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Institute of Environment, Health and Societies

Health and Environment

# Spinning around: from molecules to particles and beyond

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# Advanced Bioprocessing Centre (ABC)

## *separation technologies based centre*

**Formally opened** on April 25th 2006

**Aim:**

"To act as a focus for Brunel's research on **bioprocessing**, establishing it as an international centre of excellence and the first point of contact for both academia and industry when facing challenging separation problems"

**Core technology:**

Liquid-liquid dynamic extraction in fluctuating *g*-field known as *Counter-current Chromatography & Centrifugal partition Chromatography*

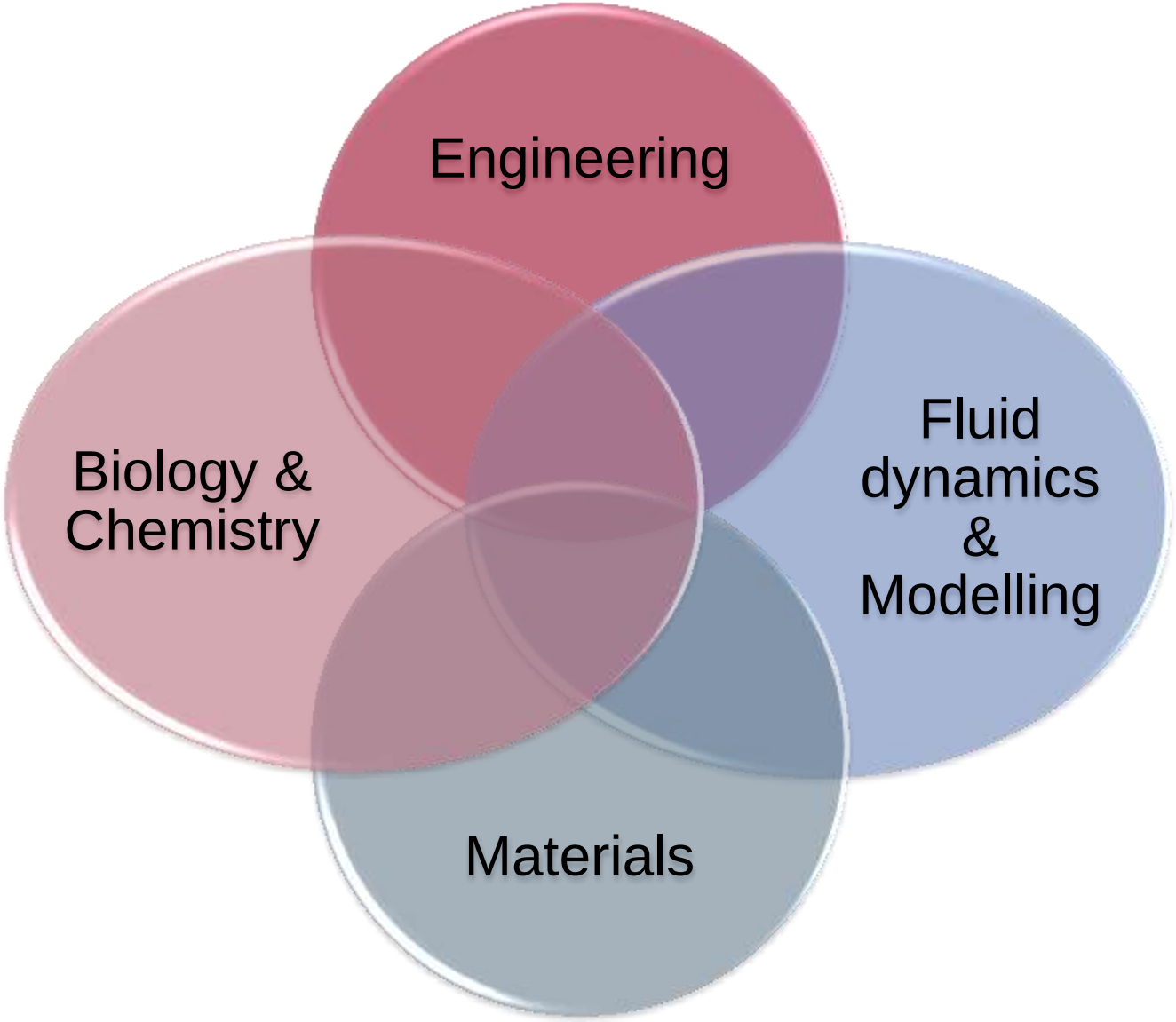
**Facilities:** 4 Labs

(Analytical, Applications' , Biological, Hazards' Labs)

