High throughput protein analysis and characterisation for stability studies

Daniel Lund, Avacta Analytical

Avacta ANALYTICAL

Avacta Analytical

- Purveyors of fine analytical instrumentation
- Biophysics specialists with track record of excellence in contract research and development
- Part of the healthcare and diagnostics conglomerate Avacta Group plc
- Major application of our technology is high throughput screening for formulation and stability studies…

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High throughput screening? I must be dreaming…

Novel in-process applications of spectroscopic analyses

Avacta ANALYTICAL

Coming up today…

1. Who are Avacta
2. The importance of protein conformation and stability
3. The Optim protein characterisation and stability platform
4. Where is high throughput stability analysis being used
5. Novel in-process applications
6. What's next?

Protein conformation and stability

- Protein function depends on the conformation
- Over time proteins can degrade, via various potential pathways
- ‘Real time’ measurements can take many years
- Predictive tools available such as Tm, Tagg, Arrhenius kinetics etc
- Fluorescence offers an accessible probe of the tertiary structure of the protein

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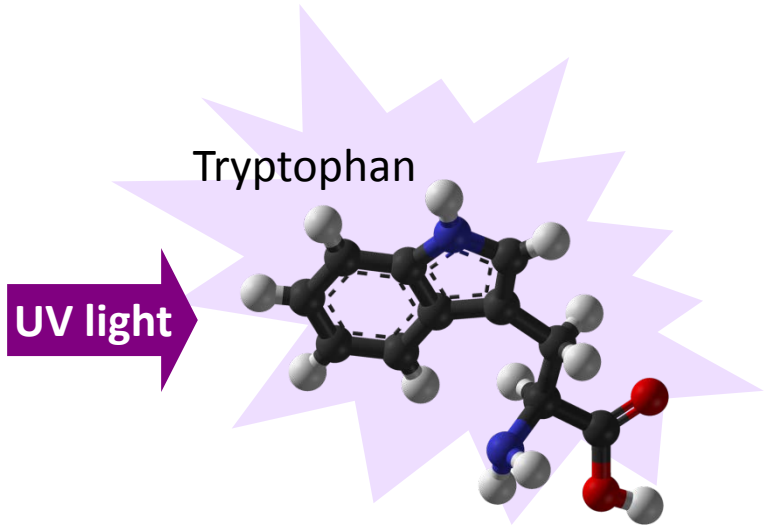
Intrinsic protein fluorescence



- Intensity – quenching
- Peak position - polarity

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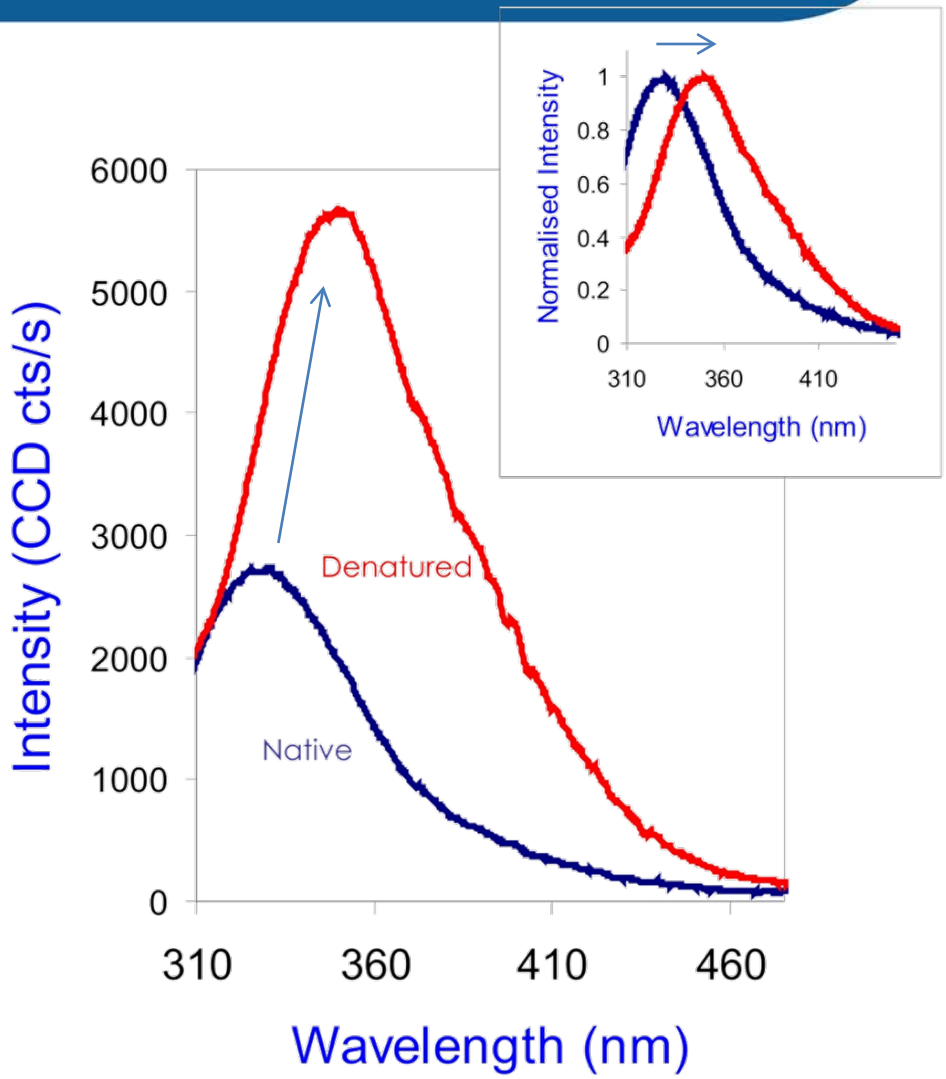
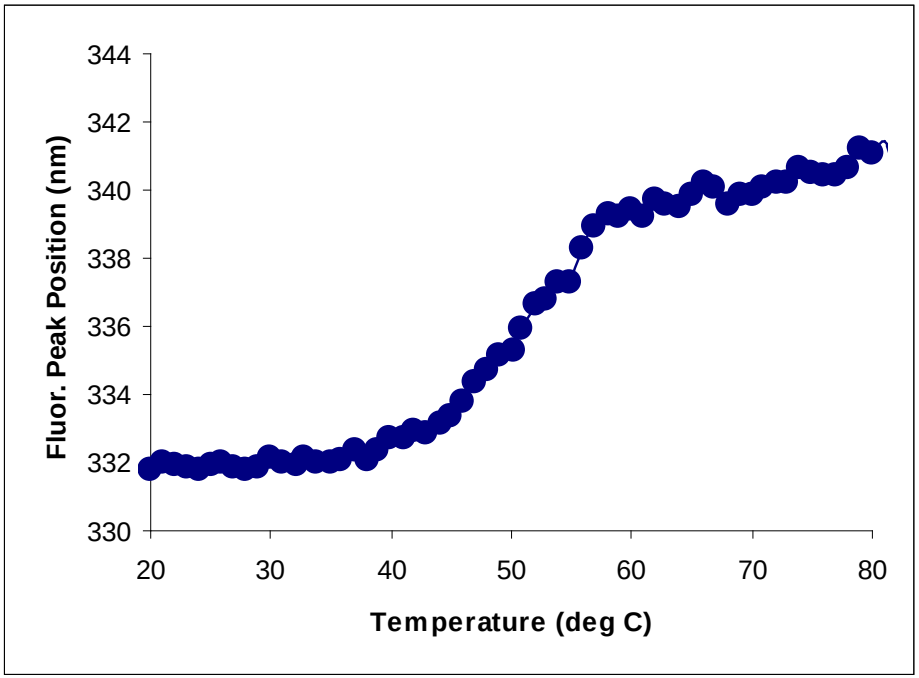


Polarity

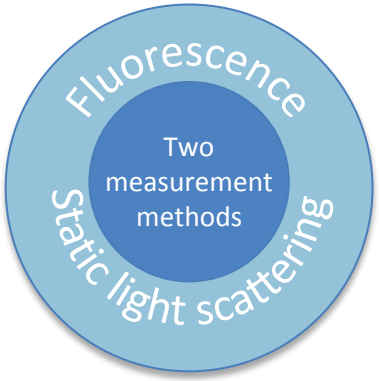
- This is saying that the tryptophans are going from a relatively non polar environment to a more polar environment
- We correlate that with unfolding

