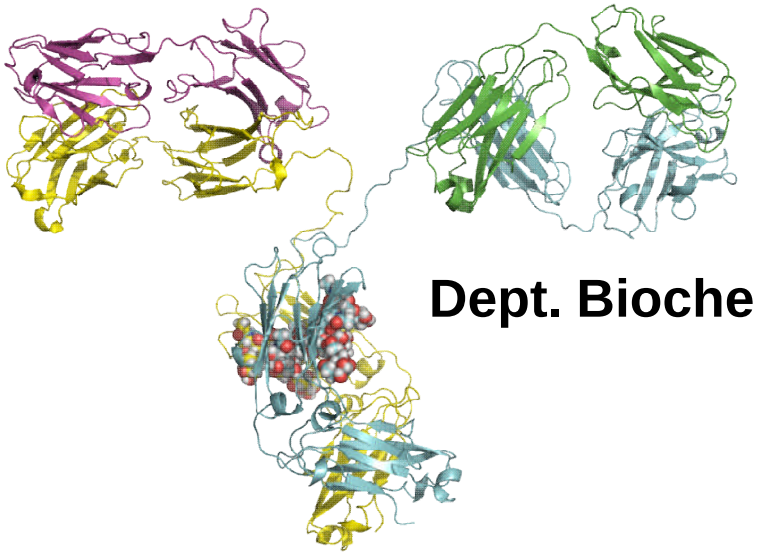
UCL

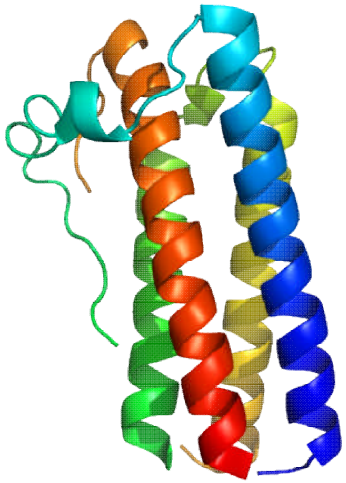
EPSRC CENTRE for Innovative Manufacturing in Emergent Macromolecular Therapies

Screen early - fail early: Rapid analytical screens for protein formulation



Paul Dalby

Dept. Biochemical Engineering, UCL



EPSRC CENTRE for Innovative Manufacturing in Emergent Macromolecular Therapies

EPSRC Centre for Innovative Manufacturing

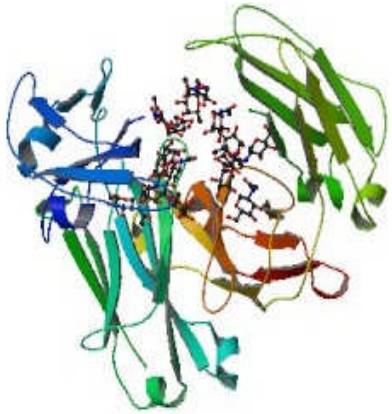
UCL

The School of Pharmacy University of London

Imperial College London

Creating manufacturing innovations so as to deliver affordable next generation advanced therapies to the UK healthcare system

Biopharmaceutical Lifecycle Optimisation



Complex macromolecular candidates

Optimise manufacturability and lifecycle costs

Affordable advanced therapies

Healthcare delivery to patient

UCL

The School of Pharmacy University of London

Imperial College London

Project Partners. Industry Associations: ABPI, BIA, HealthTech & Medicines KTN. **Companies:** Aegis Analytical, Avacta, BioPharm Services, BTG Plc, Eli Lilly, Francis BioPharma Consulting, Fujifilm Diosynth Biotechnologies, GE Healthcare, GlaxoSmithKline, Lonza Biologics, MedImmune, Merck & Co, MSD Biologics, Novo Nordisk, Novozymes Biopharma, Office of Health Economics (OHE), Pfizer, Syntaxin, The Automation Partnership (TAP), UCB Pharma. **Government-related:** Health Protection Agency, NIBSC, NHS QIPP.

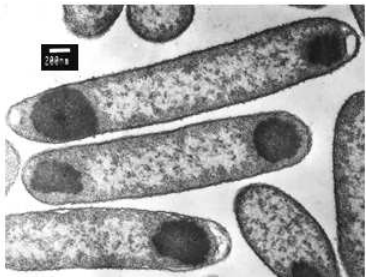
Centre Team & Consortium

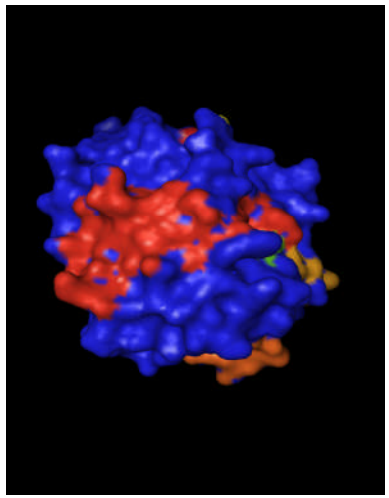
- *Collaboration initiated by an academic core*:-
  UCL – Biochemical Engineering (Lead)
  Chemical Engineering
  Health Economics
  LSoP – Formulation Engineering
  ICL – Chemical Engineering

- *Supported by a group of*:-
  25 industrial users including SMEs,
  7 NGOs / Industry Associations

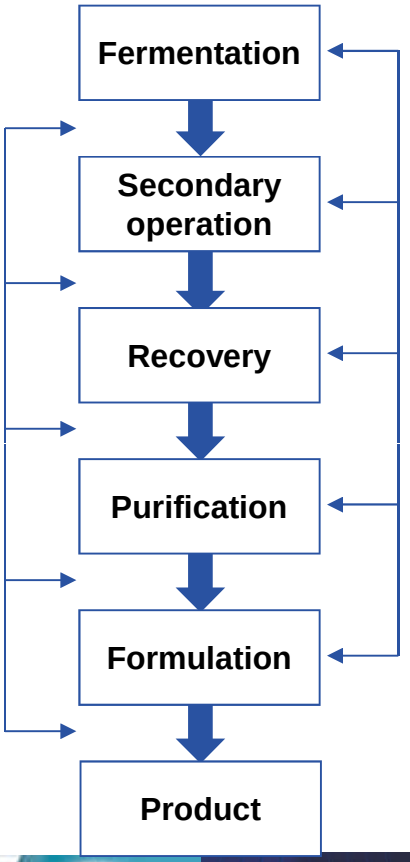
- *Extended by a network of*:-
  23 national & international academics

When does formulation start?

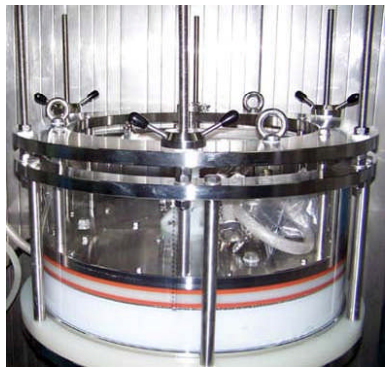


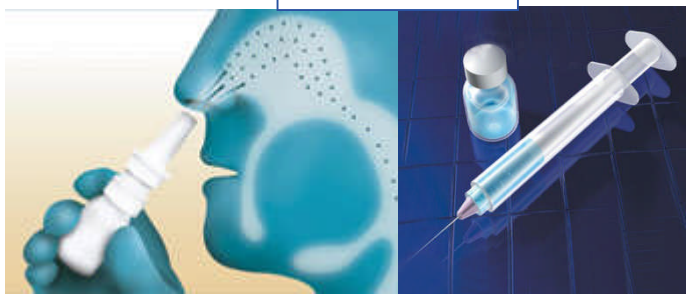


Biomaterial parameters



Operational parameters





Paradigms to improve “formulatability”

1. “Screen early: fail early”

- Find "troublemaker" proteins earlier
- Provide appropriate “stress tests" for formulation engineers

2. “Better by design”

- Develop robustly manufacturable protein scaffolds
- Establish predictive protein design evaluation tools
- Understand protein aggregation better

Formulation development

Design space

Protein variants | [Protein] | pH

[Buffer] & type | Ionic strength | GRAS excipients

Design of Experiment (DoE) - Stress and analyse

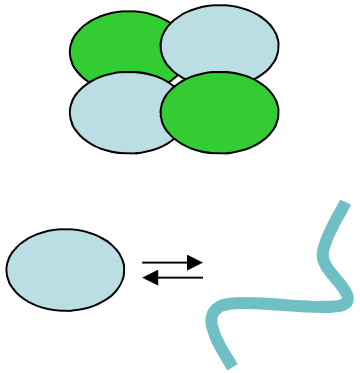
High T, low t? | Freeze-thaw? | T_m or T_{agg}? | Agitation?