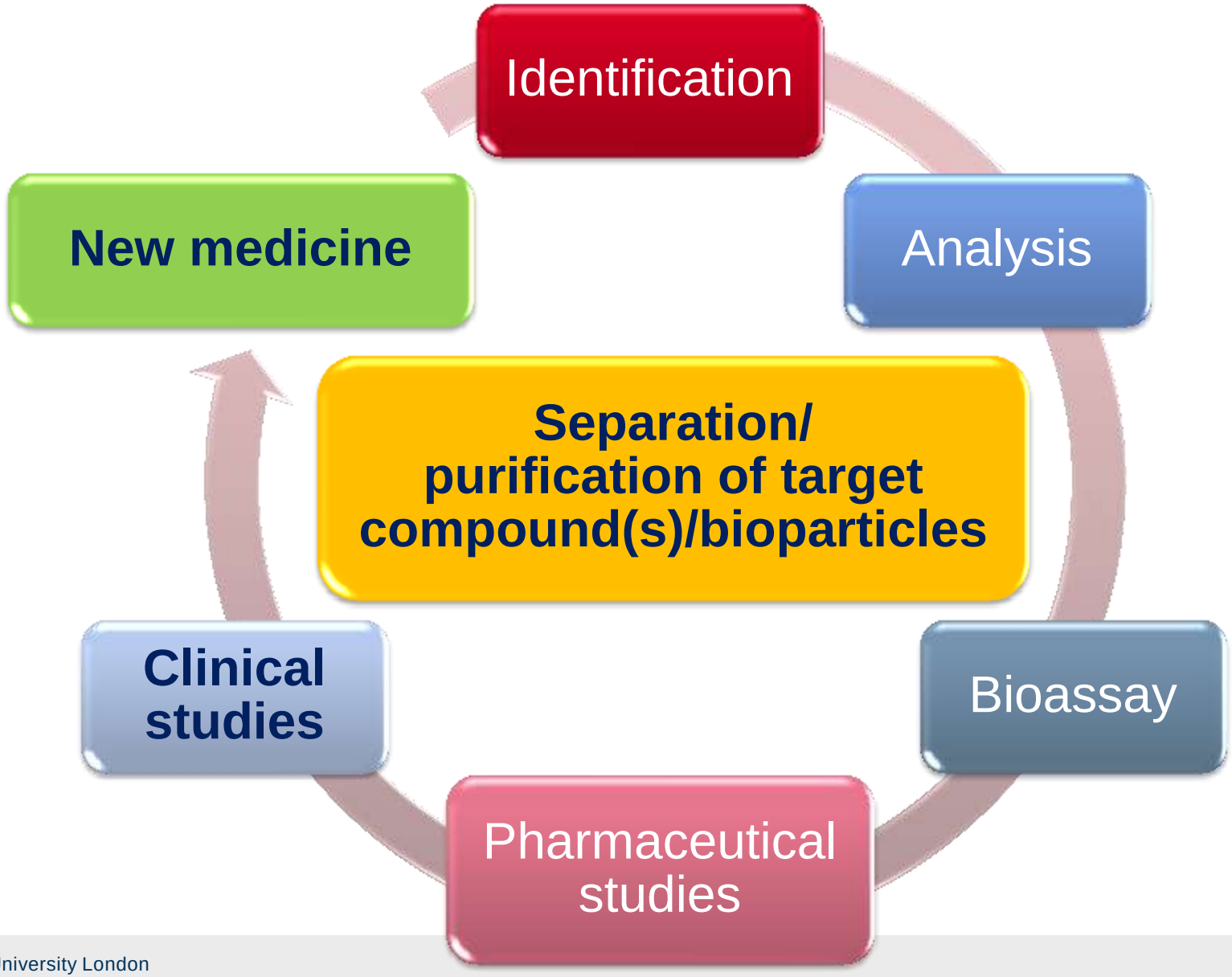
**Dynamic Extractions Ltd (Wales, UK) since 2003**