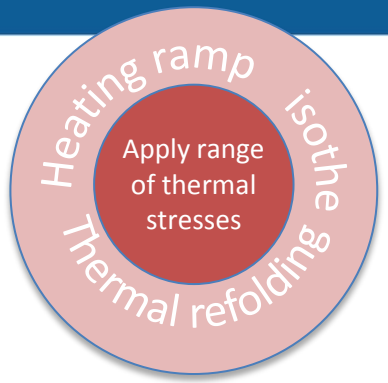


Right now

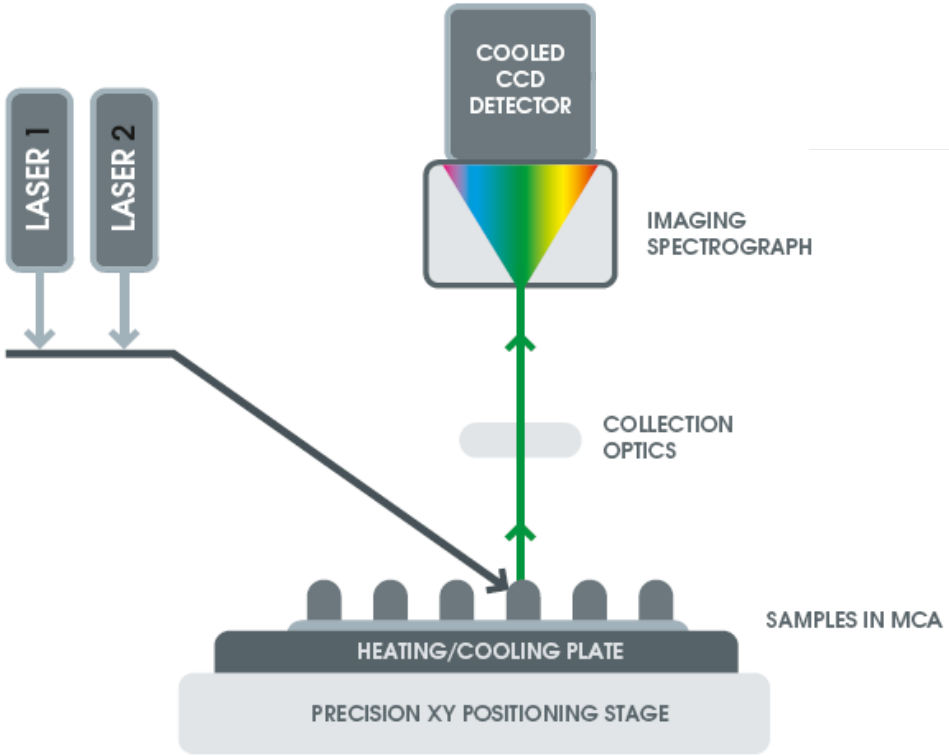
The Optim protein characterisation and stability platform

What technology does it use?





What's in the box



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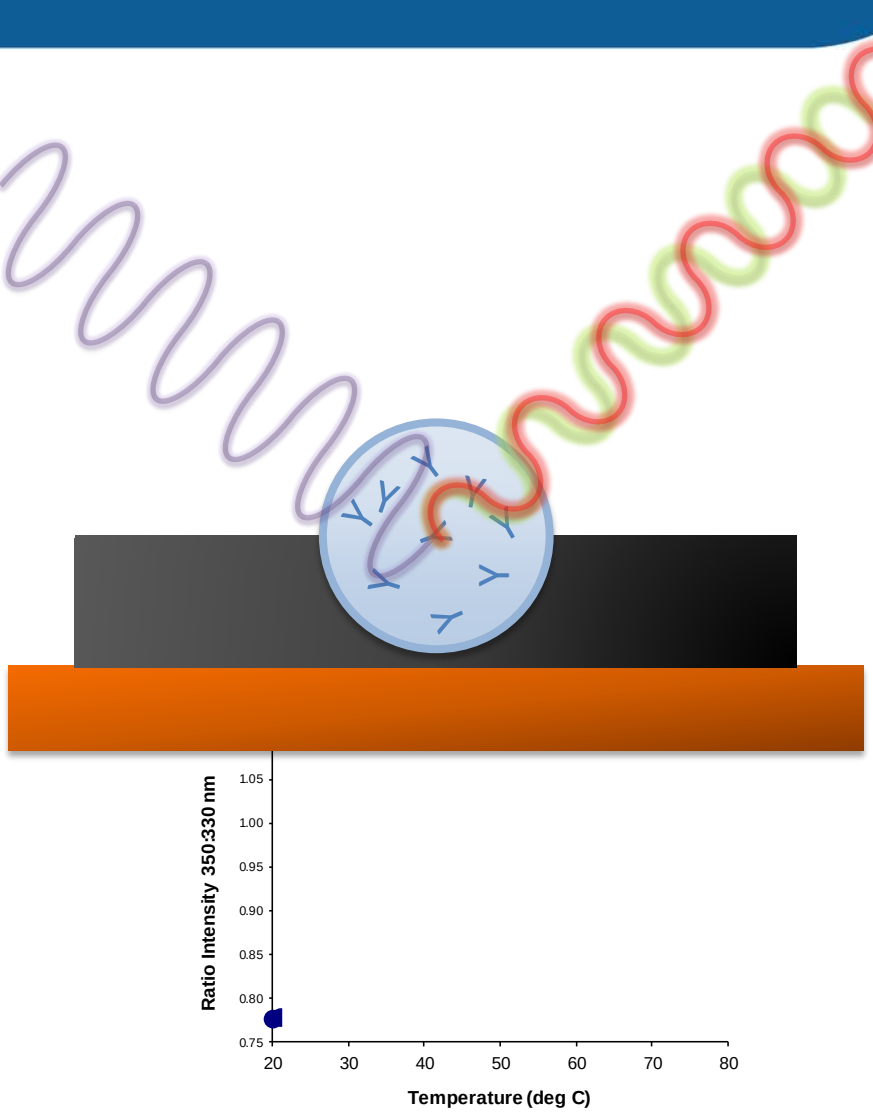
How does Optim work

- Light from the **lasers** is illuminating the **samples** in the **MCA**
- The samples are initially **cold**
- Light of a particular **wavelength** is emitted by the sample and detected
- Heat is being applied from **underneath**
- As the samples get **hotter** they change their conformation
- The colour of the light emitted **changes** – this can be detected

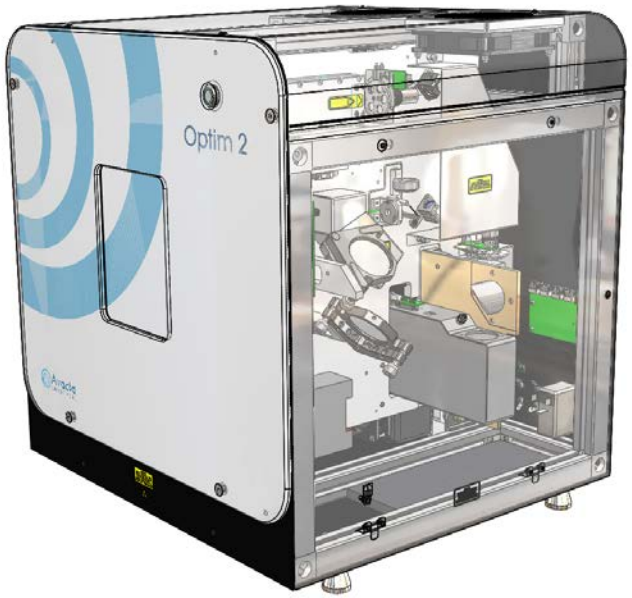


What makes Optim so special?

System designed for high sensitivity

less sample + more speed

- Superior optical performance due to:
  - Optimised optical design – using lasers allows tight focusing of light into small volume
  - High performance components increases signal to noise ratio
  - Proprietary micro cuvette array (MCA) designed to give optimum quality optical data from small sample volumes

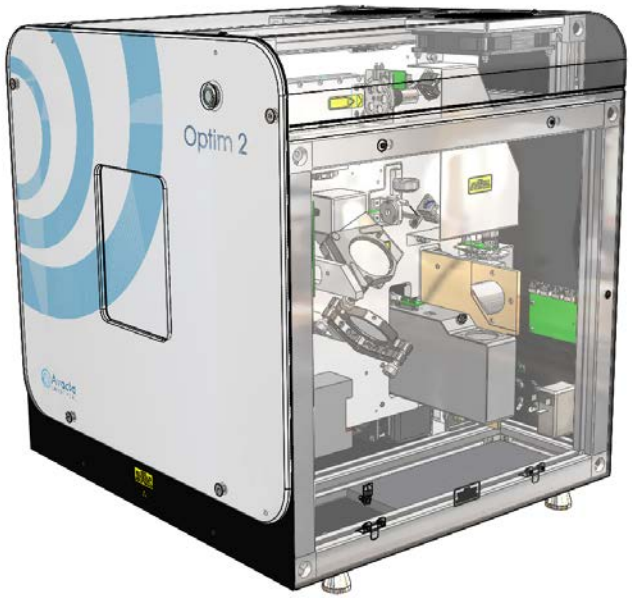


What makes Optim so special?

Full spectrum acquisition

information + speed + versatility

- Imaging spectrograph and array detection rapidly acquires information rich data (whole spectrum for every measurement)
- Design allows multiple measurement types to be made simultaneously



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See the big picture!

Isothermal
Rates of unfolding and aggregation

Debye plots
Protein – solvent interactions and colloidal stability

Dye probes
Conformation, membrane proteins, viscosity, fibrilation

A_{266}, ε_{266}
Mass attenuation coefficient or absorbance based on inner filter fluorescence

Tagg
Protein aggregation stability

ΔG, $[D]_{1/2}$
Thermodynamic information, indication of Solvent accessible surface area

Tm
Protein conformational stability

What is Optim and what does it do

- Make measurements that predict what the **ACTIVITY** and **AGGREGATION** of their products will be in years
  - **Faster** than current technologies allow
  - With **higher throughput** that current technologies
  - With **less** sample
  - Provide **more** information

Right now

Where is high throughput stability screening being used now?

