

Functional Proteomics of Membrane Protein Targets - Company Presentation -

 **LOGOPHARM**
Services for the Pharmaceutical and Biotech Industry

Uwe Schulte

BIOPRO Stuttgart 29.10.2009

LOGOPHARM Company Profile



GmbH, founded in 2005 as a privately held biotechnology company located in March, Germany

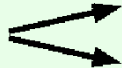
Founders and Shareholders

Dr. U. Schulte, Prof. B. Fakler, Prof. H.G. Knaus, J. Hausner

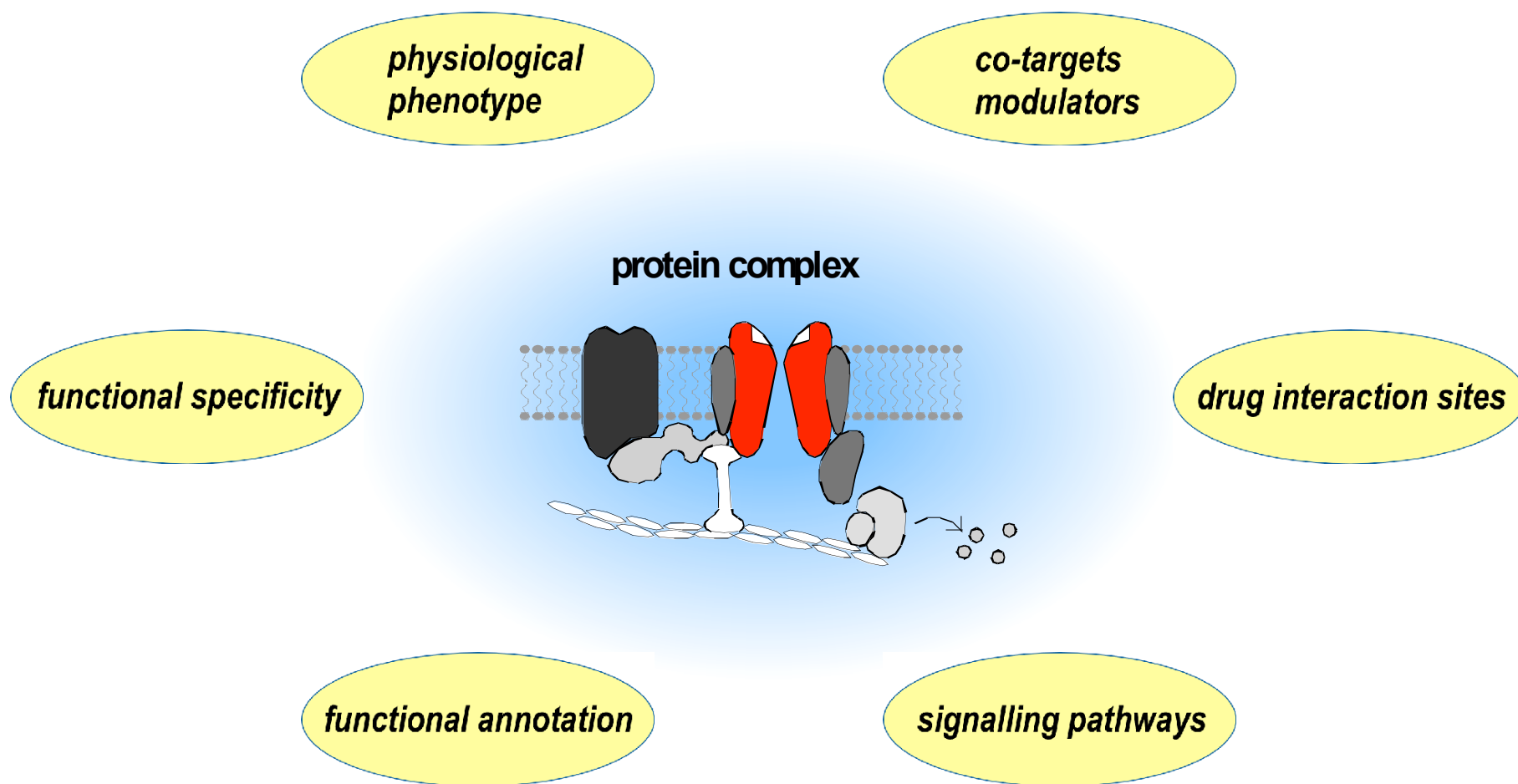
Areas of expertise and infrastructure

Biochemistry, mass spectrometry, molecular physiology, antibody techniques, electrophysiology, molecular biology, pharmacology, NMR

