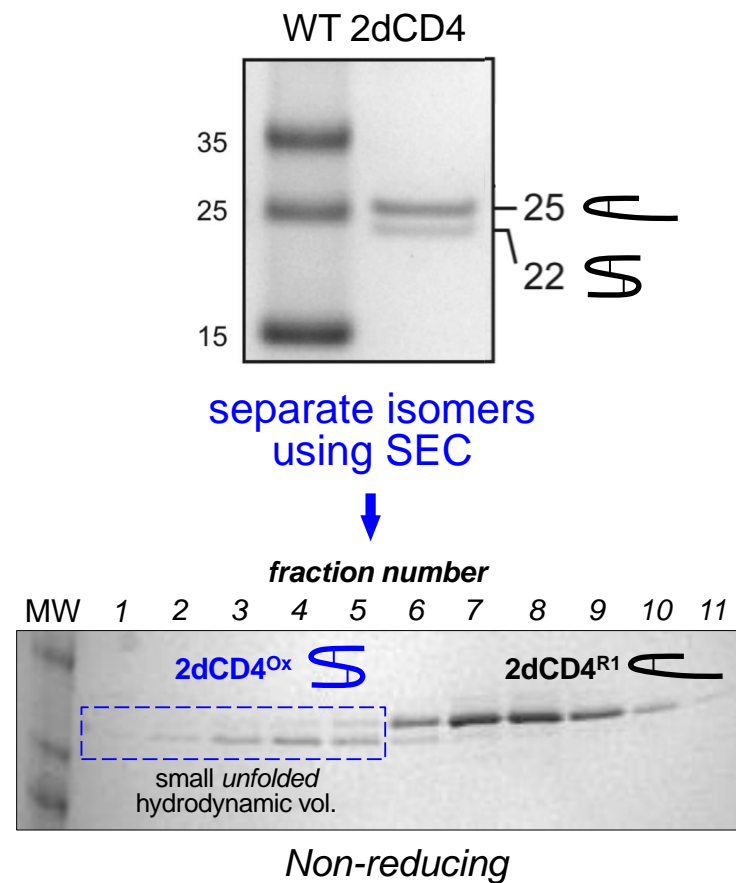
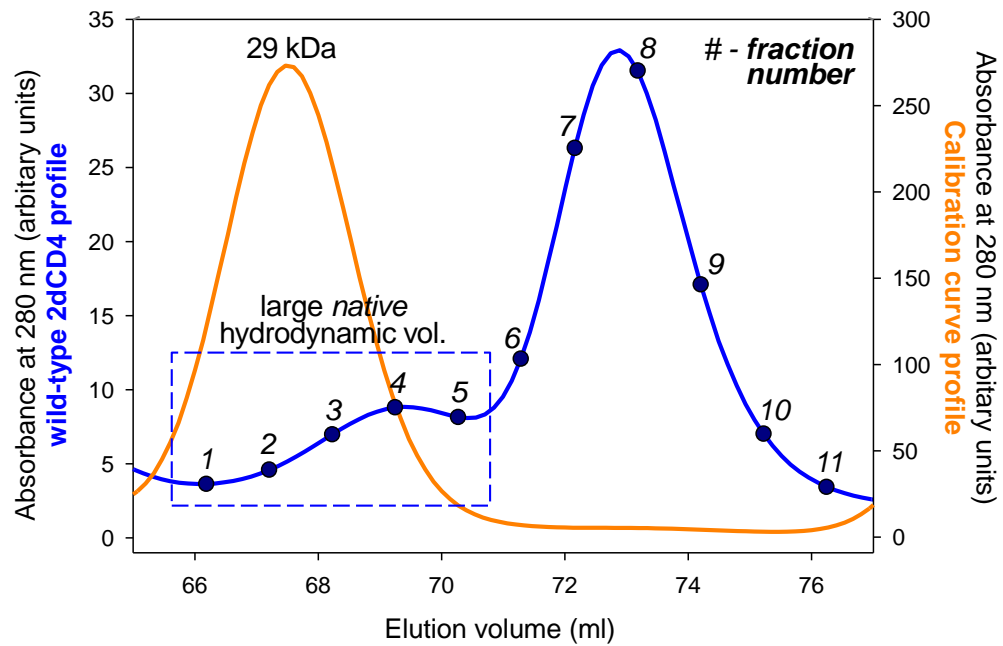
Gavin R. Owen,^{*,†} Jennifer A. Channell,^{‡,§,||} V. Trevor Forsyth,^{‡,§} Michael Haertlein,[§]
Edward P. Mitchell,^{‡,||} Alexio Capovilla,[†] Maria Papathanasopoulos,[†] and Nichole M. Cerutti[†]

[†]HIV Pathogenesis Research Unit, Department of Molecular Medicine and Haematology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown, 2193, Johannesburg, South Africa

