The EMBL protein expression and purification core facility

Kim Remans

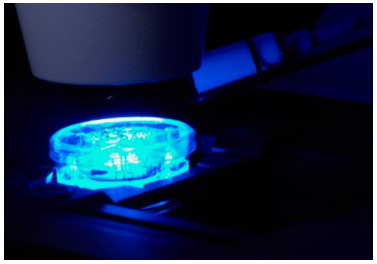
P4EU meeting June 2016

EMBL

CTLS2016

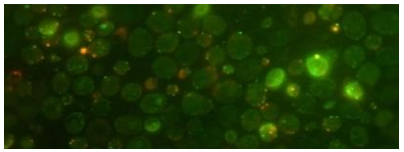
EMBL´s scientific core facilities

Advanced light microscopy
Rainer Pepperkok

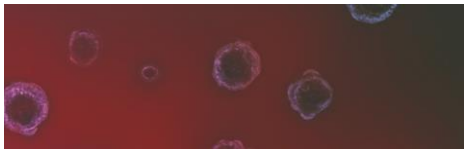


Chemical biology
Joe Lewis

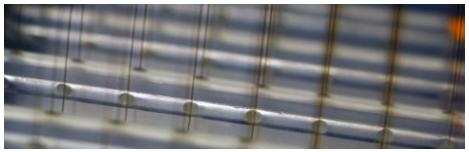
Electron microscopy
Yannick Schwab



Flow cytometry
Malte Paulsen

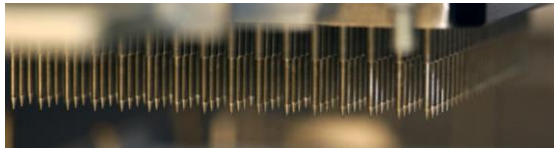


Genomics
Vladimir Benes

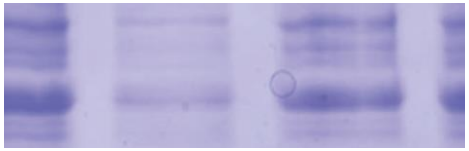


Metabolomics
Theodore Alexandrov

Protein expression & purification
Kim Remans



Proteomics
Mikhail Savitski

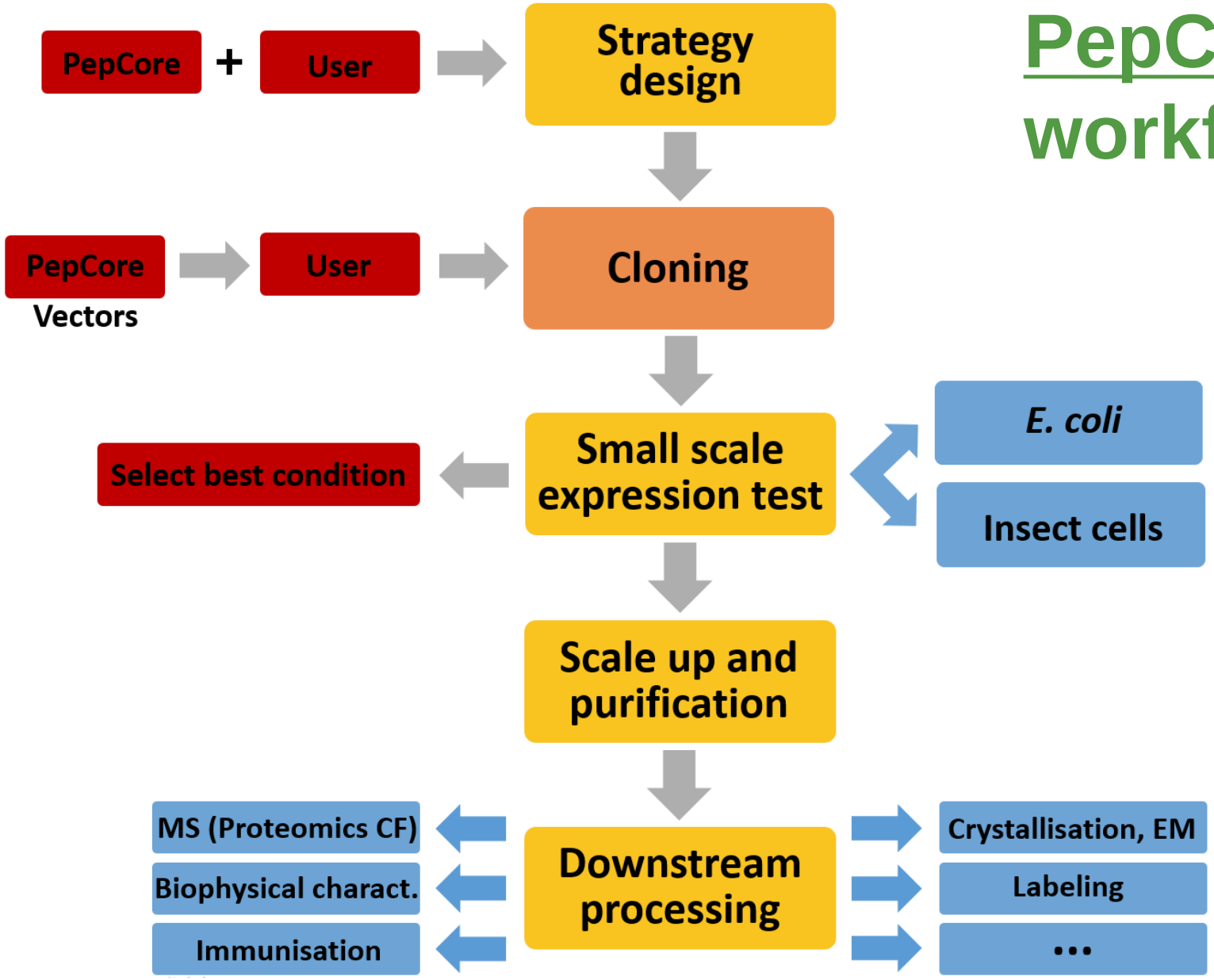