Some applications

Formulating DS/DP

determine a number of parameters that correlate with **long term activity** and **stability** of biopharmaceuticals

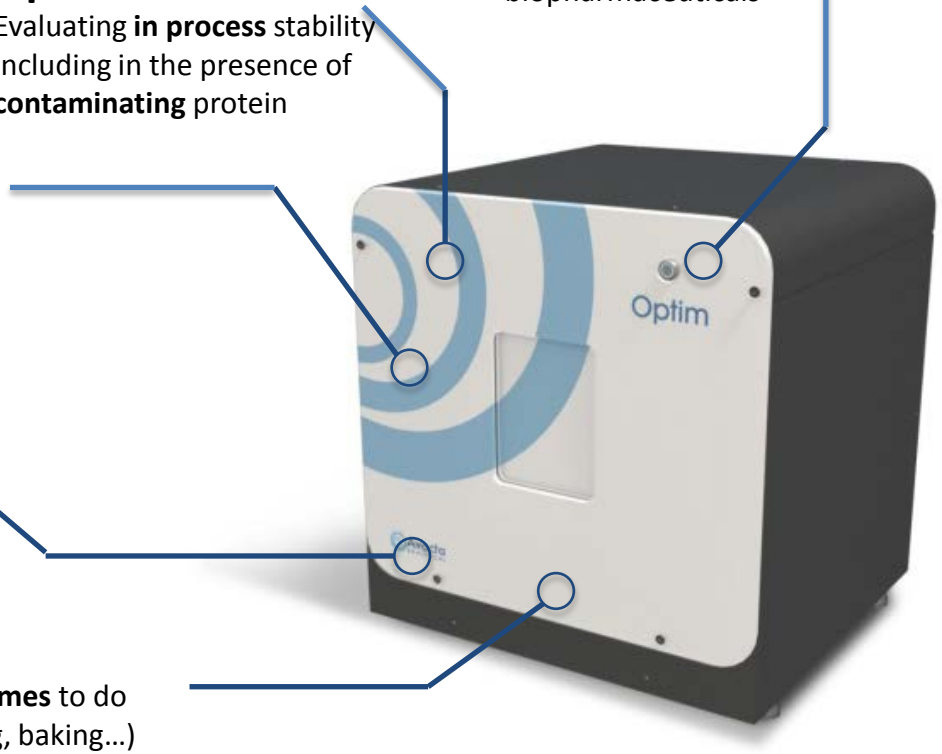
Synthon

Bioprocess Optimisation

Evaluating **in process** stability including in the presence of **contaminating** protein

Yes/No Binding

assess and **optimise binding** of proteins and ligands including **annealing protein and oligos**, and **transcription factor binding to phosphorolated peptide**



Vaccines

determine stability of adjuvant bound vaccines

Enzymology

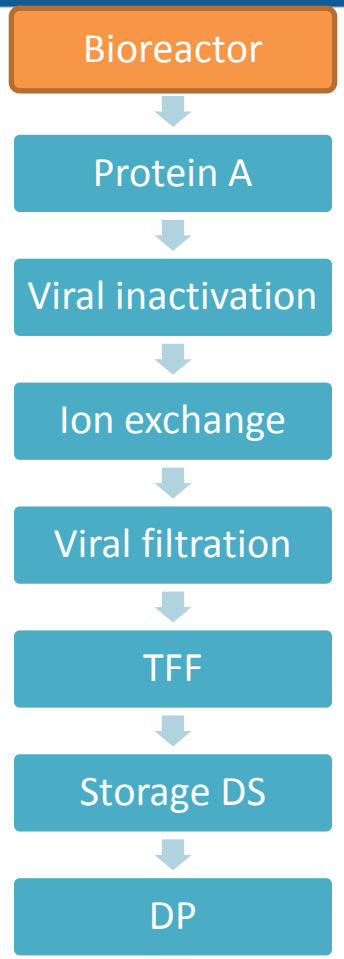
optimise design of **enzymes** to do stuff with food (brewing, baking…)

Right now

Novel applications – within the bioprocess

a) Screening for higher producer clones/media and feed optimisation
b) Bioprocess optimisation

In-process applications of spectroscopy



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- Non invasive in line monitoring (bioreactor)
  - Raman shown to correlate using PLS with for example (Abu-Absi et al (2011) *Biotech. and Bioeng.* **108**;1215)
    - Glutamine
    - Glutamate
    - Glucose
    - Lactate
    - Ammonium
    - Viable cell density
    - Total cell density
- More difficult with fluorescence – lots of different contributing factors, but...

Cunningly

- Intracellular reduced pyridine nucleotides NAD(P)H primary suppliers of reducing power to anabolic and catabolic pathways
- Fluorescence caused by presence of reduced nucleotides NADH and NADPH (jointly refered to as NAD(P)H)
- Absorb in a wide band around 340 nm, reemit around 460 nm
- Concentration of reduced and oxidised pyridine nucleotides vary in different cultures and cell types
- This fluoresence can be related to metabolic state of cells

Mainly used for

- Biomass estimation
  - Correlation between fluorescence and biomass conc in exponential growth phase
- Substrate addition/depletion responses
  - Drop in fluor during depletion
  - Response due to addition depends on metabilic state and subtrate
- Aerobic-anaerobic transitions
  - Fluor increase as dO_2 decrease

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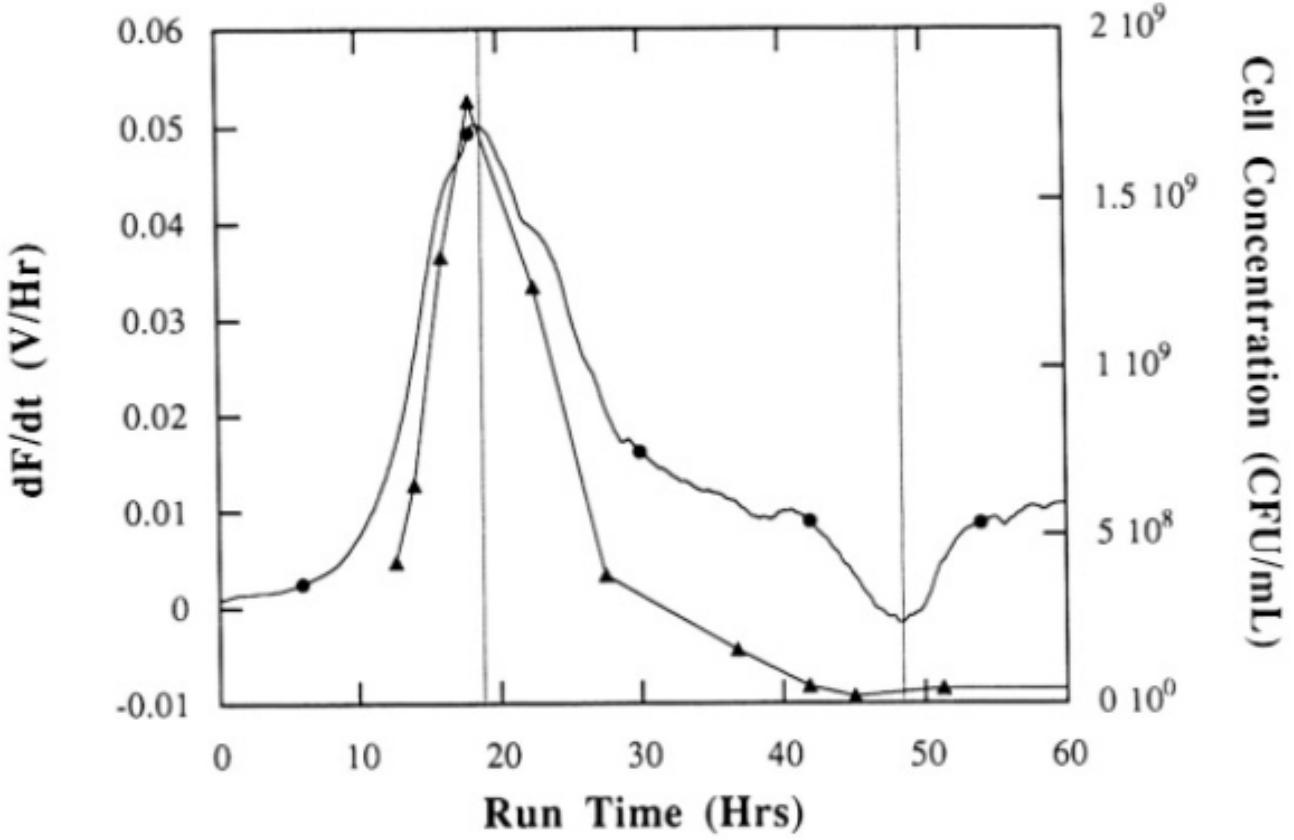
Example



Kwong et al. (1993) *Appl. Environ. Microbiol.* **59** 604-606

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In process – novel applications

- Bioreactor
- Protein A
- Viral inactivation
- Ion exchange
- Viral filtration
- TFF
- Storage DS
- DP

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Journal of Biotechnology 151 (2011) 255–260

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Ana P. Teixeira[a,b,**], Tiago M. Duarte[b], Rui Oliveira[c], Manuel J.T. Carrondo[a,b], Paula M. Alves[a,b,*]

- Three cell clones
- Viable cells and titre measured
- Correlation sought with various fluorophores

