MOLECULAR BIOPHYSICS SUITE
DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF OXFORD

STRUBI FACILITY TALK

David Staunton

27th January 2022

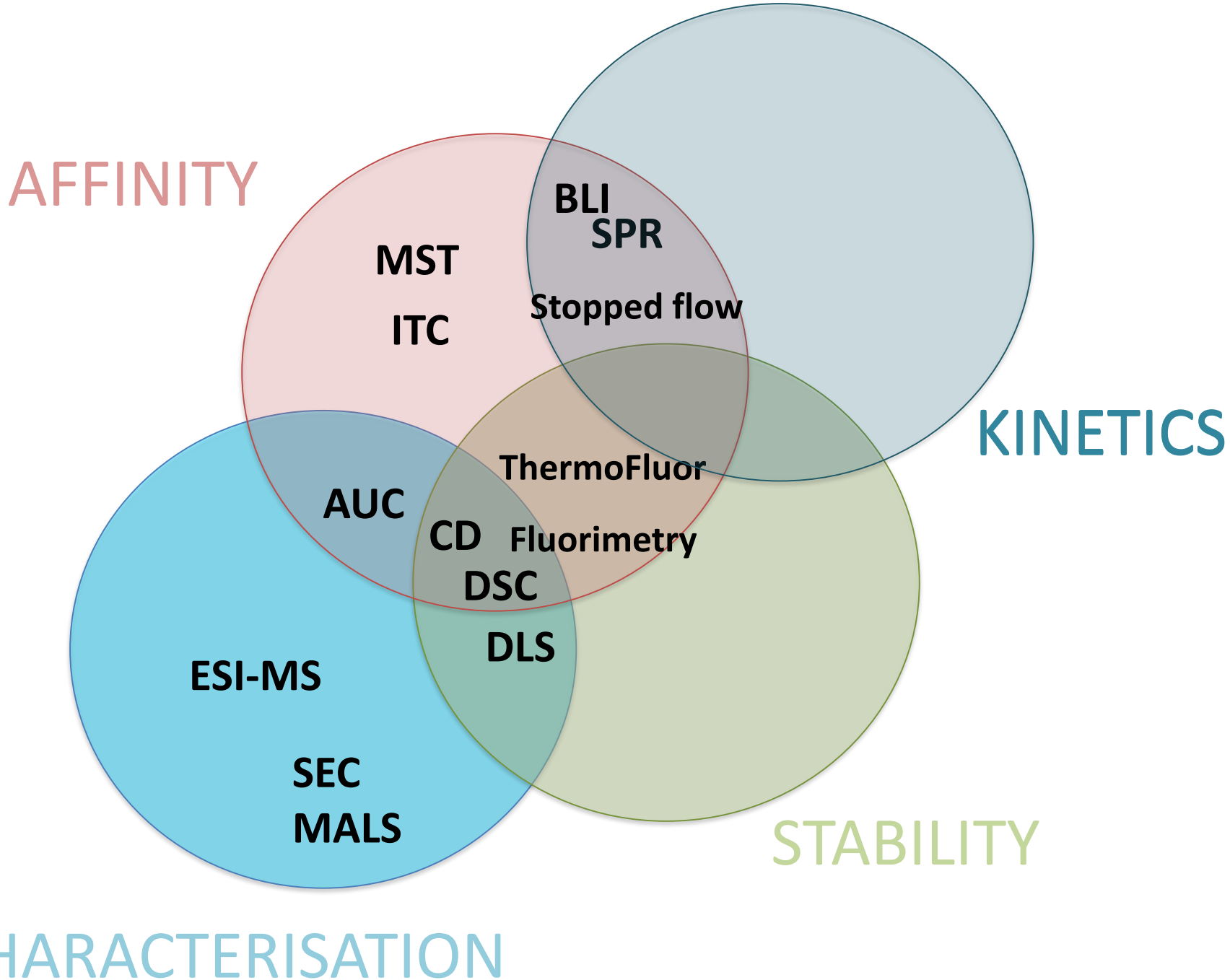
Molecular Biophysics Suite

- Organised by Dept and STRUBI and funded by Fell Fund in 2008
- Brought together equipment from various sites to one location in New Biochemistry
- Set up booking system and charging
- Maintenance and servicing
- Training and workshops

Instrument	Technique	Abbreviation
Wyatt HELEOS	Size exclusion chromatography Multiple Angle Light Scatter	SEC MALS
Beckman XL-I	Analytical ultracentrifugation	AUC
Viscotek 802	Dynamic light scatter	DLS
Malvern Vcap	Differential scanning calorimetry	DSC
JASCO 815	Circular Dichroism	CD
Stratagene MC3005PCR	Differential scanning fluorimetry	Thermofluor
Monolith 11.5	Microscale Thermophoresis	MST
Biacore T200	Surface plasmon resonance	SPR
Malvern PEAQ	Isothermal titration calorimetry	ITC
Horiba FluoroMax-4	Fluorimetry	Fluorimetry
Applied Photophysics SX	Stopped flow	Stopped flow
OctetRed 384	Biolayer interferometry	BLI
Agilent Q-TOF	Electrospray ionisation mass spectrometry	ESI-MS

Facility to move in February

- The facility will move temporarily to Phase 2 Biochemistry in February while basement redeveloped
- It will return to its new location in the basement in late summer 2022
- During the moves the facility will be closed for a week


AFFINITY
KINETICS
STABILITY
CHARACTERISATION
BLI
SPR
MST
Stopped flow
ITC
ThermoFluor
AUC
CD
Fluorimetry
DSC
DLS
ESI-MS
SEC
MALS

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Analytical Ultracentrifugation XL-I

Absolute measurement of mass and protein conformation in solution

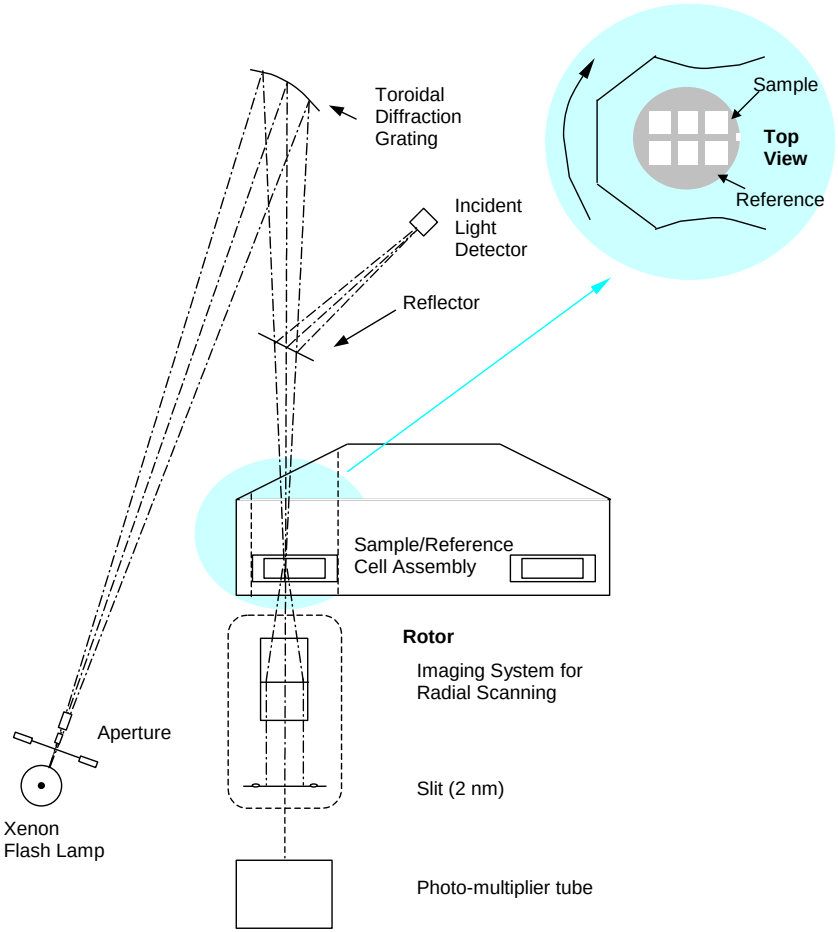
Sedimentation equilibrium gives the true MW of the macromolecule, and information on solution behaviour e.g. polydispersity

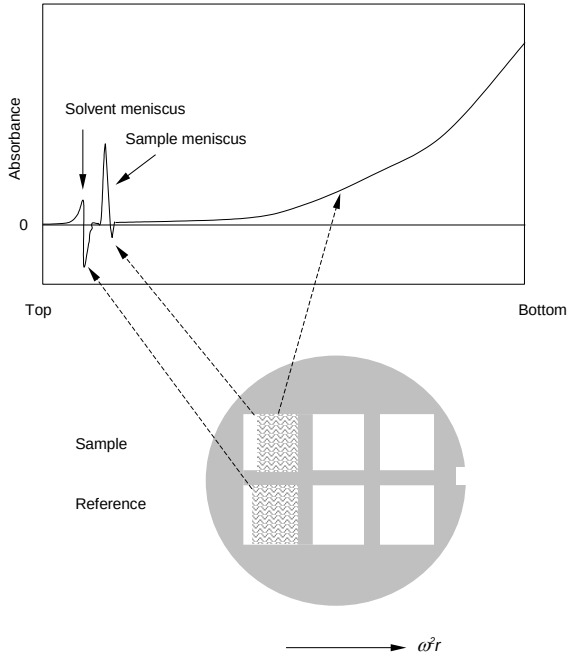
Sedimentation velocity gives information on mass, shape and size distribution

Require 100-400ul of sample and buffer control.

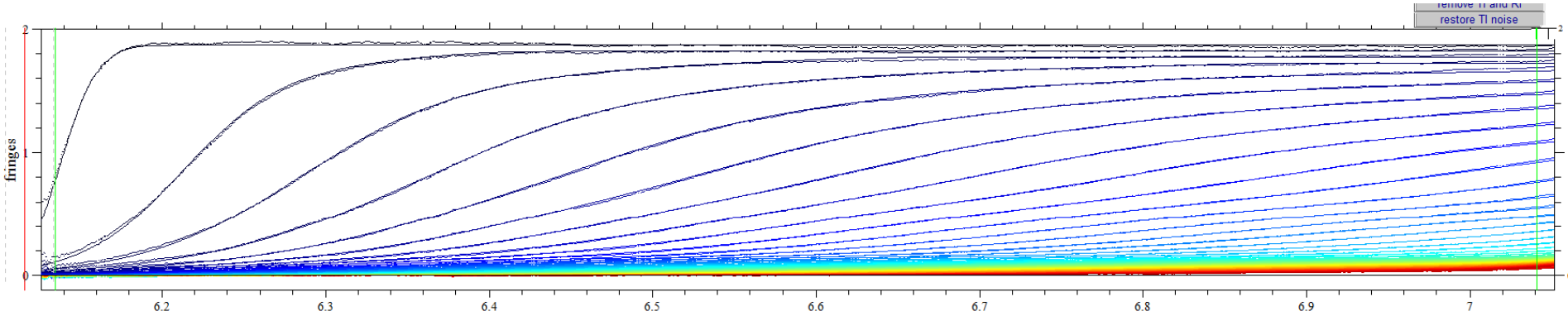
Concentration range uM (230nm) to mM (interference)