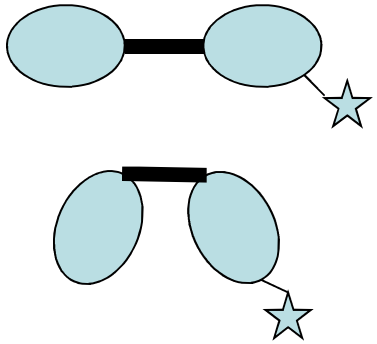
“Optimum” formulation

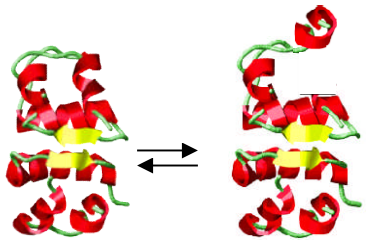
Shelf-life study

High end biophysics

Biophysical analysis of proteins







- Size
- Structure content
- Composition
- Folding extent

- Chemical modifications
- Local structure content
- Shape and conformation
- Folding dynamics

- Atomic structure
- Bond formation
- Molecular interactions
- Structural dynamics

Low resolution

CD
Fluorescence spectroscopy
DLS / particle imaging
Size exclusion chromatography
Analytical Ultracentrifugation

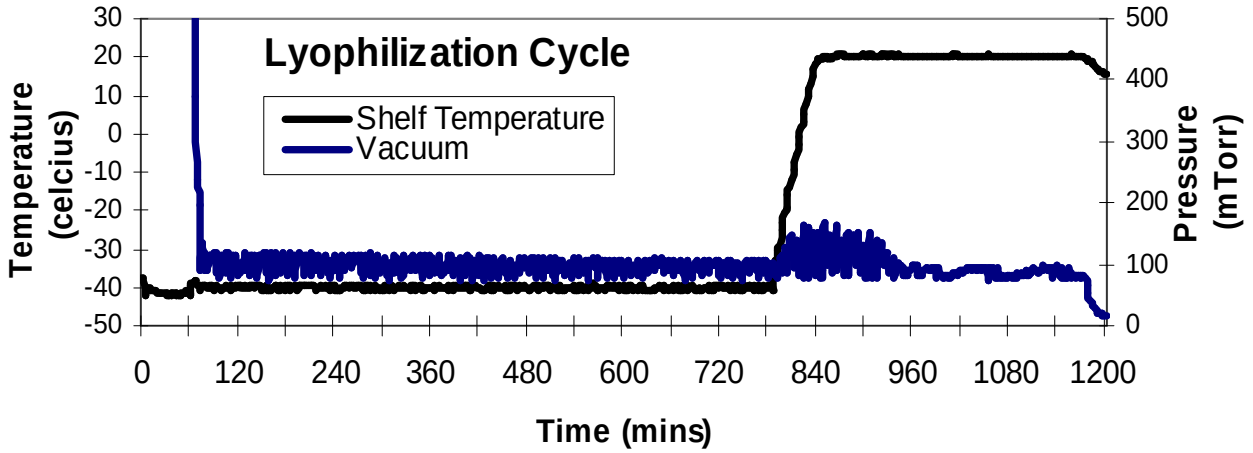
LCMS
Small angle X-ray scattering

NMR

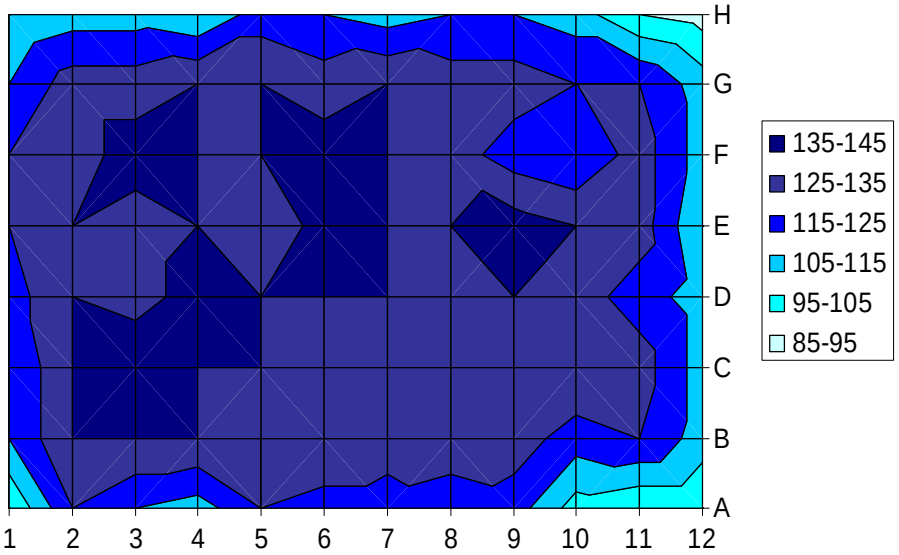
Challenges and possible solutions

- Too little material available at early bioprocess development stages
- Design space is very large for new entities
- Formulations are at high concentration (10-200 mg/ml)
- Many biophysical analyses use 0.1-2ml, 0.01-1 mg/ml
- Forced degradation is not the same as unforced degradation
  - which degradation species are a problem?
- Predict outside of measurement range
- Create lower volume analytics
- Higher throughput ***with*** high accuracy ***and*** high sensitivity
- Improve predictability of shelf-life and degradation pathways

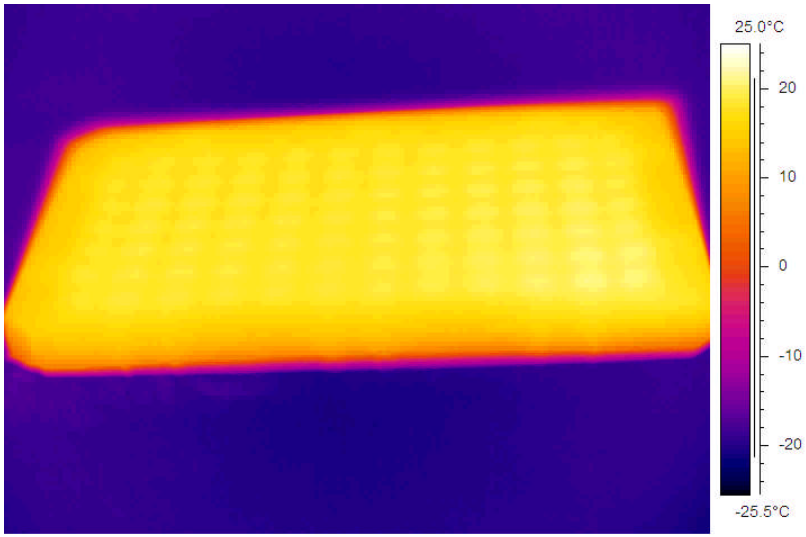
Freeze-drying in microplates



Rate of sublimation in microplate

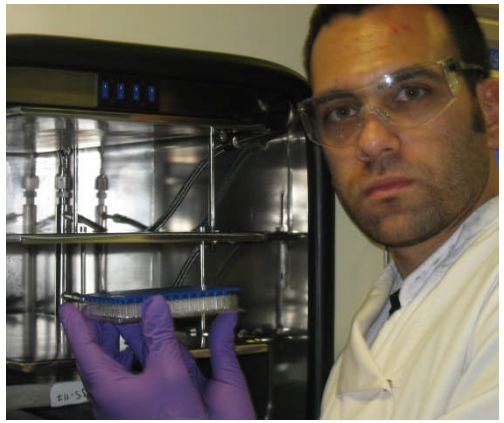


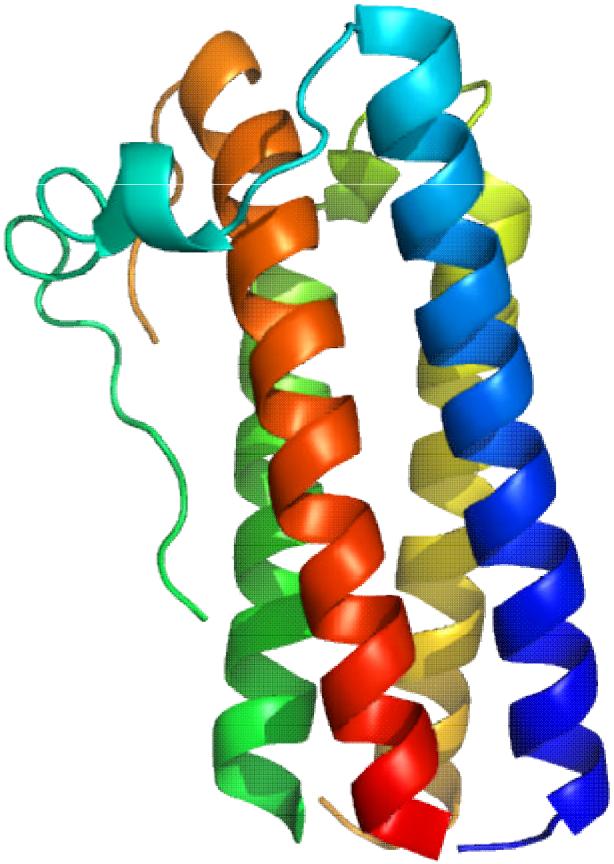
Thermal imaging of microplate freezing



Grant Y, Matejtschuk P, Dalby PA (2009) *Biotech. Bioeng.* 104:957-964. Rapid optimisation of protein freeze-drying formulations using ultra scale-down and factorial design of experiment in microplates.

