TNT 2011 Tenerife

Microscopic modeling of charge transport in sensing proteins

Lino Reggiani
Eleonora Alfinito, Jean-Francois Millithaler,
and Cecilia Pennetta

Dipartimento di Ingegneria dell'Innovazione, Università del Salento, Lecce, Italy

Research icarried out within the bioelectronic olfactory neuron device (BOND) project sponsored by the CE

CONTENT

1 - INTRODUCTION AND MOTIVATION

2 – PHYSICAL SYSTEM OF INTEREST

3 – AVAILABLE EXPERIMENTS

4 - THEORETICAL MODEL

5 - RESULTS AND DISCUSSION

6 – CONCLUSIONS AND PERSPECTIVES

INTRODUCTION AND MOTIVATION

- CHARGE TRANSPORT IN BIOLOGICAL MATERIALS : WHICH MECHANISMS ?
- MONITORING SENSING PROTEIN OF THE GPCR FAMILY THROUGH ITS ELECTRICAL PROPERTIES
- CORRELATION BETWEEN PROTEIN STRUCTURE AND ELECTRICAL PROPERTIES
- CONVERTING A BIOLOGICAL CHAIN OF DETECTION INTO A CHANGE OF AN ELECTRICA SIGNAL
- WIDE APPLICATIONS IN MOLECULAR DEVICES CONTROLLING THE QUALITY OF LIFE

PHYSICAL SYSTEM OF INTEREST

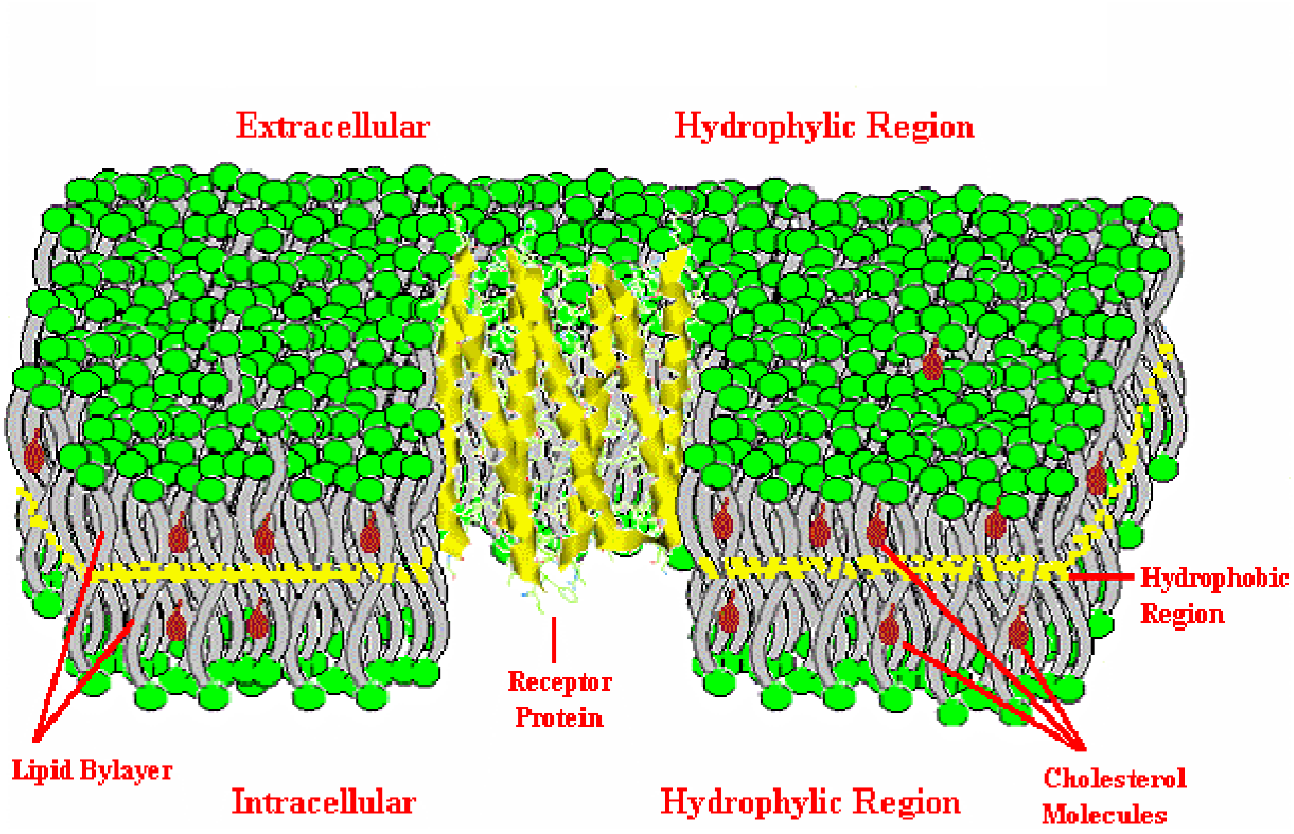
G Protein Coupled Receptor (GPCR) as sensing protein

Typical sensing action to: light , odours

For light: Bovine Rhodopsin, Bacterio-Rhodopsin

For odours: rat OR-I7, Human OR 1740, Scimpanzee OR-7D4

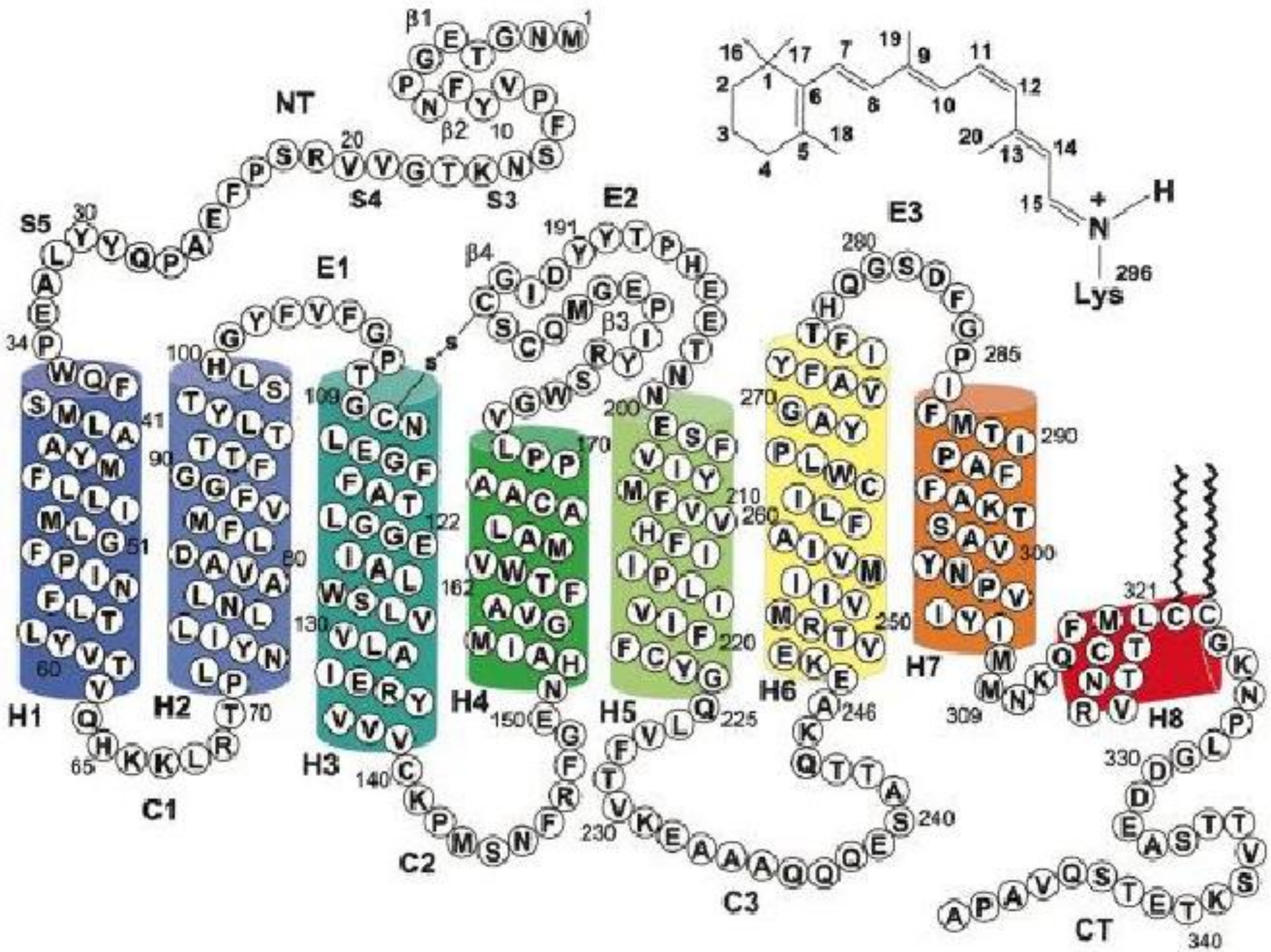
GPCR AS A TRANSMEMBRANE PROTEIN WITH 7 HELICS