In process – novel applications

- Growth rate and max cell density similar
- Wide range of titre

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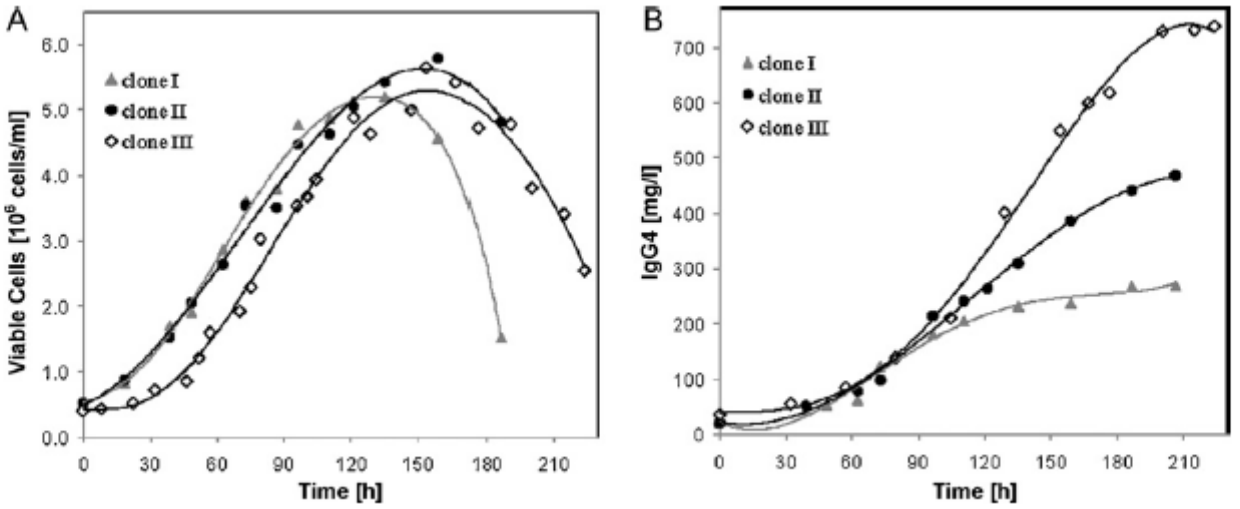

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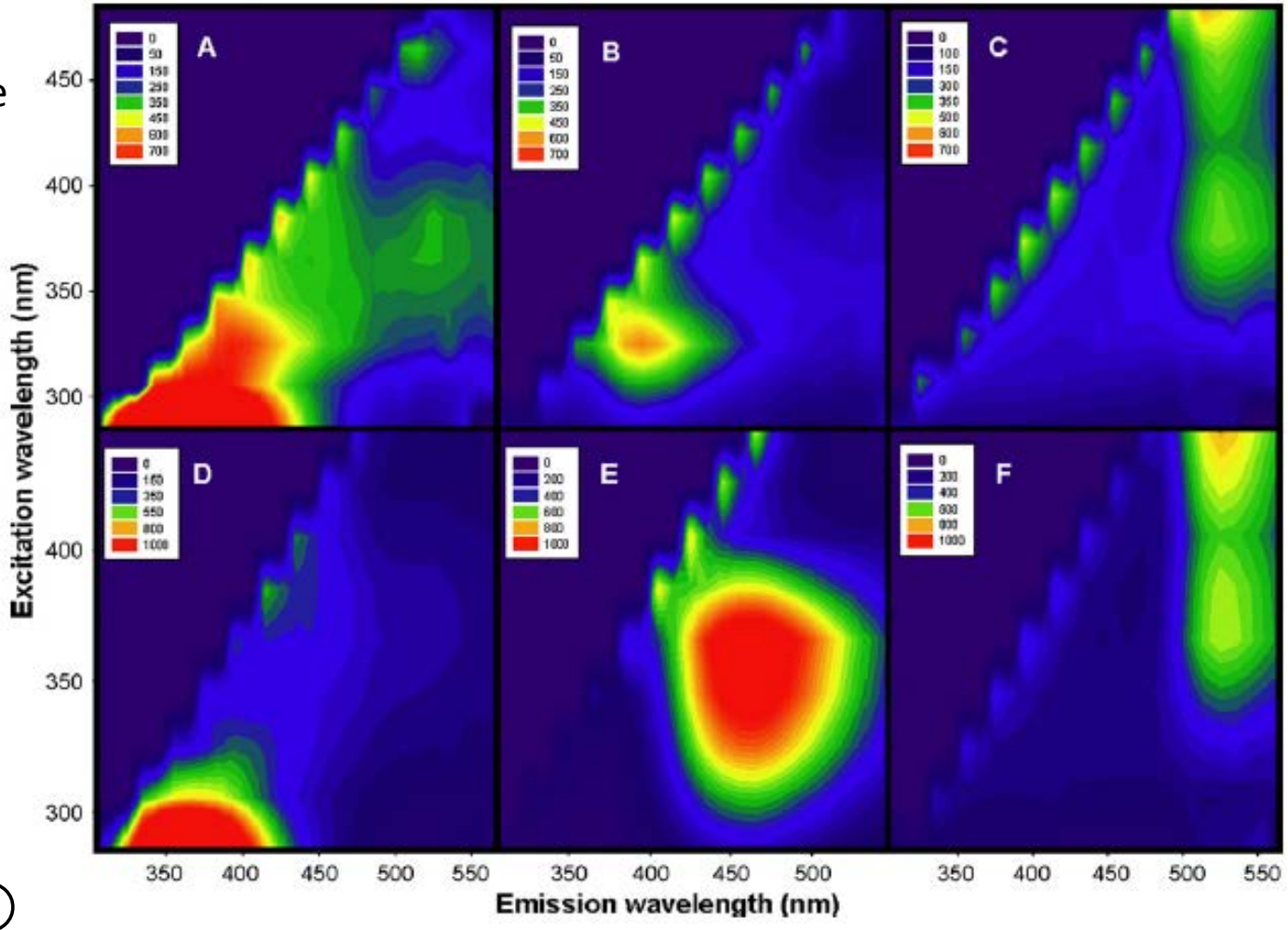
In process – novel applications

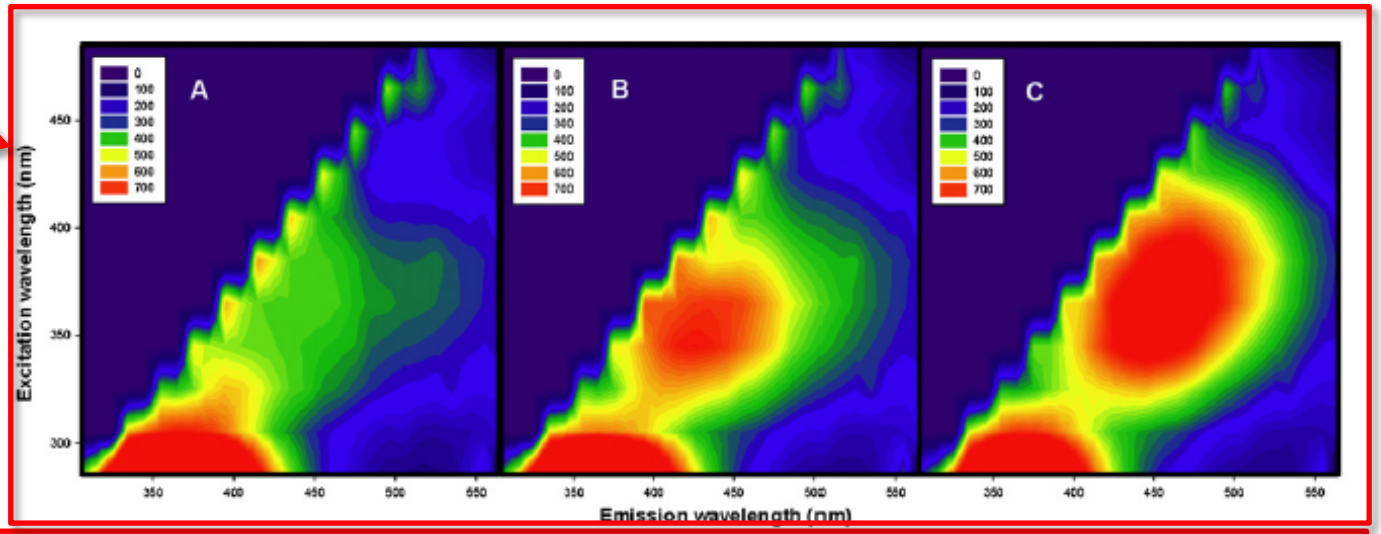
Test fluorescence map

A – media
B – pryidoxine
C – riboflavin
D – W
E – NADH
F – FAD

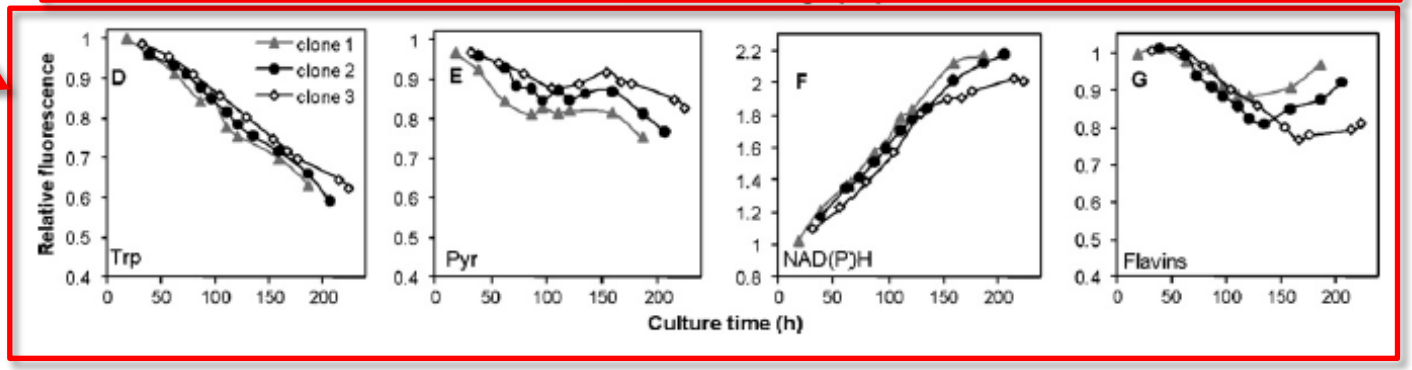


In process – novel applications

1 clone over time



3 clones Different fluorophores



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In process – novel applications