Can identify complex formation for weak interactions

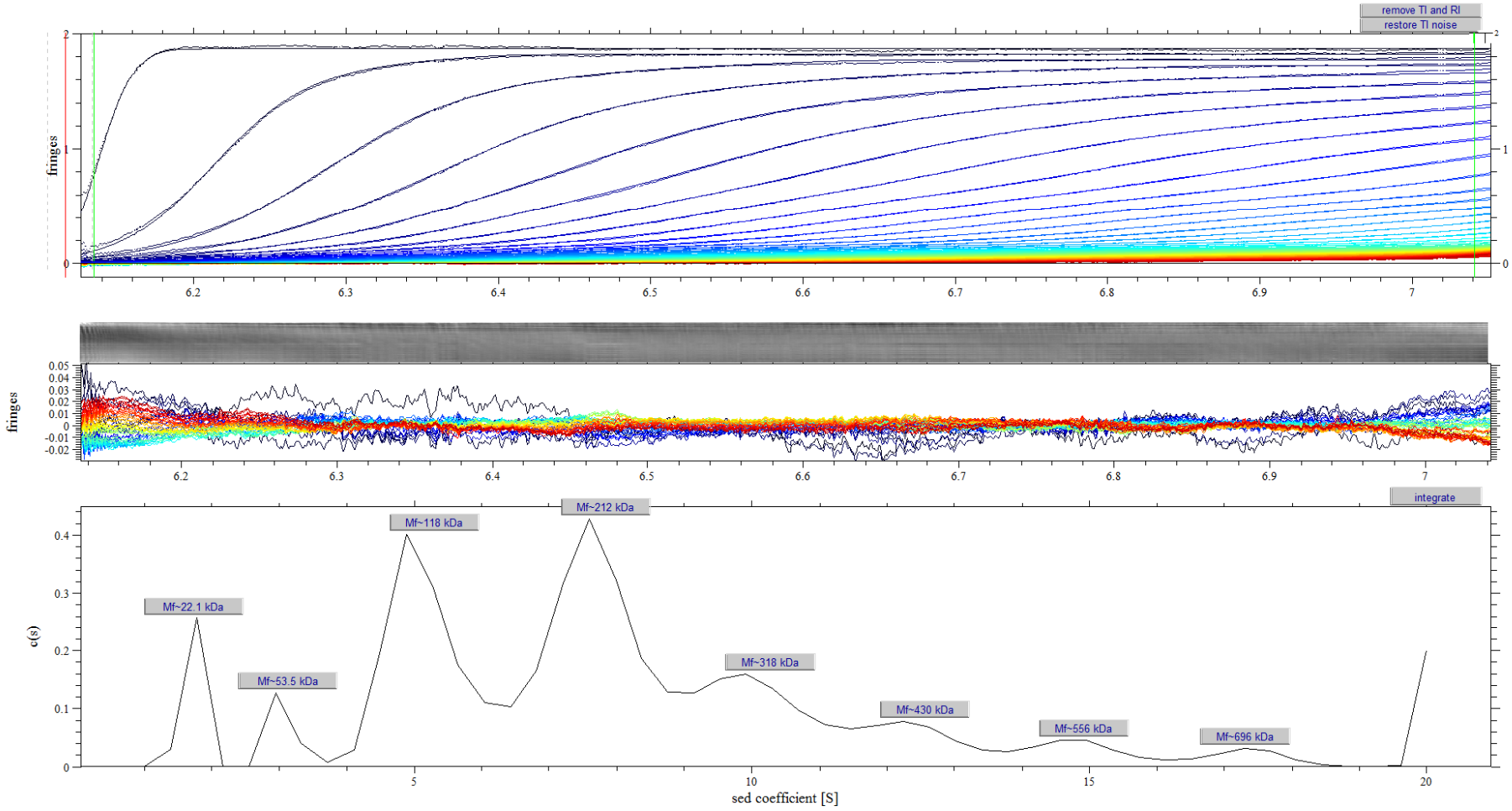

Toroidal
Diffraction
Grating
Sample
Top
View
Reference
Incident
Light
Detector
Reflector
Sample/Reference
Cell Assembly
Rotor
Imaging System for
Radial Scanning
Aperture
Slit (2 nm)
Xenon
Flash Lamp
Photo-multiplier tube


Absorbance
Solvent meniscus
Sample meniscus
0
Top
Bottom
Sample
Reference
$\omega^2 r$

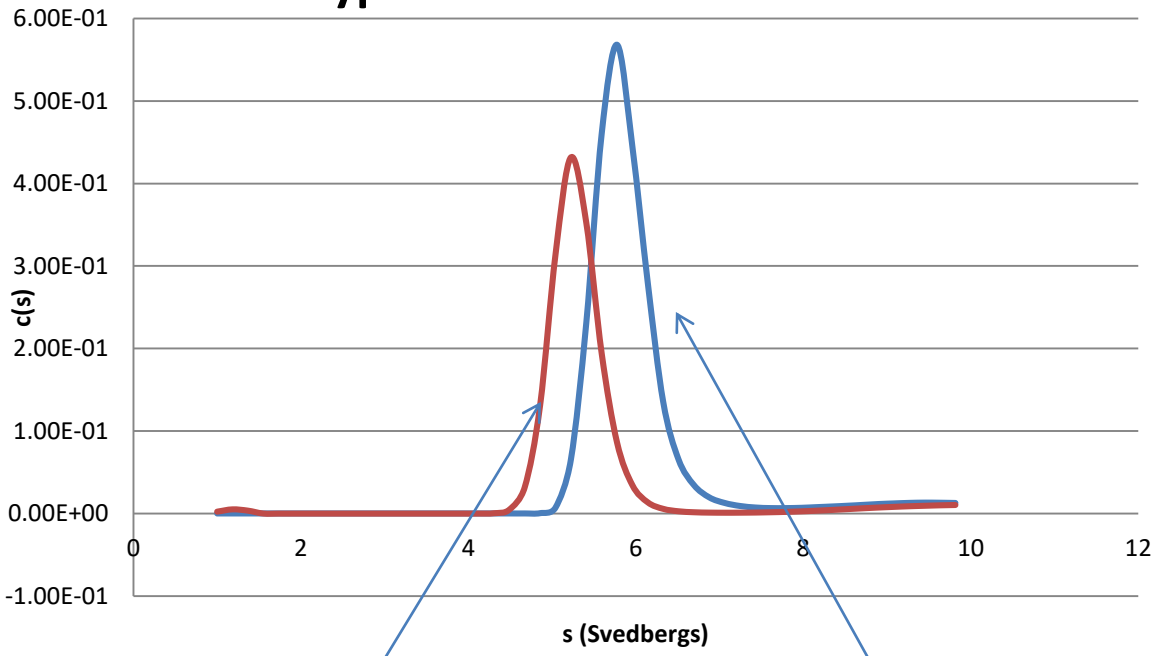
Sedimentation Velocity Experiment analysed by Sedfit

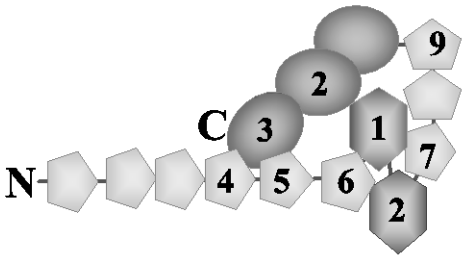


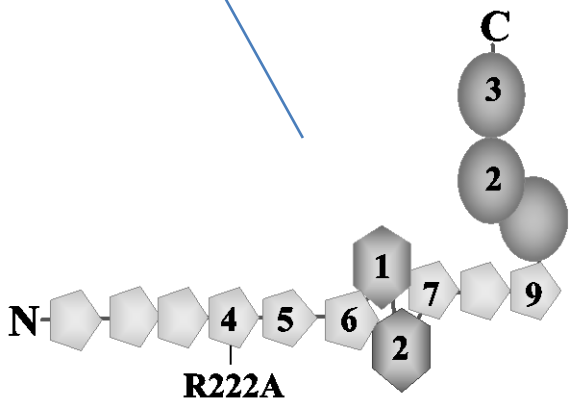
"Gel filtration without the gel"

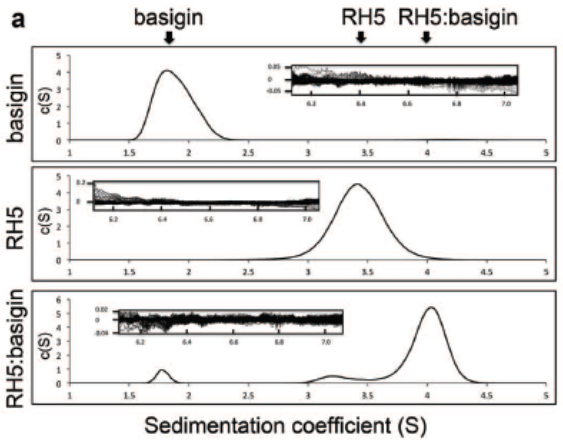

remove TI and RI
restore TI noise
fringes
fringes
integrate
Mf~22.1 kDa
Mf~53.5 kDa
Mf~118 kDa
Mf~212 kDa
Mf~318 kDa
Mf~430 kDa
Mf~556 kDa
Mf~696 kDa
c(s)
sed coefficient [S]

Velocity sedimentation analysis of Wild type and R222A 100kDa Fn







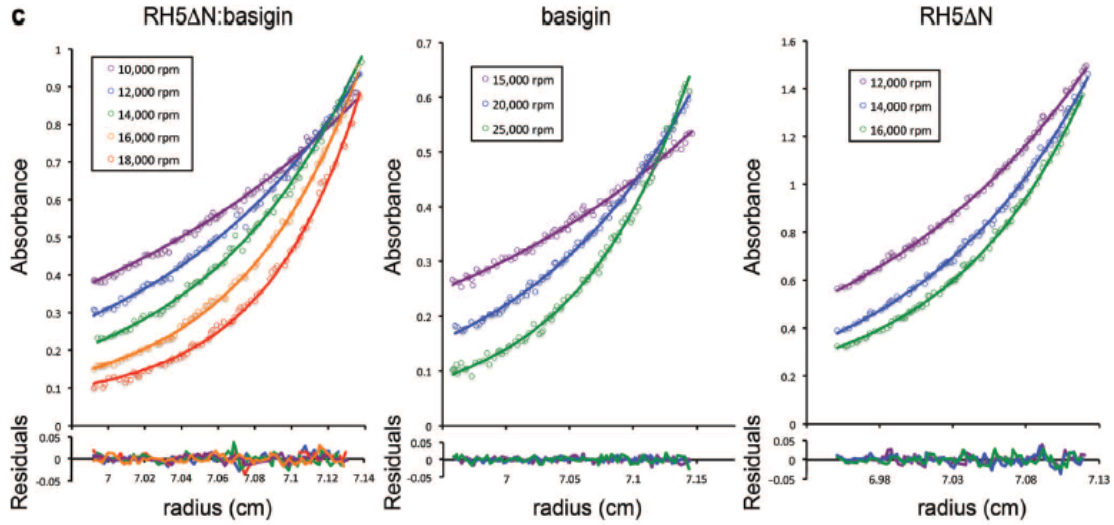


b

	Frictional coefficient (f/f_0)	Sedimentation coefficient (S)	MW_{app} (kDa)
basigin	1.37	2.0	21.5
PfRH5	1.36	3.7	51.7
PfRH5:basigin	1.58	4.3	80.2

d

	MW_{app} (kDa)	Reduced χ^2
basigin	21.0	1.52
PfRH5ΔN	51.4	4.68
PfRH5ΔN:basigin	70.2	5.11



Extended Data Figure 7 | Analysis of the PfRH5–basigin complex using analytical ultracentrifugation. a, **b**, Sedimentation velocity analysis. The continuous sedimentation coefficient distributions that best fit the data are shown for basigin (top), full-length PfRH5 (middle), and a gel-filtered PfRH5–basigin complex (bottom). The inset shows the fitting residuals. **c**, **d**, Sedimentation equilibrium analysis. PfRH5ΔN (residues 140–526), basigin, and a gel-filtered PfRH5ΔN–basigin complex were analysed. The runs lasted 20 h at different speeds, as indicated in the inset legends. Ultraviolet absorbance was monitored at 280 nm. The residuals are shown below fitted data. The calculated molecular weights are consistent with the formation of a 1:1 complex between PfRH5ΔN and basigin.