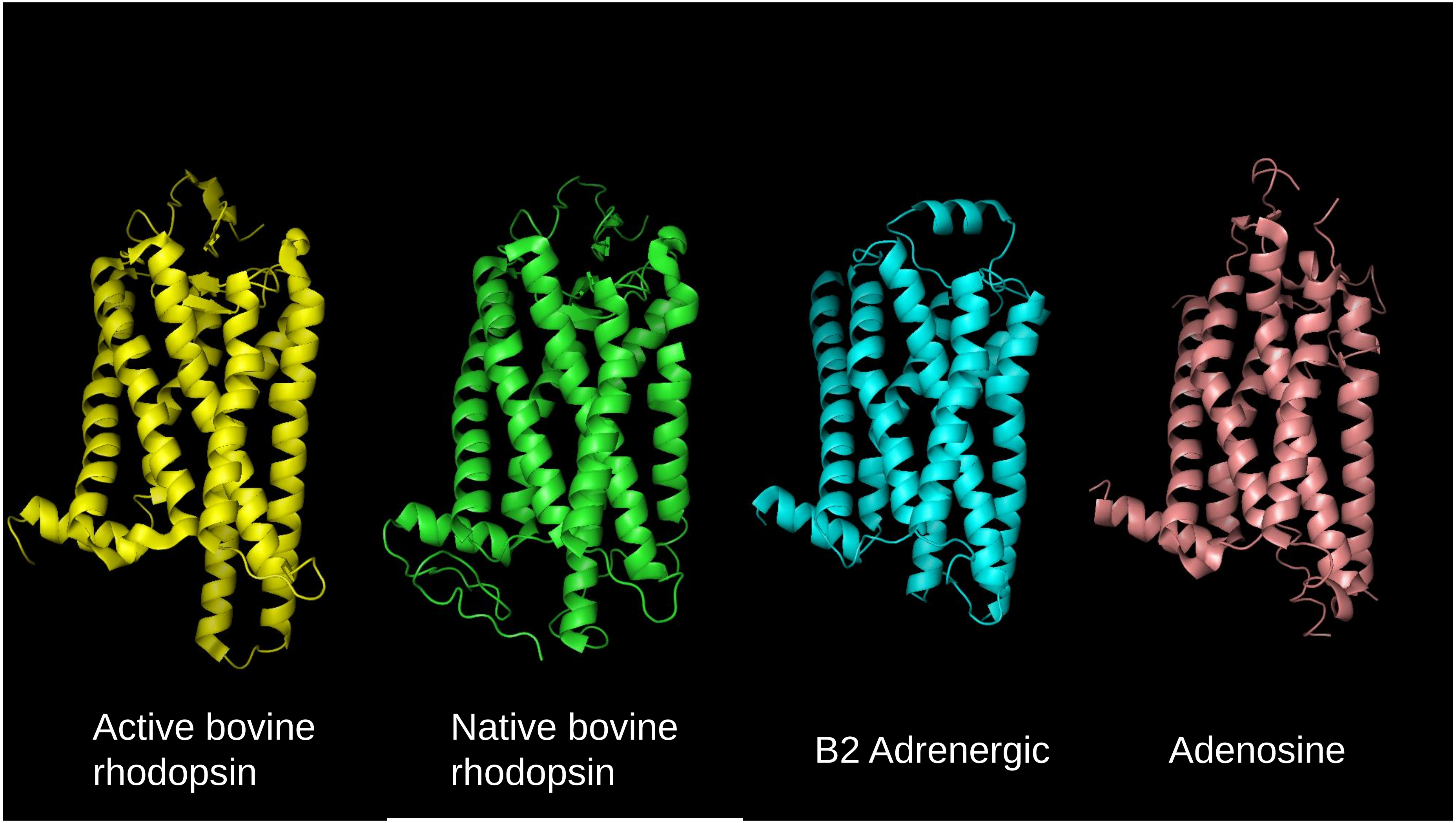
OR 7D4 HUMAN - Protein code

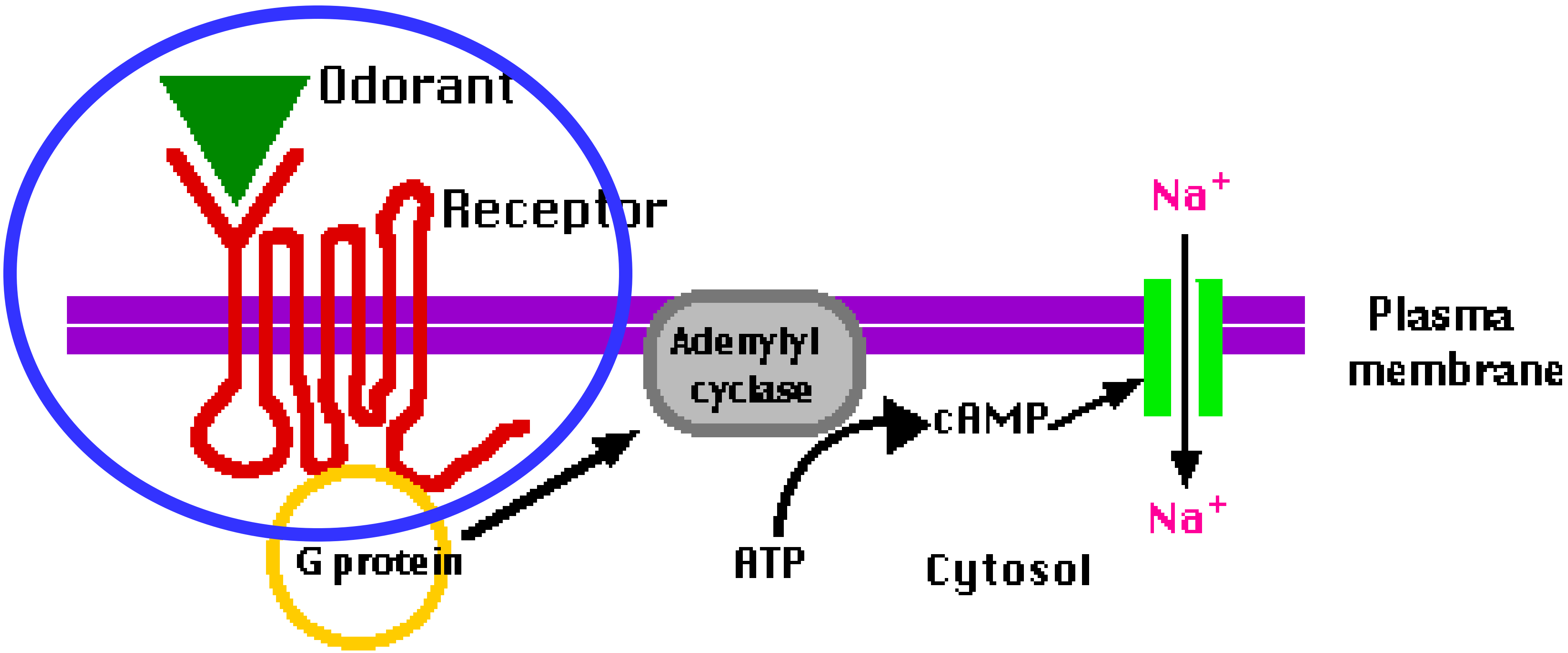
MEAENLTELSKFLLLGLSDDPELQPVLFGLFLSMYLVTVLGNLLIILAVSSDSHLH
TPMYFFLSNLSFVDICFISTTVPKMLVNIQARSKDISYMGCLTQVYFLMMFAG
MDTFLLAVMAYDRFVAICHPLHYTVIMNPCLCGLLVLASWFIIFWFSLVHILLM
KRLTFSTVTEIPHFFCEPAQVLKVACSNTLLNNIVLYVATALLGVFPVAGILFSYSQ
IVSSLMRMSSTEGKYKAFSTCGSHLCVVSLFYGTGLGVYLSSAVTHSSQSSSMA
SVMYAMVTPMLNPFIYSLRNKDVKGALERLLSRADSCP

SCHEMATIC OF BOVINE RHODOPSINE AMINOACID STRUCTURE

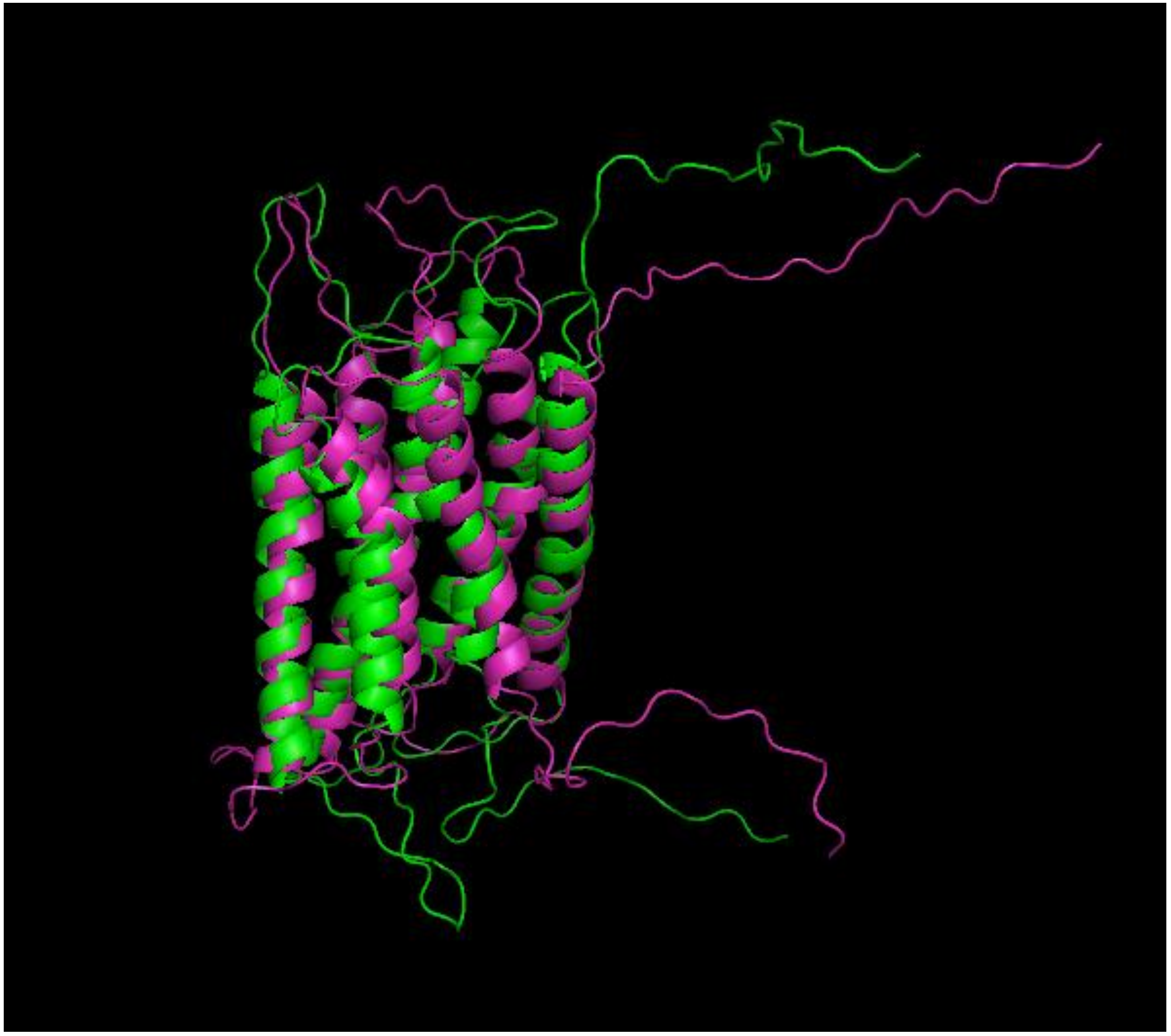


A REALISTIC VIEW OF KNOWN GPCRs





Transduction cascade of the signal.
Capture of the ligand leads to a conformational change - monitored by a G protein - that initiates a biological chain of detection – finally collected by the brain.



