HelmholtzZentrum münchen
German Research Center for Environmental Health

Scientific Highlight Januar 2012

☐ co-ordinated with the Director of the Institute / Research Unit

Institute / Research Unit / Clinical Co-operation Group / Junior Research Group:
Department of Protein Science

PSP-Element:
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Title of the Highlight:
Structural and functional protein network analyses predict novel signaling functions for rhodopsin

Keywords:
Rhodopsin, multiscale protein network, systems biology, mass spectrometry, protein complexes, structural modelling

Central statement of the Highlight in one sentence:
Work on modeling the protein network of rhodopsin was selected and highlighted by the Faculty of 1000 (http://f1000.com) and placed in their library of the top 2% of published articles in biology and medicine.

Text of the Highlight:
Understanding complex signal transduction networks is one of the big challenges in modern biology. Traditionally protein interactions that provide a physical architecture to such networks have been studied by combining biochemical and genetic experiments. Large throughput experiments using different techniques like two hybrid, or pull downs have added a new level of complexity. However in the majority of these studies network dynamics, the simultaneous regulation of several distinct higher order biological outputs by one network, and the fact that many interactions detected for a particular protein are not compatible simultaneously could not be tackled. In consequence, information on networks remains static, often fails to represent physiology and in many cases lead to wrong interpretations.

By integrating proteomic datasets, literature mining, computational analysis, and through structural information a team around Marius Ueffing and Andreas Vogt, PROT, has generated an approach that tackles some of these problems. Conceptually, this work describes rhodopsin protein network architecture and behaviour presents a general strategy applicable to the analysis of any cellular pathway.

The article, published in Molecular Systems Biology in November 2011, has been selected and highlighted by the Faculty of 1000 (http://f1000.com) a few weeks after its publication and was placed in their library of the top 2% of published articles in biology and medicine.

Rhodopsin, the major visual pigment of the retina belongs to the G-protein-coupled receptor (GPCR) family and is extremely sensitive to light, enabling vision under low-light conditions. This GPCR is tightly packed into stacks of membranes ("discs") in the outer portion of rod photoreceptor cells. Photon-activated rhodopsin translates light into a biochemical signal followed by an electrical cue that is transmitted through the neuronal network of the retina. Mutations in rhodopsin and other proteins in the signal transduction cascade of light cause severe blinding diseases such as retinitis pigmentosa, rod-cone dystrophies, and congenital stationary night blindness.

Identifying protein interactions and their networks is therefore an important step towards improving our understanding of the molecular defects that underlie blinding diseases and may directly lead to identifying new disease-associated genes.

Using proteomic methods the group cataloged the proteins involved in the rod outer segment signaling pathway of mammalian photoreceptors. By applying a new conceptual strategy the group then generated a comprehensive multiscale protein interaction network. Together with the groups of Luis Serrano at the CRG in Barcelona (Spain) and Gianni Cesareni at the University of Rome (Italy) the group combined experimental proteomics data with literature mining and structural information to develop a structural model allowing discrimination between protein interactions that are compatible and those that are mutually exclusive.

Computational analysis was combined with experiments to test and validate the models generated. These experiments provided evidence for rhodopsin interactions with small GTPases involved in cytoskeleton assembly/disassembly and dynamics, as well as vesicle and Golgi trafficking. Taken together this work suggests a new functional role for rhodopsin in self-regulating and fine-tuning the structural and functional integrity of photoreceptors.

Publication:

Kiel C*, Vogt A*, Campagna A*, Chatr-aryamontri A, Swiatek-de Lange M, Beer M, Bolz S, Mack AF, Kinkl N, Cesareni G, Serrano L, Ueffing M. Structural and functional protein network analyses predict novel signaling functions for rhodopsin. Molecular Systems Biology. 2011 Nov 22;7:551. doi: 10.1038/msb.2011.83.