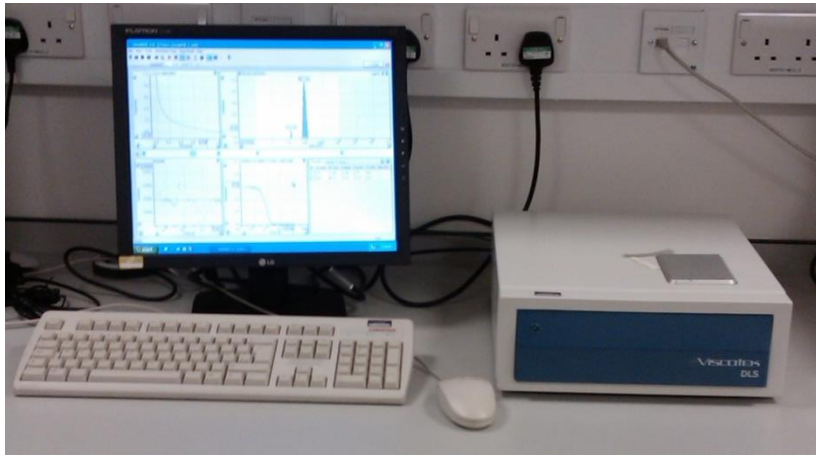
Wright et al., Nature 2014

Dynamic Light Scatter Viscotek

Measure the time dependant fluctuations in scattered light intensity to calculate the translational diffusion coefficient D and hence the hydrodynamic radius Rh using the Stokes Einstein equation:

$$D = \frac{kT}{6\pi\eta Rh}$$

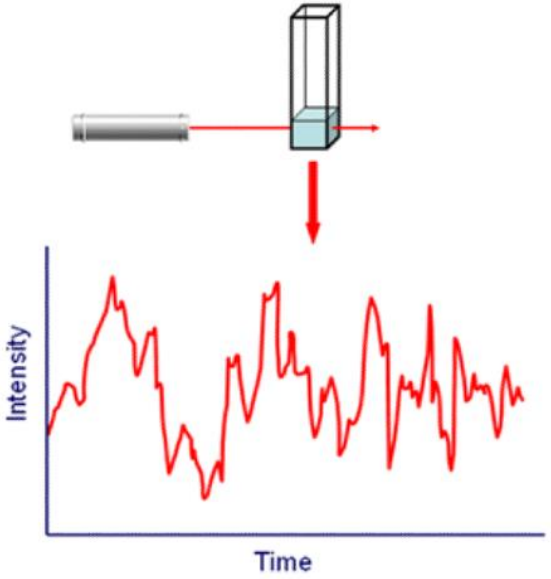
where η= viscosity

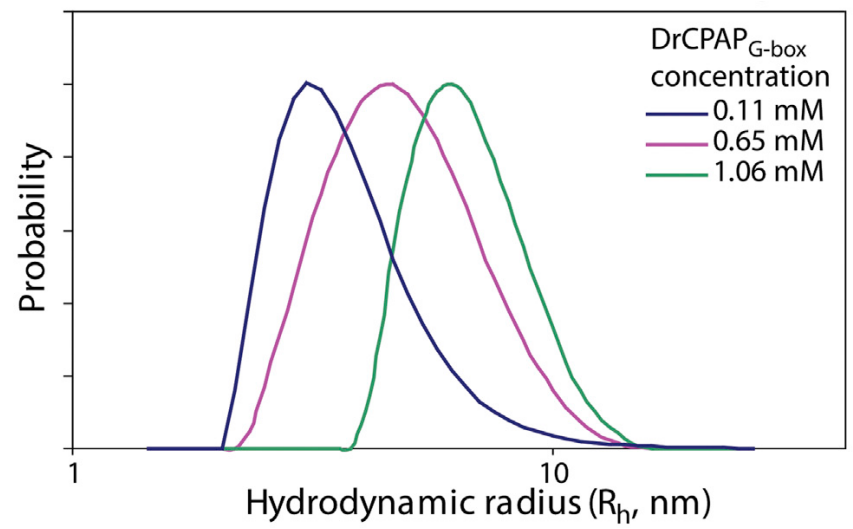


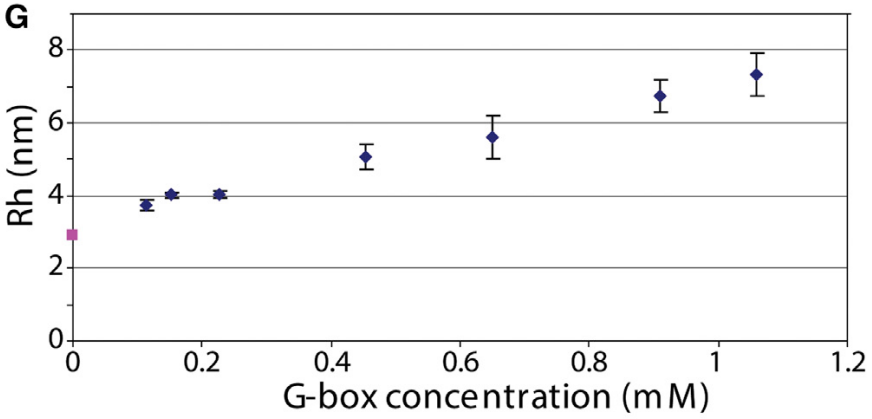
12 µl sample volume, best for 100nm range of particle size.

Signal strength inversely proportional to size therefore need higher concentrations for small proteins and macromolecules