www.dynamicextractions.com

# Multidisciplinary Research @ ABC



# From Bioactive Compounds to a Medicine



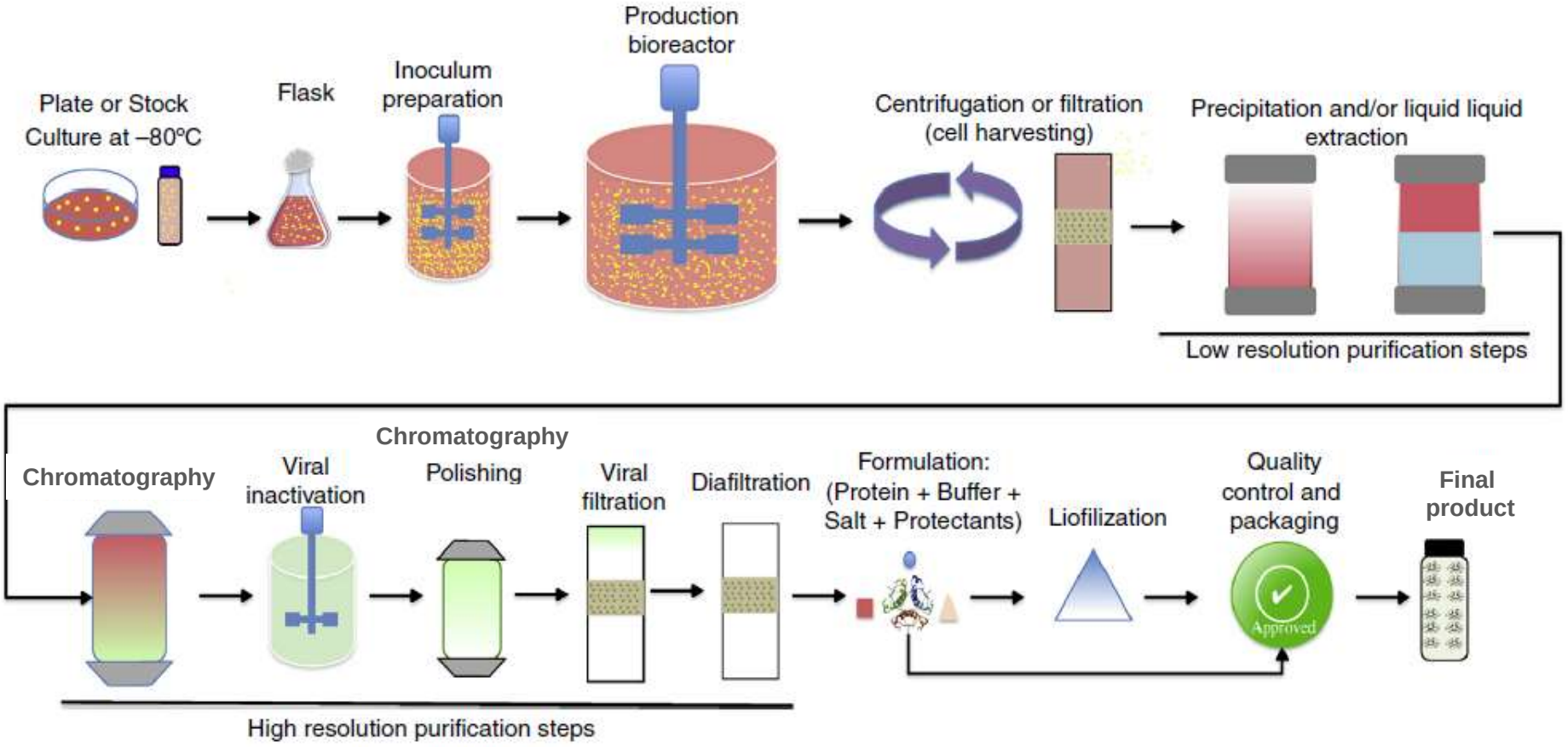
# Target purification & impurities removal

| Natural product |
| --- |
| Target concentration is low/medium/high |
| Crude Stable in Organic solvents |
| Extract is well balanced buffer system |
| Easy recovery from fractions |
| Bioactivity might be affected |

| Pharmaceuticals |
| --- |
| Target concentration is high |
| Stable in Organic solvents |
| Easy recovery from fractions |
| Bioactivity is not affected |

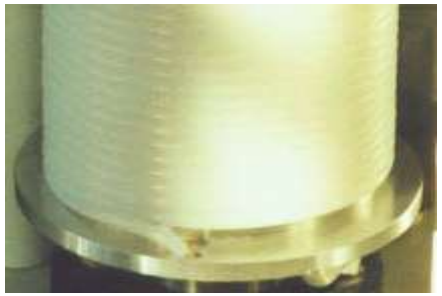
| Biopharmaceuticals |
| --- |
| Target concentration is low |
| Stability has to be carefully guided |
| Recovery can be complicated |
| Bioactivity can be compromised |