OR-7D4: native state in red and active state in green

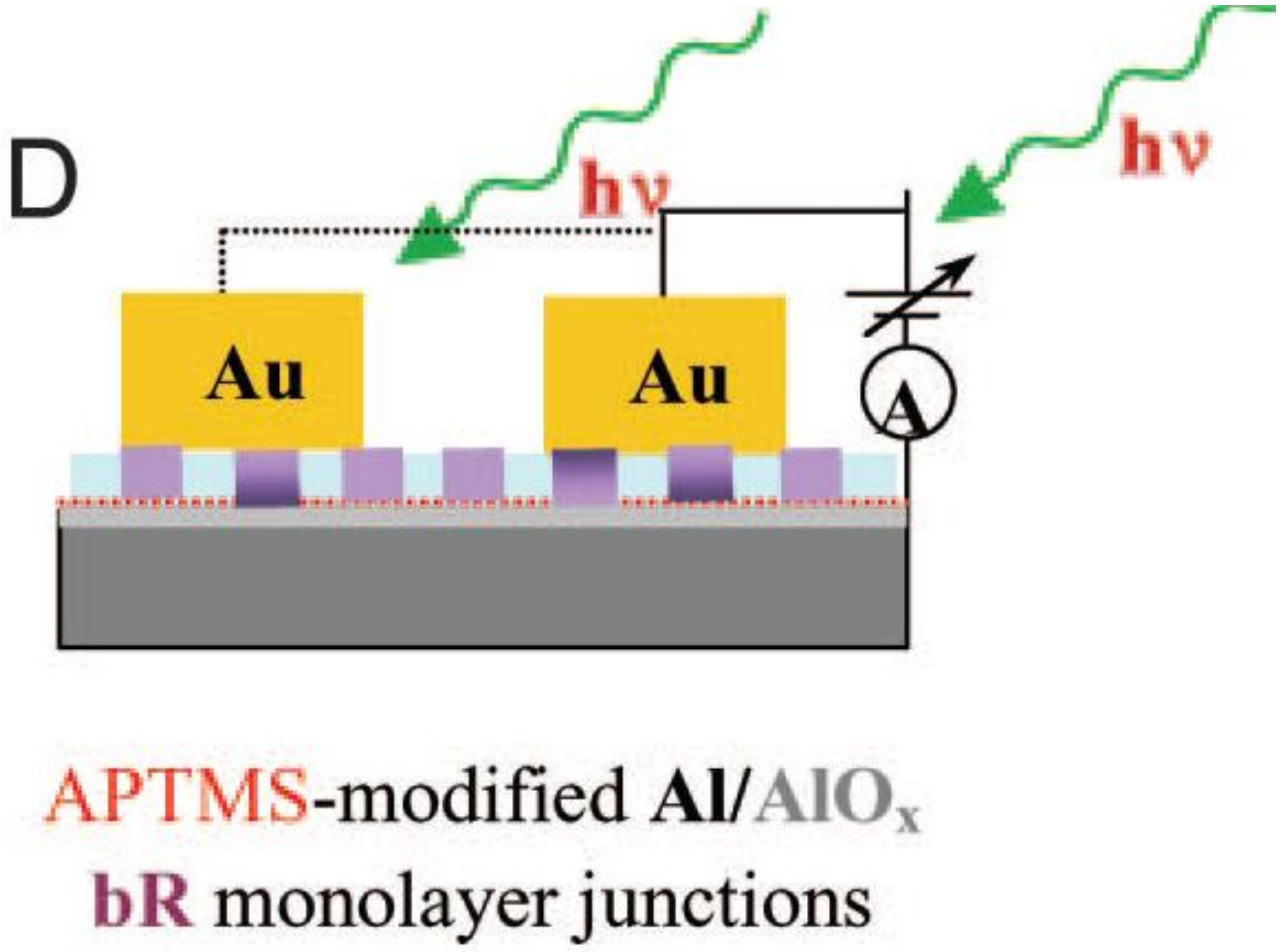
AVAILABLE EXPERIMENTS ON THE ELECTRICAL PROPERTIES OF SENSNG PROTEINS

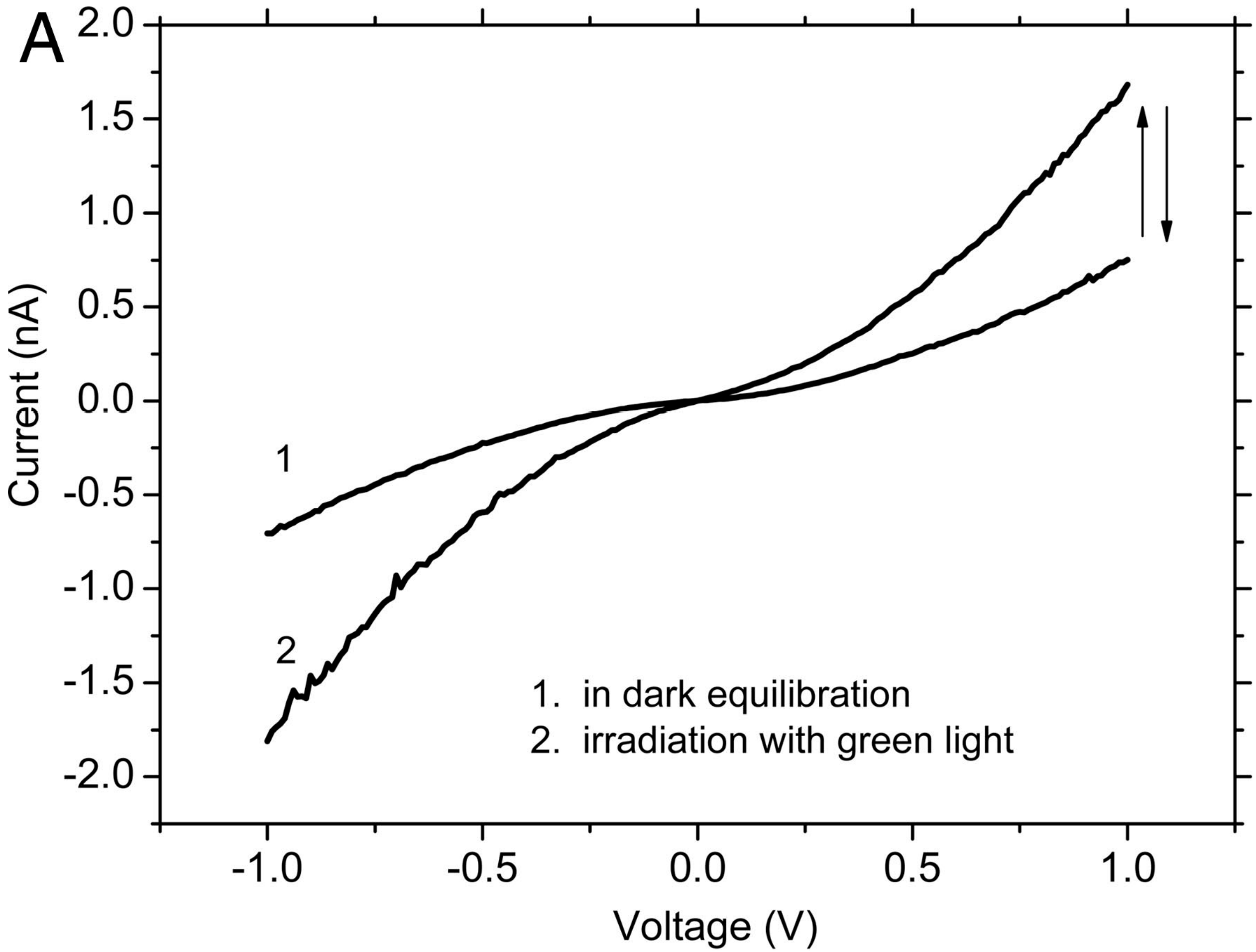
CURRET VOLTAGE CHARACTERISTICS ON NANOLAYERS OF MACROSCOPIC CROSS SECTIONAL AREA (-1 +1 V)

CURRENT VOLTAGE CHARACTERISTICS ON NANOLAYERS CARRIED OUT WITH AN AFM TECHNIQUE ON NANO AREA (-10 +10 V)

ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY ON SELF ASSEMBLED MONOLAYERS OF MACROSCIPC AREA (mV at 0.1 – 10^5 Hz)