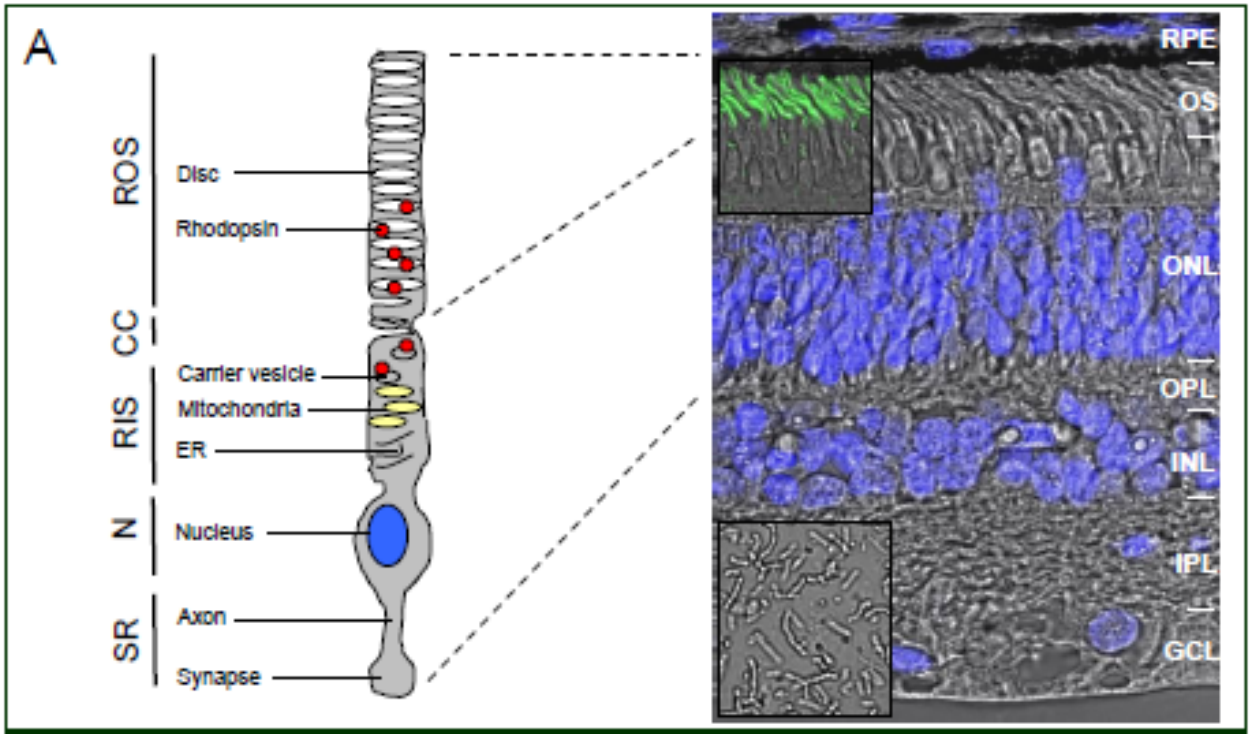
Taking account of the HMGU mission:

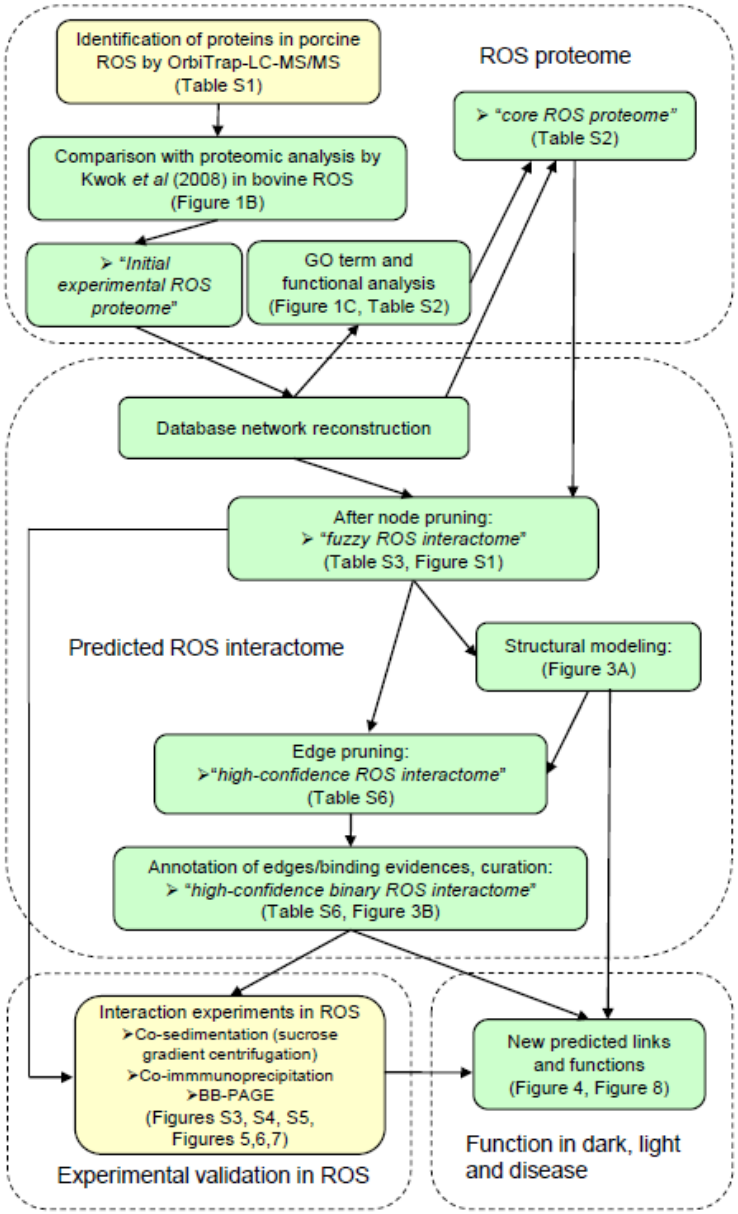
This study presents a systems biology approach and demonstrates a new conceptual strategy for generating comprehensive multiscale protein networks,