# Biopharmaceutical manufacturing technology flowchart (upstream and downstream bioprocesses)



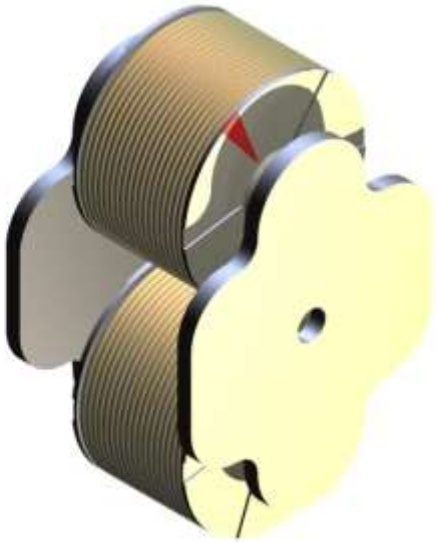

*A. Faustino Jozala, et al. Brazilian Journal of Microbiology, 47S, 2016, 51–63*

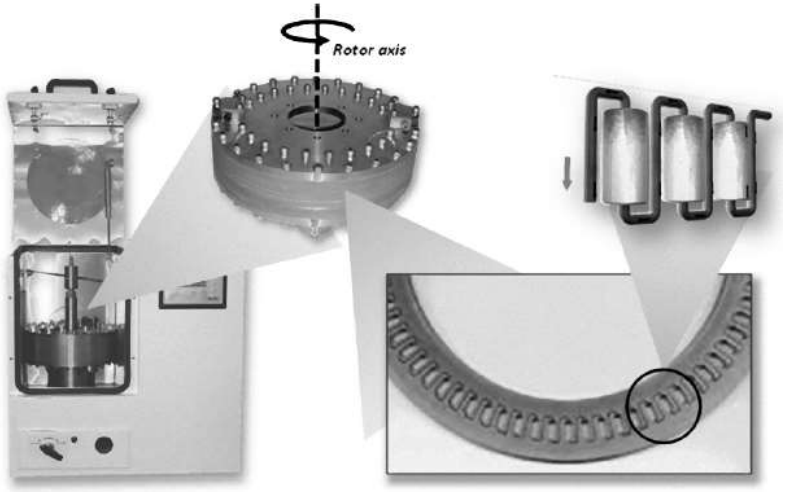
# CCC/CPC Instrument schematics



Multilayer column

CPC rotor
&
chambers





# ABC facilities:
# Dynamic Extractions CCC instruments

| | Mini | Spectrum | Midi | Maxi |
|---|---|---|---|---|
| Coil volume (mL) | 20 | 22 and 132 | 1000 | 4600 or 18000 |
| Flow rate (mL/min) | 0.5-2 | 0.5 - 12 | 10-80 | 500 - 1500 |
| Loading (g/run) | Up to 200mg | Up to 2 | 5 to 40 | 20 to 700 |
| Rotational speed @240g (RPM)* | 2100 | 1600 | 1400 | 600* |
| Elution time for D=1 component (min) | 20 | 20 | 20 | 20 |

*Maxi currently runs at 120g

# ABC facilities: from analytical to pilot scale, 4.6 & 18L MAXI-centrifuges

# ABC:
# Contract Research & Academic Investigations

- Natural product purifications
- Continuous processing & Scale up
- Small molecules
- Screening for novel metabolites
- Pharmaceutical waste stream purification
- Cell, Virus & other Bioparticles separations
- Biopharmaceuticals (incl Antibody-based products)
- Bioreactor/separator for enantiomer conversion
- Biofuels from non-food resources
- Nanomaterials
- Biorefinery (monosaccharides separation from SBP)
- Technology development for novel applications

# Liquid nature of stationary phase: removal of bulk impurities

## Fractionation of Brazilian red propolis crude extracts

***with***

***Dr Begoña Gimenez-Cassina &***
***Prof Alexandra C H F Sawaya at the University of Campinas (UNICAMP, Brazil)***

UNICAMP

Brunel University London

# Combination of CCC capacity & HPLC resolution power

- Difficulties with HPLC separations at preparative scale (presence of waxes, emulsification, very low loading).
- Removal of bulk impurities with CCC for further purification with HPLC.
- Further UPLC-MS analysis and preparative HPLC separation
- Bioassays and Toxicology studies

# Combination of CCC capacity & HPLC resolution power

- CCC step gradient in normal phase:
  1-25min with hexane-methanol-water (5:4:1)
  25-60min with hexane-ethyl acetate-methanol-water (1:1:1:1).
- Sample dissolved in methanol (higher loading, 2.5g/1L column)

# Bio-applications using CCC as extraction technology

## Capture and fractionation of Antibody-based product using Counter Current Extraction and ATPS

*with*

*Will Lewis, Peter Kampalume (GSK)*


# Purification of e-mAb using CCC
## (analogy to capture (bind/elute) chromatography)

1) Sample loading

Crude material is a part of the mobile phase

Impurities that partition into the mobile phase

2) Washing the impurities from the column

Pure mobile phase

Impurities that partition into both phases

3) Liquid stationary phase (SP) extrusion

Product (e-mAb) in SP

# Selection of ATPS parameters

Partitioning of e-mAb and impurities were tested in a range of aqueous two-phase systems (ATPS) to identify those which provided differential partitioning.
Used Protein A to quantify e-mAb partitioning and SEC and SDS-PAGE to check selectivity.