Business activities

Technology development  R&D services and tools
Independent projects

Focus: Protein Complexes in Drug Development



- ➔ *improved screening (meaningful assays, reduced risk)*
- ➔ *targeted mode of action and functional specificity*
- ➔ *novel (co)targets and compound families*

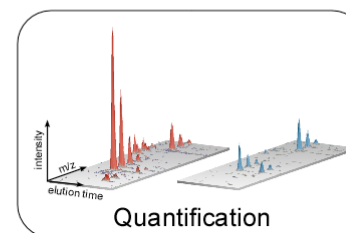
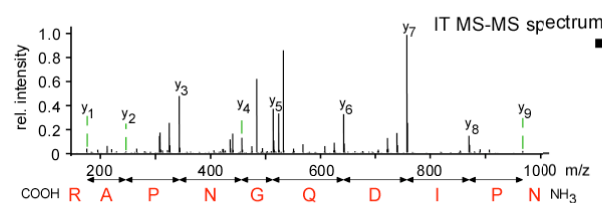
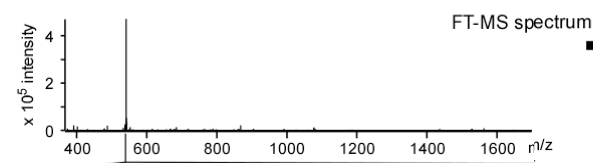
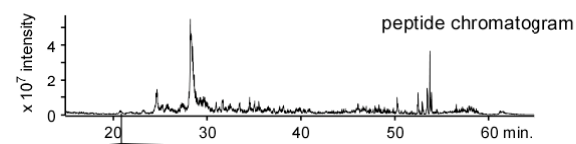
Functional Proteomic Strategy

Native tissue or cells



Comprehensive mass spectrometric analysis

nanoLC-MS/MS analysis



Masses Search Results

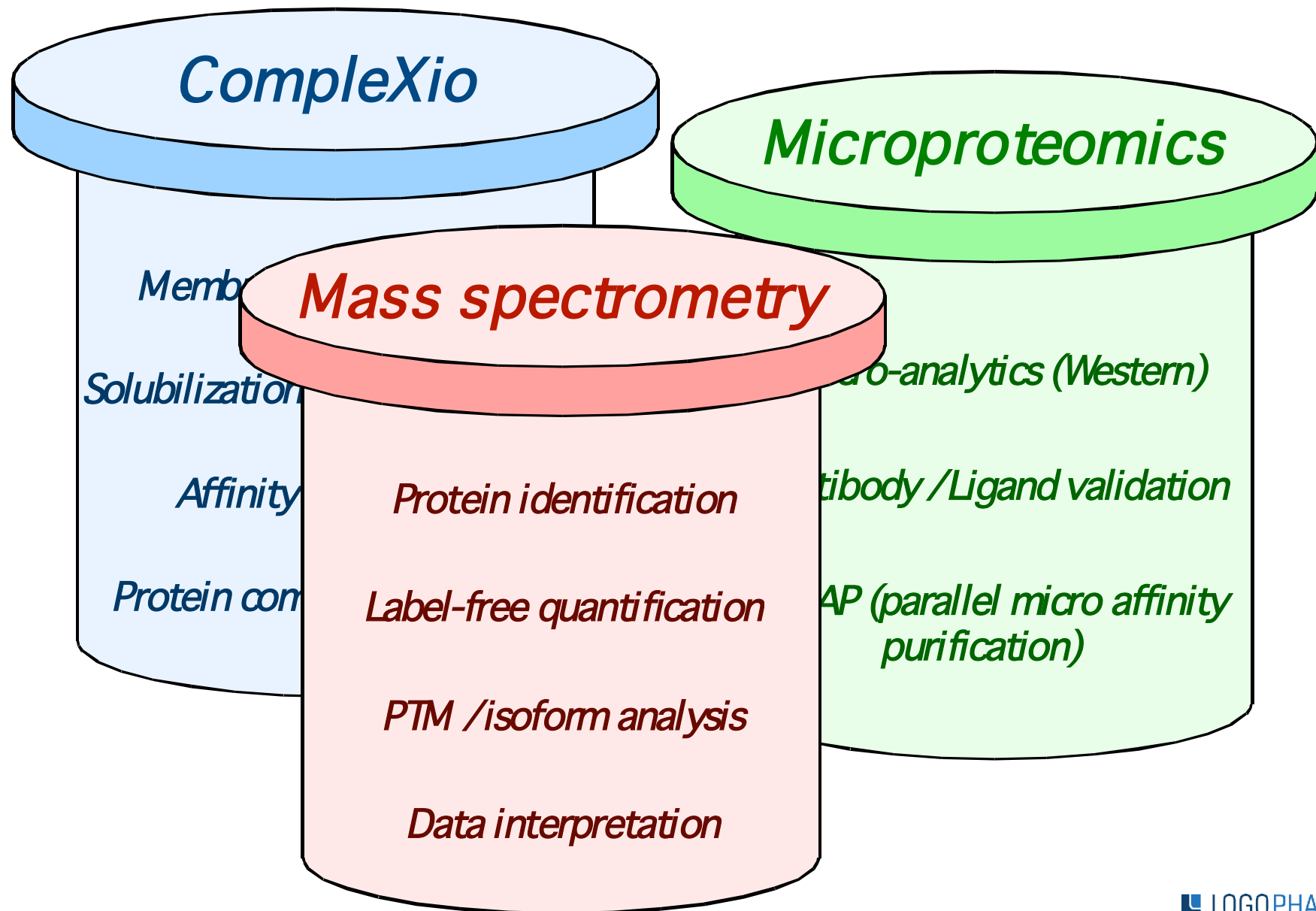
Rank	Protein Name	Accession	Score	Length	Score/Length	Score/Length	Score/Length	Score/Length	Score/Length	Score/Length
1	Protein X	Protein X	1000	100	10.0	10.0	10.0	10.0	10.0	10.0
2	Protein Y	Protein Y	950	95	10.0	10.0	10.0	10.0	10.0	10.0
3	Protein Z	Protein Z	900	90	10.0	10.0	10.0	10.0	10.0	10.0
4	Protein A	Protein A	850	85	10.0	10.0	10.0	10.0	10.0	10.0
5	Protein B	Protein B	800	80	10.0	10.0	10.0	10.0	10.0	10.0
6	Protein C	Protein C	750	75	10.0	10.0	10.0	10.0	10.0	10.0
7	Protein D	Protein D	700	70	10.0	10.0	10.0	10.0	10.0	10.0
8	Protein E	Protein E	650	65	10.0	10.0	10.0	10.0	10.0	10.0
9	Protein F	Protein F	600	60	10.0	10.0	10.0	10.0	10.0	10.0
10	Protein G	Protein G	550	55	10.0	10.0	10.0	10.0	10.0	10.0

Identification

Functional characterization of novel partners

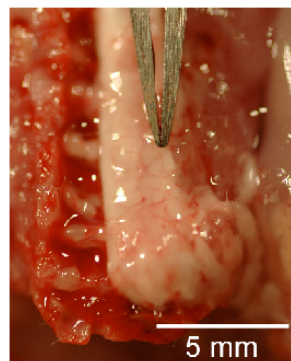
Comprehensive mass spectrometric analysis