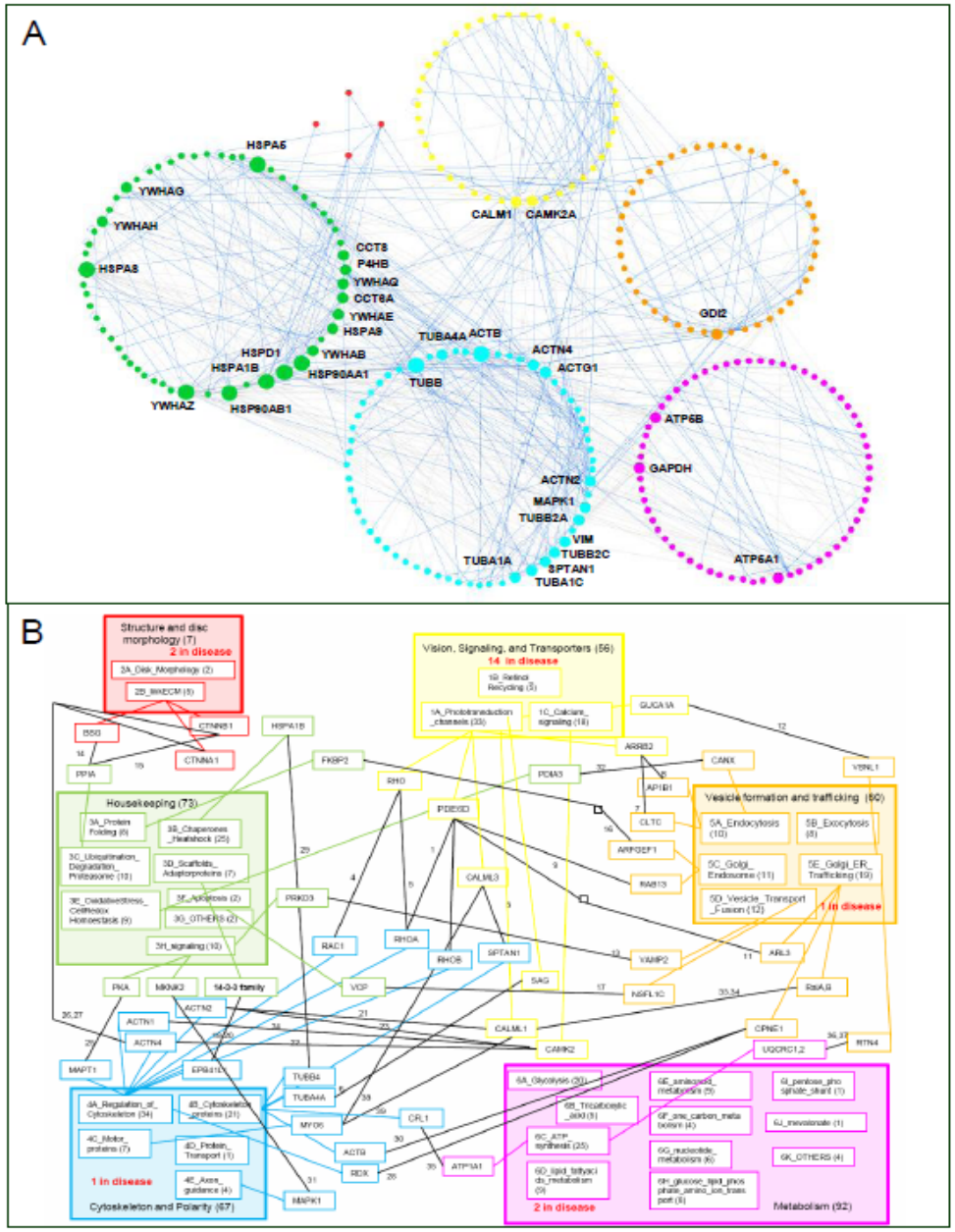
applicable for any cellular pathway, by combining experimental data with literature mining and structural information. The identification of protein interactions and their networks is an important step towards improving the understanding of the molecular defects that underlie genetically-inherited and age-related diseases, and may directly lead to the identification of disease-associated genes.