The ATPS variables were:

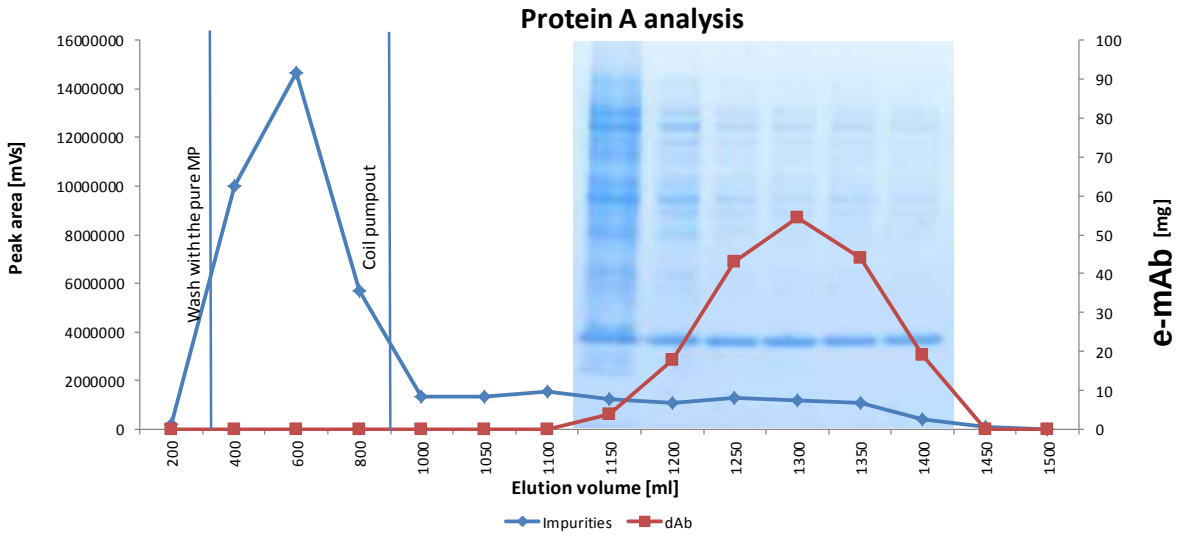
A) PEG molecular weight (400, 1000 and 3350Da)
B) PEG and salt concentration (14/14 and 20/20% w/w)
C) Presence of an additional salt (none, 5% NaCl and 5% $NaClO_4$)
D) Salt type (ammonium sulphate, di-potassium phosphate and sodium citrate)

Although pH was not tested as a separate factor, each of the three salts had different pH (potassium phosphate (pH 9.0-9.5), sodium citrate (pH 8.6-10.1) and ammonium sulphate (pH 5.5-6).

# Optimisation of the CCE conditions
## with 14/14/5% w/w/w/ PEG1000/ sodium citrate /NaCl

To maximise the product yield and purity by testing:

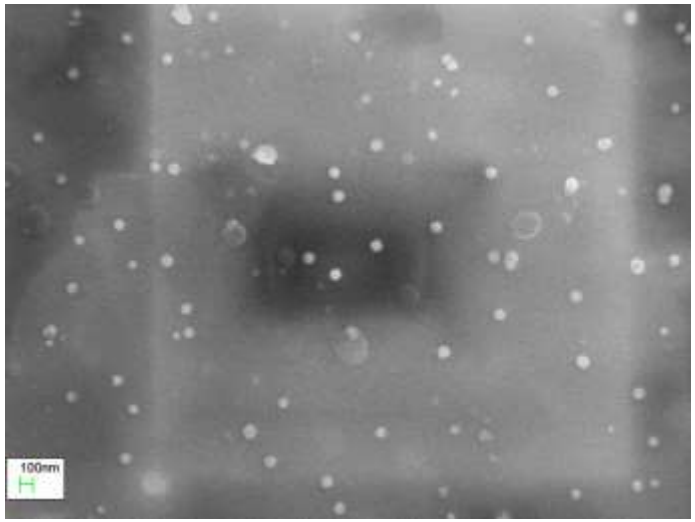
- Sample loading (volume and concentration fermentation crude)
- Volume of the mobile phase to wash the impurities from the column
  Mobile phase flow rate & type of mixing