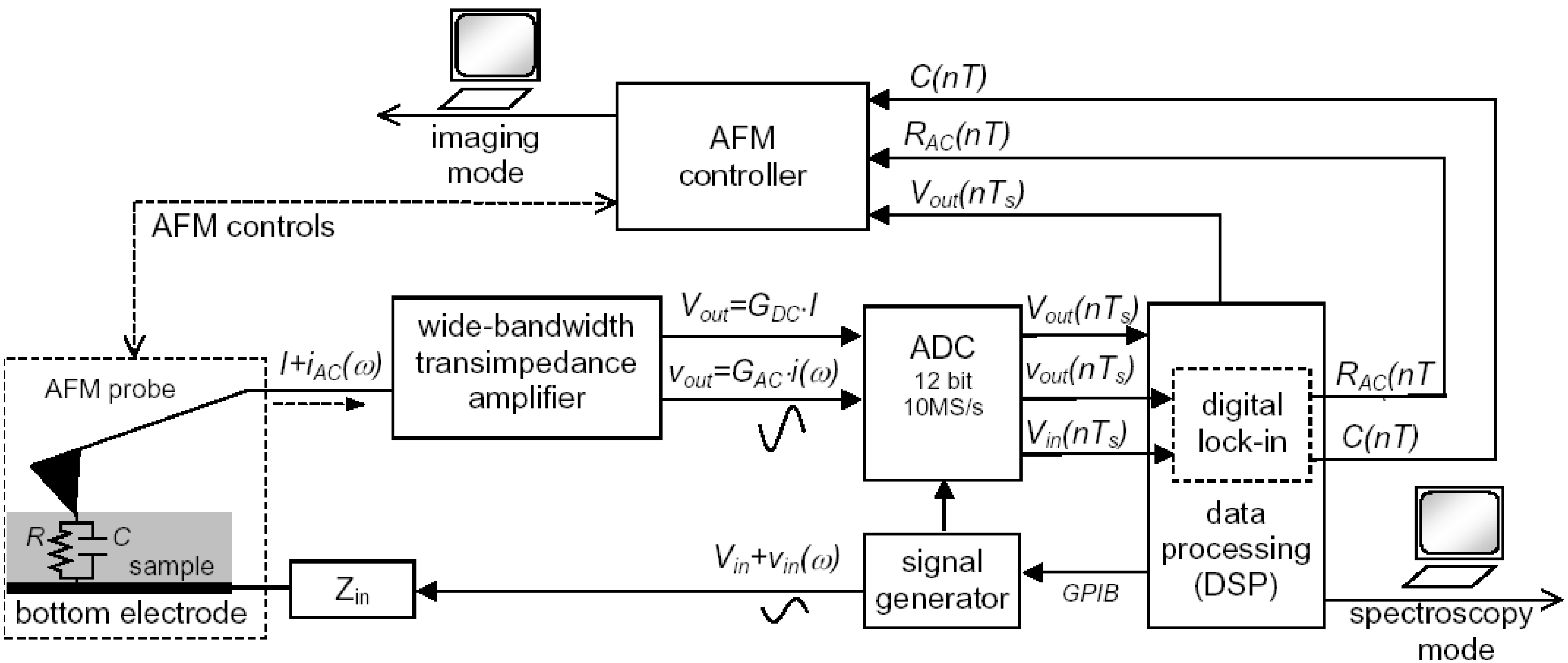
I-V ON MACRO AREA



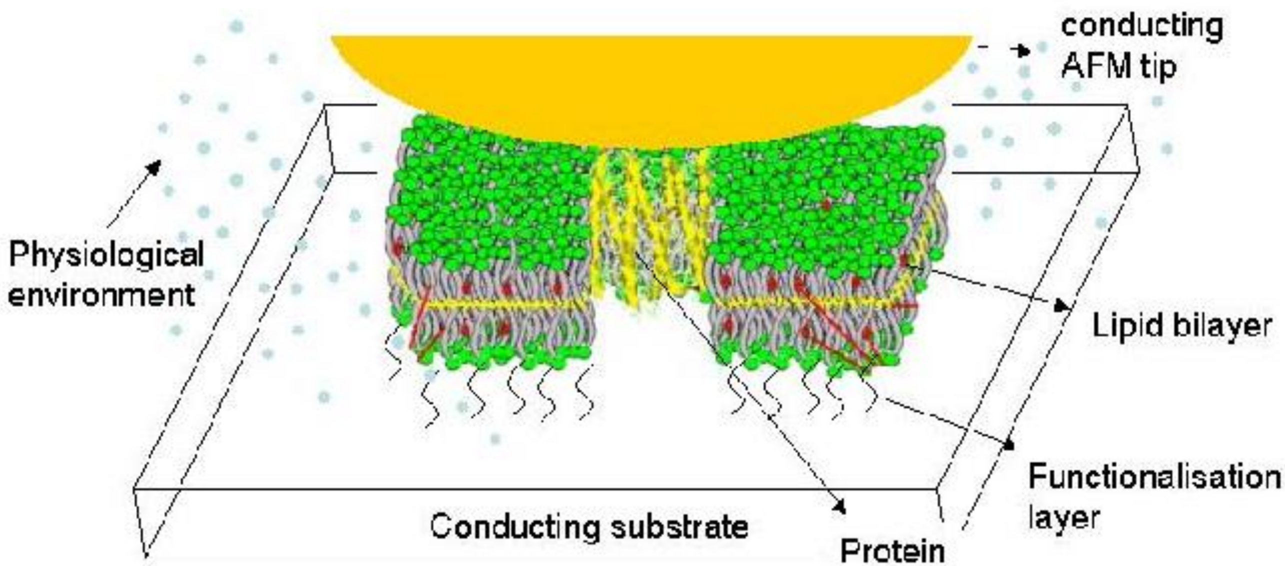


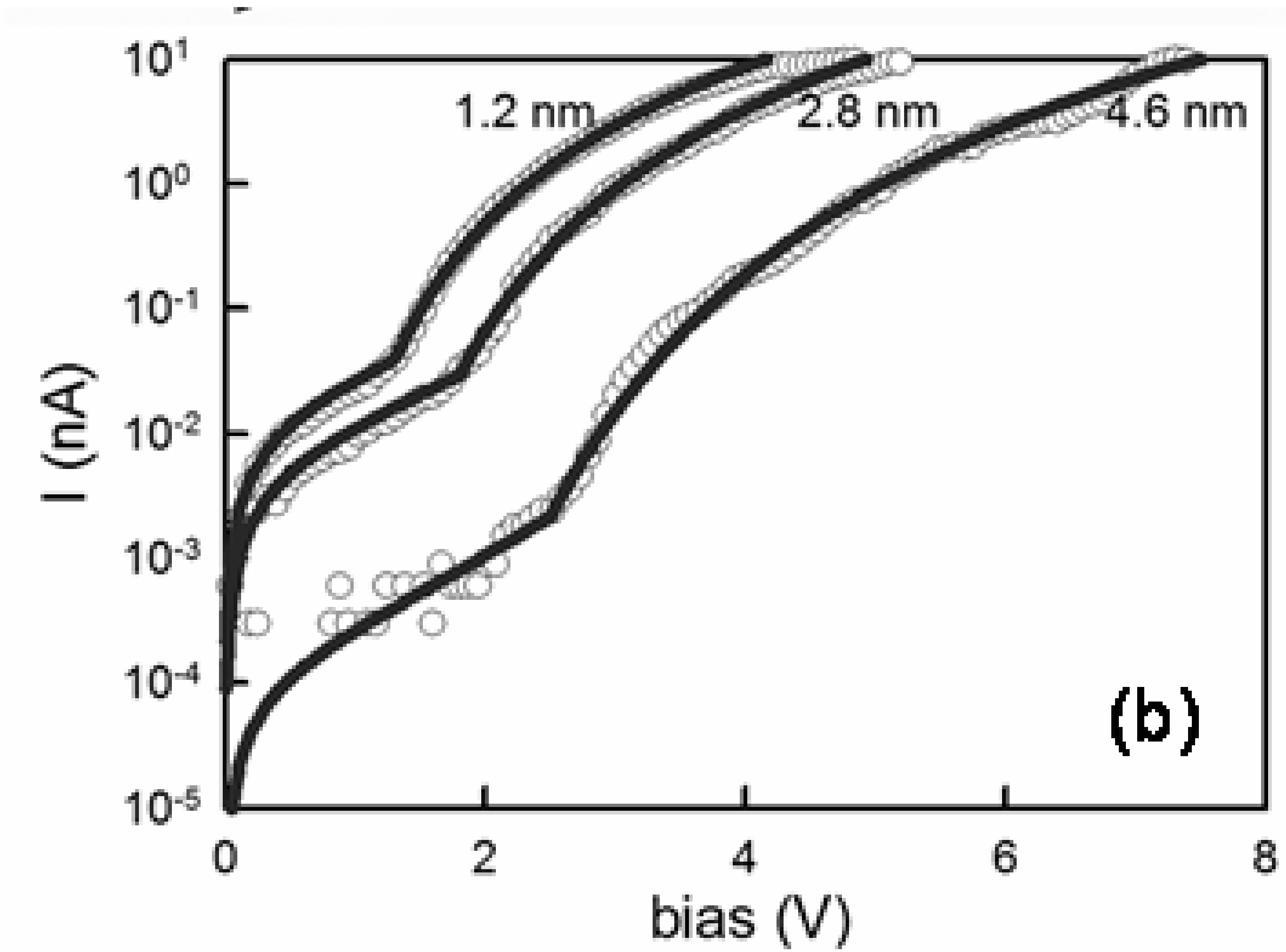
Jin et al 2006
A=2 10^{-3} cm^2
h = 5 nm

I-V ON NANO AREA (AFM)

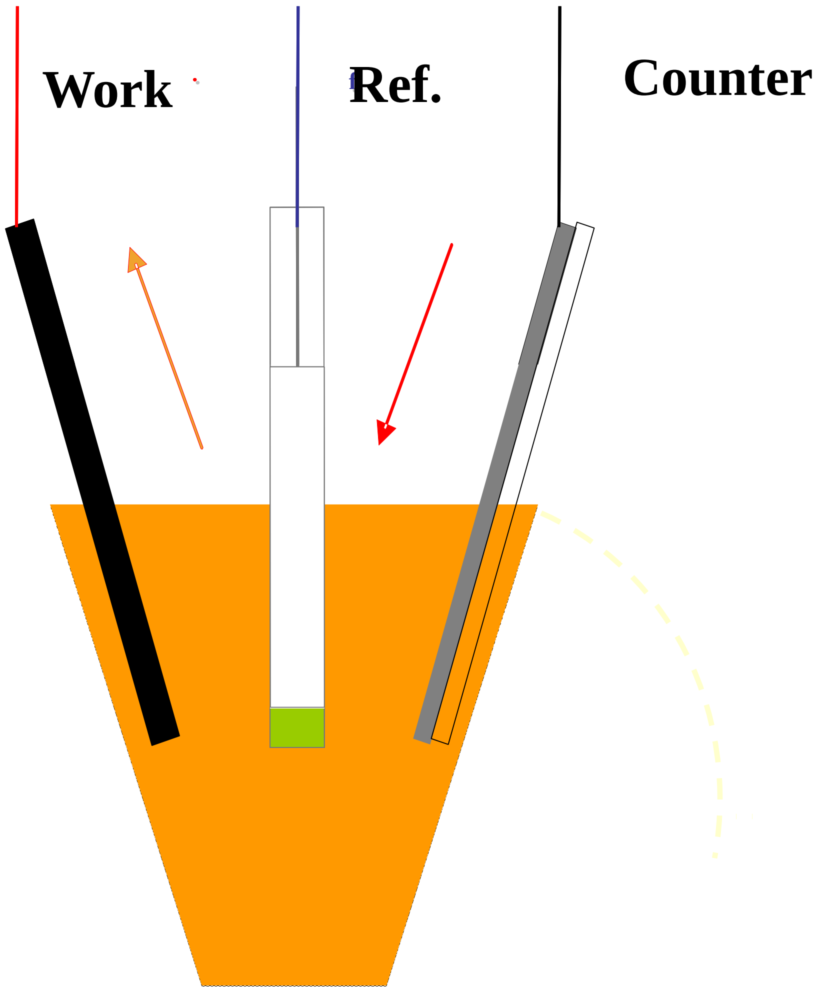


Schematic of the AFM technique and of the measurement chain


conducting
AFM tip
Physiological
environment
Lipid bilayer
Functionalisation
layer
Conducting substrate
Protein