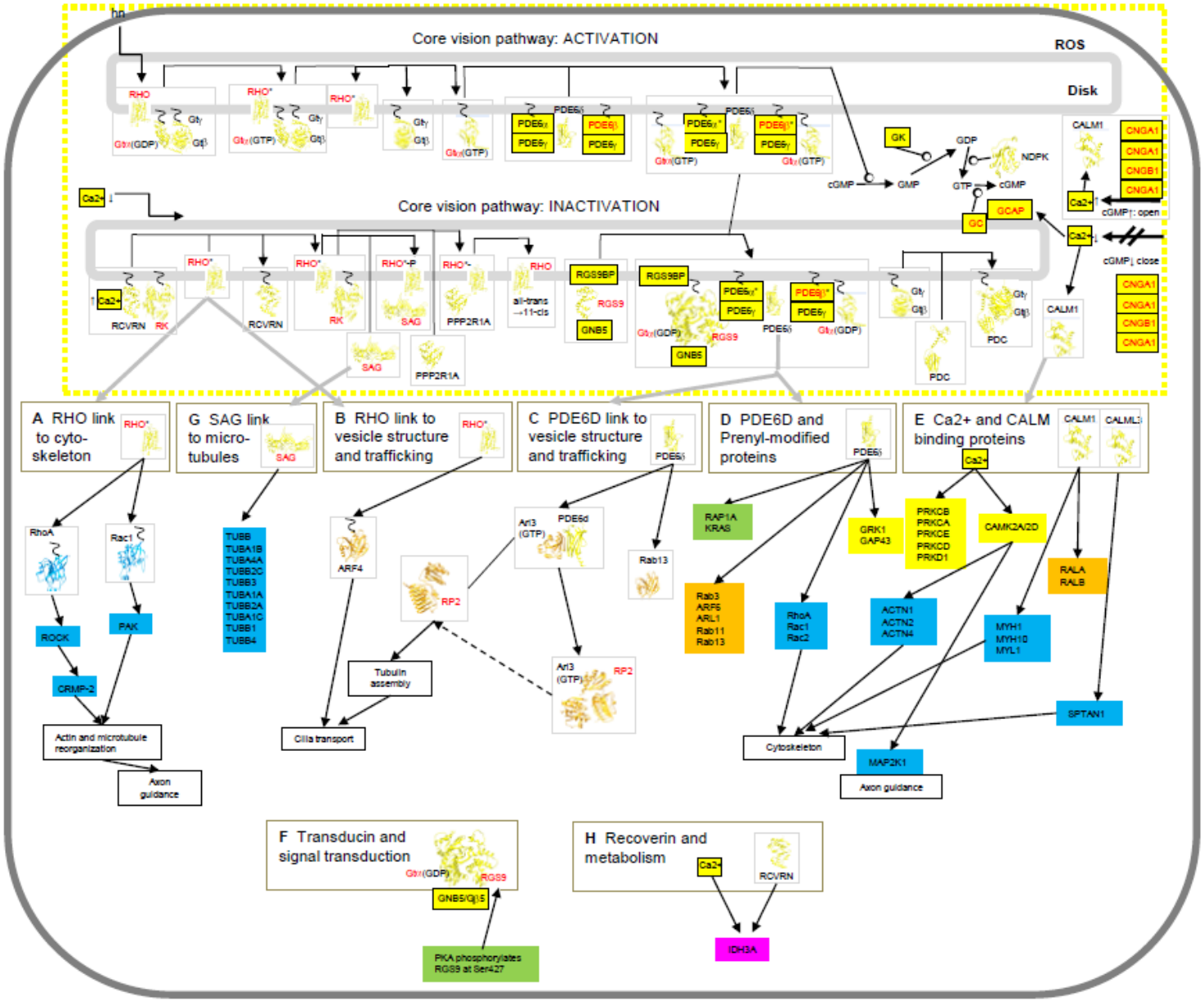
The internal HMGU co-operation partners with whom the Highlight was compiled, if appropriate:

CRG-EMBL System Biology Program, Centre de Regulació Genòmica (CRG) Barcelona, Spain; [4] University of Rome Tor Vergata, Rome, Italy; [5] Institució Catalana de Recerca I Estudis Avançats (ICREA).