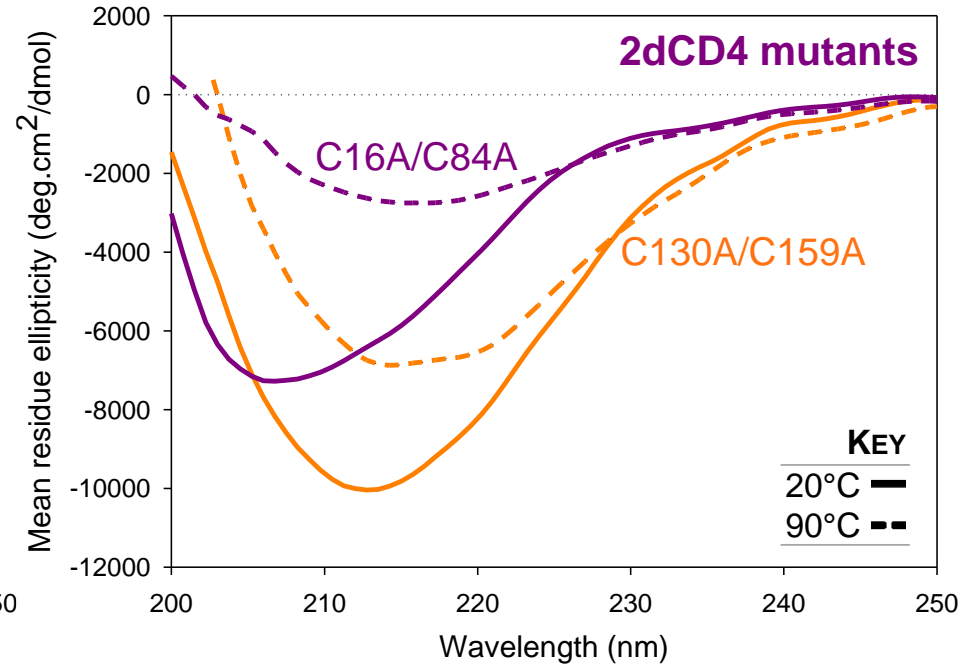
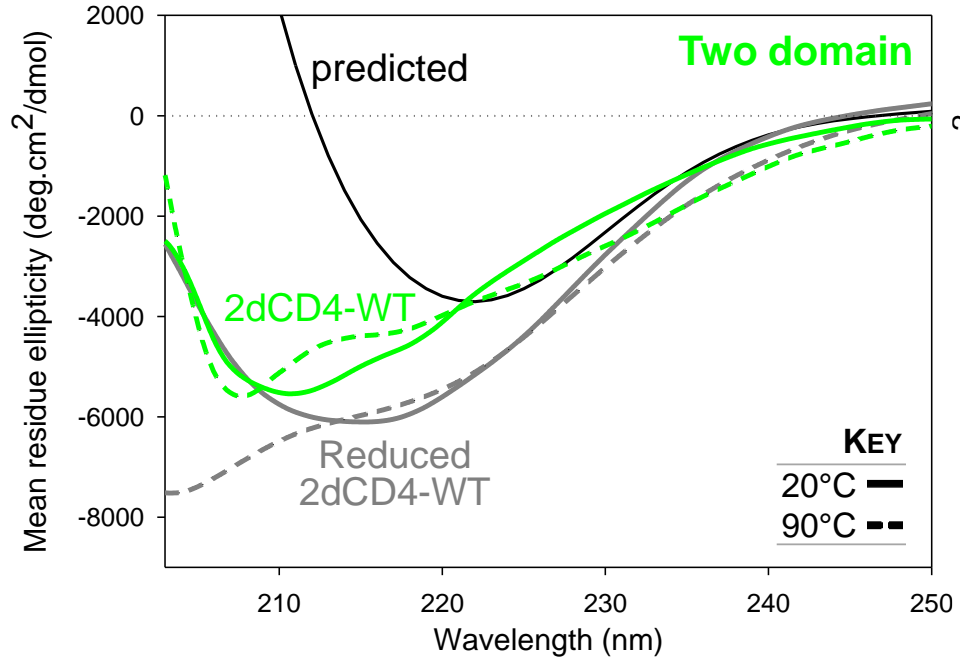
[‡]Faculty of Natural Sciences, Keele University, Keele, Staffordshire ST5 5BG, United Kingdom

[§]Life Sciences Group, Institut Laue-Langevin, 71 Avenue des Martyrs, 38042, Grenoble, France

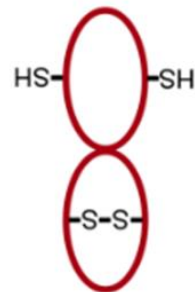
^{||}European Synchrotron Radiation Facility, 71 Avenue des Martyrs, 38042, Grenoble, France