Technology Platforms



Microproteomic Expression Profiling of GPCRs

Source material preparation



spinal cord (SC)

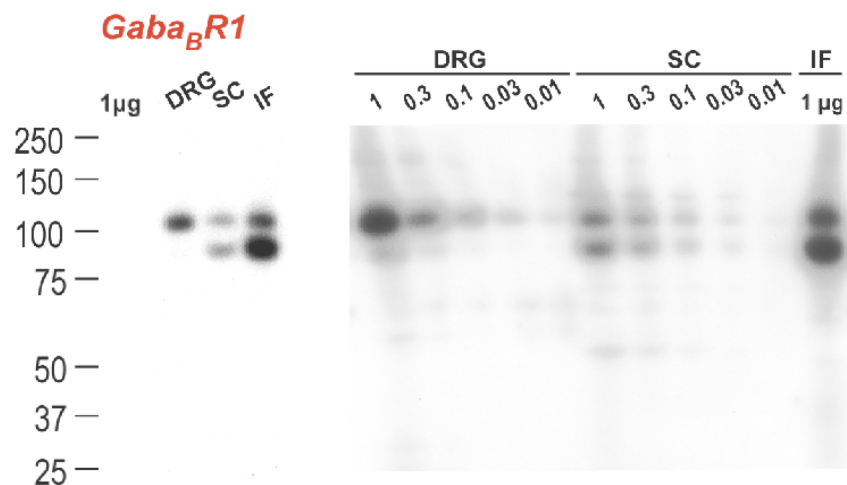
(1 mg SC ~ 10 µg membrane)



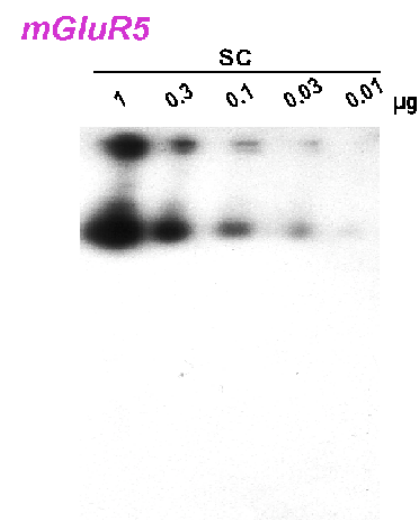
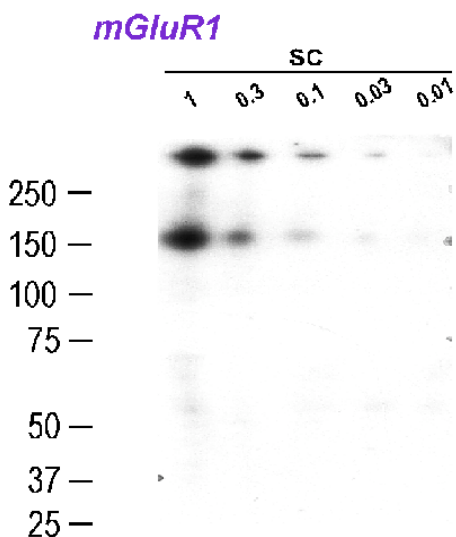
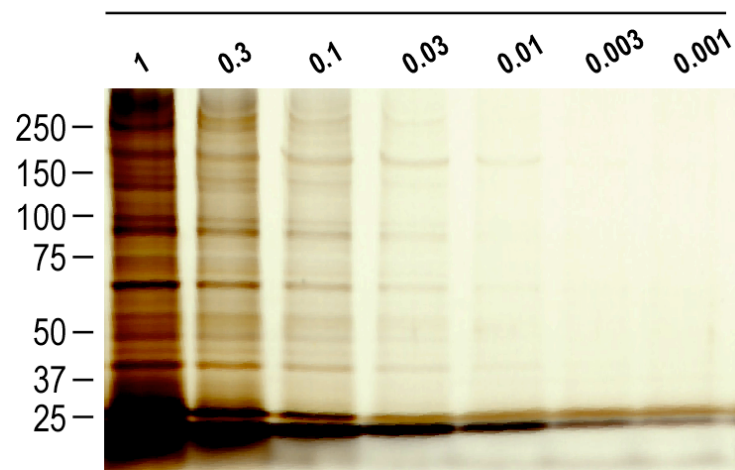
dorsal root ganglion (DRG)