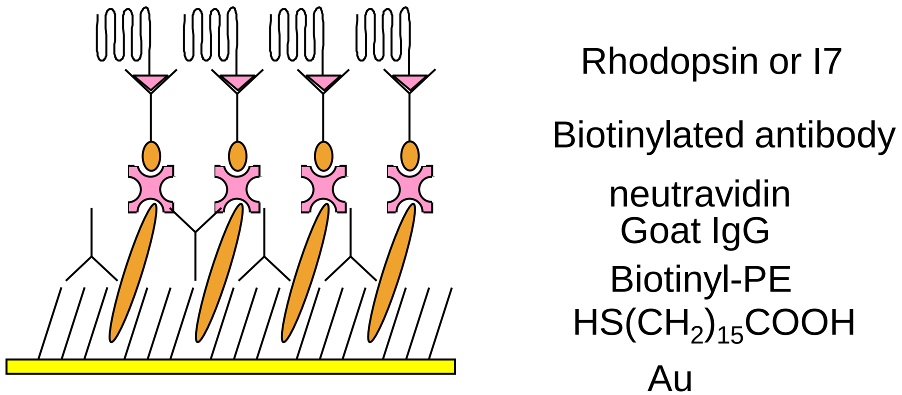


Gomila et al at IBEC Barcelona 2007
A=0.1- 0.01 nm^2
h in figure

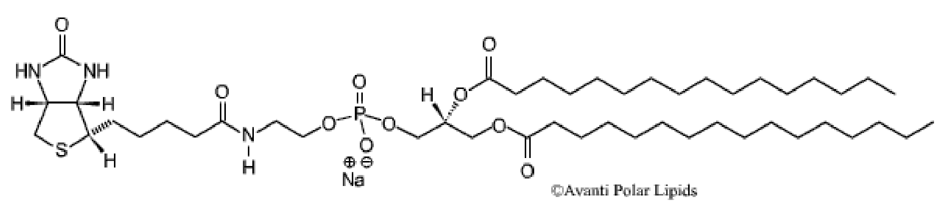


Schematic of a three electrode electrochemical cell

Formation of self-assembled multilayer

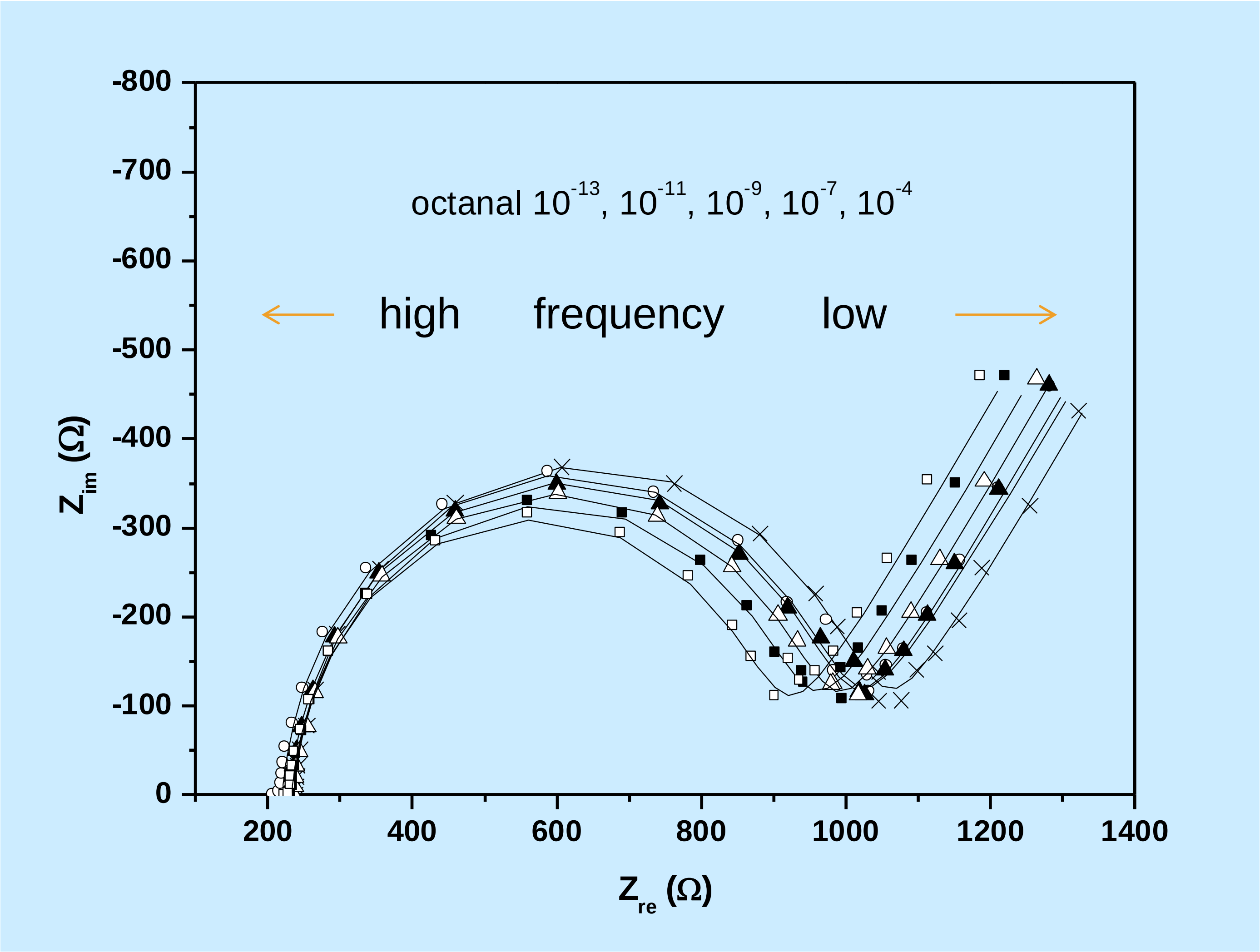


Biotinyl-PE



Thiol

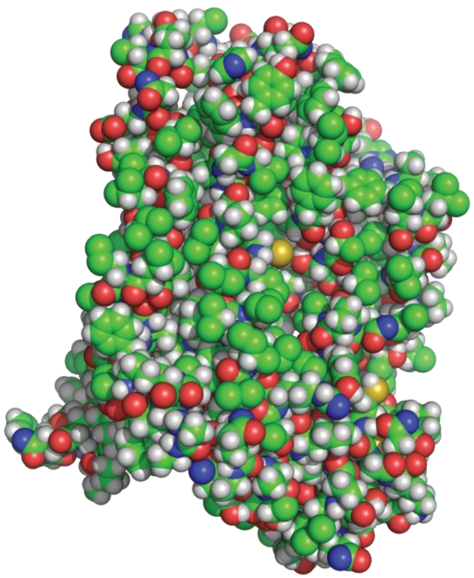
$$HSCH_2(CH_2)_{13}CH_2-\overset{\overset{\displaystyle O}{||}}{C}-OH$$

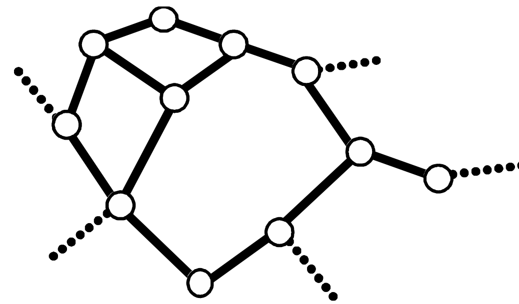


Nyquist plot of a functionalized layer with rat OR I7 in the presence of the specific odorant octanal at different concentrations

THEORETICAL MODEL

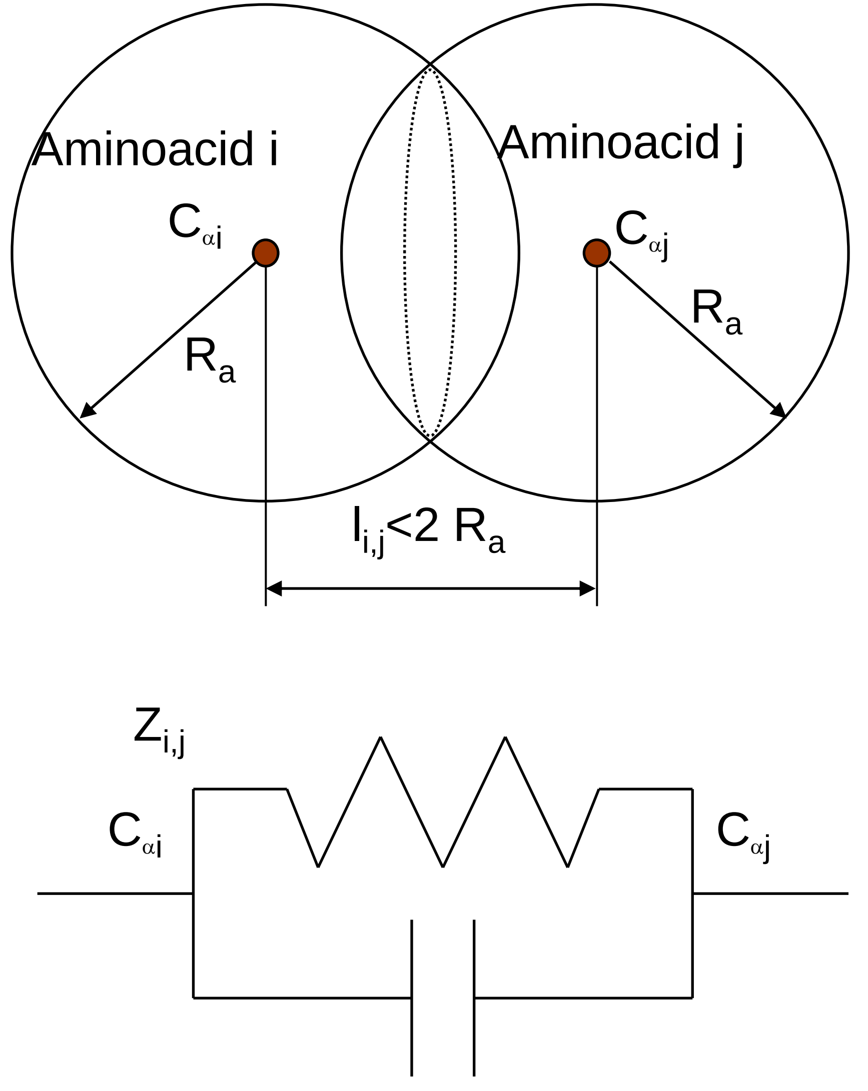
Protein Data Base (www.pdb.org)
Reconstruction from a known model (GPCRAutomodel@INRA)