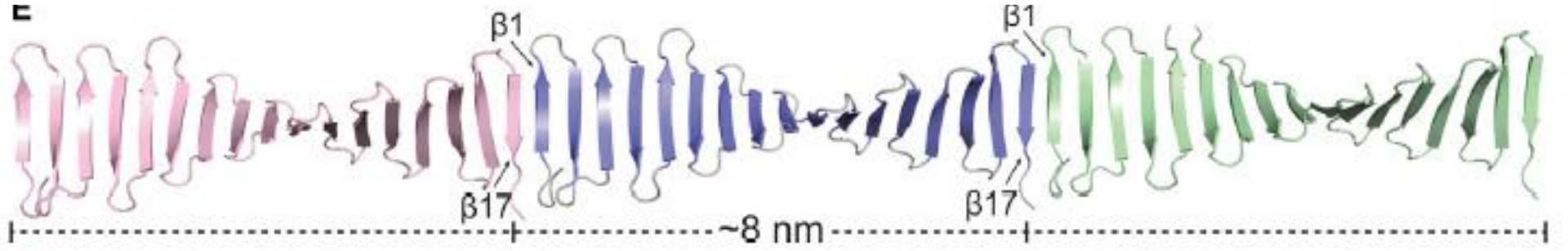
Analysis software available







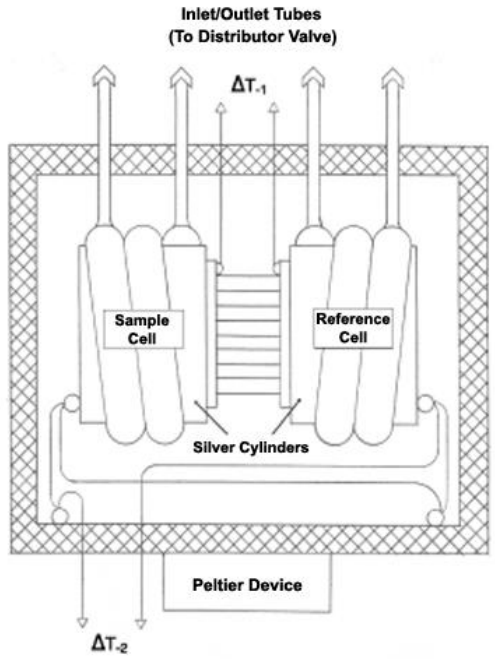
Hatzopoulos et al., 2014,

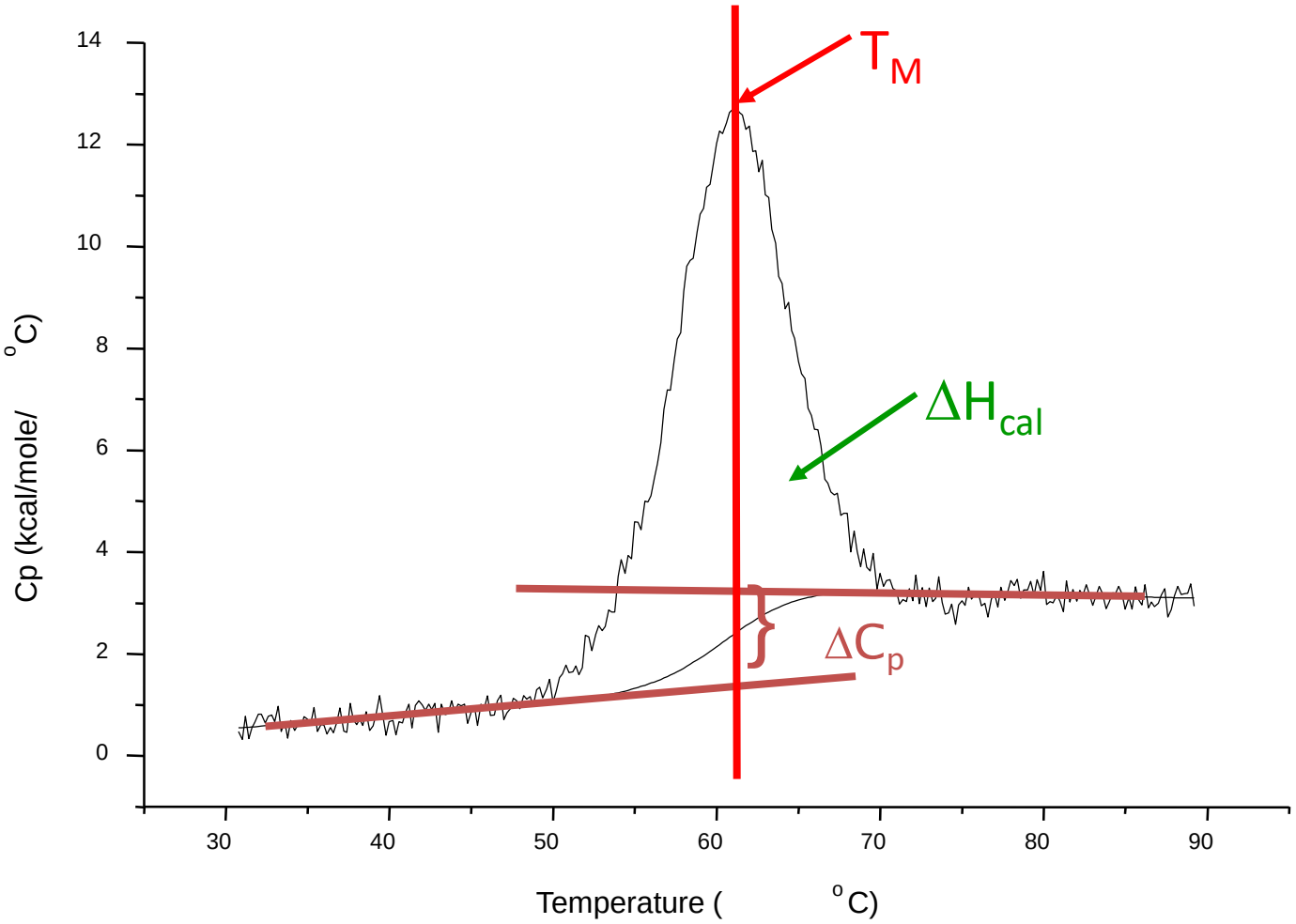


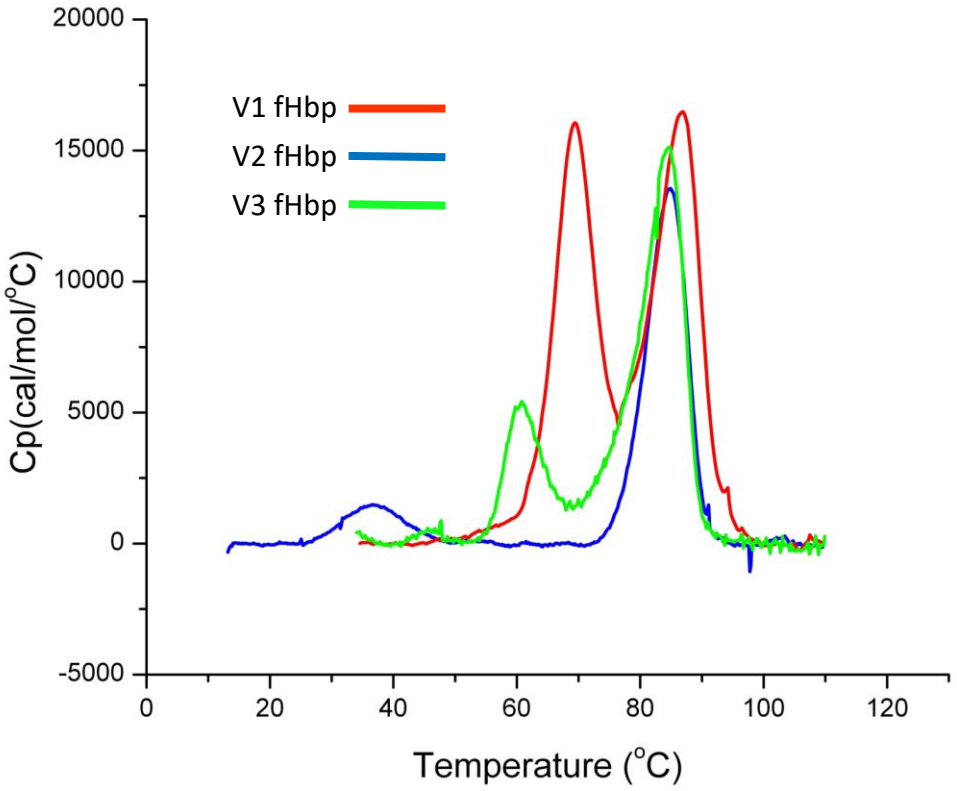
VP-Capillary DSC

Measures heat energy uptake during controlled temperature change-heat capacity
Output is a plot of heat capacity vs temperature
Capillary cells are used for protein studies

Temperature range -10 to 130 °C
Volume of cell 130ul
Sample size 400ul
Sample concentration 100-500 ug/ml
Pressurised at 30 psi
50 samples per day




14
12
10
8
6
4
2
0
Cp (kcal/mole/ °C)
30
40
50
60
70
80
90
Temperature (°C)
T_M
ΔH_cal
ΔC_p



DSC used to routinely screen fHbp mutants so that potential vaccine candidates that do not bind fH and are stable can be identified.

Stability can now be routinely engineered into the vaccines by identifying the key residues in each module.

Implications for formulation of these vaccines

Variant	Tm of N domain °C	Tm of C domain °C
V1	69.5	86.8
V2	36.6	84.9
V3	60.6	84.5

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Jasco Circular Dichroism spectrometer

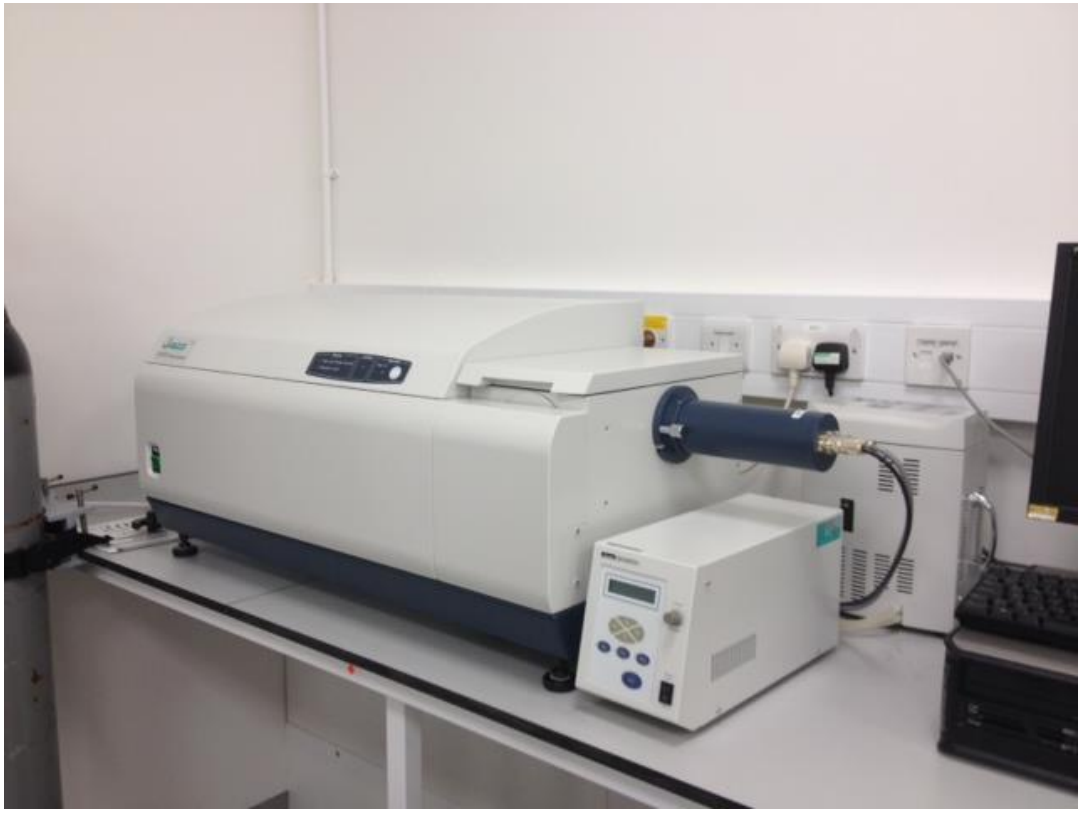
Absorption of polarised light

Path length; 1mm to 0.05mm (thin cuvettes)

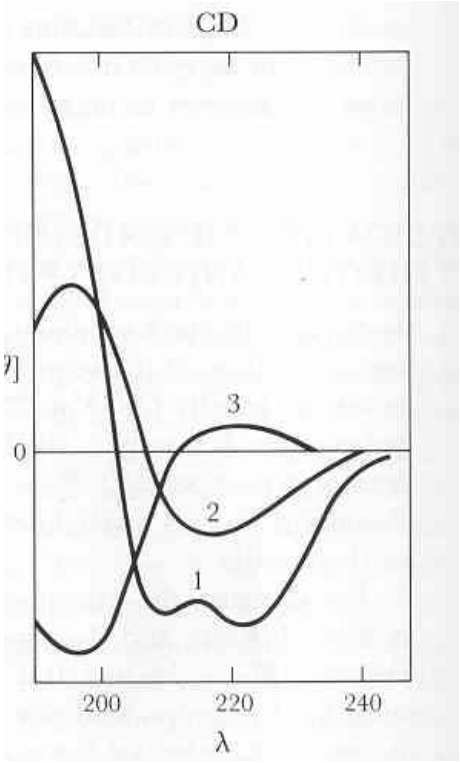
Protein concentrations; 0.1 - 0.05mg/ml, approx 200µl sample, for 1mm cuvette.

Buffers: low buffer concentration preferable. Usually 10mM phosphate buffer + 20mM NaCl