(1 DRG ~ 10 µg membrane)

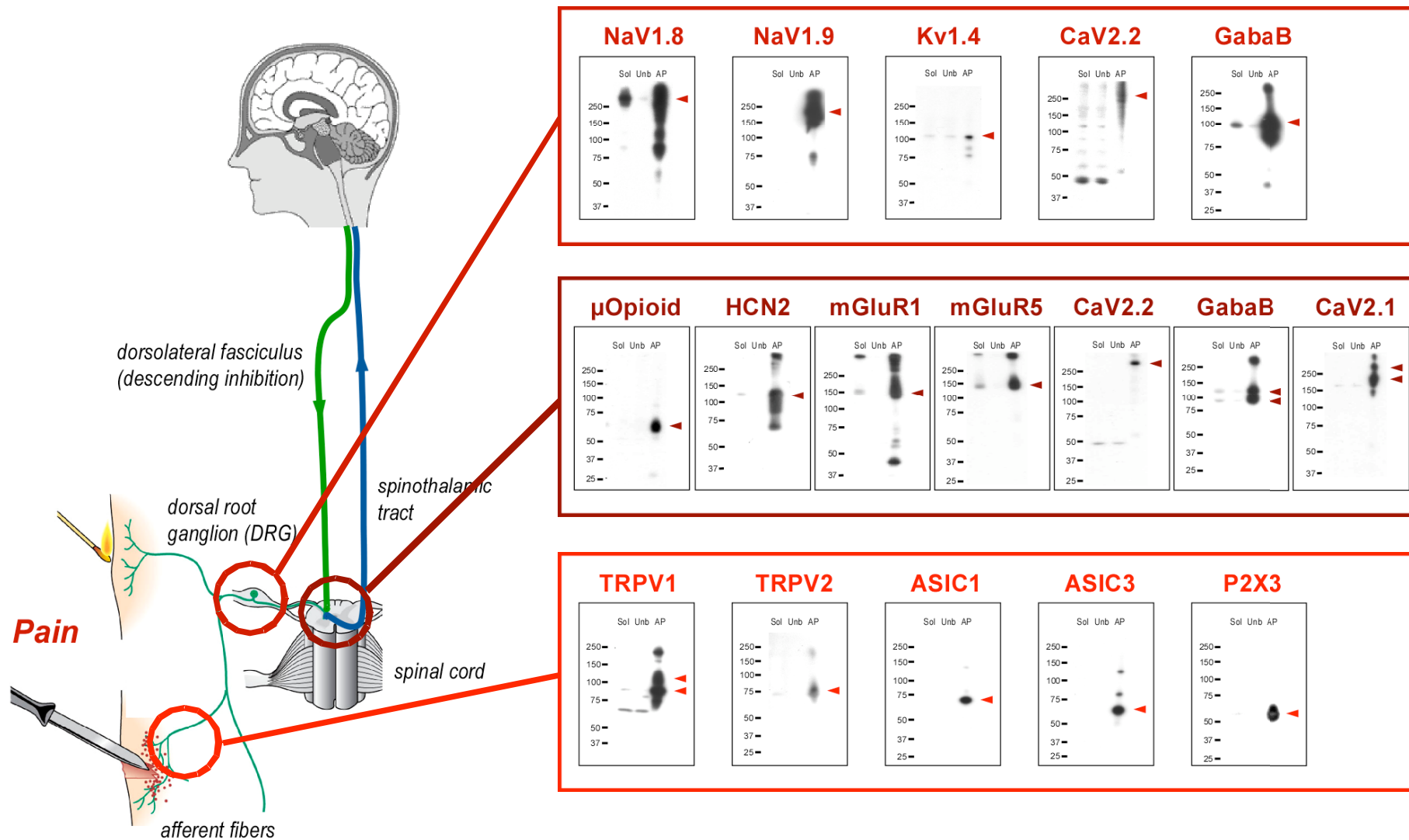
Tissue-specific expression



DRG membrane dilution series



Proteomic Analysis of Putative Pain Targets



Antibody Profiling by PMAP

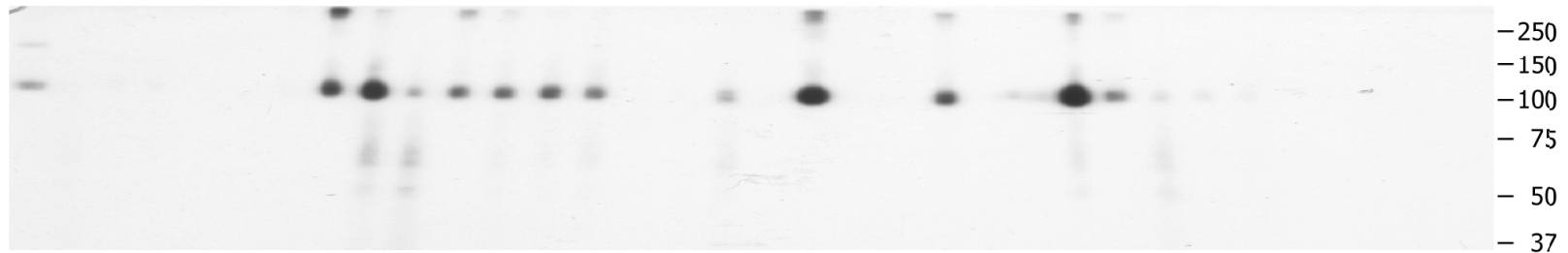
experimental setup

4 mg IF membrane
2 µg antibody each
1 h / RT
10% for Western
80% for silver-stained Gel

68	30	48/1	C	Ab	30	43/1	o
166	304	43/1	o	47	166	C	o
45	303	23/1	304	45	303	48/1	o
47	Alo	60/1	68	60/1	23/1	o	C

anti-BK Westernblot

Sol unb 68 166 45 47 30 304 303 Alo 48/1 43/1 23/1 60/1 IgG o 304 68 Alo 47 45 60/1 30 166 303 23/1 43/1 IgG 48/1 o o o o IgG