Trp

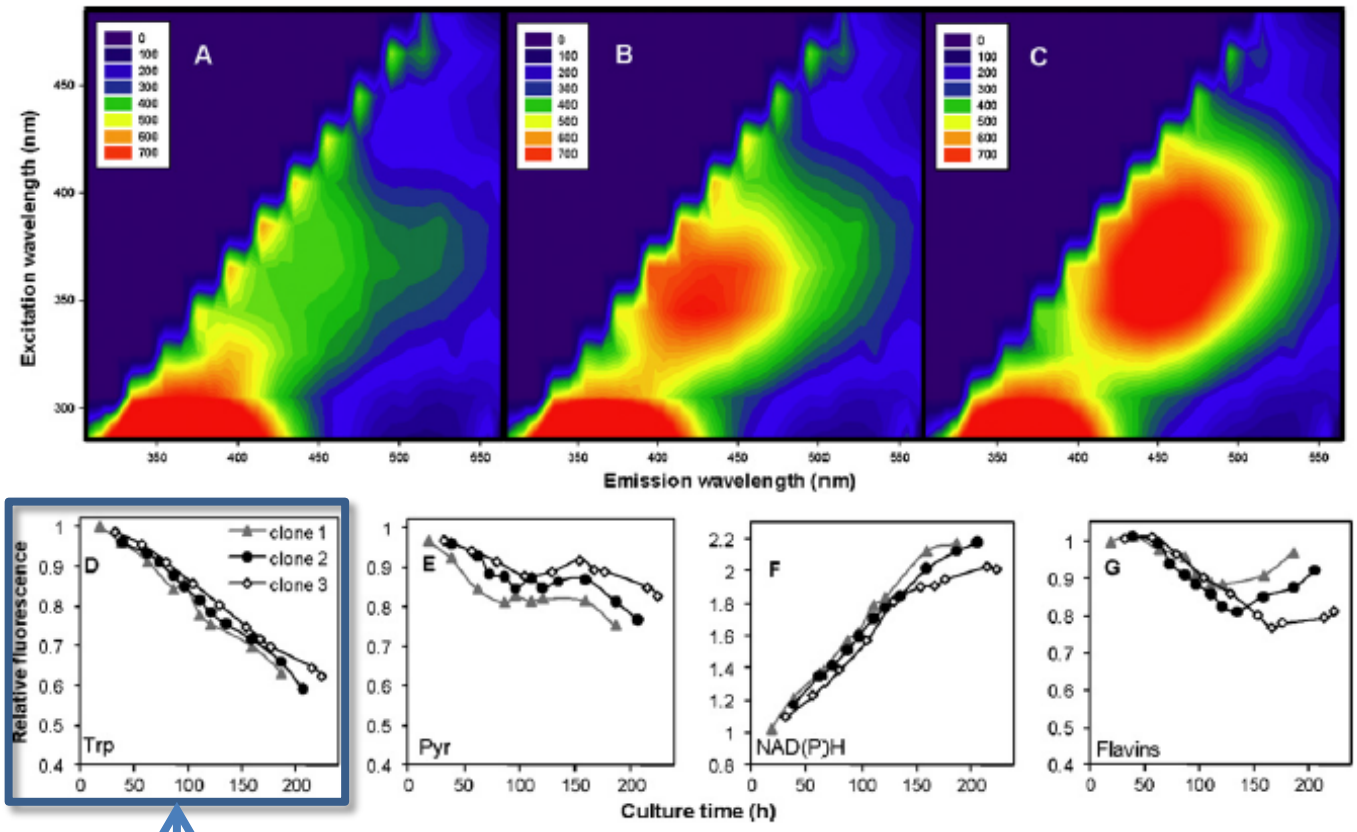
taken up by cells,

put into proteins

and quenched by

neighbouring

amino acids



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In process – novel applications

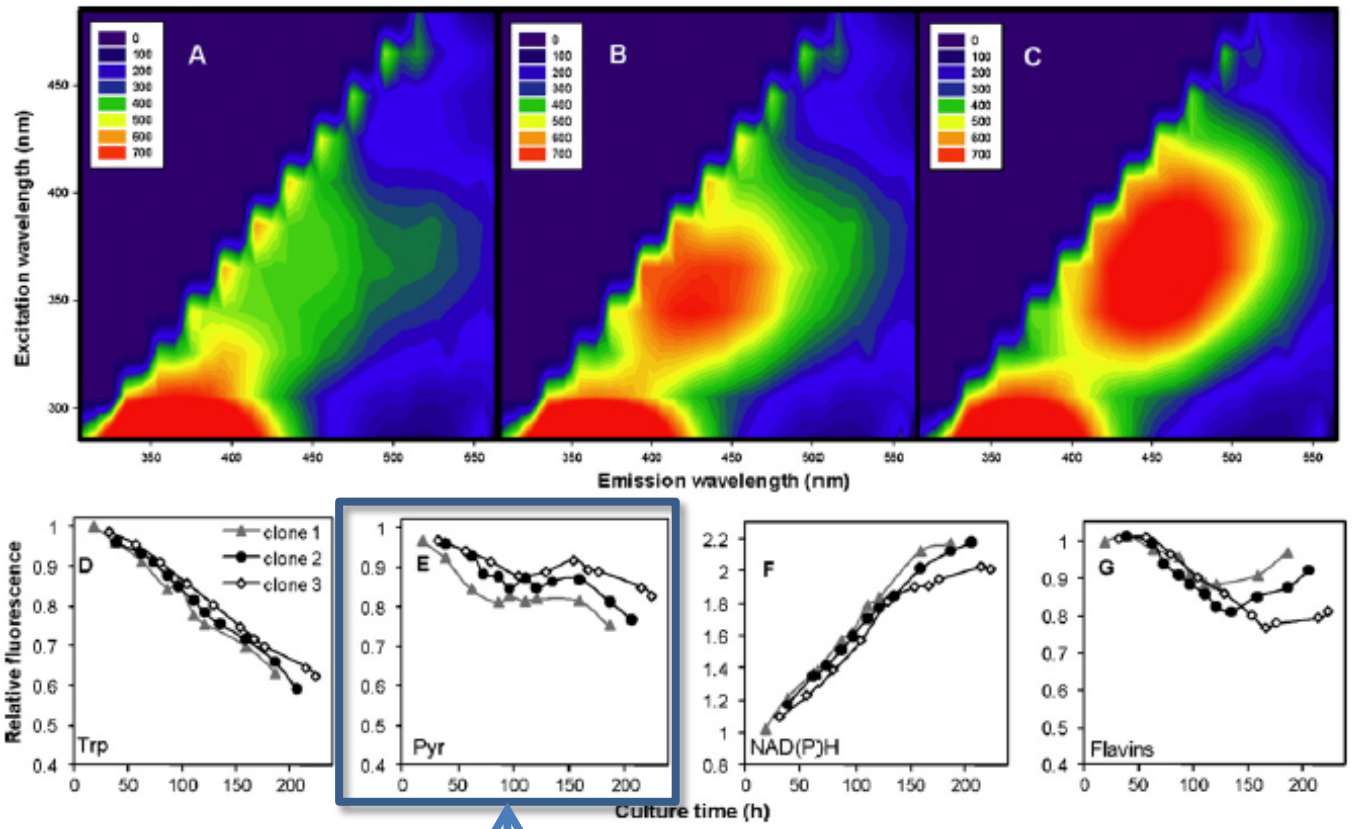
Pyr

reduce during

exp growth then

stops during

stationary phase



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In process – novel applications