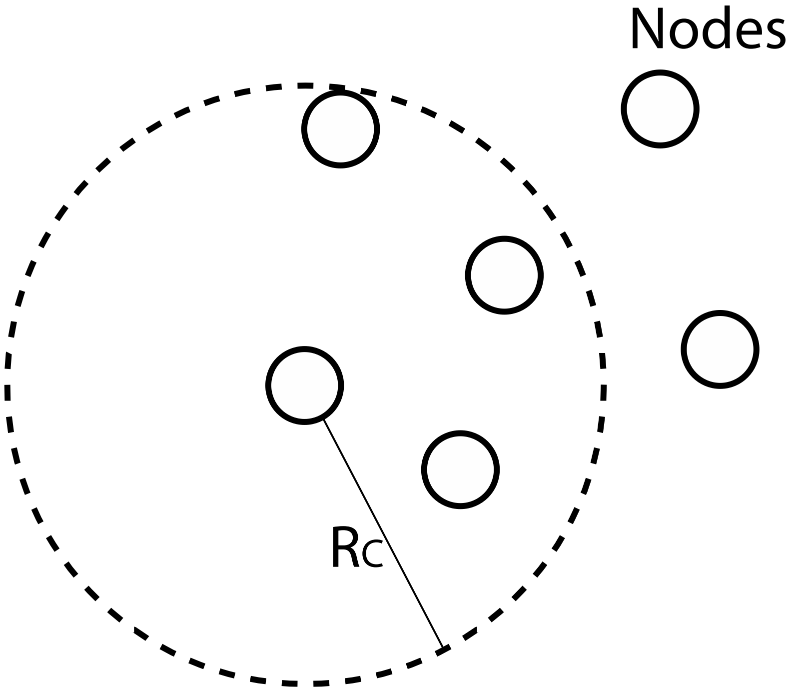


Full protein

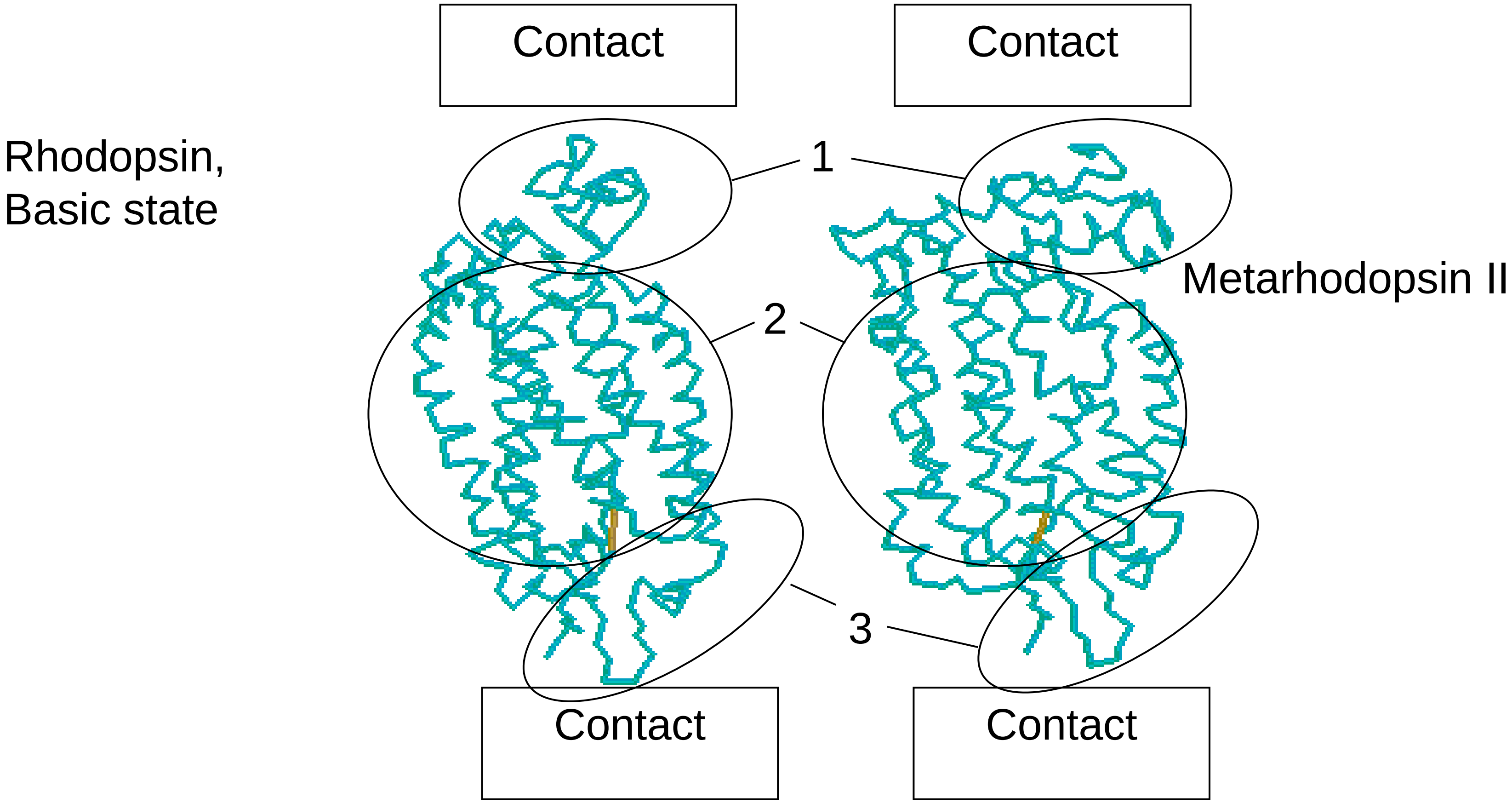
Equivalent network
(Nodes and links)



Elementary mechanism of charge transfer is the overlap between two aminoacids and the equivalent circuit element. $C_{\alpha j}$ identifies the center of the sphere corresponding to the alpha carbon atom of the *i*-th aminoacid.



Interaction radius Rc to determine the connection between nodes
The network is solved with a standard procedure based on Kirchhoff' law



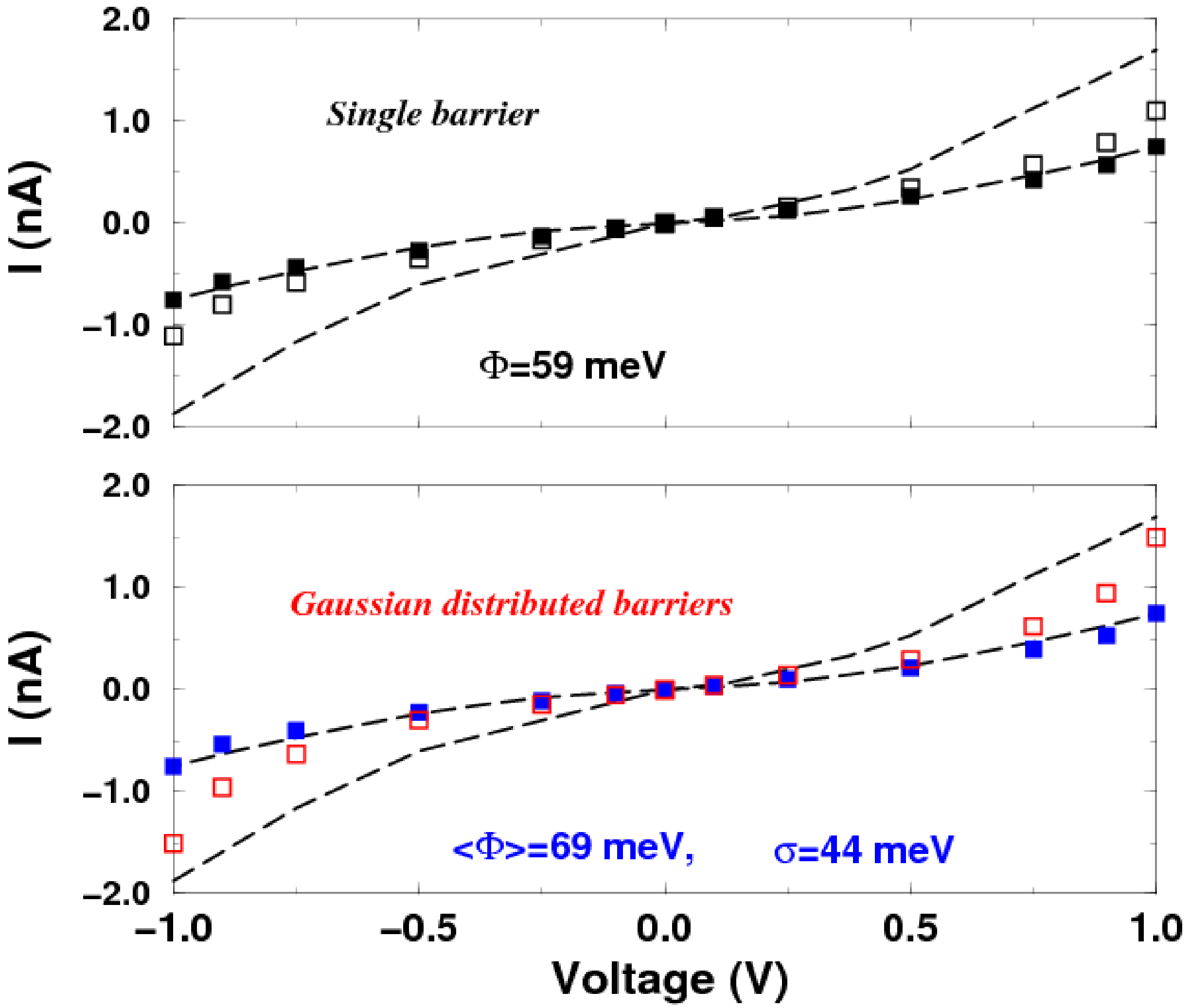
Basic state of Rhodopsin and Metarhodopsin II as constructed from the protein data base (PDB): backbones in scale:

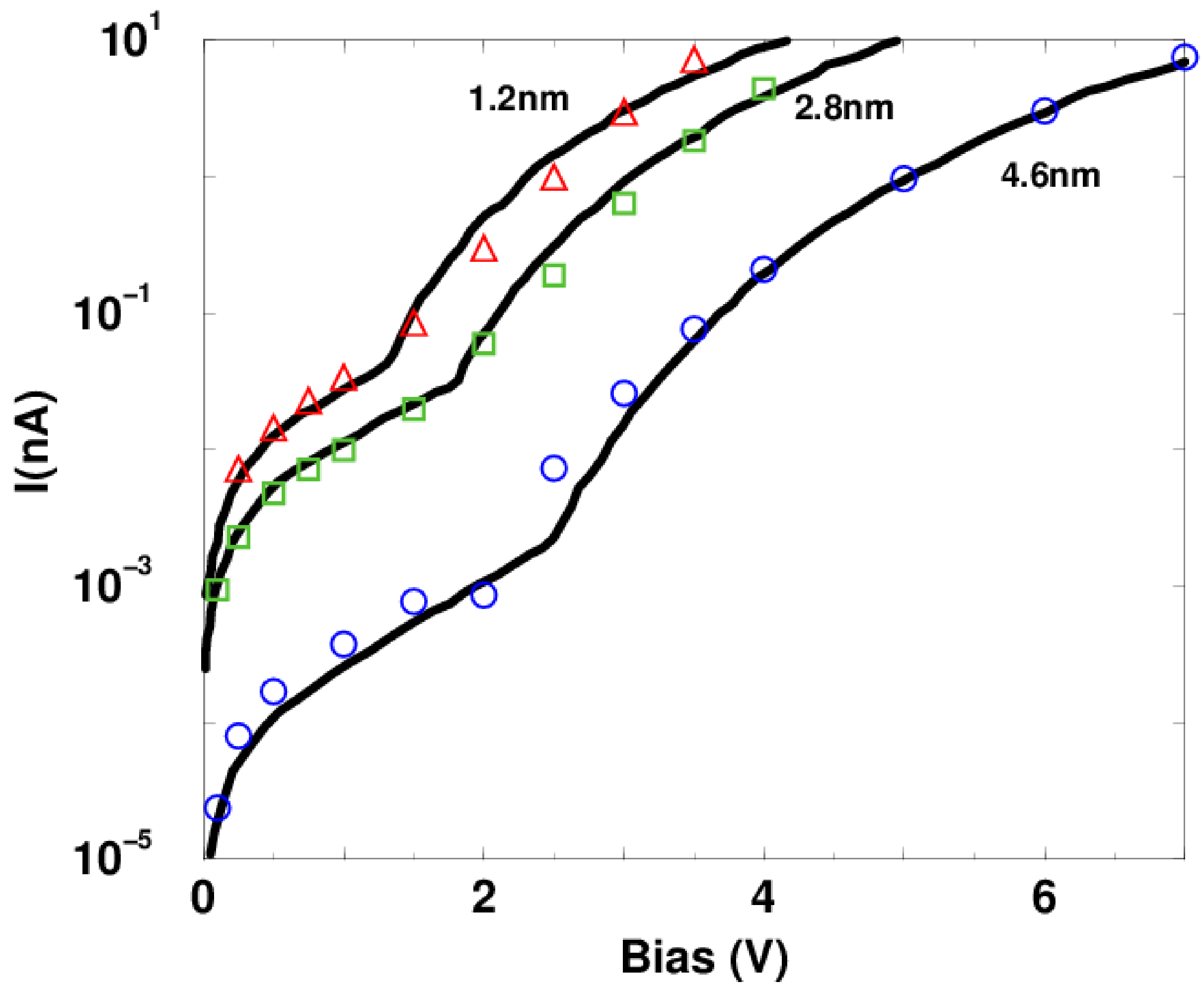
1 – C-terminus.

2 – Transmembrane core

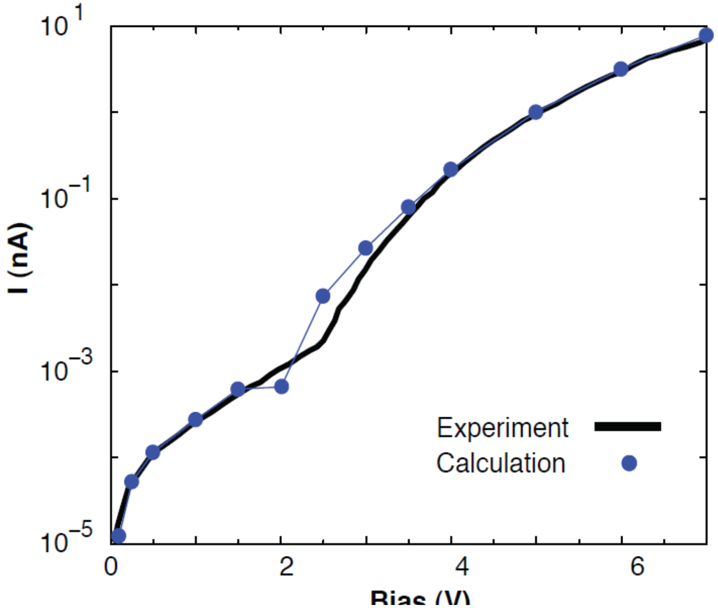
3 – N-terminus

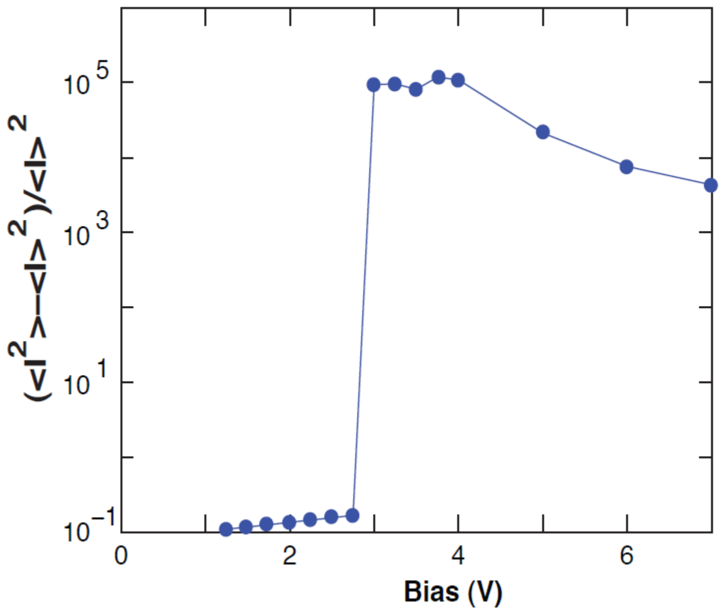
RESULTS AND DISCUSSION


2.0
1.0
0.0
−1.0
−2.0
I (nA)
Single barrier
Φ=59 meV
Gaussian distributed barriers
<Φ>=69 meV,
σ=44 meV
−1.0
−0.5
0.0
0.5
1.0
Voltage (V)



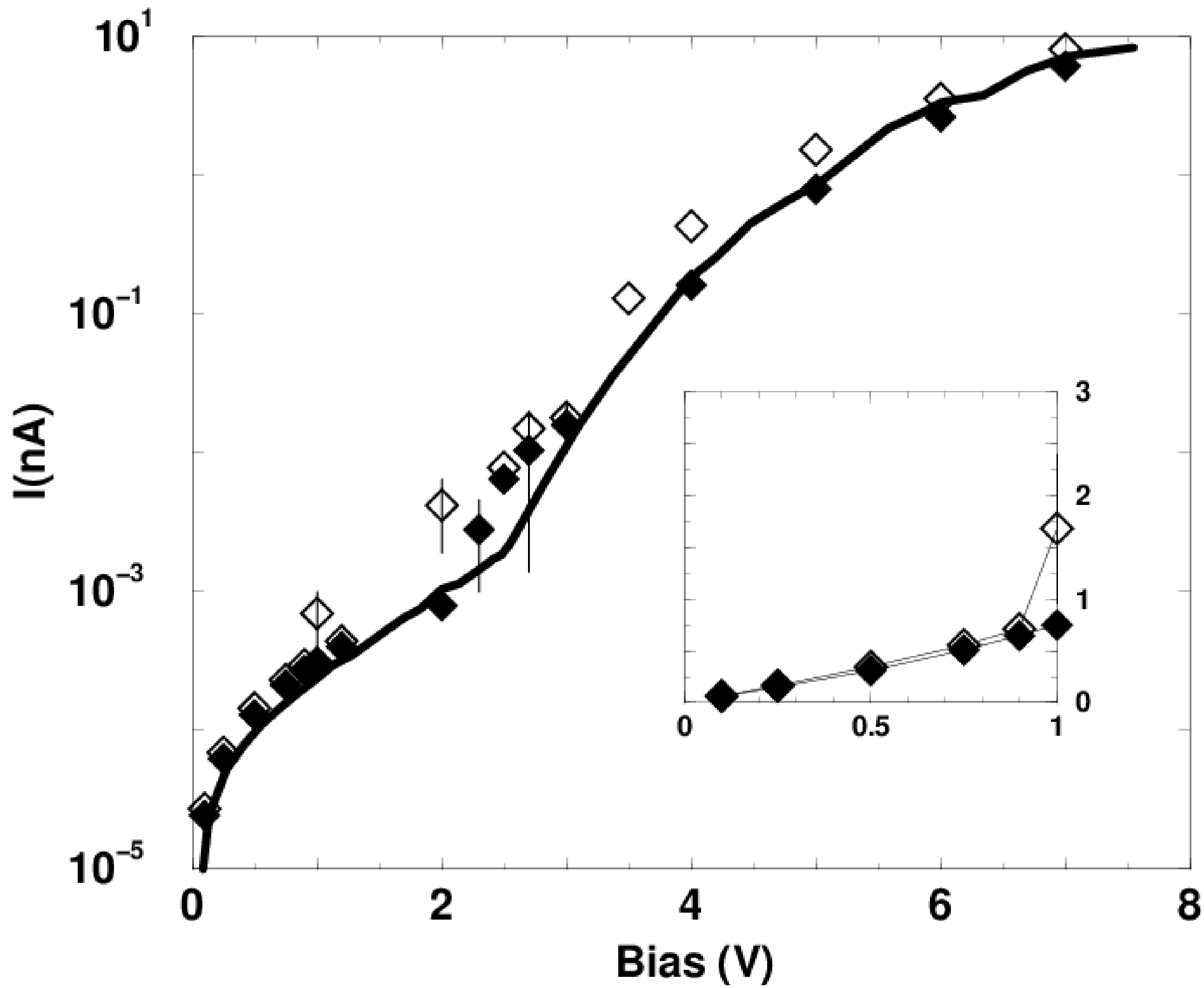
1.2nm
2.8nm
4.6nm
I(nA)
Bias (V)
10^1
10^-1
10^-3
10^-5
0
2
4
6



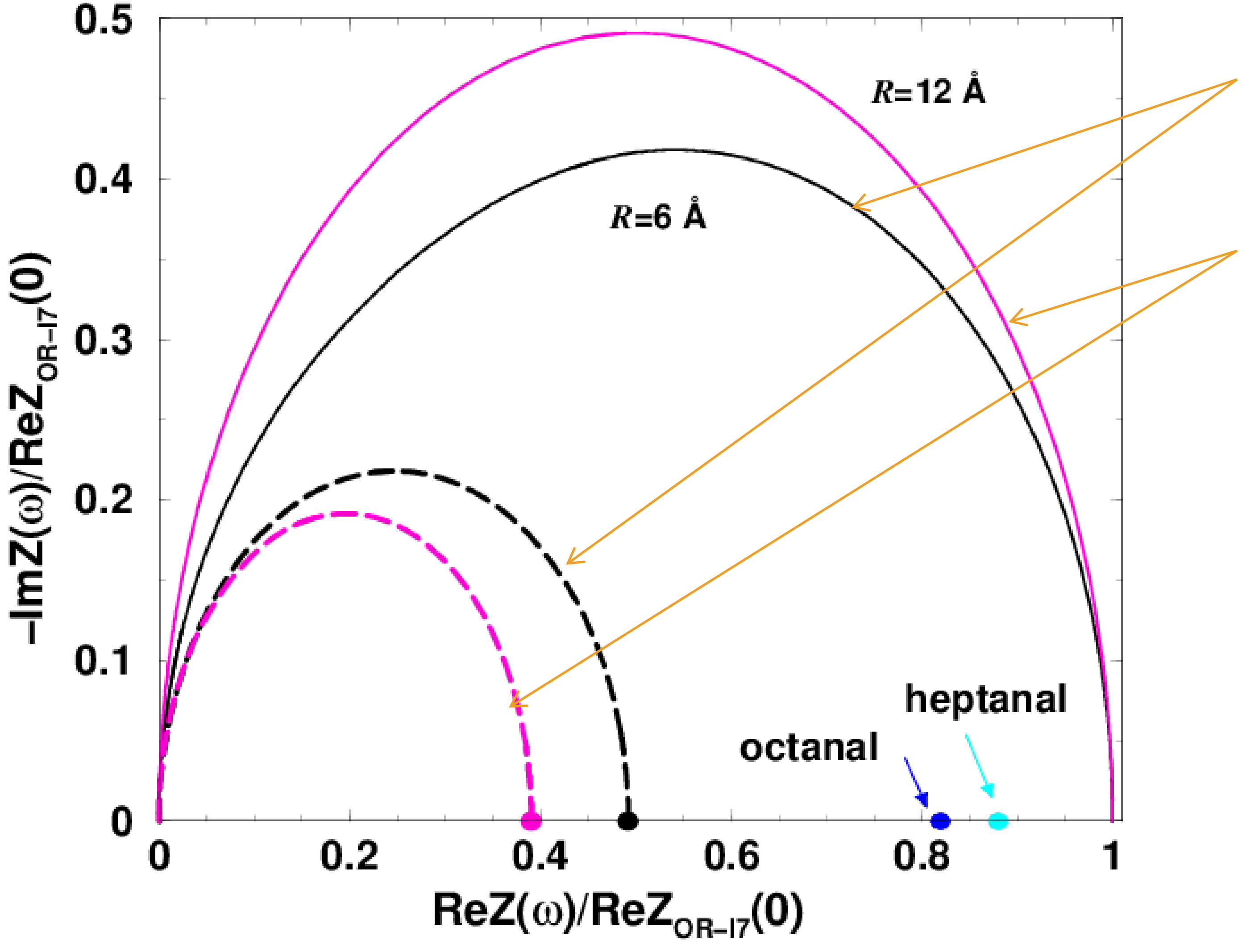


Correlation between I-V and the associated Variance of current fluctuations.