Protein expression and purification core facility (PepCore)

- Scientific advice and assistance regarding protein expression and purification
- Service facility: expression tests, scale-up and purification
- Biophysics: characterisation of proteins and their interactions with different types of molecules
- Teaching & training: courses, visitors, ...
- Frequently used proteins:
  - ✓ proteases (TEV, 3C, SenP2)
  - ✓ polymerases (Taq, Pfu, T7 RNA polymerase)
  - ✓ others (Cas9, LIF, Cre, RNAsin, BirA, anti-eGFP Nb)
- Open to internal and external users

PepCore: the team

PepCore: workflow



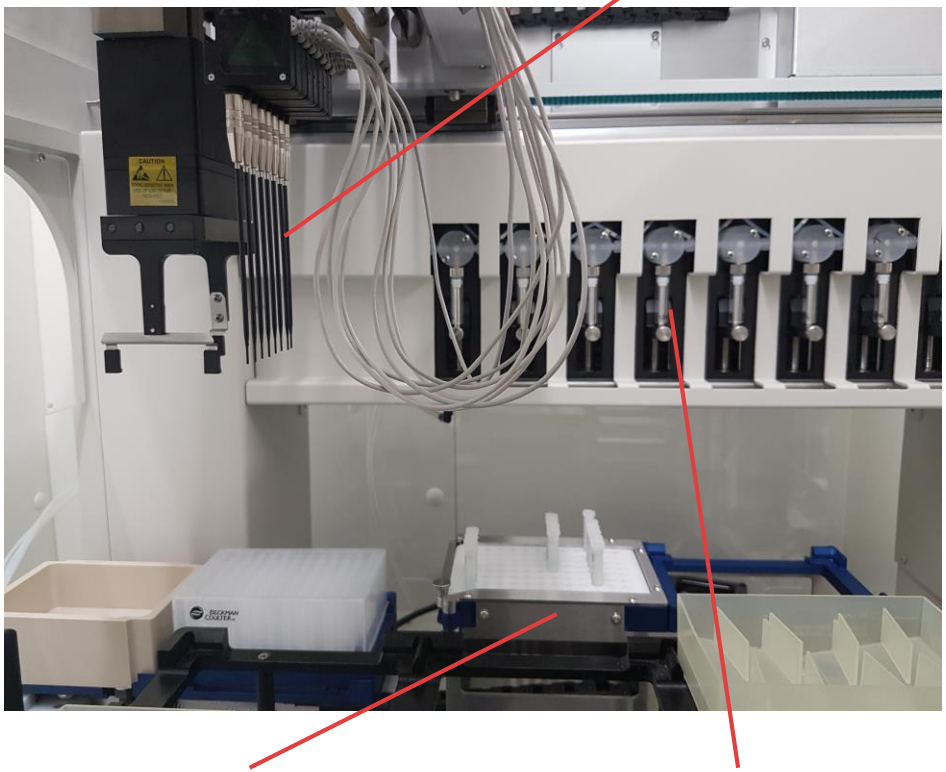
PepCore: equipment

Protein purification

- 3 Akta Purifiers (GE)
- 1 Akta Pure (GE)
- 1 NGC Quest (Biorad)