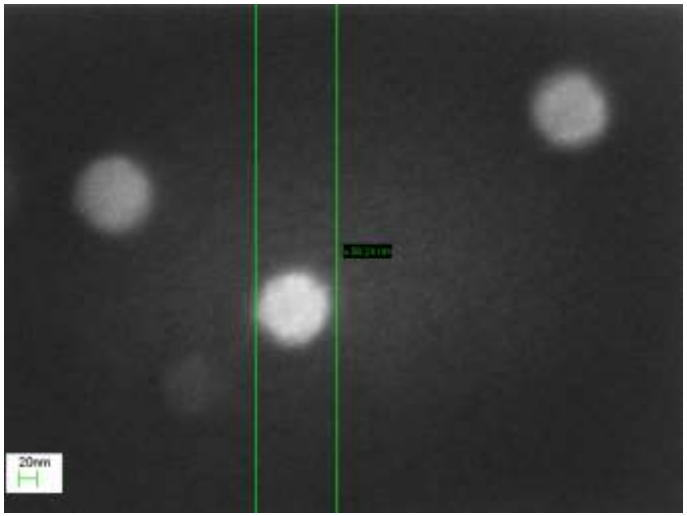


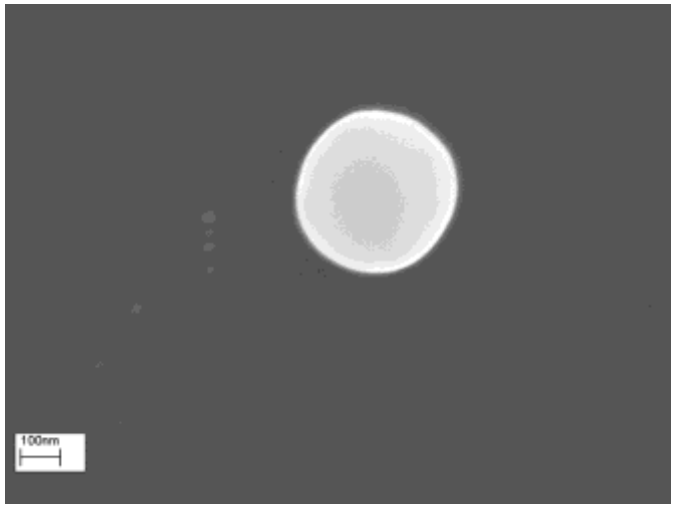

Purification of e-mAb on Midi

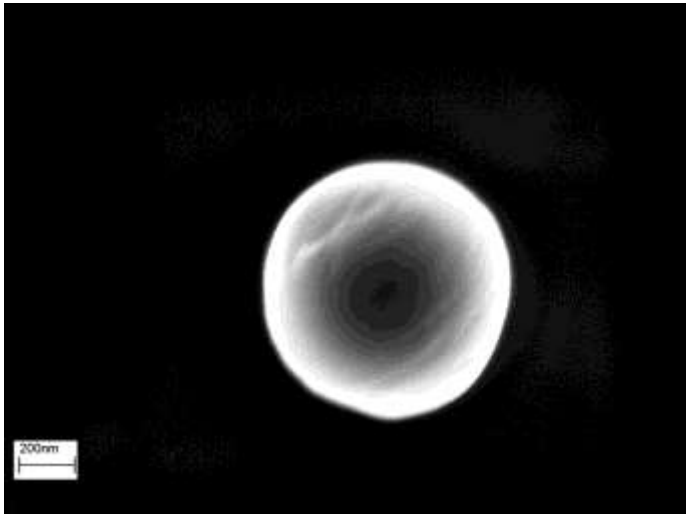
| Volume [ml] | | | e-mAb [mg] | | e-mAb yield | Flow rate | Run time |
|---|---|---|---|---|---|---|---|
| Coil | Sample | Crude | Loaded | Recovered | [%] | [ml/min] | [min] |
| 450 | 225 | 137 | 261 | 182 | 70 | 10 | 80 |