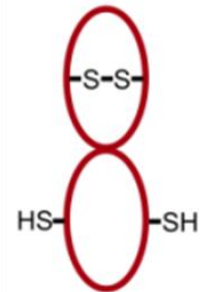
Circular Dichroism



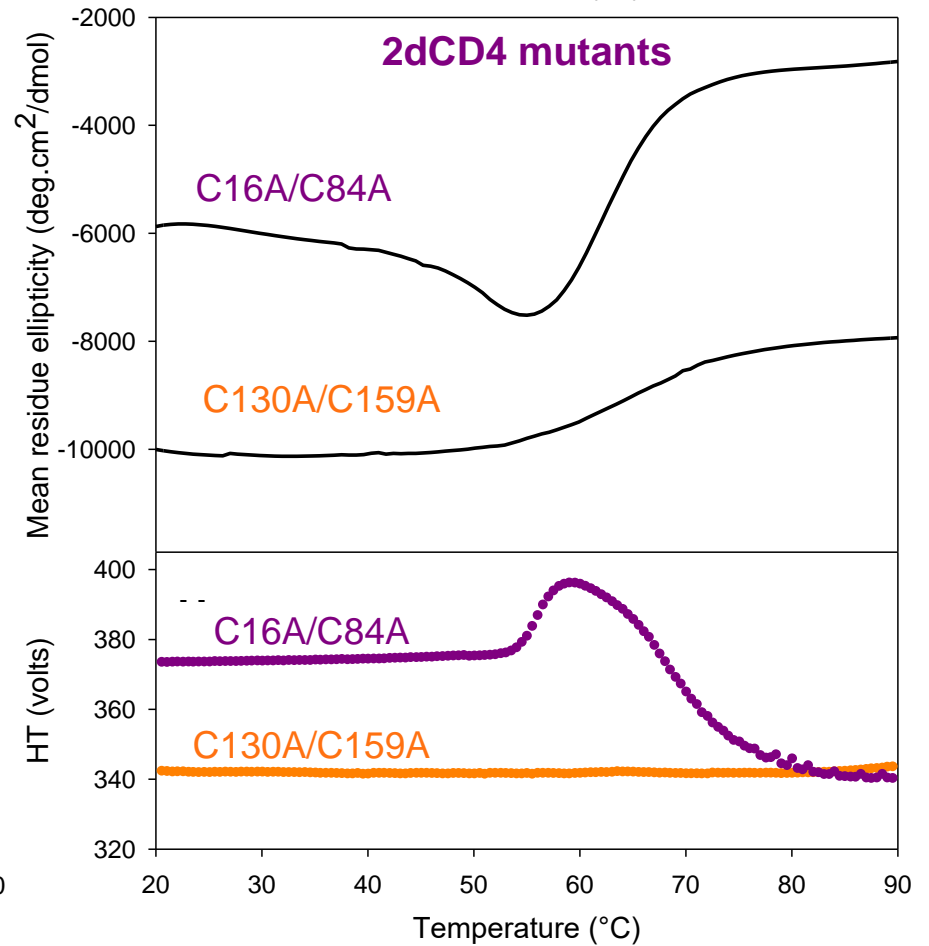
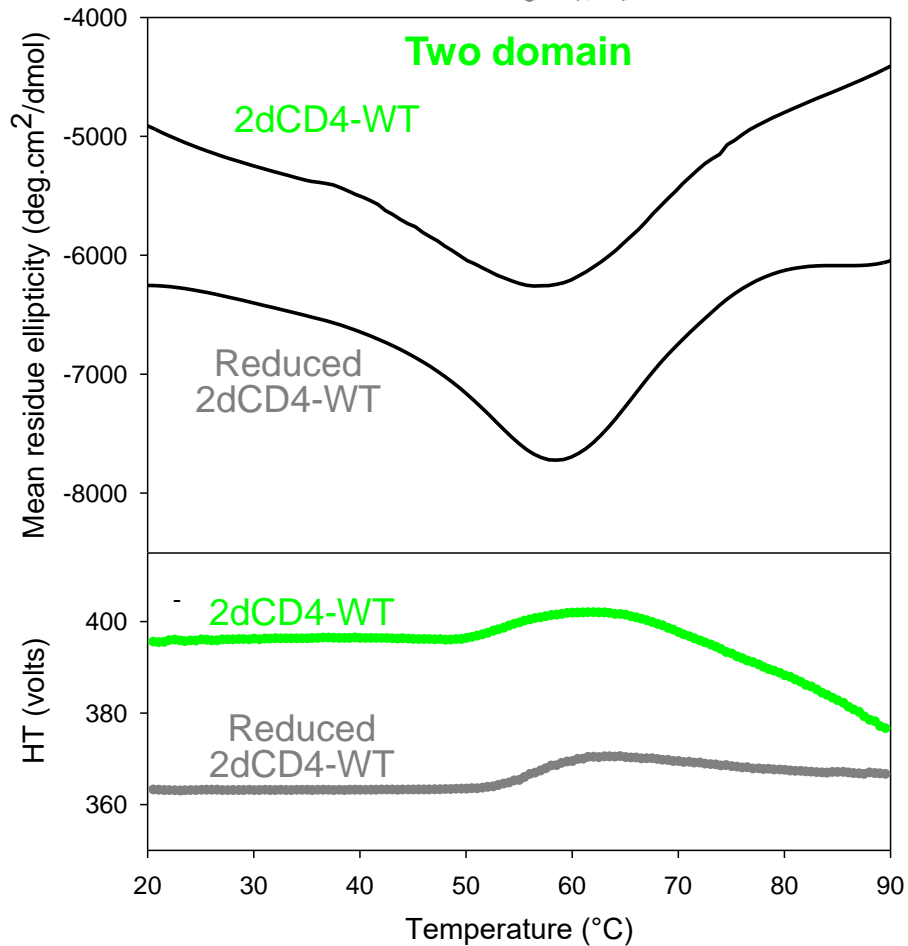
C16A-C84A



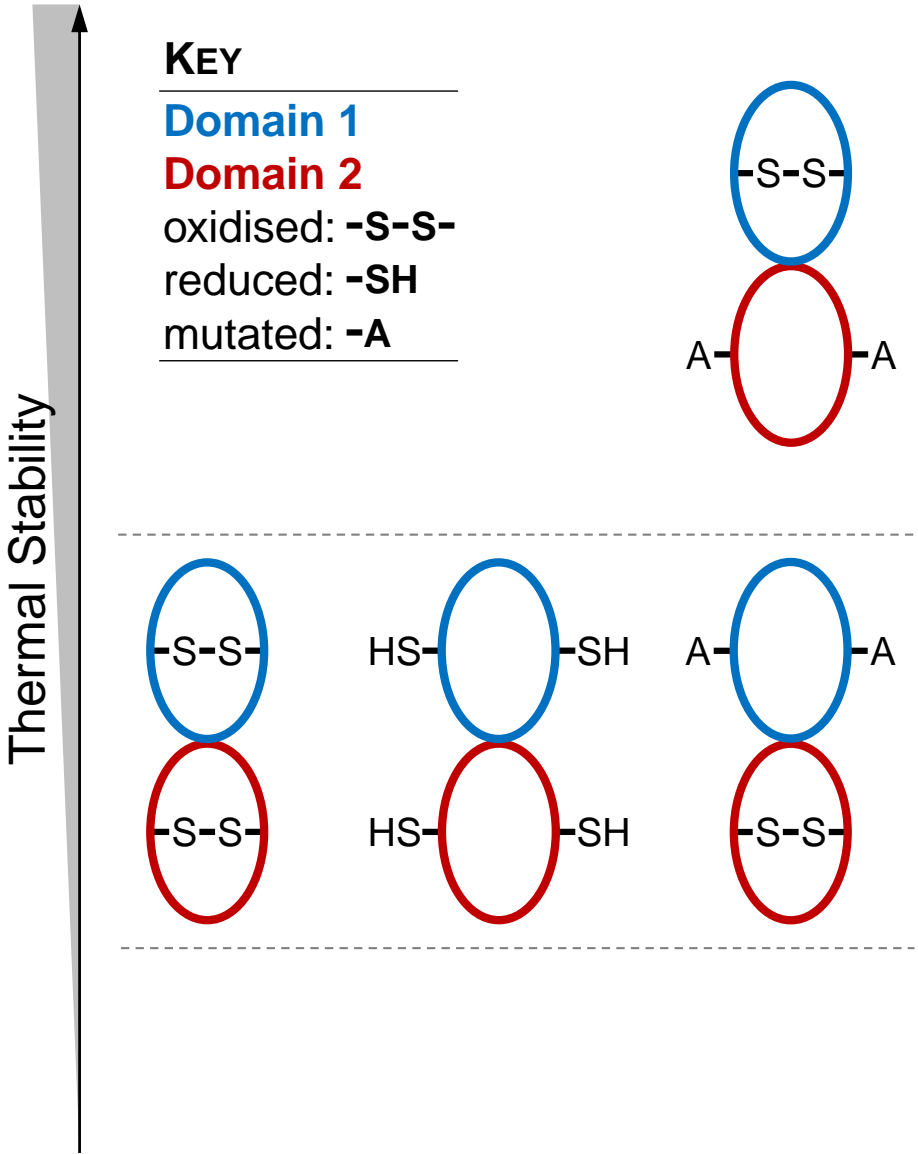
C130A-C159A



Thermal Unfolding



two
domain





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Redox exchange of the disulfides of human two-domain CD4 regulates the conformational dynamics of each domain, providing insight into its mechanisms of control