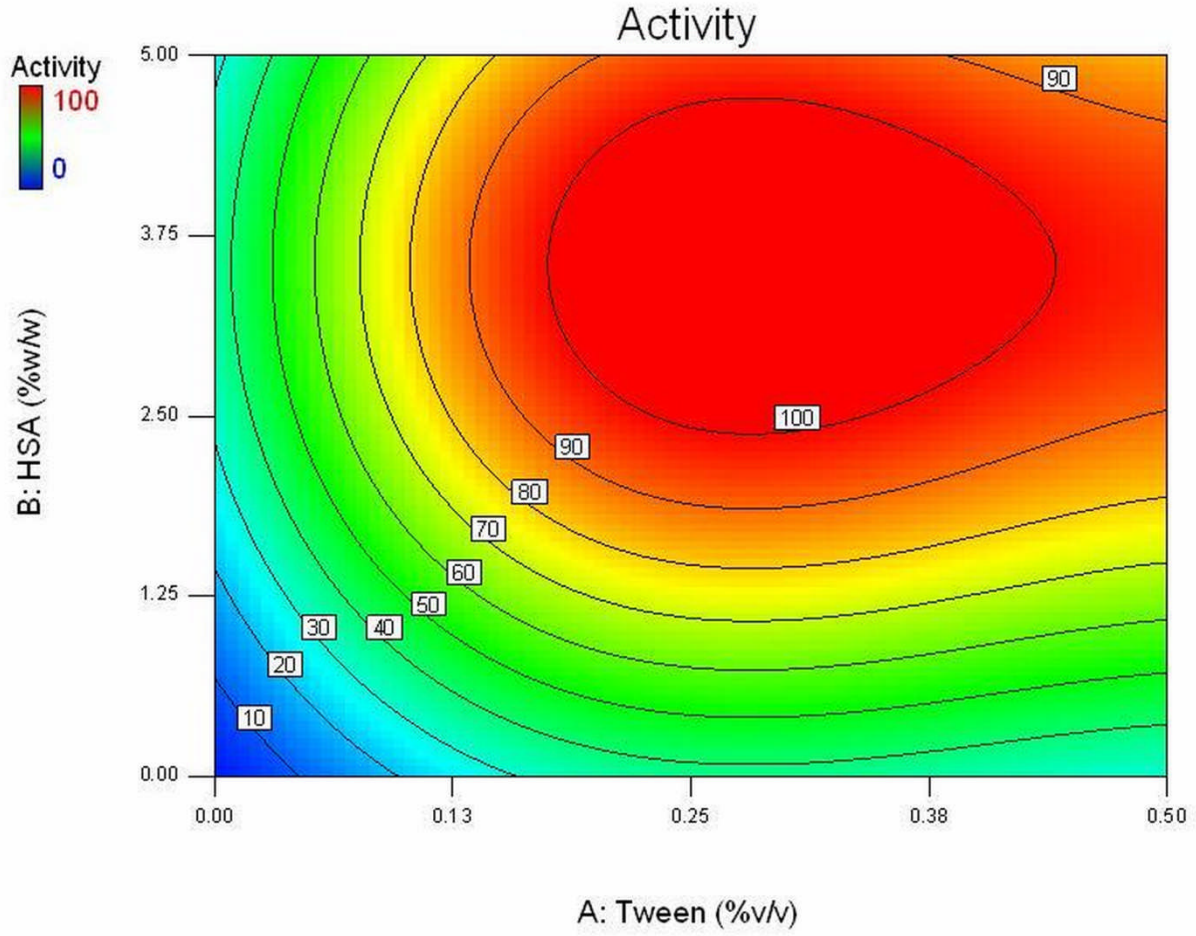
Freeze-drying optimisation - GCSF





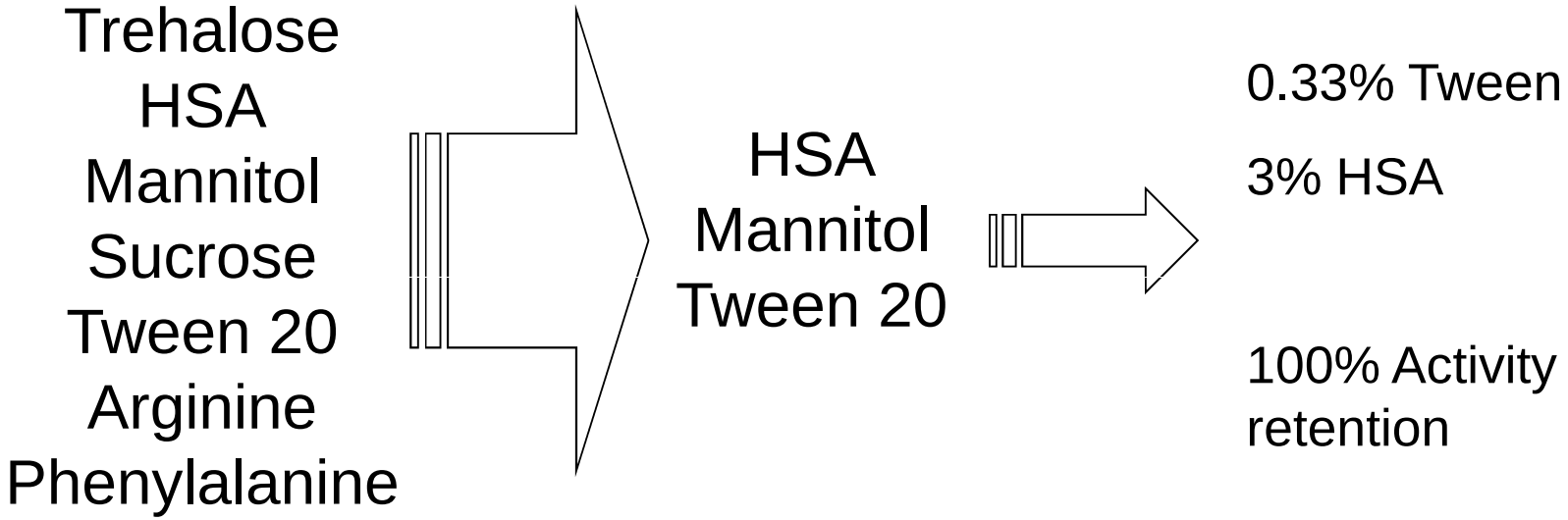
Scott Grant & Paul Matejtschuk (NIBSC)

0.1 ug/ml GCSF, pH 7



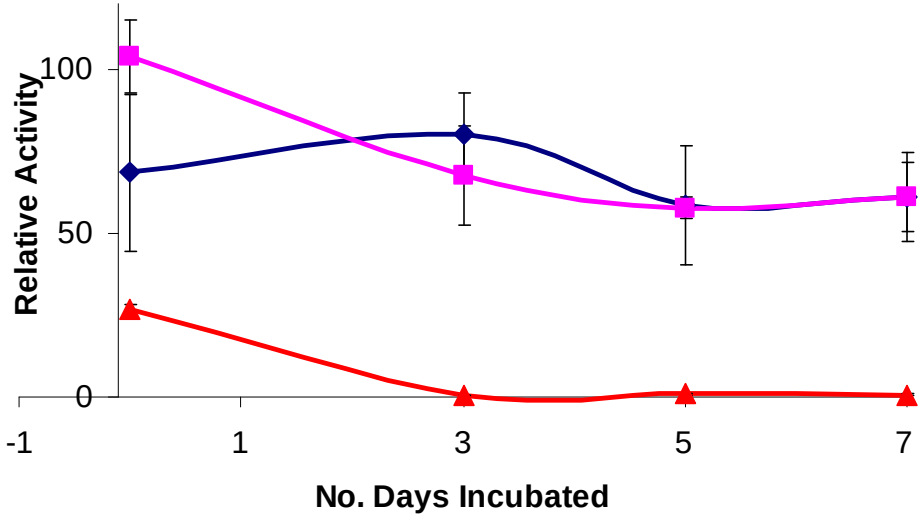
- stimulates white blood cell production
- improves recovery post-chemotherapy
- assayed by cell count after growth stimulation

Freeze-drying optimisation - GCSF



- Took two weeks and used 370 ng GCSF!

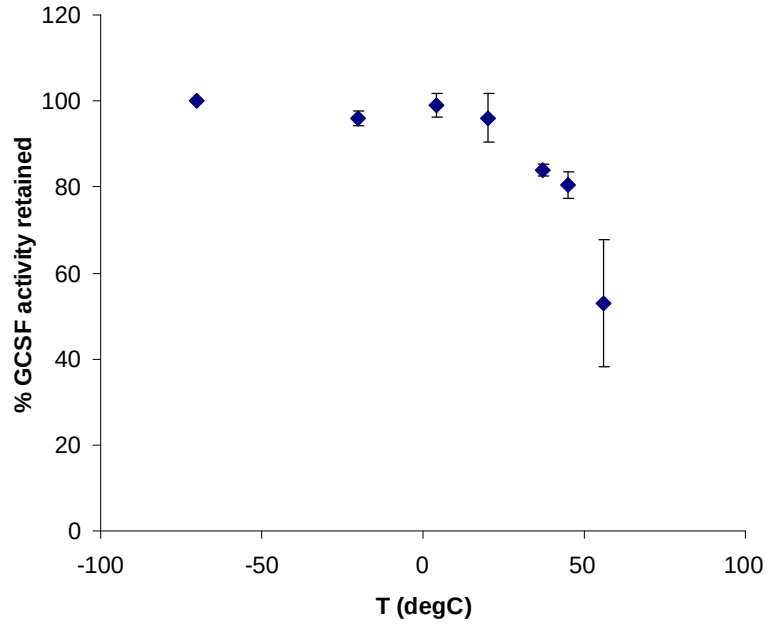
GCSF forced degradation studies

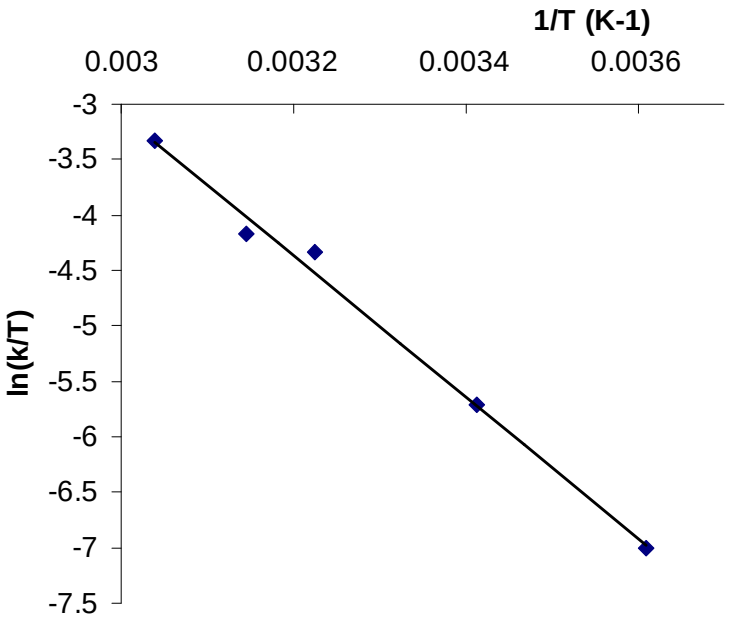


Initial comparison to unformulated GCSF

- ♦ lyophilized formulation
- ■ unlyophilized formulation
- ▲ PBS

Resuspended lyophilisates in vials: after 6 month storage at various T

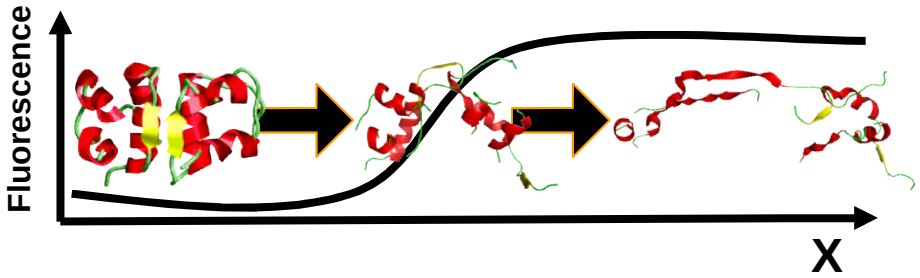




Scott Grant & Paul Matejtschuk (NIBSC)

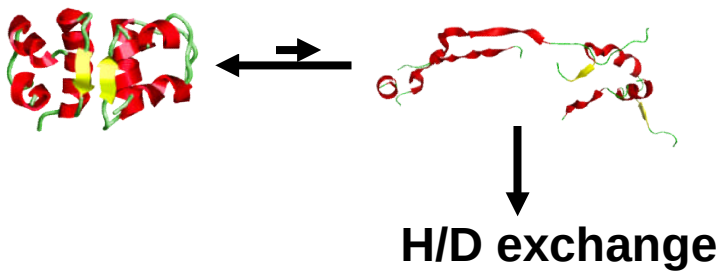
Some thermostability methods

Equilibrium denaturation:



- Capillary DSC (eg. MicroCal)
- Autotitrating fluorimeter/circular dichroism
- ANS/SYPRO binding (eg. ThermoFluor / DSF)

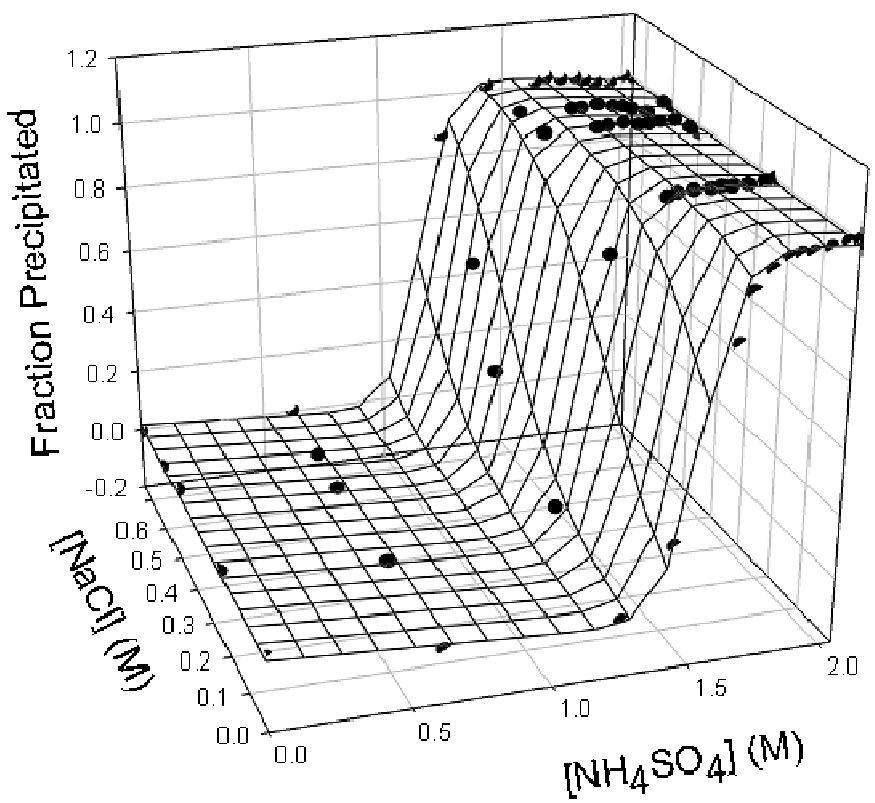
Equilibrium exchange kinetics:



- MALDI-TOF
- NMR

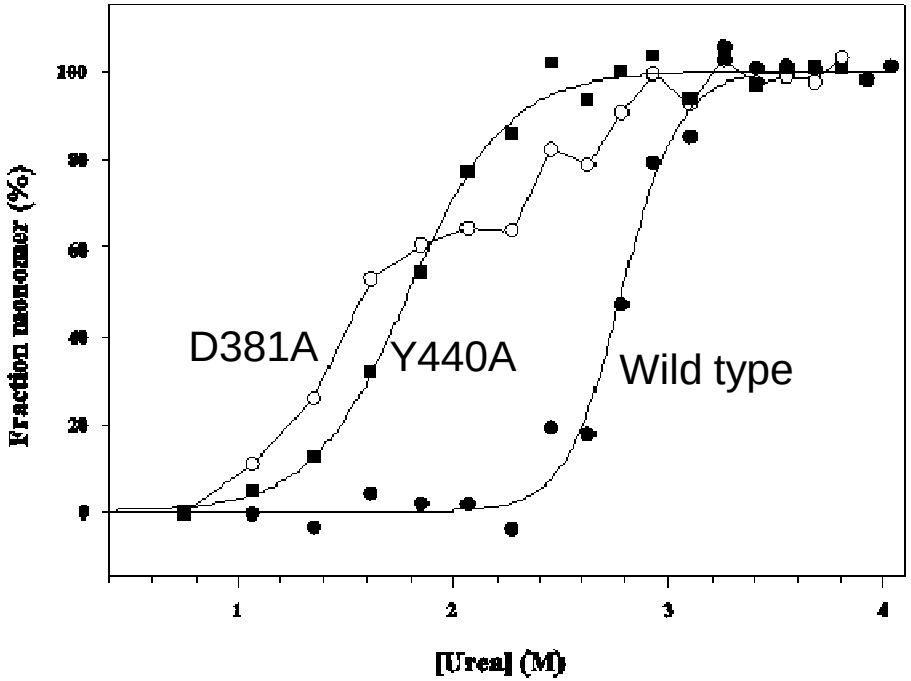
Early microplate-based screens

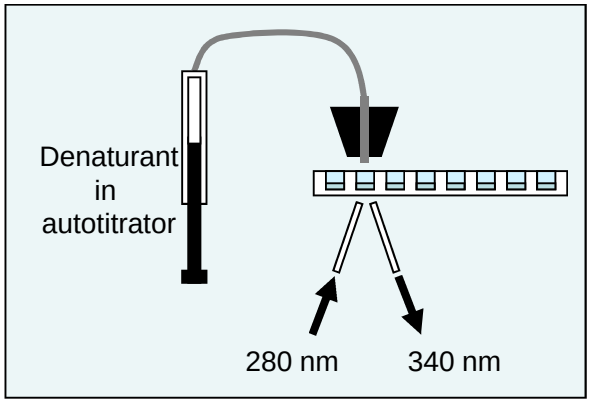
Protein precipitation / solubility



Ahmad SS, Dalby PA (2011) *Biotechnol. Bioeng.* 108:322-332. Thermodynamic parameters for salt-induced reversible protein precipitation from automated microscale experiments.

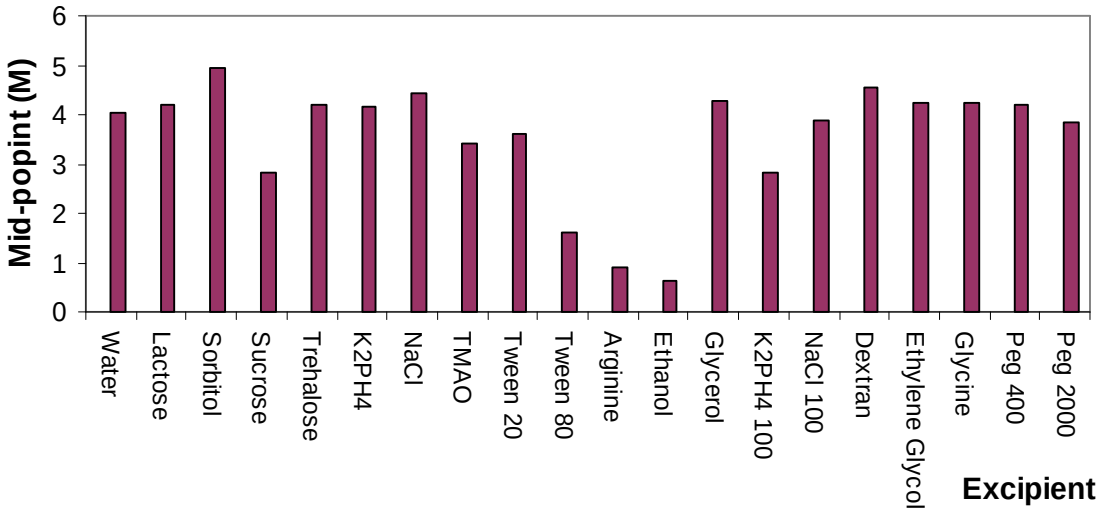
Protein stability

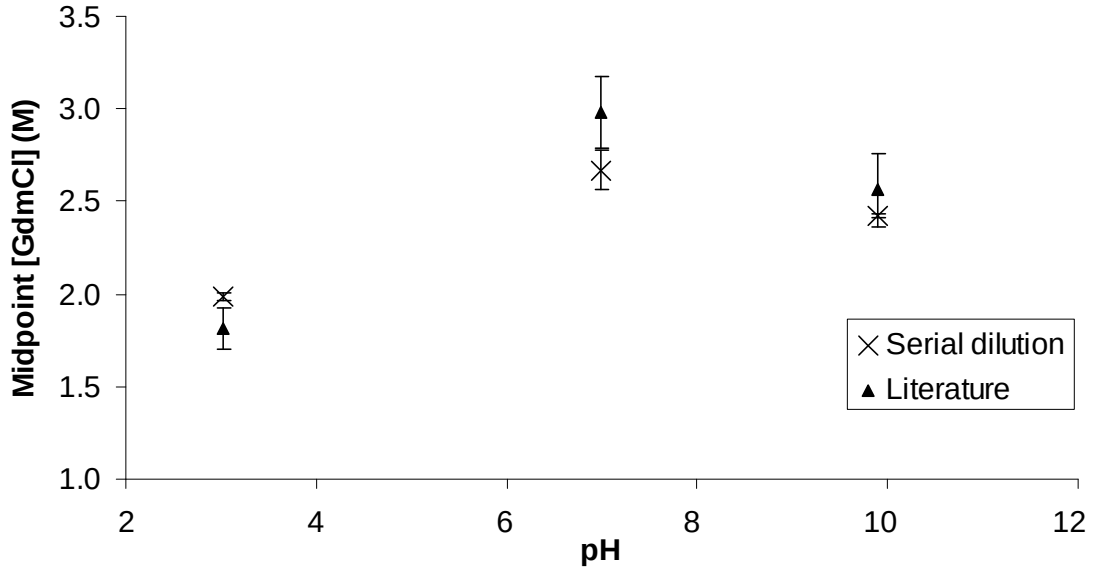




Aucamp, J. P. (2008) *Biotech Bioeng.* 99, 1303-1310
Aucamp, J. P. (2005) *Biotech Bioeng.* 89 599-607

