Dresden, 24 August 2010

ESF Unit	**LESC**
EUROCORES Programme:	**Membrane Architecture and Dynamics**
EUROCORES Acronym:	**EuroMEMBRANE**
Reference N°:	**216**

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a) Scientific report

Summary

The objective of this course was to give an introduction to the methods that can be used to analyze lipid rafts in cells and model membranes. The challenge was to provide a practical guide to this complex field in order to promote progress in understanding membrane subcompartmentalization. The course covered use of detergents and pitfalls, preparation of plasma membrane vesicles and spheres, lipid-protein interaction in model membrane systems, membrane order measurement techniques, lipid mass spectrometry, single molecule microscopy techniques (STED, Scanning FCS). We had a good response with 70 applications. We selected 16 participants with a spread around Europe as well as participants from Singapore and USA (see list). We had impressive speakers who really did a great job and were enthusiastically received by the participants. The practical course was also a success (see programme). The course was funded not only by the EUROCORE programme but also by EMBO. The accounts are enclosed.

Scientific Program

Sunday, 23 May

Arrival at "Hotel am Blauen Wunder"

Loschwitzer Straße 48

01309 Dresden

Tel. +49-351-33660, Fax: +49-351-3366299

19:00 Dinner at restaurant Schillergarten

Program **Monday, 24 May**

9:00	Opening lecture Kai Simons	Small Auditorium
9:50	Short talks: Participants	
11:20	Pinning up of posters	
12:00	Lunch at MPI-CBG canteen	
13:00	Poster Session	
14:30	Participant meeting	Galleria 2nd floor
16:00	Dimitrios Stamou	Small Auditorium
	"Bending cell membranes"	
17:00	Ilpo Vattulainen:	Small Auditorium
	"Modeling lipid-lipid and protein interactions"	

Program **Tuesday, 25 May**

9:00	Christian Eggeling:	Small Auditorium
	"STED microscopy in analyzing membrane dynamics"	
10:00	Experiments in the lab	
12:30	Lunch at MPI-CBG canteen	
13:00	Experiments in the lab	
17:00	Hai-Tao He:	Small Auditorium
	"How do lipid rafts facilitate signaling	
	at the cell surface"	

Program **Wednesday, 26 May**

9:00	Christoph Thiele:	Small Auditorium
	"Use of fluorescent lipids"	
10:00	Experiments in the lab	
12:30	Lunch at MPI-CBG canteen	

13:00 Experiments in the lab

17:00 Felix Wieland: Small Auditorium

"Lipid –protein interactions"

Program **Thursday, 27 May**

9:00 Sarah Veatch: Small Auditorium

"Is the plasma membrane positioned close to a critical point and if yes what does this imply?

10:00 Experiments in the lab

12:30 Lunch at MPI-CBG canteen

13:00 Experiments in the lab

17:00 Gerhard Schütz: Small Auditorium

"Single molecule methods to analyze membrane functions"

Program **Friday, 28 May**

9:00 Ari Helenius: Small Auditorium

"Lipid-mediated endocytosis"

10:00 Experiments in the lab

12:30 Lunch at MPI-CBG canteen

13:00 Experiments in the lab

17:00 Petra Schwille: Small Auditorium

"Spectroscopic imaging methods to analyze membrane functions"

Program	**Saturday, 29 May**	
9:00	Tobias Baumgart:	Small Auditorium
	"Sorting of lipids and proteins by membrane domains and curvature"	
10:00	Experiments in the lab	
12:30	Lunch	
13:00	Experiments in the lab	

Program	**Sunday, 30 May**
9:45	Boat tour to Meissen
12:15	Guided tour through the porcelain manufactury
3:00	Wine tasting at vineyard Prinz zur Lippe, Proschwitz
	http://www.schloss-proschwitz.de/
19:00	Concert at castle Wackerbarth
	http://www.mikhailsimonyanviolin.com/

Program	**Monday, 31 May**
10:00	Finishing experiments in the lab
12:00	Summarizing meeting with participants, organizer and host lab
13:00	Lunch at MPI-CBG canteen
Departure	

Program for the Participants

All 16 participants were distributed into 4 groups with 4 participants each. The work packages (WP) 1-4 were offered on daily from Tuesday to Friday, allowing every group to pass through each WP. The special WP by Ana Stevanovic was optional and offered on Saturday only. More than 50% of all participants brought own samples to measure. For one participant we have designed a series of new experiments.