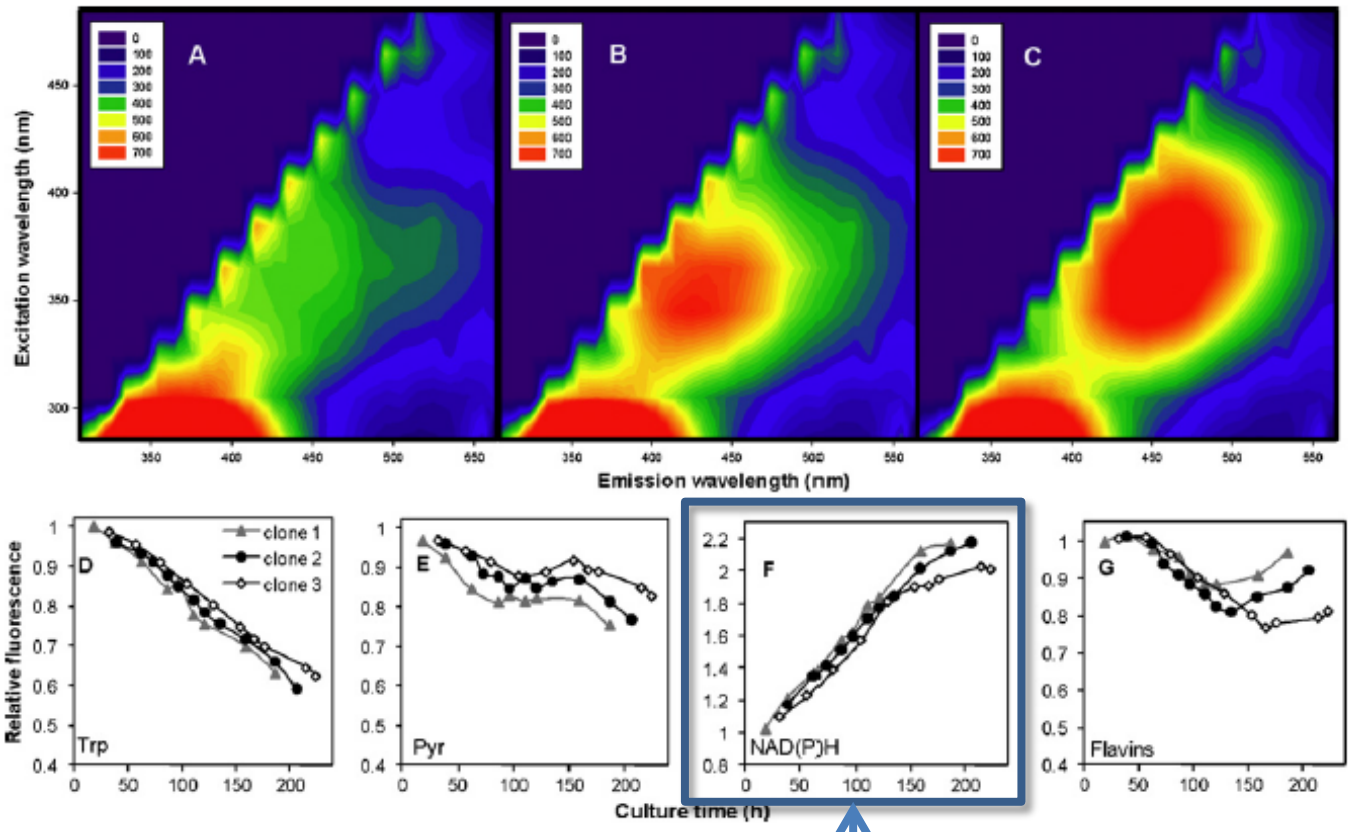
NAD(P)H

negligible at start

and increases as

cell lysis dumps

NADH into media



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In process – novel applications

Flavins

correlate well

showing

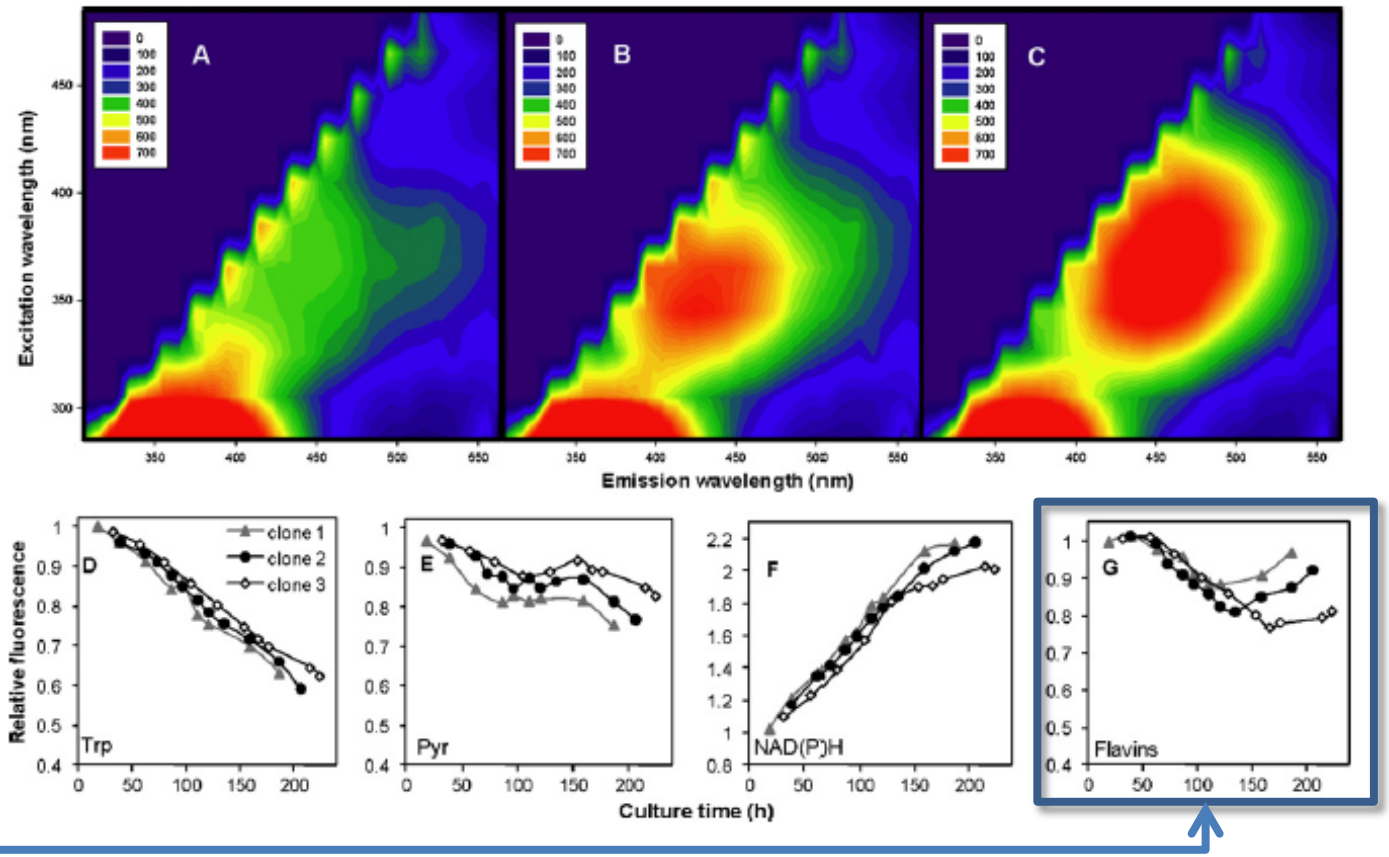
decrease during

growth, flat

during stationary

then increasing

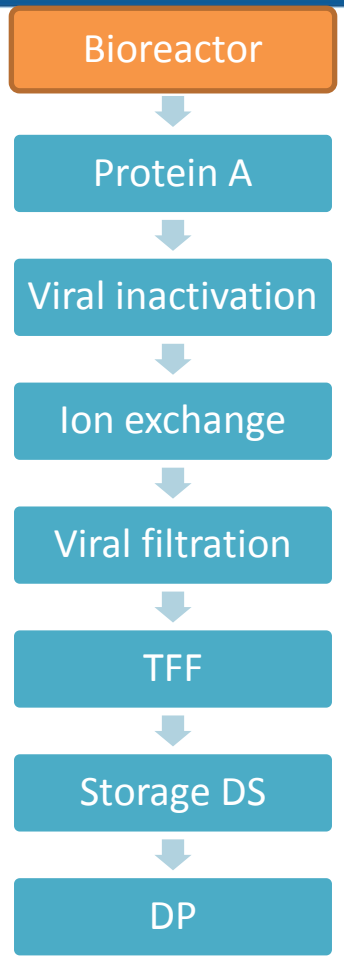
again



In process – novel applications

- Partial Least Squares to find relevant spectral regions
- Predicted antibody conc and cell density and validated with 3rd clone
- W negatively correlated with titre
- NAD(P)H also correlated with concentration
- Cell density correlated with NAD(P)H and Try but stronger negative correlation with the flavins

In process – novel applications



- Can screen for higher producer clones
- Media optimisation
- Cell culture process optimisation
- In combination with something like ambr or micro24

In process – novel applications

- Bioreactor
- Protein A
- Viral inactivation
- Ion exchange
- Viral filtration
- TFF
- Storage DS
- DP

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The use of temperature dependent intrinsic fluorescence for the analysis of the in-process stability of a protein

A study of the manufacturability of a monoclonal antibody using the Optim®1000

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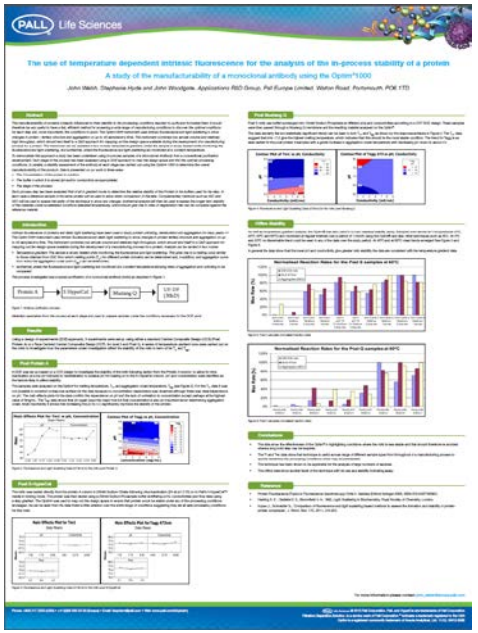
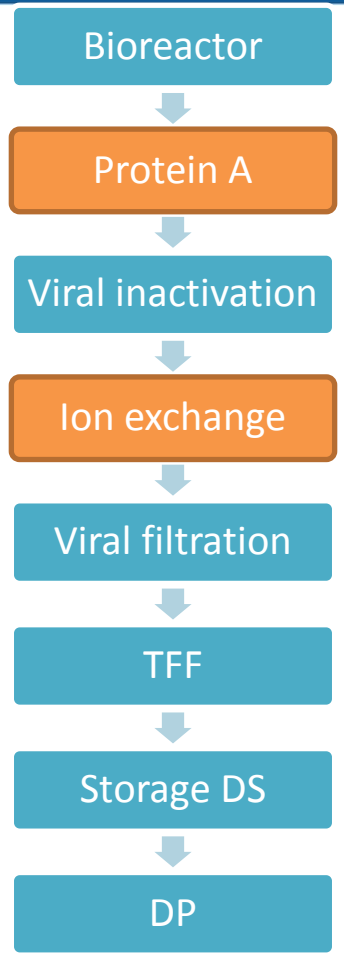


