

SBMP Workshop 2010:

Cell-Free Expression of Membrane Proteins

Principles

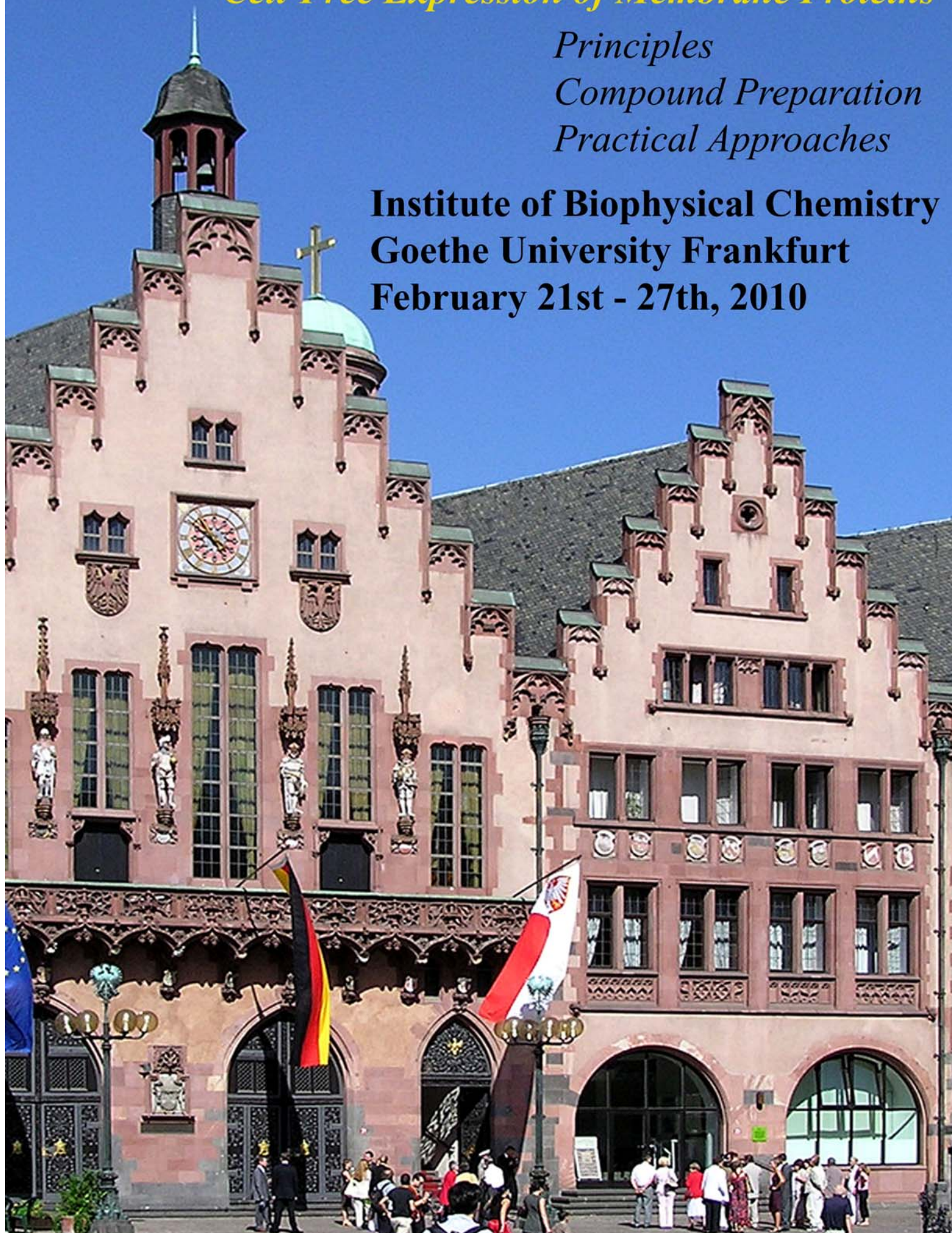
Compound Preparation

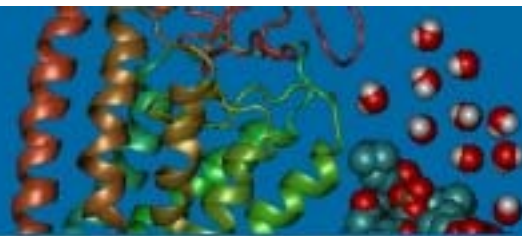
Practical Approaches

Institute of Biophysical Chemistry

Goethe University Frankfurt

February 21st - 27th, 2010





Cell-Free Expression of Membrane Proteins

Principles, Compound Preparation and Practical Approaches

Course outline:

- S30 cell-free extract preparation
- Wheat germ extract preparation
- Characteristics of bacterial and eukaryotic cell-free expression systems
- How to establish efficient cell-free expression systems
- Design and calculation of cell-free reaction protocols
- Expression modes for cell-free membrane protein production
- Compound screening and yield optimization in throughput scales
- Strategies for membrane protein expression screening
- Set-up of cell-free expression reactions in analytical and preparative scales
- Quality control of membrane proteins by modulation of expression conditions
- Specific labelling of membrane proteins by cell-free expression
- Bring your own template: Expression screening of new targets possible

Date: February 21st – 27th, 2010

Venue: Institute of Biophysical Chemistry, Biocentre of the Goethe University Frankfurt/Main

Instructors: Vladimir Shirokov, Institute of Protein Research, Pushchino, Russia

Frank Bernhard, Institute of Biophysical Chemistry, Goethe University Frankfurt, Germany

Lei Kai, Institute of Biophysical Chemistry, Goethe University Frankfurt, Germany

Davide Proverbio, Institute of Biophysical Chemistry, Goethe University Frankfurt, Germany

Number of participants: Limited to six students

Costs: Admission is free to SBMP members. Travel costs should be covered by the participants. Hotel accommodation close to the campus can be organized at approx. 60 €/per room. Rooms may be shared by two persons.

Registration deadline is December 31st 2009.

Please register by email to: fbern@bpc.uni-frankfurt.de.

Workshop Agenda

- Sunday 21st:** **Arrival at hotel**
17.30 – 18.30 Meeting and dinner at IBPC/University
18.30 – 20.00: Introduction and workshop overview
Lecture: Applications of cell-free expression systems in protein research
- Monday 22nd:** **Eukaryotic cell-free (CF) expression systems**
8.30 – 9.30: **Lecture:** Eukaryotic CF expression systems
9.30 – 12.00: **Demonstration:** Wheat germ embryo and extract preparation
12.00 – 13.00 **Lunch**
13.00 – 17.00 **Lab:** Wheat germ expression
19.00 - Get-together in an authentic Frankfurt Äppelwoi tavern
- Tuesday 23rd:** **CF expression of membrane proteins**
8.30 – 9.30: **Lecture:** CF production of membrane proteins
9.30 – 12.00 **Lab:** Evaluation of wheat germ expression
12.00 – 13.00 **Lunch**
13.00 – 14.00: **Lecture:** Compounds/configurations/modes for CF expression
14.00 – 18.00: **Instruction:** Design/set-up of CF expression reactions
Lab: P-CF and D-CF mode expression of transporters
Lab: D-CF detergent screen
Lab: P-CF expression of individual targets
- Wednesday 24th:** **Detergent screen and evaluation of CF expression reactions**
8.00 – 10.00: **Lab:** P-CF detergent screen
10.00 – 12.00: **Lab:** SDS-PAGE analysis, GFP quantification
12.00 – 13.00: **Lunch**
13.00 – 15.00: **Lab:** CF reaction analysis
15.00 – 18.00: **Lab:** Preparation of 2 x 10 l fermenter and starter cultures
- Thursday 25th:** ***E. coli* S30 extract preparation**
7.30 – 11.00: **Lab:** *E. coli* fermentation, recording of growth curves
8.00 – 9.00: **Instruction:** S30 extract and enzyme preparation
11.00 – 14.00: **Lab:** Cooling and harvesting of cells
Lunch time depends on cell growth
14.00 – 19.00: **Lab:** S30 extract preparation
Overnight: Dialysis of extract
- Friday 26th:** **Analysing S30 extract preparations**
8.30 – 10.00: **Lab:** Harvesting and analysing S30 extract
10.00 – 12.00 **Lab:** Analysing and optimization of S30 extract quality
12.00 – 13.00: **Lunch**
13.00 – 15.00: **Demonstration:** Throughput screening of CF expression conditions
15.00 – 17.00: **Final discussion group** with German Glühwein and snacks
- Saturday 27th:** **Departure**

Venue:

Goethe-University Frankfurt, Campus Riedberg

Institute of Biophysical Chemistry

Max-von-Laue Str. 9, 60438 Frankfurt

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Phone: 0049 (0)69 79829620

Cell phone: 0151 28088204

Direction to the Goethe-University/Campus Riedberg:

- with S-Bahn 1/2/3/4/5/6/8/9 to “Hauptwache”

then either:

- with U-Bahn, line 2, to “Sandelmühle”

- then with bus, line 26, to “Uni Niederurseler Hang

or:

- with U-Bahn, line 3, to “Niederursel”

- turn right to “Kreuzer Hohl”, walk uphill for ~ 5 minutes until you reach the Campus.

Hotel:

