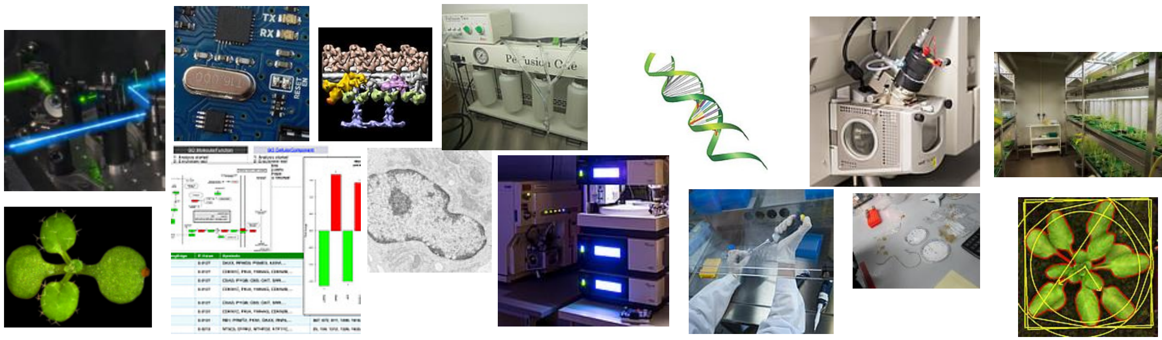

Benchmarking of biomolecular interaction methods

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CEITEC BIC

VBCF VIENNA BIOCENTER CORE FACILITIES

EUROPEAN UNION

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Interreg Austria-Czech Republic
European Regional Development Fund

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Pilot project introduction

***Project partners**:*

- *CEITEC MU – Biomolecular Interaction and Crystallization Core Facility*
- *VBCF – Protein Technologies Core Facility*

***Problem**: Results obtained by biophysical techniques used for biomacromolecular (protein) characterization frequently do not meet expectations, low reproducibility → increased demand in time, money and manpower*

***Main causes**:*

1) Sample quality is not corresponding to the minimal requirements of experiment
2) Experiments using different machines in different laboratories are not conducted the same way

***Partial goals**:*

- *Define the minimal description of biomacromolecular samples*
- *Create standardized service for characterization of user samples*
- *Develop standard operation procedures (SOPs) for main biophysical techniques used in partnering facilities*
- *Set-up mechanisms for continuous quality improvement of services via know-how exchange*

***Project goal**: To give users of both involved facilities an easy tool to characterize their samples and ensure access to standardized methods*

Pilot project introduction

Broad range of equipment available in both partner labs is used for the project:

- ***CF BIC**: Monolith NT.115 (MST), DelsaCore (DLS), VP-DSC (DSC), Jasco J-815 (CD), XL-I (AUC), Prometheus (DSF), AutoITC200 (ITC), VP-ITC (ITC), Biacore T200 (SPR), Rigaku Minstrel*

- ***ProTech CF**: Monolith NT.115 (MST), Monolith NT.LabelFree (MST), DynaPro PlateReader (DLS), VP-DSC (DSC), Chirascan Plus (CD), Akta micro (SEC-MALLS)*

Project implementation

Main steps in collaboration:

Staff exchange

- *Know-how sharing*
- *Development*
- *Optimization*

Experimental system testing and optimization

- *PAIIL – fucose*
- *Chymotrypsin – soybean trypsin inhibitor*
- *Mannose-binding lectin – maltose*
- *Lysozyme – NAG3*

Protocols and procedures development

- *Based on current state of problematics*
- *Implementing new approaches*
- *Combining precision with simplicity*

Testing of created procedures

- *Internal – Core Facilities' staff*
- *External – users*

Date	ProTech → Brno	BIC → Vienna
7.6.2017	1	
3.-4.8.2017	18	
27.4.2018	2	
23.-24.5.2018	2	
6.-7.6.2018		6
20.-21.6.2018		14
4.-6.9.2018	3	

Number of person*days/visit

Project results

Standard operating procedures (SOP)

User benefits:
- *Step-by-step description of experimental set-up*
- *Reduces user-based mistakes*
- *Ensures higher data reproducibility*
- *Improves lab-to-lab agreement in results*