In process – novel applications



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- In process samples assessed for biophysical stability using DOE approach and Optim 1000 to find optimal conditions of a monoclonal antibody – T_m and T_{agg}
- Parallel stability assessment carried out which correlated with in process ramp data
- DOE allows build quality into product

Bringing it all together: Manufacturability

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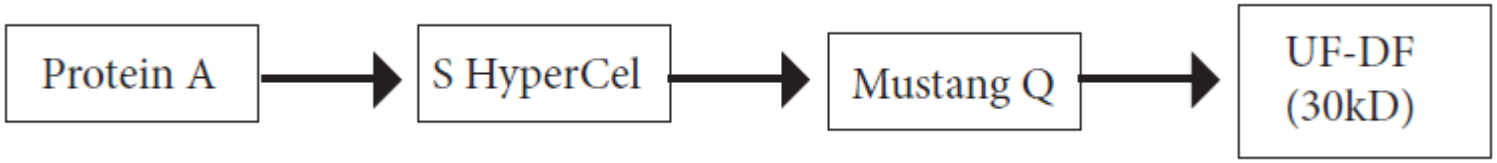

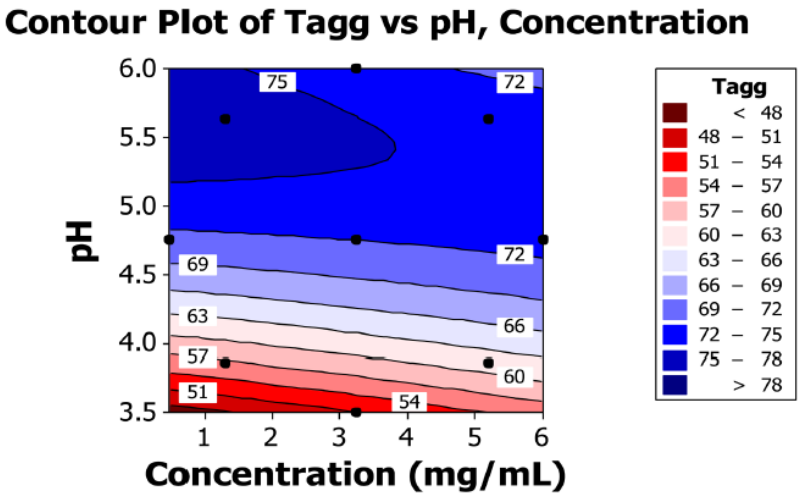

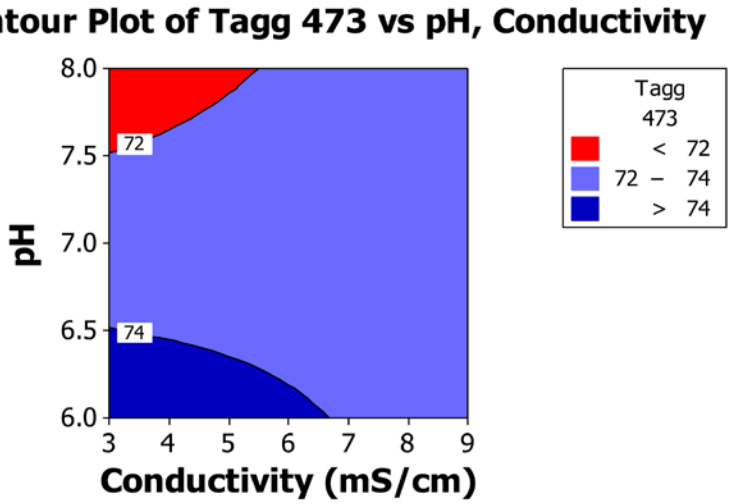

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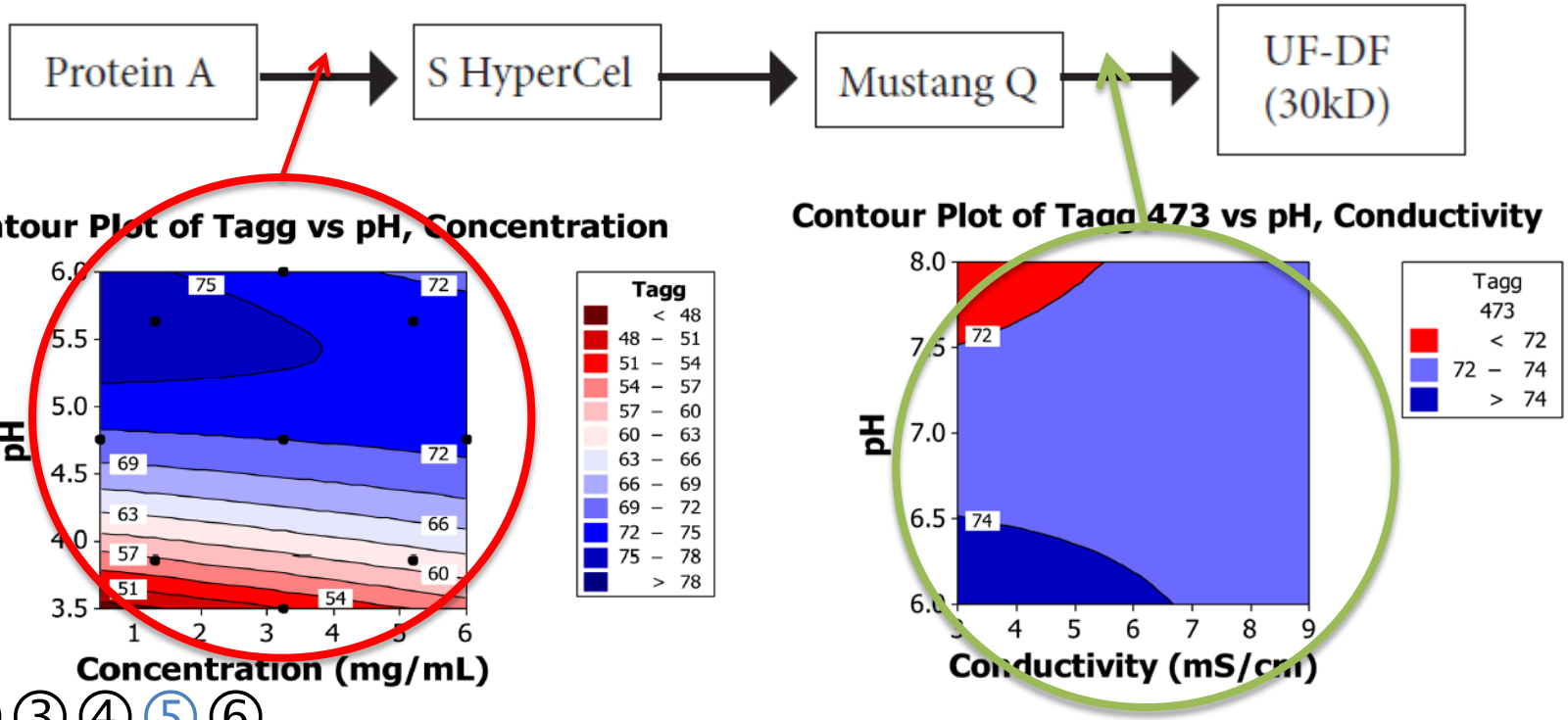

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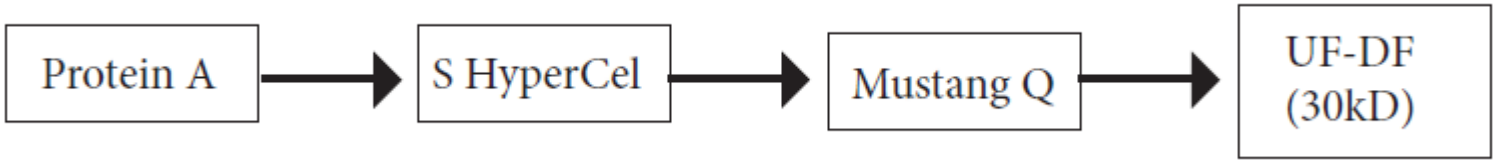