A
ROS
CC
RIS
N
SR
Disc
Rhodopsin
Carrier vesicle
Mitochondria
ER
Nucleus
Axon
Synapse
RPE
OS
ONL
OPL
INL
IPL
GCL


ROS proteome
Identification of proteins in porcine ROS by OrbiTrap-LC-MS/MS (Table S1)
➢ "core ROS proteome" (Table S2)
Comparison with proteomic analysis by Kwok et al (2008) in bovine ROS (Figure 1B)
➢ "Initial experimental ROS proteome"
GO term and functional analysis (Figure 1C, Table S2)
Database network reconstruction
After node pruning:
➢ "fuzzy ROS interactome"
(Table S3, Figure S1)
Predicted ROS interactome
Structural modeling: (Figure 3A)
Edge pruning:
➢"high-confidence ROS interactome"
(Table S6)
Annotation of edges/binding evidences, curation:
➢ "high-confidence binary ROS interactome"
(Table S6, Figure 3B)
Interaction experiments in ROS
➢Co-sedimentation (sucrose gradient centrifugation)
➢Co-immmunoprecipitation
➢BB-PAGE
(Figures S3, S4, S5, Figures 5,6,7)
Experimental validation in ROS
New predicted links and functions (Figure 4, Figure 8)
Function in dark, light and disease


A
HSPA5
YWHAG
YWHAH
HSPA8
CCT8
P4HB
YWHAQ
CCT6A
YWHAE
HSPA8
HSPD1
HSPA1B
YWHAB
HSP90AA1
YWHAZ
HSP90AB1
CALM1
CAMK2A
GDI2
TUBA4A
ACTB
ACTN4
ACTG1
TUBB
ATP6B
GAPDH
ACTN2
MAPK1
TUBB2A
VIM
TUBB2C
TUBA1A
SPTAN1
TUBA1C
ATP6A1
B
Structure and disc morphology (7)
2 in disease
Vision, Signaling, and Transporters (56)
14 in disease
Housekeeping (73)
Vesicle formation and trafficking (60)
1 in disease
Cytoskeleton and Polarity (67)
1 in disease
Metabolism (92)
2 in disease


Core vision pathway: ACTIVATION
ROS
Disk
hR
RHO
RHO*
Gtγ
Gtβ
Gtα(GDP)
Gtα(GTP)
PDE6γ
PDE6β
PDE6γ*
PDE6β*
PDE6α
PDE6α*
GK
GDP
NDPK
GTP
cGMP
GMP
CALM1
CNGA1
CNGB1
Ca2+
cGMP↑: open
cGMP↓ close
GC
GCAP
Core vision pathway: INACTIVATION
RHO*-P
RHO*-
all-trans →11-cis
RCVRN
RK
SAG
PPP2R1A
RGS9BP
RGS9
GNB5
PDE6γ*
PDE6β*
PDC
A RHO link to cyto-skeleton
G SAG link to micro-tubules
B RHO link to vesicle structure and trafficking
C PDE6D link to vesicle structure and trafficking
D PDE6D and Prenyl-modified proteins
E Ca2+ and CALM binding proteins
CALML3
RhoA
Rac1
ROCK
PAK
CRMP-2
Actin and microtubule reorganization
Axon guidance
TUBB
TUBA1B
TUBA4A
TUBB2C
TUBB3
TUBA1A
TUBB2A
TUBA1C
TUBB1
TUBB4
ARF4
RP2
Tubulin assembly
Cilia transport
Arl3 (GTP)
PDE6d
Rab13
RAP1A
KRAS
Rab3
ARF6
ARL1
Rab11
Rab13
GRK1
GAP43
PRKCB
PRKCA
PRKCE
PRKCD
PRKD1
CAMK2A/2D
RhoA
Rac1
Rac2
ACTN1
ACTN2
ACTN4
MYH1
MYH10
MYL1
RALA
RALB
SPTAN1
Cytoskeleton
MAP2K1
Axon guidance
F Transducin and signal transduction
GNB5/Q15
PKA phosphorylates RGS9 at Ser427
H Recoverin and metabolism
IDH3A