A colossal increase of current fluctuations is predicted at the cross over between direct and Fowler Nordheim tunnel regimes.



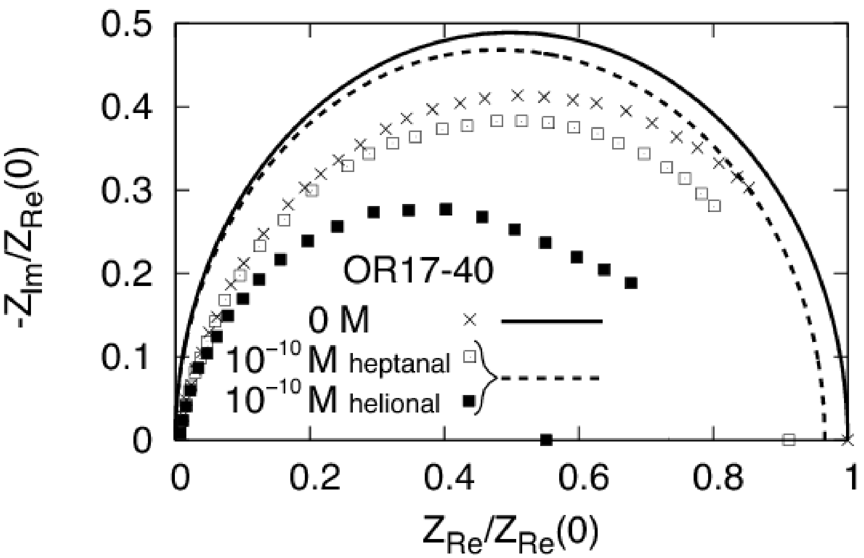
Prediction of the sensitvity of the AFM measuremts to the presence of green light


0.5
0.4
0.3
0.2
0.1
0
0
0.2
0.4
0.6
0.8
1
-ImZ(ω)/ReZ_OR-I7(0)
ReZ(ω)/ReZ_OR-I7(0)
R=12 Å
R=6 Å
heptanal
octanal

Rat OR I7

D5.5: Modeling Nyquist plots on the basis of the conformation of a given sensing protein

Available data: OR I7, OR 17-40



E. Alfinito, J-F. Millithaler, L. Reggiani, N. Zine, N. Jaffrezic-Renault, submitted to JAP

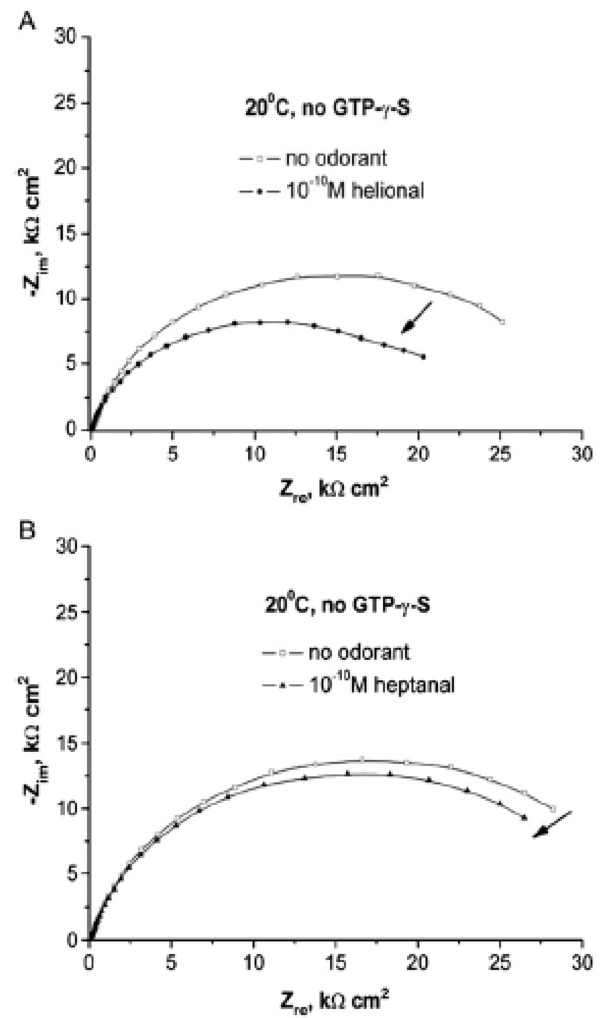
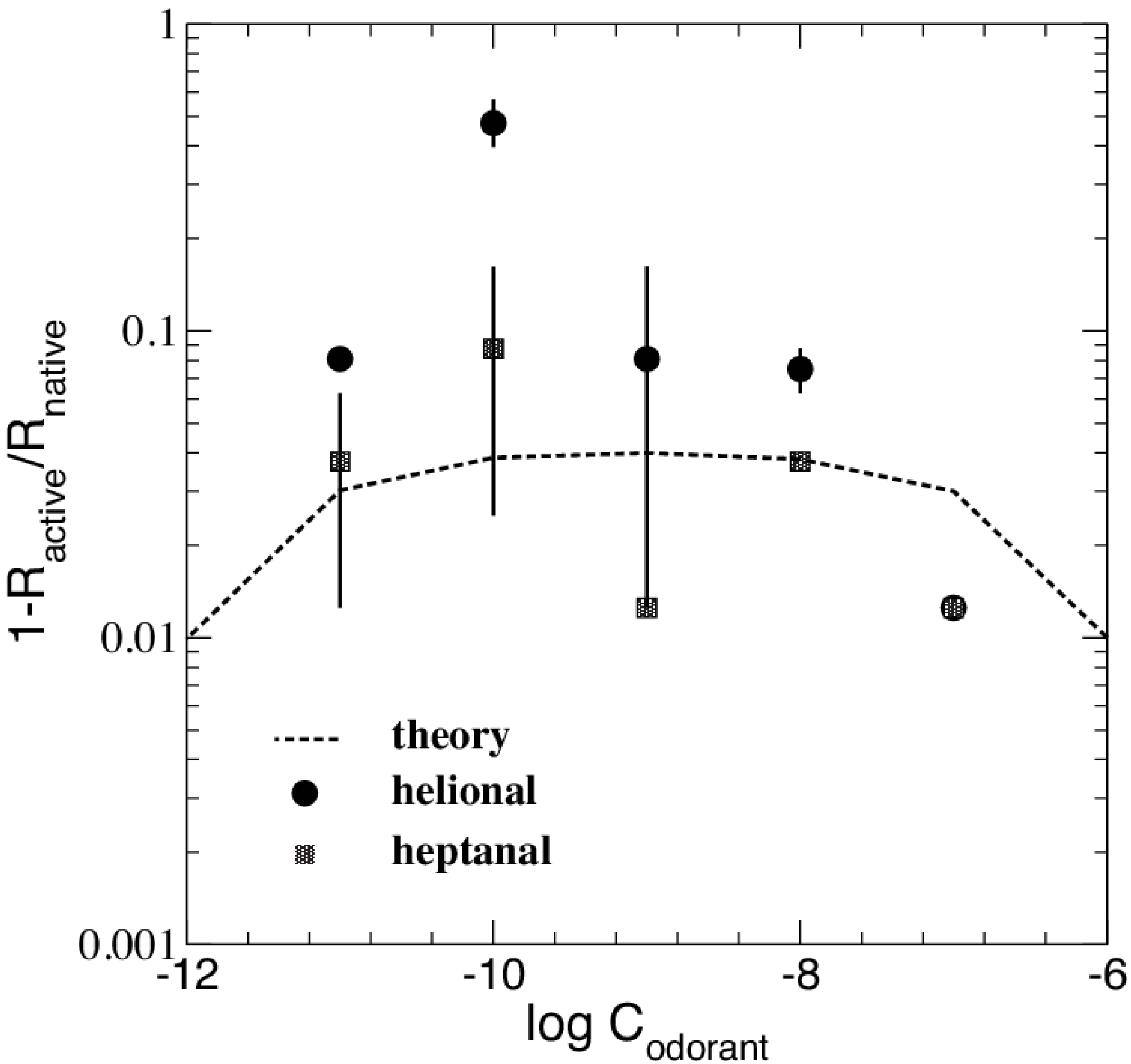


Fig. 3. Sensor responses to the injection of 10^{-10} M helional (A) and 10^{-10} M heptanal (B). Measurements were performed in PBS pH 7.0 at 20 °C.

I.V. Benilova et al. / Materials Science and Engineering C 28 (2008) 633–639



1
0.1
0.01
0.001
-12
-10
-8
-6
log C odorant
1-R active/R native
theory
helional
heptanal

OR 1740

CONCLUSIONS AND PERSPECTIVES

Sensing proteins exhibit detectable charge transfer properties

As microscopic mechanisms we suggest overlap between neighbouring aminoacids

Static I-V are dominated by tunneling mechanism of charge tansfer, direct at low voltages Fowler Nordheim at high voltages

Conformational change due to capture of the ligand leads to a detectable change of the electrical properties both as I-V and EIS characteristics

The change of electrical response of a protein due to its sensing action is promising for the developent of a new familiy of sensors which mimics the mammalian light and olfact senses carried out at a cellular (nanosize) level

The Impedance Network Protein Analogue (INPA) we have developed has been validated by comparison with experiments and provied to be a valuable first step towards a microscopic interpretation of the electrical properties of a given protein

Acknowledgments

Gabriel Gomila **Universitat de Barcelona**
Nicole Jaffrezic **Universitè Claude Bernarde 1 - Lyon**
Edith Pajot-Augy **INRA-CNRS- Paris**
Josep Samitier **Universitat de Barcelona**
Marco Sampietro **Politecnico di Milano**

THANKS FOR THE ATTENTION