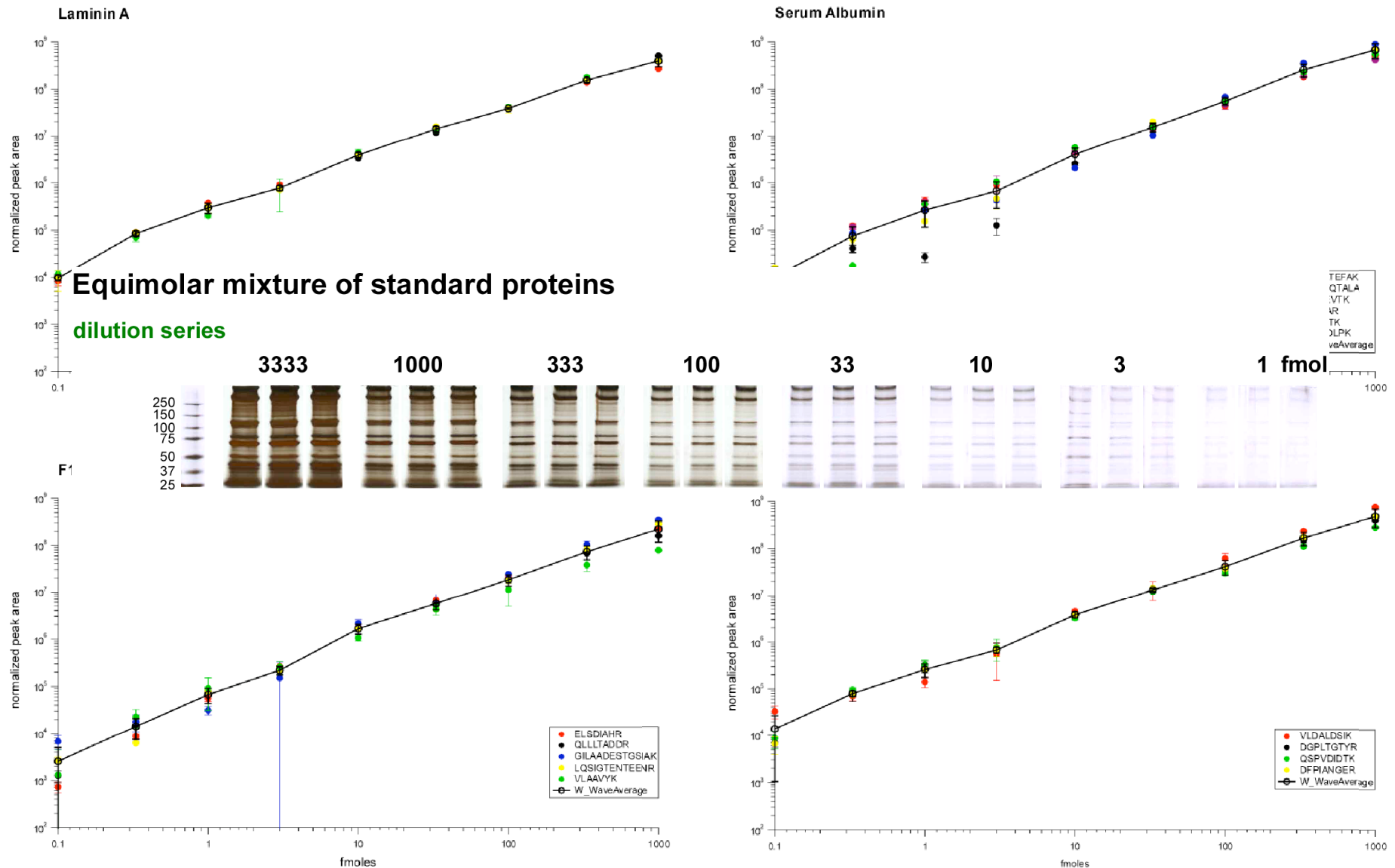


silver stained SDS-Page

68 166 45 47 30 304 303 Alo 48/1 43/1 23/1 60/1 304 68 Alo 47 45 60/1 30 166 303 23/1 43/1 48/1



Mass Spectrometric Quantification of Proteins



Detailed Characterization of Native Membrane Proteins (BK α)

1 MDALIIPTVM EVPCDSRGQR MWWAFLASSM VTFFGGLFII LLWRTLKYLW TVCCHCGGKT KEAQKINNGS SQADGTLKPV DEKEEVVAEE VGWMTSVKDW
 101 AGVMISAQTL TGRVLVVLVF ALSIGALVIY FIDSSNPIES CQNFYKDFTL QIDMAFNVEF LLYFGLRFIA ANDKLWFWLE VNSVVDFTV PPVFVSVYLN
 201 RSWLGLRFLR ALRLIQFSEI LQFLNILKTS NSIKLVNLLS IFISTWLTAA GFHILVENSG DPWENFQNNQ ALTYWECVYL LMVTMSTVGY GDVYAKTTLG
 301 RLFMVFFILG GLAMFASYVP EIELIGNRK KYGGSYSAVS GRKHIVVCGH ITLESVSNFL KDFLHKDRDD VNVEIVFLHN ISPNLELEAL FKRHFTQVEF