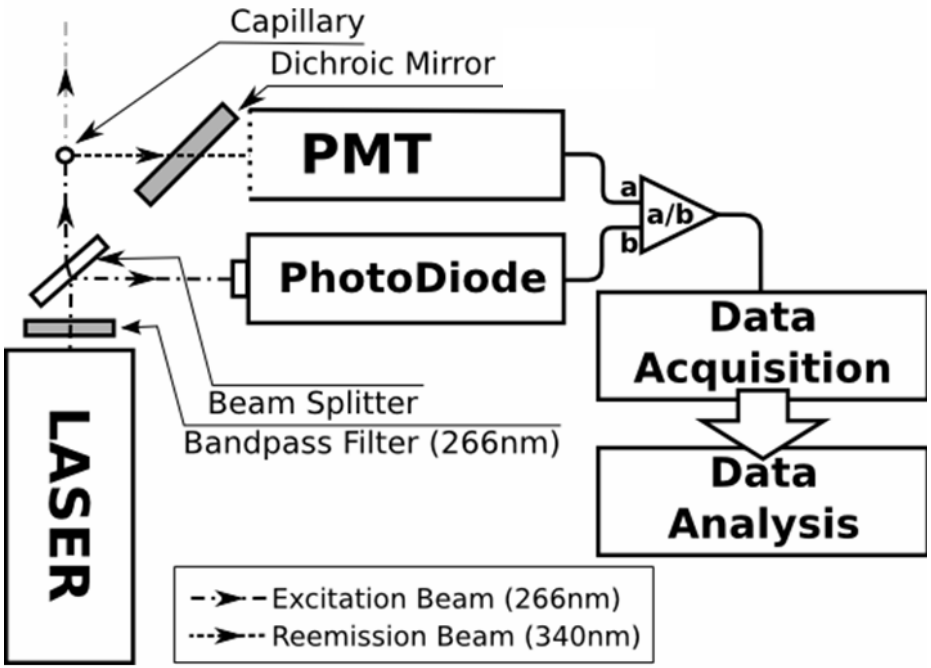
Effect of excipients on $G_{1/2}$

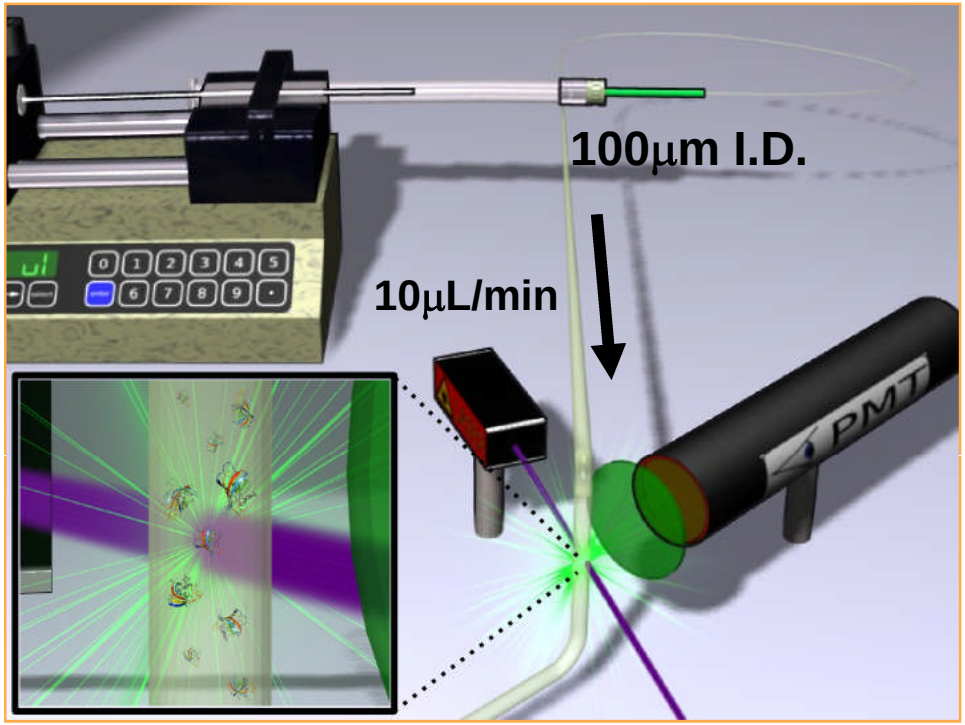


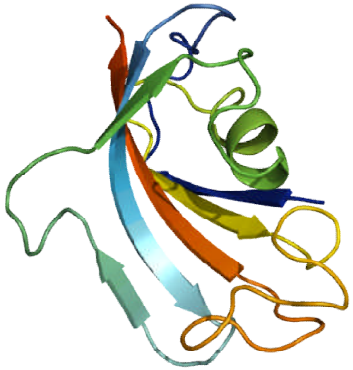


RNaseA in 50 mM formate pH3, MOPS pH7 or glycine pH9.9
Literature values from Pace *et al* 1990 *Biochemistry* 29:2564-2572

Protein stability in microfluidics



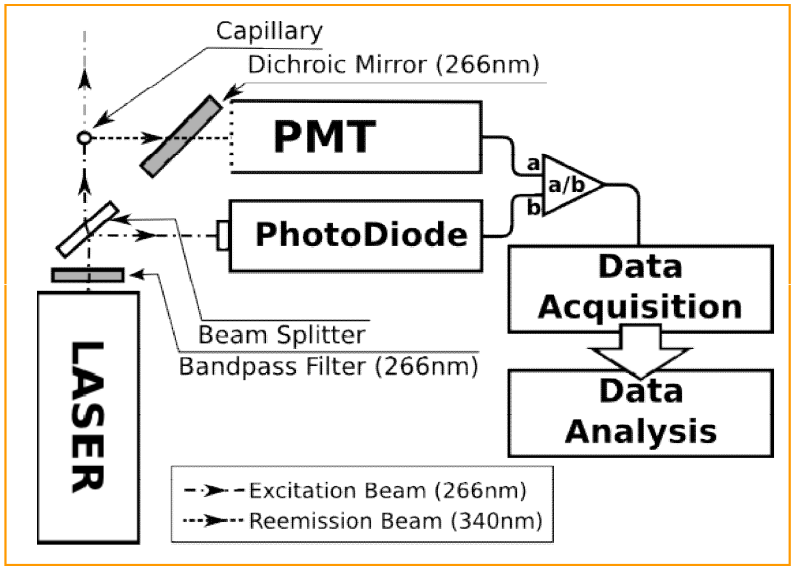




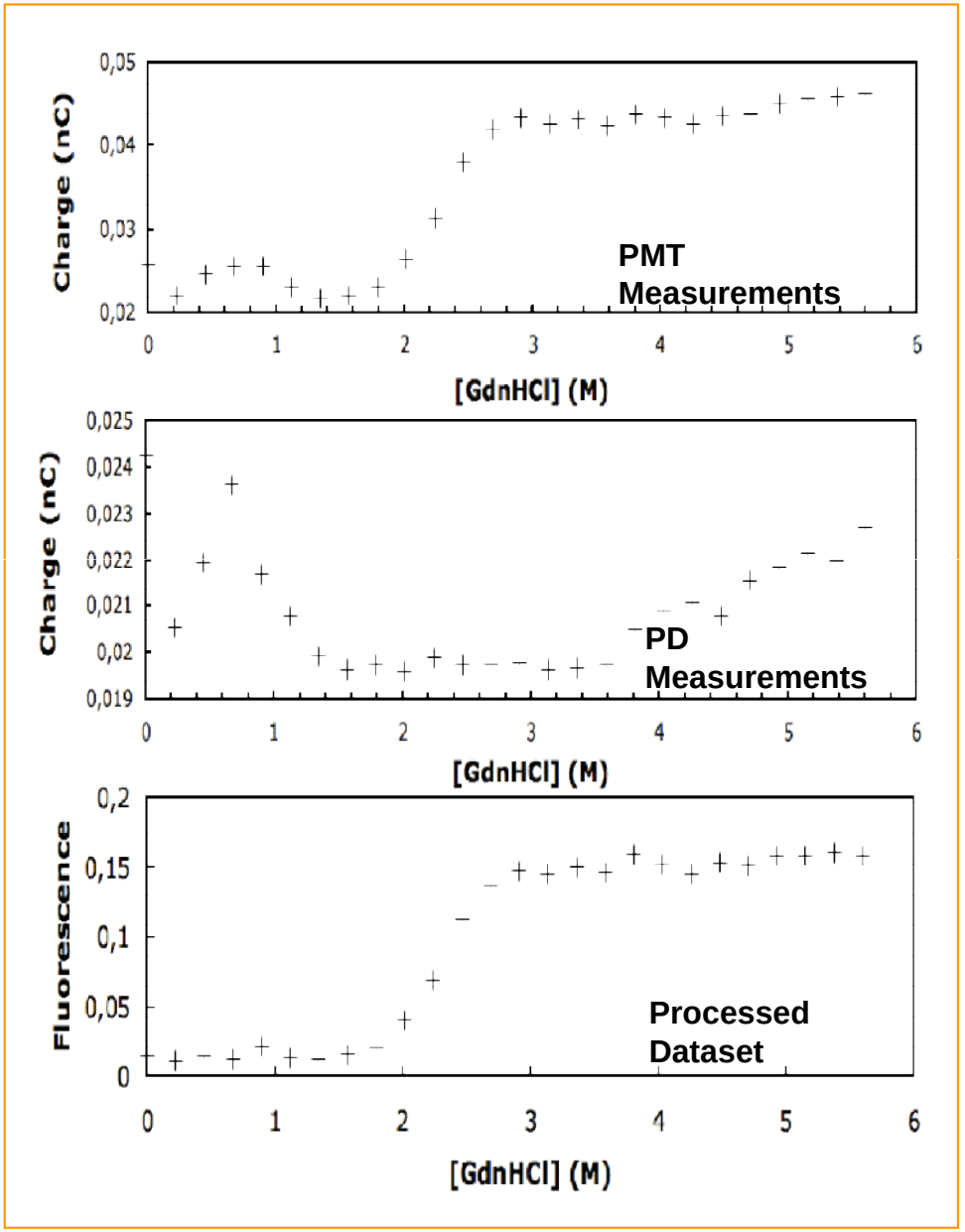
- 266 nm, 5 mW, 1 kHz pulsed UV laser
- fused-silica glass micro-capillary (ID=100 mm, OD=300 mm)
- Emission filtered by 320-400 nm dichroic mirror
- Measurement volume of 1.5 nL minimum