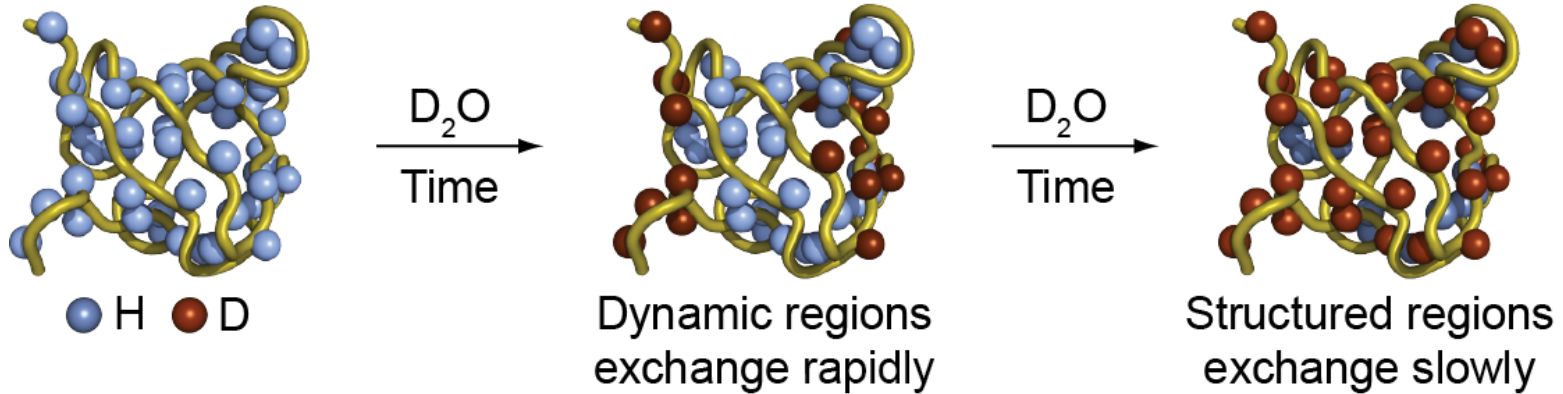
Gavin R. Owen^{a,*}, Doris Le^a, Stoyan Stoychev^b, Nichole M. Cerutti^a,
Maria Papathanasopoulos^a

^a HIV Pathogenesis Research Unit, Department of Molecular Medicine and Haematology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown, 2193, Johannesburg, South Africa

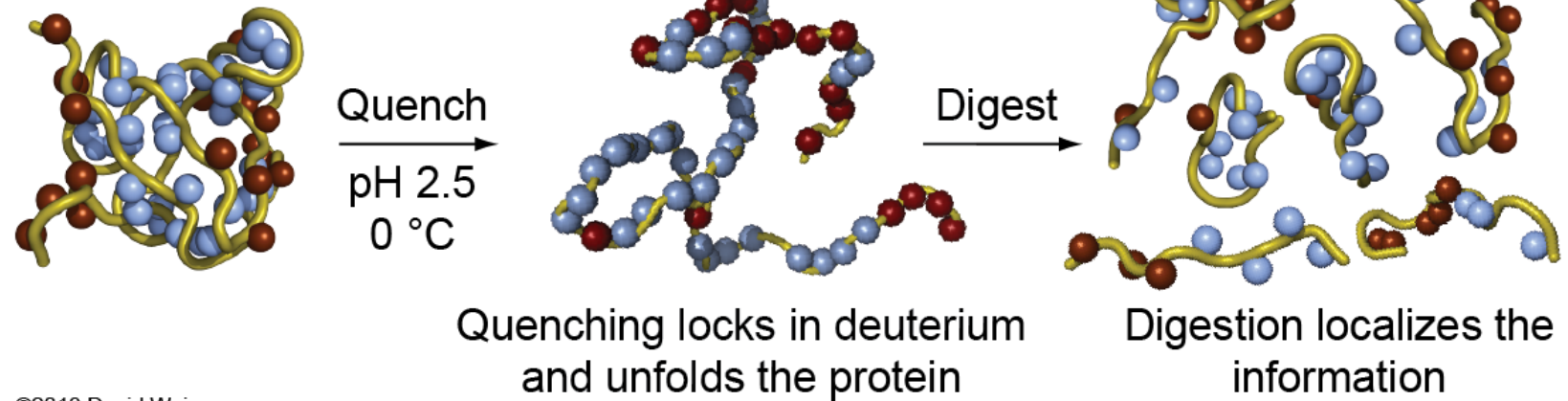
^b Council for Scientific and Industrial Research, Biosciences, Pretoria, 0001, South Africa