Nitrogen: need to purge sample compartment and lamp



Circular Dichroism – protein secondary structure



1 = helical

2 = sheet

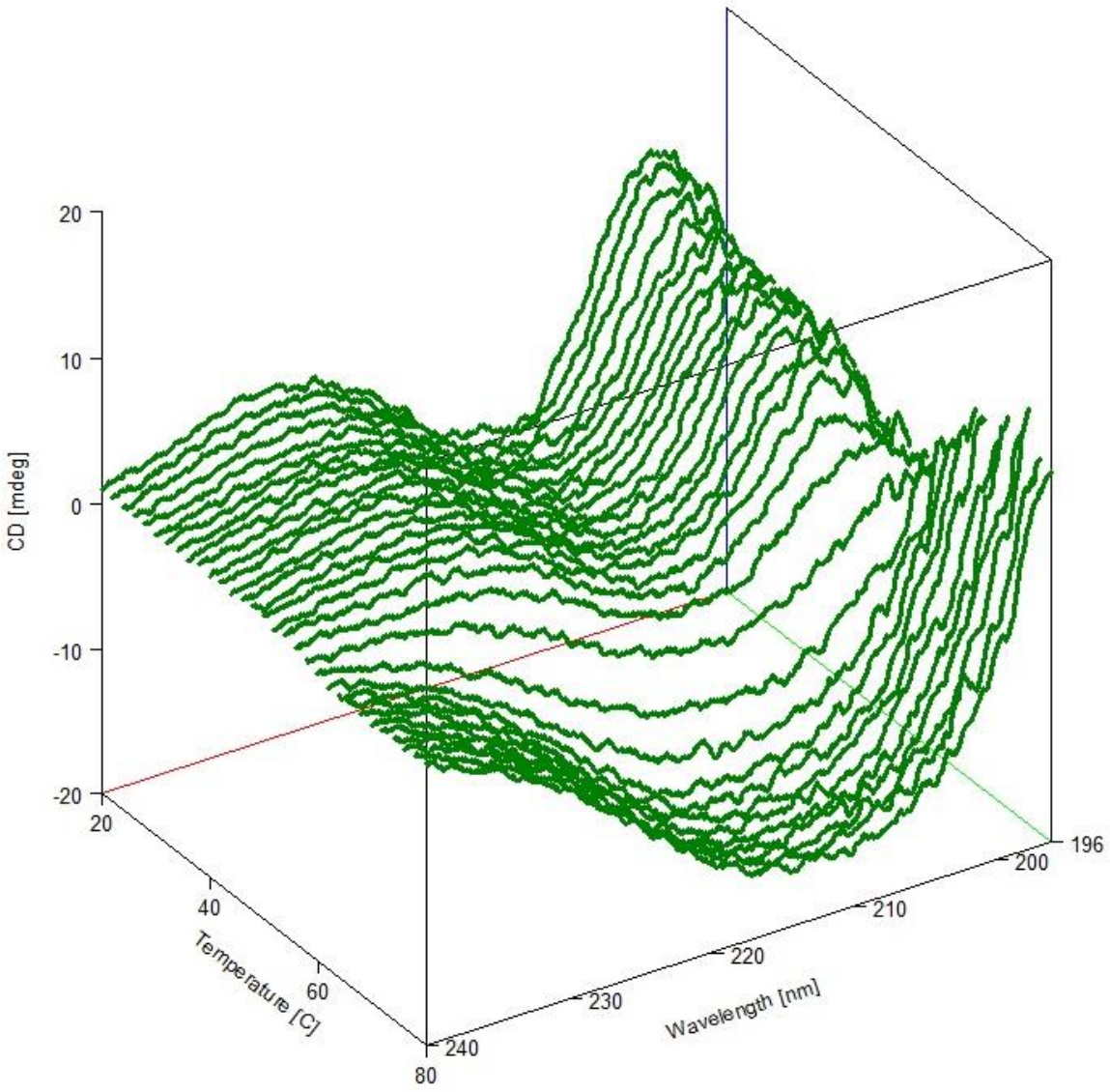
3= random coil

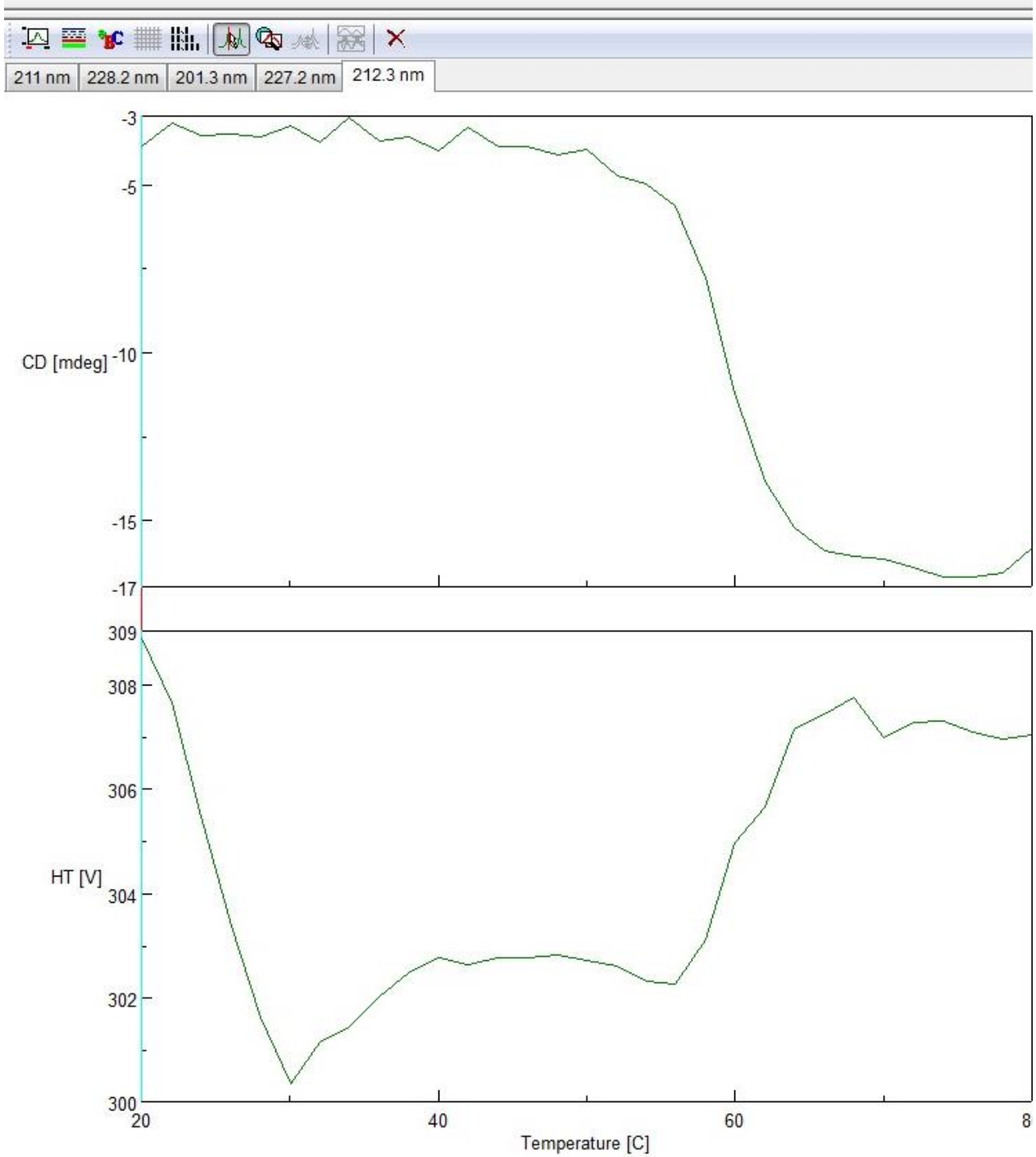
All data should be saved as mean residues ellipticities prior deconvolution.
The mean residue molar ellipticity [Φ] is:

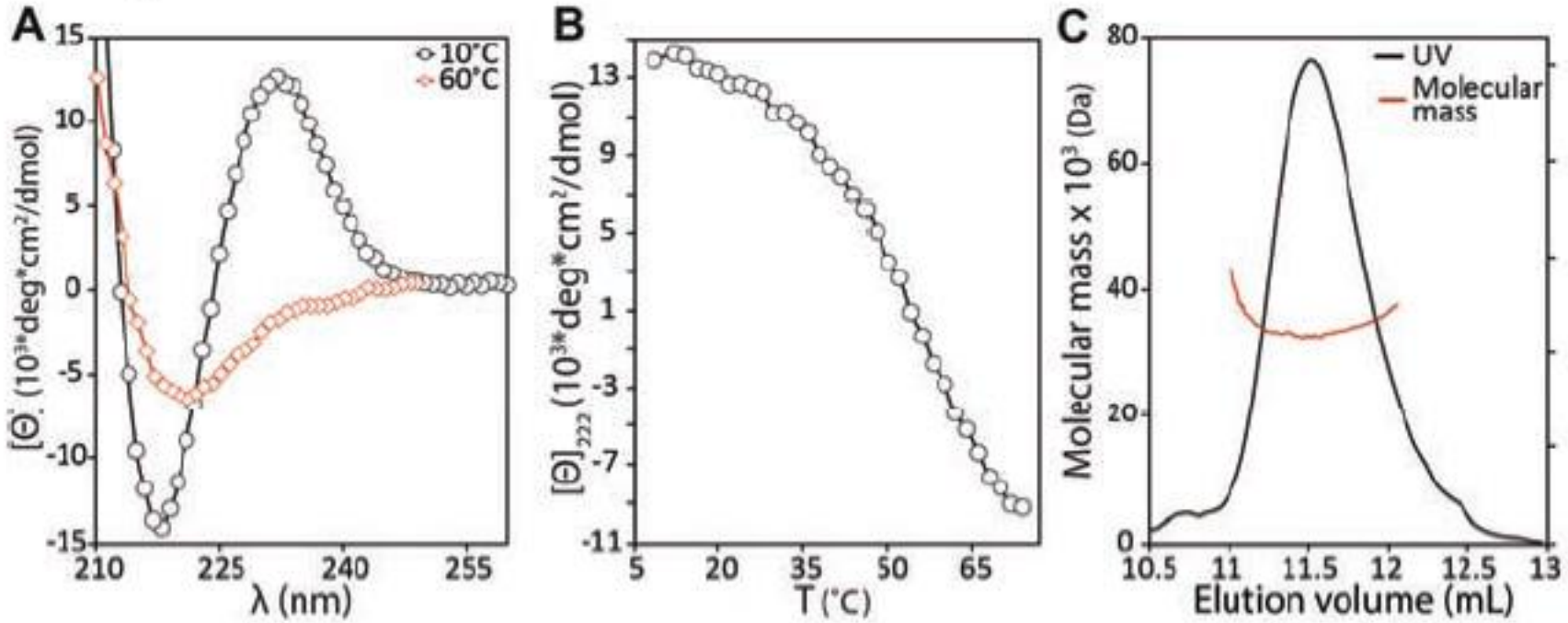
$$[\Phi] = \frac{100\Phi}{l\,c\,n} = 3298\Delta\varepsilon$$

in which
l is the optical width in cm
n is the number of residues
c is the molar concentration of protein
$\Delta\varepsilon$ is the molar circular dichroism
Φ is the ellipticity

Must be calculated accurately!


20
10
0
-10
-20
CD [mdeg]
20
40
60
80
Temperature [C]
240
230
220
210
200
196
Wavelength [nm]


211 nm
228.2 nm
201.3 nm
227.2 nm
212.3 nm
CD [mdeg]
-3
-5
-10
-15
-17
HT [V]
309
308
306
304
302
300
20
40
60
8
Temperature [C]



Structural Analysis of the G-Box Domain of the Microcephaly Protein CPAP Suggests a Role in Centriole Architecture

Georgios N. Hatzopoulos,[1] Michèle C. Erat,[1] Erin Cutts,[1] Kacper B. Rogala,[1] Leanne M. Slater,[1] Philip J. Stansfeld,[1] and Ioannis Vakonakis[1,*]

Microscale Thermophoresis

The Monolith NT.115 can measure KDs between 1nM and 500mM.

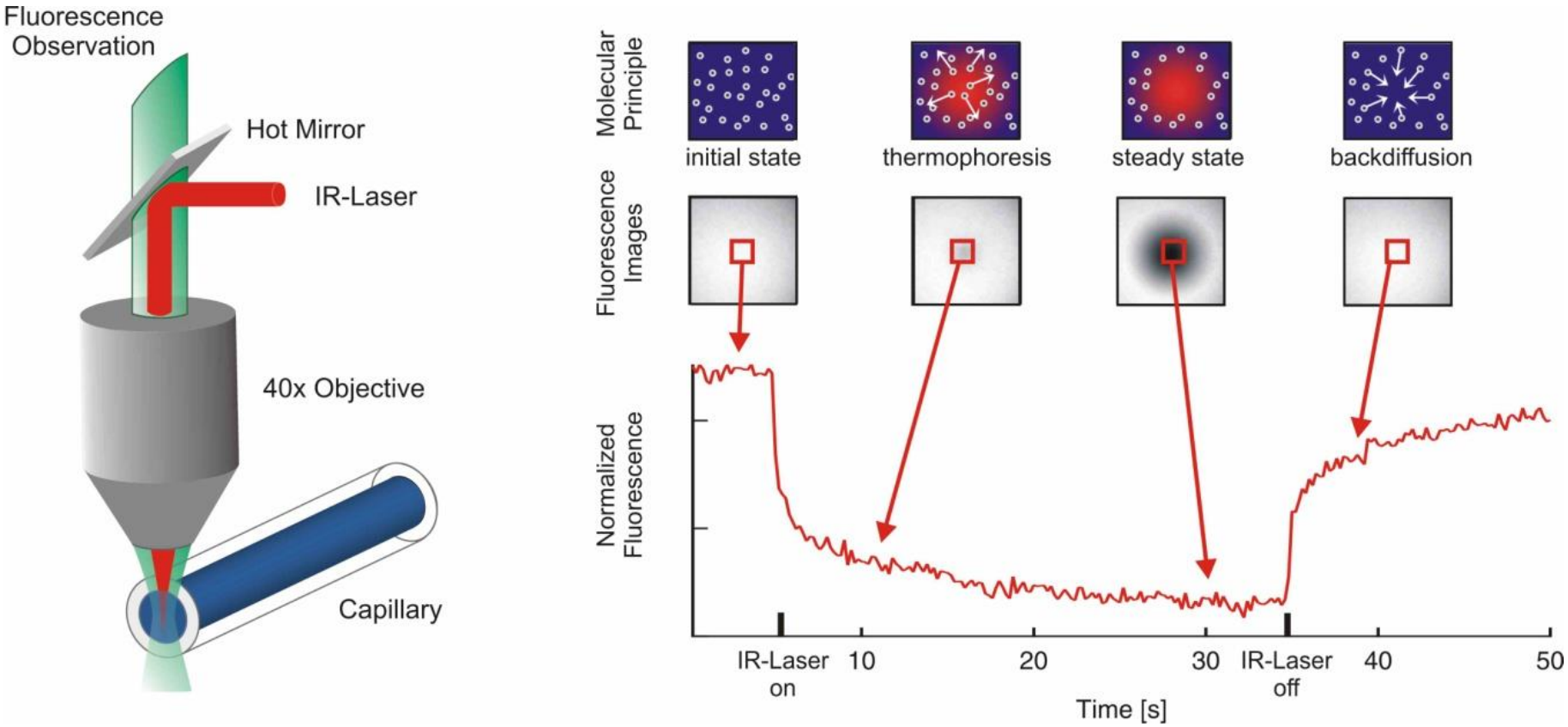
The fluorescence label can be on either component.