Gaudet M, Remtulla N, Jackson SE, Main ERG, Bracewell DG, Aeppli G, Dalby PA (2010) *Protein Science.* 19: 1544-1554. Protein denaturation and protein:drug interactions from intrinsic protein fluorescence measurements at the nanolitre scale.

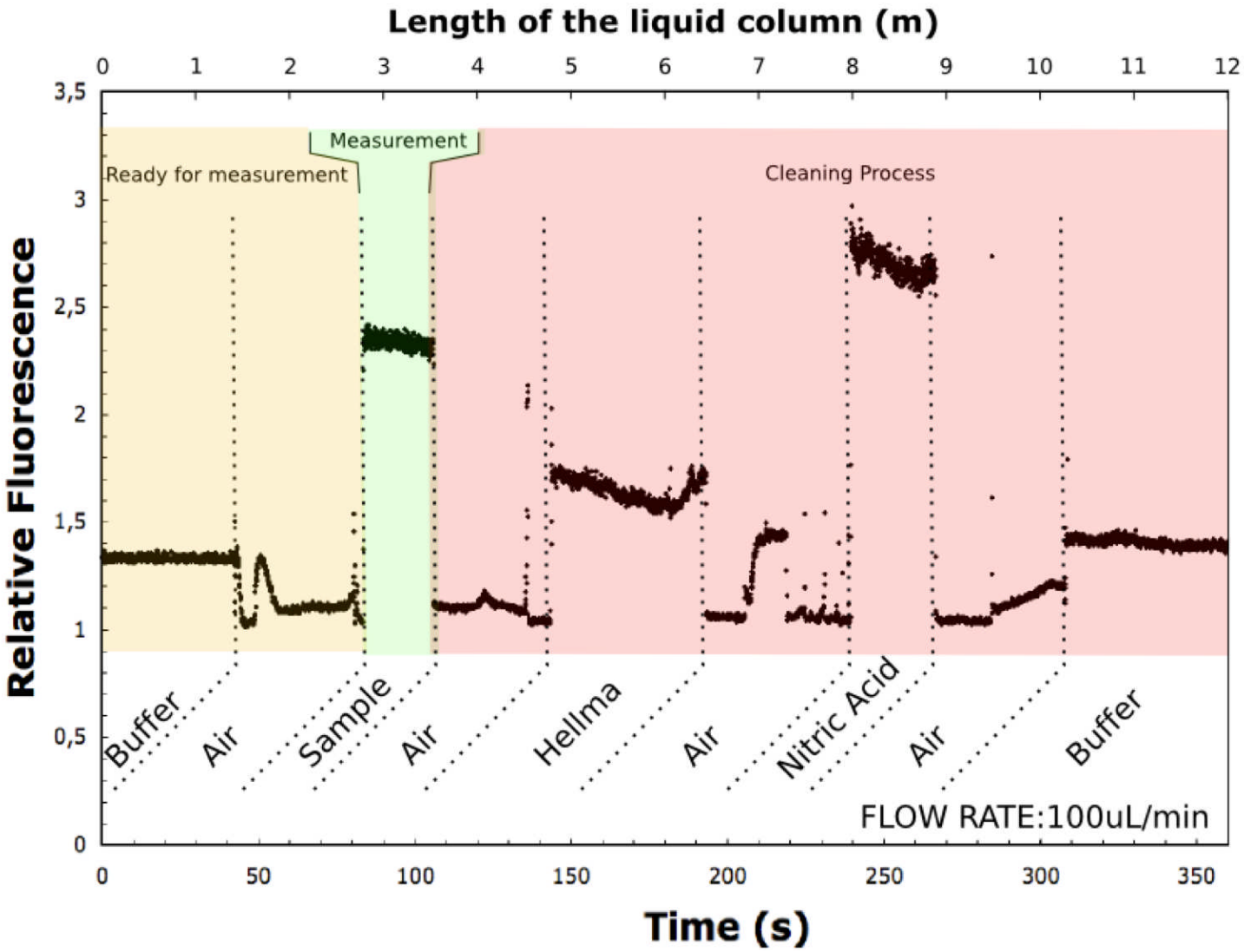
Signal processing



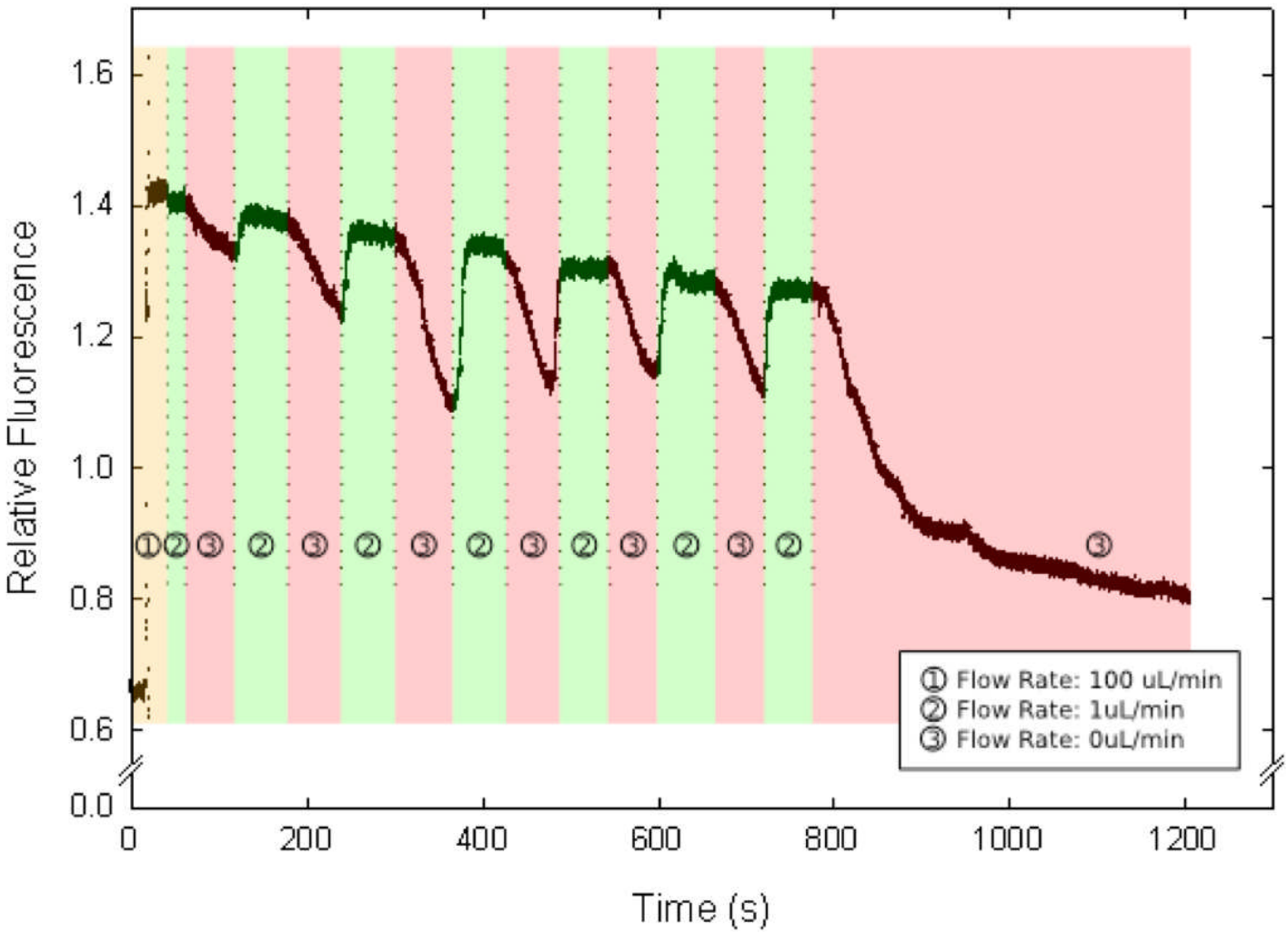
- **Beam splitter and photodiode measurement used as a reference**