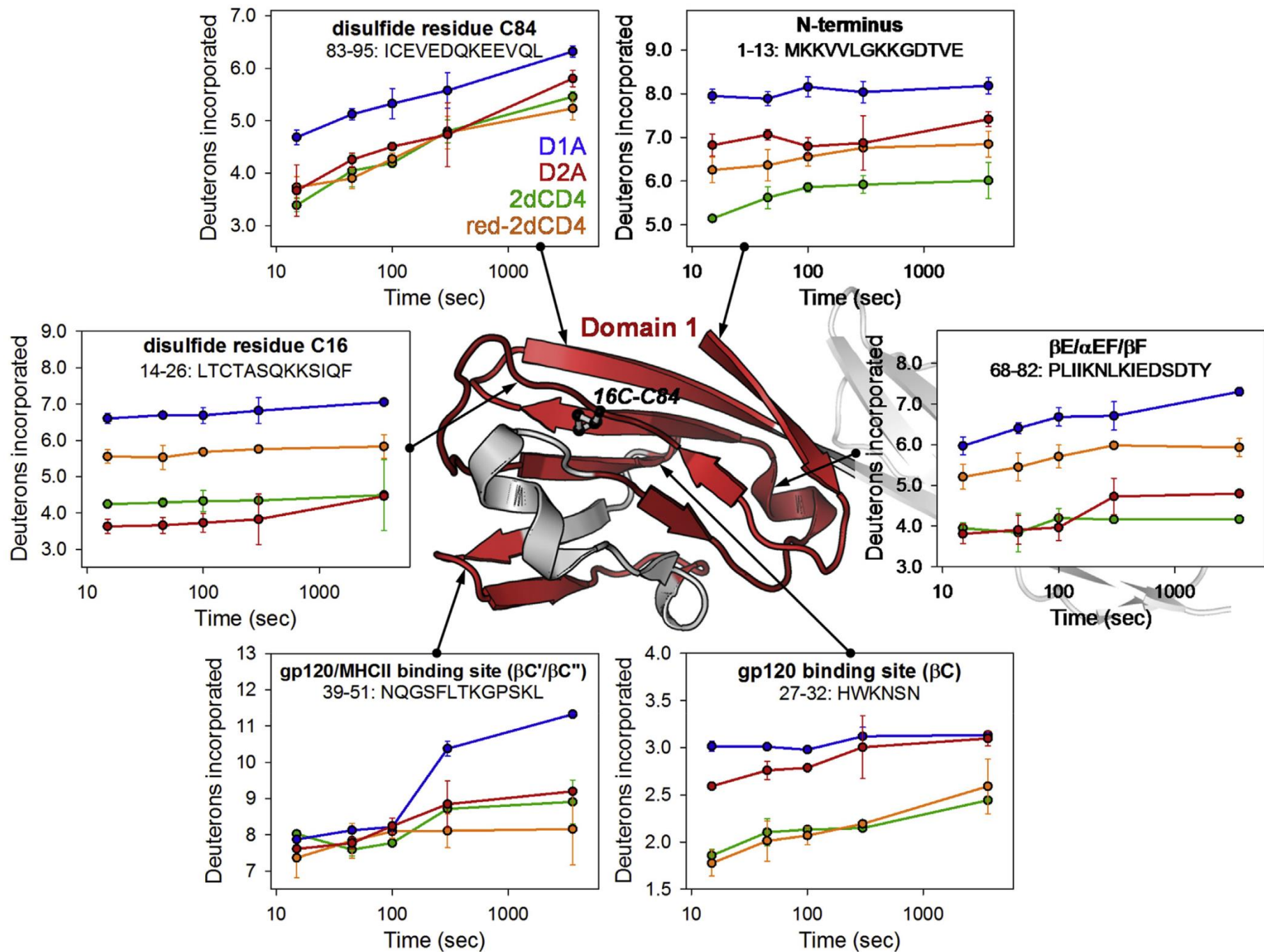
Hydrogen-Deuterium Exchange

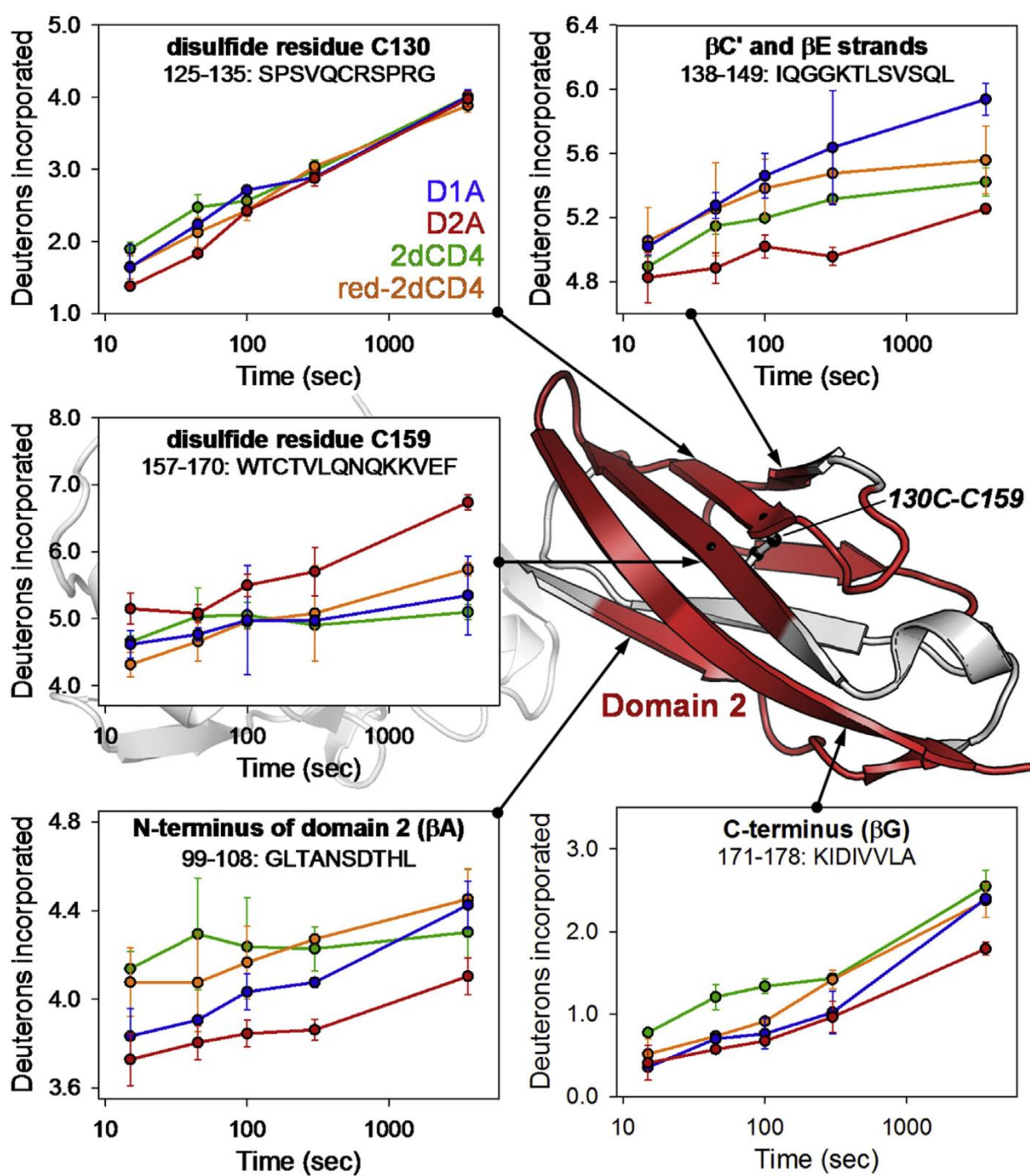
H/D Exchange

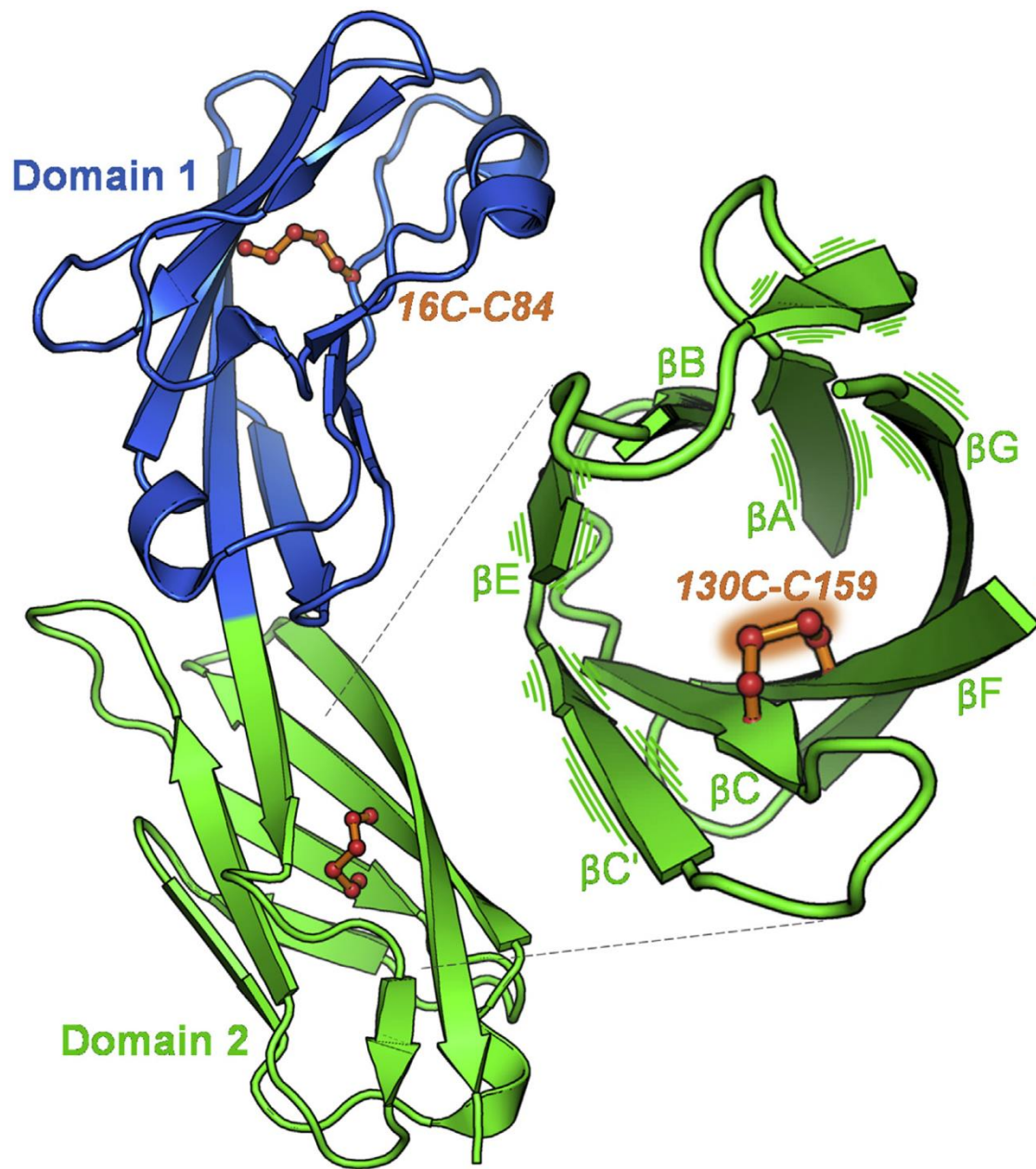


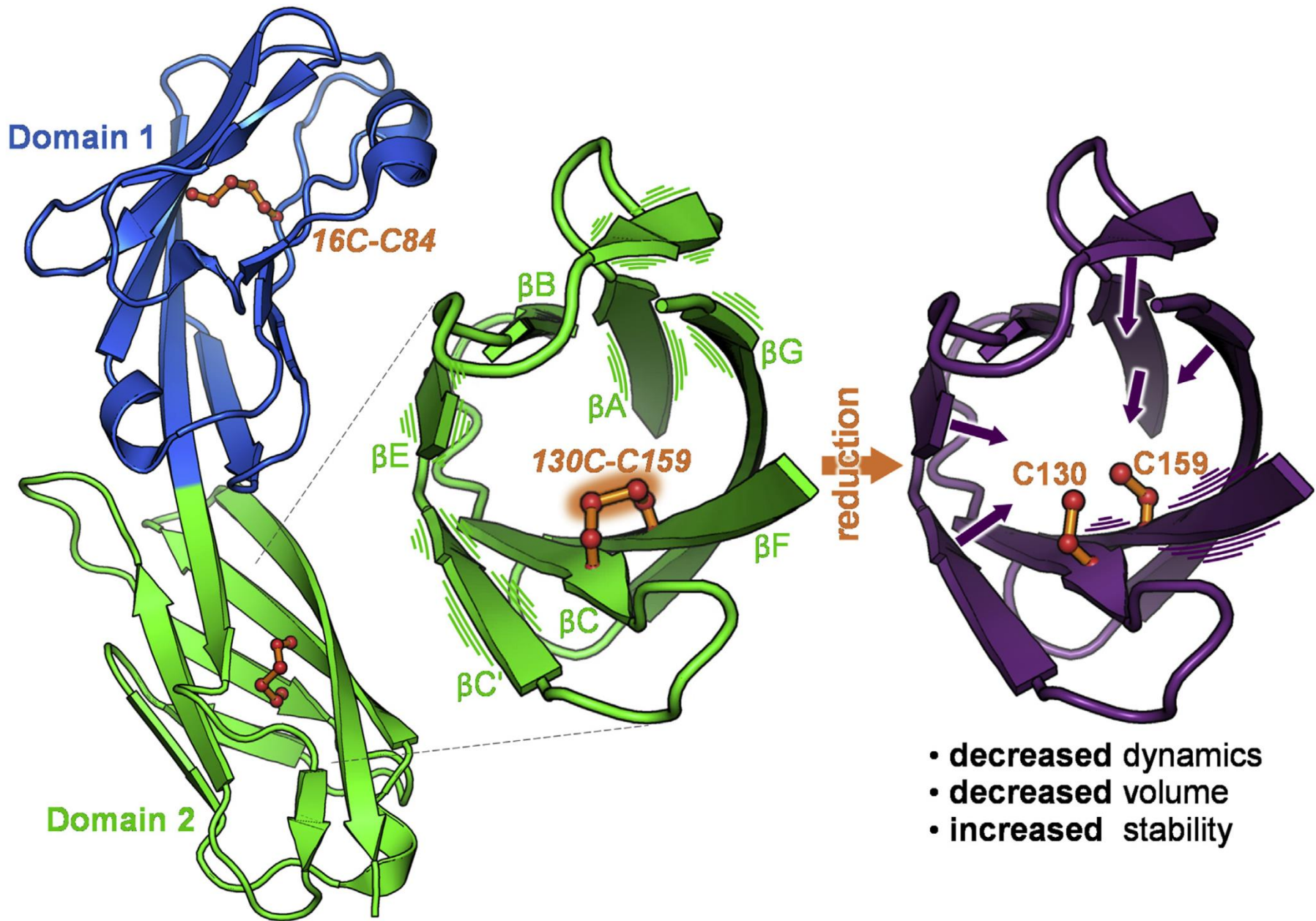
Quench & Digest











Small-Angle Scattering

Powerful technique allowing determination of:

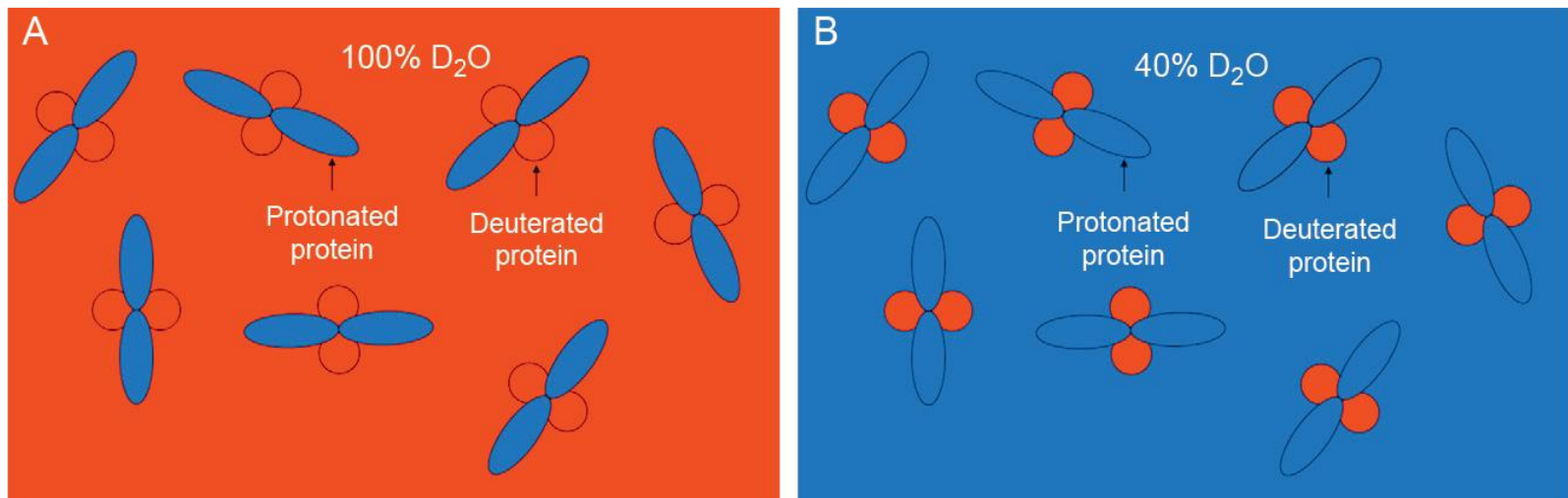
- **shape/size** and **interaction of particles in solution**
- e.g. SAXS (x-rays) and **SANS (neutrons)**

SANS - Small-Angle Neutron Scattering

- Neutrons are scattered by the **nucleus of atom**, but is not proportional to the atomic number of the atom. SANS is rather concerned with the **scattering length density (SLD)** of the atom.
- Each atom has unique scattering lengths - SLD of a molecule is unique to its molecular composition within its volume.
- **Contrast variation** is a powerful feature of SANS which allows the **isolation of the scattering of a single component in a complex.**
(SAXS can't achieve this)
- Contrast arises from molecules' unique scattering lengths.