Developed for several techniques
- *AUC, CD, DLS, ITC, MST*

Currently under testing and optimization

STANDARD OPERATING PROCEDURE (SOP)

Analytical ultracentrifugation – Sedimentation equilibrium

Sample preparation

Selection of rotor speeds

STANDARD OPERATING PROCEDURE (SOP)

Analytical ultracentrifugation – Sedimentation velocity

Sample preparation

Cell assembly and filling the cells

STANDARD OPERATING PROCEDURE (SOP)

Isothermal titration calorimetry – AutoITC200

TURNING ON THE INSTRUMENT:

BEFORE MEASUREMENT:

SAMPLE:

SETING-UP THE MEASUREMENT:

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Austria-Czech Republic
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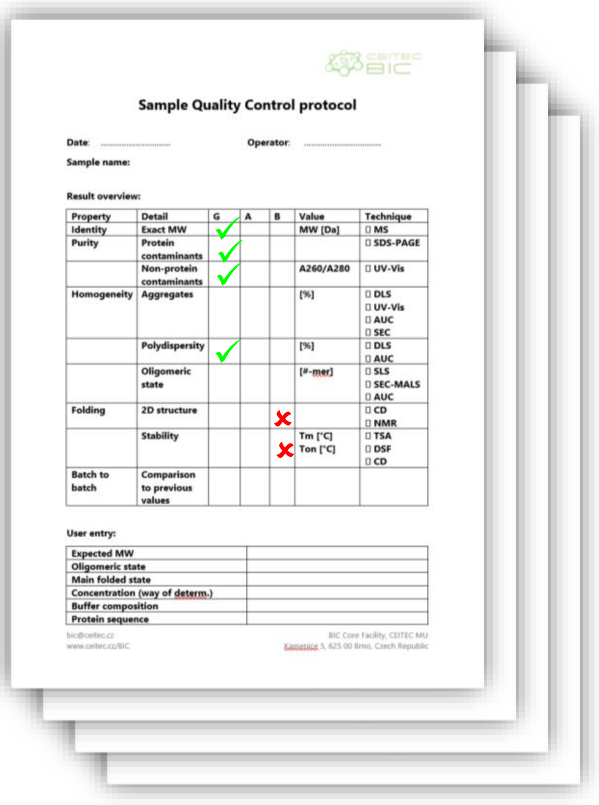
Project results

Sample quality protocol

User benefits:

- *Standardized way of sample characterization*
- *Defined criteria of sample quality*
- *Clear one-page summary*
- *Quick response from facility*

Currently under optimization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Sample Quality Control protocol

Date: .... **Operator:** ....

Sample name:

Result overview:

Property	Detail	G	A	B	Value	Technique
Identity	Exact MW	✓			MW [Da]	☐ MS
Purity	Protein contaminants	✓				☐ SDS-PAGE
	Non-protein contaminants	✓			A260/A280	☐ UV-Vis
Homogeneity	Aggregates				[%]	☐ DLS ☐ UV-Vis ☐ AUC ☐ SEC
	Polydispersity	✓			[%]	☐ DLS ☐ AUC
	Oligomeric state				[#-mer]	☐ SLS ☐ SEC-MALS ☐ AUC
Folding	2D structure			✗		☐ CD ☐ NMR
	Stability			✗	Tm [°C] Ton [°C]	☐ TSA ☐ DSF ☐ CD
Batch to batch	Comparison to previous values					

User entry:

Expected MW	
Oligomeric state	
Main folded state	
Concentration (way of determ.)	
Buffer composition	
Protein sequence	

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Kamenice 5, 625 00 Brno, Czech Republic

Project results

***Workshops** organized with support of RIAT-CZ project:*

- *Modern techniques in Molecular Biology, 18.-20.7.2017, Vienna*
  - *76 person*days*
- *Differential scanning calorimetry workshop, 3.-4.10.2017, Brno*
  - *61 persons*
- *Biomolecular crystallization workshop, 23.-26.10.2017, Brno*
  - *46 persons (lectures), 26 persons (practicals)*
- *How to characterize your sample and check its quality, 23.-25.5.2018, Brno*
  - *36 persons (lectures), 17 persons (practicals)*

Acknowledgement

All members of VBCF ProTech CF and CEITEC BIC CF

Does your facility require specific sample characterization? Contact us!