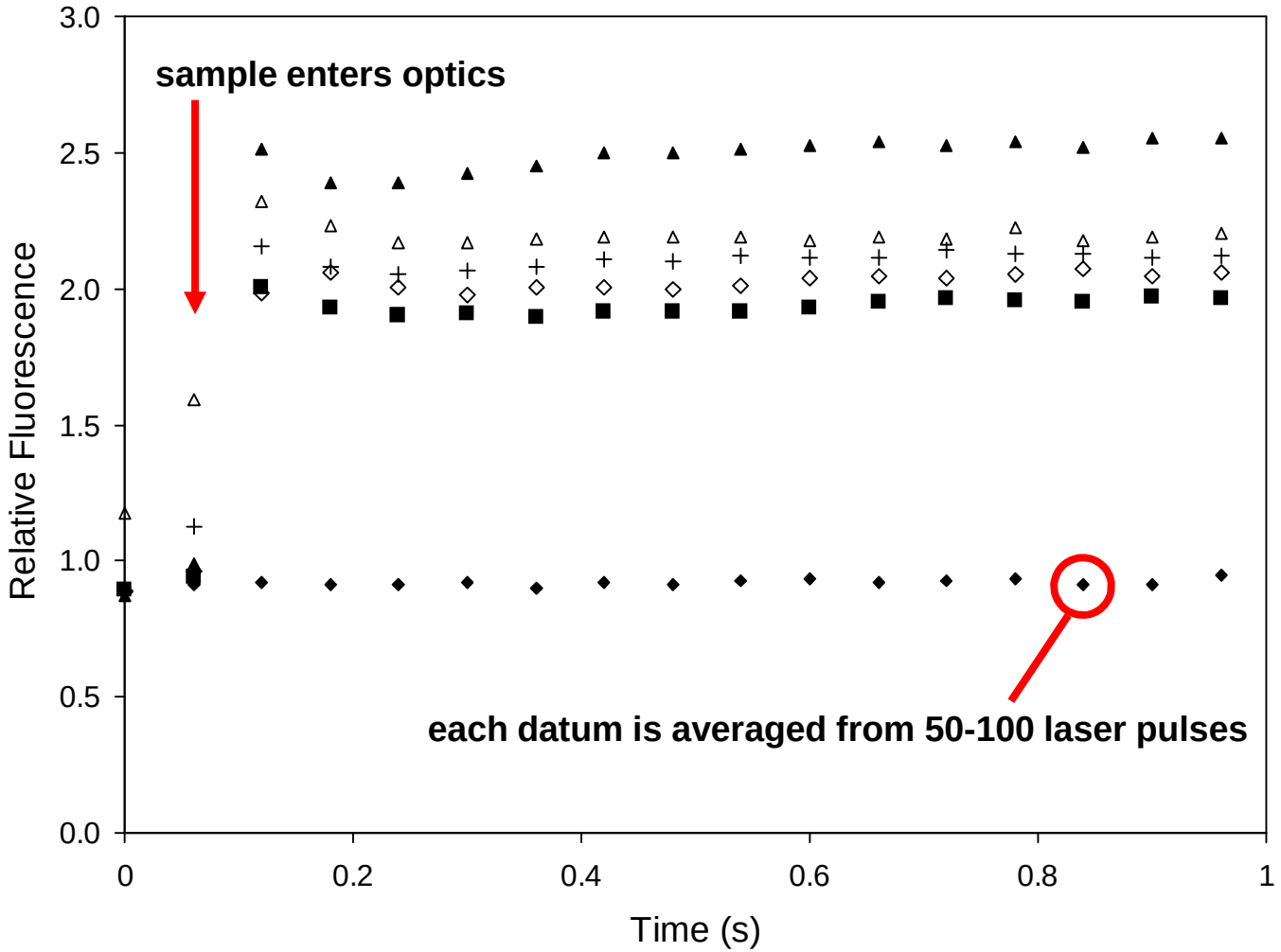
Measurement stability and cleaning



Sample flow removes optical bleaching

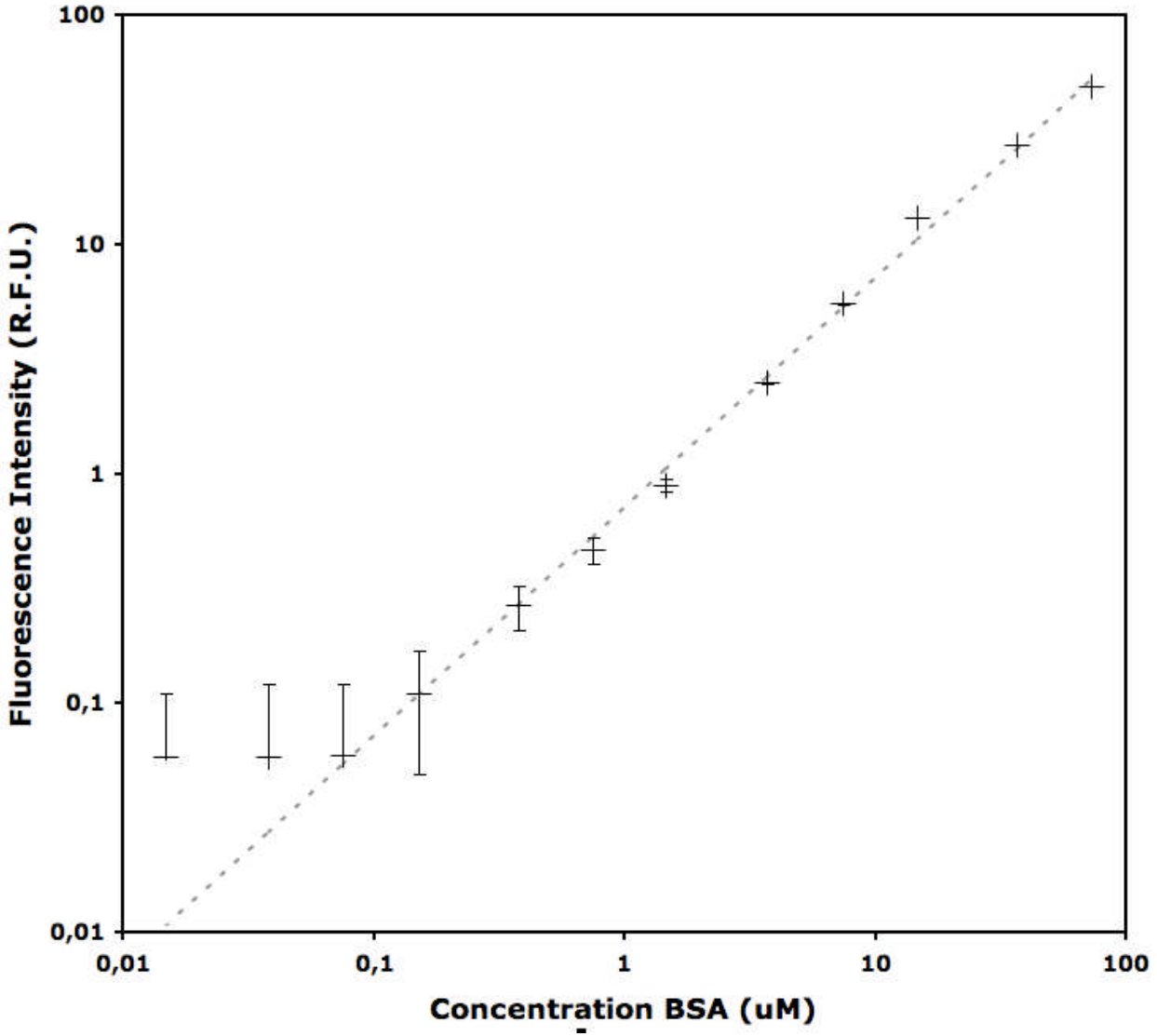


Acquiring the fluorescence measurement



- **Signal is stable (photobleaching avoided)**
- **Measurement is rapid (50-100ms to average 50-100 pulses)**

Sensitivity of fluorescence measurement



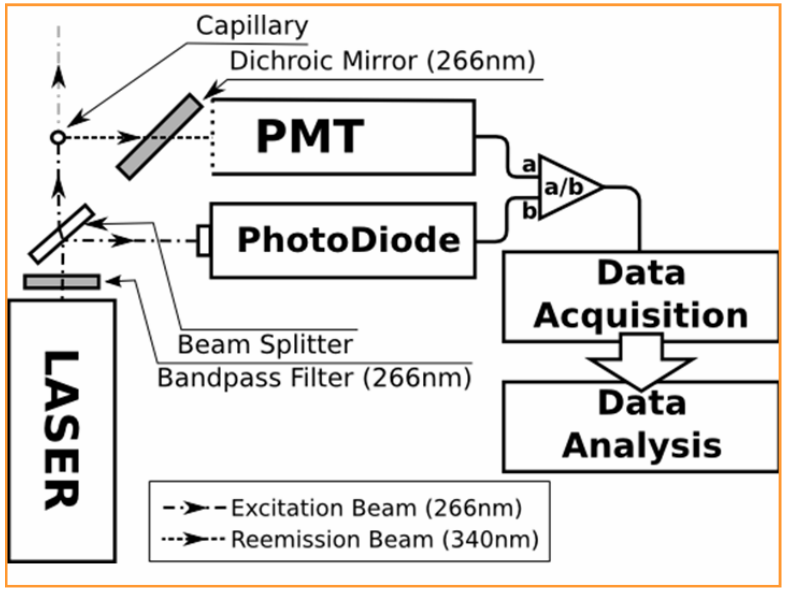
- **Signal response is linear: 0.15 μM to 1.5 mM (0.01-100 mg/ml) BSA**