It can measure the binding of small molecules (300Da) or ions to a target and it can be used with big complexes e.g. ribosomes.

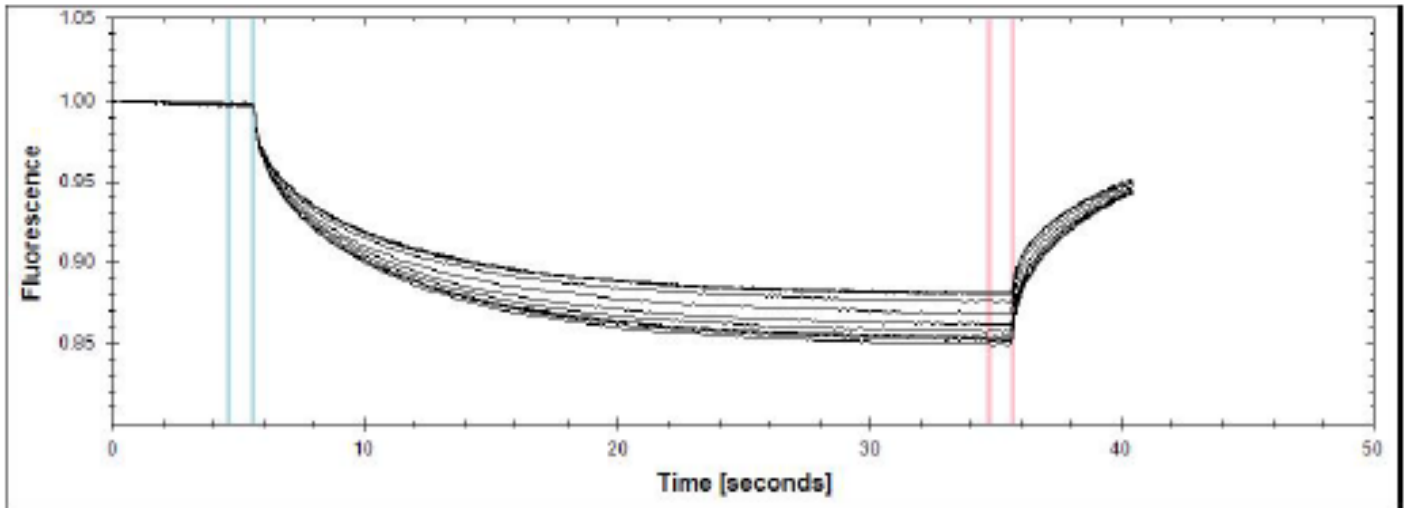
The principal is similar to shift mapping in NMR where you are looking at multiple effects (mass, electrostatics, etc) that give you an indication of the bound vs unbound state.

For an experiment you need as much of the labelled material as you can detect (~1nM) and then the unlabelled component straddles the dissociation constant by a factor of 10.

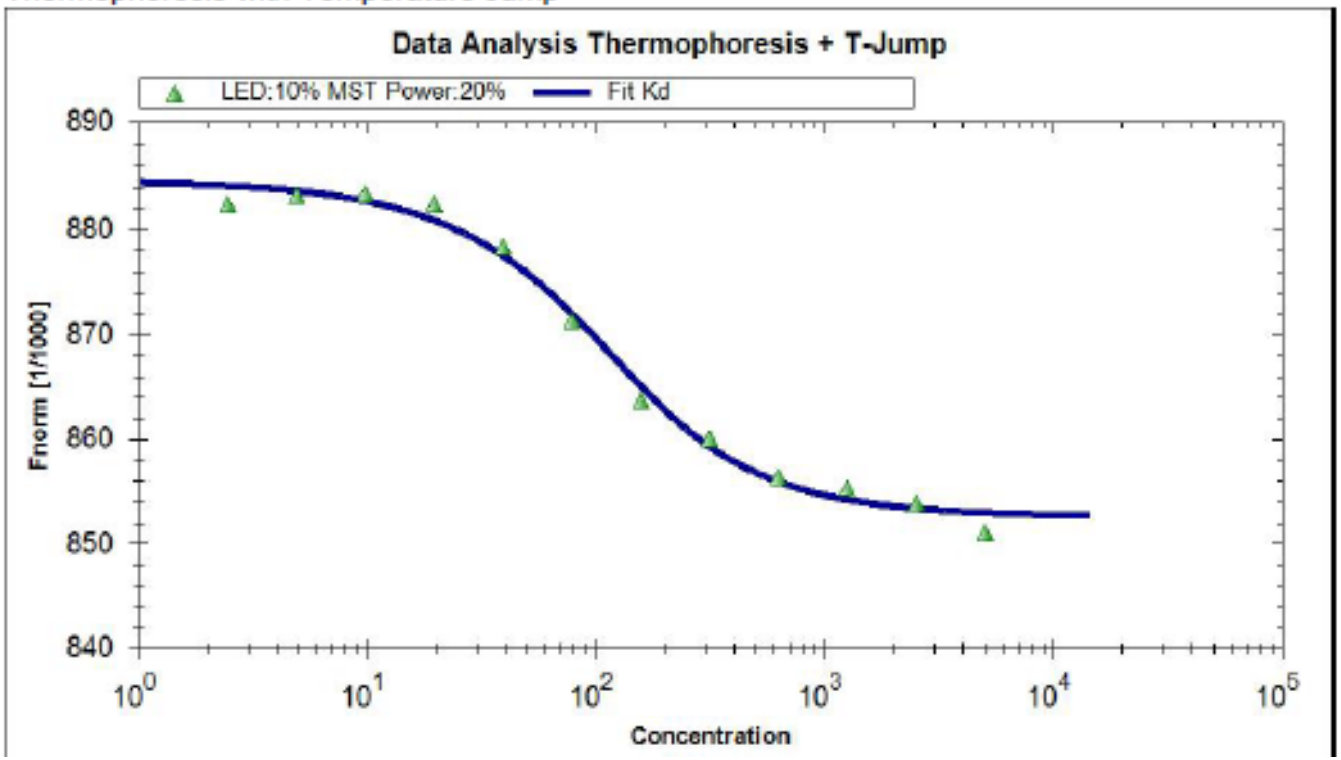
Although you only use 4ul per capillary in the instrument you need 20ul for each titration point just to avoid pipetting errors.


Fluorescence
Observation
Hot Mirror
IR-Laser
40x Objective
Capillary
Molecular
Principle
initial state
thermophoresis
steady state
backdiffusion
Fluorescence
Images
Normalized
Fluorescence
IR-Laser
on
10
20
30
IR-Laser
off
40
50
Time [s]

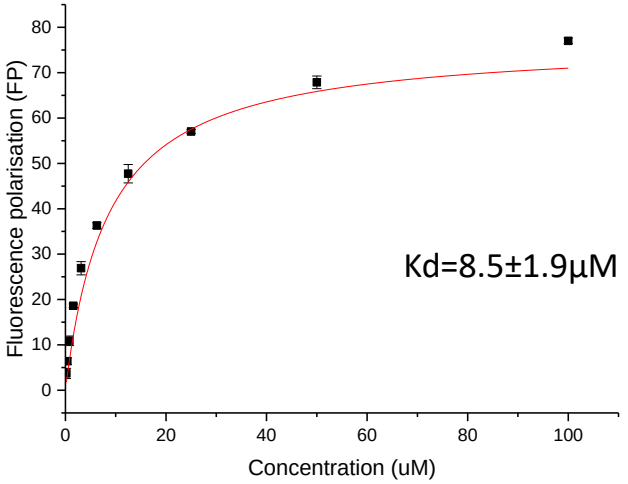
Normalized Fluorescence Timetrace

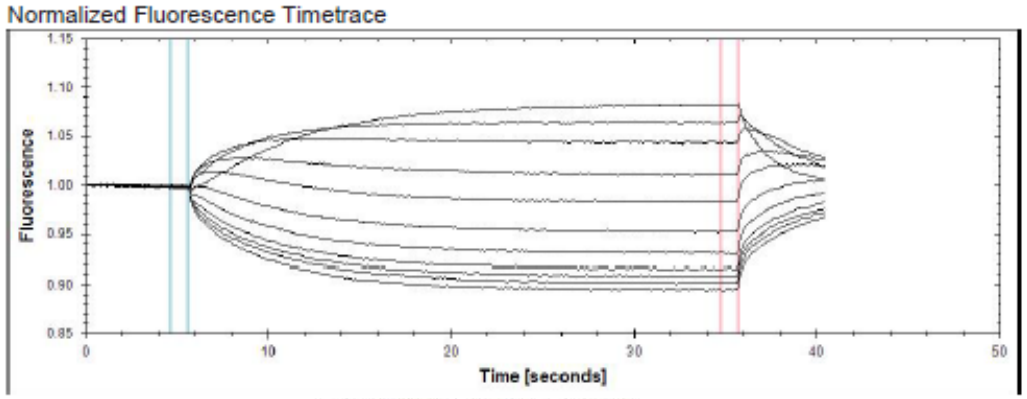


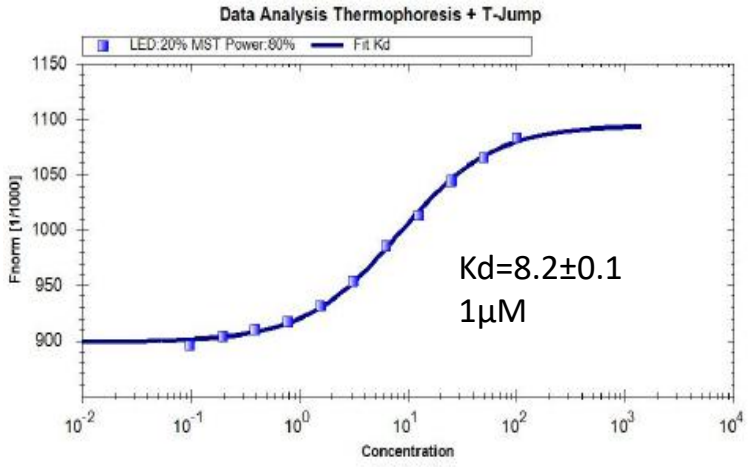
Thermophoresis with Temperature Jump

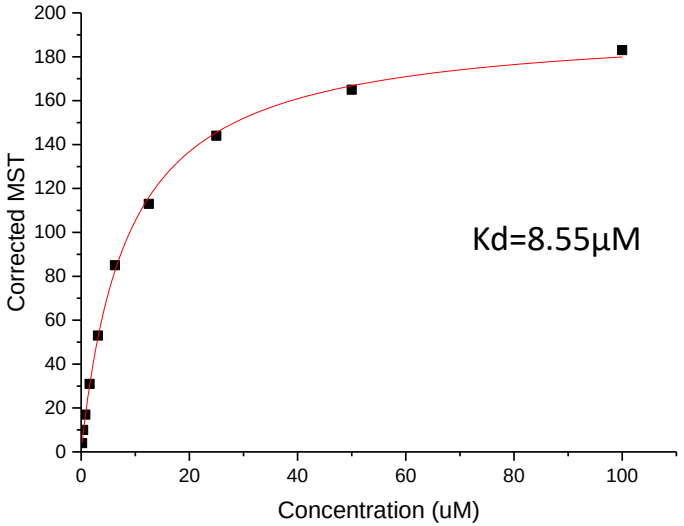


Use the same samples in another technique e.g. FP and MST.
Fluorescein type label used in FP. Samples in the multi well plate can be directly loaded into capillaries for MST analysis.
Note that discrepancies in KD values can arise from analysis method. Try to analyse identically.