WP 1: Plasma membrane vesicle and spheres for phase separation studies

Instructors: Hermann-Josef Kaiser, Christian Lange, Ilya Levental, Daniel Lingwood

Theory:

1. Why model membranes systems and not living cells only?
2. nGPMV vs. pdGPMV vs. GUV, properties, pitfalls
3. Lipid and protein marker (to be shared with module 4)

Experiments:

1. Isolate nGPMVs and pdGPMVs from A431 or BHK and MDCK basolateral and apical.
2. Prepare GUVs
3. Compare lipid and protein markers
4. Impact of cholesterol removal and titration
5. Impact of clustering

WP 2: Lipid protein interactions using reconstituted liposomes vesicles

Instructors: Uenal Coskun, Michal Grzybek

Theory:

1. Pitfalls in lipid binding detection techniques: Lipid Strips vs. Pelleting vs. Floatation vs. MSD Chemiluninescence Assay

Experiments:

1. Compare lipid binding and possibly curvature selectivity of FAPP1-PH domain and FAPP2
2. Producing liposomes with varying diameter (30-200nm) and lipid compositions
3. Lipid binding assays: Floatation & MSD Assay

WP 3: Florescence correlation- and STED microscopy

Instructors: Jonas Ries, Erdinc Sezgin

Theory:

1. The need for high resolution methods
2. FCS vs. scanning FCS on biological membranes
3. STED
4. Lipid markers

Experiments:

1. Use vesicles from module 1 for sFCS
2. Test lipid and Proteinmarkers

Module 4: Lipid analysis by mass spectrometry

Instructors: Julio Sampaio, Mathias Gerl

Theory:

1. Lipid extraction Methods
2. Lipid MS
3. Relative vs. absolute quantification

Experiments:

1. Use Vesicles from Module 1
2. Lipid extraction
3. Lipid MS
4. Comparison to MDCK and A431 or BHK total

Special WP: Fluorescent lipids in cell biology

Instructor: Ana Stevanovic

Theory:

1. Study of lipids in cell biology by fluorescent microscopy
2. Methods available for fluorescent labeling of lipids, with emphasis on the labeling methods that were developed in the Thiele lab (polyene lipids, LD540 dye)
1. Advantages and disadvantages of live cell and fixed cell microscopy
2. and discussion on other technologies developed in the lab for studies of lipids (photocholesterol; polyene lipids in applications

Experiments:

1. demonstrate fluorescent labeling methods, example: labeling with small fluorescent dye LD540
2. LD540 staining in both fixed and living cells
3. sample preparation and fluorescent laser scanning confocal microscopy

Assessments of the results

The course was evaluated by the participants. They filled out the EMBO evaluation form and the result was a smashing success (see hardcopy).

Thus the course served the purpose set. European membrane research was boosted by the success. The practical guide that the participants received is bound to show up in increased quality of future publications. This not only concerns the experimental proficiency but also the many discussions between the organizers, speakers and participants on the theoretical foundations of this upcoming field will sow its seeds into the minds of those who were there. This is an area where Europe is at the top and thus we want to keep it this way.

b) List of speakers and participants

List of speakers

Last name	First name	Title	Gender	Position/Job title
Schwille	Petra	Mrs.	Female	Professor
Vattulainen	Ilpo	Mr.	Male	Professor
Ries	Jonas	Mr.	Male	Postdoc
Eggeling	Christian	Mr.	Male	Postdoc
Schütz	Gerhard	Mr.	Male	Postdoc
Baumgart	Tobias	Mr.	Male	Professor
Veatch	Sarah	Mrs.	Female	Assistant Professor
Helenius	Ari	Mr.	Male	Professor
Thiele	Christoph	Mr.	Male	Professor
Wieland	Felix	Mr.	Male	Professor
He	Hai-Tao	Mr.	Male	Postdoc
Stamou	Dimitrios	Mr.	Male	Professor
Stevanovic	Ana	Mrs.	Female	Predoc

