The EMBL protein expression and purification core facility

Kim Remans
P4EU meeting June 2016
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: equipment

Protein purification

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

Automated liquid handling: Biomek NX⁺ Span8

- Fixed needles
- RoboColumn ALP
- 1 ml syringes
- 15 deck spaces
- Washing station
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 NX<sup>P</sup> Span8 and RoboColumns

50 µl Ni-NTA

- TagRFP-sfGFP
- MBP-RNAsin
- TagRFP-sfGFP
- Sumo3-GFP
- MBP-eGFP Nb

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100 µl glutathione-sepharose

- GST-3C
- GST-TEV
- GST-3C
- GST-NLS
- GST-Tiggrin
- GFP-GST-NLS

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**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

![qPCR Image]
Thank you for your attention!