PepCore: equipment

Automated liquid handling: Biomek NX^{P} Span8

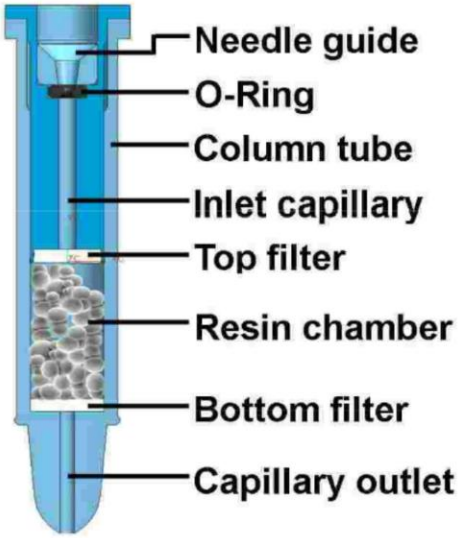


PepCore: equipment

High throughput screening with RoboColumns

Analytical scale:

- 50 µl affinity columns (Ni-NTA & GSH-sepharose)
- Screen entire protein family
- Screen homologs from different organisms
- Large complexes: screen single components or subcomplexes

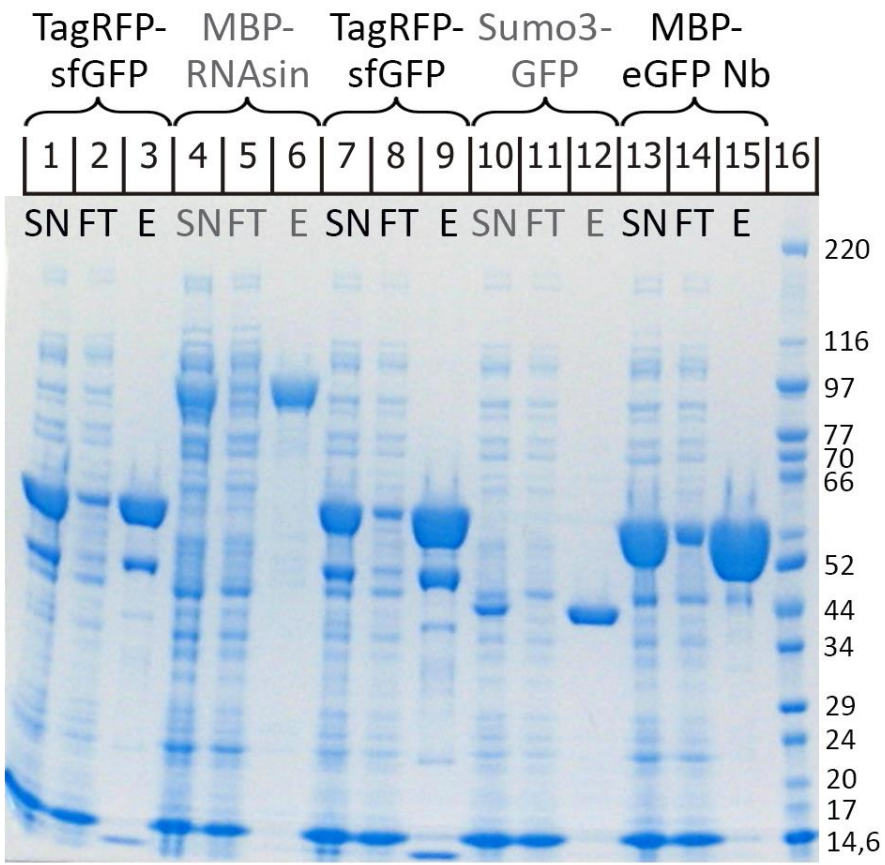


Semi-preparative scale:

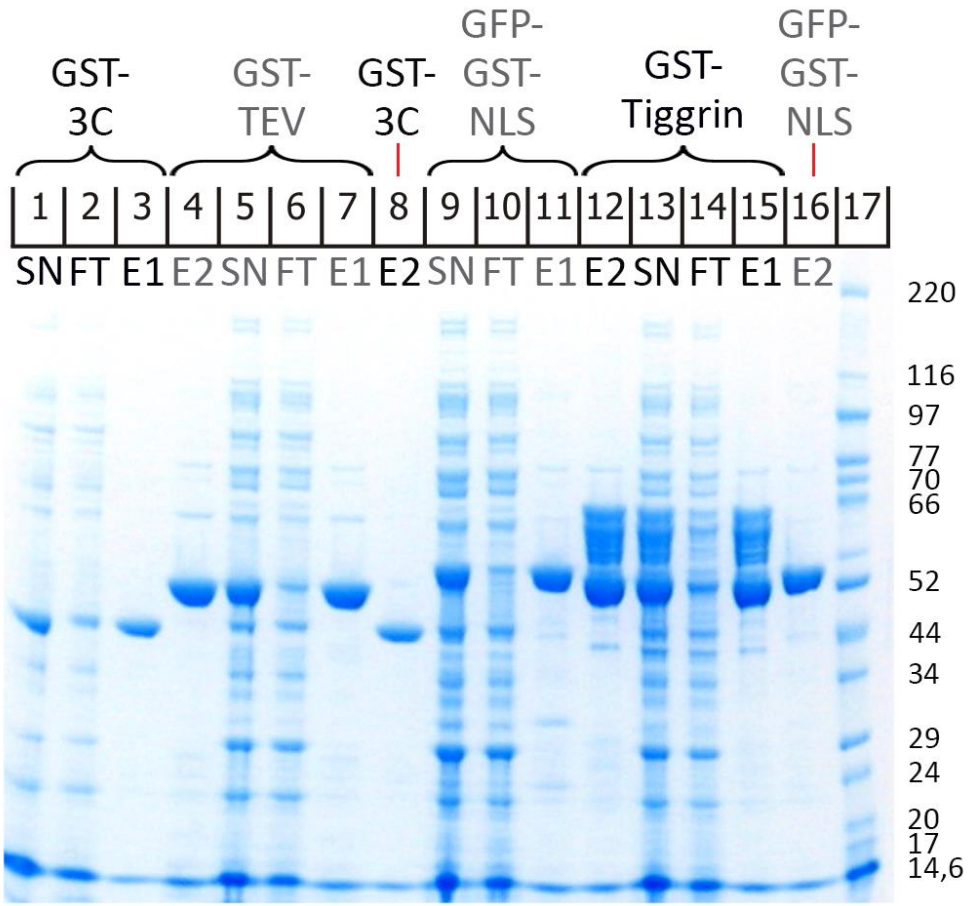
- 600 µl affinity columns
- Scale up best hits from analytical screen
- Purify a couple of mg´s of protein
- Tandem purifications of multi-component complexes

1st test runs of Biomek NXP Span8 and RoboColumns

50 µl Ni-NTA



100 µl glutathione-sepharose



SN: supernatant (cleared lysate)
FT: flow through column

E & E1: elution
E2: elution 2nd run (regenerated column)

Biophysics

Senior biophysics officer: Vladimir Rybin

- Advice on experimental set-up
- Data analysis
- Maintenance of equipment, training of new users

Equipment: shared with units

- MicroCal ITC200
- Jasco815 CD spectrometer
- PTI spectrofluorometer
- Agilent Cary60 UV-VIS spectrophotometer
- ~~Beckman Optima XL-A Analytical Ultracentrifuge~~
- NanoTemper Monolith NT.115 (MicroScale Thermophoresis)
- Malvern Zetasizer μV (dual function light scattering detector)
- Malvern Viscotek (multi-detector GPC/SEC system)
- BioLogic Stopped-Flow system

Biophysics

THERMOFLUOR: optimise buffer conditions to improve stability, solubility or folding

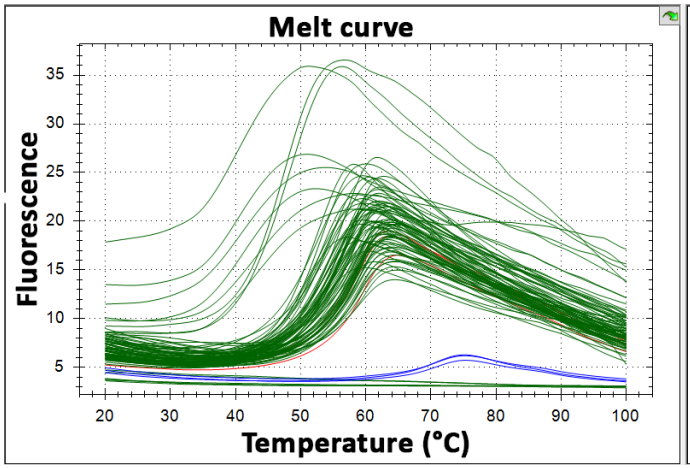
- Molecular Dimensions RUBIC buffer screen:

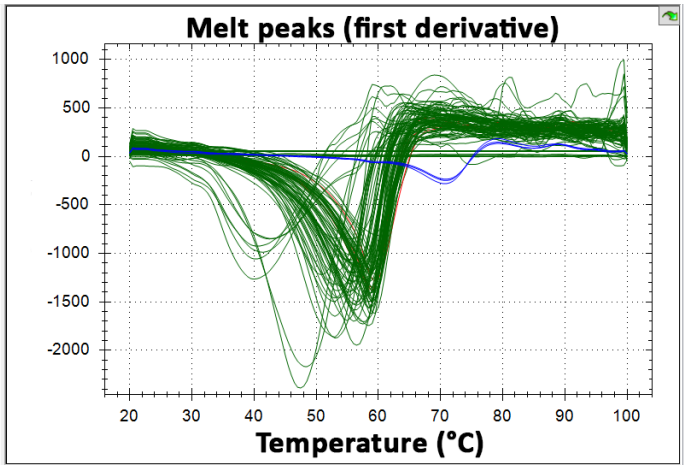
  pH, buffering component, salt concentration

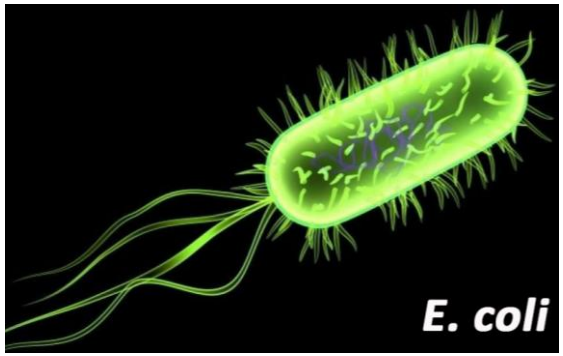
- Molecular Dimensions RUBIC additive screen:

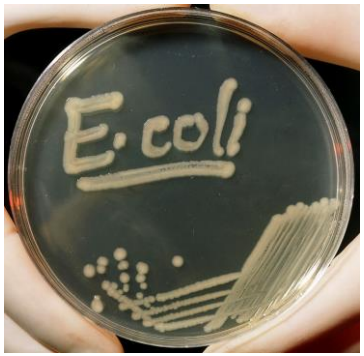
  salts, ions, chaotropes, detergents, nucleotides, sugars, co-factors, glycerol, ...

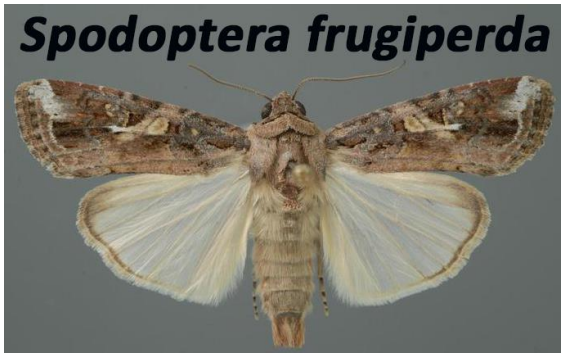
- SYPRO orange dye: fluoresces upon binding to hydrophobic patches

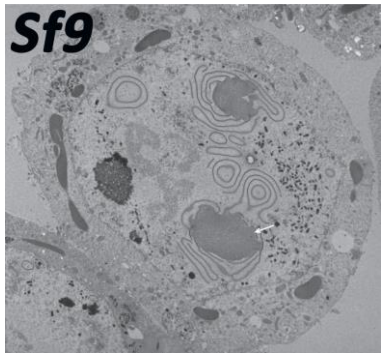




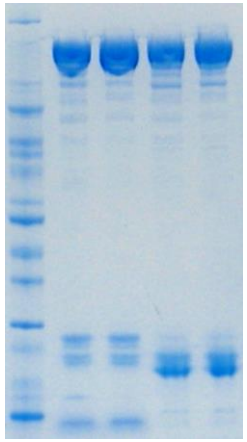








Thank you for your attention!



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