List of Participants

Last name	First name	Title	Gender	Position/Job title
Almsherqi	Zakaria	Mr.	Male	PhD candidate
Balogh	Andrea	Ms.	Female	PhD student/Research Assistant
Beck-García	Katharina	Mrs.	Female	PhD
Bissig	Christin	Mrs.	Female	Graduate student
Blaskovic	Sanja	Ms.	Female	Doctoral student
Drayman	Nir	Mr.	Male	PhD student
Ernst	Andreas	Mr.	Male	PhD student
Ezrielev	Eli	Mr.	Male	PhD student
Koivuniemi	Artturi	Mr.	Male	PhD student
Larsen	Jannik	Mr.	Male	PhD student
Lechtreck	Karl	Dr.	Male	Research Assistant Prof.
Santos	Aline	Ms.	Female	PhD student
Steringer	Julia	Mrs.	Female	PhD student
Stjepanovic	Goran	Ing.	Male	PhD student
Sunzenauer	Stefan	Mr.	Male	PHD Student
Thaa	Bastian	Mr.	Male	PhD student

c) Detailed report of Expenditures

		Teilbrutto	
Accommodation			
Invoice 1 (01.06.2010, Hotel am Blauen Wunder, double room		8,499.70	
Guesthouse Jonas Ries		155.00	**8,654.70**
Travel participants (for those who wanted money)			
Jannik Larsen		208.23	
Andreas Ernst		388.26	
Katharina Beck		182.40	
Eli Ezrielev		464.11	
Nir Drayman		400.00	
Stefan Sunzenauer		161.67	
Aline Santos		296.87	
Andrea Balogh		65.41	
Christin Bissig		251.85	
Sanja Blaskovic		261.18	
Artturi Koivuniemi		509.09	
Amount travel costs students			**3,189.07**
Travel speaker			
Ilpo Vattulainen		873.80	
Jonas Ries		313.24	
Christian Eggeling		80.29	
Gerhard Schuetz		236.19	
Tobias Baumgart		708.23	
Sarah Veatch		202.34	
Ari Helenius		649.35	
Christoph Thiele		230.90	
Felix Wieland		110.94	
Hai-Tao He		416.92	
Dimitrios Stamou		132.06	
Ana Stevanovic		242.72	
Amount tavel costs speaker			**4,196.98**
Travel other			
Transport to and from airport speakers		540.38	
public transport for students and speaker		433.65	
Amount travel other			**974.03**
Meals	number of people		
Coffee, water, soft drinks, snacks during the course		637.90	
Welcome Dinner 23.05.10	26	663.52	
Lunch 24.05.10	30	238.51	
Dinner 24.05.10	32	504.66	
Dinner 25.05.10	22	372.49	
Dinner 26.05.10	24	480.97	
Dinner 27.05.10	29	495.47	
Dinner 28.05.10	31	569.03	
Lunch at Canteen MPI-CBG (25.05.-28.05.)	112	734.40	
Lunch 29.05.10	2	18.35	
Dinner 29.05.10	18	276.08	
Lunch im BIOTEC		142.32	
Amount Meals			4,495.80
Social event and excursions			
Weingut Schloß Proschwitz		2,023.26	
Concert Schloß Wackerbarth		580.84	
Ticket return		-15.00	
Meissen Manufactur		364.09	
River tour		416.29	
Other administrative costs		674.53	
Course Materials			
Audiovisual support		1,091.18	
Chemicals		12,717.36	
AMOUNT		**40,001.03**	
Total Amount ESF=Accommodation+Amount travel costs students+Amount travel costs speaker+travel other		**20,100.92**	

***costs paid from ESF marked in red**