# PLA & PCL nanoparticles manufacture in CCC



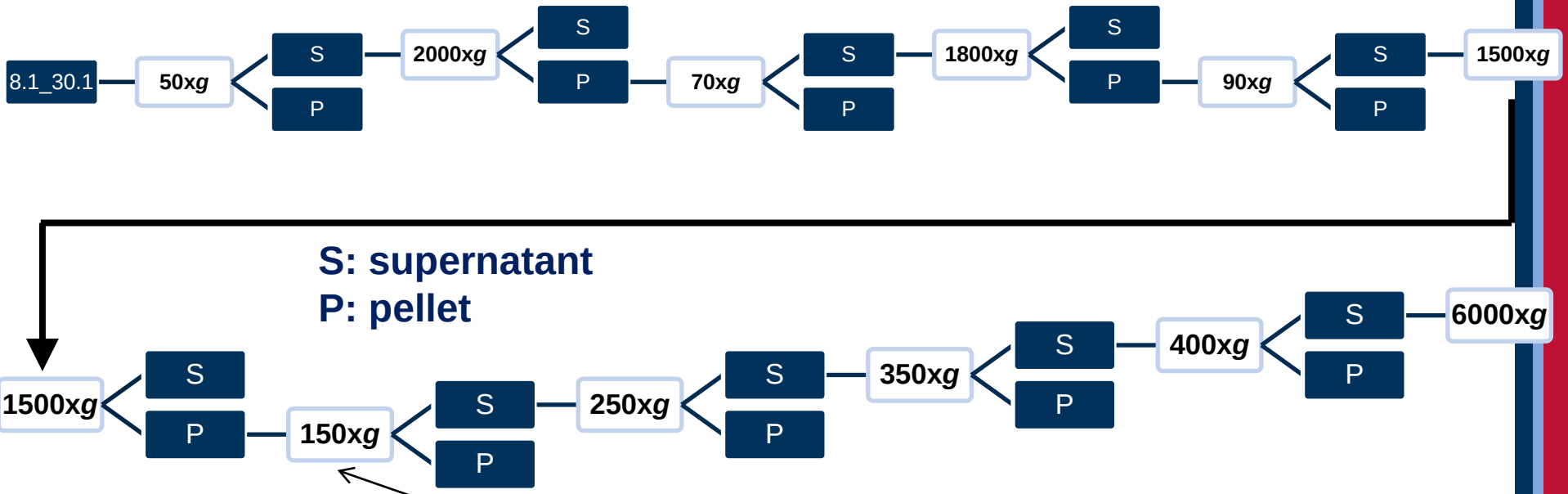



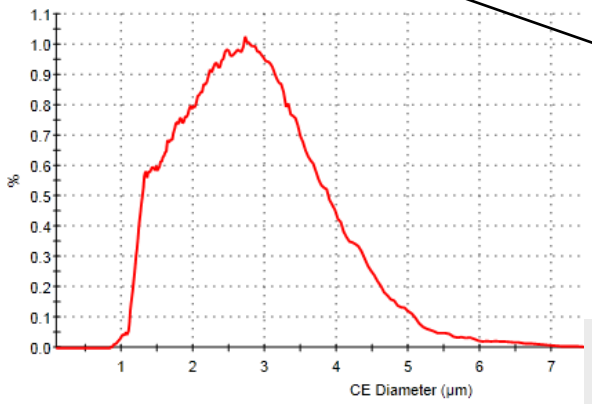




Flow rate 0.5 ml/min
Pumping direction – from Head (Centre) to Tail (Periphery)
Sample concentration:10mg/ml PLA in DCM; 5mg/ml PCL-HPG(Na) in Acetone

# Agarose crude fractionation by centrifugation - *workflow*

centrifugation of crude lot8.1 with stepwise and alternating *g*-forces
*aim:* removing big particles by low *g*-force and small particles by high *g*-force