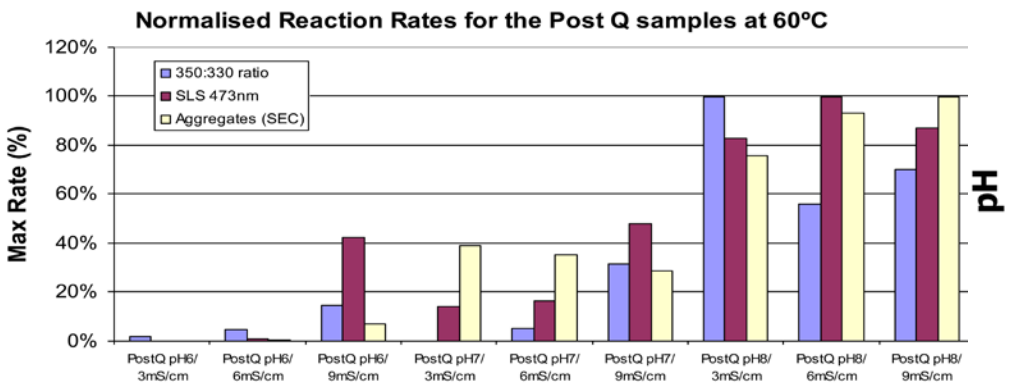

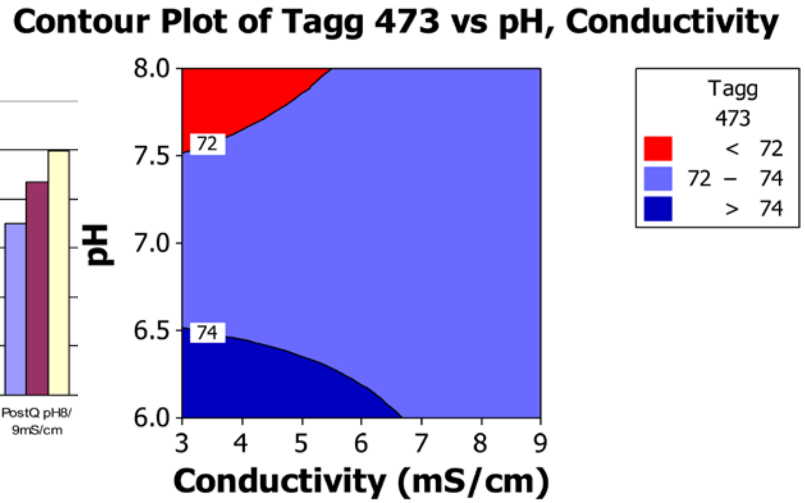

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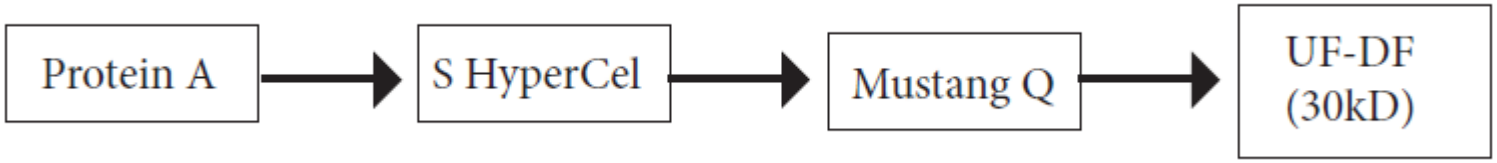

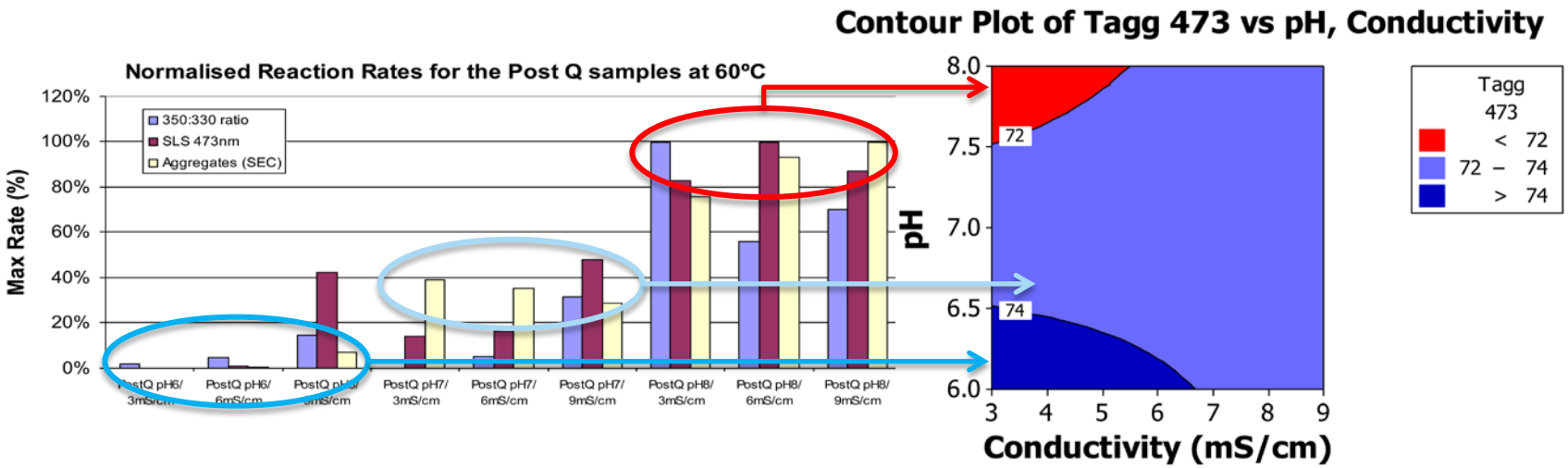

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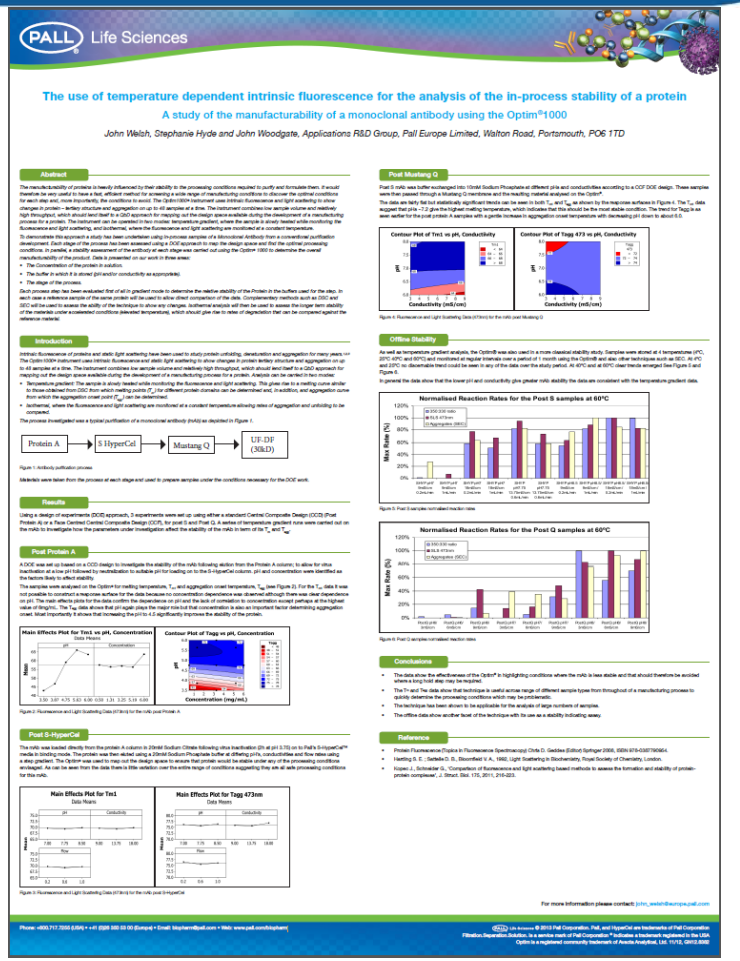
Bringing it all together: Manufacturability

- Optim effective at highlighting conditions where the mAb is less stable
- T_m and T_{agg} data show technique is useful across range of different sample types from throughout of a manufacturing process to quickly determine the processing conditions which may be problematic.
- Technique has been shown to be applicable for the analysis of large numbers of samples.
- The offline data show another facet of the technique with its use as a stability indicating assay.



PALL Life Sciences

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Abstract

Introduction

Results

Post Protein A

Post S-HyperCel

Post Mustang Q

Offline Stability

Conclusions

Reference

Well would you believe it?!

- High throughput screening not just for prediction of long term storage stability
- Also great for characterisation tertiary structure
- And aggregation characteristics
- And at-line characterisation of cell culture for clone selection/media optimsation
- And optimisation of bioprocess

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FOR OUR **WEBINAR** ON **ADVANCES IN HIGH THROUGHPUT FORMULATION** IN PARTNERSHIP WITH **EUROPEAN BIOPHARMACEUTICAL REVIEW**

Presentation 1:

Advances in high throughput formulation screening: Look past the conventional and see what you can really do.

Charlotte Dodd, Application Scientist, Avacta Analytical

Presentation 2 - Case study:

Selection and preformulation of an antibody.

Guy De Roo, Senior Scientist, Synthon Biopharma

Synthon

Date: Wednesday **20th November 2013**

Start time: 3pm GMT (UK) 4pm CET (Europe) 10am EST (US)

To register for the webinar, go to http://bit.ly/avactawebinar or for more information contact See Mun Li on email: seemunli@samedanltd.com or tel: +44 (0)20 7724 3456

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