 401 YQGSVLNPHD LARVKIESAD ACLILANKYC ADPAEDASN IMRVISIKNY HPKIRIITQM LQYHNKAHL NIPSWNWKEG DDAICLAELEK LGFIAQSCLA
 501 QGLSTMLANL FSMRSFIKIE EDTWQKYILE GVSNEMYTEY LSSAFVGLSF PTVCELCFVK LKLLMIAIEY KSANRESRIL INPGNHLKIQ EGTLGFFIAS
 601 DAKEVKRAFF YCKACHDDVT DPKRIKKCGC RRLEDEQPPY LSPKKKQKNG GMRNSPTSP KLMRHDPLLI PGNDQIDNMD SNVKKYDSTG MFHWCAPKEI
 701 EKVILTRSEA AMTVLSGHVV VCIFGDVSSA LIGLRNLVMP LRASNPHYHE LKHIVFVSGI EYLKREWETL HNFPKVSILP GTPLSRADLR AVNINLCDCMC
 801 VILSANQNNI DDTSLQDKEC ILASLNKISM QFDDSIGVLQ ANSQGFTPPG MDRSSPDNSP VHGMRLQPSI TTGVNIFIIT ELVNDTNVQF LDQDDDDDPD
 901 TELYLTQFFA CGTAFVSVL DSLMSATYFN DNILTLIRTL VTGGATPELE ALIAEENALR GGYSTPQTLA NRDRCRVAQL ALLDGPFFADL GDGGCYGDLF
 1001 CKALKTYNML CFGIYRLRDA HLSTPSQCTK RYVITNPPYE FELVPTDLIF CLMQFDHNAG QSRASLSHSS HSSQSSSKKS SSVHSIPSTA NRPNRPKSRE
 1101 SRDKQ