Surface Plasmon Resonance Biacore T200

Same principles as 2000 but greater sensitivity, throughput, sample temperature control.

Allows small molecule analytes

Software idiot proof

Sensor chips expensive but once immobilisation and regeneration conditions established very cost effective.

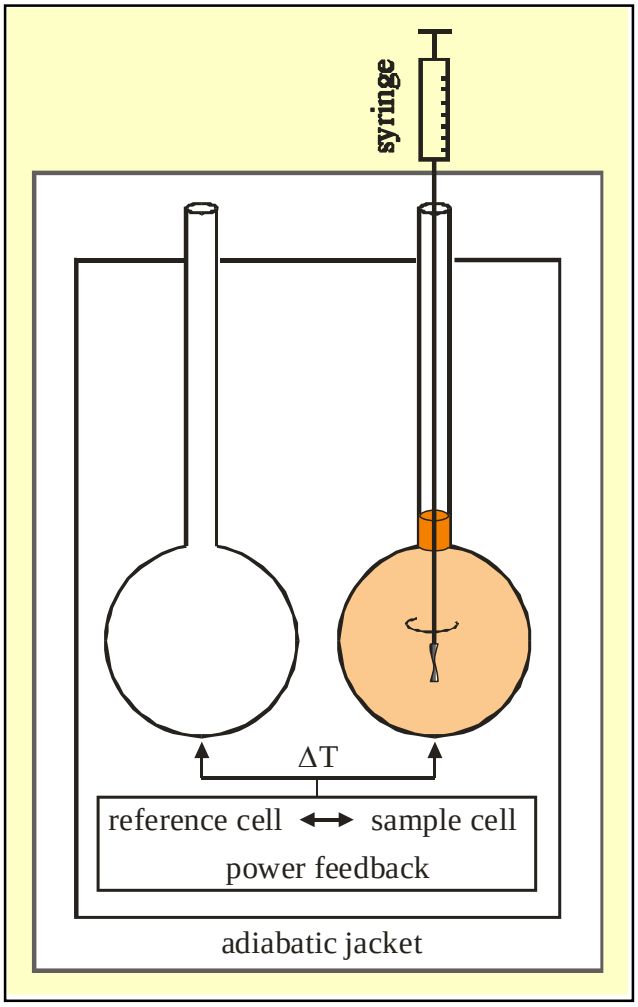
Comparison of binding

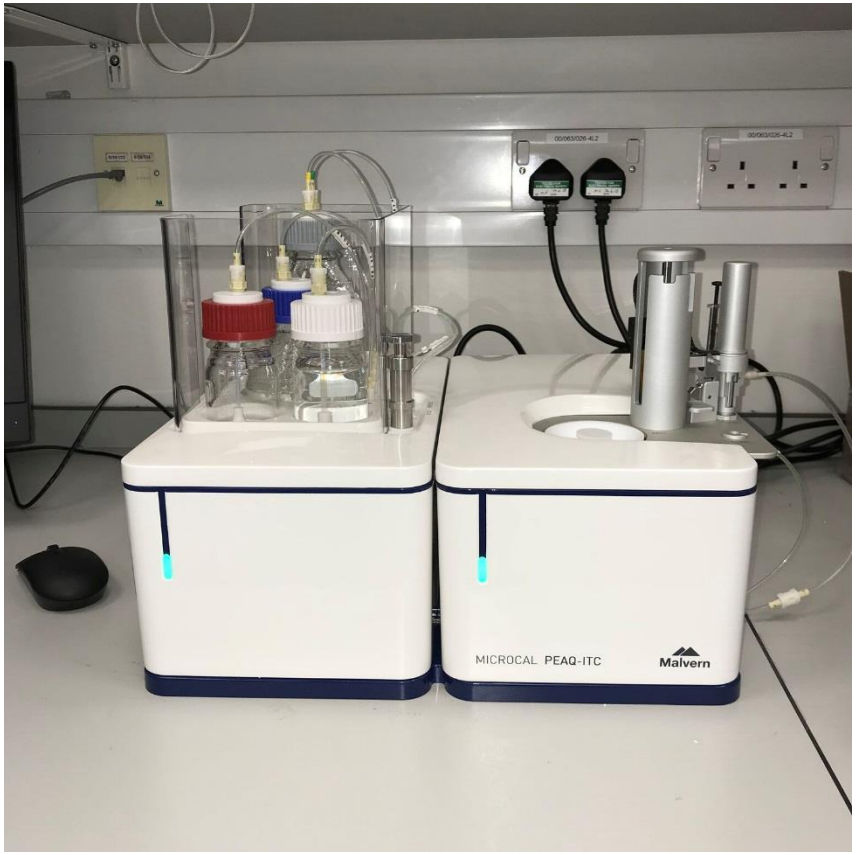
Determination of binding constants

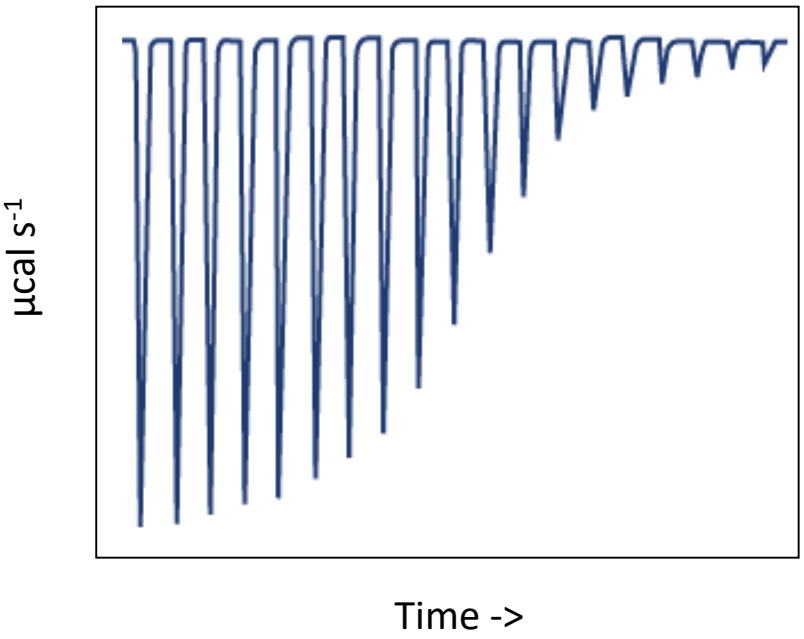
Small molecule screening

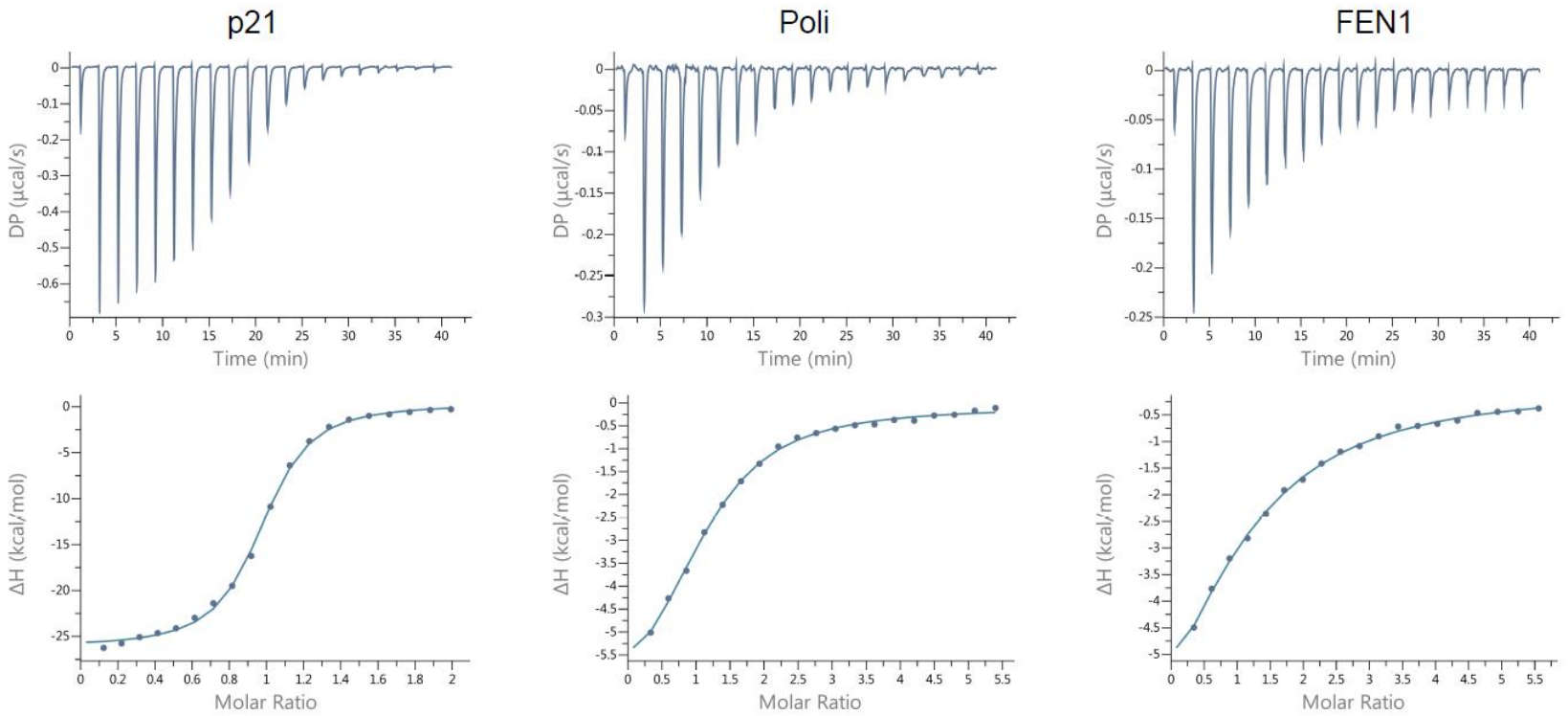
Usually require ug of material

PEAQ-ITC


syringe
ΔT
reference cell ↔ sample cell
power feedback
adiabatic jacket


MICROCAL PEAQ-ITC
Malvern


µcal s-1
Time ->
0
-2
-4
kcal mol-1 of injectant
ΔH
Kd
N
0
0.5
1.0
1.5
2.0
Molar ratio