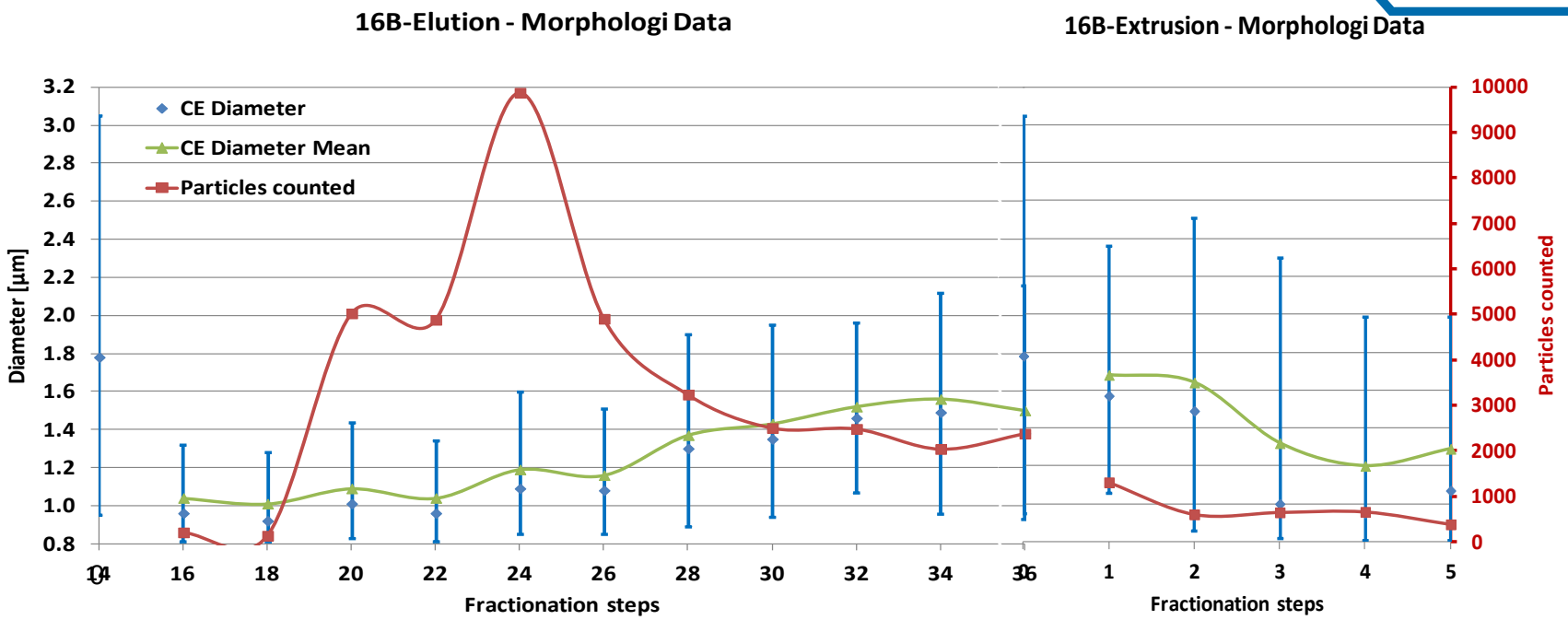


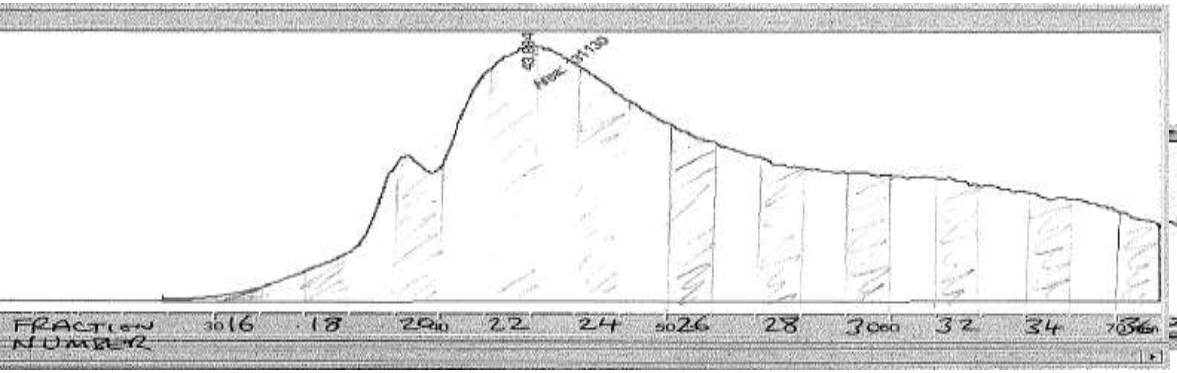



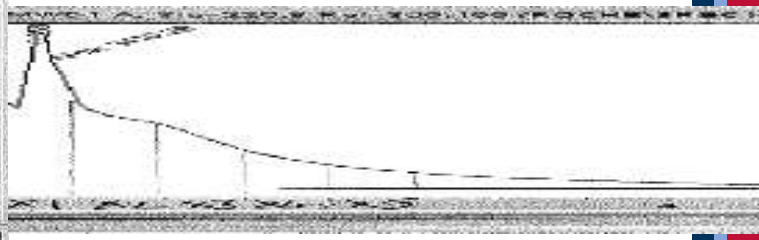

*xg* : centrifugation *g*-force 20 min 8 °C

most of small particles < 1µm are removed

# Fractionation by CCC (ABC)



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# Collaborative research with Biosciences…

Dr Michael Themis

Dr Evgeny Makarov & Dr Terry Roberts

Dr Joanna Bridger (Interdisciplinary award)

Brunel University London

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# Our research is a team work!

Prof Svetlana Ignatova

Dr Peter Hewitson

Dr Ian Garrard

Dr Jonathan Huddleston

Prof Ian Sutherland

Prof Derek Fisher