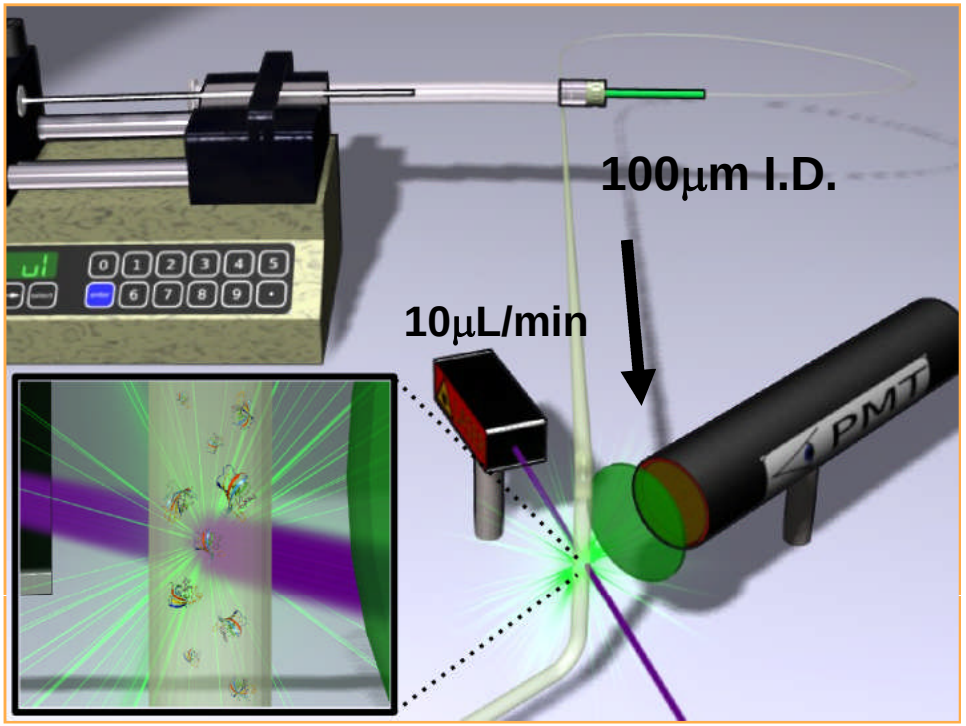
Comparison to microplates

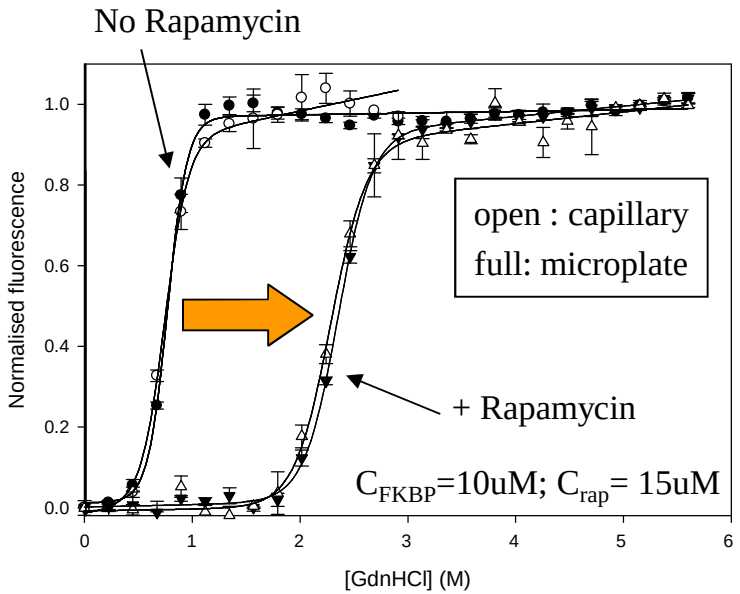
Method	Limit of detection (mg/ml)	Minimum [Protein] (uM)	Volume (L)	Number of Proteins
Microplate				
BSA	0.005	0.076	$2.6x10^{-4}$	**1.16×10^{13}**
RNaseA	0.0001	0.007	$2.6x10^{-4}$	1.14×10^{12}
Microfluidics				
BSA	0.01	0.15	$1.5x10^{-9}$	**1.4×10^{8}**

- 2x greater minimum concentration required
- 80,000x less protein required
- concentration range: 0.15 uM to 1.5 mM (0.01-100 mg/ml)

Nanolitre stability & ligand affinity screening



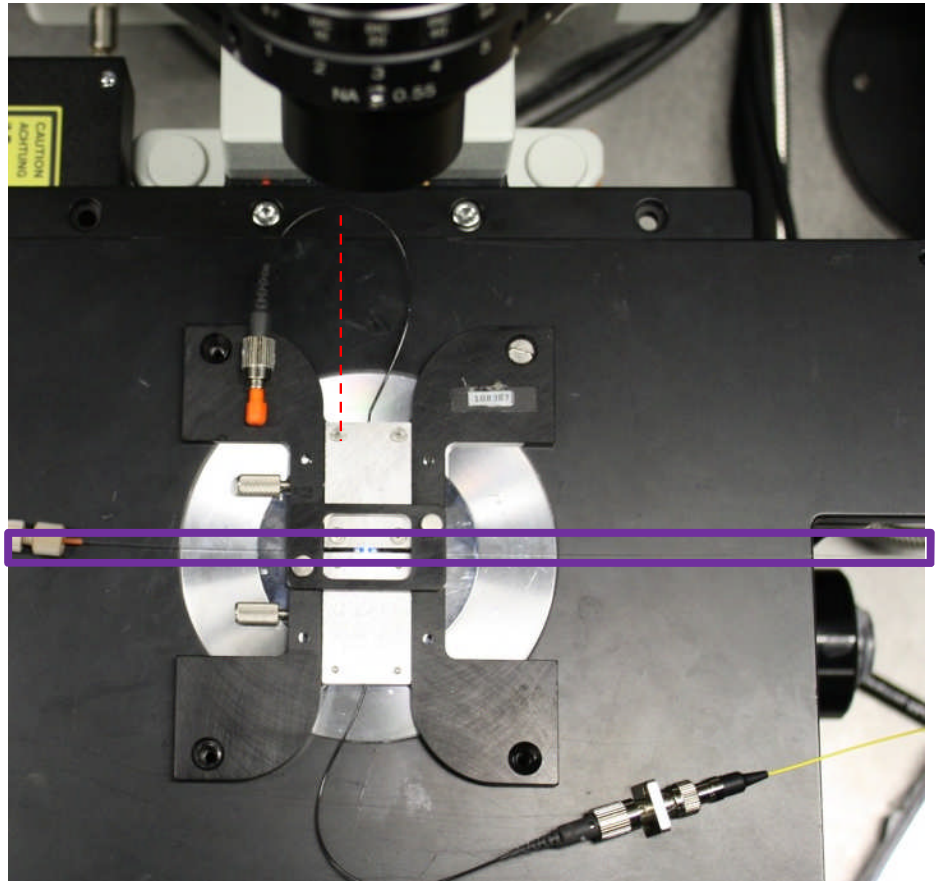


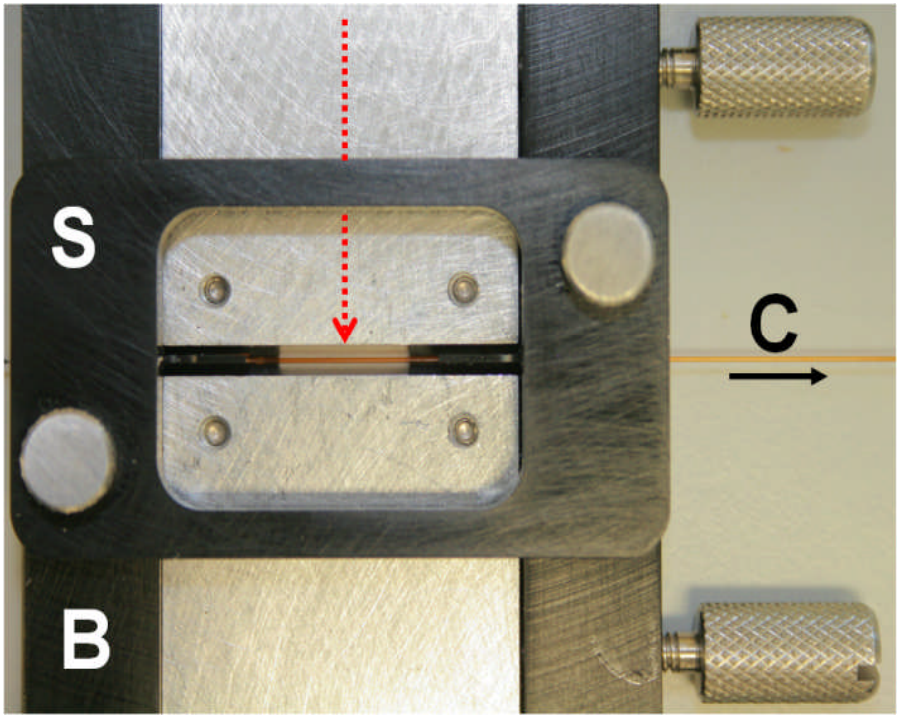


- 80,000x less protein than 96-well
- 0.15 uM to 1.5 mM (0.01-100 mg/ml)
- accurate $\Delta G_{D\text{-}N}$, $C_{1/2}$, K_d

Gaudet M, Remtulla N, Jackson SE, Main ERG, Bracewell DG, Aeppli G, Dalby PA (2010) *Protein Science.* 19: 1544-1554.

Optical control of heat in microfluidics





Acknowledgements

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Precipitation	Shahina Amhad
Freeze-drying	Scott Grant
Microfluidics	Matthieu Gaudet, Sagar Dodderi, Samir Aoudjane

Collaborators:

Microfluidics	Gabriel Aeppli (LCN)
	Dan Bracewell (UCL)
FKBP-12	Ewan Main (QMUL)
	Sophie Jackson (Cambridge)
Freeze-drying	Paul Matejtschuk (NIBSC/ HPA)

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