Sebesta et al (2017) Structural insights into the function of ZRANB3 in replication stress response. *Nat Commun*. **8**, 15847

Fluorescence

Protein concs – about 0.05 mg/ml or 1µM
Buffers – do not absorb light at wavelengths used
Inner filter effects
- Becomes significant at absorptions above 0.05 at excitation wavelength
- Check that other proteins, nucleotides and buffer do not exceed this level
- Equation to calculate
  - Kubista, M., Sjoback, R., Eriksson, S., and Albinsson, B. (1994) *Analyst 119*, 417-419

Accurate temperature control important

Instruments daily rate	Current charges for University users £
ESI-MS (per sample)	25
SPR	150
ITC	150
DSC (per sample)	25
AUC	85
CD	50
Thermofluor (per run)	25
DLS	10
Fluorimetry	50
SEC-MALS (per sample)	50
Stopped flow	100
Thermophoresis (per run)	25

http://www.bioch.ox.ac.uk/molecular_biophysics_suite/

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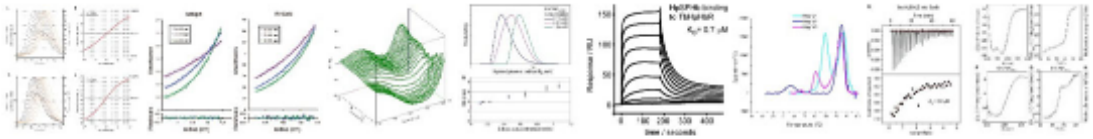

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Monolith 11.5	Microscale Thermophoresis	MST
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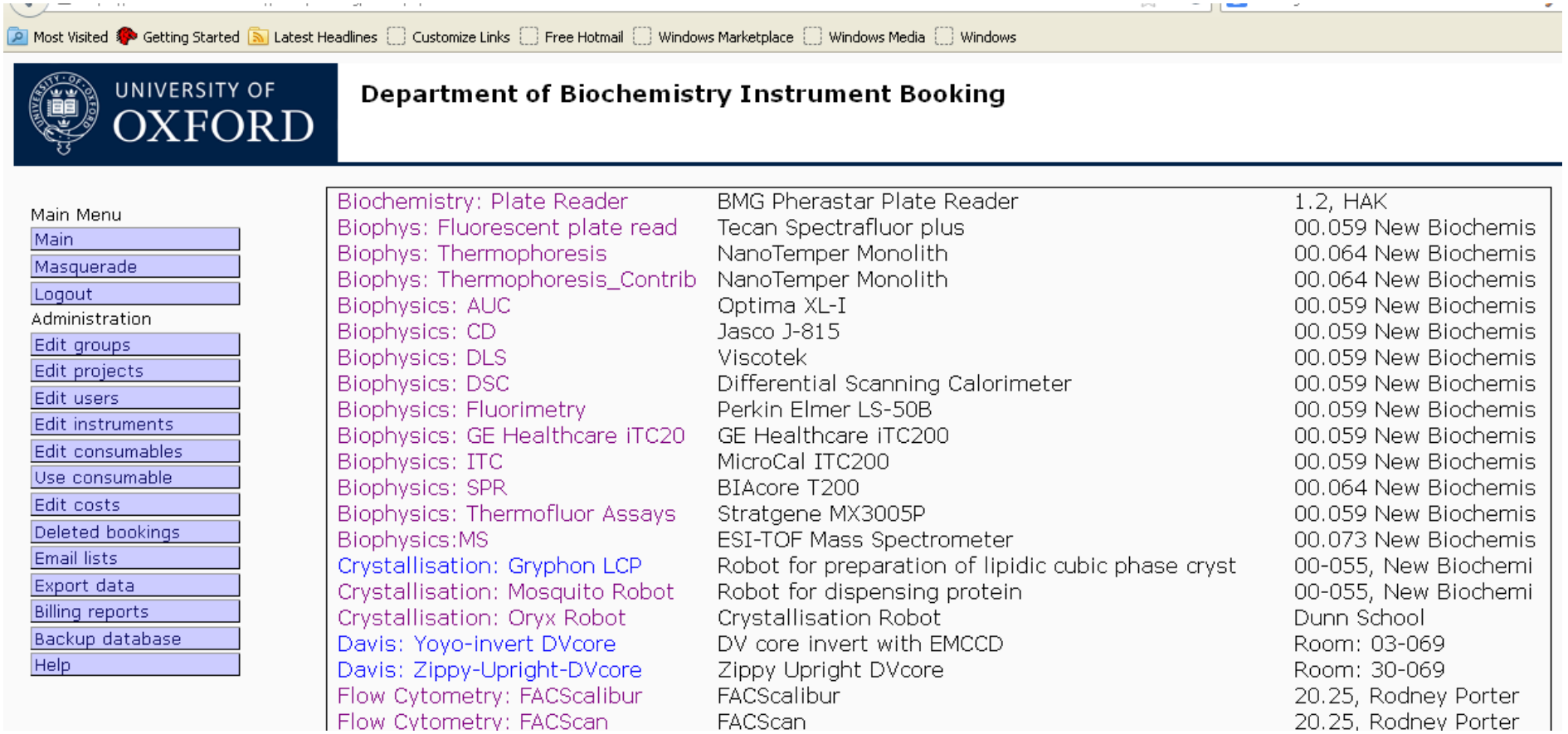
Horiba FluoroMax-4	Fluorimetry	Fluorimetry
Applied PhotophysicsSX	Stopped flow	Stopped flow


Most Visited
Getting Started
Latest Headlines
Customize Links
Free Hotmail
Windows Marketplace
Windows Media
Windows
UNIVERSITY OF OXFORD
Department of Biochemistry Instrument Booking
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Biochemistry: Plate Reader BMG Pherastar Plate Reader 1.2, HAK
Biophys: Fluorescent plate read Tecan Spectrafluor plus 00.059 New Biochemis
Biophys: Thermophoresis NanoTemper Monolith 00.064 New Biochemis
Biophys: Thermophoresis_Contrib NanoTemper Monolith 00.064 New Biochemis
Biophysics: AUC Optima XL-I 00.059 New Biochemis
Biophysics: CD Jasco J-815 00.059 New Biochemis
Biophysics: DLS Viscotek 00.059 New Biochemis
Biophysics: DSC Differential Scanning Calorimeter 00.059 New Biochemis
Biophysics: Fluorimetry Perkin Elmer LS-50B 00.059 New Biochemis
Biophysics: GE Healthcare iTC20 GE Healthcare iTC200 00.059 New Biochemis
Biophysics: ITC MicroCal ITC200 00.059 New Biochemis
Biophysics: SPR BIAcore T200 00.064 New Biochemis
Biophysics: Thermofluor Assays Stratgene MX3005P 00.059 New Biochemis
Biophysics:MS ESI-TOF Mass Spectrometer 00.073 New Biochemis
Crystallisation: Gryphon LCP Robot for preparation of lipidic cubic phase cryst 00-055, New Biochemi
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Crystallisation: Oryx Robot Crystallisation Robot Dunn School
Davis: Yoyo-invert DVcore DV core invert with EMCCD Room: 03-069
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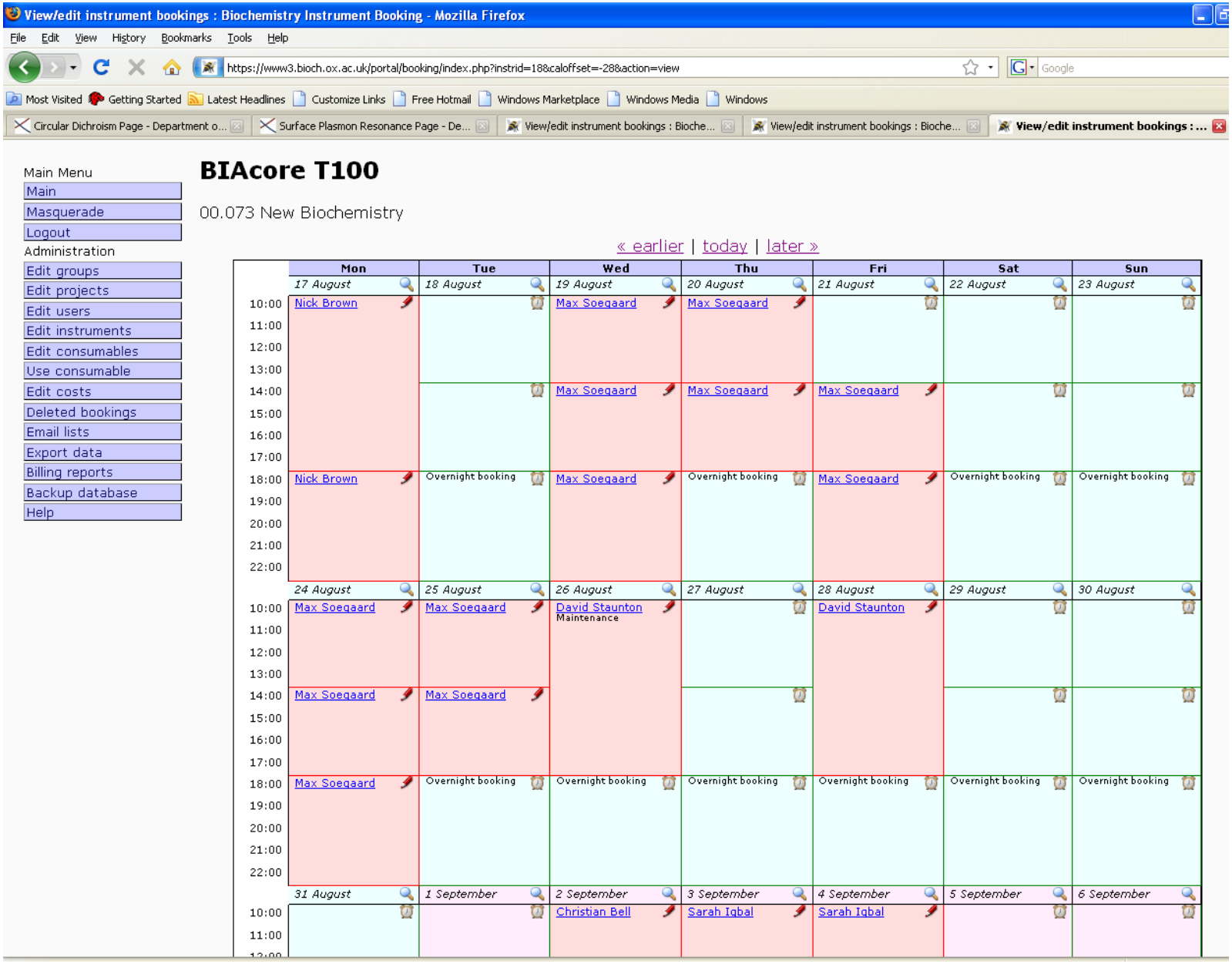
Flow Cytometry: FACScalibur FACScalibur 20.25, Rodney Porter
Flow Cytometry: FACScan FACScan 20.25, Rodney Porter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17 August 18 August 19 August 20 August 21 August 22 August 23 August
10:00 Nick Brown Max Soegaard Max Soegaard
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31 August 1 September 2 September 3 September 4 September 5 September 6 September
10:00 Christian Bell Sarah Iqbal Sarah Iqbal
11:00

Introduction to MBS

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