MANGGGGGG GSSGSSGGGG GGGGGETALR MSSNIHANHL SLDASSSSSS SSSSSSSSS SVHEPK (N-terminal extension
 by alt. start of translation)

IYF (IYF)
 IYSKMSIYKR MSRACCFDCG RSERDCSCMS GRVRGNVDTL ERNFPLSSVS VND CSTSFRA F (STREX)

AKPGKLPL VSVNQEKNSG THILMITEL (Ca27)

KYVQEERL (C-ERL)

NRKEMVYR (C-YVR)

NANQKRNLQ MRRIMPIPET FKSSP (C-SSP)

NATRMTRMGQ AEKKWFTDEP DNAYPRNIQI KPMSTHMANQ INQYKSTSSL IPPIREVEDE C (C-DEC)

Track Record of Published Projects

<i>SK channel complex</i>	Ca ²⁺ -dependent gating	<i>general</i>	<i>Neuron 2004; 43(6): 847-58</i>
<i>Kv1 channels - LGI</i>	inactivation gating	<i>brain</i>	<i>Neuron 2006; 49(5):697-706</i>
<i>HCN channels - PEX5R</i>	cAMP-dependent modulation	<i>brain</i>	<i>Neuron 2009; 62(6):814-25</i>
<i>NPC1L1 transporter</i>	ezetimibe target validation	<i>intestine</i>	<i>Proc Natl Acad Sci 2008; 105(32):11140-5</i>
<i>BK channel core</i>	phosphoproteome	<i>brain</i>	<i>Mol Cell Proteomics 2008; 7(11):2188-98</i>
<i>BK-CaV supercomplexes</i>	Ca ²⁺ nanodomain coupling	<i>brain</i>	<i>Science 2006; 314(5799):615-20</i>
<i>AMPA receptor - CNH2/3</i>	novel auxiliary subunit	<i>brain</i>	<i>Science 2009; 323(5919):1313-9</i>
<i>GABAB receptor - GIPs</i>	novel auxiliary GPCR subunit	<i>brain</i>	<i>submitted</i>
<i>CaV2 channel networks</i>	framework of local Ca ²⁺ signaling	<i>brain</i>	<i>submitted</i>

Competitive Advantages

Focus on **membrane proteins** and protein **complexes**

Native source-based approach to targets and biomarkers

Proprietary technology (biochemistry, mass spectrometry)

Scientific excellence, strong academic network

Internal database of >2000 target protein purifications



Services for the Pharmaceutical and Biotech Industry

Discovery R&D Services (Targets, Biomarkers)

Joint Development, Research Collaborations

Outlicensing / Technology Implementation

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