SANS

- Experimentally, neutron scattering by **D** contributes greater to the signal with less noise, whereas **H** contributes strongly to the background.
- At a given solvent **H₂O/D₂O ratio**:
 - SLD of **macromolecule** = SLD of **solvent**
 - macromolecule is rendered **invisible** to neutrons (i.e. match-out point)
- **Deuterium labelling** can then also be exploited for isolating the scattering of molecules within a complex.
 - the solvent H/D ratio can be adjusted to look at the isolated scattering from a **protiated** protein in complex with a **deuterated** protein



SANS of gp120-CD4 complex

3 SANS experiments were carried out to determine:

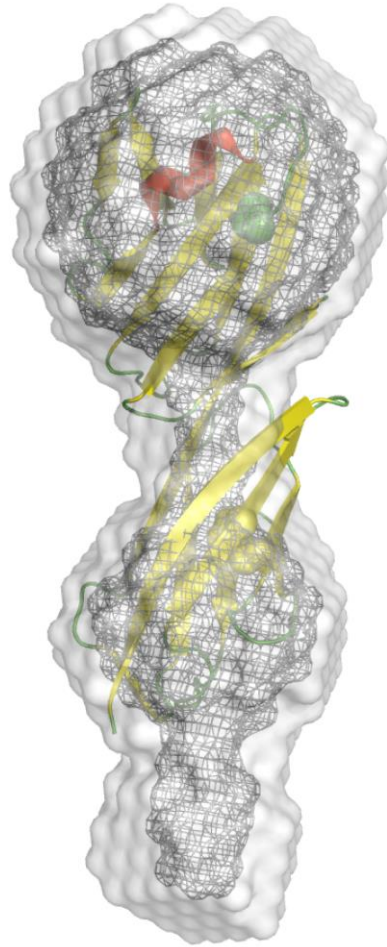
1. contrast match-out point of the **protiated, h-gp120**
2. contrast match-out point of the **deuterated, d-2dCD4-WT**
3. the scattering structure of **h-gp120**, **d-2dCD4-WT**, and the **complex** (using the match-out points to exploit contrast variation)

- h-gp120 CMP = 45.2% D₂O
- d-2dCD4-WT CMP = 90.5% D₂O

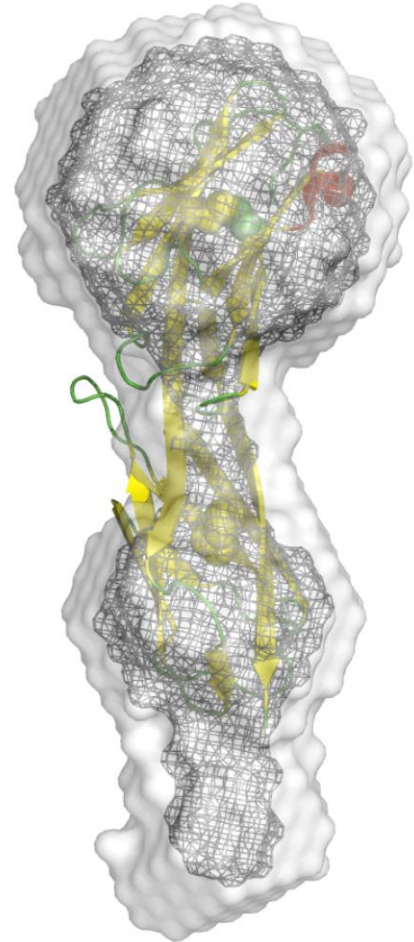
gp120-CD4 complex then measured by SANS in 45.2% = gp120 is invisible
(*Could not measure in 90.5% - where CD4 would be invisible*)

ab initio model
d-2dCD4
(in 45% D₂O)

(A) Front-view



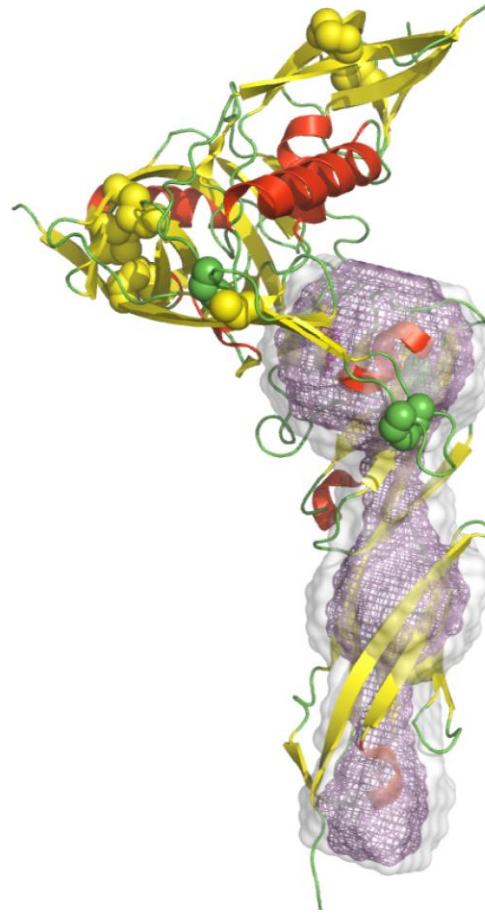
(B) Rotation by 90°



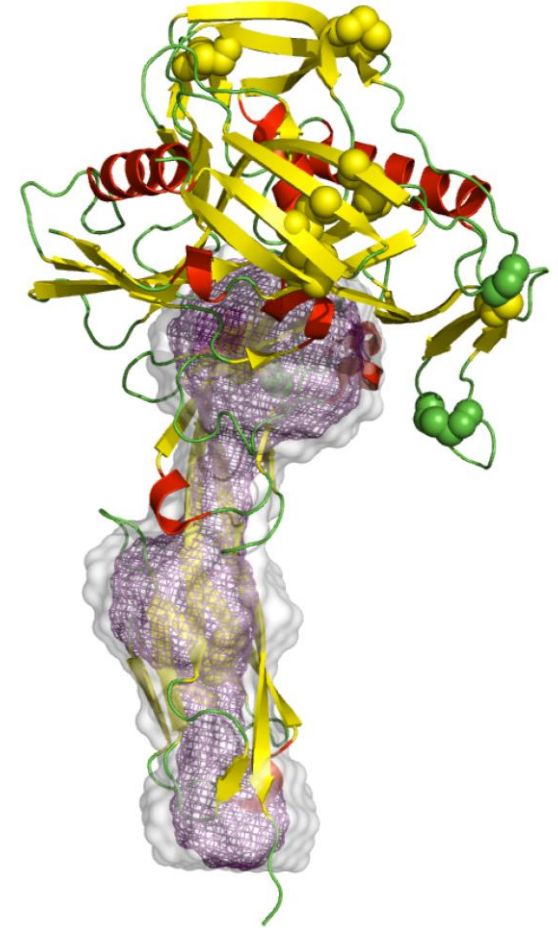
ab initio model
d-2dCD4 in
complex with **h-gp120**

(in 45% D₂O =
gp120 invisible)

(A) Front-view



(B) Rotation by 90°



Thank you